CATIONIC THIAZINE AND AZO DYE ALONG WITH PETROLEUM HYDROCARBON MICROBIAL DEGRADATION AND ASSESSMENT OF PHYSIOCHEMICAL AND RADIONUCLIDES PARAMETERS OF TARGETED POINT SOURCE (WATER)

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Biotechnology

Department of Mathematics and Natural Science
BRAC University
August 2025

Declaration

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Abstract

Background: Water pollution remains a critical environmental and public health issue in Bangladesh, particularly in Dhaka and its surrounding areas. The continuous discharge of untreated sewage, industrial effluents, and chemical wastes has led to the deterioration of aquatic ecosystems, contributing to the emergence of fish and human diseases. Regular monitoring is essential to assess the extent of pollution, track microbial adaptation, and detect potential outbreaks of novel pathogens.

Method: Water samples were systematically collected from multiple locations and transported at 4°C to preserve microbial integrity. Selective and differential media were employed to isolate bacteria resistant to hydrocarbons (petrol, kerosene) and azo dyes. Enrichment cultures in Bushnell-Haas broth, supplemented with pollutants as carbon sources and ammonium nitrate as a nitrogen source, facilitated the degradation process over seven days. Gas chromatography was utilized to analyze the breakdown of hydrocarbon pollutants. Molecular identification of isolates was performed using 16S rDNA gene sequencing. For heavy metal and radionuclide analysis, samples were acidified and analyzed using Atomic Absorption Spectroscopy (AAS) for metal quantification, while High-Purity Germanium (HPGe) detectors were used for radionuclide profiling.

Results: Radionuclide analysis of surface and groundwater samples from Dhaka showed Th-232, Ra-226, and K-40 levels within safe limits, indicating no significant radiological risk. Molecular characterization identified *Pseudomonas aeruginosa* with notable hydrocarbon-degrading abilities. Gas chromatography confirmed biodegradation of hydrocarbons like toluene, naphthalene, and hexadecane. However, AAS detected Pb toxic heavy metal exceeding permissible limits in Signboard River and Shitalakha River, indicating localized heavy metal pollution.

Conclusions: Overall, the combined microbial and chemical analyses underscore the natural bioremediation potential of indigenous microbes in mitigating hydrocarbon and azo dye pollution in the aquatic environment of Dhaka and its surroundings.

Keywords: Gamma spectrometry, Absorbtion spectrometry, Gas Chromatography

Chapter 1: Introduction

Industrial contamination of aquatic and terrestrial environments has emerged as one of the most pressing environmental challenges of the 21st century, posing significant threats to human health, aquatic ecosystems, and environmental sustainability. The discharge of diverse pollutants, including synthetic dyes, heavy metals, petroleum hydrocarbons, and radionuclides, from various industrial activities has created a complex matrix of environmental contamination that requires urgent remediation strategies (Newar et al., 2025).

The textile and dye manufacturing industries represent major contributors to environmental pollution, releasing substantial quantities of synthetic dyes into water bodies and soil systems. Among these pollutants, azo dyes constitute approximately 70% of all textile dyes used globally, with methylene blue and methyl red being prominent examples of cationic and azo dyes, respectively (Lellis et al.,2019). These xenobiotic compounds are of particular concern due to their recalcitrant nature and persistence in the environment. Methylene blue, a heterocyclic aromatic compound with molecular formula $C_{16}H_{18}N_3SCl$, forms quaternary ammonium cations in aqueous solutions and exhibits high chroma, causing severe aesthetic and environmental pollution(Negi &Verma, 2023). Similarly, methyl red, as an azo dye, contains the characteristic azo linkage (–N=N–) that imparts intense coloration and contributes to its environmental persistence (Ikram et al., 2022; Pal & Patel, 2022)

The environmental and health implications of textile dye pollution are multifaceted and severe. These dyes significantly alter water quality by reducing light penetration, which is essential for photosynthetic activities of aquatic flora and fauna, thereby disrupting entire aquatic ecosystems. The presence of azo dyes in water bodies has been linked to various adverse health effects, including angioedema, nasal congestion, dermatitis, hepatic carcinogenicity, and tumor formation (Tohamy et al.,2022). Furthermore, these compounds affect the synthesis of chlorophyllase and abscisic acid in plants, leading to chlorophyll breakdown and reduced plant productivity (Khan et al., 2022)

Concurrent with organic pollutant contamination, heavy metal pollution, particularly from lead and cadmium, poses additional environmental and health concerns. Lead contamination results in neurological disorders, developmental delays in children, cardiovascular diseases, and kidney dysfunction. Cadmium exposure is associated with kidney damage, bone disease (itai-itai disease), respiratory problems, and various forms of cancer (Mood et al., 2021). In aquatic environments, these heavy metals accumulate in the food chain through bioaccumulation and biomagnification, affecting fish physiology, reproduction, and survival while ultimately threatening human consumers of contaminated seafood (Proshad et al., 2021).

The environmental burden is further compounded by radionuclide pollution from naturally occurring radioactive materials (NORM), including radium-226, thorium-232, and potassium-40. These radionuclides, often concentrated in industrial processes such as oil and gas extraction, phosphate mining, and coal combustion, present long-term environmental and health risks. Chronic exposure to these radionuclides can lead to increased cancer risk, genetic mutations, and radiation-induced tissue damage. In aquatic systems, radionuclides can accumulate in sediments and biota, resulting in persistent contamination that impacts ecosystem integrity for extended periods (World Nuclear Association, 2024; Ibrahim & Al-Mashhadani, 2024).

Adding to this complex pollution matrix, petroleum hydrocarbon contamination from oil spills, pipeline leakages, and industrial effluents introduces another category of persistent organic pollutants. These hydrocarbons, including alkanes, aromatics, asphaltenes, and resins, are known carcinogens and neurotoxic compounds that affect both terrestrial and aquatic environments. Continuous exposure to petroleum hydrocarbons can cause respiratory problems, cardiovascular diseases, and various cancers in humans. In marine environments, oil contamination leads to temperature fluctuations, blindness, dehydration, and organ dysfunction in aquatic organisms (Ravi et al., 2022).

Given the complexity and severity of these contamination issues, conventional physical and chemical treatment methods have proven inadequate, often failing to achieve complete pollutant removal and sometimes generating secondary pollutants that require costly disposal. High operational costs, limited efficiency, and incomplete mineralization of complex organic compounds frequently characterize these traditional approaches. Consequently, there has been a paradigm shift toward biological remediation technologies, particularly microbial degradation, which offers environmentally friendly, cost-effective, and comprehensive solutions for pollutant removal (Kumari, 2023).

Bacterial biodegradation has emerged as a promising biotechnological approach for environmental remediation due to the remarkable metabolic versatility of microorganisms. Various bacterial species possess specialized enzyme systems, including azoreductase, laccase, peroxidase, and monooxygenases, that enable them to break down complex pollutants into less toxic or completely mineralized end products. The degradation process typically involves the reduction of azo bonds in synthetic dyes, bioaccumulation and transformation of heavy metals, and metabolic breakdown of petroleum hydrocarbons through oxidative pathways (Ikram et al., 2022; Sarkar et al., 2022).

