ISOLATION AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS-DEGRADING BACTERIA FROM MANGROVE HABITAT'S SEDIMENTS IN MAKUPA CREEK, MOMBASA COUNTY, KENYA

Mlaghui Florah Mshai (BSc Biochemistry and Molecular Biology)

156/CE/28500/2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (BIOTECHNOLOGY) OF THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY.

JUNE, 2025

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature---------- Date----------

Mlaghui Florah Mshai (BSc Biochemistry and Molecular Biology)
156/CE/28500/2013

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the student under our supervision.

Signature ..... Date.....
Dr. David Mburu
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

Signature ..... Date.....
Prof Joseph Mwafaida
Department of Biological Sciences
Pwani University

Signature .....Date.....
Prof. Mathew Ngugi
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

DEDICATION

To my late father, my late mother, and my late grandmother Florah Mwalimo, who taught me the value of hard work, discipline, humility, and a fear of God.

ACKNOWLEDGEMENTS

Thank Lord God for enabling me to be where I am now.

I want to thank Prof. Joseph Mwafaida, Dr. David Mburu, and Dr. Mathew Ngugi for their guidance, support, and encouragement during my research and thesis writing. I am grateful to the Biological sciences department at Pwani University for letting me utilize their labs and equipment while studying there. Prophet Ingosi, Scolastica Otieno, Joel Kalama, Felistus Mwania, Benson Odera, David Kaimenyi, and Constance Sifa are among the laboratory technical staff members for whom I am truly grateful.

I also want to thank my family, especially Evans who believed in me and supported me emotionally and financially. I want to thank my friends Betty Vulule and Zonia Mupe for their guidance and help with the data analysis. To My sons and siblings,I am grateful for your prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

BLAST	Basic Local Alignment Search Tool
dsDNA	Double-Stranded Deoxyribonucleic Acid
NCBI	National Center for Biotechnology Information
PAH	Polycyclic Aromatic Hydrocarbon
PCR	Polymerase Chain Reaction
RDP	Ribosomal Database Project
rRNA	Ribosomal Ribonucleic Acid
SWNA	Sea Water Nutrient Agar
TE	Tris-EDTA
TSA	Tryptic Soy Agar

ABSTRACT

In humans and animals, polycyclic aromatic hydrocarbons (PAHs) have a long and notorious history of being recognized as potent endocrine system disruptors, cancers, and mutagens. Their lipophilic nature and the unique chemical structure of fused aromatic rings allow them to disseminate swiftly throughout the environment. The biological degradation of PAHs is the natural ecosystem primary remediation mechanism, where microorganisms are essential to PAH metabolism. The objective of the study was to isolate, characterize and identify bacteria capable of degrading PAHs from sediment samples collected in a mangrove habitat. Twelve sediment samples were collected from various sections within the mangrove region of Makupa Creek, Mombasa, County. Anthracene and Naphthalene-supplemented marine agar were used to isolate the bacteria that broke down PAHs. Biochemical assays were conducted to assess the activities of amylase, oxidase, and catalase. Molecular methods to identify the isolated bacterium were achieved by amplifying the 16S rRNA followed by sequencing using dye terminator technique. BLAST and the RDP's SeqMatch technique were used to search the NCBI database using sequence data. ClustalW 1.6 program was then used to align the 16S rRNA gene sequence. Determination of isolates' evolutionary connection was achieved using a maximum likelihood algorithm on MEGA 6 software. Twenty-one of the 44 bacterial samples isolated from the sediments were viable. The isolates had anthracene and naphthalene degradation efficiencies ranging from 93.8% to 99.5% and 79.1% to 99.39%, respectively. Biochemical tests showed that all isolates were positive for the catalase test, while 90% and 95% had oxidase and amylase activity, respectively. The genomic DNA from each bacterial isolate was extracted. The bacterial universal primers 27F and 1492R were then used to amplify the 16S rRNA gene, yielding an amplicon of about 1500 bp. Compared to the other isolates, S3B01, S2A01 A, and S1A01 produced less DNA. Blast searches indicated that the isolates shared a sequence similarity index of between 81% - 100% with those of other existing taxon, 60% of which were *Pseudomona*s and the rest were *Bacillus, Ralstonia, Enterobacter*, and *Exiguobacterium*. Ten isolates had a similarity score of less than 97% with other species, indicating that they are novel strains. The prominence of *Pseudomonas* reinforces its significance in PAH degradation. Furthermore, the emergence of unclassified isolates suggests the exciting possibility of novel bacterial strains that can be targeted for developing anti-pollution agents. In conclusion Mangrove sediments from Makupa Creek, Mombasa County, harbor diverse bacteria capable of degrading PAHs, particularly naphthalene and anthracene. The enzymatic; catalase, amylase, oxidase activity of these isolates supported microbial degradation. The 16S rRNA analysis identified *Pseudomonas, Bacillus, Ralstonia, Enterobacter*, and *Exiguobacterium* spp as the key hydrocarbon-degrading genera. The presence of other genera undocumented isolates suggested the presence of potentially novel strains. There is need therefore, to develop the bacterial consortia from Makupa Creek sediments for hydrocarbon bioremediation.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Xenobiotic accumulation in the environment is currently one of the most pressing environmental problems of our day. This chemical stability of these compounds is brought on by the intricacy of their molecular structure, accounts for their accumulation and persistence in various environmental compartments. Polycyclic aromatic hydrocarbons (PAHs) are xenobiotics that comprise of organic molecules with several bonded benzene rings. They found in a variety of media, including soil, water, sediments, and air(Mallah *et al*., 2022). Low volatility, high molecular weight, and insoluble in water are characteristics of PAHs that greatly contribute to their persistence in the environment(Venkatraman *et al*., 2024).

These organic micropollutants are used in vast numbers in the global economy and industry, and their production is increasing quickly. The primary sources of PAH emissions into the environment include industrial waste, cooking, fuel combustion, and agricultural fires(Vijayanand *et al*., 2023a). PAHs provide negative environmental and health risks because of their toxicity. PAHs have the ability to bioaccumulate across trophic levels. However, their toxicity is modest(H. Wang *et al*., 2025). The most prevalent entry points into the body include direct touch, seafood,

grilled meat, PAH inhalation, and polluted water. The USEPA has recently recognized 16 PAHs as priority pollutants due to their potential negative effects on aquatic and terrestrial ecosystems(Jesus *et al*., 2022). Furthermore, numerous PAHs have been identified as possible carcinogens capable of inducing DNA alterations with sustained exposure(Ewa & Danuta 2022). High levels of PAHs in the body raise the risk of cardiovascular disease, chronic renal disease, chronic obstructive pulmonary disease, diabetes, child neurodevelopmental impairments, obesity, and adult depression(Hao *et al*., 2021). The substantial accumulation of PAHs in marine ecosystems, especially those near industrial cities, has been caused by several harmful human activities, including oil spills, incomplete combustion of fossil fuels, ship traffic, urban runoff, and industrial operations (Dai *et al*., 2022a).

With an aim of reducing or eradicating PAHs from the environment, numerous researches have been conducted with an aim of investigating and developing strategies of removing and degrading PAHs from various contaminated environmental compartments. This has contributed to the development of several physical, biological and chemical remediation strategies(Aparicio *et al*., 2022). However, among these approaches, bioremediation has received extensive attention. Bioremediation is based on the fundamental principle of utilizing the metabolism of microorganisms, fungi and plants to degrade pollutants(Ayilara & Babalola, 2023). These converts hazardous pollutants into less hazardous or non-hazardous ones by mineralizing, converting, or immobilizing them. Optimizing the capacity of these organisms for biodegradation ensures that pollutants are removed quickly and with the least negative environmental

impact. According to Vijayanand *et al.,* (2023a) ,biodegradation has eliminated soil pollutants, including PAHs with three or fewer aromatic rings. *Comamonas testosterone*,a gram positive bacterium and *Pseudomonas putida* have been reported to effectively degrade aromatic hydrocarbons(Xu *et al*., 2022). Additionally, *Rhodococcus.Sp* and *Pseudomonas stutzeri* have also been reported to degrade various aromatic compounds such as PAHs (Kumari & Chandra, 2023). This implies that the cooperation of bacteria in PAHs degradation is a promising remediation tool of removing PAHs from the environment.

Mangrove ecosystems are densely inhabited with microorganisms that can degrade PAHs. However, most bacteria are uncultivable, severely limiting the isolation of PAH degraders(Liu *et al*., 2023). Additionally, there is limited research focused on the isolation and utilization of mangrove bacteria in the degradation of PAHs in Kenya. Therefore, this research aimed to isolate Mangrove bacteria and investigate their ability in breaking down PAHs and utilizing them in biodegradation in marine habitats.

1.2 Statement of the problem

Estuarine and coastal ecosystems (ECEs) are among the most heavily exploited and ecologically vulnerable natural systems worldwide(Liu *et al*., 2021). In Kenya's coastal region, Makupa Creek hosts biologically significant habitats such as mangrove forests, seagrass beds, and coral reefs. However, these ecosystems are under increasing threat from polycyclic aromatic hydrocarbons (PAHs), a class of persistent

organic pollutants introduced through various anthropogenic activities(Dai *et al*., 2022b).

Makupa Creek is particularly susceptible to PAH contamination from two major sources. First, the Kibarani area serves as a dumping site for municipal solid waste, much of which is transported into the creek through surface runoff and atmospheric deposition. Second, the rapid growth of industrial activity around the creek has exacerbated the influx of hydrocarbons and other pollutants into the marine environment (Wanjeri *et al*., 2023). The accumulation of PAHs in sediments and water poses significant health risks to both aquatic organisms and humans, including carcinogenicity, mutagenicity, and developmental toxicity(Ghosh & Mukherji, 2023). Consequently, several PAHs are classified as priority pollutants by the United States Environmental Protection Agency (USEPA).

While physical and chemical methods have been employed for PAH removal, these approaches are often economically burdensome and environmentally unsustainable(Eldos *et al*., 2022). In contrast, biological degradation through microbial activity offers a cost-effective and eco-friendly alternative. Microorganisms play a central role in the natural decontamination of ecosystems by breaking down PAHs into less harmful substances(Raganati *et al*., 2023). Mangrove sediments, particularly those in Makupa Creek, represent ideal environments for sourcing such microorganisms due to their rich microbial diversity and prolonged exposure to hydrocarbons(Mondal *et al*., 2024). However, there is still limited knowledge about the identity and capabilities of indigenous PAH-degrading bacteria in this region. A

detailed investigation into these microbial communities is essential for advancing bioremediation efforts and supporting the ecological recovery of contaminated coastal habitats.

1.3 Justification of the study

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants widely distributed in estuarine and coastal ecosystems due to various anthropogenic activities, including industrial discharge, maritime transport, and improper waste disposal. These compounds pose severe ecological and public health risks due to their toxic, mutagenic, and carcinogenic properties. In Kenya, Makupa Creek in Mombasa is particularly vulnerable to PAHs contamination because of its proximity to industrial and urban activities.

Various methods have been employed to degrade PAHs in the environment, including both physicochemical and biological approaches(Vijayanand *et al*., 2023b). However, physicochemical methods are often limited by high operational costs, energy demands, and the risk of secondary pollution. As a result, biological methods, particularly microbial bioremediation, have gained prominence as cost-effective and environmentally sustainable alternatives. Bioremediation using naturally occurring microorganisms offers the potential for complete mineralization of PAHs into harmless end-products (Premnath *et al*., 2021)

Mangrove ecosystems are known reservoirs of diverse microbial communities, many of which have adapted to degrade hydrocarbons under harsh environmental conditions(Siddique *et al*., 2024). Makupa Creek's mangrove sediments, therefore, present a promising site for isolating indigenous PAH-degrading bacteria. This study aims to determine whether these sediments harbor naturally occurring bacterial strains

with inherent PAH-degrading capabilities, contributing to the natural purification of municipal and industrial wastewater. The findings will not only enhance our understanding of microbial diversity and function in contaminated coastal environments but also support the development of locally adapted, microbe-based bioremediation strategies for effective environmental restoration.

1.4 Hypotheses

i. Mangrove sediments from Makupa Creek do not contain bacterial strains capable of degrading polycyclic aromatic hydrocarbons (PAHs).
ii. The PAH-degrading bacterial isolates from Makupa Creek do not exhibit distinct biochemical characteristics associated with hydrocarbon degradation.
iii. The PAH-degrading bacteria isolated from Makupa Creek are not phylogenetically related to known hydrocarbon-degrading genera, and no novel strains are present.

1.5 Objectives

1.5.1 General objective

To isolate, identify and characterize PAH-degrading bacteria from mangrove sediments collected from Makupa Creek.

1.5.2 Specific objectives

i. To isolate bacteria that degrade PAHs from the mangrove sediments of Makupa creek.
ii. To characterize the PAHs degrading bacteria isolates using biochemical techniques
iii. To determine the phylogeny of the PAHs degrading bacteria isolates

1.6 Significance of the Study

This study has significant implications for environmental management, public health, and scientific advancement in the context of coastal pollution. Makupa Creek, located along Kenya's shoreline, is becoming more contaminated by polycyclic aromatic hydrocarbons (PAHs), which are persistent, poisonous, and probably carcinogenic substances. These pollutants endanger the ecological integrity of critical habitats including mangrove forests, seagrass beds, and coral reefs, as well as the health and livelihoods of the local communities that rely on these ecosystems.

The goal of this study is to identify and characterize native bacteria capable of degrading PAHs by examining the indigenous microbial communities in Makupa Creek's mangrove silt. The identification of such microbes will help us better understand the natural attenuation mechanisms that occur in tropical coastal areas. Furthermore, it will pave the way for the development of long-term, microbe-based bioremediation techniques that are both locally relevant and environmentally friendly.

Given the limitations of traditional physicochemical remediation approaches, which are frequently expensive, energy-intensive, and environmentally unsustainable, the study of biological alternatives is both scientific and practical. The findings from this study could influence policy and environmental restoration practices by providing evidence-based techniques to reducing PAH pollution in estuarine and coastal ecosystems. It will also contribute to global research efforts focusing on the ecological significance and biotechnological potential of microbial communities in contaminated marine habitats, filling a significant knowledge gap in the fields of environmental microbiology and coastal ecosystem restoration.

CHAPTER TWO

LITERATURE REVIEW

2.1 Polycyclic aromatic hydrocarbons

2.1.1 Physical characteristics

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds composed of two or more fused benzene rings organized in linear, angular, or clustered shapes. Their main structural unit is the benzene ring, and they can contain a variety of substituents, including alkyl, nitro, or amino groups(Altarawneh & Ali, 2024).

The PAHs' aromatic structure contributes to their great chemical stability and longevity in the environment. PAHs are divided into two types based on their molecular weight: low-molecular-weight (LMW) PAHs, which have two to three aromatic rings (e.g., naphthalene, anthracene), and high-molecular-weight (HMW) PAHs, which have four or more rings (e.g., fluoranthene, chrysene). The LMW PAHs are often more volatile and present in the environment as vapors, whereas HMW PAHs are less volatile and are largely linked with airborne particulate matter. The HMW PAHs with five or more rings, such as benzo[g,h,i]perylene, are predominantly found in particulate matter(Sivaram *et al*., 2019).

The physical and chemical features of PAHs have a substantial impact on their environmental behavior and toxicological profiles. They have high melting and boiling temperatures, low vapor pressure, and restricted solubility in water properties

that increase with molecular weight. PAHs, on the other hand, are well-soluble in organic solvents, have a high lipophilicity, and tend to adsorb on organic debris and sediments. These characteristics enhance their environmental persistence and bioaccumulation potential(Sivaram *et al.,* 2019).

Analytically, PAHs are distinguished by their unique ultraviolet (UV) absorbance and fluorescence, which enable detection by excitation and emission at specified wavelengths. Because of their limited aqueous solubility, PAHs can travel through soils and potentially contaminate groundwater systems.

The US Environmental Protection Agency (US EPA) has classified sixteen PAHs as priority pollutants because of their hazardous, mutagenic, and carcinogenic qualities. While the International Agency for Research on Cancer (IARC) classifies many PAHs as likely or suspected human carcinogens, benzo[a]pyrene is the only one that has been definitively identified as a human carcinogen(Tchounwou *et al*., 2022).

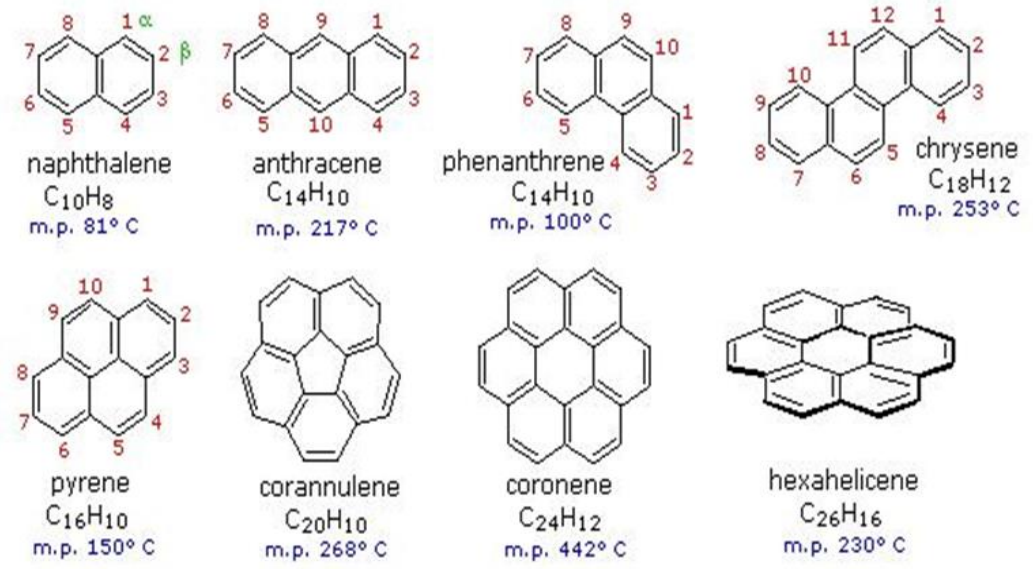


Figure 2.1 Examples of Polycyclic Aromatic Hydrocarbons (PAHs)

2.1.2 Sources of Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) enter the environment through both natural and anthropogenic sources, however anthropogenic contributions are substantially more prevalent(Balmer *et al*., 2019). These sources are roughly divided between petrogenic and pyrogenic origins. Petrogenic PAHs are naturally occurring and emerge from geochemical processes over millions of years. They typically form at low temperatures (100-150 °C) and high pressure during the maturation of organic materials in fossil fuels. Natural sources of PAHs include oil seepage, volcanic eruptions, and lightning-caused forest and rangeland fire. Although these natural processes emit PAHs into the environment, their influence is negligible when compared to human-caused sources(Frapiccini *et al*., 2024).

Pyrogenic PAHs, on the other hand, are typically produced by incomplete burning of organic molecules and are largely linked to human activity. These PAHs are created by the combustion of fossil fuels and organic materials such coal, wood, petroleum, rubber, paper, and tobacco. Waste incineration, iron and steel manufacture, aluminium smelting, cement manufacturing, and coal-tar pitch and asphalt processing are all major industrial operations that emit PAHs(Abdel-Shafy & Mansour, 2016). Furthermore, enterprises involved in dye manufacturing, pesticide production, and petroleum refining discharge considerable amounts of PAHs into the surrounding ecosystem.

