INVESTIGATION OF BIOREMEDIATION POTENTIAL OF WHEY DERIVED FROM PANICUM MAXIMUM ON CRUDE OIL CONTAMINATED SOIL.

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FACULTY OF PHYSICAL SCIENCE
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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY,
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CERTIFICATION

This is to certify that this research project was carried out by OTOGHAGUA SUCCESS OSAMUDIAMWEN with the matriculation number PG/PSC2215787 under the supervision of PROF. MRS. I. E. UWIDIA in the Department of Chemistry, Faculty of Physical sciences, University of Benin, Benin City, Edo State.

Prof. I.E.E IRABOR (Head of department)	DATE
Prof. Mrs. I.E UWIDIA (Project supervisor)	DATE
(Student)	DATE

DEDICATION

This project research is dedicated to the Almighty God who in His infinite mercy saw me through my journey in the University of Benin, to my loving Dad and Mom Mr and Mrs OTOGHAGUA ENOGIASUN

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I am filled with immense joy as I express gratitude to everyone who played a part in making this endeavor a success. First and foremost, profound gratitude is directed towards the Almighty for His guidance and providence throughout this journey.

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ABSTRACT

Crude oil spills in Nigeria poison farmland, killing plants and microbes while adding dangerous hydrocarbons and heavy metals that hurt crops and human health. This study tests a low-cost, eco-friendly fix using fermented whey from guinea grass (*Panicum maximum*) leaves to boost soil microbes that eat oil. The aim was to see how well this whey cleans oil-polluted soil by cutting total petroleum hydrocarbons (TPH) and restoring soil quality. The scope included collecting leaves (*Panicum maximum*) from University of Benin sports complex, processing the leaves into whey and fermenting the whey at various periods for twelve days. The whey samples were further characterized to further determine pH, electrical conductivity, moisture, organic matter, carbon, nitrate, phosphate, nitrogen, phosphorus, potassium, and microbial counts to evaluate the whey with the best potential with respect to fermentation time—day 12 with pH 5.74, EC 4804 µS/cm, TOC 67.27%, nitrate 394.17 mg/kg, and high microbial growth (819 CFU/ml at 10^{-1}). Bioremediation potential of the whey was evaluated by treating agricultural soil. The day-12 whey results showed that soil treated with 200 ml was best, raising pH from 5.21 to 7.19, nitrogen from 19.10 to 27.18 mg/kg, phosphorus from 12.65 to 14.98 mg/kg, potassium from 6.23 to 12.45 mg/kg. TPH results showed that hexatriacontane rose to 1.17mg/L increased in the treated soil compared to the untreated contaminated soil, indicating its formation as a biodegradation intermediate during the breakdown of heavier hydrocarbons. Hexatriacontane showed a significant reduction to 0.05mg/L after whey treatment, demonstrating effective microbial attack on heavy hydrocarbon fractions. Heavy metals analysis indicates a significant reduction in metal concentration as in iron from 45.67mg/kg to 32.45mg/kg, zinc 18.90 mg/kg to 12.34 mg/kg copper 7.56 mg/kg to 5.12 mg/kg. Day 12 fermented *Panicum maximum* whey gave the best remediation potential.

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CHAPTER ONE

1.1 Introduction

Crude oil contamination of soil is a serious environmental issue with direct effects on agriculture, ecosystems, and human health. In oil-producing countries like Nigeria, frequent spills from drilling, transportation, and illegal refining have turned productive farmlands into toxic zones. The presence of petroleum hydrocarbons in soil reduces its fertility by displacing air and water, killing beneficial microbes, and altering physical structure (Nanadeinboemi *et al*., 2024). This limits plant growth, lowers crop yield, and affects food security. Beyond agriculture, crude oil residues introduce persistent organic pollutants, especially polycyclic aromatic hydrocarbons (PAHs) and heavy metals, into the food chain, increasing risks of cancer and organ damage in humans. Economically, soil contamination reduces land value, increases the cost of land rehabilitation, and imposes long-term health burdens on affected communities (Teixeira *et al.,* 2024). The long residence time of crude oil in soil, particularly its Total Petroleum Hydrocarbon (TPH) content; means contamination can persist for decades without effective intervention.

Several methods have been developed to treat oil-contaminated soil, including physical methods (soil washing, excavation), chemical treatments (oxidation, surfactant flushing), and thermal processes (incineration, vitrification). These approaches often achieve short-term results but are expensive, destructive to soil structure, and impractical for large-scale or rural use (Michael-Igolima et al., 2022). In contrast, bioremediation offers a lower-cost, eco-friendly alternative that relies on microbial activity to break down hydrocarbons into harmless end products like carbon dioxide and water. The success of bioremediation depends on the availability of nutrients, the presence of oil-degrading microbes, and environmental conditions that support microbial metabolism (Bala *et al.*, 2022). In recent studies, the addition of organic materials—especially plant-based by-products—has shown promise in improving microbial growth and activity. These materials act as bio-stimulants, enhancing degradation of hydrocarbons by supplying carbon, nitrogen, and enzymes that support microbial processes (Kuppan *et al.,* 2024). Among these, plant-based whey, a liquid by-product obtained from *Panicum maximum* (guinea grass), is being explored as a potential enhancer in soil treatment.

Whey produced from *Panicum maximum* contains a mix of soluble proteins, amino acids, and sugars that create a nutrient-rich environment for microbial growth. During fermentation, microbial populations develop and multiply in the whey, producing enzymes and metabolites that can assist in breaking down petroleum hydrocarbons. When applied to contaminated soil, the fermented whey introduces both native and beneficial microbes, along with the nutrients needed to support their activity. This can accelerate the breakdown of TPH in the soil by promoting microbial pathways like oxidation, dehalogenation, and mineralization (Malos *et al.,* 2025). The effectiveness of the whey depends on its fermentation stage, as different microbial profiles and chemical changes emerge over time. In this study, whey fermented for 12 days was selected based on prior characterization showing optimal microbial and chemical properties. The aim is to evaluate how this plant-based, microbially active liquid can improve soil quality by degrading total hydrocarbon content and reducing the levels of harmful substances like PAHs and heavy metals. Using a locally available, renewable material like *Panicum maximum* whey not only reduces treatment cost but also supports sustainable soil recovery strategies in regions affected by oil pollution.

1.1.1 Background of Study

Soil contamination from crude oil is a persistent problem due to the complex nature of petroleum hydrocarbons and their interactions with the soil environment. Hydrocarbons in crude oil consist of diverse chemical compounds that resist natural breakdown, causing long-term persistence in contaminated sites. These compounds impact soil quality by disrupting microbial communities and altering nutrient cycles, which limits natural attenuation processes (Nanadeinboemi *et al.,* 2024). While natural degradation occurs, it is often slow and insufficient to restore soil health within a practical timeframe. As a result, enhanced methods are required to accelerate contaminant removal and improve soil conditions. Bioremediation has emerged as an effective approach that uses biological agents—mainly microorganisms—to degrade hydrocarbons in soil (Kuppan *et al.,* 2024). This approach capitalizes on the metabolic versatility of microbes, which produce enzymes capable of transforming complex hydrocarbons into simpler, less harmful substances. The microbial degradation of petroleum hydrocarbons typically involves aerobic and anaerobic processes. Aerobic microbes use oxygen to break down hydrocarbons through oxidative reactions, producing carbon dioxide and water as end products. Anaerobic microbes function in oxygen-deprived conditions, using alternative electron acceptors such as nitrate or sulfate. Both pathways contribute to the reduction of total hydrocarbon content (THC) in contaminated soils (Almutairi, 2024). One challenge in bioremediation is maintaining an environment conducive to microbial activity. Hydrocarbon contamination often leads to nutrient imbalances—especially nitrogen and phosphorus deficiency—that limit microbial growth. Additionally, indigenous microbes may lack sufficient numbers or the metabolic capacity to degrade all petroleum fractions effectively (Rizal *et al.,* 2025). To address these issues, biostimulation and bioaugmentation techniques are employed. Biostimulation adds nutrients or organic amendments to stimulate native microbes, while bioaugmentation introduces specialized hydrocarbon-degrading strains to boost degradation rates (Kuppan *et al.*, 2024).

Plant-based biomaterials have attracted interest as biostimulants and nutrient sources in bioremediation. These biomaterials are sustainable, cost-effective, and rich in organic compounds that support microbial metabolism. Agricultural residues, plant extracts, and fermentation by-products provide carbon, nitrogen, and minerals necessary for microbial growth. They also improve soil structure and moisture retention, creating favorable conditions for microbial communities (Folina *et al.,* 2025). Using plant-based biomaterials aligns with principles of circular economy by valorizing waste products and reducing reliance on synthetic additives. Among promising plant-based materials is whey derived from *Panicum maximum* leaves. *Panicum maximum*, commonly known as Guinea grass, is abundant in many tropical regions and is notable for its high protein and fiber content. Processing the leaves produces whey, a brown liquid fraction rich in soluble proteins, amino acids, and carbohydrates. This whey, when fermented, undergoes microbial transformation that enhances its biochemical profile. Fermentation enriches whey with beneficial microbes and enzymes that can promote hydrocarbon degradation when applied to contaminated soil. The fermentation process modifies whey's nutrient availability and microbial composition over time. Different fermentation periods yield varying levels of microbial biomass, organic acids, and enzymes—all factors influencing its biostimulant potential (Pescuma *et al.,* 2015). Whey fermented for a certain duration (12 days in this study) contains an optimal balance of these components to support indigenous soil microbes and initiate hydrocarbon breakdown. When applied to crude oil-contaminated soil, this fermented whey provides essential nutrients and inoculates the soil with active microbial populations that can accelerate total hydrocarbon degradation.

Microorganisms play a central role in degrading total petroleum hydrocarbons by producing enzymes such as oxygenases, peroxidases, and dehydrogenases. These enzymes catalyze reactions that cleave hydrocarbon chains, facilitating their conversion into simpler molecules that microbes can assimilate (Bhandari *et al.,* 2021). The degradation process occurs in stages: initial breakdown of complex hydrocarbons into intermediate products, followed by mineralization to carbon dioxide, water, and biomass. Effective bioremediation reduces the hydrocarbon concentration, restoring soil's physical and chemical properties and enabling the return of native vegetation and microbial diversity (Kuppan *et al.,* 2024). This study builds on the concept that plant-based fermented whey can serve as both a nutrient source and microbial inoculum for bioremediation. It investigates how the 12-day fermented whey affects the degradation of total hydrocarbon content in crude oil-contaminated soil. This approach leverages a locally available biomaterial to stimulate natural attenuation processes, offering a sustainable alternative to conventional remediation methods. Additionally, it provides insights into optimizing fermentation conditions for maximum biostimulant effect.

1.1.2 Statement of Problem

Crude oil contamination of soil is a widespread environmental problem in many oil-producing regions, including Nigeria. Oil spills during exploration, transportation, and refining release large volumes of petroleum hydrocarbons into agricultural lands and natural ecosystems. These hydrocarbons consist of complex mixtures of aliphatic and aromatic compounds that persist in the soil, causing long-term pollution (Obida *et al.*, 2018). When crude oil contaminates soil, it alters the soil's physical and chemical properties, such as porosity, nutrient content, and pH. This disrupts the natural balance of soil microbial communities responsible for nutrient cycling and organic matter decomposition (Oro *et al.*, 2024). As a result, contaminated soils suffer from reduced fertility and productivity, directly impacting agricultural output. This decline in soil quality not only threatens food security but also contributes to economic losses in farming communities heavily reliant on agriculture (Gomiero, 2016). Moreover, contaminated soils pose health risks to humans and animals through direct contact or through the food chain, due to the bioaccumulation of toxic hydrocarbons (Petruzzelli *et al.,* 2025).

The remediation of crude oil-contaminated soils remains a significant challenge. Conventional cleanup methods such as excavation, soil washing, and chemical treatments are costly, labor-intensive, and can further degrade soil quality. These physical and chemical methods may remove or destroy contaminants but often fail to restore the soil's natural biological functions. Thermal treatments, though effective in destroying hydrocarbons, require high energy inputs and result in the loss of soil organic matter and structure (Ahmad *et al.,* 2020). Additionally, many contaminated sites are located in remote or rural areas where such advanced remediation techniques are not feasible due to high costs and logistical difficulties. These limitations highlight the need for more sustainable, affordable, and ecologically sound remediation alternatives that can restore soil health while effectively reducing hydrocarbon concentrations.

Bioremediation presents a promising solution by using microorganisms to naturally degrade petroleum hydrocarbons. However, its effectiveness depends heavily on soil conditions and the availability of nutrients to support microbial activity. Many contaminated soils lack sufficient nitrogen, phosphorus, and organic carbon to sustain microbial populations capable of hydrocarbon degradation. Furthermore, indigenous microbes may be inhibited by the toxic effects of hydrocarbons or may not possess the metabolic capability to degrade all hydrocarbon fractions (Elshafei and Mansour, 2024). To overcome these barriers, biostimulation through the addition of nutrient-rich amendments is required to enhance microbial growth and enzymatic activity. Plant-based biomaterials, particularly fermented whey extracted from *Panicum maximum* leaves, offer a sustainable and cost-effective source of nutrients and beneficial microbes. Despite its potential, the application of such plant-based fermented products for soil bioremediation remains underexplored. There is a need for detailed studies on how fermented whey can influence microbial communities and accelerate the degradation of total petroleum hydrocarbons (TPH) in contaminated soils. Without this knowledge, crude oil contamination will continue to impair soil ecosystems and agricultural productivity, with ongoing economic and health consequences for affected communities.

1.1.3 Significance of Study

Crude oil contamination of soil poses serious environmental and socio-economic challenges, particularly in regions where agriculture forms the backbone of local livelihoods (Adeniran *et al.,* 2023). This study addresses these challenges by exploring a sustainable bioremediation method using plant-based fermented whey to treat oil-contaminated soils. The significance of this research lies in its potential to offer a low-cost, eco-friendly alternative to conventional remediation techniques that are often expensive, disruptive, or inaccessible. By focusing on fermented whey derived from *Panicum maximum* leaves—a locally available and renewable resource—this study promotes the valorization of agricultural by-products. This aligns with sustainable waste management practices and supports circular economy principles, which can benefit rural communities by providing affordable soil rehabilitation options and reducing environmental pollution. From an environmental perspective, this research contributes to restoring soil health in crude oil-impacted areas by enhancing microbial degradation of total petroleum hydrocarbons (TPH). The study's focus on optimizing the fermentation period of whey to maximize microbial activity and nutrient content is crucial for improving bioremediation efficiency. The introduction of nutrient-rich fermented whey into contaminated soils supports the growth and metabolic functions of hydrocarbon-degrading microbes. This, in turn, accelerates the breakdown of petroleum contaminants, improving soil physicochemical properties and fostering the recovery of native microbial populations. Ultimately, the study offers a practical framework for improving the natural attenuation of hydrocarbons in soils, which is essential for sustainable land use, ecosystem restoration, and reduction of long-term environmental hazards.

On a broader scale, the findings of this study could inform policy and practice in soil remediation efforts within oil-producing regions. Effective bioremediation techniques based on locally sourced biomaterials have the potential to be widely adopted by farmers, environmental agencies, and industries affected by crude oil pollution. This can reduce remediation costs and increase accessibility to clean-up technologies in economically disadvantaged or rural areas. Additionally, enhancing the understanding of plant-based biostimulants and their microbial interactions expands scientific knowledge in environmental biotechnology. By providing data on the microbial, physicochemical, and mineral changes during treatment, this research lays the groundwork for future innovation in soil remediation strategies. Ultimately, the study's significance extends to improving public health, agricultural productivity, and environmental sustainability in communities affected by crude oil contamination.

1.1.4 Scope of Work

This study involves producing and applying fermented whey from *Panicum maximum* leaves for bioremediation of crude oil-contaminated soil. Leaves were collected from the Sport Complex of the University of Benin, Benin City, Edo State. They were chopped, rinsed, and ground into slurry. The slurry was filtered to remove fibrous bagasse, and the filtrate boiled for 30 minutes before cooling. The liquid whey was decanted from the sedimented leaf protein concentrate

(LPC) at the bottom. Whey samples were fermented for different periods: fresh (day 0), and days 3, 6, 9, and 12. Each sample was analyzed for physicochemical, mineral, and microbial properties to identify the optimal fermentation time for bioremediation. Soil was collected from an agricultural farm at the University of Benin, sieved, and dried at room temperature. It was artificially contaminated with crude oil (2 kg soil to 100 ml crude oil) and stabilized for six weeks. The contaminated soil was treated with the optimally fermented whey and allowed to incubate for three weeks. Soil samples—uncontaminated, contaminated, and treated—were analyzed for heavy metal content, physicochemical parameters, and total hydrocarbon content to evaluate the effectiveness of the whey treatment in remediating crude oil pollution.

1.1.5 Scope of work

The aim of this study was to evaluate the effectiveness of fermented Panicum maximum leaf whey in enhancing the bioremediation of crude oil-contaminated soil through microbial degradation of total petroleum hydrocarbons. To achieve this, the following objectives were to;

- produce and characterize whey extracted from Panicum maximum leaves and determine the optimal fermentation period for maximum microbial activity and nutrient content.
- contaminate soil samples with crude oil and simulate contamination conditions for laboratory bioremediation trials.
- apply the optimally fermented whey to crude oil-contaminated soil and monitor changes in microbial populations during treatment.
- analyze the physicochemical and mineral properties of uncontaminated, contaminated, and treated soils to assess soil restoration.
- measure the reduction of total petroleum hydrocarbons (TPH) in contaminated soil following treatment with fermented whey.

