The Efficiency of Naturally Derived Pigments from Microorganisms, Fungi, and Plants in Dyeing Fabric

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Abstract

Artificial dyes have become the normal process for the commercial fashion industry. Due to heavy and universal use, the synthetic dyes have contributed to an increase in negative environmental effects including pollution, and colored waste water. As a more eco-friendly society continues to rise up, demand for a new pigment source that is naturally produced has increased. In turn, researchers have begun to develop theories and hypotheses on the potential of biopigments, specifically from plants, bacteria, and fungi. This study presents research to show the potential comparison between the different pigment origins as well as how they oppose the standard synthetic dye used. The organisms used for pigment production included two plant origins *Allium cepa* peels and *Quercus robur* leaves, two bacteria *Monascus sanguineus* in the form of monascus red pigment and *Aspergillus carbonarius* in the form of melanin pigment, and two fungi *Scytalidium cuboideum* and *Chlorociboria aeruginosa*, which produce draconin red and xylindein, respectively. The data measured for each pigment included color produced, yield of pigment after production, light absorbance and transmittance percentage. The pigments were able to dye the preferred fabric with the fungal pigments having best results. Overall, biopigments show great promise for commercial use and can potentially limit the environmental impacts of dye as more research is conducted and technology is advanced.

Keywords: dyes, biopigments, pigments, textiles, fibers, dyeing, bacterial pigments, fungal pigments, plant pigments, synthetic dye.

The Efficiency of Naturally Derived Pigments from Microorganisms, Fungi, and Plants in Dyeing Fabric

Color has been a factor in human evolution since the beginning of time. Whether through communication or symbolism, color has allowed humanity to further its technology and improve society overall. Before modern science, color was derived from natural open commodities like soil, clay, and rocks mixed with other materials like blood and water (Ardila-Leal et al., 2021). The first pigments were mainly used for rituals and traditions but soon became significant in society's attire through dyes (Ardila-Leal et al., 2021). However, during this beginning of history, colors were simple and made based on efficiency (Ardila-Leal et al., 2021). These colors had a small variety of yellows, browns, blacks, browns, and reds, not any of the vibrant colors made today (Ardila-Leal et al., 2021). The more complex colors were either more challenging to obtain or were only found in low quantities, such as from rare minerals, like the Egyptian Blue pigment produced from lapis lazuli (Ardila-Leal et al., 2021). Even so, the frequency of colors led to increased symbolism for the pigments, an example being the color purple representing wealth and royalty due to being so expensive to produce that those of the monarchy could wear it (Ardila-Leal et al., 2021). Ultimately, the advancement of color has gone hand-in-hand with the establishment of dyes.

Furthermore, as time and technology advanced, pigment research shifted to other natural mediums, including insects, fruit, vegetables, and minerals, as well as improving the processing of extraction and dyeing (Ardila-Leal et al., 2021). Consequently, this continuance of research encouraged cost-effectiveness and production efficiency, leading to the creation of the first synthetic dye, Mauveine, in the 1850s (Ardila-Leal et al., 2021). After the creation of Mauveine, artificial dyes became the suitable option for manufacturing textiles due to their expansive range

of color variety, greater reproducibility, economical efficiency, and consistency; natural pigment analysis soon fell to the way-side (Bechtold et al., 2005). Despite the innovation and capability of synthetic dyes, after long-term use, their environmental impacts are detrimental. The severe impacts that manufactured dyes have been shown to produce are colored wastewater and non-biodegradable (Ardila-Leal et al., 2021). The effect of colored wastewater is evident as 20% of the dye is distributed into water sources, causing high levels of pollution, including fluctuating pH levels, a lowered concentration of dissolved oxygen, a decreasing chemical content in the water, and an increase of non-biodegradable particles (Ardila-Leal et al., 2021). These environmental changes, brought on by synthetic dyes, have been tested and shown to potentially cause carcinogenic, recalcitrant, and toxic effects on the present ecosystem and humans (Ardila-Leal et al., 2021). Additionally, the commercial use of synthetic pigments requires using finite natural resources for energy usage in production (Gong et al., 2018; Carvalho & Santos, 2015). Therefore, the demand for an eco-friendly and sustainable alternative to synthetic dyes has become more focused (Jha et al., 2017; Aman et al., 2022). Biopigments are considered non-toxic, non-polluting, and less hazardous to health while having the potential for high productivity due to rapid growth, seemingly a better option environmentally (Jha et al., 2017). The natural/biopigments that have developed a helpful impression are those from microorganisms, plants, and fungi dyes. Hence, this resurgence of biopigments has driven new research and questions about their industrial performance. Specifically, the goal is to determine if natural pigments can be used to dye textiles and provide characteristics such as proficiency in yield of dye output, cost-effectiveness, reproducibility on a commercial scale, and quality color saturation while comparing the results to the universal synthetic dyes. Overall, this research aims to further develop the yield of pigment and quality of saturation to determine if pigment

produced from fungi, microorganisms, and plants can be considered a better alternative to synthetic dyes.

Research Questions and Hypotheses

RQ1: Can microbial and other natural pigments be used to dye textiles based on quantity yield produced, and quality of saturation?

H_0: Microbial, fungal, and natural pigments cannot be used to dye a textile based on yield of pigment, and quality of saturation.

H_A: Microbial, fungal, and natural pigments can be used to dye a textile based on yield of pigment, and quality of saturation.

RQ2: What biopigments produce the most yield of pigment during application when dyeing textiles?