The significance of physicochemical testing in pollution assessment cannot be overstated, as these parameters provide critical insights into the extent and nature of contamination. Key physicochemical parameters, including pH, temperature, dissolved oxygen, conductivity, turbidity, and pollutant concentrations, serve as indicators of water quality and ecosystem health. pH affects the bioavailability and toxicity of pollutants, with many bacterial degradation processes being pH-dependent. Temperature influences microbial metabolic rates and pollutant solubility, while dissolved oxygen levels determine the aerobic or anaerobic nature of biodegradation processes (Syeed et al., 2023). Conductivity measurements indicate the presence of dissolved ions, including heavy metals, while turbidity can reflect the presence of suspended pollutants, including dyes and petroleum products. These physicochemical assessments are essential for monitoring pollution levels, designing remediation strategies, and evaluating the effectiveness of treatment processes (Vaidya & Kadam, 2022).

The optimization of bacterial degradation processes requires a comprehensive understanding of the physicochemical factors that influence microbial activity and the bioavailability of pollutants. Parameters such as nutrient availability, redox conditions, ionic strength, and the presence of co-substrates have a significant impact on bacterial growth and metabolic efficiency (Chakraborty, 2025). Moreover, characterizing bacterial isolates through morphological, biochemical, and molecular techniques, including 16S rRNA sequencing, is crucial for identifying the most effective strains for specific pollutant degradation applications.

The present research focuses on the isolation, characterization, and optimization of bacterial strains capable of degrading multiple categories of industrial pollutants, including synthetic dyes (methylene blue and methyl red), petroleum hydrocarbons, and potentially heavy metals and radionuclides. This comprehensive approach addresses the growing need for integrated bioremediation strategies that can handle complex, multi-component pollution scenarios commonly encountered in industrial contamination sites. The study emphasizes the importance of understanding the synergistic effects of various physicochemical parameters on bacterial degradation efficiency and the development of optimized conditions for maximum pollutant removal.

By investigating the biodegradation potential of indigenous bacterial strains isolated from contaminated environments, this research contributes to the development of sustainable, cost-effective remediation technologies that can address the multifaceted challenges of industrial pollution. The integration of advanced analytical techniques, including spectroscopic and chromatographic methods, enables comprehensive monitoring of degradation processes and identification of metabolic pathways, providing valuable insights for scaling up bioremediation applications. This holistic approach to environmental contamination addresses the urgent need for practical, environmentally sustainable solutions to protect both human health and ecosystem integrity in an increasingly industrialized world.

Research objectives:

1. To check whether *Pseudomonas aeruginosa* degrade petrol or not.
2. To check the efficiency of *Bacillus spp* to biodegrade cataionic thiazine dye.
3. To check Cd and Pb pollution status in Shitalakha River and Signboard.
4. To check whether *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *streptomyces* break down azo dye.
5. To check the physiochemical parameters of water

Chapter 2: Literature Review

Introduction

Water pollution, a significant environmental challenge, is being addressed with the potential of bioremediation. The contamination of water bodies with petroleum hydrocarbons, synthetic dyes, heavy metals, and organic pollutants poses significant threats to both environmental and human health. However, the exploration of biological remediation strategies as sustainable alternatives offers hope for a cleaner future (Varjani, 2017).

Bioremediation harnesses the metabolic capabilities of microorganisms to transform pollutants into non-toxic compounds. Bacteria, in particular, have shown remarkable adaptability in degrading complex organic compounds and immobilizing heavy metals through various enzymatic and biochemical processes (Goveas et al., 2022; Zargar et al., 2022). This literature review examines the current state of knowledge regarding the biodegradation potential of specific bacterial species against petroleum hydrocarbons, cationic thiazine dyes, azo dyes, and heavy metals, while also addressing critical parameters in water quality assessment.

Petroleum Biodegradation by Pseudomonas aeruginosa

Biodegradation Mechanisms and Efficiency

Pseudomonas aeruginosa has emerged as one of the most extensively studied hydrocarbon-degrading microorganisms, demonstrating exceptional versatility in metabolizing petroleum components. Research by Hentati et al. (2021) confirmed that *P. aeruginosa* san ai, an alkaliphilic, metallotolerant strain, achieved impressive biodegradation efficiencies of 80% for n-hexadecane, 98% for n-nonadecane, 96% for fluorene, 50% for phenanthrene, and 41% for pyrene at initial concentrations of 20 mg/L within seven days. The strain demonstrated a remarkable capacity for degrading both aliphatic and aromatic fractions from crude oil, reaching stage 3-4 on the oil biodegradation scale (0-10 scale).

The biodegradation process involves terminal oxidation pathways where *P. aeruginosa* produces rhamnolipids, which serve as biosurfactants that enhance the bioavailability of hydrophobic compounds (Zhang et al., 2005). Studies have shown that the addition of 1 g/L glycerol or 0.22 g/L rhamnolipid can facilitate biodegradation, with over 58% of crude oil degraded and converted into accumulated cell biomass and rhamnolipids (Zhang et al., 2005). The bacterium's ability to produce rhamnolipids in two distinct stages reflects its adaptive metabolic strategy, initially utilizing readily available carbon sources before switching to petroleum compounds.

Co-culture Enhancement Strategies

Recent advances in biodegradation technology have focused on co-culture systems to enhance degradation efficiency. The work of Tao et al. (2023), which involves a defined co-culture of *Bacillus subtilis* SL and *Pseudomonas aeruginosa* WJ-1, achieves approximately 75% removal of saturated fractions from crude oil, serving as a testament to the innovative approaches in bioremediation.

The co-culture system showed enhanced cell density and cell surface hydrophobicity compared to monocultures, suggesting improved microbial community performance through metabolic cooperation. This finding aligns with the concept that petroleum biodegradation requires a consortium of microorganisms with complementary metabolic capabilities to effectively degrade the wide range of hydrocarbon compounds present in crude oil (Muthukumar et al., 2022).

Cationic Thiazine Dye Biodegradation by Bacillus Species

Methylene Blue Degradation Mechanisms

Cationic thiazine dyes, particularly methylene blue, pose a significant environmental threat due to their extensive use in the textile industry and their resistance to conventional treatment methods. *Bacillus* species have shown considerable promise in degrading these recalcitrant compounds through both biosorption and enzymatic mechanisms.

Research conducted by Sani et al. (2022) demonstrated that *Bacillus megaterium* and *Bacillus velezensis* achieved dye removal efficiencies of 49.3-92.5% and 73.2-93.4% respectively, for various reactive dyes within 48 hours at pH 11.3 and temperature 37°C. The biodegradation process followed Freundlich's isotherm model, with strong linear correlation coefficients (R^2 = 0.923-1.000), indicating that multilayer biosorption occurred at numerous sites on the bacterial cell surface.