Mobile sources, particularly emissions from internal combustion engines in vehicles, contribute significantly to atmospheric PAHs levels, especially in urban areas. In these conditions, incomplete combustion of gasoline and diesel fuels causes the emission of both low- and high-molecular-weight PAHs(Ali *et al*., 2021). Domestic activities such as biomass cooking, heating with kerosene or wood stoves, waste disposal, and the use of gas burners all contribute significantly, particularly in low- and middle-income communities where these behaviors are common. Agricultural sources, especially open-field burning of crop residues and biomass, add to PAHs pollution in rural and peri-urban contexts(Zabaniotou & Stamou, 2020).

Overall, pyrogenic PAHs are dominant due to the extensive and diversified character of human combustion activities. These chemicals, particularly the high-molecular-weight PAHs generated at high temperatures, are more ecologically persistent and hazardous. PAHs sources are ubiquitous and complex, emphasizing the need for

comprehensive monitoring and tailored remediation techniques to reduce their impact on both ecosystems and public health(Vijayanand *et al.,* 2023b).

2.1.3 Routes of Exposure to Polycyclic Aromatic Hydrocarbons (PAHs)

Human exposure to polycyclic aromatic hydrocarbons (PAHs) occurs through a variety of environmental pathways, the most common of which are inhalation, ingestion, and skin contact. These exposures come from a range of sources, including outdoor and indoor air, contaminated soil and dust, drinking water, and nutrition(Mallah *et al*., 2022b). The degree and route of exposure are determined by environmental concentrations, lifestyle behaviors, occupation, and geographical location.

Inhalation is one of the most significant exposure mechanisms, particularly in urban and industrial areas where airborne PAH concentrations are high due to vehicle emissions, fossil fuel combustion, biomass burning, and industrial activities(Sekar & T R, 2024). Indoor air, in particular, offers a significant concern because people spend 85-90% of their time indoors. Common indoor sources include tobacco smoke, cooking with biomass fuels, heating appliances, the burning of candles or incense, and the intrusion of outdoor contaminants(Agathokleous *et al*., 2021).

Environmental tobacco smoke (ETS) remains a significant indoor source of PAHs. According to (Zena Bukowska *et al*., 2022) cigarette smoking is one of the most common exposure routes for the ordinary person. A single cigarette can emit 20-40 ng of benzo[a]pyrene (BaP), and smoking a pack of unfiltered cigarettes per day can

result in an exposure of around 0.7 µg/day. Side stream smoke, which comes from the burning end of the cigarette, includes higher quantities of PAHs than mainstream smoke (the portion inhaled by the smoker), providing a health concern even to nonsmokers through passive exposure(Torres *et al*., 2018).

Ambient PAHs concentrations vary with location and activities. Priority PAHs concentrations in air range from 0.02 to 1.2 ng/m³ in rural areas and up to 19.3 ng/m³ in urban environments (Kramer *et al*., 2025). Although these levels are lower than those found in the workplace, long-term exposure in large populations can pose serious public health risks(Cheng & Cheng, 2024).

Ingestion is another significant route of exposure, mainly through eating and drinking water. PAHs are typically found in grilled, charred, and smoked meals, particularly meats and fish. Cooking procedures that include direct contact with flame or smoke can drastically raise PAHs levels in food(Duedahl-Olesen & Ionas, 2022). Furthermore, fruits, vegetables, and cereals cultivated in contaminated soil or irrigated with polluted water can increase dietary PAHs intake. Drinking water contamination is typically caused by industrial discharges, oil spills, and leaching from contaminated soils. Although concentrations in treated drinking water are normally far below the US Environmental Protection Agency's maximum contamination level (MCL) of 0.2 parts per billion (ppb) for benzo[a]pyrene, the ingestion route remains essential, particularly in regions with inadequate water quality management(Ziyaei *et al*., 2024).

Dermal contact is caused by direct exposure to PAH-contaminated soil, dust, or water, particularly in occupational contexts such as construction, mining, and petroleum sectors. In the majority population, cutaneous exposure occurs during leisure or gardening activities in polluted locations. PAHs are largely deposited in soils through air fallout, and they tend to collect near sources such as oil refineries and heavily trafficked roads(Tumelo Monty *et al*., 2024). Urban soil samples typically contain fewer than 2,000 µg/kg of PAHs, whereas concentrations near industrial operations can reach 200,000 µg/kg.

PAHs exposure usually occurs concurrently through various routes. Individuals living in high-traffic metropolitan areas, for example, may be exposed by inhalation and ingestion, as well as skin contact with contaminated surfaces. This combined exposure can raise the total absorbed dose and pose health hazards. Furthermore, lifestyle factors such as smoking, food choices, and the use of solid fuels for cooking or heating have an impact on the degree of exposure (S. Lee & Hyun, 2021).

2.1.4 Effects of Polycyclic Aromatic Hydrocarbons (PAHs) on Human Health

The effect of polycyclic aromatic hydrocarbons (PAHs) on human health is determined by a complex interaction of factors such as exposure route and duration, PAHs content and chemical composition, and their respective toxicological profiles(Das & Ravi, 2022). According to (Sun et al., 2021) three parameters exposure pathway, intensity, and chemical properties are the key indicators of health effects after PAHs exposure. Age, genetic predisposition, underlying medical

conditions, and immunological status may all have an impact on individual susceptibility (Yu *et al.*, 2022a).

Short-term exposure to high amounts of PAHs or PAH combinations can have serious health consequences. These include mucosal and cutaneous irritation, nausea, vomiting, diarrhea, migraines, and dizziness (Feng *et al.*, 2025). Sub-chronic exposure has been linked to symptoms such as respiratory problems, asthma-like illnesses, and changes in pulmonary function, especially in sensitive populations such children and those with pre-existing respiratory diseases (Kim *et al*., 2021)

Prolonged exposure to PAHs has been strongly associated to chronic health consequences, the most notable of which is carcinogenesis. Because of their potent mutagenic and carcinogenic effects, PAHs such as benzo[a]pyrene (BaP) have been extensively researched(Kumari & Chandra, 2023). BaP is a frequent reference chemical for measuring PAH exposure and risk, and the International Agency for Research on Cancer (IARC) classifies it as a Group 1 carcinogen—carcinogenic to humans (Tilton et al., 2015). Other PAHs, such as naphthalene, chrysene, benz[a]anthracene, and benzofluoranthenes, are classified as Group 2B (probably carcinogenic to humans), based on evidence from both animal research and limited human data(Mallah *et al*., 2022c)(Mallah *et al*., 2022c).

Epidemiological studies have consistently found a link between long-term PAHs exposure and an elevated risk of lung cancer. Zhang *et al*., (2024) found that when PAHs exposure reaches a threshold of 10^{-6} , there is a 45% likelihood of acquiring

carcinogenic consequences. This highlights the importance of chronic low-dose exposure in population-level risk assessment.

PAHs are primarily hazardous because they activate cytochrome P450 enzymes, resulting in the creation of reactive intermediates such as diol epoxides. These metabolites have a strong affinity for DNA, forming DNA adducts that can cause mutations, chromosomal abnormalities, and, eventually, carcinogenesis. PAHs accumulate in lipid-rich tissues and organs, which contributes to prolonged systemic retention and higher internal exposure (Zhang *et al*., 2016)

Long-term PAHs exposure can cause cancer in a variety of organs, including the lungs, skin, liver, pancreas, colon, esophagus, bladder, and female breast tissue. The risk is particularly high in industrial situations such as coke production, asphalt paving, and aluminum smelting, where persons are consistently exposed to higher PAH concentrations(Vandana *et al.,* 2022).

Beyond carcinogenicity, there is mounting evidence associating PAHs exposure to cardiovascular disease (CVD). Chronic exposure has been linked to an elevated risk of atherosclerosis, hypertension, myocardial infarction, and thrombosis. These effects are mediated by mechanisms involving oxidative stress, systemic inflammation, and endothelial dysfunction(Zheng *et al.*,2022). Epidemiological studies have established correlations between PAHs biomarkers (e.g., urinary 1-hydroxypyrene) and elevated levels of inflammatory markers such as CRP, interleukins, and tumor necrosis factor-alpha (TNF-α), which all indicate cardiovascular risk (Yang *et al*., 2017)

PAHs have also been linked to liver and kidney damage, immunological suppression, and endocrine disruption. Chronic exposure may cause liver enzyme changes, renal damage, and impaired detoxification pathways, as well as impact hormone control and reproductive health(Yu et al., 2022).

Certain populations, such as children, the elderly, pregnant women, and those with weakened immune or respiratory systems, are especially vulnerable to the negative health impacts of PAHs(Drwal et al., 2019). Socioeconomic characteristics, as well as closeness to industrial sources or high-traffic regions, might exacerbate exposure and health concerns in vulnerable groups. These discrepancies highlight the significance of strong environmental legislation and public health actions to reduce PAH exposure.

2.2 Remediation of PAHs

2.2.1 Overview

As one of the most common environmental toxicants, polycyclic aromatic hydrocarbons have been the target of several cleanup attempts. The three main components of these technologies are transfer, degradation, and sequestration. There are three ways in which PAHs may be removed: transfer, degradation, and sequestration.Transfer involves moving PAHs to a new location without changing their structure, degradation is where the PAHs structures are altered from their original form whereas sequestration involves removing toxicants from bioavailable pools and storing them for a long time. Many different remediation methods exist,

including the more conventional physicochemical, chemical, thermal, and biological approaches.

2.2.2 Traditional remediation

Traditional remediation techniques to polycyclic aromatic hydrocarbon (PAH) pollution entail physically removing polluted soils or sediments, followed by off-site treatment via incineration or secure disposal in landfills (Eom & Park, 2021). These strategies intend to remove the immediate point source of contamination while reducing environmental and human health hazards. While adept at removing PAHs from contaminated sites, they are frequently expensive, energy-intensive, and environmentally harmful. Furthermore, they do not contribute to the degradation of PAHs, and in the case of landfilling, the contaminants remain in a sequestered but persistent state, creating possible long-term environmental concerns(Kapley et al., 2020).

PAHs exhibit strong environmental persistence due to their chemical stability and low water solubility, leading to their long-term retention in soil and sediment matrices(Maletić et al., 2019). Adsorption and diffusion are the two main mechanisms that determine their persistence. Adsorption occurs when PAHs molecules bond to soil components, primarily organic matter and clay minerals. This interaction is influenced by a variety of soil properties, including clay type, organic carbon concentration, and moisture level(Saeedi et al., 2020). Adsorption lowers PAHs' mobility and bioavailability, lowering the chance of microbial breakdown. Diffusion, on the other hand, refers to the progressive movement of PAHs into soil micropores, where they become physically trapped and inaccessible to degrading microbes. PAHs' hydrophobic nature increases their attraction for the non-aqueous phases

within soil pores, effectively protecting them from microbial attack and environmental degradation processes(W. Zhang et al., 2023).

Overall, while traditional remediation techniques offer immediate containment or removal of PAHs, they often fall short in promoting long-term environmental recovery. Their high operational costs, limited sustainability, and potential for residual contamination have spurred growing interest in alternative methods such as physicochemical remediation, bioremediation and phytoremediation, which focus on in situ degradation and ecosystem restoration.

2.2.3 Physicochemical remediation

Physical remediation approaches provide efficient first techniques for eliminating polycyclic aromatic hydrocarbons (PAHs) from contaminated media, particularly water and soil. These approaches are generally based on the concept of phase separation or pollutant transfer, and do not chemically modify the PAH structure(Drwal et al., 2019). Among the most notable physical procedures is soil washing, which uses organic solvents such as acetone, alcohol, hexane, dichloromethane, methyl ethyl ketone, and toluene to remove hydrophobic PAHs due to their high solubility in such compounds (Von Lau et al., 2014). Soil washing is very effective at removing high molecular weight (HMW) PAHs, which have a significant attraction for soil particles but limited bioavailability (Greish et al., 2018). Biodegradable and non-toxic extractants such as cyclodextrins, vegetable oil, humic acid, and supercritical or subcritical fluids are being investigated to improve

sustainability (Gitipour et al., 2018). Solvent regeneration is possible through distillation, which normally results in around 10% solvent loss. Surfactants added to soil washing solutions can greatly improve PAH desorption and solubilization, although the efficacy is dependent on the physicochemical features of the PAHs, surfactant structure, and soil composition (Tiwari & Tripathy, 2023).

Membrane-based filtration technologies, including ultrafiltration, nanofiltration, and reverse osmosis, have shown excellent efficiency in removing PAHs from aqueous systems (Gong et al., 2024). Likewise, many adsorption-based approaches employ materials such activated carbon, charcoal, biochar, modified clays, magnetic nanomaterials, graphene oxide, and nano-sulfonated graphene (SGE) for PAH capture from both soil and water ((Lamichhane et al., 2016; GAN et al., 2017). Notably, SGE has demonstrated excellent effectiveness in in situ soil washing, removing up to 80% of PAHs under ideal conditions because to its large surface area, dispersibility, and ease of separation (GAN et al., 2017). Biochar, while successful for HMW PAHs due to its high-temperature activation, is less suited for low molecular weight (LMW) PAHs, which may volatilize during the activation process (Sullivan et al., 2019).

Electro-kinetic remediation is another potential physical approach, especially for soils with poor permeability. This in situ approach uses a low-intensity electric current to move PAHs to collection electrodes. Its efficacy can be greatly increased when paired with solubilizing agents or biodegradation techniques (Kuppusamy et al., 2017).Furthermore, thermal incineration at temperatures ranging from 900 to 1,200°C can totally eliminate or volatilize PAHs in polluted matrices (Busari, 2024). In situ

thermal incineration can be accomplished safely and with minimum emissions by leveraging vacuum systems or carrier gases connected to gas treatment units (Kuppusamy et al., 2017). However, the high energy demand associated with this method remains a critical drawback.

Despite their value, most physical remediation techniques just transfer PAHs rather than chemically degrade them, raising questions about long-term efficacy and environmental safety. As a result, these technologies are commonly used in conjunction with biological or chemical procedures to obtain more comprehensive and long-term repair results.

2.2.4 Biological remediation

This is a process that use microorganisms or plants to detoxify or remove organic or inorganic compound from the environment. When microorganisms are used, the process is specifically known as bioremediation and it is further discussed below.

2.3 Bioremediation of PAHs

2.3.1 Polycyclic aromatic hydrocarbons degrading microorganisms

Bioremediation is a biological process that involves degradation of PAHs by microbes that convert pollutants into innocuous chemicals (Lawal., 2017). Degraders of PAH chemicals include bacterial, fungal, and algal species; nevertheless, bacteria are the most significant (Arora et al., 2016). Microorganisms can transform PAHs into other organic molecules or inorganic substances like carbon (IV) oxide and water (Ghosal

*et a*l., 2016). Although several bacterial species and genera have demonstrated the ability to break down PAHs with two or three rings, few bacteria can do so with a significant molecular weight. However, some *Pseudomonas* species may degrade PAHs with four or five rings, according to Lawal (2017). Table 2.1 lists the bacteria that break down PAHs and the compounds they target.

Table 2.1: Some PAH-degrading bacteria and the compounds degraded.

Bacteria species	**Polycyclic aromatic hydrocarbon compounds degraded**
Acidovorax	phenanthrene, anthracene
Alcaligenes	phenanthrene, fluorene, fluoranthene
Arthrobacter	benzene, naphthalene, phenanthrene
Mycobacterium	phenanthrene, pyrene, benzo[α]pyrene
Pseudomonas	phenanthrene, fluoranthene, fluorene, benzo[α]pyrene
Rhodococcus	pyrene, benzo[α]pyrene
Sphingomonas	phenanthrene, fluoranthene, anthracene

Source: (Lawal, 2017).

2.3.2 Factors Influencing Biodegradation

For biodegradation to occur, the quantity and condition of nutrients are crucial. An increase in biodegradation may be achieved by adding nutrients via fertilizers (Chiaregato et al., 2021). The community makeup and the amount of activity of microbes are both affected by soil pH. According to (Naylor et al., 2022), bacteria typically thrive in a pH range of 6 to 8, while fungi are the primary degrading organisms at pH levels below 5.5.

Temperature is also an influential factor that influences biodegradation. The optimal temperature ranges for biodegradation in soil, as (Pischedda et al., 2019), is 15 °C to 45 °C, with the highest rates of biodegradation happening between 25°C and 35 °C. According to (Gou et al., 2023), the potential biodegradation of PAHs and the activity of microbes in the process doubles every 10 degrees Celsius when the temperature rises to 45 degrees Celsius. Salts in dredge sediments hinder biodegradation because they kill off microbes. According to Díaz *et al*., (2002), the rates of hydrocarbon metabolism decline as saline levels rise. Leaching the soil is a way to get rid of too much salt(Abou Khalil et al., 2021).

2.3.3 Metabolic pathways in PAH microbial degradation

Several academic articles have extensively described the many metabolic routes bacteria employ to degrade PAHs. The main enzymes that degrade PAHs include lignolytic enzymes, phosphatases, oxygenase, and dehydrogenase (Dong *et al.*, 2023). According to (Fillat et al., 2017), degradative enzymes are most active at the

mesophilic temperature, which is between 80 - 90°C. While most enzymes are substrate-specific, lignolytic enzymes can break down phenolic and non-phenolic organic molecules by oxidizing one electron and producing cation radicals (Ravichandran & Sridhar, 2017)

The presence or absence of oxygen determines the main mechanism for PAH breakdown. Oxygen is the most critical electron acceptor in aerobic aromatic catabolism and a co-substratum for the aromatic ring's hydroxylation and oxygenolytic ring breakage (Nzila, 2018). However, anaerobic degradation of aromatic compounds mostly relies on reductive reactions to break down the aromatic ring (Rabus et al., 2016).

The metabolic processes of bacteria and fungi differ from one another. PAHs are commonly used as a carbon and energy source by bacteria that break them down (Yamini & Rajeswari, 2023). However, fungi convert PAHs into molecules that are more soluble in water, making it easier to remove them. The fungus cytochrome P-450 enzyme oxidizes PAHs to produce phenols and trans-dihydrodiols, which can be conjugated and excreted from the organism(Al-Hawash, 2018). Dioxygenase targets one of the aromatic rings in PAHs when they are broken down by bacteria, resulting in the dehydration of cis-dihydrodiol to catechol(Nienke & Volkerink,.2023). According to (Padilla-Garfias et al., 2024), cytochrome P450 monooxygenase, epoxide hydrolase, and several conjugating enzymes are the main players in the PAH-metabolic pathways.

