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Exploring of Bacterial Pigments from Thamirabarani River Alluvial Soil: Harnessing their Versatility in Dye and Paint Manufacturing

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Abstract:- Color is a fundamental aspect of human life, playing a vital role in culture and industry. The paints and coatings industry, a significant sector within global manufacturing, has seen increased scrutiny due to its environmental and health impacts. Conventional paints contain harmful chemicals like volatile organic compounds (VOCs) and solvents, which pose risks to both workers and the environment. Recent shifts towards natural and bio-based pigments, particularly those derived from microorganisms, offer a promising alternative. Microbial pigments, such as those produced by chromogenic bacteria, provide eco-friendly, non-toxic colorants with additional benefits like antioxidant and antimicrobial properties. This review highlights the potential of bacterial pigments in various applications, particularly in the textile industry as natural dyes, and discusses their role in reducing the environmental footprint of synthetic pigments. The study also emphasizes the importance of further research into improving the production and stability of these bio-pigments for broader commercial use in industries such as food, cosmetics, and pharmaceuticals.

Keywords:- *(Microbial Pigments, Natural Colorants, Bacterial Pigments, Bio-Paints, Synthetic Dyes, Volatile Organic Compounds (VOCs), Eco-Friendly Dyes, Textile Industry, Environmental Impact, Antimicrobial Properties, Antioxidant Properties, Chromogenic Bacteria, Natural Dyes, Bio-Colours, Non-Toxic Pigments, Microbial Autecology.)*

I. INTRODUCTION

Colour is an integral aspect of human experience, deeply embedded in culture and everyday life. Among the many ways color is utilized, paint plays a vital role, encompassing a variety of products such as varnishes, lacquers, coatings, and primers. Paint is not only used to enhance the appearance of surfaces but also to protect and preserve materials, both natural and man-made. The paint and coatings industry is a significant segment of global manufacturing, providing essential products for a wide range of applications. Despite facing challenges during the COVID-19 pandemic, which saw a temporary decline in the market, the industry is projected to grow substantially by 2029, driven by increasing demand.

However, the widespread use of paint comes with significant health and environmental concerns. Traditional paints contain a complex mixture of chemicals, including resins, pigments, solvents, and various additives. Workers in the paint industry, particularly painters, are frequently exposed to these substances, which can lead to serious health risks. Research has shown that exposure to volatile organic compounds (VOCs) and other chemicals in paints is linked to chronic health issues such as respiratory problems, skin allergies, and neurological damage. Moreover, the environmental impact of these chemicals is profound, as VOCs contribute to air pollution and pose risks to both human health and the ecosystem.

In response to these concerns, there has been a growing shift towards the development of safer, more environmentally friendly paints. Water-based paints, which contain fewer harmful solvents, have emerged as a preferable alternative. However, the need for even more sustainable solutions has led to increased interest in natural pigments and bio-paints. These alternatives are derived from natural sources, including microorganisms, and offer a promising way to reduce the environmental footprint of the paint industry.Microbial pigments, produced by bacteria, are particularly noteworthy for their potential applications in various industries. Unlike synthetic pigments, which have been associated with toxicity and environmental harm, microbial pigments are biodegradable and can be produced sustainably. Additionally, these natural pigments possess valuable bioactive properties, such as antibacterial and antioxidant effects, making them suitable for use in food, cosmetics, textiles, and pharmaceuticals. As the demand for eco-friendly products grows, microbial pigments represent a promising solution that aligns with the goals of sustainability and health protection.

This research aims to explore the production and application of microbial pigments as a viable alternative to synthetic pigments. By investigating the potential of these bio-paints and bio-dyes, this study seeks to contribute to the development of safer, more sustainable products that minimize environmental impact while meeting the demands of modern industry.

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➢ *Aim and Objectives:*

The aim of this study is to isolate, identify, and characterize pigment-producing bacteria from the alluvial soil of the Thamirabarani River for the production of dyes and bio-paints. The specific objectives are:

- To isolate pigment-producing microbes from the alluvial soil sample.
- To identify the isolated microbes through staining and biochemical tests.
- To determine microbial species using 16S rRNA gene sequencing.
- To screen pigments from selected microbes via submerged fermentation.
- To extract pigments using centrifugation.
- To characterize pigments through qualitative and quantitative analyses.
- To assess the antibacterial activity of pigments against test pathogens.
- To prepare dyes and paints using the extracted color pigments.

II. LITERATURE REVIEW

- **Title:** The Role of Natural and Synthetic Pigments in Various Life Forms and Industrial Applications
- Authors: Cristea, D., Moisescu, G., & Nicolescu, I. 2006

This study explores the presence and significance of pigments across various life forms, including plants, animals, bacteria, and fungi. It highlights the natural roles of pigments in processes such as photosynthesis, respiration, and protection against environmental stress. Additionally, the study discusses the industrial applications of both natural and synthetic pigments in products ranging from food and cosmetics to textiles and medicines. It emphasizes the pharmacological properties of natural pigments, such as their anticancer and cardiovascular benefits.

