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De Novo Synthesis of Biopaint Using Transformed Bacteria: Analysis of Spectral Intensity Trends and Comparison to Commercial Paint

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Abstract

Background: Commercial paint pigments contain toxic heavy metals that harm humans and pollute the environment. To mitigate these harms, ecologically safe pigments are necessary. **Objective:** This experiment aims to create a biopaint de-novo using transformed *Escherichia coli* bacteria and compare it to commercial paint. **Methods:** Genetically engineered *E. coli* bacteria producing magenta pigment were grown in petri dishes. The pigment protein was extracted, filtered, and dehydrated into a crystalline powder. This was mixed with acrylic medium to make biopaint. The biopaint and commercial paint were applied on acrylic paper; red, green, blue, and total spectral intensities were measured daily under different testing conditions. Spectral intensity variability was measured and compared using the Coefficient of Variation (CV). Trends in spectral intensity were analyzed using regression analysis. **Results:** The differences in the CV of biopaint to commercial paint were less than 20% under all testing conditions. Spectral intensities for both biopaint and commercial paint did not show any significant change during the testing period under the conditions of room temperature, heat, and humidity. However, under the cold testing condition, biopaint showed a slight but statistically significant (p-value < 0.05) decline in total, red, and blue spectral intensities. **Conclusion:** This experiment proves that E. coli-derived pigments can be used to make biopaint which has a similar durability to commercial paint as measured by the spectral intensities.

Keywords

Synthetic Biology, *E. coli* Protein Pigments, Sustainable Paint, Biomaterials,

Microbial Pigments, Biogenic Paint Development

1. Introduction

The utilization of mineral pigments across various industrial sectors has been standard practice due to their appealing characteristics of vibrant colors, stability, and consistency [1]. Paint use has been increasing annually with the global pigment consumption reaching approximately 9.7 million metric tons in 2014 [2] [3]. In addition, around 700,000 to 800,000 tons of pigment are used in the dye industry and a significant amount is used in the cosmetics industry [4]-[8]. Pigment compounds are typically comprised of inorganic materials and are largely used in paints and coatings [5] [9]. Inorganic dyes derived from coal-tar derivatives have applications in textiles, food and beverages, paints and coatings, cosmetics, and personal care [5].

The adverse health and environmental ramifications of these inorganic colorants have raised significant concerns [10] [11]. Toxic heavy metals, including lead, titanium, arsenic, cadmium, cobalt, nickel, zinc, and manganese, are integral components of inorganic pigments and have been associated with considerable risks to both human and environmental health [12]-[16]. The detrimental effects of heavy metal pigments on human health are profound [16]-[20]. Research indicates that exposure to heavy metals in paint correlates with a significantly higher risk of developing certain types of cancers [21] [22]. The National Institute of Health (NIH) has classified several of these metals as carcinogens and a Chinese study concluded that the total carcinogenic risk posed by heavy metals in paints can reach close to 1%, highlighting the significant threat they represent [21]-[23]. Exposure to these metals, whether through skin absorption, ingestion, or inhalation, can lead to severe outcomes, including organ damage, neurological disorders, and death [20] [24]-[26]. Children are notably at risk from lead exposure from paints, which is linked to developmental delays, behavioral issues, and learning disabilities [20] [27]. There is a 1.16% increase in the incidence of childhood developmental disorders attributable to lead exposure [20] [28]-[30].

The environmental ramifications of inorganic pigments are equally concerning [10] [31]. The pigment production cycle, encompassing mining, milling, extracting, and grinding, contributes to environmental degradation, releasing toxic substances into the air, water, and soil [32]. This pollution adversely affects ecosystems and wildlife, with aquatic life being particularly vulnerable to the toxic effluents from pigment production processes, leading to significant water pollution and habitat destruction [10] [31] [32]. The bioaccumulation of heavy metals through the food chain can result in devastating impacts on biodiversity and ecosystem health [33]. Processes and procedures to maximize the utilization of mineral resources and minimize the environmental, social, and economic impacts associated have shown promise [34]. Studies have been done to guide urban

planning taking into account the influence of emissions on weather conditions [35].