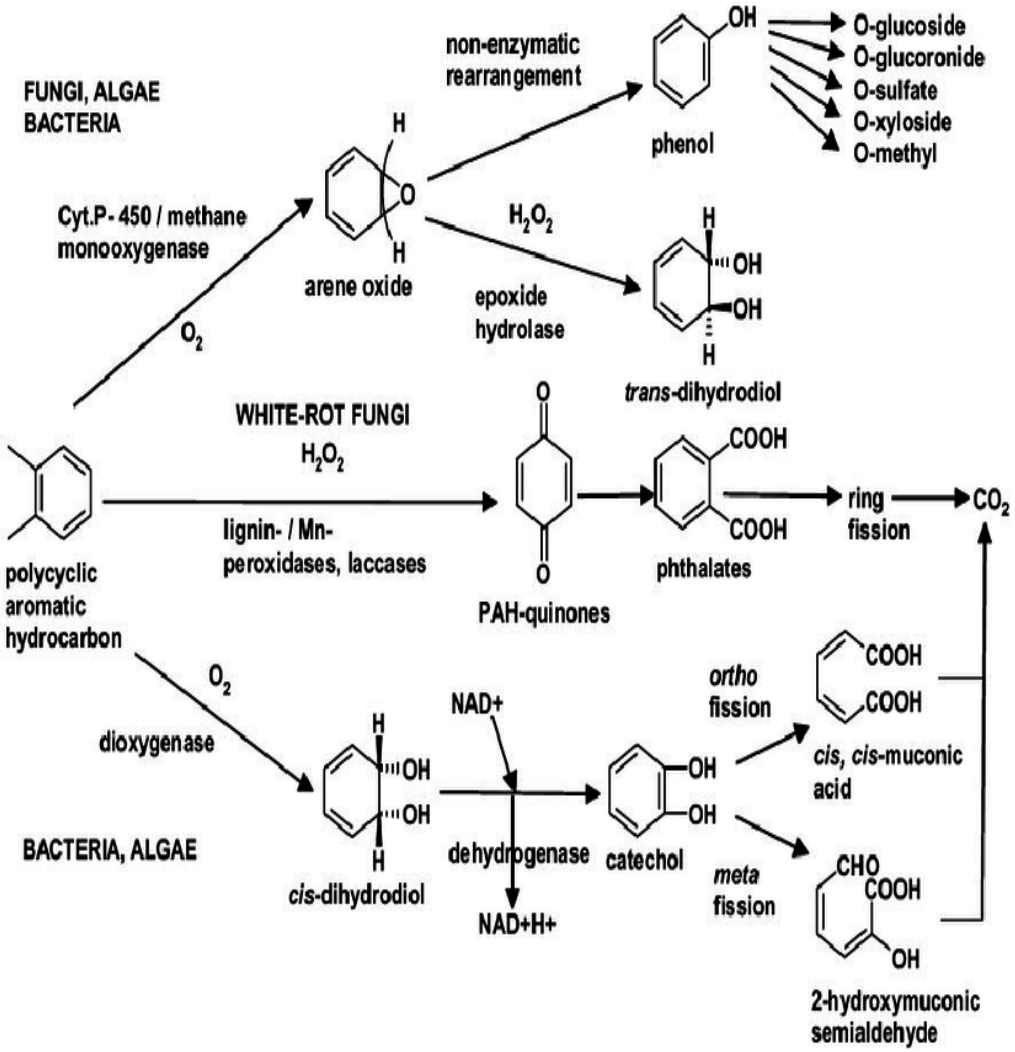


Figure 2.2: Pathways of microbial degradation of PAHs (Obayori et al., 2010)

2.4 Genes in PAH biodegradation

The genetic diversity of organisms that can degrade PAHs is enormous. Naphthalene degradation is generally linked to *Pseudomonas* bacterial species, which typically contain the well-known and widely reported *nah*-type genes(C. Wang et al., 2017). Examples of genes that are identical but have different roles include the *nid* gene in

Mycobacterium vanbaalenii, the *phn* gene in *Burkholderia sp.* strain RP007, and the *nag* gene in *Polaromonas naphthalenivorans* and *Ralstonia sp*. strain U2(Alam et al .2021)

The genetic information needed for the hydrocarbon breakdown process is provided by bacterial plasmids. The cloned genes from *P. putida* NCIB 9816-4 are present in plasmid pDTG112 (Varner et al., 2022). An incomplete 3.6-kb SalI fragment encodes naphthalene dioxygenase, which lacks the ferredoxin (*nahAb*), reductase (*nahAa*), and major subunit (*nahAc*) genes. (Alam et al.,2021)

Additionally, *Ralstonia sp.* U2 and *Comamonas testeroni* strain GZ42 share similar naphthalene catabolic genes (Chakraborty, 2022). It has been shown that the nucleotide sequences of the genes encoding the enzymes for the upper pathway are approximately 90% similar across several *Pseudomonas* strains (Alam et al., 2021). All strains of *Pseudomonas*, including *P. putida* G7, NCIB9816-4, ND6, and *P. stutzeri* AN10, have the same genes that encode the lower pathway of naphthalene catabolism (Madamwar et al., 2021). These organisms may include a naphthalene chemotaxis gene (*nahY*) among eleven others in sequence no GTHINLOMKJY, the lowest route of the naphthalene operon. However, in the AN10 and ND6 strains, a distinct salicylate hydroxylase gene (*nahW*) was found to be near the sal operon but outside of it.

The genetic sequences of *Pseudomonas* strains that express the enzymes involved in the upper pathway have been disclosed by two separate investigations (Fischer et al.,

2021). *Pseudomonas putida, Pseudomonas stutzeri, and Pseudomonas aeruginosa* are some of these bacteria.

The *Rhodococcus* genus is unique among bacteria that degrade PAHs because of its exceptional catabolic flexibility (Krivoruchko et al., 2023). Krivoruchko et al (2023)) discovered that, in contrast to Gram-negative bacteria like *Pseudomonas,* which usually include clusters of naphthalene catabolic genes, Gram-positive *Rhodococcus* strains often only have three structural genes—*narAa, narAb,* and *narB*—necessary for naphthalene breakdown. The α- and β-subunits of naphthalene dioxygenase (NDO) are encoded by the genes *narAa* and *narAb*. Only 30% of *Rhodococcus sp.* strain NCIMB12038's NDO subunits resembled those of *P. putida*. (Incha, 2023) demonstrated that naphthalene-induced cells produce a single mRNA encoding two putative regulatory genes (*narR1* and *narR2*, *GntR*-like transcriptional regulator).

2.5 Empirical research on PAH biodegradation

Numerous studies have examined how microorganisms break down PAHs, most of which have concentrated on bacterial species in maritime settings. Hydrocarbon utilizers constituted as much as one hundred percent of the total heterotrophic bacteria in sediments and waters from the maritime shorelines of Bermuda and Canada, according to research by (Peng et al., 2023). (Saravanan et al., 2021) discovered that the microbes tested exhibited a wide range of enzyme capabilities for hydrocarbon breakdown, suggesting that they may be able to extract or transform oil from the habitats they were studied.

(Vala et al., 2021) pointed out that microorganisms isolated from soils contaminated with creosote in the US, Norway, and Germany were capable of decomposing PAHs. The strongest strains of *Sphingomonas* were able to degrade 4- and 5-ring PAH. (Logeshwaran et al., 2022) reported that samples supplemented with *M. flavescens* had a higher biodegradation rate. The isolation of pyrene bacteria was also reported in two further studies, one by (Zada et al., 2021) and one by (Liang et al., 2023), reported a discovery of 53 strains of bacteria that break down PAHs from mangrove sediments in China. The breakdown of pyrene (Pyr) was found to be accomplished by thirteen strains, benzo[a]pyrene (Bap) by thirteen strains, phenanthrene (Phe) by fourteen strains, and a combination of these three PAHs (Phe + Pyr + Bap) by thirteen strains. All the individual colonies were identified by16S rDNA sequencing. After three days of testing, the researchers discovered that Phe and mixed PAH-degrading consortia degraded Phe in a liquid media at a rate of more than 91%.

(Revathy et al., 2015) found that *Burkholderia cocovenenans* BU-3 species could degrade phenanthrene at neutral pH and concentrations as high as 1000 mg/L after isolating PAH-degrading bacteria from petroleum-contaminated soil. After 40 days of initial treatment, (L. Zhang et al., 2021) reported six gram-negative bacterial strains from a petrochemical waste disposal site that could degrade acenaphthene, fluorene, phenanthrene, anthracene, and pyrene 70–100%. Two of the strains were fluorescent bacteria (*Pseudomonas fluoresens*), and one was a type of *Haemophilus* species. In the other four, the bacteria were rod-shaped.

Eighty to ninety percent of the anthracene and naphthalene were broken down in PAH-friendly soil, according to (Yemele et al., 2024), who recovered five bacterial and one endophytic strain from the uncontaminated rhizosphere of *P. deltoids*. (Li et al., 2020) reported that PAHs can be oxidized by the bacterial laccases CueO from *Escherichia coli* and CotA from *Bacillus subtilis*. The main problems in using them to clean up PAH-contaminated soil are their slow oxidation rate and dependence on copper.

Using numerical taxonomy, the phenotypic similarity of bacteria that break down petroleum has been examined. 80–85% of the bacteria in the US Chesapeake Bay were discovered to break down petroleum, according to (Herath et al., 2016), who used 14 distinct phenetic categories to analyze the bacteria. Among the groups mentioned are *Actinomycetes, Micrococcus sp., Nocardia sp., Pseudomonas sp., Sphaerotilus natans, Klebsiella aerogenes, Enterobacteriaceae, and Comeforms.* Despite having the identical 16S rRNA gene sequence, the strains of *Pseudoalteromonas* studied by (Bisht et al., 2015) differed significantly in their capacity to break down PAHs. This implies that the bacteria may have horizontally transmitted the gene for an enzyme that degrades naphthalene.

Research like this highlights the significance of bioremediation of PAHs as a remediation strategy and delves into the factors that may influence it. It is primarily a question of genetics as to whether bacteria may use metabolites produced by PAH degradation. Numerous studies, including genetic markers of *Pseudomonas* species, have focused on the decomposition of naphthalene. Additionally, research has

primarily focused on Asian and Western nations. The breakdown of PAHs by microbes in Kenya requires further research. This study aimed to isolate and identify the bacteria that degrade PAHs (anthracene and naphthalene) from Makupa Creek mangrove sediments.

2.6 Biochemical Bacteria Characterization

Biochemical tests continue to be essential for characterizing and identifying bacterial isolates. Pure cultures are often subjected to a battery of conventional microbiological tests, beginning with Gram staining to establish cell wall properties, followed by morphological examination and evaluation of catalase and oxidase enzyme production. Motility is assessed using either motility agar or phase-contrast microscopy. Biochemical studies that examine metabolic skills, such as the oxidative and fermentative consumption of carbohydrates like glucose and lactose, help to differentiate further(Shoaib et al., 2021). During sugar fermentation, gas production is monitored, including H_2 S and CO_2 . Additional tests include urease activity, indole synthesis, and the presence of tryptophan deaminase, all of which help to creating a metabolic profile required for bacterial identification(Campus et al.,2021). When analyzed combined, these conventional assays provide a valid method for discriminating between bacterial taxa, especially in clinical and environmental microbiology.

2.7 Molecular Bacterial Identification

2.7.1 In Situ Hybridization (ISH)

In situ hybridization (ISH) is a molecular technique that detects specific nucleic acid sequences, primarily RNA, within intact cells or tissue samples via complementary DNA probes. When these probes are labeled with fluorophores, the process is known as fluorescence in situ hybridization (FISH). Because of the inherent sequence specificity of the genetic code, ISH enables precise taxonomic identification at the genus, species, subspecies, or even strain level, making it especially useful in epidemiological investigations(Veselinyová et al., 2021). The approach works with a variety of cell types, including bacterial, fungal, viral, parasitic, and host cells. Advances in probe synthesis have made bespoke design and labeling more accessible. However, successful application requires on prior knowledge of the target sequence and validation of probe specificity. ISH is a complementing tool that bridges morphological, immunological, and molecular diagnostics, with high sensitivity and specificity, low cost, and compatibility with formalin-fixed, paraffin-embedded samples allowing for retrospective analysis(Monné Rodríguez et al., 2023). Ribosomal targets, such as 16S rRNA, are often utilized in diagnostics because of their high copy number, which increases detection sensitivity.

2.7.2 API (Analytical Profile Index) System

The Analytical Profile Index (API) system is a standardized and efficient biochemical approach. It is commonly used to identify and classify bacteria based on their metabolic activity. The API20E strip is designed to identify Enterobacteriaceae and other Gram-negative rods. It includes 20 miniaturized and dehydrated biochemical

test wells with assays for citrate consumption, Voges-Proskauer reaction, and β-galactosidase activity, among others(Ramatla et al., 2021). These wells are inoculated with a bacterial suspension and incubated, and colorimetric changes are analyzed either manually or using automated systems. The tests are organized into sets of three, with each positive result assigned a number value (1, 2, or 4). The sum of each triplet provides a seven-digit profile number, which is compared to a commercial database to determine the bacterial species(Luca, 2025). An oxidase test, which confirms coliform bacteria, can be used as a 21st test. Initially limited to Enterobacteriaceae, the API system has now expanded to incorporate specialized strips for identifying fastidious and non-enteric organisms, making it more useful in clinical and environmental microbiology.

2.7.3 16S rRNA Gene Sequencing

Bacterial identification is essential for clinical microbiology, environmental surveillance, and microbial ecology. Conventional phenotypic approaches, such as microscopy, culture, biochemical profiling, and serological assays, may be limited by low discriminatory power, subjectivity, and the inability to identify fastidious or unculturable organisms. Molecular approaches, particularly 16S ribosomal RNA (rRNA) gene sequencing, have developed as superior alternatives, delivering high accuracy and greater applicability(Abellan-Schneyder et al., 2021).

All bacteria have the 16S rRNA gene, which has conserved sections that allow for universal primer binding as well as hypervariable areas that allow for taxonomic

differentiation. DNA extraction, gene amplification by PCR, amplicon purification, and Sanger sequencing are all steps in the sequencing process(Kapustina et al., 2021). Taxonomic identification is accomplished by comparing the sequence to reference databases like as SILVA or NCBI, utilizing both similarity and phylogenetic analysis. In a 30-month clinical research conducted by , this method identified 83.1% of clinical isolates to the species level and 15.8% to the genus level, exceeding traditional methods, notably for Gram-positive rods and cocci(Ahmed & Mohammed, 2018).

When many species share substantially similar sequences, such as *Streptococcus, Campylobacter, and Enterobacteriaceae,* 16S rRNA sequencing species-level resolution becomes difficult(Church et al., 2020). According to Mignard et al., 67% of genus-level-only identifications were due to sequence overlap, while the rest were due to poor-quality sequences or a lack of reference sequences in databases.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

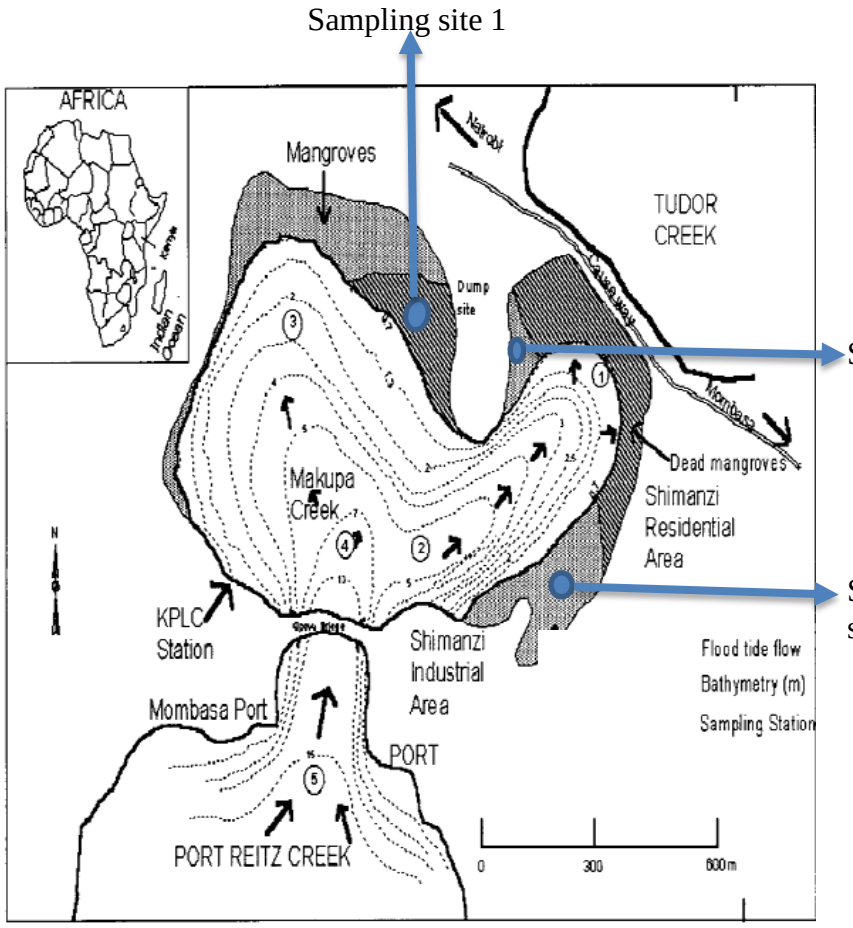


Figure 1.1: Study sites in Makupa Creek, Mombasa, Kenya

Makupa Creek on Mombasa Island, situated on the Kenyan coast at 39°64' E and 4°04' S, served as the study site. According to (Lyons et al., 2018), the site was divided into three blocks, each 10 meters apart, with only minimal modifications. The first block(site), closest to the Kibarani dumpsite, contained the fewest mangroves. The second block (site 2) had a medium population of mangrove plants and was situated

halfway between the shore and the dump site. The most prosperous mangrove plant ecology was found in the third block (site 3), which was nearer the shore.

3.2 Sampling

Sampling was conducted using a stratified random sampling approach, with each block serving as a distinct stratum, as described by (Pagliarella et al., 2018). From each block, four composite sediment samples (10 g each) were randomly collected during low tide. The samples were obtained using a core sampler with an internal diameter of 8 cm to ensure uniformity in sample size and depth. To minimize contamination, all samples were handled aseptically following the procedures outlined by (Douksouna et al., 2019). After collection, samples were placed in sterile, labeled containers and immediately transported to the laboratory for analysis. Additional samples intended for delayed processing were stored at 4°C to preserve their integrity.

3.3 Isolation of bacteria that break down PAHs from mangrove sediments

Polycyclic aromatic hydrocarbon (PAH)-degrading bacteria were isolated from mangrove sediments using an enrichment and dilution plating method. Initially, 5 grams of sediment were suspended in 50 milliliters of sterile seawater and agitated in a rotary shaker for 30 minutes to dislodge microbial cells. A 10^{-1} dilution was prepared by transferring 1 milliliter of the suspension into a tube containing 9 milliliters of sterile seawater, following the method described by (Acer et al., 2021). Subsequent serial dilutions (10^{-2} and 10^{-3}) were prepared in the same manner. From

each dilution, 100 µl aliquots were spread onto seawater nutrient agar (Oxford, UK) and incubated at 37°C for 24 hours.

To screen for PAH-degrading bacteria, a stock solution of anthracene and naphthalene was prepared by dissolving 5 milligrams of each compound in 1 milliliter of hexane. A 0.5 milliliter aliquot of this solution was evenly spread over marine agar plates to create a thin PAH layer and allowed to evaporate briefly. After 24 hours of incubation, transparent colonies were carefully selected and sub-cultured onto fresh marine agar plates supplemented with the PAH stock solution. These plates were incubated at 37°C for an additional 24 hours to confirm PAH degradation activity.

3.4 Screening for PAHs degraders

Twenty-one isolates that showed remarkable growth in PAH-supplemented marine agar were introduced into Luria Bertani (LB) broth (Kobian Scientific, Kenya) and incubated at 37^{o}C for 24 hours. After that, the cell mass was spun in a centrifuge at 4 °C for 10 minutes at 2000 rpm. The supernatant was removed, and the pellets were mixed with 1 milliliter of new LB broth. A 100 µl volume of the suspended cell mass was transferred to 5 ml of minimal salt media (MSM) tubes supplemented with anthracene and naphthalene. The pellets were then incubated at 37°C in a rotary shaker for seven days. The remaining levels of anthracene and naphthalene PAHs were then extracted and quantified.