Staphylococcus aureus isolated from textile wastewater treatment facilities achieved 88% COD removal and 98% color removal efficiency when degrading methylene blue under optimized conditions (pH 5, 35°C temperature, 150 ppm concentration, and an 8-hour treatment period) (Ayed et al., 2022). Spectroscopic analysis confirmed complete biodegradation rather than simple biosorption, with metabolites showing reduced toxicity in phytotoxicity and mutagenicity assays.

Enzymatic Pathways and Metabolite Formation

The degradation of cationic thiazine dyes involves multiple enzymatic systems, primarily azoreductases and peroxidases. FTIR analysis of methylene blue metabolites revealed the formation of various intermediate compounds, including metal ions at octahedral sites, N-H bending of primary amines, and H-bonded alcohols and phenols (Ayed et al., 2022). The presence of free NH groups and OH bending vibrations indicates a systematic breakdown of the thiazine ring structure, leading to the formation of less toxic aromatic compounds.

The biodegradation mechanism involves initial electron transfer reactions followed by ring cleavage and subsequent mineralization. *Bacillus* species produce extracellular enzymes that facilitate dye decolorization without requiring the dyes as sole carbon sources. This process, known as cometabolism, suggests that the bacteria are using the dyes as a secondary source of energy, rather than their primary food source (Sani et al., 2022).

Heavy Metal Pollution Assessment in River Systems

Contamination Status of Bangladeshi River Systems

Heavy metal contamination in riverine environments has become a critical concern, particularly in developing countries where industrial development often lacks adequate environmental protection measures. The Shitalakshya River system in Bangladesh exemplifies this challenge, with multiple studies documenting significant contamination levels that exceed international safety standards. Assessment of the Shitalakshya River revealed severe pollution conditions, particularly in downstream areas receiving industrial effluents (Rahman et al., 2021). The River Pollution Index (RPI) analysis, which measures the overall pollution level based on multiple parameters, indicated that upstream areas scored 3.1-6.0 (moderately polluted), while downstream areas reached scores of 10 (severely polluted). Heavy metal concentrations followed the trend: Fe > Pb > Zn > Cr > Cu > Cd, with iron concentrations ranging from 9.129 mg/L and lead concentrations of 0.033 mg/L exceeding WHO and USEPA guidelines (Bhuyan et al., 2024).

Azo Dye Biodegradation by Multiple Bacterial Species

Pseudomonas aeruginosa Azo Dye Degradation

Pseudomonas aeruginosa has demonstrated significant potential for azo dye remediation through multiple degradation pathways. The bacterium's azoreductase enzyme system facilitates the reductive cleavage of azo bonds under both aerobic and anaerobic conditions (Mahmood et al., 2016).

Escherichia coli Azo Dye Metabolism

Escherichia coli degradation mechanism involves three distinct pathways: bacterial-induced precipitation, cell wall adsorption, and enzymatic metabolism. The relative contribution of each pathway depends on the chemical nature of the specific dye compound. For basic azo dyes, enzymatic metabolism dominated the degradation process, with reductive cleavage of azo groups confirmed through metabolite analysis (Barra Caracciolo et al., 2015).

Bacillus cereus Azo Dye Remediation

Bacillus cereus strains have emerged as particularly effective agents for biodegrading azo dyes under both aerobic and anaerobic conditions. Strain ROC, isolated from dye-contaminated industrial soil, demonstrated the ability to degrade Reactive Orange 16 and Reactive Black 5 with high efficiency within five days (Ahmad et al., 2022). *Bacillus cereus* strain BPL achieved 78% decolorization of Deep Red GLX dye within four days under alkaline conditions (pH 9), demonstrating adaptation to high-alkaline textile effluent conditions (Vasniwal et al., 2016). The strain's tolerance to alkaline conditions, combined with supplementary carbon and nitrogen sources (maltose and peptone), significantly enhanced degradation performance.

Streptomyces Azo Dye Biodegradation

Streptomyces degradation mechanism involves laccase enzyme activity (5.96 U/mg specific activity) combined with azoreductase systems, which facilitate the systematic breakdown of azo bonds. GC-MS analysis revealed the formation of metabolites including benzene, biphenyl, and naphthalene derivatives, indicating complete structural modification rather than simple decolorization (El-Naggar et al., 2024).

Physicochemical Water Quality Parameters

Essential Water Quality Indicators

Physical parameters include temperature, pH, electrical conductivity, total dissolved solids (TDS), turbidity, and color. Chemical parameters encompass dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and various ionic species concentrations (Atlas Scientific, 2025).

Temperature significantly influences other water quality parameters, with increasing temperature generally decreasing dissolved oxygen levels and affecting bacterial metabolic rates. The pH range of 6.5-8.5 is typically considered optimal for aquatic life, while values outside this range can indicate pollution or natural geological influences (APHA, 1995). Dissolved oxygen concentrations above 6.5-8 mg/L are essential for maintaining healthy aquatic ecosystems, with levels below 5-6 ppm causing stress to most fish populations.

International Water Quality Standards and Guidelines

The Environmental Protection Agency (EPA) establishes comprehensive water quality standards encompassing Maximum Contaminant Levels (MCLs), Total Maximum Daily Load (TMDL) calculations, numeric criteria for pollutants, and designated use classifications (EPA, 2000). These standards provide frameworks for assessing water quality and implementing appropriate treatment strategies based on intended use categories.

World Health Organization (WHO) guidelines for drinking water quality establish specific limits for various contaminants, with particular attention to heavy metals, microbial contaminants, and chemical pollutants that pose immediate health risks (WHO, 2011). The guidelines emphasize risk-based approaches that consider local conditions, exposure patterns, and available treatment technologies.

For developing countries like Bangladesh, adapting international standards to local conditions remains challenging due to resource limitations and constraints on enforcement capacity. The Bangladesh National Environmental Quality Standards (NEQS) provide locally relevant criteria; however, many water bodies continue to exceed these standards due to inadequate treatment infrastructure and ineffective regulatory enforcement.

Conclusion

Water pollution caused by petroleum hydrocarbons, synthetic dyes, and heavy metals remains a persistent environmental concern, particularly in developing regions where industrial discharge and inadequate wastewater management prevail. The reviewed literature highlights the significant potential of bioremediation as a sustainable and practical approach to mitigate these pollutants. Microorganisms such as *Pseudomonas aeruginosa*, *Bacillus* species, *Escherichia coli*, *Bacillus cereus*, and *Streptomyces* have demonstrated remarkable capabilities in biodegrading complex organic pollutants—including petroleum hydrocarbons, cationic thiazine dyes, and azo dyes—through diverse enzymatic pathways and metabolic strategies. Their abilities to produce biosurfactants, extracellular enzymes, and to function synergistically in co-culture systems underscore the adaptability and efficiency of microbial bioremediation.

Furthermore, bioremediation not only reduces the toxicity and environmental persistence of hazardous compounds but also aligns well with eco-friendly and cost-effective remediation goals. However, the success of these biological treatments hinges on optimizing operational parameters such as pH, temperature, carbon and nutrient supplementation, and microbial community interactions. Additionally, the persistent heavy metal contamination in river systems, such as Bangladesh's Shitalakshya River, emphasizes the urgent need for integrated monitoring and pollution control strategies, supported by rigorous assessments of physicochemical water quality indicators aligned with international and national standards.