Considering these adverse effects of inorganic pigments, a pursuit of sustainable and non-toxic alternatives is emerging. Sajjad *et al*. have suggested pigment production by cold-adapted bacteria and fungi can have wide-ranging applications and can represent a promising solution [36]. These organisms can produce a broad spectrum of colors without the negative environmental and health impacts associated with inorganic pigments [37] [38]. Non-toxic and sustainably sourced biopigments reduce the dependence on hazardous chemicals and processes. The potential applications of bacterial pigments are extensive and diverse [36] [39]-[41]. In the textile industry, which accounts for significant global water pollution, biopigments could offer an eco-friendly alternative to these inorganic dyes [42]-[44]. In the food industry, where consumers increasingly demand natural and safe products, natural colorants from bacteria could replace artificial additives [44]-[46]. Moreover, in cosmetics, bacterial pigments could provide safer, more sustainable coloring options, minimizing the risk of skin irritations and allergic reactions linked to inorganic colorants [44] [47] [48]. This research addresses the feasibility of utilizing *Escherichia coli* bacteria to produce sustainable pigments for paint applications. By leveraging specific bacterial strains known for their pigment-producing capabilities, this experiment aims to develop a viable method for generating safer and more environmentally friendly alternatives to commercial paint pigments. This innovation could pave the way to biopaint production and significantly reduce the environmental footprint of the paint, dye, and cosmetic industries.

2. Methods

Genetically engineered *Escherichia coli* bacteria capable of expressing a magenta pigment were cultured in petri dishes (plasmid vectors sourced from Amino Labs) (**Figure 1**). The bacterial cells were subjected to a lysis process to release the pigment. This was achieved through the application of Triton X-100, a nonionic surfactant, alongside Hen Egg White Lysozyme (HEWL), an enzyme that breaks down bacterial cell walls. The contents were allowed to settle for 24 hours at room temperature, to facilitate complete cell lysis and pigment release. The resultant solution underwent centrifugation at 15,000 revolutions per minute (RPM). This step facilitates the separation of the lysed cellular components from the soluble pigment. Subsequently, the supernatant was filtered through a 0.22 μm pore-size membrane filter, ensuring the removal of all cellular debris and leaving behind a pigment solution. This filtrate was then subjected to a dehydration process to harvest crystalline pigment powder (**Figure 2**). The pigment was then formulated into a biobased paint by amalgamating with an acrylic medium, creating a homogeneous mixture suitable for application (**Figure 3**).

For the experimental evaluation, eight one-centimeter by one-centimeter squares of acrylic painting paper served as a testing surface; four were coated with the

biopaint and four with a commercially available paint (Liquitex Acrylic Magenta). The testing conditions were exposure to room temperature (20˚C), heat (40˚C), cold (−10˚C), and humidity. The assessment of the paint's durability and color stability was conducted over a 10-day period. Spectral intensities (red, green, blue, total) of the painted squares were measured daily using the ColorPicker software application, which provided a quantitative measure of color retention and fading.

The variability in spectral intensity was quantified through the calculation of the coefficient of variation for both the biopaint and the commercial paint under each testing condition. A linear regression analysis was performed and analyzed to identify any significant trends over time. This approach allowed for a visualization of the biopaint's performance against that of commercial paint, aiming to highlight the potential benefits and limitations of biobased pigments in commercial applications.

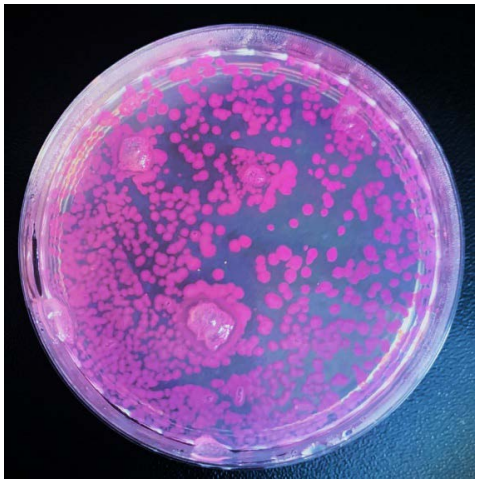

Figure 1. *Escherichia coli* bacterial colonies expressing pigment protein coded by jellyfish gene.

Figure 2. Extracted bacterial pigment.

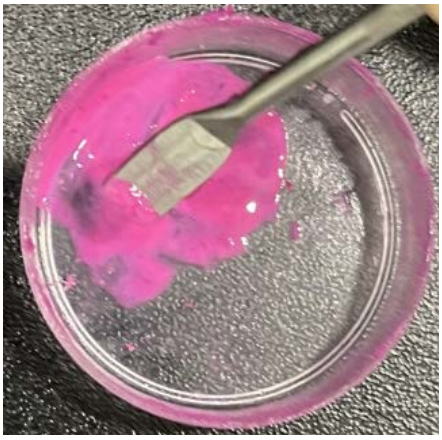

Figure 3. Amalgamate bacterial pigment with acrylic binder.