3.4.1 Extraction of anthracene and naphthalene

After 7 days of shaking, residual PAHs was extracted from 5ml of culture. For extraction, equal volume of dichloromethane was added to the test tubes containing the cultures. The tubes were then vortexed for 10 minutes and left for a few minutes to separate aqueous and organic phases. The upper aqueous layer was removed leaving the organic phase. The organic phase was then left to dry overnight to be used for further analysis.

3.4.2 Quantification of anthracene and naphthalene

Five milliliters of hexane were used to re-suspend the dried PAH residue after extraction. Using UV- spectrophotometry, the absorbance of naphthalene and anthracene in the hexane solution was determined at their respective absorbance maxima.

The residual levels of naphthalene and anthracene were measured using conventional calibration curves. To do this, a range of known concentrations of each PAH in hexane typically between 0.5 and 10 mg/L were prepared. The same spectrophotometric conditions were employed to determine the absorbance of each standard solution, and the results were utilized to create linear regression equations.

To create standard calibration curves, the absorbance values (y) were plotted against the known amounts (x) using linear regression analysis. With an R2 value of 0.99, the regression equations that were obtained showed a significant linear correlation: y =

0.0043x + 0.129 for anthracene and y = 0.0032x + 0.1051 for naphthalene. By entering the sample absorbance values into the corresponding regression calculations, these equations were then utilized to determine the concentration of residual PAHs in the experimental samples.

3.5 Characterization of the isolates by biochemical methods

3.5.1 Catalase test

Bacterial isolates were initially scattered onto tryptic soy agar (TSA) plates (Kenya - ERWEKA GmbH) and incubated for 24 hours at 37°C in order to measure catalase activity (Elly Kipchumba BVM, 2022). A small amount of the bacterial growth was moved to a sterile glass slide following incubation. Following that, the bacterial smear was treated with two drops of 3% hydrogen peroxide (H_2O_2). A positive catalase reaction was demonstrated by the bubbling, which indicated that the isolate generated the catalase enzyme that could convert hydrogen peroxide into oxygen gas and water. On the other hand, the lack of bubble formation indicated a negative reaction, meaning that there was no catalase activity in the isolate. It is important to note that the test was performed using TSA rather than blood agar to avoid false positives caused by catalase present in red blood cells.

3.5.2 Oxidase test

Cytochrome oxidase production was measured using oxidase discs containing tetramethyl-p-phenylenediamine dihydrochloride. After applying a sterile toothpick to

the discs, each isolate was selected and allowed to sit for fifteen minutes. A positive test was shown by blue or purple coloring, while no coloration indicated a negative test (Lee et al., 2024).

3.5.3 Starch hydrolysis test

To assess the amylase activity, the isolates were streaked on TSA plates enriched with soluble starch and incubated for 24 hours at 37 °C. Next, drops of the iodine solution were applied to the plates' surface. The excess iodine was then discarded. Blue-black staining of the medium denoted a negative result for amylase activity, but clear zones surrounding the bacterial growth line indicated a positive result.

3.6 Characterisation of isolates by Molecular techniques

3.6.1 Extraction of genomic DNA

Pure bacterial isolates were cultured in 20 ml of freshly prepared nutrient broth and incubated for 24 hours at 30°C in a shaker incubator at 200 rpm. After incubation, 1.5 ml of each culture was transferred to sterile Eppendorf tubes and centrifuged at 13,000 rpm for 5 minutes to pellet the bacterial cells. The supernatant was discarded, and the pellet was resuspended in 200 µl of lysis buffer (Solution 1), consisting of 50 mM Tris-HCl, 50 mM EDTA, and 25% sucrose.

To enzymatically weaken the bacterial cell wall, 5 µl of lysozyme (20 mg/ml) was added, and the mixture was gently mixed and incubated at 37°C. Lysozyme specifically hydrolyzes the β-1,4-glycosidic bonds in the peptidoglycan layer, making

it particularly effective for lysing Gram-positive bacteria. To eliminate RNA contaminants, 5 µl of RNase A (20 mg/ml) was subsequently added, and the suspension was incubated at 37°C for one hour.

Following enzymatic treatment, 600 µl of Solution 2 (10 mM Tris-HCl, pH 8.5; 5 mM EDTA, pH 8.0; and 1% SDS) was added to disrupt the cytoplasmic membrane and solubilize cellular components. SDS is an anionic detergent that facilitates cell lysis by dissolving membrane lipids and denaturing proteins. To further degrade residual proteins, including nucleases and histones, 10 µl of proteinase K (20 mg/mL) was added, and the mixture was incubated at 50°C for 30 minutes.

Cell lysis was achieved through a combination of enzymatic (lysozyme, RNase A, and proteinase K) and chemical (EDTA and SDS) treatments, enabling the effective release of high-molecular-weight genomic DNA from bacterial cells. The lysate was centrifuged at 13,000 rpm for 20 minutes to pellet cellular debris. The supernatant containing the DNA was subjected to extraction with an equal volume of phenol: chloroform to remove residual proteins and lipids. DNA was precipitated from the aqueous phase by adding 0.1 volumes of 3 M sodium chloride (NaCl) and an equal volume of cold 100% ethanol. The mixture was incubated overnight at –20°C to enhance DNA precipitation. The following day, the DNA was pelleted by centrifugation, washed with 70% ethanol to remove salts, air-dried at room temperature, and finally resuspended in 200 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The purified DNA was stored at –20°C until further use (Lehto, 2021).

3.6.2 16S rRNA PCR amplification

Using universal primers 27F (5′-AGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GTTACCTTGTTACGACTT-3′), which target conserved sections of the 16S rRNA gene were acquired from Inqaba Biotec. The bacterial isolates were identified by amplification of the 16S rRNA gene(Sharma et al., 2021).

A total reaction volume of 50 µl was used for the PCR amplification. The combination included 5 µl of 1× PCR buffer (75 mM Tris-HCl, pH 8.8 at 25°C, 20 mM $(NH_4)_2SO_4$, and 0.01% Tween 20), 5 µL of 2.5 mM dNTPs, 5 µL of 10 µM of each forward and reverse primer, 5 µl of 25 mM $MgCl_2$, and 5 µl of template DNA with roughly 50 ng of genomic material. The final volume was adjusted with nuclease-free water.

Amplification was carried out in a thermal cycler (MRC, model unidentified) with the following cycling parameters: five minutes of initial denaturation at 94°C, thirty cycles of denaturation at 94°C for 30 seconds, one minute of annealing at 58°C, and one minute of extension at 68°C. The last extension phase was performed for 10 minutes at 68°C to finish the amplification.

3.6.3 Gel electrophoresis of PCR products

According to (Arunachalam & Sasidharan,(2021), 2 grams of agarose gel were boiled in 100 milliliters of 1x TBE buffer to separate the samples by gel electrophoresis. The solution was supplemented with 1µl of 1000x gel red nucleic acid dye and mixed

thoroughly. After a short cooling period, the mixture was placed onto gel casting trays. Following the gel set, 1µl of 6x loading gel and 5µl of PCR products were added to the gel's wells. The size of the pieces was ascertained by running the 100bp+DNA ladder. Following a 45-minute electrophoresis at 70 volts using MRC scientific equipment, the samples were visualized using a UVP photo DOC imaging system.

3.6.4 Sequencing and Phylogenetic Analysis

Sequencing was performed by a commercial service provider (inqaba biotec,n.d) using dye terminator sequencing technique(Sager et al,1977).The dye terminator Sanger sequencing employs fluorescently labeled dideoxynucleotides (ddNTPs) in a cycle sequencing procedure. During sequencing, each ddNTP is randomly integrated into freshly produced DNA strands, thus terminating synthesis. These terminated fragments were then separated using capillary electrophoresis, and the emitted fluorescence was measured and converted into nucleotide sequence data using automated DNA sequencers (e.g., ABI 3500 Genetic Analyzer).

The generated chromatograms were visually reviewed and adjusted with Chromas version 2.6.6, and any low-quality base calls were eliminated. The forward and reverse sequences were aligned and assembled into consensus sequences with BioEdit version7.2.5.

3.7 Data analysis

Data collected on degradation of naphthalene and anthracene on the three sites was analyzed by Minitab 19 software using one-way ANOVA. A search for similarities was carried out by aligning the partial sequences of 16S rRNA genes from different bacterial strains with the 16S rRNA sequences that were available in the public nucleotide database of the National Centre for Biotechnology (NCBI) through its worldwide website. The search also used the BLAST and seqmatch algorithms developed by the Ribosomal Database Project (RDP). The 16S rRNA sequences of the bacterial strains were then aligned using the ClustaI W 1.6 algorithm (Zhang *et a*l., 2024). A maximum likelihood approach based on the Tamura-Nei technique was used to determine the evolutionary history (Pazos *et al*., 2010). The MEGA 6 program was used to perform the evolutionary analyses. The *Trichoderma sp*. gene sequence (HQ630962.1) was used as a negative control group. The NCBI libraries for the 16S rRNA gene were then used to create a phylogenetic tree for the isolates that biodegrade PAHs.

CHAPTER FOUR

RESULTS

4.1 Isolation and screening of PAHs degrading bacteria

A total of 44 bacterial isolates were obtained from 12 mangrove sediment samples taken from three (3) different sites along Makupa Creek in Mombasa County. The isolates were first tested for their capacity to use polycyclic aromatic hydrocarbons (PAHs) as the only carbon source on marine agar enriched with anthracene and naphthalene. Following incubation, 21 isolates showed viability and observable growth in the PAH-enriched medium, showing hydrocarbon-degrading capacity. As shown in Table 4.1, seven isolates came from Site 1, two from Site 2, and the remaining twelve from Site 3. These findings suggest that Site 3 may have a greater diversity or abundance of PAH-degrading bacteria than the other examined locations.

4.2 Degradation of polycyclic aromatic hydrocarbons by bacterial isolates found in mangrove sediments

4.2.1 Anthracene degradation

The 21 viable isolates were tested for their ability to utilize PAH anthracene as their only carbon source. After 7 days of incubation on a minimal salt media supplemented with PAH, the S3D05 isolate degraded anthracene at 93.84%, while S1C01 degraded it at 99.54%. These differences were not statistically significant ($p > 0.05$). Table 4.2 documents the specifics of the isolates' anthracene breakdown.The one-way ANOVA test comparing the anthracene degradation abilities of all isolates resulted in a p-value

of approximately 2.98×10^{-10}. This very small p-value indicates that there is a statistically significant difference in degradation abilities among the bacterial isolates.

Table 4.1: Growth of Makupa Creek sediment isolates on marine agar supplemented with naphthalene and anthracene

Isolate	Sampling site	Activity	Isolate	Sampling site	Activity	Isolate	Sampling site	Activity
S1A 01	MC site 1	+++	S2A 03	MC site 2	-	S3A 08	MC site 3	-
S1A 02	MC site 1	-	S2A 04	MC site 2	+++	S3A 09	MC site 3	+++
S1A 03	MC site 1	+++	S2C 01	MC site 2	-	S3A 10	MC site 3	+++
S1B 01	MC site 1	-	S2C 02	MC site 2	-	S3B 01	MC site 3	+++
S1B 02	MC site 1	-	S2C 03	MC site 2	-	S3B 02	MC site 3	+++
S1B 03	MC site 1	+++	S2D01	MC site 2	-	S3B 03	MC site 3	+++
S1C 01	MC site 1	+++	S2D02	MC site 2	-	S3C 01	MC site 3	-
S1C 02	MC site 1	+++	S2D03	MC site 2	-	S3C 02	MC site 3	+++
S1C 03	MC site 1	+++	S3A 01	MC site 3	+++	S3C 03	MC site 3	-
S1D 01	MC site 1	+++	S3A 02	MC site 3	-	S3D 01	MC site 3	-
S1D 02	MC site 1	-	S3A 03	MC site 3	-	S3D 02	MC site 3	+++
S1D 03	MC site 1	-	S3A 04	MC site 3	-	S3D 03	MC site 3	+++
S1D 04	MC site 1	-	S3A 05	MC site 3	+++	S3D 04	MC site 3	-
S2A 01	MC site 2	+++	S3A 06	MC site 3	+++	S3D 05	MC site 3	+++
S2A 02	MC site 2	-	S3A 07	MC site 3	-			

MC = Makupa creek ; +++ = excellent growth ; - = no growth

Table 4.2: Percentage of degraded anthracene by bacterial isolates from Makupa Creek.

Isolates	Sampling site	Percentage degradation	Isolates	Sampling site	Percentage degradation
S1A01	MC1	98.60±1.16[a]	S3A06	MC3	96.98±0.13[a]
S1A03	MC1	98.95±0.35[a]	S3A09	MC3	99.30±0.26[a]
S1C01	MC1	99.54±0.00[a]	S3A10	MC3	97.21±0.33[a]
S1C02	MC1	98.95±0.58[a]	S3B01	MC3	96.75±0.23[a]
S1C03	MC1	98.02±0.12[a]	S3B02	MC3	94.53±1.51[a]
S1D01	MC1	96.98±0.23[a]	S3B03	MC3	98.14±0.23[a]
SIB03	MC1	98.84±0.47[a]	S3C02	MC3	99.07±0.24[a]
S2A01	MC2	95.12±2.09[a]	S3D02	MC3	98.72±1.05[a]
S2A04	MC2	98.26±1.28[a]	S3D03	MC3	94.42±0.23[a]
S3A01	MC3	98.26±1.05[a]	S3D05	MC3	93.84±1.98[a]
S3A05	MC3	94.77±1.98[a]			

The superscript "a" (e.g., 99.54 ± 0.00[a]) typically implies statistical grouping—meaning that all values with the same letter "a" are not statistically distinct from one another at a given confidence level (commonly $p > 0.05$), based on post-hoc tests such as Tukey's HSD.

4.2.2 Naphthalene degradation

The S1C03 isolate showed the lowest naphthalene degradation rate (79.09%) when tested for the ability to use naphthalene as their only carbon source. At 99.39%, the isolates S1A01 and S2A04 had the highest. Compared to the 17 other isolates, the rates at which S1C03 (79.09%) and S2A01 (88.47%) degraded naphthalene were significantly lower ($p < 0.05$). Details of the isolates' breakdown of naphthalene are given in Table 4.3.

Table 4.3: Percentage of degraded naphthalene by bacterial isolates from Makupa Creek.

Isolates	Sampling site	Percentage degradation	Isolates	Sampling site	Percentage degradation
S1A01	MC1	99.39±0.33[a]	S3A06	MC3	96.59±3.13[a]
S1A03	MC1	90.35± 0.63[ab]	S3A09	MC3	98.00±0.78[a]
S1C01	MC1	91.13±0.16[ab]	S3A10	MC3	95.03±2.19[a]
S1C02	MC1	97.05±2.02[a]	S3B01	MC3	91.59±0.31[a]
S1C03	MC1	79.09±8.12[b]	S3B02	MC3	98.78±0.00[a]
S1D01	MC1	97.83±0.96[a]	S3B03	MC3	95.19±0.16[a]
SIB03	MC1	96.25±0.00[a]	S3C02	MC3	95.50±0.47[a]
S2A01	MC2	88.47±1.25[b]	S3D02	MC3	96.59±3.13[a]
S2A04	MC2	99.39±0.33[a]	S3D03	MC3	96.25±0.00[a]
S3A01	MC3	98.78±0.00[a]	S3D05	MC3	96.58±2.48[a]
S3A05	MC3	96.59±3.13[a]			

MC = Makupa creek. Values with the same superscript indicate insignificant difference between each other at $p = 0.05$.

4.3 Biochemical characteristics

Biochemical assays for catalase, oxidase, and amylase activities were conducted on all bacterial isolates to assess their metabolic characteristics and potential involvement in PAH degradation. These tests provided insight into the enzymatic capabilities of the isolates, which may relate to their ability to metabolize structurally complex compounds such as polycyclic aromatic hydrocarbons (PAHs). The results are summarized in Table 4.4 and illustrated in Figure 4.1

Catalase activity was evaluated by adding hydrogen peroxide to bacterial cultures and observing the formation of oxygen bubbles, which indicates the enzymatic breakdown of hydrogen peroxide into water and oxygen. All 21 isolates tested positive for catalase, as evidenced by immediate bubble formation, confirming the presence of the catalase enzyme.

Oxidase activity was assessed using oxidase reagent, which detects the presence of cytochrome C oxidase, an enzyme involved in the electron transport chain. A positive result is indicated by the appearance of a dark blue or purple coloration. Nineteen isolates showed a color change, indicating positive oxidase activity. Two isolates, S1C01 and S3C02, exhibited no color change and were classified as oxidase negative.

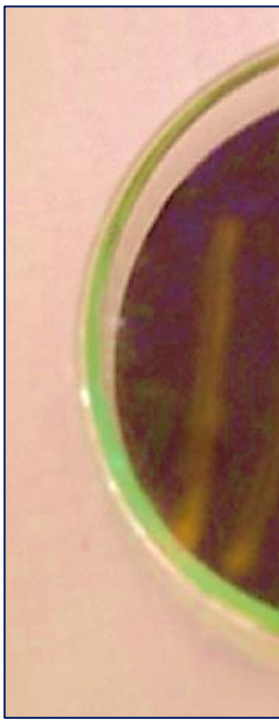
Amylase activity was determined using the starch hydrolysis test on tryptic soy agar (TSA) plates. After incubation, the plates were flooded with iodine solution, and the presence of a clear halo around colonies against a dark blue background indicated starch degradation by amylase. Of the 21 isolates, 20

demonstrated positive amylase activity, while isolate S1D01 was negative, showing no clear zone formation.

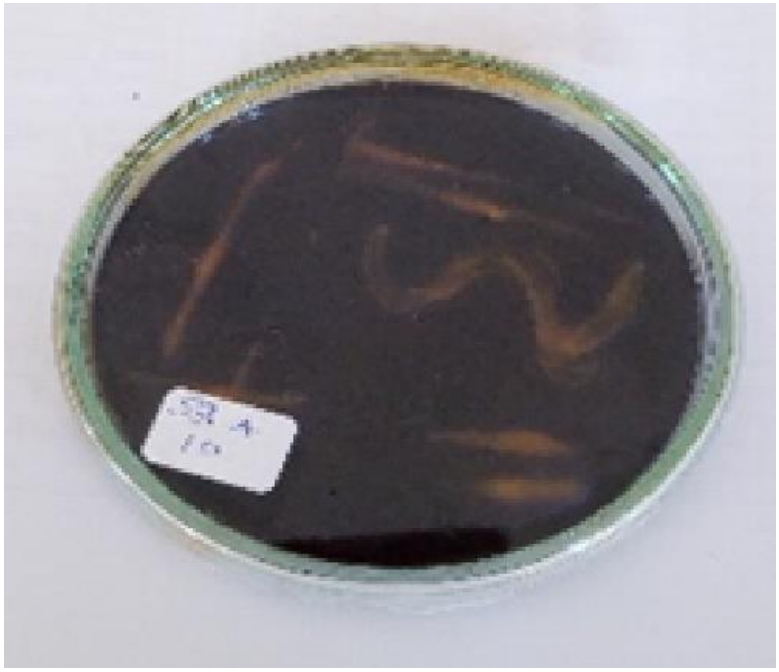
These biochemical profiles not only characterize the metabolic capabilities of the isolates but also suggest potential functional roles in the breakdown of complex organic compounds such as PAHs.