- **Title:** The Historical Context and Decline of Synthetic Colorants in Food, Medicine, and Cosmetics
- **Authors:** Freedman, D.A., Cowan, N.J., & O'Connell, P.- 1978

This study provides a historical overview of the introduction and widespread adoption of synthetic colorants in the 19th century. Initially celebrated as a technological breakthrough, synthetic colorants were preferred over natural ones due to their stronger tinctorial properties and perceived safety. However, over the past five decades, concerns about the health impacts of synthetic additives, including colorants, have led to a decline in their usage, especially in the food industry. The study also discusses the regulatory measures implemented to ensure the safety of food color additives.

- **Title:** Natural Colorants in Food: Their Origin, Classification, and Application
- **Authors:** Delgado-Vargas, F., Jiménez, A.R., & Paredes-López, O.-2002

This study examines the sources, classification, and applications of natural colorants in food. It categorizes natural pigments based on their origin (plant, animal, or microbial) and chemical structure. The study highlights the instability of natural colorants and their sensitivity to storage and processing conditions. It also addresses the growing interest in natural pigments as alternatives to synthetic colorants, due to their potential health benefits and eco-friendly attributes. The study discusses the challenges in using natural colorants and the ongoing research to improve their stability and production efficiency

- **Title:** Developing Concern on the Use of Consumable Coloring Agents and Their Natural Alternatives
- **Authors:** Eyal, J., et al.- 1992

This study reports the growing concerns over the use of consumable synthetic coloring agents, many of which have been prohibited due to their potential carcinogenic and teratogenic effects. The authors discuss the shift towards natural colors, also known as biochromes, which are produced by living organisms and exhibit specific color due to selective light absorption. The study emphasizes the longstanding debate over synthetic food colors and the increasing consumer interest in natural coloring alternatives, sourced from minerals, plants, microalgae, and pigment-producing microorganisms like fungi, yeast, and bacteria. These natural colors are highlighted for their ability to absorb specific wavelengths of light, with the reflected wavelengths determining the color perceived by the human eye.

- **Title:** Enhancing Music Streaming Experiences with Predictive Analytics: An Indian Perspective.
- **Authors:** Iyer, S., Kulkarni, V., & Rao, M.
- **Year:** 2018

This study uses predictive analytics to enhance user experiences in Indian music streaming platforms. It focuses on predicting user actions such as song skips and playlist modifications using machine learning techniques, with the aim of creating more personalized and engaging music streaming experiences.

III. METHODOLOGY

➢ *Soil Sample Collection:*

Soil samples were collected from the alluvial soil near the Thamirabarani River at Sivalaperi village, Palayamkottai taluka, Tirunelveli district, Tamil Nadu, India. The samples were stored in airtight zip-lock bags under sterile conditions and transported to the laboratory. After each use, the containers were sealed. The soil samples were thoroughly washed with sterile distilled water to remove any floating dust and debris.

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Fig 1 Soil Sample Collection

➢ *Serial Dilution Method:*

Serial dilution is a method used to estimate the concentration of microorganisms in a sample by creating stepwise dilutions and counting the colonies that grow from each dilution. This helps in determining the microbial load in the original sample.

