

## Article

# Evaluation of Phycocyanin Extract from *Limnothrix planctonica* (KU.B3) as a Natural Blue Color for Textile Screen Printing: Effects of Additive Compounds on Colorfastness and UV Stability

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## Abstract

Natural dyes are attracting increasing attention owing to their environmental compatibility and safety profile, particularly in contrast to synthetic dyes, which may contain hazardous compounds posing risks to human health and ecosystems. In this study, crude phycocyanin extract from the cyanobacterium *Limnothrix planctonica* (KU.B3) was evaluated as a natural blue color for textile screen printing. The investigation encompassed the optimization of curing temperatures and assessment of colorfastness under simulated-use conditions, including washfastness, lightfastness, and rubfastness. The results indicated that a curing temperature of 110 °C represented the practical upper limit for maintaining phycocyanin chromophore stability during the screen printing process. Among the additive compound evaluated, copper sulfate conferred the greatest resistance to UV-induced fading; the compound-treated fabric retained a K/S value of  $0.83 \pm 0.03$  following 5 h of UV exposure, representing a decline of approximately 16% compared with approximately 25% in the untreated control. However, washfastness was poor across all treatment conditions (grey scale score 1), indicating that under the binder system investigated in this study phycocyanin may be more suitable for decorative rather than washable textile applications.

**Keywords:** phycocyanin; natural dye; screen printing; metallic additive compound; colorfastness; textile



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## 1. Introduction

Synthetic dyes are currently the preferred colorants in the global textile industry, owing to their broad color spectrum, superior colorfastness, and the ability to produce consistent, reproducible results at industrial scale [1]. Nevertheless, significant environmental and public health concerns have been raised regarding the manufacture and application of synthetic dyes. Textile dyeing effluents frequently contain toxic compounds that contaminate aquatic environments and soil, posing health risks to humans and ecosystems. Of particular concern is the biodegradation of certain azo dyes into carcinogenic aromatic amines, which can be released during dyeing or upon textile-water contact. In response, the European Union has enacted stringent regulations prohibiting specific azo dyes identified

as precursors to harmful aromatic amines, reflecting a broader commitment to reducing hazardous chemical inputs in textile manufacturing [2].

Natural pigments or dyes are derived from diverse biological sources, including plants [3], fungi [4,5], animals [6], and algae [7–12]. Algae-based dyes are gaining attention as a more sustainable and environmentally friendly alternative to traditional synthetic dyes in the textile industry [13]. Algae present several advantages as natural dye sources. Their rapid growth rates, relative to terrestrial plants, enable more frequent harvesting and consistent pigment production. Many algal species accumulate high concentrations of pigments suitable for textile dyeing across a broad spectral range, which offer considerable potential for textile colorant applications. These pigments are broadly classified into water-insoluble (chlorophylls (green) and carotenoids (orange to yellow)) and water-soluble compounds (phycobiliproteins, which comprise phycocyanin (blue), phycoerythrin (red), and allophycocyanin (blue-green)). These algal pigments can be produced and extracted from various species and have been studied for use in textile dyeing [7–12].

Algae can be cultivated in controlled systems such as photobioreactors, ponds, or tanks. This process requires significantly less land and water than conventional crops. As a result, algae-based pigment production has a much smaller environmental footprint compared with synthetic dye manufacturing [7]. Algal cultivation also enables CO<sub>2</sub> sequestration and nutrient recycling. The biomass can be utilized for multiple purposes beyond pigment extraction, including the production of biofuels, nutraceuticals, and pharmaceuticals, making it relevant within a circular bioeconomy framework [14–16]. Phycocyanin, a blue phycobiliprotein pigment, functions as an accessory pigment in cyanobacterial photosynthesis. *Arthrospira platensis*, a primary commercial source, is widely studied for its antioxidant, anti-inflammatory, and coloring properties [17].

Despite these advantages, the application of algal-based pigments in textile systems remains limited by several challenging issues, including pigment stability, durability, and compatibility with conventional textile processing methods. Natural pigments or dyes are also a concern due to limitations such as lower colorfastness and low affinity to textile fibers, resulting in low dye uptake and poor bonding between the pigment and fabric. This can lead to pigment loss during washing and wearing. Thus, mordants, such as natural and metallic compounds, are often used to improve colorfastness and help retain the dye on the fiber. Additionally, natural pigments can be sensitive to changes in environmental conditions during the dyeing process, leading to variations in color and dye uptake, which makes achieving consistent results more difficult [18]. Unlike conventional dyeing, screen printing is a textile coloration technique that can utilize either dyes or pigments, depending on the intended application. In pigment printing, the colorant does not chemically bond to the textile fiber and therefore requires a polymeric binder to achieve adhesion and fixation on the fabric surface. Consequently, the curing process is a critical step because it promotes binder film formation and directly influences the durability of the printed pattern [19].

In particular, limited studies have systematically evaluated the performance of phycocyanin in textile screen printing techniques, where pigment fixation relies on binder-pigment interactions rather than direct fiber–dye affinity [19]. Some of which also study bio-based colorants and use coating techniques, but are challenged in controlling color and are contaminated with other pigments, such as green color, when using whole algal biomass [20,21]. For this reason, finding alternative extractions is also crucial to ensure pigment consistency and leveled compositions.

Achieving consistent and reproducible outcomes with natural dyes requires a thorough understanding of pigment source characteristics, extraction parameters, and the intricacies of the dyeing or printing process. Ongoing research and technological innovation are therefore essential for improving the stability, durability, and applicability of

natural dye systems [22]. Additionally, while most studies have focused on *Arthrospira platensis* as a source of phycocyanin, alternative cyanobacterial strains such as *Limnothrix planctonica* remain underexplored. Therefore, this study addressed the compatibility challenges between the crude phycocyanin extract from *Limnothrix planctonica* (KU.B3) as a natural blue dye and conventional textile screen printing. Unlike previous studies that primarily employed *Spirulina* biomass or dyeing techniques [10,20,23–25], this study utilized a simple water-based extraction method to obtain a more consistent pigment composition, with the specific objective of assessing its curing limitations, colorfastness properties, and additive compound-assisted stabilization on color outcomes and pigment durability.