H_0: The microbial pigments will not produce a bigger yield of pigment compared to the other natural alternatives by milligrams/milliliter.

H_A: The microbial pigments will produce a bigger yield of pigment compared to the other natural alternatives by milligrams/milliliter.

RQ3: Which biopigment has the best color output?

H_0: The plant pigments will not produce the best color output compared to the other biopigments based on the absorbance and transmittance percentage from a visible spectrophotometer.

H_A: The plant pigments will produce the best color output compared to the other biopigments based on the absorbance and transmittance percentage from a visible spectrophotometer.

Methods

Different methods were created to evaluate the independent variables to test the predicted hypotheses. The four categories of pigments centered on the control synthetic dye, plant dyes, bacterial dyes, and fungal dyes. The plant pigments were derived from *Allium cepa* (onion) peels (P1) and *Quercus robur* (oak) leaves (P2). The bacterial pigments originated as secondary

metabolites from the bacteria *Monascus sanguineus* (B1) in the form of monascus red pigment and *Aspergillus carbonarius* (B2) in the form of melanin pigment (Kramer & Kostic, 2022; Amen et al., 2022). The fungi biopigments that were desired originated from the *Scytalidium cuboideum* (F1) and *Chlorociboria aeruginosa* (F2), which produce draconin red and xylindein, respectively (Hinsch et al., 2015). The standard synthetic dye was used to compare it with the natural alternatives. As a constant, 100% wool was the textile preference because of its porous texture and lack of need for mordants. Seventy, six by four-inch sections of wool were cut and dyed; ten strips were used for each pigment. These strips were held in each dye bath for 30 seconds and left to dry untouched for 24 hours or more for each pigment. The control was synthetic dye as the uptake and production efficiency are already known, and the differences will be observed against the natural alternatives. The color was observed, and each pigment was measured by its yield ratio in milligrams per milliliter to test the efficiency of usage, average absorbance, and average transmittance percentage to evaluate the color. A chi-squared test of association to determine the correlation between the average absorbance value and average transmittance percentage for each corresponding pigments, as well as a paired t-test on the average color analysis values, and ANOVA tests with the raw data were used on to determine the significance of the correlations. Then finally after all the data was collected the biopigments were compared to each other and how they measure to the synthetic dye.

Synthetic Dye Bath Method

A standard bottle of red concentrated dye was used as a control group. Following the instructions on the back calling for the desired concentration, 16.56 millimeters of the liquid dye and 600 milliliters of distilled water constituted the dye bath. The dye concentration was

heated until boiling, then the strips were inserted in the dye for 30 seconds each. After 30 seconds, the wool was dried on a wire hanger with a clothespin. Then, the fabric sections were evaluated using the spectrophotometer after thoroughly drying.

Onion Peel Dye Bath Method

Allium cepa (onions or P1) was used as a sample of plant pigments. Only the peels were used to extract the pigment. 700 mL of distilled water made up the base of the dye bath. 28800 mg of peels were used in boiling water and left to simmer for 60 minutes, being stirred periodically. After 60 minutes, each strip was dyed and hung on wire hangers to dry. Furthering, the color evaluations were performed in the spectrophotometer.

Oak Leaf Dye Bath Method

Quercus robur (oak or P2) was the other plant pigment used to show variety, and the leaves were collected from the surrounding area of Keystone College. The oak leaves were rinsed in preparation for the dye bath to limit the outside factors from the environment in dye production. 28800 mg of leaves were used in 700 mL of boiled distilled water and simmered for 60 minutes. The wool was then dyed, and the observed color analysis was recorded.

Melanin Growth

The growth of *Aspergillus carbonarius* (B1) would then lead to melanin production in the solid-submerged fermentation of fruit and vegetable pulps (Aman et al., 2022). To produce this

pigment, the bacteria was set to grow in 15 tubes filled with a yeast nutrient broth for a month (Aman et al., 2022). After fermentation, the solution was sent through the centrifuge to solidify and filter out the necessary pigment (Aman et al., 2022). The solid pigment is then filtered from the yeast broth, purified, and extracted using sonication-assisted extraction with water (Aman et al., 2022). The yield output would then be weighed. However, although the necessary steps and procedures were taken, fermentation was not observed. To further complete the study's research questions, an alternative was needed to test the color and output. Instead of using naturally derived melanin, an alternative to synthetic melanin was used. Synthetic melanin is the same compound as the naturally derived pigment and was used to imitate the effects and characteristics of natural melanin exactly.

Melanin Dye Bath Method

As stated, fermentation was not observed to create the natural melanin produced by B1, so synthetic was used. Melanin has very low solubility in most solvents, yet a concentration was made to keep consistency with the other dye baths (Bronze-Uhle et al., 2015). The concentration was a ratio of 50 mL of room temperature distilled water with 8700 mg of the melanin pigment to stay consistent with some of the other biopigments. The wool strips were then dyed and set to dry on a sterile surface. Color evaluations were completed after drying and recorded.

Monascus Red Dye Bath & Dye Method

Another variety of bacteria pigment, *Monascus sanguineus* (B2), was used to produce a pigment called monasus red. This pigment is created from the breakdown of the solid-state

fermentation of broken rice (Aman et al., 2022). Due to the isolated origin of this species, a B2 strain could not be found or purchased legally in the United States. To get around this issue, a less pure alternative was used instead. Red yeast rice is a vitamin supplement prevalent throughout Asia and although less pure contains mainly the monascus red pigment (Dufossé et al., 2023). 29400 mg of the red yeast rice powder was combined with 700 mL of boiled distilled water and left to simmer for 60 minutes. The wool strips were dyed and hung on a wire hanger to dry. After 24 hours, the color was analyzed and recorded.

