


UNIVERSITY OF BIRMINGHAM

# Phytoremediation of crude oil contaminated soil using *Chrysopogon zizanioides*

By

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**Abstract**

Oil spills pollute the environment, which affects human health and the ecosystem due to the presence of harmful substances such as petroleum hydrocarbons. We investigated the efficiency of biosurfactants 95% Di-Rhamnolipid and 95% Mono-Rhamnolipid in extracting total petroleum and hydrocarbons (TPH) from crude oil-contaminated soil using mild shaking. The capacity of *Chrysopogon zizanioides* to extract polycyclic aromatic hydrocarbons (PAHs) while being influenced by fertiliser and biosurfactant was also investigated. The results showed that 61% and 53.1% of TPH were removed by 95% di-Rhamnolipid and 95% mono-Rhamnolipid, respectively. The combined effect of fertiliser and biosurfactant resulted in the highest reduction of PAHs compared to fertiliser alone, biosurfactant alone, and the controls. The results suggest that fertiliser application promoted the growth of *C. zizanioides* and root turnover, which in turn increased the soil organic matter (SOM). The impact of biosurfactant, which solubilised the pollutants, may have facilitated the sorption, immobilisation, and degradation of the HMW PAHs in the soil. The results have also shown that the growth of the plants in soils treated with fertiliser alone, which was 63.4 cm, was slightly higher than that of plants grown in soils treated with a combination of fertiliser and biosurfactant, which was 56.9 cm. However, the combined effect of fertiliser and biosurfactant was most effective in removing 86% of TPH from the oil-contaminated soil. The results suggest that the combined effect of fertiliser and biosurfactant can potentially be used to aid the phytoremediation of crude oil-contaminated soil.

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**LIST OF ABBREVATIONS**

| Abbreviation | Definition |
|---|---|
| TPH | Total Petroleum and Hydrocarbons |
| PAH | Polycyclic Aromatic Hydrocarbons |
| SOM | Soil organic Matter |
| HMW | High Molecular Weight |
| PTDF | Petroleum Technology Development Fund |
| SPE | Solid Phase Extraction |
| GC | Gas Chromatography |
| MS | Mass Spectrometry |
| QA | Quality Assurance |
| QC | Quality Control |
| BCF | Bioconcentration Factor |
| TF | Translocation Factor |
| XRF | X-ray Fluorescence |
| FTIR | Fourier Transform Infrared |
| XRD | X-ray Diffraction |
| NPK | Nitrogen Phosphorus and Potassium |
| SD | Standard Deviation |
| USA | United States of America |
| EPA | Environmental Protection Agency |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| CRM | Certified Reference Material |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| WC | Water Content |
| WHO | World Health Organisation |
| CO | Carbon Monoxide |
| CO2 | Carbon Dioxide |
| SO2 | Sulphur Oxide |

| | |
|---|---|
| NO | Nitrogen Oxide |
| $NO_2$ | Nitrogen Dioxide |
| O3 | Ozone |
| $PM_{10}$, | Particulate matter less than 10 μm in diameter |
| $PM_{2.5}$ | Particulate matter less than 2.5 μm in diameter |
| $PM_{0.1}$ | Particulate matter up to 0.1 μm |
| BP | British Petroleum |
| NNPC | Nigerian National Petroleum Corporation |
| UNEP | United Nations Environment Programme |
| $CH_2$ | Methylene group |
| NORM | Naturally occurring radioactive materials |
| BTEX | Benzene toluene, ethylbenzene, and xylene |
| POP | Persistent organic pollutant |
| NAPL | Non-aqueous phase liquids |
| MCL | Maximum contaminant level |
| UK | United Kingdom |
| CLEA | Contaminated Land Exposure Assessment |
| NOAA | National Oceanic and Atmospheric Administration |
| KI | Potassium iodide |
| EDTA | Ethylenediamine tetra acetic acid |
| HCL | Hydrochloric acid |
| $HNO_3$ | Nitric acid |
| $H_2SO_4$ | Sulfuric Acid |
| $H_3PO_4$ | Phosphoric Acid |
| $Na2S_2O_3$ | Sodium thiosulfate |
| $H_2O_2$ | Hydrogen peroxide |
| $S_2O_8^2$ | Peroxydisulfate (VI) ion |
| $H_2O$ | Water |
| COC | Chemicals of concern |
| GST | Glutathione *S*-transferases |
| TCE | Trichloroethylene |
| TNT | Trinitrotoluene |
| RDX | Royal Demolition Explosive |
| HMX | High Melting Explosive |
| PCB | Polychlorinated biphenyls |
| MTBE | Methyl tert-butyl ether |
| FW | Fresh weight |
| CEC | Cation exchange capacity |
| BAF | Bioaccumulation factor |

| | |
|---|---|
| TOG | Total oil and grease |
| VGT | Vetiver grass technology |
| VS | vetiver system |
| C4 | Carbon 4 compound |
| EC | Electric conductivity |
| LOI | Loss on ignition |
| DW | Dry weight |
| CGASPL | Chhajed Garden Agro Services Private Limited |
| HPLC | High Performance Liquid Chromatography |
| DCM | Dichloromethane |
| TIC | Total Ion Current |
| SIM | Selected Ion Monitoring |
| ID | Internal Diameter |
| IS | Internal Standard |
| RFF | Relative response factor |
| SS | Sample size |
| OSX | Apple MacIntosh operating system |
| PPMCC | Pearson's product moment correlation coefficient |
| EU | European union |
| CMC | Critical micelle concentration |
| LMW | Low Molecular Weight |
| MMW | Medium Molecular Weight |
| p.l.c. | Public limited company |
| ND | Not Detected |

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background

Environmental pollution can be defined as the introduction of contaminants (natural or anthropogenic) into the environment that can harm humans and the ecosystem (Perk, 2013, p.4). The World Health Organisation (WHO) reported on the 15th of March 2016 that an estimated mortality of 12.6 million people occurred in 2012, which is attributed to working or residing in harmful environments. This accounts for one in four deaths that occur globally due to environmental risk factors such as air, water, and land pollution, as well as exposure to chemicals, climate change, and ultraviolet radiation, which together cause over 100 illnesses (WHO, 2016).

Soil contamination is generally linked to rapid population growth combined with a lack of awareness of the negative effects of contaminants (Gupta, 2006; Ashraf and Hanfiah, 2017). It is also linked with rapid growth in industrial, agricultural, and domestic activities that has led to an increase in environmental contamination (Singh and Jain, 2003; Gupta, 2006; Kang, 2014; Ashraf and Hanfiah, 2017). Since the industrial revolution and the introduction of the internal combustion engine, there has been a rapid increase in the use of natural resources, resulting in many health and environmental consequences, such as the introduction of toxic substances such as PAHs, carbon monoxide (CO), carbon dioxide (CO2), sulphur oxides (SO2), nitrogen oxides (NO and $NO_2$), ozone (O3) and particulate matter ($PM_{10}$, $PM_{2.5}$, and $PM_{0.1}$) (Longman et al., 2018; Ukaogo et al., 2020). The current global population is estimated to be 7.3 billion, with projections of 8.5 billion by 2030, 9.7 billion by 2050, and 11.2 billion by 2100 (United Nations, 2019). Consequently, the rapid increase in global population has also generated a continuous growth in energy demand and use, putting more pressure on resources such as petroleum (Asif and Muneer, 2007; Scheffran et al., 2020). For instance, in 1990, global energy consumption relied heavily on fossil fuel sources, and is also projected to increase by 2035 (Karakurt and Aydin, 2013). Similarly, between 1980 and 2001, global consumption of fossil fuels such as petroleum, coal, and natural gas increased by 22, 27, and 71%, respectively. According to a recent energy industry report, the proportion of the world's primary energy consumption in 2022 that came from petroleum, coal, and natural gas was around 31%, 28%, and 25%, respectively (BP p.l.c, 2022).

The petroleum industry is generally responsible for environmental damage, which often occurs through oil spills because of the exploration, extraction, production, transportation, and use of petroleum (Schmidt-Etkin, 2011; Anejionu et al., 2015; Zhang et al., 2019). According to Chilvers et al., (2021), more than 1,700 acute oil spills have been documented between 1970 and 2018. The major causes of oil contamination have been attributed to accidental oil spills from ruptured pipes and oil wells, and improper disposal of oil waste (Brandt et al., 2006; Jernelöv, 2010; Galuzzi-Silva and Mata-Lima, 2021). Soil contamination that occurs through the release of petroleum hydrocarbons is a global environmental issue that can affect human health and the surrounding ecosystem (Banks and Schultz, 2005; Osuji and Nwoye, 2007; Lee, 2011). For example, the exploration and exploitation of petroleum has created a lot of negative impacts on the socio-physical environment of the Niger Delta region of Nigeria (Uwakonye et al., 2006; Sakib, 2021). There are currently 606 oil fields in the Niger Delta region of Nigeria, of which 355 are onshore and 251 are offshore, with a total of 193 functional as of 2015 (NNPC, 2015). Furthermore, an average of 240,000 barrels of crude oil is reported to be spilt annually in the Niger Delta region of Nigeria (Ordinioha and Brisibe, 2013). However, despite the environmental impact caused by the oil industry, it has also played a major role in the growth of Nigeria's economy. Petroleum is the major source of income for Nigeria and the country has risen to the position of the tenth largest oil producer in the world since oil was first discovered in 1956 (Uwakonye et al., 2006). As a result of the importance of oil extraction, oil pollution has become a significant environmental factor within the Niger Delta, affecting vegetation and making the soil infertile for agricultural production and posing a threat to human health (Brown and Tari, 2015). Furthermore, it has been estimated that it may take up to twenty-five to thirty years to restore some of the contaminated lands in the Niger Delta region of Nigeria (UNEP, 2011a).

Historical records have shown that soil pollution has always been ignored, which has resulted in severe, widespread environmental pollution (Scullion, 2006). However, the dangers of exposure to contaminants such as Polycyclic Aromatic Hydrocarbons (PAH) and heavy metal contamination from oil spills are now well-known (Khan et al., 2004; Wei et al., 2015; Zychowski and Godard-Codding, 2017; Qu et al., 2020). It is estimated that about 0.08-0.46% of the total global oil

produced is spilt into the environment, causing severe environmental contamination (Bognolo, 1999; Kostecki, 1999). Thus, concern over human and environmental health is raised as petroleum contaminants are believed to contain carcinogenic, mutagenic, and growth-inhibiting chemicals (Singh and Lin, 2008; Reátegui-Zirena et al., 2014; Ramirez et al., 2017; Koukoulakis et al., 2020). Generally, land-based oil spills affect soil and groundwater through the distribution and transformation of petroleum hydrocarbons in the environment (Menendez-Vega et al., 2007). When crude oil is released onto land surfaces, the petroleum hydrocarbons are distributed in various forms (Coulon et al., 2010). The organic matter in the soil absorbs some petroleum hydrocarbons and volatilisation of the extremely volatile hydrocarbons also takes place while others migrate into the groundwater (Huesemann et al., 2005; Molins et al., 2010). The PAHs component of crude oil is usually absorbed by organic matter present in the soil (Plant et al., 2012; Ukalska-Jaruga et al., 2019; Diagboya et al., 2021). PAHs normally persist in the environment with a potential for bioaccumulation and are considered and classified as carcinogenic compounds (Rajendran et al., 2013; Wang et al., 2020; Anae et al., 2021). The United States Environmental Protection Agency (US EPA) has classified 16 PAHs as priority pollutants with regards to obtainable information including toxicity, possible human contact, and persistence in contaminated environments. Therefore, to ensure the protection of human health and the surrounding ecosystem, appropriate soil remediation is essential. Regardless of the major achievements recorded in terms of the conventional techniques used for soil remediation, most of the technologies seem unsustainable (Batty and Dolan, 2013; Novo et al., 2018). The reason is that they often cause more harm to the environment in several ways. These include the use of toxic chemicals for treating or transforming contaminants in the soil, mechanical means for soil excavation to landfill or thermal treatment that may affect soil fertility (Batty and Dolan, 2013).

Globally, researchers are trying to explore sustainable approaches for treating harmful environmental contaminants (Srivastava et al., 2008). Phytoremediation, which involves the efficient use of plants for treating environmental contaminants, is a cost-effective and sustainable approach (Novo et al., 2018). It involves the use of plants and soil microorganisms for the treatment of contaminated environments such as soil, water, and air (Mench et al., 2009; Thijs et al., 2017

Dhanwal et al., 2017; Dhanwal et al., 2017; Odoh et al., 2019). It also serves as an alternative to engineering methods that are capital-intensive and cause damage to the environment in many ways as mentioned above. Phytoremediation can be used for the treatment of contaminants such as crude oil, poly-aromatic hydrocarbons, heavy metals, pesticides, solvents, explosives, and landfill leachate (Zhang et al., 2012; Novo et al., 2013; Hou et al., 2015; Brown et al., 2017; Dhanwal et al., 2017). There are various types of phytoremediation that can be applied depending on the contaminants involved, including phytoextraction, phytostabilisation, phytovolatilisation, phytodegradation, rhizodegradation, or phytostimulation (Sharma and Pandey, 2014; Chandra et al., 2017; Cristaldi et al., 2020). Among the processes involved in the phytoremediation of organic compounds such as petroleum contaminants, rhizodegradation, which involves the degradation of organic contaminants through mutual interaction between plants and soil microorganisms, is considered more efficient, amendable and can potentially improve the degradation of organic contaminants (Dzantor, 2007 ; Badri et al., 2009; Sharma and Pandey, 2014; Dessaux et al., 2016; Ling et al., 2022). However, the process known as phytodegradation seems faster than all the methods mentioned above and is also capable of directly eliminating contaminants from the environment (D'Orazio et al., 2013; Sharma and Pandey, 2014; Houshani et al., 2021). Phytoremedition involves the absorption and degradation of organic contaminants within the tissues of plants (Abdel-Shafy and Mansour, 2018; Houshani et al., 2021). This normally occurs when plants absorb and metabolise chemical compounds such as organic contaminants present in their growing environment and then break them down into smaller molecules which are stored within the tissues of the plants (Sharma and Pandey, 2014; Hyland et al., 2015; Zhou et al., 2015; Christou et al., 2019).

According to Wittig et al. (2003) and Ferro et al. (2013), certain species of plants such as the poplar tree have successfully been used for the phytoremediation of organic contaminants. However, grasses are more suitable for use in phytoremediation because of their ability to easily grow without requiring maintenance, the presence of fibrous and deep roots that deeply penetrate the soil, and the ability to survive under unfavourable soil conditions (Sinha et al., 2013; Pidlisnyuk et al., 2014; (Kassaye et al., 2017; Mirzaee et al., 2021). For instance, vetiver grass (*C. zizanioides*), a non-

invasive native plant to Southeast Asia, is known to have certain special characteristics that are considered ideally suited for phytoremediation (Zhang et al., 2014; Suelee et al., 2017; Patel and Patra, 2017; Ramos-Arcos et al., 2021). These characteristics include the ability to tolerate harsh environmental conditions (drought, floods, and high temperatures), possession of intensive and deep root systems, and the ability to tolerate acidic, alkaline, and saline soils, and soils contaminated with heavy metals, including Pb, Cu, Zn, and Cd Truong et al., 2010; Kiiskila et al., 2019; Wijitkosum, 2020; Islam et al., 2020). *C. zizanioides* has generally been used across the globe for various environmental protection purposes, including the control of erosion and treatment of contaminated areas such as industrial and landfill sites (Bwire et al., 2011; Yeboah et al., 2015; Nero, 2021; Islam et al., 2020). Furthermore, *C. zizanioides* has also been proven to tolerate soils contaminated with PAHs (Li et al., 2006; Nisa and Rashid, 2015; Alsghayer et al., 2020). Thus, *C. zizanioides* can be considered as a potentially suitable plant for conducting the phytoremediation of crude oil contaminated soil.

The limitations associated with phytoremediation usually include factors such as the rooting depth of plants, leaching of contaminants, disposal of plants material (by-products), availability of nutrients and water, as well as atmospheric, physical, and chemical conditions (Banuelos et al., 2015; Zand and Hoveidi, 2016a; Tripathi et al., 2020). Similarly, phytoremediation can be affected by the interaction of plants with other organisms (pests and pathogens) as well as contact with the contaminants (Susarla et al., 2002; Ortega et al., 2020). Thus, the major limiting factors of phytoremediation result from the physiochemical properties of the compounds, such as contaminant bioavailability and toxicity, environmental factors (Chaudhry et al., 2002; Liao et al., 2016), soil type, plant species, availability of nutrients (Chaudhry et al., 2002; Zand and Hoveidi, 2016), long duration and suitability of the method (Singh et al., 2003; Banuelos et al., 2015). However, the advantages of this technology outweigh its limitations because it is cost-effective, environmentally friendly and conserves natural resources.

Phytoremediation of petroleum hydrocarbons is limited by their low solubility, which affects their bioavailability and absorption by plants (Kuyukina et al., 2005; Sharma and Pandey, 2014; Liao et

al., 2016). However, efficient phytoremediation can be achieved through the introduction of microorganisms such as contaminant degrading bacteria, including *Pseudomonas putida*, *Flavobacterium* sp., and *Pseudomonas aeruginosa* (Huang et al., 2004a; Liao et al., 2016; Wang et al., 2017; Liduino et al., 2018). For example, microorganisms belonging to *P. aeruginosa* species are known to release bio-surfactants (rhamnolipids) which help in solubilizing extremely hydrophobic organic compounds, thereby increasing the bioavailability of these compounds (Kuyukina et al., 2005; Ram et al., 2019; Zhen et al., 2019; Jia et al., 2020). Another limiting factor that affects the efficiency of phytoremediation is the phytotoxicity of petroleum contaminants, which affect the growth of plants (Agnello et al., 2016; Nero, 2021). This issue, however, can be addressed by applying fertiliser, which is known to improve plant growth, during the phytoremediation process (Cartmill et al., 2014; Yu et al., 2019).

### 1.2. Research gaps

Previous studies on phytoremediation of petroleum-contaminated soils using *C. zizanioides* have indicated that effective reduction of petroleum contaminants can only be achieved through compost amendment (Nisa and Rashid, 2015; Nero, 2021). However, soil composting may encourage more microbial activity than plant-based remediation. Also, as many sources of animal manure contain antibiotics, composting can potentially pose a threat to human health by introducing pathogenic and antibiotic-resistant bacteria and genes into the environment (Soobhany, 2018; Cunningham et al., 2020). As recent research on phytoremediation of petroleum hydrocarbons using *C. zizanioides* have not focused on PAHs, which normally persist in the environment (Dudai et al., 2018; Rajaei and Seyedi, 2018; Nero, 2021), the ability of the plant to extract persistent organic pollutants such as PAHs from the soil is still unclear. Furthermore, previous studies on the use of biosurfactants to enhance phytoremediation of persistent organic pollutants such as petroleum hydrocarbons have focused on the use of plants such as *Medicago sativa, Zea mays,* and *Ludwigia octovalvis* (Almansoory et al., 2015; Liao et al., 2016; Benson et al., 2017). However, these plants that have short roots which restricts the treatment of

contaminants to the upper layers of the soil (Ansari et al., 2014; Zand and Hoveidi, 2016). Additionally, the use of invasive plant species that may cause negative impacts on the environment also constitutes part of the limitations of phytoremediation (Zand and Hoveidi, 2016; Oyuela Leguizamo et al., 2017; Keeran et al., 2019). Hence, there is a need to investigate the potential of *C. zizanioides* as a deep-rooted, tolerant, non-invasive, perennial plant under the influence of fertiliser and biosurfactant to address the problems mentioned above. This will help in creating a sustainable and holistic approach to addressing the problem of crude oil contamination in the soil.

### 1.3. Aim and Objectives of the Research

Aim

The overall aim of this research is to determine the effectiveness of *C. zizanioides* in the phytoremediation of crude oil contaminated soil, with particular emphasis on PAHs due to their toxicity and persistence in the environment.

The research has the following objectives:

1. To determine the general ability of *C. zizanioides* to phytodegrade petroleum-derived hydrocarbons in the soil.
2. To determine the ability of *C. zizanioides* to immobilise and contain crude oil contaminants in the soil.
3. To assess the level of tolerance of *C. zizanioides* to crude oil contaminated soil.
4. To identify the effect of biosurfactants (rhamnolipids) on the bioavailability of petroleum hydrocarbons for uptake by *C. zizanioides*.
5. To assess the effect of NPK fertiliser on the growth of *C. zizanioides* and dissipation of PAHs in a crude oil contaminated soil.

6. To assess the effect of soil chemistry (minerals and elements) on the tolerance of *C. zizanioides* to crude oil contaminated soil

## 1.4. Structure of the thesis

This thesis is structured as a thesis by publication, and as such is composed of a series of draft publications. Hence, some of the methods utilised are similar across the chapters. The chapters of the thesis are presented as follows:

- Chapter 2: describes the composition and global distribution of petroleum crude oil as well as the impact and current state of the art in remediation of crude oil contaminated soil.
- Chapter 3: describes the materials and methods utilised in this thesis.
- Chapter 4: describes the removal of removal developed in this thesis, utilising *C. zizanioides* for removal of PAHs from the soil
- Chapter 5: describes the immobilisation of petroleum hydrocarbons in the soil and the effect of biosurfactant on PAH extraction from the soil.
- Chapter 6: describes the effect of petroleum contaminants such as PAHs on the growth of *C. zizanioides* and on the soil microbial quantity during phytoremediation.
- Chapter 7: describes the summary of the main outcomes of the research, the conclusions, and the future directions of the approach developed in this PhD research.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Description and Composition of Crude Oil

The term petroleum is derived from the Latin words petra and oleum, meaning rock oil (Groysman, 2014; Moustafa, 2014). It is also termed as crude oil, formed from a mixture of decomposed organic matter over millions of years (Speight, 2020). Petroleum may appear as a whitish or blackish liquid that flows freely or becomes immobile at room temperature (Speight, 2016).