Chapter 3: Materials and Methods

Fig1: Atomic Absorption Spectrophotometer

Fig2: High-Purity Germanium (HPGe) detector

Fig3: pH, DO, EC/TDS, and Salinity meter

Fig4: GC-MS

Fig5: Water bath

Heavy metals and Radio nuclides determination

Sampling Location

The study area was Signboard River (23.694671,90.481309) and Shitalakha River (23.703160,90.516517). These locations were selected due to continuous industrial discharge and deposition of municipal wastewater effluents. The toxic components of this waste can have detrimental effects on both humans and the aquatic habitat.

Sample preparation and analysis for heavy metals and radionuclides

Two (02) sampling points in the Narayanganj area were sampled using acid-washed plastic bottles of quantity 1 liter. A Whatman 41 filter paper was used to remove unwanted particles. Then, concentrated nitric acid amount of 3 ml per liter was added. The solution was then transferred to a 1 L beaker and allowed to evaporate at 80 C to 100 ml in a water bath. For heavy metal analysis, Flame Atomic Absorption Spectroscopy (FAAS) by Varian Spectra AA220 was used (see fig1). Next, for radionuclides, activity analysis HPGe instrument by Kolga was used (see Fig2).

Physiochemical Parameters

For the above locations, Dissolved oxygen (DO), pH, Total dissolved solids (TDS), and Salinity were measured. For measurements, DO (Lutron PDO-519), pH (Milwaukee), TDS (HANNA Grow line), and Salinity (HANNA Salinity tester) brands were used (see Fig3). The tests were done in the sample location, during collection, in a 250 ml beaker.

Bacteria Identification

Bacillus cereus agar, cetrimide agar, EMB agar, and Starch caesin agar were used for *B.cereus*, *P.aeruginousa*, *E.coli*, and *Streptomyces*. Gram's staining procedure was followed for morphological identification.

Petrol degrading bacteria:

The collected strain (*Pseudomonas aeruginosa*) sample was first enriched in a nutrient media containing petrol for 24 hrs. In two 50ml sterilized Falcon tubes, 25ml of Bushnell Hass broth (BHB broth) media was taken. The falcons were labeled Control and Sample. 6% petrol samples were prepared and filtered with a 0.45 μm hydrophobic syringe filter. Afterwards, 1ml of this was added to each tube. For the Sample, 1ml of the cultured sample was also added. Then the tubes were kept in a shaker incubator at 37 °C for 24 hrs.

GC-MS analysis of petroleum hydrocarbons:

From the culture tube in the shaker incubator, after 24 hrs, 2 ml of Control and Sample were taken and mixed with 13ml n of hexane. The mixed samples were then analyzed by GC-MS with fused silica capillary column (30m x 0.25mm ID x 0.25 μm). Following the analysis, an internal library search was performed to classify the petroleum metabolites.

Azo dye degradation:

In two sterilized 50 ml Falcon tubes labelled with Ca and Sa, i.e., Ca for control and Sa for sample, 30 ml BHB broth mixed with 2ml of 0.5 g/l methyl red dye were added. 1ml of each sample culture of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *streptomyces* grown 24 hrs in the incubator was vortexed after addition to S. Following that, the tubes were kept in a shaker incubator at 30 °C for 48 hrs.

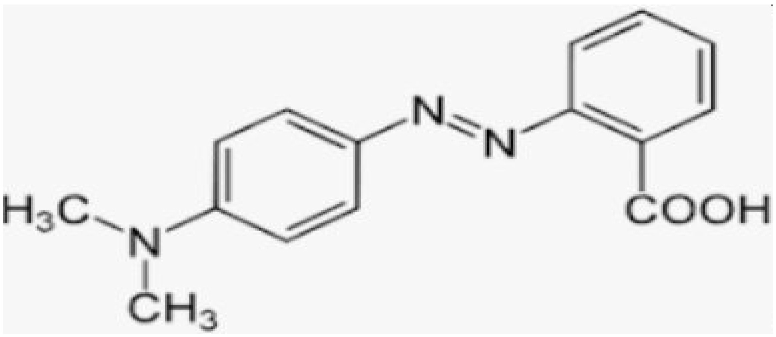


Fig6: Structure of Methyl red

Cationic thiazine dye:

In a 250ml conical flask containing 100 ml of nutrient broth 0.1g of methylene blue dye was added. Afterwards, 1 ml of *Bacillus spp.* grown in nutrient broth for 24 hrs was added to the flask. The flask was kept in the shaker incubator for 5 days at 37 °C at 120 RPM. A UV spectrophotometer was used to measure absorbance. The efficiency of degradation of the dye was calculated using the formula below.

$$\%\ Degradation = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}}\ x\ 100$$

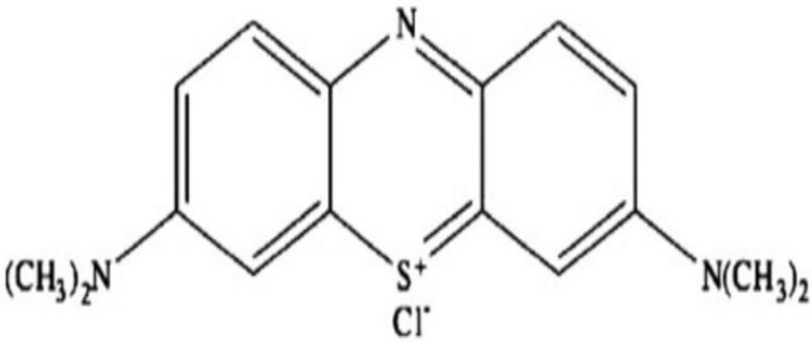


Fig7: Structure of Methylene blue.

GC-MS analysis of azo dye:

From the culture tube in the shaker incubator, after 7 days, 1ml of culture from Ca and Sa was transferred to the same labeled 15 mL Falcon tube, and 13ml of n-hexane was added to each. GC-MS with a fused silica capillary column (30m x 0.25mm ID x 0.25 μm) was used for mixture analysis. Then, an internal library search was performed to obtain the dye-degraded metabolite.

Sampling collection and preparation for microbial testing

The chosen location was Signboard River (23.694671,90.481309), the sample was collected in a 50 ml autoclaved Falcon tube and transported in a cool box maintaining a temperature range from 4 °C to 10 °C. The processing was done within 6 hrs. Spread plating was done using a dilution factor of 10-7, and incubated at 37 °C for 24 hrs.

Molecular confirmation of Pseudomonas aeruginosa based on 16s rDNA sequence by PCR

For this, the primer used was PA-SS-F 5’ GGGGGTCTTCGGACCTCA 3’ and PA-SS-R 5’ TCCTTAGAGTGCCCACCCG 3’. The PCR tubes containing the DNA extracted by the boiling method were placed in BIORAD T100TM Thermal cycler (see Fig8).