## 2. Materials and Methods

### 2.1. Chemicals and Materials

The chemicals used for *Limnothrix planctonica* (KU.B3) cultivation were purchased from the following suppliers: The BG-11 medium included  $K_2HPO_4 \cdot 3H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $NaNO_3$  (KemAus™, Elago Enterprises Pty Ltd., Cherrybrook, NSW, Australia), Citric acid ( $HOC(COOH)(CH_2COOH)_2 \cdot 2H_2O$ ) (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $CaCl_2 \cdot 2H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $MgSO_4 \cdot 7H_2O$  (Merck KGaA, Darmstadt, Germany), Ethylenediamine tetra acetic acid (EDTA; Ajax Finechem Pty Ltd., Taren Point, NSW, Australia), and Ferric ammonium citrate green ( $C_6H_8O_7 \cdot Fe_3 \cdot NH_3$ ; CARLO ERBA Reagents SAS, Val de Reuil, France), and the trace element chemicals included  $Co(NO_3)_2 \cdot 6H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $NaMoO_4 \cdot 2H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $CuSO_4 \cdot 5H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $H_3BO_3$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia),  $MnCl_2 \cdot 4H_2O$  (CARLO ERBA Reagents SAS, Val de Reuil, France), and  $ZnSO_4 \cdot 7H_2O$  (Ajax Finechem Pty Ltd., Taren Point, NSW, Australia). The standard phycocyanin (C-phycocyanin) was purchased from Sigma-Aldrich, St. Louis, MO, USA. A hot-air oven (Mettler, Meditop Co., Ltd., Bangkok, Thailand) was used for drying. A desiccator (Bossman, Shanghai Bosman Industrial Co., Ltd., Shanghai, China) was used for storage. Centrifugation was performed using a Centrifuge 5430 (Eppendorf AG, Hamburg, Germany). Spectrophotometric measurements were performed using a UV-visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Chemicals and materials used in the screen printing experiments were sourced as follows: Thickener (Acracore® FN01, Tanatex Co., Ltd., Rayong, Thailand), Binder (Zenatex binder A1, Selitex Co., Ltd., Bangkok, Thailand), Urea ( $NH_2CONH_2$ , KemAus™, Elago Enterprises Pty Ltd., Cherrybrook, NSW, Australia). Additive compounds were used as follows: Aluminum sulfate ( $Al_2(SO_4)_3 \cdot 18H_2O$ , Fisher Scientific, Thermo Fisher Scientific (Thailand) Co., Ltd., Bangkok, Thailand), copper sulfate ( $CuSO_4$ , KemAus™, Elago Enterprises Pty Ltd., Cherrybrook, NSW, Australia), ferrous sulfate ( $FeSO_4$ , Fisher Scientific, Thermo Fisher Scientific (Thailand) Co., Ltd., Bangkok, Thailand), and tannin powder was used as a tannic acid CAS 1401-55-4. Dye staining was evaluated using a grey scale conforming to ISO 105 A03:1987 [26], BS 1006 A03 1990 [27], and were obtained from SDC Enterprises Ltd. (Holmfirth, West Yorkshire, UK). Color change was assessed using a grey scale conforming to ISO 105 A02:1987 [28], BS 1006 A02 1990 [29], and were obtained from SDC Enterprises Ltd. (Holmfirth, West Yorkshire, UK). Mixing was performed using an overhead stirrer (IKA Works GmbH & Co. KG, Staufen, Germany). Color measurements were performed using a Datacolor 550 spectrophotometer (Datacolor Inc., Lawrenceville, NJ, USA). ECE phosphate reference detergent (B) (SDC Enterprises Co., Ltd., Manchester, UK) was used for all washfastness tests. Washing tests were conducted using a Lab-IR dyeing machine (Daelim Starlet Co., Ltd., Siheung-si, Republic of Korea).

## 2.2. Cyanobacterial Strain and Cultivation

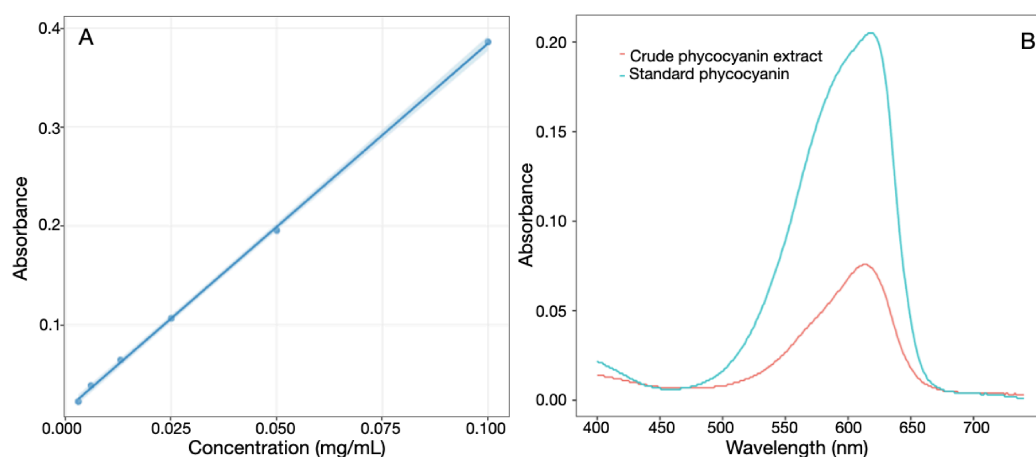
The cyanobacterium *Limnothrix planctonica* (KU.B3), an unbranching filamentous strain isolated from a vegetable plant drainage system in Nonthaburi, Thailand, was used as the pigment source in this study. Strain identity was confirmed by 16S rRNA gene sequencing showing 98% similarity to *Limnothrix planctonica* (Wołoszyńska) Meffert 1988, as previously described [30]. For pigment production, the strain was cultivated in 3 L of BG-11 medium under white LED illumination at 70  $\mu\text{mol photons/m}^2/\text{s}$  with a 12:12 h light:dark photoperiod and continuous aeration for 18 days. Medium pH was maintained at 7.4 using 1 N HCl and 1 N NaOH.

## 2.3. Extraction of the Crude Phycocyanin Extract

To prepare cyanobacterial powder, harvested biomass was centrifuged at 3000 rpm for 10 min, or until complete cell sedimentation was achieved. The pellet was retained, and the supernatant was carefully decanted to minimize cell loss. The wet biomass was spread evenly on a tray lined with a silicone mat to prevent adherence and dried in a hot-air oven at 60 °C for 12–24 h until completely desiccated. The dried biomass was ground to a fine powder using a mortar and pestle, transferred to foil-wrapped containers, and stored in a desiccator at room temperature to prevent light exposure and moisture absorption.