Table 4.4: Biochemical characteristics of PAHs degrading isolates.

Isolate	Catalase	Oxidase	Amylase
S1 A 01	+	+	+
SIA 03	+	+	+
S1 B 03	+	+	+
S1 C 01	+	-	+
S1 C 02	+	+	+
S1 C 03	+	+	+
S1 D 01	+	+	-
S2 A 01	+	+	+
S2 A 04	+	+	+
S3 A 01	+	+	+
S3 A 05	+	+	+
S3 A 06	+	+	+
S3 A 09	+	+	+
S3 A 10	+	+	+
S3 B 01	+	+	+
S3 B 02	+	+	+
S3 B 03	+	+	+
S3 C 02	+	_	+
S3 D 02	+	+	+
S3 D 03	+	+	+
S3 D O5	+	+	+



a)



b)

Figure 4.1: Positive amylase test of isolates (a) and (b)

4.4 Molecular characterization

4.4.1 PCR amplification of 16S rRNA gene from the isolates

Genomic DNA was extracted from each bacterial isolate, and the 16S rRNA gene was subsequently amplified using universal bacterial primers 27F and 1492R, yielding an amplicon approximately 1500 base pairs in length. The DNA yield was low with S3B01, S2A01 A and S1A01 isolates but was higher with other isolates (Figure 4.3)

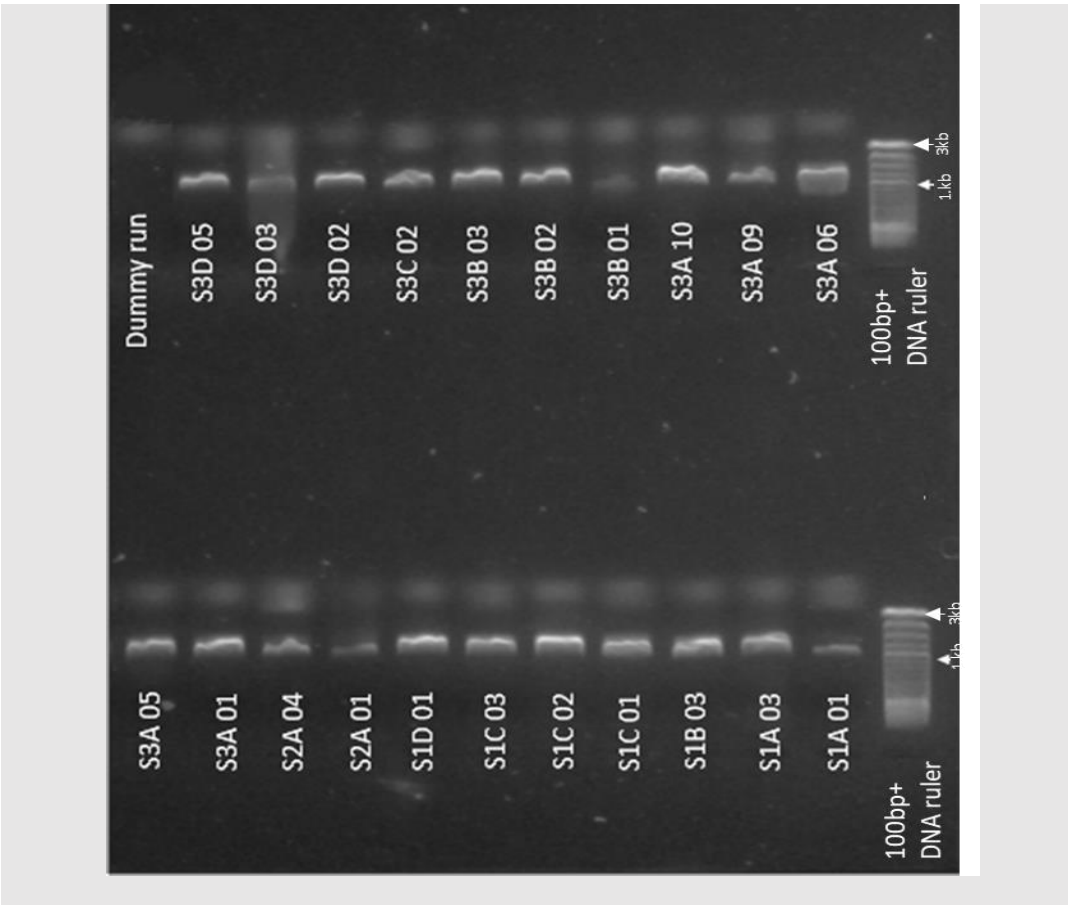


Figure 4.2: UVP photo DOC imaging of PCR Gel Electrophoresis

4.4.2 Sequencing and Phylogenetic Analysis

Out of the 21 bacterial isolates that were characterized, 20 were sequenced. The sequencing of isolate S3D03 was unsuccessful due to technical issues. Blast searches of DNA databases indicated that the 20 isolates had a sequence similarity index of 81.23% - 100% with those of another known taxon (Table 4.5).

Molecular phylogenetic analysis was done using *Trichoderma* species as the out-group, and the dendrogram tree showed that the 20 isolates were clustered into six groups (Figure 4.5). Cluster I had 13 isolates, while Clusters III (S3A06; S1A01) and VI (S1C01; S3B02) had two isolates each. The rest of the clusters had one isolate that was distributed as follows: Cluster II (S3A05), Cluster IV (S3A10), Cluster V (S1B03)

The tree also revealed that the 20 PAHs degrading isolates were phylogenetically related to bacterial from five genera, namely *Pseudomonas*, *Bacillus*, *Enterobacter*, *Exiguobacterium* and *Ralstonia* (Figure 4.3).

Thirteen out of the 20 isolates were clustered together with 11 *Pseudomonas* strains, and the sequence similarity index between them ranged from 83.41% to 100% (Figure 4.3: Table 4.5)

The study also showed that three isolates (S3A06, S1A01, S1B03) were clustered together with five strains of *Bacillus*, and the sequence similarity homology between them ranged from 81.33% and 92.55%. Isolate S3A05 was closely related to *Enterobacter hormaechei* (93.33%) and *Enterobacter cloacae* (93.22%) species. Meanwhile, isolate S3A10 was closely related to two

Exiguaobacterium strains (89%). Isolate S1C01 and S3B02 were closely related to *Ralstonia* strains (85%)

It is important to note that ten isolates (S3A05, S3A06, S3A10, S3B02, S3B03, S1A01, S1A03, S1B03, S1C01, and SIC03) had a gene sequence similarity value of less than 97% with other existing species therefore indicating that they are novel strains (Table 4.5).

Table 4.5: Blast searches of DNA databases showing shared sequence

Isolate	Next neighbour	% ID	Accession number
S1C-02	*Pseudomonas guguanenis strain*	99.44	MK544130.1
	Pseudomonas sp. strainR11	99.44	MH773373.1
S2A-01	*Pseudomonas pseudoalcaligenes strain IN83*	98.86	KY511069.1
S2A-04	*Pseudomonas stutzeri strain M4*	97.23	MH665736.1
	Pseudomonas mendocina strain SCAU-025	97.23	MF155917.1
S3A-01	*Pseudomonas sp. strainA110*	100	MH773199.1
S3A-05	*Enterobacter hormaechei strain IAE47*	93.33	MK414992.1
	Enterobacter cloacae strain IAE2004	93.22	KY285198.1
S3A-06	*Bacillus cereus strain 20.SH.1*	92.55	MG776355.1
S3A-09	*Pseudomonas guguanenis strain FX03*	99.79	KX585259.1
S3A-10	*Exiguobacterium sp.strain 201709CJKYOP17*	88.89	MH093774.1
	Exiguobacterium sp.strain 201709CJKYOP16	88.9	MH093773.1
S3B-01	*Pseudomonas guguanenis strain VKA3*	99	MK544130.1
S3B-02	*Ralstonia mannitolilytica strain TS 28*	90.18	MN022579.1
	Ralstonia mannitolilytica strain TS 30	90.18	MN022574.1
S3B-03	*Bacillus firmus strain XJSL1-3*	89.54	GQ903382.1
	Bacillus oceanisediminis strain XJSL1-3	89.46	MF163139.1
S3C-02	*Pseudomonas pseudoalcaligenes strain NRSS3*	99	MF992192.1
S3D-02	*Pseudomonas mendocina strain SCAU-025*	97.19	MN096694.1
S3D-05	*Pseudomonas pseudoalcaligenes strain NRSS3*	99.31	MF9921992.1
SIDI-01	*Pseudomonas guguanensisstrain VKA3*	98.73	MK544130.1
	Pseudomonas sp.strain A211	98.73	MH773242.2
S1A-01	*Bacillus sp. Strain BP-Nof-AY*	81.23	KX644098.1
S1A-03	*Pseudomonas mendocina strain Y20*	94.64	MH997639.1
SIB-03	*Bacillus aquimaris strain AHR4-1*	93	KF551974.1
SIC-01	*Ralstonia pickettii strain LB95*	84.93	MT394008.1
	Ralstonia mannitolilytica strain MB98	84.93	MT377813.1
SIC-03	*Pseudomonas plecoglossicida strain MP6-0205*	83.41	MH174322.1

homology between the isolates and known taxon

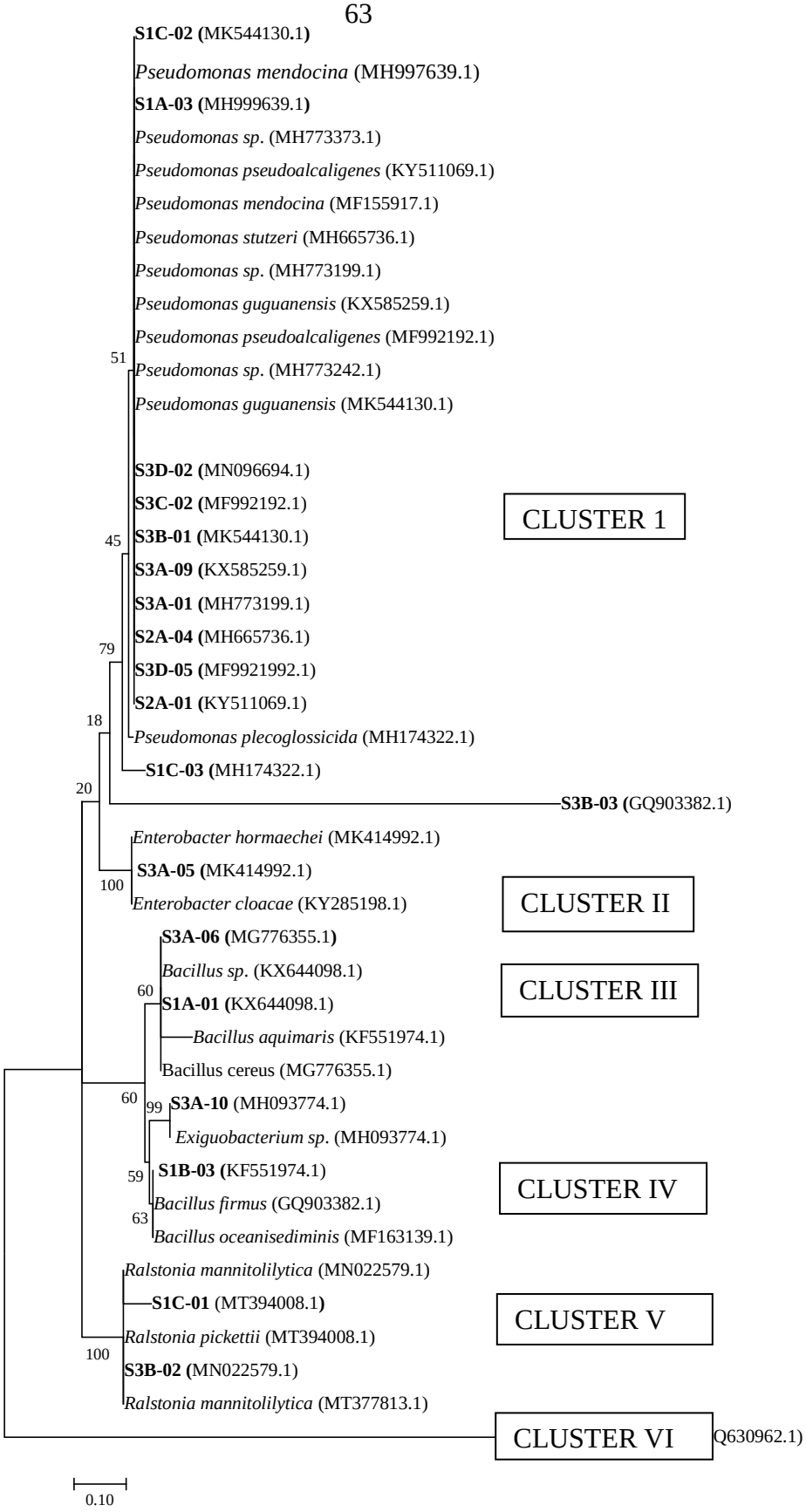

S1C-02 (MK544130.1)
Pseudomonas mendocina (MH997639.1)
S1A-03 (MH999639.1)
Pseudomonas sp. (MH773373.1)
Pseudomonas pseudoalcaligenes (KY511069.1)
Pseudomonas mendocina (MF155917.1)
Pseudomonas stutzeri (MH665736.1)
Pseudomonas sp. (MH773199.1)
Pseudomonas guguanensis (KX585259.1)
Pseudomonas pseudoalcaligenes (MF992192.1)
Pseudomonas sp. (MH773242.1)
Pseudomonas guguanensis (MK544130.1)
S3D-02 (MN096694.1)
S3C-02 (MF992192.1)
S3B-01 (MK544130.1)
S3A-09 (KX585259.1)
S3A-01 (MH773199.1)
S2A-04 (MH665736.1)
S3D-05 (MF9921992.1)
S2A-01 (KY511069.1)
Pseudomonas plecoglossicida (MH174322.1)
S1C-03 (MH174322.1)
S3B-03 (GQ903382.1)
Enterobacter hormaechei (MK414992.1)
S3A-05 (MK414992.1)
Enterobacter cloacae (KY285198.1)
S3A-06 (MG776355.1)
Bacillus sp. (KX644098.1)
S1A-01 (KX644098.1)
Bacillus aquimaris (KF551974.1)
Bacillus cereus (MG776355.1)
S3A-10 (MH093774.1)
Exiguobacterium sp. (MH093774.1)
S1B-03 (KF551974.1)
Bacillus firmus (GQ903382.1)
Bacillus oceanisediminis (MF163139.1)
Ralstonia mannitolilytica (MN022579.1)
S1C-01 (MT394008.1)
Ralstonia pickettii (MT394008.1)
S3B-02 (MN022579.1)
Ralstonia mannitolilytica (MT377813.1)
CLUSTER 1
CLUSTER II
CLUSTER III
CLUSTER IV
CLUSTER V
CLUSTER VI
Q630962.1)
51
45
79
18
20
100
60
60
99
59
63
100
0.10

Figure 4.3: Evolutionary relationships of taxa

A Maximum Likelihood approach based on the Tamura-Nei technique was used to estimate the evolutionary history (Tamura et al., 2011). The branch length measure is the number of substitutions per site, and the tree is displayed at an exact scale. 32 distinct nucleotide sequences were employed in this investigation. MEGA 6 was used to produce evolutionary study results expressed in base substitutions per site. *Trichoderma sp*. gene sequence HQ630962.1 was employed as a negative control in this experiment.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, RECOMENDATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

5.1 Discussion

The choice of mangrove environments, particularly Makupa Creek in Mombasa, Kenya, for the isolation of polycyclic aromatic hydrocarbon (PAH)-degrading bacteria is ecologically and scientifically rational. Mangrove environments are distinguished by their unique physicochemical characteristics, such as changing salinity, tidal influence, and high organic matter concentration, which promote the formation of varied and metabolically adaptable microbial communities(Palit *et al.,* 2022). Mangroves are good habitats for microbial bioremediation investigations, particularly those targeting resistant contaminants such as PAHs. Additionally, the limited structure of Makupa Creek promotes PAH retention in sediments. This extended exposure exerts a selective pressure on microbial populations that can use PAHs as carbon and energy sources(Premnath *et al.,* 2021). As a result, microbial consortia in this habitat are more likely to house specialized PAH-degrading bacteria than less contaminated or more dynamic aquatic systems.

Twenty-one isolates of PAH-degrading bacteria were found in Makupa Creek's mangrove sediments. The isolates' anthracene degradation efficiency ranged from 93.8% to 99.5%, which is different from previous studies that showed an

anthracene degradation efficiency of 24% to 83%. (Bibi *et al.,* 2018; Clement Akinola, 2022). It is in line with the results of (Salam et al., 2015), however, who achieved 90% and 95%, respectively. Similar to the 79% to 99% degradation rates displayed above, Mortazavi Mehrizi *et al*.,(2022) and Rabani *et al*., (2020) reported 75% to 96% degradation rates for naphthalene.

Several factors influence Anthracene and Naphthalene degradation in bacteria. The first is the bacterial factors, including microbial consortia, metabolic potential, population density, biosurfactant production, high pollution factor in mangrove habitant and microbial competition (Mohapatra & Phale, 2021). The others are environmental factors, including temperature, pH, aeration, light intensity and availability of some nutrients in culture (Hazaimeh *et al.,* 2021). Makupa Creek has a high annual average temperature of 27.9℃. Therefore, the high anthracene and naphthalene degradation rates reported in this study could be attributed to the study area's high temperatures and the type of microbial consortia isolated. Furthermore, because of their inherent physical and chemical features, mangrove inhabitants have an exceptional ability to accumulate toxins, making them extremely polluted and containing bacteria capable of decomposing PAHs(Billah *et a*l., 2022). This makes Makupa Creek's mangrove habitat a perfect location for PAH microbial breakdown. This may explain the remarkable breakdown efficiency of anthracene and naphthalene by bacteria isolated from Makupa Creek.

Biochemical characterization revealed that all isolates obtained from Makupa Creek were catalase-positive, while 95% and 90% were positive for amylase

and oxidase, respectively. According to studies, organisms with high catalase activity have a greater capacity for antioxidants, which leads to the effective breakdown of polyaromatic hydrocarbons, (Na *et al*., 2020). The current investigation found a positive correlation between catalase and the breakdown of naphthalene and anthracene hydrocarbons. Recently, scientists have focused on the amylase enzyme's ability to break down PAHs across various pH and temperature conditions (Na *et al*., 2020). This suggests that amylase-positive isolates in various environments may break down hydrocarbons. Additionally, the isolates generated cytochrome oxidase, an enzyme that stimulates microbial growth and metabolic capacities to facilitate the biodegradation of hydrocarbons.

The 16S rRNA was the target gene for the isolates' molecular characterization. Due to its excellent conservation and presence in all bacterial species, this gene is the most widely used genetic marker for bacterial categorization. The samples' conventional PCR amplification produced amplicons of roughly 1500 bp, the anticipated size of 16S rRNA (Kai *et al.,* 2019.). Because 16S rRNA markers are conservative, they are essential for determining the evolutionary and phylogenetic relationships between different biological species, including bacteria that break down PAHs. This helps to clarify the evolution and catabolic capacity of these bacteria (Sakdapetsiri *et al*., 2021).