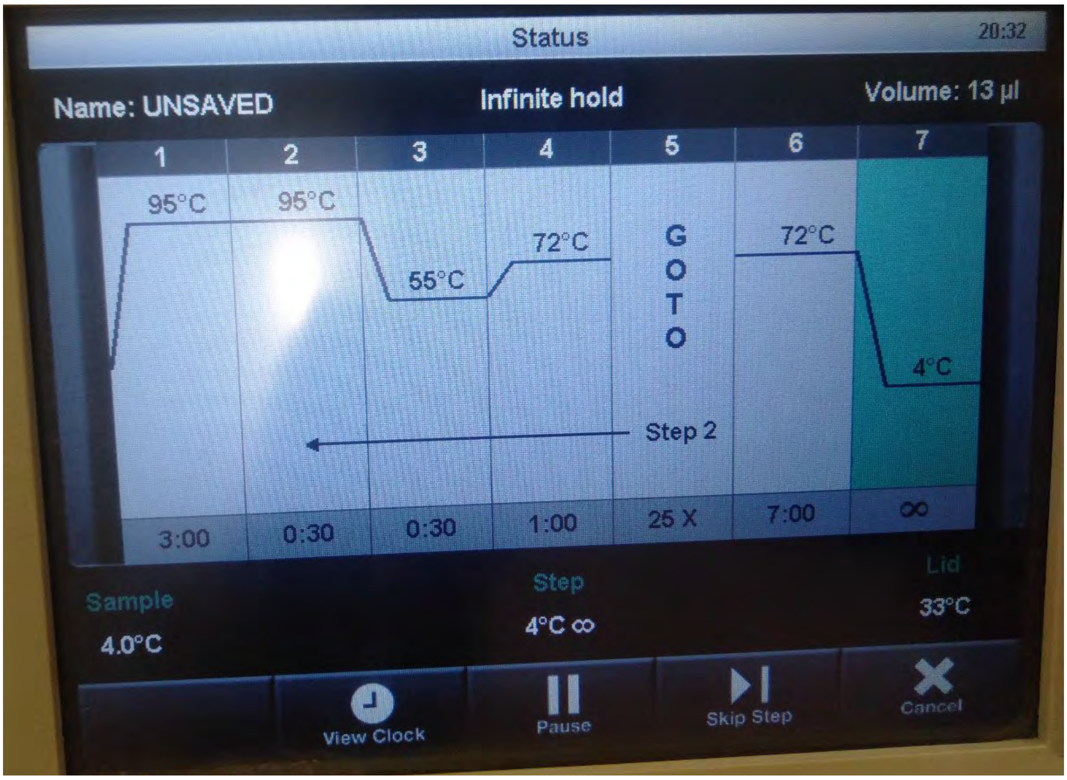
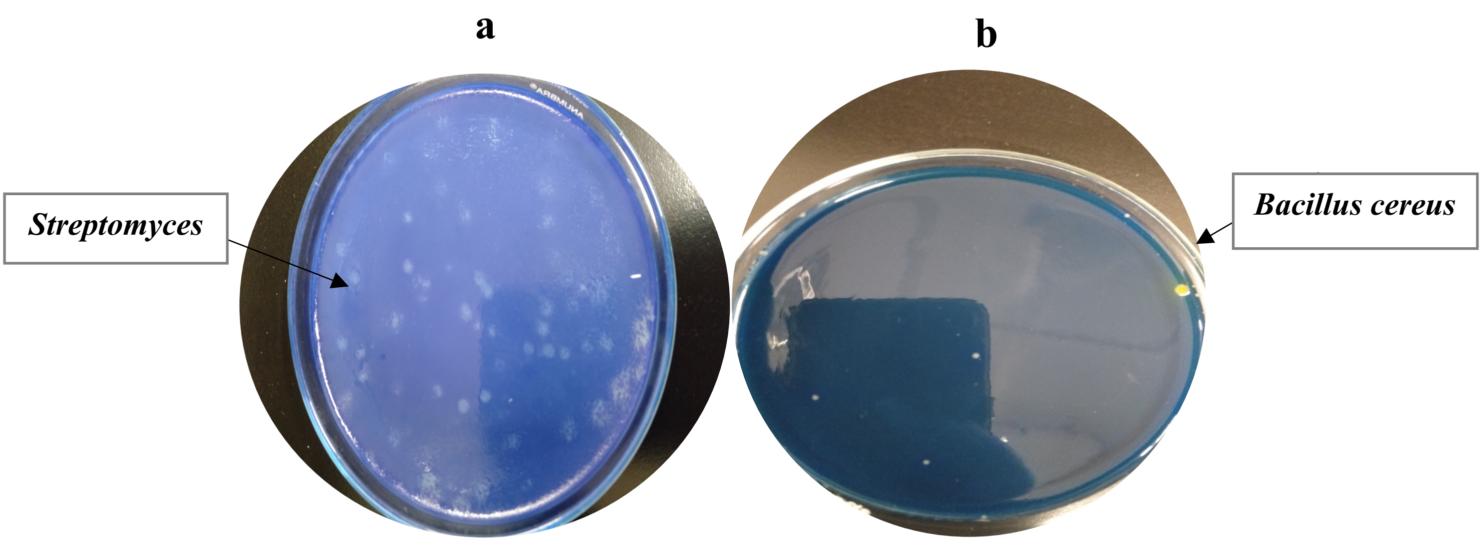
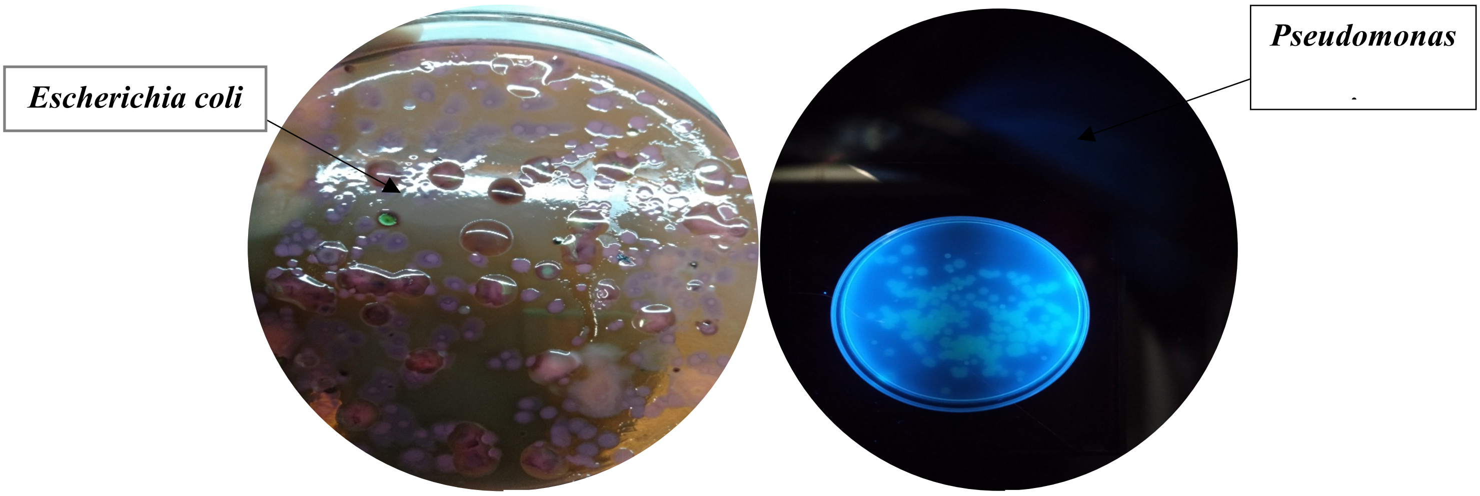