## 2.4. Standard Calibration Curve of Phycocyanin

Phycocyanin concentration was quantified using a standard calibration curve established by linear regression of absorbance at 620 nm against known pigment concentrations. The standard phycocyanin was prepared at a concentration of 0.1 mg/mL. The regression equation is  $y = 3.705x + 0.014$  with  $R^2 = 1.000$  (Figure 1). The crude phycocyanin extract, obtained after harvesting on the 18th day in a 3 L culture, was dried and ground into a powder, yielding approximately 1.32 g. The phycocyanin content in this extract was calculated using the standard phycocyanin, which was approximately 10.39 mg phycocyanin per 1 g of dry weight.



**Figure 1.** (A) The standard calibration curve for measuring the concentration of standard phycocyanin at an absorbance of 620 nm. Blue dots represent the experimental measurements, and the blue line represents the linear regression used for calibration. (B) Comparative spectra of the crude phycocyanin extract (red line) and standard phycocyanin (blue line) illustrating a prominent peak around 620 nm.

## 2.5. Preparation of Screen Printing Pigment Paste

The substrate used for screen printing was a 100% cotton fabric with a mass per unit area of 125 g/m<sup>2</sup>. The print paste was prepared according to the formulation given in

Table 1. All components were mixed thoroughly with an overhead stirrer until a homogeneous, viscous consistency was obtained. Cotton fabric samples were secured on the screen printing table, and the screen frame was positioned over the fabric. During printing, the pigment paste was applied using a squeegee with 2 passes bidirectional squeegee motion (one forward pass followed by one backward pass) to ensure uniform distribution of the print paste across the fabric surface, and the printed samples were subsequently cured at the specified temperatures.

**Table 1.** Paste preparation with crude phycocyanin extract.

| Components         | Amount (100 g)          |                            |
|--------------------|-------------------------|----------------------------|
|                    | With Additive Compounds | Without Additive Compounds |
| Thickener          | 6                       | 6                          |
| Binder             | 15                      | 15                         |
| Urea               | 1                       | 1                          |
| Water              | 63                      | 68                         |
| Pigment            | 10                      | 10                         |
| Additive compounds | 5                       | -                          |

## 2.6. Printing Experiment

### (a) Without additive compounds

The print paste composition is shown in Table 1. Printed fabrics were subjected to curing temperature screening to identify the optimal condition for phycocyanin pigment fixation.

#### (i) Curing temperature assessment

The effectiveness of the binder activity was evaluated at curing temperatures of 90, 100, 110, 120, and 130 °C, with each condition being applied for 2 min. Although the acrylic binder is recommended to be cured at approximately 150 °C to achieve complete cross-linking, preliminary experiments showed that curing at temperatures above 130 °C resulted in severe discoloration resulting from phycocyanin degradation. Therefore, curing temperatures above 130 °C (from 140 to 150 °C) were not included in this study.

#### (b) With additive compounds

Four different additive compounds were utilized, derived from both natural and synthetic sources. Tannin was selected as a natural additive compound, whereas aluminum sulfate, ferrous sulfate, and copper sulfate were used as inorganic metallic additive compounds. Each of the compounds was incorporated into the print paste (Table 1) at a concentration of 5% (*w/w*). Nevertheless, these compounds were selected based on their common use in textile applications and their potential to form coordination complexes with a natural pigment used in this study.

However, these additive compounds were investigated for their potential stabilization effect on phycocyanin rather than their conventional mordanting function in textile dyeing.

## 2.7. Color Measurement

Colorimetric parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C$ , and  $K/S$ ) were determined for all printed fabric samples. The  $L^*$  value denotes lightness, ranging from 0 (black) to 100 (white);  $a^*$  denotes the red ( $+a^*$ ) to green ( $-a^*$ ) axis;  $b^*$  denotes the yellow ( $+b^*$ ) to blue ( $-b^*$ ) axis; and  $C$  represents chroma (color saturation). The  $K/S$  value, which reflects color strength based on light reflectance, was determined using a reflectance spectrophotometer [31]. The evaluated color strength values are background-corrected  $K/S$  values.

## 2.8. Colorfastness Tests

Colorfastness assessments were conducted to evaluate resistance to rubbing and washing under conditions simulating routine daily use, as described below.

### 2.8.1. Fastness to Rubbing

Dry and wet rubbing tests were conducted on white standard fabric using a crocking machine. Color staining was assessed against a grey scale [26,27] under a D65 illuminant. Scores ranged from 1 (severe staining or bad) to 5 (no staining or good).

### 2.8.2. Fastness to Washing

The samples were subjected to a standard multifiber washing test for 30 min at 40 °C in a washing machine. 4 g/L of detergent (pH 9.7) was utilized in the washing process of a liquor ratio (L:R) at a 1:50. A color assessment of the samples was performed with multifiber to observe color staining occurrence.

## 2.9. UV Light Exposure Test

(a) The print-compound samples were exposed to UV light for 1 and 5 h, and color changes were evaluated using K/S values. The evaluation used the grey scale to assess color change.

(b) The best of the print-compound sample, the CuSO<sub>4</sub>-printed sample, was chosen to test the extent of UV light exposure durations, including 0, 1, 2, 3, 5, and 8 h, and to compare with the control (without treated sample).