Petroleum is generally made up of a complex mixture of different types of hydrocarbons (compounds of hydrogen and carbon) and other non-hydrocarbons, as well as traces of metals (Speight and Ozum, 2001; Overton et al., 2016; Bolade et al., 2021). The hydrocarbon group includes paraffins, cycloparaffins, aromatics and the unsaturated hydrocarbons (Speight, 2014; Sarıkoç, 2020). The paraffin hydrocarbons are composed of saturated chain-shaped molecules that are an extension of methane with each member of the family having an additional $CH_2$. Naphthenes, also known as cycloparaffin or alicyclic hydrocarbons, are saturated ring-shaped hydrocarbons that are an extension of volatile liquids like naphtha. Aromatic hydrocarbons are a class of unsaturated ring-shaped hydrocarbons that have benzene as the main compound (Speight, 2014; Moustafa, 2014), The non-hydrocarbons consist of compounds of sulphur, oxygen, and nitrogen. They may also contain carbon dioxide, naphthenic acids, Naturally Occurring Radioactive Materials (NORM) and inorganic salts such as sodium chloride, magnesium chloride, calcium chloride, and trace metals including copper, nickel, iron, arsenic, and vanadium (Elsamani and Abdel Aziz, 2016; Gupta, 2019). The form and amount of chemicals in petroleum crude oil vary greatly (Overton et al., 2016). Crude oil normally contains an average of 84% carbon, 14% hydrogen, 1-3% sulphur and a very small amount (less than 1%) of nitrogen, oxygen, metals, and salts (Nasirpouri et al., 2014; Coker, 2018). However, the issue of primary concern is the toxicity of some of the chemicals present in crude oil that can cause harm to human health and the surrounding ecosystem because of spills or accidental releases into the environment.

Total Petroleum Hydrocarbons (TPH) refers to a complex mixture of chemical compounds dominated by hydrocarbons, aromatic compounds, asphaltenes and resins (Tang et al., 2012;

Pinedo et al., 2013). Thus, it is not possible to quantify each TPH individually, but the TPH at a site can be quantified as a general indication of the extent of contamination by petroleum (Rauckyte et al., 2010; Pinedo et al., 2013). However, there should be more concern over the potential environmental and health risks of petroleum hydrocarbons than just measuring the TPH at a site (ATSDR, 1999; Brewer et al., 2013; Alinnor et al., 2014; Adipah, 2019). Aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are specific constituents of TPH considered to pose high health risks to humans (Pinedo et al., 2013; Bolade et al., 2021). However, benzene is the only compound that has been classified as carcinogenic (cancer-causing) by the U.S. Environmental Protection Agency (EPA) and other health and environmental agencies (Smith et al., 2016; Elliott et al., 2017; Masekameni et al., 2019). Benzene is first in the group of aromatic compounds; it is a colourless liquid with a distinct odour, is slightly water soluble and mixes readily with organic solvents (Patnaik, 2007).

Polycyclic Aromatic Hydrocarbons (PAHs) are also components of TPH that are considered to pose health hazards (Rengarajan et al., 2015). Unlike aromatic hydrocarbons, PAHs are made up of multiple rings (Tiwari et al., 2013; Wang et al., 2018). PAHs with three or more rings have low water solubility and low vapour pressure, which increase with increasing molecular weight (Nwaichi et al., 2016; Jameson, 2019). They are mostly located in soil and sediment, the atmosphere, and on water surfaces (Dong and Lee, 2009). They tend to be immobile and persist in the environment, which lead to excessive levels of toxicity (Nwaichi et al., 2016). PAHs normally bio-accumulate in the food chain, causing biochemical and physiological effects on many living organisms including human beings (Octaviani et al., 2017; Omwoma et al., 2019). PAHs have been classified as carcinogens, mutagens, teratogens, and toxic compounds (Haleyur et al., 2016; Petrovic et al., 2018). The US EPA has recognized specific 16 categories of PAHs as priority pollutants based on reasons such as the available information on their toxicity, possible human contact, and the duration for which they exist in a contaminated environment (Bojes and Pope, 2007; Morakinyo et al., 2020). The PAHs of concern (classified as carcinogenic) are characterised by a very low aqueous solubility, higher molecular mass, low bioavailability, and high numbers of aromatic rings

(Johnsen et al., 2005; Duan et al., 2015). Table 2.1 shows the 16 PAHs selected by the US EPA as priority pollutants based on their physical and chemical properties.

Table 2.1 US EPA's 16 priority-pollutant PAHs and selected physical–chemical properties of these PAHs (Lee, 2010; ATSDR in Bojes and Pope, 2007).

| Polycyclic aromatic hydrocarbons | Potential Human Carcinogens | No. of Rings | Mol. wt. (g/mole) | Solubility (mg/L) | Vapour pressure (mm Hg) |
|---|---|---|---|---|---|
| Naphthalene | - | 2 | 128.17 | 31 | 8.89E−02 |
| Acenaphthene | - | 3 | 154.21 | 3.8 | 3.75E−03 |
| Acenaphthylene | - | 3 | 152.20 | 16.1 | 2.90E−02 |
| Anthracene | - | 3 | 178.23 | 0.045 | 2.55E−05 |
| Phenanthrene | - | 3 | 178.23 | 1.1 | 6.80E−04 |
| Fluorene | - | 3 | 166.22 | 1.9 | 3.24E−03 |
| Fluoranthene | - | 4 | 202.26 | 0.26 | 8.13E−06 |
| Benzo(a)anthracene | Yes | 4 | 228.29 | 0.011 | 1.54E−07 |
| Chrysene | Yes | 4 | 228.29 | 0.0015 | 7.80E−09 |
| Pyrene | - | 4 | 202.26 | 0.132 | 4.25E−06 |
| Benzo(a)pyrene | Yes | 5 | 252.32 | 0.0038 | 4.89E−09 |
| Benzo(b)fluoranthene | Yes | 5 | 252.32 | 0.0015 | 8.06E−08 |
| Benzo(k)fluoranthene | Yes | 5 | 252.32 | 0.0008 | 9.59E−11 |
| Dibenz(a,h)anthracene | Yes | 6 | 278.35 | 0.0005 | 2.10E−11 |
| Benzo(g,h,i)perylene | - | 6 | 276.34 | 0.00026 | 1.00E−10 |
| Indeno[1,2,3-cd]pyrene | Yes | 6 | 276.34 | 0.062 | 1.40E−10 |

## 2.2. Distribution of Crude Oil in the Environment

Following a spill, the distribution of petroleum compounds in the soil usually depends upon the processes of evaporation, dispersion, and microbial degradation that vary due to hydrocarbons having different physical and chemical properties such as vapour pressure and solubility (CRAM et al., 2004). The moment petroleum oil enters the environment, certain physiochemical and biological processes occur that alter the composition, toxicity, distribution, and bioavailability of the oil (Brassington et al., 2007). The collective term used for these physiochemical and biological processes is referred to as weathering (Williams et al., 2006, Mishra and Kumar, 2015). These

processes mainly involve volatilisation, sorption (e.g. onto soil organic matter), biodegradation, and dissolution (Williams et al., 2006; Zhao et al., 2016; Mishra and Kumar, 2015; Kuppusamy et al., 2020). For example, when crude oil is spilt onto land, the hydrocarbons with fewer carbon and hydrogen atoms are volatilised, leaving behind the heavier and less volatile hydrocarbons (Fingas, 2014; US EPA, 2022).

The distribution of petroleum hydrocarbons in the soil is normally affected by certain factors, such as the soil properties including soil texture, moisture, and the presence of organic matter in the soil (Reid et al., 2000; Chen and Tien, 2020; Cao et al., 2021). Similarly, contaminant properties and environmental factors such as temperature and rainfall can also affect the fate of contaminants in the soil (Reid et al., 2000; Cao et al., 2021). The major weathering processes that occur in the soil are summarised in Figure 2.1.

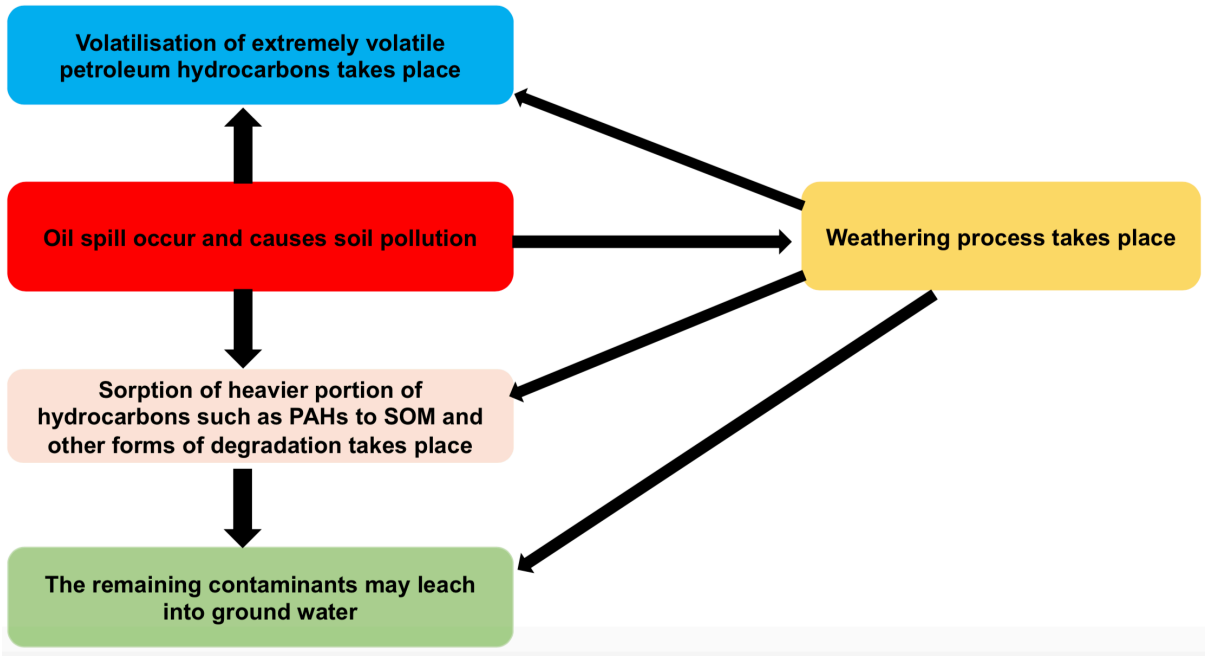



Figure 2.1 The stages of weathering of petroleum hydrocarbons in the environment.

### 2.2.1. Volatilisation

When petroleum contaminants enter the environment, the extremely volatile hydrocarbons are usually emitted through volatilisation, and subsequently some of the volatilised hydrocarbons are dispersed in the atmosphere (Halmemies et al., 2003 Ryerson et al., 2011; Afshar-Mohajer et al., 2019). Similarly, the density and viscosity of the heavier and less volatile hydrocarbons becomes altered because of the volatilisation of lighter and extremely volatile petroleum hydrocarbons (Williams et al., 2006; Fingas, 2014). The rate of volatilisation of petroleum hydrocarbons in the environment generally depends on temperature and vapour pressure as well as the concentration of gas and liquid phases of the substance (Halmemies et al., 2003 Kar et al., 2009; Mackay and van Wesenbeeck, 2014). A practical example is the research conducted by Han et al., (2018) on the environmental impacts of the Chennai oil spill in India. The result of their weathering experiment revealed that volatilisation under at a temperature of 29°C had played a significant role in degrading the oil within the early hours of the spill. Guo et al., (2019) also studied the weathering characteristics of the spilt crude oil and discovered that temperature above 32 °C increased the volatilisation of the crude oil. Bykova et al., (2021) also conducted research on the thermal desorption treatment of petroleum hydrocarbon contaminated soil under temperatures ranging from 25 to 250 °C. The results revealed that reduction in the concentration of petroleum hydrocarbons below the acceptable values was only achieved under an optimal temperature of 250 °C. Li et al., (2008) have also investigated a microwave induced thermal treatment for petroleum contaminated soil and discovered that 99% removal of contaminants within 10 mins at a microwave power of 800 W and temperature of 500 °C was achieved.

### 2.2.2. Sorption

Sorption is the distribution of compounds from one phase to another by absorption or partitioning, which can affect the movement and chemical and biological reactivity of hydrophobic organic contaminants such as polynuclear aromatic hydrocarbons (Huang et al., 2003). Sorption of organic

compounds to constituents of soil is an essential factor in the fate of organic contaminants in the environment (Huang et al., 2003; Akram et al., 2018). It is mainly controlled by soil organic matter (SOM) such as decaying plants and soil microorganisms (Gunasekara and Xing, 2003; Cornelissen et al., 2005; Akram et al., 2018). When petroleum contaminants are spilt onto the land surface, a large quantity of the heavier non-volatile portion gets trapped and absorbed by organic matter in the soil, which affects their distribution, bioaccumulation, bioavailability, and rate of mass transfer for biodegradation (Lai et al., 2009; Akram et al., 2018). While the spilt oil persists in the environment over extended periods some changes may occur due to weathering, causing more damage to the surrounding ecosystem (Huang et al., 2004; Omwoma et al., 2019). Some persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), usually get absorbed onto soil organic matter, which stops them from migrating to the groundwater. For example Chen et al., (2019) explored the effect of natural organic matter on the bioavailability of petroleum hydrocarbons in the soil and discovered a significant effect of 20% SOM on their degradation than 2% SOM. Liu et al., (2013) also studied the effect of SOM on bioremediation of diesel contaminated soil and discovered a reduction in the rate of degradation with increase in SOM. John et al., (2016) investigated the weathering pattern of PAHs based on the detection of residues along the shoreline of Alabama from the Deepwater Horizon oil spill and discovered large amount of hydrocarbons including PAHs within SOM.

### 2.2.3. Biodegradation

Biodegradation of petroleum contaminants can be described as the breakdown of complex petroleum hydrocarbons to simpler compounds through microbial processes (Varjani, 2017). It plays a major role in the fate and distribution of petroleum hydrocarbons in the environment as most of them get degraded by microorganisms in the subsurface (Youdeowei, 2012). Hence, it is considered as an important process of weathering that helps in the removal of toxic petroleum contaminants in the environment (Venosa and Zhu, 2003). For instance, the catabolic activities of soil microorganisms serve as a major device for the removal of organic contaminants such as

PAHs in the soil (Semple et al., 2003). These microorganisms mainly consist of species such as bacteria, yeast and fungi that utilize organic compounds for food through catabolism that produce smaller compounds (Al-Majed et al., 2012; Varjani, 2017). A typical example is shown in the work conducted by Das and Mukherjee, (2007), which revealed that *Pseudomonas aeruginosa* M and NM, isolated from a petroleum-contaminated site in India, reduced the TPH level in the soil from 84 to 21 g/kg, and *Basillus subtilis* DM-04 decreased the TPH level in the soil from 84 to 39 g/kg, as compared to control soil, where TPH was lowered from 84 to 80 g/kg after 120 days. Yanto and Tachibana, (2014) studied the potential of fungal co-culturing in enhancing the biodegradation of petroleum hydrocarbons in the soil. It involves the use of filamentous *Pestalotiopsis* sp. NG007 consisting of four basidiomycetes, namely *Trametes versicolor* U97, *Pleurotus ostreatus* PL1, Cerena sp. F0607, and Polyporus sp. S133. The results indicated a high level of biodegradation by the fungal co-culture in all fractions of petroleum hydrocarbons in the contaminated soil samples. Similarly, Gojgic-Cvijovic et al., (2012) found that a bacterial consortium from various genera, including *Pseudomonas, Achromobacter, Bacillus,* and *Micromonospora,* isolated from petroleum sludge resulted in degrading 82 to 88% of TPH in petroleum sludge and 86 to 91% of TPH in contaminated soil in a period of over 12 weeks.

### 2.2.4. Dissolution

During the process of crude oil weathering in the soil, some portions of petroleum hydrocarbons dissolve and infiltrate into the groundwater (Nadim et al., 2000; Mao et al., 2009). Thus, it often becomes a source of groundwater contamination  Teng et al., 2013). These petroleum hydrocarbons are referred to as non-aqueous phase liquids (NAPL) because they are not soluble or easily mixed with water (Youdeowei, 2012). The migration of the NAPL in the saturated zone usually occurs vertically because of gravitational and capillary forces depending on the water solubility of the NAPL (Müller and Sedláčková, 2003). Moreover, the fate of petroleum hydrocarbons in the unsaturated zone during the process of leaching can generally be affected by factors such as sorption, volatilisation, dissolution, biodegradation, and general composition of the

hydrocarbons (Dror et al., 2002; Akram et al., 2018). Certain portions of crude oil known as aromatic hydrocarbons, particularly benzene, toluene, ethylbenzene and xylene (BTEX), are considered as the most highly soluble and mobile compounds (Njobuenwu et al., 2005). Benzene is the primary contaminant of concern, typically found in groundwater near oil spill sites, and is more mobile and toxic than other petroleum hydrocarbons (Williams et al., 2006). A typical example is shown by Lindén and Pålsson, (2013) who studied the level and impact of oil contamination in rivers, creeks, and groundwater in Ogoniland, Nigeria. The results showed severe, widespread contamination caused by oil spills in the rivers, creeks, groundwater, and the entire vegetation with a level of benzene found at 9000 $mgL^{-1}$, considered to be 900 times more than the WHO approved standard. Doherty and Otitoloju, (2013) also assessed the extent and nature of soil and groundwater contamination by oil spills from the NNPC pipeline explosions in Lagos state, Nigeria and discovered high levels of petroleum hydrocarbons in the soil and groundwater above the W.H.O. approved limit of 0.1 mg/L, and the level of BTEX found was more than the 0.2 mg/L limit approved by the Environmental Guideline for the Petroleum Industry in Nigeria. Gross et al., (2013) also assessed the concentration of BTEX in groundwater from surface spills at a drilling site in the United States of America by analysing publicly available data from 2010 to 2011 on 77 reported cases of BTEX in groundwater from surface spills that exceeded the National Drinking Water Maximum Contaminant Level (MCLs) in Weld County and discovered groundwater contamination with benzene. Similarly, Rego and Netto, (2007) discovered high levels of benzene contamination in groundwater above the limit approved by the Brazilian regulations in research conducted at gasoline stations in Rio de Janeiro, Brazil between the years 2003 and 2004.

### 2.3. Environmental Risk Assessment

Risk assessment involves evaluating the potential threat of contaminants on humans and the environment that may result from chemical incidents leading to exposure (Fryer et al., 2006). It helps in managing potential risks of contaminants and determines if soil remediation is needed or

not (Lai et al., 2010; Damian et al., 2019). Thus, assessment of health effects arising from an oil spill is normally preceded by assessing the amount and extent of petroleum contaminants that can affect the sub-soil and groundwater, and whether this exceeds safe limits for key ecosystem organisms and/or humans, which also helps to determine the level of remediation to be carried out (Iturbe et al., 2005). Following the completion of the risk assessment, remediation can be carried out by severing the connection between the source, pathway and receptor (Catherine et al., 2000; Lai et al., 2010). This can be done by reducing the quantity, concentration, toxicity, and mobility of contaminants (Caliman et al., 2011; Radziemska et al., 2019).

Risk can be managed through relevant legislation, and structures consisting of laws, policies, regulations, and procedures primarily designed for environmental protection (Fingas, 2016). For instance, according to the UK Contaminated Land Exposure Assessment (CLEA) model, the need for soil remediation solely depends on the relationship that links the source of contaminants, pathway of exposure (inhalation, ingestion and contact with skin) and the receptor (Gay and Korre, 2006; Thornton et al., 2008).

The fundaments of risk assessment involves analysing the potential risks that may arise from incidents by gathering information from hazard identification, which determines the potential human health and ecological risks due to exposure to contaminants, the exposure assessment, which evaluates the concentration, extent, and frequency of human and environmental exposure to contaminants, dose-response/toxicity assessment, which describes the harmful effect of dosage on humans or the environment and risk characterisation that involves assessing the number of incidences related to health and environmental effects due to exposure to contaminants (Öberg and Bergbäck, 2005; Carlon et al., 2008; Dixit, 2018).

The level of concentration of the contaminants and uncertainty values are also taken into consideration during the process of conducting a risk assessment (Fryer et al., 2006; Carlon et al., 2008). Additionally, the quantity, identity, distribution, toxicity, chemical reactivity, and by-products of contaminants that could be present in the environment from chemical incidents can also be

evaluated by assessing the contaminated land (Catherine et al., 2000). Likewise, information on the level of contamination and potential effects of the contaminants can be obtained through environmental monitoring, which also helps with decisions on human health issues (Wyke et al., 2014). Similarly, the CLEA model also considers the uncertainly values that may result from the absence of information on the risks arising from the source, fate, and transport of contaminants in the environment due to parameter uncertainty, model uncertainty and scenario uncertainty (Jeffries and Martin, 2008). Hence, it is essential to obtain information on the type, chemical composition, and the depth to which hydrocarbons and other petroleum components have penetrated the soil in the case of an oil spill contamination.

The major routes of transport of oil contaminants into the environment is through the air, soil, water, and the food chain, where the soil plays a major role, serving as both a direct and intermediate pathway to human exposure and an intermediary to other sources of contamination (Cocarta et al., 2015). For instance, when contaminants get deep down into the soil or settle at the surface of the soil, they can potentially leach and contaminate groundwater and the atmospheric air, respectively (Pierzynski et al., 2005). Therefore, humans become exposed to petroleum contaminants through different routes, such as ingestion of contaminated soil (common for children and unusual in adults), breathing contaminated air, or having skin contact with the contaminated soil, dust, or water at different concentrations, lengths, and frequencies (Cocarta et al., 2015). Figure 2.3 below shows a summary diagram of the multiple pathways via which humans can become exposed to contaminants in the environment.