According to blast searches of DNA databases, the isolates shared a sequence similarity of 81% to 100% with an established taxon. According to the dendrogram tree analysis, the isolates were phylogenetically related to bacteria

from five genera: *Pseudomonas, Bacillus, Ralstonia, Enterobacter, and Exiguobacterium.*

According to Li *et al*., (2020), 60% of the isolates were closely related to *Pseudomonas spp*., making them the most common PAH degraders due to their ability to use them as the sole carbon source. The efficiency of *Pseudomonas* in degradation of PAH is due to the enzyme naphthalene dioxygenase which is known to break down naphthalene, phenanthrene and anthracene, Acenaphthene and fluorene hydrocarbons(Sivasamy et al., 2024). According to recent research, *P. fluorescens, P. putida, P. aeronosa, P. veronii, and P. gessardii species* dominate the breakdown of polyaromatic hydrocarbons. *Pseudomonas veronii* is unique because it can break down naphthalene fully and change the four-ring hydrocarbons (Mullaeva *et al*., 2022).

Four of the isolates in this study are linked to *Bacillus species,* which are known to use the enzymes dioxygenase, hydrogenase, and monooxygenase to break down naphthalene and phenanthrene hydrocarbons. Two *Ralstonia* and one isolate related to *Enterobacter* were also discovered in this investigation. The two species can also break down polycyclic aromatic hydrocarbons, according to earlier findings. (Li *et al*., 2020).

Isolate S3A10 is closely related to the *Exiguobacterium* family, which plays a significant role in the degradation of PAHs in extremely saline environments. According to Sakdapetsiri *et al*.,(2021), PAH elimination peaked at 7.0 and was maintained in alkaline circumstances (pH = 8.0–9.0), while

Exiguobacterium exhibited the highest degradation at a salinity of 15 g/L. Because of this finding, they can generally be used in alkaline seawater.

The evidence supporting the viability of employing bacteria to biodegrade PAH chemicals which pose a health risk to the general public has increased with the shown breakdown of anthracene and naphthalene. Finding new strains of bacteria that can degrade PAHs is a step in the right direction, increasing the number of microorganisms that can be used to create antipollution products.

5.2 Conclusions

i. The mangrove sediments in Makupa Creek, Mombasa County, contain many bacteria that effectively degrade polycyclic aromatic hydrocarbons (PAHs), specifically naphthalene and anthracene. This efficient degradation is likely due to the area's unique microflora and environmental conditions.
ii. Biochemical analysis revealed that the bacteria had catalase, amylase, and oxidase activities, which enhance their ability to degrade PAHs by boosting microbial growth and metabolic functions.
iii. Molecular and phylogenetic analysis of 16S rRNA identified that half of the bacterial isolates were related to known hydrocarbon-degrading genera like *Pseudomonas, Bacillus, Ralstonia, Enterobacter*, and *Exiguobacteria*, with *Pseudomonas* being particularly notable for its role in PAHs degradation. Additionally, unclassified isolates indicate potential novel bacterial strains that warrant further study.

5.3 Recommendations

i. The bacterial consortia isolated from the mangrove sediments of Makupa Creek should be further explored and developed as potential bioremediation agents for the degradation of hydrocarbons in contaminated environments.

ii. The ten novel isolates identified in this study require further characterization and accurate taxonomic identification through advanced molecular and biochemical techniques.

5.4 Suggestion for future research

i. Functional genomics be employed to understand the mechanism of action that the isolates employ to degrade the anthracene and naphthalene hydrocarbons.

ii. Whole genome sequencing be used to identify the distinct microbial consortia isolated for this investigation.

REFERENCES

Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*(1), 107–123. https://doi.org/10.1016/J.EJPE.2015.03.011

Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M., & Neuhaus, K. (2021). Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. *MSphere*, *6*(1). https://doi.org/10.1128/msphere.01202-20

Abou Khalil, C., Prince, V. L., Prince, R. C., Greer, C. W., Lee, K., Zhang, B., & Boufadel, M. C. (2021). Occurrence and biodegradation of hydrocarbons at high salinities. *Science of The Total Environment*, *762*, 143165. https://doi.org/10.1016/J.SCITOTENV.2020.143165

Acer, Ö., Johnston, G. P., Lineman, D., & Johnston, C. G. (2021). Evaluating degradation of polycyclic aromatic hydrocarbon (PAH) potential by indigenous bacteria isolated from highly contaminated riverbank sediments. *Environmental Earth Sciences*, *80*(23), 1–15. https://doi.org/10.1007/S12665-021-10070-5/METRICS

Agathokleous, E., Barceló, D., & Calabrese, E. J. (2021). US EPA: Is there room to open a new window for evaluating potential sub-threshold effects and ecological risks? *Environmental Pollution*, *284*, 117372. https://doi.org/10.1016/J.ENVPOL.2021.117372

Ahmed, M., & Mohammed, M. (n.d.). *Superiority of partial 16s rRNA gene sequencing for identification of bacterial strains isolated from meat products*.

Alam, K., Islam, M. M., Li, C., Sultana, S., Zhong, L., Shen, Q., Yu, G., Hao, J., Zhang, Y., Li, R., & Li, A. (2021). Genome mining of pseudomonas species: Diversity and evolution of metabolic and biosynthetic potential. *Molecules*, *26*(24). https://doi.org/10.3390/molecules26247524

Al-Hawash, A. B. (2018). Fungal Degradation of Polycyclic Aromatic Hydrocarbons. *International Journal of Pure & Applied Bioscience*, *6*(2), 8–24. https://doi.org/10.18782/2320-7051.6302

Ali, M. U., Siyi, L., Yousaf, B., Abbas, Q., Hameed, R., Zheng, C., Kuang, X., & Wong, M. H. (2021). Emission sources and full spectrum of health impacts of black carbon associated polycyclic aromatic hydrocarbons (PAHs) in urban environment: A review. In *Critical Reviews in Environmental Science and Technology* (Vol. 51, Issue 9, pp. 857–896). Bellwether Publishing, Ltd. https://doi.org/10.1080/10643389.2020.1738854

Altarawneh, M., & Ali, L. (2024). Formation of Polycyclic Aromatic Hydrocarbons (PAHs) in Thermal Systems: A Comprehensive Mechanistic Review. In *Energy and Fuels*. American Chemical Society. https://doi.org/10.1021/acs.energyfuels.4c03513

Amadi, I. E., Chukwura, E. I., Umeoduagu, N. D., & Anene. (2024). Isolation and Characterization of Multidrug resistant Enterococcus faecium isolated from sputum specimen. *International Journal of Advanced Research in Biological Sciences*, *11*(9), 56–67. https://doi.org/10.22192/ijarbs

Aparicio, J. D., Raimondo, E. E., Saez, J. M., Costa-Gutierrez, S. B., Álvarez, A., Benimeli, C. S., & Polti, M. A. (2022). The current approach to soil remediation: A review of physicochemical and biological technologies, and the potential of their strategic combination. *Journal of Environmental Chemical Engineering*, *10*(2), 107141. https://doi.org/10.1016/J.JECE.2022.107141

Arora, P. K., Kaestner, M. E., Van Hullebusch, E. D., Dutta, T. K., Ahn, Y., Ghosal, D., & Ghosh, S. (2016). *Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review*. https://doi.org/10.3389/fmicb.2016.01369

Arunachalam, K., & Sasidharan, S. P. (2021). *Gel Electrophoresis and PCR Amplification*. 241–258. https://doi.org/10.1007/978-1-0716-1233-0_23

Ayilara, M. S., & Babalola, O. O. (2023). *Bioremediation of environmental wastes: the role of microorganisms*. https://doi.org/10.3389/fagro.2023.1183691

Balmer, J. E., Hung, H., Yu, Y., Letcher, R. J., & Muir, D. C. G. (2019). Sources and environmental fate of pyrogenic polycyclic aromatic hydrocarbons (PAHs) in the Arctic. *Emerging Contaminants*, *5*, 128–142. https://doi.org/10.1016/J.EMCON.2019.04.002

Bibi, N., Hamayun, M., Khan, S. A., Iqbal, A., Islam, B., Shah, F., Khan, M. A., & Lee, I. J. (2018). Anthracene biodegradation capacity of newly isolated rhizospheric bacteria Bacillus cereus S13. *PLoS ONE*, *13*(8). https://doi.org/10.1371/journal.pone.0201620

Billah, M. M., Bhuiyan, M. K. A., Amran, M. I. U. Al, Cabral, A. C., & Garcia, M. R. D. (2022). Polycyclic aromatic hydrocarbons (PAHs) pollution in mangrove ecosystems: global synthesis and future research directions. In *Reviews in Environmental Science and Biotechnology*. Springer Science and Business Media B.V. https://doi.org/10.1007/s11157-022-09625-0

Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V., & Krishan, D. (2015). Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. *Brazilian Journal of Microbiology*, *46*(1), 7–21. https://doi.org/10.1590/S1517-838246120131354.

Busari, M. (2024). *Thermal Decomposition Pathways of Emerging Contaminants in Waste Incineration*.

Campus, M., St, A.-N., Mahmood, B., Al-Joda, S., & Jasim, A. H. (n.d.). *Studying the Efficiency Dependence of CR-39 Detector on the Chamber Height*. *29*(2), 168–176. www.journalofbabylon.com

Chakraborty, I. (2022). *STRUCTURAL INSIGHTS AND MOLECULAR DOCKING STUDIES FOR POLYCYCLIC AROMATIC HYDROCARBON DEGRADATION IN NOVEL BACTERIAL STRAINS FOR BIOREMEDIATION: AN IN-SILICO MODELLING STUDY*.

Cheng, J. J., & Cheng, J. (2024). *Spatiotemporal Trends and Environmental Inequity of Polycyclic Aromatic Hydrocarbon (PAH) Air Pollutants*.

Chiaregato, C. G., Souza, C. F., & Faez, R. (2021). The fertilizer release into water and soil as the biodegradation process in the sustainable material enhancing the fertilizer efficiency. *Environmental Technology & Innovation*, *22*, 101417. https://doi.org/10.1016/J.ETI.2021.101417

Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. In *Clinical Microbiology Reviews* (Vol. 33, Issue 4, pp. 1–74). American Society for Microbiology. https://doi.org/10.1128/CMR.00053-19

Clement Akinola, O. (n.d.). *Naphthalene and Anthracene Degrading Potentials of Bacteria Naphthalene and Anthracene Degrading Potentials of Bacteria Isolated from Soil Samples with Evidence of Polycyclic Aromatic Isolated from Soil Samples with Evidence of Polycyclic Aromatic*. https://digitalscholarship.tsu.edu/theses/6

Dai, C., Han, Y., Duan, Y., Lai, X., Fu, R., Liu, S., Leong, K. H., Tu, Y., & Zhou, L. (2022a). Review on the contamination and remediation of polycyclic aromatic hydrocarbons (PAHs) in coastal soil and sediments. *Environmental Research*, *205*, 112423. https://doi.org/10.1016/J.ENVRES.2021.112423

Dai, C., Han, Y., Duan, Y., Lai, X., Fu, R., Liu, S., Leong, K. H., Tu, Y., & Zhou, L. (2022b). Review on the contamination and remediation of polycyclic aromatic hydrocarbons (PAHs) in coastal soil and sediments. *Environmental Research*, *205*, 112423. https://doi.org/10.1016/J.ENVRES.2021.112423

Das, D. N., & Ravi, N. (2022). Influences of polycyclic aromatic hydrocarbon on the epigenome toxicity and its applicability in human health risk assessment. *Environmental Research*, *213*, 113677. https://doi.org/10.1016/J.ENVRES.2022.113677

Douksouna, Y., Masanga, J., Nyerere, A., Runo, S., & Ambang, Z. (2019). *Towards Managing and Controlling Aflatoxin Producers Within Aspergillus Species in Infested Rice Grains Collected from Local Markets in Kenya*. https://doi.org/10.3390/toxins11090544

Drwal, E., Rak, A., & Gregoraszczuk, E. L. (2019). Review: Polycyclic aromatic hydrocarbons (PAHs)—Action on placental function and health risks in future life of newborns. *Toxicology*, *411*, 133–142. https://doi.org/10.1016/J.TOX.2018.10.003

Duedahl-Olesen, L., & Ionas, A. C. (2022). Formation and mitigation of PAHs in barbecued meat–a review. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 13, pp. 3553–3568). Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2020.1867056

Eldos, H. I., Zouari, N., Saeed, S., & Al-Ghouti, M. A. (2022). Recent advances in the treatment of PAHs in the environment: Application of nanomaterial-based technologies. *Arabian Journal of Chemistry*, *15*(7), 103918. https://doi.org/10.1016/J.ARABJC.2022.103918

Elly Kipchumba BVM, K. (2022). *Phenotypic and Molecular Characterization of Antimicrobial Resistant Non-pathogenic Staphylococcus Species in Raw Camel Milk From Garissa County, Kenya*. https://erepository.uonbi.ac.ke/handle/11295/161798

Ewa, B., & Danuta, M.-Š. (n.d.). *Polycyclic aromatic hydrocarbons and PAH-related DNA adducts*. https://doi.org/10.1007/s13353-016-0380-3

Feng, Y., Li, Z., & Li, W. (2025). Polycyclic Aromatic Hydrocarbons (PAHs): Environmental Persistence and Human Health Risks. In *Natural Product Communications* (Vol. 20, Issue 1). SAGE Publications Inc. https://doi.org/10.1177/1934578X241311451

Fillat, Ú., Ibarra, D., Eugenio, M. E., Moreno, A. D., Tomás-Pejó, E., & Martín-Sampedro, R. (2017). *fermentation Laccases as a Potential Tool for the Efficient Conversion of Lignocellulosic Biomass: A Review*. https://doi.org/10.3390/fermentation3020017

Fischer, J., Hu, B., Xin, X., Zou, L., Yang, R., Li, S., Li, Y., Yan, Y., Fang, Y., & Chen, G. (2021). *Bactericidal Effect of Pseudomonas oryziphila sp. nov., a Novel Pseudomonas Species Against Xanthomonas oryzae Reduces Disease Severity of Bacterial Leaf Streak of Rice*. https://doi.org/10.3389/fmicb.2021.759536

Frapiccini, E., De Marco, R., Grilli, F., Marini, M., Annibaldi, A., Prezioso, E., Tramontana, M., & Spagnoli, F. (2024). Anthropogenic contribution, transport, and accumulation of Polycyclic Aromatic Hydrocarbons in sediments of the continental shelf and slope in the Mediterranean Sea. *Chemosphere*, *352*, 141285. https://doi.org/10.1016/J.CHEMOSPHERE.2024.141285

GAN, X., TENG, Y., REN, W., MA, J., CHRISTIE, P., & LUO, Y. (2017). Optimization of Ex-Situ Washing Removal of Polycyclic Aromatic Hydrocarbons from a Contaminated Soil Using Nano-Sulfonated Graphene. *Pedosphere*, *27*(3), 527–536. https://doi.org/10.1016/S1002-0160(17)60348-5

Ghosh, P., & Mukherji, S. (2023). Fate, detection technologies and toxicity of heterocyclic PAHs in the aquatic and soil environments. *Science of The Total Environment*, *892*, 164499. https://doi.org/10.1016/J.SCITOTENV.2023.164499

Gitipour, S., Sorial, G. A., Ghasemi, S., & Bazyari, M. (2018). Treatment technologies for PAH-contaminated sites: a critical review. *Environmental Monitoring and Assessment*, *190*(9). https://doi.org/10.1007/s10661-018-6936-4

Gong, W., Bai, L., & Liang, H. (2024). Membrane-based technologies for removing emerging contaminants in urban water systems: Limitations, successes, and future improvements. *Desalination*, *590*, 117974. https://doi.org/10.1016/J.DESAL.2024.117974

Gou, Y., Song, Y., Yang, S., Yang, Y., Cheng, Y., Wu, X., Wei, W., & Wang, H. (2023). Low-temperature thermal-enhanced anoxic biodegradation of polycyclic aromatic hydrocarbons in aged subsurface soil. *Chemical Engineering Journal*, *454*, 140143. https://doi.org/10.1016/J.CEJ.2022.140143

Greish, S., Rinnan, Å., Marcussen, H., Holm, P. E., & Christensen, J. H. (2018). Interaction mechanisms between polycyclic aromatic hydrocarbons (PAHs) and organic soil washing agents. *Environmental Science and Pollution Research*, *25*(1), 299–311. https://doi.org/10.1007/s11356-017-0374-7

Hao, G., Liu, F., Zhang, Q., Ali Mallah, M., Ali Mallah, M., Liu, Y., Xi, H., Wang, W., & Feng, F. (2021). Relationship Between Polycyclic Aromatic Hydrocarbons and Cardiovascular Diseases: A Systematic Review. *Frontiers in Public Health | Www.Frontiersin.Org*, *9*, 763706. https://doi.org/10.3389/fpubh.2021.763706

Hazaimeh, M., Pollution, E. A.-E. S. and, & 2021, undefined. (2021). Bioremediation perspectives and progress in petroleum pollution in the marine environment: A review. *Springer*, *28*(39), 54238–54259. https://doi.org/10.1007/s11356-021-15598-4

Herath, A., Wawrik, B., Qin, Y., Zhou, J., & Callaghan, A. V. (2016). Transcriptional response of Desulfatibacillum alkenivorans AK-01 to growth on alkanes: Insights from RT-qPCR and microarray analyses. *FEMS Microbiology Ecology*, *92*(5). https://doi.org/10.1093/femsec/fiw062

Incha, M. R. (2023). *Excavating the genome mine of Pseudomonas putida KT2440*.