## 2.10. Statistical Analysis

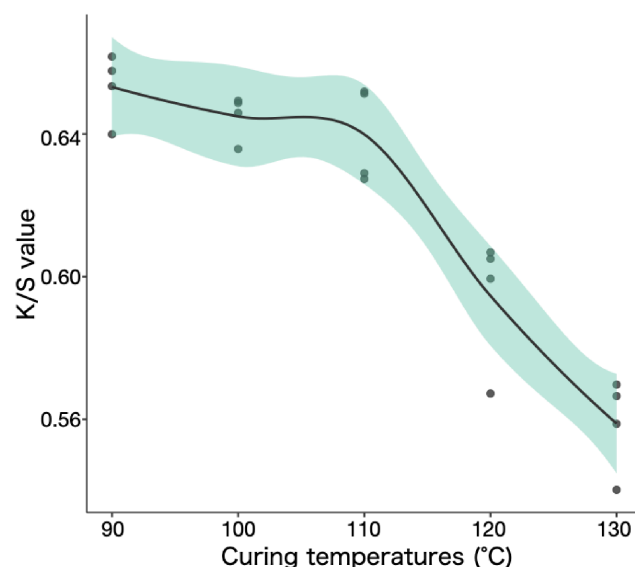
All data were analyzed using RStudio version 2024.09 (Posit Software, PBC, Boston, MA, USA) and R version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Differences among treatment groups were assessed by one-way analysis of variance (ANOVA), with statistical significance set at  $p \leq 0.05$ . Post hoc multiple comparisons were conducted using Tukey's HSD test. Data are expressed as mean  $\pm$  standard deviation ( $n = 4$ ) unless otherwise stated.

# 3. Results

## 3.1. Effect of Curing Temperatures on K/S Value

The results showed that the K/S values at 90 and 100 °C did not exhibit significant differences and yielded the highest K/S value of approximately 0.65. Upon increasing the curing temperature to 110 °C, the K/S value remained relatively stable at approximately 0.64, similar to the previous temperatures. However, at 120 °C, a reduction in the K/S value to 0.60 was observed. Subsequently, at the final temperature of 130 °C, the K/S value decreased to 0.56, and the fabric color displayed a diminished intensity of blue shades, indicating pigment degradation at high temperatures. Consequently, it can be concluded that elevated curing temperatures beyond 110 °C adversely impact the stability and performance and reflect a thermal denaturation of the phycocyanin pigment. At 110 °C, the K/S value remained comparable to that at 90 and 100 °C, making it the highest practically acceptable curing temperature for subsequent experiments (Figure 2).

A curing temperature of 110 °C thus offered the best compromise between adequate binder activity and phycocyanin chromophore preservation and was therefore adopted for all subsequent experiments.



**Figure 2.** Effect of curing temperature on K/S during screen printing. The solid line indicates the smoothed trend, and the shaded area represents the standard error.

### 3.2. Screen Printing with Additive Compounds

A curing temperature of 110 °C for 2 min was selected as the optimal condition for this experiment. The results revealed distinct color variations associated with different additive compounds (Figure 3 and Table 2). Specifically, the aluminum sulfate yielded a light blue shade, while the ferrous sulfate produced a browner shade ( $a^*$  and  $b^*$  value at 0 h was  $-4.56$  and  $9.05$ , respectively). The tannin exhibited a deeper shade ( $a^*$  and  $b^*$  value at 0 h was  $-2.20$  and  $10.50$ , respectively), according to the lowest lightness value ( $L^*$  value at 0 h approximately 67.68). The copper sulfate sample retained blue shades ( $a^*$  and  $b^*$  value at 0 h was  $-18.83$  and  $-5.01$ , respectively), although it appeared slightly different from the control sample ( $a^*$  and  $b^*$  value at 0 h was  $-17.01$  and  $-7.54$ , respectively).

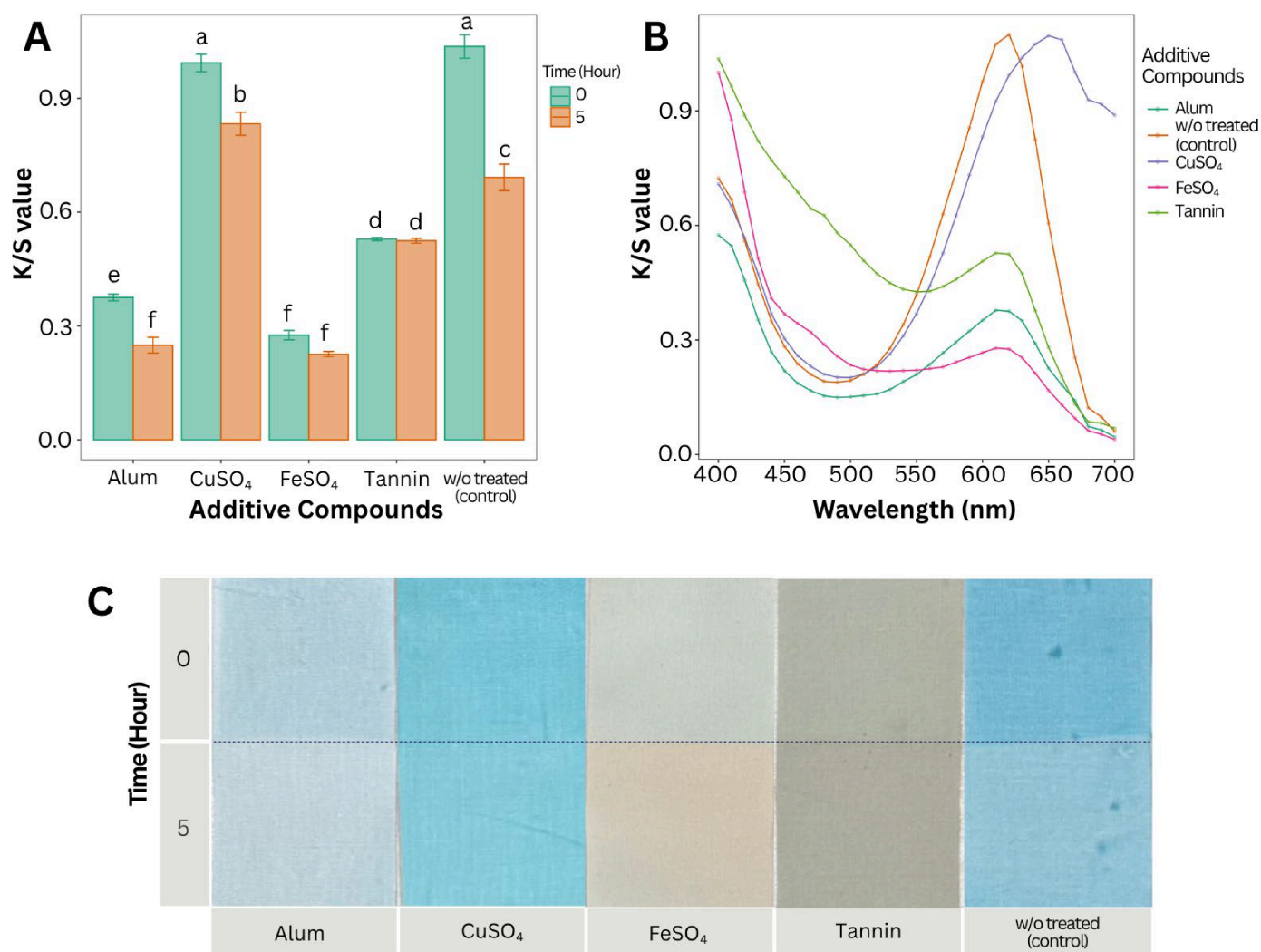
**Table 2.** Colorimetric parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and K/S) of compound-treated phycocyanin-printed fabrics before (0 h) and after (5 h) UV light exposure.