3. Results

The baseline spectral intensity characteristics (Mean ± SD) for biopaint and commercial paint along with the CV under the testing conditions are shown in Table 1. The total spectral intensity Mean with Standard Deviation (SD) is 164 ± 3.5 for the biopaint with a CV of 2.1 and 184.6 ± 6.1 for the commercial paint with a CV of 3.3. A determination was made *a priori*, for the purposes of this study, a difference in CV% of ≥20% is considered significant. As shown in Table 1, the CV variance between the biopaint and commercial paint ranged from 0.0% to 14.5% across all tested conditions.

Table 1. Baseline spectral intensity comparison.

	Biopaint		Commercial Paint		
	Room Temperature (20˚C)				
	Mean ± SD	CV	Mean ± SD	CV	CV Diff
Red	105.5 ± 1.1	1.0	106.4 ± 1.3	1.2	0.2
Green	10.6 ± 2.8	26.4	19.3 ± 3.2	16.6	9.8
Blue	47.9 ± 1.6	3.3	59.1 ± 2.0	3.4	0.1
Total	164 ± 3.5	2.1	184.8 ± 6.1	3.3	1.2
	Heat (40˚C)				
Red	106.3 ± 2.8	2.6	106.2 ± 1.8	1.7	0.9
Green	22.3 ± 6.7	30.0	19 ± 4.0	21.1	8.9
Blue	55.9 ± 4.8	8.6	58 ± 2.4	4.1	4.5
Total	184.5 ± 13.2	7.2	183.2 ± 7.7	4.2	3.0
	Cold (−10˚C)				
Red	140.9 ± 4.5	3.2	106.9 ± 1.4	1.3	1.9
Green	15.6 ± 5.6	35.9	19.2 ± 4.1	21.4	14.5
Blue	61.5 ± 6.4	10.4	59.3 ± 1.7	2.7	7.7
Total	218 ± 15.2	7.0	185.4 ± 5.9	3.2	3.8
	Humidity				
Red	116.3 ± 3.2	2.8	104.8 ± 1.5	1.4	1.4
Green	15.7 ± 5.0	31.8	18.5 ± 4.3	23.2	8.6
Blue	49.4 ± 6.4	13.0	57.2 ± 2.9	5.1	7.9
Total	181.4 ± 11.4	6.3	180.5 ± 8.2	4.5	1.8

The changes in spectral intensity values, including Total, Red, Green, and Blue, over time for each testing condition, are presented in Figures 4-7. These figures indicate the durability of the paints under different testing scenarios.

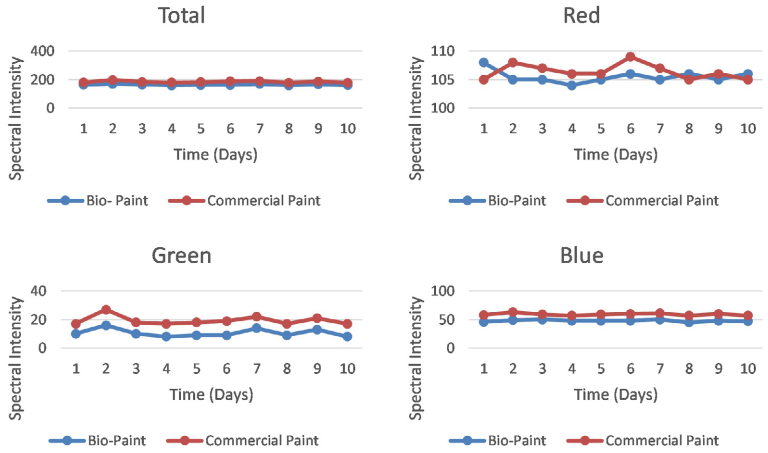

Figure 4. Testing condition: Room temperature.

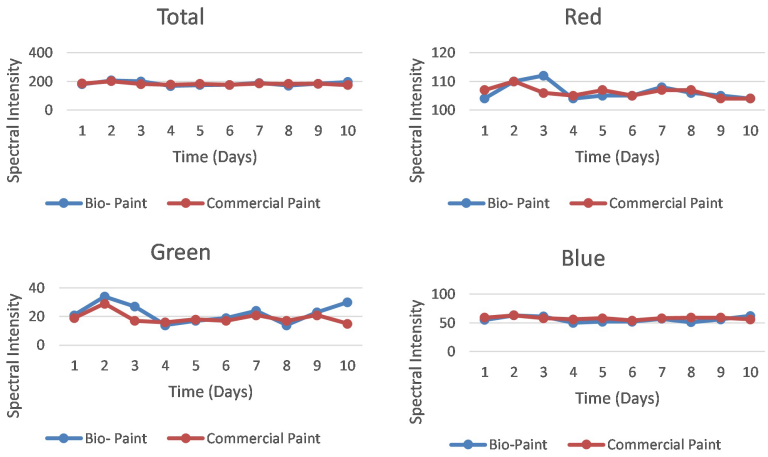

Figure 5. Testing condition: Heat.