Fungal Pigment Growth Method

As for the fungal pigments, *Scytalidium cuboideum* (F1) produces the pigment draconin red, while *Chlorociboria aeruginosa* (F2) produces the pigment xylindein. Draconin red is a pigment certain fungi produces during wood coloration or spalting (Lin & Xu, 2022). Xylindein varies from yellow and green to blue and is also produced during wood coloration or spalting (Lin & Xu, 2022). To stimulate these pigmentation processes, the bacteria were cultivated on 30 plates, or 15 plates each, of malt agar, mixed with wood chunks for one month (Hinsch et al., 2015). After the growth duration ended, the samples were left to dry under a fume hood for 24 hours (Hinsch et al., 2015). 1-2 cm-sized pieces of the Petri plates would be weighed and recorded as the pigment yield, then placed into beakers of dichloromethane (DCM) and stirred for 30 minutes (Hinsch et al., 2015). The DCM would have been evaporated using a Buchi RE 111 Rotovapor at 25 psi with no heat (Hinsch et al., 2015). The extraction and evaporation processes would be repeated twice (Hinsch et al., 2015). Although, similarly to the melanin pigment, no pigmentation was observed in any of the samples. Due to the lack of pigmentation, concentrated alternatives were used that matched the characteristics of each

pigment. The alternatives used were Lawsone (F1), simulating *Scytalidium cuboideum*'s draconin red pigment, and p-Benzoquinone (F2), simulating *Chlorociboria aeruginosa*'s xylindein pigment.

Lawsone Dye Bath Method

The alternative to draconin red in this study was established to be Lawsone. Lawsone is a component from the leaves of the *Lawsonia inermis* or henna plant (Bonev & Cartwright-Jones, 2003). Draconin red is a type of pigment called quinone based on its molecular structure. Lawsone is another variety of a quinone pigment that produces an orangish-red color close to draconin red and is easy to find (Prabhu & Bhute, 2012). Lawsone is used heavily in cosmetic products like henna gel (Bonev & Cartwright-Jones, 2003). To create the dye bath for the Lawsone concentration, 17.22 mL of 95% ethanol was used at room temperature because Lawsone is insoluble in water (Bonev & Cartwright-Jones, 2003). The chemical provider already established the solvent ratio, which shifted with the study's desired amount of 2.996 mg added to the ethanol. The wool was then dyed and laid to dry on a sterile surface. After drying, the strips of wool were then evaluated with color analysis through the spectrophotometer

p-Benzoquinone Dye Bath Method

Due to the lack of growth from the F2 bacteria, an alternative was chosen, this being p-Benzoquinone. P- Benzoquinone is a comparable alternative as it is also a naphthoquinone pigment with a similar molecular structure and initial pigment color to xylindein, the desired pigment (Prabhu & Bhute, 2012). The compound of p-Benzoquinone is water soluble when at room temperature. However, it is incredibly toxic. The chemical provider has already established

the ratio of the solvent. Under a fume hood, 5.145 mg/mL p-Benzoquinone was mixed into 350 mL of distilled water to dilute the powder, following the safety water solubility ratio. The ten strips were then dyed separately and set to dry on a sterile surface. After drying, a spectrophotometer evaluated the strips with a color analysis.

Color Evaluations

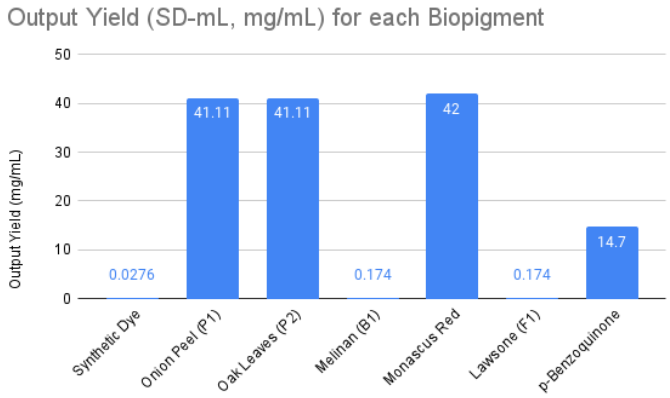
The color evaluations were done through observation and the fixed lens of a Thermo Scientific GENESYS 30 Visible Spectrophotometer with an equation of the default ABS(λ) x F1, where λ= 500 and F1= 1.000. To calibrate the machine a plain segment of wool was used as a control. Each of the 70 strips was cut into five fourth-of-an-inch segments spaced uniformly across the six inches used as separate trials to measure the color. Overall, each pigment had 50 trials of color analysis and was measured through the spectrophotometer one at a time. Both the absorbance number (ABS) and transmittance percentage (T%) were recorded for each strip and averaged .