| Additive Compounds                  | Time (h) | $L^*$            | $a^*$             | $b^*$            | C                | K/S             |
|-------------------------------------|----------|------------------|-------------------|------------------|------------------|-----------------|
| w/o additive compound (Control)     | 0        | $72.08 \pm 0.31$ | $-17.01 \pm 0.39$ | $-7.54 \pm 0.23$ | $18.60 \pm 0.41$ | $0.92 \pm 0.03$ |
|                                     | 5        | $73.35 \pm 0.28$ | $-13.17 \pm 0.37$ | $-4.68 \pm 0.31$ | $13.97 \pm 0.51$ | $0.69 \pm 0.04$ |
| Aluminum sulfate                    | 0        | $77.15 \pm 0.27$ | $-9.53 \pm 0.19$  | $0.67 \pm 0.12$  | $9.55 \pm 0.19$  | $0.38 \pm 0.01$ |
|                                     | 5        | $80.35 \pm 0.42$ | $-7.12 \pm 0.56$  | $1.85 \pm 0.57$  | $7.83 \pm 0.40$  | $0.25 \pm 0.02$ |
| Copper sulfate ( $\text{CuSO}_4$ )  | 0        | $70.65 \pm 0.25$ | $-18.83 \pm 0.34$ | $-5.01 \pm 0.10$ | $19.49 \pm 0.33$ | $0.99 \pm 0.02$ |
|                                     | 5        | $73.16 \pm 0.25$ | $-18.66 \pm 0.40$ | $-4.44 \pm 0.19$ | $19.16 \pm 0.43$ | $0.83 \pm 0.03$ |
| Ferrous sulfate ( $\text{FeSO}_4$ ) | 0        | $76.53 \pm 0.42$ | $-4.56 \pm 0.18$  | $9.05 \pm 0.34$  | $10.14 \pm 0.30$ | $0.28 \pm 0.01$ |
|                                     | 5        | $75.72 \pm 0.78$ | $-0.94 \pm 0.96$  | $13.53 \pm 1.67$ | $13.60 \pm 1.58$ | $0.23 \pm 0.01$ |
| Tannin                              | 0        | $67.68 \pm 0.27$ | $-2.20 \pm 0.05$  | $10.50 \pm 0.16$ | $10.53 \pm 0.15$ | $0.53 \pm 0.04$ |
|                                     | 5        | $68.84 \pm 0.12$ | $-0.74 \pm 0.27$  | $9.42 \pm 0.09$  | $9.67 \pm 0.09$  | $0.53 \pm 0.01$ |

Note: The values are expressed as mean  $\pm$  standard deviation of  $n = 4$ .

The K/S values, reflecting color strength based on fabric light reflectance, were highest in the copper sulfate-treated samples, followed in decreasing order by tannin, aluminum sulfate, and ferrous sulfate; the copper sulfate produced color strength comparable to the non-treated control (Figure 3A). Analysis of the wavelength and phycocyanin pigment demonstrated that all compound-treated fabrics exhibited a strong peak at 620 nm, characteristic of the phycocyanin pigment responsible for the observed blue shades in the

samples. The highest peak was observed in the non-treated (control) sample, followed by the tannin, aluminum sulfate, and ferrous sulfate compounds. Moreover, the copper sulfate exhibited a slight bathochromic shift, resulting in brighter blue shades compared to the control (Figure 3B). Based on these findings, it is evident that adding various additive compounds led to distinct effects on the stability and color strength of the pigment on the printed fabrics. Each compound resulted in different color shades, with aluminum sulfate producing light blue and the ferrous sulfate and tannin yielding olive shades. The copper sulfate maintained blue shades but slightly differed from the control (Figure 3C).



**Figure 3.** Effects of UV light exposure on additive compound-treated printed samples: (A) color strength (K/S value) before (0 h; green bars) and after (5 h; orange bars) UV exposure; (B) influence of additive compound type on pigment stability and color strength in printed fabrics; and (C) visual appearance of the printed samples. Different letters denoted statistically significant differences among groups (Tukey's HSD,  $p \leq 0.05$ ).

These findings prompted further investigation of the copper sulfate-treated samples under extended UV exposure and multifiber washing conditions.

### 3.3. Color Evaluations

#### 3.3.1. Fastness to Rubbing

The efficacy of curing temperatures (90–130 °C) in fixing phycocyanin pigment within the binder-fabric matrix was assessed using wet and dry rubbing tests performed with

a crocking machine. All samples cured across the temperature range showed excellent dry-rubbing resistance (score 5), with no color staining observed on the white reference fabric. In contrast, wet rubbing revealed temperature-dependent variation: samples cured at 90 and 100 °C scored 3 (moderate staining), whereas those cured at 110, 120, and 130 °C scored 4 (slight staining) (Table 3).

**Table 3.** Colorfastness to rubbing of phycocyanin-printed fabrics cured at different temperatures.

| Curing Temperature (°C) | Wet Rubbing | Dry Rubbing |
|-------------------------|-------------|-------------|
| 90                      | 3           | 5           |
| 100                     | 3           | 5           |
| 110                     | 4           | 5           |
| 120                     | 4           | 5           |
| 130                     | 4/5         | 5           |

Notably, samples cured at 130 °C exhibited the least pigment staining in the wet rubbing test, receiving a score of 4/5 (Table 3), suggesting partial benefit from the higher curing temperature despite associated pigment degradation.