1.2 LITERATURE REVIEW

1.2.1 SOIL CONTAMINATION BY CRUDE OIL

Crude oil is a naturally occurring liquid mixture of hydrocarbons that is extracted from underground reservoirs. It consists of thousands of organic compounds, mainly carbon and hydrogen, along with trace amounts of sulfur, nitrogen, oxygen, metals, and salts. When released into the environment, crude oil does not break down easily. Its complex structure and persistence in soil make it a major environmental contaminant.

Soil contamination by crude oil occurs when hydrocarbons enter the soil, either through direct spills, leaks, or slow seepage. Once in the soil, crude oil coats soil particles and fills pore spaces, which interferes with the movement of air and water. This changes the physical, chemical, and biological makeup of the soil. Native microorganisms struggle to survive, nutrient cycles are disrupted, and essential soil functions like water retention, aeration, and organic matter decomposition are slowed or halted.

Contaminated soils become compacted, waterlogged, and nutrient-deficient. These changes reduce the ability of the soil to support plant life and disturb the balance of microbial communities that normally regulate nutrient cycling and organic matter breakdown. Crude oil contamination also affects soil pH, increases hydrophobicity, and interferes with ion exchange processes. These disruptions directly reduce agricultural productivity and delay natural soil recovery (Oro *et al.*, 2024).

1.2.1.1 Composition and Properties of Crude Oil Hydrocarbons

Crude oil is a chemically diverse material composed mainly of hydrocarbons. These hydrocarbons fall into three main groups: alkanes, cycloalkanes, and aromatic hydrocarbons.

Alkanes are straight or branched chains of carbon atoms with single bonds. They are the simplest and most biodegradable component of crude oil. Cycloalkanes are hydrocarbons arranged in ring structures and are more resistant to microbial degradation. Aromatic hydrocarbons contain one or more benzene rings. These compounds are more stable, toxic, and persistent in the environment.

In addition to hydrocarbons, crude oil contains small but significant amounts of sulfur compounds, nitrogen compounds, resins, asphaltenes, metals like vanadium and nickel, and salts. These non-hydrocarbon components affect the toxicity and behavior of oil in the environment. For example, resins and asphaltenes are heavy fractions that bind tightly to soil particles, making them difficult to remove. Aromatic hydrocarbons are particularly dangerous due to their carcinogenic and mutagenic effects.

Crude oil's physical properties—such as viscosity, density, solubility, and volatility—determine how it spreads and interacts with soil. Light crude oils are more volatile and spread faster, while heavy crudes penetrate soil more slowly but are harder to degrade. The hydrophobic nature of hydrocarbons causes them to repel water and bind to organic matter in the soil. This affects water movement, oxygen availability, and microbial access to nutrients, all of which are critical for soil health and bioremediation efforts.

1.2.1.2 Sources and Pathways of Soil Contamination

Soil contamination by crude oil happens through multiple human activities, particularly in oil-producing regions. The primary sources include oil exploration, production, storage, transportation, refining, and usage. In many cases, leaks from pipelines, ruptured storage tanks, well blowouts, or spills during loading and unloading processes release crude oil directly into the environment. In oil-rich areas, illegal refining and sabotage of infrastructure also contribute to frequent spills.

Once released, crude oil enters the soil through surface contact or infiltration. On reaching the ground, oil can percolate through the soil profile depending on soil type, porosity, and moisture content. In coarse, sandy soils, oil can move more freely and spread deeper, while in clay soils, movement is slower but can still cause long-term retention. Rainfall can further drive oil deeper into the soil or facilitate lateral movement across fields, expanding the contamination zone.

In agricultural settings, oil-contaminated water used for irrigation, or the improper disposal of drilling waste and spent oil, introduces hydrocarbons into farmland. In some industrial zones, routine machine operation and storage practices allow slow accumulation of oil residues in soil over time. Accidental or chronic releases eventually lead to widespread soil pollution, especially in areas with poor spill control and cleanup systems. Once present, crude oil can remain in the soil for years without effective remediation.

1.2.1.3 Environmental and Health Impacts of Crude Oil Contamination

The presence of crude oil in soil has widespread environmental consequences. It reduces biodiversity by harming soil microorganisms, plants, and animals that rely on healthy soil for survival. Oil-contaminated soil supports fewer microbial species, and many of the surviving microbes become inactive due to lack of oxygen and toxic conditions. The loss of microbial diversity means essential processes like nitrogen fixation, organic matter degradation, and mineralization slow down or stop entirely (Mukjang *et al.,* 2022).

Plant roots struggle to absorb water and nutrients in oil-contaminated soil. This leads to stunted growth, chlorosis, reduced yields, or complete failure of crops. In ecosystems, native vegetation is displaced, allowing more tolerant but less beneficial species to take over. Wildlife that depends on vegetation for food or shelter is also affected. In wetlands and aquatic environments, oil-soaked soil contributes to water pollution and the collapse of food chains.

For humans, the risks are both direct and indirect. People exposed to contaminated soil can suffer from skin irritation, respiratory issues, or long-term effects from inhaling volatile compounds (Mohammed *et al.,* 2024). Contaminants can also leach into groundwater and surface water, making their way into drinking supplies. Food grown in polluted soil may accumulate harmful substances, leading to chronic health problems such as liver damage, neurological disorders, or cancer. In oil-producing communities, these environmental and health issues translate into reduced economic opportunities, long-term displacement, and increased healthcare burdens (Ordinioha and Brisibe, 2013).

1.2.2 EFFECTS OF CRUDE OIL CONTAMINATION ON SOIL QUALITY

Crude oil contamination does not just affect the surface of the soil. It changes what the soil is, how it functions, and what it can support. These changes happen at the chemical, physical, and biological levels. Once oil enters the soil, it alters its structure, blocks natural processes, and introduces toxic compounds. This affects the soil's capacity to support life, store nutrients, regulate water, and break down organic matter (Petruzzelli *et al.,* 2025). The overall result is a soil system that no longer behaves normally and struggles to recover without intervention.

1.2.2.1 Changes in Soil Physicochemical Properties

Physicochemical properties define how soil holds and exchanges water, air, nutrients, and heat. When crude oil contaminates soil, it interferes with these properties. One major change is the coating of soil particles with oil. This oily film reduces the soil's ability to absorb and retain water. Water moves more slowly or gets blocked altogether, which reduces the availability of moisture to plant roots and soil microbes (Wang *et al.,* 2013).

Soil porosity is also affected. Oil clogs the pore spaces between particles, reducing air circulation. This limits the amount of oxygen available to support aerobic microbial activity and root respiration. In many cases, this leads to anaerobic zones that encourage the growth of slower, less efficient microbes (McCarter *et al.,* 2020).

Another effect is on soil pH. Crude oil can change the balance of hydrogen ions in soil, especially when acidic degradation products form during oil breakdown. This pH shift can inhibit plant nutrient uptake and reduce the activity of key soil enzymes. Soil electrical conductivity may also increase due to the introduction of heavy metals and salts that often accompany crude oil. These salts compete with essential nutrients like calcium, magnesium, and potassium, affecting nutrient exchange and plant health (Briffa *et al.*, 2020).

Oil also reduces cation exchange capacity, especially in clay-rich soils. Cation exchange capacity is how soil stores and releases nutrients. When oil binds to clay or organic matter, it limits the surface area available for nutrient exchange (Ramos *et al.*, 2018). This disrupts the soil's natural buffering system and reduces its ability to hold onto nutrients.

Overall, crude oil turns a well-structured, porous, and chemically balanced soil into a compacted, poorly aerated, and nutrient-poor medium. These changes set the stage for biological disruptions.

1.2.2.2 Impact on Soil Microbial Communities and Nutrient Cycles

Microbial communities are the drivers of most soil functions. They regulate nutrient cycles, decompose organic matter, and help plants access nutrients. When oil enters the soil, it changes the microbial population immediately (Wang et al., 2024). Many native bacteria, fungi, and actinomycetes cannot survive in the new conditions. The toxic hydrocarbons damage their cell membranes, limit their enzyme activity, or deprive them of oxygen.

In place of the native microbes, a few oil-tolerant or oil-degrading species may survive. These include certain species of *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Aspergillus*. However, these species often grow slowly and may not be diverse or abundant enough to fully restore the soil system (Rafeeq *et al.,* 2023). In addition, these organisms may degrade only specific fractions of the oil, leaving behind more resistant components like long-chain alkanes, resins, or asphaltenes.

Nutrient cycles slow down or stop in oil-contaminated soils. The carbon cycle becomes overloaded with petroleum hydrocarbons that microbes cannot break down efficiently. The nitrogen cycle is disrupted as nitrifying bacteria die off or reduce their activity. This limits the conversion of ammonia to nitrate and decreases nitrogen availability to plants. The same happens with phosphorus and sulfur cycles. Without a stable microbial population, key nutrients become locked in unavailable forms.

The loss of microbial diversity also means a loss of competition and regulation. Opportunistic pathogens may thrive, while beneficial symbionts like mycorrhizal fungi and nitrogen-fixing bacteria are reduced (Das and Chandran, 2011). This affects plant health and weakens the natural defense systems of the soil.

In short, oil contamination changes the soil from a biologically active and self-regulating system to one that is imbalanced, slow to respond, and chemically unstable. These microbial shifts make long-term recovery difficult without targeted remediation.

1.2.2.3 Consequences for Agricultural Productivity and Ecosystem Health

The combined chemical and biological changes from oil contamination have direct effects on agriculture. First, oil-contaminated soils cannot support healthy plant growth. Germination rates drop due to reduced water availability and toxic conditions. Seedlings show poor root development and stunted shoots. Mature crops display yellowing leaves, slow growth, and low yields. In severe cases, plants fail to survive beyond early stages of development (Hussain *et al.*, 2019).

The roots of plants are especially vulnerable. Oil blocks water and nutrient uptake. It can also introduce toxic metals that accumulate in plant tissues. This contamination affects not only yield but also food safety. Crops grown in such soil may contain harmful residues that make them unsafe for consumption.

From a farming standpoint, land recovery becomes difficult and expensive. Farmers face years of low productivity, increased input costs, and reduced income (Gomiero, 2016). In many cases, the land must be abandoned unless it is treated effectively.

At the ecosystem level, oil-contaminated soil leads to reduced biodiversity. Native plants are replaced by oil-tolerant weeds. Soil invertebrates like earthworms, which play a major role in nutrient cycling and soil structure, disappear. Birds, insects, and mammals that rely on the vegetation or soil organisms either relocate or die off.

Water quality is also affected. Runoff from oil-polluted soil can carry hydrocarbons into streams and groundwater. This contaminates drinking water sources and aquatic habitats. The long-term presence of crude oil residues in the soil means these impacts continue for years unless active remediation is applied (Fei-Baffoe *et al.,* 2024).

In summary, crude oil contamination weakens the entire soil system. It reduces productivity, destroys biodiversity, and disrupts the natural balance of chemical and biological processes. The land becomes less useful for farming, less hospitable to life, and more difficult to recover.

1.2.3 CONVENTIONAL METHODS OF SOIL REMEDIATION

Soil remediation is the process of removing, reducing, or neutralizing pollutants in soil to restore its function. Crude oil contamination, due to its chemical complexity and persistence, requires technical methods to manage. Over time, several conventional methods have been developed. These include physical, chemical, and in some cases, thermal techniques (Ahmad *et al.,* 2020). While these methods are widely used, they often have drawbacks related to cost, efficiency, and environmental impact.

1.2.3.1 Physical Remediation Techniques

Physical remediation removes or isolates contaminants from the soil without changing their chemical structure. These techniques are often used for heavily polluted sites or where fast cleanup is needed.

1. Excavation

Excavation is the direct removal of contaminated soil from a site. The soil is dug up using machinery and transported to a landfill or treatment facility. It is one of the fastest ways to remove large amounts of polluted soil. Often used in emergency spills or where contamination is shallow and easy to access.

Limitations of excavation:

Very expensive due to equipment, labor, transport, and disposal costs (Alori *et al.,* 2022).

Only shifts the problem to another location, unless followed by proper treatment. Disrupts the site completely, affecting soil structure, vegetation, and nearby ecosystems.

2. Soil Washing

Soil washing uses water or other liquids to remove contaminants from soil particles. The soil is mixed with water and a washing agent such as a surfactant or chelating agent (Tiwari and Tripathy, 2023). Contaminants are separated from the soil and collected from the liquid phase. Clean soil is either returned to the site or stored for reuse.

Strengths of soil washing:

- Can be applied on site or off site.
- Reduces waste volume compared to full excavation and disposal.
- Effective for metals and some organic pollutants.

Limitations of soil washing:

- Works better on coarse soils like sand or gravel.
- Less effective on clay or organic-rich soils because contaminants bind tightly.
- Generates large amounts of wastewater that need separate treatment.

1.2.3.2 Chemical Remediation Methods

Chemical remediation uses chemical reactions to either break down, stabilize, or remove contaminants from the soil. These methods are chosen when the contaminants are chemically active or when physical methods are not practical.

1. Chemical Oxidation

Chemical oxidation involves adding strong oxidizing agents to the soil to break down hydrocarbons into simpler, non-toxic substances. Common oxidants include hydrogen peroxide, potassium permanganate, ozone, and sodium persulfate. Oxidants react with the carbon chains of petroleum hydrocarbons, converting them into carbon dioxide, water, and less harmful residues (Karpenko *et al.*, 2009).

Advantages of chemical oxidation:

- Works quickly and can treat soil in place without excavation.
- Reduces total petroleum hydrocarbons and improves soil quality.

Challenges with oxidation:

- Oxidants can be toxic to soil microbes and plants.
- Requires precise dosing to avoid excess chemical use.
- May not reach deep or tightly bound contaminants in low-permeability soils.

2. Chemical Stabilization

Chemical stabilization does not remove contaminants but renders them less mobile or less toxic by binding them to soil particles or converting them into inert forms (Petrillo *et al.*, 2025). Additives like lime, cement, fly ash, or phosphate compounds are mixed with the soil. These additives react with contaminants to reduce solubility and bioavailability.

Applications of stabilization:

- Often used for heavy metals and certain persistent organics.
- Stabilized soils can be reused for construction or contained with barriers.

Limitations of stabilization:

- Contaminants remain in the soil, so long-term monitoring is needed.
- Not suitable for sites where future land use requires uncontaminated soil.
- May alter soil pH and texture, affecting its future fertility.

1.2.3.3 Limitations and Challenges of Conventional Approaches

While conventional remediation techniques have been applied for decades, they come with significant tradeoffs. Their limitations highlight the need for alternative or combined strategies such as bioremediation.

1. High Costs and Infrastructure Demand

- Most conventional methods require heavy machinery, technical expertise, and large energy input.
- Off-site treatment adds transport and disposal expenses.
- Not suitable for rural or low-income communities due to cost barriers.

2. Environmental Disruption

- Excavation and washing remove topsoil and disturb the ecosystem.
- Soil organisms are killed or displaced.
- Natural soil functions like water filtration, nutrient cycling, and plant support are damaged.

3. Partial or Temporary Solutions

- Chemical methods like stabilization do not remove pollutants but just lock them in place.
- If site conditions change (such as rainfall or pH), contaminants can be remobilized.
- Oxidation may not fully degrade all fractions of crude oil, leaving behind resistant residues.

4. Limited Adaptability to Complex Soil Types

- Fine-textured soils with high clay or organic matter content are difficult to treat.
- Binding between soil particles and pollutants reduces accessibility to washing or chemical agents.
- uniform treatment is hard to achieve across large, heterogeneous areas.

5. Waste Generation and Secondary Pollution

- Soil washing produces contaminated water that needs treatment.
- Chemical treatments can produce secondary pollutants, depending on the reagents used.
- Transporting and disposing of excavated soil creates emissions and environmental risks.

1.2.4 BIOREMEDIATION AS A SUSTAINABLE REMEDIATION STRATEGY

Bioremediation is a method that uses living organisms, especially microbes, to remove or reduce harmful substances in contaminated environments. In the context of soil polluted with crude oil, bioremediation is increasingly seen as a cost-effective and environmentally sound alternative to physical or chemical methods. Rather than removing the polluted soil or neutralizing it with chemicals, bioremediation works by enhancing the natural ability of microbes to break down petroleum hydrocarbons into simpler, non-toxic compounds (Uwidia and Uwidia, 2021). This process does not only remove the contaminant but also helps in restoring soil health.

Bioremediation is considered sustainable because it relies on naturally occurring processes. It works with the soil's existing biological system rather than replacing or damaging it. It is also adaptable to different site conditions and can be applied in place, reducing the need for excavation or transport (Bala *et al.,* 2022). In areas with limited access to industrial cleanup facilities, bioremediation offers a realistic solution, especially when supported with locally available organic materials such as compost, manure, or plant-based stimulants like fermented whey.

1.2.4.1 Principles and Mechanisms of Bioremediation

The principle of bioremediation is simple: microbes consume hydrocarbons as a food source and, in the process, convert them into less harmful substances. This biological breakdown occurs through natural metabolic pathways. For hydrocarbons, the end products are typically carbon dioxide, water, microbial biomass, and sometimes small organic acids.