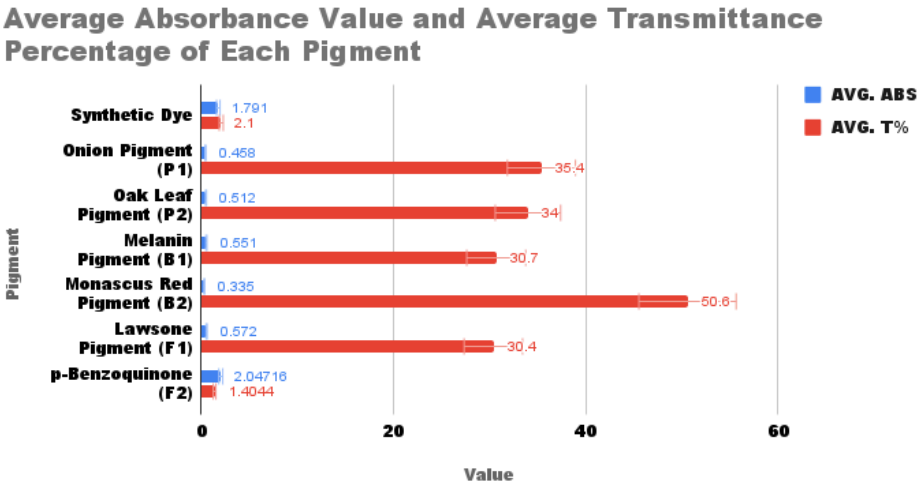
Results

The results of this study centered around the visible color of the pigment, yield of pigment quantity (mg/mL), absorbance value on fabric, and transmittance percentage of the fabric. As for color, there was a variety. The synthetic red dye produced a red-colored fabric. The P1 and P2 dyes produced a tan color, the onion peels being more yellow to orangish, while the oak leaves were more brown. As for the bacterial biopigments, the B1 wool

	Visible Color on Fabric
Synthetic Dye (Liquid)	Red
Onion Peel (P1)	Yellow/Tan
Oak Leaves (P2)	Brown/Tan
Melinan (B1)	Black
Monascus Red (B2)	Light Red
Lawsone (F1)	Orange/Yellow
p-Benzoquinone (F2)	Brown/Tan

segments were black, and the B2 pigment formed a light red pigment. Lastly, the color rendered by the fungal pigments included the F1 being orange and the F2 being brown. The yield of pigment was also measured by a ratio of milligrams per milliliter for each pigment. The values ranged from 0.174 mg/mL from B1 and F1 to 42 mg/mL of B2 for the tested biopigments. The other yield values included P1 and P2 with 41.11mg/mL, F2 with 14.7 mg/mL, and the exception of the liquid synthetic dye, which had a measurement of .0276 mL. Absorbance value (ABS) and transmittance percentage (T%) were taken from 50 segments from each pigment. The control and calibration constant was a plain wool section, which produced an ABS of 0 and a T% of 100. The average absorbance values were 1.79, .458, .512, .551, .335, .572, and 2.047 which correlated with the synthetic dye, P1, P2, B1, B2, F1, and F2 pigments respectfully. As for the average transmittance percentage, the synthetic dye produced an average of 2.1, the plant pigments being 35.4 (P1) and 34 (P2), the bacterial pigments being 30.7 (B1) and 50.6 (B2), as well as the fungal pigment's T% being 30.4 (F1) and 1.04 (F2).





The ABS and T% values were then put into an Unbalanced Two Way ANOVA test, a chi-squared test of association, and a paired t-test. The Unbalanced Two Way ANOVA test was done on the raw data of each pigment, with Factor A being the pigments and Factor B being the absorbance value and the transmittance percentage. The analysis part of the study showed that when comparing the data through ANOVA, the data is less than .05 p-value of less than 2.2e^(-16) with a degree of freedom of 6 for Factor A and Factor B being 1. As for the chi-squared test of the association between ABS and T%, it was shown that the chi-square statistic was 58.854, which is out of the region of acceptance (< 12.592). Continuing with the chi-squared results, the p-value equaled .000128. Finally, a paired t-test was also run on both average color analysis measurements per pigment, which also detailed that the p-value was less than .0001 with a degree of freedom of 349. The other values determined by the paired t-test were the standard deviation of the ABS as .6989 and the T% as 20.1623, with a standard error difference of 1.110.

ANOVA table (Type II)

Hover over the cells for formulas and calculation.

Source	DF	Sum of Square (SS)	Mean Square (MS)	F Statistic (df_1,df_2)	P-value
Factor A - rows (A)	6	45707.066	7617.844	120.773 (6,686)	< 2.2e-16
Factor B - columns (B)	1	112788.729	112788.729	1788.155 (1,686)	< 2.2e-16
Interaction AB	6	53068.959	8844.827	140.226 (6,686)	< 2.2e-16
Error	686	43269.772	63.075		
Total	**699**	**254834.526**	**364.57**		

Discussion

Although natural dyeing of fabric has been around since ancient times, as time has continued, society has become reliant on the most efficient methods of procedure, which has shifted the industry to more artificial methods. These artificial methods are reliable; however, they have come at the cost of more pollution and negative environmental effects due to universal use (Ardila-Leal et al., 2021). As the demand for more environmentally friendly methods has increased, other methods have been researched to determine the best scenario for both desires,