### 3.3.2. Fastness to Washing

Washfastness assessment revealed a critical limitation of phycocyanin-based screen printing. Prior to washing, all compound-treated samples displayed distinct color appearances, confirming successful pigment deposition. Following detergent washing, however, all samples, regardless of compound treatment, lost their blue phycocyanin coloration entirely (grey scale score 1) (Table 4). In the tannin-treated samples, a residual brown coloration from the tannin persisted, but the characteristic blue of phycocyanin was absent. The multifiber reference strip confirmed total pigment loss, indicating irreversible denaturation or leaching of phycocyanin under the alkaline washing conditions employed (pH 9.7). These findings demonstrate that none of the additive compound systems tested provided sufficient stabilization to protect phycocyanin against detergent-induced degradation.

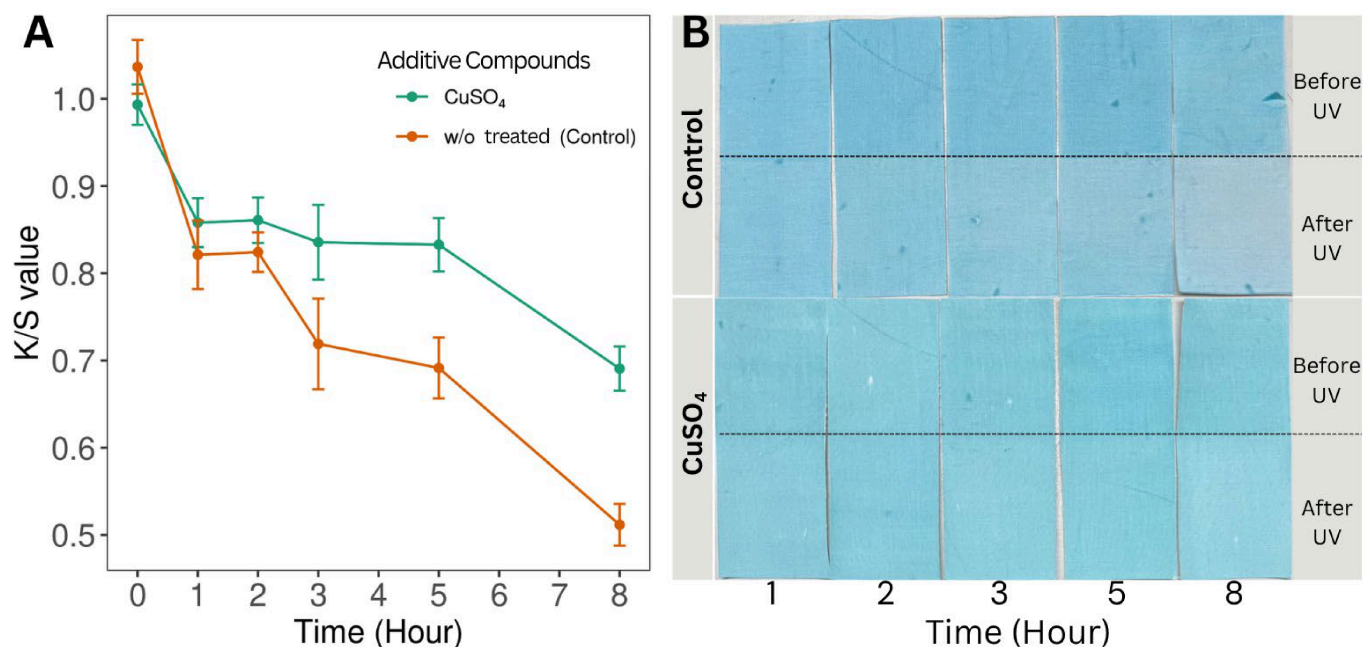
**Table 4.** Colorfastness to UV light (5 h exposure) and washing of phycocyanin printed fabrics treated with different compounds.

| Additive Compounds                   | Colorfastness  |         |
|--------------------------------------|----------------|---------|
|                                      | UV Light (5 h) | Washing |
| w/o additive compound (Control)      | 1/2            | 1       |
| Aluminum sulfate                     | 1              | 1       |
| Copper sulfate (CuSO <sub>4</sub> )  | 3/4            | 1       |
| Ferrous sulfate (FeSO <sub>4</sub> ) | 1              | 1       |
| Tannin                               | 2/3            | 1       |

### 3.3.3. Fastness to UV Light

Colorfastness to UV light was evaluated for compound-treated and untreated control samples at defined exposure intervals. At 0 h UV light exposure, the copper sulfate-treated and untreated control samples showed no statistically significant difference in color strength ( $K/S = 0.99$  vs.  $0.92$ , respectively;  $p > 0.05$ ), both exhibiting high initial color retention (Figure 4). After 5 h of UV light exposure, the untreated control experienced a significant decline in color strength (~25% reduction in  $K/S$ ), whereas the copper sulfate-treated sample showed a significantly smaller decrease (~16%;  $p \leq 0.05$ ) (Figure 3A), demonstrating the protective effect of the copper compound against UV-induced phycocyanin degradation. Some additive compounds affected the color appearance and interfered with the blue color

of the phycocyanin pigment. For example, the tannin produced a brown color, while the ferrous sulfate resulted in a pale blue to light brown color, both leading to a reduction in the K/S value compared to the control after 0 h of exposure (approximately K/S = 0.53 and 0.28, respectively) (Figure 4). The aluminum sulfate caused a partial loss of the phycocyanin pigment, resulting in decreased K/S value after 0 h of exposure; the fabric appeared a light blue color in the case of the aluminum sulfate (Figure 3C).



**Figure 4.** Time-dependent effects of UV light exposure on color strength (K/S value) of printed fabrics, comparing the untreated control and copper sulfate (CuSO<sub>4</sub>)-treated samples: **(A)** K/S values across UV exposure durations. The green line represents the copper sulfate-treated samples, whereas the orange line represents the untreated control.; **(B)** visual appearance of phycocyanin-printed fabrics before and after UV exposure. Values are expressed as mean  $\pm$  standard deviation.

Overall, the copper sulfate conferred the highest UV resistance (grey scale score 3/4), reflecting effective color retention under UV irradiation. The tannin showed moderate UV resistance (grey scale score 2/3), though their inherent brown color likely influenced the perceived final blue shade. The untreated control exhibited poor UV durability (grey scale score 1/2), with substantial color fading over the exposure period. Both aluminum sulfate and ferrous sulfate performed poorly (grey scale score 1), with rapid color loss observed in both cases (Table 4).