Fig8: PCR condition for ***Pseudomonas aeruginosa.***

Chapter 4: Results and Discussions:

Isolation of Biodegrading bacteria





c d

Fig9: (a) White colony in starch caesin agar represent ***Streptomyces*** it has released amylase and broken-down starch. (b) Yellow colony in bacillus cereus agar represent ***Bacillus cereus***.(c) Green sheen in EMB agar represent ***E.coli*** (d) Florescence (neon green) given out by ***Pseudomonas aeruginosa*** under U.V light in cetrimide agar.

The figure above shows four different isolated colonies in different media after spread plating; the serial dilution factor was 10^{-7} of the location Signboard.

Detection of the PASS gene through PCR and Visualization of bands through gel electrophoresis

PASS gene is a housekeeping gene for *Pseudomonas aeruginosa.* The figure below shows control, c, and sample, s at 956 bp position signifying a confirmed culture isolate of *Pseudomonas aeruginosa.*

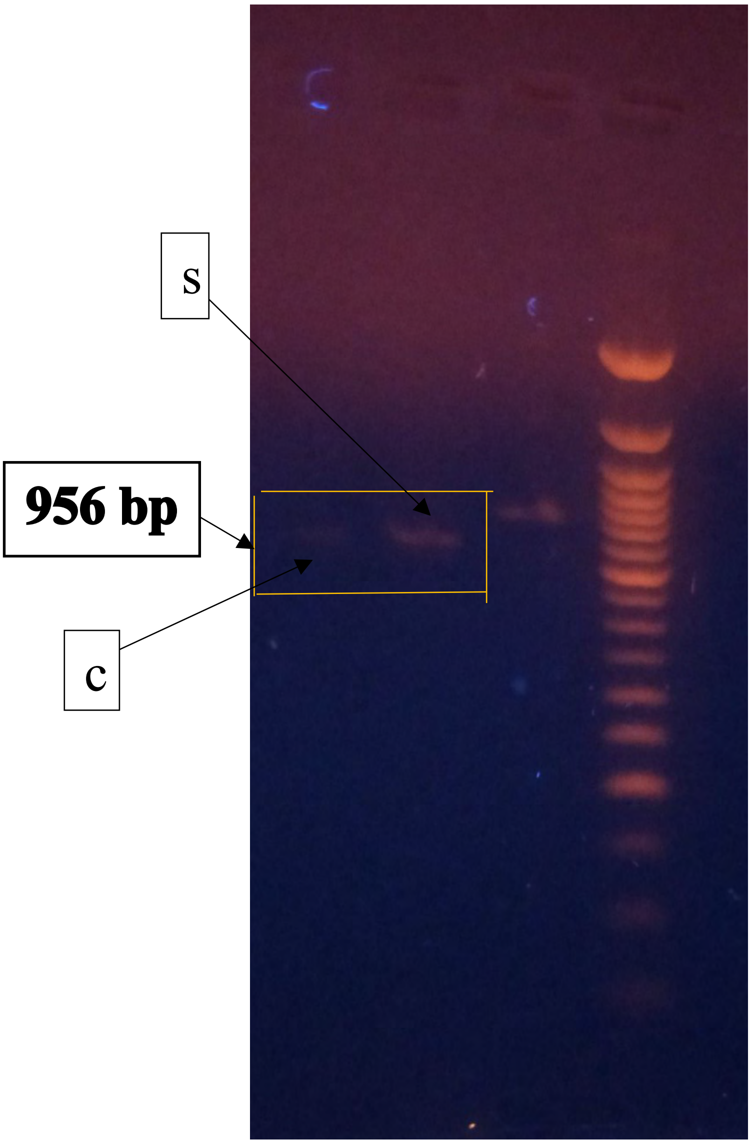


Fig10: Gel-Image of PCR products for ***Pseudomonas aeruginosa*** band showed at 956 bp (marked under orange box)

GCMS (Petrol degradation)