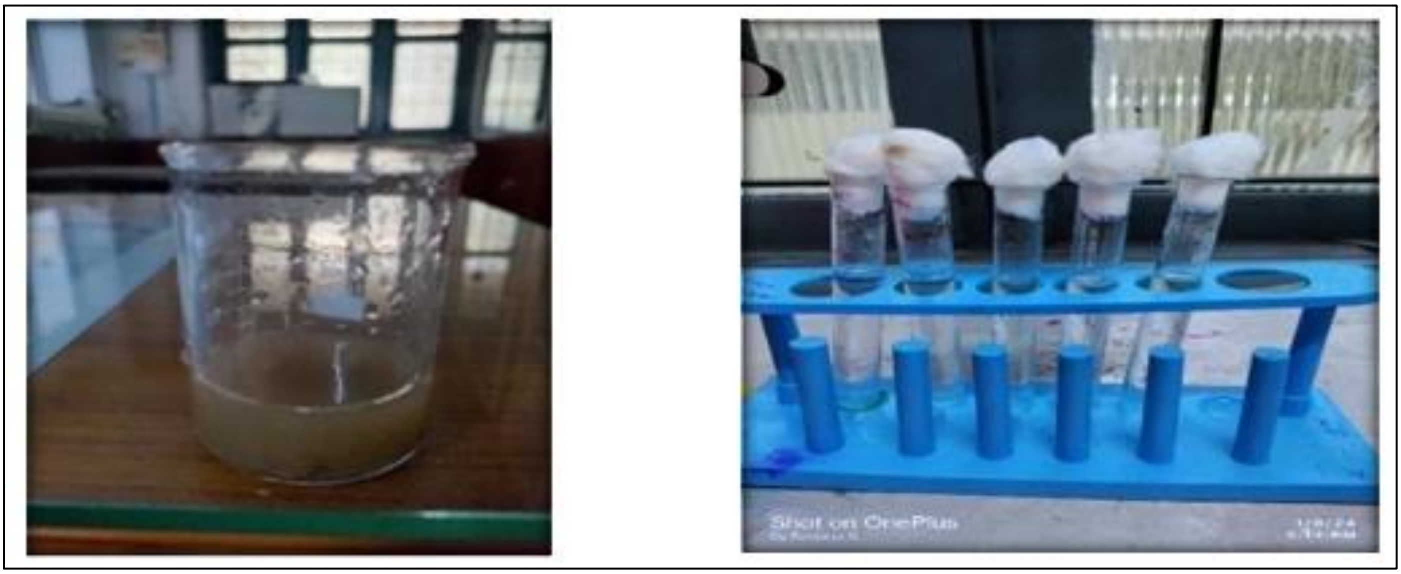

Fig 2 Serial Dilution Method

For each composite soil sample, 1g was added to 9ml of distilled water, shaken at room temperature, and used as a stock culture. A 1ml aliquot from this stock was transferred to 9ml of sterile saline, mixed, and further diluted in a series up to 10^{-6}. After dilution, 0.1ml of each sample was plated using the spread plate technique and incubated at 37°C for 24 hours. Bacterial colonies were identified by their smooth, opaque appearance and were sub-cultured for further incubation at 37°C for 24 hours.

➢ *Identification of Pigment-Producing Bacteria*

After incubation, bacterial isolates were identified by their smooth, opaque colonies partially submerged in agar. These colonies were sub-cultured and incubated at 37°C for 24 hours. Identification was carried out using Bergey's manual and biochemical assays.

➢ *Identification Tests*

- **Gram Staining:** Differentiated Gram-positive from Gram-negative bacteria using standard staining procedures.
- **Motility:** Hanging drop technique was used to observe motility in organisms with flagella, pseudopodia, or cilia.
- **Biochemical Analysis:** Included tests such as KOH, indole, methyl red, Voges-Proskauer, citrate utilization, catalase, oxidase, urease, triple sugar iron, nitrate reduction, and carbohydrate fermentation to confirm bacterial identification.
- **Extracellular Enzyme Production:** Tests for starch, casein, and lipid hydrolysis were performed to assess the ability of bacteria to degrade these substances.

➢ *Molecular Characterization of Bacterial Species*

- **PCR Amplification of 16S rRNA:** Bacterial colonies were directly used for PCR with specific primers. The PCR products were analyzed using agarose gel electrophoresis.

- **DNA Sequencing and BLAST Analysis:** Sequences were compared with those in the NCBI database using BLAST to confirm species identification with 98% or greater similarity.

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- **Phylogenetic Analysis:** 16S rRNA sequences were aligned and analyzed using software to construct a phylogenetic tree.

➢ *Screening of Pigment-Producing Microorganisms*

Microorganisms were cultured in nutrient broth with 2% glycerol. After 5 days, colonies displaying pigment production were selected for further study.

➢ *Extraction and Purification of Pigments*

Pigments were extracted using ethyl acetate and separated from cells via centrifugation. The supernatant containing the pigment was collected and stored for further use.

➢ *Antibacterial Activity of Pigments*

Antibacterial activity was tested against two bacterial strains using the Kirby-Bauer and well diffusion methods. Plates were incubated, and zones of inhibition were measured.

➢ *Characterization of Pigments*

- **Spectrophotometric Analysis:** Pigment absorption was measured in UV-visible ranges to determine maximum absorption spectra.
- **Paper Chromatography:** Pigments were separated and analyzed using Whatman No. 1 filter paper, with results interpreted using retention factors after Ninhydrin reagent spraying
- **Fourier Transform Infrared Spectroscopy (FTIR):** Functional groups in pigments were identified using FTIR. Purified pigments were encapsulated in KBr, and spectra were measured in the IR range of 4000 to 200 cm^{-1} to analyze key functional groups (OH, N-H, C=C, etc.).

➢ *Preparation of Bio-Paint*

Bio-paint was made using hydrated lime, ammonium carbonate, acetic acid, casein, and colored pigments. The process involved mixing casein in water, adding acetic acid and ammonium carbonate, then blending in the pigment to create a paste. This paint was applied to cardboard and clay for artistic purposes.

➢ *Dye Preparation*

- **Cloth and Thread Dyeing:** Cotton cloth and thread were pre-treated with mordants, dyed with bio-pigments for 60 minutes, then washed and dried.
- **Paper Dyeing:** Paper was cut into circles, dipped in pigments for 30 minutes, and allowed to dry. The paper absorbed the dye more effectively than cloth and thread.

IV. RESULTS

In this study, two pigment-producing bacterial species were isolated from the alluvial soil of the Thamirabarani River near Sivalaperi using the spread plate technique. The isolated bacteria were identified based on their morphological and biochemical characteristics as *Pseudomonas* and *Xanthomonas*, with the details provided in Table 1 and Table 2.