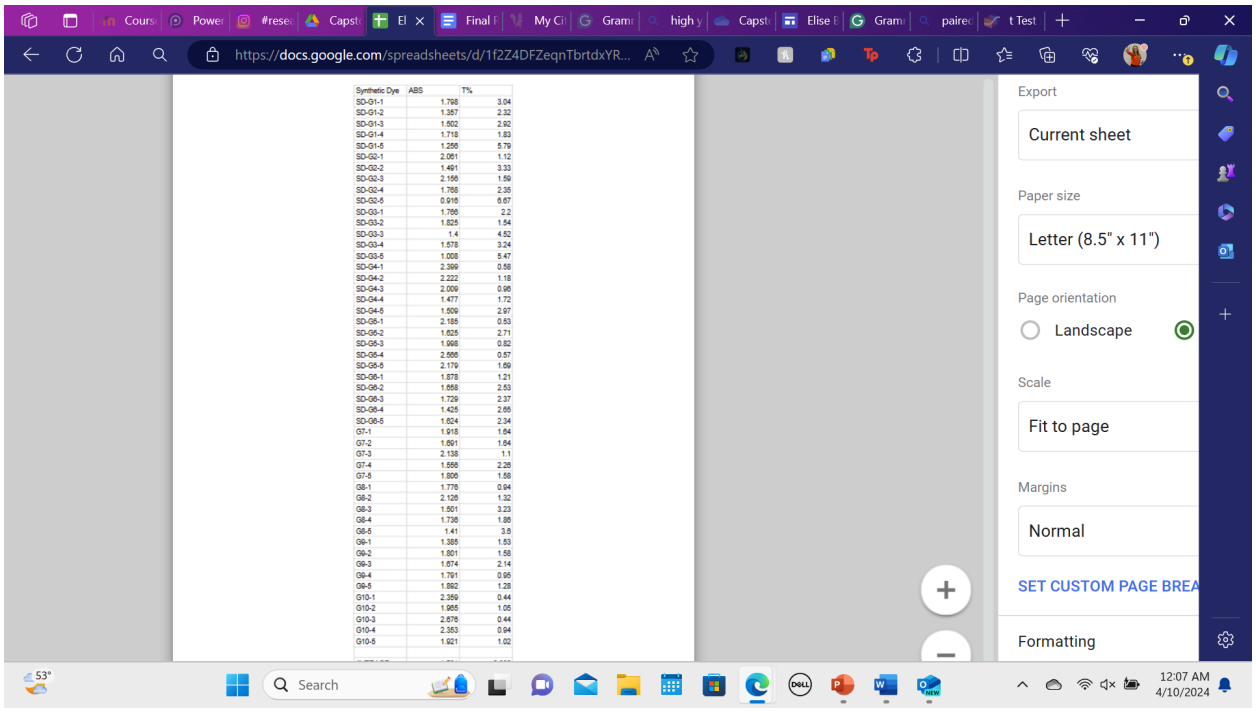
which are environmentally friendly and easy for commercial production. The most recent research has determined that natural resources like plants, bacteria, and fungi could produce pigments with the ability to dye fabric. Furthering, to evaluate this hypothesis that biopigments can dye fabric, the ABS and T% from a spectrophotometer were used. Further research on biopigments determined that each pigment could be used to dye the fabric. This is due to the observed change in the ABS and T% compared to the plain white wool at 0 and 100, respectively. Each pigment produced a higher ABS and a lower T% than the plain white wool, showing a change in color and dyeability. This validates this study's first hypothesis, as the data was partly used to establish that natural dyes could be a source of color. This hypothesis is also supported heavily by not only all the articles used in this study, but some of the first studies to demonstrate this idea were the teachings of ancient peoples for plants, *Let's Try Mushrooms for Color* by Miriam C. Rice using fungal pigments and the pioneer bacterial pigment study by A. Shirata, T. Tsukamoto, H. Yasui, and H. Hata called "Isolation of bacteria producing bluish-purple pigment and use for dyeing" (Ardila-Leal et al., 2021; Rice, 1974; Kramer & Kostic, 2022, & Shirata et al., 2000). Each rhetoric details the use of biopigments to produce color on fabric and serves as stepping stones to further advance the technology and knowledge in the field (Ardila-Leal et al., 2021; Rice, 1974; Kramer & Kostic, 2022, & Shirata et al., 2000). As the ABS increased, the T5 became lower than the control plain wool values. However, the plant dyes could be produced when observing the yield quantity for the second part of the first hypothesis. However, the sources could not produce the bacterial and fungal pigments. This goes against part of the hypothesis as no yield quantity was established. Nevertheless, all the studies referenced in this research could conceive colorant production, so human error or strain difference could have been at play. When evaluating the 350 trials of ABS and T%, the less than