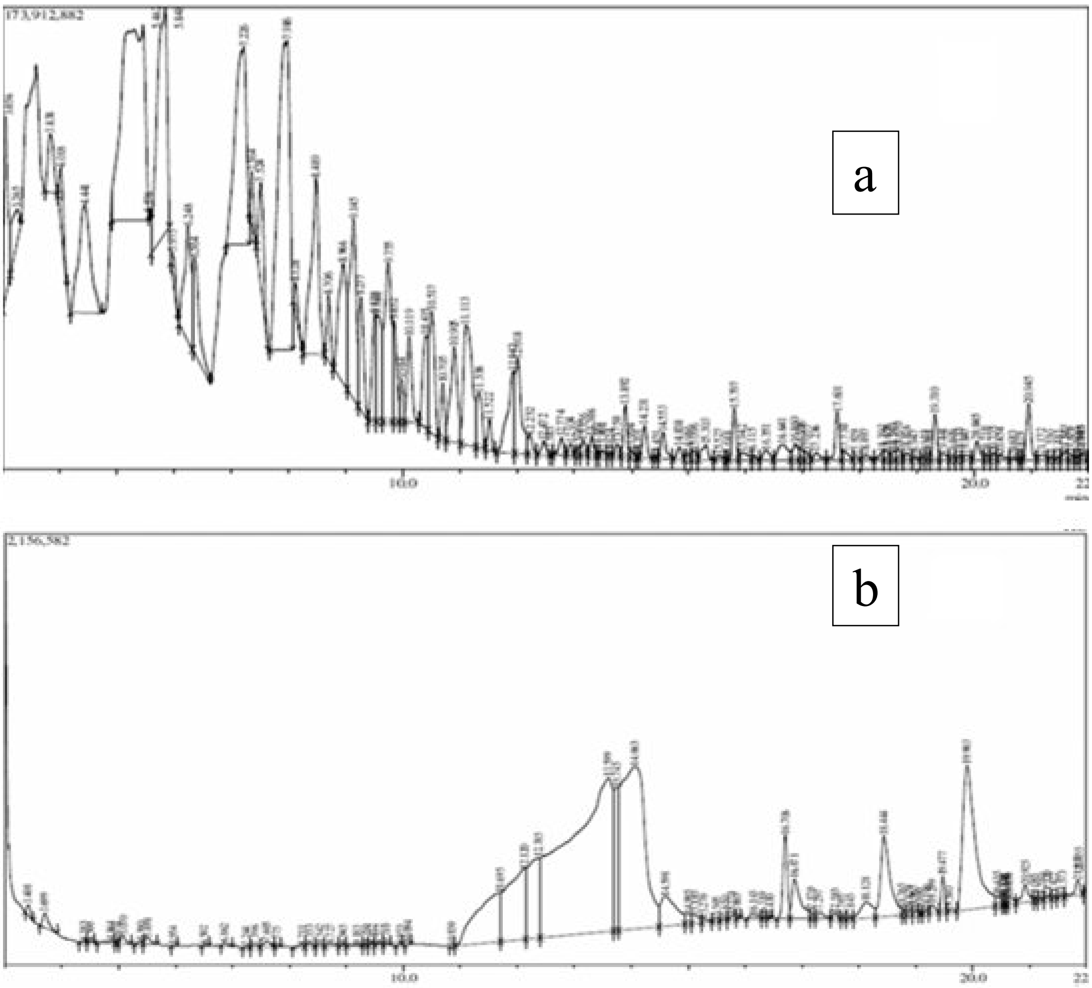


Fig11: GC-MS of degraded petroleum products. (a) Control; (b) Pseudomonas aeruginosa

In this part of the study, the sole source of the carbon source for the culture of bacterial isolates is petroleum. The nitrogen source of the bacteria given by Ammonium nitrate. When comparing (a) and (b) it could be seen in a, control, hydrocarbons such as cosane, docosane, tetracontane, hexadecane, and naphthalene is present (large carbon compounds). Additionally, it has pentene and cyclohexane (low carbon compounds). (b) The higher alkanes are completely broken down. The aliphatic and aromatic compounds present in the hydrocarbons was degraded via the aerobic routes. The intermediates such as alcohol, and aldehydes in degradation of aliphatic compounds and esters and diols in degradation of the petroleum products found indicate this bacteria's successful breakdown. According to Luo et al. (2012), the degrading efficiency of ***Pseudomonas ssp.*** was analyzed to be 75% in one week. Thus, prove this bacterium as a potent degrader of petroleum hydrocarbons.

Azo dye degradation:

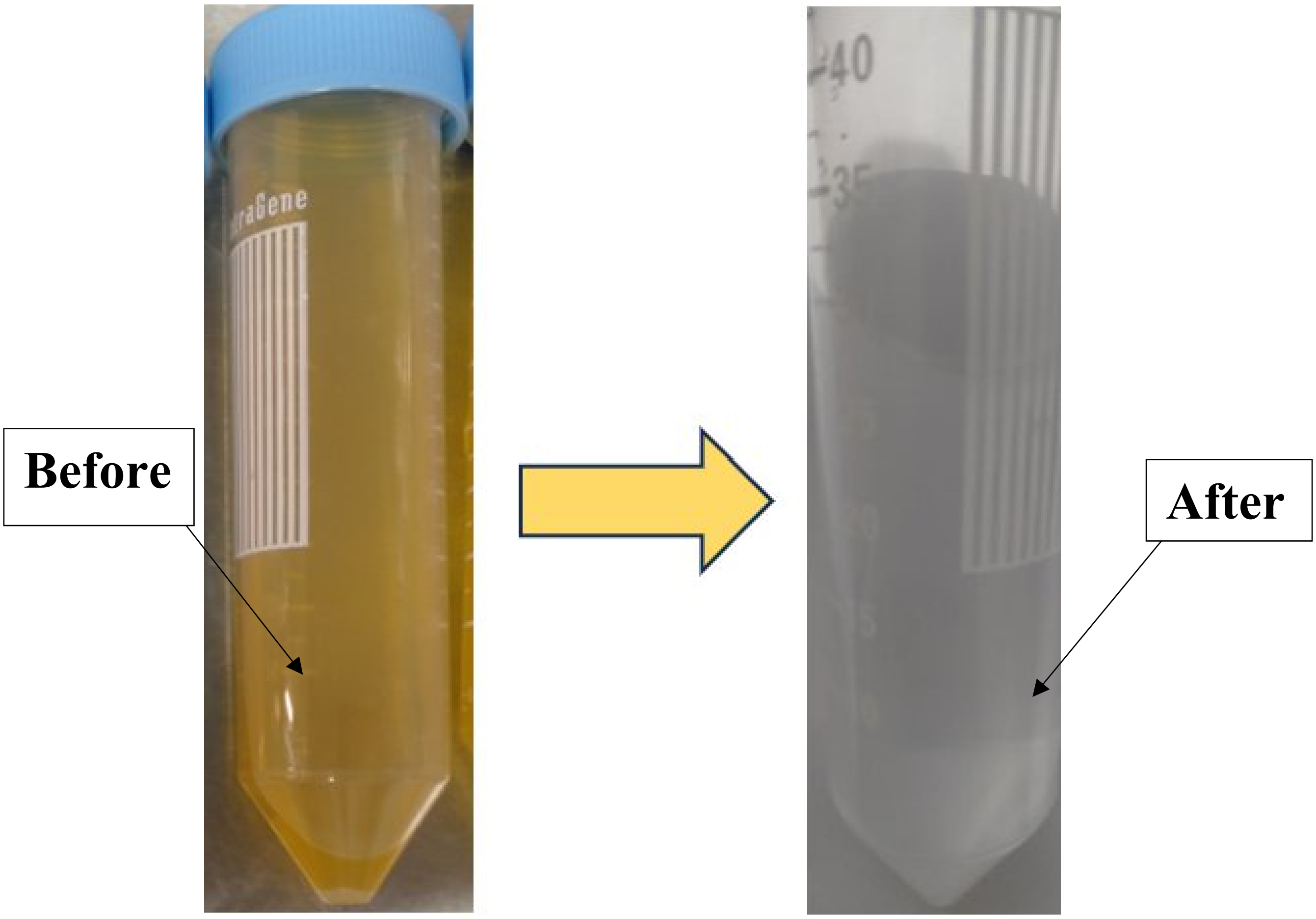


Fig12: Before (Sa) and after (Sa) after 48 hrs, azo compound in methyl red broken down.