Table 1 i. Colony Morphology

S.no	Sample	Media	Color of the colonies	Morphology
1.	Alluvial soil	Nutrient agar	Yellowish orange	Rough, Thick, circular, group of colonies
2.			White	Smooth, Creamy white, round, thin colonies

Table 1 ii. Microscopic Examination

S. no	Organisms	Characteristics
1.	*Pseudomonas fluorescens*	Rod shaped, gram negative, motile bacteria
2.	*Xanthomonas axonopodis*	Short rod, gram negative, motile bacteria

Fig 3 Isolation of Bacterial from Alluvial Soil

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Fig 4 Coloured Colonies

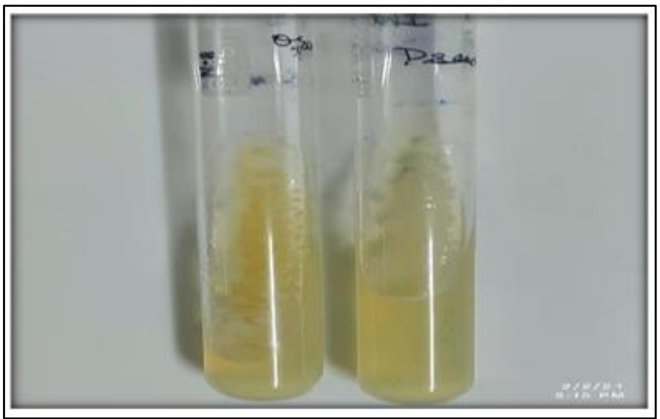
Fig 5 Sub-Culture of Coloured Colonies

- *Biochemical Results:*

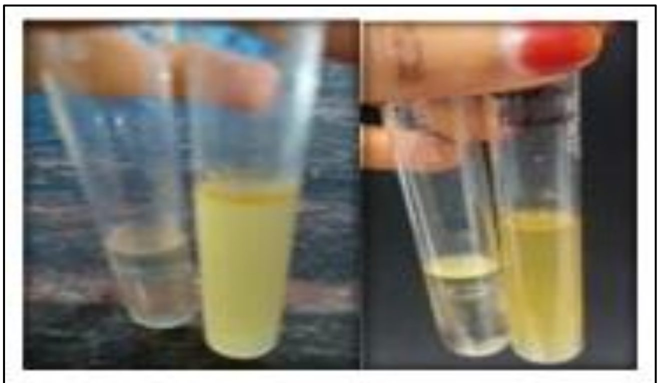
Fig 6 Indole Test


Fig 7 KOH Test


Fig 8 Methyl Red Test


Fig 9 Voges Proskauer Test

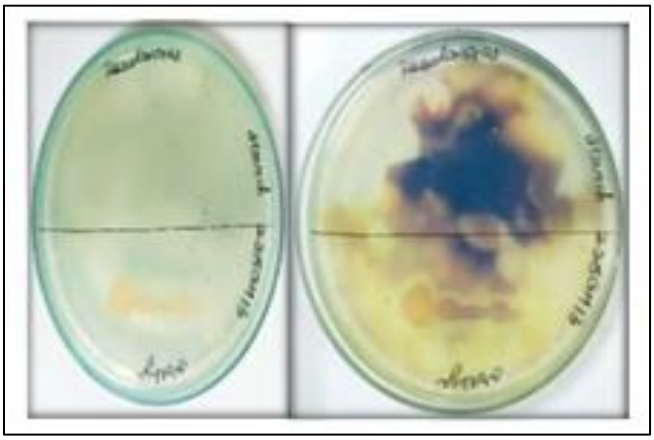
Fig 10 Starch Hydrolysis

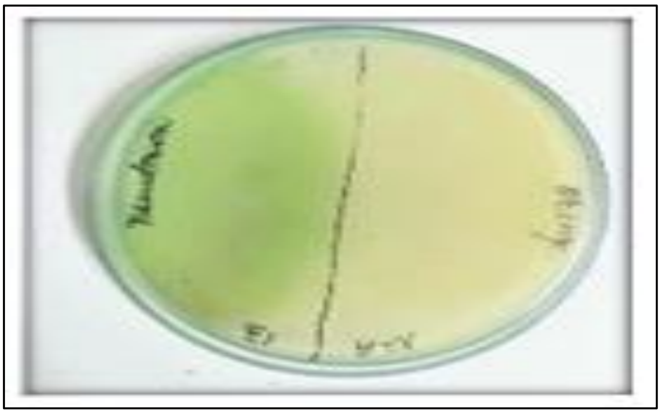
Fig 11 Lipid Hydrolysis

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Fig 12 Pseudomonas

Fig 13 Xanthomonas

Fig 14 Gram Staining

Fig 15 TSI Test

Fig 16 Fluorescence of Pseudomonas under UV

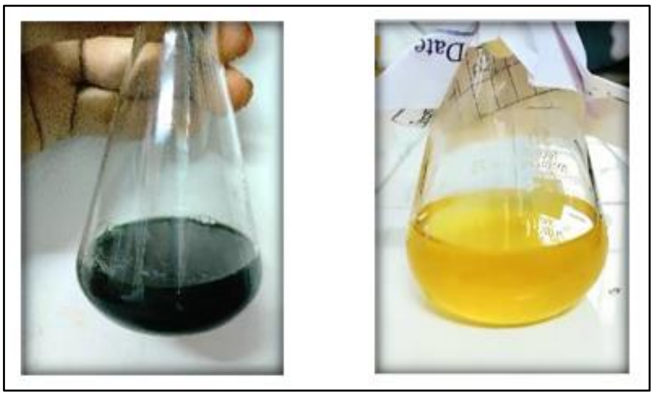

Fig 17 Pigment Production

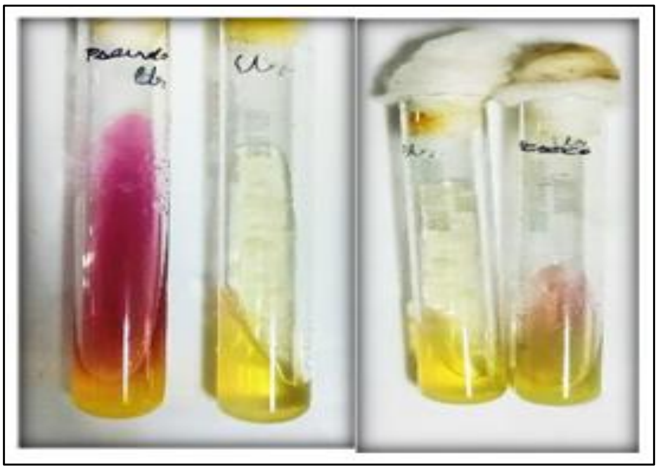

Fig 18 Urease Test

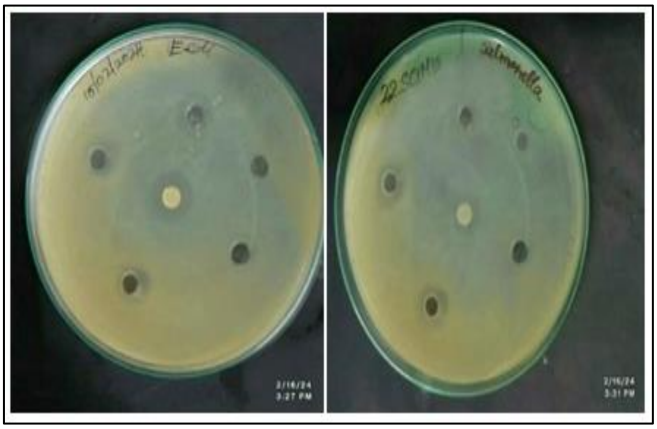

Fig 19 Disc Diffusion (Pyocyanin against Pathogens)

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Fig 20 Xanthomonadin against Pathogens