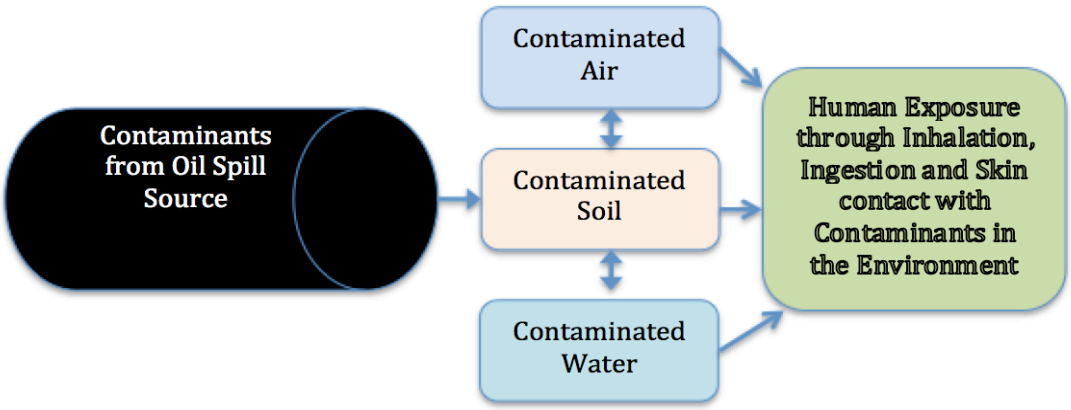



Figure 2.2 Multiple pathways of human exposure to environmental contaminants from oil spill.

Generally, the main chemicals of concern to human health that can be deposited in the environment from an oil spill include the TPHs, PAHs, BTEX and metals such as cadmium, lead, copper, zinc, and arsenic (Swartjes, 2011). For example, the anthropogenic activities of oil operations in Ogoni land in Rivers state, in the Niger Delta region of Nigeria have created devastating effects in the region (UNEP, 2011; Ite et al., 2018). An assessment carried out by UNEP as indicated in its 2011 report on Ogoni land in Rivers State, Nigeria, has revealed the presence of high levels of benzene (a known carcinogen) to have contaminated the source of drinking water in one of the communities in the area. The level of benzene discovered was 900 times more than the World Health Organisation (WHO) approved standard. Similarly, high levels of hydrocarbons were discovered within the drinking water in at least about 10 different communities. The highest concentration of TPHs in groundwater was discovered to have exceeded 1 million milligrams per litre (mg/L) in five samples, which contradicted the Nigerian approved standard of 600 mg/L even though there is no set standard by the WHO on TPHs in groundwater. However, it also exceeded the 0.5 mg/L detection limit set by the US EPA 5030B (WHO, 2008). Consequently, the United Nations Environment Programme (UNEP) report has recommended emergency action on Ogoni land and has also advocated the cessation of all sources of contamination before commencing any process of remediation (UNEP, 2011).

## 2.4. Impacts of Petroleum Contamination

Petroleum contaminants often enter the environment through oil spills from industrial activities as well as through transportation and other use of petroleum products (Fingas, 2010). The presence of petroleum contaminants in the environment can become a threat to both human health and the surrounding ecosystem due to the complex mixtures of hydrocarbons with negative impacts on the economy, biodiversity, and human health (Onwurah et al., 2007). The details of these negative impacts of contamination are discussed below.

### 2.4.1. Impact on Economy

Environmental contamination from oil spills can cause a major setback to some viable economic industries. For example, when spills occur in a fishing zone, the consumption of fish may become undesirable for fear of contaminated fish until the region is restored and considered safe by the relevant health authorities (Phillips, 2017). A good example is the Exxon Valdez oil spill that occurred on March 24, 1989, in Alaska, which caused the death of thousands of sea animals within days, leading to thousands of jobs being lost and costing billions of dollars spent on restoration (Hunt, 2010; Magris and Giarrizzo, 2020). Similarly, the UNEP has also indicated in its 2011 report that the Ogoni land oil pollution could be the world's most extensive and require the longest remediation time, with remediation efforts expected to last 25 to 30 years (UNEP, 2011). Furthermore, the initial fund for the first five years of cleaning and restoration is estimated to be $1 billion (Etemire and Muzan, 2017; Nwozor, 2020).

### 2.4.2. Impacts on Biodiversity

The occurrence of oil spill incidents either onshore or offshore can affect living organisms with devastating consequences for biodiversity. These incidences are usually linked with spills and leaks from various upstream and downstream oil and gas activities such as production, refining, storage, and transportation (Onwurah et al., 2007). Magris and Giarrizzo, (2020) investigated the impact of the oil spill contamination that occurred in the southern Atlantic ocean and revealed that a total of 27 coastal species were affected by the incident including estuaries, mangroves, and seagrass meadows. Abbasian et al., (2016) researched biodiversity changes the microbial population in crude oil contaminated soil. Their results revealed that higher concentrations of crude oil contamination resulted in decreased ratios of chemoheterotrophic bacteria, which were replaced by photoheterotrophic bacteria mostly Rhodospirillales. Aishatu et al., (2016) studied the environmental impact of oil pipeline vandalism with particular focus on the challenge to biodiversity in Port Harcourt, Rivers State, Nigeria. Their results revealed a huge damage to biodiversity including on cash crops such as coffee, cocoa, rubber, and palm trees caused by oil spill from the vandalism.

Marine biodiversity also suffers major effects from petroleum contaminants when an oil spill occurs in the marine environment. A typical example is the British Petroleum (BP) Gulf oil spill that occurred in April 2010, killing a wide range of marine organisms (Larramendy and Soloneski, 2015). Research conducted by the National Oceanic and Atmospheric Administration (NOAA) on the BP Gulf oil spill revealed that many dolphins were discovered to have died because of severe lung disease linked to petroleum contaminants (McWhirter, 2015). Similarly of petroleum contaminants on aquatic biota in three different ways (Leifert, 2012). These effects are presented in Table 2.3.

Table 2.2 Effects of Petroleum Contaminants on Marine Organisms (Leifert, 2012).

| Type of Pollutant | Effect | Effect on Organisms |
|---|---|---|
| Petroleum Hydrocarbons | Physical | Causes death by suffocation and hypothermia. Blocks air passageways or gills and cause numbing of sensory organs (affects ability to find food and detect predators). |
| Petroleum Hydrocarbons | Toxic | Causes skin ulceration, immune system suppression, damages spleen, adrenal tissues, lungs, liver, and other organs. |
| Petroleum Hydrocarbons | Indirect | Reduces growth rate and fecundity, increases larval mortality, and affects the food chain in marine environment. |

### 2.4.3. Impacts on Human Health

In recent years, there has been increased concern regarding the global impact of environmental pollution on human health. The WHO has estimated that almost a quarter of the diseases currently suffered by humans are environmentally related, the majority of which manifest later in adulthood (Pruss-Ustun et al., 2006). Environmental pollution in the air, water and land are the main cause of human health problems (Briggs, 2003). These sources of pollutions can potentially expose humans to toxic chemicals or biological agents such as carcinogens, heavy metals, aerosols, or endocrine distributors that often cause diseases such as cancers, respiratory diseases, cardiopulmonary diseases, and mental disorders (Entwistle et al., 2019; Wu, 2020; Alengebawy et al., 2021).

The presence of petroleum hydrocarbons in the environment thus affect human health through different routes of exposure such as inhalation, ingestion, and absorption through the skin (Kuppusamy et al., 2020b). For instance, land-related oil spills often occur near residential and agricultural areas where petroleum contaminants tend to percolate and contaminate sources of water supply such as groundwater, streams, or rivers that are often used for domestic and livestock consumption (Gay et al., 2010). Sebastian et al., (2001) investigated exposure to environmental

contaminants and cases of cancer in Ecuador's oil producing zone San Carlos from 1989 to 1998. Their results revealed the presence of TPH ranging from 10 to 288 more than the limit allowed by European Community Regulations. Furthermore, ten patients were diagnosed with cancer and evidence was established that could be linked to the presence of toxic petroleum contaminants. Villeneuve et al., (2010) also studied the occupational exposure to petroleum products among motor mechanics and discovered a consistent risk of male breast cancer among them. Similarly, exposure to petroleum contaminants has been reported in Nigeria and Kuwait through the consumption of contaminated water and livestock, which resulted in an increased rate of cancer cases and intestinal problems in those places (Gay et al., 2010). However, the health effects resulting from these contaminants depend on factors such as the chemical composition of the contaminants, the duration of exposure, and the quantity that enters the human body (Ferguson et al., 2020). The maximum contamination level (MCL) and health effects of human exposure to some of the toxic compounds in petroleum compounds are presented in parts per million (ppm) or milligrams per litre (mg/L) below.

Table 2.3 Health effects based on maximum concentration levels of toxic petroleum contaminants (Todd et al., 1999; MDH, 2005; Calabrese, 2017).

| **Compounds** | **MCL (ppm/mg/L)** | **Health Effect** |
|---|---|---|
| Benzene | 0.005 | Can cause cancer in humans. |
| Ethylbenzene | 0.7 | Affects liver, kidney, and nervous system. |
| Toluene | 1 | Affects liver, kidney, nervous and circulatory systems. |
| Benzo(a)pyrene | 0.0002 | Affects red blood cells and immune system. Causes reproductive and developmental disorder and cancer. |
| n-hexane | 2500 | Causes nerve disorder and paralysis in severe cases. |

## 2.5. Techniques for Soil Remediation

Soil remediation involves the use of both in-situ and ex-situ techniques for the treatment and restoration of contaminated soils using various approaches such as physical, chemical, and biological processes (Hakeem et al., 2014). These remediation techniques normally depend on the nature of the pollutants, environmental conditions, and soil microbial biomass (Megharaj et al., 2011; Paul et al., 2021). The common soil remediation methods are summarised in table 2.5.

Table 2.4 Common soil remediation techniques (Liao et al., 2016a; Abdullahi et al., 2022; Teng et al., 2020; Wiśniowska and Włodarczyk-Makuła, 2018; Gómez et al., 2019; L. Chen et al., 2020; Sanchez-Hernandez, 2021; Ali et al., 2021; Georgees and Hassan, 2022; Li et al., 2023; Kristanti and Hadibarata, 2023).

| | Technology | Operation | Reagent/organism involved |
|---|---|---|---|
| Physical remediation | Soil replacement | Cleaning soil replaces contaminated soil | – |
| | Soil vapour extraction | Reduction of the vapor pressure of soil pores | – |
| | Thermal desorption | Separation of pollutants from soil by heating | – |
| | Electric remediation | Establish electric field gradient | KI, EDTA, $H_2SO_4$ etc. |
| Chemical remediation | Soil washing | Extraction and separation of contaminants from soil by eluent | HCl, $HNO_3$, $H_2SO_4$, $H_3PO_4$, NaCl, $Na_2S_2O_3$, KI, etc. |
| | Chemical stabilisation | Addition of chemical reagents or chemical materials | $FeH_4O_6S^{+2}$, CaO, $CH_3COOOH$, $H_3PO_4$, $Ca_3(PO_4)_2$, $C_3H_5NO$ etc. |
| Biological remediation | Phytoremediation | The use of plants and their associated rhizospheric microorganisms to remove contaminants | e.g., trees, grasses, aquatic plants, chelating agents, biosurfactants, microbial inoculants. |
| | Microbial remediation | Control contaminants in soil by introducing microorganisms | Oxygen and nutrients such as carbon sources, nitrogen, and phosphorus. Living organisms such as bacteria, fungi, and algae. |
| | Animal remediation | Utilizing the activities of some lower animals to enhance bioremediation | Earthworm, crustaceans and plastivores. |

### 2.5.1. Physical remediation

Physical remediation involve the use of physical techniques mainly soil replacement and thermal treatment for removal or treatment of soil contaminants (Sidhu, 2016; Qayyum et al., 2020; Riser-Roberts, 2020). This process generally depends on the particle size distribution of the contaminants, and the removed contaminants are usually subject to further treatment (Sharma et al., 2018).

(a) Soil replacement

Soil replacement is the process of replacing contaminated soil with uncontaminated soil to reduce the concentration of contaminants in the soil Khalid et al., 2017). This technique is effective in reducing the impact of contaminants such as heavy metals on the environment (Jiang et al., 2022). Soil replacement is carried out by three main processes including (i) soil replacement which involves the complete removal of contaminated soil and replacing it with a treated one (Sharma et al., 2018). (ii) Soil spading, which involves deep burrowing of contaminated soil to reduce the concentration of contaminants (Rajendran et al., 2022). (iii) Soil importation which involves mixing contaminated soil with a new soil from a different location to lower the concentration of contaminants (Li et al., 2019; Rajendran et al., 2022). However, the major setback associated with this technique is that it is very expensive, requires high labour, and is only suitable for small areas (Sidhu, 2016; Sharma et al., 2018; Li et al., 2019; Rajendran et al., 2022).

(b) Thermal Treatment

Thermal treatment involves the use of heat above 1000ºC (incineration) to remove the volatile portion of contaminants from the soil (Scullion, 2006; Samolada and Zabaniotou, 2014; Li et al., 2019; Lane et al., 2020). It may also involve the use of temperatures below 600 °C (thermal desorption) whereby soil contaminants with lower boiling points are desorbed before being treated further by other means (Khan et al., 2004; Zhao et al., 2019). Vitrification is also a technique that uses thermal energy for solidification or stabilisation of soil contaminants (Mulligan et al., 2001; Pei et al., 2020). During the process of vitrification, the contaminated soil is solidified by placing electrodes into the soil or mixing with materials such as cement and the heat generated by the electrodes volatilizes the contaminants where the end products are disposed of in landfills Sobiecka and Szymanski, 2014; Sharifikolouei and Ferraris, 2021). However, the disadvantage of this technique is the high increase in the volume of contaminants during the process of vitrification (Shu et al., 2021). Also, air pollution control may be required when poisonous gases such carbon monoxide (CO) and volatile hydrocarbons are emitted during the process of thermal treatment (Mulligan et al., 2001; Vidonish et al., 2016; Zhang et al., 2021). The technique also requires high energy input and it is costly (Zhao et al., 2019;  Garcia-Vaquero et al., 2020).

### 2.5.2 Chemical Processes

Chemical treatment involves the use of chemical processes to destroy contaminants or convert them to harmless compounds where they are subsequently removed or immobilised within the soil (Koul and Taak, 2018). It mainly consists of processes that include oxidation/reduction, soil flushing, and solvent extraction (Bhandari et al., 2007  Trojanowicz et al., 2018; Garcia-Vaquero et al., 2020). The details of chemical processes are discussed below.

(a) Chemical oxidation/Reduction

This method involves the application of chemicals known as oxidants to convert toxic compounds into harmless ones for the treatment of contaminated soil and water (EPA, 2001; Moussavi and Rezaei, 2017; Bell, 2018). The common oxidants used for this technique include chemical reagents such as hydrogen peroxide ($H_2O_2$), ozone ($O_3$), hypochlorite ($ClO^-$), permanganate ($MnO_4^-$), and per-sulphate ($S_2O_8^{2-}$) (Islam, 2015; Bell, 2018). The oxidation/reduction reaction is generally described as a redox reaction where electrons are either gained or lost when substances are oxidized or reduced, respectively (Nathanail and Bardos, 2005). For instance, the remediation of organic compounds such as petroleum hydrocarbons through chemical oxidation commonly involveS the application of $H_2O_2$ and other substances to oxidize organic compounds into carbon dioxide ($CO_2$) and water ($H_2O$) or other forms of harmless compounds (Jafarinejad, 2016). However, one of the technique's limitations is that some chemicals of concern (COCs) such as PAHs may be resistant to oxidation Rengarajan et al., 2015). This technique may also potentially transform compounds into more poisonous forms, produce heat and vapour, and reduce soil permeability (Caliman et al., 2011). Furthermore, the technique also requires a large quantity of oxidizing agents that are considered very expensive (Besha et al., 2018).

(b) Solvent extraction

This technique involves mixing contaminated soil with chemical solvents to separate them from the contaminants (Xie et al., 2014; Garcia-Vaquero et al., 2020). The chemical solvents usually dissolve complex molecular substances into simple components for the extraction of organic contaminants for treatment or recycling using other techniques (Islam, 2015). However, the efficiency of this technique can be hindered by soil type and moisture content (Lau et al., 2010). This technique involves the use of detergents and emulsifiers (Basu et al., 2015). Another setback is the concern over the toxicity of the solvents waste used for the treatment process (Magangana et al., 2021).

(c) Soil Flushing

This method involves the separation of contaminants from the soil using flushing solutions such as chemical surfactants to increase the solubility and mobility of the contaminants (Trellu et al., 2016). Subsequently, the contaminants and the flushing solutions are recovered and treated using other techniques, after which the solutions may be reused or disposed (Khan et al., 2004; Senevirathna et al., 2021). However, the shortcoming of this method is the plume of contaminants that may extend outside the recovery area (Bhandari et al., 2007; Senevirathna et al., 2021). Also, the use of acids and solvents to enhance the process causes damage to the soil (Lee et al., 2011). The process also requires high energy, consumes high amounts of reagents, and may produce toxic substances during the degradation of the target pollutant (Trellu et al., 2016). The flushing solutions used may also react with contaminants and cause a reduction in soil permeability and hydraulic conductivity (Bhandari et al., 2007; Senevirathna et al., 2021).

### 2.5.3 Biological Process

Biological treatment or bioremediation generally involves the use of microorganisms, enzymes, or plants to breakdown, immobilise or transmute environmental contaminants into harmless products (Martín et al., 2004; Hazen, 2018; Hakeem et al., 2020). This technology is considered safe and sustainable because it requires low energy inputs mainly provided through the metabolic action of bacteria, fungi, plants, and cellular free enzymes (Zhou and Hua, 2004; Megharaj and Naidu, 2017; Tyagi and Kumar, 2021). Hence, the process may involve the augmentation of certain indigenous microorganisms or manipulation of environmental conditions to improve the bioremediation efficiency (Scullion, 2006b; Pande et al., 2020). Basically, bioremediation consists of three key processes, which include microbial remediation, phytoremediation, and cellular free enzyme

phytoremediation (Zhou and Hua, 2004; Ojuederie and Babalola, 2017; Guo et al., 2021). These treatment processes usually involve both in situ or ex situ techniques, and they comprise of technologies such as land-farming, composting, bioreactors, bioventing, bio-filters, bio-augmentation, bio-stimulation, and pump and treat (Boopathy, 2000; Francis and Nancharaiah, 2015; Wadgaonkar et al., 2019; Paul et al., 2021). The main technique of concern to this thesis is phytoremediation, which is discussed in detail in section 2.6.

## 2.6 Phytoremediation

The word phytoremediation is derived from the Greek and Latin words *phyto* meaning (plants) and *remedium* meaning (cure or restore). It was first invented by Ilya Raskin in 1991, where it was used to refer to plants with remediation capabilities (Beans, 2017). Phytoremediation can be described as the effective use of plants in contaminated areas for decontamination through the removal, detoxification, or containment of contaminants by natural processes of the plants (Jadia and Fulekar, 2009).

Tolerant plant species such as trees, grasses and legumes have a good system of absorption, metabolism, and transportation that allows them to absorb nutrients or contaminants where they are grown (Danh et al., 2009; Hao et al., 2014; Doty et al., 2017; Tripathi et al., 2021). The metabolism of xenobiotic compounds in plants is like that of the mammalian liver as described in the green liver concept (Zand and Hoveidi, 2016; He et al., 2017). The similarities that exist between the metabolism of organic compounds in plants and the function of the mammalian liver include the detoxification of contaminants, the classes of enzymes involved and the elimination of contaminants to protect vulnerable organelles (Burken, 2003a). However, the distinction between plants and mammals is that while plants can store contaminants in a less toxic form (compartmentalisation) within their tissues, such as in cell walls and vacuoles, mammals tend to expel the contaminants through the process of excretion (Pflugmacher et al., 2015a). The idea

behind the green liver concept is primarily connected with the natural transformation of compounds through the activities of enzymes that can conduct the transformation process (Burken, 2003a). The sequence of metabolism in both plants and animals involves three main stages, namely transformation, conjugation, and compartmentalisation (He et al., 2017) The transformation stage occurs through the activities of enzymes such as cytochrome P450 mono-oxygenases (Pflugmacher et al., 2015a). It also involves various reactions of organic contaminants such as oxidation-reduction reactions and hydroxylation which involves the addition of hydroxyl ion [$OH^-$], which makes the next stage known as conjugation possible (Burken, 2003a). Conjugation, occurs through the activities of enzymes such as Glutathione S-transferases (GSTs) that use compounds including amino acids and sugars for conjugation reactions (Pflugmacher et al., 2015a). At this stage, some of the compounds resulting from the transformation stage are metabolised, leading to the formation of water-soluble conjugates, which serves as a major instrument for deactivating many toxic chemical contaminants (Burken, 2003a). The third stage, which is referred to as compartmentalisation, involves the isolation of conjugated compounds formed during the conjugation stage (Burken, 2003; He et al., 2017). However, the distinction between plant and mammalian liver function at this stage is that while plants store the conjugates within their tissues, mammals tend to remove them through excretion (Pflugmacher et al., 2015a). Figure 2.6 shows the diagrammatic representation of the processes involved in phytoremediation.