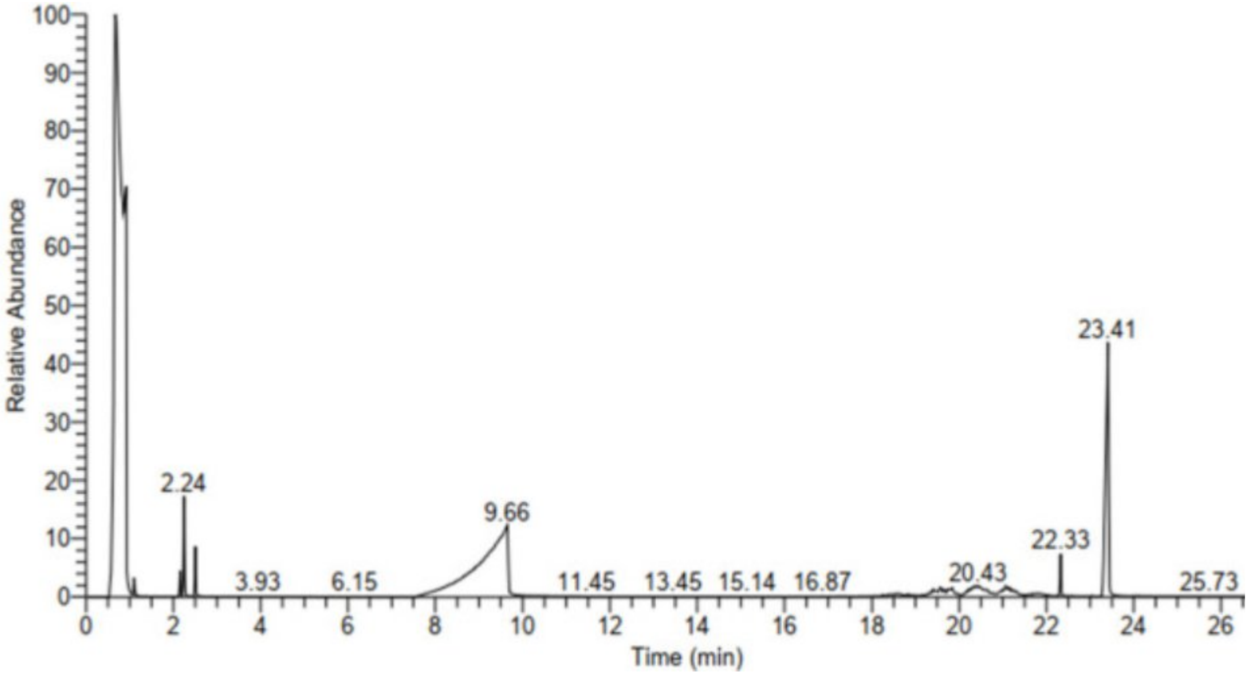


Fig13: GC-MS of Sa after degradation

From the figure above the metabolic products of Sa was found at retention time 2.24 and 9.56 min the mass to charge ratio closely matches with benzoic acid and ortho-xylene. The azo bond of the dye was broken down by bacteria consortium (*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *streptomyces*) producing azo-reductase enzyme. The molecular weight of the metabolites was 122 (benzoic acid) and 106 (ortho-xylene) respectively.

Cationic thiazine dye degradation by spectrophotometric analysis:

The study was carried out for 5 days with *Bacillus spp* and the percentage of decolorization was monitored every one-day gap using UV-visible spectrophotometer. The maximum absorption wavelength was found to be 665nm for methylene blue. The gradual decrease in absorbance is shown in the table1 below.

Table1: Absorbance value of dye decolorization

Days	Blank OD (Nm)	Sample OD (Nm)	Decolorization (%)
Day 1	2.142	1.739	18.81
Day 3	2.142	1.546	27.82
Day 5	2.142	1.226	42.76

Physiochemical Analysis of water samples from Signboard River and Shitalakha River

Table2: Physical testing

Location	**pH**	**EC (mS/cm)**	**Salinity (ppt)**	**DO (mg/L)**	**Temperature (C)**
Signboard River	7.3	0.24	0.07	6.8	24.6
Shitalakha River	7.3	0.28	0.07	6.6	30.2

The Table2 above shows two sampling locations, namely Signboard River and Shitalakha River. The dissolve oxygen concentration (DO) is within a 6.5-8 mg/l range for a healthy river system. Electrical conductivity (EC), Salinity, and other components are within normal range.

Table3: Cd and Pb concentrations in ppb

Location	Cd/ppb	Pb/ppb
Signboard River	0.2	68.1
Shitalakha River	0.5	109.3

The Cd concentrations of 0.2 ppb and 0.5 ppb are below the WHO guideline for drinking water of 3 ppb (see Table3). It is within the regulatory limit. However, even this concentration can cause risk to aquatic life and affect humans eventually through bioaccumulation in the food chain. Next, the Pd concentration at both locations is significantly high. The water is significantly polluted and pose a health risk. It is well above 10 ppb limit of the WHO. Therefore, consumption of this water may lead to lead poisoning.

Table4: Activity of Th-232, Ra 226, and K-40 in (Bq/l)

Location	Th-232 (Bq/l)	Th-232 Uncertainty	Ra-226 (Bq/l)	Ra-226 Uncertainty	K-40 (Bq/l)	K-40 Uncertainty
Signboard River	1.8	0.21	1.51	0.15	42	0.14
Shitalakha River	2.1	0.2	1.22	0.17	43	0.13

The activity of radionuclides in surface waters (Signboard River and Shitalakha River) overall is low, and no radiological contamination has occurred (see Table4). Although the results in both rivers fell well within recognized safety criteria, they were at the top end (Th-232, Ra-226) 1.8 and 1.51 for Signboard River and 2.1 and 1.22 for Shitalakha River or above (K-40) 42 Bq/l for Signboard River and 43Bq/L for Shitalakha River, of the usual stated range for drinking water. For particular alpha-emitting radionuclides like Ra-226 and Th-232, the World Health Organization (WHO) and UNSCEAR propose guideline values of 1 Bq/L; for K-40, this recommendation is less strict due to its modest radiological impact and nutritional significance (Najeba F. Salih, 2022).

Chapter 6: Limitations and Future works:

The n-hexane extraction of petrol sample evaporates at room temperature so some of the molecules may have escaped to prevent it the procedure can be carried on ice in future. Other than that, species such as *Bacillus licheniformis* which breaks higher hydrocarbons and naptheline more efficiently could be tried out (Ravi et al., 2022). Additionally, combination of bacteria could be tried out to observe efficiency of breakdown. Lastly, fungus incorporation can also be down.

Chapter 7: Conclusion

The present study determines that degradation of petroleum compounds by *Pseudomonas aeruginosa*, azo dye by *Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, and streptomyces*, and cationic thiazine dye by *Bacillus spp* isolated from the contaminated Signboard River. Additionally, it looked at the radioactive profiling of radionuclides and physicochemical properties of water, including heavy metal pollution from Cd and Pd from industrial activities sampled from Shitalakha river and Signboard River. The GC-MS data showed petroleum compounds containing large hydrocarbons broken down successfully by *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus and streptomyces* broken down harmful carcinogenic methyl red dye. Enzyme name azo-reductase was responsible producing intermediates name benzoic acid and ortho xylene from the dye. Decolorization of methylene blue by *Bacillus spp.* with an efficiency of 42.76% in 5 days conclude that this strain of bacteria is also a capable degrader. The DO, EC, and Salinity are in the normal range for aquatic river water life. Following that, it is found no or low Th-232, Ra-226, and K-40 radionuclide pollution. Even though these parameters are found to be normal within the range of international standards the Pb pollution from both sources are significantly high and need to be addressed as this is likely to cause serious health risks such as cancer.

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