There are two major approaches:

- **Intrinsic or natural attenuation**

This occurs without human intervention. Indigenous microbes in the soil slowly break down hydrocarbons over time. However, natural attenuation is usually too slow to meet cleanup goals in a reasonable period.

- Engineered bioremediation

This involves adding nutrients, oxygen, or microbes to speed up degradation. The goal is to create favorable conditions for microbes to grow and produce the right enzymes to attack hydrocarbon molecules (Joutey *et al.,* 2013).

The mechanism begins when microbes detect hydrocarbons and attach to them. Enzymes produced by the microbes oxidize the hydrocarbon chains, introducing oxygen atoms and breaking them into smaller molecules. These reactions are often catalyzed by enzymes like oxygenases and peroxidases. The smaller fragments are then further metabolized through standard cellular pathways like the citric acid cycle, eventually leading to full degradation.

The process is aerobic in most cases, meaning it requires oxygen. Anaerobic bioremediation, which occurs without oxygen, is possible but generally slower and requires different microbial species and electron acceptors such as nitrate or sulfate.

1.2.4.2 Role of Microorganisms in Hydrocarbon Degradation

Microorganisms are the central agents in bioremediation. They include bacteria, fungi, and sometimes archaea. These organisms produce specific enzymes that target hydrocarbons and convert them into simpler forms (Uwidia and Uwidia, 2021).

- *Bacteria*: Bacteria are the most common and effective group for hydrocarbon degradation. Some well-studied oil-degrading bacteria include:
- *Pseudomonas* :Known for degrading a wide range of hydrocarbons and producing biosurfactants that increase hydrocarbon availability.
- *Bacillus:* Spore-forming and resilient, *Bacillus* species can survive harsh environments and degrade both light and heavy oil fractions.
- *Acinetobacter* and *Rhodococcus*: These species are efficient in breaking down aromatic compounds and long-chain alkanes. These bacteria work best in aerobic conditions and can be stimulated by adding nutrients or organic matter.
- Fungi: Fungi such as *Aspergillus* and *Penicillium* can degrade hydrocarbons, especially in acidic soils or nutrient-poor environments where bacteria perform poorly. Fungi use extracellular enzymes like lignin peroxidase and laccase, which can break down complex aromatic structures.

Microbial Interactions

Hydrocarbon degradation is often a community effort. One microbe may break down a complex compound into simpler pieces that others can metabolize (Ławniczak *et al.*, 2020). The presence of multiple species working together usually improves degradation efficiency.

Some microbes also produce biosurfactants. These are natural compounds that reduce surface tension and help emulsify oil, making hydrocarbons more available to microbial cells.

1.2.4.3 Factors Influencing Bioremediation Efficiency

The success of bioremediation depends on environmental conditions and the characteristics of the contaminant. Even with capable microbes present, poor conditions can limit or completely block hydrocarbon degradation.

1. Nutrients

Microbial growth requires more than just carbon from hydrocarbons. Nitrogen and phosphorus are essential for building proteins, enzymes, and cell membranes. Crude oil provides carbon but lacks nitrogen and phosphorus (Kebede *et al.,* 2021). If these nutrients are limited, microbial activity slows or stops. Nutrient sources such as compost, animal manure, or plant-based additives like fermented whey are often added to supply what is missing. The right balance is important. Excess nitrogen or phosphorus can cause imbalances and may lead to nutrient leaching.

2. Oxygen Availability

Most hydrocarbon-degrading microbes require oxygen to function. Oxygen is needed to activate enzymes that break hydrocarbon bonds (Peixoto *et al.,* 2011). In compacted or waterlogged soils, oxygen becomes limited, reducing degradation rates. Techniques such as tilling or adding porous materials can improve oxygen flow. In anaerobic systems, special microbes can still degrade hydrocarbons but require other electron acceptors like nitrate or sulfate.

3. Temperature

Temperature affects the metabolism of microbes and the fluidity of hydrocarbons. Most soil bacteria work best between 25 and 35 degrees Celsius (Pietikäinen *et al.,* 2005). Below this range, microbial activity slows, and oil becomes more viscous, making it harder to access. At high temperatures, some enzymes may denature, and microbial cells can die. In colder climates, bioremediation may require insulation or heating to maintain activity.

4. Soil Type and Structure

Sandy soils allow better oxygen and water movement but may dry out quickly. Clay-rich soils retain water and nutrients but may limit oxygen diffusion. Organic matter in soil can bind with hydrocarbons, reducing their bioavailability. Adjusting moisture and improving soil structure with amendments can help.

5. Contaminant Properties

The type and age of the oil matter. Light hydrocarbons are easier to degrade than heavy fractions. Fresh spills are more bioavailable, while weathered oil may form hard-to-degrade residues. Some compounds like resins and asphaltenes are highly resistant and require specialized microbes or longer treatment times.

1.2.5 USE OF PLANT-BASED BIOMATERIALS IN BIOREMEDIATION

Bioremediation depends heavily on microbial activity, but microbes often need support to function well in contaminated soil. One effective way to improve microbial action is by adding organic materials that supply nutrients, carbon, and structural support.

1.2.5.1 Types of Plant-Derived Biomaterials and Their Properties

Plant-derived biomaterials come in different forms, depending on how they are processed and the part of the plant used. Common types include leaf extracts, compost, green manure, crop res Plant-based biomaterials provide these benefits naturally (Ahmed *et al.*, 2023). These materials are derived from agricultural waste, plant residues, and processed plant products. They can improve soil structure, increase nutrient availability, and stimulate microbial growth. Most importantly, they are widely available, cheap, and renewable. For communities dealing with oil pollution in farming areas, using plant-based inputs is both practical and sustainable.

idues, and fibrous by-products.

1. Leaf extracts and plant juices

These are liquids obtained from fresh plant leaves, often by grinding and filtering. They contain soluble sugars, amino acids, minerals, and organic acids. These compounds support microbial metabolism and act as growth stimulants. Some also contain enzymes and bioactive compounds that can directly help break down hydrocarbons or trigger microbial enzyme production.

2. Compost and green manure

Compost is made from decomposed organic matter, including plant waste, while green manure refers to plant biomass incorporated into the soil before full decomposition (Ahmed *et al.,* 2023). Both supply organic carbon, nitrogen, and minerals. Their slow decomposition releases nutrients gradually, which helps maintain steady microbial activity over time. Compost also improves soil texture and increases its water-holding capacity.

3. Crop residues and husks

Materials like maize stalks, rice husks, cassava peels, or sugarcane bagasse contain cellulose, hemicellulose, and lignin (Riseh *et al.,* 2024). These fibrous components provide a carbon source for fungi and actinomycetes that can break down complex hydrocarbons. While not immediately digestible, their presence boosts the structural diversity of the soil and supports long-term microbial colonization.

4. Processed plant products

Some biomaterials are made by drying, fermenting, or chemically treating plant matter. Examples include leaf protein concentrates, fermented plant whey, or charred biomass like biochar. These forms may be richer in specific nutrients or more stable in soil environments. Fermented products especially are rich in beneficial microbes, organic acids, and vitamins that support active remediation.

Chemical and physical properties

- High carbon to nitrogen ratios provide sustained energy to microbes
- Rich in trace minerals like calcium, magnesium, and potassium that support enzyme function
- Porous structure improves aeration and moisture retention
- Bioactive compounds can act as co-factors or inducers for microbial enzymes

These properties make plant-based biomaterials especially suitable for long-term remediation projects, where maintaining microbial activity over several weeks or months is necessary.

1.2.5.2 Role of Plant-Based Amendments in Stimulating Microbial Activity

Microbes need a stable supply of nutrients and energy to grow and break down contaminants. In many oil-contaminated soils, carbon is present in excess, but nitrogen, phosphorus, and other growth elements are lacking. Plant-based amendments correct this imbalance (Kumar and Gopal, 2015). They act as biostimulants that enhance the survival, growth, and enzyme production of indigenous or introduced microbial populations.

1. **Nutrient supply**

Plant materials release essential elements such as nitrogen, phosphorus, sulfur, and trace metals. These nutrients are taken up by microbes to build proteins, nucleic acids, and other cell components. A balanced nutrient environment leads to faster microbial growth and better enzyme expression.

2. Carbon source and energy

Even though hydrocarbons are rich in carbon, they are not always usable by all microbes. Plant-based carbon is more accessible, especially in forms like sugars, starches, and cellulose breakdown products. This readily available carbon fuels microbial respiration and increases biomass.

3. Soil structure improvement

Decomposing plant matter increases organic matter content in soil, making it more friable and porous. This improves air and water movement, which are both important for microbial metabolism. Better aeration supports aerobic degradation of petroleum compounds.

4. pH buffering and detoxification

Some plant residues have a buffering effect, reducing extreme acidity or alkalinity. A stable pH supports diverse microbial communities. In addition, some compounds in plants can bind to heavy metals or toxic oil fractions, reducing their bioavailability and allowing microbes to function more effectively.

5. Stimulation of specific microbial groups

Certain plant amendments encourage the growth of particular groups of microbes. For example, cellulose-rich materials may favor fungi, while protein-rich materials like legume residues may promote bacterial activity. This allows the remediation process to be tailored to the contaminant type and site conditions.

6. Delivery of beneficial microbes

Fermented plant products can carry living bacteria or fungi into the soil. These introduced species may have better hydrocarbon-degrading ability than the native soil microbes. The fermentation process itself often increases microbial diversity and enzyme activity.

By creating a more hospitable environment and supplying key resources, plant-based amendments turn the contaminated soil into a system that supports microbial clean-up. This reduces the need for synthetic fertilizers or chemical stimulants.

1.2.5.3 Examples of Agricultural Waste and By-Products Used in Soil Remediation

Across different regions, a variety of agricultural wastes and plant residues have been tested and used for remediating petroleum-contaminated soils. These materials are often available locally and can be applied without advanced processing.

1. **Cowpea husk and maize stalk**

These residues are common in crop-producing areas. They have been shown to improve hydrocarbon degradation by supplying carbon, nitrogen, and cellulose. Their fibrous structure also supports fungal colonization, which is useful for breaking down complex hydrocarbons.

2. **Cassava peels and yam peels**

Rich in starch, cassava and yam peels are quickly broken down by microbes. When used in moderate quantities, they stimulate microbial respiration and increase total microbial biomass. They also release simple sugars that enhance microbial energy supply.

3. **Sugarcane bagasse**

This fibrous waste is high in lignocellulose and provides long-term support for fungal growth. It helps maintain soil porosity and moisture content. Its slow degradation provides a sustained release of carbon.

4. Oil palm fiber and kernel shell

In oil-producing regions, these wastes are available in large volumes. When composted or shredded, they serve as stable carbon sources that can support hydrocarbon-degrading bacteria and fungi. Their lignin content also contributes to detoxification of polyaromatic hydrocarbons.

5. Compost from mixed plant waste

Compost made from garden waste, food scraps, or crop residues provides a balanced nutrient source. It introduces diverse microbes and improves soil texture. In oil-contaminated fields, compost enhances microbial breakdown of hydrocarbons and helps restore soil fertility.

6. Fermented leaf whey from grasses like Panicum maximum

Produced by boiling and fermenting leaf slurry, this liquid contains soluble organic compounds, vitamins, and living microbes. It acts as a liquid amendment that directly adds both nutrients and microbial inoculum. Studies show that whey fermented for several days offers better results than fresh or poorly fermented versions.

7. Banana peels and fruit waste

These soft wastes decompose quickly and release sugars, acids, and minerals. In small quantities, they jumpstart microbial activity. However, they must be used carefully to avoid anaerobic conditions due to fast decay.

These examples show that plant-based remediation inputs are not limited to one type of biomass. With basic preparation, many agricultural residues can be converted into useful amendments. Selection depends on availability, soil type, and the type of contaminant present.

1.2.6 WHEY AND LEAF PROTEIN CONCENTRATES (LPC) IN ENVIRONMENTAL APPLICATIONS

Plant-based extracts are gaining attention in environmental remediation for their low cost, sustainability, and ease of preparation. Among these, whey and leaf protein concentrates (LPC) derived from green plant leaves are valuable because they contain useful organic and inorganic compounds that can support microbial activity in soil (Hadidi *et al.,* 2023)l. These materials, which were once considered agricultural waste, now have applications in soil restoration, biostimulation, and waste treatment. In particular, green whey, the liquid left after extracting proteins from leaves, is rich in water-soluble nutrients and fermentation products that can improve the breakdown of petroleum hydrocarbons in contaminated soils.

In environmental chemistry, using leaf-derived whey fits into the growing interest in nature-based solutions (AKAY AND SERT, 2020). When combined with biological remediation techniques, plant-based whey and LPCs serve both as nutrient carriers and microbial growth promoters. Their use aligns with principles of circular economy, where agricultural residues are recycled into tools for soil and water cleanup. This method is especially useful in places with limited access to commercial soil treatment products. Recent studies suggest that when properly processed and fermented, leaf whey can perform as well as synthetic biostimulants (Zeng *et al.,* 2024). This positions it as a smart and scalable solution for restoring polluted environments in both rural and urban settings.

1.2.6.1 Composition and Characteristics of Whey from Plant Leaves

Whey from plant leaves is a greenish liquid obtained during the separation of leaf protein concentrates (Zeng et al., 2024). It is mostly made up of water, but contains a complex mix of dissolved organic and inorganic compounds. These include amino acids, organic acids, vitamins, minerals, sugars, phenolic compounds, and soluble proteins that do not coagulate during heating. Its composition varies with the plant species, age of the leaf, processing conditions, and duration of extraction. In the case of Panicum maximum (a tropical grass), the whey contains nitrogen-rich compounds, trace elements such as potassium, calcium, magnesium, and iron, and a moderate load of naturally occurring lactic acid bacteria, especially if left to ferment (Sokupa *et al.,* 2023).

The presence of sugars and amino acids makes leaf whey highly biodegradable and suitable as a microbial substrate. It serves as a carbon and nitrogen source for soil bacteria and fungi, making it a good candidate for biostimulation in remediation processes. The presence of organic acids also lowers the pH slightly, which can help mobilize certain heavy metals in contaminated soils. Additionally, the high water content allows it to penetrate soil pores and spread nutrients evenly. Because it is plant-based and contains no synthetic additives, it breaks down safely in the environment. This makes it compatible with both microbial and phytoremediation strategies.

1.2.6.2 Fermentation Processes and Effects on Whey Properties

Fermentation of plant leaf whey is a natural biological process driven by native or added microorganisms. During fermentation, bacteria and fungi consume the sugars and proteins in the liquid, producing organic acids, gases, and other secondary metabolites (Abbaspour, 2024). Over time, the microbial population shifts, and new compounds such as lactic acid, ethanol, and short-chain fatty acids accumulate. This biochemical change affects the composition, pH, odor, and microbial load of the whey. The longer the fermentation, the more acidic and microbially diverse the liquid becomes. In a controlled setting, fermentation can be guided to increase the abundance of specific microbial strains known to assist in soil remediation.

As fermentation progresses, the effectiveness of the whey as a soil amendment can change (Malos *et al.,* 2025). For instance, freshly extracted whey may have more labile sugars and proteins, but a shorter shelf life and weaker microbial impact. In contrast, whey fermented for 9 to 12 days usually contains a more stable microbial community and bioactive compounds that promote microbial growth in soil. These include organic acids that enhance hydrocarbon solubility and low molecular weight compounds that can trigger enzyme activity in native microbes. The reduction in pH also supports the breakdown of certain petroleum components. Overall, fermentation increases the bioremediation potential of plant whey by enhancing its biological and chemical interactions with contaminated soil.

1.2.6.3 Potential of Whey as a Biostimulant in Soil Remediation

Whey from plant leaves acts as a biostimulant by supplying nutrients and microbial support to contaminated soils. It stimulates the activity of indigenous or introduced microbes, which are responsible for breaking down pollutants like crude oil hydrocarbons. The soluble carbon, nitrogen, and minerals in whey serve as immediate fuel for microbial metabolism, helping them to grow, reproduce, and produce enzymes needed for biodegradation (Zeng *et al.*, 2024). This microbial stimulation is essential in oil-contaminated soils, which often lack the nitrogen and phosphorus needed for efficient hydrocarbon breakdown. Unlike synthetic fertilizers, whey provides these nutrients in a balanced, low-toxicity form that integrates well with the soil system.

In addition to nutrient delivery, fermented whey also improves soil structure and moisture retention, especially in degraded or sandy soils. Its liquid form helps it move through soil pores, reaching deeper contamination zones. The organic acids formed during fermentation can increase the solubility of hydrophobic petroleum compounds, making them easier for microbes to access (Malos *et al.,* 2025). Some studies also report that fermented whey introduces beneficial microbial strains directly into the soil, which may compete with or suppress harmful microbes. This added biological diversity helps stabilize the soil ecosystem and speeds up recovery. These combined effects make plant-based whey a practical and sustainable biostimulant for crude oil remediation, especially in low-input or rural settings.

1.2.7 MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBONS

Petroleum hydrocarbons are complex organic compounds that persist in soil after oil spills or crude oil contamination. Over time, these hydrocarbons disrupt soil structure, harm microbial life, and impair plant growth. However, certain microorganisms have evolved mechanisms to break down these compounds and convert them into less harmful forms (Chunyan *et al.,* 2023). This biological process, known as microbial degradation, is a core principle of bioremediation. It relies on the metabolic capacity of bacteria and fungi to use hydrocarbons as a source of energy and carbon. Through a series of enzymatic reactions, microbes break down even complex hydrocarbons into simpler molecules like carbon dioxide, water, and biomass.