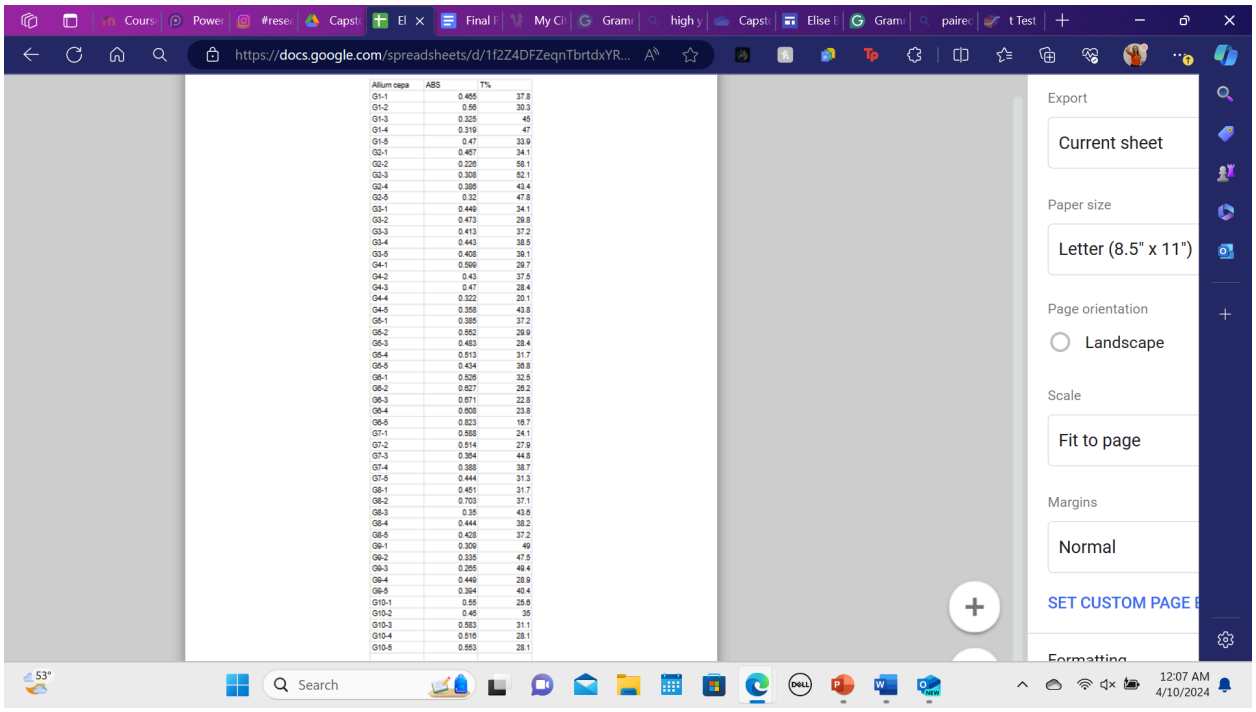
.05 p-values of ANOVA (2.2e^(-16)), chi-squared (.00128), and the paired t-test (.001) all determined significance in the values compared to the pigments they originated from as well as both values coordinating conversely with each other. Based on the data and analyzing the averages, the highest quality of color saturation came from the fungal alternatives, specifically the F2 pigment, which had the highest average ABS value of 2.047 and lowest average T% value of 1.4044, even compared to the synthetic comparison. The F1 pigment was not as extreme but produced a higher ABS and lower T% than the rest of the biopigments. The results differed mostly from the third research question hypothesis, as it was predicted that the plant pigments would have the best color pay-off compared to the other natural pigments. Lawsone is technically derived from the henna plant, but because of its molecular structure, it was used to simulate the fungal colorant *Scytalidium cuboideum,* which partly supports the hypothesis. In spite of this, the hypothesis is still invalid because it has not been completely demonstrated. However, the study called "A recent (2009–2021) perspective on sustainable color and textile coloration using natural plant resources" by Jiangning Che and Xu Yang states that the pay-off of plant dyes has been seen to achieve dark colors even on structured textiles giving potential of plant dyes being the most likely to produce a higher color saturation (Che & Yang, 2022). Lastly, the quantity yield results showed B1 and F1 having the lowest ratio of pigment to solvent for all biopigments, .174 mg/mL. When evaluating the data, both B1 And F1 would be able to create more dye with a set quantity than the other biopigments. This validates the second research question hypothesis as one of the microbial pigments could produce the most quantity yield during application based on the lower ratio. This is also true for other research done, as detailed in the study "The realm of microbial pigments in the food color market" by Babita Rana, Malini Bhattacharyya, Babita Papni, Mamta Arya, and Gopal K. Joshi presented that compared to plants

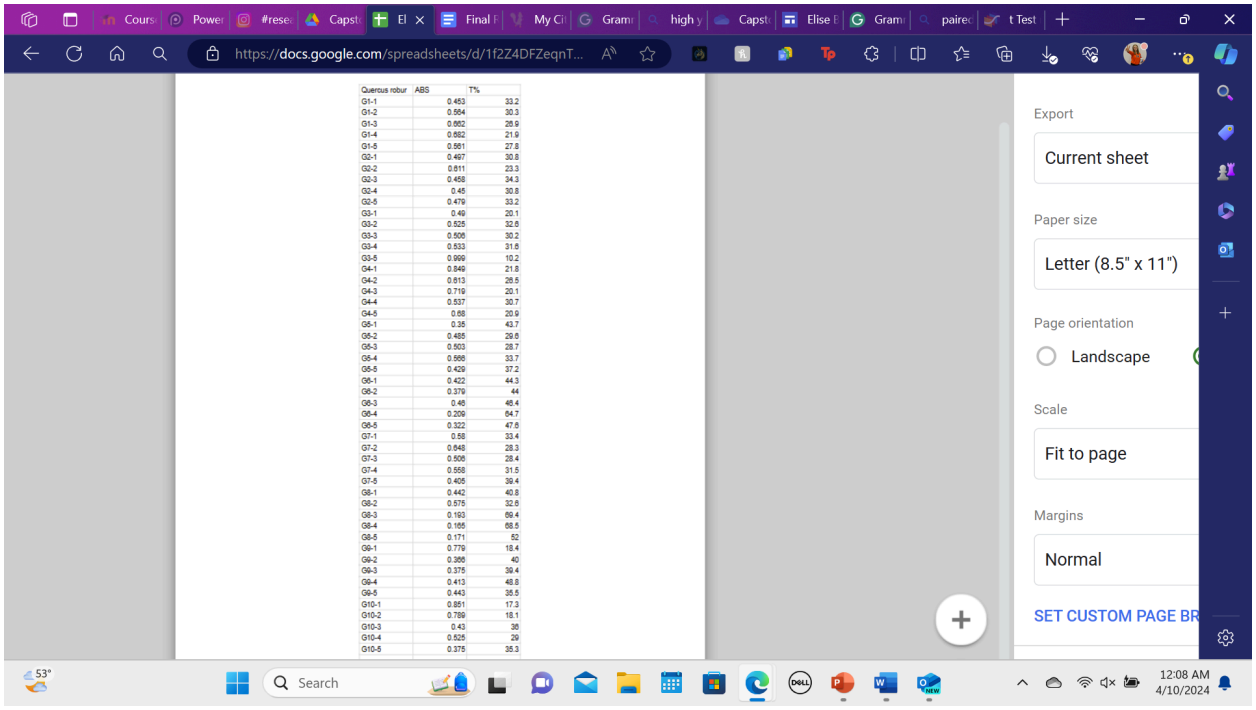
microbial colorants can produce improved yields predicting a higher quantity with more technology research (Rana et al., 2021). Overall, natural colorants are extremely promising for the future of sustainable products. These biopigments can produce stable color quantity and a variety of colors with the benefit of being biodegradable and organic. Despite knowing these pigments can be used, there is a lack of research comparing the standard synthetic dye to natural products. Other future study topics could include more knowledge on other species of organisms that produce colorants, mixtures of biopigments to provide new color options, or the possibility of using different biowaste as a natural colorant source. There is a whole realm of possibilities with natural pigments, and with the advancement of technology and research, biopigments could become the future of commercial dyeing.