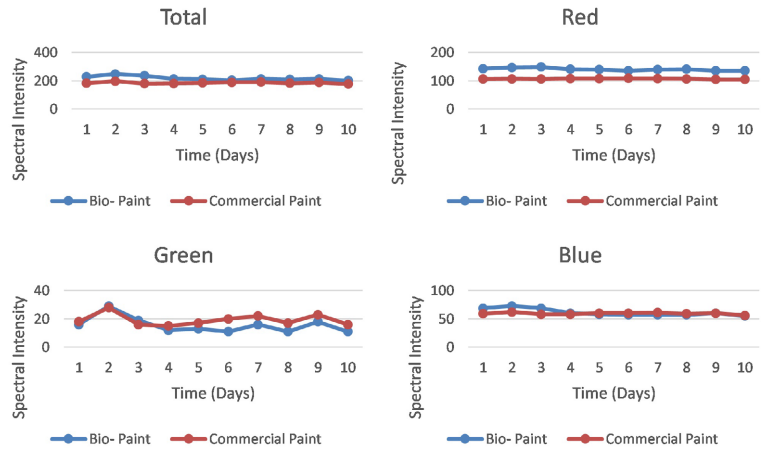

Figure 6. Testing condition: Cold.

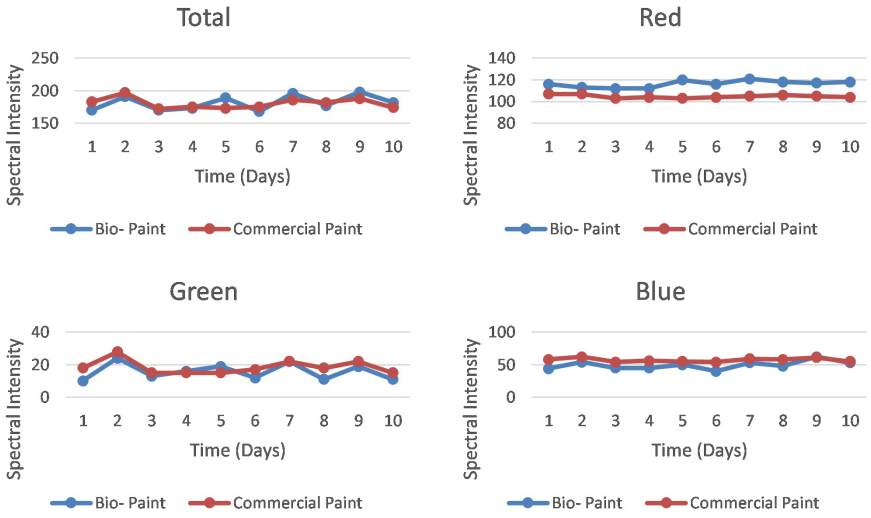

Figure 7. Testing condition: Humidity.

Table 2 shows the mean estimates of spectral intensity alongside their 95% confidence intervals (CI) and associated p-values. The mean estimate for biopaint's total spectral intensity at room temperature is 165.73 with a 95% CI 160.06 - 171.40 (p = 0.45). The mean estimate for commercial paint's total spectral intensity at room temperature is 187.33 with a 95% CI 177.43 - 209.50 (p = 0.52). As noted in Table 2, spectral intensity trends showed significant p-values for total, red, and blue spectral intensities for biopaint under the cold testing condition.

Table 2. Spectral intensity estimates, 95% confidence interval, and p-values testing for trends of significance.

Testing Condition - Room Temperature	Estimate	95% CI	p-Value
Total Spectral Intensity - Bio	165.73	160.06 - 171.40	0.45
Total Spectral Intensity - Commercial	187.33	177.43 - 209.50	0.52
Red Spectral Intensity - Bio	105.8	104.02 - 107.58	0.67
Red Spectral Intensity - Commercial	107	104.81 - 109.18	0.5
Green Spectral Intensity - Bio	11.47	6.93 - 16	0.63
Green Spectral Intensity - Commercial	20.33	15.01 - 25.65	0.63
Blue Spectral Intensity - Bio	48.47	45.85 - 51.08	0.59
Blue Spectral Intensity - Commercial	60	56.82 - 63.18	0.48
Testing Condition - Heat	Estimate	95% CI	p-Value
Total Spectral Intensity - Bio	187.73	165.96 - 209.5	0.71
Total Spectral Intensity - Commercial	189.4	177.95 - 200.85	0.2
Red Spectral Intensity - Bio	108.06	103.70 - 112.43	0.32
Red Spectral Intensity - Commercial	108.2	105.8 - 110.61	0.06
Green Spectral Intensity - Bio	23.27	12.16 - 34.37	0.82
Green Spectral Intensity - Commercial	21.6	15.31 - 27.89	0.31
Blue Spectral Intensity - Bio	56.4	48.44 - 64.36	0.87
Blue Spectral Intensity - Commercial	59.6	55.86 - 63.34	0.3
Testing Condition - Cold	Estimate	95% CI	p-Value
Total Spectral Intensity - Bio	238.89	222.32 - 255.41	0.01*
Total Spectral Intensity - Commercial	187.67	177.98 - 197.35	0.56
Red Spectral Intensity - Bio	147.13	142.31 - 151.95	0.01*