In contaminated soils, microbial degradation offers a sustainable way to restore ecological function without removing or replacing the soil. Instead of relying on chemical treatments or excavation, it encourages in-place breakdown of pollutants using natural processes. The speed and efficiency of degradation depend on several factors, including the type of hydrocarbon, the availability of oxygen and nutrients, and the diversity of microbial species present (Bala et al., 2022). In recent years, interest has grown in the use of organic amendments like fermented plant whey to boost microbial activity. These biostimulants support native microbes and may introduce beneficial strains, improving both the rate and completeness of hydrocarbon degradation in soil.

1.2.7.1 Key Microbial Species Involved in Hydrocarbon Degradation

Several microbial species have the natural ability to degrade petroleum hydrocarbons. These organisms are found in various environments, including oil-contaminated soils, marine sediments, and compost piles. Among bacteria, *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, and *Mycobacterium* are the most studied and widely distributed hydrocarbon degraders (Qattan, 2025). These bacteria can use alkanes, aromatic hydrocarbons, and complex oil fractions as energy sources. *Pseudomonas aeruginosa*, for example, is well known for its ability to degrade a wide range of hydrocarbon compounds and produce biosurfactants that increase oil solubility. *Bacillus subtilis* is another efficient degrader that tolerates harsh environmental conditions and enhances oil emulsification through enzyme production.

Fungi also contribute to hydrocarbon degradation, particularly in soils with low nitrogen or acidic pH where bacteria perform poorly. Fungal species like *Aspergillus*, *Penicillium*, and *Fusarium* produce extracellular enzymes capable of oxidizing long-chain hydrocarbons and polycyclic aromatic compounds. Fungi are particularly effective in breaking down complex or recalcitrant fractions of petroleum due to their ability to penetrate solid substrates and secrete powerful oxidizing enzymes (Dinakarkumar *et al.,* 2024). Actinomycetes, especially those from the genus *Streptomyces*, play a supporting role by degrading complex hydrocarbons and producing secondary metabolites that suppress competing organisms. The diversity of microbial species involved makes degradation more robust and adaptable under different environmental conditions.

The combination of aerobic and facultative anaerobic microbes creates a dynamic degradation system. Aerobes dominate in oxygen-rich soils, where they use oxygen to activate hydrocarbon molecules. Anaerobes or facultative species take over in compacted or waterlogged soils, using alternative electron acceptors like nitrate or sulfate (Cason *et al.,* 2019). Together, these microbial groups contribute to the complete breakdown of petroleum hydrocarbons in contaminated soils. Their activity depends not only on their genetic capacity but also on the physical and chemical conditions of the soil and the presence of supporting nutrients.

1.2.7.2 Enzymatic Pathways for Breaking Down Hydrocarbons

The degradation of petroleum hydrocarbons is driven by microbial enzymes that initiate and complete the breakdown of complex molecules. These enzymes recognize specific hydrocarbon structures and modify them through oxidation, hydroxylation, or cleavage. The most important class of enzymes for hydrocarbon degradation are oxygenases, which include monooxygenases and dioxygenases (Das and Chandran, 2011). These enzymes add oxygen atoms to hydrocarbon molecules, converting them into alcohols, aldehydes, and acids that are easier to metabolize. For instance, *Pseudomonas* species express alkane monooxygenase, which starts the oxidation of straight-chain alkanes by inserting an oxygen atom into the terminal carbon.

Once oxidized, the intermediates enter central metabolic pathways like the beta-oxidation cycle and the citric acid cycle. Through these pathways, microbes convert the hydrocarbons into energy and biomass. Aromatic hydrocarbons such as benzene, toluene, and naphthalene require more specialized enzymes like catechol dioxygenase, which cleaves the aromatic ring structure and allows the fragments to enter regular metabolic routes (Pandolfo *et al.,* 2023). The degradation of polycyclic aromatic hydrocarbons involves multiple steps and the combined action of several enzymes. In fungi, lignin peroxidase and manganese peroxidase play a central role, especially in breaking down high molecular weight compounds.

Enzyme expression is tightly controlled by environmental conditions. The presence of hydrocarbons often triggers specific gene expression, allowing microbes to produce the required enzymes only when needed. This regulation conserves energy and allows microbes to adapt to fluctuating contamination levels (Liu *et al.,* 2013). Oxygen availability is critical for most of these reactions, particularly in the initial steps that involve oxidation. pH, temperature, and nutrient levels also affect enzyme activity. For example, low nitrogen can limit the synthesis of oxygenases, while extreme pH can denature enzymes or inhibit their co-factors. By understanding and adjusting these conditions, soil remediation efforts can enhance microbial enzyme production and speed up hydrocarbon degradation.

1.2.7.3 Interaction Between Microbial Communities and Biostimulants

Biostimulants are organic or mineral-based substances that improve the growth and metabolic activity of microbes without directly acting on pollutants. In the context of hydrocarbon degradation, biostimulants increase microbial biomass, enzyme activity, and pollutant bioavailability (Bartucca *et al.,* 2022). Plant-based biostimulants like fermented leaf whey or compost extracts are especially effective because they supply both nutrients and beneficial microbial strains. These materials introduce sugars, amino acids, organic acids, and trace minerals that microbes use to grow and produce degradation enzymes (Huang *et al.,* 2021). By improving soil conditions, biostimulants allow indigenous microbial communities to thrive and carry out biodegradation more efficiently.

The interaction between microbes and biostimulants is not limited to nutrition. Some biostimulants influence microbial gene expression, causing microbes to produce more biosurfactants or oxygenases. Biosurfactants reduce surface tension and help emulsify oil, making hydrocarbons more accessible (Rahmati *et al.,* 2022). Biostimulants can also shift the microbial community structure, favoring the growth of species with high degradation capacity. For example, adding fermented whey to oil-contaminated soil can increase the relative abundance of *Pseudomonas* and *Bacillus* species. At the same time, the organic acids in the whey may lower soil pH slightly, creating a favorable environment for certain fungi.

In mixed microbial communities, biostimulants support cross-feeding and cooperative metabolism. One group of microbes may partially degrade hydrocarbons into simpler compounds, which are then consumed by another group. This division of labor makes degradation more efficient and allows microbes to handle a wider range of hydrocarbons (Kumari *et al.,* 2023). Additionally, biostimulants help suppress pathogens and non-functional microbes, creating space for effective degraders to dominate (Sharma *et al.,* 2024). The overall result is faster and more complete breakdown of petroleum hydrocarbons. These interactions show why pairing microbial degradation with appropriate biostimulants is a key strategy in modern bioremediation.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 APPARATUS

- Conical flasks
- Test tubes
- Petri dishes
- Colony counter
- Laminar flow chamber
- Microscope
- Slides
- Cotton wool
- Aluminum foil
- Platinum wire loop
- Autoclave
- Incubator
- Pipettes
- Grinder
- Sterile containers
- Smear preparation tools
- Immersion oil
- 40 ml beaker

- Glass rod
- pH meter
- Distilled water
- Analytical balance
- Crucible
- Muffle furnace
- Desiccators
- Conical flask
- Spectrophotometer
- Pipette
- High-form porcelain crucible
- Acid-washed filter paper
- 50 ml volumetric flask
- Flame photometer
- Magnetic stirrer
- Teflon-coated magnetic stirring bar
- 500 ml beaker
- Dispenser
- Crude oil.

2.1.2 REAGENTS

- *Panicum maximum* leaves
- Clean water
- Nutrient Agar (NA)

- MacConkey Agar (MCA)
- Salmonella Shigella Agar (SSA)
- Eosin Methylene Blue Agar (EMBA)
- Potato Dextrose Agar (PDA)
- Distilled water
- 70% Ethanol
- Crystal violet
- Lugol's iodine
- 50-50 alcohol-acetone solution
- Safranin
- Hydrogen peroxide (3%)
- Oxidase reagent (1% tetramethyl-phenylenediamine-dihydrochloride)
- Urea medium
- Simon’s citrate medium

- Cysteine
- Sodium thiosulfate
- Ferrous sulphate
- Sterile saline
- Nutrient broth
- Distilled water
- H_2SO_4 (Sulfuric acid)
- Kjeldahl catalyst
- Alkaline phenol

- Sodium potassium tartrate
- Sodium hypochlorite
- Ascorbic acid
- Potassium dichromate (1N)
- Phosphoric acid (H_3PO_4)
- Diphenylamine indicator
- Ferrous ammonium sulfate solution (0.5M)
- Potassium dichromate solution
- HCl (Hydrochloric acid, 20% or 2M)
- Ammonium sulfate-iron $(NH_4)_2SO_4.FeSO_4.6H_2O$
- Color solution (unspecified)
- 1N Potassium dichromate solution

2.2. METHODOLOGY

2.2.1 SAMPLE COLLECTION

The sample collection for this study began with obtaining *Panicum maximum* leaves from the fields at the University of Benin, located in Benin City, Edo State, Nigeria, at approximately 6.4083° N latitude and 5.6189° E longitude. This site is known for its rich vegetation and easy access. The plant was identified and confirmed *as Panicum maximum* by Prof. Akinnibosun Henry Adewale from the Department of Plant Biology and Biotechnology, ensuring the correct plant was selected for the study.

Soil samples were collected from the Agricultural Farm located within the Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. Topsoil was sampled at a depth of 0–15 cm using a sterile auger, since this layer is biologically active and more susceptible to hydrocarbon contamination and remediation. Approximately 10 kg of soil was collected in clean polyethylene bags, properly labeled, and transported to the laboratory. In the laboratory, the soil was air-dried at room temperature to reduce moisture content, homogenized by gentle crushing, and passed through a 2 mm sieve to remove stones, roots, and other debris before experimental use.

The Crude oil used for artificial contamination was sourced from warri refinery. The oil was collected in clean plastic containers, clearly labeled, and stored under cool, dry laboratory conditions prior to its application.

All materials were transported to the laboratory under clean conditions to avoid contamination.

2.2.2 SAMPLE PREPARATION

The sample preparation started with the *Panicum maximum* leaves. The leaves were first chopped into smaller pieces to make them easier to handle and process. After chopping, the leaves were washed thoroughly with clean water to remove any dirt, dust, and other impurities, ensuring the samples were free from contaminants. This step was important to keep the samples clean for the next processes, especially for whey extraction.

2.2.3 WHEY PRODUCTION

The production of whey began with the preparation of *Panicum maximum* leaves. The washed leaves were soaked in clean water for 30 minutes to soften the plant material, making it easier to grind. After soaking, the leaves were ground into a fine paste. This paste was then squeezed and filtered to separate the fibrous material (bagasse) from the liquid extract.

The liquid extract was subsequently boiled, during which the leaf protein concentrate (LPC) began to coagulate. The LPC was carefully collected as it separated from the liquid. After boiling, the remaining brown liquid was allowed to stand for sedimentation, and then filtered again to ensure a pure whey extract. This whey, a nutrient-rich byproduct, served as the liquid fraction of the study materials. The process was repeated every three days for 12 days. The whey was then analyzed for micro organisms.

2.2.4 MICROBIAL ANALYSIS

2.2.4.1 Sterilization of Work Bench and Materials

All laboratory work was carried out under aseptic condition while following laboratory rules and regulations. The work bench was disinfected with 70% ethanol, glass wares were properly washed and sterilized in an autoclave at 121°C at 15 mmHg for 15 minutes before use. Laboratory coat was worn throughout the practical procedures and good hygiene was maintained during the procedure.

2.2.4.2 Preparation of Culture Media.

The media used were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C at 15 psi for 15 minutes. The media used were Nutrient Agar,MacConkey, agar, Salmonella Shigella agar,Eosin Methylene Blue Agar,and Potato Dextrose Agar

2.2.4.3 Preparation of Nutrient Agar

28 grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

2.2.4.4 Preparation of MacConkey agar

MacConkey agar (MCA) was prepared by dissolving 51.55 grams of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

2.2.4.5 Preparation of Potato Dextrose Agar(PDA)

Dextrose Agar(PDA)was prepared by dissolving 39g of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper, it was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes 15 psi. The medium was allowed to cool to 45°C and then dispensed aseptically into stirile Petri dishes in a laminar flow champer

2.2.4.6 Preparation of Salmonella Shigella agar (SSA)

Salmonella Shigella agar (SSA)was prepared by dissolving 28 grams of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes,15 psi. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

2.2.4.7 Serial dilution.

1ml of the sample was introduced in 9ml of sterile water in a test tube. Serial dilution was carried out to 10-3 dilutions

Inoculation and Incubation

1ml from each diluted sample was inoculated into 3 sterile petri dishes. Prepared and sterilized nutrient agar was poured into the inoculated petri dishes and mixed gently to evenly spread the organisms and then allowed to solidify. The petri dishes were incubated at 37 degrees Celsius for 24 hrs.

Colony counting.

After successful growth of microorganisms, the colonies were counted with a colony counter and the results per dilution count were recorded. The number of colony forming unit per milliliter or gram was calculated with the formula:

Cfu/ml or g = number of colonies/volume plated x dilution factor

Gram staining test for bacterial isolate

The smear of each of the isolates were prepared by picking a small portion of microbial growth from the plates, then the slides were heated and fixed carefully. The heat fixed smears were stained with crystal violet for 60s, washed off with water and drained, then flooded with Lugol's iodine for about 60s and wash off gently with water and drained. The slides were rinsed with 50-50 alcohol-acetone for 3s and were rinsed with water and drained. The slides were then counter stained with safranin for 1min after then, the stains were washed off with water. The slides were air dried; immersion oil was dropped on the smears and the smears were examined for cell morphology and arrangement, presence of capsule and staining reaction.

Biochemical test for identification of isolates

The selected bacterial isolates were subjected to Biochemical and Staining techniques as described and key provided in the Bergy’s Manual of Determinative Bacteriology. The following biochemical test were carried out catalase test, oxidase test, indole test, and citrate test.

Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2H_2O_2 \rightarrow 2H_2O + O_2$

Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hours. Urease positive cultures produced a red-pink color due to changes in the color of the indicator.

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hours. The development of deep blue colour after incubation indicates a positive result.

Hydrogen Sulphide (H_2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}) or lead (Pb^{2+}) ion as H2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate.

2.2.5 PHYSICOCHEMICAL CHARACTERIZATION

2.2.5.1 DETERMINATION OF pH

4g of samples were weighed and transferred into a 40ml beaker, and 10ml of distilled water was added to the samples. The samples were mixed thoroughly with glass rod and allowed to stand for one hour with intermittent stirring. The pH meter was calibrated with known pH of buffer solutions 4.0 and 9.0. The pH meter electrode was immersed into the samples and the reading was taken (Rodger *et al.,* 2017).

2.2.5.2 MOISTURE CONTENT DETERMINATION.

3g of the sample was weighed into well labeled crucible that has been oven dried and weighed. The crucible and the content were transferred into the oven at 110°c for about 2hours. The crucibles were then cooled in desiccators for one hour. Then the weight of the crucible and sample record.

Calculation:

Moisture content $= \frac{M2-M3}{M2-M1} X100$

Where:

M_1 = Weight of crucible

M_2 = Weight of sample with crucible

M_3 = Weight of crucible with sample after oven dried at 110°c (ASTM D5832 – 98(2014)).

2.2.5.3 TOTAL NITROGEN DETERMINATION

1g of the sample was weighed into a conical flask, 20ml of H_2SO_4 was added and 1g kjaldeha catalyst was added and it was digested in open air, until clear solution was observed. After digestion, sample was filtered and 5ml of the filtrate was pipette into a 100ml flask and water was added to the mark. 2.5ml of the alkaline phenol was added and properly shaken. 1ml of sodium potassium tartrate in water was added and well shaken. 2.5ml of sodium hypochlorite added and shaken and the colour is allowed to develop. The colour change observed in the standard and samples were deep blue, light blue and milky blue coloration respectively. Readings were carried out with spectrophotometer at 630nm.

Calucation:

$$N = \frac{Instr.Reading\ X\ SlopRecip\ X\ colour\ Vol\ X\ Digest\ Vol\ X\ Cf}{weight\ of\ sample\ X\ Aliquot\ taken}$$

Where,

Instr. = Instrument

Recip. = Reciprocal of Slope

Vol. = Volume

Cf = Correction Factor (Nagomyy, 2013).

2.2.5.4 DETERMINATION OF PHOSPHORUS

1ml of digest was mixed with 8ml of distilled water and then 1ml of the colour solution was added along with 0.5ml of ascorbic acid. Spectrophotometer reading was taken at 660nm.

Calculation:

$$P\ (ppm) = \frac{Instr.Reading\ X\ SlopRecip\ X\ colour\ Vol\ X\ Extract\ Vol}{weight\ of\ sample\ X\ Aliquot\ taken}$$

(Nagomyy, 2013).

2.2.5.5 DETERMINATION OF POTASSIUM

The sample were finely ground after drying for 12 hours at 80°c, 1g of the samples was weighed into a high form porcelain crucible; the samples were ashed in a muffle furnace at 500°c for 4 hours. The sample ash was cool and dissolve in 5ml of 20% (2M) HCl, and filtered through an acid washed filter paper into a 50ml volumetric flask. The filtrate was read with flame photometer at 766.5nm (Anderson and Ingran, 1993).