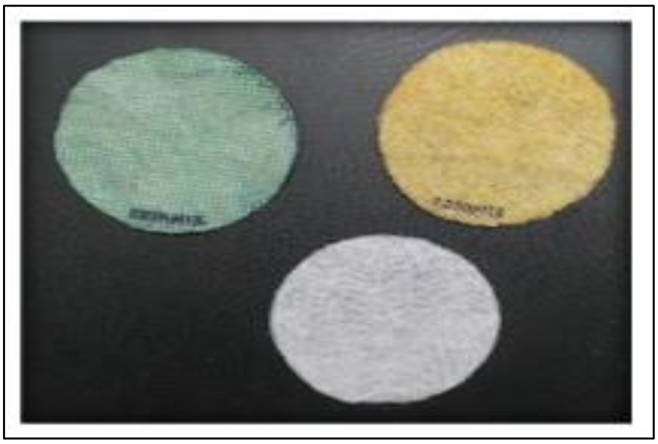

Fig 21 Cloth Dye

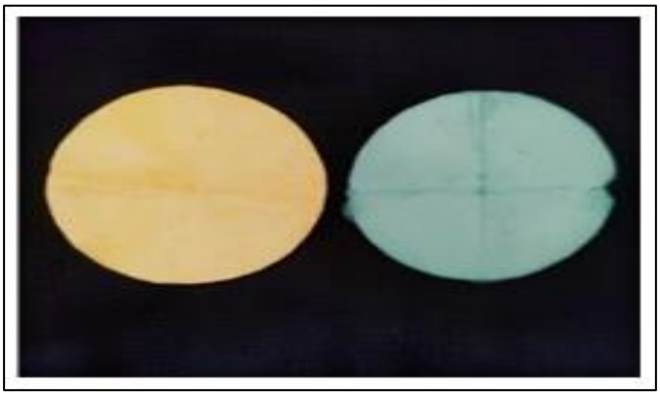

Fig 22 Paper Dye

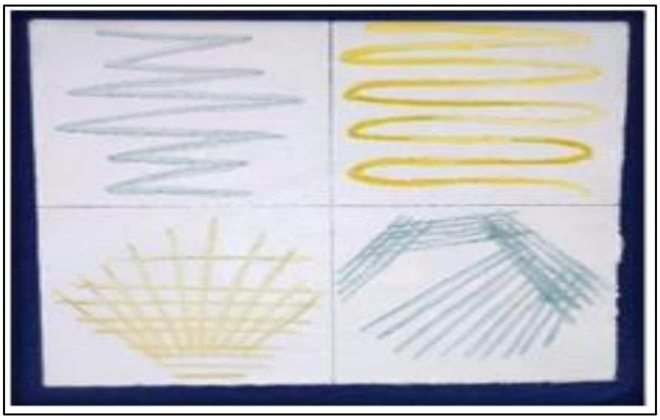

Fig 23 Water Colorings

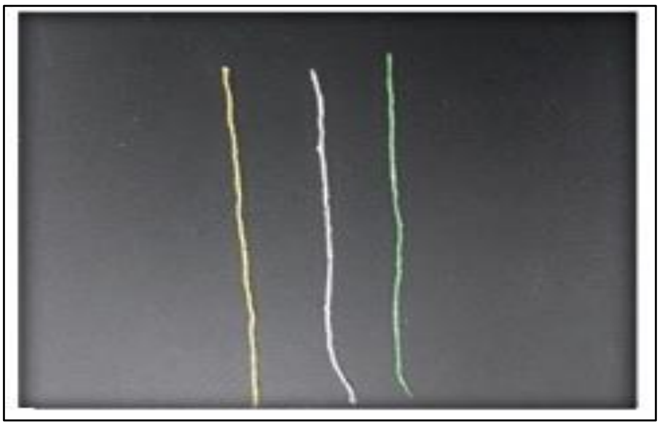

Fig 24 Thread Dye

Fig 25 Cardboard Painting

Fig 26 Diya Paintings

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➢ *Blast Analysis*

Table 2 Results of BLAST Analysis. Name of the Closest species with its Genbank Accession Number and Corresponding Percentage of Similarity (in %) was Tabulated.

S.No.	Identification Strains	(%) Similarity	Accession number
1.	*Xanthomonas axanopodis* (RanjF1)	100%	PP460555.1
2.	*Pseudomonas fluorescens* (RanjF2)	100%	PP460554.1