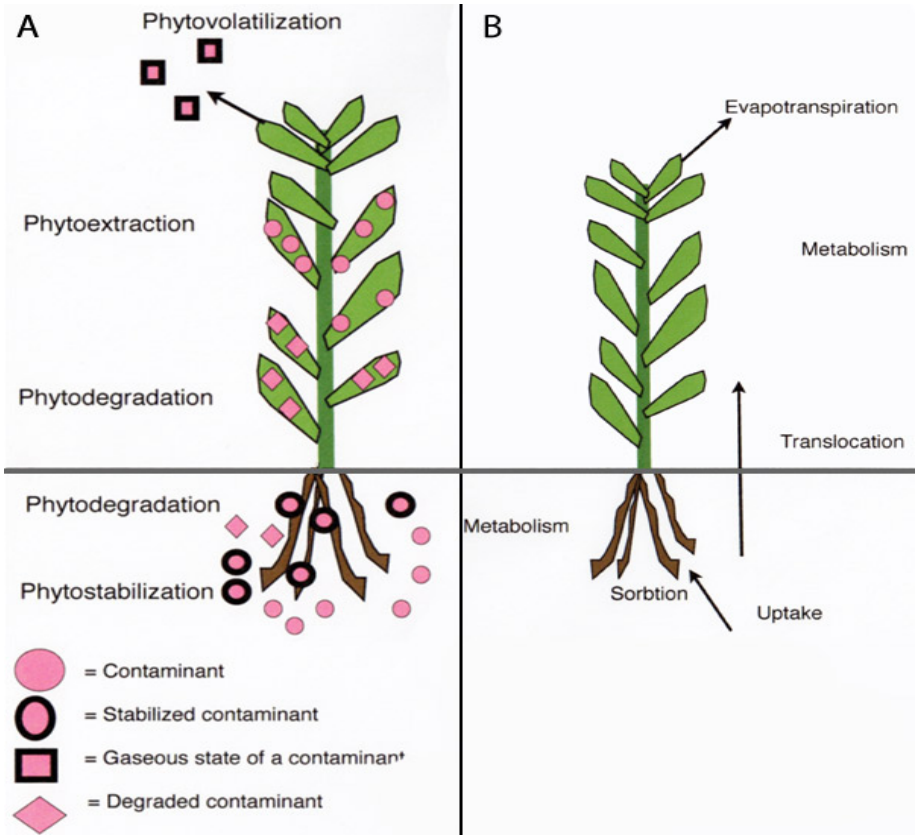



Figure 2.3 The process of phytoremediation (Greipsson, 2011).

(A) Schematic diagram of various phytoremediation techniques

(B) Plant physiological mechanisms involved in phytoremediation

The technical concepts used in phytoremediation were originally obtained from very old techniques practiced by scientists in other fields, such as land reclamation, wastewater treatment, oily waste land farming, soil chemistry, and plant physiology, and applied to pesticides used in agriculture (Cunningham et al., 1996; Chen et al., 2011). It generally involves growing plants in a contaminated area for a certain period to enhance the biodegradation, detoxification, or containment of environmental contaminants such as metals, pesticides, solvents, explosives, crude oil, PAHs, and landfill leachates (Abdel-Shafy and Mansour, 2018). Consequently, the plants are harvested, treated, and properly disposed (Chen et al., 2011). Phytoremediation is mainly targeted at treating organic pollutants and toxic heavy metals that often persist in the soil at mining locations, refineries, gas plants, landfills, and other waste sites (Singh and Jain, 2003; Mohan et al., 2021; Adnan et al.,

2022). The benefit of using phytoremediation for controlling environmental pollution is that it is a cost-effective and environmentally friendly approach. Moreover, it also helps in the control of soil erosion, provides wildlife habitat, increases biodiversity, and restores the ecosystem (Ghavzan and Trivedy, 2005; Russell, 2005; Kozdrój and van Elsas, 2001; Hakeem et al., 2014). It is generally considered ideal to use plant species that grow rapidly, such as grasses, for performing phytoremediation (Brandt et al., 2006; Sinha et al., 2013). The overall benefit of using grasses is centred around their perennial nature and possession of a broad fibrous root system that penetrates deep into the soil (Ma et al., 2018; Sinha et al., 2013).

### 2.7. Types of Phytoremediation

There are different types of phytoremediation processes that are used for soil remediation based on the nature of contaminants present in the soil. These processes include the in-situ stabilisation, degradation, and removal (volatilisation or extraction) of the contaminants (Wu et al., 2006; Azubuike et al., 2016). Furthermore, these processes can be divided into various categories such as phytoextraction, phytostabilisation, phytovolatilisation, phytodegradation and rhizodegradation, (Susarla et al., 2002). Table 2.5 below shows a comparison between the various phytoremediation technologies while Table 2.6 shows a summary of the various types of phytoremediation techniques applied to different contaminants using different plant types.

Table 2.5 Summary of phytoremediation technologies

| Technique | Description | Contaminant | References |
|---|---|---|---|
| Phytostabilization | Retained in situ, preventing migration, and reducing bioavailability of contaminants | Organics and metals | Wang et al., 2017, Barbosa and Fernando, 2018; Awa and Hadibarata, 2020 |
| Phytodegradation | Attenuated in situ, breaking down organic contaminants through metabolic processes | Organics | Riser-Roberts, 2020, Ossai et al., 2020, Tyagi and Kumar, 2021 |
| Phytovolatilization | Removed from soil and released into the atmosphere through plant processes | Organics and metals | Arya et al., 2017, Muthusaravanan et al., 2018, Awa and Hadibarata, 2020 |

| | | | |
|---|---|---|---|
| Phytoextraction | Removed from soil, accumulating contaminants in plant tissues for harvest or disposal | Metals | Ali et al., 2013, Yuan et al., 2019, Shah and Daverey, 2020 |
| Rhizodegradation | Degradation of organic compounds through enzymatic activities facilitated by root exudates and associated microorganisms | Organic compounds | Lu et al., 2017, Turkovskaya and Muratova, 2019 |

Table 2.6 Summary of plants normally used for phytoremediation

| Method | Substrate | Contaminant | Common Plants | References |
|---|---|---|---|---|
| Phytoextraction | Soil | Heavy metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) | *Brassica juncea*, *Helianthus annuus*, *Populus spp.*, *Thlaspi caerulescens* | Lee, 2013, Mahar et al., 2016, Haq et al., 2020, Pouresmaieli et al., 2022 |
| Phytostabilisation | Soil | Heavy metals, metalloids (Pb, Cd, Zn, Ni, Cu) | *Festuca arundinacea, Agropyron elongatum, Lolium perenne* | Ghosh et al., 2017, Guterres et al., 2019, Gavrilescu, 2022 |
| Rhizodegradation | Soil, water | Organic compounds (Herbicide, chlorinated aliphatics (e.g., TCE), aromatics (e.g., BTEX), ammonium waste (TNT, RDX, HMX, perchlorate), nutrients (nitrite, ammonium, phosphate), biodegradable organics (BTEX, TPH, PAHs, PCBs, pesticides) | *Populus spp., Salix spp., Zea mays*, *Phragmites australis* | Hussain et al., 2018, Abdullah et al., 2020, Hano, 2022, Haokip et al., 2023 |
| Phytodegradation | Soil, water | Organic compounds (Herbicide, chlorinated aliphatics (e.g., TCE), aromatics (e.g., BTEX), ammonium waste (TNT, RDX, HMX, perchlorate), nutrients (nitrite, ammonium, phosphate), biodegradable organics (BTEX, TPH, PAHs, PCBs, pesticides) | *Populus spp., Salix spp., Zea mays*, *Phragmites australis* | Prusty et al., 2017, Hussain et al., 2018, Hoang et al., 2021, Rakshit et al., 2021 |
| Phytovolatilisation | Soil, sediments | Volatile organic compounds (MTBE), metals (Selenium, arsenic, mercury) | *Populus spp., Salix spp., Pinus spp., Eucalyptus spp.* | Gaspard and Ncibi, 2013, Jain et al., 2022, Guidi Nissim et al., 2023 |

### 2.7.1 Phytoextraction

Phytoextraction is the process of extracting contaminants such as metals from soil using plant species capable of producing adequate biomass and accumulating high concentration of metals (Lee, 2013; Eissa, 2017). The plants usually absorb contaminants from the soil through their roots and then concentrate them in their harvestable parts such as the shoots and leaves (Lorestani et al., 2013; Lee, 2013b; Sharma and Pandey, 2014). The plants are harvested and treated by incineration and the process is repeated to reduce the amount of the contaminants to the lowest possible level. However, the efficiency of this technique can be affected by plant species with low biomass and by the bioavailability of the contaminants (Sheoran et al., 2016).

### 2.7.2 Phytostabilisation

Phytostabilisation is the process of limiting contaminant mobility where absorption and precipitation of contaminants take place through plant roots and rhizosphere, respectively (Vangronsveld et al., 2009; Bolan et al., 2011; Malik et al., 2022). The growth of plant roots increases microbial activity, which enhances the degradation of hydrocarbons and thus increases soil organic matter (Phillips et al., 2012; Bolan et al., 2011). This in turn reduces the bioavailability of contaminants and lowers their migration into air and groundwater and thus, safeguards the food chain (Hakeem et al., 2014; Radziemska et al., 2019). Phytostabilisation of contaminants such as metals can be improved by using tolerant plant species and soil amendments such as the application of organic fertilisers, biochar and limestone (Meeinkuirt et al., 2016; Li et al., 2019). This process also helps in regenerating the vegetation cover in areas affected by high concentrations of metal contaminants (Sharma and Pandey, 2014). However, one of the disadvantages of this method is the frequent application of soil amendments to stabilise the contaminants that may have potential negative environmental effects (Meeinkuirt et al., 2016; Li et al., 2019; Xiang et al., 2021). The complexity

of monitoring the root zone, where the interaction between plant roots and contaminants occurs, along with the intricate biochemical processes involving root exudates and soil amendments, contributes to the challenge of evaluating the effectiveness of phytostabilisation (Bolan et al., 2011; Sharma and Pandey, 2014; Shackira and Puthur, 2019).

### 2.7.3 Phytovolatilisation

This process involves the use of plants to absorb contaminants from the soil and subsequently release them into the atmosphere through the process of volatilisation (Trapp and Karlson, 2001; Pilon-Smits, 2005). Thus, the contaminants are initially transformed into a volatile state before being released into the atmosphere by the plants (Sharma and Pandey, 2014). This occurs when the contaminants are absorbed by the plants and transported to the leaves before being released in the form of vapour (Limmer and Burken, 2016). This method has been used to treat contaminants such as mercury, trichloroethene, selenium, and arsenic. For example, it has been reported that the poplar tree was able to volatilise about 90% of the trichloroethene (TCE) absorbed from the environment (Gupta et al., 2017). However, the disadvantage of this process is that the compounds released into the atmosphere may be deposited back into the environment (Sharma and Pandey, 2014; Rahman et al., 2016).

### 2.7.4 Rhizodegradation

This process is also called phytostimulation, which involves the breakdown of contaminants around the root region of plants (Etim, 2012). It is achieved through the interaction between plants and soil microorganisms which helps in stimulating microbial biomass in the rhizosphere that acts as a driving force for the degradation of contaminants (Hakeem et al., 2014). This occurs because of the nutrients supplied to the microbial community through the release of natural substances such

as acids, alcohols, and sugars by the roots of plants (Wang et al., 2017; Yang et al., 2022). Similarly, the additional surface-area provided by the roots of plants favours microbial growth and supplies oxygen, which enhances the degradation of the contaminants (Sharma and Pandey, 2014). The plant-microbial relationship creates favourable conditions for the microorganisms to convert contaminants into less toxic products through bioremediation (Pandotra et al., 2018; Viesser et al., 2020). This method has successfully been used for the treatment of organic compounds such as petroleum contaminants and some solvents (Sharma and Pandey, 2014). Despite some of the successes recorded, this method is still considered much slower than phytodegradation (dos Santos and Maranho, 2018; Kanwal and Shad, 2018).

### 2.7.5 Phytodegradation

Phytodegradation, also referred to as phytotransformation, involves the use of plants to breakdown complex organic contaminants into simpler molecules within the tissues of plants (Kumar et al., 2018; Lee et al., 2020). This is achieved through the absorption and subsequent metabolism and degradation of contaminants by plants within the area where they grow (Al-Baldawi et al., 2015). Similarly, certain enzymes produced by plants such as dehalogenase, peroxidase, nitroreductase, laccase, and nitilase play major roles in catalysing the chemical reactions that occur during the process of phytodegradation (Morikawa and Erkin, 2003; Hazarika et al., 2022). Furthermore, this process has been used successfully for the treatment of certain organic contaminants such as chlorinated solvents, herbicides and wastes from weapons of defence (Sharma and Pandey, 2014). The effectiveness of this process is linked with the ability of plants to effectively absorb and degrade contaminants, which depends on the level of hydrophobicity and the solubility of the contaminants (Sharma and Pandey, 2014; Muthusaravanan et al., 2018).

The focus of this research is mainly on phytodegradation as an approach for the treatment of petroleum hydrocarbon contaminants in soil. Phytodegradation of organic compounds normally occurs within or outside the plant in the region of the rhizosphere (Newman and Reynolds, 2004).

Thus, it may involve the direct absorption and degradation of organic compounds through the metabolic and enzymatic activities of plants that play a major role in the dissipation of organic compounds (Spaczynski et al., 2012). During this process, complex organic compounds are taken up by the roots of plants and broken down into simpler and less toxic substances, which are stored within the tissues of plants (Sharma and Pandey, 2014; Lee et al., 2020). Furthermore, this method has been used successfully for the treatment of several contaminants in the environment, including petroleum hydrocarbons, groundwater solvents, and volatile compounds in the atmosphere (Newman and Reynolds, 2004).

## 2.8 Limitations and Advantages of Phytoremediation

The effectiveness of phytoremediation normally depends on favourable conditions that support the natural activities of plants and the soil microorganisms involved in the process (Pilon-Smits, 2005). The employment of plants in this process not only remove contaminants, but it also helps to preserve and restore the surrounding ecosystem. This comprehensive strategy encourages biodiversity, improves soil quality, improves air and water quality, and delivers aesthetic benefits. Furthermore, phytoremediation is a non-intrusive technology that does not require substantial excavation or the disruption of engineering structures. Phytoremediation offers considerable promise as an environmentally conscious remediation approach because of its ability to reduce pollution and recover damaged environments as compared to other methods such as physical and chemical processes (Wan et al., 2016; Bharti et al., 2017; Horne, 2020; Futughe et al., 2020). However, there are certain limitations that can affect the efficiency of this technology, such as the depth of the plant roots, the availability of nutrients, moisture, as well as the atmospheric and physiochemical conditions (Zand and Hoveidi, 2016; Kuppusamy et al., 2017). Details of the factors that limit the effectiveness of phytoremediation are discussed in the sections below.

Despite the limitations of phytoremediation, the advantages outweigh its limitations as it offers a cost-effective versatile, aesthetically pleasing, environmentally friendly and sustainable approach for environmental decontamination as presented in Table 2.7.

Table 2.7 Advantages and disadvantages of phytoremediation over traditional soil remediation technologies (Susarla, et al., 2002; Kamath et al., 2004; Abioye, 2011).

| Advantages | Limitations |
|---|---|
| Relatively low cost | Requires longer time |
| Easily implemented and maintained | Limited to shallow ground water, soils, and sediments |
| Several mechanisms for removal | Results are variable |
| Environmentally friendly | Influenced by soil and climate conditions of the site. |
| Aesthetically pleasing | May affect the food web (when toxic substances accumulate in plants and animals consume them) |
| Reduces landfilled wastes | Effective only on moderately hydrophobic compounds |
| Harvestable plant materials | Toxicity and bioavailability of biodegradation products is not known. |
| Costs 10 – 20% of conventional methds | Contaminants may leach into the ground water |
| Faster than natural attenuation | Associated with mass transfer limitations like other bio-treatments |
| High public acceptance | - |
| Few air and water emissions | - |
| Conserves natural resources | - |
| Driven by solar energy | - |
| Increases microbial biomass in the soil | - |

### 2.8.1 Concentration of contaminants

The success of phytoremediation depends largely on the concentration of the contaminants in the soil, as plants may be affected by the phytotoxic effects of the contaminants which in turn affect

the efficiency of the process (Patra et al., 2020; Wei et al., 2019; Yadav et al., 2019; Rizvi et al., 2020). Ding et al., (2019) assessed the ability of the grass *Paspalum distichum* to phytoremediate mercury (Hg) contaminated soil. The results revealed 17 down-regulated proteins and a slight reduction in root growth and weight of the plant in response to Hg stress over 60 days. Su et al., (2018) also discovered growth inhibition caused by aluminium (Al) toxicity on rice plants in their response to gallium (Ga) and indium (In) acidic contaminated soil. Mathur et al., (2016) revealed a significant growth inhibition of wheat plants caused by chromium phytotoxicity. Tang et al., (2011) reported inhibition of germination of both maize and wheat by TPHs at a concentration higher than 0.1% while studying the eco-toxicity of petroleum contaminated soil using earthworm (*Eisenia fetida*) luminescent bacteria, and plants, which included wheat (*Triticum aestivum*), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), tall fescue (*Festuca arundinacea*), and Mexican corn grass (*Euchlaena mexicana*).

### 2.8.2 Pollutant Bioavailability

Bioavailability can be described as the portion of organic compounds in the soil that can be absorbed or transformed by living organisms, which is determined by the rate of mass transfer, uptake, and metabolism of the compounds (Semple et al., 2003; van Gestel, 2008; Prokop et al., 2016; Kuppusamy et al., 2020). Bioavailability helps in determining the toxicity and risk posed by contaminants and how they can be accessible to both plants and soil microorganisms (Allard et al., 2000; Harmsen and Naidu, 2013; Petruzzelli et al., 2015). The bioavailability of organic contaminants normally declines because of the aging process of compounds in the soil caused by sorption, biodegradation, and reaction of the compounds that occur over time (Tang et al., 2012; Li et al., 2016). The more the organic compounds age in the soil, the more they become less available they become for absorption by living organisms in the soil (Alexander, 2000; Tang et al., 2012; Zhang et al., 2016), which affects the efficiency of phytoremediation, which relies on the ability of the living organisms (plants and associated microorganisms) to absorb and act on the

contaminants in the soil (Zand and Hoveidi, 2016; Li et al., 2016). For example, the absorption and translocation of organic contaminants in the soil may occur only in compounds that are not hydrophobic in nature (Chaudhry et al., 2002; Wang and Kelly, 2017). This is due to the insolubility of some hydrophobic organic compounds classified as "recalcitrant pollutants" such as PCBs, PAHs, and other hydrocarbons that persistently bind to soil organic matter, which affects their bioavailability and limits the efficiency of phytoremediation (Zand and Hoveidi, 2016). Chen et al., (2019) studied the effect of natural organic matters on the bioavailability of petroleum hydrocarbons in a soil-water system. The results revealed a significant reduction in the hydrocarbon degradation rate caused by low bioavailability due to the soil organic matter (SOM) content. Ramadass et al., (2015) also investigated the factors affecting bioremediation (biopile) of arid soils contaminated with weathered petroleum hydrocarbons and discovered reduced degradation of TPH due to their low bioavailability. Ma et al., (2010) studied the interaction between plant growth and PAH dissipation in freshly spiked and aged contaminated soils, which revealed a higher rate of PAH dissipation in freshly spiked soil samples than the later soil. Esteve-Turrillas et al., (2005) studied the uptake of persistent organic pollutants (POPs) including α-endosulfan, β-endosulfan and endosulfan sulfate by lettuce plants and discovered that most of the endosulfan compounds were less bioavailable for uptake by the plants after a period of 10, 20 and 33 days.

### 2.8.3 Soil Properties

Phytoremediation can be affected by soil type, as variation in soil properties such as organic matter, pH, soil aeration, as well as other physical and chemical characteristics, often affects the rhizosphere and microbial biomass, thus affecting the efficiency of phytoremediation (Zand and Hoveidi, 2016; Gil-Martínez et al., 2021). For example Cui et al., (2021) investigated the effect of soil properties on the efficiency of phytoremediation, which revealed that soil cation exchange capacity (CEC) significantly affected the growth of plants and total Cd extraction. Afzal et al., (2011) also studied the effect of soil types on plant activities and bacterial strains during the

phytoremediation of diesel under the influence of three different soil types, including sand, loamy sand, and loam. This research particularly investigated the survival, abundance, and catabolic gene expression of bacterial strains in the roots and shoots of an Italian ryegrass in diesel contaminated soil and discovered the highest levels of plant colonisation, microbial activities, and degradation of hydrocarbons in loamy soils. Sun et al., (2010) assessed the roles of factors such as abiotic losses, microbes, plant roots and root exudates on the phytoremediation of PAHs in a barren soil. Pot experiments were conducted to investigate the effectiveness of phytoremediation under the influence of phenanthrene and pyrene in soil samples with low organic matter content of 3.75$kg^{-1}$. The results indicated a lower rate of PAH removal from the soil samples containing plant roots than samples with root exudates due to low soil organic matter, which created competition for nutrients between the roots and soil microorganisms.

### 2.8.4 Plant species

Phytoremediation techniques normally involve different plant processes that aid the treatment of contaminants in the soil (Cherian and Oliveira, 2005; Vishnoi and Srivastava, 2007). Moreover, phytoremediation also depends on the species of plants used due to variation in plant features such as root exudation, microbial biomass, and morphology that can affect the effectiveness of the process (Susarla et al., 2002; Patra et al., 2020; Gil-Martínez et al., 2021). The main plant characteristics that support phytoremediation of organic pollutants include fast growth, extensive root systems, bioaccumulation factor (BAF), bioconcentration factor, translocation factor, microbial biomass, viability, resistance, and tolerance to contaminants (Zand and Hoveidi, 2016; . Ghazaryan et al., (2019) investigated the phytoremediation potentials of 16 native wild plant species growing in copper contaminated soil in a mining area in Armenia. The results revealed that three plant species, including *Thymus kotchyanus, Phleum pratense***,** and *Achillea millefolium,* are most suitable for cleaning the contaminated soil due to the high bioconcentration factor of their roots and their low translocation factor. Ikeura et al., (2016) investigated the potential of 33 plant

species for phytoremediation of oil contaminated soil in Japan. However, only four plant species, including Mimosa, Zinnia, Gazania, and Cypress, were selected for further assessment based on their initial growth. The results revealed that only the Gazania plant survived after 180 days of phytoremediation based on its high tolerance to the petroleum contaminated soil. Parihar et al., (2021) studied the bioaccumulation potential of 23 indigenous plants species including trees, shrubs, and herbs for phytoremediation of heavy metals in India and discovered the highest bioaccumulation of the heavy metals in the tree species.