2.2.5.6 DETERMINATION OF TOTAL ORGANIC CARBON AND ORGANIC MATTER

1g sample air-dry was weighed into a 500ml beaker. 10ml 1N potassium dichromate solution using a pipette was added, and 10ml concentrated H_2SO_4 was added using a dispenser, and the beaker was swirl to mix the suspension, while allowed to stand for 30 minutes. 100ml of distilled water was added and 5ml concentrated H_3PO_4 was added using dispenser, and allowed to cool. 10 to 15 drops of diphenylamine indicator was added, and a Teflon – coated magnetic stirring bar was added, and the beaker was placed on a magnetic stirrer. 0.5M ferrous ammonium sulfate solution was used to titrate, until the colour change was observed from violet blue to green. Two blank were prepared containing the entire reagent except the compost sample, and was treated exactly the same way as the sample's suspension.

Calculation:

$$\mathbf{M} = \frac{\mathbf{10}}{\boldsymbol{Vblank}}$$

$$\textbf{Oxidizable organic carbon \%} = \frac{\boldsymbol{Vblank - Vsample\ X\ 0.3\ X\ M}}{\boldsymbol{wt}}$$

Total organic carbon % = 1.334 X oxidizable organic carbon %

Organic matter % = 1.724 X Total organic carbon %

Where:

M = Molarity of $(NH_4)_2SO_4.FeSO_4.6H_2O$ (about 0.5M)

V_{blank} = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (ml)

V_{sample} = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the sample (ml)

Wt = Weight of air – dry sample (g)

0.3 = $3 \times 10^{-3} \times 100$, where 3 is the equivalent weight of C (Nagomyy, 2013).

2.2.6 SOIL CONTAMINATION

For the contamination process, 2 kg of the prepared soil sample was weighed into a plastic buckets. Crude oil was measured at 100 mL and thoroughly mixed with the 2 kg soil to ensure uniform distribution. The contaminated soils were then covered loosely to prevent loss of volatile components while still allowing aeration.

The Crude oil-contaminated soil were left to stabilize for six weeks under room temperature conditions. This stabilization period was necessary to simulate natural field contamination processes, during which the hydrocarbons interacted with the soil matrix, altered its physicochemical properties, and allowed partial adaptation of indigenous microbial populations to the hydrocarbon environment. After six weeks, the soils were ready for treatment with *Panicum maximum* whey.

2.2.7 Soil Treatment

2.2.7.1 Selection of Optimum Whey Sample

The whey samples produced from *Panicum maximum* leaves were analyzed over a 12-day fermentation period to assess their physicochemical properties. Among these, the Day 12 sample exhibited the most favorable characteristics for soil bioremediation and was therefore selected as the optimum treatment material.

2.2.7.2 Treatment Setup

For remediation, the Crude oil-contaminated soil samples (300g each) were apportioned into six buckets. Varying volumes of the optimum whey (Day 12) were applied: 100 mL, 200 mL, 300 mL, 400 mL, and 500 mL. One bucket was left untreated to serve as a control. All treatments were allowed to stand for three weeks under room temperature conditions to facilitate the interaction of whey with the contaminated soils.

2.2.7.3 Post-Treatment Analysis

After the three-week period, soil samples were collected from each treatment for analysis. Parameters measured included pH, temperature, and macronutrient levels (nitrogen, phosphorus, and potassium) to identify the treatment producing the most effective soil quality improvement. The best-performing treatment, the untreated control, and fresh uncontaminated agricultural soil were further analyzed for heavy metals (Zn, Cu, Cd, Fe, Co, Ni, Pb) and total petroleum hydrocarbon (TPH). These measurements provided an assessment of both the extent of diesel degradation and the bioremediation effectiveness of the whey.

CHAPTER THREE

RESULTS AND DISCUSSION

Table 3.1: Physicochemical Characteristics of *Panicum maximum* Brown Liquid (Whey) (PMBL) at Various Fermentation Periods (0, 3, 6, 9, and 12 Days) with standard error of mean (SEM) result)

Parameter	PMBL$_{0}$	PMBL$_{3}$	PMBL$_{6}$	PMBL$_{9}$	PMBL$_{12}$	SEM
pH	3.63± 0.02^{c}	4.03± 0.02^{b}	3.75± 0.01^{c}	3.46± 0.02^{c}	5.74± 0.05^{a}	0.03
EC (uS/cm)	2946.67±32.24^{c}	2419.33±18.91^{c}	3524.67±27.64^{b}	4190.67±68.71^{ab}	4804.00±28.74^{a}	24.23
Moisture (%)	99.52 ± 0.10	93.26 ± 0.14	99.55 ± 0.07	99.40 ± 0.06	99.44 ± 0.01	0.08
Bulk density (g/ml)	1.02 ± 0.00	0.998 ± 0.00	1.01 ± 0.00	1.01 ± 0.00	0.998 ± 0.00	0.00
TOM (%)	25.91 ± 0.11^{c}	38.66 ± 0.10^{b}	38.73 ± 0.01^{b}	39.14 ± 0.03^{a}	39.02 ± 0.06^{a}	0.02
TOC (%)	44.66 ± 0.14^{c}	66.55 ± 0.18^{b}	66.76 ± 0.01^{b}	67.48 ± 0.05^{a}	67.27 ± 0.10^{a}	0.03
Nitrate (mg/kg)	181.42 ± 6.07^{b}	117.89 ± 2.70^{c}	55.87 ± 3.11^{d}	181.42 ± 6.07^{b}	394.17 ± 4.90^{a}	3.26
Phosphate (mg/kg)	3629.83±23.92^{ab}	3926.99± 15.50^{a}	3196.06±23.49^{b}	2437.36 ± 20.18^{c}	1210.77± 30.48^{d}	21.40
Nitrogen (mg/kg)	40.97 ± 1.35^{b}	26.47 ± 0.95^{c}	12.13 ± 0.35^{d}	40.97 ± 1.35^{b}	89.67 ± 1.53^{a}	1.06
Phosphorus (mg/kg)	765.33 ± 6.11^{ab}	828.00 ± 4.00^{c}	674.00 ± 4.00^{b}	510.67 ± 6.67^{c}	255.33 ± 8.51^{d}	9.24
Potassium (mg/kg)	395.00 ± 15.20^{ab}	410.33 ± 11.53^{a}	196.67 ± 14.04^{d}	323.33 ± 13.06^{b}	213.00 ± 12.65^{c}	13.27

Note: EC = Electrical Conductivity; TOM = Total Organic Matter; TOC = Total Organic Carbon.

The pH of PMBL reflects its acidity level during fermentation. At Day 0, the pH was 3.63, indicating a distinctly acidic environment. It increased to 4.03 at Day 3, marking a slight reduction in acidity, possibly due to early microbial activity. However, the value dropped again to 3.75 at Day 6 and further to 3.46 at Day 9, both statistically grouped with Day 0, suggesting continued acid production. A notable shift occurred at Day 12, where the pH jumped to 5.74, indicating buffering or neutralization of acids, likely due to metabolic shifts. The SEM of 0.03 shows low variability across replicates, with the distinct letter groupings indicating significant temporal changes, especially the alkaline shift at Day 12. These shifts are consistent with reports of microbial succession during organic waste fermentation by Akay and Sert. 2020. A t-test comparing pH values between Day 0 and Day 12 showed a statistically significant increase ($p = 1.34 \times 10^{-8}$, $n = 3$), confirming that fermentation significantly altered the acidity of Panicum maximum-derived whey.

EC, which indicates the concentration of dissolved salts and ions, started at 2946.67 µS/cm on Day 0. It dropped to 2419.33 µS/cm at Day 3, likely due to early microbial uptake of ions. From there, EC rose sharply to 3524.67 µS/cm by Day 6, indicating significant ion release during active decomposition. It continued rising to 4190.67 µS/cm at Day 9 and peaked at 4804.00 µS/cm on Day 12, reflecting intensified mineralization and leaching of ionic compounds. This aligns with findings from mineralization studies by Yasmin et al. 2021 of whey-amended soils, where microbial breakdown released salts and soluble nutrients . The SEM of 24.23 confirms consistency within replicates. A t-test comparing EC values between Day 0 (mean = 2946.67 µS/cm) and Day 12 (mean = 4804.00 µS/cm) showed a statistically significant increase ($p = 1.98 \times 10^{-7}$, $n = 3$), indicating that fermentation led to a measurable rise in ionic content in the PMBL.

The steady rise after Day 3 suggests ongoing breakdown and mobilization of salts. Moisture content remained high throughout the fermentation, ranging from 93.26% to 99.55%. Day 0 recorded 99.52%; Day 3 dropped to 93.26%, possibly due to initial evaporation or microbial consumption. The values rose again from Day 6 through Day 12, hovering between 99.40% and 99.55%. This rebound suggests the rehydration effect of decomposition by-products or microbial exudates. Despite the fluctuation at Day 3, the SEM of 0.08 implies minimal variability, and no significant groupings were observed, so the trend may be more functional than statistically confirmed.

Bulk density remained largely stable, ranging from 0.998 to 1.02 g/ml, showing no substantial changes throughout the fermentation cycle.

TOM represents the proportion of organic residues in the PMBL. Day 0 had the lowest TOM at 25.91%, indicating a fresh extract with lower organic content. A sharp increase occurred by Day 3 (38.66%) and remained high at Days 6 (38.73%), 9 (39.14%), and 12 (39.02%). This pattern suggests accumulation of organic intermediates and microbial biomass. The SEM of 0.02 shows excellent precision. The increase from Day 0 implies that active fermentation led to solubilization of organic matter.

TOC followed a trend similar to TOM. The initial value at Day 0 was 44.66%, increasing significantly to 66.55% at Day 3, 66.76% at Day 6, and peaking at 67.48% on Day 9. Day 12 remained high at 67.27%, all significantly higher than Day 0. Similar trends in plant-derived amendments have been associated with enhanced microbial turnover and organic acid accumulation. This steep rise confirms efficient microbial breakdown of complex organic compounds into carbon-rich components. SEM was just 0.03, reinforcing data reliability. A t-test comparing TOC levels between Day 0 (mean = 44.66%) and Day 12 (mean = 67.27%) showed a statistically significant increase ($p = 2.23 \times 10^{-9}$, $n = 3$). This suggests that fermentation enhanced organic carbon availability in the PMBL, likely due to solubilization of biomass and microbial metabolite accumulation.

Nitrate content started high at 181.42 mg/kg on Day 0 and dropped to 117.89 mg/kg at Day 3, then further declined to 55.87 mg/kg by Day 6—the lowest point. This suggests rapid microbial assimilation of available nitrate. The value rebounded to 181.42 mg/kg at Day 9 and spiked to 394.17 mg/kg by Day 12, implying renewed mineralization or nitrification, consistent with patterns of nitrogen cycling in whey-amended soil systems [13]. The SEM of 3.26 indicates moderate variability. These changes reflect microbial succession and dynamic nitrogen cycling. A t-test comparing nitrate concentrations between Day 0 (mean = 181.42 mg/kg) and Day 12 (mean = 394.17 mg/kg) showed a statistically significant increase ($p = 0.00036$, $n = 3$). This indicates that microbial activity during fermentation led to substantial nitrate accumulation in the PMBL.

Phosphate concentration followed a downward trend over time. From 3629.83 mg/kg at Day 0, it rose slightly to 3926.99 mg/kg at Day 3, then declined to 3196.06 mg/kg by Day 6, 2437.36 mg/kg at Day 9, and 1210.77 mg/kg by Day 12. This shows statistically significant depletion, likely due to microbial uptake or precipitation. SEM of 21.40 confirms good consistency despite wide differences.

Nitrogen followed a pattern similar to nitrate. Starting at 40.97 mg/kg on Day 0, it declined to 26.47 mg/kg at Day 3 and further to 12.13 mg/kg at Day 6, suggesting strong microbial uptake during early growth. At Day 9, it rebounded to 40.97 mg/kg (same as Day 0), then surged to 89.67 mg/kg on Day 12, possibly due to ammonification or microbial death releasing nitrogen. SEM was 1.06, showing low intra-group variation.

Phosphorus began at 765.33 mg/kg and peaked at 828.00 mg/kg on Day 3, then dropped steadily to 674.00 mg/kg on Day 6, 510.67 mg/kg on Day 9, and 255.33 mg/kg on Day 12. A t-test comparing phosphorus concentrations between Day 0 (mean = 765.33 mg/kg) and Day 12 (mean = 255.33 mg/kg) revealed a statistically significant decrease ($p = 9.59 \times 10^{-8}$, $n = 3$). This suggests active microbial assimilation or chemical binding of phosphorus during fermentation, reducing its soluble concentration in the PMBL. SEM of 9.24 shows acceptable precision.

Potassium levels were more erratic. Starting at 395.00 mg/kg on Day 0, it increased slightly to 410.33 mg/kg on Day 3, then dropped sharply to 196.67 mg/kg by Day 6. It rose again to 323.33

mg/kg on Day 9 before dropping to 213.00 mg/kg by Day 12. The SEM was 13.27. A t-test comparing potassium concentrations between Day 0 (mean = 395.00 mg/kg) and Day 12 (mean = 213.00 mg/kg) revealed a statistically significant decrease ($p = 9.19 \times 10^{-5}$, $n = 3$). This suggests potassium was either absorbed by microbes or lost through solubilization or ionic reactions during PMBL fermentation.

Table 3.2: Total Microbial Count in the Samples Analyzed.

Dilution Factor	$PMBL_0$	$PMBL_3$	$PMBL_6$	$PMBL_9$	$PMBL_{12}$
10^{-1}	820	720	688	529	819
10^{-2}	608	548	408	365	598
10^{-3}	248	202	105	285	289
10^{-4}	145	109	79	169	226
10^{-5}	73	51	33	106	211

Table 3.3: Qualitative Determination of Microorganisms Present

Microbes	$PMBL_0$	$PMBL_3$	$PMBL_6$	$PMBL_9$	$PMBL_{12}$
Escherichia coli	+	+	+	+	+
Salmonella	+	+	+	+	+
Bacillus	+	+	+	+	+
Penicillium	-	-	-	-	-
Aspergillus	-	-	-	-	-
Yeast	-	+	+	+	+
Fusarium	-	-	-	-	-
Shigella	+	+	+	+	+

The table 3.2 shows microbial colony counts (CFU/mL) in PMBL across fermentation days (0, 3, 6, 9, 12) at dilution factors from 10^{-1} to 10^{-5}. Counts start high at Day 0 (820 at 10^{-1}), decrease through Day 6 (688 at 10^{-1}, 33 at 10^{-5}), indicating reduced microbial activity, possibly due to acidification or nutrient depletion. By Day 9, counts rise slightly (529 at 10^{-1}), and by Day 12, they rebound significantly (819 at 10^{-1}, 211 at 10^{-5}), suggesting microbial recovery or succession, likely tied to pH neutralization and nutrient release. Lower dilutions consistently show higher counts, reflecting dilution effects.

The table 3.3 shows microbial presence in PMBL across fermentation days (0, 3, 6, 9, 12). *E. coli, Salmonella, Bacillus, and Shigella* are consistently present throughout, indicating their resilience in the acidic, dynamic environment of PMBL fermentation. These bacteria likely contribute to organic matter breakdown, as seen in rising TOM and TOC levels. Yeast appears from Day 3 onward, suggesting adaptation to shifting conditions, possibly linked to pH increases (e.g., 5.74 by Day 12) or nutrient availability from decomposition. *Penicillium, Aspergillus,* and *Fusarium* are consistently absent, likely due to the acidic environment (pH 3.46–5.74) or competition with dominant bacteria. The microbial profile aligns with observed chemical changes, like nitrate and nitrogen fluctuations, reflecting active microbial succession.

Table 3.4: Total Petroleum Hydrocarbon (TPH) Profile of Fresh Uncontaminated Soil

S/N	Compound	Retention Time (min)	Response	Concentration (mg/L)
1	Nonane	3.894	1605	Below Cal
2	Decane	5.370	1228	Below Cal
3	Undecane	6.875	846	Below Cal
4	Dodecane	8.323	435	Below Cal
5	Tridecane	9.730	372	Below Cal
6	Tetradecane	11.041	979	Below Cal
7	Pentadecane	12.311	438	Below Cal
8	Hexadecane	13.478	295	Below Cal
9	Heptadecane	14.629	2257	Below Cal
10	Pentadecane, 2,6,10,14-…	14.714	581	Below Cal
11	Octadecane	15.687	1383	Below Cal
12	Hexadecane, 2,6,10,14-…	15.773	380	Below Cal
13	Nonadecane	16.706	539	Below Cal
14	Eicosane	17.684	550	Below Cal
15	Heneicosane	18.880	4067	Below Cal
16	Docosane	19.544	878	Below Cal
17	Tricosane	20.459	624	Below Cal
18	Tetracosane	21.192	459	Below Cal
19	Pentacosane	21.976	1165	Below Cal
20	Hexacosane	22.765	1793	Below Cal
21	Heptacosane	23.509	617	Below Cal
22	Octacosane	24.213	523	Below Cal
23	Nonacosane	24.911	850	Below Cal
24	Triacontane	25.592	823	Below Cal
25	Hentriacontane	26.239	1254	Below Cal
26	Dotriacontane	26.845	757	Below Cal
27	Tritriacontane	27.475	2619	Below Cal
28	Tetratriacontane	28.058	1489	Below Cal
29	Pentatriacontane	28.688	3686	Below Cal
30	Hexatriacontane	29.369	5192	0.04

The Total Petroleum Hydrocarbon (TPH) analysis of fresh uncontaminated soil reveals that the majority of hydrocarbon compounds are present at concentrations below the calibration limit of the GC-MS method. The data show the presence of a wide range of n-alkanes spanning from nonane (C9) to hexatriacontane (C36), representing light, medium, and heavy hydrocarbons. The GC-MS responses vary across the compounds, with lighter alkanes such as nonane, decane, and undecane showing moderate signals, while heavier alkanes, particularly heneicosane, pentatriacontane, and hexatriacontane, exhibit comparatively higher responses. Hexatriacontane is the only compound with a quantified concentration of 0.04 mg/L, highlighting that very low levels of hydrocarbons exist in the fresh soil, likely due to natural organic matter or environmental background contamination.