➢ *Graphical Presentation of Pigment Production*

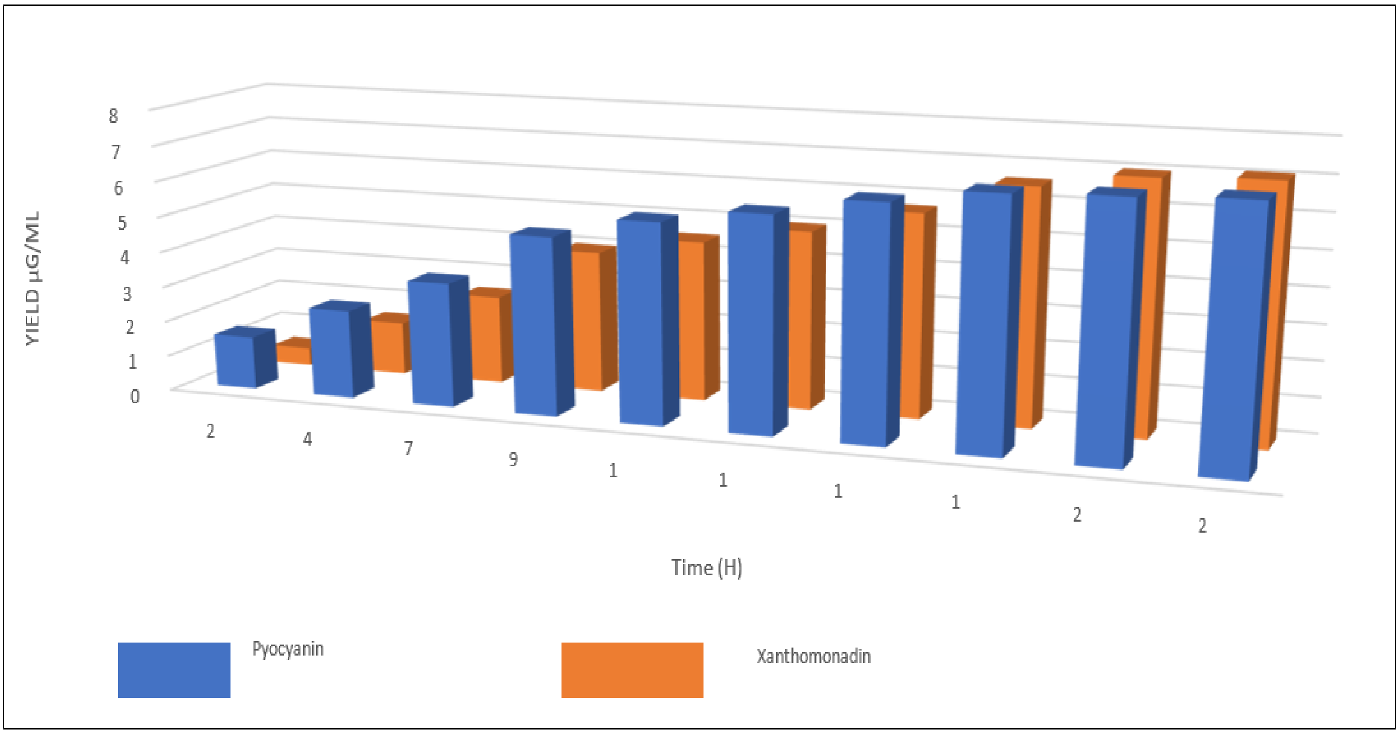

Fig 27 Shows the Incubation Period of Bacteria Producing Pigments

Secondary metabolites, including microbial pigments, are produced during the stationary phase of microbial growth, often in response to stress. In this study, two bacterial species, *Pseudomonas fluorescens* and *Xanthomonas axonopodis*, were observed for pigment production during incubation. *Pseudomonas fluorescens* began producing the green pigment pyocyanin after 48 hours, with the color intensifying to bluish-green by the fifth day. In contrast, *Xanthomonas axonopodis* exhibited a slower growth rate and delayed pigment production, starting with a yellowish color after five days and transitioning to a yellowish-orange by the eighth day.