Continued

Red Spectral Intensity - Commercial	107.47	105.24 - 109.7	0.53
Green Spectral Intensity - Bio	20.67	12.59 - 28.75	0.14
Green Spectral Intensity - Commercial	20	13.23 - 26.77	0.77
Blue Spectral Intensity - Bio	71.07	65.1 - 77.04	0.01*
Blue Spectral Intensity - Commercial	60.2	57.48 - 62.92	0.42
Testing Condition - Humidity	Estimate	95% CI	p-Value
Total Spectral Intensity - Bio	173.4	155.84 - 190.96	0.27
Total Spectral Intensity - Commercial	187.67	177.98 - 197.35	0.56
Red Spectral Intensity - Bio	113	108.67 - 117.34	0.08
Red Spectral Intensity - Commercial	105.53	103.16 - 107.91	0.44
Green Spectral Intensity - Bio	16.53	8.17 - 24.91	0.8
Green Spectral Intensity - Commercial	19.53	12.41 - 26.65	0.72
Blue Spectral Intensity - Bio	43.87	34.53 - 53.20	0.16
Blue Spectral Intensity - Commercial	57.4	52.63 - 62.18	0.92

4. Discussion

The key advantage of biopigments lies in their minimal environmental and health impact. Unlike synthetic pigments, biopigments are typically biodegradable, non-toxic, and sustainable, making them an eco-friendly alternative. Their production and use do not involve harmful chemicals, ensuring that they are safer for both human health and the environment. As a result, they are gaining traction as a viable replacement in industries seeking to reduce their ecological footprint while maintaining vibrant and stable color properties [1].

This experiment has demonstrated that protein pigments synthesized by genetically modified *Escherichia coli* bacteria can be extracted, dehydrated, and subsequently formulated into a functional biogenic paint. Testing conditions in this experiment were chosen to reflect real-world environmental conditions. In the comparative analysis using the Coefficient of Variation to evaluate the distribution of spectral intensity across both biopaint and commercial paints, we observed that all resultant values were below the 20% threshold. This suggests consistency in spectral behavior, underscoring the biopaint's potential as a viable alternative to its commercial counterparts. In this experiment, a p-value <0.05 indicates the paint did lose or gain spectral intensity of statistical significance over the testing period. Conversely, a p-value >0.05 indicates the paint did not lose or gain spectral intensity of statistical significance over the testing period. The spectral intensity profiles of commercial paint remained largely unaltered across a spectrum of testing conditions over the ten-day period, as evidenced by p-values exceeding the 0.05 significance level. Notably, the biopaint exhibited similar stable spectral intensities under diverse environmental conditions, including Room Temperature, Heat, and Humidity. However, the biopaint's spectral intensities for total, red, and blue under cold conditions exhibited statistically significant deviations, with p-values below 0.05. Despite this statistical significance, the actual magnitude of these differences was relatively minor (3.79 for total spectral intensity, 1.13 for red, and 1.74 for blue), likely imperceptible to the human eye. The significant p-

values observed are potentially attributable to multiple comparisons. The family-wise error rate indicates an 81% chance of finding at least one false positive p-value.

We would like to acknowledge several study limitations. One limitation is the small sample size, necessitating future experimentation with a large sample size to validate these findings. Additionally, during the application, biopaint exhibited a more globular texture in comparison to commercial paint, potentially attributable to insufficient biopigment drying and grinding. It needs to be seen whether longer drying and finer grinding of the biopigment will enhance usability characteristics. The drying time for biopaint was observed to be longer than that of commercial paint, indicating potential practical implications. The extraction of pigments from bacterial sources also presents logistical and economic hurdles, given the labor-intensive process and the relatively low yield of pigment, raising questions about its scalability and commercial viability. Furthermore, the paint's durability over extended periods remains unexamined, as does its performance under a wider array of environmental conditions. This study also focused exclusively on magenta pigments, limiting the generalizability of these findings to other pigmentary formulations.

Despite these challenges, the potential applications for biogenic pigments in areas where commercial pigments pose environmental, or health risks, are promising. The production of pigments through bacterial synthesis can be a costly endeavor due to the relatively low yield associated with microbial processes. However, this method holds significant promise in niche applications, particularly in the generation of unique or exotic colors that are difficult to achieve through conventional methods. Moreover, bacterial pigments offer a sustainable alternative in cases where synthetic pigments pose substantial environmental risks. The field of cosmetology may benefit from the development of biopigments as safer alternatives to inorganic pigments. Additionally, advances in synthetic biology, including the utilization of fungal systems for pigment production, offer exciting avenues for overcoming current limitations and enhancing the economic feasibility of biogenic pigment production.

One limitation affecting the usability of biopaint is its extended drying time. Several biologically derived drying agents, such as eucalyptus oil, chitosan, starch, and fruit stone powders, are available on the market and have shown potential in accelerating the drying process in various applications. However, further research is necessary to evaluate whether the incorporation of these agents can enhance the performance characteristics of biopaints, particularly with respect to drying efficiency, surface finish, and durability. Exploring these additives could improve the practicality of biopaints for broader commercial use.

In conclusion, this exploratory study substantiates the feasibility of creating a biopaint from bacterial pigments, which closely mirrors the spectral characteristics of traditional commercial paints under various conditions over 10 days. Despite its preliminary nature and the obstacles identified, this research paves the

way for further innovations in the development of sustainable and environmentally friendly pigment sources.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Barreto, J.V.O., Casanova, L.M., Junior, A.N., Reis-Mansur, M.C.P.P. and Vermelho, A.B. (2023) Microbial Pigments: Major Groups and Industrial Applications. *Microorganisms*, **11**, Article 2920. https://doi.org/10.3390/microorganisms11122920

[2] Statista (2024) Market Volume of Paints and Coatings Worldwide from 2015 to 2019. https://www.statista.com/statistics/1042799/paints-and-coatings-market-volume-worldwide/#:~:text=The%20total%20global%20market%20volume,help%20to%20optimize%20many%20industries

[3] European C OATINGS (2016) Titanium Dioxide Remains the Most-in-Deman d Type of Pigments. https://www.european-coatings.com/news/raw-materials/titanium-dioxide-remains-the-most_in_demand-type-of-pigments/

[4] Slama, H.B., Chenari Bouket, A., Pourhassan, Z., Alenezi, F.N., Silini, A., Cherif-Silini, H., *et al*. (2021) Diversity of Synthetic Dyes from Textile Industries, Discharge Impacts and Treatment Methods. *Applied Sciences*, **11**, Article 6255. https://doi.org/10.3390/app11146255

[5] Synthetic Dye and Pigment Global Market Report 2024—By Type (Synthetic Dye, Synthetic Pigments), by Dye Type (Acid Dye, Reactive Dye, Disperse Dye, Basic Dye, VAT Dye, Other Dye Types), by Pigment Type (Organic, Inorganic), by Application (Textiles, Food and Beverage, Paint and Coatings, Cosmetics and Personal Care, Other Applications)—Market Size, Trends, and Global Forecast 2024-2033.

[6] (2024) Synthetic Dye and Pigment Global Market Report 2024. The Business Research Company. https://www.thebusinessresearchcompany.com/report/synthetic-dye-and-pigment-global-market-report

[7] Yadav, S., Tiwari, K.S., Gupta, C., Tiwari, M.K., Khan, A. and Sonkar, S.P. (2023) A Brief Review on Natural Dyes, Pigments: Recent Advances and Future Perspectives. *Results in Chemistry*, **5**, Article 100733. https://doi.org/10.1016/j.rechem.2022.100733

[8] Bhatia, D., Sharma, N.R., Singh, J. and Kanwar, R.S. (2017) Biological Methods for Textile Dye Removal from Wastewater: A Review. *Critical Reviews in Environmental Science and Technology*, **47**, 1836-1876. https://doi.org/10.1080/10643389.2017.1393263

[9] Jamee, R. and Siddique, R. (2019) Biodegradation of Synthetic Dyes of Textile Effluent by Microorganisms: An Environmentally and Economically Sustainable Approach. *European Journal of Microbiology and Immunology*, **9**, 114-118.

[10] American Coatings Association (2018) The Demand for Coatings Raw Materials to 2022.

https://www.paint.org/coatingstech-magazine/articles/demand-coatings-raw-materials-to-2022/

[11] Ardila-Leal, L.D., Poutou-Piñales, R.A., Pedroza-Rodríguez, A.M. and Quevedo-Hidalgo, B.E. (2021) A Brief History of Colour, the Environmental Impact of Synthetic Dyes and Removal by Using Laccases. *Molecules*, **26**, Article 3813. https://doi.org/10.3390/molecules26133813

[12] Kim, J. (2023) The Environmental and Health Impact of Paint Products. https://nhsjs.com/2023/the-environmental-and-health-impact-of-paint-products/

[13] Rebelo, A., Pinto, E., Silva, M.V. and Almeida, A.A. (2015) Chemical Safety of Children's Play Paints: Focus on Selected Heavy Metals. *Microchemical Journal*, **118**, 203-210. https://doi.org/10.1016/j.microc.2014.09.008

[14] Khan, M.R., Ahmad, N., Ouladsmane, M. and Azam, M. (2021) Heavy Metals in Acrylic Color Paints Intended for the School Children Use: A Potential Threat to the Children of Early Age. *Molecules*, **26**, Article 2375. https://doi.org/10.3390/molecules26082375

[15] Skocaj, M., Filipic, M., Petkovic, J. and Novak, S. (2011) Titanium Dioxide in Our Everyday Life; Is It Safe? *Radiology and Oncology*, **45**, 227-247. https://doi.org/10.2478/v10019-011-0037-0

[16] New Jersey Department of Health (2016) Hazardous Substance Fact Sheet. https://nj.gov/health/eoh/rtkweb/documents/fs/1861.pdf

[17] Rashid, M.M., Forte Tavčer, P. and Tomšič, B. (2021) Influence of Titanium Dioxide Nanoparticles on Human Health and the Environment. *Nanomaterials*, **11**, Article 2354. https://doi.org/10.3390/nano11092354

[18] Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N. (2014) Toxicity, Mechanism and Health Effects of Some Heavy Metals. *Interdisciplinary Toxicology*, **7**, 60-72. https://doi.org/10.2478/intox-2014-0009

[19] Witkowska, D., Słowik, J. and Chilicka, K. (2021) Heavy Metals and Human Health: Possible Exposure Pathways and the Competition for Protein Binding Sites. *Molecules*, **26**, Article 6060. https://doi.org/10.3390/molecules26196060

[20] Briffa, J., Sinagra, E. and Blundell, R. (2020) Heavy Metal Pollution in the Environment and Their Toxicological Effects on Humans. *Heliyon*, **6**, e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

[21] World Health Organization (2023) Lead Poisoning. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

[22] Kim, H.S., Kim, Y.J. and Seo, Y.R. (2015) An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. *Journal of Cancer Prevention*, **20**, 232-240. https://doi.org/10.15430/jcp.2015.20.4.232

[23] Wang, B., Su, Y., Tian, L., Peng, S. and Ji, R. (2020) Heavy Metals in Face Paints: Assessment of the Health Risks to Chinese Opera Actors. *Science of the Total Environment*, **724**, Article 138163. https://doi.org/10.1016/j.scitotenv.2020.138163

[24] Guha, N., Merletti, F., Steenland, N.K., Altieri, A., Cogliano, V. and Straif, K. (2010) Lung Cancer Risk in Painters: A Meta-Analysis. *Environmental Health Perspectives*, **118**, 303-312. https://doi.org/10.1289/ehp.0901402

[25] Cleveland Clinic (2024) Heavy Metal Poisoning (Heavy Metal Toxicity): Symptoms, Causes & Treatment.

[26] (2024) Understanding Cobalt's Human Cost. ScienceDaily. https://www.sciencedaily.com/releases/2021/12/211217113232.htm

[27] Medscape (2023) Heavy Metal Toxicity. https://emedicine.medscape.com/article/814960-overview?form=fpf

[28] Delgado, C.F., Ullery, M.A., Jordan, M., Duclos, C., Rajagopalan, S. and Scott, K. (2018) Lead Exposure and Developmental Disabilities in Preschool-Aged Children. *Journal of Public Health Management and Practice*, **24**, e10-e17. https://doi.org/10.1097/phh.0000000000000556

[29] Geier, D., Kern, J. and Geier, M. (2017) Blood Lead Levels and Learning Disabilities: A Cross-Sectional Study of the 2003-2004 National Health and Nutrition Examination Survey (NHANES). *International Journal of Environmental Research and Public Health*, **14**, Article 1202. https://doi.org/10.3390/ijerph14101202

[30] Centers for Disease Control and Prevention (2024) Health Effects of Lead Exposure.

[31] Mayans, L. (2019) Lead Poisoning in Children. *American Academy of Family Physicians*, **100**, 24-30.

[32] Ali, H., Khan, E. and Ilahi, I. (2019) Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, **2019**, 1-14. https://doi.org/10.1155/2019/6730305

[33] O'Brien, S. and Buckling, A. (2015) The Sociality of Bioremediation. *EMBO Reports*, **16**, 1241-1245. https://doi.org/10.15252/embr.201541064

[34] Almeida, V.O.D. and Schneider, I.A.H. (2022) Hydrometallurgical Processing of Brazilian Iron Ore Tailings for the Synthesis of Pigments. *Geomaterials*, **12**, 30-36. https://doi.org/10.4236/gm.2022.122003

[35] Henninger, S. (2012) Biogenic Isoprene and Its Impact on Human Health in Dependence on Meteorological Conditions. *Journal of Environmental Protection*, **3**, 1206-1212.

[36] Bałdowska-Witos, P., Tomporowski, A. and Bieliński, M. (2023) Using the LCA Method to Develop the Production of Pigment for Processing Plastics. *Materials*, **16**, Article 5524. https://doi.org/10.3390/ma16165524

[37] Sajjad, W., Din, G., Rafiq, M., Iqbal, A., Khan, S., Zada, S., *et al*. (2020) Pigment Production by Cold-Adapted Bacteria and Fungi: Colorful Tale of Cryosphere with Wide Range Applications. *Extremophiles*, **24**, 447-473. https://doi.org/10.1007/s00792-020-01180-2

[38] Orlandi, V.T., Martegani, E., Giaroni, C., Baj, A. and Bolognese, F. (2021) Bacterial Pigments: A Colorful Palette Reservoir for Biotechnological Applications. *Biotechnology and Applied Biochemistry*, **69**, 981-1001. https://doi.org/10.1002/bab.2170

[39] Amorim, L.F.A., Fangueiro, R. and Gouveia, I.C. (2022) Characterization of Bioactive Colored Materials Produced from Bacterial Cellulose and Bacterial Pigments. *Materials*, **15**, Article 2069. https://doi.org/10.3390/ma15062069

[40] Numan, M., Bashir, S., Mumtaz, R., Tayyab, S., Rehman, N.U., Khan, A.L., *et al*. (2018) Therapeutic Applications of Bacterial Pigments: A Review of Current Status and Future Opportunities. 3 *Biotech*, **8**, Article No. 207. https://doi.org/10.1007/s13205-018-1227-x

[41] Narsing Rao, M.P., Xiao, M. and Li, W. (2017) Fungal and Bacterial Pigments: Secondary Metabolites with Wide Applications. *Frontiers in Microbiology*, **8**, Article 1113. https://doi.org/10.3389/fmicb.2017.01113

[42] Kumar, S., Kumar, V., Ambika, A.A.A., Nag, D., Kumar, V., Darnal, S., *et al*. (2022) Microbial Pigments: Learning from Himalayan Perspective to Industrial Applications. *Journal of Industrial Microbiology and Biotechnology*, **49**, kuac017. https://doi.org/10.1093/jimb/kuac017

[43] Li, F., Guo, Z., Mao, L., Feng, J., Huang, J. and Tao, H. (2023) Impact of Textile Industries on Surface Water Contamination by Sb and Other Potential Toxic Elements: A Case Study in Taihu Lake Basin, China. *International Journal of Environmental Research and Public Health*, **20**, Article 3600. https://doi.org/10.3390/ijerph20043600

[44] Lellis, B., Fávaro-Polonio, C.Z., Pamphile, J.A. and Polonio, J.C. (2019) Effects of Textile Dyes on Health and the Environment and Bioremediation Potential of Living Organisms. *Biotechnology Research and Innovation*, **3**, 275-290. https://doi.org/10.1016/j.biori.2019.09.001

[45] Di Salvo, E., Lo Vecchio, G., De Pasquale, R., De Maria, L., Tardugno, R., Vadalà, R., *et al*. (2023) Natural Pigments Production and Their Application in Food, Health and Other Industries. *Nutrients*, **15**, Article 1923. https://doi.org/10.3390/nu15081923

[46] Novais, C., Molina, A.K., Abreu, R.M.V., Santo-Buelga, C., Ferreira, I.C.F.R., Pereira, C., *et al*. (2022) Natural Food Colorants and Preservatives: A Review, a Demand, and a Challenge. *Journal of Agricultural and Food Chemistry*, **70**, 2789-2805. https://doi.org/10.1021/acs.jafc.1c07533

[47] Sen, T., Barrow, C.J. and Deshmukh, S.K. (2019) Microbial Pigments in the Food Industry—Challenges and the Way Forward. *Frontiers in Nutrition*, **6**, Article 7. https://doi.org/10.3389/fnut.2019.00007

[48] Kiki, M.J. (2023) Biopigments of Microbial Origin and Their Application in the Cosmetic Industry. *Cosmetics*, **10**, Article 47. https://doi.org/10.3390/cosmetics10020047