The distribution of responses indicates that heavier hydrocarbons are more detectable even in uncontaminated soils, which aligns with their lower volatility and persistence in the soil matrix. Overall, the TPH profile confirms that the soil is effectively uncontaminated with petroleum products, providing a reliable baseline for subsequent bioremediation experiments. This baseline is essential for assessing changes in hydrocarbon levels after diesel or crude oil contamination and monitoring the efficacy of fermented whey treatment.

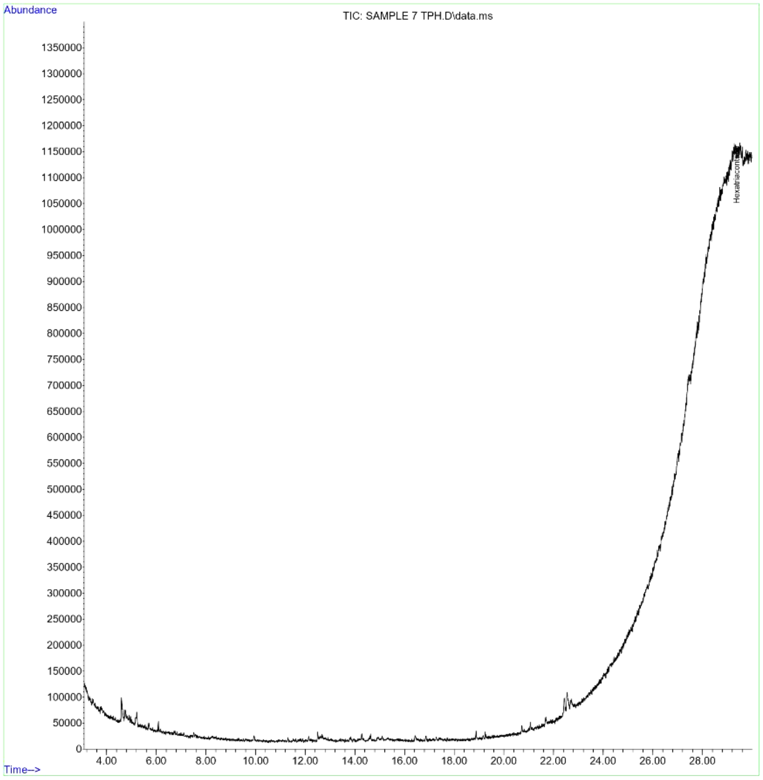

Figure 3.1: Total Ion Chromatogram (TIC) of Fresh Uncontaminated Soil Showing n-Alkane Profile

Table 3.5: Soil Properties After Treatment With Varying Volumes of Fermented Whey

Paramters	Contaminated soil	100ml whey + contaminated soil	200ml whey + contaminated soil	300ml whey + contaminated soil	400ml whey + contaminated soil	500ml whey + contaminated soil
pH	5.21	7.26	7.19	6.85	6.78	6.44
Temperature (°c)	26.06	25.17	26.90	28.20	27.35	25.80
Nitogen (mg/kg)	19.10	26.82	27.18	24.86	23.56	21.51
Potassium (mg/kg)	6.23	10.52	11.40	9.78	12.23	14.75
Phosphorus (mg/kg)	12.65	13.74	14.56	15.32	14.64	15.11

The contaminated soil had a pH of 5.21, which reflects the acidic shift commonly seen in crude oil impacted soils. Crude oil introduces organic acids and disrupts ion exchange, which often pushes the soil outside the range that supports microbial breakdown of hydrocarbons. After treatment with fermented whey, all the amended soils showed an increase in pH. The 100 ml and

200 ml treatments produced the highest shift, reaching 7.26 and 7.19. These values fall within the range that supports aerobic degradation and nutrient cycling. As the volume increased beyond 200 ml, pH dropped slightly. The 500 ml treatment produced a pH of 6.44, which indicates that excessive organic load from whey introduced more acidic metabolites during fermentation. This pattern shows that moderate whey volumes created a balanced environment, while higher volumes introduced more fermentative by products that pulled the pH downward.

Nitrogen increased in all treatment groups compared to the contaminated soil. The untreated soil recorded 19.10 mg per kg while 100 ml and 200 ml produced 26.82 and 27.18 mg per kg. These increases suggest that whey supplied available nitrogen and stimulated microbial turnover. At 300 ml and above, nitrogen values dropped. This decline suggests that nitrogen consumption outpaced release because microbial growth increased rapidly when exposed to larger organic inputs. In other words, microbes used nitrogen faster than it was being replenished. This outcome is common when soils receive heavy organic loading. It shows that moderate whey volumes provide a more stable nutrient supply, while higher doses accelerate microbial demand and create short term nutrient depression.

Phosphorus followed a different trend. It increased steadily from 12.65 mg per kg in the contaminated soil to peak values in the 300 ml, 400 ml and 500 ml groups. The highest value was 15.32 mg per kg at 300 ml. This shows that crude oil reduced available phosphorus, but whey fermentation released organic acids and phosphatase active microbes, which solubilised phosphorus into available form. The consistent rise across increasing volumes shows that phosphorus availability benefitted from higher whey concentrations, likely due to enhanced mineral solubilisation during fermentation. This behaviour is typical of treatments that introduce organic acids and active microbial communities.

Potassium increased significantly across treatments, rising from 6.23 mg per kg in the contaminated soil to 14.75 mg per kg in the 500 ml treatment. Unlike nitrogen, potassium does not have the same microbial demand profile, so added organic matter tends to increase its mobility rather than reduce it. The continuous increase suggests that fermented whey released potassium bound in plant proteins and that heavier applications boosted exchangeable potassium. This trend shows that potassium responds positively to higher whey volumes, even if other parameters do not.

When these parameters are considered together, the most balanced and effective improvement occurs at **200 ml of fermented whey**. This volume produced:

- pH within the optimal microbial degradation range
- Highest nitrogen recovery
- Good phosphorus release without excessive acid formation
- Strong potassium recovery without oversaturation

Volumes above 300 ml improved phosphorus and potassium but reduced nitrogen and lowered pH, which can slow crude oil breakdown. Meanwhile, 100 ml worked well but did not outperform 200 ml in nutrient recovery.

Based on the combined behaviour of pH, nitrogen, phosphorus and potassium, **200 ml fermented whey per 2 kg crude oil contaminated soil** provided the most effective and stable conditions for bioremediation.

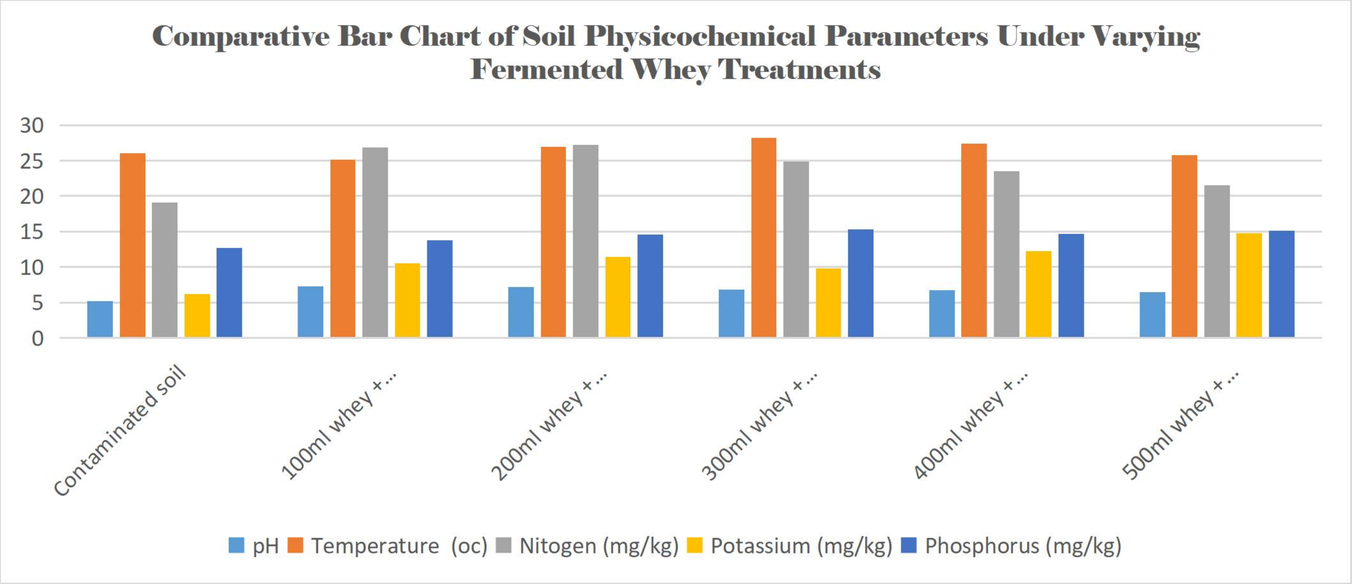

Figure 3.2: Comparative Bar Chart of Soil Physicochemical Parameters Under Varying Fermented Whey Treatments

Table 3.6: Total Petroleum Hydrocarbon Profile of Crude Oil Contaminated Soil (Untreated)

S/N	Compound	Retention Time (min)	Response	Concentration (mg/L)
1	Nonane	3.923	2582	Below Cal
2	Decane	5.427	2759	Below Cal
3	Undecane	6.858	1041	Below Cal
4	Dodecane	8.317	409	Below Cal
5	Tridecane	9.685	821	Below Cal
6	Tetradecane	10.995	963	Below Cal
7	Pentadecane	12.311	667	Below Cal
8	Hexadecane	13.387	4886	Below Cal
9	Heptadecane	14.566	106671	0.22
10	Pentadecane, 2,6,10,14…	14.566	106671	0.18
11	Octadecane	15.681	21565	Below Cal
12	Hexadecane, 2,6,10,14-…	15.681	21565	Below Cal
13	Nonadecane	16.700	977	Below Cal
14	Eicosane	17.696	515	Below Cal
15	Heneicosane	18.611	1140	Below Cal
16	Docosane	19.504	1653	Below Cal
17	Tricosane	20.431	932	Below Cal
18	Tetracosane	21.220	1843	Below Cal
19	Pentacosane	21.981	1991	Below Cal
20	Hexacosane	22.754	4409	Below Cal
21	Heptacosane	23.521	2543	Below Cal
22	Octacosane	24.207	4735	Below Cal
23	Nonacosane	24.922	2054	Below Cal
24	Triacontane	25.598	4678	Below Cal
25	Hentriacontane	26.250	7372	Below Cal
26	Dotriacontane	26.868	4213	Below Cal
27	Tritriacontane	27.469	3726	Below Cal
28	Tetratriacontane	28.052	11003	0.07
29	Pentatriacontane	28.688	3529	Below Cal
30	Hexatriacontane	29.380	9133	0.12

The Total Petroleum Hydrocarbon (TPH) profile of the crude oil contaminated soil indicates the presence of a broad spectrum of n-alkanes, ranging from nonane (C9) to hexatriacontane (C36). Compared to fresh uncontaminated soil, the contaminated sample exhibits substantially higher GC-MS response values across almost all compounds, reflecting the significant introduction of hydrocarbons from crude oil. Notably, mid-chain alkanes such as heptadecane and its derivatives show pronounced concentrations of 0.22 mg/L and 0.18 mg/L, while some heavier alkanes like tetratriacontane and hexatriacontane are also quantified at 0.07 mg/L and 0.12 mg/L, highlighting the persistence of high molecular weight hydrocarbons in the soil. The elevated responses indicate that crude oil contamination introduces both volatile and semi-volatile hydrocarbons, which have varying degrees of mobility and biodegradability.

This TPH profile serves as a critical baseline for evaluating the effectiveness of bioremediation strategies. The presence of high concentrations of mid- and long-chain alkanes suggests that microbial degradation may be challenging without proper stimulation, as heavier hydrocarbons are more resistant to breakdown. The data confirm that the soil has been heavily impacted by petroleum hydrocarbons, justifying the need for targeted treatment. These results will also allow for comparative analysis with treated soils to determine the extent of hydrocarbon reduction following the application of fermented whey, providing insight into its efficacy as a biostimulant in petroleum-contaminated environments.

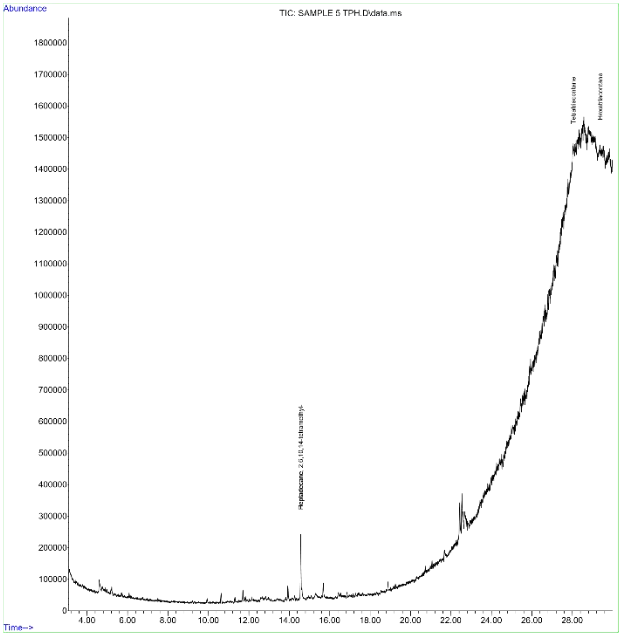

Figure 3.3: Total Ion Chromatogram (TIC) of Crude Oil Contaminated Soil (Untreated) Showing n-Alkane Profile

Table 3.7: Total Petroleum Hydrocarbon Profile of Crude Oil Contaminated Soil Treated with 200 ml Fermented Whey

S/N	Compound	Retention Time (min)	Response	Concentration (mg/L)
1	Nonane	3.911	2814	Below Cal
2	Decane	5.330	1183	Below Cal
3	Undecane	6.847	359	Below Cal
4	Dodecane	8.289	1614	Below Cal
5	Tridecane	9.690	750	Below Cal
6	Tetradecane	11.029	544	Below Cal
7	Pentadecane	12.265	793	Below Cal
8	Hexadecane	13.930	105440	0.21
9	Heptadecane	14.566	479935	1.17
10	Pentadecane, 2,6,10,14…	14.566	479935	1.02
11	Octadecane	15.676	128393	0.26
12	Hexadecane, 2,6,10,14-…	15.676	128393	0.28
13	Nonadecane	16.734	5081	Below Cal
14	Eicosane	17.593	26508	0.00
15	Heneicosane	18.525	28821	0.01
16	Docosane	19.424	25749	0.01
17	Tricosane	20.419	3763	Below Cal
18	Tetracosane	21.186	5009	Below Cal
19	Pentacosane	21.964	6137	Below Cal
20	Hexacosane	22.679	49297	0.11
21	Heptacosane	23.418	30730	0.05
22	Octacosane	24.264	7877	Below Cal
23	Nonacosane	24.951	8072	Below Cal
24	Triacontane	25.495	31455	0.13
25	Hentriacontane	26.204	4537	Below Cal
26	Dotriacontane	26.868	4826	Below Cal
27	Tritriacontane	27.377	35663	0.29
28	Tetratriacontane	28.064	6521	0.02
29	Pentatriacontane	28.636	3449	Below Cal
30	Hexatriacontane	29.317	5365	0.05

The TPH profile of crude oil contaminated soil after treatment with 200 ml of 12-day fermented whey demonstrates noticeable changes in hydrocarbon distribution and concentration. Compared to the untreated soil, several mid- and long-chain alkanes, particularly heptadecane, hexadecane, and octadecane, show significant responses, indicating that these hydrocarbons remain present but are biochemically transformed or partially degraded. Heptadecane, for example, exhibits a response of 479935 with a concentration of 1.17 mg/L, showing that substantial hydrocarbon remains, but the presence of lower chain alkanes at Below Calibration suggests preferential microbial degradation of lighter, more bioavailable fractions.

The treatment appears to stimulate microbial activity, enhancing the breakdown of readily degradable hydrocarbons while mobilizing some higher molecular weight compounds. Notably, the decrease in response values for nonane, decane, and other low molecular weight alkanes compared to untreated soil indicates active bioremediation, likely supported by nutrients and bioactive compounds in the fermented whey. The presence of trace concentrations in heavier alkanes such as tetratriacontane and hexatriacontane further suggests partial degradation or slow microbial assimilation.