Jesus, F., Pereira, J. L., Campos, I., Santos, M., Ré, A., Keizer, J., Nogueira, A., Gonçalves, F. J. M., Abrantes, N., & Serpa, D. (2022). A review on polycyclic aromatic hydrocarbons distribution in freshwater ecosystems and their toxicity to benthic fauna. *Science of The Total Environment*, *820*, 153282. https://doi.org/10.1016/J.SCITOTENV.2022.153282

Kai, S., Matsuo, Y., Nakagawa, S., Kryukov, K., Matsukawa, S., Tanaka, H., Iwai, T., Imanishi, T., Hirota, K., Matsuo, C. Y., & Hirota, K. (n.d.). *Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION TM nanopore sequencer*. https://doi.org/10.1002/2211-5463.12590

Kapley, A., Kjellerup, B. V, Saxena, G., Teng, Y., Desai, C., Madamwar, D., Patel, A. B., Shaikh, S., & Jain, K. R. (2020). *Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches*. https://doi.org/10.3389/fmicb.2020.562813

Kapustina, Ž., Medžiūnė, J., Alzbutas, G., Rokaitis, I., Matjošaitis, K., Mackevičius, G., Žeimytė, S., Karpus, L., & Lubys, A. (2021). High-resolution microbiome analysis enabled by linking of 16S rRNA gene sequences with adjacent genomic contexts. *Microbial Genomics*, *7*(9). https://doi.org/10.1099/MGEN.0.000624

Kim, M. J., Kim, S., Choi, S., Lee, I., Moon, M. K., Choi, K., Park, Y. J., Cho, Y. H., Kwon, Y. M., Yoo, J., Cheon, G. J., & Park, J. (2021). Association of exposure to polycyclic aromatic hydrocarbons and heavy metals with thyroid hormones in general adult population and potential mechanisms. *Science of The Total Environment*, *762*, 144227. https://doi.org/10.1016/J.SCITOTENV.2020.144227

Kramer, A., Vivanco, S., Bare, J., & Panko, J. (2025). Analysis of EPA air toxics monitoring data and tools for use in general population exposure assessments: Using acrylonitrile as a case study. *Journal of the Air and Waste Management Association*. https://doi.org/10.1080/10962247.2024.2438793

Krivoruchko, A., Kuyukina, M., Peshkur, T., Cunningham, C. J., & Ivshina, I. (2023). *molecules Rhodococcus Strains from the Specialized Collection of Alkanotrophs for Biodegradation of Aromatic Compounds*. https://doi.org/10.3390/molecules28052393

Kumari, B., & Chandra, R. (2023). Benzo[a]pyrene degradation from hydrocarbon-contaminated soil and their degrading metabolites by Stutzerimonas stutzeri (LOBP-19A). *Waste Management Bulletin*, *1*(3), 115–127. https://doi.org/10.1016/J.WMB.2023.07.006

Kuppusamy, S., Thavamani, P., Venkateswarlu, K., Lee, Y. B., Naidu, R., & Megharaj, M. (2017). Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. *Chemosphere*, *168*, 944–968. https://doi.org/10.1016/J.CHEMOSPHERE.2016.10.115

Lamichhane, S., Bal Krishna, K. C., & Sarukkalige, R. (2016). Polycyclic aromatic hydrocarbons (PAHs) removal by sorption: A review. *Chemosphere*, *148*, 336–353. https://doi.org/10.1016/J.CHEMOSPHERE.2016.01.036

Lee, C. P., Le, X. H., Gawryluk, R. M. R., Casaretto, J. A., Rothstein, S. J., & Millar, A. H. (2024). EARLY NODULIN93 acts via cytochrome c oxidase to alter respiratory ATP production and root growth in plants. *The Plant Cell*, *36*(11), 4716–4731. https://doi.org/10.1093/PLCELL/KOAE242

Lee, S., & Hyun, J. (2021). *MEASUREMENT AND ANALYSIS OF TOXIC VOLATILE ORGANIC COMPOUNDS (VOC) AND POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AT SINGAPORE'S INTEGRATED TRANSPORT HUBS (ITH).*

Lehto, R. (2021). *LONG TERM DNA STORAGE EVALUATION AND METHODOLOGY*.

Liang, C., Ye, Q., Huang, Y., Zhang, Z., Wang, C., Wang, Y., & Wang, H. (2023). Distribution of the new functional marker gene (pahE) of aerobic polycyclic aromatic hydrocarbon (PAHs) degrading bacteria in different ecosystems. *Science of The Total Environment*, *865*, 161233. https://doi.org/10.1016/J.SCITOTENV.2022.161233

Li, T., Huang, L., Li, Y., Xu, Z., Ge, X., Zhang, Y., Wang, N., Wang, S., Yang, W., Lu, F., & Liu, Y. (2020). The heterologous expression, characterization, and application of a novel laccase from Bacillus velezensis. *Science of The Total Environment*, *713*, 136713. https://doi.org/10.1016/J.SCITOTENV.2020.136713

Liu, C., Liu, G., Yang, Q., Luo, T., He, P., Franzese, P. P., & Lombardi, G. V. (2021). Emergy-based evaluation of world coastal ecosystem services. *Water Research*, *204*, 117656. https://doi.org/10.1016/J.WATRES.2021.117656

Liu, R., Zhao, S., Zhang, B., Li, G., Fu, X., Yan, P., & Shao, Z. (2023). Biodegradation of polystyrene (PS) by marine bacteria in mangrove ecosystem. *Journal of Hazardous Materials*, *442*, 130056. https://doi.org/10.1016/J.JHAZMAT.2022.130056

Logeshwaran, P., Subashchandrabose, S. R., Krishnan, K., Sivaram, A. K., Annamalai, P., Naidu, R., & Megharaj, M. (2022). Polycyclic aromatic hydrocarbons biodegradation by fenamiphos degrading Microbacterium esteraromaticum MM1. *Environmental Technology & Innovation*, *27*, 102465. https://doi.org/10.1016/J.ETI.2022.102465

Luca, C. (2025). *Comparative Evaluation of Biochemical and Molecular Techniques for Bacterial Identification*.

Lyons, M. B., Keith, D. A., Phinn, S. R., Mason, T. J., & Elith, J. (2018). A comparison of resampling methods for remote sensing classification and accuracy assessment. *Remote Sensing of Environment*, *208*, 145–153. https://doi.org/10.1016/J.RSE.2018.02.026

Madamwar, D., Liu, X., Mohapatra, B., & Phale, P. S. (2021). *Microbial Degradation of Naphthalene and Substituted Naphthalenes: Metabolic Diversity and Genomic Insight for Bioremediation*. https://doi.org/10.3389/fbioe.2021.602445

Maletić, S. P., Beljin, J. M., Rončević, S. D., Grgić, M. G., & Dalmacija, B. D. (2019). State of the art and future challenges for polycyclic aromatic hydrocarbons is sediments: sources, fate, bioavailability and remediation techniques. *Journal of Hazardous Materials*, *365*, 467–482. https://doi.org/10.1016/J.JHAZMAT.2018.11.020

Mallah, M. A., Changxing, L., Mallah, M. A., Noreen, S., Liu, Y., Saeed, M., Xi, H., Ahmed, B., Feng, F., Mirjat, A. A., Wang, W., Jabar, A., Naveed, M., Li, J. H., & Zhang, Q. (2022a). Polycyclic aromatic hydrocarbon and its effects on human health: An overeview. *Chemosphere*, *296*, 133948. https://doi.org/10.1016/J.CHEMOSPHERE.2022.133948

Mallah, M. A., Changxing, L., Mallah, M. A., Noreen, S., Liu, Y., Saeed, M., Xi, H., Ahmed, B., Feng, F., Mirjat, A. A., Wang, W., Jabar, A., Naveed, M., Li, J. H., & Zhang, Q. (2022b). Polycyclic aromatic hydrocarbon and its effects on human health: An overeview. *Chemosphere*, *296*, 133948. https://doi.org/10.1016/J.CHEMOSPHERE.2022.133948

Mallah, M. A., Changxing, L., Mallah, M. A., Noreen, S., Liu, Y., Saeed, M., Xi, H., Ahmed, B., Feng, F., Mirjat, A. A., Wang, W., Jabar, A., Naveed, M., Li, J. H., & Zhang, Q. (2022c). Polycyclic aromatic hydrocarbon and its effects on human health: An overeview. *Chemosphere*, *296*, 133948. https://doi.org/10.1016/J.CHEMOSPHERE.2022.133948

Mohapatra, B., & Phale, P. S. (2021). Microbial Degradation of Naphthalene and Substituted Naphthalenes: Metabolic Diversity and Genomic Insight for Bioremediation. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/FBIOE.2021.602445/FULL

Mondal, S., Biswas, B., Chowdhury, R., Sengupta, R., Mandal, A., Nath Kotal, H., Kumar Giri, C., Ghosh, A., Saha, S., Momtaj Begam, M., Mukherjee, C., Das, I., Kumar Basak, S., Mitra Ghosh, M., Ray, K., Fernandez, C., Jiang, C., & Bhadury, P. (2024). Estuarine mangrove niches select cultivable heterotrophic diazotrophs with diverse metabolic potentials-a prospective cross-dialog for functional diazotrophy OPEN ACCESS EDITED BY. *Front. Microbiol*, *15*, 1324188. https://doi.org/10.3389/fmicb.2024.1324188

Monné Rodríguez, J. M., Frisk, A. L., Kreutzer, R., Lemarchand, T., Lezmi, S., Saravanan, C., Stierstorfer, B., Thuilliez, C., Vezzali, E., Wieczorek, G., Yun, S. W., & Schaudien, D. (2023). European Society of Toxicologic Pathology (Pathology 2.0 Molecular Pathology Special Interest Group): Review of In Situ Hybridization Techniques for Drug Research and Development. In *Toxicologic Pathology* (Vol. 51, Issue 3, pp. 92–111). SAGE Publications Inc. https://doi.org/10.1177/01926233231178282

Mortazavi Mehrizi, M., Yousefinejad, S., Jafari, S., Baghapour, M. A., Karimi, A., Mahvi, A. H., & Jahangiri, M. (2022). Bioremediation and microbial degradation of benzo[a]pyrene in aquatic environments: a systematic review. *International Journal of Environmental Analytical Chemistry*, *102*(15), 3508–3523. https://doi.org/10.1080/03067319.2020.1770743

Mullaeva, S. A., Delegan, Y. A., Streletskii, R. A., Sazonova, O. I., Petrikov, K. V., Ivanova, A. A., Dyatlov, I. A., Shemyakin, I. G., Bogun, A. G., & Vetrova, A. A. (2022). Pseudomonas veronii strain 7–41 degrading medium-chain n-alkanes and polycyclic aromatic hydrocarbons. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-25191-5

Na, G., Gao, Y., Li, R., Gao, H., Hou, C., Ye, J., Jin, S., & Zhang, Z. (2020). Occurrence and sources of polycyclic aromatic hydrocarbons in atmosphere and soil from 2013 to 2019 in the Fildes Peninsula, Antarctica. *Marine Pollution Bulletin*, *156*,111173. https://doi.org/10.1016/J.MARPOLBUL.2020.111173

Naylor, D., Mcclure, R., & Jansson, J. (2022). *microorganisms Review Trends in Microbial Community Composition and Function by Soil Depth*. https://doi.org/10.3390/microorganisms10030540

Nienke, S., & Volkerink, J. (n.d.). *Bacterial metabolic networks channeling the biodegradation of PAHs in contaminated soils. Oxy-PAHs as catabolic nodes*. www.tdx.cat

(No Title). (2024). https://doi.org/10.3390/w16172520

Nzila, A. (2018). Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons under anaerobic conditions: Overview of studies, proposed pathways and future perspectives. *Environmental Pollution*, *239*, 788–802. https://doi.org/10.1016/J.ENVPOL.2018.04.074

Obayori, O. S., Salam, L. B., Obayori, O. S., & Salam, L. B. (2010). Degradation of polycyclic aromatic hydrocarbons: Role of plasmids. *Scientific Research and Essays*, *5*(25), 4093–4106. http://www.academicjournals.org/SRE

Padilla-Garfias, F., Araiza-Villanueva, M., Calahorra, M., Sánchez, N. S., & Peña, A. (2024). Advances in the Degradation of Polycyclic Aromatic Hydrocarbons by Yeasts: A Review. In *Microorganisms* (Vol. 12, Issue 12). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/microorganisms12122484

Pagliarella, M. C., Corona, P., & Fattorini, L. (2018). Spatially-balanced sampling versus unbalanced stratified sampling for assessing forest change: evidences in favour of spatial balance. *Environmental and Ecological Statistics*, *25*(1), 111–123. https://doi.org/10.1007/S10651-017-0378-Y/METRICS

Palit, K., Rath, S., Chatterjee, S., & Das, S. (2022). Microbial diversity and ecological interactions of microorganisms in the mangrove ecosystem: Threats, vulnerability, and adaptations. *Environmental Science and Pollution Research*, *29*(22), 32467–32512. https://doi.org/10.1007/S11356-022-19048-7

Pazos, M., Rosales, E., Alcántara, T., Gómez, J., & Sanromán, M. A. (2010). Decontamination of soils containing PAHs by electroremediation: A review. *Journal of Hazardous Materials*, *177*(1–3), 1–11. https://doi.org/10.1016/J.JHAZMAT.2009.11.055

Peng, X., Amend, A. S., Baltar, F., Blanco-Bercial, L., Breyer, E., Burgaud, G., Cunliffe, M., Edgcomb, V. P., Grossart, H.-P., Mara, P., Masigol, H., Pang, K.-L., Retter, A., Roberts, C., Bleijswijk, J. van, Walker, A. K., & Whitner, S. (2023). *Planktonic marine fungi: A review*. https://doi.org/10.22541/essoar.169903594.45892424/v1

Pischedda, A., Tosin, M., & Degli-Innocenti, F. (2019). Biodegradation of plastics in soil: The effect of temperature. *Polymer Degradation and Stability*, *170*, 109017. https://doi.org/10.1016/J.POLYMDEGRADSTAB.2019.109017

Premnath, N., Mohanrasu, K., Guru Raj Rao, R., Dinesh, G. H., Prakash, G. S., Ananthi, V., Ponnuchamy, K., Muthusamy, G., & Arun, A. (2021). A crucial review on polycyclic aromatic Hydrocarbons - Environmental occurrence and strategies for microbial degradation. *Chemosphere*, *280*. https://doi.org/10.1016/j.chemosphere.2021.130608

Rabani, M. S., Habib, A., & Gupta, M. K. (2020). Polycyclic Aromatic Hydrocarbons: Toxic Effects and Their Bioremediation Strategies. *Bioremediation and Biotechnology, Vol 4*, 65–105. https://doi.org/10.1007/978-3-030-48690-7_4

Rabus, R., Boll, M., Heider, J., Meckenstock, R. U., Buckel, W., Einsle, O., Ermler, U., Golding, B. T., Gunsalus, R. P., Kroneck, P. M. H., Krüger, M., Lueders, T., Martins, B. M., Musat, F., Richnow, H. H., Schink, B., Seifert, J., Szaleniec, M., Treude, T., … Wilkes, H. (2016). Anaerobic microbial degradation of hydrocarbons: From enzymatic reactions to the environment. *Journal of Molecular Microbiology and Biotechnology*, *26*(1–3), 5–28. https://doi.org/10.1159/000443997

Raganati, F., Markou, G., De, C. E., Silva, F., & Alaidaroos, B. A. (2023). *Advancing Eco-Sustainable Bioremediation for Hydrocarbon Contaminants: Challenges and Solutions*. https://doi.org/10.3390/pr11103036

Ramatla, T., Ngoma, L., & Mwanza, M. (2021). The Utility of MALDI-TOF-Mass Spectrometry, Analytical Profile Index (API) and Conventional-PCR for the Detection of Foodborne Pathogens from Meat. *Journal of Food and Nutrition Research*, *9*(8), 442–448. https://doi.org/10.12691/jfnr-9-8-7

Ravichandran, A., & Sridhar, M. (2017). Insights into the mechanism of lignocellulose degradation by versatile peroxidases. In *REVIEW ARTICLES CURRENT SCIENCE* (Vol. 113, Issue 1).

Revathy, T., Jayasri, M. A., & Suthindhiran, K. (2015). Biodegradation of PAHs by Burkholderia sp. VITRSB1 Isolated from Marine Sediments . *Scientifica*, *2015*, 1–9. https://doi.org/10.1155/2015/867586

Saeedi, M., Li, L. Y., & Grace, J. R. (2020). Effect of co-existing heavy metals and natural organic matter on sorption/desorption of polycyclic aromatic hydrocarbons in soil: A review. In *Pollution* (Vol. 6, Issue 1, pp. 1–24). University of Tehran. https://doi.org/10.22059/POLL.2019.284335.638

Sakdapetsiri, C., Kaokhum, N., & Pinyakong, O. (2021). Biodegradation of crude oil by immobilized Exiguobacterium sp. AO-11 and shelf life evaluation. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-92122-1

Salam, L. B., Obayori, O. S., & Raji, S. A. (2015). Biodegradation of used engine oil by a methylotrophic bacterium, Methylobacterium mesophilicum isolated from tropical hydrocarbon-contaminated soil. *Petroleum Science and Technology*, *33*(2), 186–195. https://doi.org/10.1080/10916466.2014.961610

Saravanan, A., Kumar, P. S., Vo, D. V. N., Jeevanantham, S., Karishma, S., & Yaashikaa, P. R. (2021). A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. *Journal of Hazardous Materials*, *419*, 126451. https://doi.org/10.1016/J.JHAZMAT.2021.126451

Sekar, M., & T R, P. (2024). Critical review on the formations and exposure of polycyclic aromatic hydrocarbons (PAHs) in the conventional hydrocarbon-based fuels: Prevention and control strategies. *Chemosphere*, *350*, 141005. https://doi.org/10.1016/J.CHEMOSPHERE.2023.141005

Sharma, A., Dev, K., Sourirajan, A., & Choudhary, M. (2021). Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 99. https://doi.org/10.1186/S43141-021-00186-3

Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z. A., & Yaseen, I. (n.d.). *A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria*.

Siddique, A., Al Disi, Z., AlGhouti, M., & Zouari, N. (2024). Diversity of hydrocarbon-degrading bacteria in mangroves rhizosphere as an indicator of oil-pollution bioremediation in mangrove forests. In *Marine Pollution Bulletin* (Vol. 205). Elsevier Ltd. https://doi.org/10.1016/j.marpolbul.2024.116620

Sivaram, A. K., Logeshwaran, P., Lockington, R., Naidu, R., & Megharaj, M. (2019). Low molecular weight organic acids enhance the high molecular weight polycyclic aromatic hydrocarbons degradation by bacteria. *Chemosphere*, *222*, 132–140. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.110

Sivasamy, S., Rajangam, S., Kanagasabai, T., Bisht, D., Prabhakaran, R., & Dhandayuthapani, S. (2024). Biocatalytic Potential of Pseudomonas Species in the Degradation of Polycyclic Aromatic Hydrocarbons. *Journal of Basic Microbiology*. https://doi.org/10.1002/JOBM.202400448

Sullivan, G. L., Prigmore, R. M., Knight, P., & Godfrey, A. R. (2019). Activated carbon biochar from municipal waste as a sorptive agent for the removal of polyaromatic hydrocarbons (PAHs), phenols and petroleum based compounds in contaminated liquids. *Journal of Environmental Management*, *251*, 109551. https://doi.org/10.1016/J.JENVMAN.2019.109551

Sun, K., Song, Y., He, F., Jing, M., Tang, J., & Liu, R. (2021). A review of human and animals exposure to polycyclic aromatic hydrocarbons: Health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. *Science of The Total Environment*, *773*, 145403. https://doi.org/10.1016/J.SCITOTENV.2021.145403

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Academic.Oup.Com*. https://doi.org/10.1093/molbev/msr121

Tchounwou, P. B., Zhao, L., Zhou, M., Zhao, Y., Yang, J., Pu, Q., Yang, H., Wu, Y., Lyu, C., & Li, Y. (2022). Academic Editors: Daniela Varrica Potential Toxicity Risk Assessment and Priority Control Strategy for PAHs Metabolism and Transformation Behaviors in the Environment. *Public Health*, *19*, 10972. https://doi.org/10.3390/ijerph191710972

Tilton, S. C., Siddens, L. K., Krueger, S. K., Larkin, A. J., Löhr, C. V, Williams, D. E., Baird, W. M., & Waters, K. M. (n.d.). *Mechanism-based classification of PAH mixtures to predict carcinogenic potential Downloaded from*. http://toxsci.oxfordjournals.org/

Tiwari, M., & Tripathy, D. B. (2023). *Soil Contaminants and Their Removal through Surfactant-Enhanced Soil Remediation: A Comprehensive Review*. https://doi.org/10.3390/su151713161

Torres, S., Merino, C., Paton, B., Correig, X., & Ramírez, N. (2018). Biomarkers of exposure to secondhand and thirdhand Tobacco smoke: Recent advances and future perspectives. In *International Journal of Environmental Research and Public Health* (Vol. 15, Issue 12). MDPI AG. https://doi.org/10.3390/ijerph15122693

Vala, A. K., Dudhagara, D. R., & Dave, B. P. (2021). Marine Microbial Bioremediation. In *Marine Microbial Bioremediation*. CRC Press. https://doi.org/10.1201/9781003001072