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➢ *Ftir Analysis*

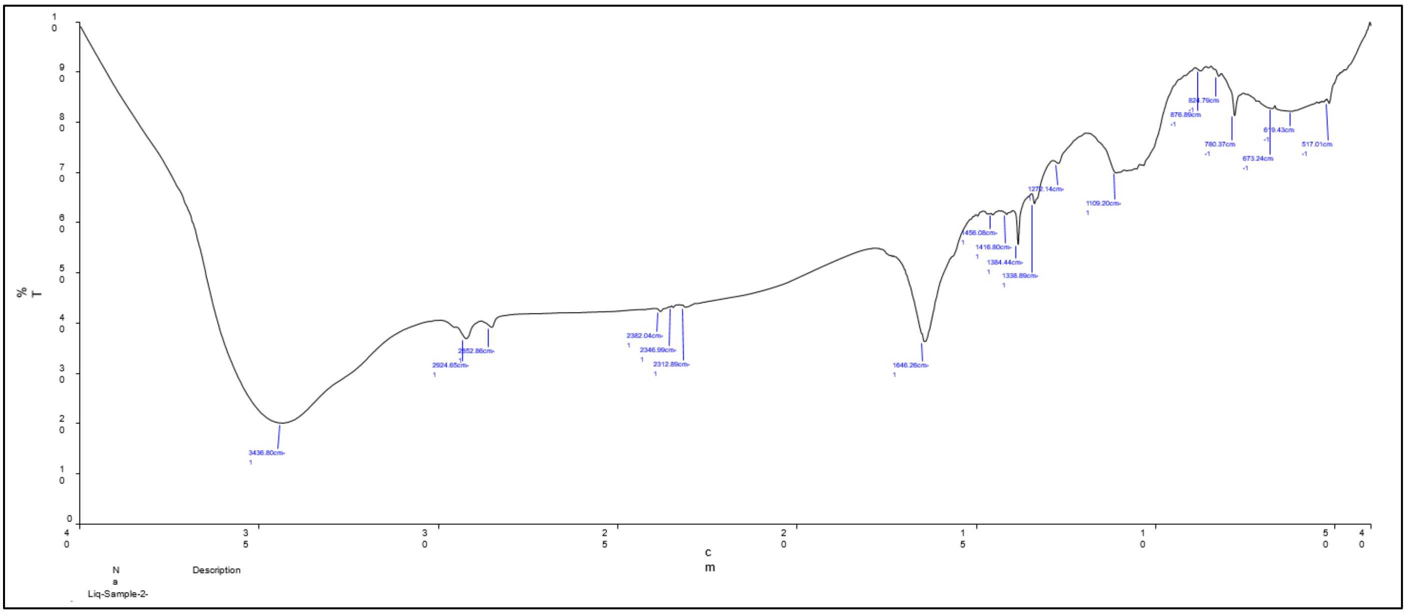

Fig 28 FTIR Analysis of Xanthomonadin

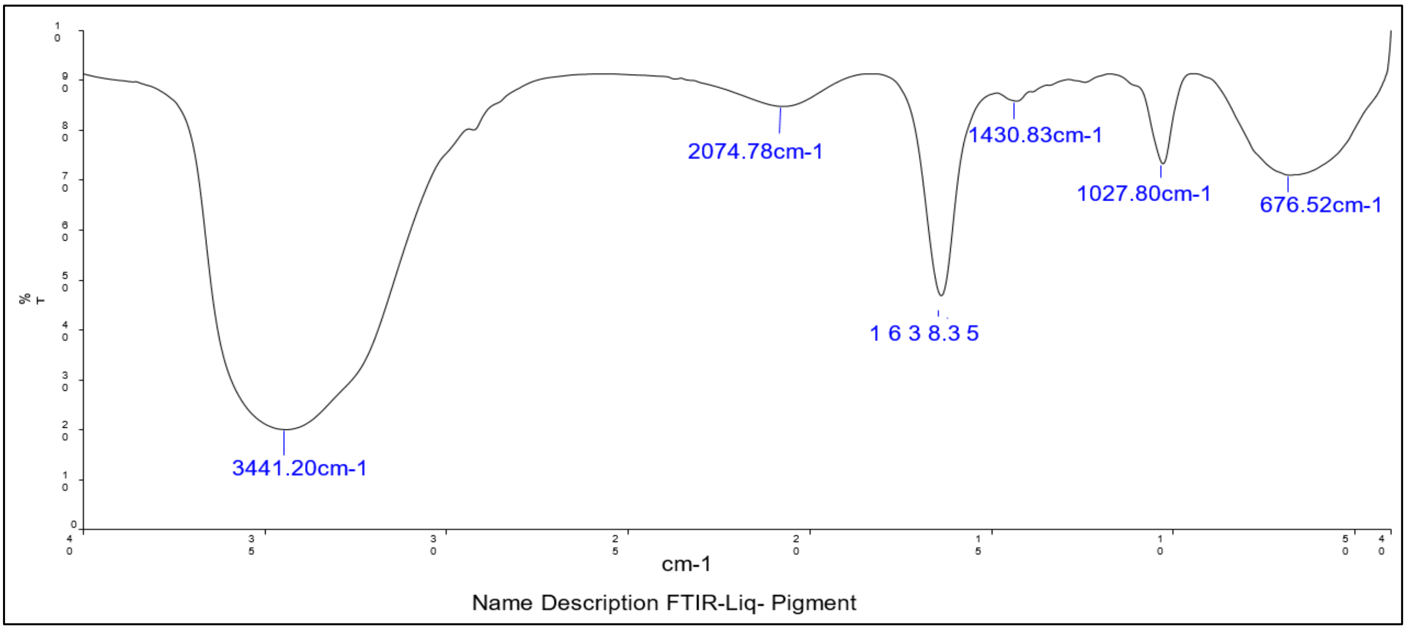

Fig 29 FTIR Analysis of Pyocyanin

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups in pigments produced by *Xanthomonas axonopodis* and *Pseudomonas fluorescens*. The FTIR spectrum for the yellow xanthomonadin pigment revealed key functional groups such as O-H stretching (3436 cm^{-1}), C=O ketone stretching (1646 cm^{-1}), and C-Cl stretching of alkyl halides (876 cm^{-1}). It confirmed the presence of a methyl ring (4-bromo-3-methoxyphenyl) associated with the pigment. For the pyocyanin pigment, the FTIR spectrum identified functional groups like O-H stretching of polyphenols (3441 cm^{-1}) and C=O stretching of the ketone group (1638 cm^{-1}), confirming the presence of an aromatic ring typical of pyocyanin. The spectra matched known standards, verifying the pigment compositions.

V. DISCUSSION

The study highlights the growing interest in natural pigments, particularly those produced by microorganisms, as sustainable alternatives to synthetic dyes. Historically, natural sources like plants were used for colorants, but microbial pigments offer new opportunities, especially in industries such as textiles and cosmetics. Microbial pigments, known as bio-colors, are safer, biodegradable, and environmentally friendly compared to synthetic dyes. The research focused on isolating pigment-producing bacteria from alluvial soil, specifically *Pseudomonas fluorescens* and *Xanthomonas axonopodis*, for use in bio-paint and dye production.

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Pigments like pyocyanin and xanthomonadin were utilized in various applications, including watercolor paintings and dyeing of cotton, paper, and thread. The study found that paper absorbed the pigments more effectively than cloth, indicating potential uses in various industries. The pigments also showed antimicrobial properties, suggesting their suitability for food colorants and cosmetic products.