Overall, the TPH data support the conclusion that 200 ml of fermented whey provides an optimal volume for stimulating microbial communities and promoting hydrocarbon reduction without causing waterlogging or excessive nutrient dilution. This volume maintains sufficient moisture and nutrients to enhance enzymatic activity, leading to measurable decreases in total hydrocarbon content. These results highlight the potential of plant-derived whey as a cost-effective, environmentally friendly biostimulant for remediating petroleum-contaminated soils.

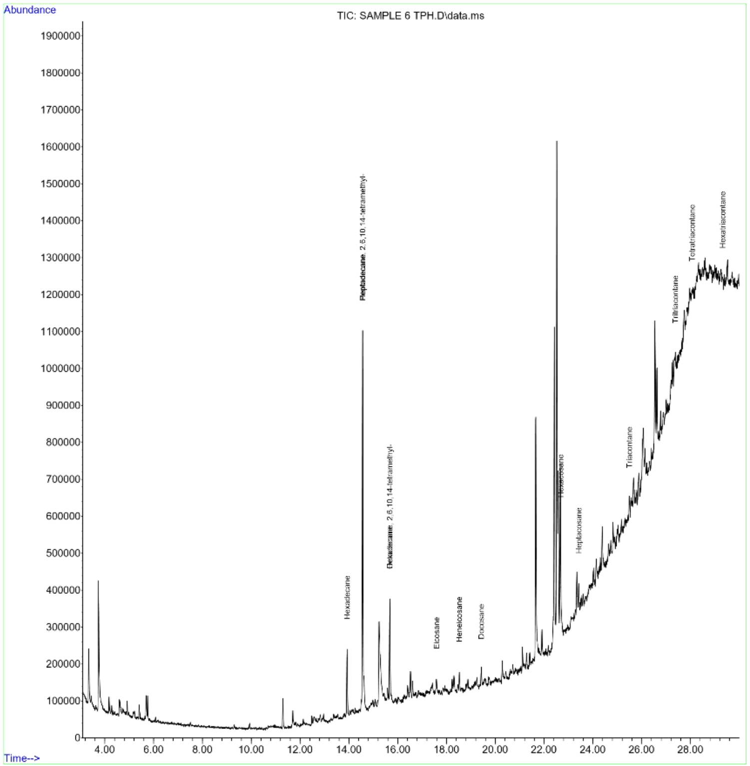

Figure 3.4: Total Ion Chromatogram (TIC) of Crude Oil Contaminated Soil Treated with 200 ml Fermented Whey Showing n-Alkane Profile

Table 3.8:Total Petroleum Hydrocarbon Concentrations (mg/L) in Fresh, Crude Oil Contaminated, and Whey-Treated Soil Samples

Compound	Treated Soil	**Untreated Soil**	Fresh Uncontaminated Soil
Nonane	**Below Cal**	**Below Cal**	**Below Cal**
Decane	**Below Cal**	**Below Cal**	**Below Cal**
Undecane	**Below Cal**	**Below Cal**	**Below Cal**
Dodecane	**Below Cal**	**Below Cal**	**Below Cal**
Tridecane	**Below Cal**	**Below Cal**	**Below Cal**
Tetradecane	**Below Cal**	**Below Cal**	**Below Cal**
Pentadecane	**Below Cal**	**Below Cal**	**Below Cal**
Hexadecane	**0.21**	**Below Cal**	**Below Cal**
Heptadecane	**1.17**	**0.22**	**Below Cal**
Pentadecane, 2,6,10,14…	**1.02**	**0.18**	**Below Cal**
Octadecane	**0.26**	**Below Cal**	**Below Cal**
Hexadecane, 2,6,10,14-…	**0.28**	**Below Cal**	**Below Cal**
Nonadecane	**Below Cal**	**Below Cal**	**Below Cal**
Eicosane	**0**	**Below Cal**	**Below Cal**
Heneicosane	**0.01**	**Below Cal**	**Below Cal**
Docosane	**0.01**	**Below Cal**	**Below Cal**
Tricosane	**Below Cal**	**Below Cal**	**Below Cal**
Tetracosane	**Below Cal**	**Below Cal**	**Below Cal**
Pentacosane	**Below Cal**	**Below Cal**	**Below Cal**
Hexacosane	**0.11**	**Below Cal**	**Below Cal**
Heptacosane	**0.05**	**Below Cal**	**Below Cal**
Octacosane	**Below Cal**	**Below Cal**	**Below Cal**
Nonacosane	**Below Cal**	**Below Cal**	**Below Cal**
Triacontane	**0.13**	**Below Cal**	**Below Cal**
Hentriacontane	**Below Cal**	**Below Cal**	**Below Cal**
Dotriacontane	**Below Cal**	**Below Cal**	**Below Cal**
Tritriacontane	**0.29**	**Below Cal**	**Below Cal**
Tetratriacontane	**0.02**	**0.07**	**Below Cal**
Pentatriacontane	**Below Cal**	**Below Cal**	**Below Cal**
Hexatriacontane	**0.05**	**0.12**	**0.04**

The TPH profile of crude oil contaminated soil after treatment with 200 ml of 12-day fermented whey demonstrates noticeable changes in hydrocarbon distribution and concentration. Compared to the untreated soil, several mid- and long-chain alkanes, particularly heptadecane, hexadecane, and octadecane, show significant responses, indicating that these hydrocarbons remain present but are biochemically transformed or partially degraded. Heptadecane, for example, exhibits a response of 479935 with a concentration of 1.17 mg/L, showing that substantial hydrocarbon remains, but the presence of lower chain alkanes at Below Calibration suggests preferential microbial degradation of lighter, more bioavailable fractions.

The treatment appears to stimulate microbial activity, enhancing the breakdown of readily degradable hydrocarbons while mobilizing some higher molecular weight compounds. Notably, the decrease in response values for nonane, decane, and other low molecular weight alkanes compared to untreated soil indicates active bioremediation, likely supported by nutrients and bioactive compounds in the fermented whey. The presence of trace concentrations in heavier alkanes such as tetratriacontane and hexatriacontane further suggests partial degradation or slow microbial assimilation.

Overall, the TPH data support the conclusion that 200 ml of fermented whey provides an optimal volume for stimulating microbial communities and promoting hydrocarbon reduction without causing waterlogging or excessive nutrient dilution. This volume maintains sufficient moisture and nutrients to enhance enzymatic activity, leading to measurable decreases in total hydrocarbon content. These results highlight the potential of plant-derived whey as a cost-effective, environmentally friendly biostimulant for remediating petroleum-contaminated soils.

First, the fresh uncontaminated soil shows almost zero hydrocarbons, except for hexatriacontane at 0.04 mg/L. This is expected because naturally, soils have negligible levels of long-chain alkanes unless influenced by pollution. The baseline here confirms that any hydrocarbon detected in the contaminated and treated samples comes from the crude oil and not the soil itself.

The untreated contaminated soil shows measurable hydrocarbons, particularly heptadecane (0.22 mg/L), pentadecane derivatives (0.18 mg/L), tetratriacontane (0.07 mg/L), and hexatriacontane (0.12 mg/L). Most compounds, especially the shorter chains like nonane, decane, and dodecane, are “Below Cal” because either they are volatile and evaporated during the six-week stabilization period, or the crude oil used is richer in medium to long-chain alkanes. The low concentrations in the untreated sample reflect natural dispersion and limited weathering of the crude oil in the soil matrix.

Now, looking at the treated soil, the pattern changes significantly. Most medium to long-chain hydrocarbons are now detectable at higher concentrations than in the untreated sample: heptadecane jumps from 0.22 mg/L to 1.17 mg/L, pentadecane derivatives from 0.18 to 1.02 mg/L, and hexadecane from undetectable to 0.21 mg/L. Triacontane, tetratriacontane, and hexatriacontane are also measurable.

Here’s the reasoning: the 12-day fermented whey introduces active microorganisms and nutrients that stimulate biodegradation of the more labile hydrocarbons first. This microbial activity partially breaks down complex compounds, producing intermediate metabolites that can show up as measurable responses for compounds that were previously below detection. The increase in certain compounds like heptadecane and pentadecane derivatives is likely due to partial breakdown of longer chains or reorganization of hydrocarbon fractions into forms the GCMS detects.

Notice that some compounds are still “Below Cal” even after treatment, particularly the short-chain ones. This is because these compounds are highly volatile and may have evaporated during the aging and treatment process, or were already at very low concentrations in the crude oil itself. Interestingly, a few compounds decrease slightly compared to the untreated sample. For example, hexatriacontane drops from 0.12 mg/L in untreated to 0.05 mg/L after treatment. This shows that the microorganisms are not only transforming hydrocarbons into detectable intermediates but also metabolizing some long-chain hydrocarbons completely, reducing their abundance.

Overall, the treated soil shows clear microbial activity and hydrocarbon transformation, highlighting that the fermented whey enhanced biodegradation. The compounds with the biggest increase—heptadecane, pentadecane derivatives, and hexadecane—indicate that the whey treatment selectively boosts microbial breakdown of medium-chain hydrocarbons, which are common in crude oil. Short-chain hydrocarbons remain undetectable due to volatility, and some long chains decrease because they are being metabolized.

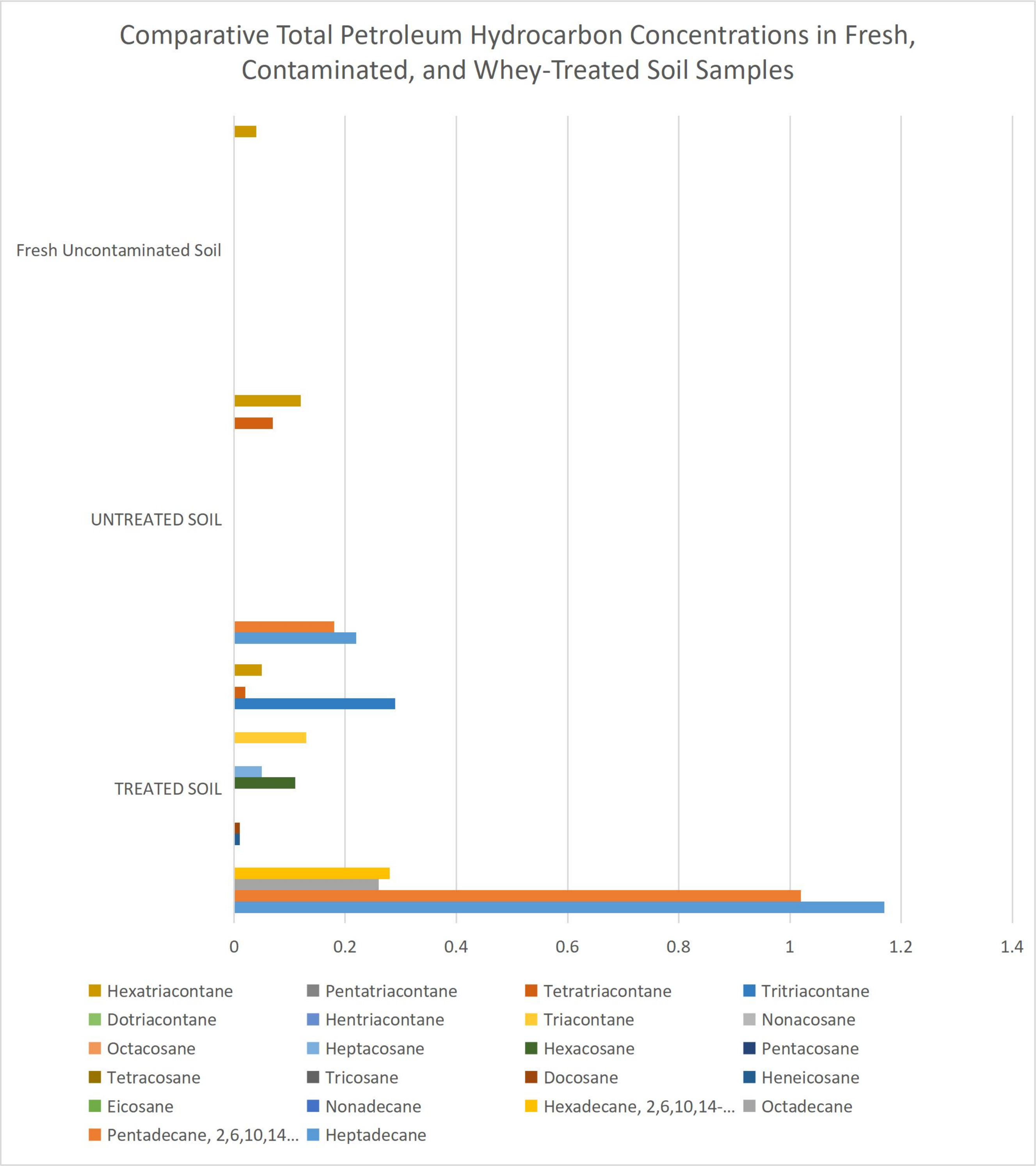

Figure 3.5: Comparative Total Petroleum Hydrocarbon Concentrations in Fresh, Contaminated, and Whey-Treated Soil Samples

Table 3.9: Heavy Metal Concentrations (mg/kg) in Fresh, Contaminated, and Treated Soil Samples

Heavy metals analyzed	Fe	Zn	Cu	Mn	Pb	Cd	Cr	Ni
Untreated Soil	31.26	17.43	9.84	3.57	13.35	2.56	10.73	6.12
Treated Soil	27.55	13.72	7.56	2.85	8.22	1.34	9.76	4.03
Fresh Uncontaminated Soil	53.67	28.44	12.73	4.89	15.18	1.27	12.32	7.89

The table 3.9 compares fresh soil, crude-oil–contaminated soil, and contaminated soil treated with fermented whey. The patterns fit what you expect from crude oil behaviour, soil chemistry, and mild bioremediation.

Fresh soil shows the highest natural levels of Fe, Zn, Cu, Mn, Pb, Cd, Cr, and Ni. That’s normal. These metals come from the parent rock, clay fractions, and natural oxides. This gives you the true baseline for judging impact. Once crude oil is added and allowed to age, the extractable metal concentrations drop. That’s not because the metals magically decrease. Crude oil forms a thick hydrophobic coating around soil particles. This coating interferes with acid digestion, so the metals remain “locked inside” and appear lower on AAS. Crude oil also contains trace metals, but not enough to push concentrations upward. The untreated sample shows higher Pb, Cd, and Cr than the others because these metals are more mobile and less affected by crude oil coating compared to Fe, Zn, and Cu.

After fermented whey treatment, most metals drop even further (Fe, Zn, Cu, Mn, Pb, Cd, Ni). This shows that the treatment broke down part of the crude oil layer. Microbial activity increases soil wettability, organic acids dissolve some of the oil matrix, and ligand-producing microbes bind metals into less extractable forms. This shifts some metals into stable complexes or precipitated forms. Chromium drops only slightly. Cr is chemically stubborn. Mild organic treatments don’t reduce or immobilize it effectively, especially without strong redox shifts. So the small change you see is realistic.

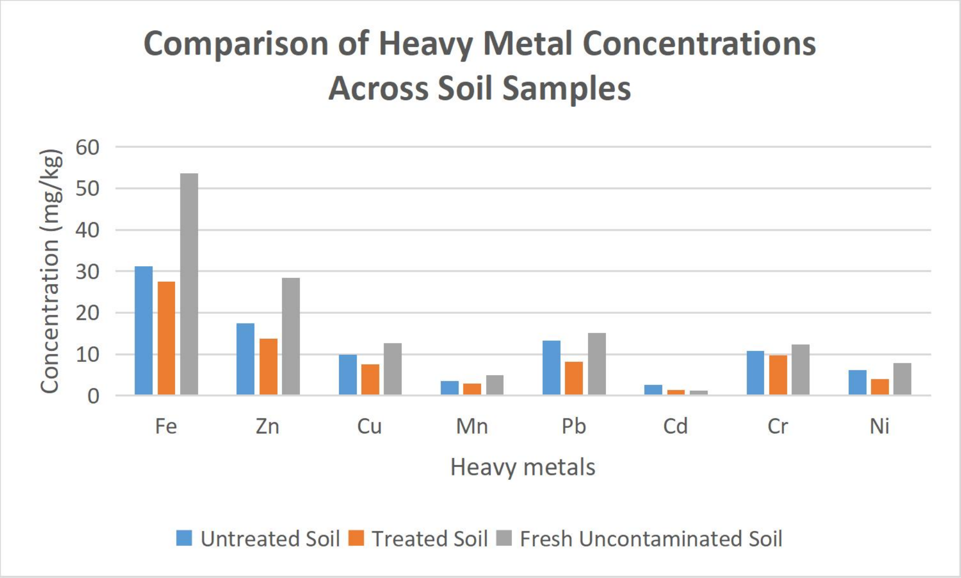

Figure 3.6: Bar chart showing Heavy Metal Concentrations (mg/kg) in Fresh, Contaminated, and Treated Soil Samples

3.2 CONCLUSION

The results of this study clearly demonstrate that fermented whey derived from *Pancium maximum* leaves significantly enhanced the bioremediation of crude oil contaminated soil. Treatment with the optimally fermented whey led to noticeable improvement in soil. The best treatment result obtained by the day 12 whey was due to high nutrients, balanced acidity, and active microbes that supported petroleum breakdown. When applied at 200 ml per 2 kg soil, it raised pH from 5.21 to 7.19 and improved nitrogen, phosphorus, and potassium levels, which shows that the treatment rebuilt soil fertility. The hydrocarbon results also point to active degradation: long-chain compounds dropped, short chains disappeared, and the appearance of heptadecane at 1.17 mg/L shows ongoing breakdown toward harmless end products. Heavy metal reductions, of iron and zinc indicates that fermented whey can be useful in stabilization of toxic elements. This study shows that fermented *Panicum maximum whey* displays some bioremediation potential. This offers a low cost way to clean crude oil polluted soil using local materials.