### 2.8.5 Supply of Nutrients

Most petroleum contaminated sites lack adequate nutrients such as nitrogen and phosphorus, that are required for enhancing plant growth and microbial quantity (McCutcheon and Schnoor, 2004; Chorom et al., 2010). This brings about competition between plants and soil microorganisms during the microbial degradation of the carbon source (Merkl et al., 2005; Sun et al., 2010). However, the limited supply of nutrients can be amended through fertiliser application, which minimises the plant/microbial competition and improves plant growth and microbial quantity for effective degradation of organic contaminants in the rhizosphere (Hutchinson et al., 2001; Basumatary et al., 2012). Nero, (2021) studied phytoremediation of petroleum hydrocarbon contaminated soils using *Jatropha curcas* and *Vetiveria zizanioides*, which revealed reduction of hydrocarbons in the soil through soil composting. Brown et al., (2017) investigated the impact of land-farming amendments on the bioremediation of petroleum hydrocarbon-contaminated soil in Nigeria's Niger Delta region and discovered a 23% reduction in TPH in samples amended with nitrogen, phosphorous, and potassium (NPK) fertiliser. Similarly, Merkl et al., (2005) studied the influence of fertilizer levels on phytoremediation of crude oil contaminated soil using *Brachiaria brizantha,* grown on the oil contaminated soil and then amended twice with different concentrations of nutrients that included 200, 300, and 400 mg of N, P, and K per 1kg of soil and discovered improvement in root growth with a high rate of PAH dissipation of up to 18.4% in the presence of 300 mg fertilizer after 22 weeks.

## 2.9 Features, Form and Functions of Vetiver Grass

Vetiver grass, shown in Figure 2.6, is a fast-growing perennial tussock grass that belongs to the family Poaceae and is native to Southeast Asia (Dalton et al., 1996; Srivastava et al., 2008; Patel and Patra, 2017). A recent reclassification has combined the genus Vetiver with Chrysopogon and it is now called *C. zizanioides* (L.) Roberty (Srivastava et al., 2008; Banerjee et al., 2016; Panja et al., 2020). There are ten different species of vetiver grass, but *C. zizanioides* is the most widely known (Maffei, 2002). The plant is sterile and non-invasive in nature and does not compete for nutrients with native species as it propagates through small offset than subterranean stolons, which act as a growth stimulant for other native plants (Akpona, 2013; Effendi et al., 2020; Mondal and Patel, 2020). It grows up to 2 meters high and forms clumps having erect and stiff stems with rigid leaves measuring up to 120-150 cm long and 0.8 cm wide (Borin et al., 2011; Gingade et al., 2015; Gnansounou et al., 2017). It has brownish purple pinacles measuring 15-30cm with long whorled branches of 2.5-5.0 cm long and has pairs of spikelets with three stamens. The plant also has a very strong fibrous root system with fine structure having no stolons or rhizomes, which grow down deeply to a depth of about 7 meters (Danh et al., 2009). The roots also contain fragrant oil mainly composed of benzoic acid, vetiverol, furfurol, vetivone, and vetivene (Gingade et al., 2015).



Figure 2.4 Vetiver grass *C. zizanioides* (defeatingpain, 2014)

The best method of propagating *C. zizanioides* involves splitting the parent plant to obtain small slips while the use of seeds is considered slow and difficult (Truong et al., 2008; Gingade et al., 2015). The photosynthetic nature of the plant often makes it difficult for it to grow under shade, but it always recovers quickly and grows normally when removed from the shade (Truong et al., 2008; Gingade et al., 2015; Kumar and Nikhil, 2016). Furthermore, the ideal time for planting in India, is from February to April (Gingade et al., 2015). Despite the ability of the plant to grow on all soil types, the use of well-drained sandy loam and lateritic soils is preferable because light soils may cause low production of fragrant oil in the roots. Furthermore, a better yield of the plant is normally favoured by humid conditions, and it flourishes with an annual rainfall of 1,000 to 2,000 mm (Maffei, 2002; Gingade et al., 2015). However, the plant can still grow well even under a condition of low humidity (Maffei, 2002). Interestingly, the plant is capable of tolerating temperatures ranging from 15 °C to 55 °C with an ideal temperature of 25 °C for normal growth of the plant in any part of the world. For example, it has been confirmed that *C. zizanioides* was able to survive at a temperature

of -11 °C during snowfall for 18 days and under rainfall of more than 1100 mm with a minimum temperature that occasionally dropped below freezing point for 8 months (Maffei, 2002). It was also able to survive in poorly fertile soil in the north of the Rome Himalayas (India) as well as in extremely cold temperatures coupled with severe grazing by animals (Maffei, 2002). Similarly, the plant has been confirmed to survive for up to three months when completely submerged in a water body (Gingade et al., 2015). The plant is also considered to be highly resistant to pests, making diseases and infestations of no serious concern (Gingade et al., 2015; Prakash et al., 2021).

## 2.10 Selection of plants and biosurfactant for phytoremediation

### 2.10.1 Plant selection

As mentioned in section 2.8.4, the ability of plants to tolerate environmental contaminants can determine their potential for phytoremediation. Hence, the selection of an appropriate plant and a biosurfactant that can solubilise and aid the uptake of contaminants such as petroleum hydrocarbons are important elements for effective phytoremediation. Generally, the ability to tolerate the toxicity of petroleum contamination varies between plant species (Pérez-Hernández et al., 2013; Balasubramaniyam, 2015). For example, Merkl et al., (2005) studied the ability of different conditions of legumes and grasses, including *Calopogonium mucunoides, Centrosema brasilianum***,** and *Stylosanthes capitata* and *Brachiaria brizantha***,** *Cyperus aggregatus***,** and *Eleusine indica* respectively, to degrade 5% (w/w) heavy crude oil contaminated soil. They discovered the inability of the legumes to tolerate petroleum toxicity which resulted in their death within a period of six to eight weeks. A reduced biomass was observed in the grasses, but a significant reduction in the concentration of oil and aromatics was observed in soils vegetated with *B. brizantha*. Interestingly, among the grasses that have been studied for their ability to remediate petroleum contaminated soils, the potential of *C. zizanioides* has been discovered to be relevant for that purpose. The plant has shown the ability to tolerate a concentration of 5% heavy chain oil

contaminants, increase their bioavailability and biodegradability, and reduce TPH and total oil and grease (TOG) by 51.1 and 39.7%, respectively (Brandt et al., 2006a; Rajaei and Seyedi, 2018; Dudai et al., 2018; Nero, 2021). However, soil composting was used to aid the removal of the petroleum hydrocarbons from the soil (Nero, 2021). Furthermore, it has been generally indicated that the biodegradation of petroleum contaminants in soil using *C. zizanioides* is worthy of further research and development (Dudai et al., 2018). Additionally, *C. zizanioides* has been shown to remove toxic heavy metals such as arsenic, cadmium, copper, lead, mercury, and zinc from the environment (Danh et al., 2009). *C. zizanioides* is among the first grasses recognised for soil conservation in Fiji in the early 1950s (Srivastava et al., 2008a). The plant was promoted by the World Bank in the 1980s in India for soil and water conservation, and since then, several other projects have also been initiated to develop vetiver grass technology (VGT), popularly known as the vetiver system (VS), for environmental protection (Lavania, 2004; Srivastava et al., 2008). Since the introduction of the VS, it has provided solutions to many environmental problems such as soil and water conservation, wastewater treatment, stabilisation of embankments, flood control, pollution control, and agroforestry management (Xu et al., 2003; Lavania, 2004; Srivastava et al., 2008).The unique morphological and physiological characteristics of *C. zizanioides* makes the plant a suitable choice for phytoremediation. These characteristics include the possession of high biomass, high C4 photosynthetic efficiency, long massive roots, ability to withstand harsh environmental conditions, and high tolerance to acidic, alkaline, and saline growth media as well as the ability to survive in every soil type, including flooded or waterlogged conditions (Truong, 2000; Lavania, 2000; Lavania, 2004; Srivastava et al., 2008).

### 2.10.2 Biosurfactants

Biosurfactants can be described as a group of low molecular weight surface active compounds produced naturally by certain microorganisms that are capable of synthesizing organic compounds in the environment (Gkorezis et al., 2016a). Biosurfactants tend to assume a detergent-like shape, having a hydrophobic head and a hydrophilic tail (Johnsen et al., 2005; Ribeiro et al., 2019). The

microorganisms that produce biosurfactants include bacteria, fungi, and yeasts such as *Pseudomonas aeruginosa, Bacillus subtilis* and *Candida bombicola*, which produce rhamnolipids, lipopeptides known as (surfactin) and sophorolipids respectively (Mulligan, 2005; Patel et al., 2019; Borah et al., 2021). In addition, these microbial biosurfactants normally help in the removal of contaminants through the enhancement of their solubility and bioavailability, thereby facilitating the biodegradation of the contaminants (Das and Chandran, 2010; Fenibo et al., 2019).

The classification of biosurfactants is usually based on their chemical composition, molecular weight, origin, physiochemical properties, and type of action, which differentiates them from synthetic chemical surfactants (Pacwa-Płociniczak et al., 2011). Moreover, biosurfactants are further divided into low molecular mass biosurfactants, which include glycolipids, phospholipids and lipopeptides and high molecular mass biosurfactants that contain amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins, or a complex mixture of these (Padmanabhan et al., 2014; Bhattacharya et al., 2017; Karlapudi et al., 2018). Similarly, surfactants are generally used for the reduction of the interfacial tension that exists between liquid interfaces such as oil and water and between liquid and solid interfaces such as oil and rocks (Sen, 2008). However, the key benefits of using biosurfactants over their synthetic chemical counterparts for the treatment of contaminants include the fact that they are biodegradable, environmentally friendly, cost-effective, function actively under high temperatures, pH, and salinity, produce better foams, and their production normally utilizes industrial wastes (Kang et al., 2010; Pacwa-Płociniczak et al., 2011; Sarubbo et al., 2022; Rajkhowa and Sarma, 2022).

Basically, the mechanism that aids the active treatment of organic contaminants by biosurfactants is linked to their amphiphilic structure (hydrophobic and hydrophilic parts), which helps to increase the surface area of the organic compounds and improves the ability of the biosurfactants to emulsify, form and disperse the hydrophobic organic contaminants in the environment (Mulligan, 2005; Pacwa-Płociniczak et al., 2011; Fenibo et al., 2019). Thus, the main purpose of introducing microbial biosurfactants during the process of treating organic contaminants such as petroleum hydrocarbons is to increase their bioavailability, mobility, and removal through the processes of

solubilisation and emulsification (Banat et al., 2010; Fenibo et al., 2019). Similarly, the addition of biosurfactants can also address the difficulty experienced during the remediation of polycyclic aromatic hydrocarbons (PAHs) due to their insolubility in water, which affects their bioavailability for microbial degradation (Ron and Rosenberg, 2002; Bezza and Chirwa, 2017; Zang et al., 2021). Biosurfactants produced by *Pseudomonas* bacteria have demonstrated tremendous potential for remediation of petroleum hydrocarbons among the numerous biosurfactants that have been extensively researched for their capacity to breakdown petroleum hydrocarbons (Obayori et al., 2009; Liao et al., 2016; Patowary et al., 2017; Rehman et al., 2021; Varjani and Upasani, 2021). However, the effectiveness of biosurfactants to aid in the phytoremediation of petroleum-contaminated soil using *C. zizanioides*, a tolerant and non-invasive plant with a robust root system capable of reaching leachate at soil depth, is unknown.

### 2.11 Synopsis of research gaps and novelty statement

Phytoremediation has evolved over the years as researchers have explored plant species such as trees, hyperaccumulators, native plants, tolerant plants, fast-growing plants, and plants that have long roots as a means to restore the environment (Dudai et al., 2018; Rajaei and Seyedi, 2018; Gómez et al., 2019; Hasnaoui et al., 2020). The plant *C. zizanioides* has exhibited promising potential among various fast growing plants that have been studied for phytoremediation of petroleum hydrocarbon contaminated soils (Dudai et al., 2018; Nero, 2021). However, soil composting was used to aid the degradation of the petroleum hydrocarbons rather than directly utilising the plant-based remediation. This raises concern over the introduction of pathogenic and antibiotic-resistant bacteria and genes into the environment, which may pose a threat to human health as many sources of animal manure contain antibiotics (Soobhany, 2018; Cunningham et al., 2020; Nero, 2021). Also, there are concerns over the persistence of organic contaminants such as PAHs in the soil due to their insolubility and low bioavailability. As a result, researchers have been exploring the use of biosurfactants to enhance the solubility and bioavailability of organic contaminants to aid their removal from the environment. Among the biosurfactants that have been researched for improving the breakdown of petroleum hydrocarbons in the soil, the biosurfactants

produced by *Pseudomonas* bacteria have shown enormous promise (Varjani and Upasani, 2016; Gao et al., 2017; Ojewumi et al., 2018; Olowomofe et al., 2019). Consequently, there are concerns that petroleum contaminants may potentially leach into groundwater due to the increased mobility caused by biosurfactants. However, research on biosurfactant assisted phytoremediation of petroleum hydrocarbon contaminated soil using a non-invasive, tolerant, and deep-rooted plant such as *C. zizanioides* that can reach out and contain or treat soil leachates is currently lacking.
Plants are generally capable of accumulating contaminants through absorption, translocation, and metabolism of compounds in the environment where they grow (Paz-Alberto and Sigua, 2013; Gunathilakae et al., 2018; Antoniadis et al., 2021). However, the accumulation of petroleum hydrocarbons from the soil normally affects the growth of plants due to the toxicity of the contaminant (Tang et al., 2011; Xun et al., 2015; Ahmad, 2022). Yet, the effect of petroleum toxicity on the morphological structure of *C. zizanioides* is still unclear.

The novelty of our research is based on addressing the challenges and research gaps mentioned above. The research is aimed at determining the ability of *C. zizanioides* to phytodegrade petroleum hydrocarbons in the soil with particular focus on PAHs. The objective of the research is to determine the influence of biosurfactants (rhamnolipids) on the bioavailability of PAHs to aid their uptake by the plant. It is also to determine the ability of *C. zizanioides* to immobilise and contain crude oil contaminants in the soil. We also assessed the level of tolerance of *C. zizanioides* to crude oil contaminated soil and the toxic effect of the contaminant on the morphological structure of the plant. Additionally, we also looked at the effect of NPK fertiliser on the growth of *C. zizanioides* and dissipation of PAHs in a crude oil contaminated soil. The effect of soil chemistry (minerals and elements) on enhancing the tolerance of *C. zizanioides* to crude oil contaminated soil was also assessed.

This research will be highly beneficial for countries like Nigeria that have been affected by oil pollution with a devastating effect on their vegetation, making the soil infertile for agricultural production and posing a threat to human health. It will go a long way toward providing the country with a cost-saving, environmentally friendly, and sustainable approach to environmental

restoration. The first phase of cleaning up oil pollution in Nigeria is estimated to cost $1 billion and it is projected to last for 30 years and may cost many more billions of dollars (UNEP, 2011b).

# CHAPTER THREE

# METHODOLOGY

## 3.1 Materials and methods

The main methodology of this research involved a glasshouse experiment conducted to determine the effectiveness of *C. zizanioides* in treating a crude oil contaminated soil. The research explored the tolerance and ability of *C. zizanioides* to contain the heavy chain oil contaminant and to degrade petroleum hydrocarbons with the aid of NPK fertiliser and biosurfactant. The level of degradation of total petroleum and hydrocarbons (TPH) was gravimetrically analysed.

## 3.2 Soil Properties

A sandy loam soil was purchased from Singletons Plant Centre in the United Kingdom. 35 kg of sandy loam soil was air dried in a sedimentology laboratory without sunlight exposure for 72 hours at a temperature of 20 °C before sieving with a 2 mm metal sieve (Zornoza et al., 2006; Moritsuka et al., 2019). The properties of the soil were analysed to determine pH, electric conductivity, moisture content and SOM content.

### 3.2.1 Soil pH

The pH of the dried and sieved soil was determined using the RAPIDE pH indicator test kit manufactured by Tenax UK Ltd. About 5 g of the sandy loam soil was dried in an oven at a constant temperature of 60 °C for 24 hours. 1 mL of Barium Sulphate was added per dried soil sample in a clean test tube to a level of about 3 mL. This mixture was then added to 7 mL of pH indicator solution in another test tube which was covered and mixed properly by shaking at 100 rpm for 3 minutes (mins). The mixture was then allowed to stand for 30 mins until the soil settled at the

bottom of the tube. Finally, the colour of the supernatant solution was compared against a colour reading chart provided by the manufacturer from which the soil pH was determined. The experiment was repeated thrice, making a total of four replicates and the same result was obtained.

### 3.2.2 Electrical Conductivity

The electrical conductivity (EC) of the soil was determined by suspending 10 g of the dried and sieved soil in a 50 mL of deionised water in the ratio of 1:5 for one hour. Following this, EC was measured using a portable calibrated HANNA EC instrument model number (HI98129). A total of four independently prepared replicates were measured and the standard deviation of the results was calculated.

### 3.2.3 Soil Moisture

The soil moisture was determined using the method described by Smith, (2000). 10 g of the dried and sieved sandy loam soil was placed in a weighted heat-resistant crucible and placed in an oven for 24 hours at a constant temperature of 105 °C. Thereafter, the dried sample was placed in a desiccator using tongs and allowed to cool and the weight was measured again. The moisture content of the soil was then calculated and expressed in percentage using the formula presented by Pansu and Gautheyrou, (2007) as illustrated below.

Equation (1). Percentage of the soil water content (WC).

$$\%WC \text{ at } 105^{O}C = (M_1 - M_2) \div (M_1 - M_O) \times 100.$$

Where $M_1$ = mass of air-dried soil, $M_2$= mass of oven dried soil and $M_O$= mass of the crucible.

### 3.2.4 Soil Organic Matter Content

The soil organic matter (SOM) content was determined using the loss on ignition (LOI) method described by Heiri et al (2001). 10 g of the dried and sieved soil was placed in clean crucibles and dried in an oven at 105 °C for 24 hours, from which the weight of the soil was determined and designated as $DW_{105}$. Following that, the soil samples were measured and added to the crucibles in replicates of three and then heated in a furnace at 550 °C for 5 hours after, which the weight of the samples was determined and designated as $DW_{550}$. The organic matter content of the soil was then determined and expressed as a percentage using the following formula:

$$\mathrm{LOI} = (\mathrm{DW}_{105} - \mathrm{DW}_{550}) \div \mathrm{DW}_{150}) \times 100.$$

Equation (2). Organic matter content

where $DW_{105}$ = weight of soil dried at 105 °C, $DW_{550}$ DW = weight of soil dried at 550 °C.

Table 3.1 Physicochemical parameters of soil values are means ± SD (n=3).

| **Parameters** | **Values** |
|---|---|
| EC (µS/cm) | 0.04 |
| pH | 7.5 |
| Soil moisture content (%) | 2.74 ± 0.15 |
| SOM (%) | 1.18 ± 0.04 |

## 3.3 Soil Spiking

The soil was spiked with crude oil in the ratio of 70:1 (soil and crude oil) respectively where about 70kg of soil was spiked with 1kg of crude oil in accordance with the method described by Brown et al., (2017). Thereafter, the spiked soil was manually mixed thoroughly with a hand shovel and then allowed to weather for 100 days (from March to June 2018). 1 kg of the weathered soil was

transferred into each of 20 terracotta pots, where 16 of the pots are to be planted with *C. zizanioides* in a greenhouse experiment.

### 3.4 Preparation and Transplantation of plants

Seedlings of *C. zizanioides* supplied by Chhajed Garden Agro Services Private Limited (CGASPL, Pune, India; https://www.chhajedgarden.com/) were prepared for transplanting by cutting them into smaller pieces, known as slips, of similar heights (Kumar and Nikhil, 2016b). Slips were then washed with deionized water before transplanting into the terracotta pots at a depth of 5 cm. The application of fertilizer (from Garten Shop GREEN24, Germany, https://www.green24.de/) was carried out according to the manufacturer's instructions (GREEN24) Germany by diluting 5 mL of liquid fertilizer with 2.5 L of deionised water. 10 mL of the resulting solution was gradually added to relevant pots (8 replicates, see details in section 3.5) that require fertiliser treatment in the experiment every 3 days for a period of 3 months (from June to August 2018).

### 3.5 Experimental Set-up

After the soil spiking stage, the 100-day weathered soil was split into 5 groups of 4 replicates of terracotta pots for subsequent treatments (see schematic in Figure 6.2). The pot dimensions are: 11.5 cm height, 13 cm top diameter, 7 cm base diameter and 1.5 cm drainage hole at the base. The treatments were: (A) crude oil spiked soil, *C. zizanioides,* fertiliser, and biosurfactant, (B) crude oil spiked soil, *C. zizanioides* and fertiliser, (C) crude oil spiked soil and *C. zizanioides*, (D) crude oil spiked soil, *C. zizanioides* and biosurfactants. Controls were also prepared: (E) uncontaminated soil and *C. zizanioides*, (F) crude oil spiked soil only and (G) uncontaminated soil only making a total of 28 pots. The experiment was conducted in a glasshouse located in the Winterbourne House and Garden of the University of Birmingham (Figure 6.1) under an average temperature of 11 °C

with daily minimum temperature of 5.3 °C and daily maximum temperature of 30.8 °C. The experiment lasted for 8 months from June 2018 to February 2019 during which an average of 60 g of each of the soil samples were collected monthly from each of the 28 pots and stored in a freezer at -3 °C. Thereafter, the samples were freeze dried and used for various analysis.