Appendix

Raw Data:



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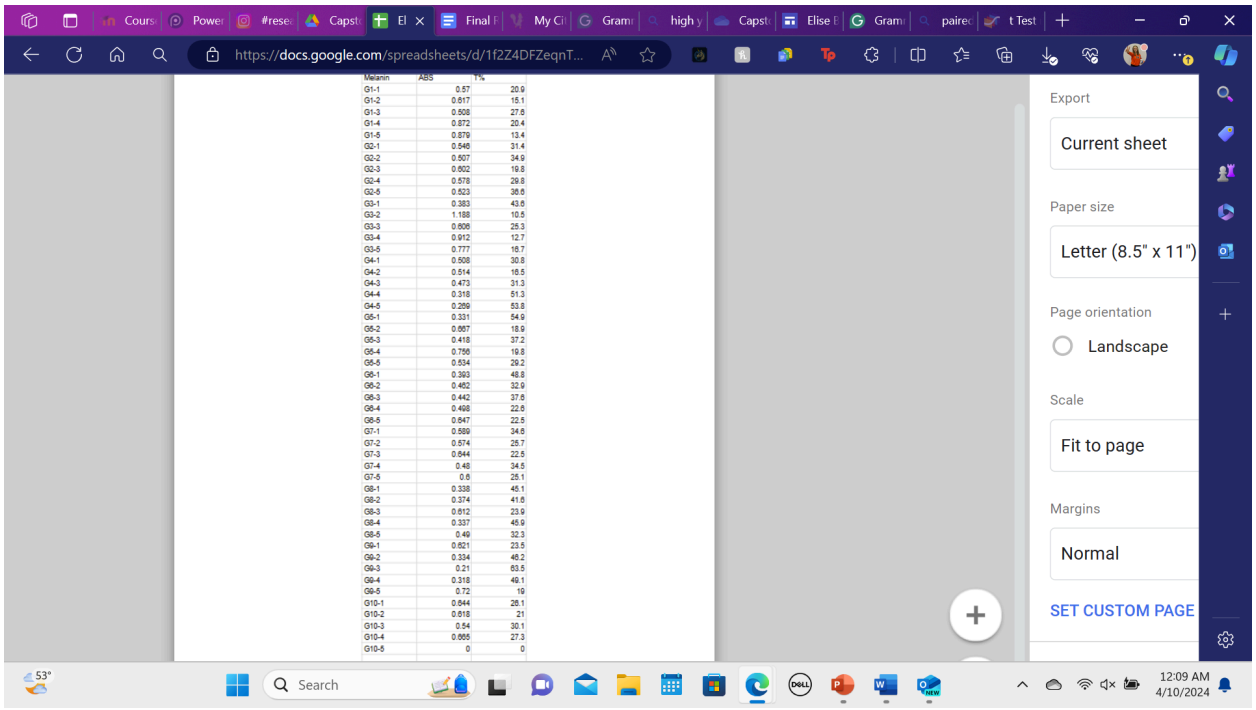
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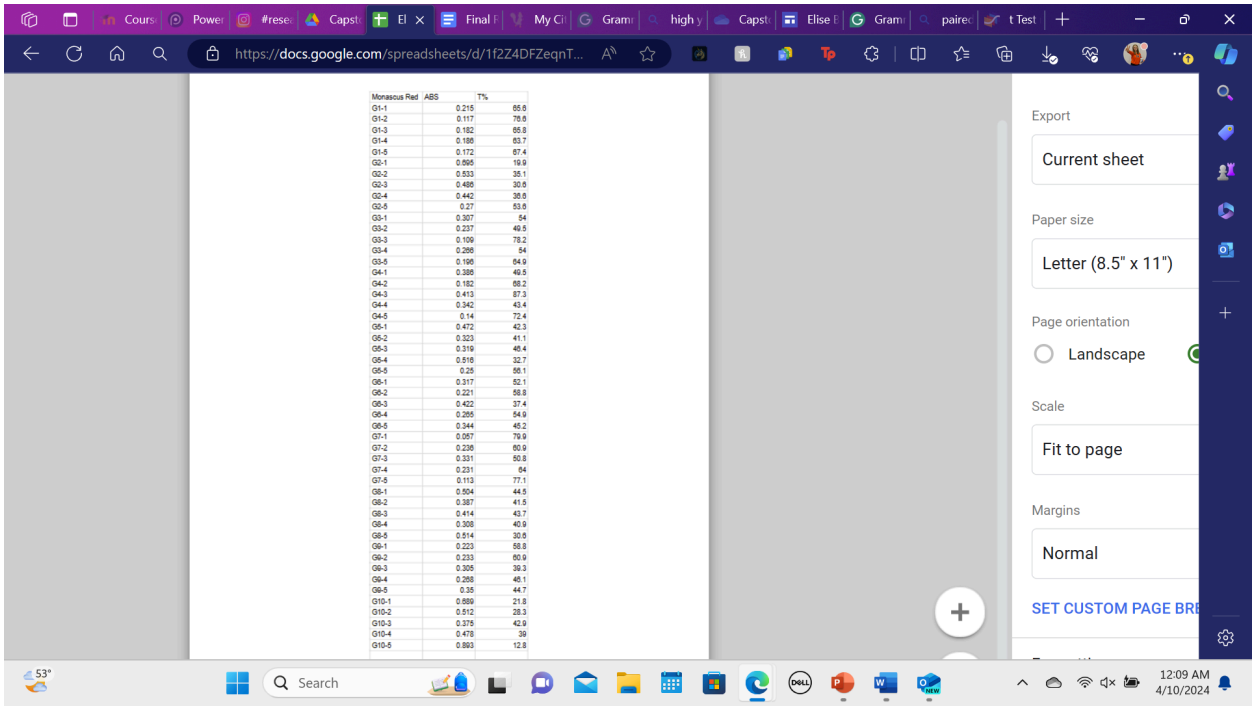
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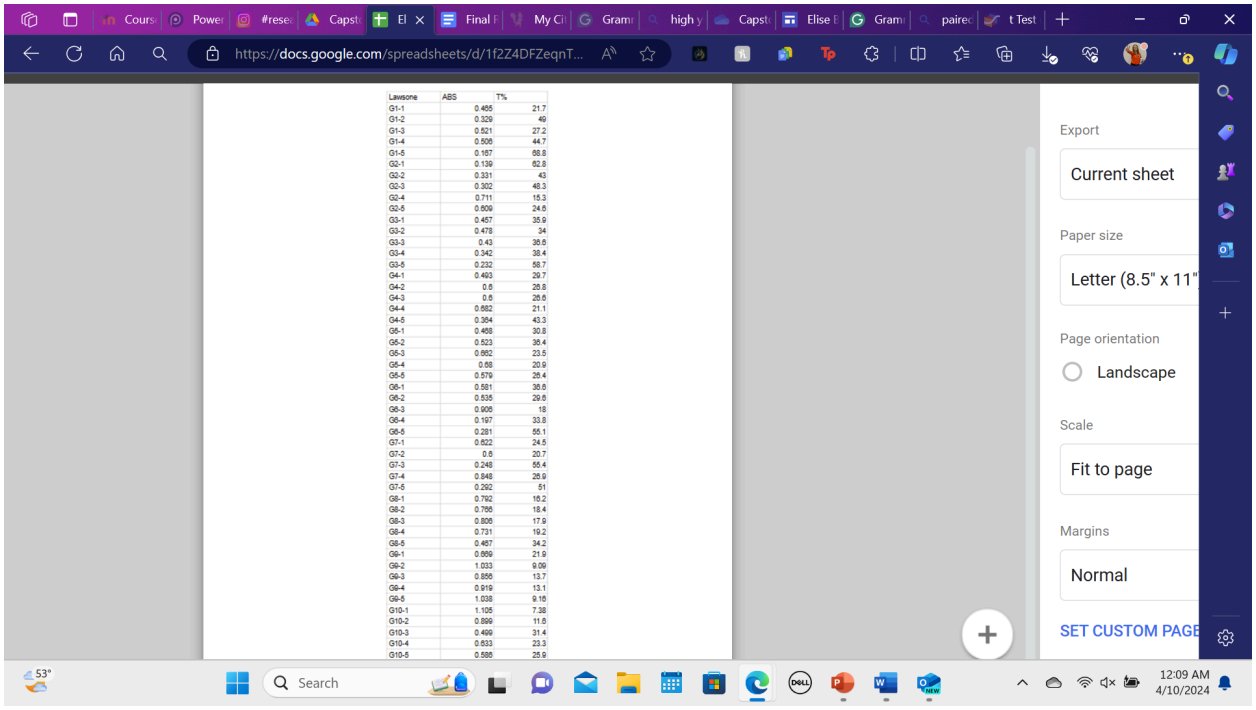
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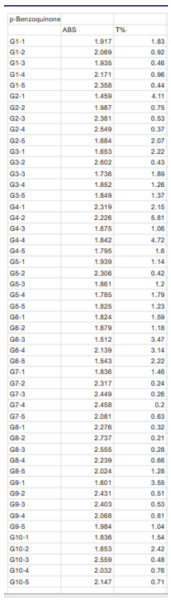

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