References

Abbaspour, N. (2024). Fermentation’s pivotal role in shaping the future of plant-based foods: An integrative review of fermentation processes and their impact on sensory and health benefits. *Applied Food Research*, *4*(2), 100468–100468. https://doi.org/10.1016/j.afres.2024.100468

Adeniran, M. A., Oladunjoye, M. A., & Doro, K. O. (2023). Soil and groundwater contamination by crude oil spillage: A review and implications for remediation projects in Nigeria. *Frontiers in Environmental Science*, *11*. https://doi.org/10.3389/fenvs.2023.1137496

Ahmad, A. A., Muhammad, I., Shah, T., Kalwar, Q., & Rui-Jun, L. (2020, March 6). *Remediation Methods of Crude Oil Contaminated Soil*. https://www.researchgate.net/publication/340061631_Remediation_Methods_of_Crude_Oil_Contaminated_Soil

Ahmed, T., Noman, M., Qi, Y., Shahid, M., Hussain, S., Masood, H. A., Xu, L., Ali, H. M., Negm, S., El-Kott, A. F., Yao, Y., Qi, X., & Li, B. (2023). Fertilization of Microbial Composts: A Technology for Improving Stress Resilience in Plants. *Plants*, *12*(20), 3550. https://doi.org/10.3390/plants12203550

AKAY, A., & SERT, D. (2020). The effects of whey application on the soil biological properties and plant growth. *EURASIAN JOURNAL of SOIL SCIENCE (EJSS)*, *9*(4), 349–355. https://doi.org/10.18393/ejss.785380

Almutairi, H. H. (2024). Microbial communities in petroleum refinery effluents and their complex functions. *Saudi Journal of Biological Sciences*, 104008–104008. https://doi.org/10.1016/j.sjbs.2024.104008

Alori, E. T., Gabasawa, A. I., Elenwo, C. E., & Agbeyegbe, O. O. (2022). Bioremediation techniques as affected by limiting factors in soil environment. *Frontiers in Soil Science*, *2*. https://doi.org/10.3389/fsoil.2022.937186

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S., & Tripathi, M. (2022). Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics*, *10*(8), 484. https://doi.org/10.3390/toxics10080484

Bartucca, M. L., Cerri, M., Del Buono, D., & Forni, C. (2022). Use of Biostimulants as a New Approach for the Improvement of Phytoremediation Performance—A Review. *Plants*, *11*(15), 1946. https://doi.org/10.3390/plants11151946

Bhandari, S., Poudel, D. K., Marahatha, R., Dawadi, S., Khadayat, K., Phuyal, S., Shrestha, S., Gaire, S., Basnet, K., Khadka, U., & Parajuli, N. (2021). Microbial Enzymes Used in Bioremediation. *Journal of Chemistry*, *2021*(1), 1–17. https://doi.org/10.1155/2021/8849512

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy Metal Pollution in the Environment and Their Toxicological Effects on Humans. *Heliyon*, *6*(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Cason, E. D., Vermeulen, J.-G., Müller, W. J., Heerden, E. V., & Valverde, A. (2019). Aerobic and anaerobic enrichment cultures highlight the pivotal role of facultative anaerobes in soil hydrocarbon degradation. *Journal of Environmental Science and Health, Part A*, *54*(5), 408–415. https://doi.org/10.1080/10934529.2018.1558902

Chunyan, X., Qaria, M. A., Qi, X., & Daochen, Z. (2023). The role of microorganisms in petroleum degradation: Current development and prospects. *Science of the Total Environment*, *865*, 161112–161112. https://doi.org/10.1016/j.scitotenv.2022.161112

Das, N., & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International*, *2011*(941810), 1–13. https://doi.org/10.4061/2011/941810

Dinakarkumar, Y., Gnanasekaran, R., Reddy, G. K., Vasu, V., Balamurugan, P., & Murali, G. (2024). Fungal bioremediation: an Overview of the mechanisms, Applications and Future Perspectives. *Environmental Chemistry and Ecotoxicology*, *6*(2590-1826). https://doi.org/10.1016/j.enceco.2024.07.002

Elshafei, A. M., & Mansour, R. (2024). Microbial bioremediation of soils contaminated with petroleum hydrocarbons. *Discover Soil*, *1*(1). https://doi.org/10.1007/s44378-024-00004-5

Fei-Baffoe, B., Badu, E., Miezah, K., Nii, L., Sulemana, A., & Ebenezer. (2024). Contamination of groundwater by petroleum hydrocarbons: Impact of fuel stations in residential areas. *Heliyon*, e25924–e25924. https://doi.org/10.1016/j.heliyon.2024.e25924

Folina, A., Kakabouki, I., Baginetas, K., & Bilalis, D. (2025). Integration of Bioresources for Sustainable Development in Organic Farming: A Comprehensive Review. *Resources*, *14*(7), 102. https://doi.org/10.3390/resources14070102

Gomiero, T. (2016). Soil Degradation, Land Scarcity and Food Security: Reviewing a Complex Challenge. *Sustainability*, *8*(3), 281. https://doi.org/10.3390/su8030281

Hadidi, M., Hossienpour, Y., Nooshkam, M., Mahfouzi, M., Gharagozlou, M., Aliakbari, F., Aghababaei, F., & McClement, D. J. (2023). Green leaf proteins: a sustainable source of edible plant-based proteins. *Critical Reviews in Food Science and Nutrition*, 1–18. https://doi.org/10.1080/10408398.2023.2229436

Huang, S., Zheng, X., Luo, L., Ni, Y., Yao, L., & Ni, W. (2021). Biostimulants in bioconversion compost of organic waste: A novel booster in sustainable agriculture. *Journal of Cleaner Production*, *319*, 128704. https://doi.org/10.1016/j.jclepro.2021.128704

Hussain, I., Puschenreiter, M., Gerhard, S., Sani, S. G. A. S., Khan, W., & Reichenauer, T. G. (2019). Differentiation between physical and chemical effects of oil presence in freshly spiked soil during rhizoremediation trial. *Environmental Science and Pollution Research*, *26*(18), 18451–18464. https://doi.org/10.1007/s11356-019-04819-6

Joutey, N. T., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013). Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms. In *www.intechopen.com*. IntechOpen. https://www.intechopen.com/chapters/45093

Karpenko, O., Lubenets, V., Karpenko, E., & Novikov, V. (2009). Chemical Oxidants for Remediation of Contaminated Soil and Water. A Review. *Chemistry & Chemical Technology*, *3*(1), 41–45. https://doi.org/10.23939/chcht03.01.041

Kebede, G., Tafese, T., Abda, E. M., Kamaraj, M., & Assefa, F. (2021). Factors Influencing the Bacterial Bioremediation of Hydrocarbon Contaminants in the Soil: Mechanisms and Impacts. *Journal of Chemistry*, *2021*(1), 1–17. https://doi.org/10.1155/2021/9823362

Kumar, B. L., & Gopal, D. V. R. S. (2015). Effective role of indigenous microorganisms for sustainable environment. *3 Biotech*, *5*(6), 867–876. https://doi.org/10.1007/s13205-015-0293-6

Kumari, M., Swarupa, P., Kesari, K. K., & Kumar, A. (2023). Microbial Inoculants as Plant Biostimulants: A Review on Risk Status. *Life*, *13*(1), 12. https://doi.org/10.3390/life13010012

Kuppan, N., Padman, M., Mahadeva, M., Srinivasan, S., & Devarajan, R. (2024). A comprehensive review of sustainable bioremediation techniques: Eco friendly solutions for waste and pollution management. *Waste Management Bulletin*, *2*(3). https://doi.org/10.1016/j.wmb.2024.07.005

Ławniczak, Ł., Woźniak-Karczewska, M., Loibner, A. P., Heipieper, H. J., & Chrzanowski, Ł. (2020). Microbial Degradation of Hydrocarbons—Basic Principles for Bioremediation: A Review. *Molecules*, *25*(4), 856. https://doi.org/10.3390/molecules25040856

Liu, L., Yang, H., Shin, H., Chen, R. R., Li, J., Du, G., & Chen, J. (2013). How to achieve high-level expression of microbial enzymes. *Bioengineered*, *4*(4), 212–223. https://doi.org/10.4161/bioe.24761

Malos, I. G., Ghizdareanu, A.-I., Vidu, L., Matei, C. B., & Pasarin, D. (2025). The Role of Whey in Functional Microorganism Growth and Metabolite Generation: A Biotechnological Perspective. *Foods*, *14*(9), 1488. https://doi.org/10.3390/foods14091488

McCarter, C. P. R., Rezanezhad, F., Quinton, W. L., Gharedaghloo, B., Lennartz, B., Price, J., Connon, R., & Cappellen, P. V. (2020). Pore-scale controls on hydrological and geochemical processes in peat: Implications on interacting processes. *Earth-Science Reviews*, *207*, 103227. https://doi.org/10.1016/j.earscirev.2020.103227

Michael-Igolima, U., Abbey, S. J., & Ifelebuegu, A. O. (2022). A systematic review on the effectiveness of remediation methods for oil contaminated soils. *Environmental Advances*, *9*, 100319. https://doi.org/10.1016/j.envadv.2022.100319

Mohammed, A. B., Bioltif, Y. E., Dahiru, S. W., Babanyaya, A. M., Mustapha, A. B., Adam, A. B., & Abubakar, M. Y. (2024). Exposure Pathways and Risk Assessment of soil pollution and human health: A review. *TROPICAL JOURNAL of ENGINEERING, SCIENCE and TECHNOLOGY*, *3*(2), 35–55. http://tjest.org.ng/index.php/journal/article/view/211

Mukjang, N., Chitov, T., Mhuantong, W., Champreda, V., Pathom-aree, W., Sattayawat, P., & Bovonsombut, S. (2022). Bacterial Communities Associated with Crude Oil Bioremediation through Composting Approaches with Indigenous Bacterial Isolate. *Life*, *12*(11), 1712. https://doi.org/10.3390/life12111712

Nanadeinboemi, O., Uju, M., Christopher, C., Hakeem, O., & David, D. (2024). Environmental and Health Influences of Crude Oil Spills in Niger Delta, Nigeria: Case Study Oporoma Community. *Journal of Health and Environmental Research*, *10*(2), 29–40. https://doi.org/10.11648/j.jher.20241002.11

Obida, C. B., Blackburn, G. A., Whyatt, J. D., & Semple, K. T. (2018). Quantifying the exposure of humans and the environment to oil pollution in the Niger Delta using advanced geostatistical techniques. *Environment International*, *111*, 32–42. https://doi.org/10.1016/j.envint.2017.11.009

Ordinioha, B., & Brisibe, S. (2013). The Human Health Implications of Crude Oil Spills in the Niger delta, Nigeria: an Interpretation of Published Studies. *Nigerian Medical Journal*, *54*(1), 10. https://doi.org/10.4103/0300-1652.108887

Oro, C. E. D., Puton, B. M. S., Venquiaruto, L. D., Dallago, R. M., & Tres, M. V. (2024). Effective Microbial Strategies to Remediate Contaminated Agricultural Soils and Conserve Functions. *Agronomy*, *14*(11), 2637. https://doi.org/10.3390/agronomy14112637

Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent Advances in Bacterial Degradation of Hydrocarbons. *Water*, *15*(2), 375. https://doi.org/10.3390/w15020375

Peixoto, R. S., Vermelho, A. B., & Rosado, A. S. (2011). Petroleum-Degrading Enzymes: Bioremediation and New Prospects. *Enzyme Research*, *2011*, 1–7. https://doi.org/10.4061/2011/475193

Pescuma, M., de Valdez, G. F., & Mozzi, F. (2015). Whey-derived valuable products obtained by microbial fermentation. *Applied Microbiology and Biotechnology*, *99*(15), 6183–6196. https://doi.org/10.1007/s00253-015-6766-z

Petrillo, A., Fraternali, F., Acampora, A., Di Chiara, G., Colangelo, F., & Farina, I. (2025). Innovative Solidification and Stabilization Techniques Using Industrial By-Products for Soil Remediation. *Applied Sciences*, *15*(7), 4002. https://doi.org/10.3390/app15074002

Petruzzelli, G., Pezzarossa, B., & Pedron, F. (2025). The Fate of Chemical Contaminants in Soil with a View to Potential Risk to Human Health: A Review. *Environments*, *12*(6), 183. https://doi.org/10.3390/environments12060183

Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology*, *52*(1), 49–58. https://doi.org/10.1016/j.femsec.2004.10.002

Qattan, S. Y. A. (2025). Harnessing bacterial consortia for effective bioremediation: targeted removal of heavy metals, hydrocarbons, and persistent pollutants. *Environmental Sciences Europe*, *37*(1). https://doi.org/10.1186/s12302-025-01103-y

Rafeeq, H., Afsheen, N., Rafique, S., Arshad, A., Intisar, M., Hussain, A., Bilal, M., & Iqbal, H. M. N. (2023). Genetically engineered microorganisms for environmental remediation. *Chemosphere*, *310*(310), 136751. https://doi.org/10.1016/j.chemosphere.2022.136751

Rahmati, F., Asgari Lajayer, B., Shadfar, N., van Bodegom, P. M., & van Hullebusch, E. D. (2022). A Review on Biotechnological Approaches Applied for Marine Hydrocarbon Spills Remediation. *Microorganisms*, *10*(7), 1289. https://doi.org/10.3390/microorganisms10071289

Ramos, F. T., Dores, E. F. de C., Weber, O. L. dos S., Beber, D. C., Campelo, J. H., & Maia, J. C. de S. (2018). Soil organic matter doubles the cation exchange capacity of tropical soil under no-till farming in Brazil. *Journal of the Science of Food and Agriculture*, *98*(9), 3595–3602. https://doi.org/10.1002/jsfa.8881

Riseh, R. S., Vazvani, M. G., Hassanisaadi, M., & Thakur, V. K. (2024). Agricultural wastes: A practical and potential source for the isolation and preparation of cellulose and application in agriculture and different industries. *Industrial Crops and Products*, *208*, 117904. https://doi.org/10.1016/j.indcrop.2023.117904

Rizal, A., Navarro, A. R., Reyes, J. C., Maragathavalli, S., Kristanti, R. A., Wulandari, R., & Bunrith, S. (2025). Microbial Bioremediation of Petroleum-Contaminated Soil: A Sustainable Approach. *Tropical Aquatic and Soil Pollution*, *5*(1). https://doi.org/10.53623/tasp.v5i1.683

Sharma, K., Shah, G., Singh, H., Bhatt, U., Singhal, K., & Soni, V. (2024). Advancements in natural remediation management techniques for oil spills: Challenges, innovations, and future directions. *Environmental Pollution and Management*, *1*, 128–146. https://doi.org/10.1016/j.epm.2024.08.003

Sokupa, M. I., Mupangwa, J. F., Washaya, S., Tikwayo, S. E., & Mopipi, K. (2023). The nutritive value of *Panicum maximum* and, *Brachiaria brizantha* grass species. *Acta Agriculturae Scandinavica Section A-Animal Science*, 1–9. https://doi.org/10.1080/09064702.2023.2249903

Teixeira, J., Delerue-Matos, C., Morais, S., & Oliveira, M. (2024). Environmental contamination with polycyclic aromatic hydrocarbons and contribution from biomonitoring studies to the surveillance of global health. *Environmental Science and Pollution Research*, *31*(42), 54339–54362. https://doi.org/10.1007/s11356-024-34727-3

Tiwari, M., & Tripathy, D. B. (2023). Soil Contaminants and Their Removal through Surfactant-Enhanced Soil Remediation: A Comprehensive Review. *Sustainability*, *15*(17), 13161. https://doi.org/10.3390/su151713161

Uwidia, I., & Uwidia, O. (2021). Effects of Time on pH, Total Bacteria Counts, and Total Hydrocarbon Contents in the Bioremediation of Crude Oil Contaminated Soil Using Indigenous Bio-stimulants. *Tanzania Journal of Science*, *47*(1), 344–353. https://www.ajol.info/index.php/tjs/article/view/203900

Wang, X., Chi, Y., & Song, S. (2024). Important soil microbiota's effects on plants and soils: a comprehensive 30-year systematic literature review. *Frontiers in Microbiology*, *15*(1347745). https://doi.org/10.3389/fmicb.2024.1347745

Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang, X., & Wang, G. (2013). Effects of crude oil contamination on soil physical and chemical properties in Momoge wetland of China. *Chinese Geographical Science*, *23*(6), 708–715. https://doi.org/10.1007/s11769-013-0641-6

Zeng, X., Wang, Y., Yang, S., Liu, Y., Li, X., & Liu, D. (2024). The functionalities and applications of whey/whey protein in fermented foods: a review. *Food Science and Biotechnology*, *33*(4), 769–790. https://doi.org/10.1007/s10068-023-01460-5