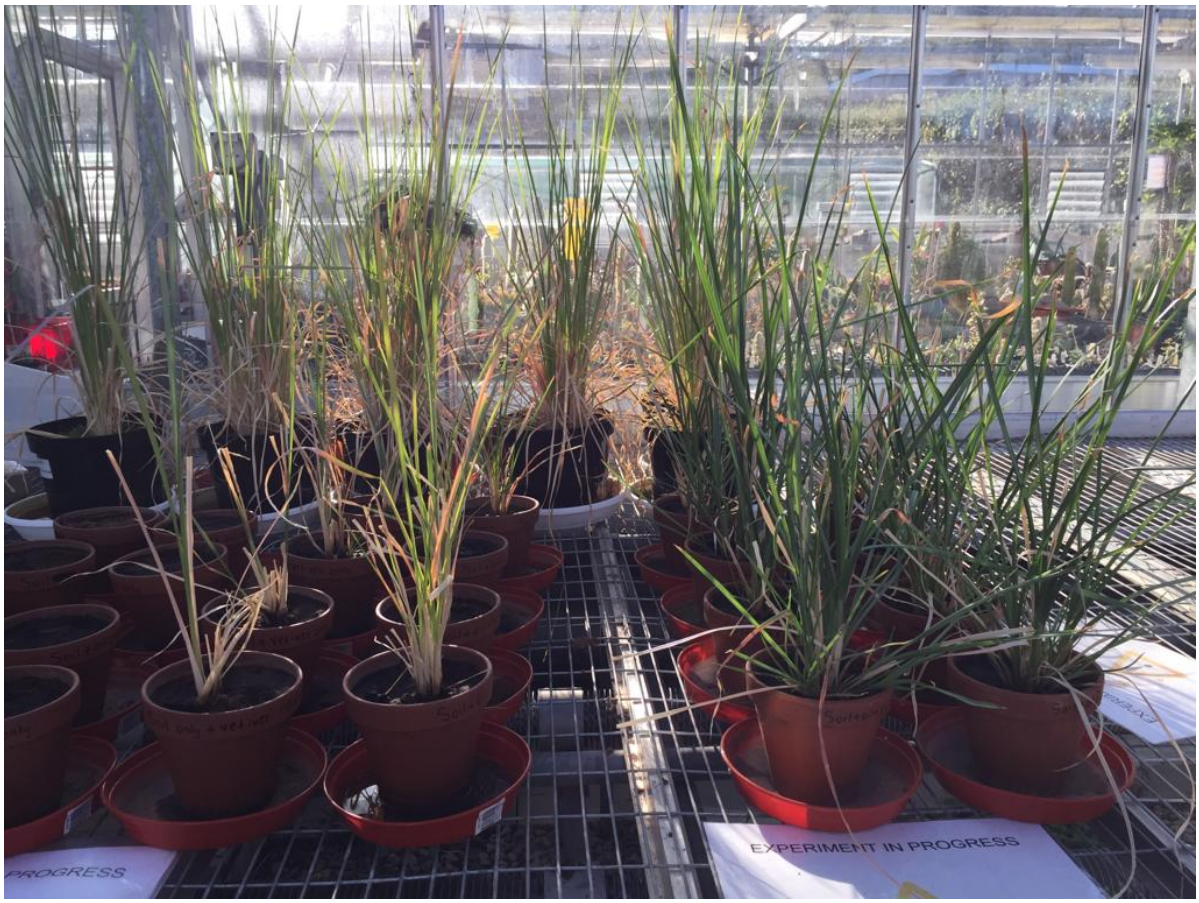
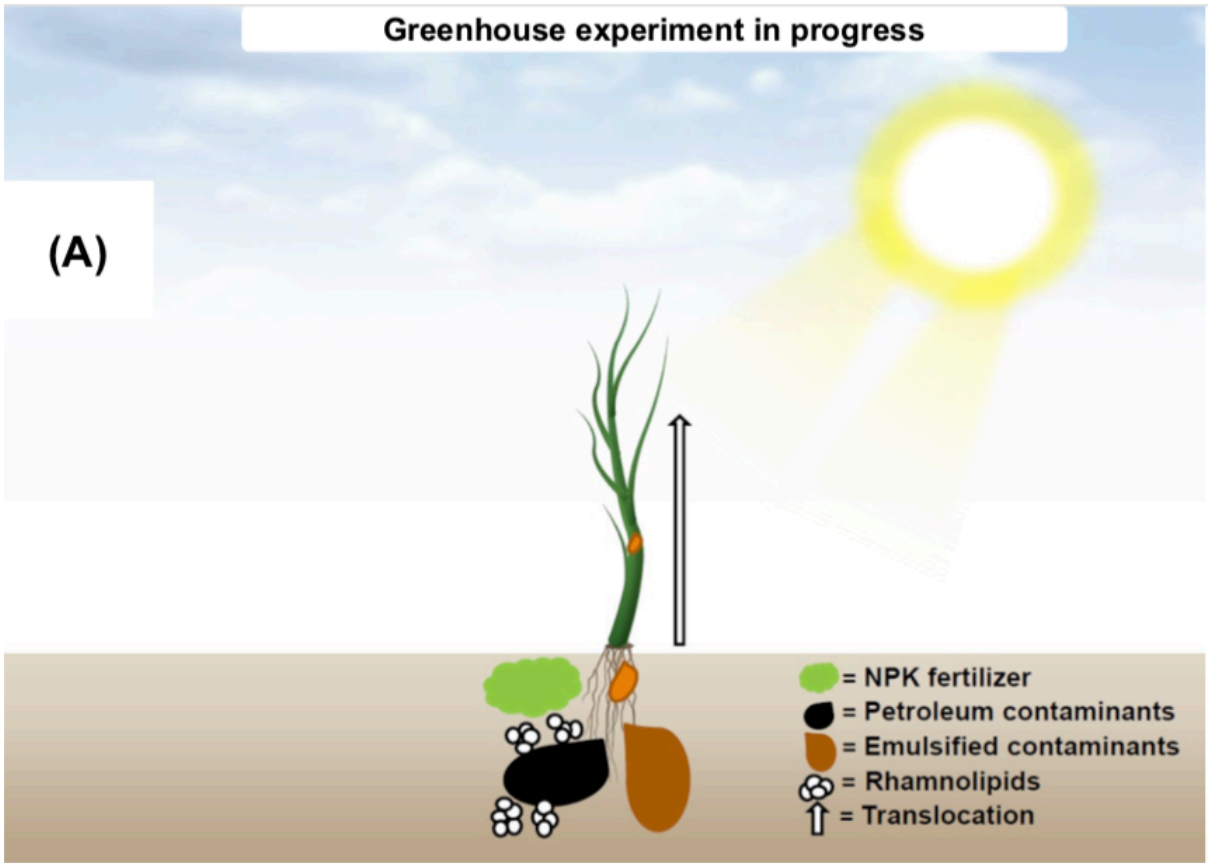



Figure 3.1 Photograph of phytoremediation experiment in progress in the greenhouse – the groups four replicates for each condition can be observed


Greenhouse experiment in progress
(A)
= NPK fertilizer
= Petroleum contaminants
= Emulsified contaminants
= Rhamnolipids
= Translocation

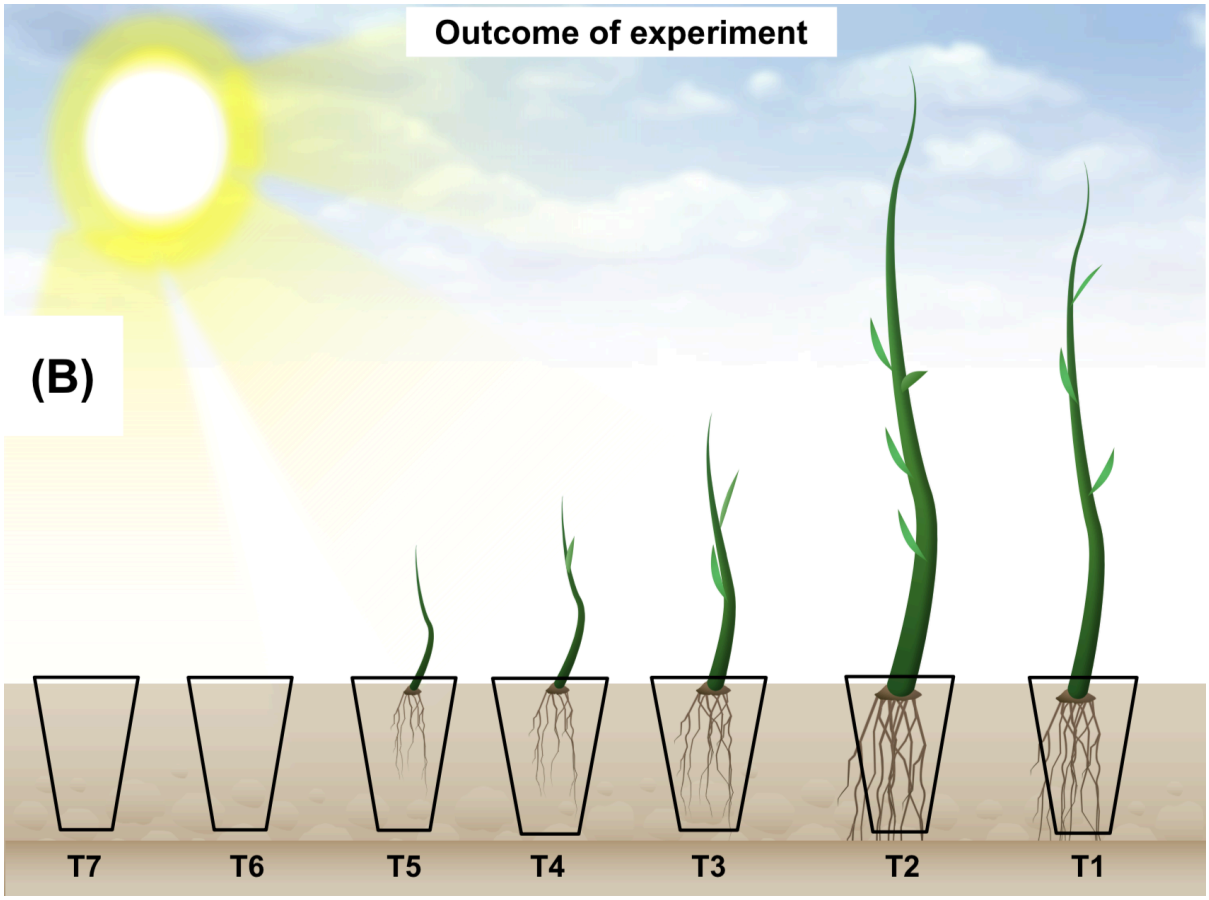

Outcome of experiment
(B)
T7
T6
T5
T4
T3
T2
T1

Figure 3.2 Graphical configuration of the greenhouse experiment exploring the impact of biosurfactant and NPK fertilisation on the mobilisation and uptake of crude oil contaminants such as PAHs by *C. zizanioides.*

Exploring the impact of biosurfactant and NPK fertilisation on the mobilisation and uptake of crude oil contaminants such as PAHs by *C. zizanioides.* Translocation indicates the uptake and movement of the crude oil or its constituents within the plants, while rhamnolipids are the biosurfactants used to solubilise the contaminants.

(A) The mechanisms by which petroleum contaminants are emulsified by rhamnolipids and absorbed and translocated by a fertilised *C. zizanioides* are being explored.

(B) The five samples vegetated with *C. zizanioides* and amended with various treatments, and the two unplanted control samples (T1 = plant + soil + oil + fertiliser + biosurfactant), (T2 = plant + soil + oil + fertiliser), (T3 = plant + soil + oil), (T4 = plant + oil + soil + rhamnolipid), (T5 = plant + soil only), (T6 = Crude oil contaminated soil only) and (T7 = uncontaminated soil only).

Biosurfactant (95% Di Rhamnolipid dominant) purchased from Sigma Aldrich was used to aid the desorption of hydrocarbons from the soil particles using the method described by Liao et al., (2015). This involved dissolving the rhamnolipids in deionized water to obtain a concentration of 10,0000 mg/L. This was followed by the addition of 100 mL of the solution to each relevant pot (treatments A and D as described in section 3.5 above) and the process was repeated every month for gradual uptake by the plants (Liao et al., 2016). All pots were irrigated during the experimental period by gradually adding 20 mL of deionised water daily during the summer and twice a week during winter to maintain adequate soil moisture.

## 3.7 Extraction and Analysis of PAHs

Soil samples were collected in a 20 mL glass bottles monthly during the greenhouse experiment and stored in sealable plastic bags at a freezer temperature of -3 °C and freeze dried before carrying out other analyses. At the end of the greenhouse experiment the roots and shoots of *C. zizanioides* were divided and washed with deionised water to get rid of dust (Novo et al., 2013a). The plant samples were air dried and ground separately (each replicate of each treatment was ground individually) using an electric coffee grinder (BOSCH TS6A013B). The extraction and analysis of PAHs from soil and plant samples was carried out in the following stages.

### 3.7.1 Sonication/Ultrasonic Extraction

The PAHs in soil and plant samples were determined using a method adopted from Martinez et al., (2004). 5 g of the sampled soil (2 g of ground plant) was placed in a 50 mL centrifuge tube and spiked with 10 µL of surrogate internal standards of the target compounds purchased from RESTEK (UK) using an electrical syringe (eVol XR). 30 mL of 1:1 mixture of HPLC grade dichloromethane (DCM) and n-Hexane was added to samples, which were then sonicated for 10 minutes and centrifuged for 5 minutes, and the process was repeated 3 more times. Following this the samples were purified using florisil tubes (Solid phase Extraction) as explained in section 3.7.2.

### 3.7.2 Solid Phase Extraction (SPE)

The SPE is done with the aim of purifying the extracts by getting rid of moisture, colour interferences, polar hydrocarbons and all impurities before the Gas Chromatography Mass Spectrometry (GC MS) analysis (Okop and Okorie, 2013). This is done in accordance with the method described by Guerriero et al., (2009) using dual layer Florisil/$Na_2SO_4$ SPE tubes (2 g / 2 g

/ 6 mL) supplied by Supelco USA. The first step involved the use of 4 mL of DCM/Hexane (1:1) to wash the SPE cartridges after which the cartridges were conditioned with 4 mL of hexane to activate the cartridges. Thereafter, the extract obtained from sonication in step 3.7.1 was concentrated to 0.5 mL through evaporation under nitrogen gas and then purified using SPE cartridges loaded with 20 mL 10:1 n-Hexane and DCM for conditioning and activation of the cartridges. The analyte was loaded onto the SPE column and eluted with 100 mL of n-Hexane and DCM (10:1) and then 100 mL of 2:1 n-Hexane and DCM, and the eluate was collected and evaporated using nitrogen gas at room temperature to dryness, and then reconstituted to a volume of 250µL with n-Hexane (Martinez et al., 2004). Finally, the extract was transferred into 1.5 mL amber vials for analysis using GC MS.

### 3.7.3 Analysis of PAHs using GC MS

The level of PAHs dissipated from soil during phytoremediation was analysed using an Agilent GC (6890N) coupled with an Agilent MS (5973). The MS was set and operated in Total Ion Current (TIC) and Selected Ion Monitoring (SIM) modes (Skoczynska et al., 2013). The oven temperature conditions were set from 40 °C (2 min hold) to 330 °C at 10 °C per minute (10 mins hold). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector and detector temperatures were set at 250 °C and 330 °C respectively. A fused silica column Rxi-5m (30m x 0.25mm Internal Diameter (ID) x 0.25 µm film thickness) was used. The identification and quantification of PAHs in samples was done in Selected Ion Monitoring (SIM) mode. Batches of 20 samples were analysed together with one Certified Reference Material (CRM) namely 104 sediment purchased from Sigma Aldrich, the middle concentration of the calibration standard, solvent blanks, internal standard spiked blanks and matrix spikes (Harrad, 2005; Qiao et al., 2006; Suman et al., 2016).

### 3.7.4 Method validation, quality assurance and quality control (QA/QC)

The QA/QC of the method used in this experiment was ensured in accordance with the procedures described by Harrad, (2005). The instrument calibration was done using a calibration standard (SV Calibration Mix # 5 / 610 PAH Mix), (Catalog # 31011) to create a five-point calibration curve including concentrations of 5 ppm, 10 ppm, 20 ppm, 40 ppm and 50 ppm. The calibration curve has produced a strong linear range with $R^2$ values ranging between 0.9993 to 0.9998. Instrument tuning was performed daily to maximise the sensitivity and performance of the instrument for analysing the target compounds. All samples were spiked with a known amount of method 525.2 internal standard mix (3 components) purchased from RESTEK (Catalog # 31825) and the mean value of recovery of the internal standard was determined. Additionally, the use of plastics was avoided throughout the experiment to prevent contamination from the release of plasticizers when in contact with solvents including DCM and Hexane, as plasticizers a known to contain organic contaminants including PAHs (Teuten et al., 2009).

#### 3.7.4.1 Precision and accuracy

A Certified Reference Material (CRM)-104 sediment purchased from Sigma Aldrich was used to evaluate the precision and accuracy of the method. The extraction and purification of the CRM was done using the methods described in 3.7.1 and 3.7.2 above. The extract was analysed using GC MS and the target compounds (16 PAHs) were quantified. The certified values were then compared with mean concentration of target compounds and was found to be within the ranges of the certified values, which validates the method used in the experiment as shown in Table 3.2.

Table 3.2 Mean measured values of PAHs in CRM-104 compared to certified values (n=5).

| Target compounds | Certified value (ng/g) | Certified confidence interval | Mean measured value (ng/g) | ±SD |
|---|---|---|---|---|
| Naphthalene | 435 ± 339 | 212 to 659 | 280.63 | 2 |
| Acenaphthylene | 386 ± 257 | 37.7 to 192 | 186.366 | 1.4 |
| Acenaphthene | 115 ± 164 | 188 to 583 | 405.736 | 0.4 |
| Fluorene | 187 ± 71.1 | 119 to 254 | 221.49 | 1.2 |
| Phenanthrene | 187 ± 43.7 | 127 to 247 | 217.264 | 1.4 |
| Anthracene | 53.3 ± 56.1 | 21.2 to 225 | 98.998 | 2 |
| Fluoranthene | 481 ± 154 | 330 to 632 | 384.586 | 1.2 |
| Pyrene | 220 ± 69.3 | 155 to 285 | 231.272 | 1.1 |
| Benzo[a]anthracene | 53.7 ± 73.3 | 18.9 to 276 | 78.296 | 1.9 |
| Chrysene | 206 ± 66.2 | 141 to 271 | 220.368 | 1.7 |
| Benzo[b]fluoranthene | 167 ± 50 | 120 to 213 | 179.868 | 1.2 |
| Benzo[k]fluoranthene | 139 ± 26.1 | 122 to 155 | 139.966 | 0.5 |
| Benzo[a]pyrene | 20.6 ± 24.4 | 2.05 to 96.2 | 30.586 | 1.5 |
| Indeno[1,2,3-cd]pyrene | 75.8 ± 34.3 | 42.5 to 109 | 89.286 | 1.6 |
| Dibenzo[a,h]anthracene | 109 ± 61.6 | 56.9 to 161 | 153.758 | 2 |
| Benzo[ghi]perylene | 147 ± 80.1 | 76.9 to 218 | 188.668 | 1.5 |

#### 3.7.4.2 Recoveries of internal standard

The percentage of Internal Standard (IS) recoveries was calculated to determine the amount of target compounds lost during sample extraction and purification as a means of QA/QC check. The IS recovery of each sample was calculated using the following equation:

$$\%\ \mathrm{IS} = \left[\left(\frac{A_{IS}}{A_{RDS}}\right)_{S} \times \left(\frac{A_{RDS}}{A_{IS}}\right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}}\right)_{S}\right] \times 100.$$

Equation (3). Percentage of Internal Standard.

where $\left(\frac{A_{IS}}{A_{RDS}}\right)_{S}$ is the ratio of IS peak area to recovery determination standard peak area in the sample; $\left(\frac{A_{RDS}}{A_{IS}}\right)_{STD}$ is the ratio of recovery of determination standard peak area to IS peak area in the calibration standard; $\left(\frac{C_{IS}}{C_{RDS}}\right)_{STD}$ is the ratio of the concentration of IS to the concentration of the recovery determination standard in the calibration standard and $\left(\frac{C_{RDS}}{C_{IS}}\right)_{S}$ is the ratio of the concentration of recovery determination standard to the concentration of IS in the sample. The

percentage of IS recovery was discovered to be within the range of 73.7% to 117.5% as shown Table 3.3.

Table 3.3 Percentage of Internal standard recovery.

| Internal Standard | Mean recovery rate (%) | ± SD | Min | Max |
|---|---|---|---|---|
| Acenaphthene-d10 | 79.21 | 5.39 | 73.68 | 87.83 |
| Phenathrene-d10 | 91.77 | 8.12 | 75.6 | 100 |
| Chrysene-d12 | 101.24 | 10.49 | 90.1 | 117.49 |

#### 3.7.4.3 Identification and quantification of target compounds

PAHs in soil and plant were identified based on the retention time and quantification ions in relation to respective IS (Qiao et al., 2006). The IS Acenaphthene-d10, was used to quantify 4 target compounds namely Naphthalene, Acenaphthylene, Acenaphthene and Fluorene. Phenathrene-d10 was used to quantify 4 other compounds including Phenanthrene, Anthracene, Fluoranthene and Pyrene. Chrysene-d12 was used to quantify 8 compounds namely Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene, and Benzo[ghi]perylene (table 3.4). The quantity of PAH in soil or plant samples was calculated using the formula described by Harrad, (2005) below.

$$\text{Conc.} = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS}$$

equation (4). Concentration of PAH.

Where $A_{IS}$ is peak area of IS in the sample, $A_{NAT}$ is the peak area of the target compound in the sample, $M_{IS}$ is mass of IS in the sample (pg), RFF is the relative response factor for the compound and SS is the sample size (g). The RFF was calculated using the following equation:

$$RFF = \frac{A_{NAT}}{A_{IS}} \times \frac{C_{IS}}{C_{NAT}}$$

equation (5). Relative response factor.

where $A_{NAT}$ is the peak area of native compound in standard, $A_{IS}$ is the peak area of IS in standard, $C_{NAT}$ is the concentration of native compound in standard and $C_{IS}$ is concentration of IS in standard.