### 3.4. Effect of UV Exposure Durations Between Copper Sulfate (CuSO<sub>4</sub>) Compound and Control

This experiment examined the time-dependent effect of UV exposure on color strength, comparing copper sulfate-treated samples with the untreated control across durations of 0, 1, 2, 3, 5, and 8 h. The copper sulfate-treated samples maintained relatively stable color retention between 1 and 5 h of UV exposure (K/S = 0.76–0.86), followed by gradual decline at 5 h and a cumulative pigment degradation of ~30% after 8 h. In the untreated control, phycocyanin was well retained up to 2 h (K/S = 0.82) but showed a marked decline from 3 h onwards (K/S = 0.72), reaching ~45% degradation after 8 h (Figure 4 and Table 5). Visual assessment confirmed that copper sulfate-treated fabrics retained their blue appearance more effectively than the untreated control, which showed progressive fading throughout the exposure period.

**Table 5.** Colorimetric parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and  $K/S$ ) of the untreated control and copper sulfate ( $\text{CuSO}_4$ )-treated phycocyanin-printed fabrics at different UV light exposure duration (0–8 h).

| Additive Compounds                 | Durations (h) | $L^*$            | $a^*$             | $b^*$            | C                | K/S             |
|------------------------------------|---------------|------------------|-------------------|------------------|------------------|-----------------|
| w/o additive compound<br>(Control) | 0             | $72.08 \pm 0.31$ | $-17.01 \pm 0.39$ | $-7.54 \pm 0.23$ | $18.60 \pm 0.45$ | $0.92 \pm 0.03$ |
|                                    | 1             | $73.14 \pm 0.38$ | $-15.98 \pm 0.43$ | $-6.90 \pm 0.10$ | $17.41 \pm 0.41$ | $0.82 \pm 0.04$ |
|                                    | 2             | $72.38 \pm 0.17$ | $-15.11 \pm 0.31$ | $-6.18 \pm 0.17$ | $16.32 \pm 0.35$ | $0.82 \pm 0.02$ |
|                                    | 3             | $73.55 \pm 0.43$ | $-14.02 \pm 0.65$ | $-5.33 \pm 0.56$ | $15.17 \pm 0.80$ | $0.72 \pm 0.05$ |
|                                    | 5             | $73.35 \pm 0.28$ | $-13.17 \pm 0.37$ | $-4.68 \pm 0.31$ | $13.97 \pm 0.45$ | $0.69 \pm 0.04$ |
|                                    | 8             | $75.01 \pm 0.24$ | $-9.62 \pm 0.42$  | $-2.72 \pm 0.39$ | $10.00 \pm 0.51$ | $0.51 \pm 0.02$ |
| Copper sulfate ( $\text{CuSO}_4$ ) | 0             | $70.65 \pm 0.25$ | $-18.83 \pm 0.34$ | $-5.01 \pm 0.10$ | $19.42 \pm 0.33$ | $0.99 \pm 0.02$ |
|                                    | 1             | $73.10 \pm 0.25$ | $-18.49 \pm 0.50$ | $-4.88 \pm 0.22$ | $19.14 \pm 0.49$ | $0.81 \pm 0.02$ |
|                                    | 2             | $72.48 \pm 0.30$ | $-18.14 \pm 0.43$ | $-5.21 \pm 0.15$ | $18.87 \pm 0.45$ | $0.86 \pm 0.03$ |
|                                    | 3             | $73.86 \pm 0.12$ | $-17.54 \pm 0.10$ | $-4.64 \pm 0.14$ | $18.14 \pm 0.13$ | $0.76 \pm 0.01$ |
|                                    | 5             | $73.16 \pm 0.25$ | $-18.66 \pm 0.40$ | $-4.44 \pm 0.19$ | $19.16 \pm 0.43$ | $0.83 \pm 0.03$ |
|                                    | 8             | $74.62 \pm 0.31$ | $-16.64 \pm 0.33$ | $-4.12 \pm 0.16$ | $17.23 \pm 0.33$ | $0.69 \pm 0.03$ |

Note: The values are expressed as mean  $\pm$  standard deviation of  $n = 4$ .

## 4. Discussion

### 4.1. Effect of Curing Temperature on Phycocyanin Screen Printing

Elevated curing temperatures are essential for effective pigment fixation in screen printing. Insoluble printing pigments lack inherent affinity for textile fibers and must be encapsulated by a binder through crosslinking reactions, resulting in a film-forming element that fixes and holds pigment inside. In acrylic binder systems, these crosslinking reactions typically proceed optimally at  $150^\circ\text{C}$  [22,32]. In this study,  $110^\circ\text{C}$  was identified as the practical upper limit for phycocyanin stability during curing; however, this temperature was insufficient to fully activate the acrylic binder crosslinking mechanism (which requires  $150^\circ\text{C}$ ), resulting in incomplete pigment fixation and consequent staining during wet rubbing tests [22]. Preliminary trials at 140 and  $150^\circ\text{C}$  confirmed complete phycocyanin degradation at the higher temperature, highlighting the fundamental incompatibility between the thermal requirements of conventional acrylic binders and the thermolability of phycocyanin.

This study is limited by using only one fabric type and binder system, which may affect the curing temperature constraints observed. High curing temperatures can cause protein denaturation, degrade chromophore structure, and break down tetrapyrrole rings [33–37]. The poor pigment retention is attributable not only to the low substantivity of phycocyanin but also to the incompatibility between the high curing temperature required by the conventional acrylic binder and the thermolabile nature of phycocyanin. Therefore, future studies should investigate commercially available low-temperature curing-type binders ( $100$ – $110^\circ\text{C}$ ), which may provide sufficient film formation while minimizing thermal degradation of phycocyanin, testing alternative curing agents, or applying UV curing technology [38–40] to enhance pigment stability.

### 4.2. Effect of Additive Compounds on Color Strength and Colorfastness

In a comparable screen printing study by [20], *Spirulina* whole cells were incorporated into a print paste applied to cotton and linen fabrics. The addition of aluminum sulfate and ferrous sulfate altered the final color hue. Although the *Spirulina*-printed samples showed some residual coloration after washing (appearing green), the initial blue pigment was lost, leading the authors to conclude that *Spirulina*-based dyes exhibit low affinity for textile fibers and are poorly suited for applications involving repeated laundering [20,25].