Despite the advantages, microbial pigment production faces challenges, such as low yields from natural sources and the high cost of synthetic media. However, fermentation techniques, particularly submerged fermentation, can enhance production efficiency. The study emphasizes the need for further research to optimize microbial pigment production and reduce reliance on synthetic dyes, which are harmful to the environment. Natural colorants from microbes could play a crucial role in addressing environmental pollution caused by synthetic dye effluents.

VI. CONCLUSION

The study involved collecting alluvial soil from the Thamirabharani River to isolate pigment-producing bacteria. These bacteria were identified through microscopic and biochemical analysis, followed by 16s rRNA sequencing. The closest related organisms were found using NCBI BLASTN, and a phylogenetic tree was constructed with MEGA X software. The bacteria were then cultured in a nutrient broth with 2% glycerol for eight days to produce pigments. The pigments, identified as pyocyanin and xanthomonadin, were extracted using ethyl acetate and characterized through UV-Visible spectrophotometry, FTIR analysis, and paper chromatography. Antimicrobial tests showed that pyocyanin had higher microbial activity against *Escherichia coli* and *Salmonella sp.* compared to xanthomonadin.

The research emphasized the growing preference for natural over synthetic products, particularly in industries like textiles, where synthetic dye pollution is a concern. The study suggests that bacterial pigments offer a sustainable alternative, reducing environmental impact while providing durable and stable colorants for use in paints and dyes. The research also highlights the need for further studies, particularly in genetic engineering, to enhance bacterial pigment production and stability. The results indicate that bacterial pigments can be effectively used in various applications, including fabric and paper dyes, with potential improvements in formulation through the use of natural additives.

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