Table 3.4 GC MS condition: retention time, ions monitored and related internal standards.

| Internal Standard | Retention time (min) | Target compounds | Retention time (min) | Quantification ion (m/z) | Qualifier ion (m/z) |
|---|---|---|---|---|---|
| Acenaphthene-d10 | 14.55 | Naphthalene | 6.84 | 162 | 128 |
| | | Acenaphthylene | 13.71 | 162 | 152 |
| | | Acenaphthene | 14.7 | 162 | 153 |
| | | Fluorene | 17.41 | 162 | 166 |
| Phenathrene-d10 | 22.54 | Phenanthrene | 22.65 | 188 | 178 |
| | | Anthracene | 22.9 | 188 | 178 |
| | | Fluoranthene | 29.47 | 188 | 202 |
| | | Pyrene | 30.66 | 188 | 202 |
| Chrysene-d12 | 37.81 | Benzo[a]anthracene | 37.73 | 240 | 228 |
| | | Chrysene | 37.93 | 240 | 228 |
| | | Benzo[b]fluoranthene | 43.59 | 240 | 252 |
| | | Benzo[k]fluoranthene | 43.72 | 240 | 252 |
| | | Benzo[a]pyrene | 45.13 | 240 | 252 |
| | | Indeno[1,2,3-cd]pyrene | 50.24 | 240 | 276 |
| | | Dibenzo[a,h]anthracene | 50.46 | 240 | 278 |
| | | Benzo[ghi]perylene | 51.25 | 240 | 276 |

#### 3.7.4.4 Analysis of sample blanks

Matrix blank samples spiked with IS were prepared to detect PAHs that may be present in blank samples. The blanks were extracted, cleaned, and analysed using the methods described in sections 3.7.1, 3.7.2 and 3.7.3. However, none of the target compounds was found in the blanks. Therefore, there was no subtraction of blank concentrations from concentrations determined in the main samples.

#### 3.7.4.5 Limit of detection and limit of quantification

The instrument Limit of Detection (LOD) for each target compound was calculated using a signal-to-noise ratio of 3:1. The Limit of Quantification (LOQ) was calculated using the equation:

$$\mathrm{LOQ} = \frac{\mathrm{LOD} \times \mathrm{F_{EV}}}{\mathrm{V_{FEI}} \times \mathrm{SS}} \times \frac{100}{\%\ \mathrm{IS\ Recovery}}$$

Equation (6). Limit of Quantification.

where $F_{EV}$ is the final extract volume (µL), $V_{FEI}$ is the volume of final extract injected (µL), SS is the sample size ($m^3$ or g), and the % IS recovery indicates the percentage of recovery of the IS used to quantify the target pollutant in a particular sample. The instrument LOD and sample LOQ are presented in Table 3.5.

Table 3.5 LOD and LOQ of the target compounds using the Agilent GC MS system.

| **Compounds** | **LOD (ng/g)** | **LOQ (ng/g)** |
|---|---|---|
| Naphthalene | 0.31 | 1.94 |
| Acenaphthylene | 0.14 | 0.91 |
| Acenaphthene | 0.18 | 1.15 |
| Fluorene | 0.24 | 1.54 |
| Phenanthrene | 0.16 | 0.85 |
| Anthracene | 0.39 | 2.13 |
| Fluoranthene | 0.11 | 0.61 |
| pyrene | 0.09 | 0.51 |
| benzo[a]anthracene | 0.09 | 0.42 |
| chrysene | 0.09 | 0.44 |
| benzo[b,k]fluoranthene | 0.06 | 0.28 |
| benzo[a]pyrene | 0.09 | 0.44 |
| indeno[1,2,3-cd]pyrene | 0.13 | 0.66 |
| dibenzo[a,h]anthracene | 0.23 | 1.13 |
| benzo(ghi)perylene | 0.1 | 0.48 |

### 3.8 Quantification of TPH

The TPH in soil was determined using a modified gravimetric method adopted from Tang et al., (2010). 5 g of the soil stored in a freezer at -3 °C was freeze dried and then extracted by adding 20 mL of DCM in a 50 mL glass centrifuge tube. The tube was placed in ultrasonic bath for 15 mins and centrifuged at 2500 rpm for 10 mins where upon the extract was transferred into a turbo Vap vial. The process was repeated three more times and the solvent was evaporated from the extract using a turbo Vap evaporator at 40 °C. The TPH was determined gravimetrically by calculating the weight difference between the empty vial and the vial post-evaporation. Subsequently, the TPH extract was separated into different fractions including aromatic, saturated and polar fractions of hydrocarbons using column chromatography. The precipitate of insoluble asphaltene fraction was removed using 50 mL n-Hexane and the soluble fraction was loaded onto a silica gel column (Peng et al., 2009). The saturated hydrocarbon was eluted with 50 mL n-Hexane, aromatic fraction eluted with 50 mL n-Hexane and dichloromethane (1:1) and the polar hydrocarbon fraction was eluted with 50 mL methanol. The solvents were evaporated from the eluents and the masses of the various fractions were gravimetrically determined.

### 3.9 Statistical analysis

Data analysis was carried out using R studio version 1.2.5033 for Mac OSX. A total of four replicates were used for each treatment. The Shapiro-Wilk method (normality test) was used to analyse the distribution of the data. The homogeneity of variance was also tested using Levene's test and residual plots. Log transformation of non-normally distributed data was performed before using the ANOVA test of variance to examine significant differences in the mean values of the effects of crude oil, fertilizer, and biosurfactant on the growth of *C. zizanioides,* the plant biomass, proline accumulation, chlorophyll level, and PAH dissipation in the soil. A Tukey post-hoc test was used to determine if there was a significant difference between the variables ($p < 0.05$). The t-test

was used to carry out a single pairwise comparison between variables such as the roots and shoots of *C. zizanioides* and the plant biomass ($p < 0.05$). The Pearson's Product-Moment Correlation Coefficient (PPMCC) was used to determine the relationship between the effects of the elements like phosphorous (P), iron (Fe), calcium (ca), calcium (Ca), sulphur (S), and magnesium (Mg) present in the soil and the growth of plants, and TPH reduction.

# CHAPTER FOUR

# REMOVAL OF PAHs FROM THE SOIL

## 4.0. BIOACCUMULATION AND REMOVAL OF PAHs BY *C. ZIZANIOIDES* UNDER THE INFLUENCE OF BIOSURFACTANT AND FERTILISER.

**Abstract**

The environment is affected by oil spills and leaks that result from the considerable pressure that industrialisation and population growth have placed on petroleum resources. As a result, both human health and the ecosystem are negatively impacted by the presence of harmful substances like polycyclic aromatic hydrocarbons (PAHs). Over the years, phytoremediation has been investigated as a sustainable approach to cleaning the environment. In this study, we investigated the efficiency of biosurfactants 95% Di-Rhamnolipid and 95% Mono-Rhamnolipid in extracting total petroleum and hydrocarbons (TPH) from crude oil-contaminated soil using mild shaking. We also investigated the capacity of *C. zizanioides* to extract and bioaccumulate PAHs from the oil-contaminated soil while being influenced by fertiliser and biosurfactant. The results showed that 95% di-Rhamnolipid removed 61% of TPH and 95% mono-Rhamnolipid removed 53.1% of TPH. PAHs were bioaccumulated within the plant's shoots and roots. However, the concentration of PAHs in the soil was higher than in the plant's tissues. The combined effect of fertiliser and biosurfactant resulted in the highest reduction of PAHs compared to fertiliser alone, biosurfactant alone, and the untreated controls. The BCF levels were found to be generally low, ranging from 0.21 to 0.55 in all samples. The TF levels in plants growing in all treated samples were likewise seen to closely converge. Our findings suggest that the application of a combination of fertiliser and biosurfactant can potentially be used to aid the phytoremediation of crude oil-contaminated soil using *C. zizanioides.*

## 4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of multiple-ringed compounds that form part of the components of crude oil and are toxic (carcinogenic) to humans (Rengarajan et al., 2015; Hussain et al., 2018). These compounds enter the environment through oil spills and leaks as a result of human anthropogenic activities such as the exploration and exploitation of petroleum resource (Hussain et al., 2018; Brittingham et al., 2014). A total of number 16 PAHs have been categorised as priority pollutants by the World Health Organisation (WHO) and the United States (US) Environmental Protection Agency (EPA) according to the level of their occurrence in the environment, toxicity and human exposure (Concetta Bruzzoniti et al., 2010a; Hussain et al., 2018).

Table 4.13 US EPA 16 priority-pollutant PAHs and selected physical–chemical properties (ATSDR 1995, ATSDR in Bojes and Pope, 2007).

| Compound | Potential Carcinogens | No of Rings | Mol. wt. (g/mole) | Solubility (mg/L) | Vapour pressure (mm Hg) |
|---|---|---|---|---|---|
| Naphthalene | - | 2 | 128.17 | 31 | 8.89E−02 |
| Acenaphthylene | - | 3 | 152.2 | 16.1 | 2.90E−02 |
| Acenaphthene | - | 3 | 154.21 | 3.8 | 3.75E−03 |
| Fluorene | - | 3 | 166.22 | 1.9 | 3.24E−03 |
| Phenanthrene | - | 3 | 178.23 | 1.1 | 6.80E−04 |
| Anthracene | - | 3 | 178.23 | 0.045 | 2.55E−05 |
| Fluoranthene | - | 4 | 202.26 | 0.26 | 8.13E−06 |
| Pyrene | - | 4 | 202.26 | 0.132 | 4.25E−06 |
| Benzo(a)anthracene | √ | 4 | 228.29 | 0.011 | 1.54E−07 |
| Chrysene | √ | 4 | 228.29 | 0.0015 | 7.80E−09 |
| Benzo(b)fluoranthene | √ | 5 | 252.32 | 0.0015 | 8.06E−08 |
| Benzo(k)fluoranthene | √ | 5 | 252.32 | 0.0008 | 9.59E−11 |
| Benzo(a)pyrene | √ | 5 | 252.32 | 0.0038 | 4.89E−09 |
| Benzo(g,h,i)perylene | - | 6 | 276.34 | 0.00026 | 1.00E−10 |
| Dibenz(a,h)anthracene | √ | 6 | 278.35 | 0.0005 | 2.10E−11 |
| Indeno[1,2,3-cd]pyrene | √ | 6 | 276.34 | 0.062 | 1.40E−10 |

The EPA has classified 7 of the 16 PAHs mentioned in table 4.13 as potential human carcinogens, including Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Chrysene, Dibenzo[a,h]anthracene, and indeno[1,2,3,c-d]pyrene (Rengarajan et al., 2015). It is for this reason that the presence of PAHs at polluted sites has sparked widespread public concerns (Nganje et al., 2007; Liu et al., 2015). Hence, the urgent need for environmental restoration through remediation is due to the persistence of PAHs in the environment, which may cause a threat to human health.

The moment crude oil is spilt onto the surface of the land the distribution of petroleum hydrocarbons occurs in various forms a process known as weathering (Coulon et al., 2010). The volatile portion of the spilt petroleum hydrocarbons evaporates into the atmosphere while the non-volatile and denser portion becomes absorbed by soil organic matter. Furthermore, some portion of the non-volatile spilt petroleum hydrocarbons leaches and contaminates the groundwater (Huesemann et al., 2005; Paria, 2008). PAHs form part of the portion of petroleum hydrocarbons that normally become absorbed by soil organic matter (Martens et al., 1997, Plant et al., 2012). As a result, these compounds persist in the environment with potential carcinogenic effect on humans. The pathways of exposure to PAHs in the environment in humans usually occurs through ingestion, inhalation and skin contact (Lang et al., 2012; Wang et al., 2013; Yang et al., 2014).

Over the years there have been rising concerns over the cost and harmful effect of conventional techniques used for soil reclamation. For example, the European Union (EU) has recently reported an estimate of more than one hundred billion dollars for reclamation of contaminated sites (Saier and Trevors, 2010). Additionally, the use of mechanical, thermal and chemical methods often harm the environment through excavation that causes erosion and prolonged effect of heat and toxic chemicals on the soil respectively (Batty and Dolan, 2013; Ndimele et al., 2018). As a result, researchers are studying plant-based techniques for *in situ* soil reclamation known as phytoremediation.

Phytoremediation is an *in situ* technology that uses plants to remove, detoxify, degrade, transform or immobilise contaminants within the soil and groundwater (Novo et al., 2018). This plant-based technology helps in overcoming the issues identified in the conventional methods above, as it is inexpensive, treats pollution *in situ* and does not require addition of strong oxidising or reducing chemicals. Overall, the technology is considered simple to operate, eco-friendly, cost saving, viable and widely accepted by the public (Liu et al., 2018).

Plants generally have the ability to bioaccumulate and metabolise nutrients and contaminants from the soil and groundwater and bio-transform them into by-products (Van Aken, 2008). The ability of plants to effectively bioaccumulate and translocate contaminants can be evaluated using the bioaccumulation factor of the plants (Aladesanmi et al., 2019). There are similarities between the process of metabolism of xenobiotic compounds in plants and the mammalian liver as described in the green liver concept (Sandermann, 1999; Zand and Hoveidi, 2016). These include the ability of both plants and mammals to detoxify contaminants and protect vulnerable organelles by getting rid of harmful substances (Burken, 2003b). Nonetheless, whilst mammals are capable of expelling contaminants through excretion, plants can only store contaminants (less toxic form) within their tissues such as the cell walls and vacuoles (Pflugmacher et al., 2015b). The mechanism of metabolism in both plants and mammals involves transformation and conjugation aided by enzymes such as cytochrome P450 mono-oxygenases and glutathione S-transferases respectively and compartmentalisation or separation of compounds during conjugation (Sandermann, 1994; Burken, 2003). However, many studies have indicated negative effects from the toxicity of petroleum contaminants on the growth of plants (Besalatpour et al., 2008; Peng et al., 2009; Han et al., 2016). Hence, the plant candidate commonly known as vetiver grass was selected for this research based on outstanding morphological and physiological characteristics. These include high biomass content, high photosynthetic efficiency, high tolerance to harsh environmental conditions, and ability to survive in various soil types including flooded/waterlogged conditions (Greenfield, 1995; Dalton et al., 1996; Mucciarelli et al., 1998; Truong, 2000; Lavania, 2004; Srivastava et al., 2008).

Despite the successes recorded on phytoremediation of organic contaminants aided by soil microorganisms particularly in the rhizosphere (Kirk et al., 2005; Millioli et al., 2009). Effective treatment of contaminants is limited to moderately hydrophobic compounds due to their insolubility, high sorption to soil organic matter (SOM) and low bioavailability (Xia et al., 2014; Liao et al., 2015; Chaudhary and Kim, 2019; Chen et al., 2019). Thus, researchers have resorted to the use of biosurfactants to enhance the solubility, bioavailability and plant bioaccumulation of organic compounds which also facilitates their biodegradation in the environment (Ron and Rosenberg, 2002; Das and Chandran, 2011, Xia et al., 2014). This occurs because of the amphiphilic structure of microbial bio-surfactants comprising of hydrophobic and hydrophilic parts which emulsifies and disperse organic contaminants by increasing their surface area.

Biosurfactants are a group of low molecular weight surface active compounds produced by microorganisms that are capable of degrading organic compounds in the environment (Gkorezis et al., 2016b). These microbial biosurfactants are produced by bacteria, fungi and yeasts such as *Pseudomonas aeruginosa, Bacillus subtilis* and *Candida bombicola,* that produce rhamnolipids, lipopeptides known as (surfactin) and sophorolipids respectively (Mulligan, 2005b). Microbial biosurfactants generally enhance the solubility and bioavailability of organic compounds to facilitate their biodegradation in the environment (Das and Chandran, 2011). Their ability to treat organic contaminants is linked to their amphiphilic structure that consist of hydrophobic and hydrophilic parts which increases the surface area, emulsifies and disperse organic contaminants in the environment (Pacwa-Płociniczak et al., 2016). Previous research on biosurfactant assisted phytoremediation of organic contaminants have demonstrated good results on treating organic contaminants in the soil (Liao et al., 2016; Pacwa-Płociniczak et al., 2016; Liduino et al., 2018). However, there is a lack of complete elucidation on biosurfactant, and fertiliser assisted phytoremediation of crude oil contaminated soil using *C. zizanioides,* which is non-invasive, fast growing, and has a robust root structure capable of using potential leachate at great depths in the soil.

The availability of nutrients such as nitrogen, phosphorus and potassium (NPK) is known to play an important role in promoting the degradation of organic contaminants through improved growth

of plants (Basumatary et al., 2012; Titah et al., 2013; Brown et al., 2017). Yet, the combined effect of fertiliser and biosurfactant in enhancing the degradation of PAHs during phytoremediation of crude oil contaminated soil using *C. zizanioides* is yet to be elucidated. Phytoremediation of toxic pollutants has also generated public concerns about the accumulation of substances like PAHs in plant tissues, which produces harmful waste and has an impact on the food web (Fismes et al., 2002, Szczygłowska et al., 2011; Liao et al., 2016). Therefore, there is a need for full elucidation of PAH accumulation in the tissues of *C. zizanioides* under the influence of fertiliser and biosurfactant during phytoremediation of crude oil polluted soil.

This research is aimed at investigating the effect of fertiliser and biosurfactant in enhancing the removal of PAHs during the process of phytoremediation of crude oil contaminated using *C. zizanioides*. The objectives are: (1) to investigate the efficiency of biosurfactants in solubilising PAH in a crude oil contaminated soil, (2) to investigate the ability of *C. zizanioides* in extracting the US EPA 16 during the process of phytoremediation and (3) to determine the level of bioaccumulation of PAHs when the plant is exposed to crude oil-contaminated soil.

## 4.2 Materials and method

### 4.2.1 Experimental design and sample analysis

As previously explained in detail in sections 3.2, 3.3, 3.4, 3.5 and 3.6, a sandy loam soil was air-dried and homogenised using a 2 mm steel sieve (Zornoza et al., 2006; Moritsuka et al., 2019). The soil pH, EC, moisture content and SOM were evaluated. The homogenised soil was spiked with crude oil and weathered for 100 days and vegetated with *C. zizanioides*. Relevant samples were treated with fertiliser and biosurfactant to improve the growth of plants and microbial quantity and to enhance the desorption of petroleum contaminants in the soil, respectively.

### 4.2.2 TPH removal efficiency of biosurfactants

The efficiency of biosurfactants in removing TPH from crude oil contaminated soil was determined using a method adapted from Lai et al., (2009). 50g of contaminated soil was measured and placed in a 500mL flask. Biosurfactants (95% Di Rhamnolipid dominant and 95% Mono Rhamnolipid dominant) purchased from Sigma Aldrich were separately dissolved in deionised water to obtain a high critical micelle concentration (CMC) of 10,000 mg/L (Liao et al., 2015). 100mL of each of the solutions of dissolved biosurfactants were added to the samples and deionised water was used as control blank. The flask was gently shaken at 50 rpm at room temperature for 24 hours and the solution was removed from the soil and the extraction of TPH was carried out as explained below. The TPH removal efficiency was evaluated using the equation below.

$$TPH\ removal\ effieciency = \frac{TPH\ from\ liquid\ extraction}{TPH\ from\ liquid\ extraction + TPH\ from\ soil\ extaction}\ \text{x}\ 100\%$$

Equation (7). TPH removal efficiency

### 4.2.3 Extraction of TPH

The extraction of TPH was carried out using a modified gravimetric method adapted from Tang et al., (2010) as previously explained in detail in section 3.9.

### 4.2.4 Extraction and analysis of target compounds (PAHs)

The extraction and analysis of the target compounds of interest (PAHS) was carried out to determine their level of reduction using the methods described in detail in section 3.7.

### 4.2.6 Accumulation of PAHs in plant tissues

The ability of plants to absorb, transport, and accumulate contaminants is determined by the bioconcentration factor (BCF) and translocation factor (TF), which also determines their phytoremediation ability (Jayampathi et al., 2019; Gajić et al., 2020). Accordingly, the bioremediation capacity of *C. zizanioides* as the plant candidate for phytoremediation of PAHs in crude oil contaminated soil was evaluated using the (BCF and TF). The BCF can be described as the ratio of concentration of compounds in plants divided by the concentration of compounds in the contaminated environment (Jayampathi et al., 2019). When the bioconcentration factor (BCF) is less than 1, it is considered low, and when the BCF is greater than 1, it is considered high (Pachura et al., 2016). The BCF can be calculated using the equation described by Novo et al., (2013) below:

$$\text{BCF} = \frac{Cp}{Cs}$$

Equation (8). Bioconcentration factor.

Where *Cp* is the concentration of compounds in plant part and *Cs* is the concentration of compounds in soil.

Translocation factor (TF) can be defined as the ability of plants to translocate compounds from the roots of plants to the shoots Majid et al., 2014). The translocation factor can be calculated using the equation described by Novo et al., (2013) as shown below:

$$\text{TF} = \frac{Cs}{Cr}$$

Equation (9). Translocation factor

Where *Cs* is concentration of compounds in shoot and *Cr* is concentration of compounds in root.

### 4.2.7 Statistical analysis

The statistical analysis was carried out using R studio version 1.2.5033 for Mac OSX as explained previously in section 3.9. ANOVA test of variance was used to examine significant differences in the mean values of TPH removal, dissipation of PAHs, accumulation of PAHs in plant tissues, BCF, and TF. A Tukey post-hoc test was used to determine if there was a significant difference between the variables ($p < 0.05$).

## 4.3 Results

### 4.3.1 Soil Characterisation

The results obtained from analysis of the original soil before the greenhouse (table 4.2).