Vandana, Priyadarshanee, M., Mahto, U., & Das, S. (2022). Mechanism of toxicity and adverse health effects of environmental pollutants. *Microbial Biodegradation and Bioremediation: Techniques and Case Studies for Environmental Pollution*, 33–53. https://doi.org/10.1016/B978-0-323-85455-9.00024-2

Varner, P. M., Allemann, M. N., Michener, J. K., & Gunsch, C. K. (2022). The effect of bacterial growth strategies on plasmid transfer and naphthalene degradation for bioremediation. *Environmental Technology & Innovation*, *28*, 102910. https://doi.org/10.1016/J.ETI.2022.102910

Venkatraman, G., Giribabu, N., Mohan, P. S., Muttiah, B., Govindarajan, V. K., Alagiri, M., Abdul Rahman, P. S., & Karsani, S. A. (2024). Environmental impact and human health effects of polycyclic aromatic hydrocarbons and remedial strategies: A detailed review. *Chemosphere*, *351*. https://doi.org/10.1016/j.chemosphere.2024.141227

Veselinyová, D., Mašlanková, J., Kalinová, K., Mičková, H., Mareková, M., & Rabajdová, M. (2021). *molecules Review Selected In Situ Hybridization Methods: Principles and Application*. https://doi.org/10.3390/molecules26133874

Vijayanand, M., Ramakrishnan, A., Subramanian, R., Issac, P. K., Nasr, M., Khoo, K. S., Rajagopal, R., Greff, B., Wan Azelee, N. I., Jeon, B. H., Chang, S. W., & Ravindran, B. (2023a). Polyaromatic hydrocarbons (PAHs) in the water environment: A review on toxicity, microbial biodegradation, systematic biological advancements, and environmental fate. *Environmental Research*, *227*, 115716. https://doi.org/10.1016/J.ENVRES.2023.115716

Vijayanand, M., Ramakrishnan, A., Subramanian, R., Issac, P. K., Nasr, M., Khoo, K. S., Rajagopal, R., Greff, B., Wan Azelee, N. I., Jeon, B. H., Chang, S. W., & Ravindran, B. (2023b). Polyaromatic hydrocarbons (PAHs) in the water environment: A review on toxicity, microbial biodegradation, systematic biological advancements, and environmental fate. *Environmental Research*, *227*, 115716. https://doi.org/10.1016/J.ENVRES.2023.115716

Von Lau, E., Gan, S., Ng, H. K., & Poh, P. E. (2014). Extraction agents for the removal of polycyclic aromatic hydrocarbons (PAHs) from soil in soil washing technologies. *Environmental Pollution*, *184*, 640–649. https://doi.org/10.1016/J.ENVPOL.2013.09.010

Wang, C., Guo, G., Huang, Y., Hao, H., & Wang, H. (2017). Salt Adaptation and Evolutionary Implication of a Nah-related PAHs Dioxygenase cloned from a Halophilic Phenanthrene Degrading Consortium. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-12979-z

Wang, H., Shu, Y., Kuang, Z., Han, Z., Wu, J., Huang, X., Song, X., Yang, J., & Fan, Z. (2025). Bioaccumulation and potential human health risks of PAHs in marine food webs: A trophic transfer perspective. *Journal of Hazardous Materials*, *485*, 136946. https://doi.org/10.1016/J.JHAZMAT.2024.136946

Xu, M., Wu, M., Zhang, Y., Zhang, H., Liu, W., Chen, G., Xiong, G., & Guo, L. (2022). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial mixture. *International Journal of Environmental Science and Technology*, *19*(5), 3833–3844. https://doi.org/10.1007/s13762-021-03284-4

Yamini, V., & Rajeswari, V. D. (2023). Metabolic capacity to alter polycyclic aromatic hydrocarbons and its microbe-mediated remediation. *Chemosphere*, *329*, 138707. https://doi.org/10.1016/J.CHEMOSPHERE.2023.138707

Yang, L., Hou, X. Y., Wei, Y., Thai, P., & Chai, F. (2017). Biomarkers of the health outcomes associated with ambient particulate matter exposure. *Science of The Total Environment*, *579*, 1446–1459. https://doi.org/10.1016/J.SCITOTENV.2016.11.146

Yemele, O. M., Zhao, Z., Nkoh, J. N., Ymele, E., & Usman, M. (2024). A systematic review of polycyclic aromatic hydrocarbon pollution: A combined bibliometric and mechanistic analysis of research trend toward an environmentally friendly solution. *Science of The Total Environment*, *926*, 171577. https://doi.org/10.1016/J.SCITOTENV.2024.171577

Yu, Y. yiyi, Jin, H., & Lu, Q. (2022a). Effect of polycyclic aromatic hydrocarbons on immunity. *Journal of Translational Autoimmunity*, *5*, 100177. https://doi.org/10.1016/J.JTAUTO.2022.100177

Yu, Y. yiyi, Jin, H., & Lu, Q. (2022b). Effect of polycyclic aromatic hydrocarbons on immunity. *Journal of Translational*

Autoimmunity, *5*, 100177. https://doi.org/10.1016/J.JTAUTO.2022.100177

Zabaniotou, A., & Stamou, K. (n.d.). *Balancing Waste and Nutrient Flows Between Urban Agglomerations and Rural Ecosystems: Biochar for Improving Crop Growth and Urban Air Quality in The Mediterranean Region*. https://doi.org/10.3390/atmos11050539

Zada, S., Zhou, H., Xie, J., Hu, Z., Ali, S., Sajjad, W., & Wang, H. (2021). Bacterial degradation of pyrene: Biochemical reactions and mechanisms. *International Biodeterioration & Biodegradation*, *162*, 105233. https://doi.org/10.1016/J.IBIOD.2021.105233

Zena Bukowska, B., Mokra, K., & Michałowicz, J. (2022). *International Journal of Molecular Sciences Benzo[a]pyrene-Environmental Occurrence, Human Exposure, and Mechanisms of Toxicity*. https://doi.org/10.3390/ijms23116348

Zhang, H., Wang, X., Chen, A., Li, S., Tao, R., Chen, K., Huang, P., Li, L., Huang, J., Li, C., & Zhang, S. (2024). Comparison of the full-length sequence and sub-regions of 16S rRNA gene for skin microbiome profiling. *MSystems*, *9*(7). https://doi.org/10.1128/msystems.00399-24

Zhang, L., Qiu, X., Huang, L., Xu, J., Wang, W., Li, Z., Xu, P., & Tang, H. (2021). Microbial degradation of multiple PAHs by a microbial consortium and its application on contaminated wastewater. *Journal of Hazardous Materials*, *419*, 126524. https://doi.org/10.1016/J.JHAZMAT.2021.126524

Zhang, W., Wu, W., Wu, J., Liu, X., Tian, J., Li, H., Li, Q., & Zheng, Y. (2023). Surfactant enhanced thermally activated persulfate remediating PAHs-contaminated soil: Insight into compatibility, degradation processes and mechanisms. *Chemosphere*, *335*, 139086. https://doi.org/10.1016/J.CHEMOSPHERE.2023.139086

Zhang, X., Gao, H., Qi, A., Duan, S., Zhang, W., Zhang, Y., Huang, Q., Zhao, T., Han, G., Wang, W., & Yang, L. (2024). Origins and health risk assessment of PAHs/APAHs/NPAHs/OPAHs in PM2.5 at a background site in North China Plain: Implications for crude oil emissions. *Atmospheric Pollution Research*, *15*(5), 102081. https://doi.org/10.1016/J.APR.2024.102081

Zhang, Y., Dong, S., Wang, H., Tao, S., & Kiyama, R. (2016). Biological impact of environmental polycyclic aromatic hydrocarbons (ePAHs) as endocrine disruptors. *Environmental Pollution*, *213*, 809–824. https://doi.org/10.1016/J.ENVPOL.2016.03.050

Zheng, J., Guo, C., Chen, H.-Z., Zhang, Y., Copyright, fnut, Fu, C., Li, Y., Xi, H., Niu, Z., Chen, N., Wang, R., Yan, Y., Gan, X., Wang, M., Zhang, W., & Lv, P. (n.d.). *Benzo(a)pyrene and cardiovascular diseases: An overview of pre-clinical studies focused on the underlying molecular mechanism*. www.iarc.who.int

Ziyaei, K., Mokhtari, M., Hashemi, M., Rezaei, K., & Abdi, F. (2024). Association between exposure to water sources contaminated with polycyclic aromatic hydrocarbons and cancer risk: A systematic review. *Science of The Total Environment*, *924*, 171261. https://doi.org/10.1016/J.SCITOTENV.2024.171261

APPENDICES

Appendix I: Composition of media

A) Sea water nutrient agar

0.5% Peptone, 0.3% Yeast Extract, 1.5% Agar, 0.5% NaCl, Distilled Water at pH 6.8

B) Marine agar

5g Peptone, 1g Yeast Extract, 0.1g Ferric Citrate, 19.45g NaCl, 8.8g Mg/Cl, 3.24g Na_2SO_4, 1.8g $CaCl_2$, 0.55g KCl, 0.16g Na_2CO_3, 0.08g KBr, 0.034g $SrCl_2$, 0.022g Boric Acid, 0.004g Sodium Silicate, 0.0024 NaF, 0.0016g NH4MO3, and 15g Agar in1L of Distilled Water

C) Luria Bertani

10g Peptone, 5g Yeast Extract, 10g NaCl in 1L Distilled Water

D) Minimal salt media

1g $(NH_4)SO_4$, 0.8g K_2HPO_4, 1g $MgSO_4$, 0.1g $CaCl_2.H_2O$, 5mg $FeCl_3.6H_2O$ per liter

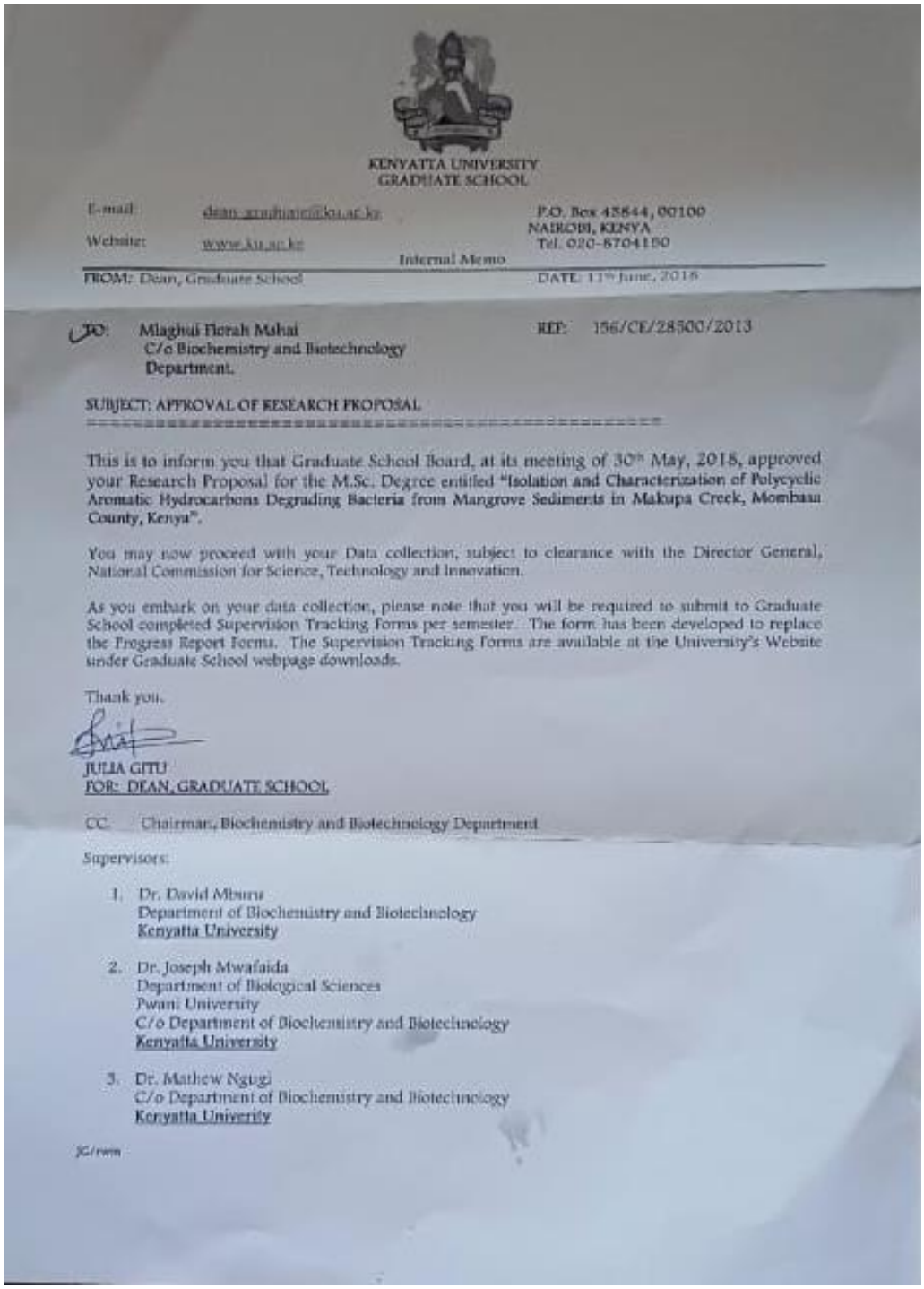
E) Tryptic soy agar

15g pancreatic digest of Casein, 5g pancreatic digest of Soy Bean, 5g Sodium Chloride, 15g Agar, 1L Distilled Water

F) Nutrient broth

0.5% Peptone, 0.3% Yeast Extract, 0.5% Nacl, and Distilled Water at pH 6.8

Appendix II: Research Approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean_graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School

DATE: 11th June, 2018

TO: Mlaghui Florah Mshai
C/o Biochemistry and Biotechnology
Department.

REF: 156/CE/28500/2013

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 30th May, 2018, approved your Research Proposal for the M.Sc. Degree entitled "Isolation and Characterization of Polycyclic Aromatic Hydrocarbons Degrading Bacteria from Mangrove Sediments in Makupa Creek, Mombasa County, Kenya".

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

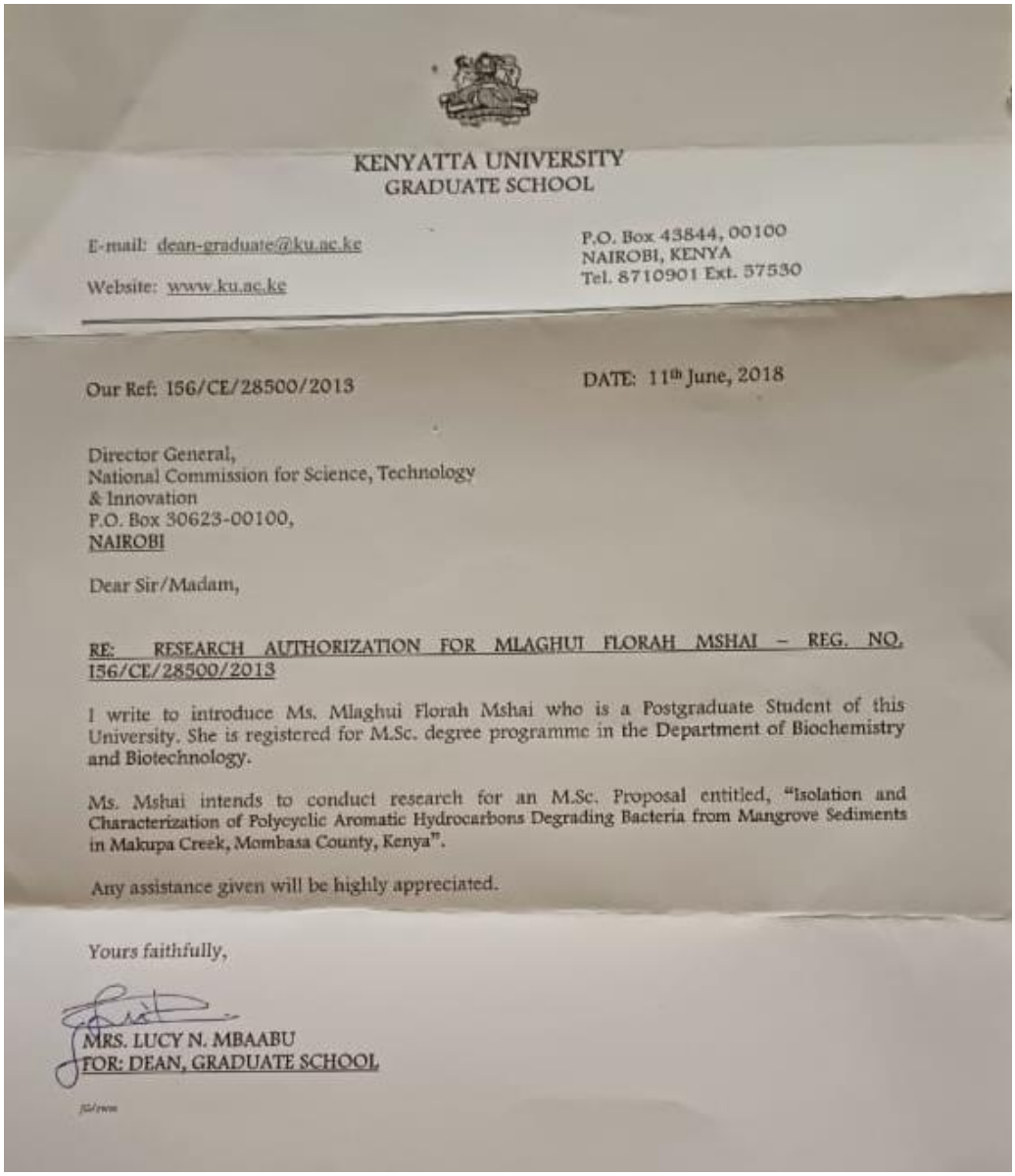
CC. Chairman, Biochemistry and Biotechnology Department

Supervisors:

1. Dr. David Mburu
   Department of Biochemistry and Biotechnology
   Kenyatta University
2. Dr. Joseph Mwafaida
   Department of Biological Sciences
   Pwani University
   C/o Department of Biochemistry and Biotechnology
   Kenyatta University
3. Dr. Mathew Ngugi
   C/o Department of Biochemistry and Biotechnology
   Kenyatta University

JG/rwm

Appendix III: Research Authorization



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: 156/CE/28500/2013

DATE: 11th June, 2018

Director General,
National Commission for Science, Technology
& Innovation
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MLAGHUI FLORAH MSHAI – REG. NO. 156/CE/28500/2013

I write to introduce Ms. Mlaghui Florah Mshai who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the Department of Biochemistry and Biotechnology.

Ms. Mshai intends to conduct research for an M.Sc. Proposal entitled, "Isolation and Characterization of Polycyclic Aromatic Hydrocarbons Degrading Bacteria from Mangrove Sediments in Makupa Creek, Mombasa County, Kenya".

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

/lc/rwm

Appendix IV: NACOSTI Research Authorization

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **457915**

Date of Issue: **24/July/2024**

RESEARCH LICENSE

This is to Certify that Ms.. flora mshai mlaghui of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Mombasa on the topic: ISOLATION AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS DEGRADING BACTERIA FROM MANGROVE SEDIMENTS IN MAKUPA CREEK, MOMBASA COUNTY, KENYA for the period ending : 24/July/2025.

License No: **NACOSTI/P/24/38030**

457915

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions