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¨DYEING AND PRINTING NATURAL FIBERS WITH ALGAE-BASED COLORANTS¨

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Abstract

The crucial need for sustainable development has an immense impact on industrial evolution paving the path for a progressive transition towards a sustainable bioeconomy. Considering the pollutant evolution of the textile industry, approaches to exploring environmentally friendly alternatives to fossil-based resources and polluting processes have become the most current practice nowadays. In this sense, this study proposes the exploration of a sustainable alternative colorant material for coloration applications, from algae-based feedstock, considering the reduced environmental pressure embroiled by algae cultivation, colorant extraction, and its possible further textile application.

This study explores the viability of the use of algae-based colorants for their application in exhaustion dyeing and pigment printing coloration techniques for the textile industry. Micro and macroalgae strains selection was performed according to criteria ensuring facile and optimized cultivation and the provision of basic colors for the textile industry as blue (C-phycocyanin), red (R-phycoerythrin), yellow (β-carotene), and green (Chlorophyll-a). Liquid colorant-rich extracts were employed in the selected textile finishing processes on natural fibers, like cotton and wool.

To approach the viability of these alternative sustainable colorants and enhance coloration properties and efficiencies, a series of auxiliaries were assessed, as conventional metallic and newly discovered mordants, and biomordants. Theoretical assumptions related to the plausible bonding among fibers-mordants-colorants were approximated. The auxiliaries implication in exhaustion dyeing and pigment printing processes were analyzed through a series of characterization tests, envisaging objective color characterization determined by the measurement of CIELab color coordinates; reflectance spectrum, and color strength approaching the depth of the coloration; completed by the calculation of the absorption coefficient, based on the remaining colorant matter in the wastewater effluents (for the dyeing process). Nevertheless, the mordants' influence on the quality of the coloration process was assessed through laundering and lightfastness measured according to European standards for textiles characterization.

The dyeing exhaustion process was also subjected to parameters optimization in terms of the definition of optimum temperature, dyeing liquor ratio, pH, and time, considering the colorants' sensibilities for degrading and processing agents.

A preliminary nearing towards the dyeing wastewater effluent quality assessment supporting the sustainability character of the process was performed through the measurement of basic water characterization indicators as BOD_5, COD, metal content, and behavior against biological wastewater treatment. Furthermore, the possible added value of the algae-based colorants was investigated by measuring the antimicrobial and solar protection capacity, enlarging the application prospects of these innovative raw materials for textile applications.

The pigment printing process approached the research of the feasibility of the colorants by employing the conventional synthetic printing paste, completed with the assessment of alternative natural printing paste, considering, at the same time the commercial availability of ingredients.

The obtained results confer a preliminary validation of the suitability of the application of algae-based colorant matter in exhaustion dyeing and pigment printing of cotton and wool, at laboratory scale**,** with key elements for textile functionalization perspectives, thus paving the path for further investigation which will make possible the upscaling of the processes and industrial use of alternative sustainable materials in the textile industry.

Resumen

La inminente necesidad de un desarrollo sostenible ha influido en el crecimiento de la industria, abriendo el camino para la transición progresiva hacia una bioeconomía sostenible. La evolución de la industria textil, caracterizada por altos niveles de contaminación, exige la continua exploración de alternativas a los recursos fósiles y procesos contaminantes. En este sentido, el presente estudio propone la exploración de una alternativa sostenible de colorantes naturales procedentes de biomasa de algas, para su aplicación en procesos de coloración, seleccionados por su reducido impacto ambiental, tanto en el cultivo de las algas, como en la extracción de colorantes, y su posible aplicación textil.

Este estudio tiene como objetivo explorar la viabilidad del uso de colorantes procedentes de algas para su aplicación en procesos de coloración textil, como la tintura por agotamiento y estampación pigmentaria. La selección de micro y macroalgas ha sido realizada según criterios que facilitan un cultivo optimizado y simple, ofreciendo diferentes colores básicos para la industria textil, como son el azul (C-ficocianina), el rojo (R-ficoeritrina), el amarillo (β-caroteno), y el verde (Clorofila a). Los extractos líquidos concentrados en colorantes se han empleado en los procesos seleccionados de acabado textil, para conferir color a fibras naturales, como el algodón y la lana.

La viabilidad de las fuentes alternativas de colorantes sostenibles mencionadas anteriormente ha sido estudiada mediante el análisis de la influencia de ciertos auxiliares de coloración, como son los mordientes. Una serie de mordientes convencionales (metálicos, y recién descubiertos) y biomordientes han sido seleccionados para investigar su capacidad de mejora de las propiedades y eficacia de coloración de los colorantes sostenibles. Asimismo, se han estudiado aproximaciones teóricas relacionados con la posible unión fibras-mordientes-colorantes. La implicación del uso de mordientes en los procesos de tintura por agotamiento y estampación pigmentaria se ha caracterizado a través de la medición de las coordenadas cromáticas del espacio de color CIELab; el espectro de reflectancia junto con el cálculo de la fuerza del color definiendo estos últimos la profundidad de la coloración. El análisis se ha completado con el cálculo del coeficiente de absorción, en base al colorante remanente en los efluentes del agua residual de tintura (para los procesos de tintura). No obstante, la influencia de los mordientes sobre la calidad de los procesos de coloración ha sido evaluada mediante ensayos de medición de solideces al lavado y a la luz realizados según normativas europeas en vigor.

El proceso de tintura por agotamiento se ha optimizado para determinar los valores óptimos para la temperatura, relación de baño de tintura, pH, tiempo de proceso, teniendo en cuenta la sensibilidad de los colorantes frente a los factores degradantes y de proceso.

Siguiendo el carácter sostenible del proceso, se ha realizado una valoración cualitativa preliminar de los efluentes de aguas residuales de tinturas, a través de mediciones de indicadores básicos de caracterización de aguas, como DBO_5, DQO, contenido en metales, y el comportamiento frente a un tratamiento biológico de aguas residuales. Igualmente, se investigó el posible valor añadido de los colorantes procedentes de algas, midiendo la capacidad de protección solar y antimicrobiana, ampliando las perspectivas de aplicación de estas materias primas innovadoras para aplicaciones textiles.

El proceso de estampación pigmentaria abordó la investigación de la viabilidad de los colorantes mediante el empleo de la pasta de estampación sintética convencional, completada con la evaluación de la alternativa representada por la pasta de estampación natural, considerando, al mismo tiempo, la disponibilidad comercial de los componentes.

Los resultados obtenidos confieren una validación preliminar de la idoneidad de la aplicación de materia colorante procedente de algas en procesos de tintura por agotamiento y la estampación pigmentaria sobre sustratos textiles de algodón y lana, a escala de laboratorio, con elementos clave para las perspectivas de funcionalización textil, abriendo así el camino para futuras investigaciones que hará posible el escalado de los procesos y el uso industrial de materiales alternativos sostenibles en la industria textil.

Resum

La creixent necessitat per a un desenvolupament sostenible ha tingut un gran impacte en el creixement de la indústria, obrint el camí per a la transició progressiva cap a una bioeconomia sostenible. L'evolució de la indústria tèxtil, caracteritzada per els alts nivells de contaminació fomenta la contínua exploració d'alternatives als recursos fòssils i processos contaminants. En aquest sentit, el present estudi proposa l'exploració d'una alternativa sostenible de colorants naturals procedents de biomassa d'algues, per a la seua aplicació en processos de coloració, seleccionats a causa de l'impacte ambiental reduït, generat pel cultiu de les algues, l'extracció de colorants, i la seua possible aplicació tèxtil.

Aquest estudi té com a objectiu explorar la viabilitat de l'ús de colorants procedents d'algues per a la seua aplicació en processos de coloració tèxtil, com la tintura per esgotament i estampació pigmentària. La selecció de micro i macroalgues ha sigut realitzada segons criteris que faciliten un cultiu optimitzat i senzill, que proveeix colors bàsics per a la indústria tèxtil, com són el blau (C- ficocianina), el roig (R-ficoeritrina), el groc (β-caroteno, i el verd (Clorofil·la a) . Els extractes líquids concentrats en colorants s'han emprat en els processos seleccionats d'acabat tèxtil, per a conferir color a fibres naturals, com el cotó i la llana.

La viabilitat de les fonts alternatives de colorants sostenibles mencionades anteriorment ha sigut abordada per mitjà de l'anàlisi de la influència de certs auxiliars de coloració, com són els mordents. Una sèrie de mordents convencionals, metàl·lics, acabats de descobrir i bio mordents ha sigut seleccionada per a investigar la seua capacitat de millora de les propietats i eficàcia de coloració dels colorants sostenibles. A més a més, es va estudiar aproximacions teòriques relacionades amb la unió plausible entre fibres-mordents-colorants. La implicació de l'ús de mordents en els processos de tintura per esgotament i estampació pigmentària s'ha analitzat mitjançantassajos de caracterització, contemplant la caracterització objectiva del color per mitjà del mesurament de les coordenades cromàtiques de l'espai de color CIELab; l'espectre de reflectància junt amb el càlcul de la força del color, definint la profunditat de la coloració; anàlisi completada pel càlcul del coeficient d'absorció, a base del colorant romanent en els efluents de l'aigua residual de tintura (per als processos de tintura). No obstant això, la influència dels mordents sobre la qualitat dels processos de coloració ha sigut avaluada per mitjà d'assajos de mesurament de solideses al llavat i a la llum realitzats segons normatives europees en vigor.

El procés de tintura per esgotament ha sigut optimitzat per a determinar els valors òptims per a la temperatura, relació de bany de tintura, pH, temps de procés, valorant el caràcter sensible dels colorants front als agents degradants i de procés.

Recolzant el caràcter sostenible del procés, s'ha realitzat una valoració qualitativa preliminar dels afluents d'aigües residuals de tintures, mitjançant mesuraments d'indicadors bàsics de caracterització d'aigües, com DBO5, DQO, contingut en metalls, i el comportament enfront d'un tractament biològic d'aigües residuals. Igualment, es va investigar el possible valor afegit dels colorants procedents d'algues, mesurant la capacitat de protecció solar i antimicrobiana, ampliant les perspectives d'aplicació d'aquestes matèries primeres innovadores per a aplicacions tèxtils.

El procés d'estampació pigmentària va abordar la investigació de la viabilitat dels colorants per mitjà de l'ocupació de la pasta d'estampació sintètica convencional, completada amb l'avaluació de l'alternativa representada per la pasta d'estampació natural, considerant, al mateix temps, la disponibilitat comercial dels ingredients.

Els resultats obtinguts confereixen una validació preliminar de la idoneïtat de l'aplicació de matèria colorant procedent d'algues en processos de tintura per esgotament i l'estampació pigmentària sobre substrats tèxtils de cotó i llana, a escala de laboratori, amb elements clau per a les perspectives de funcionalització tèxtil, obrint així el camí per a futures investigacions que farà possible l'escalat dels processos i l'ús industrial de materials alternatius sostenibles a la indústria tèxtil.

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Abbreviations and terms

Absorption coefficient	K
Absorbance value	A
Allophycocyanin	APC
Average effective UVR irradiance for the protected skin	E¢
B-phycoerythrin	B-PE
Biochemical Oxygen Demand	BOD
Biochemical Oxygen Demand of the sample during 5 days of incubation	BOD_5
Blue-yellow color space coordinate	*b**
Blue-yellow color difference	Δb
Chlorophyll a	Cl a
Chlorophyll b	Cl b
Chlorophyll c	Cl c
Chlorophyll d	Cl d
Chlorophyll f	Cl f
Color difference	ΔE
Color strength	K/S
C-phycocyanin	C-PC
Chemical Oxygen Demand	COD
Compound annual growth rate	CAGR
Difference (delta)	Δ
Dyeing liquor ratio	Rb
Effective UVR irradiance for unprotected skin	Eeff
Fourier transform infrared	FTIR
High-performance liquid chromatography	HPLC
Integrated Multi-Tropic Aquaculture	ITMA
Commission Internationale de l´Eclairage	CIELAB
Luminosity color space coordinate	*L**
Luminosity difference	ΔL
Membrane photobioreactor	MBR
Molar absorption coefficient	ε
Molar concentration	c
Optical path length	l
Photocatalytic Membrane Reactor	PMR
Phycocyanin	PC
Phycoerytrhin	PE
Plate Count Agar	PCA
Potential of Hydrogen	pH
Reflectance at maximum wavelength	R
Red-green color space coordinate	*a**
Red-green color difference	Δa
R-phycoerythrin	R-PE
Scattering coefficient	S
Scanning electron microscope	SEM
Solar spectral radiance	Sh
Trypon Soy Broth	TSB
Ultraviolet protection factor	UPF
Ultraviolet radiation	UV
Visible spectrum	VIS
Wavelength	λ
Weight of fabric	w.o.f.

Chapter 1. Introduction

This initial chapter envisages an introductory part that contextualizes the further sections, focusing on describing the context and background of the study, the problem addressed, and finally the proposed solution to the polluting character of the textile industry fossil-based colorant matter.

1.1. Context and background

The definition of sustainability indicates that a current generation should fulfill its needs without compromising the achievement of the needs of future generations (Brundtland 1987).

Sustainability has become a focus point for the last decades to the global development, thus to all industrial sectors, generating an imminent need for improvement and consideration, being at the base of the United Nations agenda (United Nations Department of Economic and Social Affairs 2015) and European Commission Circular Economy Action plan (Commission 2019) (European Commission 2015). This action plan is sustained by the Green Deal pillar, which aims at disconnecting the resource use from the economic growth, apart from social and climatic concerns (EU 2020). In this sense, the United Nations have adopted the 17 sustainable goals (SDGs) as main objectives for 2030, aiming, overall, at ending poverty, improving health and education, diminishing inequality, fostering economic growth, meanwhile tackling the major issue of climate change, and protecting oceans and forests (United Nations Department of Economic and Social Affairs 2015).

In this context, the aimed societal and industrial path is oriented towards the transition to the ultimate concept of circular bioeconomy. For a better understanding, it is worth highlighting that the bioeconomy is a developing domain fostering the employment of renewable biological resources for socio-economic and environmental benefits (F et al. 2014). Meanwhile, the circular economy can be narrowed down to the opposite concept of the linear economy, focusing on closing resource loops, for promoting maximal material resource use and minimal residues generation (Bocken et al. 2016). Therefore, the circular bioeconomy can be defined as the convergence of the described concepts focusing on the use of natural, renewable raw materials in the most efficient manner, harboring sustainability (Tan and Lamers 2021).

The textile industry represents an important waste producer, including waste types considered hazardous, which can be neither recycled nor disposed of, generating the need of encountering a viable alternative (Islam 2021) (Salvador 2021).

In this global development context, the clothing and textile industry displays an alarming increase, and its characterized by the demand for abundant amounts of fresh water and chemicals for its process, with millions of tons of CO_2 emissions and waste, accounting for 10% of the environmental impact in EU consumption (Sajn 2019). The annual worldwide production of dyes adds up to more than 10 000 types which are aimed at various industries, as textiles, food, paper, and plastics (Bhatia et al. 2017).

The negative environmental impact generated by the use of dyes in the textile industry is highly influenced by their production process, based on petrochemical fossil-based raw material and synthesized through noxious chemical reactions, on the one side (Bhute 2015). This is completed by their use, finalizing with the accumulation of different types of dyes and inorganic and organic pollutants in the textile effluents emitted to the environment (Saxena et al. 2021). Considering that approximately 10 to 15% of dyes pollute surface and underground waters after exiting the processing unit (Koroglu et al. 2018), and they are stable, recalcitrant and some of them have a toxic and carcinogenic effect on the wastewater effluent, thus consequently represent a high risk for human health (Moussavi and Mahmoudi 2009) (Ghodke et al. 2018).

In this context, the textile industry, led by consumerism, and characterized by disposable or fast replaceable items, has become an industry driven by economic sustainability at the expense of the concept of environmental sustainability, which is systematically disregarded (Islam 2021).

Sustainable feedstock as a source for industrial raw material is gaining increasing importance and support from the European community, with a special focus on the marine environment (Commission 2019). In this sense, a new industry is emerging, characterized by premature technological industrial development, and it is represented by the production of aquatic biomass, cultivation of algae, comprising micro and macroalgae, for multidisciplinary and multipurpose use.

Algae represent a large group of aquatic ancient plants, autotrophic and photosynthetic organisms, that live in water, as solitary cells, colonies, or filaments represented by the microalgae group and, on the other hand with plant-like morphologies, defined by the macroalgae group (Amulya et al. 2016) (Håkanson 2009).

These organisms (micro and macroalgae) metabolize a series of high-added value compounds, like proteins, lipids, carbohydrates, carotenoids, vitamins (Mobin and Alam 2017) (Rico et al. 2017) (Papadaki et al. 2017), for a large range of industrial applications as pharmaceutical, cosmetics, pigments, food ingredients, fertilizers, fibers, biofuels and many more (Kuddus et al. 2013) (Sharma and Sharma 2017) (Kumar et al. 2021).

The added value of the algae-based bioindustry lay on the very reduced interference with the fight over natural resources, and fertile land, water, and oxygen (Béchet et al. 2017), as algae, present rapid growth, low demand for arable areas, the capacity of reaching high yields for ingredients/elements of interest through controllable and optimized conditions and parameters (Matos et al. 2015) and representing a valuable CO_2 sink (Slade and Bauen 2013) (Campbell et al. 2019)(Kumar et al. 2021).

Even though the European algae bio-industrial scenery is in rapid development, it must be highlighted that technological evolution including pilot-scale highly cost-efficient biomass cultivation equipment and sustainable ingredients obtention processes have been developed, presenting thus, high potential for this sector industrialization (Camacho et al. 2019).

1.2. The problem

An overall overview of the historical evolution of the textile industry illustrates an accelerated development focused on rapid processing and low-cost production, with its corresponding methods and technologies, mostly disregarding environmental aspects. In this sense, the textile industry employs vast amounts of fresh water, electricity, and fuel, and releases greenhouse gas emissions and highly contaminated effluents (Hasanbeigi and Price 2015).

The contamination generated by the textile industry occurs from various perspectives, affecting environmental and human health, distributed overall the complete production chain, from the production of raw material, fibers, and dyes, to the final use (Islam 2020).

Textile industry pollution affects different dimensions starting with air pollution, accounting for approximately 10% of global carbon emissions, including Volatile organic components (Glycolic ether, detergent, combustion gases, reactive, components, and volatile molecules, among), Nitrous oxide, and Sulphur dioxide, Aniline vapors, carrier Hydrogen sulfide, Chlorine, and Chlorine dioxide, Carbon monoxide, Hydrocarbons and Ammonia, Formaldehyde, and many more, resulting from processing activities as coating, hardening, dyeing, bleaching, factory boilers, etc. (Claudio 2007) (Chron.com 2019)(Islam 2020).

Apart, the resulting wastewater from the textile activities degenerates into water pollution, due to the high level of contamination of the finishing effluents, causing reduction of oxygen concentrations, pollution through high contents of organic chemicals, hardly degradable dyes, finishing salts, Sulphur, Naphthol, vats, Nitrates, Acetic acid, soaps, Chromium compounds, very toxic materials like Copper, Arsenic, Lead, Cadmium, Mercury, Nickel, and Cobalt, completed by Formaldehyde (remaining from fixing agents), Hydrocarbons (resulting from softeners), and also colloidal matter. In addition, these effluents may often impact through high temperatures and pH, with a very negative impact on the environment (Gleeson and Labonté 2020) (Islam 2019). Additionally, maritime pollution is defined by the increased amount of microplastics affecting the aquatic environments and finally reaching the water consumed by the population (The UK Marine SACs Project 2001) (Halder and Islam 2015).

Farther, the textile industry also contaminates via high amounts of non-toxic and toxic solid waste. In this sense, elements as packaging, residual fabric scraps, machine parts represent non-toxic waste as they can be subjected to recycling procedures. Nevertheless, the toxic wastes, cannot be recycled, only with very innovative technologies, and may be represented by bleach, sludge, fiber packaging and its waste, and many more (Sobhani et al. 2019).

Direct negative impact on the environment and human health is generated by noise pollution originating (Sumardiyono et al. 2019) from processing sections as spinning, weaving, knitting, dyeing, printing, finishing, and sewing, with recorded noise levels of 70-100 dB, and additional contaminants as dust, fluids, dirt and other chemicals (Mehwish and Mustafa 2016) (ID et al. 2017) (Hinson et al. 2016). Aside, high relevance is given to the increase of CO_2 emissions, due to the need for energy for all the processes involved, with immense amounts of carbon released into the atmosphere (Pachauri et al. 2014). This is completed by the 1,2 billion tons of greenhouse gases produced every year (Sajn 2019).

Human basic needs are being affected by the development of this industry considering the increased need for fresh water for the production and processing steps, large agricultural surfaces for the production of natural fibers, followed by the release of chemicals directly into the human food chain (microplastics, chemicals, etc.) (Islam 2020).

According to various studies and assessments performed by the European Commission, it can be indicated that the textile industry represents the 2nd most contaminant industry, after the oil industry (Islam 2020) (Bhatia 2017) (Cyril Villemain-UNEP 2019) (European Commission 2020).

The contaminant effect of synthetic dyes used in the textile finishing industry represents a major concern for human and environmental health in recent years (Srivastava and Sofi 2019). Firstly, the synthesis of synthetic dyes lays on fossil-based, non-renewable hydrocarbon,

environmentally unfriendly raw materials, relying on the use of large amounts of energy (Nambela et al. 2020).

Furthermore, the resulting synthetic dyes are characterized by high stability to temperature, physicochemical and optical factors with high resistance to degradation due to their symmetrical aromatic structures, generating a complicated removal problem (Lima et al. 2019). The magnitude of the problem must be considered in the context of the use of approximately 150 liters of freshwater for each kilogram of fabrics generated through dyeing and printing processes, discarding through the washing process, a proportional amount of wastewater effluents characterized by an accumulation of dyes and auxiliaries (Sajn 2019). The proportion of wasted dyes and auxiliaries is considered to be 10-60%, generating a loss of 280,000 tons of dyes per year, with additional chemical products and dyeing and printing auxiliaries (Berradi et al. 2019). The wastewater effluents resulting from the textile dyeing and printing processes are characterized by the presence of high concentrations of diverse organic and inorganic chemical contaminants, turbidity, chrominance, high chemical oxygen demand (COD), and biochemical oxygen demand (BOD) (Meng et al. 2020). Additionally, the printing process, specifically, generates also air contamination through the evaporation into the atmosphere of compounds used in thickeners or binders, as, for example, kerosene, or formaldehyde, carbon dioxide, etc. (El-Molla and Schneider 2006).

Environmental and human health safety is endangered by the untreated dyes reaching water bodies, through coloration, with a direct impact on water transparency, interfering with the light penetration, thus disturbing the photosynthetic process, affecting the complete ecosystem (Mahdavi Talarposhti et al. 2001). Once reaching potable water, synthetic dyes are considered an indirect cancer-determining source and mutagenicity (Bhatia et al. 2017) (Berradi et al. 2019).

Remediation of the environmental pollution generated by the textiles industry has been vastly researched, mainly due to the contamination and use of potable water, and it can be approached from the perspective of a conscious selection of raw materials, chemicals, and auxiliaries used in the process; or through the development of highly efficient and sustainable processes of wastewater treatment (Yaseen and Scholz 2019).

In this sense, the present study focuses on exploring the suitability of the use of algae-based colorant matter, as a dyestuff in selected textile finishing processes, like dyeing and printing, at a laboratory scale.

1.3. Natural colorant matter in the textile industry

Textile coloration was developed based on natural dyes since historical times (Shukla and Vankar 2017a), reaching a shift in 1856, with the discovery of the first synthetic dye ¨mauve¨ by W.H. Perkin, generating an intense increase in development, production, and application of the synthetic dyes, thus oblivion of the natural ones (Shahid et al. 2013b). Nevertheless, due to the health and environmental concerns generated by this exhaustive use of synthetic dyes, natural dyes reemerged as their sustainable alternative, to some extent (Gong et al. 2019).

From a structural point of view, colorants are formed by two types of atomic groups responsible for color, defined as chromophores, with color-defining roles and auxochromes

accountable for color enhancement. These atomic groups are linked through a conjugated system, where the chromophores are commonly electron-withdrawing groups and the auxochromes are usually electron-releasing, acting within the donor/acceptor chromogen concept (Christie 2015).

The colorant matter is categorized in dyes and pigments, according to the existence or not of affinity for the substrate to be applied on. In this sense, dyes present this affinity, which is defined by interaction mechanisms relying on the physico-chemical properties of the dye and substrate. Meanwhile, pigments are characterized as colored compounds lacking affinity, thus no interaction mechanisms, and implying the necessity of the use of a binder, for coating a substrate. Additionally, the dyes and pigments, are also classified based on their origin, into natural or synthetic, being equally applied on various substrates (Gürses et al. 2016)(Nambela et al. 2020).

Natural dyes and pigments represent the coloring material obtained from natural sources without the need for chemical processing or modification (Shahid et al. 2013b). These can be obtained from conventional naturally occurring sources, classified into four categories, specifically plant/vegetable, insect/animal, mineral, and microbial (Sheikh et al. 2016) (Vankar 2016). These natural colorants are represented by secondary metabolites, synthesized by the previously mentioned organisms, representing molecules with low molecular weight, and defined by the fact that their production is unnecessary for growth and reproduction (Mosunova et al. 2020). The following table (Table 1) summarises specific sources of natural colorants for each identified category.

Type of natural colorant	***Obtention source***	***Reference***
Plants/vegetable origin	From all the parts of the plants: root or trunk, bark, seed, fruit, flower, leaf	(Bhute and S 2015)
Insect/animal origin	Organisms excretion or dried bodies	(Yusuf et al. 2017)
Mineral origin	Are inorganic colorants obtained from metal salts and metal oxides	(Tuli et al. 2015)
Microbial origin	Microorganisms such as bacteria, algae, and fungi produce stable pigments	(Yusuf et al. 2017) (Yuan et al. 2018)

Table 1. Natural colorants classification by origin

The natural dyes and pigment classification, apart from considering the origin factor, is additionally performed considering their chemical structure and application method on textiles (Yusuf et al. 2017), as detailed in Table 2 below.

Clasification criteria	***Types of natural colorants***	***Reference***
Origin	Plants/vegetable, insect/animal, mineral, and microbial	(Sheikh et al. 2016) (Vankar 2016)
Chemical structure	Indigoids, pyridine based, carotenoids, quinonoid, flavonoids, dihydropyran based, betalains, tannins	(Bhute and S 2015)
Application method	Mordant dyes, vat dyes, direct dyes, acid dyes, basic dyes, disperse dyes	(Vankar 2016)

Table 2. Natural colorants classification by chemical structure

Among the main advantages of using natural dyes and pigments, it can be identified the environmentally friendly character, easily eliminated when accumulated in the textile processes wastewater effluents, without toxic and allergic potential, complemented by the added value of reduction of additional investment processes (Shukla and Vankar 2017a).

Nevertheless, depending on the origin of the natural colorant matter, there are two big disadvantages related to the industrial application context and are represented by the competition with arable land for food and CO_2 emission throughout cultivation (Pachauri et al. 2014).

In this sense, algae biobased feedstock represents a potential alternative for sustainable textile industry, due to the specificities of this emerging industry. Algae cultivation is characterized by very reduced demand for the arable area, with the possibility of sea or aquaculture water use, rapid growth, and high yield in optimum conditions, with the main nutrient represented by CO_2, becoming a CO_2 sink (Matos et al. 2015)(Kumar et al. 2021).

Considering the presented context and main aim of this study, the following sections will approach the natural colorants array, exploring an emerging branch of an underexploited but potential sustainable alternative, algae biobased feedstock.

1.4. Algae -sustainable feedstock

Algae envisage a vast group of chlorophyll-containing photosynthetic aquatic organisms, using it for fixing atmospheric CO_2 (Chia et al. 2018). This group is mainly divided into microalgae and macroalgae, ranging from unicellular microorganisms, as diatoms to 30 meters long seaweeds, distributed among habitats ranging from the marine to rivers, ponds, lakes, reservoirs, and freshwater (Rico et al. 2017). Their growth is strongly dependent on factors as temperature, light, and nutrients (Zhang et al. 2016).

The importance of algae for sustainability is widely addressed nowadays for most of the existing industries, first of all, due to their growing conditions, on one side being beneficial for the aquatic environment, due to the production of oxygen and organic matter for other aquatic organisms (Taylor 2009), by representing a natural carbon sink; and on the other side for the minimum requirements needed for their cultivation, without competing with food, land or fresh water (Sudhakar and Viswanaathan 2019).

Microalgae are single-cell microscopic, autotrophic organisms having approximately 300,000 species, mainly divided into 4 categories, based on the main organism color: *cyanobacteria* (blue-green algae), *rhodophytes* (red algae), *chlorophytes* (green algae), and *chromophytes* (all other algae) (Mobin and Alam 2017). An important element to highlight is the continuous change in the classification system, with a continuous disagreement among taxonomists around the world (Levasseur et al. 2020).

In the following Table 3, examples of some major microalgae species per category, considering the high interest for commercial applications:

Microalgae category	***Major species***	***References***
Cyanobacteria	*Spirulina (Arthrospira) platensis* *Aphanizomenon flos-aquae* *Halomicronema sp.* *Arthospira maxima Scavenge*	(Mobin and Alam 2017) (Pagels et al. 2019)
Rhodophytes	*Porphyridium sp.* *Porphyridium cruentum* *Rhodella reticulata*	(Gaignard et al. 2019)
Chlorophytes	*Dunaliella salina* *Chlorella sp.* *Haematococcus pluvialis* *Caespitella pascheri*	(Gaignard et al. 2019) (Heimann and Huerlimann 2015) (M.D. Guiry in Guiry, M.D. & Guiry 2021)
Chromophytes (all below)		(Heimann and Huerlimann 2015) (Levasseur et al. 2020)
Haptophyta	*Isochrysis galbana* *Pavlova salina*	
Dinophyta	*Crypthecodinium cohnii*	
Stramenopiles (3 below)		
Eustigmatophyceae	*Nannochloropsis oculata* *Nannochloropsis sp.*	
Bacillariophyceae	*Skeletonema costatum,* *Chaetoceros muelleri,* *Thalassiosira pseudonana*	
Labyrinthulomycetes	*Auranthiochytrium sp.* *Schizotrichium sp.* *Thraustochytrium sp.* *Ulkenia sp.*	

Table 3. Examples of major microalgae species of commercial interest per phylum

Macroalgae are commonly known as seaweed and are organisms that showcase similar photosynthetic and structural characteristics as terrestrial plants, populating coastal areas, mainly attached to rock or hard fundament. They are mainly divided into 3 groups, based on the predominant thallus color: *Rhodophyta* (red algae), *Heterokontophyta* (known as the O*chrophyta*)(brown algae), and C*hlorophyta* (green algae) (Rindi et al. 2012).

The following Table 4 includes examples of seaweeds of commercial interest, belonging to each of the 3 groups.

Macroalgae group	*Major species*	*References*
Rhodophyta	*Gracilaria sp.* *Hypnea sp.* *Acanthophora spififer* *Laurencia papillosa*	(Chia et al. 2018) (Kumar et al. 2021) (Leong et al. 2021) (Campbell et al. 2019) (Suganya et al. 2016)
Heterokontophyta (Ochrophyta)	*Padina gymnospora* *Colpomenia sinuosa* *Sargassum sp.* *Turbinaria conoides* *Saccharina sp.* *Fucus serratus* *Laminaria digitata*	
Chlorophyta	*Ulva sp.* *Enteromorpha sp.* *Codium tomentosum* *Caulerpa racemosa* *Chaetomorpha sp.*	

Table 4. Examples of macroalgae species of commercial interest per phylum

The commercial interest in the use of this sustainable feedstock comes from the potential benefits they offer (Levasseur et al. 2020), starting from the cultivation phase, including the potential of carbon integration from combustion gases (Carbon source) (Sierra et al. 2017) (Papadaki et al. 2017) (Chia et al. 2018), the potential of adapting to wastewater cultivation media and using contaminants as a source of nutrients (Sierra et al. 2017) (Gonçalves et al. 2017)(Abdel-Raouf et al. 2012). Additionally, their potential for developing a rich macronutrient composition (carbohydrates, proteins) and micronutrient profile (nitrogen, potassium, phosphorous) (Rahman and Miller 2017) (Siqueira et al. 2018) (Suganya et al. 2016).

The main compounds with special industrial interest include pigments, lipids, and fatty acids, proteins, polysaccharides, and phenolic compounds, and are exploited in diverse industries as the pharmaceutical and cosmetic industry, followed by food and feed, and completed with ecological applications (bioenergy, water treatment, CO_2 emissions remediation, chemicals, materials, pharmaceuticals care) (Liu et al. 2016) (Kuddus et al. 2013)(Chia et al. 2018)(Sharma and Sharma 2017)(Hosseini Tafreshi and Shariati 2009) (Mobin and Alam 2017).

A summary of the actual commercial approach and interest of the algae biobased feedstock is presented in Figure 1, adapted from (Kumar et al. 2021).

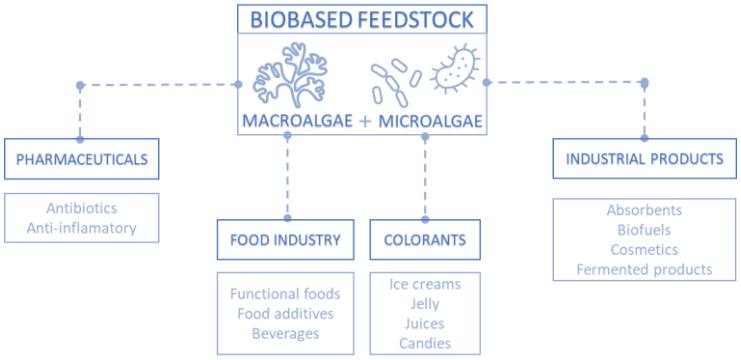


Figure 1. The commercial approach of algae biobased feedstock

1.4.1. Algae cultivation

Algae cultivation systems are characterized by the possibility of parameter optimization for yield increase according to the target application. Main cultivation parameters affecting yield and quality of algal biomass, and influencing the growth rate are represented by water (cultivation medium composition), the concentration of nutrients (nitrogen, phosphorous, etc.), oxygen, CO_2, pH of the medium, temperature, and light intensity (Béchet et al. 2017). These combined parameters influence the characteristics of the obtained biomass, in the controllable cultivation systems, nevertheless the parameter - depletion situations are used to generate ¨stressfull¨ conditions for the cells and influence the generation of primary (lipids, proteins, carbohydrates, etc.) and secondary metabolites (pigments, carotenoids, phycobiliproteins, phytosterols, phenolic compounds, etc.) in high yields (Rico et al. 2017). Nevertheless, for sea cultivation, where these parameters cannot be controlled, they must be monitored, for the decision-making process, as optimum seeding and harvesting (Kumar et al. 2021).

These systems are divided into the major category of algae type: microalgae and macroalgae, and they will be detailed accordingly below.

a) Microalgae cultivation

Microalgae cultivation is mainly focused on open systems (e.g. raceway ponds) and closed systems (photobioreactors (PBR)) (Kumar et al. 2021). Outdoor cultivation methods, through open or closed raceway ponds, are largely employed at a commercial scale (Hase et al. 2000). These may be constructed on natural waters like lakes, lagoons, or ponds, or artificially as tanks, circular or shallow ponds, and they are equipped with paddle wheels or agitation systems for medium circulation, aimed at biomass enhanced growth. The benefit generated by their cost-efficient construction and easier operation is downsized by limitations as poor light use by cells, diffusion of CO_2 into the atmosphere, requirements of large areas of land, and evaporative losses. Furthermore, external invasive factors as predators and other fast-growing heterotrophs may result in low biomass productivity (Ugwu et al. 2008). These drawbacks influence the focus on the benefits offered by photobioreactors, which may be defined as enclosed, illuminated culture vessels characterized by increased control over the cultivation parameters, thus generating higher productivities and preventing contamination (Cogne et al. 2005). PBRs can be constructed with tubular flat plate geometry, as a vertical bubble or air-lift columns or horizontal with a serpentine path. They can use membrane separation or sedimentation for containing the biomass (Znad 2020).

A large number of photobioreactors have been investigated, and some of the naturally illuminated are flat-plate, horizontal/serpentine tubular airlift, inclined tubular photobioreactors; nevertheless, artificially illuminated examples include bubble column, airlift column, helical, tubular, conical. An important hurdle is represented by the efficiency of the use of solar energy, and from this point of view, flat-plate, horizontal and inclined tubular photobioreactors are promising, except for scaling up difficulties (Ugwu et al. 2008).

b) Macroalgae or seaweed cultivation

Macroalgae cultivation is based on seawater for their growth and reproduction, as they also reproduce both asexual and sexual, by spores formation followed by fertilization and

gametes fusion (Kumar et al. 2021). The cultivation is performed onshore (on land) and offshore (open sea) through different methods, based on the type of seaweed, as rope, longline, monocline, puch, raft, tube-net, net bag, tank, and even photobioreactors for some species, and influenced by seasonality (Mantri et al. 2015) (Ugarte et al. 2008) (Mantri et al. 2017). All methods may be divided into extensive or intensive, depending on their location. Extensive methods, or seaweed farms, exploit natural waters by utilizing natural seaweed communities for enhancing productivity with the application of certain techniques for support, protection, etc.; or by introducing new cultures of species into the ecosystem. Intensive methods include the cultivation of one or several species in tanks with controlled parameters (natural or artificial light, nutrients, phytohormones), or cultivation in small natural water bodies (ponds, lakes, lagoons) with the application of agronomic techniques (weeding, light regulation, water mix, etc.). Another intensive cultivation method is considered the spray cultivation system (Titlyanov and Titlyanova 2010).

Due to the advantageous possibility of using seawater, the Integrated Multi-Tropic Aquaculture (ITMA) and polyculture systems for seaweed cultivation along with cage culture fisheries and other aquatic animals, have gained popularity for economic commercial application (Buschmann and Camus 2019).

1.4.2. Extraction of algae-based colorants

There is a large variety of extraction methods used for the obtention of natural colorants, applicable also to algae-based colorants, and their selection is influenced by the characteristics of the cellular wall that protects these secondary metabolites. Among these methods are included aqueous, non-aqueous, alkaline or acid extraction, fermentation, microwave-assisted or ultrasonic energy, enzymatic, supercritical fluid extraction, soxhlet extraction (Nambela et al. 2020).

The aqueous extraction is suitable for water-soluble colorants, as it implies using water as a solvent. For increased efficiency, the process involves the preparation of the biomass, if dry by cutting it into smaller pieces or ground in powder form, and if paste ground with glass spheres. The prepared biomass is soaked with water, at specific temperatures and times, depending on the solubility of the colorant. The resulting mixture is filtered, for supernatant separation then concentrated to remove the solvent, and further purified. This is a green method, with limitations as water and time consumption, and selective applicability (Bechtold et al. 2006) (Haddar et al. 2018)(Chattopadhyay et al. 2018).

Fermentation and enzymatic extraction follow the same procedural steps as the aqueous extraction, by replacing the solvent with enzymes. The main advantages of the method imply enhanced efficiency and less energy use, nevertheless, the drawback of the method is represented by an increased time consumption (Sierra et al. 2017).

The solvent extraction method is mainly based on the use of organic or aqueous/organic mixtures as the solvent, depending on the colorant solubility, with the possible addition of alkali or acid for increased efficiency. Solvent removal is facilitated by the volatility of the organic solvent, but the organic solvent contaminant character is an important drawback of the method (Nambela et al. 2020).

Acid or alkaline extraction is a suitable method for a large variety of colorants, except pH-sensitive ones. It involves water as a solvent, following the steps of the aqueous extraction, but based on the pH of the extraction bath, therefore is needed the addition of acid or alkali. The main advantage is increased yield, but the disadvantage is represented by the negative influence on the environmental pH, potentially negatively influencing the aquatic ecosystems (Muthu 2017).

Sonicator and microwave extraction are used for the optimization of the aqueous extraction limitations, where the ultrasonic and microwave energy enhances the yield of the extraction. Benefits include low temperature, less time, and solvents usage, however, the purification process still needs to be applied (Naveed et al. 2020)(Amin et al. 2020)(İlter et al. 2018)(Chuyen et al. 2018).

The supercritical fluid extraction method consists of using supercritical CO_2 as a solvent. Carbon dioxide gas condenses and liquefies under high pressure, and it further returns into the gaseous state after decreasing the pressure, resulting in precipitate obtention in the separator. Filtration of the extract (precipitate) obtained is required after this sustainable method application. Additionally, this extraction involves reduced energy use, but the only inconvenience is represented by the costly equipment installation (McHugh Mark and Krukonis Val 2013).

The soxhlet extraction method is used for the separation of semi-volatile colorants from solid natural materials. It is an efficient method using the soxhlet equipment, with the employment of organic solvents. The environmental impact is determined by the use of contaminant solvents and high energy costs for heating and condensing of the solvent (Luque de Castro and Priego-Capote 2010).

The general natural colorant extraction scheme consists of preparing the biomass, for subjection to the selected extraction method, followed by filtration and purification processes.

Preparation involves collecting, drying, size reduction, and homogenization, depending on the state of the source material. After subjecting it to the selected extraction process, filtration may be performed via membrane, gravitational, or by using a centrifuge (Yusuf et al. 2017). The last step is represented by purification, if needed, which can be done by recrystallization or chromatography (Thin Layer Chromatography or High-Performance Liquid Chromatography). After the obtention of the natural colorant, this is characterized via spectrophotometric methods (infraRed, Ultraviolet, Gas Chromatography, and Nuclear Magnetic Resonance) (Forgacs and Cserhati 2002) (Buchweitz 2016)(Pagels et al. 2019)(Mir et al. 2019)(Vankar 2017).

A summary of the general approach of an extraction process from algae biomass, adapted from (Nambela et al. 2020), is presented in Figure 2.

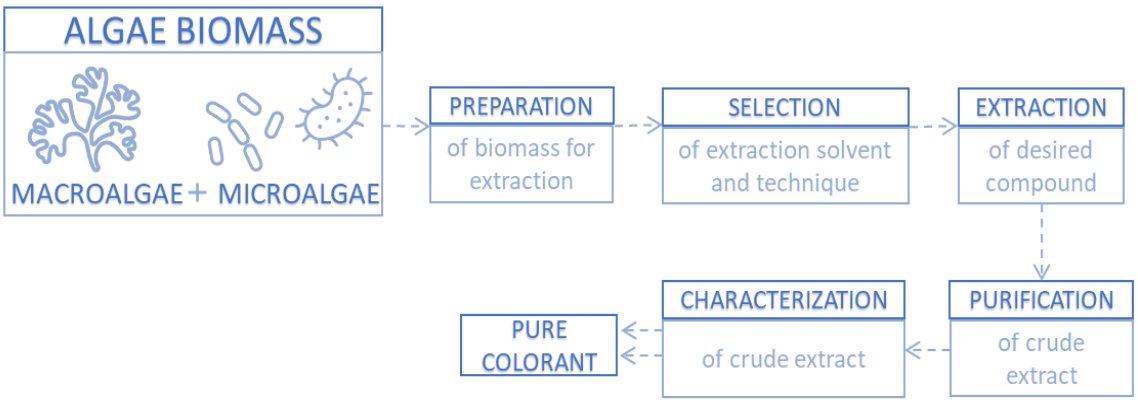


Figure 2. General colorant extraction scheme from algae biomass

New approaches towards cost-efficient exploitation of natural biomass may be considered through cascading biorefinery approach, as to use the maximal part of biomass through extraction and generating zero wastes. This implies cascading subsequent extraction processes from one biomass, always using the residual one for the further extraction step, with a sustainable approach in the selection of the solvent. In this sense, a bioactive high-added value compound is obtained at the end of each extraction step with various industrial applications (BIOSEA 2019).

1.4.3. Algae-based dyes and pigments

Algal potential for a low-carbon circular bioeconomy has determined extensive research on specific algae species due to the vast commercial potential, generated by primary and secondary metabolites composition, which can be enhanced through cultivation parameters control (Leong et al. 2021).

Phycocyanin (blue colorant) sourced from cyanobacteria *Spirulina platensis*

Spirulina sp. belongs to the phylum *Cyanobacteria* and is a multicellular and filamentous organism that may appear in two different shapes, disk or spiral rod (Mobin and Alam 2017).

Spirulina platensis is the most popular non-toxic cyanobacteria (blue-green algae), commercially cultivated on large scale, for application in various industries as medical, health care, cosmetics, food additives, and more (Zhang et al. 2016), due to its content in phycobiliproteins, carotenoids, fatty acids, minerals, vitamins, polysaccharides, etc. (İlter et al. 2018).

Its proteic content and structure are of great interest for coloration applications, being determined by phycobiliproteins, which represent 50% of the total cellular protein (Bryant et al. 1979). These colored molecules are part of a family of light-harvesting water-soluble proteins classified into 4 main types, considering their maximum absorption wavelength: phycocyanin, allophycocyanin, phycoerythrin, and phycoerythrocyanin (Antelo et al. 2008). The abundance among these types in cyanobacteria distributed decreasingly is as follows: phycocyanin -the majority, followed by phycoerythrin and allophycocyanin (Pagels et al. 2019). Table 5 details the maxima light absorption peak of the 4 types of phycobiliproteins.

Phycobiliprotein type	*Maximum absorption (nm)*
Phycocyanin	650-660
Allophycocyanin	610-625
Phycoerythrin	490-570
Phycoerythrocyanin	560-600

Table 5. Phycobibiproteins absorption peak maxima. Source: (Pagels et al. 2019)

The structure of the phycobiliproteins is based on several covalently bond subunits with a central protein and a phycobilin. Phycobilins are the light-harvesting chromophores, at the basis of photosynthesis (Gwizdala et al. 2018).

The most common phycobiliprotein in cyanobacteria is **phycocyanin,** containing the blue **phycocyanobilin** chromophore ($C_{33}H_{38}N_4O_6$) (see Figure 3 below) (Pagels et al. 2019).

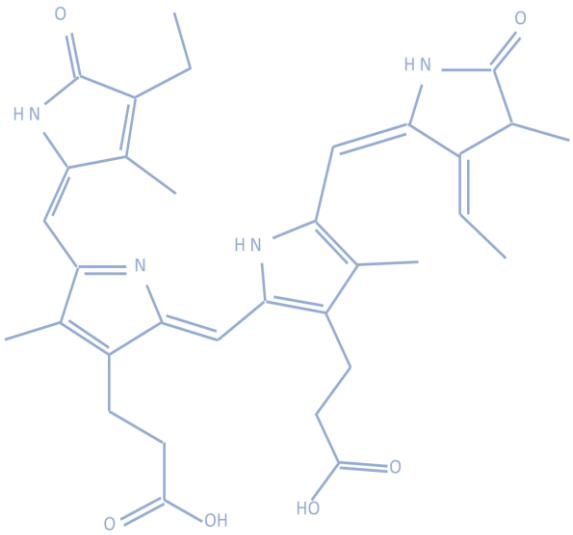


Figure 3. Phycocyanobilin chemical structure (Pagels et al. 2019)

Phycocyanin, blue colorant matter, is widely used in various industrial applications as in the analysis of cells and molecules, as a fluorescent marker (Kuddus et al. 2013), medical (e.g. cancer research) (Liu et al. 2016), blue colorant in food (Buchweitz 2016), cosmetics, nutraceutical, pharmaceutical, etc. (Sonani 2016) (Mobin and Alam 2017). This use has generated the extensive characterization of this blue coloring protein.

From a structural point of view, blue phycocyanins contain the phycocyanobilin chromophore and are defined as C-phycocyanins (C-PC). The monomer of C-PC is composed of dissimilar polypeptide units (α- and β-), spontaneous aggregates generating trimers and hexamers, which cluster into phycobilisomes. This structure defines its composition in solution as oligomers with molecular weight comprised between 44 and 260 kDa (Buchweitz 2016).

Stability-wise tests have revealed sensitivity to pH, temperature, and exposure time. Significant color stability, up to 80-95%, has been demonstrated in conditions characterized with pH ranging from 5 to 7, heating up to 65°C for over 30 minutes (Patel et al. 2004)(Antelo et al. 2008). Food industries applied thermal stabilizers include sugar, citric acid (Chaiklahan et al. 2012), or polyhydric alcohols (Antelo et al. 2008).

Photobioreactor cultivation conditions for increased yield of C-PC, applying fed-batch strategy include Nitrogen depletion, light intensity control, and exposure time, meaning optimum harvesting time (Xie et al. 2015), and CO_2 feeding rate (Soletto et al. 2008).

Phycoerythrin (red colorant) sourced from macroalgae *Gracilaria gracilis*

Gracilaria sp. belongs to the phylum *Rhodophyta*, the red macroalgae family, distributed in tropical waters of the Atlantic ocean and presents commercial interest due to its high agar content (Kazir et al. 2019) and rapid growth rates, extensive tolerance to external factors, and species diversity (Dawes et al. 1998).

From a composition point of view, *Gracilaria sp.* presents high proteic content reaching up to 10% of dry weight (Rasyid et al. 2019), and growth seasonality covering most of the year (Fleurence 2004).

Among the proteins of the *Gracilaria sp.* seaweed, phycoerythrin is abundant, and of interest due to the red color. Phycoerythrins may be divided into two types: R-phycoerythrin encountered in most of the red algae and B-phycoerythrin encountered in a particular class of the red seaweed, specifically *Bangiales* (Fleurence 2004).

R-phycoerythrin (R-PE) is a protein responsible for light harvesting, specialized in the energy transfer chain, water-soluble, with a relative mass ranging from 240.000 to 260.000 (Liu et al. 2005). The R-PE contains a covalently bond bilin (phycoerythrobilin), represented by an open chain tetrapyrrole, to the apoprotein, which possesses physio-biochemical properties. R-PE absorption maxima peaks at 497-498 nm, with a red fluorescence emission at a maximum wavelength ranging from 572 to 578 nm (Parodi 2011). The adaptation of the chemical structure of phycoerythrobilin from (Pagels et al. 2019) is presented in Figure 4.

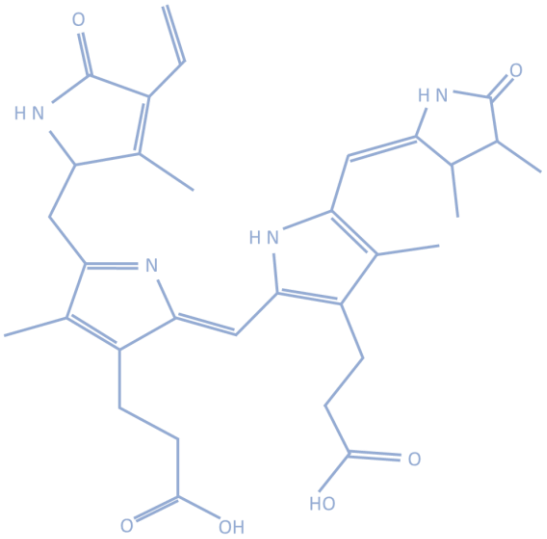


Figure 4. Phycoerythrobilin chemical structure(Pagels et al. 2019)

Commercial processing of R-PE is oriented towards food colorant, photosensitizer in photodynamic therapy, flow cytometry, immunoassays, solar cell, and more, considering the thermal stability at approximately 45^0C, the high molar absorption coefficient ($\sim 1.96 \times 10^6 M^{-1} cm^{-1}$), and emission quantum yield of >80% (Bharmoria et al. 2020). Nevertheless, limitations as poor photochemical stability, involve the need for use of preservatives and osmolytes, in

aqueous solutions (for a maximum of 67 days at temperatures ranging from 4 to 35^{0}C), as sucrose, sodium chloride, ascorbic acid, glucose, citric, and benzoic acid, sodium azide and butyl hydroxyl toluene, 5% Sodium chloride in distilled water (Hsieh-Lo et al. 2019). Even so, the chemical stability for a long period needs improvements (Bharmoria et al. 2020).

Gracilaria sp. may be cultivated onshore (raft) (Mantri et al. 2017) and offshore (tube net) (Mantri et al. 2015), and the induction of R-phycoerythrin has been demonstrated to be influenced by salinity and light irradiance(Dawes et al. 1998), and the fluctuations of the culture medium pH = 8 (Lee et al. 2019).

Carotenoids (yellow colorant) sourced from microalgae *Dunaliella salina*

Dunaliella sp. belongs to the phylum *Chlorophyta,* with ovoid, spherical, fusiform, ellipsoid or pyriform cells, ranging in size from 5 to 25 µm (length) and 3 to 13 µm (width) (Hosseini Tafreshi and Shariati 2009), and it can be found in marine and fresh habitats (Mobin and Alam 2017).

Dunaliella salina, is a green halophilic microalga, with increased resistance to extreme pH, salinity, and temperature, stressful conditions that conduct to the generation of protective secondary metabolites in high quantities, the yellow-orange colorants, β-carotenoids, which may represent up to 14% of dry biomass, commercially valuable due to antioxidant properties (Pisal and Lele 2005) (Borovkov et al. 2020).

Commerical interest for β-carotene obtained from *Dunaliella salina,* arose due to the antioxidant high-added value, complemented by enhancing human body functions and anticarcinogenic significance, with application in cosmetics, food and feed supplements, pharmaceutical industry, etc. (Hosseini Tafreshi and Shariati 2009). Additionally, these microalgae can accumulate, apart from carotenoids, glycerol, vitamins, lipids, proteins, and minerals (Britton 2020).

Carotenoids are a large group with more than 600 different chemicals and are classified into two categories: carotenes, which are oxygen free hydrocarbons (e.g. α – and β – carotene), and xanthophylls, which are oxygenated derivatives of carotenes (lutein, violaxanthin, fucoxanthin, astaxanthin, etc.)(Robert Edward Lee 2018).

Structurally, carotenoids are formed of linearly conjugated polyene tetraterpenes, characterized by a system of conjugated electrons, visually resulting in colors as yellow, orange, and red (Bhute 2015). They are constituted by 8 isoprenoid units joined head to tail (Levasseur et al. 2020).

β-carotene (Figure 5) sourced from *Dunaliella sp.* composition is defined by a combination of cis- and trans- isomers, as it follows: 9-cis (41%), all-trans (42%), 1- cis (10%), and other (6%), being influencing solubility and crystallization capacity (Hosseini Tafreshi and Shariati 2009).

Yellow carotenoids, including β-carotene, have a broad spectrum with absorption wavelength ranging from 400 to 500 nm, and absorption maxima peaking at 470 nm (Lichtenthaler and Buschmann 2005).

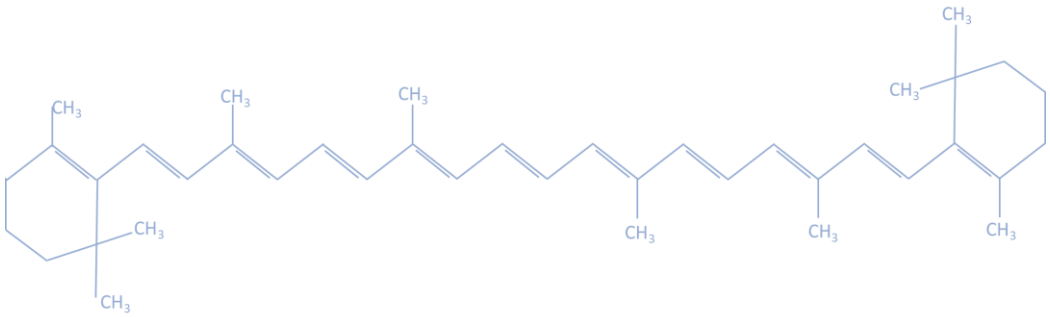


Figure 5. β-carotene chemical structure (Suganya et al. 2016)

Degradation analysis of β-carotene has revealed the lipophylic character due to the long unsaturated aliphatic chains, making them insoluble in water and likely to deteriorate due to heat or light (Lu et al. 2020). Thermal degradation has been analyzed, and prolonged subjection to high processing temperatures (e.g. 1,5 hours at 120^0C) destabilizes β-carotene (Aparicio-Ruiz et al. 2011), nevertheless, storage produces the same effect, affecting its structure after a time of 2 months of storage, and at a temperature higher than 25^0C (Bollinedi et al. 2019).

β-carotene induction covers several cultivation strategies, either in open ponds or photobioreactors, including control of severe conditions, as high salinities, low nutrient level (as Nitrogen starvation), high temperatures, and high irradiance, generating thus, growth inhibition and production of the colorant (Pisal and Lele 2005)(Hosseini Tafreshi and Shariati 2009) (Kim et al. 2017).

Chlorophylls (green colorant) sourced from microalgae *Caespitella pascheri*

Caespitella pascheri is a fresh water *Chlorophyceae*, belonging to the *Chaetophorales* order, characterized by their growth on support, either a plant (epiphytic) or rock (epilithic). It is defined by mononuclear cells, grouped into branched or unbranched filaments. The cell size does not exceed 10 µm (Park et al. 2019). The chloroplast, representing the photosynthetic apparatus contains one or two pyrenoids, important for the atmospheric carbon concentration mechanism, found in many algae but not in the majority of terrestrial plants (Michetti et al. 2010).

Overall the green microalgae *Chlopophyceae* which includes the strain *Caespitella pascheri* are characterized by a protein content that ranges from 15.2–25.6% of dry weight, a lipid content varying from 8.5–18.4% of dry weight among the species; completed by chlorophyll a from 0.23–1.54% of dry weight (Brown and Jeffrey 1992).

Caespitella pascheri presents commercial interest due to its renewable source viability characters and composition for biofuels, nutraceutical, pharmaceutical industries (Baudelet et al. 2017), due to the content in phenolic compounds, with effective antioxidant activity (Vidhyanandan et al. 2020). The high content of lipids and β-carotene is an added value for these *Chlorophyceae* microalgae (Park et al. 2019).

Chlorophylls are green tetrapyrrole colorants, necessary for photosynthesis, encountered in the majority of photoautotrophic organisms (Levasseur et al. 2020). The commercial interest is raised due to the intense green coloration, as a dye agent in pharmaceutical, cosmetics, and food applications (Odjadjare et al. 2017) (Yaakob et al. 2014), as deodorant, a component of

dentifrice, antitumor agent, due to anti-inflammatory effects and antioxidant activities, etc. (da Silva Ferreira and Sant'Anna 2017).

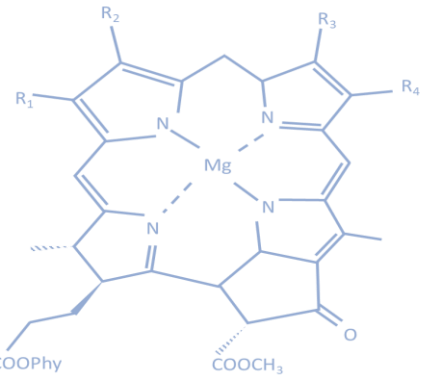
Chlorophyll content in photosynthetic organisms may vary depending on the environmental conditions, and strain type, ranging from 0,5 to 4% dry weight (Levasseur et al. 2018) (Spolaore et al. 2006). Structurally the chlorophyll includes an aromatic ring with a tetrapyrrole, in which the central Magnesium ion is linked to 4 pyrrole rings and a hydrocarbon tail connected to chlorin (Mulders et al. 2014).

Chlorophylls are classified into 5 types: chlorophyll a, b, c, d, and f, (with absorption maxima, and abundance in an organism, presented in Table 6) and similar molecular structure, differentiated by macrocycle perimetral groups, interfering with their absorption spectra (Li and Chen 2015).

Chlorophyll's type	***Maximum absorption (nm)****	***Abundance organism***
Chlorophyll a	665	1st (most) abundant all photosynthetic organisms
Chlorophyll b	652	2nd (most) abundant in plants and green microalgae
Chlorophyll c	630	3rd (most) abundant in chromophytes
Chlorophyll d	696	Identified in *Rhodophyta*
Chlorophyll f	707	Identified in cyanobacteria

*Table 6. Chlorophyll absorption peak maxima and abundance organism. Source *(Chen 2014), ** (da Silva Ferreira and Sant'Anna 2017)*

The different types of chlorophylls are mainly synthesized from chlorophyll a, with more clarifications needed for the process of chlorophyll c and chlorophyll f (Chen 2014) (Ho et al. 2016). The chemical structure of the different types of chlorophylls is identified in Figure 6 and adapted from (Voloshin et al. 2015).



Chlorophyll type	R_1	R_2	R_3	R_4
a	CH_3	$CH=CH_2$	CH_3	CH_2-CH_3
b	CH_3	$CH=CH_2$	CHO	CH_2-CH_3
c	CH_3	CHO	CH_3	CH_2-CH_3
f	CHO	$CH=CH_2$	CH_3	CH_2-CH_3

Figure 6. Chlorophylls (a,b,c,f)chemical structure (Voloshin et al. 2015)

Chlorophylls processing must consider their sensitivity to environmental stress, based on oxygen, as they react when in contact, light, enzymes, high temperature, acidic or alkaline pH, leading to their degradation and color loss (Kang et al. 2019).

In cultivation systems chlorophyll inducing is reached by the creation of stress conditions through reduction of light, using red light spectrum, increased temperature, and Nitrogen and phosphorus depletion (da Silva Ferreira and Sant'Anna 2017) (He et al. 2015).

Commercially cultivated algae with a high content of chlorophylls are, mainly microalgae: *Chlorella vulgaris* (Levasseur et al. 2018), *Ankistrodesmus sp., Chlorella sp., Monoraphidium dybowskii, Scenedesmus dimorphus, Pavlova lutheri* (da Silva Ferreira and Sant'Anna 2017), and macroalgae, as *Ascopyllum nodosum, Fucus vesiculosus, Polysiphonia lanosa, Palmaria palmata, Chondrus crispus, Ulva intestinalis, Coryphaenoides rupestris, Ulva sp.,* etc.(Baumann et al. 2009)(Martins et al. 2021).

1.4.4. Quantification of algae-based colorants

Quantification of the extraction yield of the algae-based colorants is calculated considering the chromophores´ absorption maxima, based on their structural complementarity and production in the algal organism.

In this sense, phycobiliproteins are calculated using the equations proposed by Bennett and Bogorad (Bennett and Bogorad 1973), with the application of the extinction coefficients defined by Bryant in 1979 (Bryant et al. 1979).

Phycobiliproteins purity is assayed as a function of the purity ratio (R), according to the method of Haris and Angal (1989) (Wood 1991) representing the absorbance ratio: A_{619}/A_{280}, and A_{565}/A_{280}, where R=0 represents pure extract.

Considering the conjugated inclusion in algal organisms of the chlorophylls and carotenoids, their concentration is calculated using the Wellburn method (Lichtenthaler and Wellburn 1983).

Chlorophylls and carotenoids purification, at a laboratory scale, is determined mainly instrumental, with the use of chromatographic techniques (HPLC) (Eghbali Babadi et al. 2020) (Fernandes et al. 2021).

1.5. Use of natural dyes in the textile industry

Natural colorants were used in the textile industry since the ancient stone age, with discoveries of excellent application of natural colorants on textiles by the Egyptians (Yusuf et al. 2017). Considering the shift towards sustainability, increased research has been oriented towards the use of natural colorants in finishing textile applications (Nambela et al. 2020).

Nevertheless, the major drawback imposed by the use of natural colorants is mainly characterized by the low textile fibers-natural colorant affinity (Shukla and Vankar 2017a) (Shahid et al. 2013c). In this sense, a conventional solution is represented by the use of mordants, usually derived from metallic salts, and it has been demonstrated to improve dye uptake and color fastness, thus increasing competitivity with synthetic colorants (Shukla and Vankar 2017b).

1.5.1. Mordanting methods

The word mordants´ significance originates from the Latin word ¨*mordere¨,* which means ¨*to bite¨.* A mordant is a chemical auxiliary used in the textile industry for its capacity of fixing itself on the fiber, meanwhile forming a bond with the natural colorants. Among the benefits of its usages are included: increased absorption and fixation of the colorant, improved fastness, etc.(Bhute 2015).

There is a wide variety of mordants, classified into 3 conventional main categories, due to their content, as metallic or tannic (metallic salts, as Alum, Ferrous sulphate, Aluminum triformate, Aluminum acetate, Aluminum sulphate, Tartaric acid, and its salt Cream of tartar) tannins (as Tannic acid, Myrobalan, Oak gall) (Bhute 2015), and oil mordants (fatty acids as palmetic, stearic, oleic, etc.) (Vankar 2000).

Nevertheless, a series of novel mordants have been invented, and some of them are Stannic chloride, Calcium chloride, Magnesium sulphate, Aluminum sulphate, Lanthanum oxide, Ferric chloride, Neodymium trichloride, and many more (Shahid et al. 2013c).

Mordanting processes are differentiated according to the application time, as pre-, meta-, and post- mordanting, according to the dyeing process.

The pre-mordanting process involves the development of the textile treatment prior to the dyeing process, as a separate procedure, conferring sufficient and exclusive time and site for the bonding accomplishment, and facilitating an appropriate layering of the dye, mordant, and textile. Through this type of mordanting metal complexation occurs, and it is complemented by dye complexation resulting in increased resistance to light, rubbing, and wash fastness. The increased light fastness is conferred by the chelating complexation, influencing the proper energy dissipation of protons of light into the complex. This is considered one of the most sustainable approaches due to the optimum utilization of resources (Yusuf et al. 2017).

Meta-mordanting represents a simultaneous mordanting with the dyeing process, where the colorant and the mordant are dissolved into the dyeing bath, during the dyeing process. This is considered a resource waste generating process, due to the various types of complexation that occur, as textile and colorant, textile and mordant, and colorant and mordant. This results in unevenly dyed textiles, specifically due to these bondings, as some sites of the textile materials are occupied with the colorant, meanwhile the other with the mordant (Nambela et al. 2020).

Post-mordanting process is applied on the previously dyed textile, thus forming complexation with the colorant molecules on the surface of the textile, for shade range broaden purposes (Gürses et al. 2016) (Bhute 2015).

1.5.2. Dyeing process (interaction of dyes with natural fibers)

Considering the natural colorant application process in the textile industry, throughout time, vast research on improvements of the processes has been performed, thus facilitating their classification into conventional and advanced or innovative dyeing systems (Ding and Freeman 2017)(Bhute and S 2015). The textile dyeing industry is characterized by an increasing market forecast with a CAGR of 8,2% until 2026 (Choudhary and Prasad 2020).

Therefore, the conventional and innovative/improved dyeing systems are briefly approached below.

Conventional dyeing systems involve the aqueous application of colorants to textile substrates, after their cleaning and preparation phase, at specific temperatures and pressure. The mechanism of dye transfer occurs firstly through the diffusion of the colorant molecules into the liquid phase, establishing a monomolecular state, followed by its adsorption onto the surface of the textile fibers, to adsorption, and finally diffusion within the interior of the fibers (Vassileva and Valcheva 2008). For the coloration process the improvement and facilitating the creation of bonds among the textile substrate and dye, auxiliaries, as mordants may be applied (Moore and Ausley 2004). This dyeing system can be applied as a continuous or batch process, the selection depending on various factors as material, fiber type, and more (Drumond Chequer et al. 2013).

Continuous dyeing involves the use of rolls, through which heat and steam are applied on the fabric substrate meanwhile it transits various concentrated chemical solutions. In this process, the fabric absorbs a great amount of the chemicals, while rinsing removes part of them. Each passing through a solution requires water in the equivalent of the weight of the fabric (Moore and Ausley 2004).

Batch dyeing, it is also called exhaust dyeing, due to the gradual transfer of the colorant matter from the dye bath to the textile substrate over a long period of time (up to hours), in the presence of auxiliaries. In this process, the textile substrate is maintained in an equipment, with the possibility to alternately fill and drainage of water, at each process step. These types of equipments can operate at high temperatures (above 100^0C) and pressurized ambients. In this case, each alternate bath involves approximately 5 to 10 times the fabric´s weight in water (Moore and Ausley 2004).

Fixing the colorant matter onto the fiber, in an aqueous solution involves mainly 4 types of interactions: ionic, Van der Waals, hydrogen interaction, and covalent bonds (Christie 2015).

Ionic interlinkage occurs among oppositely charged ions existing in colorants and fibers, such as the ones generated by natural fibers functionalities as amino and carboxyl (–NH2, –COOH, and –OH) and dyes containing anionic and cationic water-soluble groups as –NR3 + and –SO3. This interaction mainly materializes within fibers like wool, cotton, silk (Zollinger 2003) (Ketema and Worku 2020).

Van der Waals interactions describe a group of interactions generated by a close approach between the π orbitals of the colorant molecule and the fiber, which results in a strong fixing of the colorant molecule onto the fiber, due to an affinity process. Examples of this interaction may be the dyeing of wool with dyes with high affinity (Drumond Chequer et al. 2013)(Ketema and Worku 2020).

Hydrogen bonds take place between covalently bonded hydrogen atoms in the colorant and free oxygen and nitrogen atoms in the fiber, and they may be found in the dyeing of wool and silk (Drumond Chequer et al. 2013)(Ketema and Worku 2020).

Covalent interactions are specific to the cotton fiber and occur among the reactive groups (electrophilic groups) of the colorant molecule (e.g. carbon atom) and nucleophilic groups on

the fiber (e.g. oxygen, nitrogen, or sulfur atom of a hydroxy or amino group) (Drumond Chequer et al. 2013).

Advanced/innovative dyeing systems have resulted from the need of encountering alternative and efficient sustainable solutions for the dyeing industry, and they involve various studies on technological approaches, apart from the use of environmentally friendly raw materials.

The innovation approach is divided into textile surface substrate modification for dye-fiber affinity increase and technological innovation in the application of dye, for facilitating the absorption of dye into the substrate (Berradi et al. 2019). The following paragraphs present some of the innovative examples, inter alia.

Technologies for dyeing systems improvement:

- Ultrasonic energy technology: ultrasonic energy is characterized by a frequency between 20 kHz–500 MHz, and its chemical strength derives from the potential of cavitation, representing the creation of microbubbles in a liquid where negative pressure is sustained. In the textile finishing sector, its use improves the dyeing process through an increase of the rate of diffusion of dye to fibers, through dye aggregates or fiber liquid boundary breakage, fostering fiber-liquid contact, by eliminating the air between the fibers and expansion of fiber-liquid interface by improving the swelling of fibers. In the dyeing process, the sustainable character is given by the low temperature use, short dyeing periods, low auxiliaries, and colorant matter needed (Muthu 2017) (Drumond Chequer et al. 2013) (Shahid et al. 2013a) (Shukla and Vankar 2017b) (Haddar et al. 2015).
- Microwave energy technology: microwave energy is characterized by the frequency comprised between 300–300.000 MHz, being non-ionized electromagnetic radiation, and its industrial use is due to the potential of heating of materials, generating a reduction in process duration and energy use. Low-temperature microwave processing is used in textile applications, with dye uptake and fastness properties improvements, with facilitation of dye penetration into fibers (Muthu 2017) (Adeel et al. 2020) (Shukla and Vankar 2017b).
- Liposome-based technology: liposomes are vesicular structures with encapsulation capacities, composed of lipid vesicles bilayers. They are used in dyeing processes due to their carrier role, as transporters of auxiliaries, generating benefits as nontoxicity, biodegradability, reduction in dyeing temperature, improved quality of the produced textiles (Drumond Chequer et al. 2013).
- Ozone after treatment with natural dyes on cotton fabrics, for the substitution of the use of mordants (Benli and Bahtiyari 2018).
- Sustainable colorants dyeing, sourced from green algae *Cladophora glomerate L.*, represents one of the very few experiments approaching aquatic biomass for cotton dyeing (Mir et al. 2019), completed by the use of extracts from red algae *Laurencia obtusa* and extracts from brown algae as *Lyengaria stellata, Sargassum muticum, Colpomenia sinuosa* (Adeel et al. 2017).

Textile substrate modification through:

- Plasma technology: plasma is an ionized gas consisting of a combination of neutrons, photons, electrons, free radicals, negative and positive ions, and metastable excited species, being considered an environmentally friendly approach for various industries. The textile substrate (cotton, wool) cold plasma treatment is used for etching, cleaning, cross-linking, functionalization of the surface of the materials (Shukla and Vankar 2017a) (Shahid et al. 2013b) (Haddar et al. 2018), generating an increase of fiber roughness, thus improving dyeability (Haji and Payvandy 2020), shrink resistance, hydrophilicity, improvement of water and oil repellent properties, scour (Muthu 2017) (Haji 2020) (Haji 2019).
- Enzymatic treatment: employing a high variety of enzymes (as protease, transglutaminase, laccase, amylase, trypsin) on natural fibers for their surface preparation, with results in an improvement in dye uptake, enhance shrink-resistance properties. Nevertheless, enzyme-dye specificity may be a limitation in upscaling their application (Shahid et al. 2013a).
- Other treatments (Shahid et al. 2013a):
    - Incorporation of statistically studied models of the surface response methodology (Aminoddin et al. 2018), for example, when developing non-aqueous dyeing of wool with decamethyl cyclopentasiloxane (Alebeid et al. 2020).
    - Cationization (Baaka et al. 2019), and citric acid crosslinking (Haji et al. 2018), of the cotton fiber surface, have been proven to sustainably increase the natural dyeing efficiency.
    - Pretreatment with TiO_2 nano-sol was proven to increase the dyeing efficiency and provide ultraviolet protection capacity (Alebeid et al. 2015).
    - Pretreatment with ultrasonic (Naveed et al. 2020)(Amin et al. 2020) and microwave irradiation on proteinic and cellulosic fibers have been proven to improve the dyeing process efficiency (Adeel et al. 2020).
    - Use of natural polymers for increasing absorption capacity, for example, one successful application has been demonstrated with naturally occurring polymer, chitosan (Janhom et al. 2006).
    - Pre-mordanting, followed by dyeing with sustainable dyes and analysis of the complexation process produced among fiber-mordant-dye (Kiumarsi et al. 2017).

Recently the **modification of the structure of natural colorants** is being considered in various research studies, through addition or change of auxochromes, obtaining a change of the shade, or increasing the affinity of the colorants, thus improving shade reproducibility and dye performance (Nambela et al. 2020).

1.5.3. Interaction of mordants with the natural fibers (cotton and wool)

In the context of sustainability, the possibility of the application of natural colorants in the textile industry has encountered hurdles generated by their low affinity to the textile substrates. For that, studies on the possible interaction among mordants-textiles substrates and natural

colorants have been performed. These investigations considered types of mordants and textile substrates interconnections and the influence on the dyeing process efficiency.

In this sense, tannins and metallic mordants' interaction with cellulosic (cotton) and proteinic (wool) textile substrates can encompass various bonding types, occurring between specific groups of the mordant structure and respectively groups of the textile substrate. The following Table 7 summarizes the possible bond types among molecular groups of mordants and corresponding groups of fibers (Bhute and S 2015).

Bond type	**Mordant**		**Textile substrate**
Hydrogen bonds	Phenolic hydroxyl groups	and	Free amino and amido group
Ionic bonds	Anionic groups	and	Cationic groups
*Covalent bonds**	Quinine or semi-quinone	and	Suitable reactive group

Table 7. Bond types among mordants and cellulosic and proteinic textile substrates

*applies only to cellulosic fibers

Nevertheless, in the case of cotton (cellulosic fiber) for the covalent or coordination bonding with a tannin mordant, a metal ion is necessary, for the creation of the attachment to the oxygen molecule of the connecting elements (Ding and Freeman 2017).

1.5.4. Printing process

Textile finishing processes encompass the application of dyes through processes that consider fiber affinity properties and facilitate the physical or chemical bonding, and processes that consider coloration through the application of pigments with lack of affinity to the fiber, with the need of the accompany of binders (which provide their fixation on the fibers) (Drumond Chequer et al. 2013). The latter being referred to as pigment printing and is considered one of the easiest methods (El-Molla and Schneider 2006).

Printing is the process of application of colorant matter on the textile substrates with the support of binding agents and other auxiliaries, without considering the affinity of the colorant to the fiber, thus offering the possibility of application on all types of substrates (Bahtiyari et al. 2013).

Textile printing occupies a strong position and shows high demand within the textile industry, with a forecast increase of 2,8% CAGR by 2026 (Business Wire 2020) of the Global Textile Printing Industry. It was initially performed in its traditional manner as block or engraved copper printing, becoming nowadays almost obsolete. Nevertheless, the industrial approach has driven this textile finishing practice to diversification into **direct printing,** further divided based on the type of colorant matter use, as a pigment, reactive dye, disperse dye, vat dye, acid dye, and digital inkjet printing, and **other types of printing** determined by the type of technique applied, as resist, discharge or burn-out printing, depending on the reaction occurring in the process (Ujiie 2015).

One of the most used techniques is pigment printing due to its main advantages which include simplicity, versatile character, reduced costs, and many more (El-Molla and Schneider 2006). However, there are some disadvantages such as the color fastness mainly to rubbing.

Natural colorants application in the textile printing as coloration technique reveals limited research, nevertheless, studies approach mostly the pigment printing technique on natural

fabrics with or without the textile substrate pretreatment, as mordanting and cleaning processes (Chattopadhyay et al. 2018) (Rekaby et al. 2009).

Pigment printing is mostly performed through the screen-printing technique, involving the application of the printing paste over the prepared textile substrate. The printing paste is mainly constituted of three main elements: binder, thickener, and colorant matter (Abdou et al. 2016). This printing paste is applied with a scraper onto the previously cleaned and pretreated textile substrate and subjected to the curing and drying process.

The binder´s or fixing agent´s function in the process is to physically link the colorant matter to the textile substrate, by forming a superficial, adhesive film, influencing, in the last instance the fastness and mechanical properties. The binding occurs by subjection to a suitable fixing process, represented by the curing step, by dry hot air, generating the crosslinking reaction, which produces covalent bonds (Iqbal et al. 2012). Binders are mainly synthetic polymers based on kerosene and acrylate copolymers, characterized as hydrocarbons contaminants, and recent studies propose chitosan and starch-chitosan as valuable sustainable alternatives (Sonani 2016) (El-Molla and Schneider 2006) (Teli et al. 2013b).

Thickener´s function in a printing paste is to provide an adequate, pasty viscosity to an aqueous printing solution, conferring sharp patterns, by preventing dye migration and providing homogeneous distribution of the printing paste (Fijan et al. 2009). The conventional synthetic thickeners are mainly acrylic-based or polyurethane-based components characterized as non-biodegradable, corrosive contaminants, thus generating the imperative need for the identification of sustainable alternatives (Abdelrahman et al. 2020). Sustainable thickener alternatives are represented by starch, sodium alginate, British gum, meypro gum, carboxymethyl cellulose (CMC) sodium salt, carboxymethyl starch (CMS) (Rekaby et al. 2009) (Eid and Ibrahim 2021).

Innovation in textile printing processes include, on the one side, already mentioned sustainable alternatives in the printing paste components, and serious consideration and exploration of natural colorants with the use of resource efficient technologies, and on the other hand promising horizons seem to open for digital ink jet printing (Ujiie 2015).

1.6. Natural dyes role in functional finishing

An important added value generated by the use of natural dyes and pigments in the textile finishing industry, apart from the crucial health and environmental benefits, is represented by the functionalization conferred to the colored textile substrate as antimicrobial, UV protective, and insect repellent finishing (Shahid et al. 2013a).

Antimicrobial functionalization is a finishing practice of great interest and wide application in the textile industry due to the inherent capacity of the textile substrates for facilitating a growth environment to health-threatening microorganisms. Natural fibers, especially cotton and wool, due to their absorption capacity of water, nutrients, and oxygen, confer the optimum conditions for the growth of microbes and bacteria, and the contingency measures are mainly represented by metal-based finishes (Joshi et al. 2009). Various studies have demonstrated this functional capacity provided by natural dyes such as curcumin (from turmeric, naphthoquinones), lawsome (*Lawsonia inermis)*, juglone (walnut), lapachol (taigu), catechin

(Acacia catechu), anthraquinones (Rubia tinctorum, Rubia cordifolia, Rheum emodi. Punica granatum, Quercus infectoria) (Yusuf et al. 2017). Algae-based colorants have also proved enormous antimicrobial capacity, as identified and demonstrated in studies for pharmaceutical, medical and cosmetic applications, as chlorophylls (*Chlorella sp., Mono- raphidium dybowskii*) (da Silva Ferreira and Sant'Anna 2017), carotenoids (*Arthrospira plantensis, Dunaliella salina)* (Camacho et al. 2019), phycobiliproteins, including phycocyanin and phycoerythrin (*Arthrospira platensis, Anabaena oryzae, Nostoc sp., Synechococcus sp.)* (Pagels et al. 2019). Specific antimicrobial behavior of phycocyanin has been successfully validated by several researchers (Chiniforush et al. 2020) (Davaeifar et al. 2019).

Ultraviolet protection functionalization has been proven to be automatically provided by natural colorants due to their intrinsic absorption of light in the ultraviolet region. In this sense, the use of tannins has been validated by various researchers, completed by pomegranate extracts (Muthu 2017), *Cinnamomum Camphora* leaves extracts applied on wool and silk (Gong et al. 2019), orange peel natural extracts (Shahid et al. 2013b), henna extract dyeing of cotton (Alebeid et al. 2015) and many more examples. Algae and microbial organisms have also been demonstrated to poses UV protection function in the colorant molecules, as red colorant from *Steptomyces spp.* (Tuli et al. 2015), most carotenoids and chlorophylls provide UV screening (Suganya et al. 2016), complemented with various types of protection offered by phycobiliproteins (Levasseur et al. 2020).

Insect repellency functionalization is crucial for the textile industry due to the possible damages produced by these organisms to garments, carpets, or upholstery items. Several natural colorants have been proved to provide these properties to dyed textile substrates, as lac dye, madder, cochineal, indigo, and many more against several insects as black carpet beetle (Shahid et al. 2013b). Considering that algae bioactive compounds have been studied for food and feed applications, properties as antioxidant, antibacterial, antiviral, and many more high added functionalities have been demonstrated (Sharma and Sharma 2017)(Camacho et al. 2019), further properties as insect repellency are potential and should be subjected to future studies.

1.7. Textile industry wastewater treatment

The textile industry processing systems contaminate greatly by the discharge of chemicals in the wastewater effluents. In this sense, wastewater discharge management focuses on their analysis and characterization, and it has been widely researched considering the Chemical and Biochemical Oxygen Demand, metals content, and coloration, as the most popular parameters defining the process and wastewater quality (Bisschops and Spanjers 2003). These parameters define quantitatively the content of contaminant elements in the discharge effluents and the necessity of the type of water treatment before releasing into the environment or reuse possibility. Main polluting elements are represented by organic and inorganic matter, unfixed dyes, metals, and other auxiliary recalcitrant compounds defining thus, the importance of the awareness in the selection process of the raw materials to be used in the textile dyeing and printing processes (Correia et al. 1994) (Samsami et al. 2020).

The classification of the primary pollutants types of the wastewater streams from the textile industry is represented by biodegradable organic compounds, heavy metals, suspended

solids, pathogens, nutrients, toxic organic and inorganic compounds (priority pollutants), refractory organic compounds, and dissolved inorganics (Pal 2017).

The biodegradable organic compounds are mainly represented by carbohydrates, proteins, and fats. The presence of these pollutants in the environment, through their stabilization, can generate depletion of natural oxygen resources and lead to unsanitary conditions, affecting thus the aquatic environs. The measurement of the amount of these organic compounds is performed through BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) (Chakraborty 2010).

The COD test is mainly performed for the measurement of the number of oxidizable pollutants in the water or liquid waste, being expressed in milligrams /Liter (mg/L) or parts per million (ppm). This parameter indicates the amount (mass) of oxygen to be consumed per liter of solution, to fully oxidize its components (Wellinger et al. 2013).

BOD measurement methodology refers to the quantity of dissolved oxygen required by aerobic biological organisms to disintegrate the organic material from a water sample in specific conditions of temperature and time. The most common test used is BOD_5, expressed in mg/L of consumed O_2/L of the sample during 5 days of incubation at 20^0C (Yu and Brooks 2016).

The two oxygen demand tests previously described, are similar in function, by measuring the organic compounds in water, differentiated by the specificity of the measurements, as BOD measures biodegradable organic matter, meanwhile COD measures all chemically oxidizable matter (Li and Liu 2018).

Wastewater effluents may contain heavy metals in the composition, defined as elements with five times higher atomic weight and density when compared to water. They are characterized by their toxic nature, even at very low concentrations, thus needing to be identified and eliminated before reaching a water body or reuse of the wastewater effluent after treatment (Masindi and Muedi 2018).

The following Table 8 summarizes accepted limitations for the previously mentioned parameters as discharges contents of the textile finishing effluents towards the wastewater treatment plants.

Parameter	***Limitation***	***Source***
BOD_5 average weekly limitation	45 mg/L	EPA (Environmental protection agency) regulations (Zhang et al. 2015)
COD average weekly limitation	Regulation permits substitution with BOD_5 % correlation	
COD average daily maximum	1000 mg/L	Spanish national limitations (Alcaldesa et al. 2003)
BOD_5 average daily maximum	500 mg/L	
Aluminum content average daily maximum	10 mg/L	
Metal content: Iron content average daily maximum	5 mg/L	

Table 8. Industrial wastewater effluents discharge limitations into wastewater treatment plants

Due to the high dependency of the textile industry on potable water (Yaseen and Scholz 2019), international and national regulations regarding the limitation of these contaminants are imposed, for possible reuse of this wastewater effluents, thus great importance is given to the

raw materials employed, and in the same proportion, to the treatments applied for their cleansing and purification processes (Samsami et al. 2020).

Discharge of textile finishing process effluents into wastewater treatment plants are strictly limited (United States Environmental Protection Agency 2010), thus this sector has achieved vast developments of their treatment, for ensuring further use of these waters. The wastewater treatment methods can be divided into biological, chemical, physical, hybrid, or various combinations (Bhatia et al. 2017) (Kishor et al. 2021).

Initial development of these treatments corresponded to the physico-chemical approach, generating occasionally, unsustainable approaches due to the use of chemicals, energy, sludge quantity generation, etc. (De Gisi et al. 2016), leading to the use of biological methods based on the degradation capacity of an organism as algae, yeast, fungi, bacteria, or enzymes (Ali 2010). These organisms can mineralize dyes and decolorize the effluents under specific conditions (Pandey et al. 2007)(Samsami et al. 2020).

Due to the variability of the previously mentioned organisms, their use and efficiency analysis reveal fungi higher effectiveness in favor of bacteria, due to the generation of bioactive products as a result of the degradation process, offering the possibility of reuse of the treated water in food and feed industries, and increased efficacy in degrading recalcitrant compounds (Sankaran et al. 2010). Nevertheless, highly contaminated, acidic wastewater is more suitable to be treated with the use of yeasts (Wang et al. 2018). Microalgae, due to their capacity of using Nitrogen and Phosphorous for their growth are used in tertiary and quinary treatment processes (Abdel-Raouf et al. 2012). Enzymes are efficient and reliable industrial solutions for wastewater treatment, but their cost represents the main drawback in the application in this sector (Nguyen et al. 2016).

Hybrid or combined treatment methods represent an innovation in the sector and involve a combination of two or more treatment steps applied sequentially, leaning on benefiting from their advantages, expected to be cost and time-saving processes (Samsami et al. 2020). Main approaches with future prospects are represented by MBR (Membrane bioreactor) and PMR (Photocatalytic membrane reactor) technologies. MBR technology uses the combined membrane unit approach (Ćemanović et al. 2019), meanwhile, PMR focuses on the photocatalytic reactor approach (Karabelas et al. 2018).

A brief and visual proposal of the plausible complete life cycle of natural dyes derivated from microalgae and macroalgae biomass and employed in the textile finishing application is presented in Figure 7, adapted from (Muthu 2017) and (Kumar et al. 2021).

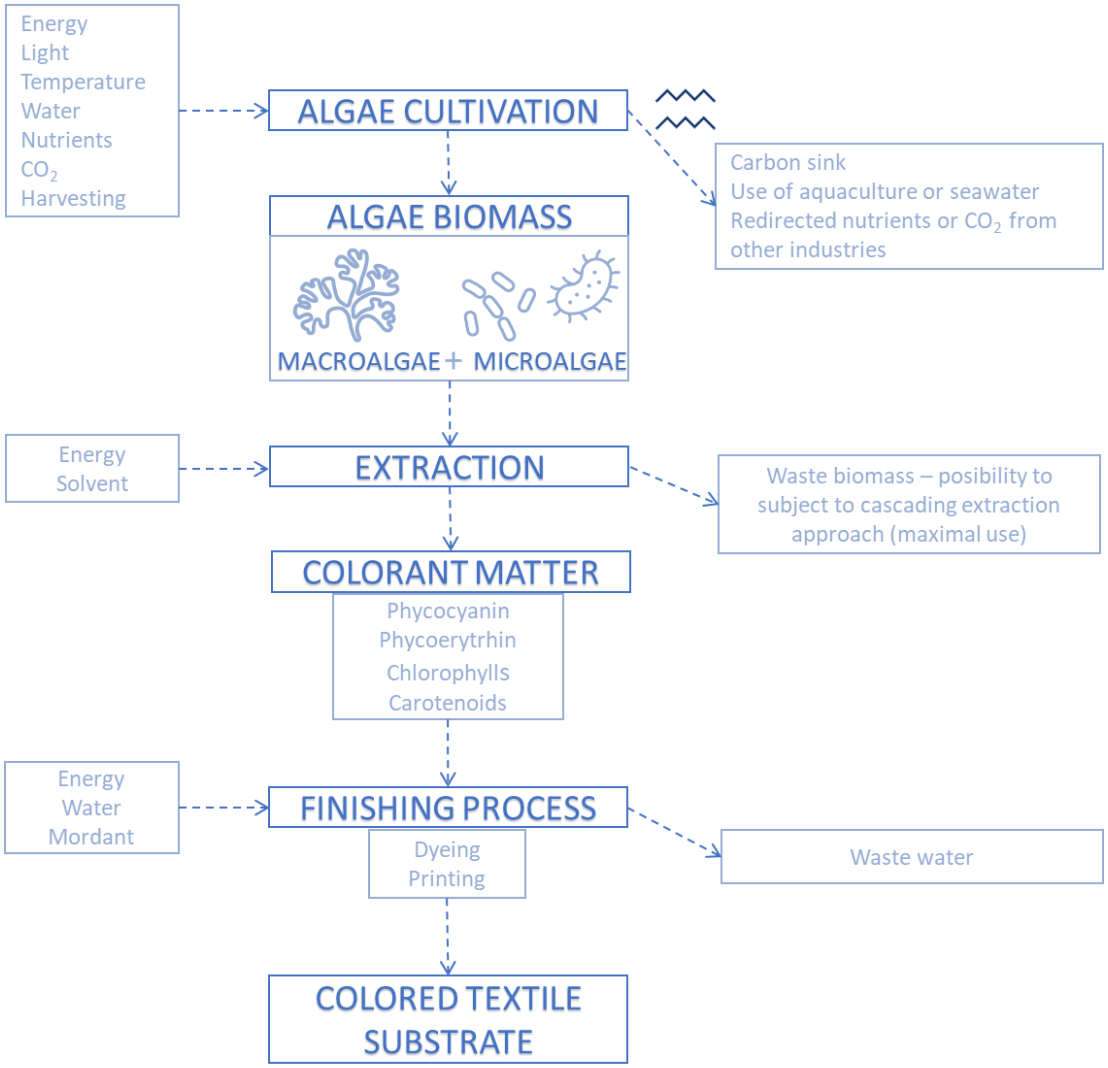


Figure 7. Proposal of a plausible complete-life cycle of algae sourced colorants and their use in textile dyeing and printing processes

Chapter 2. Hypotheses and objectives

This chapter describes thoroughly the main objective of the study and the hypotheses aimed to be validated and completed with the specific objectives, that ensure the achievement of the general scope.

Considering the sustainability context and the grave impact of the textile finishing industry on human and environmental health and safety, completed by its fast evolution and demand increase, researchers have focused their attention on alternative solutions that aim to reduce its impact. In this sense, the focus is oriented towards, on the one hand, the identification of sustainable raw materials that do not compete with vital resources as water, land, and air, and, on the other hand, the development and innovation of methodologies and technologies that reduce the impact or treat effectively the wastewater effluents, that characterize this industry. The natural colorants approach in the textile industry has revealed promising results, but the commercial application is not yet available (Komboonchoo and Bechtold 2009).

This study aims at proposing a solution for the perspective of a conscious selection of sustainable colorants for textile dyeing and printing processes, through the development of a laboratory-scale exploration of the suitability of algae-based colorant matter for selected textile finishing applications. The main objective is represented by the validation of the application of various natural colorants obtained from macroalgae and microalgae biomass, onto natural textile substrates, like cotton and wool, through conventional exhaustion dyeing and pigment printing processes. The verification of the applicability of these natural dyes and pigments is performed through the analysis of the results obtained through the variation of different process parameters, and the application of different auxiliaries. Additionally, these variations of the process parameters aim at improving the sustainability of the conventional processes and also the detection of sustainable alternatives of the commercially used auxiliaries. The objective validation of the proposed hypothesis of exploration of the suitability of algae-based sustainable alternatives for the conventional textile finishing processes is performed by means of colored textile characterization and analysis of the wastewater effluents.

The main focus of this study is to propose a sustainable alternative natural colorant resource for the textile industry by approaching a laboratory-scale exploration of the suitability of algae-based colorant matter for selected textile finishing applications, starting from the following hypotheses:

Hypothesis 1: Natural colorants represented by plant-based secondary metabolites have been proven as suitable candidates for textile coloration, as should be the ones proceeding from the aquatic photosynthetic organisms (micro and macroalgae feedstock).

Hypothesis 2: Micro and macroalgae feedstock is a valuable source for blue (phycocyanins), red (phycoerythrins), yellow(carotenoids), and green(chlorophylls) added-value colorant matter extracts suitable for employment in textile substrate conventional coloration processes.

Hypothesis 3: Colorfastness limitations of natural dyestuff may be surpassed with the optimization of the coloration process conditions or the use of mordants as coloration enhancers.

Hypothesis 4: Algae-based colorant matter represents a sustainable alternative for conventional synthetic and another natural dyestuff with an increased possibility of environmental impact reduction of the textile finishing industry.

OBJECTIVES

A series of objectives were proposed for reaching the main focus of this study oriented towards exploring the textile coloration suitability of algae-based colorant matter.

General objective: Explore the suitability and applicability of an alternative source of natural colorant matter, obtained from algae-based feedstock, for the textile coloration of natural fibers.

For the achievement of the general objective and validation of the hypothesis, secondary objectives were proposed.

Secondary objectives:

1) Select micro and macroalgae strains with the potential of a high yield of colorant matter.

The suitable screening of the algae-based feedstock will provide the basis for the selection of the most adequate strains with the highest yield colorant delivery potential.

2) Obtain colorant-rich extracts providing the primary colors (red, blue, yellow, and green) for coloration.

The possibility of obtention of the complete set of primary colors from algae-based feedstock ensures the future provision of a wide range of coloration possibilities for the textile industry.

3) Verify the colorants´ applicability through textile finishing processes, via exhaustion dyeing and pigment printing, on cotton and wool textile substrates.

To test the application possibility of algae-based colorants in the textile industry, the conventional coloration methods exhaustion dyeing, and pigment printing were selected, for the exploration of compatibility and manipulation possibility of these colorants in textile finishing processes.

4) Optimize the coloration process by process parameters variation.

The influence of various process parameters, as temperature, time, pH, bath ratio, the composition of dyeing liquor and printing paste are elements defining the quality and efficiency of the coloration process.

5) Assess the influence of different mordants on the coloration process efficiency.

Natural dyes do not present intrinsic affinity to natural fibers; thus efficiency and coloration enhancers must be explored. A screening, selection, and application of mordants as substrate textile treatment could improve the dyeability or printability of the algae-based colorants.

6) Test sustainable alternative ingredients for the selected finishing processes.

For increased sustainability of the proposed solution, alternative auxiliaries, biomordants, or natural components of the printing pastes behavior and compatibility with the overall process must be assessed.

7) Characterize the finished samples in terms of:
    a. Objective color assessment through measurement of CIElab color space.
    b. Color strength of the colored samples through measurement of the reflectance spectrum.
    c. Added-value capacity: antimicrobial and solar protection capacity assessment.

The colored samples' quality and process efficiency must be validated through objective surface color and color depth assessment with the use of color measurement instruments, as spectrophotometers.

8) Measure the fastness of the finished samples.

Laundering and lightfastness assessment according to European norms must be performed for the validation of the efficiency and quality of the coloration process, via the resistance of color against common external degrading agents.

9) Assess plausible theoretic interactions among the algae-based dyestuff-mordants and natural fibers.

The possible bonding among the colorants, mordants, and fibers should be assessed from a theoretical point of view, considering the characteristics and properties of each of the components of the equation.

10) Analysis of the coloration of the dyeing wastewater effluents to measure the unfixed remaining colorant matter in the effluents and calculate the dye uptake percentage for the coloration efficiency assessment.

11) Explore the sustainability of the dyeing process through the analysis of the quality of the dyeing wastewater effluents through:
    a. Measurement of organic matter (represented by the colorant matter employed in the process) via parameters as COD and BOD_5.
    b. Measurement of metallic content (due to metallic-based auxiliary inputs in the process) via parameters as Aluminum and Iron.

A preliminary approach on the quality analysis of the dyeing effluents considering parameters as COD or BOD_5, and metal content would represent a sufficient starting step due to the reduced ingredients input in the dyeing liquor (organic colorant matter, mordants, water).

    c. Experimental preliminary biological treatment approach of the dyeing effluents.

A sustainable added value of the process could be confirmed by the facileness and aggressivity level of the treatment necessary for wastewater effluents cleaning and preparation for possible reuse. The biological wastewater treatment represents a common industrial approach, and an initial laboratory assessment could validate this character of the proposed solution.

In this sense, the overall approach of this study is distributed among the main experimental chapters: Chapter 3, Chapter 4, and Chapter 5, describing the materials, methods, completed with the obtained results, their discussion, and finalizing with the main drawn conclusions. Chapter 6 focuses on recommending future actions based on the valuable results obtained.

Chapter 3 describes the raw materials used for the development of the experimental phase, including the innovative and sustainable algae-based colorant matter, natural textile substrates, and conventional and natural alternatives auxiliaries. Furthermore, it defines processes' optimum conditions, starting with pre-treatments and concluding with finishing processes and characterization methods.

Chapter 4 encompasses the complete experimental work results and their interpretation, starting with the selection criteria for the specific algae strains used in this study and the colorant matter obtained and used for the coloration processes. All the experimental results presented in this chapter focus on finishing processes applied on natural textile substrates, as standardized cotton and wool, for coloration with algae-based phycocyanin (blue color), phycoerythrin (red color), carotenoids (yellow color), and chlorophylls (green-color). Sections 4.1. and 4.2. focus on describing algae selection and the colorant-rich extracts obtained. Sections 4.3. and 4.4. tackle the results obtained with the exhaustion dyeing process application considering also the analysis of the influence of the conventional mordanting treatment on the dyeability efficiency, through objective color characterization focusing on both dyed textiles and wastewater effluent analysis, completed with fastness to light and laundering assessment. Nevertheless, initial approaches on the wastewater effluents quality (via parameters as BOD_5, COD, and metal content), and high-added value assessments through antimicrobial or solar protection capacity were performed. Section 4.5. addresses a theoretical proposal of possible interactions among the cotton and wool natural fibers, mordants, and the natural colorants. Section 4.6. focuses on the description and analysis of the results obtained through the pigment printing process with the algae-based colorants on natural fibers, indicating their compatibility with the process, the influence of mordanting, and natural alternatives to the conventional synthetic printing paste. The printed textiles were also subjected to light and laundering fastness characterization. Finally section 4.7. explores colorants´ sensitivity to pH and the additional applicability of the colored fabrics.

Chapter 5 highlights the most relevant results obtained from the experimental setup presented in this study.

Chapter 6 proposes future recommendations, based on the obtained results, representing the basis for further investigation, according to the innovations discovered through desk research, most of the latest briefly explained in Chapter 1. Introduction, proposing a future implementation of this innovative coloring solution in the textile industry.

Chapter 3. Materials and Methods

This chapter focuses on the description of the materials and methods used for performing the experimental section of the present study, represented by the laboratory exploration of dyeing and printing processes with naturally sourced colorant mater from micro and macroalgae.

3.1. Materials

a. Algae-based colorant matter

Phycocyanin-rich liquid extract obtained from *Spirulina platensis*, (procured from Banco Español de Algas (BEA), Gran Canarias, Spain) was employed as a blue natural colorant in dyeing and printing experiments.

Phycoerythrin-rich liquid extract obtained from the red macroalgae *Gracilaria gracilis* procured from Algaplus, Portugal, was used as a red natural colorant in dyeing and printing experiments.

Carotenoid-rich liquid extract sourced from microalgae *Dunaliella salina* (cultivated by Banco Español de Algas (BEA) from Gran Canarias, Spain) was used as a yellow-orange natural colorant in this study for dyeing and printing experiments.

Chlorophyll-rich liquid extract sourced from microalgae *Caespitella pascheri* (cultivated by Banco Español de Algas (BEA) from Gran Canarias, Spain) was used as a green natural dye in this study for dyeing and printing experiments.

b. Colorant matter extraction ingredients

Solvents used in the extraction process include laboratory distilled water, Hexane (Sigma Aldrich, Spain), Acetone (Sigma Aldrich, Spain), and Potassium hydroxide (Sigma Aldrich, Spain).

A protein stabilizer was used for increasing the resistance of phycobiliproteins to temperature and other factors, by using Ammonium sulphate (supplied by Sigma-Aldrich, Spain).

Consumables used in the extraction process are represented by Whatman filter paper (Sigma Aldrich, Spain), and glass spheres (Sigma Aldrich, Spain).

c. Textile substrates

Cotton: Standard commercial undyed, bleached 200 g/m^2 cotton fabrics, EMPA221 (Intexter UPC, Spain), in compliance with ISO 105-F01 were used as dyeing and printing substrates. The fabric was *2/1* twill, ends, and picks 33×27 per cm.

Wool: Standard wool fabrics (James Heal, England), complying with ISO 105-F01 were used as dyeing and printing textile substrates. The textiles are produced for tests for color fastness, by the supplier, for Part F01: Specification for wool adjacent fabric. It is a 100 % wool plain weaved fabric with 125 g/m^2.

d. Mordants

A total of **10 different mordants**, as indicated in Table 9 divided into synthetic and biomordants were used for the mordanting process in textile dyeing and printing processes.

Mordant			*Supplier*	*Chemical formula*	*% (w.o.f)*
Synthetic mordants	1	Cream of tartar (Potassium Acid Tartar or potassium bitartrate)	Gran velada, Spain Sigma Aldrich, Spain	$KC_4H_5O_6$	3,6,12
	2	Alum (potassium alum)	Gran velada, Spain Sigma Aldrich, Spain	$AlK(SO_4)_2$	10,20,30
	3	Ferrous sulphate	Sigma Aldrich, Spain	$FeSO_4$	3
	4	Tartaric acid	Sigma Aldrich, Spain	$C_4H_6O_6$	6
	5	Aluminum triformate	Sigma Aldrich, Spain	$C_3H_3AlO_6$	10
	6	Aluminum acetate	Greening, France	$C_6H_9AlO_6$	20
	7	Aluminum sulphate	Greening, France	$Al_2(SO_4)_3$	20
	8	Titanyl potassium oxalate	Greening, France	$C_4K_2O_9Ti$	20
Biomordants	9	Myrobalan-tannin	Greening, France	$C_{76}H_{52}O_{46}$	25
	10	Oak gall-tannin	Greening, France	$C_{76}H_{52}O_{46}$	25

Table 9. Selected synthetic and biomordants for the study

The selection of mordants was focused on covering a variety of the conventional natural, biomordants, and synthetic mordants, encompassing a selection of conventional metallic mordants and newly discovered.

Differentiation in the use of mordants for the experimental exploration among the dyeing and printing coloration method was applied, considering that for the dyeing experimental cases a total of 10 mordants was employed. Nevertheless, due to the consideration that for the printing technique a white textile base was necessary, the mordants characterized by an intrinsic natural coloration were not considered, as Ferrous sulphate, and the biomordants Myrobalan and Oak gal.

e. Bleaching ingredients

Oxidative bleaching was conducted with nonionic detergent (Clarite, Huntsman, Spain), Pyrophosphate tetrasodium (Sigma Aldrich, Spain), Hydrogen peroxide 30% (Fisher Scientific, Spain), and Ammonia 25% (PanReac, Spain).

f. Dyeing process ingredients

Distilled laboratory water was used as the aqueous base for the experimental dyeing liquors, and pH levels were controlled with 5 and 7 pH buffers (Hach-Lange, Spain). The final phase included the washing of the dyed textiles with non-ionic textile detergent (Clarite, Huntsman, Spain).

g. Printing process ingredients

Table 10 details the ingredients needed for the synthetic and natural mother printing paste preparation.

Paste element	***Synthetic mother paste***	***Natural mother paste***
Binder	Resin STK-100 (Color-Center, Spain)	Resin AC-60 (Color-Center, Spain)
Fixer	Color Center MC-LF (Color-Center, Spain)	Color Center MC-LF (Color-Center, Spain)
Thickener	Clear HC-35 (Color-Center, Spain)	CMC (Sigma-Aldrich, Spain)
Laboratory distilled water		

Table 10. Synthetic and natural mother printing paste ingredients

h. Wastewater biological treatment for reuse

An industrial wastewater plant-sourced fungi mixture was provided by a local wastewater treatment plant (Muro de Alcoy, Spain), for the experimental biological treatment approach. The fungi solution was represented by a combination of different anaerobic microorganisms as heterotrophic bacteria and protozoan species capable of degrading organic matter.

i. Additional applicability assessment for the colored fabrics ingredients

pH indicator capacity assessment of the dyed fabrics was performed with Sodium hydroxide 50^0Be 50% (Sigma Aldrich, Spain) for the preparation of an alkaline solution, and Acetic acid (Sigma Aldrich, Spain), was used as acid pH. The assessment of pH values was performed with pH indicator paper (Sigma Aldrich, Spain).

3.2. Methods

a. Cultivation of algae

Microalgae (*Cyanobacteria*) *Arthrospira platensis (Spirulina)* cultivated by BEA (Banco Español de Algas, Gran Canarias, Spain). The tailored cultivation started with strains from their bank collection, into an open raceway pond, developed under stress conditions (Nitrogen depletion), for the obtention of an enhanced yield of phycocyanin. The complementary conditions for the obtention of the maximal yield of colorant matter included optimization of parameters as amount of CO_2 and light. The obtained biomass was harvested, further filtered for separating from the culture medium. The storage conditions are defined by -20^0C under dimmed light.

Microalgae (*Chlorophytes*) *Dunaliella salina* cultivated by BEA (Banco Español de Algas, Gran Canaria, Spain), from the proprietary strain collection, into an indoor cultivation chamber, under controlled conditions, with parameters adjustment for carotenoids yields increase. These parameters control include high salinities, Nitrogen starvation, increased irradiance, and temperature. The biomass was harvested in the carotenogenic phase (carotenoids accumulation), then filtered, and maintained at -20^0C before being subjected to the colorant extraction process.

Microalgae (*Chlorophytes*) *Caespitella pascheri* was cultivated by BEA (Banco Español de Algas, Gran Canaria, Spain), selected from their strain bank, and cultivated into their internal cultivation chamber, with the possibility of vital parameters control. For the increase of chlorophyll yield, stress conditions were created by light reduction completed with the influence of red color, temperature increase, Nitrogen, and Phosphorous depletion. Additionally, CO_2 bubbling was an element of high importance. The biomass was then harvested, filtered, and frozen at -20^{0}C for storage purposes prior to extraction.

Macroalgae (*Rhodophytes*) *Gracilaria gracilis* was cultivated at Algaplus, Ilhao, Portugal in an Integrated Multi-Trophic Aquaculture (IMTA) onshore, in large-scale cultivation tanks, in an open pond system. The controlled conditions for determining R-phycoerythrin increased yield was represented by high salinity and light irradiance, complete by pH fluctuations of the culture medium (around pH=8). Biomass was harvested and stored at -20^{0}C before subjection to the colorant matter extraction process.

b. Extraction and quantification of algae-based colorant matter

Phycobiliproteins, represented by **phycoerythrin and phycocyanin** were subjected to similar mechanical extraction methods, due to their complementary presence in the cellular wall. In this sense, in the initial pretreatment phase, fresh algal biomass was submitted to 3 freeze-defrost cycles, where the subjection temperatures were -20^{0}C and 4^{0}C respectively, for a duration of 12h for each step. All the freeze-thaw processes were performed in full darkness conditions. The following mechanical phase was differentiated, for each colorant matter, due to the breakage capacity and rigidity of the cellular wall. In this sense, the second phase is separately described for each colorant below.

Phycocyanin: The phase 1 resulting *Spirulina platensis* biomass was subjected to a mechanical homogenization process, with a mortar and pestle, with the addition of laboratory distilled water and Phosphate Buffer at pH=7. Glass spheres were added for increased cellular wall breakage, resulting in the separation of the supernatant from the residual biomass.

Phycoerythrin: The initial phase resulting *Gracilaria gracilis* biomass was submitted to magnetic stirring for 5 hours, with the addition of laboratory distilled water at pH=7 (leveled with Phosphate Buffer), and glass spheres under dimmed light.

The final separation and extract concentration phase of the phycobiliproteins was the same and was performed through centrifugation cycles (30 minutes at 4000 rpm) followed by filtration, with filtration paper. To the final obtained colorant-rich liquid extract, protein stabilizer was added, 20% (with respect to the biomass weight) of Ammonium sulphate and was employed as obtained in the further textile finishing process.

The resulting phycocyanin-rich extract represented the blue colorant matter of this exploratory study, respectively the resulting phycoerythrin-rich liquid extract represented the red colorant matter, for the further dyeing and printing experiments.

The liquid phycocyanin-rich extract, the blue colorant, and phycoerythrin -rich extract, the red colorant matter in this study, were quantified through the analysis of UV spectral absorbance, due to the wavelength-based absorbance capacity of these chromophores. Spectral visible absorbance (UV, Thermoscientific Evolution 60S) was measured for the quantification of

the mg/mL of phycocyanin, respectively phycoerythrin in solution, via Bennett and Bogorad (1973) (Bennett and Bogorad 1973) equation, and applying the extinction coefficients defined by Bryant et al (1979) (Bryant et al. 1979).

Equation 1. Phycocyanin quantification

$$PC = {A615 - 0{,}474(A652)}/{5{,}34}$$

Equation 2. Allophycocyanin quantification

$$APC = {A652 - 0{,}208(A615)}/{5{,}09}$$

Equation 3. Phycoerythrin quantification

$$PE = {A562 - 2{,}41(PC) - 0{,}849(APC)}/{9{,}62}$$

Where PC represents phycocyanin, APC represents allophycocyanin, PE phycoerythrin, and A represents the value of absorbance at the indicated wavelength.

Considering the intimate location of the carotenoids and chlorophyll in the cellular structure, it must be highlighted, that even though the extraction follows different procedures, the quantification approach follows the same equation, thus reflected in the following paragraphs.

Carotenoids: The extraction process followed BEA internal procedures, using organic solvent Hexane for carotenoids extraction and Potassium Hydroxide for the separation of chlorophyll, considering that both chromophores are intimately connected, thus the separation phase was needed. The process involved maintaining the thawed biomass in Borosilicate bottles with Hexane, completed with glass pearls for 12h in an obscure environment. Further, the elimination of chlorophylls was executed with the addition of Potassium Hydroxide, in shaking condition at 40^0C for 40 min. The separation of the supernatant was performed through centrifugation at 600 rpm for 15 min and then filtered. The resulting biomass was further extracted with Hexane, and the second supernatant was mixed with the first one obtained before the chlorophyll elimination phase. This carotenoid-rich solution was concentrated in a rotary evaporator (Lamers et al. 2012).

Chlorophylls: The extraction process internalized by BEA implies the use of Acetone (100%) in Borosilicate bottles and obscure environments, due to the light sensitivity of this secondary metabolite. For enhancing the maceration process were added glass spheres, and after a 4h extraction phase, the residual biomass is separated from the extract solution via centrifugation. Further, the precipitate is subjected to consecutive extractions with Acetone, until the obtention of a white, colorless precipitate. The previously obtained chlorophyll-rich extracts are combined into a unique solution and further concentrated in a rotary evaporator.

The visible absorbance spectrums of the carotenoid-rich extract, respectively chlorophyll-rich extract were measured through UV spectrophotometry analysis, by subjecting the liquid extracts to the spectrophotometer (UV, Thermoscientific Evolution 60S), for absorbance measurements, using the corresponding quartz cells.

Extract concentration was calculated with the use of the Welburn method (1994) (Sumanta et al. 2014), employing UV-VIS spectrophotometer (UV, Thermoscientific Evolution 60S), for the measurement of absorbance values:

Equation 4. Chlorophyll a quantification

$$Cl\ a = 16{,}72\ A665{,}2 - 9{,}16\ A652{,}4$$

Equation 5. Chlorophyll b quantification

$$Cl\ b = 34{,}09\ A652{,}4 - 15{,}28\ A665{,}2$$

Equation 6. Total carotenoids quantification

$$Total\ Carotenoids = (1000\ A470 - 1{,}63\ Cla - 104{,}96\ Clb)/221$$

where Cl a= Chlorophyll a, Cl b= Chlorophyll b, A= absorbance.

For all colorant matter obtained, the mg/mL concentration identified, was used for the calculation of the exact quantity of extract-rich solution necessary for the coloration process, expressed as % weight of fabric (w.o.f).

c. Bleaching of wool

Prior to mordanting, the wool fabrics were subjected to an oxidative bleaching process. The bleaching solution includes decalcified water, 10 to 20 mL/L of Hydrogen peroxide 30% (Fisher Scientific, Spain), 2g/L of nonionic detergent (Clarite, Huntsman, Spain), 2g/L of Pyrophosphate tetrasodium (Sigma Aldrich, Spain). The pH adjustment to 8,5-9 was performed with Ammonia 25% (PanReac, Spain). The process was performed by immersing the wool fabric into the bleaching solution at a bath ratio of 1/15, at 60^0C for 55 min, in the dyeing machine Ugolini Redkrome (Italy), followed by rinsing and drying at room temperature.

d. Mordanting treatment

Textile substrates treatment for increasing fabrics dyeability, through increasing dyestuff fiber affinity, was applied on both cotton and bleached wool, through different processes including mainly pre-mordanting and also various tests of meta-mordanting. The same treatment was identically applied for both cotton and bleached wool study cases.

Pre-mordanting treatment was applied using the exhaustion dyeing device (Ugolini Redkrome, Italy), by immersing the fabric into the treatment liquor containing the preselected mordants in the % of w.o.f indicated in (Table 9). For each mordant, a different treatment liquor was prepared. This was conducted at a liquor-to-goods ratio of 1/40. The temperature was raised at 2°C per min to a maximum of 85°C and for 45 min. At the end of the mordanting treatment, the fabrics were rinsed and dried at ambient temperature before being subjected to the dyeing or printing process.

A preliminary study on **meta-mordanting** (mordanting and dyeing in one step) was conducted with specific mordants. In this case, the mordant was added into the dyeing liquor, and subjected to the dyeing process conditions, at 60°C for 60 min (see dyeing section for detailed information on the process parameters).

e. Exhaustion dyeing process

The wet dyeing application via exhaustion dyeing process was applied for all the textile substrates used in this study, cotton, and wool, with varying parameters such as liquor to goods ration, pH, temperature, and time, for the identification of their influence on the efficiency of the process. The constant parameter was represented by the 2% w.o.f. dyestuff applied in all the experiments. The exhaustion dyeing process involved the immersion of the textile substrate into the dyeing bath, then subjected to specific parameters combination (Figure 8) into the exhaustion dyeing device (Ugolini Redkrome, Italy), set to a temperature rise of 2°C per min.

Table 11 details the parameters combination used as analysis subjects in this exploratory study:

Fabric		***Parameter***					
Fabric pretreatment		***Bath ratio***	***Time (min)***	***Temperature (°C)***	***pH***	***Temperature raise***	***Dyestuff concentration (% w.o.f)***
Cotton	Non-mordanted	1/40 1/10 1/4	60** 90 60	65** 50 75	7*	2°C per min	2
	Pre-mordanted						
	Meta-mordanted						
Wool	Not treated	1/40 1/20 1/10 1/4			5*		
	Bleached						
	Bleached and pre-mordanted						
	Meta-mordanted						

Table 11. Summary of explored parameters in the dyeing process

*pH, adjusted with buffer by Hach- Lange

**optimum dyeing conditions

The selected optimum dyeing parameters for wool and cotton with algae-based dyestuff is indicated in the graph below:

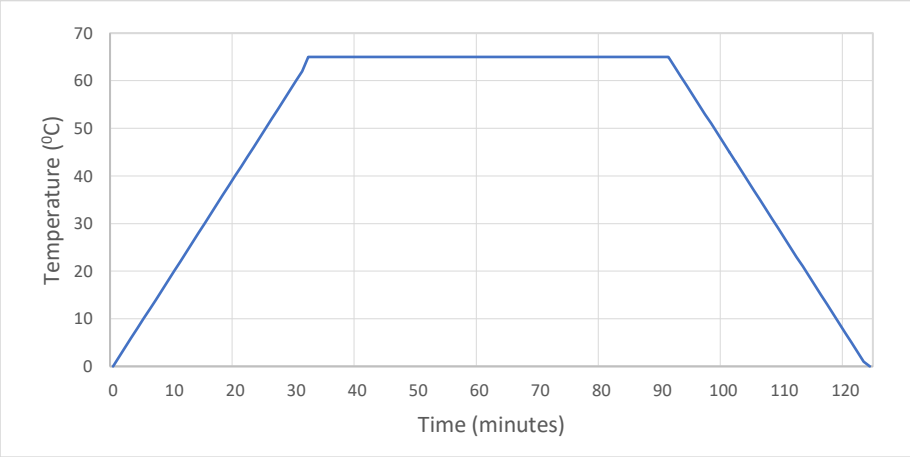


Figure 8. Optimum exhaustion dyeing conditions for wool and cotton with algae-based colorant matter

The exhaustion dyeing process was applied to the cotton and wool fabrics, where various parameters, as temperature, time, fabric treatment, and bath ratio, were modified for the identification of their influence on the quality of the dyeing effluents resulting from the process.

The fabrics were immersed in the dyeing bath liquor in the specific steel recipients, closed under pressure, and functioning in a rotative manner at a selected speed, corresponding to the laboratory exhaustion dyeing device, Ugolini Redkrome (Italy).

Posterior to the dyeing process, the samples were cold water rinsed, and further washed with non-ionic textile detergent (Clarite, Huntsman, Spain) for 30 min at a temperature of 50^{o}C, in continuous shaking, and finally dried at room temperature.

f. Wastewater sampling

The dyeing process resulting effluents were sampled, for further analysis subjection. The process involves cooling the effluents at room temperature, in the dyeing canisters after the removal of the textile substrates. The cooled wastewater samples are then transferred into a closed recipient, to be stored at 4^{o}C under dimmed light, in preparation before further analysis.

g. Wastewater fungi treatment experiments

The sustainable character of the resulting effluents from the exhaustion dyeing process of natural fibres with algae-based colorant matter was explored through a laboratory scale biological wastewater treatment approach, by analyzing the degradability of the residual matter. The action mechanism of this biological treatment is based on the fungi capacity of bio-reduction, represented by their reproduction capacity by using the sample nutrients (these (biodegradable compounds of the effluents), in the absence of solar energy.

The treatment methodology is focused on combining the treatment solution, represented by the fungal mixture, with the wastewater effluent, at stable conditions for 24h at a constant temperature of 20 ± 1^{o}C, in a closed recipient, allowing the realization of the anaerobic digestion phase.

The resulting sample is then subjected to COD and BOD_5 analysis for efficiency comparison reasons, considering the following selections of the samples: fungi solution, centrifuged fungi solution, treated wastewater with fungi, and centrifuged treated wastewater

h. Printing

The printing stock paste was always prepared with the following ingredients: binder, fixer, thickener, with 2% dye concentration rated to the weight of the printing paste.

The formulation steps involve first the thorough mixing of the binder and the fixer, preferably via blender, and secondly the addition of the respective amount of water and colorant-rich extract. Finally, the thickener is gradually added, until the formation of a consistent printing paste.

The following table (Table 12) indicates the recipes for natural and synthetic printing pastes and the corresponding temperatures and times necessary for the curing and drying phases.

Paste element	*Synthetic mother paste*		*Natural mother paste*	
Binder	Resin STK-100*	25%	Resin AC-60*	27 %
Fixer	Color Center MC-LF*	2,5 %	Color Center MC-LF*	4 %
Thickener	Clear HC-35*	2 %	CMC**	1 %
Pigment	2%			
Distilled water	(to complete)	68,5 %	(to complete)	66%
Total		1000 gr		1000 gr
Drying step	ventilation at 40ºC/80 ºC	2 min	20ºC/ Room temperature***	24 h
Curing step	150ºC/ 110 ºC	3 min 2 min		

Table 12. Printing paste recipes (synthetic and natural ingredients)

*supplied by Color-Center, Spain
**supplied by Sigma-Aldrich, Spain
*** the binder used in the printing process is self-crosslinking

The final colored printing paste was applied on the non-mordanted and white* pre-mordanted fabrics locked on the printing surface, through conventional manual printing screen and scraper, by applying three repetitions and manual uniform pressure with the elimination of the excess paste.

*It is important to highlight that from the 10 selected mordants (Table 9), a printing selection of 7 mordants was performed, due to the consideration of coloration exploring on a white base. 3 mordants are generating intense colored fabrics: brown for Myrobalan and Oak gall and yellow for Ferrous sulphate, thus these were not used for the printing experiments.

Following the manual printing phase, the curing and drying steps were executed in a laboratory-type drying oven (by Memmert, Germany), according to the temperature and time required by the ingredients included in the printing paste matrix.

i. Color characterization: CIELab coordinates, VIS spectrum, K/S

The dyed samples were subjected to the measurement of **chromatic coordinates**, with the use of Datacolor DC 650 spectrophotometer (supplied by Datacolor, Spain), applying the UNE-EN ISO 105-J01:2000 European standard for textiles characterization. The apparatus was equipped with standard components, CIE-Lab 10º observer and D65 illuminant.

The calculation of the ***color difference*** among the samples was performed according to the following equation (Equation 7) (L´Eclairage 2004), based on the mean value of the coordinates achieved on three measurements for each sample.

Equation 7. Color difference calculation

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Where,

ΔL^*=L^* mordanted dyed sample-L^*not mordanted dyed sample;

Δa^*=a^*mordanted dyed sample-a^*not mordanted dyed sample;

Δb^*=b^*mordanted dyed sample-b^*not mordanted dyed sample;

The analysis context of ΔE values considers values >3 revealing significant color differences and values <3 representing insignificant color differences.

For analysis purposes, the following relationship, generally accepted, and applied in the study indicates that the *L** value represents luminosity, the *a** value refers to red-green hues, meanwhile, the *b** value specifies blue-yellow shades.

In this sense, objectively, the following approach puts the basis for the measurements´ values interpretation:

*L**=100 indicates absolute white, meanwhile, *L**=0 is pure black;

*a**<0 refers to green color space, meanwhile *a**>0 characterizes the red shades;

*b**<0 valorizes the blue color space, meanwhile *b**>0 represents the yellow colors.

Fabrics´ **reflectance** was measured with UV-VIS spectrophotometer Lambda 950 (Perkin Elmer, Spain), and the identification of relative dye uptake, considered as color strength, K/S, was determined with the use of the Kubelka-Munk equation, given in (Equation 8)(Shen et al. 2016) (Kubelka, P. and Munk 1931). The reflectance value is considered the one corresponding to the maximum wavelength of each chromophore used in this study, as for carotenoids λ_{max}=490nm, for chlorophyll λ_{max}=665nm, for phycocyanin λ_{max}=620nm and respectively for phycoerythrin λ_{max}=560nm.

Equation 8. Color strength calculation

$$\mathrm{K/S} = \frac{(1-R)^2}{2\mathrm{R}}$$

where *K* is the absorption coefficient, *S* is the scattering coefficient and *R* reflectance at maximum wavelength.

j. Wastewater analysis: Absorption coefficient, COD, BOD_5, metals

Absorption experiments: Dye absorption assessment was performed based on the standard calibration curve plotted for the original dye liquor, prior to subjection to the coloration process. In this sense, a stock solution was prepared, containing 2% colorant matter, diluted for the further production of 4 standard solutions, to be subjected to spectrophotometric measurements. The maximum absorbance spectrum was obtained for each of the solutions and used for the plotting of the calibration curves. Further, the wastewater effluents obtained after the dyeing process were also subjected to spectrophotometric measurements, focusing on the maximum absorbance wavelength of the colorant matter, $\lambda_{max\text{-}phycocyanin}$=620 nm, $\lambda_{max\text{-}phycoerythrin}$=560nm, $\lambda_{max\text{-}chlorophyll}$ =665nm, and respectively $\lambda_{max\text{-}carotenoids}$=490nm. The calibration curve was then used for the calculation of the percentual dye uptake (dye absorption on the fabrics) of the colored samples, based on the absorbance of the resulting dyeing effluents. The difference between the initial dye concentration and the one remaining in the wastewater represents the dye uptake percentage, considering the assumption that the dye missing in the water is absorbed by the textile fibers used in this study.

Lambert-beer equation (Equation 9) was used for quantifying the colorant matter (phycocyanin, phycoerythrin, carotenoids, and chlorophyll) concentration in the remaining dyeing effluent.

Equation 9. Lambert-beer equation

$$A = \varepsilon \mathrm{cl}$$

Where A is absorbance, ε is molar absorption coefficient, c is molar concentration and l is the optical path length.

Dyeing effluents quality assessment was performed through specific ***quality parameters analysis***, following European standardized methodologies for the measurement of as Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and metal content.

Biochemical Oxygen Demand (BOD_5) measurement was performed following the pressure gauge S.M. 5210-D (Ed.22) method. The respirometric procedure involves direct measurement of the quantity of consumed oxygen, determined by the pressure difference generated by the degradation of the organic matter, by the microorganisms present in the sample, for 5 days, in darkness conditions, at constant stirring and temperature (20 ± 1^{o}C). The microorganisms of interest for this method are represented by the ones present in the ambient oxygen-rich air, and additional reactives as beer yeast, nutrients, urea, type II quality water, defined by a high quantity of microorganisms capable to oxidize the organic matter present in the samples (eg. domestic residual, non-chlorinated surface waters that receive residual water discharges and effluents from biological treatment systems), amongst others (European Environment Agency 2017) (Mckenzie 2003).

Chemical Oxygen Demand (COD) was measured according to the indications of the adapted procedure Proc.10E028. The main approach considers the colorimetric determination of the chemical oxygen demand, determined by the samples´ chemical oxidation, via spectrophotometry (Spectrophotometer DR3900). The procedure involves the reaction of the sample with a specific commercial KIT, for the digestion phase of the oxidizable substances, resulting in the green color indicating the presence of Chrome III, which is subjected to evaluation. The commercial KITs are then further used in the evaluation phase for the preparation of the blank and the dilutions, representing standard solutions, corresponding to quantifiable COD values (in ppm). Then these standard solutions will represent the references for measurement in line with the samples, via spectrophotometry, for the quantification of the COD, with the direct value detection (Service and Env 2017).

Metal content, strictly limited in this study to the quantification of the Aluminum and Iron content, via ICP.AES S.M 3120-B (ed.22). The method involves the usage of Plasma Emission Spectroscopy for the ionization process of the prepared water sample, at high temperatures. The conditioning of the wastewater sample involves the addition of Nitric acid, diluted to the required volume, and finally mixed with the sample for the proper analysis. The measurement of the prepared sample generates an emission spectrum with allows the direct quantification of the metals in the water sample (European Enviroment Agency 2021) (Wang et al. 2012).

k. Antimicrobial capacity

The **antibacterial inhibition capacity** was analyzed according to European standards ASTM E 2149-13 (ASTM E2149-13a.). The bacterial material tested was *Escherichia coli and Staphylococcus aureus,* and the analysis involved 3 repetitions for each measurement. The method includes the subjection of the sample, in the culture medium with Plate Count Agar (PCA), using Trypon Soy Broth (TSB) during a 24h contact time incubation, at a temperature of 35 ± 2ºC. Both, the dyestuff and the dyed textile were subjected to this analysis.

l. Ultraviolet solar radiation protection capacity

The **UPF (Ultraviolet factor) determination** was performed following the indications of the European standard AS/NZS 4399/1996 (Gies, P., Roy, C., Toomey, S., & Tomilinson 1999), using as reference the solar spectral radiance Sh of Noon of 17th January 1990 for Melbourne (38^0S). Its calculation is focused on the determination of the average effective UVR irradiance for unprotected skin (Eeff), based on the sum of the incident solar spectral irradiance with relative spectral effectiveness in the wavelength radius comprised between 290 and 400 nm. In order to reflect the results as the average effective UVR irradiance for the protected skin (E¢), the previous calculation is performed again considering the spectral transmittance of the fabric, then the Eeff to E E¢ ratio is then expressed as the ultraviolet protection factor (UPF).

The classification of the UPF values is done according to the indications below:

UPF Ratings	*Protection category*
15,20	*Good protection*
25,30,35	*Very good protection*
40,45,50,50+	*Excellent protection*

Table 13. Ultraviolet solar radion protection values classification

It is important to mention that the minimum value for a textile to be considered as providing ultraviolet solar radiation protection is 15.

m. Measurement of colorfastness: laundering and lightfastness

External degrading color factors as laundering and lightfastness, according to European standards were performed as it follows:

Fastness to domestic and commercial laundering was performed according to the standards regulated by UNE-EN ISO 105-C06:2010 (Spanish Standardization Organization 2010). The methodology involves the use of a Gyrowash apparatus (James Heal, UK), equipped with canisters, where the dyed samples of 10×4 cm are immersed into 150 ml of water and 0,6 gr of detergent. The processing involves the addition of 10 steel balls in the canister, a temperature of 25 ºC for 45 min, followed by a drying phase into a forced-air circulation dryer.

Change in color	*Results interpretation*
5	*Good behavior*
4-3	*Fair behavior*
2-1	*Poor behavior*

Table 14. Laundering fastness-change in color results interpretation

Staining	*Results interpretation*
5	*Very good-excellent*
4	*Good*
3	*Fair*
2	*Poor*
1	*Very poor*

Table 15.Laundering fastness-staining results interpretation

Lightfastness was performed according to the standards regulated by UNE-EN ISO B02:2014 (Spanish Standardization Organization 2014). The measurement apparatus is represented by Xenon arc fading lamp (James Heal, UK) and the methodology involves dyed samples pre-treatment by water spraying and their further exposition to the lamp for cycles of 16 hours.

Value	*Results interpretation*
8	*Excellent*
7	*Very good*
6	*Good*
5	*Moderate*
4	*Fair*
3	*Poor*
2	*Poor*
1	*Very poor*

Table 16 Lightfastness results interpretation

n. Additional applicability assessment for the colored fabrics

In order to define some additional applications of fabrics dyed with algae-based colorants, regarding their pH sensitivity, two substances were selected according to their pH. A solution of Sodium hydroxide 50^0Be (50%) was prepared for the alkaline solution, and Acetic acid was used as acid pH. A drop of the afore-mentioned products was placed on the surface of cotton dyed fabrics. Wool was not selected due to its high reactivity against alkaline products. Once the fabrics dried at ambient temperature the color coordinates were measured, for comparison reasons.

Chapter 4. Results and discussions

This chapter focuses on presenting the results obtained from the exhaustion dyeing and pigment printing experiments, using algae-based colorant matter, focusing on exploring the suitability of blue (phycocyanin), red (phycoerythrin), yellow (carotenoids), and green (chlorophyll) for textile coloration processes, considering the quality of the process and sustainability assumptions.

4.1. Algae strains selection

Algae strains selection was based on several criteria:

- To have mandatory 1 of the pigments/colorants of interest for this study (blue, red, yellow, green) in a sufficient yield.
- Their cultivation to be easily optimized, already performed by accessible providers, for facilitating an industrial scale up, in case promising results are validated.

In this sense, from the widely and industrial cultivation of the multitude of algae strains, the following (Table 17) selection was performed, all the references to the presented information were included in the desk research compiled in Chapter 1:

Type	***Strain***	***High yield matter***	***Industrial application***	***Cultivator for this study***
Macroalgae (Rhodophyta)	*Gracilaria gracilis*	R-Phycoerythrin (red)	Widely cultivated for food colorant, photosensitize in photodynamic therapy, flow cytometry, immunoassays, solar cell, and more (Fleurence 2004).	AlgaPlus
Microalgae (Cyanobacteria)	*Spirulina platensis*	C-Phycocyanin (blue)	Widely cultivated for medical, health care, cosmetics, food additives, and more (Zhang et al. 2016).	Banco Español de Algas
Microalgae (Chlorophyta)	*Dunaliella salina*	B-Carotenoids (yellow)	Cultivated for cosmetics, food, and feed supplements, pharmaceutical industry (Hosseini Tafreshi and Shariati 2009).	Banco Español de Algas
Microalgae (Chlorophyta)	*Caespitella pascheri*	Chlorophyll-a (green)	Widely used for food and medical applications (Jerez-Martel et al. 2017)(Vidhyanandan et al. 2020).	Banco Español de Algas

Table 17 Selected strains of microalgae and macroalgae for this study

4.2. Algae-based colorant rich liquid matter used in the exhaustion dyeing and pigment printing finishing processes

Phycocyanin from microalgae *Spirulina platensis*

The blue C-phycocyanin-rich extract was obtained from *Spirulina platensis* and was used in a liquid state in the finishing processes. The proteic chromophores´ fluorescent emission absorbance peaked at the interval of 615-622 nm accordingly (Pagels et al. 2019) (see Figure 9), and the extract showed a C-phycocyanin maximum concentration of 0,738 mg/mL.

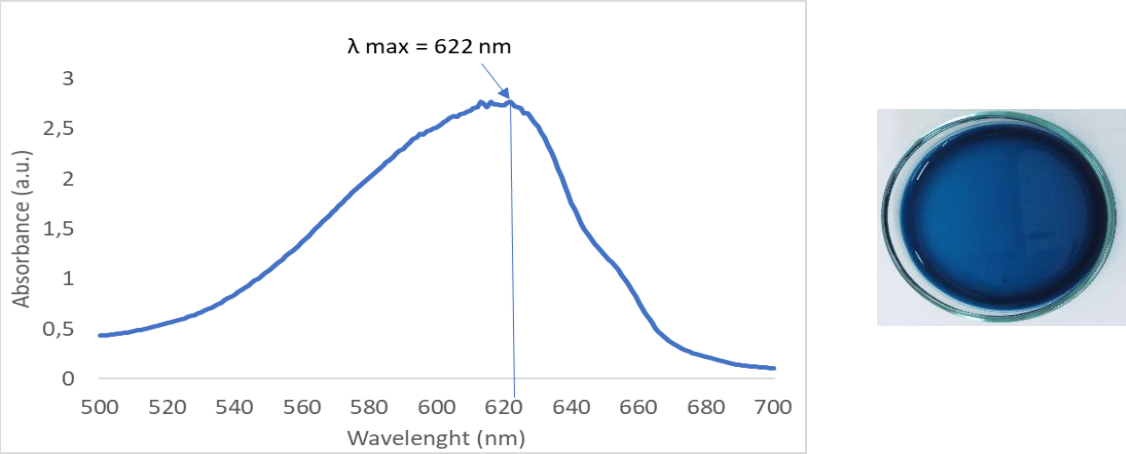


Figure 9. C-Phycocyanin rich extract spectrum and image of the blue extract

Phycoerythrin from red macroalgae *Gracilaria gracilis*

The red R-phycoerythrin-rich liquid extract was obtained from *Gracilaria gracilis* and was further used as obtained in the finishing experiments. The peak of the colorant matter corresponding to the absorption spectrum was centered at 541 nm accordingly (Parodi 2011) (see Figure 10), at a calculated concentration of R-phycoerythrin of 0,246 mg/mL.

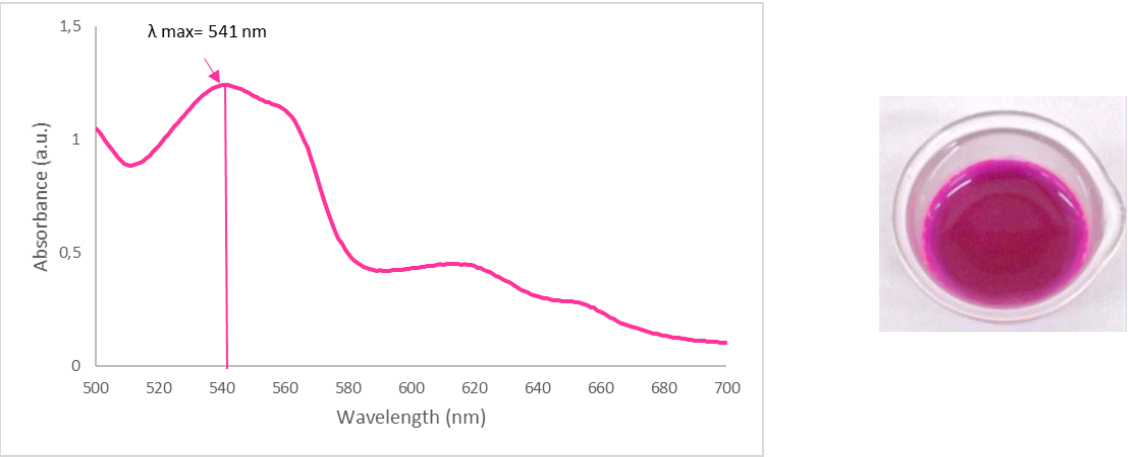


Figure 10. R-Phycoerythrin rich extract spectrum and image of the red extract

Carotenoids from microalgae *Dunaliella salina*

The yellow-orange β-carotenoids–rich liquid extract obtained from microalgae *Dunaliella salina* biomass has been employed as obtained in the finishing experiments. The absorbance of the visible spectrum reached a maximum value at 470 nm (Figure 11), as expected (Lichtenthaler and Buschmann 2005), and is characterized by a β-carotene concentration of 3,79 mg/mL.

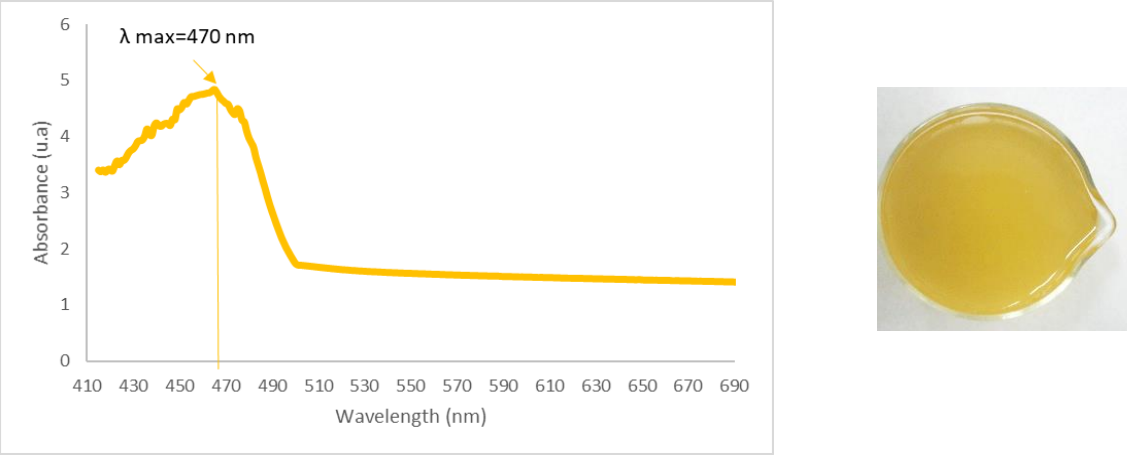


Figure 11. β- carotene-rich extract spectrum and image of the yellow extract

Chlorophyll from microalgae *Caespitella pascheri*

The green Chlorophyll a-rich liquid extract obtained from microalgae *Caespitella pascheri* biomass has been employed as obtained in the finishing experiments. The absorbance of the visible spectrum reached a maximum value at 670 nm (Figure 12), corresponding to Chlorophyll-a (Li and Chen 2015), and is characterized by Chlorophyll-a concentration of 14,4 mg/ml.

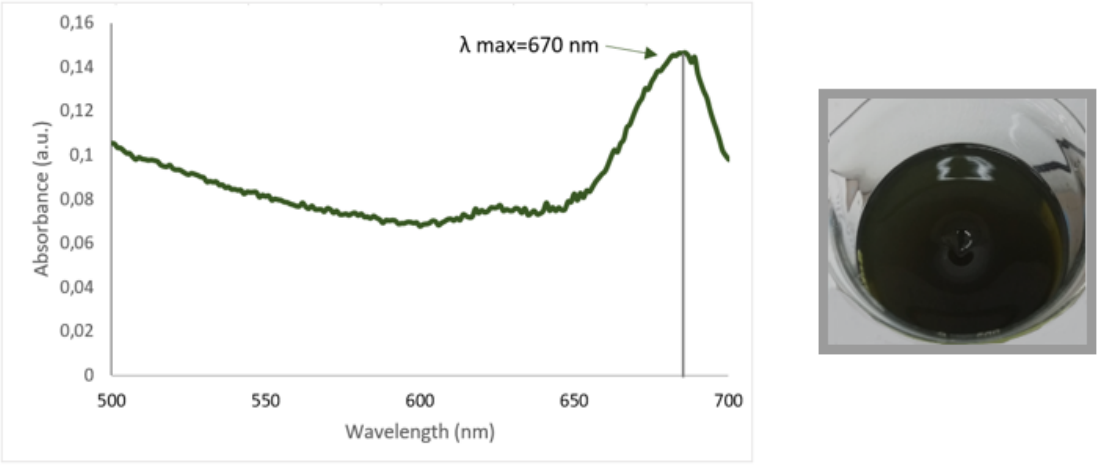


Figure 12. Chlorophyll a-rich extract spectrum and image of the green extract

4.3. Laboratory scale exploration of exhaustion dyeing process with algae-based colorant matter on cotton and wool textile substrates

The following section approaches and analyses the results obtained through the exploration of the exhaustion dyeing process with C-phycocyanin (blue), R-phycoerythrin (red), β-carotene (yellow-orange), and Chlorophyll a (green) sourced from micro and macroalgae, of natural cellulosic and proteinic textile substrates, like cotton and wool.

4.3.1. Analysis of the dyeability of natural fabrics with blue algae-based colorant matter C-phycocyanin-rich extract from microalgae *Spirulina platensis*

a) Analysis of results obtained from cotton textile substrate dyeing with C-phycocyanin

1) Color assessment: Chromatic characterization

In the following image (Figure 13) it can be visually appreciated a color difference in terms of tones and intensity obtained through the application of the 10 different mordants described in the materials and methods section, indicating thus an influence and possible compatibility with the textile substrate and the blue chromoprotein. In further applications this can be a selection criterion due to the high range of the results obtained, starting from blue shades with variations of yellow and brown.

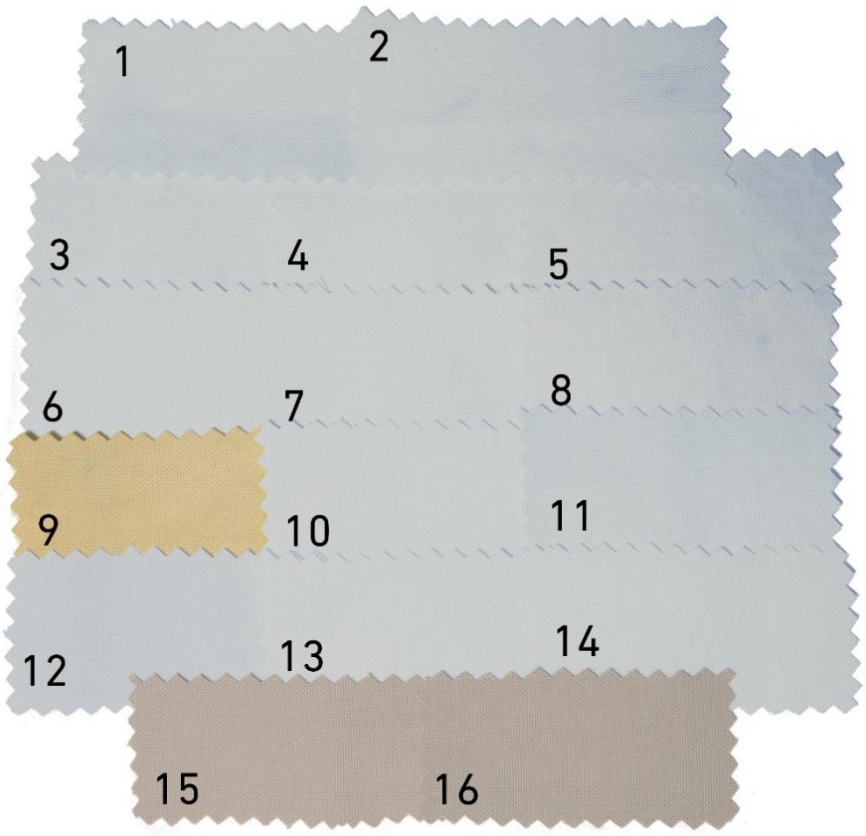


Figure 13. Dyed cotton fabrics with C-phycocyanin obtained from Spirulina platensis

1. No mordant (alkaline pH rinse); 2. No mordant (acid pH rinse); 3. Cream of tartar (3% w.o.f.); 4. Cream of tartar (6% w.o.f.); 5. Cream of tartar (12% w.o.f.); 6. Alum (10% w.o.f.); 7. Alum (20% w.o.f.); 8. Alum (30% w.o.f.); 9.Ferrous sulphate; 10. Tartaric acid; 11. Aluminum Triformate; 12. Titanyl potasium oxalate; 13. Aluminum acetate ; 14 Aluminum sulphate; 15. Myrobalan; 16. Oak gall.

Use of mordants and the influence on the dyeing process efficiency

Apart from the visual assessment, subjective color analysis was conducted, and the dyeing process effectiveness was objectively analyzed, through the measurement of the chromatic coordinates. The complete set of color space characterization (*L*, a*, b** values) measurements, completed with color difference *ΔE* and color strength *K/S* calculation, are included in Table 18. It can also be observed that the highlighted sample (no mordant with alkaline pH rinse) is considered the reference sample for further analysis. This reference sample was selected based on the high influence of the pH on the denaturation of the protein, as acidic conditions destabilize it (Patel et al. 2004)(Antelo et al. 2008). Secondly, acidic rinse water, at an industrial scale has a very negative environmental impact, considering that the limitations allow the minimum pH values starting with 6 (Berradi et al. 2019)(Islam 2020). Nevertheless, the different pH rinsing conditions were analyzed (first two lines in Table 18) for the validation of this denaturation of cyanobacterial C-phycocyanin in acidic conditions, even after the application of the dyeing process.

Sample	***Color coordinates and color strength***							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant (alkaline pH rinse)	83,69	-1,76	-1,55	-	-	-	-	33,64
No mordant (acid pH rinse)	85,98	-1,17	-0,99	2,29	0,59	0,56	2,43	34,20
Cream of tartar (3% w.o.f)	85,94	1,11	0,94	2,25	2,87	2,49	4,42	35,91
Cream of tartar (6% w.o.f)	87,01	-1,04	2,32	3,32	0,72	3,87	5,15	34,15
Cream of tartar (12% w.o.f)	84,92	0,13	1,36	1,23	1,89	2,91	3,68	34,16
Alum (10% w.o.f)	82,94	-0,44	2,11	-0,75	1,32	3,66	3,96	34,65
Alum (20% w.o.f)	87,08	-0,28	2,2	3,39	1,48	3,75	5,27	35,31
Alum (30% w.o.f)	86,37	0,74	1,05	2,68	2,5	2,6	4,49	35,33
Ferrous sulphate	82,32	1,28	13,56	-1,37	3,04	15,11	15,47	34,28
Tartaric acid	84,58	-0,44	1,2	0,89	1,32	2,75	3,18	34,29
Aluminum Triformate	83,06	-0,46	-0,02	-0,63	1,3	1,53	2,10	34,79
Titanyl potasium oxalate	87,13	0,14	1,05	3,44	1,9	2,6	4,71	34,89
Aluminum acetate	85,68	-0,14	1,39	1,99	1,62	2,94	3,90	34,38
Aluminum sulphate	86,69	-0,54	1,84	3	1,22	3,39	4,69	34,51
Myrobalan	67,21	0,85	12	-16,48	2,61	13,55	21,49	22,82
Oak gall	62,48	0,31	10,69	-21,21	2,07	12,24	24,58	21,96

Table 18. CIELab coordinates and calculations for C-phycocyanin dyed cotton

Rinsing pH value influence on the dyeing process efficiency

Considering the mandatory character of the rinsing process after the dyeing process, for unfixed dyes and auxiliaries' elimination, and the phycocyanins' sensitivity to pH, according to Table 18, it is confirmed the positive influence of the use of alkaline rinsing water, by maintaining the color intensity (*L**).

CIE*lab* color space characterization

ΔE calculated values (given in Table 18), ranging from 2,43 up to 24,58, indicate clear color differences between the non-mordanted dyed cotton fabrics (referenced in Figure 13 with number 1), considered as the reference sample in this study, and the ones where mordants were

applied (Roy Choudhury 2014). The color difference values, confirm the visual analysis, reflected in Figure 13.

One important aspect in dyeing with natural colorants is represented by the color intensity, objectively indicated through ΔL >0, confirming differences among the samples. In this sense, the reference sample (non-mordanted), shows more intense blue tones than the mordanted ones. These results are unexpected, as the effect should be the contrary, as also indicated in other studies approaching pre-mordanted cotton dyed with natural dyes (Haji 2019). Very slight differences, even considered as visually imperceptible, with less intense tones than the reference sample, were obtained with the use of the following mordants Tartaric acid, Cream of Tartar (3%, 6%, and 12% w.o.f.), Titanyl potassium oxalate, and Aluminum-based mordants as Alum (20% and 30% w.o.f.), Aluminum acetate and Aluminum sulphate represented by values ranging from 0,89 up to 3,39. This indicates a major limitation and non-effectiveness with the use of the previously mentioned mordants.

On the other hand, when referring to the values of ΔL<0 it means that the reference sample (non-mordanted and dyed) owns less intense coloration when compared with the use of Alum (10% w.o.f.), Ferrous sulphate, and Aluminum triformate.

A particular case can be assigned to the use of Myroballan and Oak gall, the biomordants used in this study, as they count with a natural intrinsic brown color, influencing the resulting coloration, into darker tones. This is supported by the obtention of extreme L^* values as 67,21 and 62,48 respectively. Apart from this, the Δb calculated values between 12,24 and 15,11 with the use of Ferrous sulphate, Myrobalan, and Oak gall, objectively confirm the brownish/yellowish tones generated by these mordants (with the highest values of b^*). This does not represent an affinity increase between the biomordants, C-phycocyanin, and cotton fibers, but the influence of the natural coloration of these specific mordants.

Figure 14 plots the CIE*Lab* color diagram, where b^*<0 refers to the blue color space, the objective of this particular set of experiments. A blueish-green color location can be observed, with reddish influence and this can be justified by the interference of the strongly connected phycoerythrin (red influence) and chlorophyll (green), due to the reduced purification level of the extract. This distribution does not confirm colorant matter-fabric affinity, as this must be validated in complementarity with the fastness results.

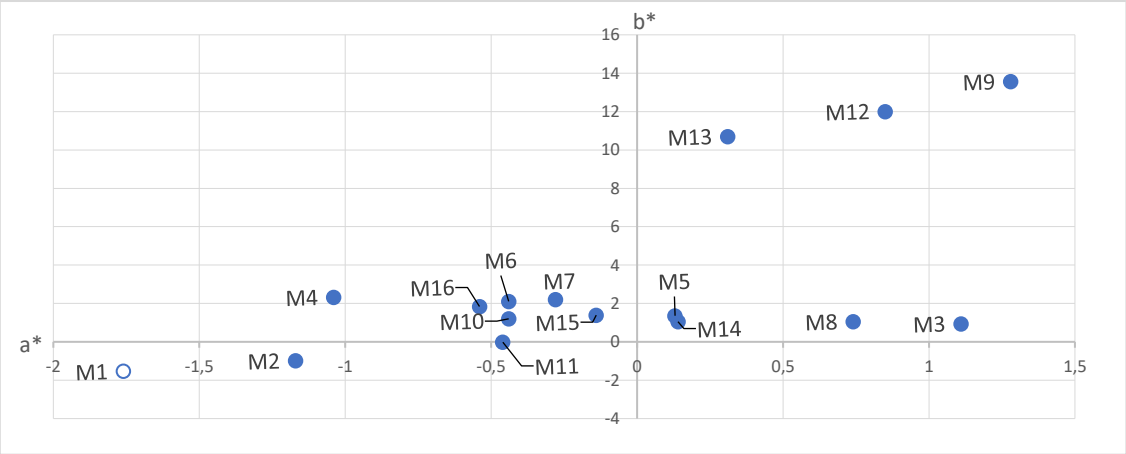


Figure 14. Color specifications in CIELab of the dyed cotton fabrics with C-phycocyanin

M1. No mordant (alkaline pH rinse; M2. No mordant (acid pH rinse); M3. Cream of tartar (3% w.o.f); M4. Cream of tartar (6% w.o.f); M5. Cream of tartar (12% w.o.f); M6. Alum (10% w.o.f); M7. Alum (20% w.o.f); M8. Alum (30% w.o.f); M9. Ferrous sulphate; M10. Tartaric acid; M11. Aluminum Triformate; M12. Myrobalan; M13. Oak gall; M14.Titanyl potassium oxalate; M15. Aluminum acetate; M16. Aluminum sulphate.

Mordant concentration influence on the dyeing process efficiency

Two conventional mordants, Alum and Cream of tartar have been used for analyzing the influence of concentration on the dyeing process efficiency (Table 18), to validate commercially used solutions. It can be stated that, in the case of Cream of tartar, increasing the concentration does not influence positively the final result, even more, the molecules of the mordant over-occupy the textile substrate molecules, generating a reduction in the blue hue intensity (*Δb>0)*. Considering the use of Alum, the increase in applied concentration (up to 30%) slightly improves the blue shade obtained, but only in comparison with the other concentration variations, and not when compared with the reference sample.

Reflectance spectrum and color strength analysis

Another means of comparison of the influence of the color depth of the dyed fabrics were performed through the measurement of the reflectance spectrum and calculation of the color strength (K/S). As it can be observed in Figure 15, the highest values in blue color uptake and strength correspond to the superior part of the graph, thus to the employment of Cream of tartar 3%, Titanyl potassium oxalate, Aluminum-based mordants as Aluminum acetate, Aluminum sulphate, concentration Alum, mainly in comparison with the brown-yellow color influencing mordants.

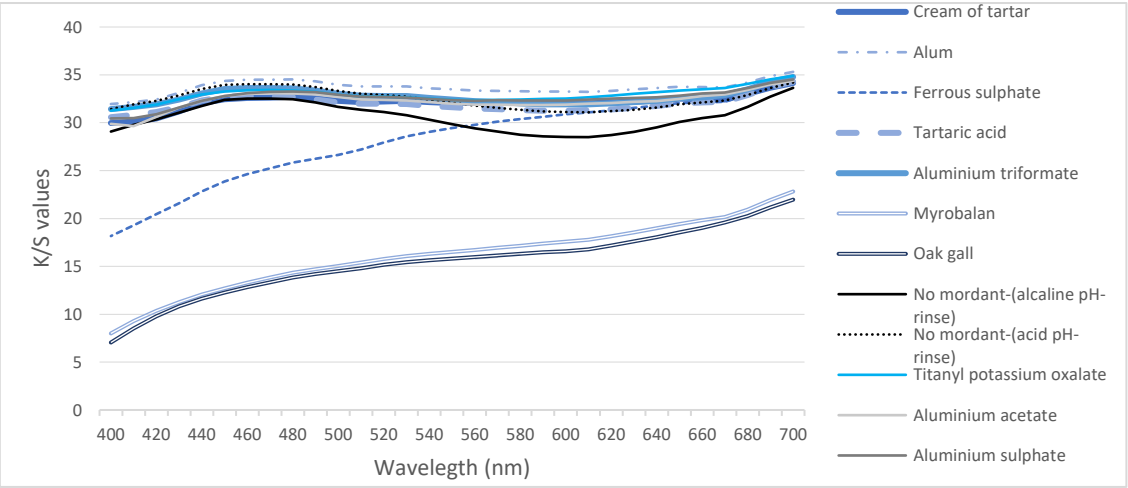


Figure 15. K/S spectrum of dyed cotton with C-phycocyanin -mordant influence analysis

The chromatic characterization of the cotton fabrics dyed with a series of mordants, reveals a negative conclusion, in terms of influence on dyeability. Nevertheless, an exception may be indicated, with the use of Aluminum triformate, accomplishing the *b*<0* slightly negative (-0,02).

To the best of my understanding, a reduced amount of studies on cotton dyeing with naturally occurring blue colorant were performed, most of them being performed with synthetic additions (Ding and Freeman 2017) or with indigo-based approaches (Luo et al. 2020)(Ben Ticha et al. 2013) (Saikhao et al. 2018).

Measurement of residual colorant matter in C-phycocyanin-cotton dyeing wastewater effluents

Dyeing wastewater effluents were characterized for the determination of the quality of the emissions generated by this particular finishing process. In this sense, it was measured the residual unfixed colorant matter in the remaining liquor after the dyeing process of cotton. Figure 16 reflects the dye uptake %, referring to the quantity of remaining unfixed dye in the wastewater effluent, thus the higher the values calculated, the higher the process efficiency, and more absorption of C-phycocyanin on the cotton substrates. Similar values of dye uptake were obtained (ranging from 46% to 56%), for the majority of mordanted fabrics and below the non-mordanted ones, peaking with the use of Cream of tartar and Alum, but not identifying a significant dyeability improvement with the use of mordants, and closely followed by the Aluminum-based mordants. Additionally, the influence of the intrinsic color of the Ferrous sulphate, Myrobalan, and Oak gall is confirmed by significantly lower values, in comparison with the set of experimental cases.

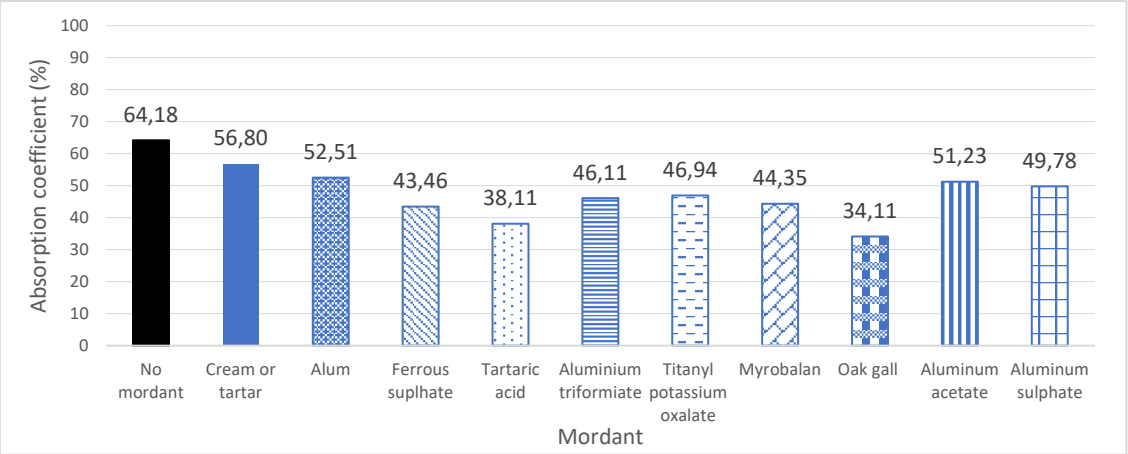


Figure 16. Colorant absorption coefficient based on dyeing of cotton with C-phycocyanin wastewater effluent

2) C-phycocyanin cotton dyeing process quality assessment through measurement of laundering and lightfastness

A thorough analysis of the reaction to domestic and commercial laundering and lightfastness of the dyed cotton fabrics was performed, and the color change and staining on a variety of different samples were measured, as indicated in Table 19.

Sample	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant (alkaline pH rinse)	1	4-5	4-5	4-5	4-5	4-5	4-5	6-7
No mordant (acid pH rinse)	1	5	5	5	5	5	5	6-7
Cream of tartar (3% w.o.f)	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Cream of tartar (6% w.o.f)	1	5	5	5	5	5	5	6-7
Cream of tartar (12% w.o.f)	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum (10% w.o.f)	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum (20% w.o.f)	1	5	5	5	5	5	5	6-7
Alum (30% w.o.f)	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Ferrous sulphate	2	5	5	5	5	5	5	6-7
Tartaric acid	1	5	5	5	5	5	5	6-7
Aluminum Triformate	1	5	5	5	5	5	5	6-7
Titanyl potassium oxalate	1	4-5	4-5	4-5	4-5	4-5	4-5	5
Myrobalan	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Oak gall	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	5
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	5

Table 19. Fastness properties of the C-phycocyanin dyed cotton influence of different mordants

Commercial and domestic laundering show a clear degradation of the color of the dyed fabrics, due to the range of values obtained (1-2), defining very poor behavior, and low color resistance on the fabric. Even though, compared to the reference samples, low improvements are observed with the use of Cream of tartar, Alum, and Ferrous sulphate. Nevertheless, the very good behavior in staining other textile substrates in laundering conditions indicates the very reduced affinity of the C-phycocyanin to the tested fabrics, due to the bonding incapability among the colorant matter and the substrates involving the need for additional treatment. In

this sense, a suitable mordanting treatment would increase the dye uptake, as demonstrated to appear in minority in the wastewater effluents. The concentration variation with the use of Cream of tartar and Alum results maintain coherence in these results also, as there is no influence on the quality of the dyeing process. The special situation of the biomordants, Myrobalan and Oak gall is identified in this set of analyses too, as their intrinsic color, brown is analyzed, and does not reflect the C-phycocyanin dyeability improvement.

Considering that the natural colorants are not characterized by high values of lightfastness, it is worth mentioning that an added value element employed in this study, which influences the lightfastness of the dyed fabrics, is represented by the use of the Ammonium sulphate, in the extraction process, a protein stabilizer, conferring thus, more stability against the degradation effect of the light factor. In the specific case of the use of Tartaric acid, Aluminum triformate, Titanyl potassium oxalate, Aluminum sulphate, and Aluminum acetate, it represents the improved effect of this combination of elements, a protein stabilizer, and a mordant bonding effect. Regarding the dyed fabrics, with no mordant and the use of Cream of tartar and Alum, the very low color intensity generates, the low appreciably color differences between the sites of the fabrics subjected to the xenon lamp and the reference ones, thus valued high lightfastness. Ferrous sulphate is a mordant that confers an inherent yellow color to the dyed fabric, canceling the blue dye and with high natural lightfastness.

Partial conclusions for dyeing of cotton with C-phycocyanin

- *Mordant concentration influence* on color intensity was analyzed among 10 mordants and reveals significant color difference with the use of **Alum (10% w.o.f)** and Aluminum Triformate (10% w.o.f.). In terms of lightness (*L**), no improvement is achieved when increasing the concentration from the conventionally used 3·% w.o.f (*L**= 85,94) of Cream of tartar up to 6% (*L**= 87,01) and 12% (*L**= 84,92) respectively, and the same conclusions were drawn in the case of the decrease of **Alum concentration** from the conventional 30% w.o.f. (*L**= 86,37) to 20% (*L**= 87,08) respectively. This means that the mordanted samples are significantly lighter than the non-mordanted ones.
    - The same non-effectiveness was observed with the use of Tartaric acid (6% w.o.f.), Titanyl potassium oxalate, Aluminum acetate, and Aluminum sulphate when compared with a non-mordanted dyed sample.
    - *Intrinsic natural yellow to brown* color of some of the tested mordants has a major color influence on the resulting dyed cotton fabrics, obtaining: yellowish fabric with the use of Ferrous sulphate (3% w.o.f) in the pre-mordanting process; and brown hues of the dyed cotton fabrics with the use of the biomordants Myrobalan (25%), and Oak gall (25%).
- *The analysis of the absorption coefficient of the wastewater effluents* concludes that the amount of dye remaining in the effluent of the pre-mordanted samples is lower than the non-mordanted samples, and even lower values with the use of Ferrous sulphate, Myrobalan, and Oak gall (43,46%, 44,35%, respectively 34,11%).
- *Laundering and lightfastness* indicate laundering defined by clear degradation of the color of the dyed fabrics, due to the range of values obtained (1-2), defining very poor behavior, and low color resistance on the fabric and good behavior in staining other textile substrates, and light characterized by acceptable behavior.

b) Analysis of results obtained from wool textile substrate dyeing with C-phycocyanin

1) Color assessment: Chromatic characterization

A visual assessment of the C-phycocyanin dyed wool substrates can be seen in the following image (Figure 17), and a direct appreciation of the influence in color tones and intensity, of the different experimental conditions, including the employment of 10 different mordants, can be performed. An interesting range of colors has been obtained from light blue to yellow and brown tones. This analysis is further deepened with objective color measurements.

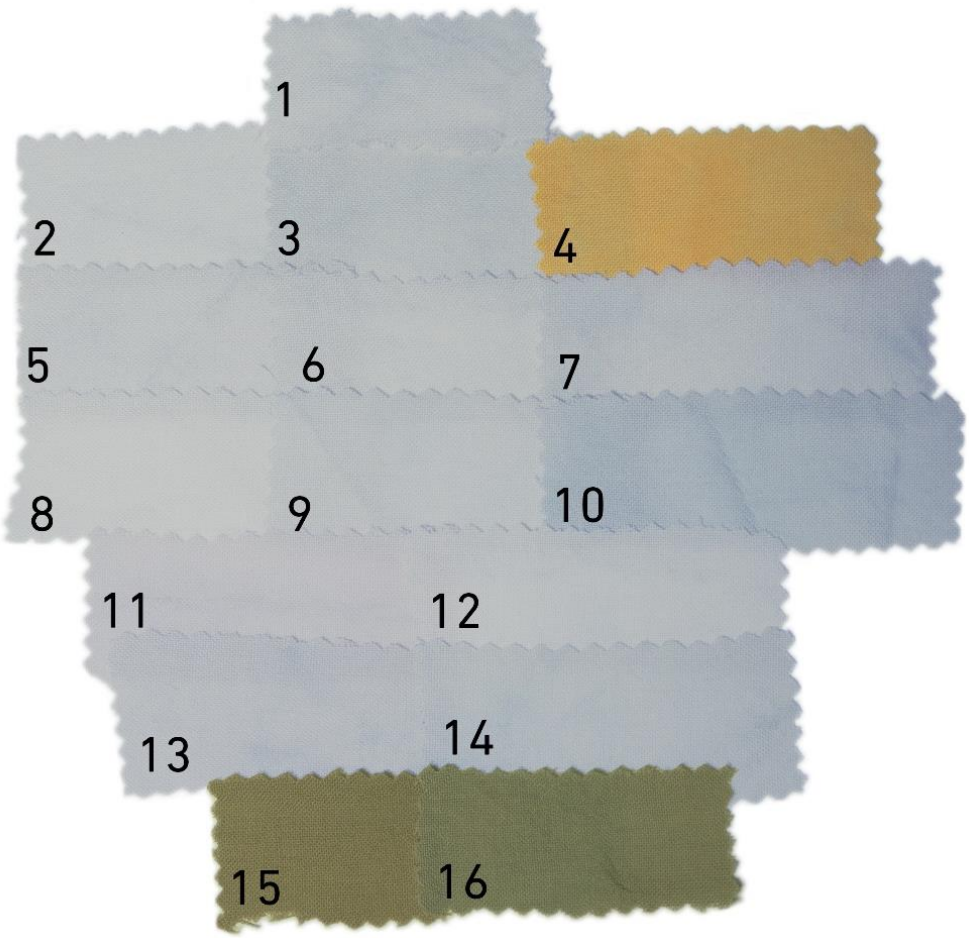


Figure 17. Dyed wool fabrics with C-phycocyanin obtained from Spirulina platensis

1.No mordant; 2. Cream of tartar (3% w.o.f); 3. Alum (10% w.o.f); 4. Ferrous sulphate; 5. Tartaric acid; 6. Aluminum Triformate; 7. Aluminum acetate; 8. Cream of tartar (3% w.o.f.; Rb=1/4); 9. Cream of tartar (3% w.o.f.; Rb=1/10); 10. Cream of tartar (3% w.o.f.; Rb=1/20); 11. Aluminum sulphate; 12. Metamordant Cream of tartar (3% w.o.f., Rb=1/10); 13. Titanyl potassium oxalate (bleached) 14. Titanyl potassium oxalate (natural, not bleached); 15.Myrobalan; 16. Oak gall.

Use of mordants influence on the dyeing process efficiency

Objective color characterization of the dyed wool substrates with C-phycocyanin was performed through CIE*Lab* chromatic coordinates measurement, completed with the color difference and color strength calculations, and the complete set of results are included in Table 20. It can be observed, as highlighted in the table, that the reference sample in this set of calculations, is considered the bleached non- mordanted C-phycocyanin dyed wool fabric, and

it was selected for the proper analysis of the influence of the mordanting process on the dyeability efficiency.

Sample	*Color coordinates and color strength*							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant	76,31	-3,43	3,56	-	-	-	-	23,69
Cream of tartar (3% w.o.f.)	80,13	-3,01	6,94	3,82	0,42	3,38	5,12	22,80
Alum	79,77	-3,63	4,7	3,46	-0,2	1,14	3,65	24,66
Ferrous sulphate	67,15	8,81	26,82	-9,16	12,24	23,26	27,83	23,22
Tartaric acid	75,76	-2,63	7,58	-0,55	0,8	4,02	4,14	20,32
Aluminum Triformate	78,03	-2,64	8,32	1,72	0,79	4,76	5,12	23,53
Aluminum acetate	65,54	-4,87	5,98	-10,77	-1,44	2,42	11,13	22,80
Aluminum sulphate	75,58	-5,74	6,88	-0,73	-2,31	3,32	4,11	23,09
Myrobalan	53,87	2,58	19,92	-22,44	6,01	16,36	28,41	16,79
Oak gall	56,7	-0,76	13,99	-19,61	2,67	10,34	22,37	17,23
Meta-mordant Cream of tartar (3% w.o.f., Rb=1/10)	73,33	0,48	6,13	-2,98	3,91	2,57	5,55	-
Cream of tartar (3% w.o.f.; Rb=1/10)	77,41	-3,3	7,03	1,1	0,13	3,47	3,64	-
Cream of tartar (3% w.o.f.; Rb=1/20)	72	-5,68	4,95	-4,31	-2,25	1,39	5,06	-
Cream of tartar (3% w.o.f.; Rb=1/4)	79,67	-2,35	9,18	3,36	1,08	5,62	6,64	-
Titanyl potassium oxalate (bleached)	72,24	-5,54	6,73	-4,07	-2,11	3,17	5,57	21,26
Titanyl potassium oxalate (natural, not bleached)	75,91	-4,55	10,3	-0,4	-1,12	6,74	6,84	23,17

Table 20. CIELab coordinates and calculations for C-phycocyanin dyed wool

The first element to be considered in this analysis is represented by the confirmation of a color difference generated due to parameter variation in the dyeing process as indicated by *ΔE* (given in Table 20) presenting values in the range 3,64 and 28,41, with lower impact generated by mordant concentration variation and diversity, followed by the type of mordant used.

In general, more intense (darker) and blueish colors in comparison with the reference sample, are defined by *ΔL* <0 and *Δb* approximating the 0 value, and are obtained with the employment of Aluminum acetate, Tartaric acid, Cream of tartar (in various approaches, meta, and pre-mordanting), Titanyl potassium oxalate, and Aluminum sulphate.

A series of mordants generated the most accentuated color differences, in terms of intensity and color shades, and they are Ferrous sulphate, Myrobalan, and Oak gall, but this does not indicate better performance in terms of increased dyeability, but the effect of the natural coloration generated by the mordant intrinsic color. This is validated by the *a** and *b** color coordinates values, locating these results in the yellowish (Ferrous sulphate), respectively brownish color space (Myrobalan and Oak gall).

Figure 18 plots the CIE*Lab* color diagram, where *b**<0 refers to the blue color space, and it reflects the green coordinate influence *a**<0, and slight reddish influence. This is expected as

in the colorant-rich solution residual phycoerythrin (red influence) and chlorophyll (green) may be contained, due to the low level of purification of the extract. Nevertheless, the extreme values are attributed to M7, M8, M9, being Myrobalan, Oak gal, respectively, Cream of tartar (meta-mordanted approach).

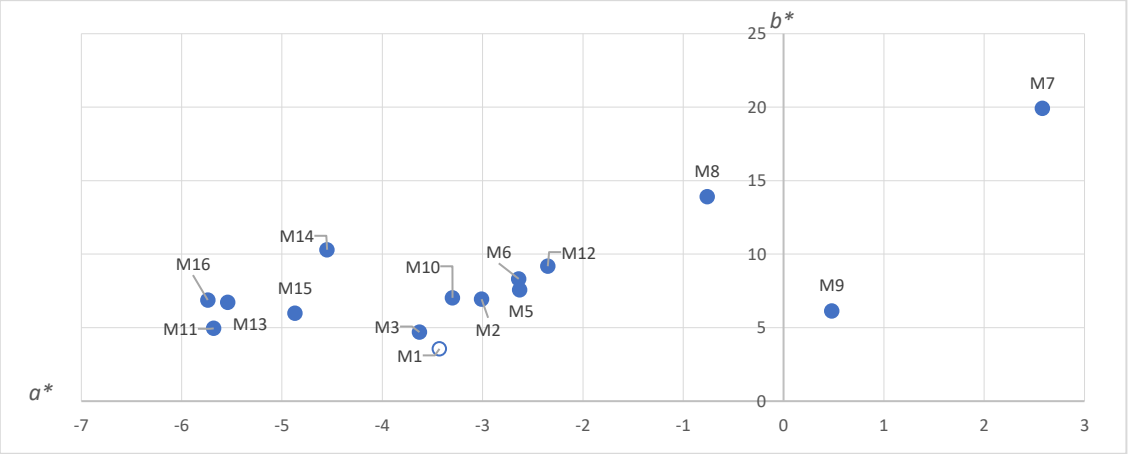


Figure 18. Color specifications in CIELab of the dyed wool fabrics with C-phycocyanin

M1. No mordant (alkaline pH rinse); M2. Cream of tartar (3% w.o.f., Rb 1/40); M3. Alum (20% w.o.f.); M4. Ferrous sulphate; M5. Tartaric acid; M6. Aluminum Triformate; M7. Myrobalan; M8. Oak gall; M9. Metamordant Cream of tartar (3% w.o.f., Rb=1/10); M10. Cream of tartar (3% w.o.f.; Rb=1/10); M11. Cream of tartar (3% w.o.f.; Rb=1/20); M12. Cream of tartar (3% w.o.f.; Rb=1/4); M13. Titanyl potassium oxalate (bleached); M14. Titanyl potassium oxalate (natural, not bleached); M15. Aluminum acetate; M16. Aluminum sulphate.

Bath ratio modification and mordanting type influence on dyeability

Considering the imperious need for sustainability, the variation of the bath ratio and mordanting momentum (pre-and meta-mordanting) influence on the process efficiency was analyzed (results in Table 20). It was observed that in the study case of employing the Cream of tartar (3% w.o.f.), the optimum bath ratio is represented by 1/20, generating the more intense and blueish results. This may be justified by a reduced dyeing dispersion space that may not allow the correct impregnation of the textile, due to reduced fabric coverage. Nevertheless, when comparing the mordanting momentum, very low differences in color tone and intensity are observed. In this sense, as a recommendation, and if the processing temperature of the mordant permits it, meta-mordanting may be applied.

Bleaching pretreatment of wool influence on dyeability

The analysis of the bleaching pretreatment of wool with the use of the efficient mordant Titanyl potassium oxalate (results in Table 20), indicates a positive impact of the process, with an intensity increase of the blue tone. This may occur by the effect of the treatment, due to the provision of a whiter base for dyeing, and modification of the fiber surface, and thus having an impact on the obtention of brighter colors.

Reflectance spectrum and color strength analysis

The color strength calculation based on the reflectance spectrum of the dyed wool substrates with C-phycocyanin is presented in Figure 19, and it indicates the efficiency and the positive effect in the dye uptake with the use of mordants. A clear difference is marked among the effective mordants and the ones influencing the coloration of the fabrics due to intrinsically

natural yellow (Ferrous sulphate) or brown (Myrobalan and Oak gall) shades, located in the lower part of the graph. The bluest results, the highest dye uptake is obtained with the use of Alum and the highest with Tartaric acid.

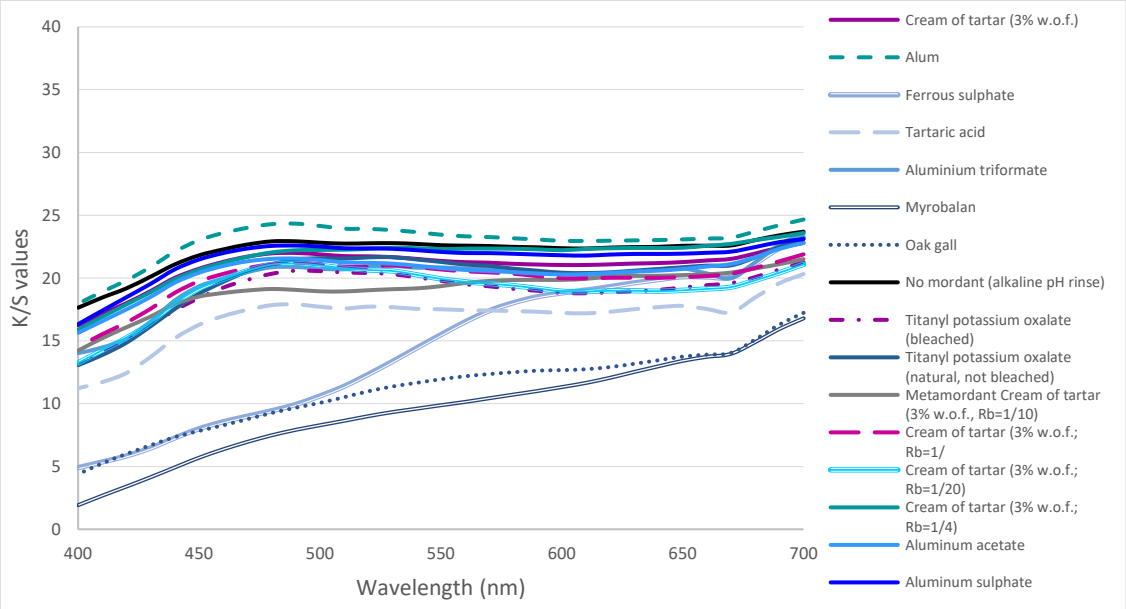


Figure 19. K/S spectrum of dyed wool with C-phycocyanin - mordant influence analysis

To the best of my understanding, alternative natural blue sources of colorants for wool substrates cover a reduced amount of research, most of them being performed with synthesized additions (Cai et al. 2020) or with indigo-based approaches (Komboonchoo and Bechtold 2009).

Measurement of residual colorant matter in C-phycocyanin-wool dyeing wastewater effluents

Temperature, mordanting, and bath ratio influence on dye uptake

Bath ratio influence on the dye uptake was analyzed on the one hand at 85^{0}C process temperature, with no mordanting application, and it was observed a doubling of the dye uptake with the dyeing liquor ratio reduction. Nevertheless, when the temperature was reduced to 65^{0}C, to avoid protein denaturation, and a Cream of tartar (3% w.o.f.) pre-treatment was applied, the dye uptake ratio increased proportionally with the decrease of the bath ratio. Overall, the temperature reduction, impedes the degradation of the chromophore, pre-mordanting facilitates dyeability and dyeing bath reduction concentrates the bonding space generating a dye uptake of >95% (Table 21).

Conditions		***Dye to bath ratio***	***%dye uptake***
Dyeing temperature	***Pretreatment of wool***		
85^{0}C	No mordant	1/40	25,31%
		1/10	43,41%
65^{0}C	Cream of tartar 3%	1/20	22,03%
		1/10	53,63%
		1/4	98,78%

Table 21. Influence of temperature and bath ratio of dye uptake %

The wastewater dyeing effluents were analyzed for the validation of the efficiency of the process, and the visible spectrum (Figure 20) was measured. Considering the Lambert-Beer law (Mäntele and Deniz 2017), the C-phycocyanin concentration in the solution is proportional with

the absorbance values, at maximum absorption wavelength for the blue chromophore. In this sense, the spectrum peaked with low values at the maximum absorbance λ=620 nm, indicating the most efficient treatment. The highest colorant uptake, meaning the lowest concentration identified in the wastewater, was identified in the following order: Tartaric acid, Aluminum sulphate, Titanyl potassium oxalate, Aluminum acetate, Aluminum triformate, non-mordanted sample, Cream of tartar, Alum, Ferrous sulphate, Myrobalan, and Oak gall.

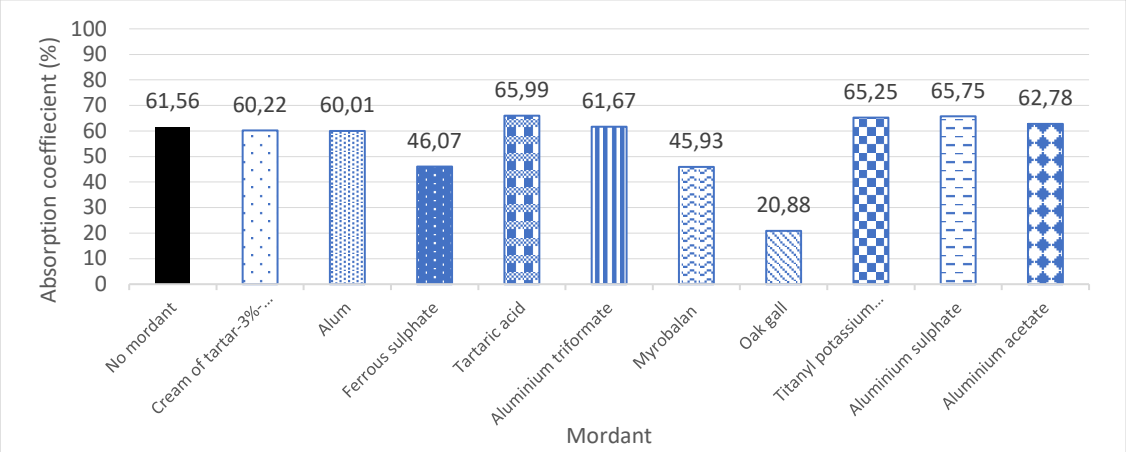


Figure 20. Colorant absorption coefficient based on dyeing of wool with C-phycocyanin wastewater effluent

2) C-phycocyanin wool dyeing process quality assessment through measurement of laundering and lightfastness

As it can be observed in Table 22, a complete set of laundering and lightfastness tests were performed, with the obtention of promising results. Regarding color degradation, the most relevant results were obtained with the use of Tartaric acid, reaching a good behavior, acceptable for natural colorants. Nevertheless, small improvements were obtained as the reference sample delivered good results too, indicating a natural affinity among the colorant molecule and the proteinic fiber. Color staining analysis on other fibers in the laundering process indicates a lack of affinity of this natural colorant with the latest, due to the lack of staining observed. One of the most important commercial features in textile coloration is represented by lightfastness, and the main results obtained are comparable with other natural colorants (Cai et al. 2020). Improvements occur with the use of Titanyl potassium oxalate, indicating a protective added value to the colorant.

Sample	Color change	Staining						Lightfastness
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
No mordant	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4
Cream of tartar (3% w.o.f.)	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Ferrous sulphate	4-5	4-5	4-5	4-5	4-5	4-5	4-5	5
Tartaric acid	4	4-5	4-5	4-5	4-5	4-5	4-5	4
Aluminium Triformate	3	4-5	4-5	4-5	4-5	4-5	4-5	4
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	4
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	4
Myrobalan	4	4-5	4-5	4-5	4-5	4-5	4-5	4
Oak gall	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Titanyl potassium oxalate (bleached)	2-3	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Titanyl potassium oxalate (natural, not bleached)	2-3	4-5	4-5	4-5	4-5	4-5	4-5	4-5

Table 22. Fastness properties of the C-phycocyanin dyed wool influence of different mordants

Partial conclusions for dyeing of wool with C-phycocyanin

- *Mordanting influence* analyzed among 10 different mordants, accounts for average improvements in coloration, objectively confirmed by ΔE with values between 3,64 and 28,41, meaning significant color differences.
- *Pre-mordanting* process (as a fabric pretreatment) outcomes display more blueish shades (Δb minimum) and higher color intensity (ΔL<0), with the application of **Aluminum acetate (20% w.o.f.**) **Titanyl potassium oxalate (20%), Cream of tartar (3% w.o.f.), Tartaric acid (6% w.o.f.),** Aluminum triformate (10% w.o.f.), Alum (30% w.o.f.). Nevertheless, the most intense shades from this batch of experiments were obtained with the added value of the intrinsic color of the use of Ferrous sulphate (3% w.o.f), Myrobalan (25% w.o.f), and Oak gall (25% w.o.f) with yellowish and brownish influence and not completed by an affinity with the chromoprotein.
- *Intrinsic natural yellow to brown* color of some of the tested mordants have influenced the most intense shades, obtaining:
    - Yellowish fabric with the use of Ferrous sulphate (3% w.o.f) in the pre-mordanting process.
    - Brown hues of the dyed cotton fabrics with the use of the biomordants Myrobalan (25%), and Oak gall (25%).
- *Metamordanting effect* was analyzed with the use of 3% w.o.f. of Cream of tartar and results reflect more efficiency in comparison with the pre-mordanted experiments, in terms of lightness ($\Delta L<0$). However, the application of mordants shows improved dyeability regardless of the momentum.
- *Bath ratio influence* was analyzed through comparison of pre-mordanted wool with 3% w.o.f. of Cream of tartar among 1/40, 1/20, respectively 1/4, with the best results obtained, with the medium ratio, 1/20, in terms of darker color and blueish tone obtained. Regardless, this ratio was inadequate for laboratory scale but is the most suitable for industrial applications.

- *Bleaching of wool* influence the dye uptake positively, by modifying the proteinic fiber and allowing the interaction with Titanyl potassium oxalate, and the C-phycocyanin, resulting in a reduction of the *b** value component, meaning a reduction of the yellow coordinate and increasing the blue one.
- *The analysis of the absorption coefficient of the wastewater effluents*, conclude improvements obtention with the remaining dye in the water effluent values decreasing in the following order (with values from 60% to 30%): Tartaric acid, **Aluminum sulphate**, **Titanyl potassium oxalate**, **Aluminum acetate.**
- Laundering and lightfastness properties show slight improvements with the employed auxiliaries.
    - *Laundering fastness* presents a good overall (4-5) response with the use of Ferrous sulphate and Myrobalan. Staining behavior (4-5) reflects the fact that, even though the degradation of the tested fabrics occurs, the staining fabrics do not get stained, meaning that mordants or other solutions are needed for fixing the C-phycocyanin on the wool fabric.
    - *Lightfastness* overall can be included in the range comprised between fair to moderate behavior (4-5).

4.3.2. Analysis of the dyeability of natural fabrics with red algae-based colorant matter R-phycoerythrin-rich extract from macroalgae *Gracilaria gracilis*

a) Analysis of results obtained from cotton textile substrate dyeing with R-phycoerythrin

1) Color assessment: Chromatic characterization

R-Phycoerythrin from macroalgae *Gracilaria gracilis* was used for cotton dyeing, and the results are presented in Figure 21, with very light intensities of red shades, and color tones variations generated by the use of 10 mordants. Visually it can be observed low effectiveness of the dyeing process, first of all, due to the diluted extract that was employed in the process, and the purification level of the R-phycoerythrin rich extract, and secondly the low dyeability of the colorant matter on the cellulosic fibers.

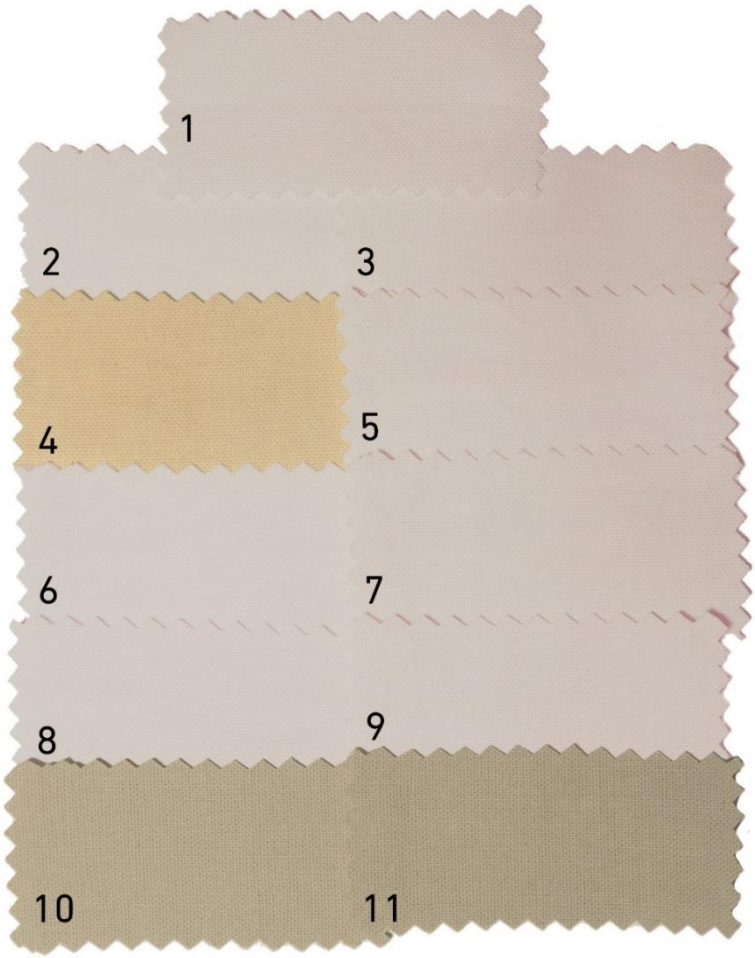


Figure 21. Dyed cotton fabrics with R-phycoerythrin obtained from Gracilaria gracilis

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

An objective analysis of the coloration of the cotton with algae-based red colorant matter, R-phycoerythrin, was performed through the measurement of the CIELab color coordinates, and the calculation of the color differences between the reference sample, represented by the dyed

non-mordanted cotton fabrics (highlighted in Table 23) and the dyed pre-mordanted ones, for the identification of the influence of the mordants on the dyeing process efficiency and quality. The complete set of results is included in Table 23 below.

Cotton sample	***Color coordinates and color strength***							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant	88,31	1,47	3,53	-	-	-	-	35,17
Cream of Tartar	89,57	1,36	4,08	1,26	-0,11	0,55	1,37	35,68
Alum	89,55	1,86	3,96	1,24	0,39	0,43	1,37	35,56
Ferrous sulphate	83,92	3,56	15,13	-4,39	2,09	11,6	12,58	35,93
Tartaric Acid	89,31	1,36	4,07	1	-0,11	0,54	1,14	35,66
Aluminum triformate	88,92	2,19	3,84	0,61	0,72	0,31	0,99	35,96
Myrobalan	74,88	2,13	9,54	-13,43	0,66	6,01	14,73	28,92
Oak Gall	74,97	2,53	8,94	-13,34	1,06	5,41	14,43	28,78
Titanyl potassium oxalate	87,71	1,94	4,79	-0,6	0,47	1,26	1,47	35,13
Aluminum acetate	84,91	3,57	6,02	-3,4	2,1	2,49	4,71	34,75
Aluminum sulphate	87,23	2,35	4,05	-1,08	0,88	0,52	1,49	35,38

Table 23. CIELab coordinates and calculations for R-phycoerythrin dyed cotton

In accordance with the visual analysis, the color coordinates indicate clear reddish tones, with intensities defined by *L** values ranging from 74,88 to 89,57, considering that the perfect white is characterized by *L**=100. It is worth mentioning that the color difference (*ΔE* ranging from 0,99 to 1,49) between the reference sample and the rest of the samples, indicates low discrepancies, except for the use of Myrobalan, Oak gall, and Ferrous sulphate, which indicate the extreme values (14,73-12,58). Nevertheless, particular values, out of the extreme ranges, are obtained with the use of Aluminum acetate (4,71) and Aluminum sulphate (1,49). More intense coloration was obtained in similar studies, but it is important to highlight the plant-based source (Jabar et al. 2021).

Considering the natural intrinsic coloration capacity, with proven interference on the dyeability of natural colorants, Myrobalan, Oak gall (both brown), and Ferrous sulphate (yellow), do not demonstrate efficiency in enhancing the reddish coloration of cotton, in this case, thus the attention should be focused on the Aluminum sulphate and Aluminum acetate.

The color intensity analysis, in the context of *ΔL*>0, in the majority of the experimental cases, proves that most of the mordants used as pre-treatment reduced the available space for colorant diffusion into the cotton fabrics, by generating less intense colored fabrics, in comparison with the reference sample. Nevertheless, the mordants identified of interest revealed expected results, in terms of intensity increase, as Aluminum acetate *ΔL*=-3,4; Aluminum sulphate *ΔL*=-1, 08, followed by Titanyl potassium oxalate *ΔL*=-0,6.

For better visualization of the color space distribution of the dyed fabrics, Figure 22 plots the CIE*Lab* diagram and indicates the complete aggregation of the fabrics in the reddish space, with yellowish influences. These influences may be generated by impurities in the colorant-rich extract, and possible chlorophyll influences, but the predominant color is red.

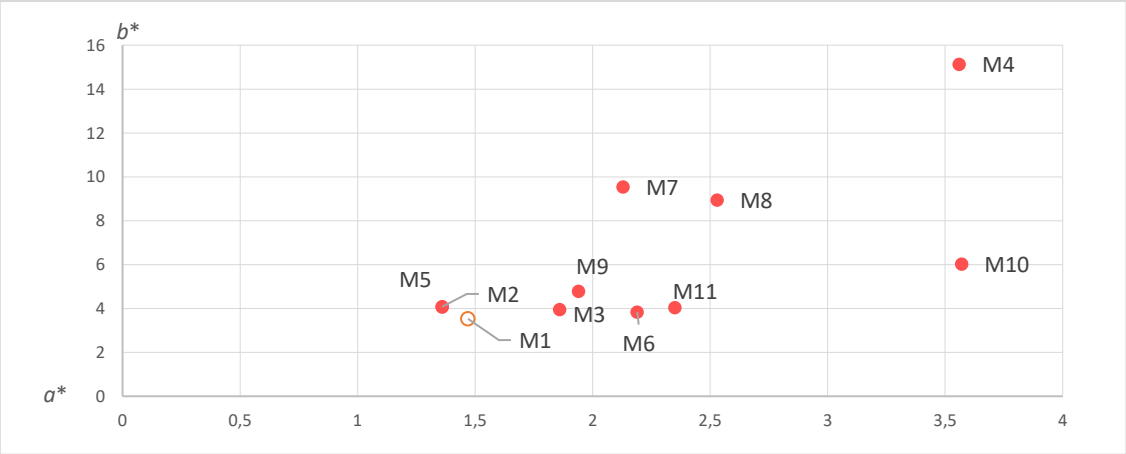


Figure 22. Color specifications in CIELab of the dyed cotton fabrics with R-phycoerythrin

M1. No mordant; M2. Cream of tartar; M3. Alum; M4. Ferrous sulphate; M5. Tartaric acid; M6. Aluminum triformate; M7. Myrobalan, M8. Oak gall; M9. Titanyl potassium oxalate; M10. Aluminum acetate; M11. Aluminum sulphate.

Reflectance spectrum and color strength analysis

Apart from the superficial coloration, the depth of the color diffusion into the fabric was analyzed through the reflectance spectrum and calculation of the K/S values. The color strength values along the reflectance spectrum are presented in a graphical form in Figure 23, and the maximum K/S indicated in Table 23 and shows the samples with the highest reflection capacity, represented by the lightest colors obtained in terms of intensity, located in the superior part of the graph, characterizing the majority of the obtained results, and on the other hand, the highest absorption capacity, defining the samples with the darkest colors. In this sense, the analysis must be performed considering the maximum absorbance wavelength of the chromophore (λ=570 nm), and the biomordants with dark brown intrinsic color, present the highest absorption capacity, meanwhile the rest of the used mordants are intimately grouped in the same part of the graph, together with the non-mordanted sample. This indicates a lack of performance of the pre-treatment applied on the cotton fabrics.

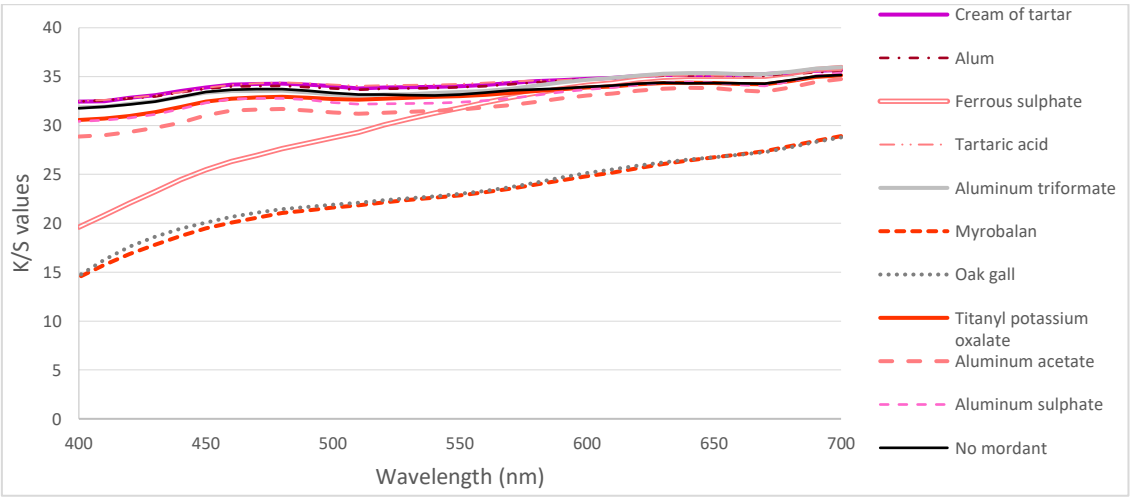


Figure 23. K/S spectrum of dyed cotton with R-phycoerythrin – mordant influence analysis

Measurement of residual colorant matter in R-phycoerythrin-cotton dyeing wastewater effluents

The residual colorant remaining in the dyeing effluent after the process execution was measured, and the obtained results are presented in Figure 24, which overall, do not show favorable results, with high quantities of residual unfixed colorant. The most efficient use of Aluminum sulphate, and Aluminum acetate was confirmed also by the previous test, but only in the case of the present study experimental approach. The very low dye uptake values confirm the lack of affinity between the cotton and the proteic chromophore and discards the option of colorant degradation by process parameters, due to the high concentrations found in the wastewater effluents.

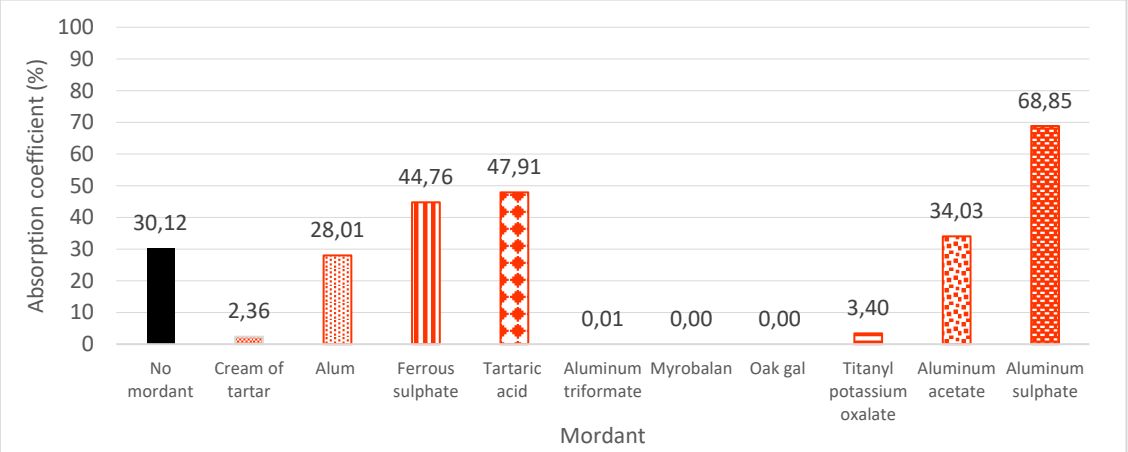


Figure 24. Colorant absorption coefficient based on dyeing of cotton with R-phycoerythrin wastewater effluent

It can be observed that many results converge around 0 up to 2, indicating a low dyeability efficiency with the use of mordants, which may also interfere with the natural colorant diffusion into the cotton fiber, by occupying the free spaces where the color molecules could have bonded. Thus, just by pre-mordanting, the cotton fabrics could have been already saturated without any free space for coloring molecules to access.

2) R-phycoerythrin cotton dyeing process quality assessment through measurement of laundering and lightfastness

Apart from color analysis, the color resistance of the dyed cotton fabric is of great interest, and for that behavior against laundering and light was analyzed, with the complete set of results included in Table 24. The most uniform results are obtained on staining onto other fibers, confirming the lack of affinity of this colorant with textile substrates, thus the need for dyeability enhancers, as the mordants. On the other hand, an improvement in color degradation was obtained with the use of Ferrous sulphate, Myrobalan and Oak gall, with almost excellent behavior, in maintaining their intrinsic yellow, and brown colors, but not the aimed red coloration. Nevertheless, Aluminum acetate reached improvements towards good behavior (grade 4) and is one of the colorants which showed higher color differences and dye uptake percentage, confirming a positive influence on the dyeing process efficiency. Apart, Titanyl potassium oxalate and Aluminum triformate, with slightly lower performance increase follow the path indicated by Aluminum acetate, with resistance improvements. The lightfastness values are similar for the majority of experimental cases, and considering the visual analysis of the color

degradation, this may be influenced by the very light shades of red obtained, thus visually indicating to not be affected by this external degrading agent.

Sample	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Cream of Tartar	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Ferrous sulphate	4-5	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Tartaric Acid	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Aluminum triformate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Myrobalan	4-5	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Oak Gall	4-5	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Titanyl potassium oxalate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Aluminum acetate	4	4-5	4-5	4-5	4-5	4-5	4-5	5-6
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	5-6

Table 24. Fastness properties of the R-phycoerythrin dyed cotton influence of different mordants

Color comparison with other similar studies, using alternative red colorant sources for the exploration of cotton dyeing efficiency reveals comparative results in terms of the quality of the dyeing process (Velmurugan et al. 2017).

Partial conclusions for dyeing of cotton with R-phycoerythrin

- *Pre-mordanting* of cotton samples with 10 different mordants did not generate significant color differences with any of the used mordants, but particular values, in terms, of the most different coloration and slightly more intense, were obtained with **Aluminum acetate (20% w.o.f.)**, and **Aluminum sulphate (20% w.o.f.)** without reaching significant impact. The highest efficacy of the previously mentioned mordants is also confirmed by a colorant absorption of approximately 35%, respectively 69%.
- *The intrinsic natural color* of mordants Myrobalan (25% w.o.f.), and Oak gall (25% w.o.f.) interfered with the coloration generating brown colors of the samples.
- *The analysis of the absorption coefficient of the wastewater effluents* indicates low efficiency and dye uptake percentages starting from 2% up to 69%.
- *The color difference* (ΔE<3) indicates that none of the mordants showed improvement of the dye absorption efficiency when applied to cotton dyeing with R-phycoerythrin. The analysis of the UV-VIS absorbance spectrum of the dyed fabrics confirms the reduced efficiency of the selected mordants as all the dyed fabrics are grouped in the same area with very light red colored non-mordanted sample, and the exception of the biomordants generating dark brown hues.
- *Laundering and lightfastness properties* do not show improvements**,** compared with the non-mordanted samples, except for Aluminum acetate, with slight influences:
  - *Laundering fastness* presents a poor overall (1-2) response with a slight increase (4). Staining behavior (4-5) reflects the fact that the degradation of the tested samples does not generate staining and does not change. *Lightfastness* overall can be defined by moderate behavior (5-6) and did not improve.

b) Analysis of results obtained from wool textile substrate dyeing with R-phycoerythrin

1) Color assessment: Chromatic characterization

The results of the wool dyeing experiments with R-phycoerythrin from macroalgae *Gracilaria gracilis* are presented in Figure 25 below, and reveal light reddish shades, with various intensities and color influences generated with the use of 10 mordants. In comparison with the previous section (where cellulosic fibers were assessed), it can be visually observed a colorant-wool-fiber affinity, starting from the non-mordanted study case.

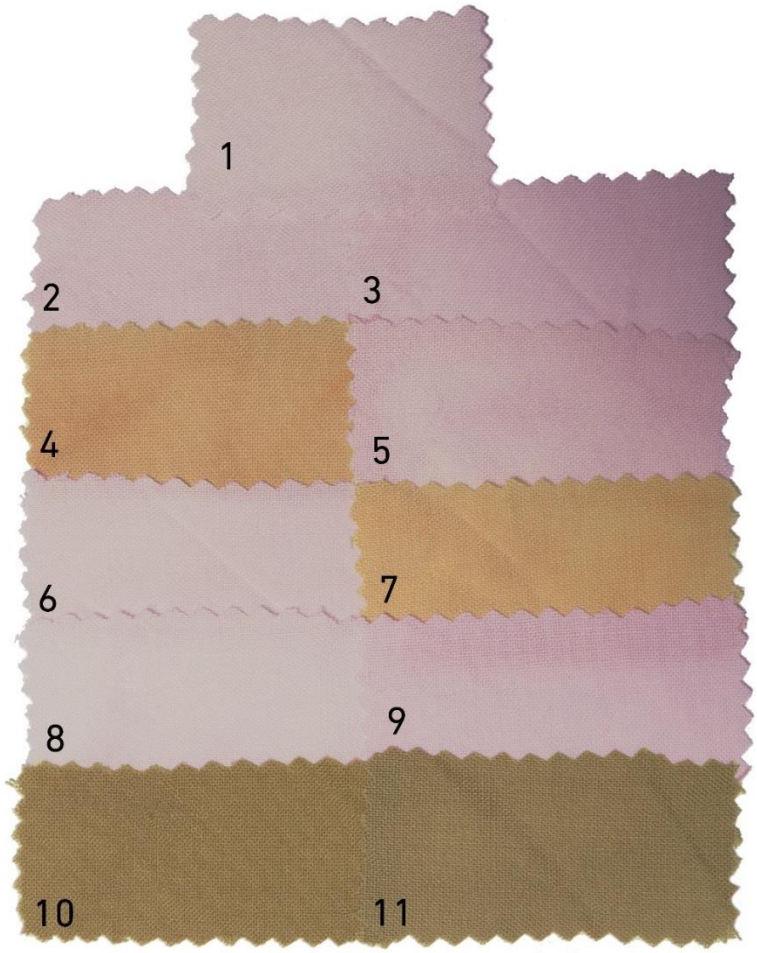


Figure 25. Dyed wool fabrics with R-phycoerythrin obtained from Gracilaria gracilis

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

An objective analysis of the coloration of the wool fabrics dyed with R-phycoerythrin was performed through the measurement of the CIE*Lab* coordinates (*L*, a*,* and *b**) and calculated color differences (*Δ*) and color strength (*K/S*). This calculus was performed on the base of the reference sample, the non-mordanted dyed wool with R-phycoerythrin, indicated in Table 25 below, for identifying the possible influence of the use of mordants in the dyeability of wool.

Cotton sample	*Color coordinates and color strength*							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant	87,56	0,11	11,3	-	-	-	-	25,28
Cream of Tartar	74,92	4,34	10,08	-12,64	4,23	-1,22	13,38	25,53
Alum	77,52	4,97	7,68	-10,04	4,86	-3,62	11,73	24,49
Ferrous sulphate	63,93	10,12	22,59	-23,63	10,01	11,29	28,04	23,16
Tartaric Acid	77,27	5,5	11,79	-10,29	5,39	0,49	11,63	25,21
Aluminum triformate	76,78	4,07	10,34	-10,78	3,96	-0,96	11,52	26,16
Myrobalan	51,62	6,44	20,89	-35,94	6,33	9,59	37,73	17,78
Oak Gall	53,32	6,91	17,64	-34,24	6,8	6,34	35,48	17,61
Titanyl potassium oxalate	67,96	12,15	30,77	-19,6	12,04	19,47	30,14	25,19
Aluminum acetate	79,93	3,38	8,83	-7,63	3,27	-2,47	8,66	26,95
Aluminum sulphate	77,09	7,59	5,58	-10,47	7,48	-5,72	14,08	25,65

Table 25. CIELab coordinates and calculations for R-phycoerythrin dyed wool

The high values of color difference (*ΔE)* among the reference sample and pre-mordanted dyed wool fabrics, with high values >8,66, clearly indicate a positive influence on the use of mordanting treatment of the textile substrate, that increases the colorant molecule diffusion into the wool structure, enhancing the coloring process.

The luminosity defining coordinates (*L*)* indicate an increase of intensity with the use of all the mordants, when compared to the reference sample, indicating thus, the possibility of fixation of dye onto the fabric. *ΔL*<0, indicates the existence of the color difference, in terms of higher intensity of the red color, when compared with the reference sample, as the obtained values range among -7,63 and -35,94. Similar results were obtained in different studies aiming at exploring alternative sources of red colorants for wool fibers dyed in mordanting conditions (Rather et al. 2020).

There are several mordants with a clear yellowish influence when interfering with the R-phycoerythrin, defined by Myrobalan (brown), Oak gall (brown), due to natural coloration and high affinity towards natural fibers. On the other hand, the Ferrous sulphate (yellowish), intrinsically possess the yellow influence and also influences the final color of the dyeing, and Titanyl potassium oxalate, considering the neutral natural coloration, it may be justified that there is a reaction with the protein-based colorant matter. These assumptions are confirmed with the dye uptake (%) calculations based on the residual colorant in the wastewater effluents, resulting from the dyeing process (Figure 28).

A visual approach of the color coordinates is plotted in Figure 26 and positions the samples in the reddish color space, with slight yellowish influence, generated either by residual carotenoids or chlorophyll in the colorant rich extract used for dyeing, or direct reaction with the red colorant matter used in this study. The mordants generating the most yellowish influences are M9. Titanyl potassium oxalate and M4. Ferrous sulphate.

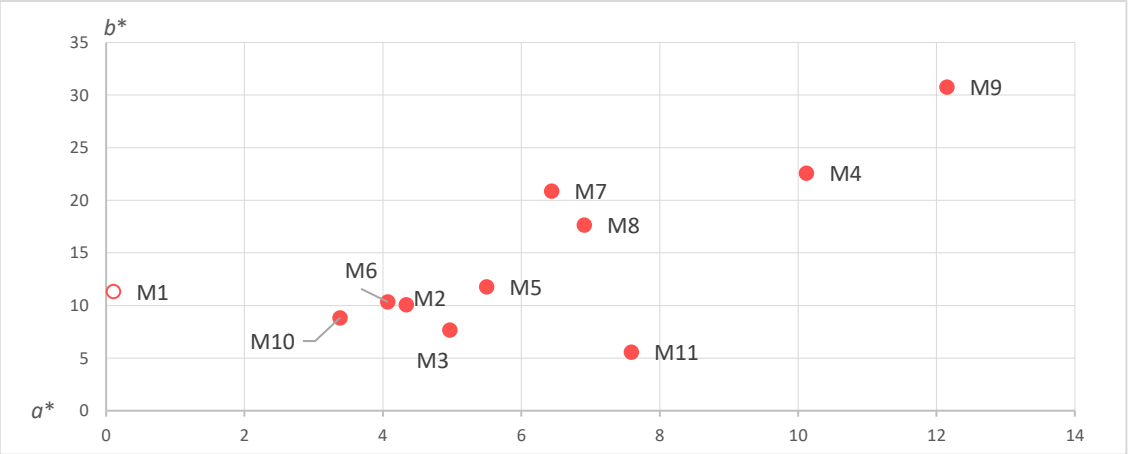


Figure 26. Color specifications in CIELab of the dyed wool fabrics with R-phycoerythrin

M1. No mordant; M2. Cream of tartar; M3. Alum; M4. Ferrous sulphate; M5. Tartaric acid; M6. Aluminum triformate; M7. Myrobalan, M8. Oak gall; M9. Titanyl potassium oxalate; M10. Aluminum acetate; M11. Aluminum sulphate.

Reflectance spectrum and color strength analysis

The reflectance spectrum of the dyed wool fabrics with R-phycoerythrin was measured, for the analysis of the color depth diffusion into the textile substrate. In this sense, Figure 27 below presents the whitest fabrics being characterized by the highest reflective strength, meanwhile, the most intense colors are prone to the highest absorbance of the light fascicle. As resulted from the previous analysis, the most intense colors have been obtained in the following order, starting with the highest color strength (as detailed in Table 25), Myrobalan, Oak gall, Ferrous sulphate, Titanyl potassium oxalate, Cream of tartar, Alum, Aluminum sulphate, Aluminum triformate, Tartaric acid, Aluminum acetate.

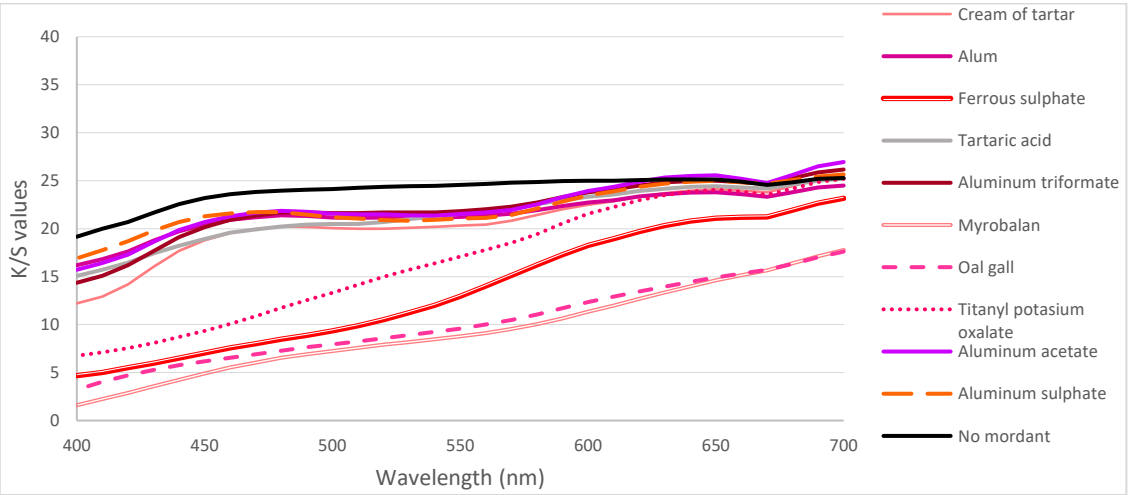


Figure 27. K/S spectrum of dyed wool with R-phycoerythrin – mordant influence analysis

Measurement of residual colorant matter in R-phycoerythrin-wool dyeing wastewater effluents

Figure 28 below summarizes the dye uptake capacity of the experimental cases regarding the dyeing of wool with R-phycoerythrin and the influence of the dyeability of the pre-mordanting process. Overall, the high dye uptake percentages, mainly ranging from 77 % up to 95%, indicate that there is a low quantity of colorant matter in the dyeing wastewater effluents,

which leads to the main assumption that, the dyeing process parameters involved sufficient dyeing time for the colorant matter to diffuse into the fabrics´ molecules, with enhancing capacity generated by the use of Tartaric acid or some Aluminum-based mordants, also acting as protectors to the temperature-sensitive colorant matter. The temperature degradation cannot be discarded, with the use of Cream of tartar, Alum, or Aluminum sulphate, as according to some R-phycoerythrin kinetic studies, completed with light degradation, this colorant maintains stability at over 45^{0}C, and pH =5 (Bharmoria et al. 2020) (Munier et al. 2014). This represents a drawback in the dyeing process at 65^{0}C, considered a low process temperature in conventional exhaustion processes, suggesting testing colder dyeing processes.

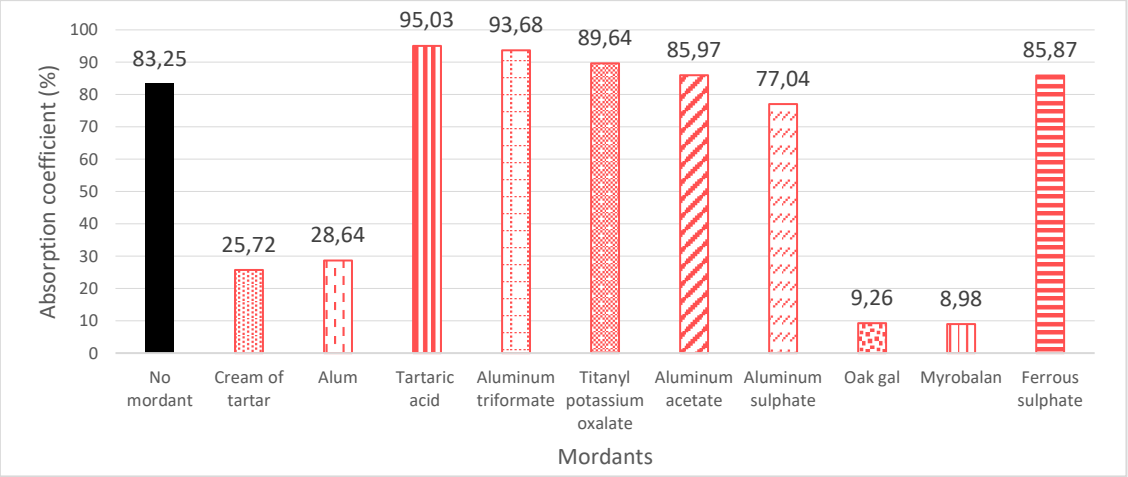


Figure 28. Colorant absorption coefficient based on dyeing of wool with R-phycoerythrin wastewater effluent

2) R-phycoerythrin wool dyeing process quality assessment through measurement of laundering and lightfastness

The characterization of the dyeing quality, through the analysis of the behavior against the laundering and light as color degrading agents, is detailed in Table 26. The results indicate a decrease in the color degradation capacity when compared with the reference sample, the non-mordanted one, suggesting the need for additional protection of the colorant, with a heat stabilizer. In terms of staining behavior, due to the low capacity of fixation to other fibers, it clearly indicates low colorant affinity, as the residual colorant would remain in the laundering water. Lightfastness also reveals low levels of improvement with the use of Cream of tartar, Alum, Aluminum triformate, and Aluminum acetate, and slightly increased with the use of Ferrous sulphate. Nevertheless, it must be confirmed the temperature and light sensitivity that characterized R-phycoerythrin, also indicated by other studies, but this is also know-how gathered in this study that allows further experiments oriented towards combating this drawback. The slight improvements obtained indicate the possible protection of this protein-based chromophore.

Sample	***Color change***	***Stain***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Cream of Tartar	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Ferrous sulphate	4-5	4-5	4-5	4-5	4-5	4-5	4-5	5
Tartaric Acid	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Aluminum triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Myrobalan	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Oak Gall	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Titanyl potassium oxalate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Aluminum sulphate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3-4

Table 26. Fastness properties of the R-phycoerythrin dyed wool influence of different mordants

Comparing the fastness values with similar studies, focusing on alternative colorant sources, reveal alike results (Rather et al. 2020)(Yusuf et al. 2016), indicating that mordanted wool dyed with R-phycoerythrin is a plausible alternative for natural red textile coloration.

Partial conclusions for dyeing of wool with R-phycoerythrin

- *Pre-mordanting* of wool with a set of 10 mordants revealed significant differences and more intense coloration, with the most efficient auxiliary as **Titanyl potassium oxalate (20% w.o.f.)** confirmed by the reflectance spectrum analysis.
- *The intrinsic natural color* of mordants, Ferrous sulphate (3% w.o.f.), Myrobalan (25% w.o.f.), and Oak gall (25% w.o.f.) influence darker coloration restricting the red dye uptake (up to 9%), as confirmed by the analysis of the wastewater effluents.
- *The analysis of the absorption coefficient of the wastewater effluents* indicates high efficiency and dye uptake percentages, with Titanyl potassium oxalate (20% w.o.f.) approximately 90%, followed closely by Tartaric acid (6% w.o.f.), and Aluminum triformate (10% w.o.f.) with an approximate 93% and 95% dye uptake.
- *The color difference* (ΔE>3) indicates that all mordants presented improved dye absorption efficiency when applied to wool dyeing with R-phycoerythrin, significantly more efficient than cotton experiments. The UV-VIS absorbance spectrum analysis of the dyed wool fabrics and the color diagram indicate that the efficient pre-mordanted dyed fabrics are grouped in the same area, like the non-mordanted samples, with darker tones. The biomordants do not improve the dyeability of the red colorant, interfering with the final results by modifying the expected color.
- *Laundering and lightfastness properties* do not show improvements**,** compared with the non-mordanted samples:
    - *Laundering fastness* behavior of red wool samples shows improvements (4-5), in comparison with cotton samples, but no significant variation when compared with the non-mordanted samples. *Lightfastness* properties count with fair behavior (4-5), determined by the natural coloration of the samples, without efficiency increase demonstrated by the use of mordants.

4.3.3. Analysis of the dyeability of natural fabrics with yellow algae-based colorant matter β-carotene-rich extract from microalgae *Dunaliella salina*

a) Analysis of results obtained from cotton textile substrate dyeing with β-carotene

1) Color assessment: Chromatic characterization

The results of the cotton textile substrates dyed with β-carotene are presented in Figure 29, and they present yellowish shades, with visual variations, in terms of tone and color intensity, influenced by the pre-treatment with a variety of 10 different mordants.

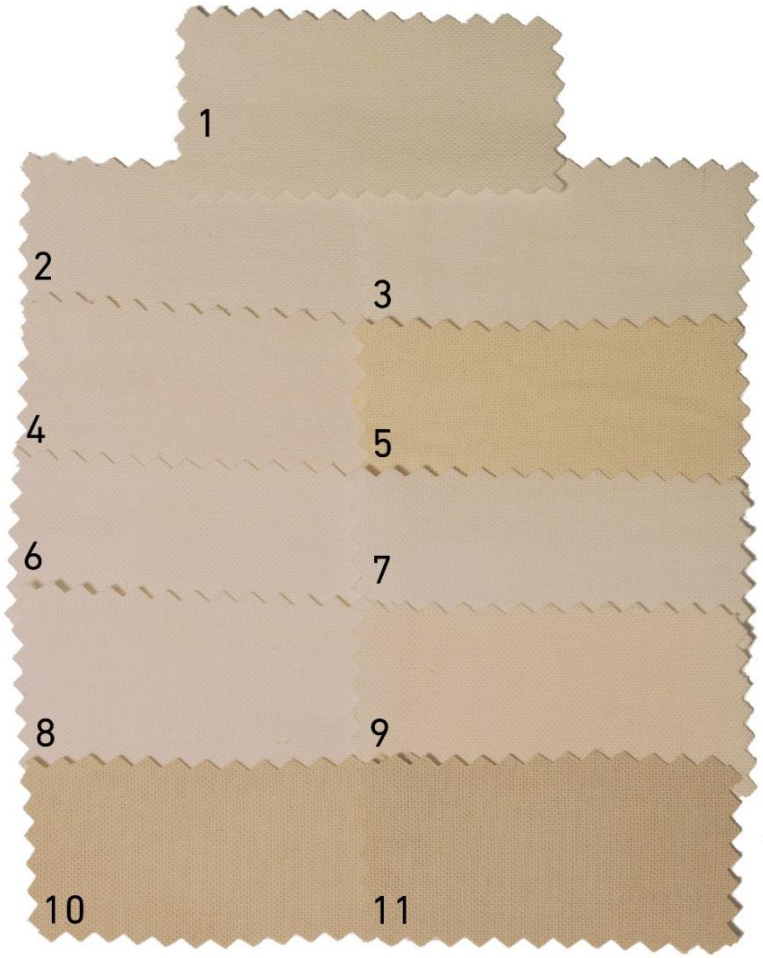


Figure 29. Dyed cotton fabrics with β-carotene obtained from Dunaliella salina

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

Apart from the visual color analysis, objective measurements of the chromatic coordinates were performed (CIE *L*, a*, b**), and the color strength was calculated, to compare the performance of the pre-treatment with mordants of the cotton fabrics on the dyeability efficiency. The reference sample is represented by the non-mordanted carotenoid dyed cotton sample. The complete set of results is presented in Table 27.

Cotton sample	***Color coordinates and color strength***							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant	91,9	-0,05	9,4	-	-	-	-	35,87
Cream of Tartar	79,76	2,29	19,35	-12,14	2,34	9,95	15,87	26,06
Alum	81,09	2,1	19,92	-10,81	2,15	10,52	15,24	25,80
Tartaric Acid	77,57	2,05	19,98	-14,33	2,1	10,58	17,94	25,01
Ferrous sulphate	62,47	9,55	25,47	-29,43	9,6	16,07	34,88	20,43
Aluminum triformate	79,82	2,25	20,5	-12,08	2,3	11,1	16,57	26,14
Myrobalan	56,37	5,49	23,21	-35,53	5,54	13,81	38,52	19,61
Oak Gall	55,03	5,36	20,13	-36,87	5,41	10,73	38,78	17,75
Titanyl potassium oxalate	75,18	5,17	35,45	-16,72	5,22	26,05	31,39	25,49
Aluminum acetate	79,83	2,51	20,52	-12,07	2,56	11,12	16,61	25,53
Aluminum sulphate	80,58	1,54	20,37	-11,32	1,59	10,97	15,84	25,50

Table 27. CIELab coordinates and calculations for β-carotene dyed cotton

The values from the upper table reflect the same tone variation and color difference, as it was possible to be appreciated in Figure 29, and confirmed by the set of values ΔE>3 in all the performed dyeing tests, confirming an increase in dyeability with the use of mordants. The most extreme differences are obtained with the use of Myrobalan, Oak gall, Ferrous sulphate, and Titanyl potassium oxalate, with ΔE> 31, and the first two are also defined by the most intense yellow colors, ΔL<0, with values of -35,53, and -36,87 respectively. The intensity of the obtained colors is comparable with studies exploring conventional mordanted cotton dyeing (Chandru and Praveena 2021) (Ayele et al. 2020).

Additionally, Δb>0, ranging from 8,71 to 26,05, clearly confirms the yellower results with the use of all of the employed mordants in this study. The efficiency of the pre-mordanting process may be enhanced by an increased affinity between the colorant matter and the textile substrate.

A thorough analysis, considering the best color coordinates combination, meaning (more intense) ΔL<0, combined with (yellower) Δb>0, and the most accentuated color difference ΔE>31 is encompassed with the use of Ferrous sulphate, Oak gall, and Titanyl potassium oxalate.

It is also important to highlight that Myrobalan and Oak gall possess brown intrinsic natural color, and Ferrous sulphate yellow intrinsic color, which interferes with yellow chromophores but appears to have a positive influence on the dyeing efficiency of the terpene-based colorant matter.

Yellow color intensity improves with the use of conventional mordants starting from the lowest, Alum, Aluminum sulphate, Aluminum acetate, Aluminum triformate, Cream of tartar, Tartaric acid, up to the highest, Titanyl potassium oxalate.

A visual distribution of the color coordinates is plotted in Figure 30, and it shows the location of the coordinates distributed in the yellowish color space, confirming the obtention of the expected color for the employment of carotene as colorant matter.

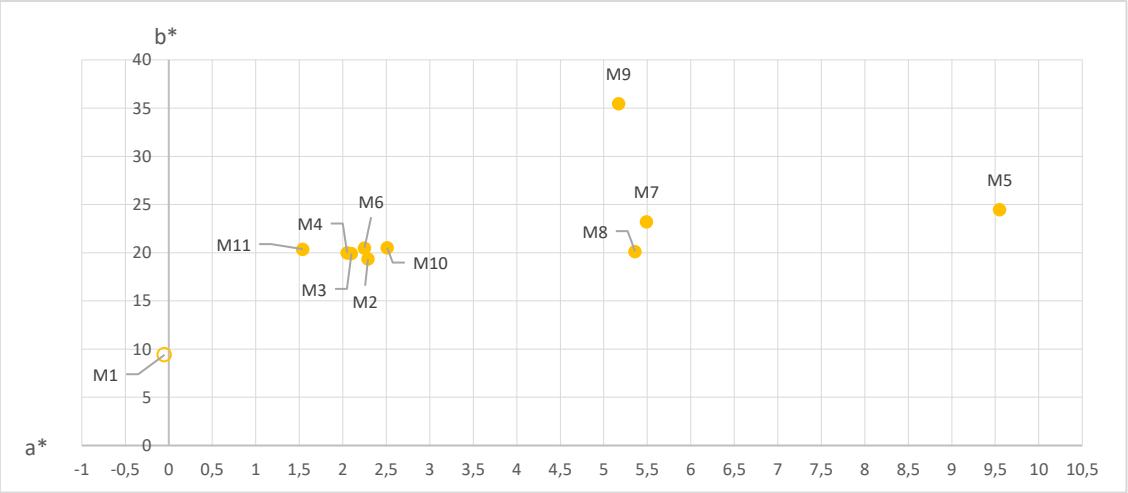


Figure 30. Color specifications in CIELab of the dyed cotton fabrics with β-carotene

M1-No mordant; M2-Cream of Tartar; M3-Alum; M4-Tartaric Acid; M5-Ferrous sulphate, M6-Aluminum triformate, M7-Myrobalan, M8-Oak Gall; M9-Titanyl potassium oxalate; M10-Aluminum acetate; M11-Aluminum sulphate

Reflectance spectrum and color strength analysis

Color strength spectrum (Figure 31), K/S, based on the dyed reflectance measurements, indicates the efficiency of the application of mordants and confirms the previous tests performed regarding the color analysis. The reflectance at maximum absorbance of β-carotene (λ= 470 nm) indicates differences among the reference sample, which is the non-mordanted dyed cotton with β-carotene, with the lowest performance, and the use of Ferrous sulphate, Myrobalan, and Oak gall, with the best performance. The other results are very similar in dyeing efficiency.

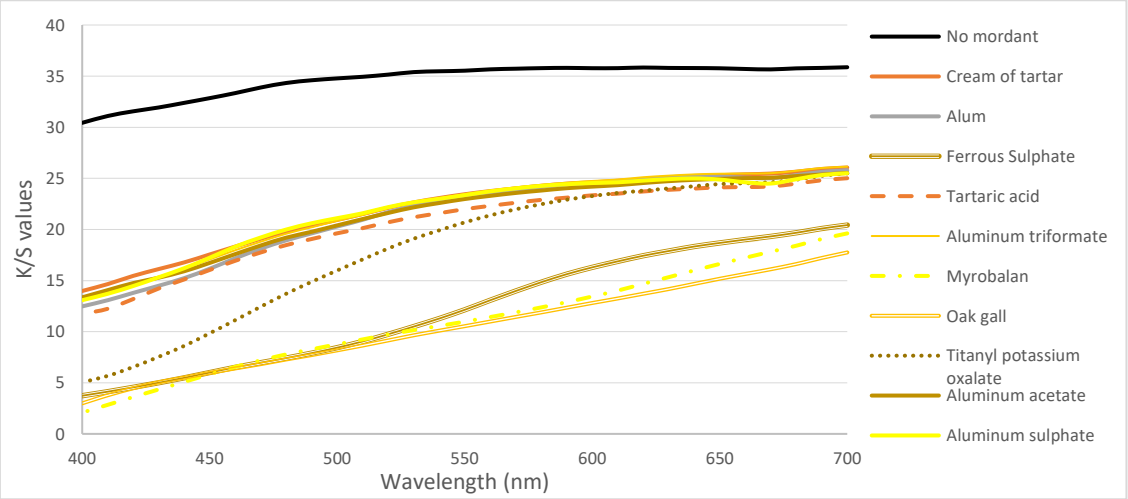


Figure 31. K/S spectrum of dyed cotton with β-carotene – mordant influence analysis

Measurement of residual colorant matter in β-carotene-cotton dyeing wastewater effluents

The fabrics color analysis reflects corresponding results with the measurements of the colorant absorption coefficient (Figure 32), analyzed based on the dyeing wastewater effluents, with the highest dye uptake attributed to Tartaric acid (88,14%), followed by Alum (83,92%), with similar results obtained with Aluminum triformate, Titanyl potassium oxalate and Cream of

tartar with approximately 60% of due uptake. These results confirm the possibility of mordant bonding with colorant matter and the cotton fabric, due to the occurrence of color fixation.

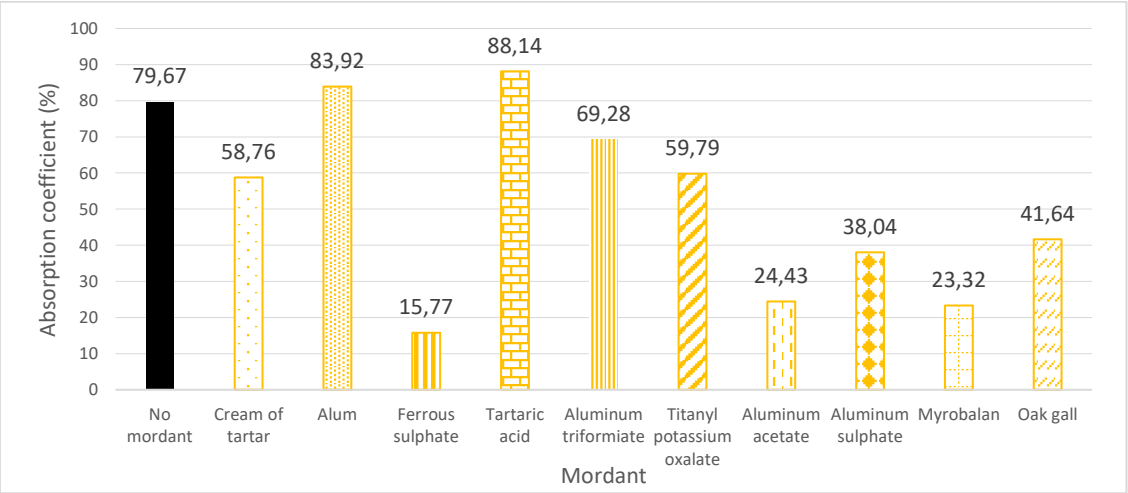


Figure 32. Colorant absorption coefficient based on dyeing of cotton with β-carotene wastewater effluent

It is very important to highlight that the interference of the natural color of the biomordants Myrobalan and Oak gall, also influenced the wastewater analysis absorbance spectrum revealing values indicating around 23% and 41%, and definitely, this represents the unfixed mordant remaining in the dyeing liquor after the process, added to the unfixed β-carotene colorant matter. In this sense, the efficiency of the Myrobalan and Oak gall in fixing the algae-based colorant matter on cotton fabrics is inconclusive. The same justification applied to the Iron based mordant Ferrous sulphate.

2) B-carotene cotton dyeing process quality assessment through measurement of laundering and lightfastness

The analysis of the quality of the cotton dyeing process with β-carotene, in terms of laundering and lightfastness, is presented in Table 28 and indicates poor behavior in terms of color degradation when subjected to fastness tests. Nevertheless, improvement in lightfastness behavior is obtained with the use of Aluminum triformate, which also showed good dye uptake values indicating the effectiveness of the mordant. Additionally, Titanyl potassium oxalate indicates very small improvements in lightfastness. The biomordant Myrobalan generates good lightfastness improvements but also possesses intrinsic coloration influence, which does not confirm improving β-carotene dyeing efficiency.

Sample	Color change	Staining						Lightfastness
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
No mordant	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Cream of Tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	4-5	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric Acid	4	4-5	4-5	4-5	4-5	4-5	4-5	1
Ferrous sulphate	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum triformate	2	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Myrobalan	4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Oak Gall	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	2
Aluminum acetate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 28. Fastness properties of the β-carotene dyed cotton influence of different mordants

Lightfastness results are not comparable, being below the efficiencies obtained within other studies approaching yellow natural dyeing of cotton yarns (Önal et al. 2021) (Chandru and Praveena 2021), indicating thus, the need for further experimental approaches on auxiliaries with the capacity of increasing light resistance.

Partial conclusions for dyeing of cotton with β-carotene

- *Pre-mordanting* of cotton with a total of 10 different mordants, indicated that **Titanyl potassium oxalate** (20% w.o.f.) has revealed as the most efficient process with a more intense and yellowish result.
- *The intrinsic natural color* of the mordants Ferrous sulphate (3% w.o.f.), Myrobalan (25% w.o.f.), and Oak gall (25% w.o.f.) boosted the yellow-brown color obtained, in terms of intensity and hue, but not colorant absorption.
- *The analysis of the absorption coefficient of the wastewater effluents* indicates that the most intense yellowish result obtained with Titanyl potassium oxalate was characterized by a 60% of dye uptake.These results are completed by Tartaric acid (6% w.o.f.), and Alum (20% w.o.f.), with dye uptakes of approximately 80%. Similar results were obtained with Cream of tartar (6% w.o.f.), and Aluminum triformate (10% w.o.f.) with dye uptakes comprised of up to 60%.
- *The color difference* (ΔE>3) confirms that all of the mordants presented improved dye absorption efficiency**.** This is additionally confirmed by the analysis of the UV-VIS absorbance spectrum of the dyed fabrics, where the majority of pre-mordanted dyed fabrics are grouped in the same area, which is significantly below non-mordanted dyed reference values, meaning darker tones. The results reveal a disposition in the range of light hues but, with a tendency to darker hues influenced by the biomordants with intrinsic natural brown color.
- *Laundering and lightfastness properties* do not show improvements with the majority of mordants employed, considering poor overall laundering response (2-3) and very poor light resistance (1-2), with the exception of Aluminum triformate and Myrobalan, and very slight improvement with the use of Titanyl potassium oxalate.

b) Analysis of results obtained from wool textile substrate dyeing with β-carotene

1) Color assessment: Chromatic characterization

Figure 33 below presents the wool textile substrates dyed with β-carotene obtained from *Dunaliella salina,* with various intensities and tones generated by the influence of the 10 different mordants analyzed in this study. Considering this visual analysis, it can be appreciated an affinity between the wool fabric and the algae-based yellow colorant matter (as per the 1st sample, non-mordanted, dyed with β-carotene).

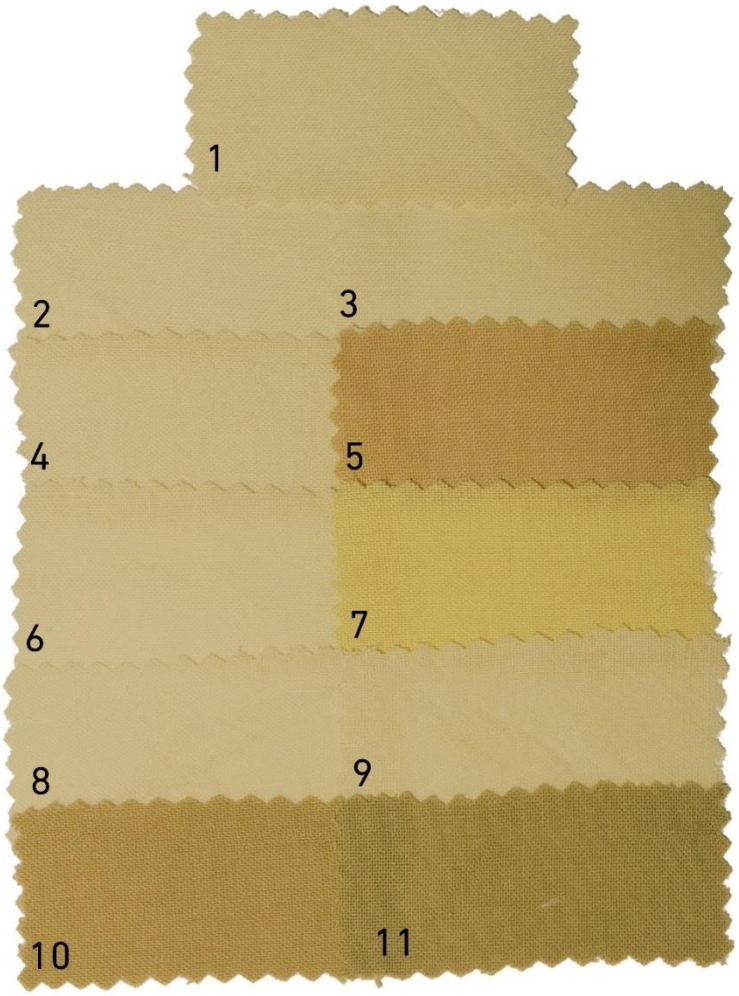


Figure 33. Dyed wool fabrics with β-carotene obtained from Dunaliella salina

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

A complete objective color analysis of the dyed wool samples is detailed in Table 29, including CIE*Lab* chromatic coordinates, completed by calculations of color difference *ΔE*, and color strength *K/S*.

Wool sample	*Color coordinates and color strength*							
	L	*a*	*b*	*ΔL*	*Δa*	*Δb*	*ΔE*	*K/S*
No mordant	76,78	0,60	23,24	-	-	-	-	22,39
Cream of Tartar	62,78	-1,15	24,24	-14	-1,75	1	14,14	19,22
Alum	50,53	-1,5	23,41	-26,25	-2,1	0,17	26,33	12,14
Tartaric Acid	58,3	-1,35	21,44	-18,48	-1,95	-1,8	18,67	13,06
Ferrous sulphate	47,75	2,3	23,52	-29,03	1,7	0,28	29,08	9,89
Aluminum triformate	48,91	2,29	22,32	-27,87	1,69	-0,92	27,94	12,54
Myrobalan	48,31	2,39	30,94	-28,47	1,79	7,7	29,55	6,94
Oak Gall	62,13	-2,05	24,15	-14,65	-2,65	0,91	14,92	14,84
Titanyl potassium oxalate	55	-0,77	24,85	-21,78	-1,37	1,61	21,88	13,60
Aluminum acetate	61,33	-2,17	23,59	-15,45	-2,77	0,35	15,70	14,79
Aluminum sulphate	51,46	-1,26	21,06	-25,32	-1,86	-2,18	25,48	11,30

Table 29. CIELab coordinates and calculations for β-carotene dyed wool

The calculations were based on the reference sample, the non-mordanted dyed wool sample, and consider all the comparisons on this basis, to identify the mordant influence on the dyeability of wool with algae-based β-carotene. In this sense, a first analysis approach is based on the values of $\Delta E>3$, clearly indicating color difference with the reference sample with the use of all mordants. The highest differences with the reference sample are obtained with the use of Ferrous sulphate and the biomordants Myrobalan and Oak gall, which are non-conclusive, due to the already identified yellow and respectively brown intrinsic color influence, but with dark brown tones results. The following ones in this classification are as follows: Aluminum-based mordants, Aluminum triformate, Alum and Aluminum sulphate, and the Titanyl potassium oxalate.

For a complete approach on color characterization, the most intense results, indicated by, $\Delta L<0$, are obtained with the use of Aluminum triformate, followed by Tartaric acid, but all the used mordants indicate significantly more intense (darker) yellow color tones. The color intensity results are comparable with studies approaching conventional mordanted wool dyeing (Chandru and Praveena 2021).

The chromatic coordinates measurements reflect the existence of an interaction among all the employed auxiliaries, the proteinic textile substrate, and the algae-based carotenoid used as colorant matter, thus increased dye uptake, meaning that the mordants are exercising the bonding among the previously mentioned components.

For a more visual approach, Figure 34 plots the color space diagram and indicates the clustering of the majority of the samples in the yellowish-greenish area. Nevertheless, some specific samples show a reddish influence as the reference sample, Aluminum triformate, and Myrobalan. All of these auxiliary influences may be generated by the possibility of residual chlorophyll (green color) in the extract or the β-carotene reddish variations, the latest possibly being triggered with the use of mordants.

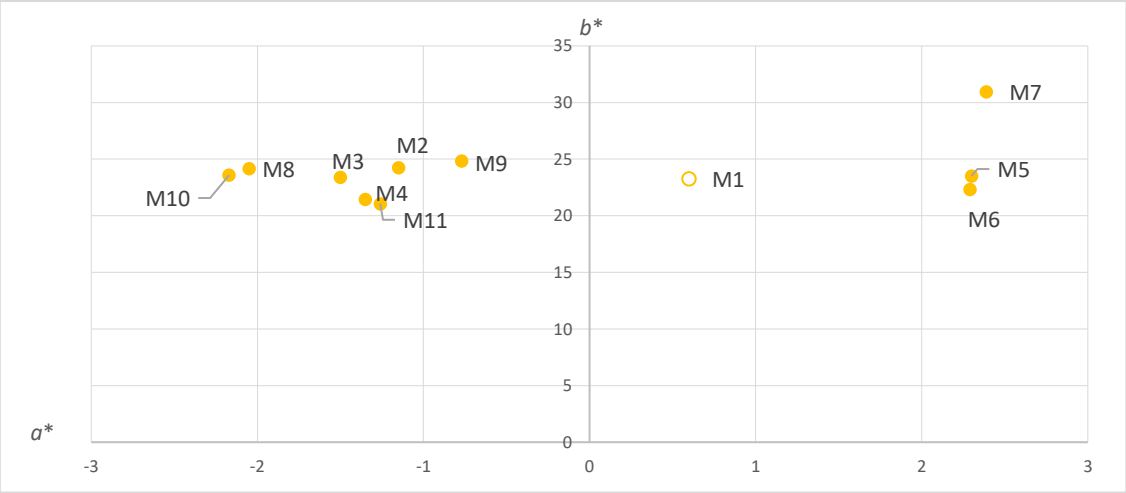


Figure 34. Color specifications in CIELab of the dyed wool fabrics with β-carotene

M1-No mordant; M2-Cream of Tartar; M3-Alum; M4-Tartaric Acid; M5-Ferrous sulphate, M6-Aluminum triformate, M7-Myrobalan, M8-Oak Gall; M9-Titanyl potassium oxalate; M10-Aluminum acetate; M11-Aluminum sulphate

Reflectance spectrum and color strength analysis

Dyed samples reflectance spectrum was measured and represented the basis for calculating the *K/S* values, presented in Figure 35 below in a percentual approach. The assumptions are made according to the carotene-based colorant matter maximum reflectance value (λ=470 nm), and must be assessed in the context of lower reflectance percentage indicating higher dye uptake, due to color absorbance, considering that a white textile has maximum reflectance capacity. In this sense, it can be assumed that most of the mordants indicate increased dyeability when compared to the reference sample and the use of Cream of tartar. Nevertheless, a clear distribution can be indicated, starting from the highest reflectance (lowest dye uptake) as Oak gall, Aluminum acetate, Tartaric acid, Titanyl potassium oxalate, Aluminum sulphate, Aluminum triformate, Ferrous sulphate, and Myrobalan.

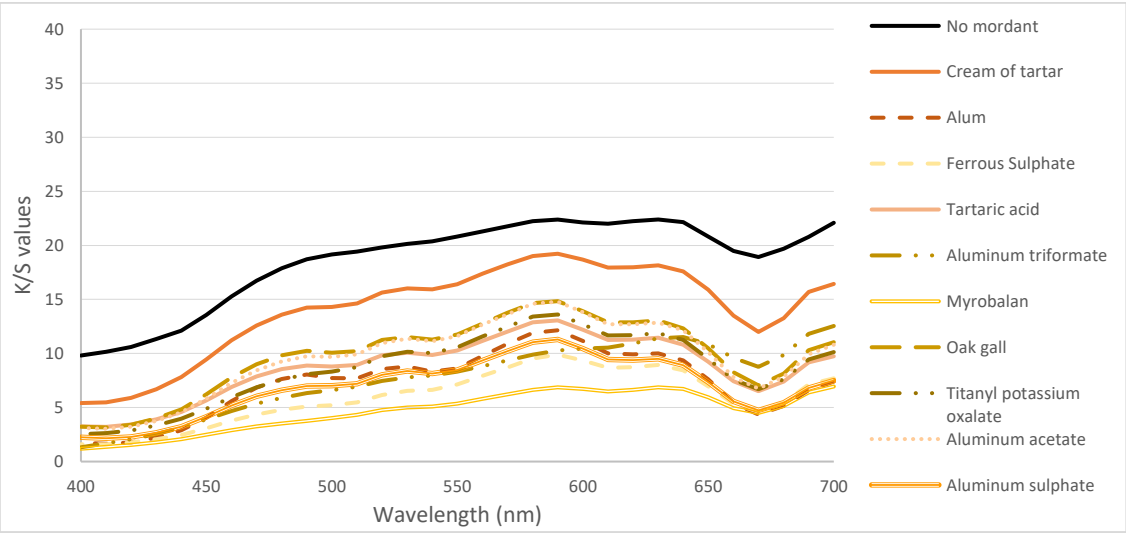


Figure 35. K/S spectrum of dyed wool with β-carotene – mordant influence analysis

Measurement of residual colorant matter in carotene-wool dyeing wastewater effluents

To confirm the dye uptake %, the wastewater effluents were subjected to spectrophotometric analysis, for the identification of the amount of unfixed dye remaining in the dyeing liquid effluent emissions. The dye uptake percentage represents the difference between the colorant matter in the dyeing bath before the dyeing process and the one remaining in the wastewater effluent. Figure 36 summarizes the colorant absorption rates and indicates values passing 95%, which represents a very high colorant absorption rate. This may be justified by the sufficient process time and optimum temperature and pH selection, permitting the proper diffusion rate of the dye molecules inside the wool fabric.

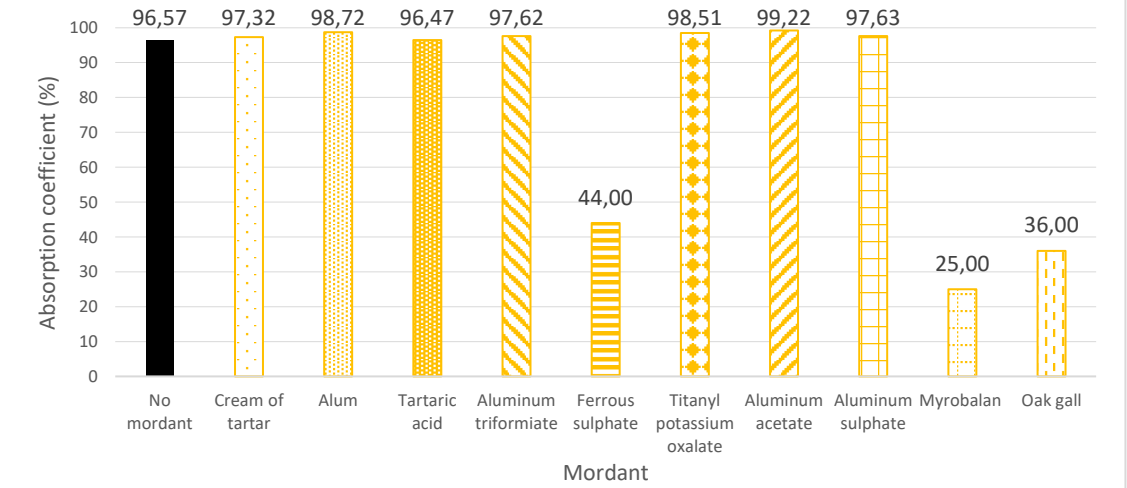


Figure 36. Colorant absorption coefficient based on dyeing of wool with β-carotene wastewater effluent

It can be observed that Ferrous sulphate and the biomordants Myrobalan and Oak gall are poorly represented in Figure 36, due to the high interference of the mordants' intrinsic natural color molecules contained in the structure, generating thus undesirable results in this set of analysis. The unfixed mordant on the fabric and the unfixed carotenoid molecules generate interference with the dye uptake capacity thus obtaining the lowest values within the analyzed range.

2) B-carotene wool dyeing process quality assessment through measurement of laundering and lightfastness

The dyed wool fabrics' color resistance against degrading agents, as laundering or light, is indicated in Table 30. With clear improvement in lightfastness with all the applied mordants, with 1 or 2 degrees. The most suitable improvements are obtained with the use of Cream of Tartar, Alum, Ferrous sulphate, and Titanyl potassium oxalate, in correlation with the dye uptake and color strength measurements, thus validating the potential of dyeing process efficiency increase.

Sample	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of Tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Tartaric Acid	4	4-5	4-5	4-5	4-5	4-5	4-5	3
Ferrous sulphate	3	4-5	4-5	4-5	4-5	4-5	4-5	4
Aluminum triformate	2	4-5	4-5	4-5	4-5	4-5	4-5	3
Myrobalan	4	4-5	4-5	4-5	4-5	4-5	4-5	3
Oak Gall	2	4-5	4-5	4-5	4-5	4-5	4-5	3
Titanyl potassium oxalate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Aluminum acetate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum sulphate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3

Table 30. Fastness properties of the β-carotene dyed wool influence of different mordants

The fastness values obtained are comparable with similar approach studies focusing on dyeing wool fabrics with natural colorants enhanced with the use of mordants, completed with the color intensity values (*L**) (Chandru and Praveena 2021) (Singh and Sheikh 2020).

Partial conclusions for dyeing of wool with β-carotene

- *Pre-mordanting* of wool with a total of 10 different mordants, indicated that **Aluminum triformate** (10% w.o.f.) has revealed as the most efficient auxiliary with a more intense and yellowish result.
- *The intrinsic natural color* of mordants Ferrous sulphate (3% w.o.f.), Myrobalan (25% w.o.f.), and Oak gall (25% w.o.f.) boosted significantly the yellow-brown color obtained, in terms of intensity and hue, but not dye absorption.
- *The analysis of the absorption coefficient of the wastewater effluents* indicates that the most intense yellowish result obtained with Aluminum triformate was characterized by a 97% of dye uptake. Followed by Alum, Aluminum sulphate, and Titanyl potassium oxalate (20% w.o.f.) Tartaric acid (6% w.o.f.), Aluminum acetate (20% w.o.f.), and Cream of tartar (6% w.o.f.), with dye uptakes surpassing 90%.
- *The color difference* (ΔE>3) confirms that all of the mordants presented improved dye absorption efficiency**.** Additionally, confirmed by the analysis of the UV-VIS absorbance spectrum of the dyed fabrics, the majority of pre-mordanted dyed fabrics are grouped in the same area, significantly below non-mordanted dyed reference values, meaning darker tones, completed with a tendency to darker hues influenced by the biomordants with intrinsic natural brown color.
- *Laundering and lightfastness properties* show slight improvements with the use of Cream of Tartar, Alum, Titanyl potassium oxalate, and Aluminum triformate:
    - *Laundering fastness* presents a poor overall (2-3) response with a slight increase (3-4). Staining behavior (4-5) reflects the fact that, even though the degradation of the tested fabrics occurs, the staining fabrics do not get stained, meaning that a mordant is needed for fixing the β-carotene on the wool fabric. *Lightfastness* overall can be defined by poor behavior (2) improved to moderate (3-4).

4.3.4. Analysis of the dyeability of natural fabrics with green algae-based colorant matter Chlorophyll-a-rich extract from microalgae *Caespitella pascheri*

a) Analysis of results obtained from cotton textile substrate dyeing with Chlorophyll-a

1) Color assessment: Chromatic characterization

Chlorophyll-a cotton dyeing experimental results, focused on the exploration of the influence of mordants, are presented in Figure 37 below, and light green shades with different tones can be observed, due to the influence of 10 mordants. Considering the very light coloration it can be indirectly assumed low efficiency and low influence of the auxiliaries.

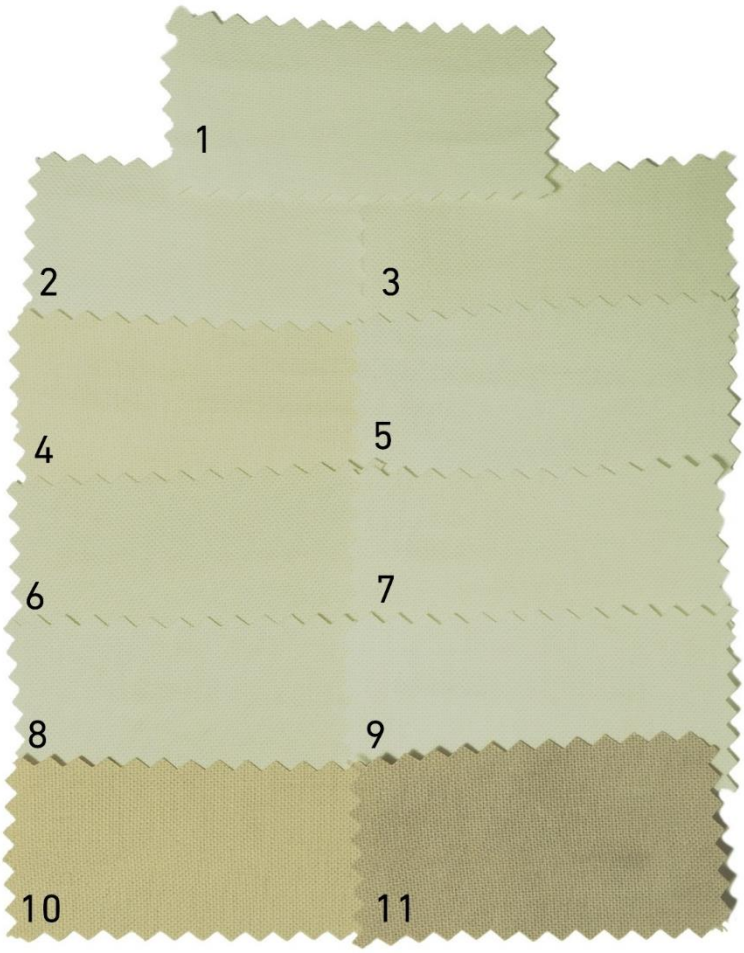


Figure 37. Dyed cotton fabrics with Chlorophyll-a obtained from Caespitella pascheri

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

The objective color characterization analysis, by measurement of CIE*Lab* color coordinates (*L*, a*,* and *b*),* color differences (*Δ*), and color strength (*K/S*) were performed for the Chlorophyll-a dyed pre-mordanted samples, for the exploration of the dyeability and influence of auxiliaries. The complete set of results is presented in Table 31. The reference sample used for comparison is the non-mordanted one, specifically for the exploration of the influence of the mordants on the process.

Cotton sample	***Color coordinates and color strength***							
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***	***K/S***
No mordant	86,99	-2,53	10,94	-	-	-	-	36,32
Cream of Tartar	86,97	-2,71	13,09	-0,02	-0,18	2,15	0,26	36,32
Alum	86,06	-2,94	9,86	-0,93	-0,41	-1,08	1,10	36,17
Ferrous sulphate	80,26	-2,84	14,06	-6,73	-0,31	3,12	6,74	33,88
Tartaric Acid	87,89	-2,25	11,13	0,9	0,28	0,19	0,98	35,78
Aluminum triformate	87,03	-2,83	13,44	0,04	-0,3	2,5	0,43	32,29
Myrobalan	70,5	-1,16	8,43	-16,49	1,37	-2,51	16,60	25,18
Oak gall	63,9	-1,03	7,73	-23,09	1,5	-3,21	23,19	24,65
Titanyl potassium oxalate	74,03	-3,12	14,91	-12,96	-0,59	3,97	12,99	35,93
Aluminum acetate	80,7	-2,97	18,67	-6,29	-0,44	7,73	6,32	34,68
Aluminum sulphate	88,61	-2,88	11,9	1,62	-0,35	0,96	1,69	36,15

Table 31. CIELab coordinates and calculations for Chlorophyll-a dyed cotton

The color difference results indicated by *ΔE*, show an interesting heterogeneity and may be analyzed in the context of *ΔE*>3 (reflecting significant color differences) and *ΔE*<3 (meaning insignificant acceptable color differences). In this sense, relevant color variation has been obtained with the use of Ferrous sulphate, Titanyl potassium oxalate, Aluminum acetate, Oak gall, and Myrobalan. This can be justified by an efficiency increase and compatibility with the cotton and natural colorant matter, also validated by a darker tone *ΔL*<0 (-6,73; -12,96; -6,20; -23,09; -16,49), and the difference of the green component *a*<0.*

The other tested mordants, Cream of tartar, Alum, Tartaric acid, and Aluminum triformate show reduced efficacy with no significant efficiency improvements, in the overall color differences analysis.

The biomordants Oak gall and Myrobalan generate significant color differences, in comparison with the reference sample, due to a natural brownish predominant coloration, resulting in an intense brown with green shades. The same justification applies to the use of Ferrous sulphate, but with intense yellow intrinsic influences.

Overall, the low color intensity of green dyeing of cotton fabrics was obtained, indicating the need of increasing the colorant concentration in the dyeing bath or exploring the coloration process with the powder form of the colorant, as by drying it a certain level of purification is obtained.

Light green colors in the dyeing of pre-mordanted cotton with chlorophyll obtained from plant leaves have been obtained in similar studies (Chandru and Praveena 2021), thus confirming the viability of the exhaustion dyeing process with the application of this colorant matter, nevertheless with further exploration for fastness behavior improvement.

Figure 38 plots the distribution of the *a** and *b** color coordinates which locate the samples in objective color space and confirm the obtention of green hues, with yellowish influences. The *Chlorophytes* microalgae family are characterized by the intimate connection of

the chlorophyll and carotene in the organism cell, thus justifying the yellowish color influence. As expected M7. Myrobalan and M8. Oak gall are located apart from the rest of the samples, due to the brownish natural influences of this biomordant, which interfere with the expected green results.

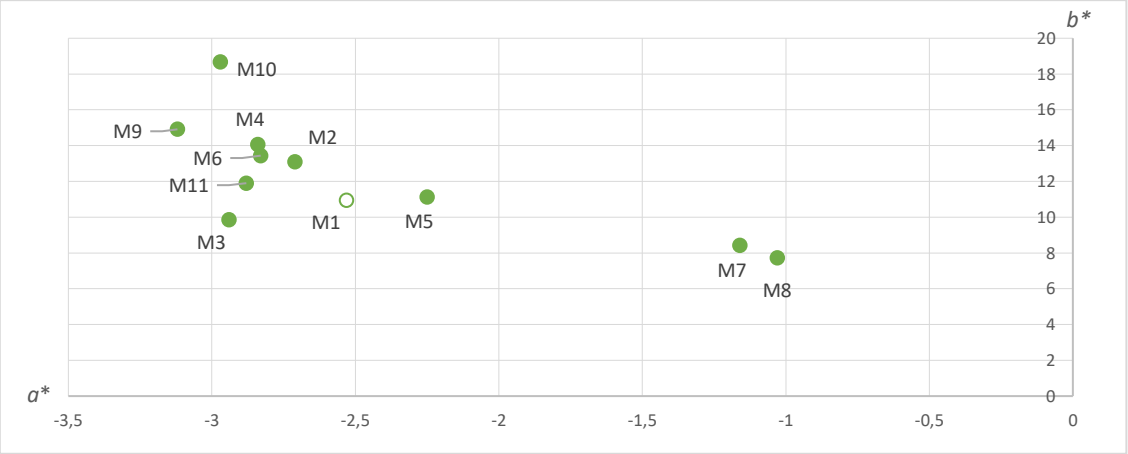


Figure 38. Color specifications in CIELab of the dyed cotton fabrics with Chlorophyll-a

M1. No mordant; M2. Cream of tartar; M3. Alum; M4. Ferrous sulphate; M5. Tartaric acid; M6. Aluminum triformate; M7. Myrobalan; M8. Oak gall; M9. Titanyl potassium oxalate; M10. Aluminum acetate; M11. Aluminum sulphate.

Reflectance spectrum and color strength analysis

The reflectance capacity of the dyed samples is an indicator of the coloration strength provided by the green natural colorant, Chlorophyll-a. Figure 39 presents the *K/S*, color strength spectrum based on the reflectance of the samples, and it positions most of the samples in the same group, with the exception of Myrobalan and Oak gall. In the context of the whitest sample reflects more light, and the darkest absorbs more light, the following classification in terms of efficiency may be done, starting with the deepest color penetration of the fabrics, at the corresponding maximum absorbance wavelength of Chlorophyll-a (λ max= 670 nm): Myrobalan, Oak gall, Aluminum triformate, Aluminum acetate, Tartaric acid, Titanyl potassium oxalate, Cream of tartar, Aluminum sulphate, Alum, No mordant, Ferrous sulphate. The Aluminum-based mordants seem to present a good influence on the dyeing of cotton with Chlorophyll-a.

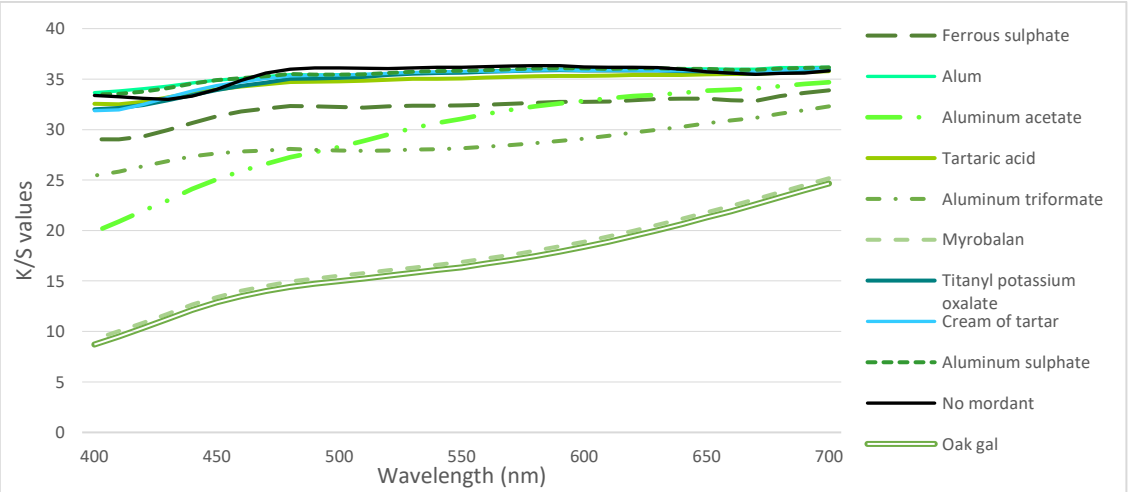


Figure 39. K/S spectrum of dyed cotton with Chlorophyll-a – mordant influence analysis

Measurement of residual colorant matter in Chlorophyll-a-cotton dyeing wastewater effluents

The colorant matter remaining after the cotton dyeing process was assessed, for the validation of the process dyeability, by measurement of the UV spectrum of the wastewater at the maximum absorbance wavelength of Chlorophyll-a. The dye uptake % is presented in Figure 40 below and confirms the good colorant uptake with the use of some Aluminum-based mordants, as Aluminum acetate and Aluminum triformate with 90,7% and 85,01% respectively. Nevertheless, it can be observed that the dye uptake rate surpasses 50% (corresponding to the non-mordanted case), in most of the dyeing experimental cases. Even though light green color shades were obtained, a potential increase in dyeability is assumed, confirmed by increased dye uptake percentages. Additionally, the colorant matter degradation can be discarded due to the resistance of the Chlorophyll-a to a temperature of 65^0C.

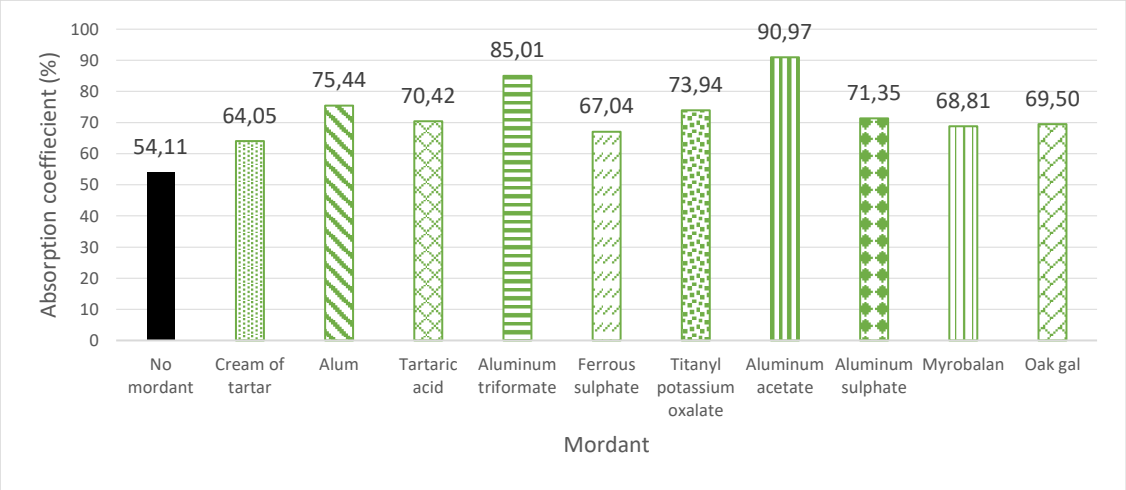


Figure 40. Colorant absorption coefficient based on dyeing of cotton with Chlorophyll-a wastewater effluent

2) Chlorophyll-a cotton dyeing process quality assessment through measurement of laundering and lightfastness

The quality of the Chlorophyll-a dyed cotton substrate textiles was analyzed through the measurement of the laundering and lightfastness, with the obtained results indicated in Table 32. The color degradation of the color of the samples presents poor behavior, and very low or no improvements are obtained with the use of mordants, when compared with the non-mordanted sample, with the exception of Myrobalan and Oak gall (influenced by the brown intrinsic coloration). Nevertheless, the very light color shade of the non-mordanted samples may influence the inefficient analysis of the results. The color staining behavior on other textile substrates is characterized by acceptable, thus indicating that the remaining colorant in the laundering water needs auxiliaries for coloration improvement. The lightfastness response is very low, thus indicating the need for exploration of additional auxiliaries for the improvement of the resistance to light of the sensitive natural colorant matter.

Sample	Color change	Staining						Lightfastness
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
No mordant	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Cream of Tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1
Ferrous sulphate	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Tartaric Acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Myrobalan	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Oak gal	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Titanyl potassium oxalate	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum acetate	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 32. Fastness properties of the Chlorophyll-a dyed cotton influence of different mordants

The unsatisfactory fastness behavior obtained in this study is not comparable with similar studies, where improvements have been demonstrated (Chandru and Praveena 2021), indicating thus the possibility for increasing the resistance of the color against light and laundering.

One of the scarce studies focusing on the use of green algae *Cladophora glomerate L* as a source for natural colorants for cotton dyeing has identified the influence of coloration according to the pH of the dyeing liquor, fastness, and mordants (Mir et al. 2019).

Partial conclusions for dyeing of cotton with Chlorophyll-a

- *Pre-mordanting* of cotton samples with 10 different mordants resulted in improvements in terms of color intensity, with greener results, with the use of **Titanyl potassium oxalate (20% w.o.f.),** and **Aluminum acetate (20% w.o.f.).**
- *The intrinsic natural color* of mordant Myrobalan (25% w.o.f.) and Oak gall (25% w.o.f.) has interfered positively with the green coloration generating darker colors of the samples located in the same color space.
- *The analysis of the absorption coefficient of the wastewater effluents* indicates high efficiency and dye uptake percentages reaching 74% for Titanyl potassium oxalate respectively 90% for **Aluminum acetate**, and Oak gall and Myrobalan by lower dye uptake (70%).
- *The color difference* (ΔE<3) indicates that heterogeneous results were obtained, on one side efficient ones, and on the other, generating insignificant color differences. The UV-VIS reflectance spectrum indicates the division among the different efficiencies of the tested mordants.
- *Laundering and lightfastness properties* do not show improvements**,** compared with the non-mordanted samples and reveal poor behavior in terms of color degradation (1-2), and do not reflect relevant improvements with the use of mordants, with the exception of Myrobalan. Meanwhile, staining is characterized by fair behavior overall (4-5).

b) Analysis of results obtained from wool textile substrate dyeing with Chlorophyll-a

1) Color assessment: Chromatic characterization

Non-mordanted and pre-mordanted wool samples were dyed with Chlorophyll-a from microalgae *Caespitella pascheri* and the resulting samples are presented in Figure 41. A total of 10 different mordants were used for the exploration of their possible influence on the process dyeability. Considering the visual analysis, it can be confirmed that more intense green shades are obtained in the wool dyeing process, when compared with the use of cotton substrates (see Figure 37). Nevertheless, color differences may be observed through this visual analysis indicating different influences of the mordant use.

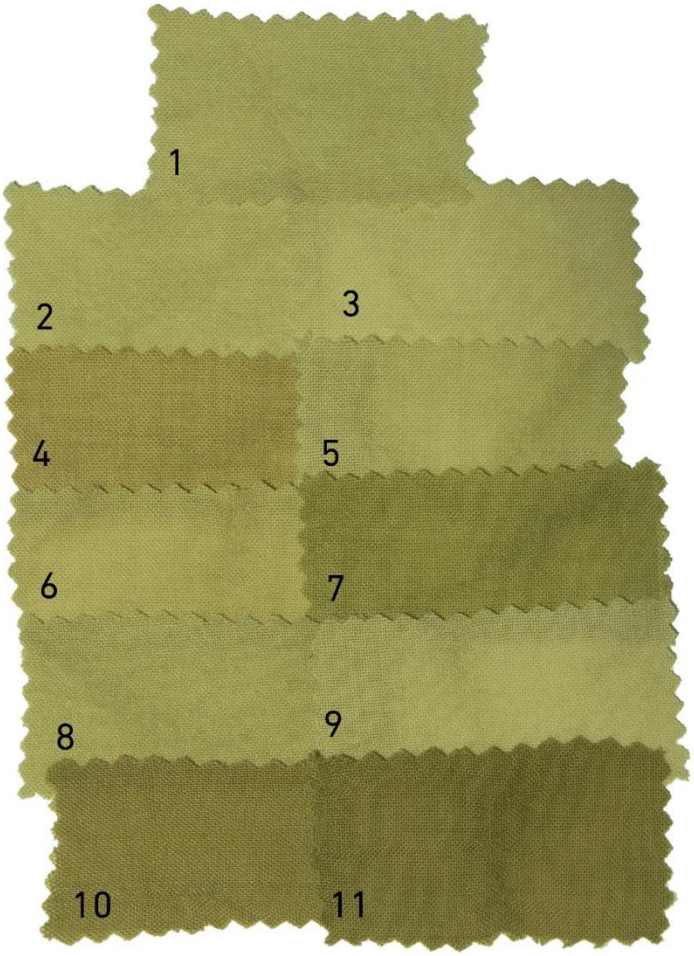


Figure 41. Dyed wool fabrics with Chlorophyll-a obtained from Caespitella pascheri

1. No mordant; 2. Cream of tartar; 3. Alum; 4. Tartaric acid; 5. Ferrous sulphate; 6. Aluminum Triformate; 7. Titanyl potassium oxalate; 8. Aluminum acetate; 9. Aluminum sulphate; 10. Myrobalan; 11. Oak gall.

Use of mordants influence on the dyeing process efficiency

The objective analysis of the green color of the dyed wool samples was performed through the measurement of the CIE*Lab* color coordinates (*L*, a*,* and *b**), and calculus of the existing color differences between the non-mordanted dyed sample, and the pre-mordanted ones, for the analysis of the influence of auxiliaries on the process efficiency. All the results are presented in Table 33, considering the non-mordanted sample as a reference, highlighted accordingly in the table.

Cotton sample	Color coordinates and color strength							
	L	a	b	ΔL	Δa	Δb	ΔE	K/S
No mordant	73,29	-3,12	18,95	-	-	-	-	14,76
Cream of Tartar	72,86	-2,21	20,66	-0,43	0,91	1,71	1,98	19,02
Alum	74,81	-1,06	19,69	1,52	2,06	0,74	2,66	14,14
Ferrous sulphate	58,23	-2,06	21,69	-15,06	1,06	2,74	15,34	24,01
Tartaric Acid	71,26	-2,18	21,89	-2,03	0,94	2,94	3,69	19,03
Aluminum triformate	65,87	-1,11	22,03	-7,42	2,01	3,08	8,28	25,58
Myrobalan	50,6	1,73	22,19	-22,69	4,85	3,24	23,43	26,56
Oak gal	43,14	1,53	17,25	-30,15	4,65	-1,7	30,55	28,11
Titanyl potassium oxalate	55,26	-2,28	23,17	-18,03	0,84	4,22	18,54	25,70
Aluminum acetate	62,6	-1,62	19,82	-10,69	1,5	0,87	10,83	25,63
Aluminum sulphate	71,82	-1,46	18,98	-1,47	1,66	0,03	2,22	21,92

Table 33. CIELab coordinates and calculations for Chlorophyll-a dyed wool

The color difference between the pre-treated samples and the mordanted ones was analyzed considering that $\Delta E>3$ indicates a significant objective color difference, meanwhile, $\Delta E<3$ indicates no significant differences. In this sense, notable differences are obtained with the use of Oak gall and Myrobalan, followed by Titanyl potassium oxalate, Ferrous sulphate, Aluminum acetate, Aluminum triformate, and Tartaric acid.

The color intensity analysis $\Delta L<0$ indicates that the samples, with the highest color differences, are also darker with greener color tones. The most intense coloration was obtained with the use of Oak gall (ΔL=-30,15) and Myrobalan (ΔL=-22,69) due to the dark brownish natural color conferred to the sample, interfering with the aimed green coloration. A yellowish influence is also provided by Ferrous sulphate (ΔL=-15,06), due to the natural intrinsic coloration of the mordant.

Similar results with the non-mordanted sample were obtained with the use of Cream of tartar, Alum, and Aluminum sulphate, indicating that their efficiency is reduced, as their activity was supposed to improve coloration and colorfastness.

Similar studies approaching the dyeing of proteinic fibers with natural chlorophyll have obtained a comparative color characterization of the dyed fibers (Gong et al. 2019).

The color diagram focused on the a^* and b^* color coordinates is plotted in Figure 42, and it locates the majority of samples in the same green space with slight yellow color influence, except samples M7. Myrobalan and M8. Oak gall, due to the brownish color influence of the mordant. This plot confirms the possibility of dyeing wool with Chlorophyll-a. Nevertheless, various levels of green can be observed, as influenced by the use of mordants, indicating the efficiency of mordants completed with a certain level of affinity between the textile substrate and colorant matter.

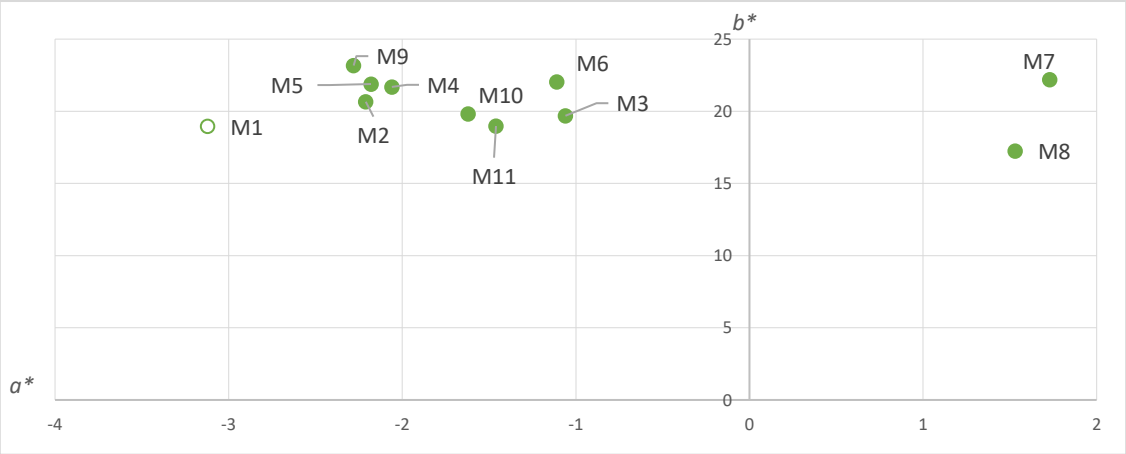


Figure 42. Color specifications in CIELab of the dyed wool fabrics with Chlorophyll-a

M1. No mordant; M2. Cream of tartar; M3. Alum; M4. Ferrous sulphate; M5. Tartaric acid; M6. Aluminum triformate; M7. Myrobalan; M8. Oak gall, M9. Titanyl potassium oxalate; M10. Aluminum acetate; M11. Aluminum sulphate.

Reflectance spectrum and color strength analysis

The color strength, K/S, of the dyed wool samples with Chlorophyll-a was calculated based on the light reflectance percentage of the samples, and it is presented in Figure 43. The whitest sample, with the highest reflectance capacity, is represented by Alum followed by the non-mordant (reference sample), meaning that the other mordants have a positive influence on improving the dyeability of the wool samples, by promoting the bonding of the colorant matter with the proteinic textile substrate. The efficiency of the mordants increased in the following order: Cream of tartar, Tartaric acid, Alum, Aluminum sulphate, Ferrous sulphate, Aluminum triformate, Aluminum acetate, Titanyl potassium oxalate, Oak gall, and Myrobalan. Even though we can attribute Myrobalan and Oak gall the brown influence, it must be indicated that it contributes to a very intense dark green result. Nevertheless, it can be confirmed that the most efficient mordant of this set of experiments was, metallic salt, Titanyl potassium oxalate.

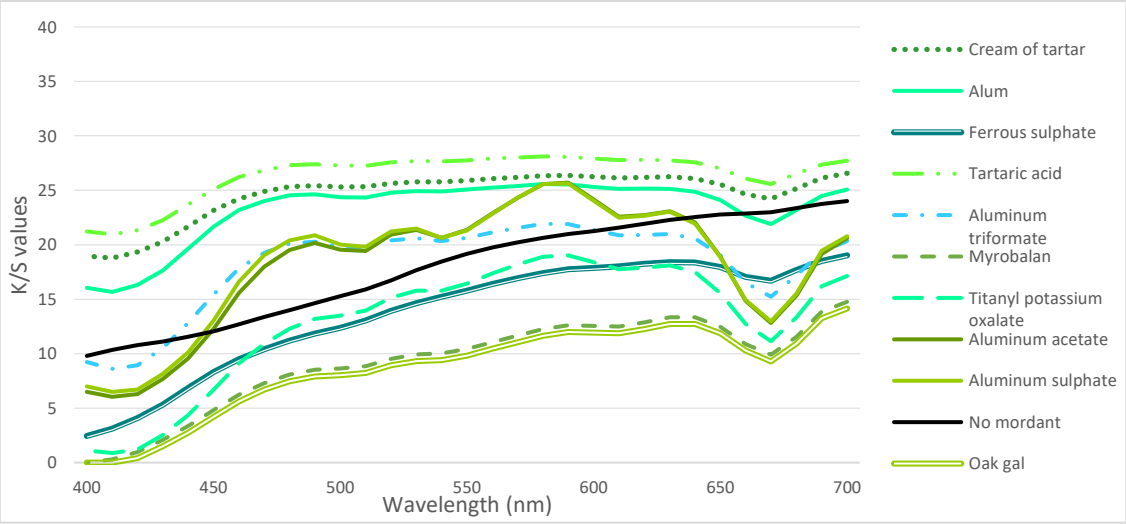


Figure 43. K/S spectrum of dyed wool with Chlorophyll-a – mordant influence analysis

Measurement of residual colorant matter in Chlorophyll-a-wool dyeing wastewater effluents

The wastewater obtained from the dyeing process of wool fabric was subjected to UV spectrum absorbance analysis, for the measurement of the remaining colorant matter in the resulting effluent, and this was reported as dye uptake percentage. The obtained results reveal high dye uptake with values surpassing 94%, indicated in Figure 44. It can be observed that most of the mordants present a higher uptake than the non-mordanted one (94,90%), thus, proving improved efficiency, except for Myrobalan and Oak gall, showing less uptake (90,99%, respectively 89,76%), but at the same time revealing the most intense color results, thus confirming the natural brown color positive influence, in this case.

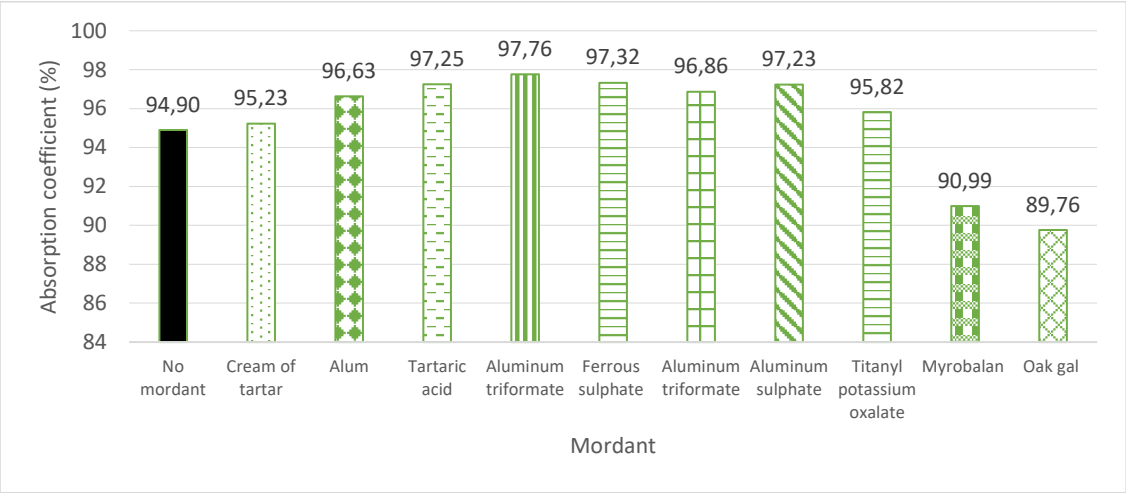


Figure 44. Colorant absorption coefficient based on dyeing of wool with Chlorophyll-a wastewater effluent

2) Chlorophyll-a wool dyeing process quality assessment through measurement of laundering and lightfastness

The quality of the dyeing process is defined by the laundering and lightfastness of the colors of the samples, and implicitly, the most adequate behavior is provided by the most efficient mordant. The complete set of results is presented in Table 34, and it can be observed that the degradation of color generated by laundering presents improvements with the use of Aluminum triformate, Aluminum acetate, Ferrous sulphate, Aluminum sulphate, Myrobalan, Oak gall, and the best results obtained with Titanyl potassium oxalate. Nevertheless, the staining of other textiles substrates through laundering reveals good behavior for all the tested samples, suggesting low affinity to natural and synthetic fibers, and the need for auxiliaries for the improvement of the diffusion of the colorant into fibers. The lightfastness response indicates a naturally small resistance (reference sample) of the colorant against light degradation, improved with the use of Ferrous sulphate, Titanyl potassium oxalate, Oak gall, and Myrobalan.

Sample	*Color change*	*Staining*						*Lightfastness*
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of Tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Ferrous sulphate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	4
Tartaric Acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Myrobalan	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Oak gal	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2
Titanyl potassium oxalate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 34. Fastness properties of the Chlorophyll-a dyed wool influence of different mordants

Studies focusing on dyeing proteinic fibers with natural chlorophyll have obtained good fastness results with the use of mordants (Gong et al. 2019), indicating the possibility of improvement of this parameter through further studies. Overall, it is worth mentioning that recent studies exploring alternatives to natural sources of colorant matter, focusing on mordanted textile substrates dyeing are relatively recent.

Partial conclusions for dyeing of wool with Chlorophyll-a

- *Pre-mordanting* of wool with 10 different mordants generated an increase in dyeability with notable color differences and more intense coloration with the use of **Titanyl potassium oxalate (20% w.o.f.),** Aluminum acetate (20% w.o.f.), Aluminum triformate (10% w.o.f.)., and Tartaric acid (6% w.o.f.). All the indicated mordants show a dye uptake surpassing 95%, as per the wastewater analysis.
- *The intrinsic natural color* of Myrobalan (25% w.o.f.) and Oak gall (25% w.o.f.) generates a rather darker green coloration of the wool substrates due to the intrinsic natural brown color compatible with the Chlorophyll-a, confirmed by a lower dye uptake (90%) than the non–mordanted samples (94,9%).
- *The analysis of the absorption coefficient of the wastewater effluents* indicates high efficiency and dye uptake percentages surpassing 90% for all the experimental cases, reaching up to 97%.
- *The color difference* (ΔE>3) indicates the same heterogeneity comparable with the cotton fabrics indicating specificity among colorants and the Chlorophyll-a structure. The metallic mordants and biomordants improve color characteristics generating more intense greener samples. This assumption is confirmed by the UV-VIS absorbance spectrum supporting the previously mentioned efficiency with a dispersion of the spectrums.
- *Laundering and lightfastness properties* show overall poor behavior in terms of color degradation to laundering (1-2) and light (1-2), and staining good behavior (4-5), and do not improve, except for Titanyl potassium oxalate and Myrobalan:
    - *Laundering fastness* reaching up to 1-2 degradation behavior improved to fair (3-4). *Lightfastness* improvement to poor-fair behavior (3-4).

4.4. Preliminary exploration of dyeing wastewater effluents quality, treatment approach, and added value assumptions

4.4.1. Wastewater effluents quality assessment

Cotton dyeing with blue C-phycocyanin from *Spirulina platensis* wastewater effluents quality analysis

a) COD and BOD_5 assessment

For the assessment of the quality of the C-phycocyanin cotton dyeing effluents, a series of tests were performed with process parameters variation, but no mordanting. In this sense, in Figure 45, the direct influence of process temperature and time are analyzed, indicating the better performance at optimum temperature (65^0C) and medium process time (60 min), with important values reduction.

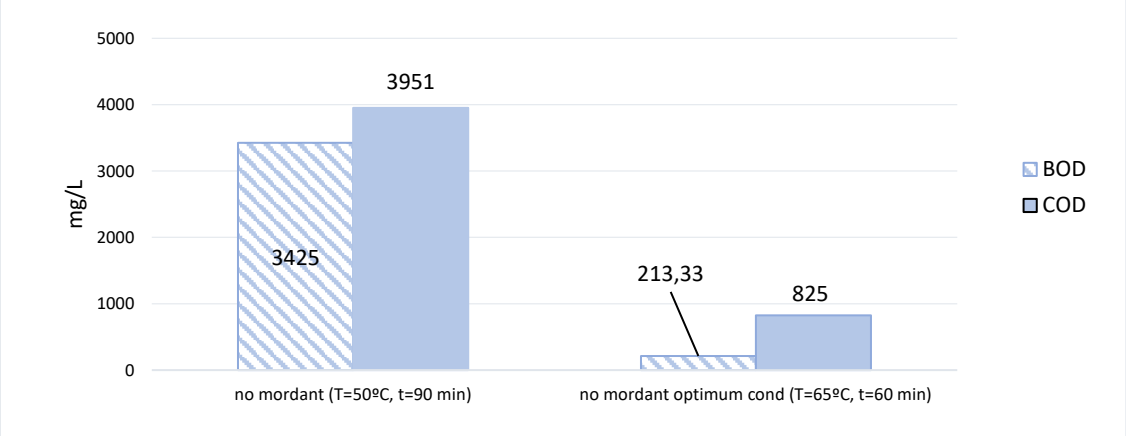


Figure 45. Influence of dyeing process temperature on COD and BOD_5 cotton dyeing wastewater effluent values

On the other hand, the inclusion of the mordanting process, has a positive impact on the wastewater effluent quality, due to an increase in dyeability, thus resulting in a lower organic residues quantity in the process emissions, as can be observed in Figure 46. Nevertheless, the meta-mordanting process seems more effective than the pre-mordanting approach, this may be justified by a positive impact of the direct interaction in solution between the mordant, colorant, and textile substrate. Nevertheless, this applies to Cream of tartar, and it cannot be extrapolated to other auxiliaries.

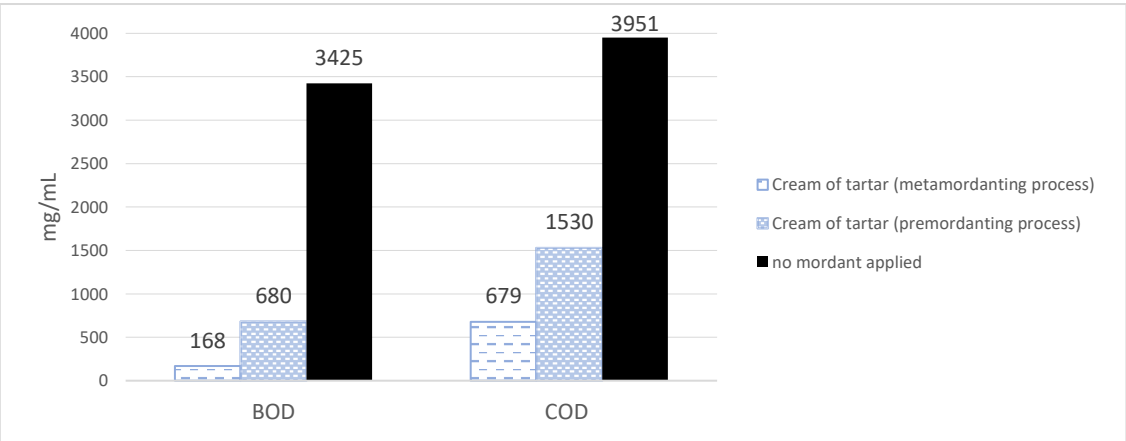


Figure 46. Influence of inclusion of Cream of tartar mordant in BOD_5 and COD levels in cotton dyeing effluents

To ensure the repeatability of the best results previously obtained, validating the optimum process temperature and time, based on color characterization results, a repeatability test was performed, and the standard error was calculated. The slight overlapping of the error bars indicates a statistically insignificant difference, which confirms the process repeatability.

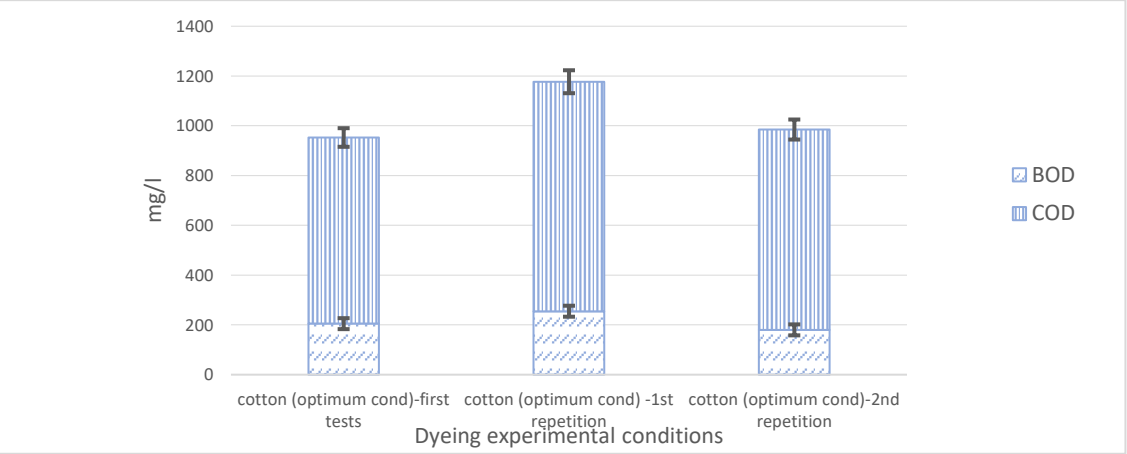


Figure 47. Validation of cotton dyeing process repeatability considering optimum conditions and influence on the wastewater effluents quality

On the other hand, the resulting values of COD and BOD_5, of the non -mordanted cotton dyeing, 1000-1200 mg/L respectively 200 mg/L are comparable with values in the cotton dyeing industry ranging from 420-1400 mg/L for COD and 80-500 mg/L for BOD_5 (Agarwal and Sonia 2021).

These favorable results can be justified by the adequate process parameters favoring an increased absorption of the C-phycocyanin on the cotton fabric. Nevertheless, considering that C-phycocyanin kinetics degradations studies indicate stability up to 65°C at pH=5 (Antelo et al. 2008) (Patel et al. 2004), it is not possible to discard chromophore degradation, due to the neutral dyeing pH 7 applied in this study. Thus, it is possible to assume that the process temperature applied, at the C-phycocyanin degradation limit, together with the pH, may slightly denature the colorant material, and that is why the use of a mordant is highly needed for fostering the colorant-cotton bonding.

b) Metal content analysis in cotton dyeing wastewater effluents

Based on the existing Spanish national limitation (Alcaldesa et al. 2003), and the use of metal and salt mordants in this study, the metal content, of Aluminum and Iron, in the wastewater effluents was analyzed.

The quality assessment of the cotton dyeing effluents, in terms of daily Aluminum and Iron limit of discharge in treatment water plants, can be analyzed in Figure 48 below. As expected, the use of Alum generates an exceeding of the Aluminum limit with approximately 11 % and the use of Ferrous sulphate, passing the daily Iron discharge limitation by 60%. These mordants are not the most sustainable auxiliaries, in this formula, but it should be highlighted that these are concentrated laboratory-scale values, which, in an industrial environment would suffer dilutions with effluents from other processes. On the other hand, the low Iron and Aluminum concentration was obtained with the use of the Cream of tartar, Tartaric acid, Aluminum triformate, Myrobalan, Oak gal, Titanyl potassium oxalate, Aluminum acetate, and

Aluminum sulphate provide evidence of exerting their proposed function in the finishing process.

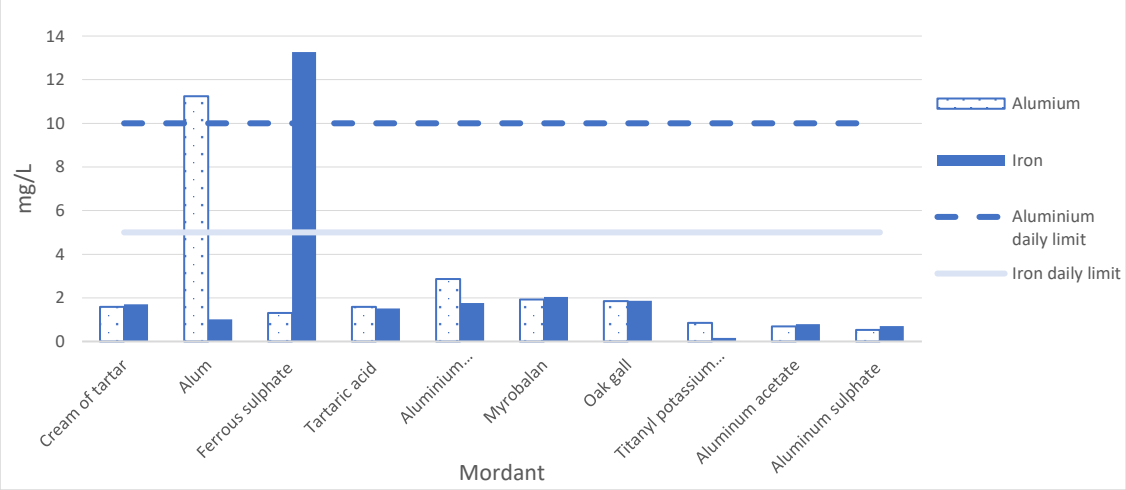


Figure 48. Influence of mordants in cotton dyeing process wastewater effluents contents of metals (Iron and Aluminum)

Wool dyeing with blue C-phycocyanin from Spirulina platensis wastewater effluents quality analysis

a) COD and BOD_5 assessment

For the assessment of the quality of the C-phycocyanin wool dyeing effluents, a series of tests were performed with parameters variation. Figure 49 reflects the effect of temperature and time on the quality of the effluents, with the most favorable performance at optimum temperature (65^0C) and medium process time (60 min), without the use of mordants. This set of parameters have been considered optimum due to the temperature sensitivity presented by the chromophore used as a dyeing agent, being stable at 65°C, in pH=5 conditions (Antelo et al. 2008) (Patel et al. 2004), also the wool dyeing conditions in this study.

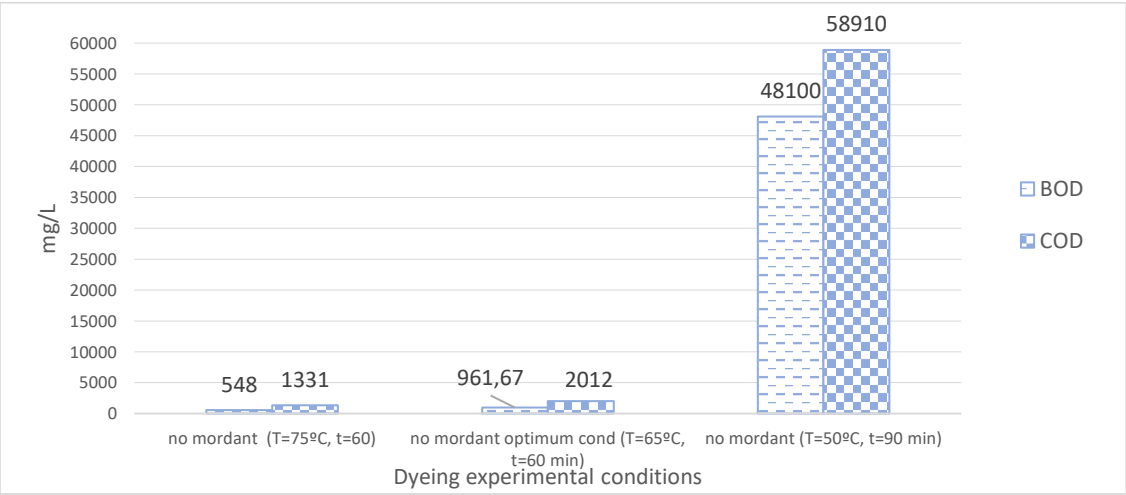


Figure 49. Wool dyeing process temperature influence on wastewater quality parameters (BOD_5 and COD)

The repeatability of the selected conditions has been verified (Figure 50), by calculating the standard error among various process repetitions, and resulting in statistically insignificant differences, thus confirming the quality of the experiments. However, the obtained results of

500-1300 mg/L for BOD_5, and 1500-2500 mg/L for COD respectively are comparable with synthetic dyeing industrial effluents, ranging between up to 1800 mg/L for BOD_5 and 1100-4600 mg/L for COD (Yaseen and Scholz 2019). This is a very promising result indicating the potential for an increased quality of the natural dyeing effluents with the use of mordants and the added value of reduced synthetic dyeing agents employed, thus increased sustainability.

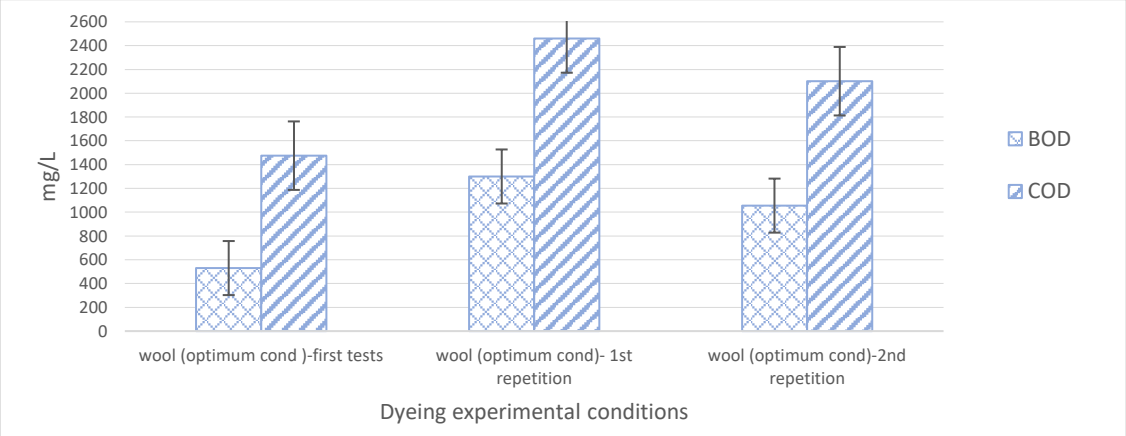


Figure 50. Validation of wool dyeing process repeatability considering optimum conditions and influence on the quality of the wastewater emissions

b) Metal content analysis in wool dyeing wastewater effluents

The compliance of the wool dyeing wastewater effluents with the Spanish national dyeing effluents daily limits discharge in wastewater treatment plants (Alcaldesa et al. 2003) was analyzed in Figure 51, due to the use of metal-based auxiliaries in the dyeing process. In this sense, it is observed that the Aluminum daily limit is exceeded by 90%, respectively 60% with the use of Alum and Aluminum triformate, meanwhile, the use of Ferrous sulphate passes de Iron daily discharge limit by 90%. The low values obtained with the use of Cream of Tartar, Tartaric acid, Myrobalan, Oak gall, Titanyl potassium oxalate, Aluminum acetate, and Aluminum sulphate indicate that they performed their function of bonding with the textile substrate, and did not remain as residue in the dyeing effluents. As in the case of cotton, it must be stated that these concentrations are obtained at a laboratory scale, where the effluents represent concentrated liquors, which, in industrial environments would suffer dilution by effluents from other processes.

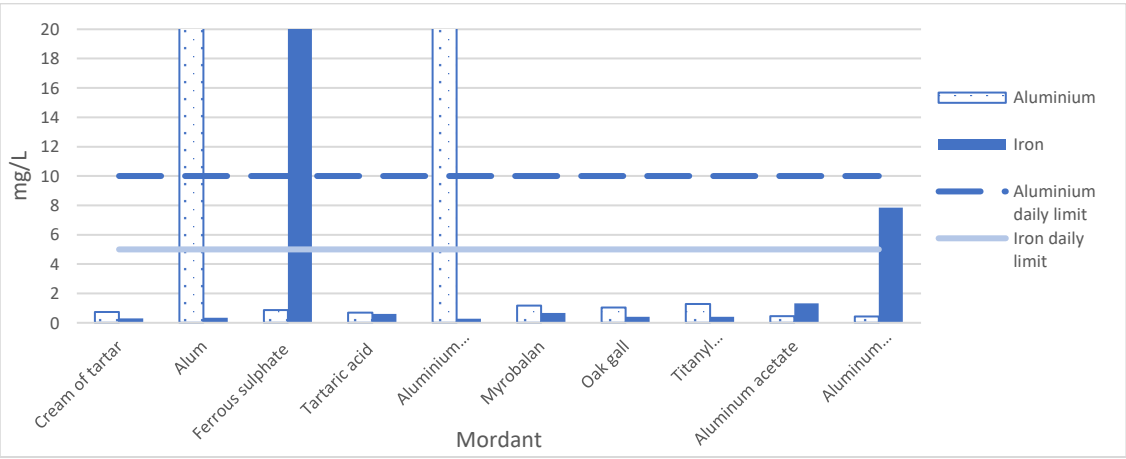


Figure 51. Influence of mordants in wool dyeing process wastewater effluents contents of metals (Iron and Aluminum)

4.4.2. Wastewater biological fungi treatment exploratory approach

To explore the sustainability character of the wastewater effluents resulting from the dyeing process of cotton and wool with C-phycocyanin was elaborated an experimental setup, at a laboratory scale, for testing the efficiency of a fungi solution biological wastewater treatment.

The main experimental setups compared are included in Table 35, focusing on the values obtained by analyzing the fungal solution, the effect of the treatment, and the sedimentation effect after treatment. The resulting BOD_5 and COD values confirm a massive treatment capacity of this conventional biologic solution. This is a successful result, considering that the main regulation, encompassing both parameters refers to EPA regulations for wastewater effluent discharge (Zhang et al. 2015), which indicates 45 mg/L for BOD_5 as weekly limitation discharge in an urban wastewater treatment plant.

Wastewater effluent	**BOD_5 (mg/L)**	**COD (mg O_2/L)**
Original fungi solution	600	10248
Fungi solution centrifuged	20	430
Wool wastewater	243	908
Wool wastewater treated with fungi	500	5728
Wool wastewater treated with fungi centrifuged	60	244
Cotton wastewater	202	664
Cotton wastewater treated with fungi	500	5234
Cotton wastewater treated with fungi centrifuged	40	220

Table 35. Dyeing wastewater treatment with fungi-quantitative analysis

From a percentual perspective, the effectiveness of the biological treatment counts with BOD_5 and COD parameters reduction with 80,2%, respectively 66,87% for cotton dyeing wastewater effluents and 75,31%, respectively 73,31% for wool wastewater effluents Figure 52. Industrial fungi wastewater treatments count with the effectivity of 70-80% for COD and BOD_5 reduction (Azab 2008). In this sense, the wastewater effluents resulting from the cotton and wool dyeing process with C-phycocyanin present a suitable level of sustainability, even in a concentrated laboratory-scale environment.

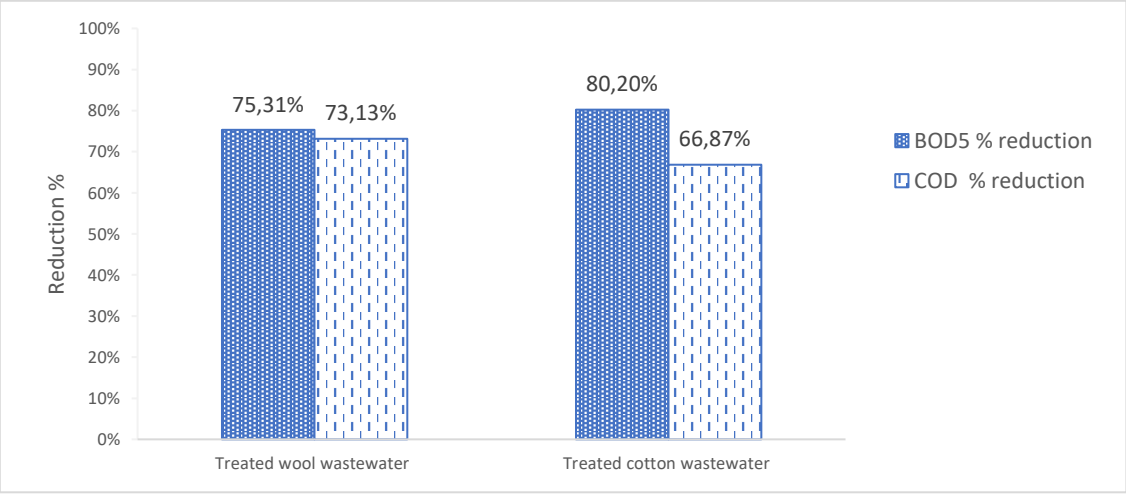


Figure 52. COD and BOD_5 % reduction with fungi wastewater treatment

4.4.3. Added-value of algae-based colorant matter

a) Antimicrobial properties analysis

The antimicrobial capacity of the algae-based colorants and non-mordanted dyed cotton and wool, against the microorganism *Escherichia coli* and *Staphylococcus aureus,* was analyzed and an increase in effectivity was observed on the finished fabrics, in comparison with the extract solely analyzed (Table 36).

Tested sample	***Escherichia coli***	***Staphhylococcus aureus***
C-phycocyanin-rich extract	46%	23%
C-phycocyanin dyed wool	99,6%	99,98%
C-phycocyanin dyed cotton	97,87%	80,23%
R-phycoerythrin-rich extract	38%	9%
R-phycoerythrin dyed cotton	99,60%	72,31%
R-phycoerythrin dyed wool	99,41%	80,83%
B-carotene-rich extract	0	0
B-carotene dyed cotton	0	0
B-carotene dyed wool	0	0
Chlorophyll-a-rich extract	56%	31,34%
Chlorophyll-a dyed cotton	99,48%	97,5%
Chlorophyll-a dyed wool	99,26%	99,9

Table 36. Antimicrobial capacity analysis results (colorant rich extract and non-mordanted dyed cotton and wool samples)

Positive improvements, in terms of reduction of bacterias, are observed with the non-mordanted dyed fabrics with C-phycocyanin, R-phycoerythrin, and Chlorophyll-a, thus validating a high-added value of these sustainable colorants. B-carotene did not show this antibacterial capacity, in any of the tested formats, extract, or dyed textile substrates.

With further optimizations, and colorant concentration increase, the antimicrobial capacity can be increased. It can be assumed that the bleached wool fabric may have an impact on this increase, but overall, this is a positive result, enlarging the potential of the use of this dyeing agent.

b) Solar protection properties analysis

The solar protection capacity of the dyed non-mordanted cotton and wool samples with the 4 selected algae-based colorant matter, C-phycocyanin, R-phycoerythrin, β-carotene, and, Chlorophyll-a were analyzed, and the results are presented in Table 37 below. In the context of solar protection capacity, it is considered effective when UPF values>15 (the minimum requirement indicated in the European characterization norm, detailed in section 3.2. Methods, part l), and it can be observed that most of the cotton fabrics treated with the 4 extracts increase their UPF value in comparison with the non treated fabric. The fabrics trated with algae extracts present a minimum of 15 UPF, except for β-carotene. Nevertheless, when analyzing the wool dyed fabrics, only R-phycoerythrin and Chlorophyll-a show this effective protection capacity.

Sample	*UPF factor Dyed textile substrate*	
Colorant -rich extract	Cotton	Wool
No extract	3	1
C-phycocyanin	15	10
R-phycoerythrin	15	15
B-carotene	10	10
Chlorophyll-a	35	15

Table 37. Solar protection capacity analysis results (non-mordanted dyed cotton and wool samples)

This is a first approach in exploring the added value of the use of the algae-based colorant matter with increased potential for the textile industry and others.

Partial conclusions for the preliminary exploration of wastewater effluents quality, wastewater biological treatment, and added value of algae-based colorant matter

- **Confirmed antimicrobial effect** against the microorganism *Escherichia coli and Staphylococcus aureus* of the non-mordanted cotton and wool dyed with C-phycocyanin, R-phycoerythrin, and Chlorophyll-a.
- **Confirmed reduced solar protection capacity**, with UPF values approximating 10-15, for dyed non-mordanted cotton and wool samples with C-phycocyanin, R-phycoerythrin, β-carotene, and Chlorophyll-a. With the interesting exception of cotton dyed with Chlorophyll-a-rich extract with UPF 35.

Cotton and wool dyeing process wastewater effluents analysis reflects a clear reduction of BOD_5 and COD with 3 respectively 4 times magnitude with the application of mordanting processes, thus validating the mordant application and its enhancing effect.

- In terms of comparison of the laboratory-scale oxygen demand values, they are slightly surpassing the average daily maximum limitation of discharge into wastewater treatment plants, according to Spanish regulations. In this sense, it is important to note that laboratory values represent an exaggeration of values, due to the more concentrated effluents generation, as in the industrial context these are mixed with other effluents resulting from rinsing, cooling, and other processes generating a considerable parameter concentration reduction through dilution.

  For comparison purposes, the Spanish average daily maximum discharge limits into wastewater plants are COD = 1000 mg/L and for BOD_5=500 mg/L. The average results obtained in the selected optimum dyeing conditions for:
  - Cotton dyeing parameters: COD = 848 mg/L and for BOD_5=203 mg/L
  - Wool dyeing parameters: COD = 2012 mg/L and for BOD_5=961 mg/L

Wastewater biological fungi treatment was applied on both cotton and wool resulting effluents from dyeing, and it has resulted in a remarkable reduction of BOD_5 and COD parameters values, reaching a decrease of the values up to:

- Cotton dyeing: reduction of 80,2% for BOD_5, respectively 66,87% for COD
- Wool dyeing: reduction of 75,31% for BOD_5, respectively 73,31% for COD

Metal content values are strictly influenced by the use of mordants, specifically the metallic ones, and in this sense, the concentration of mordant optimization applied through the pre-mordanting process is performed, for complete absorption of the auxiliary by the textile substrate to counter the lack of sustainability induced by their use.

- As expected, mordants as Alum, Ferrous sulphate, and Aluminum triformate exceed the daily limit of metals (Aluminum and Iron) discharge in wastewater plants at a laboratory scale. This represents a decision-making factor in the selection of the % of mordant used in the pre-mordanting process in correlation with performance:
    - Aluminum 10% w.o.f
    - Ferrous sulphate 3% w.o.f.
    - Aluminum triformate 10% w.o.f.

4.5. Assumptions on the algae-based colorant matter-mordant-natural fiber interaction based on the analysis of the dyeability

In the context of the lack of affinity between natural colorants and natural fibers, enhancers for intimate connection among natural colorants and natural fibers, as mordants should be applied. The analysis below presents plausible assumptions regarding interactions between molecules pertaining to the colorant matter, mordants, and cellulosic and proteinic fibers, based on literature review and the previously performed color analysis.

The selection of mordants used in this study are metallic or tannin based, and the considered context of bonding with the cellulosic and proteinic fibers should be highlighted, through the possible bonds formation (Bhute and S 2015):

3) Hydrogen: among the mordants´ phenolic hydroxyl groups and the fibers´ free amino and amido groups.
4) Ionic: connecting the mordants´ anionic groups with the fibers´ cationic groups.
5) Covalent: interconnection between the mordants´ quinine or semiquinone and the fibers´ suitable and available reactive group.

a) Cellulosic fiber approach, the cotton textile substrate

Tannin mordants interaction with cellulosic fibers and colorants requires a metal ion as an intermediary for the bond creation, as the latest is capable of attaching to the oxygens. This study focuses on the singular application of the mordants, and not in combination, thus it confirms the inefficiency of this approach.

Metallic mordants bond with cellulosic fibers through the carboxyl connection, and it is not achieved in this study, indicating the need for mixed application in the process (Ding and Freeman 2017), for enhanced dyeability obtention.

Based on the complex required by the tannin mordants, with the metallic ion intermediary, the adaptation proposed by (Bhute 2015), presented in Figure 53, was used for the interpretation of the analysis results.

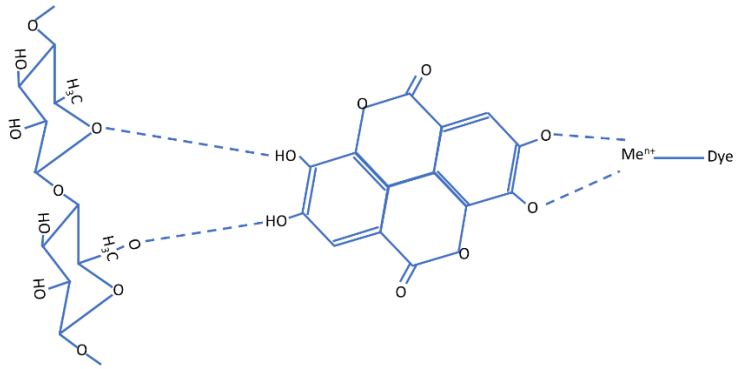


Figure 53. Cellulosic fiber theoretical bonding with tannic mordants, metallic ion, and natural colorants (Bhute 2015)

Apart, (Ding and Freeman 2017) detail the coordination bonding through dyeing of metallic mordanted cotton fibers´ molecules with natural dyes, as occurring through chelation, defined as the connection of the metal mordant with every other double-bonded oxygen.

In this sense, an adaptation to the present study, of the visual representation of the between natural fiber-mordant-natural dye, interaction by (Yusuf et al. 2017), is presented in Figure 54.

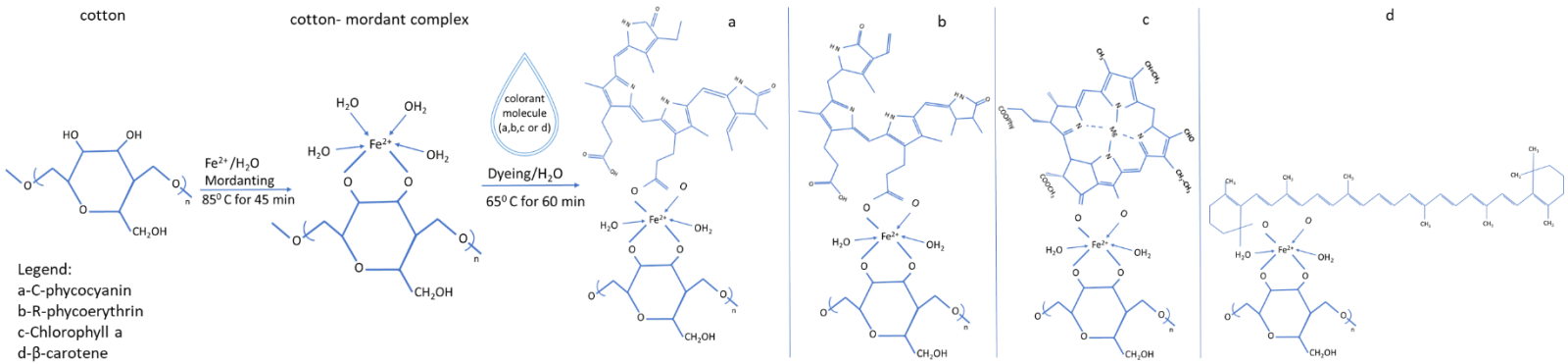


Figure 54. Possible cotton-mordant- colorant molecule interaction (Yusuf et al. 2017)

It can be concluded that the natural colorants used in this study, C-phycocyanin, R-phycoerythrin, β-carotene, and Chlorophyll-a present low affinity to cotton textile substrates, and the use of one mordant does not enhance this fiber´s dyeability. Therefore, increased mordant combinations, and dyeing techniques must be explored.

b) Proteinic fiber approach, the wool textile substrate

The previously performed color analyses indicate that wool presents an increased efficiency of the dyeing process, being more reactive to bonding with mordants, mostly due to its acid and bases absorption capability, equally.

Metallic salts applied to wool substrates as pre-treatment impregnate the capacity to hydrolyze them into a basic and an acidic element, the first one being absorbed at the carboxyl groups, and the latest easily eliminated through washing.

Due to the use of tannin and metal-based mordants used in this study, it is important to highlight the possibility of complex formation, as indicated by (Bhute and S 2015), and this theoretical approach is adapted below in Figure 55.

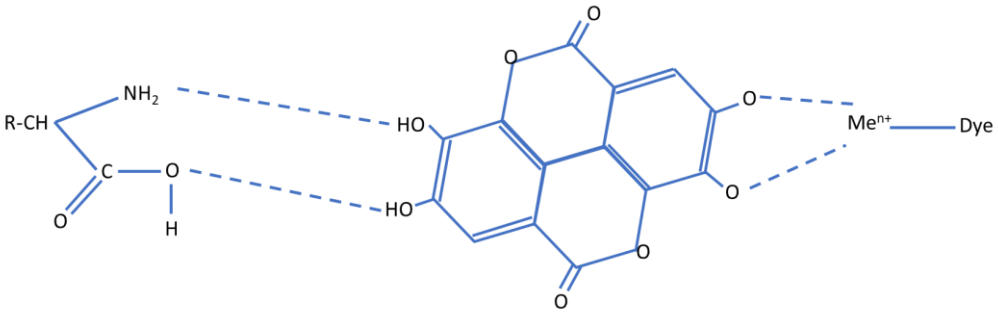


Figure 55. Proteinic fiber theoretical bonding with tannic mordants, metallic ion, and natural colorants (Bhute 2015)

(Yusuf, Shabbir, and Mohammad 2017) proposed also the wool-mordant-dye complex interaction, which seems like a plausible occurrence in this study, and in this sense Figure 56 approaches the plausible bonding with the natural algae-based colorant matter employed in this study.

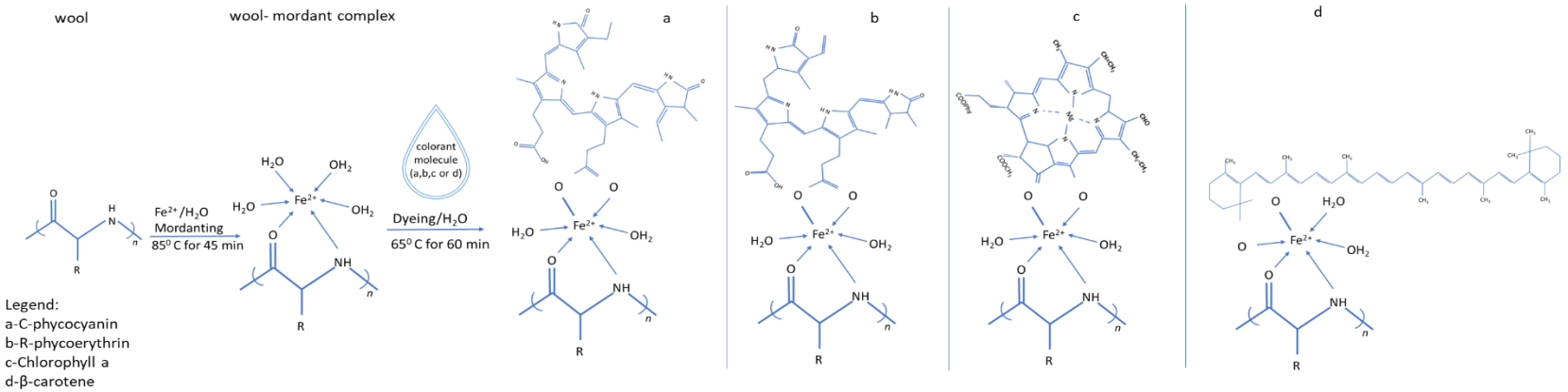


Figure 56. Possible wool-mordant- colorant molecule interaction (Yusuf, Shabbir, and Mohammad 2017)

The wool dyeing process parameters explored in this study, envisaging acidic conditions, at pH 5, impulse the creation of electrostatic synergies between the proteinic fiber and colorant matter (Ding and Freeman 2017). Mordant incorporation in the dyeing process favors the increase in dyeability and confirms the increased efficiency with these auxiliaries' addition in the process.

Overall, further experimental explorations must be performed for increasing the process dyeability, by improving the natural colorants temperature sensitivity, increase the diffusion process into the natural fibers and enhance fastness. Nevertheless, even though this study explored the conventional exhaustion dyeing process, improvements were observed, thus leading to the increased plausibility of high possibilities of process efficiency increase.

4.6. Laboratory scale exploration of pigment printing process with algae-based colorant matter on cotton and wool textiles substrates

The following section approaches and analyses the results obtained through the exploration of the pigment printing process with C-phycocyanin (blue), R-phycoerythrin (red), β-carotene (yellow-orange), and Chlorophyll-a (green) sourced from micro and macroalgae, of natural cellulosic and proteinic textile substrates, like cotton and wool.

4.6.1. Analysis of the printing capacity of natural fabrics with blue algae-based colorant matter C-phycocyanin-rich extract from microalgae *Spirulina platensis*

The experimental approach using the C-phycocyanin-rich extract for pigment printing textile substrates uses two types of printing paste, for the exploration of the printing capacity of the blue C-phycocyanin-rich extract as colorant matter. The two mother printing pastes used: printing paste based on synthetic conventional ingredients and printing paste based on natural commercial alternatives, are presented in Figure 57.

Figure 57. Printing paste with embedded C-phycocyanin, based on conventional synthetic components(left) and natural commercial alternative printing paste (right)

a) Analysis of results obtained from cotton textile substrate printing with C-phycocyanin

1) Color assessment: Chromatic characterization

Figure 58 below presents a complete set of experimental approaches employing both natural and synthetic printing pastes, completed with the analysis of the influence of the pre-treatment of fabrics with 7 different mordants. Visually, very similar results can be observed.

Figure 58. Printed cotton fabrics with C-phycocyanin obtained from Spirulina platensis (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

The influence of the type of printing paste was assessed without the interference of mordants. The CIELab coordinates (*L*, a*,* and *b**) and color differences are reflected below (Table 38). The color difference calculus considers the synthetic printing paste, as the reference sample, due to the conventional experimental case, meanwhile, the natural printing paste represents the novelty, apart from the source of this blue colorant matter. In this sense, $\Delta L<0$ indicates that the natural printing paste reveals more intense colors, with a more blueish tone. This may be generated by the lower processing temperature imposed by the natural printing paste, which does not affect the integrity of the blue chromophore.

Cotton sample	***CIELab Color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Synthetic printing paste	89,56	-1,12	2,42	-5,68	-9,53	-10,31	15,15
Natural printing paste	83,88	-10,65	-7,89				

Table 38. CIELab color differences for C-phycocyanin printed cotton with synthetic printing paste vs. natural printing paste

Figure 59 plots the CIE*Lab* color space distribution of the printed cotton with natural vs synthetic printing paste, and indicates clear blueish location, and interestingly both pastes present greenish influences. This can be justified by the residual Chlorophyll-a that may have remained in the colorant-rich extract, due to the simple, and low aggressivity properties that characterized the extraction procedure, and the low purification level.

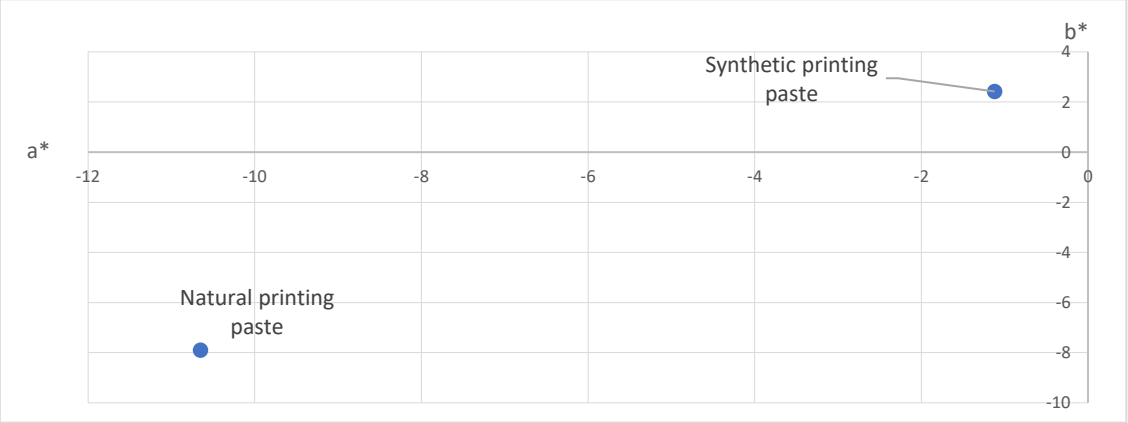


Figure 59. Color specifications in CIELab of the printed cotton fabrics with C-phycocyanin

Relevant scientific literature is very limited, but research studies focusing on sourcing natural blue colorants for the printing of cotton are mainly approaching indigo, with results comparable with the ones obtained in this study, with *L** values of 88,93 (Bahtiyari et al. 2013).

Reflectance spectrum and color strength analysis

For the analysis of the influence of the mordanting process on pigment printing with synthetic and natural printing paste, the reflectance spectrum was analyzed.

Pre-mordanted cotton fabrics printed with C-phycocyanin embedded in the synthetic paste

Figure 60 presents the reflectance spectrum of the printed samples, and accordingly, it can be confirmed that the pre-mordanting process does not positively influence the color strength of the obtained fabrics. This assumption is made in the context of the whiter the sample, the higher the reflectance value, and the majority of the samples present a higher reflectance than the non-mordanted sample, except Aluminum sulphate, Aluminum acetate, and followed by Titanyl potassium oxalate indicating a higher absorption capacity.

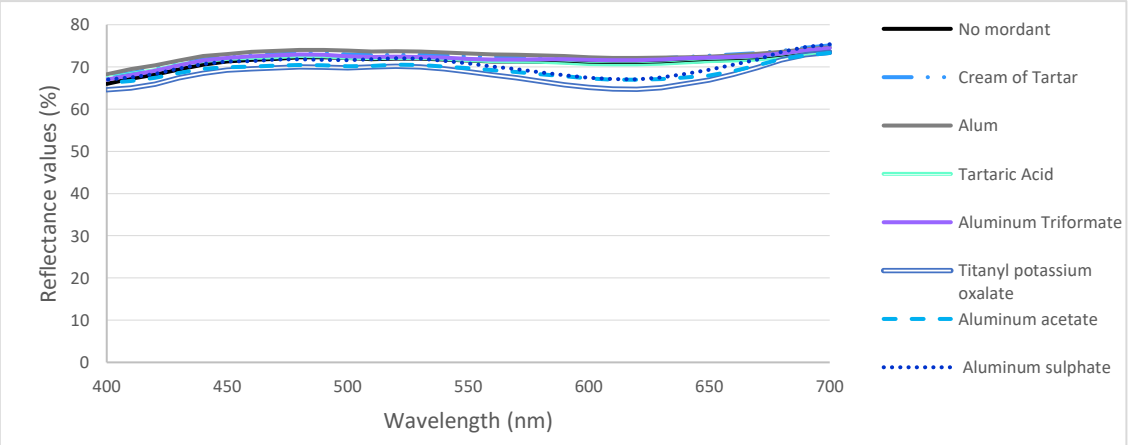


Figure 60. VIS spectrum of C-phycocyanin printed cotton with synthetic paste and the influence of the mordanting process

Pre-mordanted cotton fabrics printed with C-phycocyanin embedded in the natural paste

Natural printing of cotton with C-phycocyanin over pre-treated cotton substrates presents an influence on the color strength, as reflected in Figure 61 below, where the non-mordanted sample appears to be the most reflective sample, thus the whitest. The increasing order of the reflective strength is generated by Titanyl potassium oxalate (being the whitest, the less intense blue color), followed by Aluminum triformate, Aluminum acetate, Cream of tartar, Tartaric acid, Aluminum sulphate, and the most intense color is obtained with the employment of Alum.

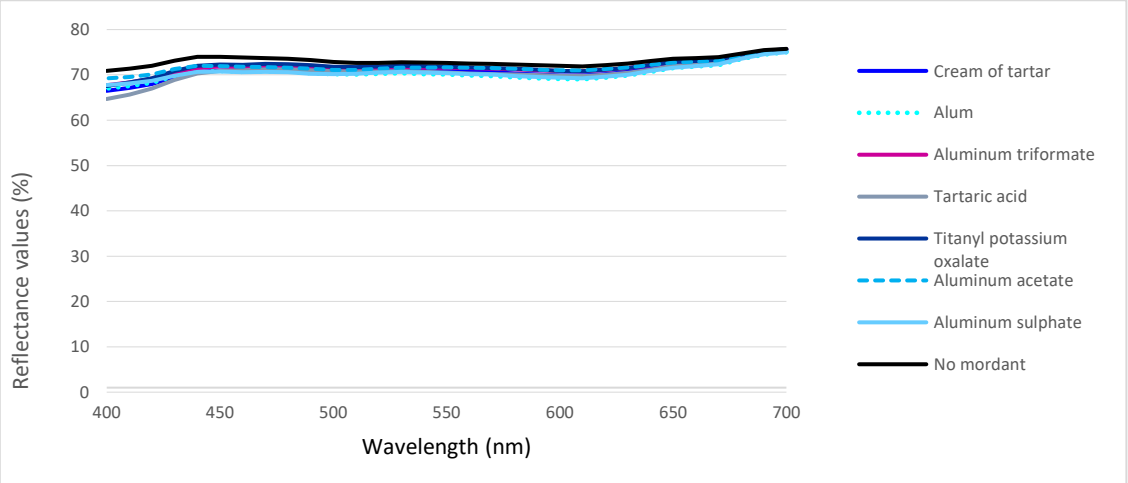


Figure 61. VIS spectrum of C-phycocyanin printed cotton with natural paste and the influence of the mordanting process

Considering the comparison of printing efficiencies generated by the use of mordants and natural and synthetic printing paste, the *K/S*, color strength values, are presented in Figure 62 below. When comparing the non-mordanted samples, the synthetic printing paste indicates the slightly higher color strength value (36,88 vs. 37,27), meanwhile, for the rest of the cases, the predominant higher values are obtained with the use of the natural printing paste, except for Aluminum acetate.

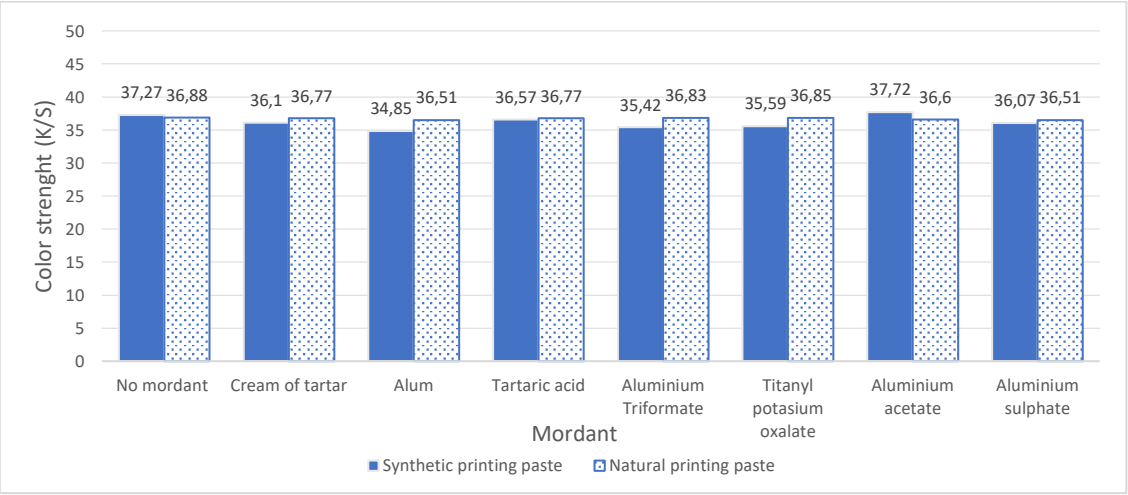


Figure 62. Cotton printing with C-phycocyanin pigment (natural vs synthetic printing paste) color strength (K/S) comparison

2) C-phycocyanin cotton printing process quality assessment through measurement of laundering and lightfastness

For a complete analysis of the efficiency of the use of mordants in the printing process, the laundering and lightfastness were assessed, and the complete set of results is presented in Table 39 below. It can be observed that there are no improvements in the color resistance against laundering and light as degrading agents. This indicates that the pre-mordanting process is not a successful treatment in the printing process, and further exploration of binders and auxiliary protection products must be explored for process optimization.

Cotton Samples Synthetic paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	3
Cream of tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Cotton samples Natural paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	3
Cream of tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2

Table 39. Fastness properties of mordanted cotton samples printed with C-phycocyanin embedded in synthetic and natural printing pastes

Studies focusing on cotton printing with indigo, for the use of a natural blue colorant source, indicate comparable laundering fastness values, but with increased resistance of the color against the light degradation (up to 6), which reflects the need for process optimization (Bahtiyari et al. 2013).

Partial conclusions on cotton printing with C-phycocyanin

- *Type of paste influence on the printing process efficiency* indicates that the **natural printing paste** generates significant color differences, with more intense blueish shades in comparison with the synthetic paste confirmed by $\Delta L<0$ (L^* *synthetic* 89,56< L^* *natural* 83,88) and Δb =-10,31.
- *Influence of pre-mordanting on the printability of C-phycocyanin* was analyzed via the measurement of the reflectance spectrum of the 7 different mordants employed, and overall indicates no significant improvements:

- *Synthetic printing paste* indicates insignificant color differences, with very slight improvements conferred by the use of Aluminum-based mordants as Aluminum sulphate (20% w.o.f), Aluminum acetate (20% w.o.f), and followed by Titanyl potassium oxalate (20% w.o.f), indicating a higher absorption capacity, with a reflectance of 64-68.
- *Natural printing paste* presents the same low coloration improvements as the synthetic printing paste, with the more blueish colors obtained with the use of Alum (10% w.o.f), and Aluminum sulphate (20% w.o.f) with reflectance percentages<70%.
- *Color strength (K/S) values comparison* indicates insignificant differences regarding the effectiveness of mordants, regardless of the type of paste used, from 34,8 up to values of 37.

- *Fastness analyses* indicate that laundering testing showed fair behavior in terms of staining (4-5) and low degrading resistance (1-2) and very poor resistance against light (1-2), without improvements generated by the use of mordants.

b) Analysis of results obtained from wool textile substrate printing with C-phycocyanin

1) Color assessment: Chromatic characterization

Wool fabrics were printed with blue colorant matter represented by C-phycocyanin, with the use of synthetic conventional printing paste, and a natural alternative, for exploration of coloration possibility. Additionally, experiments using mordants for fabrics pre-treatment were performed for the analysis of their influence on the quality of the process. The photograph of the obtained colored wool samples is presented in Figure 63 below.

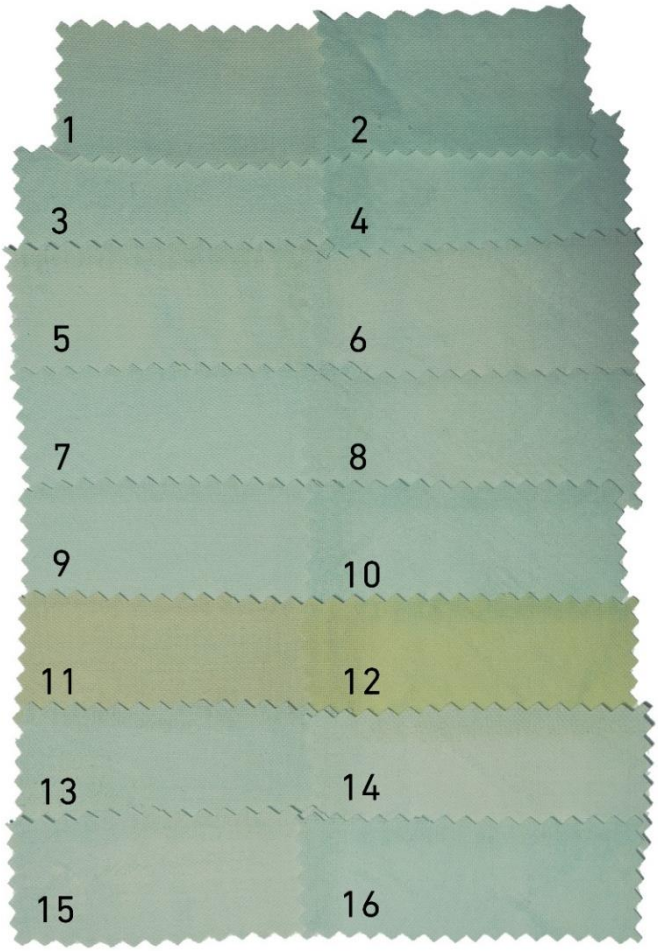


Figure 63. Printed wool fabrics with C-phycocyanin obtained from Spirulina platensis (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

The impact of using different types of printing pastes was analyzed, and the CIE*Lab* coordinates (*L*, a*, b**) were measured for the non-mordanted printed wool samples with the synthetic and natural printing pastes, and the color differences were calculated. Table 40 presents the obtained results. The color difference calculus was performed considering the synthetic printing paste values as a reference, for the analysis of the effects of the natural experimental printing paste on the coloration process. In this sense, ΔE >3 indicates a significant color difference, confirming an influence in the use of the natural printing paste. ΔL<0 is a value

indicating that the more intense color is obtained with the use of the natural printing paste, and this is due to the shorter time and lower curing temperature needed, which does not degrade the temperature-sensitive colorant matter. This is also confirmed by the *Δb*=-11,19, which indicates a more blueish coloration than with the use of the synthetic printing paste.

Sample	***CIELab color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Synthetic printing paste	82,01	-2,98	10,55	-6,84	-11,66	-11,19	17,55
Natural printing paste	75,17	-14,64	-0,64				

Table 40. CIELab color differences for C-phycocyanin printed wool with synthetic printing paste vs. natural printing paste

Figure 64 plots the color space corresponding to the printed wool with C-phycocyanin embedded in synthetic and natural printing paste, and the pattern that occurs in the cotton experimental case repeats. In this sense, both, the synthetic paste and the natural paste reflect greenish influences, probably due to the remaining Chlorophyll-a in the colorant extract, which is temperature resistant (as indicated before, the synthetic paste involves the use of increased temperatures and longer time for curing).

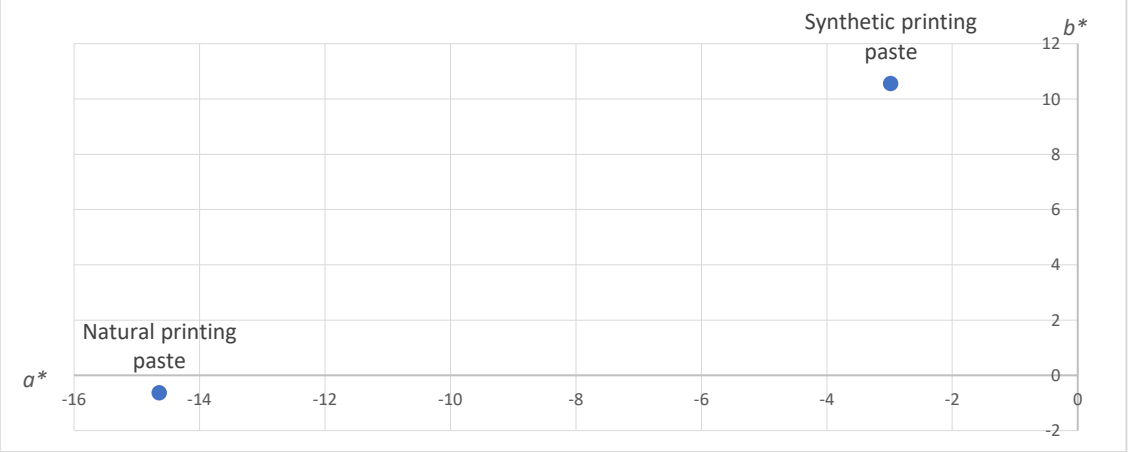


Figure 64. Color specifications in CIELab of the printed wool fabrics with C-phycocyanin

Comparable studies using indigo for the obtention of blue color on wool via printing, indicate similar results for the color intensity obtained (*L**=78,53), but less blueish than the results of this study (Bahtiyari et al. 2013).

Reflectance spectrum and color strength analysis

Wool samples printed with C-phycocyanin colorant matter and synthetic and natural paste, but also pre-treated with various mordants, were subjected to reflectance spectrum measurements, for mordant influence analysis on the coloration process.

Pre-mordanted wool fabrics printed with C-phycocyanin embedded in the synthetic paste

The influence of the pre-mordanting treatment on the coloration of wool textile substrates via pigment printing with synthetic mother paste was analyzed through the measurement of the VIS spectrum. The analysis of Figure 65, is done in the context of the whitest sample (less color intensity), reflects the most light and the darkest (most intense) absorbs the most light. In this sense, it can be observed that the darkest sample is obtained with the use of

Aluminum sulphate, demonstrating a chromophore protection action, as the rest of the mordants show less efficiency than the non-mordanted samples. The results are basically grouped in the same area of the reflectance spectrum, with similar color strength values, *K/S* ranging from 25 to 27 approximately.

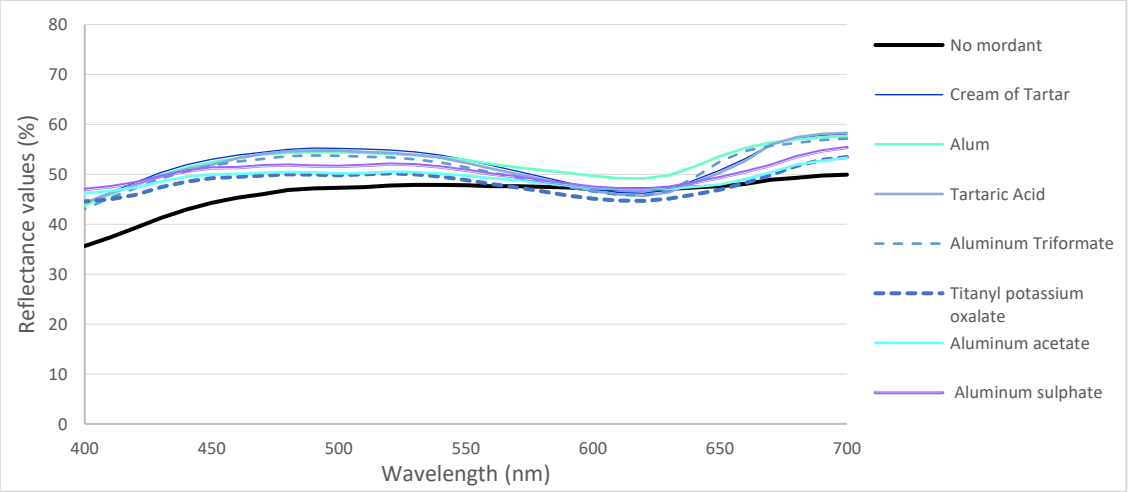


Figure 65. VIS spectrum of C-phycocyanin printed wool with synthetic paste and the influence of the mordanting process

Pre-mordanted wool fabrics printed with C-phycocyanin embedded in the natural paste

The VIS spectrum assessing the reflectance of the pre-mordanted wool fabrics printed with C-phycocyanin and natural mother paste is presented in Figure 66 below, and indicates that the most efficient mordant in this experimental study case is Aluminum acetate, results comparable with the synthetic paste analysis. In this sense, compared with the visual analysis (Figure 63), it can be confirmed the mordant influence on the obtained color, with a tendency to brownish-blue tones. In terms of comparable blue tones, it can be confirmed that the mordanting process does not influence a higher color strength, as the results present insignificant differences in *K/S* values, in the range 25-27.

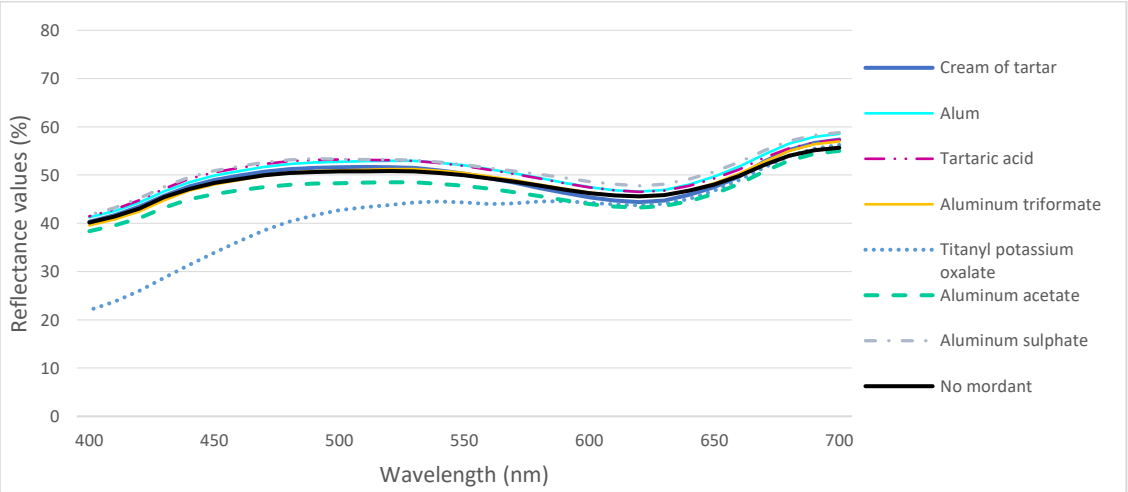


Figure 66. VIS spectrum of C-phycocyanin printed wool with natural paste and the influence of the mordanting process

Based on the reflectance spectrum, a comparison among the color strength values was performed, to explore the possible influence of the pre-mordanting process and the nature of

the printing mother paste (natural vs. synthetic). The color strength comparison is presented in Figure 67 below. It can be confirmed a similarity in the obtained results, with focus on the non-mordanted samples, where the natural printing paste, due to mild process conditions preserves the blue colorant, generating thus a *K/S* value of 26,84 in comparison with the synthetic printing paste, with *K/S*=23,98. When comparing mordants, very slight differences, mostly insignificant are observed, confirming the inefficiency of the pre-mordanting process in this coloration process.

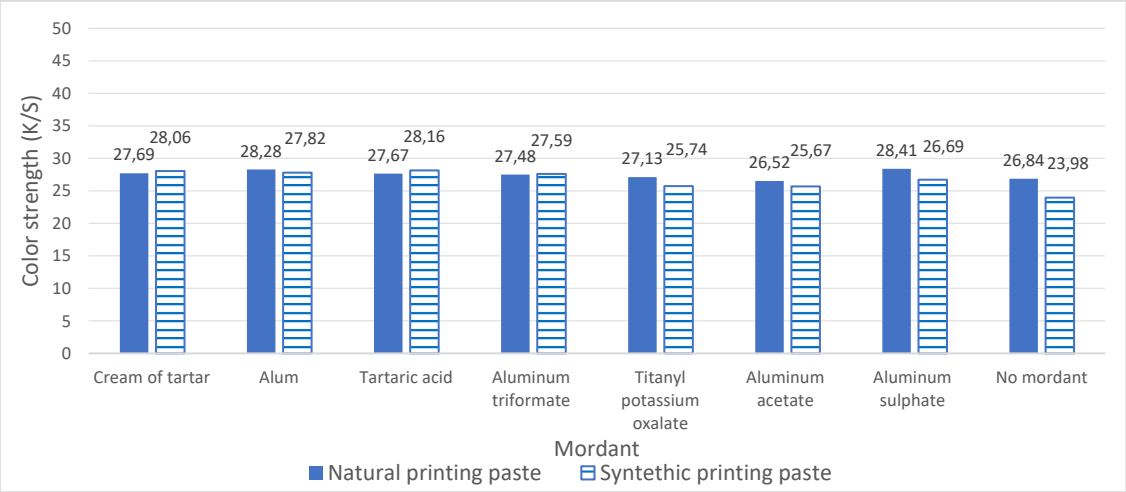


Figure 67. Wool printing with C-phycocyanin pigment (natural vs synthetic printing paste) color strength (K/S) comparison

2) C-phycocyanin wool printing process quality assessment through measurement of laundering and lightfastness

The efficiency of the pre-mordanting process was also assessed in analyzing the behavior against degrading agents as laundering or light. Table 41 presents the complete set of results obtained and indicates an overall poor behavior in color change, but good behavior in discharging over different textile substrates. This indicates the blue colorant's low affinity to textile fibers, and the necessity of specific pre-, meta- or post-treatments, and different binders, for increasing the quality of the coloration process. Lightfastness results clearly indicate, in all cases, that there are no improvements with the use of mordants when compared to the non-mordanted samples, thus additional protective methods against light, laundering, and temperature must be explored.

Wool Samples ***Synthetic paste***	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	3
Cream of tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	1

Wool samples ***Natural paste***	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	3
Cream of tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 41. Fastness properties of mordanted wool samples printed with C-phycocyanin embedded in synthetic and natural pastes

The fastness results are comparable with other studies focusing (Bahtiyari et al. 2013) on printing natural blue colorants on wool, as indigo, but differ in the lightfastness and provide evidence of the possibility of improvements for natural colorants study cases.

Partial conclusions on wool printing with C-phycocyanin

- *Type of paste influence on the printing process efficiency* indicates the obtention of significant color difference generated with the use of **natural paste**, with more intense *ΔL*=-6,84, blueish tones Δb=-11,19, as confirmed also by the color strength values with approximately 3 units difference (insignificant).
- *Influence of pre-mordanting on the printability of C-phycocyanin* over wool with the analysis of 7 different mordants presents overall insignificant efficiency, with most of the corresponding reflectance spectrums located in the same area.
    - *Synthetic printing paste* analysis indicates similar spectrums, even though without significant differences with the non-mordanted samples, is Aluminum sulphate (20% w.o.f.), in the range of 40-50% of reflectance capacity.
    - *Natural printing paste* reflectance spectrum analysis indicates slightly overlapping spectrums, with similar results as the non-mordanted samples, but the most efficient in this context appears to be the Aluminum acetate (20% w.o.f.) with 43% reflectance capacity, slightly darker than the rest of the samples, with the same range of 43-47% of reflectance.
    - *Color strength (K/S) values comparison* indicates that pre-mordanting does not generate significant differences in terms of dye uptake or process efficiency, and comparable results are obtained with the same mordant and different printing paste types. On the other hand, the obtained values, overall, are in the same range (25-27 approximately), indicating that there is no need for the pre-mordanting step.
- *Fastness analysis* indicates that laundering testing showed fair behavior in terms of staining (4-5) and degrading (1-2) and very poor resistance against light (1), similar to the cotton experimental cases, without improvements with the use of mordants as printability enhancing auxiliaries.

4.6.2. Analysis of the printing capacity of natural fabrics with red algae-based colorant matter R-phycoerythrin-rich extract from macroalgae *Gracilaria gracilis*

Pigment printing with red, R-phycoerythrin-rich extract, of cotton and wool textile substrates was performed with synthetic conventional and commercially available printing paste and natural alternative for the exploration of the compatibility of this colorant matter with the selected coloration method. The mother printing pastes used are presented in Figure 68 below.

Figure 68. Printing paste with embedded R-phycoerythrin, based on conventional synthetic components(left) and natural commercial alternatives printing paste (right)

a) Analysis of results obtained from cotton textile substrate printing with R-phycoerythrin

1) Color assessment: Chromatic characterization

The red printed cotton fabrics, with synthetic and natural printing paste, are presented in Figure 69, where light shades of red can be observed, determined by the type of paste, and its composition, as the binder and fixer, and also by the mordant used in the pre-treatment of the samples.

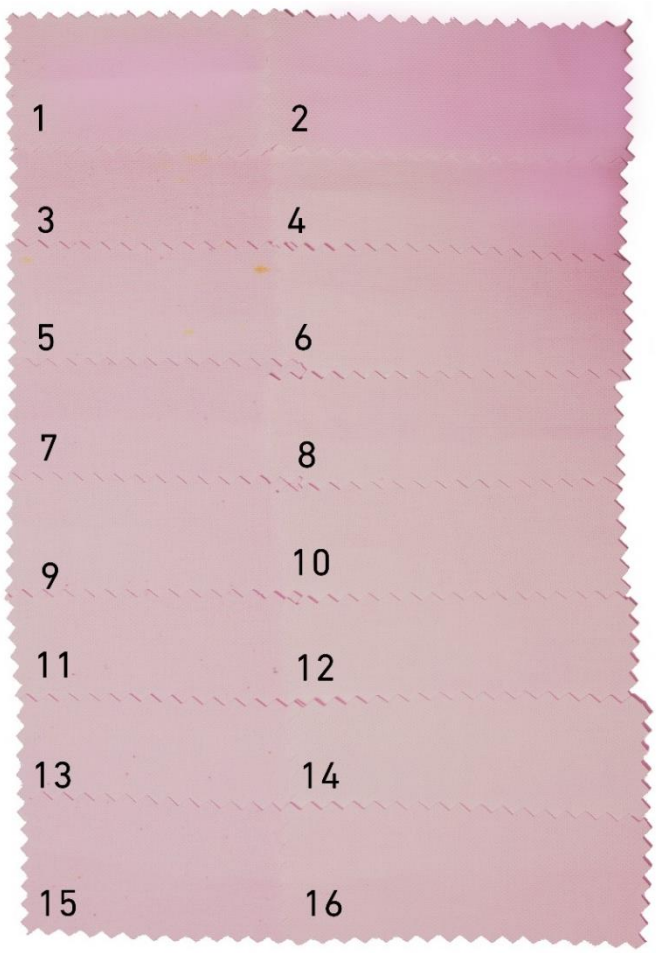


Figure 69. Printed cotton fabrics with R-phycoerythrin obtained from Gracilaria gracilis (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

The CIELab coordinates (*L*, a*, b**) were measured for the analysis of the possible effects generated by using different types of printing pastes. The color difference results are indicated in Table 42, and the color difference calculus was performed based on the reference sample, considered the printed cotton fabric with synthetic paste. In this sense, $\Delta E<3$ indicates that a color difference exists between the samples, but it is characterized by low significance. Nevertheless, it can be indicated that the more intense red color is provided by the use of natural paste, as reflected by $\Delta L<0$.

Sample	***CIELab color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Synthetic printing paste	92,99	2,61	3,91	-0,49	0,12	-1,2	1,30
Natural printing paste	92,5	2,73	2,71				

Table 42. CIELab color differences for R-phycoerythrin printed cotton with synthetic printing paste vs. natural printing paste

The color diagram plot is reflected in Figure 70, and locates the samples in the red color space with yellowish influences, fitting in the desired color area, confirming the possibility of printing with red colorant matter with the use of synthetic and natural printing paste.

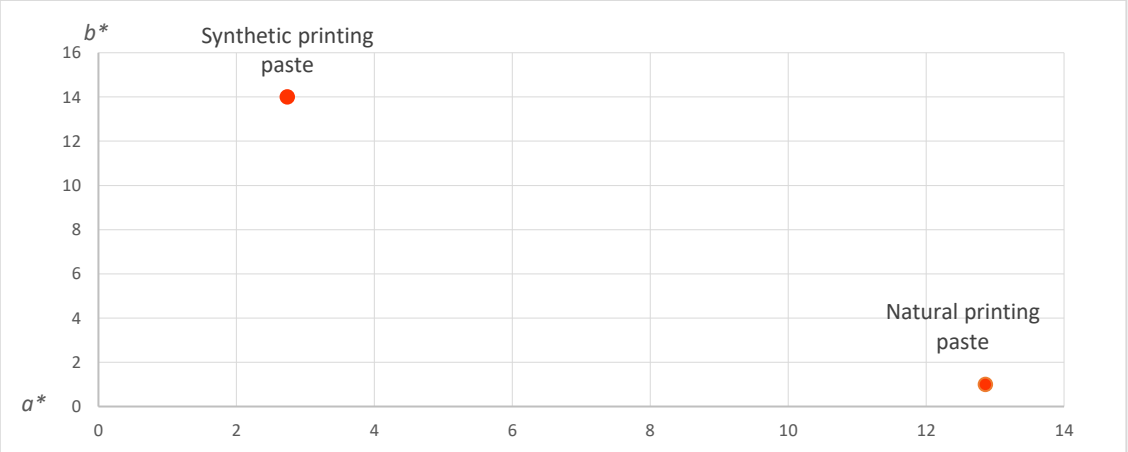


Figure 70. Color specifications in CIELab of the printed cotton fabrics with R-phycoerythrin

The color characterization of the red printed cotton fabrics is comparable with red colors obtained with alkanet, in similar studies (Rekaby et al. 2009).

Reflectance spectrum and color strength analysis

A series of mordants were used for the treatment of the cotton fabrics to analyze their influence on the coloration strength with the algae-based red colorant matter.

Pre-mordanted cotton fabrics printed with R-phycoerythrin embedded in the synthetic paste

The reflectance spectrum of non-mordanted and pre-mordanted cotton fabrics printed with synthetic paste and R-phycoerythrin is presented in Figure 71. The reflectance analysis indicates the color strength of the printed fabrics, considering that the whiter the sample, the less intense colored, reflects more light, thus with higher reflectance values, meanwhile, the darkest the samples present the lowest reflectance. It can be observed that most of the mordants generate similar coloration tones with the non-mordanted sample, indicating a lack of efficiency with their use.

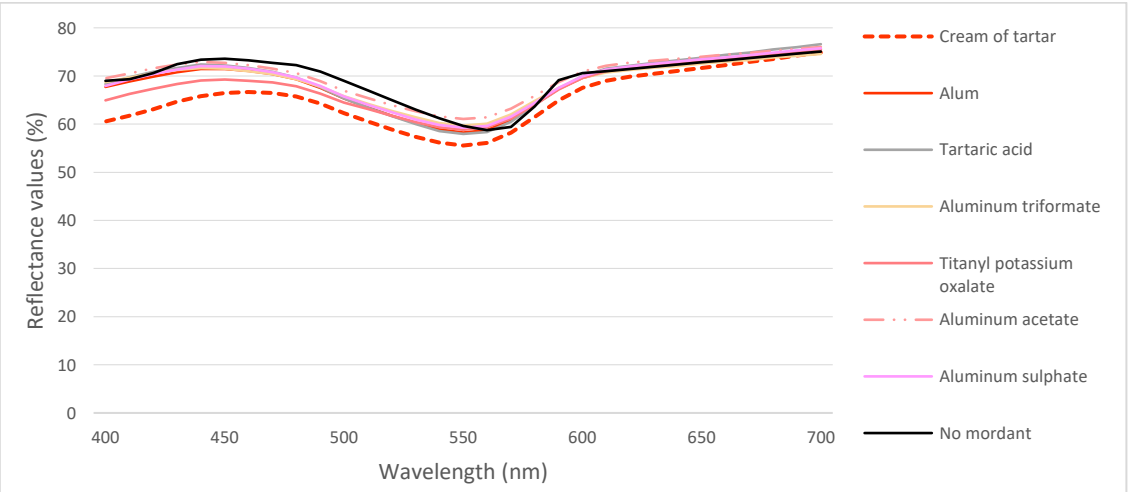


Figure 71. VIS spectrum of R-phycoerythrin printed cotton with synthetic paste and the influence of the mordanting process

Pre-mordanted cotton fabrics printed with R-phycoerythrin embedded in the natural paste

The reflectance spectrum of non-mordanted and pre-mordanted cotton samples printed with red natural colorant embedded in natural printing paste is presented in Figure 72. The reflectance values show that all the samples are located in the whiter side of the spectrum, very close to each other, indicating that there are no significant differences between the non-mordanted printed samples and the pre-mordanted ones. This indicates that the auxiliaries have reduced efficiency, and some provide reduced increase in color strength, as Alum, Tartaric acid, Aluminum sulphate, and Titanyl potassium oxalate. Interestingly, the use of Aluminum acetate generated whiter samples than the majority.

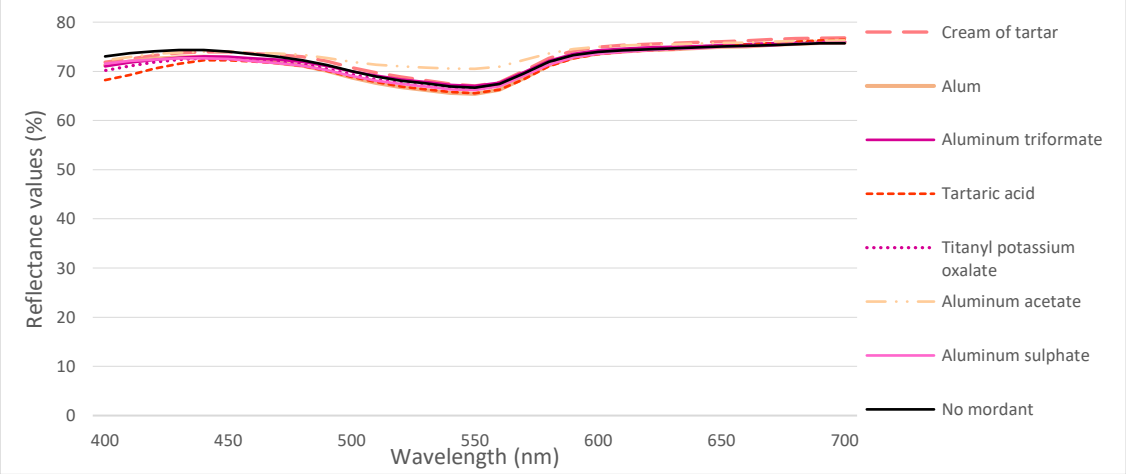


Figure 72. VIS spectrum of R-phycoerythrin printed cotton with natural paste and the influence of the mordanting process

The coloration strength of the natural and synthetic printing pastes and the comparison of the influence of the pre-mordanting process is presented in Figure 73. Very slight differences in color strength are observed, and most of them indicate that the natural printing paste is more efficient, but with less than 1% difference (average difference of 0,31). The exceptional case is represented by the use of Tartaric acid where the synthetic paste generates a slight color strength increase with 0,1 units, this being an insignificant difference. Overall, pre-mordanting does not have any influence on the pigment printing with the R-phycoerythrin of cotton fabrics.

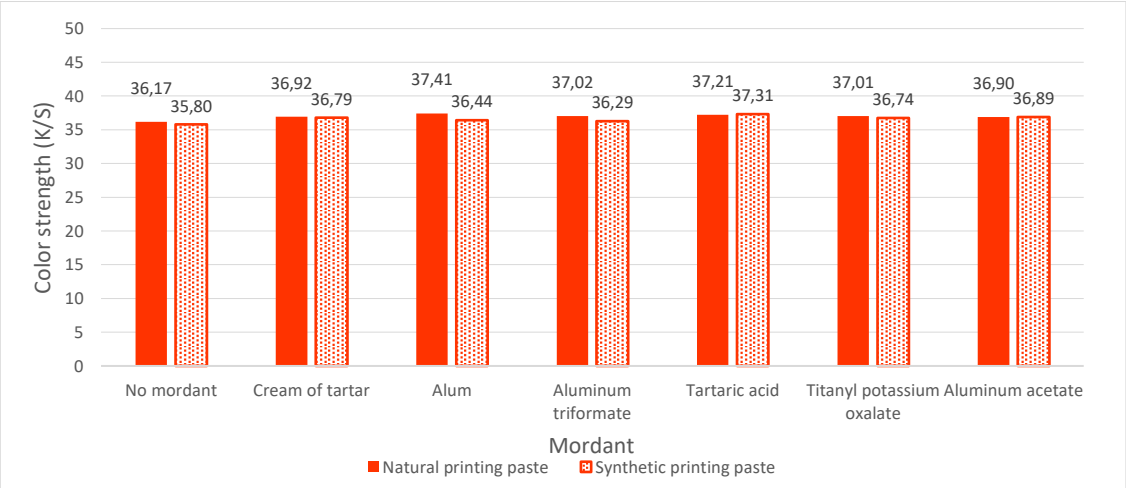


Figure 73. Cotton printing with R-phycoerythrin pigment (natural vs synthetic printing paste) color strength (K/S) comparison

2) R-phycoerythrin cotton printing process quality assessment through measurement of laundering and lightfastness

The quality of the coloration process performed through red printing with R-phycoerythrin using synthetic and natural printing paste on cotton substrates was assessed by analyzing the color resistance against laundering and light degrading agents, and the results are presented in Table 43. Not or negligible improvements are obtained with the use of the mordants, thus they are not recommended to be used for increasing the process efficiency, so other auxiliaries should be explored. Nevertheless, the most suitable behaviors are obtained in the non-mordanted cases.

Cotton Samples Synthetic paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Cream of tartar	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	3-4	1-2	4-5	4-5	4-5	4-5	4-5	2
Aluminum acetate	1	1-2	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1	1-2	4-5	4-5	4-5	4-5	4-5	1-2
Cotton samples Natural paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	2	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Cream of tartar	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 43. Fastness properties of mordanted cotton samples printed with R-phycoerythrin embedded in synthetic and natural pastes

Fastness results obtained in this study are below the ones obtained in similar studies (Rekaby et al. 2009), indicating the possibility of improvements with sustainable investigations.

Partial conclusions on cotton printing with R-phycoerythrin

- *Type of paste influence on the printing process efficiency,* revealed **very similar** (almost identical values) **overall in the color space**, with statistically insignificant color differences in the comparison of synthetic paste vs. natural paste (ΔE=1,30). Also, a slight increase in color intensity was generated by the employment of the **natural printing paste** (ΔL= -0,49).
- *Influence of pre-mordanting on the printability of R-phycoerythrin* was analyzed approaching both types of printing pastes, by assessing the color uptake capacity determined by the use of 7 different mordants.

- *Synthetic printing paste* reflectance spectrums overlap in a very small range of the absorbance values, thus a very small difference in uptake capacity was influenced by the pre-mordanting process, ranging among 58-59. Nevertheless, the most intense colored sample spectrums, with the highest light absorbance level obtained without the use of mordants.
- *Natural printing paste* presents slight influences in the spectrum's dispersion on the graph, with insignificant but higher efficiencies generated by the use of Alum (10% w.o.f.), Tartaric acid (6% w.o.f.) (both around 65%) Aluminum sulphate (20% w.o.f.) and Titanyl potassium oxalate (20% w.o.f.) (both around 66%).
- *Color strength (K/S) values comparison* indicates very slight differences in color strength, and most of them indicate that the natural printing paste is more efficient, but with less than 1% difference (average difference of 0,31).

- *Fastness results* indicate similar values for the compared experimental cases, with the most successful results obtained with the non-mordanted samples. In terms of staining (4-5) indicating relatively no staining when laundering, but with an exception in color change analysis (1), revealing more resistance with the use of synthetic printing paste (2-3). In terms of lightfastness, a very slight improvement was identified with the use of the synthetic printing paste (1-2).

b) Analysis of results obtained from wool textile substrate printing with R-phycoerytrhin

1) Color assessment: Chromatic characterization

Red wool fabrics printed with synthetic and natural printing paste were obtained with the use of the R-phycoerythrin-rich extract, with different shades, as seen in Figure 74, generated by the use of pre-mordants, and type of printing paste.

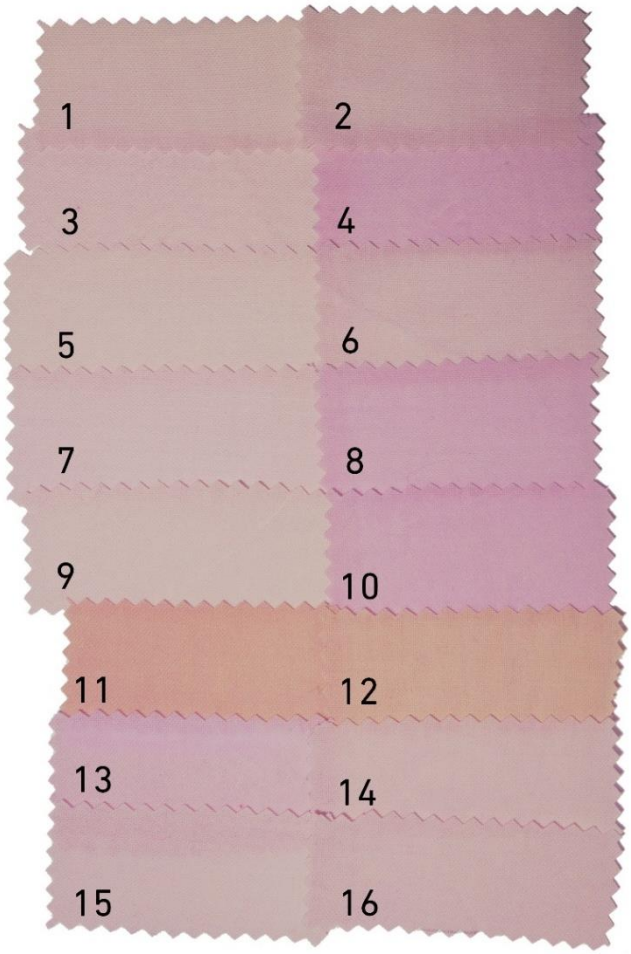


Figure 74. Printed wool fabrics with R-phycoerythrin obtained from Gracilaria gracilis (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate.

Type of paste influence on the printing process efficiency

An objective analysis of the color characterization was performed through the measurement of *L*, a*, b** coordinates of the CIE*Lab* space, for the comparison purposes of the coloration process efficiency with the use of synthetic and natural printing paste. Table 44 presents the calculation of the color difference between the two analyzed samples, considering the reference the wool fabric printed with synthetic paste, selected for the exploration of the influence of the use of the natural alternative. ΔE>3, indicates a significant color difference, with more intense results obtained with the use of the natural printing paste, confirmed by ΔL<0. This is justified by the milder conditions needed for the natural paste printing process, with lower curing time and temperature.

Sample	CIELab color coordinates			Color differences			
	L	a	b	ΔL	Δa	Δb	ΔE
Synthetic printing paste	81,59	2,74	13,99	-5,79	10,12	-13,2	17,61
Natural printing paste	75,8	12,86	0,79				

Table 44. CIELab color differences for R-phycoerythrin printed wool with synthetic printing paste vs. natural printing paste

To confirm the positioning of the samples in red color space, Figure 75 plots the *a** and *b** coordinates. It can be objectively validated that both printing pastes used in the experimental case reveal the expected results, meaning red coloration, and with good manipulating behavior of R-phycoerythrin as colorant matter.

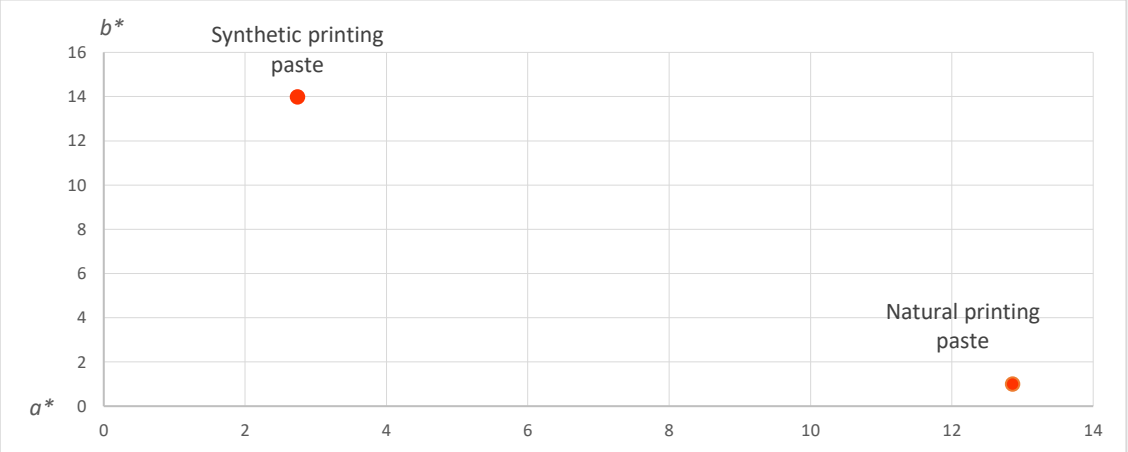


Figure 75. Color specifications in CIELab of the printed wool fabrics with R-phycoerythrin

Reflectance spectrum and color strength analysis

The influence of mordants, used for the pre-treatment of the wool fabrics, on the coloration strength with the R-phycoerythrin red colorant matter was analyzed by the measurement of the reflectance spectrum.

Pre-mordanted wool fabrics printed with R-phycoerythrin embedded in the synthetic paste

Figure 76 presents the reflectance spectrum of the pre-mordanted reddish wool fabrics. It can be observed that most of the samples are located in a relevant place of the spectrum, indicating a significant color intensity (when compared with the cotton samples Figure 71). The wool-R-phycoerythrin affinity is confirmed, and the most absorbance of light is obtained in the following order, starting with the most intense (darkest) color generated with the non-mordanted sample, followed by Titanyl potassium oxalate, the Aluminum triformate, then Aluminum acetate, Cream of tartar, Aluminum sulphate, and the whitest obtained with Alum.

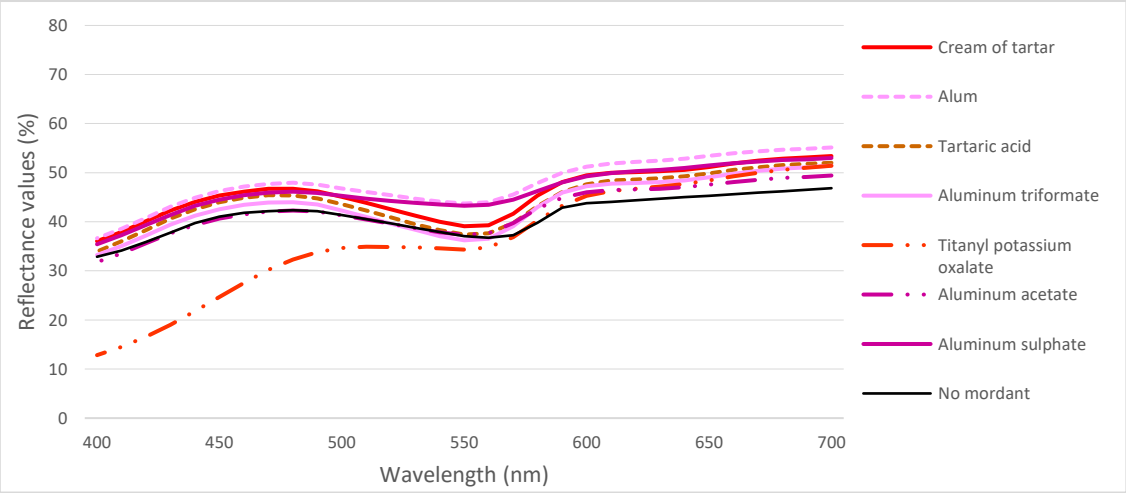


Figure 76. VIS spectrum of R-phycoerythrin printed wool with synthetic paste and the influence of the mordanting process

Pre-mordanted wool fabrics printed with R-phycoerythrin embedded in the natural paste

Figure 77 presents the reflectance spectrum of the red wool fabrics printed with natural paste and R-phycoerythrin. Considering the conclusions observed with the synthetic paste and the low influence of the mordants on the printing efficiency, the same assumptions can be inferred to the tests with the natural paste, as a similar reflectance spectrum is obtained. Nevertheless, the reflectance values seem to slightly increase indicating whiter samples, in terms of color intensity. The ranking of the mordants effect starting from the more intense towards the whites, thus higher reflectance values, is obtained as it follows: Titanyl potassium oxalate, Aluminum sulphate, Cream of tartar, Tartaric acid, Aluminum acetate, non-mordanted sample, Aluminum triformate, and the least intense color is produced with the use of Alum.

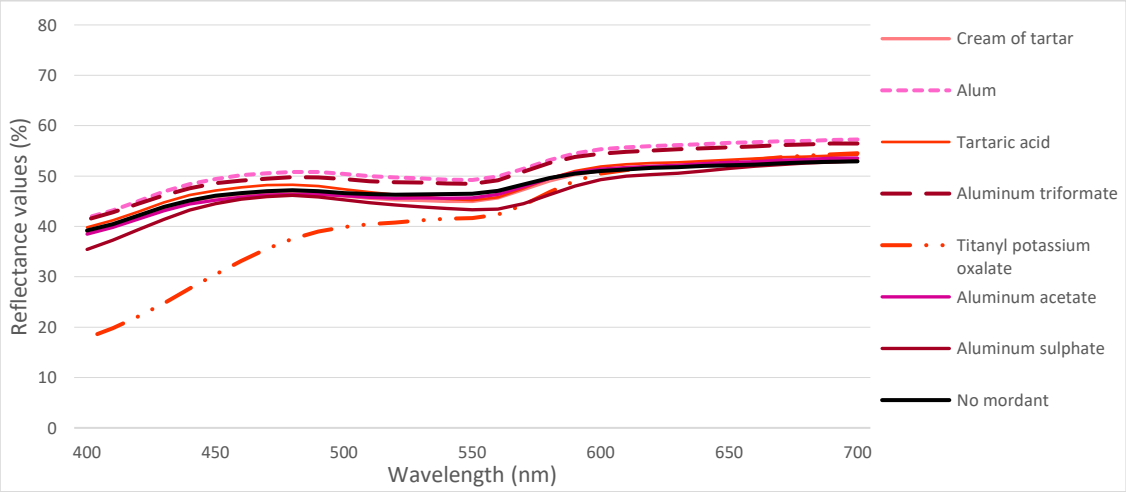


Figure 77. VIS spectrum of R-phycoerythrin printed wool with natural paste and the influence of the mordanting process

The comparative analysis of the calculated values of color strength based on the type of paste employed (natural vs. synthetic) and the influence of the mordanting process is reflected in Figure 78. It can be observed that the values corresponding to the natural paste present a slight increase, ranging from 24 to 26, and the synthetic paste ranging from 20 to 26. This is justified by the previously mentioned mild processing conditions of the printed samples, which degrade less the protein-based colorant, due to lower temperatures and less time of curing.

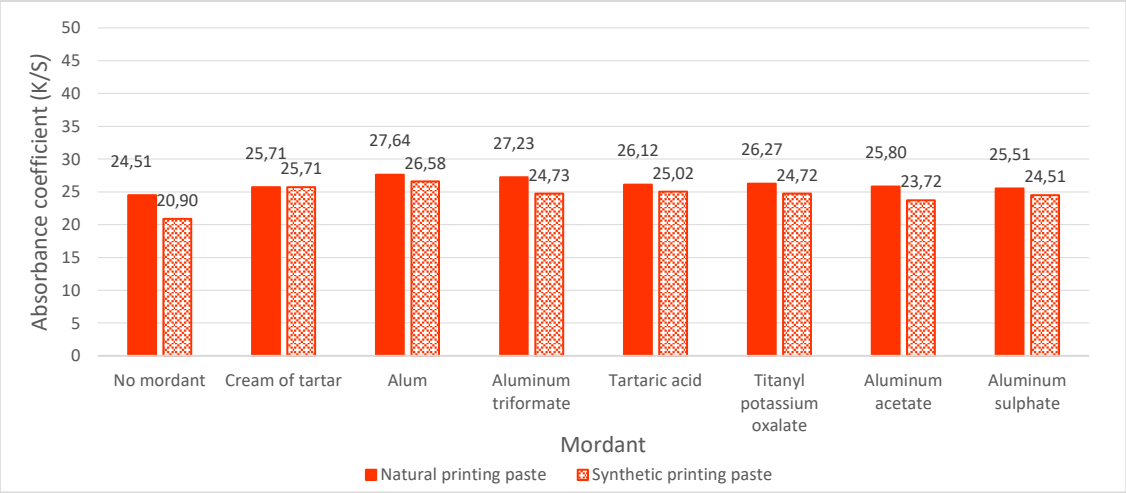


Figure 78.Wool printing with R-phycoerythrin pigment (natural vs synthetic printing paste) color strength(K/S) comparison

2) R-phycoerythrin wool printing process quality assessment through measurement of laundering and lightfastness

Fastness against common degrading agents in the textile industry, as laundering and light, were analyzed for exploring possible improvements in the coloration quality with R-phycoerythrin, and the complete set of results is presented in Table 45. Not or insignificant improvement may be observed with the use of mordants, as better results are obtained with the non-mordanted samples. The lastest are characterized by fair behavior to laundering and poor-fair against lightfastness.

Wool Samples Synthetic paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Cream of tartar	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	3	1-2	4-5	4-5	4-5	4-5	4-5	2
Aluminum acetate	1-2	1-2	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1-2	1-2	4-5	4-5	4-5	4-5	4-5	1-2
Wool samples Natural paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	4	5	5	5	5	5	5	4
Cream of tartar	2-3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2

Table 45. Fastness properties of mordanted wool samples printed with R-phycoerythrin embedded in the synthetic and natural paste

Nevertheless, further exploring with auxiliaries that may improve the behavior against degrading agents could be performed, with other possible cross-linking agents at low temperatures.

Light and laundering fastness are parameters that need to be improved, as existing similar studies indicate more efficient solutions with better results aimed at printing natural red colorants on different textile substrates (Rekaby et al. 2009).

Partial conclusions on wool printing with R-phycoerythrin

- *Type of paste influence on the printing process efficiency* analysis indicates that the **natural printing paste** generates significant color differences with the conventional synthetic printing paste ΔL=-5,79, with more intense reddish coloration Δa=10,72. Nevertheless, both printing pastes used in the experimental case reveal the expected results with good manipulating behavior of R-phycoerythrin as colorant matter.
- *Influence of pre-mordanting on the printability of R-phycoerythrin* was analyzed approaching both types of printing pastes, for identifying possible improvements in printability determined by the use of 7 different mordants.
    - *Synthetic printing paste* reflectance spectrums are grouped in the same area of the graph indicating very small differences among the samples, ranging among 37,28-43,46 reflectance capacity, measured at the maximum absorbance wavelength, with the highest dye uptake generated by the non-mordanted samples (37,28), indicating no significant effect with the use of mordants.
    - *Natural printing paste* reflectance spectrums present very small variations in their distribution with the highest efficiencies obtained with the use of Titanyl potassium oxalate (20% w.o.f.), Aluminum sulphate (20% w.o.f.), Cream of tartar (6% w.o.f.), Tartaric acid (6% w.o.f.), Aluminum acetate (20% w.o.f.) (range 42,3-49,8).
    - *Color strength (K/S) values comparison* indicates very slight differences in color strength, and most of them show that the natural printing paste is more efficient (color strength range 24,5-27,6), but comparable with the synthetic paste (range 20,8-26,5). Nevertheless, the mordant use does not prove necessary the inclusion in this type of coloration.
- *Fastness against common degrading agents* in the textile industry, as laundering and light indicate relatively no staining when laundering (4-5), and fair behavior of the non-mordanted samples to color change (3-4). In terms of lightfastness, the poor behavior characterizes the samples (3-4). Nevertheless, not or insignificant improvements were obtained with the use of mordants, as better results were obtained with the non-mordanted samples.

4.6.3. Analysis of the printing capacity of natural fabrics with yellow algae-based colorant matter β-carotene-rich extract from microalgae *Dunaliella salina*

B-carotene-rich extract was used for the coloration of cotton and wool textile substrates via pigment printing technique, by employing a conventional synthetic printing paste and also an alternative natural printing paste. Additionally, a series of mordants were used for fabric pre-treatment for their influence analysis on coloration strength and quality. The natural and synthetic printing pastes embedded with yellow carotene colorant matter, are presented in Figure 79 below.

Figure 79. Printing paste with embedded β-carotene, based on conventional synthetic components(left) and natural commercial alternatives printing paste (right)

a) Analysis of results obtained from cotton textile substrate printing with β carotene

1) Color assessment: Chromatic characterization

The printed cotton fabrics, non-mordanted and pre-treated with 7 different mordants, and colored with synthetic and natural paste are presented in Figure 80 below. Similar yellow shades can be observed as obtained through this coloration process, by visually assessing the samples.

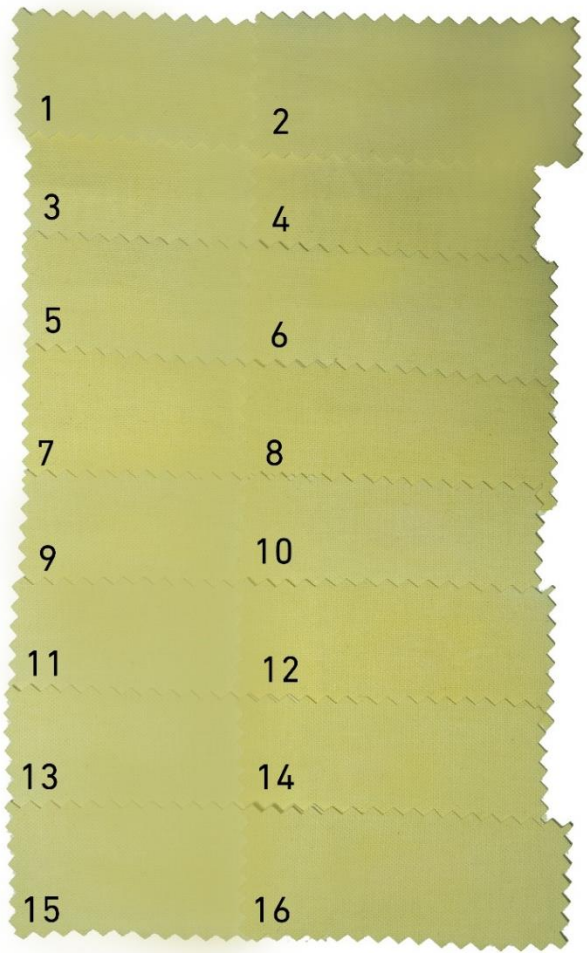


Figure 80. Printed cotton fabrics with β-carotene obtained from Dunaliella salina (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

In the context of the use of different types of printing pastes, the CIE*Lab* coordinates were measured for the calculation of the possible color differences generated by the pastes´ nature, based on the color coordinates (*L*, a*, b**). Table 46 presents the color differences results, considering the synthetic paste as the reference sample, and according to *ΔE*, it can be observed quite a low color difference between the analyzed samples. In terms of color intensity, *ΔL* >0, indicates that the reference sample, where the synthetic paste was employed, shows darker tones than the natural paste use, with less yellow influence than the naturally printed cotton.

Sample	***CIELab color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Synthetic printing paste	39,72	-0,77	2,68	1,53	-0,01	0,9	1,78
Natural printing paste	41,25	-0,78	3,58				

Table 46. CIELab color differences for β-carotene printed cotton with synthetic printing paste vs. natural printing paste

The obtained results are better than the ones obtained with alternative natural colorant yellow sources, as Buckthorn, with *L** values of 82,19 (Bahtiyari et al. 2013) and Annatto β-carotene extract printed on cellulosic fibers (*L**=58-60 range) (Chattopadhyay et al. 2018).

Figure 81 plots the distribution of the samples` coordinates and indicates their location in the yellow color space with greenish influences, this may be justified by the residual Chlorophyll-a, a chromophore found in the cell together with β-carotene, and that is resistant to the processing temperature and time.

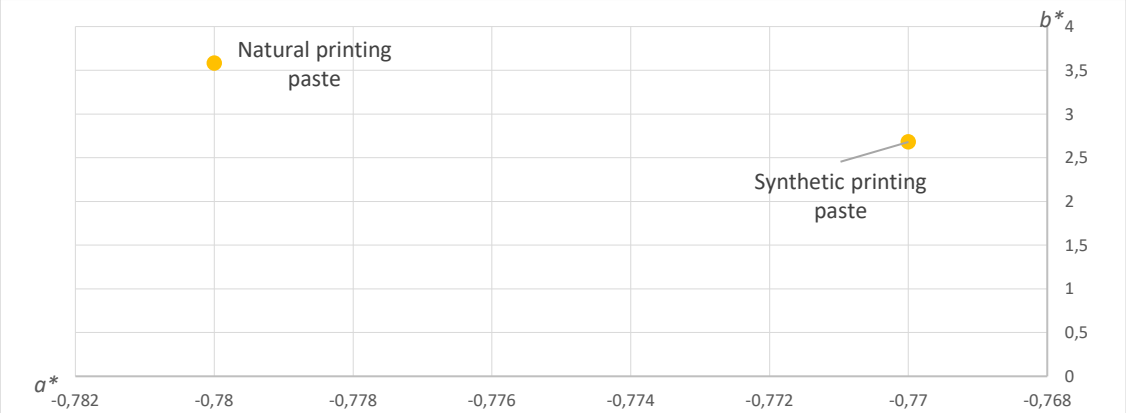


Figure 81. Color specifications in CIELab of the printed cotton fabrics with β-carotene

Reflectance spectrum and color strength analysis

The influence of the pre-mordanting process was explored by measuring the reflectance spectrum of the carotenoid printed cotton textile substrates.

Pre-mordanted cotton fabrics printed with β-carotene embedded in the synthetic paste

The reflectance spectrum of the pre-mordanted printed cotton with the synthetic paste is presented in Figure 82, and it indicates that the majority of samples almost covering the same reflectance spectrum, in the yellow intense area, except for Titanyl potassium oxalate, showing reduced reflectance at the maximum corresponding wavelength of the β-carotene (470nm). The fact that the distribution of the spectrums is almost identical with the non-mordanted samples shows that the pre-mordanting process was not efficient, and other agents for the efficiency of coloration must be used.

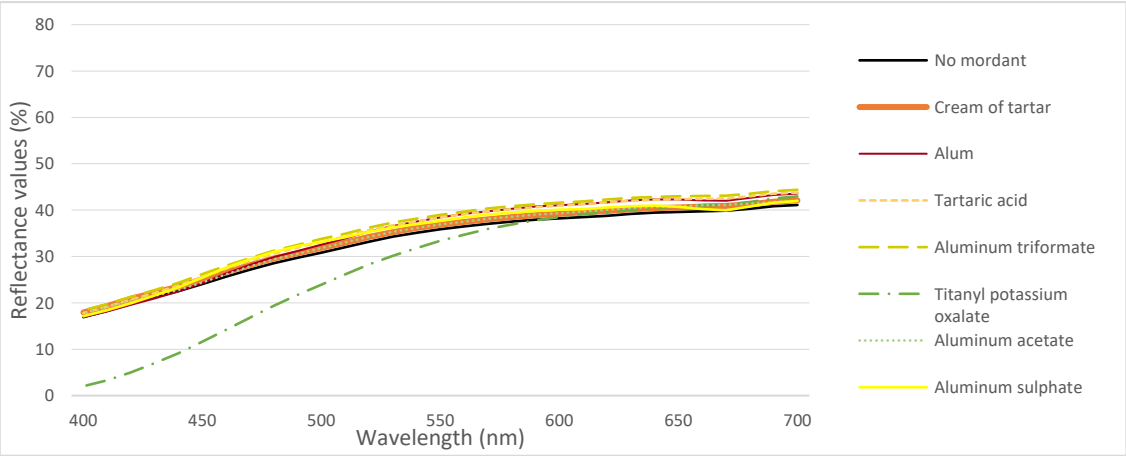


Figure 82. VIS spectrum of β-carotene printed cotton with synthetic paste and the influence of the mordanting process

Pre-mordanted cotton fabrics printed with β-carotene embedded in the natural paste

The use of natural paste as an alternative for the commercial one, for the coloration of pre-mordanted cotton substrates, was analyzed and the obtained reflectance spectrum is presented in Figure 83. Very similar results are obtained as in the case of the use of the synthetic printing paste, indicating that the natural alternative is viable, and also that the use of mordants does not generate significant improvements in the coloration process.

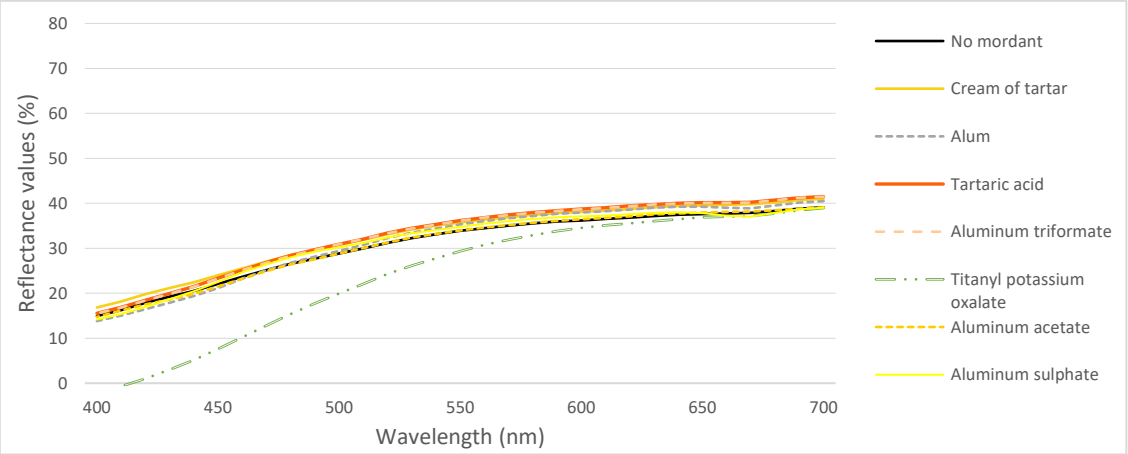


Figure 83. VIS spectrum of β-carotene printed cotton with natural paste and the influence of the mordanting process

The color strength (K/S) based on the reflectance spectrum, confirms the difference in the efficiency of the natural and synthetic printing paste (Figure 84). These differences appear due to the different processing conditions, and auxiliaries composing the mother pastes. In this sense, the natural printing paste, apart from the mild processing conditions, with lower degrading capacity, may have a different linkage with these natural colorants generating a good behavior and coloration of algae-based β-carotene.

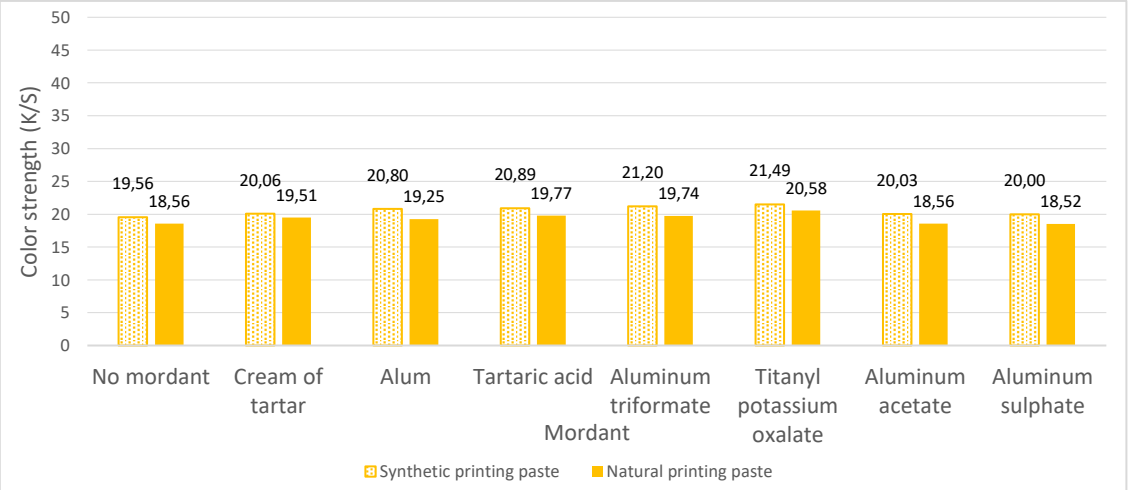


Figure 84. Cotton printing with β-carotene pigment (natural vs synthetic printing paste) color strength (K/S) comparison

2) B-carotene cotton printing process quality assessment through measurement of laundering and lightfastness

The laundering and lightfastness results of the cotton samples printed with β-carotene are presented in Table 47. The main comparison is done with the non-mordanted samples, and it can be observed that in most cases no improvements are observed, even if the color change showed the best results with the untreated fabrics, meanwhile the staining on other textiles is characterized by good behavior, confirming the need of additional elements for improving printability, as the colorant remains in the laundering wastewater. The lightfastness must also be improved, and possibilities are fostered due to the best results obtained without the use of mordants.

Cotton Samples Synthetic paste	***Color change***	***Staining***						***Lightfastness***
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
No mordant	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of tartar	3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	2	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2
Cotton samples Natural paste	***Color change***	***Staining***						***Lightfastness***
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
No mordant	3	4-5	4-5	4-5	4-5	4-5	4-5	3
Cream of tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Alum	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	3	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Titanyl potassium oxalate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum acetate	2	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	2

Table 47. Fastness properties of mordanted cotton samples printed with β-carotene embedded in synthetic and natural pastes

The laundering and lightfastness results are comparable with similar studies approaching Buckthorn sources as yellow colorant matter, printed on cotton substrates (Bahtiyari et al. 2013). Lightfastness results may be improved with natural alternatives, as indicated in similar studies with more efficient auxiliaries (Teli et al. 2013a).

Partial conclusions on cotton printing with β-carotene

- *Type of paste influence on the printing process efficiency* indicates that with the employment of the **synthetic paste**, darker samples (more intense yellow) ΔL >0 (1,53), were obtained, but with insignificant less yellow coordinate than the naturally printed cotton. This indicates slightly more compatibility of β-carotene with the synthetic printing paste.

- *Influence of pre-mordanting on the printability of β-carotene* was assessed within a range of 7 different mordants throughout the samples reflectance spectrum and it indicates very similar results in all the experimental cases:
    - *Synthetic printing paste* applied on pre-mordanted cotton substrates reveals overlapping spectrums (29-32% reflectance capacity), thus no significant difference in dye uptake, with the exception of Titanyl potassium oxalate (20% w.o.f.) with 17%.
    - *Natural printing paste* study case onto pre-mordanted cotton follows the same patterns as the synthetic printing paste (27-29% reflectance), thus validating the potential of natural printing with β-carotene. Titanyl potassium oxalate (20% w.o.f.) represents the mordant that indicates the extreme values in this range of similarity with 12%.
    - *Color strength (K/S) values comparison* indicate similar results in terms of color uptake, regardless of the type of paste used, with a slight increase generated with the use of the synthetic printing paste (K/S ranging from 19,56-21,49), nevertheless confirming the hypothesis of the potential of the use of natural printing pastes (18,56-20,58).
- *Fastness analysis* indicates that laundering testing showed fair behavior in terms of staining (4-5) and degrading (2-3), and poor resistance (2-3) against the light, with no or very low improvements generated by the use mordants, highlighting, in this case, the use of Titanyl potassium oxalate.

b) Analysis of results obtained from wool textile substrate printing with β-carotene

1) Color assessment: Chromatic characterization

The β-carotene printed wool fabrics, non-mordanted and pre-treated with 7 different mordants, with the use of synthetic and natural paste, are presented in Figure 85 below. Similar yellow shades, with slight color intensity differences, can be observed as obtained through this coloration process, through a visual analysis of the samples.

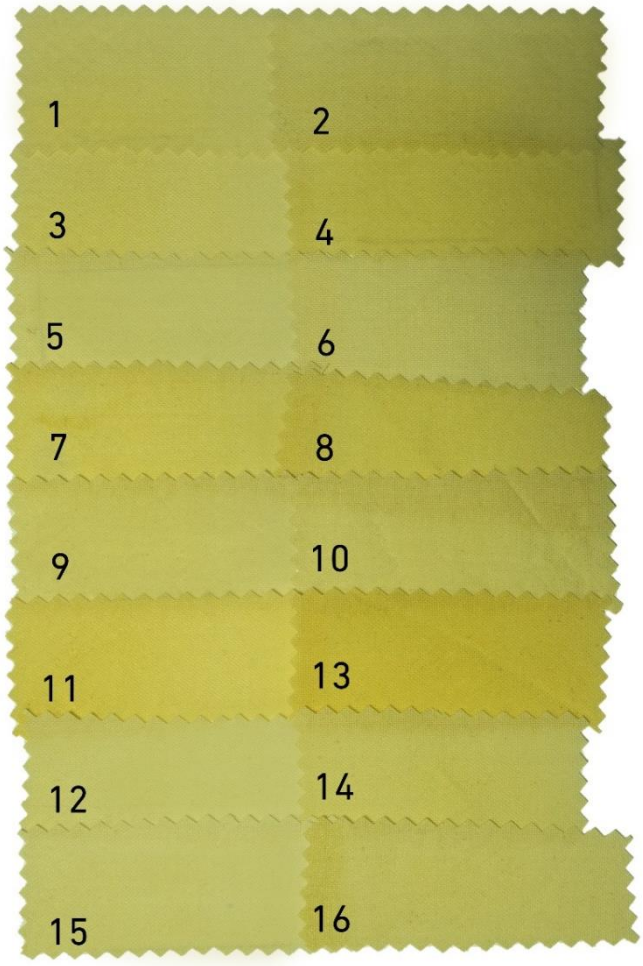


Figure 85. Printed wool fabrics with β-carotene obtained from Dunaliella salina (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate.

Type of paste influence on the printing process efficiency

The color coordinates (*L*, a*, b**) of the printed samples with β-carotene embedded in natural and synthetic printing paste were measured, and color differences were calculated, as indicated in Table 48. The reference sample is considered the one printed with synthetic paste, for exploring the natural paste behavior in pigment printing with β-carotene. According to $\Delta E<0$, it is indicated that the color differences are not significant, but the natural printing paste generates a less yellow color (according to Δb).

Sample	CIELab color coordinates			Color differences			
	L	a	b	ΔL	Δa	Δb	ΔE
Synthetic printing paste	41,55	-0,73	7,38	-1,22	-0,02	-2,68	2,94
Natural printing paste	40,33	-0,75	4,7				

Table 48. CIELab color differences for β-carotene printed wool with synthetic printing paste vs. natural printing paste

The color space plot is represented in Figure 86 and locates the sample color in the yellow space with green influences probably by remaining Chlorophyll-a, which was not sufficiently purified in the extraction process. Nevertheless, the results are satisfactory for both samples, meaning that the type of the paste does not interfere with the coloration quality of the process.

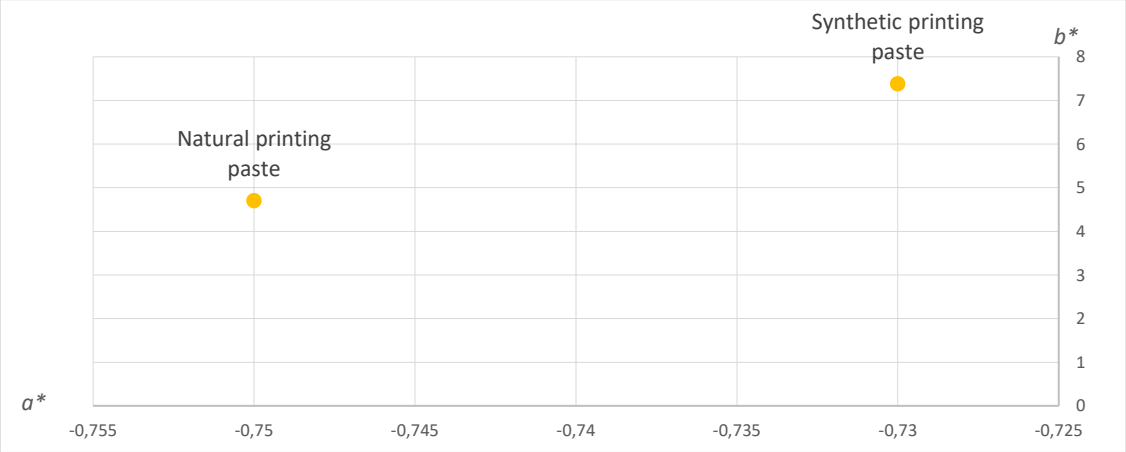


Figure 86. Color specifications in CIELab of the printed wool fabrics with β-carotene

Similar studies aiming at printing with natural sources of yellow colorants, as Buckthorn, present comparable results in terms of color characterization, with more intense color in this study (Bahtiyari et al. 2013).

Reflectance spectrum and color strength analysis

The influence of the pre-mordanting process was explored by measuring the reflectance spectrum of the β-carotene printed wool textile substrates.

Pre-mordanted wool fabrics printed with β-carotene embedded in the synthetic paste

The synthetic commercial printing paste applied on the pre-mordanted wool samples revealed similar results, and the reflectance spectrum is indicated in Figure 87. It can be observed a grouping of the individual spectrums in the same part of the graph, which does not overcome values of 25, meaning very good yellow color intensity values, considering that values of 100 represent white fabrics with increased reflection capacity, and the one tending to 0 indicate dark (intense) colors which absorb light. The most intense color is obtained with the use of Aluminum triformate and the most efficient auxiliary is represented by Alum. Nevertheless, insignificant differences are obtained among printed mordanted samples, revealing the inefficiency of the mordants in the printing process, and suggest not using them in the coloration process.

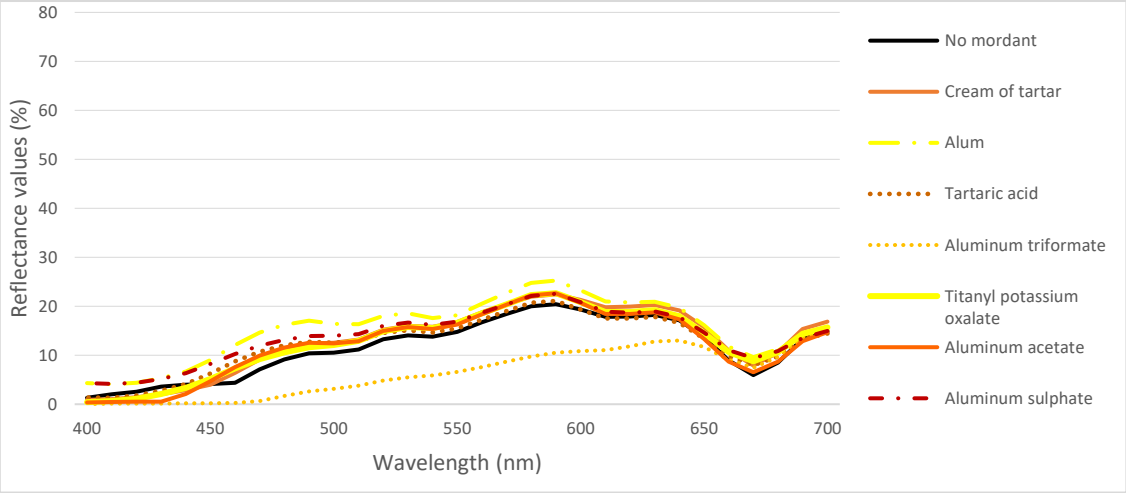


Figure 87. VIS spectrum of β-carotene printed wool with synthetic paste and the influence of the mordanting process

Pre-mordanted wool fabrics printed with β-carotene embedded in the natural paste

The natural printing paste application on the pre-mordanted wool substrates reflectance spectrum is presented in Figure 88. A very similar spectrum distribution with the synthetic printing paste approach (Figure 87) can be observed, indicating good behavior of the type of paste, wool, and colorant matter. Nevertheless, even though pre-mordanting does not show any improvements, the reflectance values indicate acceptable results in terms of yellow color intensity, thus a high possibility of printing wool with algae-based yellow colorant matter.

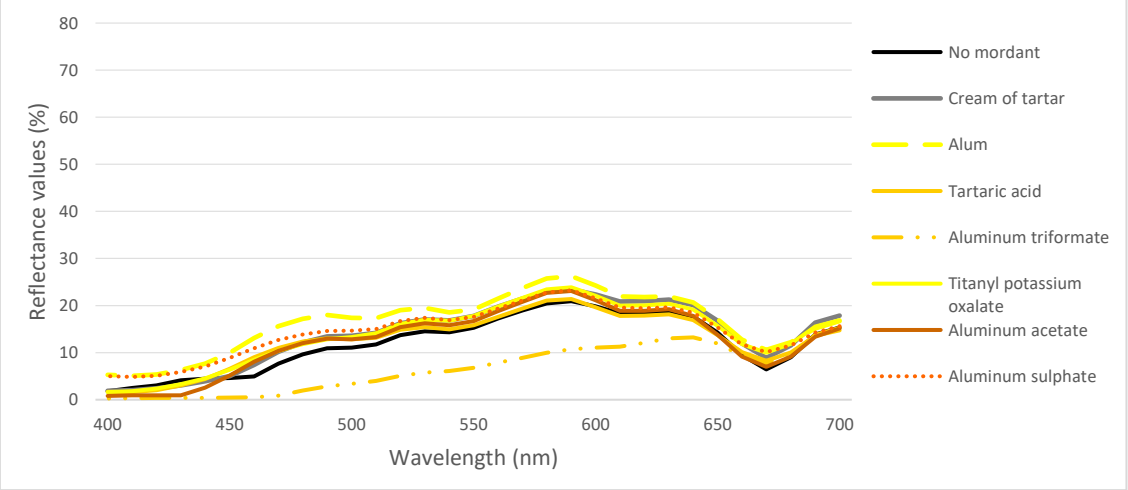


Figure 88. VIS spectrum of β-carotene printed wool with natural paste and the influence of the mordanting process

To explore the efficiency of the coloration process and the influence of the type of printing paste and pre-treatment auxiliary, the comparison of the color strength is presented in Figure 89. It can be observed a slight, but negligible higher efficiency with the use of the natural printing paste, provided by the compatibility between the paste and the coloring ingredients, and the shorter processing time, that reduces the natural color degradation capacity.

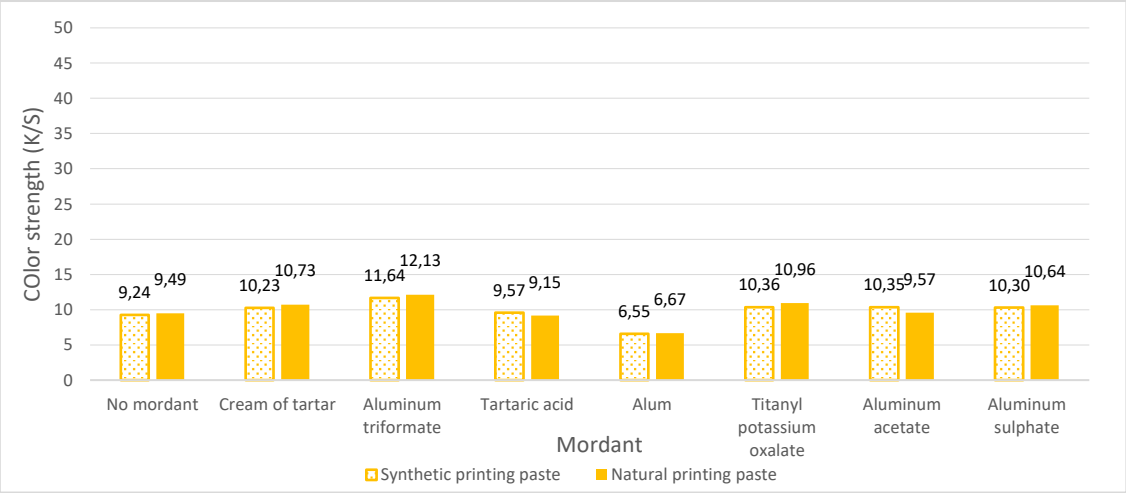


Figure 89. Wool printing with β-carotene pigment (natural vs synthetic printing paste) color strength (K/S) comparison

Overall wool substrates can be printed with β-carotene, without the need for the use of mordants, in a successful manner, from a coloration capacity point of view.

2) B-carotene wool printing process quality assessment through measurement of laundering and lightfastness

The quality of the coloration process was analyzed through measurements of the printed wool sample's resistance against laundering and light degrading agents. The complete set of results is presented in Table 49. The best results, with both types of printing pastes analyzed, were obtained with the non-mordanted samples with moderate color degradation behavior, and the rest of the samples show similar or reduced quality. All the analyzed samples show very good behavior to staining on the tested textile substrates, indicating on one side, that the colorant remains on the initial sample, but on the other hand, it could also remain in the residual laundering water, with no affinity to these substrates. The lightfastness results do not show favorable results, but even though the non-mordanted samples indicate the best behavior, it needs further exploration of possible auxiliaries for improving the resistance of the color against light degradation.

Wool Samples **Synthetic paste**	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of tartar	3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2
Tartaric acid	3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	3	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminum acetate	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2

Wool samples ***Natural paste***	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Cream of tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	3
Alum	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	3	4-5	4-5	4-5	4-5	4-5	4-5	2
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Titanyl potassium oxalate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminum acetate	2	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Aluminum sulphate	2	4-5	4-5	4-5	4-5	4-5	4-5	1

Table 49. Fastness properties of mordanted wool samples printed with β-carotene embedded in synthetic and natural pastes

The laundering and lightfastness results are comparable with studies printing non-mordanted wool with Buckthorn-based yellow colorant (Bahtiyari et al. 2013).

Partial conclusions on wool printing with β-carotene

- *Type of paste influence on the printing process efficiency* validates the use of **natural paste** for printing with β-carotene embedded in natural printing paste, with slightly more intense yellowish shades *ΔL* (-1,22), but with very similar color strength values (9,23- synthetic paste, respectively 9,48- natural paste).
- *Influence of pre-mordanting on the printability of β-carotene* over pre-mordanted wool with 7 different mordants, present overall insignificant efficiency, with very similar results for both printing pastes assessed:
    - *Synthetic printing paste* reflectance spectrums present grouped values with insignificant differences (10-17% reflectance capacity at maximum absorbance wavelength), but with more intense coloration provided by Aluminum triformate (10% w.o.f.) (1,71%), and even reduced performance generated with the use of Alum (10% w.o.f.) (17,03%).
    - *Natural printing paste* reflectance assessment follows closely the assumptions identified with the use of the synthetic printing paste (9,61-18,01%), with the most efficient being Aluminum triformate (10% w.o.f.) (1,94%) but indicating the possibility of employment of a more sustainable alternative, without the need of pre-mordanting process.
    - *Color strength (K/S) values comparison* reveals comparable efficiencies in both analyzed study cases and pre-mordanted, ranging from for synthetic paste 6,55-11,64 to 6,66-12,13 respectively for natural paste, and finally validating the use of the natural printing paste without efficiency or performance differences.
- *Fastness analysis* overall results indicate insignificant improvements generated by the use mordants, with the following conclusions:
    - *Laundering fastness* results indicate very good behavior to staining (4-5) on the tested textile substrates, meanwhile, color degradation is characterized by fair behavior (3-4).
    - Poor resistance against *the light* (2-3).

4.6.4. Analysis of the printing capacity of natural fabrics with green algae-based colorant matter Chlorophyll-a-rich extract from microalgae *Caespitella pascheri*

Natural textile substrates have been pigment-printed with Chlorophyll-a-rich extract embedded in synthetic commercial and natural alternative printing pastes. Additionally, a series of mordants were used for the fabrics pre-treatment for exploring their influence in the coloration efficiency of the selected process. The colorant matter embedded in the synthetic and natural printing pastes is presented in Figure 90, with intense green color.

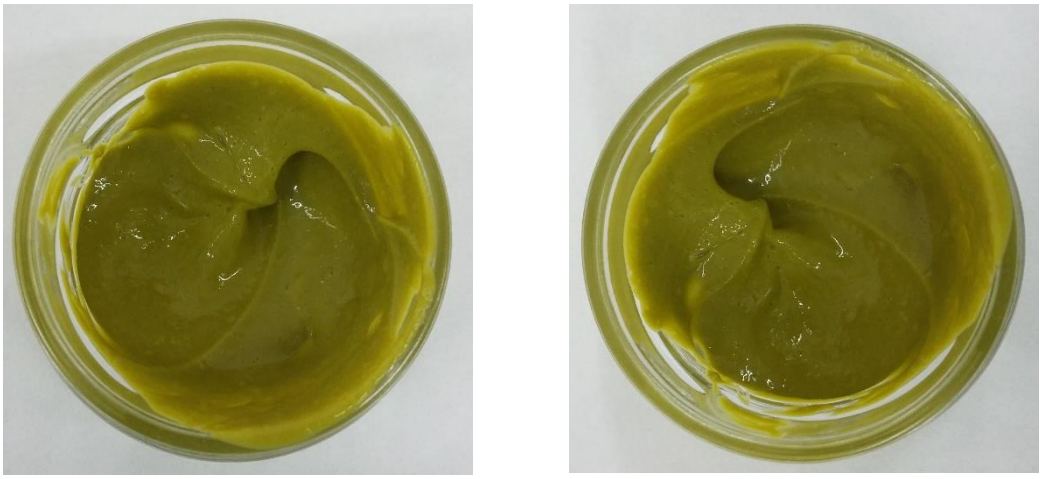


Figure 90. Printing paste with embedded Chlorophyll-a, based on conventional synthetic components(left) and natural commercial alternatives printing paste (right)

a) Analysis of results obtained from cotton textile substrate printing with Chlorophyll-a

1) Color assessment: Chromatic characterization

The greenish, Chlorophyll-a-based, non-mordanted, and pre-mordanted printed cotton samples with natural and synthetic printing pastes are presented in Figure 91. Very slight differences in the obtained color shades can be observed, which may be influenced by the type of printing paste, through binder and base, and auxiliaries.

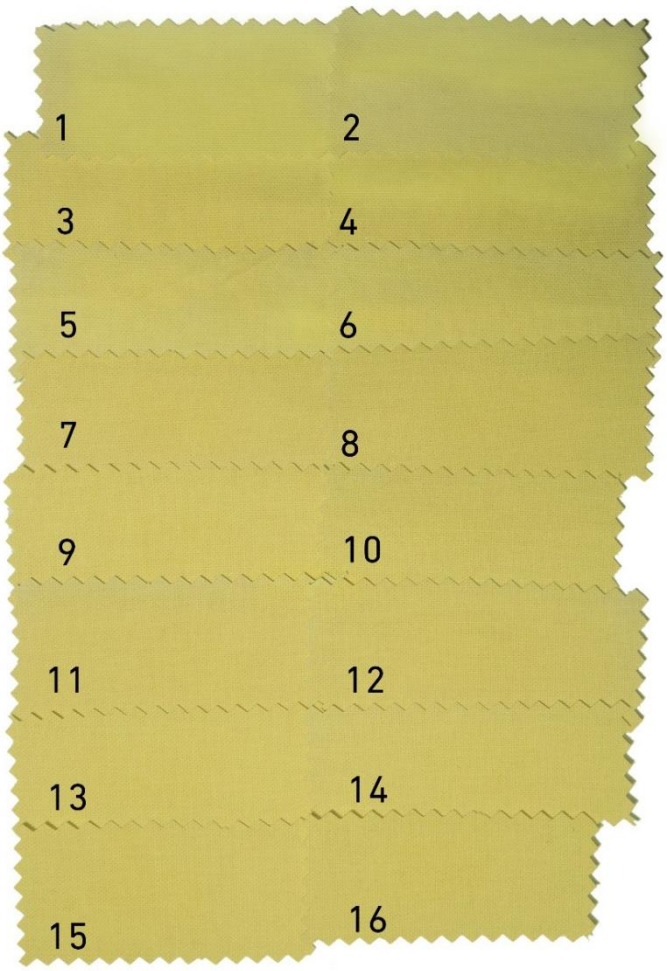


Figure 91. Printed cotton fabrics with Chlorophyll-a obtained from Caespitella pascheri (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

Objective color characterization was performed through the analysis of the green printed cotton fabrics CIE*Lab* color coordinates (*L*, a*,* and *b**), for the identification of the possible interference or efficiency of the use of the natural alternative to the synthetic commercially used printing paste. The complete set of results and color difference calculus are presented in Table 50. The analysis of the overall color difference ΔE>3 indicates significant color variation with the reference sample used in this assessment, the cotton printed with synthetic paste. The more intense results are conferred by the process involving the natural printing paste, due to the lower temperature and processing time employed. Nevertheless, the greenish coordinates indicate insignificant tones differences generated by both printing pastes. It can be assumed that both pastes reveal acceptable results for the printing of cotton with the Chlorophyll-a-rich extract.

Sample	CIELab color coordinates			Color differences			
	L	a	b	ΔL	Δa	Δb	ΔE
Synthetic printing paste	53,92	-2,95	3,71	-3,74	0,15	-0,4	3,76
Natural printing paste	57,66	-3,1	4,11				

Table 50. CIELab color differences for Chlorophyll-a printed cotton with synthetic printing paste vs. natural printing paste

The greenish color positioning is confirmed in Figure 92, plotting the *a** and *b** coordinates. This analysis confirms the expected results of printing cotton with green algae-based colorant matter, located in the same area of the graph.

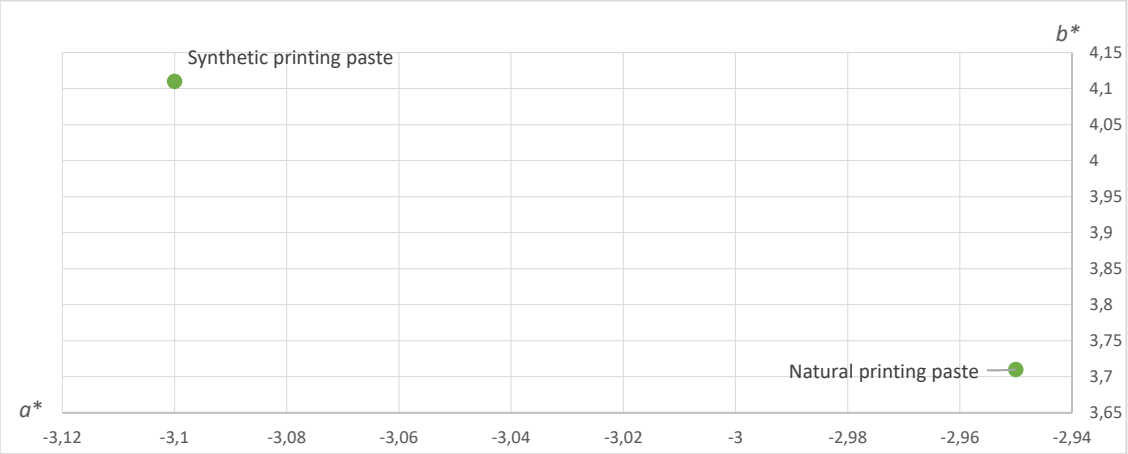


Figure 92. Color specifications in CIELab of the printed cotton fabrics with Chlorophyll-a

Reflectance spectrum and color strength analysis

Considering the employment of various mordants for the assessment of their possible influence on the coloration process, the reflectance spectrum was measured, for the identification of dye uptake through color strength analysis.

Pre-mordanted cotton fabrics printed with Chlorophyll-a embedded in the synthetic paste

The reflectance spectrum of the pre-mordanted cotton fabrics printed with Chlorophyll-a embedded in synthetic conventional printing paste is presented in Figure 93. The reflectance spectrum indicates the dye uptake of the samples with the darkest tones reflect the most coloration, thus the lowest spectrums, with the lowest values. In this sense, the darkest colors were obtained with Cream of tartar, Tartaric acid, Alum, Aluminum sulphate, Aluminum acetate, and No mordant. Even though a classification can be done, it must be highlighted that most of the samples converge around the same color and dye uptake values, meaning not or insignificant effect is obtained with the use of mordants.

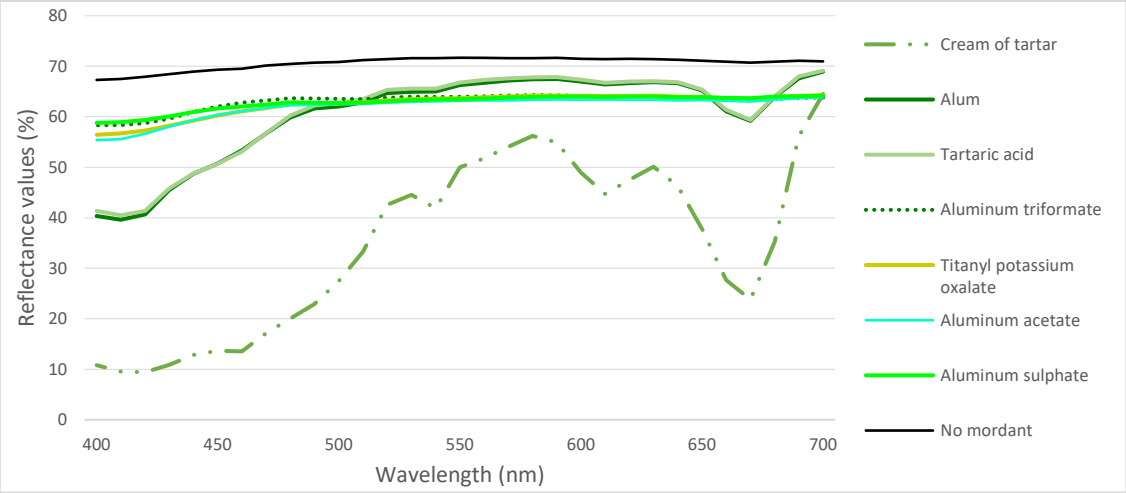


Figure 93. VIS spectrum of Chlorophyll-a printed cotton with synthetic paste and the influence of the mordanting process

Pre-mordanted cotton fabrics printed with Chlorophyll-a embedded in the natural paste

The reflectance spectrum of the printed cotton samples with the use of natural printing paste with algae-based Chlorophyll-a colorant matter is presented in Figure 94. The exploration of the influence of various mordants on the dye uptake capacity, indicated by the highest light absorption spectrum, thus represented in the graph by the lowest reflective values, was performed. In this sense, a ranking of the darkest (more intense colors, low reflective values) towards the lightest samples can be performed, according to the maximum reflectance wavelength (λ max= 670 nm): Aluminum acetate, Aluminum sulphate, No mordant, Tartaric acid, Aluminum triformate, Alum, Titanyl potassium oxalate, and Cream of tartar. This ranking and the graph indicate very small differences among the samples with most of the spectrums located in the same space, confirming the very low efficiency of the use of mordants without improvements.

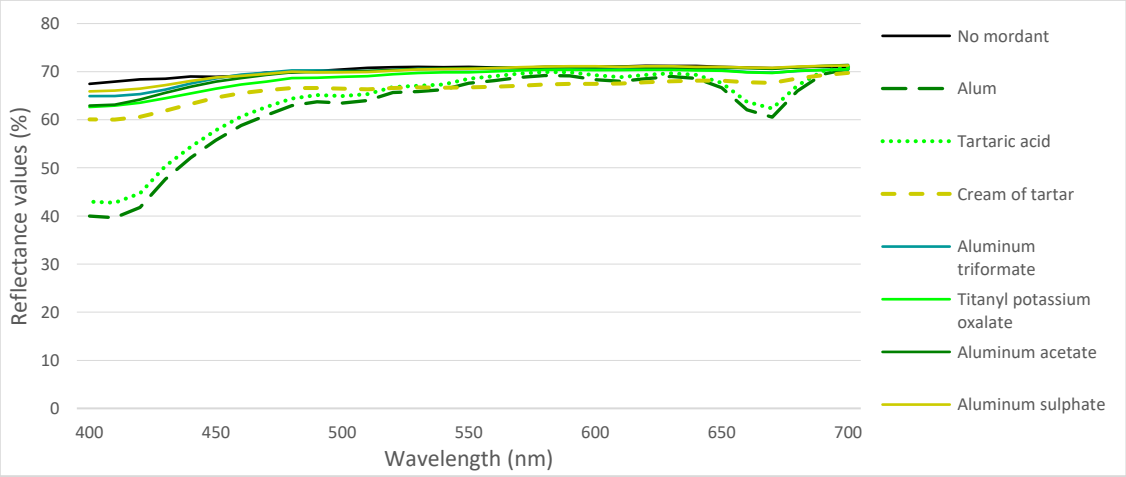


Figure 94. VIS spectrum of Chlorophyll-a printed cotton with natural paste and the influence of the mordanting process

Based on the maximum values of the reflectance spectrum of the printed cotton samples the color strength (*K/S*) was measured with the purpose of comparison of performance, on the one side of the synthetic vs natural alternative printing paste, and on the other side, effectiveness of pre-mordanting. According to Figure 95, it can be observed a slightly, but not

significant, higher efficiency with all the use cases of the natural printing paste, probably due to the paste composition, as binder and thickener, but not strictly connected to mordants efficiency.

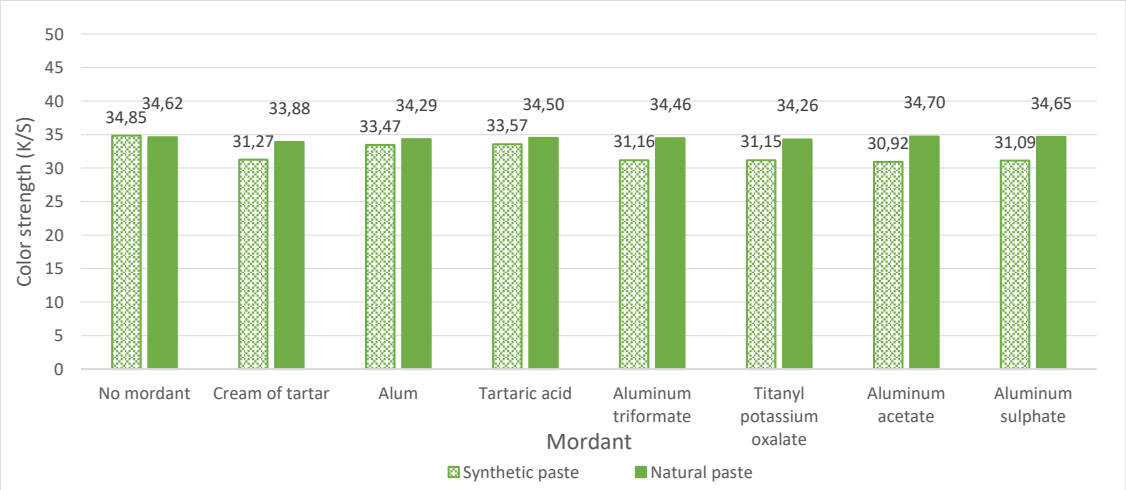


Figure 95. Cotton printing with Chlorophyll-a pigment (natural vs synthetic printing paste) color strength(K/S) comparison

2) Chlorophyll-a cotton printing process quality assessment through measurement of laundering and lightfastness

The laundering and lightfastness of the printed cotton fabrics show poor behavior overall, regardless of the used printing paste or auxiliaries (Table 51). Particularly, the color degradation shows better behavior than the one with the use of mordants. Nevertheless, the staining of color on different textiles showed good results, with values of 4-5, characterizing good to excellent behavior, indicating that special attention to the coloration must be given, due to reduced fiber colorant affinity. The lightfastness behavior is a critical issue that must be improved, due to the very poor behavior revealed, with values of 1-2, thus further auxiliaries must be explored, meanwhile considering the natural commercial alternative, due to mild process conditions, with reduced impact on colorant degradation. Nevertheless, non-mordanted samples show better results, indicating that further exploration of the natural characteristics of the colorant must be enhanced.

Cotton Samples	***Color***	***Staining***						***Lightfastness***
Synthetic paste	***change***	*Wool*	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Alum	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Tartaric acid	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	1	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Titanyl potassium oxalate	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Aluminum acetate	1	4-5	4-5	4-5	4-5	4-5	4-5	1
Aluminum sulphate	1	4-5	4-5	4-5	4-5	4-5	4-5	1

Cotton samples ***Natural paste***	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	1-2	4-5	4-5	4-5	4-5	4-5	4-5	2
Cream of tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	1
Alum	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1
Tartaric acid	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminium Triformate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1
Titanyl potassium oxalate	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Aluminum acetate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Aluminum sulphate	1-2	4-5	4-5	4-5	4-5	4-5	4-5	1-2

Table 51. Fastness properties of mordanted cotton samples printed with Chlorophyll-a in embedded in the synthetic and natural paste

Partial conclusions on cotton printing with Chlorophyll-a

- *Type of paste influence on the printing process efficiency* analysis indicates overall significant color variation among the use of natural vs. synthetic printing paste ΔE=3,76, with more intense results obtained with the **natural printing paste** ΔL=-3,74. Nevertheless, the greenish coordinates indicate insignificant tones differences generated by both printing pastes, but with acceptable results for the printing of cotton with Chlorophyll-a-rich extract.
- *Influence of pre-mordanting on the printability of Chlorophyll-a* was analyzed approaching both types of printing pastes, by assessing the color uptake capacity determined by the use of 7 different mordants.
    - *Synthetic printing paste* reflectance spectrums of the printed pre-mordanted cotton samples vary, and the results may be ranked starting with the darkest colors obtained with the use of Cream of tartar (6% w.o.f.), Tartaric acid (6% w.o.f.), Alum (3% w.o.f.), Aluminum sulphate (20 % w.o.f.), Aluminum acetate (20% w.o.f.), and non-mordanted samples, with reflectance capacity ranging among 23,83-70,70. Nevertheless, these differences are statistically insignificant.
    - *Natural printing* paste reflectance spectrums converge around similar values, with slight differences allowing the classification of the results with the indication of the less reflective (more intense) samples: **Aluminum acetate (**20 % w.o.f.) and Aluminum sulphate 20 % w.o.f.) with reflectance capacity 60,57%, respectively 62,54%.
    - *Color strength (K/S) values comparison* indicates clear difference generated by the increased dye uptake obtained with the natural printing paste, with values converging around values of 34, probably due to the paste composition, as binder and thickener, and not justifying the use of mordants.
- *Light and laundering fastness* results show poor behavior overall, regardless of the used printing paste or the auxiliaries used, but with the indication of no staining when laundering (4-5), poor degradation of 1-2, and poor light resistance (1-2). The selected mordants do not improve the behavior against color degrading agents, thus further assessments must be performed.

b) Analysis of results obtained from wool textile substrate printing with Chlorophyll-a

1) Color assessment: Chromatic characterization

Wool samples were printed with Chlorophyll-a-rich extract obtained from microalgae *Caespitella pascheri,* onto non-mordanted and pre-mordanted textile substrates. The obtained green colored fabrics are presented in Figure 96, and some slight tone variations can be observed. Nevertheless, darker colors were obtained for the coloration of wool than the cotton samples results (Figure 91).

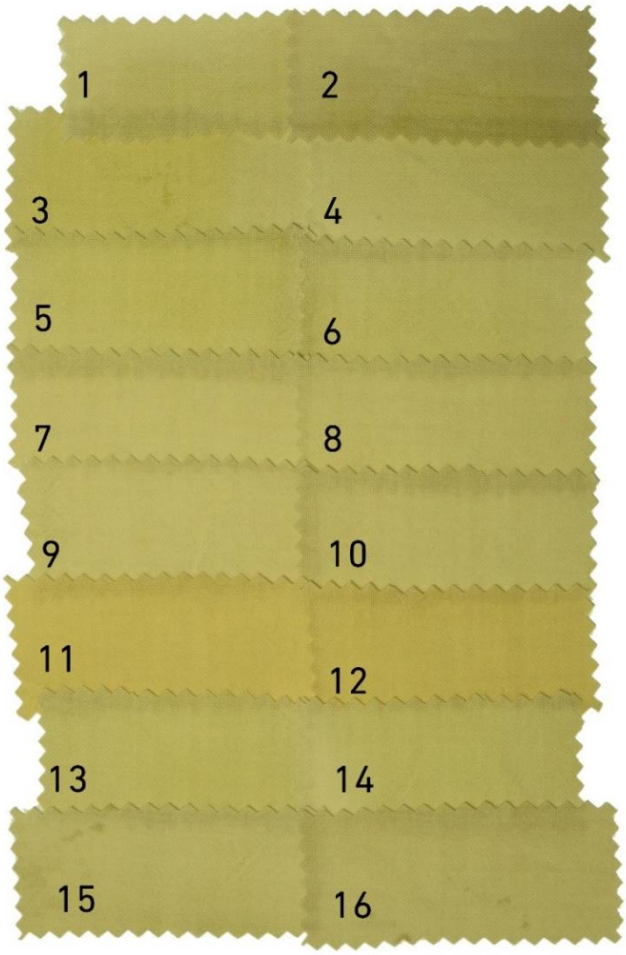


Figure 96. Printed wool fabrics with Chlorophyll-a obtained from Caespitella pascheri (synthetic paste left and natural paste right)

Uneven numbers-synthetic paste, and even numbers-natural paste: 1-2. No mordant; 3-4. Cream of tartar; 5-6. Alum; 7-8. Tartaric acid; 9-10. Aluminum Triformate; 11-12. Titanyl potassium oxalate; 13-14. Aluminum acetate; 15-16. Aluminum sulphate

Type of paste influence on the printing process efficiency

Both commercial synthetic and natural alternative printing pastes revealed a good response in terms of colorant compatibility with the paste, but a more objective analysis was performed to identify possible color differences generated by the natural printing paste. In this sense, measurements of CIE*Lab* color coordinates (*L*, a*,* and *b**) were performed and color differences calculated, as summarized in Table 52. Considering the reference sample, the synthetic printing paste, it can be observed that according to ΔE>3, there are significant color

differences with the natural printing paste, and the last shows a more intense coloration (darker) ΔL =-10,48, with a very similar green component.

Sample	*CIELab color coordinates*			*Color differences*			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Synthetic printing paste	37,07	-2,23	1,93	-10,48	0,35	-1,38	10,58
Natural printing paste	47,55	-2,58	3,31				

Table 52. CIELab color differences for Chlorophyll-a printed cotton with synthetic printing paste vs. natural printing paste

For a color space location of these two samples, Figure 97 plots the green (*a**) and yellow (*b**) coordinates. It can be observed that the synthetic printing paste presents a slighter greener coordinate, but both samples have a very similar yellow influence. This may be justified by the residual β-carotene present in the cellular wall, and the reduced purification steps when the extraction was performed. Overall, both fabrics reveal the desired coloration, validating the possibility of pigment printing with algae based-Chlorophyll-a, strictly from a color dimension approach.

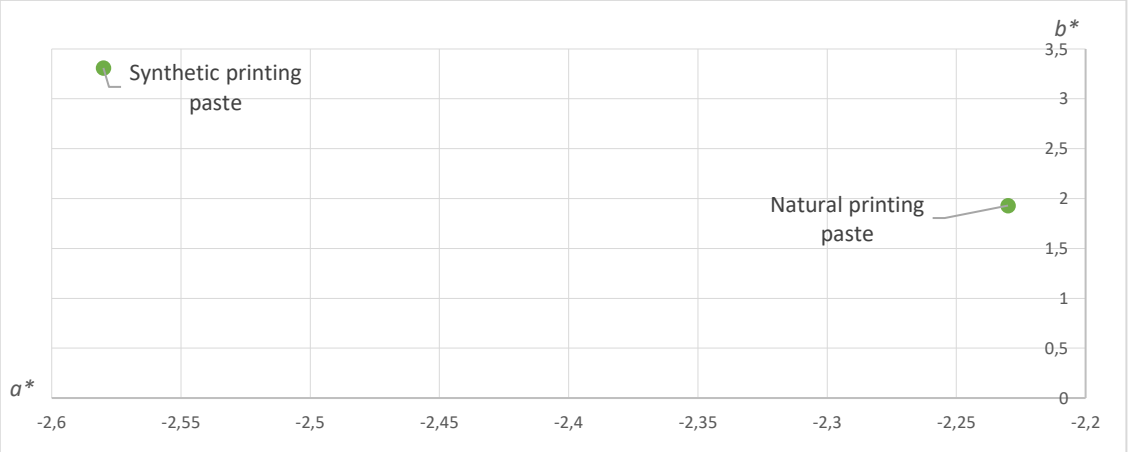


Figure 97. Color specifications in CIELab of the printed wool fabrics with Chlorophyll-a

Reflectance spectrum and color strength analysis

The possible influence of the pre-mordanting process of the wool fabrics was assessed, in terms of improvements over the dye uptake, in the printing process, and related also to the quality of the coloration through measurement of the reflectance spectrum.

Pre-mordanted wool fabrics printed with Chlorophyll-a embedded in the synthetic paste

Figure 98 presents the reflectance spectrum of the non-mordanted and pre-mordanted wool printed textiles with Chlorophyll-a embedded in the synthetic printed paste. In the context of the darkest sample absorbs more light and reflects less, it can be observed that the highest dye uptake was obtained without the use of auxiliaries, indicating the reduced efficiency of their use. Nevertheless, very little variation amongst the pre-mordanted samples may be observed. A ranking amongst mordants starting from the darkest towards the lightest samples may be Titanyl potassium oxalate, Cream of tartar, Alum, Aluminum triformate, Tartaric acid, Aluminum acetate, and Aluminum sulphate.

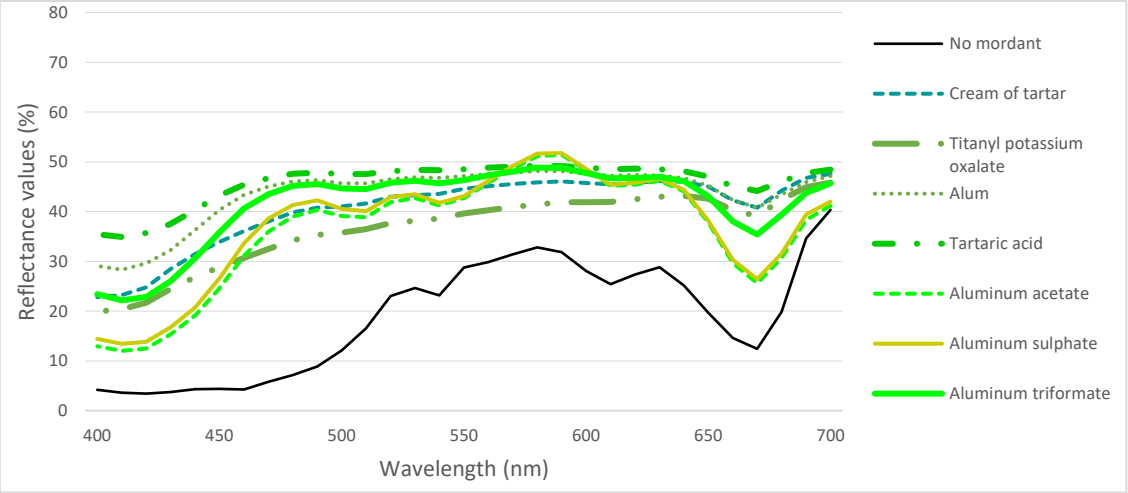


Figure 98. VIS spectrum of Chlorophyll-a printed wool with synthetic paste and the influence of the mordanting process

Pre-mordanted wool fabrics printed with Chlorophyll-a embedded in the natural paste

Figure 99 presents the reflectance spectrum of non-mordanted and pre-mordanted wool samples printed with Chlorophyll-a embedded in natural commercial printing paste. It can be observed, that contrary to the use of the synthetic printing paste use (Figure 98) there are some improvements obtained with the Aluminum-based mordants, as Aluminum triformate, Aluminum sulphate, Aluminum acetate, Titanyl potassium oxalate, and Cream of tartar, but they are considered negligible results, as the spectrums slightly overlap.

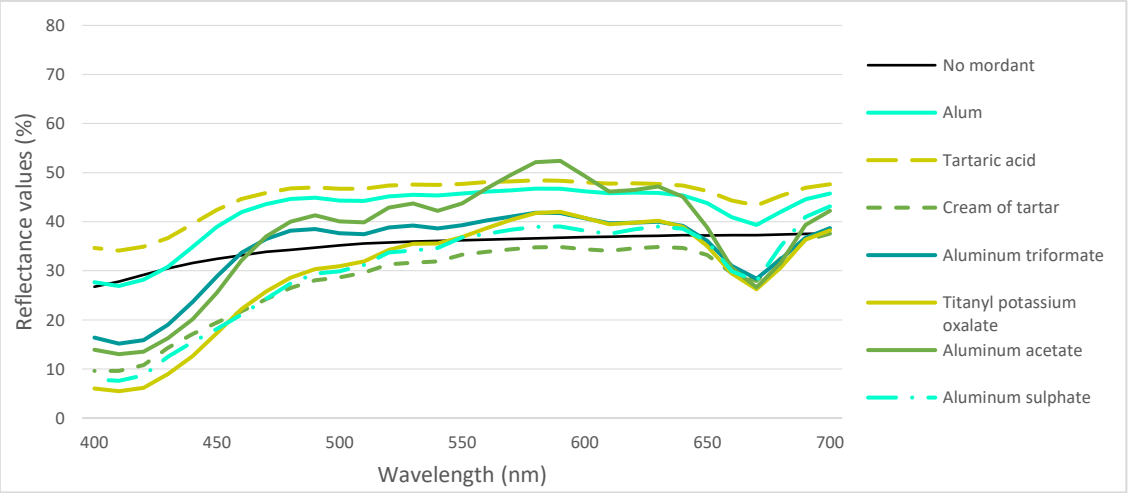


Figure 99. VIS spectrum of Chlorophyll-a printed wool with natural paste and the influence of the mordanting process

The comparison among the color strength values, referred to as *K/S*, calculated based on the maximum reflectance values, was proposed for identification of the influence of the type of paste, and mordanting on the dyeability through pigment printing, and is detailed in Figure 100 below. Overall, insignificant differences may be observed in terms of dye uptake within all the studied cases. This indicates that Chlorophyll-a obtained from microalgae presents a high potential of use via pigment printing coloration method, without significant influence of the printing paste type, or the pre-treatment of the fabrics. Nevertheless, a slight increase in *K/S* can be observed with the use of Aluminum-based mordants, for all the studies experimental cases.

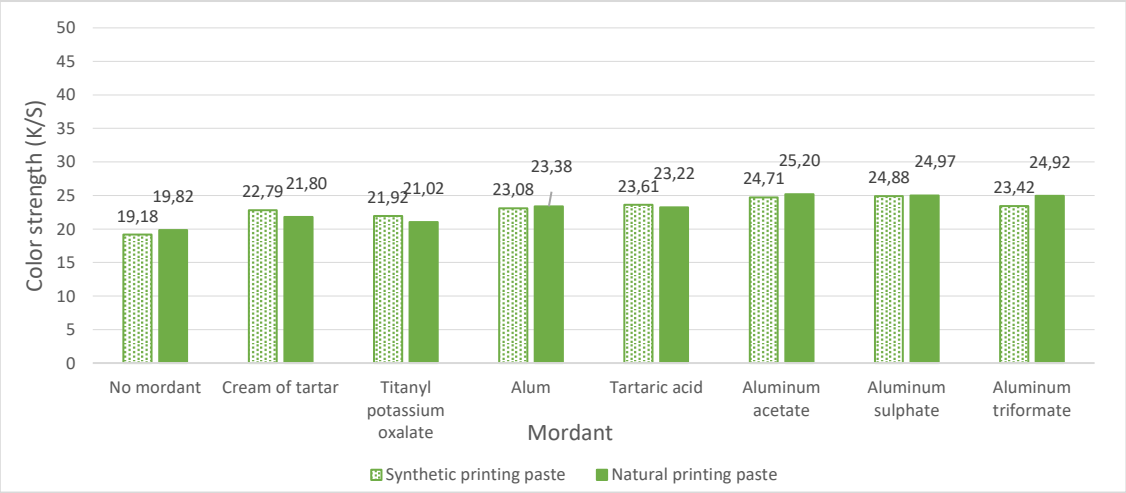


Figure 100. Wool printing with Chlorophyll-a pigment (natural vs synthetic printing paste) color strength(K/S) comparison

2) Chlorophyll-a wool printing process quality assessment through measurement of laundering and lightfastness

Table 53 details the laundering and lightfastness results of the wool samples printed with Chlorophyll-a. Overall, it can be observed an improvement, when compared with the printing of the cotton substrates (Table 51), in the same conditions. Laundering fastness is measured through the behavior against the change of color, with the best results obtained with the non-mordanted samples characterized by fair behavior (4-5), with no improvement for the other samples. The staining of other textile substrates indicates good behavior (4-5) also, but for all the samples, meaning that the residual colorant eliminated from the analyzed sample does not have an affinity for textile substrates, or it is degraded in the process. The lightfastness shows fair behavior (4) with the non-mordanted experimental case, indicating that the colorant has a certain resistance, but with the use of mordants, this behavior degrades towards poor and very poor color resistance against light. These results indicate the viability of coloration with Chlorophyll-a, but further auxiliaries must be assessed for improving the color behavior against laundering and light.

Similar studies approaching algae-based Chlorophyll-a obtained comparable lightfastness with the untreated wool substrate (El-Khatib 2016).

Wool Samples	***Color***	***Staining***						***Lightfastness***
Synthetic paste	***change***	*Wool*	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	4	4-5	4-5	4-5	4-5	4-5	4-5	4
Cream of tartar	2-3	4-5	4-5	4-5	4-5	4-5	4-5	2
Alum	2	4-5	4-5	4-5	4-5	4-5	4-5	2
Tartaric acid	3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminium Triformate	3	4-5	4-5	4-5	4-5	4-5	4-5	3
Titanyl potassium oxalate	2-3	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum acetate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Aluminum sulphate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3

Wool samples Natural paste	***Color change***	***Staining***						***Lightfastness***
		Wool	*Acrylic*	*Polyester*	*Polyamide*	*Cotton*	*Acetate*	
No mordant	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Cream of tartar	2	4-5	4-5	4-5	4-5	4-5	4-5	1-2
Alum	3-4	4-5	4-5	4-5	4-5	4-5	4-5	2
Tartaric acid	3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminium Triformate	3	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Titanyl potassium oxalate	2	4-5	4-5	4-5	4-5	4-5	4-5	2-3
Aluminum acetate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3
Aluminum sulphate	3-4	4-5	4-5	4-5	4-5	4-5	4-5	3

Table 53. Fastness properties of mordanted wool samples printed with Chlorophyll-a in embedded in the synthetic and natural paste

Partial conclusions on wool printing with Chlorophyll-a

- *Type of paste influence on the printing process efficiency* analysis indicates that the **synthetic printing paste** generates significant color differences when compared with the natural alternative ΔE=10,58. Nevertheless, both types of printing paste are located in a similar greenish color area of the CIE*lab* color space.
- *Influence of pre-mordanting on the printability of Chlorophyll-a* was analyzed with the hypothesis of efficiency and coloration quality increase to be generated by the use of 7 different mordants.
    - *Synthetic printing paste* reflectance spectrum analysis indicates very little variation amongst the pre-mordanted samples, with lower performance when compared with the non-mordanted ones, converging around 35-44 values.
    - *Natural printing paste* samples reflectance spectrum analysis shows some improvements obtained with the Aluminum-based mordants, as **Aluminum triformate (10% w.o.f.)**, Aluminum sulphate (20% w.o.f.), Aluminum acetate (20% w.o.f.), Titanyl potassium oxalate (20% w.o.f.), and Cream of tartar (3% w.o.f.), but they are considered negligible results, as the spectrums overlap, but present higher dye uptake than the non-mordanted sample. The range of reflectance capacity converges around 31-43.
    - *Color strength (K/S) values comparison* reveals insignificant differences within all the studied cases. This indicates that Chlorophyll-a obtained from microalgae presents a high potential of use via pigment printing coloration method on wool, without significant influence of the printing paste type, or the pre-treatment of the fabrics. The obtained values merge among 20-25.
- *Light and laundering fastness* do not show improvements with the use of mordants, with the best results obtained with the non-mordanted samples:
    - *Laundering fastness* characterized with the best results was obtained with the non-mordanted samples identified by fair behavior (4-5), with no improvement for the other samples. The staining of other textile substrates indicates good behavior (4-5) for all the samples.
    - The *lightfastness* shows fair behavior with the non-mordanted experimental case (4), indicating that the colorant has a certain resistance against light degradation. These results indicate the viability of coloration with Chlorophyll-

a, but further auxiliaries must be assessed for improving the color behavior against laundering and light.

Overall, according to my knowledge, there is a reduced amount of recent scientific research focused on conventional pigment printing with natural colorants, but they all suggest that natural colorants represent a viable solution for the textile industry. Nevertheless approaches on comparisons between natural and synthetic based printing pastes in printing with natural colorants on proteinic and cellulosic fibers were performed in different studies, and comparable results and conclusions were drawn, reflecting no significant differences due to the type of printing paste nature, but color influence due to some types of mordants, with no significant improvements on the laundering results (Nakpathom et al. 2011) (Rekaby et al. 2009). Nevertheless, some studies propose improvements for specific colors in terms of dye uptake and fastness (İbrahim Bahtiyari et al. 2017). Approaches on cotton printing with natural dyes, reveal light shades, and poor behavior to laundering, comparable with the results obtained in this study (Klančnik 2021).

4.7. Additional applicability assessment for the colored fabrics

The assessment of the 4 algae-based colorants to pH changes, for the identification of additional possible applications, was performed with the use of dyed cotton fabrics via direct contact with highly acidic and highly alkaline solutions. Nevertheless, the dyed wool assessment was discarded due to its high reactivity against alkaline products.

It is important to highlight that relevant results were obtained for phycobiliproteins, C-phycocyanin and R-phycoerythrin, and no results for β-carotene and Chlorophyll-a. This may be justified by the structural differences among the colorants, (as detailed in Chapter 1), and the demonstrated pH sensitivity of the protein chromophores, phycocyanins and phycoerythrins. In this sense stability tests regarding their sensitivity to pH indicate color stability at pH ranging from 5 to 7 for both phycocyanin (Patel et al. 2004)(Antelo et al. 2008), and phycoerythrin (Bharmoria et al. 2020) (Hsieh-Lo et al. 2019).

Even though there is vast literature related to the pH of Sodium hydroxide solution and Acetic acid, the pH was verified with pH indicator test paper. In the following image (Figure 101) it can be confirmed the pH of Sodium hydroxide solution with values approximating 11, and Acetic acid pH approximating 2-3.

Figure 101. Validating the pH of the treatment solutions with pH indicator test paper

The pH change analysis of the cotton dyed samples can be subjectively evaluated in the following image (Figure 102), with clear color shifts easily observed.

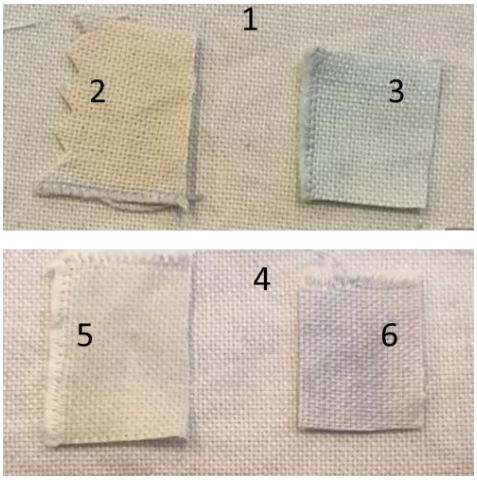


Figure 102. Visual assessment of the pH treatment behavior of the phycobiliproteins cotton dyed fabrics

M1. Cotton dyed with C-phycocyanin; M2. Cotton dyed with C-phycocyanin treated with acidic solution; M3. Cotton dyed with C-phycocyanin treated with alkaline solution; M4. Cotton dyed with R-phycoerythrin; M5. Cotton dyed with R-phycoerythrin treated with acidic solution; M6. Cotton dyed with R-phycoerythrin treated with alkaline solution

The objective analysis was performed through CIE*Lab* coordinates assessment, considering in all cases the non-mordanted dyed cotton fabrics, and the color modifications were compared to the reference sample. The reference samples are highlighted accordingly in the results table (Table 54).

Sample	***CIELab color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Cotton dyed with C-phycocyanin	78,41	-0,50	2,40	-	-	-	-
Cotton dyed with C-phycocyanin treated with acidic solution	69,15	0,99	12,94	-9,26	1,49	10,54	14,11
Cotton dyed with C-phycocyanin treated with alkaline solution	74,24	-4,54	0,58	-4,17	-4,04	-1,81	6,08
Sample	***CIELab color coordinates***			***Color differences***			
	L	***a***	***b***	***ΔL***	***Δa***	***Δb***	***ΔE***
Cotton dyed with R-phycoerythrin	78,56	1,46	3,51	-	-	-	-
Cotton dyed with R-phycoerythrin treated with acidic solution	73,26	1,87	4,20	-5,30	0,41	0,69	5,36
Cotton dyed with R-phycoerythrin treated with alkaline solution	74,27	1,23	3,00	-4,28	-0,22	-0,51	4,32

Table 54. CIElab coordinates of the pH color induced behavior on phycobiliproteins dyed cotton fabrics

CIE*Lab* measurements confirm the visual analysis results indicating clear color difference among the dyed samples and the different pH solutions treated samples. The ΔE>3 confirms significant color difference among the analyzed samples and the reference ones (the lastest highlighted in Table 54).

The luminosity coordinates (L^*) analysis indicate an increased darkening (color intensity increased) of the samples treated with the acidic solutions, with ΔL =-9,26 obtained for the treatment of C-phycocyanin dyed cotton samples, and with ΔL =-5,30 for the treatment of R-

phycoerythrin dyed samples. Nevertheless, the alkaline treatment generates also a darkening effect, but with a reduced intensity compared to the acidic experimental cases.

The specific analysis of the color coordinates (*a** and *b**) indicates a specific color behavior for both phycobiliproteins dyed cotton fabrics assessed and is visually plotted in the diagram below.

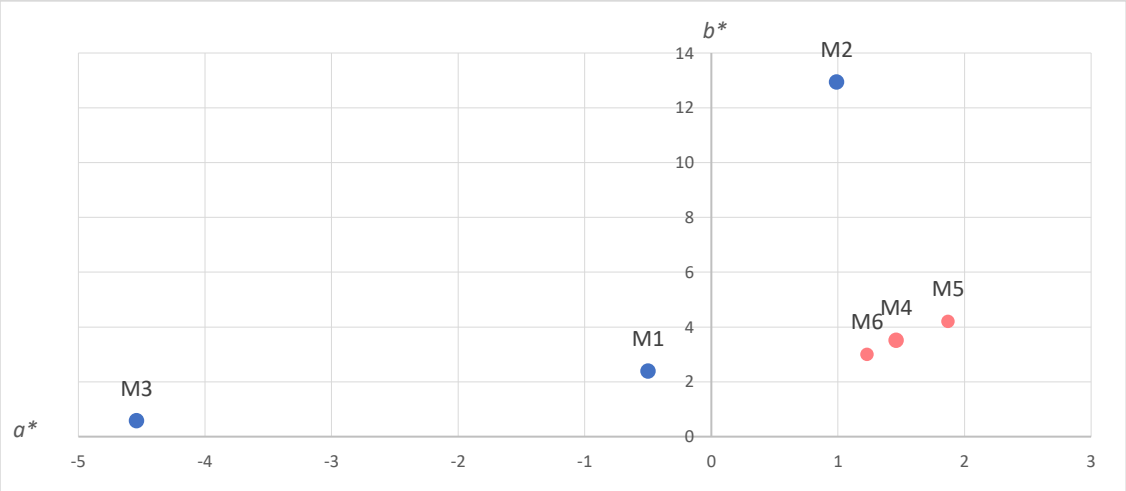


Figure 103. Color specifications in CIELab of pH color induced behavior on phycobiliproteins dyed cotton fabrics

M1. Cotton dyed with C-phycocyanin; M2. Cotton dyed with C-phycocyanin treated with acidic solution; M3. Cotton dyed with C-phycocyanin treated with alkaline solution; M4. Cotton dyed with R-phycoerythrin; M5. Cotton dyed with R-phycoerythrin treated with acidic solution; M6. Cotton dyed with R-phycoerythrin treated with alkaline solution

The acidic solution treatment generates an increase of the *a** coordinate, indicating a color switch towards the red color space; and at the same time the *b** coordinate value increases, determining the orange color space location. The alkaline experiments reveal a decreasing pattern in both analyzed coordinates, as *a** values decrease shifting towards the green color space and the *b** coordinates also decrease generating a more blueish color.

Overall, a more impacting and aggressive effect is revealed in the experimental case of treating the C-phycocyanin dyed cotton fabrics, with more intense color differences, as it can be observed in Figure 103, reaching the conclusion that the most suitable option for possible pH indicating applications would be the C-phycocyanin colorant matter applied through exhaustion dyeing on cotton textile substrate.

Chapter 5. Conclusions

The following chapter presents an extended summary of the main results obtained and draws conclusions based on the assessment of the experimental approach of exhaustion dyeing and pigment printing of cotton and wool textile substrates with algae-based colorant matter.

In the context of sustainability, the textile industry refocuses on the use and development of environmentally friendly raw materials and processes for textiles production. More specifically, the textile coloration sector has oriented the research on the use of naturally and sustainably sourced colorant matters, among other alternatives. These sources target mainly plant and animal origin with promising color provision results (Shahid et al. 2013a). Nevertheless, sustainability drawbacks are represented by limiting contaminant environmental emissions and resource consumption, as water and arable land (Muthu 2017). Apart, additional possibilities resulting from the textile industry solutions research, and widely applicable to other sectors, generated through this vast investigation oriented towards textile contamination reduction, should not be discarded.

In this sense, this study proposes a laboratory-scale exploration of the suitability, for textile coloration, of an innovative and sustainable feedstock, the aquatic biomass, represented by micro and macroalgae. This selected biomass is part of an emerging industry, developed for various sets of commercial applications, with the potential to overcome the environmental limitations faced by the naturally sourced colorants from plant and animal origin.

The present work approaches the application of algae-based natural colorant matter on natural textile substrates, like cotton and wool, through conventional processes, as exhaustion dyeing and pigment printing. Various mordants and auxiliaries were analyzed in terms of improvement of dyeability and fastness, jointly with the research of sustainable alternatives for printing auxiliaries.

For the development of this study were **successfully selected appropriate micro and macro algae strains** with a corresponding high yield of colorants, as it follows:

- **Microalgae *Spirulina platensis*** with a high yield of C-phycocyanin, used for **blue** color.
- **Macroalgae *Gracilaria gracilis*** with a high yield of R-phycoerythrin, used for **red** color.
- **Microalgae *Dunaliella salina*** with a high yield of β-carotene, used for **yellow** color.
- **Microalgae *Caespitella pascheri*** with a high yield of Chlorophyll-a, used for **green** color.

The state of the colorant extract influences the dyeing process efficiency.

Considering that there is no source of algae-based colorants for the textile industry, this study mainly focused on the use of tailored colorant extracts in a liquid state, representing the dyestuff-rich solution, which has proven reduced color intensity when applied on both cotton and wool fabrics, in comparison with lyophilized powder extract, considering the same colorant concentration application. The liquid state of the extract influences the dispersion of the chromophore in the dyeing bath and also in the printing paste generating milder tones when compared with the use of powder form.

Laboratory scale exploration of exhaustion dyeing process with algae-based colorant matter on cotton and wool (cellulosic and proteinic) textiles substrates has revealed different process efficiency and fastness, based on the substrates, applied mordants, and respectively the process conditions. Colorant manipulation and compatibility are considered acceptable.

Overall, **cotton substrates present a significantly reduced affinity to the algae-based natural colorants**, but with the possibility of improvement when mordants are conveniently included in the recipe, corresponding to each envisaged colorant matter**. Improved results are**

obtained when the same colorants are applied onto wool substrates, with visibly more intense colors, thus revealing increased dye-fiber affinity.

Among the general optimum exhaustion dyeing process conditions the following summary can be made:

- **The validated liquor ratio for laboratory studies is 1/40, but for industrial applications, a reduction up to 1/20 has been proven to be suitable.**
- **Optimum pH conditions for cotton dyeing are 7, but for wool dyeing is 5, nevertheless, intermediate values in this range are acceptable.**
- **Exhaustion dyeing optimum processing conditions are 65^0C for 60 min, with a rate increase of 2^0C/minute, while ambient temperature drying.**

The C-phycocyanin-rich solution from microalgae *Spirulina platensis* applied on cotton and wool textile substrates through the conventional exhaustion dyeing process **exhibits the potential of the chromophore for the blue textile coloration application.**

Cotton dyeing with C-phycocyanin extract results in mild blue hues, which are almost negligibly improved when the textile substrate is pre-mordanted with **Alum,** and Aluminum triformate.

- *Mordant concentration influence* on color intensity, reveal a significant color difference, with slightly more intensity with the use of **Alum,** and Aluminum triformate, but with lighter results than the non-mordanted samples, even with variation in the concentration applied.
- *Intrinsic natural yellow to brown* color of some of the tested mordants have a major color influence on the resulting dyed cotton fabrics, obtaining yellowish fabrics with the use of Ferrous sulphate and brown hues with the use of in the biomordants Myrobalan, and Oak gall in the pre-mordanting process.

Overall, none of the mordants generated improvements in the dye absorption efficiency when applied to the cotton dyeing process with C-phycocyanin dyestuff, nevertheless, the best compilation of color space coordinates is obtained by the use of Alum. This is confirmed by the analysis of the absorption coefficient of the wastewater effluents, with the dye amount remaining in the effluent similar to the non-mordanted samples. **Laundering and lightfastness properties do not show sufficient improvements with any of the employed auxiliaries.**

Wool dyeing with C-phycocyanin extract results in mild to moderate blue hues, with average improvements with the use of mordants in pre-mordanting and meta-mordanting experiments.

- *Pre-mordanting* process (as a fabric pre-treatment) outcomes display more blueish shades and higher color intensity with most of the auxiliaries employed but with the best results obtained with the application of **Aluminum acetate**, **Titanyl potassium oxalate, Tartaric acid,** and Aluminum-based mordants**.** Nevertheless, the most intense brownish shades from this batch of experiments were obtained with the added value of the intrinsic color of the use of Ferrous sulphate, Myrobalan, and Oak gall with yellowish and brownish results which are not characterized by an affinity between the chromoprotein obtaining.

- *Metamordanting effect* analysis indicated more efficiency with the use of Cream of tartar, in terms of lightness. However, the application of mordants shows improved dyeability regardless of the momentum.
- *Bath ratio influence* analysis among various ratios revealed the best results obtained, with the **medium ratio 1/20,** in terms of darker color and blueish tone obtained. Regardless, this ratio was inadequate for laboratory scale but is the most suitable for industrial applications.
- *Bleaching of wool* influences the dye uptake positively, by modifying the proteinic fiber and allowing the interaction with Titanyl potassium oxalate, and the C-phycocyanin, resulting in a reduction of the yellow coordinate and increasing the blue one.

Overall, the different explored experimental setups presented improved dye absorption efficiency when applied to wool dyeing with C-phycocyanin. This is confirmed by the analysis of the absorption coefficient in the wastewater analysis, with the dye remaining in the water effluent values decreasing in the following order Tartaric acid, Aluminum sulphate, Titanyl potassium oxalate (bleached wool and then natural), Aluminum acetate. **Laundering and lightfastness properties show slight improvements with the employed auxiliaries, being characterized by good behavior overall.**

Phycoerythrin, specifically R-phycoerythrin, obtained from the macroalgae *Gracilaria gracilis,* used for exhaustion dyeing of natural textile substrates as cotton and wool, represents **potential natural colorants for the obtention of light red hues**.

Cotton dyeing with R-phycoerythrin resulted in very light red hues characterizing the dyed fabrics, **with the relatively low improvement on color intensity with the selected mordants but presenting a possible potential for dyeability increase**.

- *Pre-mordanting* of cotton samples did not generate significant color differences with any of the mordants used, but particular values, in terms, of the most different coloration and slightly more intense, were obtained with **Aluminum acetate**, without reaching significant impact.
- *The intrinsic natural color* of mordants, Ferrous sulphate, Myrobalan, and Oak gall interfered with the coloration generating brown colors of the samples.

Overall, in terms of color difference, none of the mordants showed improvement on the dye absorption efficiency when applied to cotton dyeing with R-phycoerythrin. The analysis of the UV-VIS absorbance spectrum of the dyed fabrics confirms the reduced efficiency of the selected mordants as all the dyed fabrics are grouped in the same light-shaded area, except for the biomordants generating dark brown hues. **Laundering and lightfastness properties do not show improvements**, compared with the non-mordanted samples, **except Aluminum acetate, with slight influences**.

Wool dyeing with R-phycoerythrin revealed light reddish shades, with various intensities and color influences generated with the use of mordants. An overall increase in color intensity was observed, when compared with the cotton experimental setting, validating higher affinity of proteic colorant matter with proteinic fibers. **Significant color differences in terms of**

lightness and redness of the fabrics were obtained via the pre-mordanting process, thus validating improvement possibilities and dyeability.

- *Pre-mordanting* of wool revealed significant differences and more intense coloration, with the most efficient auxiliary as **Titanyl potassium oxalate** confirmed by the reflectance spectrum analysis and characterized by a dye uptake of approximately 90%. This efficiency is followed closely by Tartaric acid and Aluminum triformate.
- *The intrinsic natural color* of Ferrous sulphate and biomordants Myrobalan and Oak gall influence darker coloration, but unfortunately restricting the red dye uptake and do not improve the dyeability of the red colorant but interfere with the final results by modifying the expected color.

Overall, in terms of color difference, all mordants presented improved dye absorption efficiency when applied to wool dyeing with R-phycoerythrin, with significant improvement when compared to cotton experiments. The analysis of the UV-VIS absorbance spectrum of the dyed wool fabrics, and the plot of the color diagram indicate that the efficient pre-mordanted dyed fabrics are grouped in the same area, with darker tones. **Laundering fastness behavior shows improvements, in comparison with cotton samples, but no significant variation when compared with the non-mordanted samples. Lightfastness properties count with fair behavior, determined by the natural coloration of the samples, without efficiency increase demonstrated by the use of mordants.**

Carotenoids, specifically β-carotene, obtained from the microalgae *Dunaliella salina,* were applied on natural textile substrates as cotton and wool through the exhaustion dyeing process and were **validated as potential natural colorants for the obtention of yellow hues**.

Cotton dyeing with β-carotene resulted in light hues of yellow dyed fabrics, **with the validated possibility of improvement in color intensity and yellow hue with the application of textile substrate pre-treatment through pre-mordanting processes.**

- *Pre-mordanting* of cotton with **Titanyl potassium oxalate** has revealed as the most efficient process with a more intense and yellowish result, but with approximately 60% of dye uptake, resulting from the analysis of the wastewater effluent. These results are completed by Tartaric acid and Alum with higher dye uptakes.
- *The intrinsic natural color* of mordants **Ferrous sulphate, Myrobalan, and Oak gall boosted the yellow-brown color obtained**, in terms of intensity and hue.

Overall, in terms of color difference, all of the mordants presented improved dye absorption efficiency when applied to cotton dyeing with β-carotene. This is additionally confirmed by the analysis of the UV-VIS absorbance spectrum of the dyed fabrics, where the majority of pre-mordanted dyed fabrics are grouped in the same area, characterized by darker tones. The results reveal a disposition in the range of light hues but, with a tendency to darker hues influenced by the biomordants with intrinsic natural brown color. **Laundering and lightfastness properties** do not show improvements with the majority of mordants employed, **except for Aluminum triformate and Myrobalan, and very slight improvement with the use of Titanyl potassium oxalate.**

Wool dyeing with β-carotene resulted in hues of yellow dyed fabrics, with naturally more intense results, when compared to the cotton experimental setting, validating the hypothesis of increased affinity of natural colorants to proteinic fibers. **Improvements in color intensity and yellow hue with the application of textile substrate pretreatment through pre-mordanting processes are validated.**

- *Pre-mordanting* of wool with **Aluminum triformate** has revealed as the most efficient process with a more intense and yellowish result, and with an approximate 97% of dye uptake, resulting from the analysis of the wastewater effluent. Followed by similar results obtained with Aluminum-based mordants, Alum and Aluminum sulphate, and Titanyl potassium oxalate, Tartaric acid, Aluminum acetate, and Cream of tartar.
- *The intrinsic natural color* of mordants **Ferrous sulphate, Myrobalan, and Oak gall boosted significantly** the yellow-brown color obtained, in terms of intensity and hue, but not dye absorption.

Overall, in terms of color difference, all of the mordants presented improved dye absorption efficiency when applied to wool dyeing with β-carotene, with significant improvement when compared to cotton experiments. The analysis of the UV-VIS absorbance spectrum of the dyed wool fabrics indicates that the majority of pre-mordanted dyed fabrics are grouped in the same area, indicating darker tones. **Laundering and lightfastness properties** show slight improvements with the use of the majority of mordants employed, **from poor behavior towards fair behavior**.

Chlorophyll, green colorant matter, specifically Chlorophyll-a, sourced from microalgae *Caespitella pascheri* was properly employed in the exhaustion dyeing of cotton and wool**. It revealed high potential as dyestuff for natural textile substrates green coloration.**

Cotton dyeing with Chlorophyll-a colorant matter resulted in relatively light green shades, with **a potential of improvement of dyeability with the use of mordants as auxiliaries as efficiency enhancers.**

- *Pre-mordanting* of cotton samples resulted in improvements in terms of color intensity, with greener results, with the use of **Titanyl potassium oxalate,** and **Aluminum acetate,** with relevant dye uptake percentages obtained through the analysis of the wastewater effluent, reaching up to 90%.
- *The intrinsic natural color* of mordant **Myrobalan and Oak gall has interfered positively with the green coloration** generating darker colors of the samples located in the same color space.

Overall, in terms of color difference, **heterogeneous results were obtained**, with efficient ones and with other generating insignificant color differences. The UV-VIS reflectance spectrum indicates the division among the different efficiencies of the tested mordants. **Laundering and lightfastness properties reveal poor behavior in terms of color degradation and do not reflect relevant improvements with the use of mordants,** with the exception of Myrobalan and Oak gall.

Wool dyeing with Chlorophyll-a, resulted in relatively intense green tones, with slight shades variations influenced by the use of mordants. **Great potential in dyeability improvements with the use of pre-mordanting textiles substrates.**

- *Pre-mordanting* of wool generated an increase in dyeability with notable color differences and more intense coloration with the use of **Titanyl potassium oxalate**, and **Aluminum acetate**. All the indicated mordants show a dye uptake surpassing 95%, according to measurements of the wastewater analysis.
- *The intrinsic natural color* of **Myrobalan** and **Oak gall** generates a rather darker green coloration of the wool substrates due to the intrinsic natural brown color **compatible with the Chlorophyll-a.**

Overall, in terms of color difference, the same **heterogeneity** is observed as in the case of the cotton fabrics **indicating specificity among colorants and the Chlorophyll-a structure. The metallic mordants and biomordants improve color characteristics generating more intense greener samples. Laundering and lightfastness** show very poor overall behavior on the non-mordanted samples and do not reveal significant improvement with the use of mordants**, except the use of Titanyl potassium oxalate and Myrobalan, without reaching the expected results, but need more improvements.**

Preliminary exploration of dyeing wastewater effluents quality, treatment approach, and added value assumptions.

Confirmed antimicrobial effect against the microorganism *Escherichia coli and Staphylococcus aureus* of the non-mordanted cotton and wool dyed with C-phycocyanin, R-phycoerythrin, and Chlorophyll-a, exception β-carotene.

Confirmed reduced solar protection capacity for dyed non-mordanted cotton and wool samples with C-phycocyanin, R-phycoerythrin, and Chlorophyll-a, with increased values for cotton dyed with Chlorophyll-a-rich extract. The exception is represented by β-carotene, not reaching the lower limit values.

Wastewater effluents resulting from C-phycocyanin dyeing processes of wool and cotton validate the possibility of **safe discharge of the effluents into wastewater treatment plants**, based on the analysis of the quality parameters BOD_5, COD, metals (Aluminum and Iron) concentration. This is completed by **efficient and successful fungi-based wastewater treatment with an increased possibility of treated effluents reuse**.

Cotton and wool dyeing process wastewater effluents analysis reflects a clear reduction of BOD_5 and COD with 3 respectively 4 times magnitude with the application of mordanting processes, thus validating the mordant application positive and enhancing effect.

- In terms of comparison of the laboratory-scale *oxygen demand values*, they are slightly surpassing the average daily maximum limitation of discharge into wastewater treatment plants, according to Spanish regulations. In this sense, it is important to note that laboratory values represent an exaggeration of values, due to the more concentrated effluents generation, as in the industrial context these are mixed with other effluents resulting from rinsing, cooling, and other

processes generating a considerable parameter concentration reduction through dilution.

Wastewater biological fungi treatment was applied on both cotton and wool resulting effluents from dyeing, and it has resulted in a remarkable reduction of BOD_5 and COD parameters values, reaching a decrease of the values up to 80%.

Metal content values are strictly influenced by the use of mordants, specifically the metallic ones, and in this sense, the **concentration of mordant optimization applied through the pre-mordanting process is performed, for complete absorption of the auxiliary by the textile substrate to counter the lack of sustainability induced by in their use**.

- As expected, mordants as Alum, Ferrous sulphate, and Aluminum triformate exceed the daily limit of metals (Aluminum and Iron) discharge in wastewater plants at a laboratory scale, thus representing a decision-making factor in the selection of the percentage of mordant used in the pre-mordanting process in correlation with performance.

Overall, it can be confirmed that the effluents resulting from C-phycocyanin exhaustion dyeing of cotton and wool are good quality wastewaters and present a high possibility for conventional treatment and further reuse.

Assumptions on **assessing plausible theoretic interactions among mordants-natural fibers-algae-based colorant matter** indicate that the analysis of the selected mordants influence as enhancers of the dyeability of algae-based colorant on natural fibers show that is not possible to indicate a specific pattern regarding compatibilities with the selected mordants. Even so, in the context of sustainability, based on the **optimization of the concentration** of the mordant employed is possible to minimize possible wastewater contamination and the proper selection of the mordanting momentum, in this study **pre-mordanting has revealed as most beneficial**. Nevertheless, recommendations on exploring a wider set of natural mordants can be made, or even combinations among various types of auxiliaries should be performed.

Dyeing process results indicate reduced affinity of natural algae-based extracts analyzed towards cellulosic and proteinic fibers, by analyzing cotton and wool behavior, nevertheless demonstrating high potential for improvement. Consequently, solutions as the state of the colorant matter, exploring different mordants or application processes must be considered. In this sense, their application via pigment printing was analyzed. The conclusions for the printing process are discussed below.

Laboratory scale exploration of pigment printing process with algae-based colorant matter on cotton and wool textiles substrates (cellulosic and proteinic) have revealed different process efficiency and fastness, based on the substrates, the applied mordants, employed paste auxiliaries, and process conditions. Colorant manipulation and compatibility are considered acceptable. The viability of the alternative natural printing paste use with natural colorants is confirmed.

Overall, algae-based phycobiliproteins, including R-phycoerythrin and C-phycocyanin, completed with β-carotene and Chlorophyll-a, have proven good manipulation properties for printing, in terms of good combination with each of the experimental versions of the printing

pastes. Light blue, respectively light red colors, and yellow and green colors were obtained for printed cotton and wool textiles substrates. It has been observed that **pre-mordanting has a low influence on laundering and lightfastness** results.

Among the general conclusions on optimum printing process conditions, the following summary can be made:

- **For conventional synthetic printing paste maximum curing step may be performed for 3 minutes at 150° C, and the drying step could include ventilation at 40° C before significant pigment degradation occurs.**
- **For natural printing paste, all the curing and drying steps could be performed at 20° C, due to the self-crosslinking capacity of the auxiliaries. This process is most beneficial for proteic chromophores, which are temperature sensitive, nevertheless, improvement in color intensity may apply to all the natural colorants considered.**

C-phycocyanin from microalgae *Spirulina platensis* was properly applied on cotton and wool substrates via natural or synthetic printing mother paste revealing a **high potential** for its application in coloration through **printing application**.

Cotton printing with C-phycocyanin colorant matter through the use of conventional synthetic and natural printing paste revealed attractive light-blue colored textiles. Effects of the type of printing paste and influence of mordants on printability revealed the following conclusions:

- *Type of paste influence on the printing process efficiency* indicates that **the natural printing paste generates significant color differences and more intense blueish shades** in comparison with the synthetic paste, but with insignificantly lower color strength.
- *Influence of pre-mordanting on the printability of C-phycocyanin* was analyzed via the measurement of the reflectance spectrum, and overall it indicates no significant improvements:
    - *Synthetic printing paste* indicating insignificant color differences, with very slight improvements conferred by the use of Aluminum-based mordants as **Aluminum sulphate, Aluminum acetate**, and followed by **Titanyl potassium oxalate** indicating a higher absorption capacity.
    - *Natural printing paste* presents the same low coloration improvements as the synthetic printing paste, and with the more blueish colors with the use of **Alum** and **Aluminum sulphate.**
    - *Color strength (K/S) values comparison* indicate insignificant differences regarding the mordants effectiveness, regardless of the type of paste used.

Overall, similar light blue colors, in terms of intensity and hue, were obtained in all the experimental cases, with the best results obtained in the non-mordanted case, indicating that the pre-mordanting process is unnecessary for pigment printing of cotton fabrics with C-phycocyanin. **Fastness analyses** indicate that **laundering testing showed fair behavior** in terms of staining and degrading and **very poor resistance against the light**, without improvements generated by the use of mordants.

Wool printing with C-phycocyanin via synthetic commercial and natural printing paste generated more intense blue colored textiles when compared to the cotton experimental case.

The color and process efficiency analysis was oriented towards the comparison of influences generated by the type of printing paste and pre-mordanting implications:

- *Type of paste influence on the printing process efficiency* indicates the obtention of significant color difference generated with the use of **natural paste, with more intense blueish tones,** as confirmed also by the color strength values.
- *Influence of pre-mordanting on the printability of C-phycocyanin* over wool presents overall insignificant efficiency, with most of the corresponding reflectance spectrums located in the same area.
    - *Synthetic printing paste* analysis indicates similar spectrums, even though without significant differences with the non-mordanted samples, with the use of **Aluminum sulphate.**
    - *Natural printing paste* reflectance spectrum analysis indicates slightly overlapping spectrums, with similar results as the non-mordanted samples, but the most efficient in this context appears to be the **Aluminum acetate**, slightly darker than the rest of the samples.
    - *Color strength (K/S) values comparison* indicates that pre-mordanting does not generate significant differences in terms of dye uptake or process efficiency, and comparable results are obtained with the same mordant and different printing paste types. On the other hand, the obtained values overall are in the same range, indicating that there is no need for the pre-mordanting step.

Overall, the same range of light blue color, in terms of intensity and hue, are obtained in all the experimental cases, with the best results obtained with the application of the Aluminum sulphate in combination with the natural printing paste, but regardless, it is worth indicating that the pre-mordanting process is an unnecessary step for pigment printing of wool fabrics with C-phycocyanin. **Fastness analysis** indicates that **laundering testing showed fair behavior** in terms of staining and degrading and **very poor resistance against light**, similar to the cotton experimental cases, without improvements with the use of mordants as printability enhancing auxiliaries.

R-phycoerythrin from red macroalgae *Gracilaria sp.* was properly applied on cotton and wool fabrics via synthetic and natural alternative printing pastes through a pigment printing process, revealing **great potential for sustainable printing of cotton and wool fabrics.**

Cotton printed textile substrates with R-phycoerythrin embedded in commercial synthetic pastes and alternative sustainable printing pastes obtaining light red colors, compared for efficiency identification purposes.

- *Type of paste influence on the printing process efficiency,* revealed very similar (almost identical values) overall in the color space, with statistically insignificant color differences in the comparison of synthetic paste vs. natural paste. Also, a **slight increase in color intensity was generated by the employment of the natural printing paste**.
- *Influence of pre-mordanting on the printability of R-phycoerythrin* was analyzed approaching both types of printing pastes, by analyzing the color uptake capacity variation determined by the use of mordants.
    - *Synthetic printing paste* reflectance spectrums overlap in a very small range of the absorbance values, thus a very small difference in uptake capacity was

influenced by the pre-mordanting process. Nevertheless, the most intense colored sample spectrum, with the highest light absorbance level was obtained without the use of mordants.

- *Natural printing paste* presents slight influences in the spectrums dispersion on the graph, with insignificant but higher efficiencies generated by the use of **Alum**, and Tartaric acid.
- *Color strength (K/S) values comparison* indicates very slight differences in color strength, and most of them indicate that the natural printing paste is more efficient.

Overall, the same range of light pink color, in terms of intensity and hue, are obtained in both experimental cases, which indicates that the printing process is possible for pigment printing of cotton fabrics with R-phycoerythrin. Nevertheless, it is also demonstrated **the potential of sustainable alternatives of natural printing pastes, with existing commercial auxiliaries**, without efficiency alternations with the use of mordants. **Fastness results** indicate similar values for the compared experimental cases, in terms of staining indicating relatively **no staining when laundering,** but with an exception in color change analysis**, revealing more resistance with the use of synthetic printing paste. In terms of light fastness, a very slight improvement was identified with the use of synthetic printing paste.**

Wool printed textile substrates with R-phycoerythrin embedded in commercial synthetic and natural printing pastes revealed obtaining **suitable red colors,** with increased intensity when compared with the cotton experimental cases.

- *Type of paste influence on the printing process efficiency* analysis indicates that the **natural printing paste generates** significant color differences with the conventional synthetic printing paste, **with more intense reddish coloration**. Nevertheless, both printing pastes used in the experimental case reveal the expected results with good manipulating behavior of R-phycoerythrin as colorant matter.
- *Influence of pre-mordanting on the printability of R-phycoerythrin* was analyzed approaching both types of printing pastes, for identifying possible improvements in printability determined by the use of mordants.
    - *Synthetic printing paste* reflectance spectrums are grouped in the same area of the graph indicating very small differences among the samples, with the highest dye uptake generated by the non-mordanted samples, revealing no significant effect with the use of mordants.
    - *Natural printing paste* reflectance spectrums present very small variations in their distribution with the highest efficiencies obtained with the use of **Titanyl potassium oxalate**, and Aluminum sulphate.
    - *Color strength (K/S) values comparison* indicates very slight differences in color strength, and most of them show that the natural printing paste is more efficient but comparable with the synthetic paste. Nevertheless, the mordant use does not prove necessary inclusion in this type of coloration.

Overall, similar red coloration was obtained with all the experimental cases analyzed, validating the possibility of wool coloration with algae-sourced R-phycoerythrin**, with both natural and synthetic printing pastes**, eliminating the pre-treatment mordanting step, due to

reduced proven efficiency. **Fastness** against common degrading agents in the textile industry, as laundering and light indicate relatively **no staining when laundering, and fair behavior of the non-mordanted samples to color change. In terms of lightfastness, poor behavior characterizes the samples**. Nevertheless, not or insignificant improvements were obtained with the use of mordants, as better results were obtained with the non-mordanted samples.

Carotenoids from microalgae *Dunaliella salina,* specifically β-carotene, were successfully embedded in commercial synthetic and alternative natural printing pastes and applied onto cotton and wool textile substrates. Intense yellow fabrics were obtained revealing the **very high potential** for this coloration approach via **conventional pigment printing**.

Cotton printing with β-carotene embedded in synthetic and natural printing pastes revealed intense and attractive yellow colors, with certain shades and intensities variations due to the printing paste composition and the pre-mordanting processes applied.

- *Type of paste influence on the printing process efficiency* indicates that with the employment of **the synthetic paste, darker samples** (more intense yellow) were obtained, but with **insignificant less yellow color coordinates than the naturally printed cotton**. This indicates **slightly more compatibility of β-carotene with the synthetic printing paste.**
- *Influence of pre-mordanting on the printability of β-carotene* was assessed within the samples reflectance spectrum and it indicates **very similar results in all the experimental cases**:
    - *Synthetic printing paste* applied on pre-mordanted cotton substrates reveals overlapping spectrums, thus no significant difference in dye uptake, except for **Titanyl potassium oxalate**.
    - *Natural printing paste* study case onto pre-mordanted cotton follows the same patterns as the synthetic printing paste, thus validating the **potential of natural printing with β-carotene. Titanyl potassium oxalate** represents the mordant that indicates the extreme values in this range of similarity.
    - *Color strength (K/S) values comparison* indicate similar results in terms of color uptake, regardless of the type of paste used, with a slight increase generated with the use of the synthetic printing paste, nevertheless confirming the hypothesis of the potential of the use of natural printing pastes.

Overall, similar intense yellow colors, in terms of intensity and hue, were obtained in all the experimental cases, indicating that the pre-mordanting process is unnecessary for pigment printing of cotton fabrics with β-carotene. **Fastness analysis** indicates that **laundering testing showed fair behavior** in terms of staining and degrading and **poor resistance against the light**, with not or very low improvements generated by the use mordants, highlighting, in this case, the use of Titanyl potassium oxalate.

Wool printing with β-carotene using commercial synthetic and natural printing pastes resulted in intense yellow samples with a **very high potential for yellow coloration**. The use of mordants and type of printing pastes employed presented very slight influences in the yellow shades obtained, specifically:

- *Type of paste influence on the printing process efficiency* **validates the use of natural paste for β-carotene embedded printing, with slightly more intense yellowish shades**, but with very similar color strength values.
- *Influence of pre-mordanting on the printability of β-carotene* over wool present overall insignificant efficiency, with very similar results for both printing pastes assessed:
    - *Synthetic printing paste* reflectance spectrums present grouped values with insignificant differences, but with more intense coloration provided by **Aluminum triformate**, and even reduced performance generated with the use of Alum.
    - *Natural printing paste* reflectance assessment follows closely the assumptions identified with the use of the synthetic printing paste, with the most efficient being **Aluminum triformate**, but indicating the possibility of employment of a more sustainable alternative, without the need of the pre-mordanting process.
    - *Color strength (K/S) values comparison* reveals similar efficiencies in both analyzed study cases and pre-mordants, and finally validating the use of the natural printing paste without efficiency or performance differences.

Overall, similar ranges of intense yellow colors, in terms of intensity and hue, are obtained in all the experimental cases, with the best results obtained with the application of the Aluminium triformate in combination with the natural and synthetic printing paste, but regardless indicating that the pre-mordanting process is an unnecessary step for pigment printing of wool fabrics with β-carotene. **Fastness to laundering and light analysis** results indicate **very good behavior to staining** on the tested textile substrates, meanwhile, color degradation is characterized by **fair behavior. Poor resistance against the light**, with insignificant improvements generated by the use of mordants.

Chlorophyll-a from green microalgae *Caespitella pascheri* was successfully applied on cotton and wool fabrics via synthetic and natural alternative printing pastes through a pigment printing process, revealing a **very high potential for sustainable printing of cotton and wool fabrics with green colorant matter.**

Cotton printing with Chlorophyll-a embedded in commercial synthetic and natural printing pastes resulted in bright green colors and presented good manipulation and compatibility properties. Various experimental sets were compared for efficiency identification purposes:

- *Type of paste influence on the printing process efficiency* analysis indicates overall significant color variation among the use of natural vs. synthetic printing paste, with **more intense results obtained with the natural printing paste.** Nevertheless, the greenish coordinates indicate insignificant tones differences generated by both printing pastes. **It can be assumed that both pastes reveal acceptable results for the printing of cotton with the Chlorophyll-a-rich extract**.
- *Influence of pre-mordanting on the printability of Chlorophyll-a* was analyzed approaching both types of printing pastes, by analyzing the color uptake capacity variation determined by the use of mordants.
    - *Synthetic printing paste* reflectance spectrums of the printed pre-mordanted cotton samples vary, and the results may be ranked starting with the darkest

colors obtained mainly with the use of **Cream of tartar**, and non-mordanted samples. Nevertheless, these differences are statistically insignificant.

- *Natural printing* paste reflectance spectrums converge around similar values, with slight differences allowing the classification of the results with the indication of the less reflective (more intense) samples: **Aluminum acetate** and Aluminum sulphate.
- *Color strength (K/S) values comparison* indicates clear difference generated by the increased dye uptake obtained with the natural printing paste, probably due to the paste composition, as binder and thickener, and not justifying the use of mordants.

Overall, the same range of bright greenish colors is obtained in both experimental cases, validating the possibility of using algae-based Chlorophyll-a for the coloration of cotton textile substrates. Nevertheless, there is no evidence of significant process improvements with the use of mordants as coloration enhancers. **Light and laundering fastness results show poor behavior overall, regardless of the used printing paste or the auxiliaries used** but with the indication of **no staining when laundering**. The selected mordants do not improve the behavior against color degrading agents, thus further assessments must be performed.

Wool printing with commercial synthetic and natural printing pastes resulted in **satisfactory green colors, with suitable compatibility of the algae-based Chlorophyll-a** with the printing pastes and affinity with the proteinic textile substrates.

- *Type of paste influence on the printing process efficiency* analysis indicates that the **synthetic printing paste generates** significant color differences when compared with the natural alternative. Nevertheless, both types of printing paste are located in a similar greenish color area of the CIE*lab* color space.
- *Influence of pre-mordanting on the printability of Chlorophyll-a* was analyzed with the hypothesis of efficiency and coloration quality increase to be generated by the use of mordants.
    - *Synthetic printing paste* reflectance spectrums analysis indicates very little variation amongst the pre-mordanted samples, with lower performance when compared with the non-mordanted ones.
    - *Natural printing paste* samples reflectance spectrum analysis shows some improvements obtained with the Aluminum-based mordants, as **Aluminum triformate**, Aluminum sulphate, Aluminum acetate, Titanyl potassium oxalate, and Cream of tartar, but they are considered negligible results, as the spectrums overlap, but present higher dye uptake than the non-mordanted sample.
    - *Color strength (K/S) values comparison* reveals insignificant differences within all the studied cases. This indicates that Chlorophyll-a obtained from microalgae presents a high potential of use via pigment printing coloration method on wool, without significant influence of the printing paste type, or the pre-treatment of the fabrics

Overall, green coloration was successfully provided to wool samples, on all the analyzed samples, validating the possibility of wool coloration with algae-sourced Chlorophyll-a, **with the use of both natural and synthetic printing pastes.** The pre-treatment mordanting step with the

analyzed mordants may be eliminated, due to reduced efficiency, except for some Aluminum-based mordants, which deserve further exploration. **Laundering fastness** characterized with the best results was obtained with the non-mordanted samples identified by fair behavior, with no improvement for the other samples. **The staining on other textile substrates indicates good behavior** for all the samples. The **lightfastness** shows **fair behavior with the non-mordanted experimental case,** indicating that the colorant has a certain resistance. These results indicate the **viability of coloration with Chlorophyll-a**, but further auxiliaries must be assessed for improving the color behavior against laundering and light.

Additional applicability assessment for the colored fabrics oriented towards emphasizing the sensibility of the algae-based colorants to extreme pH values, revealed a very high potential of **C-phycocyanin dyed cotton as pH indicator,** due to relevant color shifts, from blue to red-orange tones due to acidic interference, and from blue to green and more intense bluer shades generated by alkaline interference. Additionally, R-phycoerythrin presented similar and relevant results, with less intensity in the coloration changes.

As a summary of the previously detailed conclusions, **it can be confirmed the initial hypotheses considering the possibility of application of algae-based natural colorants in textile coloration applications, through exhaustion dyeing and pigment printing on natural substrates as cotton and wool.**

Nevertheless, considering the natural character and temperature related sensitivity of the colorant matter, **process temperature and time** are **key points** for both considered coloration processes. In the same context, due to the previously mentioned critical parameters, **pre-mordanting is the most suitable pre-treatment for the textile substrates for exhaustion coloration,** and from a laboratory-scale exploration point of view, the following assumptions can be made with the indication of the most successful results obtained:

- Exhaustion dyeing with algae-based colorant matter is susceptible to improvements with the use of mordants, with the following highlights:
  - **C-Phycocyanin dyeing by exhaustion of cotton** pre-mordanted with **Alum** and **wool** with **Titanyl potassium oxalate**.
  - **β-carotene dyeing by exhaustion of cotton** pre-mordanted with **Titanyl potassium oxalate** and **wool** with **Aluminum triformate**.
  - **R-Phycoerythrin dyeing by exhaustion of cotton** pre-mordanted with **Aluminum acetate** and **wool** with **Titanyl potassium oxalate**.
  - **Chlorophyll-a dyeing by exhaustion of cotton and wool** pre-mordanted **with Titanyl potassium oxalate**
- Pigment printing with algae-based colorant matter with natural and synthetic printing pastes equally presents very high potential but is not susceptible to improvements with the use of explored mordants. Nevertheless, the mild conditions of the natural printing paste have an overall benefit in the efficiency of this coloration process.
- High added value for specific colors is attributed to the intrinsic natural brownish colors of bio-mordants Myrobalan and Oak gall and the yellowish mordant Ferrous sulphate, for the dyeing processes approached in this study. Nevertheless, due to the interference with the starting color base of the textile, they were eliminated in the pigment printing studies, for having a white base for this exploration study.

One of the most important highlights reflects the **lack of affinity and very low efficiency of the use of mordants in combination with natural dyes and cotton fabrics,** also validated by good staining behavior on other types of fibers. On the contrary, **wool substrates do benefit from the increased affinity, improved by the use of mordants**.

Pigment printing is suitable for the naturally sourced colorant matter, with fair behavior in the printing paste preparation (natural or synthetic) and with a confirmed sustainable alternative (natural printing paste).

Lightfastness is a great limitation faced by the use of these natural colorants and has shown slight improvements when a **protein stabilizer** was added in the extraction process of the proteic colorants, as **C-phycocyanin and R-phycoerythrin**.

The identified added value of the algae-based colorant matter based on the applications in the textile industry has been positive in terms of antibacterial and solar protection capacity, which defines the possibility of an increased range of applications, starting from coloration, with promising potential for functional finishing approaches.

Interestingly the **pH indicator character has been validated at proof-of-concept scale**, opening the way to additional applicability of the C-phycocyanin colorant matter to other relevant sectors.

Finally, this exploratory study validates at laboratory scale a possible sustainable solution for an alternative natural colorant matter for exhaustion dyeing and pigment printing process for the textile industry, with key elements for textile functionalization perspectives, and opening the path for applicability in additional sectors.

Chapter 6. Recommendations for future works

The final chapter proposes approaches for further investigations based on the results obtained and discussed in the previous sections of this study, establishing recommendations that may ensure further developments and direct the research towards processes upscaling and industrial application of the explored solution.

The laboratory explorations presented in this study have **validated the sustainable character of the algae-based dyestuff starting from raw material sourcing up to the process resulting effluents and support the continuation of studies for the coloration process efficiency improvement with a potentially positive long-term impact on the textile industry and possibly favor additional sectors**. Recommendations for future works propose improvements of light and laundering fastness, due to the imperative need of increasing competitiveness with the globally used synthetic alternatives, completed by process and applications upgrading.

In this sense proposals based on diverse approaches are recommended, as it follows:

The sourcing of the natural colorants should be reconsidered, as the initial approach was focused on tailored cultivation, considering that the liquid state of the colorant-rich extract was essential for the proof-of-concept validation. Nevertheless, tests with lyophilized, powder form revealed positive results. Commercial colorants may be purchased in higher quantities from algae farms focused on different applications, as food, feed, cosmetics. In this sense, collaboration with these producers for the definition of the characteristics of the dyestuff is of great importance for the industrial scalability of this approach.

Pretreatment of the natural textile fibers for increasing the dyestuff colorant affinity, with a focus on maintaining the sustainability character of process inputs. In this sense, *a new series of fiber-specific biomordants*, for an extension of the exploration range of auxiliaries and efficiencies analysis is proposed. Further works should approach natural mordants sourced from plants with increased tannin content and high accumulations of metal, such as pomegranate peel, natural polyphenols, banana leaves, rosemary, and thuja leaves. On the other hand, due to the very low bonding between algae-based colorants and *cotton, mordant combinations* should be tested for the analysis of the efficiency of the complexation process. Apart from the auxiliary's selection, fiber surface modifications should be considered, due to the chemicals-free approach, as cold plasma treatment, with proven capacity of increasing fibers roughness and mechanical adhesion, even though not improving chemical bonding. Thus, this treatment may have a beneficial effect when considering pigment printing as a coloring technique, as it has been demonstrated that plasma does not only affect the roughness, but some chemical species appear on the surface increasing its capability to react with some compounds. On the other hand, considering cost-related aspects, it is proposed to make a comparison of the results with the dyeing efficiency improvement produced by alternative technologies as microwave and ultrasonic radiations.

Coloring technologies selection needs high consideration of the limiting parameter, the high-temperature sensitivity of natural colorants, especially the proteinic ones. In this sense is proposed the exploration of alternative dyeing methods and self-cross-linking printing auxiliaries, with special attention to the processing temperature. Regarding the wet coloration methods, further research on low-temperature dyeing processes, like the ones based on impregnation, or even study the kinetics for bath exhaustion procedure, complemented with low-temperature curing auxiliaries is suggested. Nevertheless, alternative technologies facilitating the improvement of dye diffusion to fibers as microwaving and ultrasonication of the dyeing liquor with the immersed pretreated textile, represent an additional follow-up proposal for the coloration with algae-based natural dyestuff. Innovative and sustainable application of

dye carriers, as liposomes are suggested for the protection of the natural colorant from the degradation generated by the dyeing process, thus facilitating non-toxic transport towards the pretreated fiber, without the need for altering the conventional dyeing process.

A strong recommendation is focused on the exploration of micro-nebulization finishing, where the application process of the colorant is performed via midst generation and allows complete control over the process parameters, reduced water input, and dyestuff quantity, in comparison with a conventional dyeing process. Benefits include cost reduction generated by the current price of the colorant matter and improvement of sustainability due to reduced water use.

Considering the initial promising results concerning the pigment printing process, it is proposed a further exploration of natural alternatives for the printing process components as thickeners and binders, with emphasis on self-cross-linking finishing agents, for process temperature reduction, and implicitly energy reduction. The proposal to enlarge the analysis of natural thickening agent's efficiency as Sodium alginate, Chitosan, Guar, and Arabic gum complemented with commercial sustainable self-cross-linking binders, would emphasize the suitability of the application of these algae-based colorants in the textile finishing industry.

An additional recommendation would focus on the digital printing technique, due to the good behavior of the dyestuffs identified in this study, completed by the prospect of obtaining a suitable solution of the colorant matter for droplet application, and the possibility of fixing method control in order to avoid high temperature of the process.

Textile substrate selection revealed promising intrinsic affinity towards the proteinic fibers, specifically wool. For that, it is considered that a study of interest is represented by the exploration of this compatibility between the algae-based dyestuff and other proteinic naturally sourced fibers as silk, mohair, cashmere, or other animal and insect fibers, due to their numerous reactive functional groups content. Nevertheless, it is important to generate an extended analysis on the bonding existing between algae-based chromophores and various types of natural fibers and biopolymers, for the identification of the most efficient sustainable coloration.

Characterization studies should consider the theoretical approach on the interactions among fibers, mordants, and algae-based natural colorants in this study, thus an extension of the approach by using instrumental techniques as FTIR (Fourier Transform Infrared) for the definition of the chemical reactions occurring among mordants at fiber-colorant level and fiber-mordant-colorant scale, would be recommended. Nevertheless, SEM (Scanning Electron Microscope) analysis for the identification of possible changes on the fibers' surface, with the application of mordants and coloration techniques, would be of great interest in better understanding the interaction of these natural colorants and the textile fibers. Apart, enriching the wastewater characterization approach of this study, approaching 3 main parameters (COD, BOD_5, and metals <Aluminum and Iron>), by adding parameters that would cover the most common pollutants defining textile wastewaters biodegradable organic compounds, heavy metals, suspended solids, pathogens, nutrients, toxic organic and inorganic compounds refractory organic compounds, dissolved inorganics, considering that the use of mordants and auxiliaries may have a contaminant character, would represent a great asset.

A commercial design-oriented approach can be recommended, as an exploration for the short-term application of algae-based colorants, in the actual state of the results, considering circular economy application through a reuse concept. Taking advantage of the low laundering and lightfastness results, a garment may be dyed with algae-based colorants, with the condition of having determined the usability cycles. In this sense, it is offered to the consumers the possibility to return to the dyeing or printing facility for color reapplication, once the color has degraded thus, taking advantage of a new garment, and apply the ¨use and reuse¨ concept. Then a study on the dyes' degradability and redyeing possibilities, including techniques approach aiming at obtaining a suitable color quality could be of great interest. Even it would be worth studying the exploration of a pre-bleaching process with a wide array of bleaching agents allowing the characterization of the bleaching process and also its effect on fibers.

Apart from conventional textile applications, the promising results obtained regarding the color behavior of C-phycocyanin and R-phycoerythrin as pH indicators, reacting to both alkaline and especially acidic treatment, open the path to further deepening the analysis of the possibility of this application in various sectors. In this sense, recommendations on enlarging the analysis of the color intensities suitable for pH indicators, identification of the pH range applicability, including techniques of coloration and their influence on the process, would be of interest.

List of contributions

Research projects:

- ¨SEACOLORS¨ grant number LIFE13 ENV/ES/000445, entitled ¨*Demonstration of new natural dyes from algae as substitution of synthetic dyes actually used by textile industries*¨, within the LIFE 2013 ¨Environment 300 Policy and Governance project application¨ program.

- "GREENCOLOR" reference IMDEEA/2017/37 from the Spanish Valencian Institute of Competitive companies, for the project entitled ¨*Study on the application of natural dyes for dyeing and printing textiles*¨

Publications - Papers in Journals

-S. Moldovan, E. Franco, J. Pascual, M. Ferrandiz, Mª Bonet, J. Gisbert, E. Bou, 2021, *Laboratory studies on natural fabrics textile printing with Spirulina platensis sourced phycocyanin*, Fascicle of Textiles, Leatherwork, Annals of the University of Oradea, Vol 22, No.1, pages 53-58, ISSN 1843 – 813X.

-S. Moldovan, M. Ferrandiz, E. Franco, E. Mira, L. Capablanca, and Mª Bonet, 2017, *Printing of cotton with eco-friendly, red algal pigment from Gracilaria sp.,* IOP Conf. Ser.: Mater. Sci. Eng. 254 192011, doi:10.1088/1757-899X/254/19/192011.

- S. Moldovan, M. Ferrandiz, M. Bonet-Aracil, 2017, *Ficoeritrina: colorante rojo de macroalga Gracilaria sp. aplicado en proceso de tintura de lana,* Revista de Química e Industria Textil, N. 222, pages 12-17, ISSN: 2385-4804 (Ed. impresa) ISSN: 2385-4812 (Internet).

Review pending - Papers in Journals

- S. Moldovan, J. Pascual, E. Franco, M. Ferrandiz, M. Bonet-Aracil, J. Gisbert-Payá, E. Bou-Belda, *Laboratory studies on cotton dyeing with phycocyanin algal sourced dye*, Journal of Natural Fibers.

- S. Moldovan, J. Pascual, E. Franco, M. Ferrandiz, M. Bonet-Aracil, J. Gisbert-Payá, E. Bou-Belda, *Dyeing wool with Spirulina platensis sourced phycocyanin*, Journal of Natural Fibers.

- S. Moldovan, E. Bou-Belda, E. Franco, M. Ferrandiz, J. Gisbert-Payá, M. Bonet-Aracil, J. Pascual, *Characterization of wastewater from dyeing cotton and wool with phycocyanin*, Journal of Coloration Technology.

- S. Moldovan, J. Pascual, E. Franco, M. Ferrandiz, M. Bonet-Aracil, J. Gisbert-Payá, E. Bou-Belda, *Cotton and wool dyeing with microalgal sourced carotenoids dye*, Agronomy.

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