In contrast, the present study used an isolated crude phycocyanin extract from *Limnithrix planctonica* (KU.B3), excluding the green biomass fraction to obtain a purer blue

colorant. Incorporation of different compounds produced distinct color outcomes, as described in the Section 3. In washfastness testing, complete loss of the blue pigment was observed across all conditions, consistent with the findings of Ciptandi et al. [20], who similarly reported the absence of blue color after washing. The dry-rubbing tests demonstrated adequate pigment fixation at all curing temperatures (grey scale score 5); however, wet rubbing produced moderate staining (grey scale score 3/4), and washing under alkaline detergent conditions (pH 9.7) resulted in complete pigment loss. The alkaline environment is consistent with known phycocyanin degradation pathways, likely involving protein denaturation and chromophore cleavage. Collectively, these results indicate that curing at 110 °C was insufficient to activate the binder's crosslinking mechanism adequately, precluding strong pigment-fiber bonding. Future work should explore alternative binder systems with lower activation temperatures or microencapsulation of phycocyanin prior to incorporation into print pastes, as potential strategies to improve washfastness. Regarding UV lightfastness, copper sulfate significantly attenuated UV-induced phycocyanin degradation relative to all other treatments, potentially associated with metal-pigment interactions, as suggested in previous reports [41,42], which may stabilize the tetrapyrrole structure against photodegradation [43].

Additionally, although this study focused on crude phycocyanin to better reflect a practical and scalable extraction process, the influence of non-phycocyanin constituents on pigment stability and binder interactions cannot be excluded. Future studies comparing purified phycocyanin with crude extracts would provide further mechanistic insight.

#### *4.3. Washfastness Limitations and Future Perspectives*

Compared to conventional synthetic pigments, which typically exhibit higher washfastness (grey scale score 4 to 5) [44–46], the washfastness performance of phycocyanin as a natural pigment remains limited. The poor washfastness observed across all additive compound treatments (grey scale score 1) reflects a fundamental incompatibility between phycocyanin's protein-based chromophore structure and the alkaline conditions of standard detergent washing (pH 9.7). This finding aligns with the known thermolability and pH sensitivity of phycocyanin and indicates that conventional screen printing binder systems are insufficient to protect the pigment under aqueous stress. Future studies should explore microencapsulation of phycocyanin prior to paste preparation or the use of mild (neutral-to-mildly acidic) washing protocols as approaches to improve durability. Importantly, the current results suggest that phycocyanin-based textile applications may be most suitable for non-laundered, UV-decorative, or indoor textile contexts. Accordingly, the use of phycocyanin in screen printing is not recommended for applications involving frequent water exposure or conventional detergent washing under the binder system investigated in this study.

#### *4.4. UV Light Stability and the Role of Copper Sulfate*

Among the compounds evaluated, copper sulfate was the most effective at maintaining color strength under UV irradiation, while tannin showed moderate resistance (though its inherent brown hue confounded assessment of blue retention). Aluminum sulfate and ferrous sulfate conferred minimal UV protection, resulting in significant color fading. The superior pigment retention afforded by copper sulfate during extended UV exposure prompted its selection for detailed kinetic analysis (Section 3.4), enabling quantification of the phycocyanin degradation rate under prolonged irradiation [21,47,48]. Although CuSO<sub>4</sub> improved UV light stability, its use may partially offset the environmental advantages of phycocyanin-based coloration due to heavy metal concerns. Therefore, future work should investigate bio-based or low-impact compounds with comparable stabilizing effects.

Additionally, phycocyanin is a water-soluble phycobiliprotein that functions as a light-harvesting pigment in cyanobacteria. Its optical properties originate from phycocyanobilin (PCB), a linear tetrapyrrole chromophore covalently attached to the  $\alpha$ - and  $\beta$ -subunits of the protein. PCB is responsible for the characteristic blue color, fluorescence, and efficient energy transfer within the phycobilisome [49,50].

Recent studies have demonstrated that metal ions can influence the structural and photophysical properties of phycocyanin through multiple interaction mechanisms. Bellamy-Carter et al. [51] reported that  $\text{Cu}^{2+}$  and  $\text{Ag}^{+}$  induce structural rearrangements in phycocyanin and suggested that metal binding involves not only the phycocyanobilin chromophore but also amino acid residues located at the protein interface. Li et al. [52] further demonstrated that  $\text{Cu}^{2+}$  can bind to specific amino acid residues, promoting protein self-assembly and enhancing structural stability. In addition, Lu et al. [53] showed that  $\text{Co}^{2+}$  directly coordinates with the linear tetrapyrrole chromophore, leading to changes in its photophysical behavior and increased photodynamic activity. Collectively, these studies indicate that metal ions may interact with both the phycocyanobilin chromophore and the surrounding protein matrix, thereby altering the structural stability, fluorescence characteristics, and photostability of phycocyanin.

Therefore, the improved UV stability observed in the  $\text{CuSO}_4$ -treated samples in this study may be associated with similar metal–phycocyanin interactions. However, because no spectroscopic characterization (e.g., FTIR or metal-binding analysis) was performed in this work, the proposed interaction mechanism should be regarded as a plausible interpretation based on previous studies rather than direct experimental evidence.

## 5. Conclusions

A curing temperature of 110 °C was identified as the practical upper limit for phycocyanin stability during screen printing. Copper sulfate provided the best UV light resistance, with treated fabrics retaining a K/S value of  $0.83 \pm 0.03$  following 5 h of UV exposure; however, washfastness was poor across all conditions (grey scale score 1). Dry rubbing resistance was excellent (grey scale score 5) for all curing temperatures, whereas wet rubbing produced moderate staining (grey scale score 3/4), indicating partial binder-pigment fixation.

Overall, phycocyanin extracted from *Limnithrix planctonica* (KU.B3) shows promise as a natural blue colorant for textile applications, particularly in indoor decorative textiles, temporary prints, educational eco-printing, UV-limited display textiles, or non-laundered contexts. However, a successful application requires careful optimization of binder systems, curing conditions, and suitable stabilizing additives selection, as these factors critically govern color outcome, UV stability, and wash durability. Additionally, future development of compatible alternative techniques is essential for advancing protein-based pigments in textile applications.

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