Table 4.2 Physicochemical parameters of soil, (n=4).

| Parameters | Values |
|---|---|
| EC (µS) | 0.44 ± 0.01 |
| pH | 7.5 ± 0.06 |
| Soil moisture content (%) | 2.74 ± 0.15 |
| SOM (%) | 1.18 ± 0.04 |

### 4.3.2 Efficiency of biosurfactants on TPH removal

The removal of TPH from crude oil-contaminated soil was observed to have been enhanced through the application of biosurfactants and mild shaking. The concentrations of TPHs were reduced by 61.3%, 53.1%, and 23.1%, respectively, when biosurfactants 95% Di Rhamnolipid dominant and 95% Mono Rhamnolipid dominant and water (control) were used (Figure. 4.1). Our

results suggest that the removal of TPH from the soil that resulted from the effect of 95% Di Rhamnolipid, which increased by 11.69% was statistically significant ($p < 0.05$) when compared to the control sample that contains water alone, which increased by 5.15%. Interestingly, the removal of TPH from the soil through the application of biosurfactant (95% Mono Rhamnolipid) which increased by 10.46% was also statistically significant ($p < 0.05$) when compared to the control sample that contains water alone, which increased by 5.15%. However, there was no significant difference ($p > 0.05$) between the removal of TPHs in samples treated with biosurfactant (95% Di Rhamnolipid) and in the samples treated with 95% Mono Rhamnolipid dominant. The effect of both biosurfactants have shown effective removal of TPH from the crude oil contaminated soil. However, the results have revealed the highest removal of TPH from the crude oil contaminated soil through the effect of 95% Di Rhamnolipid dominant by 61.02% (Figure 4.1). Therefore, the 95% Di Rhamnolipid dominant was used for our experiment on the phytoremediation of PAHs in crude oil contaminated soil.

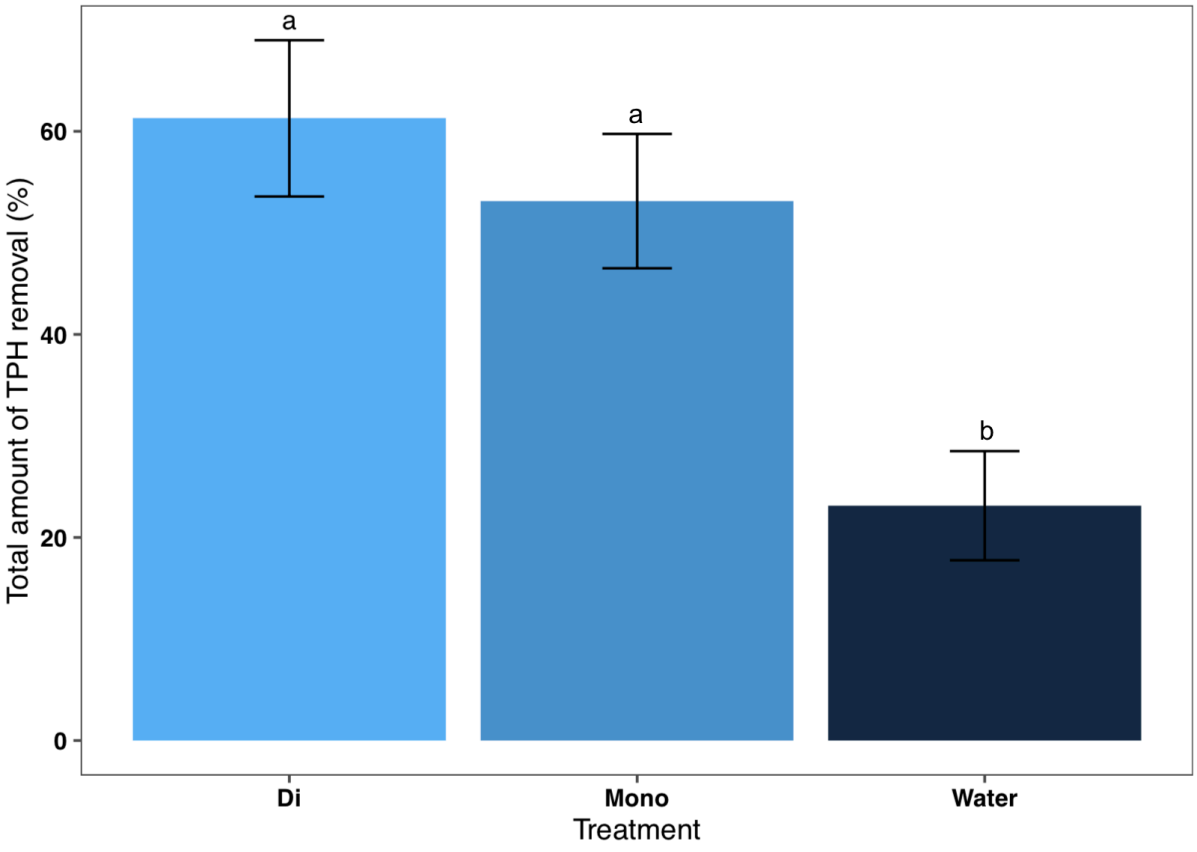



Figure 4.1 Efficiency of biosurfactants on removal of TPH in crude oil contaminated soil. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between treatments, ($p \leq 0.05$).

### 4.3.3 Dissipation of PAHs

The effect of various soil treatments has impacted the dissipation of PAHs from crude oil contaminated soils vegetated with *C. zizanioides* during our phytoremediation experiment. The highest rate of PAHs dissipation was observed in samples treated with a combination of fertiliser and biosurfactant (Figures 4.2 A, B and C). The results suggest that the combined effect of fertiliser and biosurfactant has significantly reduced ($p < 0.05$) the concentration of the LMW PAHs compared to the concentration of the LMW PAHs in samples treated with biosurfactant alone, fertiliser alone, and the untreated control samples vegetated with *C. zizanioides* as well as the unvegetated samples (Figure 4.2 A). The concentration of Naphthalene, which was reduced by 89.6%, was statistically significant ($p < 0.05$) when compared to that of Acenaphthylene, reduced by 68%, Phenanthrene by 58.3%, Acenaphthene by 44.6%, Anthracene by 38.6%, and Fluorene by 32.1%. The results also suggest that the concentration of Acenaphthylene, reduced by 68% was statistically significantly ($p < 0.05$) compared to that of Phenanthrene, which was reduced by 58.3%, Acenaphthene by 44.6%, Anthracene by 38.6%, and Fluorene by 32.1%. The concentration of Phenanthrene, reduced by 58.3% was also statistically significant ($p < 0.05$) compared to that of Acenaphthene, reduced by 44.6%, Anthracene by 38.6%, and Fluorene by 32.1%. The concentration of Acenaphthene, reduced by 44.6% was also statistically significant ($p < 0.05$) when compared to that of Anthracene, reduced by 38.6%, and Fluorene by 32.1%. Also, there was a significant difference ($p < 0.05$) between the concentration of Anthracene, reduced by 38.6%, and that of Fluorene by 32.1%.

The results have also indicated that the combined effect of fertiliser and biosurfactant has led to a significant ($p < 0.05$) reduction of the medium molecular weight (MMW) PAHs when compared to samples treated with biosurfactant alone, fertiliser alone, and the untreated control samples vegetated with *C. zizanioides* and the unvegetated samples respectively (Figure 4.2 B). The results suggest that the concentration of Fluoranthene, which was reduced by 36.1% was statistically significant ($p < 0.05$) when compared to that of Benzo[a]anthracene, reduced by 32.8%, Pyrene

by 21.2%, and Chrysene by 13.7% (Figure 4.2 B). Furthermore, the concentration of Benzo[a]anthracene, reduced by 32.8% was statistically significant ($p < 0.05$) when compared to that of Pyrene, reduced by 21.2%, and Chrysene by 13.7%. The concentration of Pyrene, reduced by 21.2% was also statistically significant ($p < 0.05$) when compared to that of Chrysene, which was reduced by 13.7%.

Our findings have also revealed that the combined effect of fertiliser and biosurfactant has resulted in dissipation of a large amount of the high molecular weight (HMW) PAHs from the soil. The results suggests that the concentrations of Benzo[k]fluoranthene which was reduced by 26.8%, Benzo[a]pyrene by 29.8%, Indeno[1,2,3-cd]pyrene by 30.5%, were all statistically significant ($p < 0.05$) when compared to that of Dibenzo[a,h]anthracene, reduced by 24.5%, and Benzo[g,h,i]perylene, reduced by 15.4% (Figure 4.2C). The concentration of Benzo[b]fluoranthene, reduced by 26.8% was also statistically significant ($p < 0.05$) when compared to that of Benzo[g,h,i]perylene, which reduced by 15.4%.

The results also revealed that large quantities of PAHs were dissipated during the phytoremediation process because of the sole usage of biosurfactant for treating the crude oil-contaminated soil. Our findings have shown that the highest dissipation of the PAHs caused by the effect of biosurfactant occurred in the LMW PAHs (Figure 4.2 A). The results suggest that the effect of biosurfactant has significantly reduced ($p < 0.05$) the concentration of the LMW PAHs compared to the concentration of the LMW PAHs in samples treated with fertiliser alone, and the untreated control samples vegetated with *C. zizanioides* as well as the unvegetated samples (Figure 4.2 A). The results suggests that the concentration of Naphthalene, which was reduced by 64.8% was statistically significant ($p < 0.05$) when compared to that of Acenaphthylene, which was reduced by 52.1%, Phenanthrene by 40.4%, Acenaphthene by 30.3%, Anthracene by 26.2%, and Fluorene by 20.7%. The concentration of Acenaphthylene, which was reduced by 52.1%, was also statistically significant ($p < 0.05$) when compared to that of Phenanthrene, reduced by 40.4%, Acenaphthene by 30.3%, Anthracene by 26.2%, and Fluorene by 20.7%. The results also revealed that the concentration of Phenanthrene, reduced by 40.4% was statistically significant when compared to that of Acenaphthene, reduced by 30.3%, Anthracene by 26.2%, and Fluorene by

20.7%. The concentration of Acenaphthene, which was reduced by 30.3% was also statistically significant ($p < 0.05$) when compared to that of Anthracene which was reduced by 26.2%, and Fluorene by 20.7%.

Our findings also showed that the exclusive effect of the biosurfactant has resulted in a reduction in the concentration of MMW PAHs in the contaminated soil (Figure 4.2 B). The results suggest that the concentration of Benzo[a]anthracene, reduced by 22.1% was also statistically significant ($p < 0.05$) when compared to that of Pyrene, reduced by 13.2%, and Chrysene by 7.7%. The concentration of Fluoranthene, reduced by 20.2% was statistically significant ($p < 0.05$) when compared to that of Pyrene, reduced by 13.2%, and Chrysene by 7.7%. Furthermore, the concentration of Pyrene, reduced by 13.2% was statistically significant ($p < 0.05$) when compared to that of Chrysene, reduced by 7.7%.

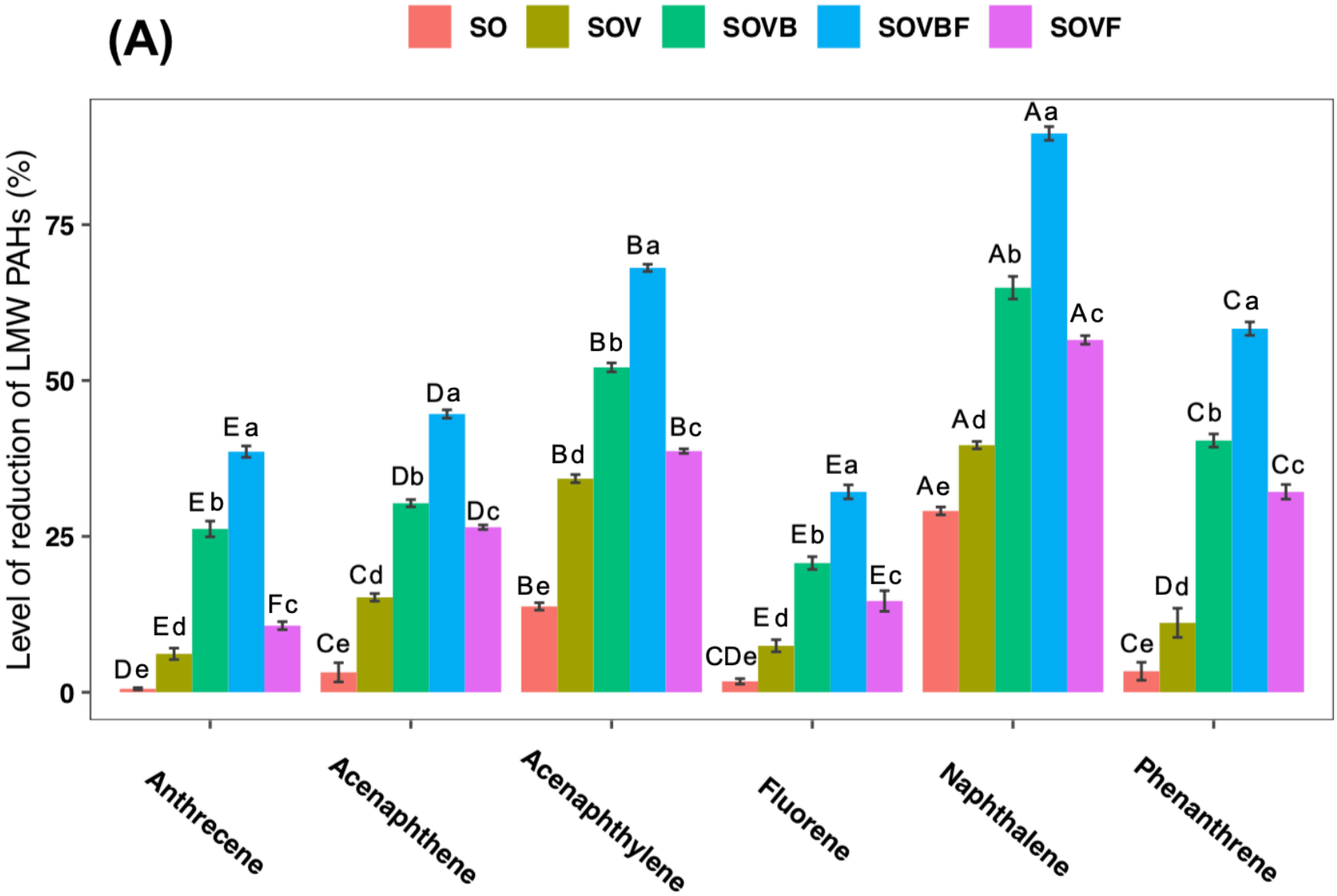
We observed that there was a little reduction in the concentration of the HMW PAHs in samples treated solely with biosurfactant. The results showed that the concentrations of Benzo[a]pyrene, reduced by 20.7%, Indeno[1,2,3-cd]pyrene by 20.2%, Dibenzo[a,h]anthracene by 20.2%, and Benzo[b]fluoranthene by 18% were all statistically significant ($p < 0.05$) when compared to that of Benzo[g,h,i]perylene, reduced by 8.6% (Figure 4.2 C).

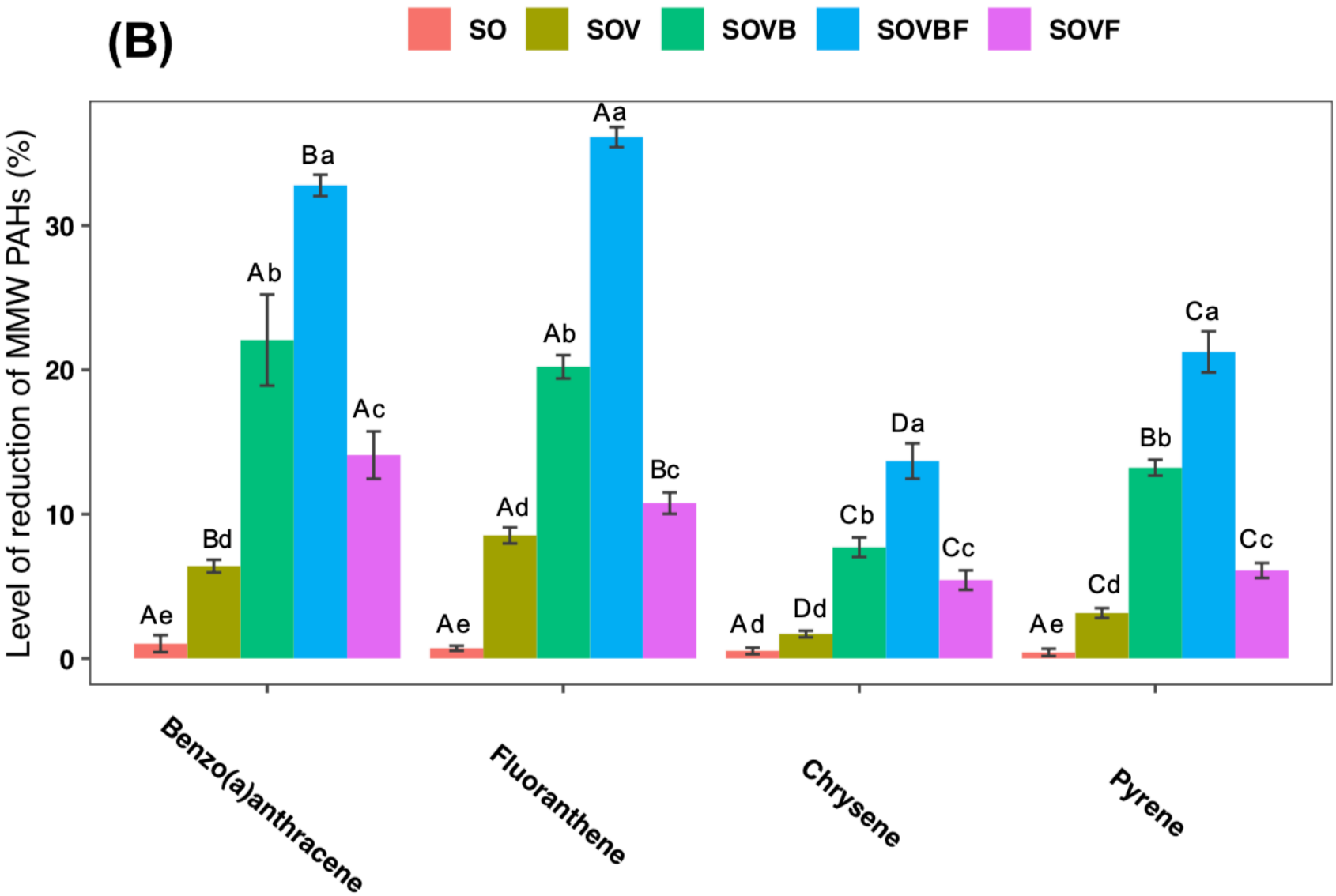
The exclusive effect of fertiliser has also led to the dissipation of PAHs during the process of phytoremediation. Our results have revealed that the highest dissipation of PAHs occurred on the LMW PAHs (Figure 4.2 A). The concentration of Naphthalene, reduced by 56.5%, was statistically significant ($p < 0.05$) when compared to that of Acenaphthylene, reduced by 38.7%, Phenanthrene by 32.1%, Acenaphthene by 26.5%, Fluorene by 14.6% and Anthracene by 10.7%. The results also suggest that the concentration of Acenaphthylene, reduced by 38.7% was statistically significant when compared to that of Phenanthrene, reduced by 32.1%, Acenaphthene by 26.5%, Fluorene by 14.6% and Anthracene by 10.7%. The concentration of Phenanthrene, reduced by 32.1% was also statistically significant ($p < 0.05$) when compared to that of Acenaphthene, reduced by 26.5%, Fluorene by 14.6% and Anthracene by 10.7%. Furthermore, the concentration of Acenaphthene was also reduced significantly ($p < 0.05$) by 26.5% when compared to that of

Fluorene, which reduced by 14.6% and Anthracene by 10.7%. The results also revealed the concentration of Fluorene, reduced by 14.6% was significant ($p < 0.05$) when compared to that of Anthracene, which reduced by 10.7%.

Our results have also shown that the sole effect of fertiliser has resulted in the dissipation of the MMW PAHs from the crude oil-contaminated soil (Figure 4.2B). The concentration of Benzo[a]anthracene, reduced by 14.1% was statistically significant ($p < 0.05$) when compared to that of Fluoranthene, reduced by 10.8%, Pyrene by 6.1% and Chrysene by 5.4%. The concentration of Fluoranthene, which was reduced by 10.8% was also statistically significant ($p < 0.05$) when compared to that of Pyrene, which was reduced by 6.1% and Chrysene by 5.4%. However, the concentration of Pyrene, which was reduced by 6.1% was not statistically significant ($p > 0.05$) when compared to that of Chrysene, which reduced by 5.4%.

The results have also revealed the dissipation of HMW PAHs in samples treated exclusively with fertiliser (Figure 4.2C). The concentration of Indeno[1,2,3-cd]pyrene, which was reduced by 16.5% was statistically significant ($p < 0.05$) when compared to that of Benzo[k]fluoranthene, which reduced by 13%, Benzo[a]pyrene by 12.7%, Dibenzo[a,h]anthracene, reduced by 11.4%, Benzo[b]fluoranthene by 11.3% and Benzo[g,h,i]perylene by 9.4%.


(A)
SO
SOV
SOVB
SOVBF
SOVF
Level of reduction of LMW PAHs (%)
0
25
50
75
De
Ed
Eb
Ea
Fc
Ce
Cd
Db
Da
Dc
Be
Bd
Bb
Ba
Bc
CDe
Ed
Eb
Ea
Ec
Ae
Ad
Ab
Aa
Ac
Ce
Dd
Cb
Ca
Cc
Anthrecene
Acenaphthene
Acenaphthylene
Fluorene
Naphthalene
Phenanthrene


(B)
SO
SOV
SOVB
SOVBF
SOVF
Level of reduction of MMW PAHs (%)
0
10
20
30
Ae
Bd
Ab
Ba
Ac
Ae
Ad
Ab
Aa
Bc
Ad
Dd
Cb
Da
Cc
Ae
Cd
Bb
Ca
Cc
Benzo(a)anthracene
Fluoranthene
Chrysene
Pyrene

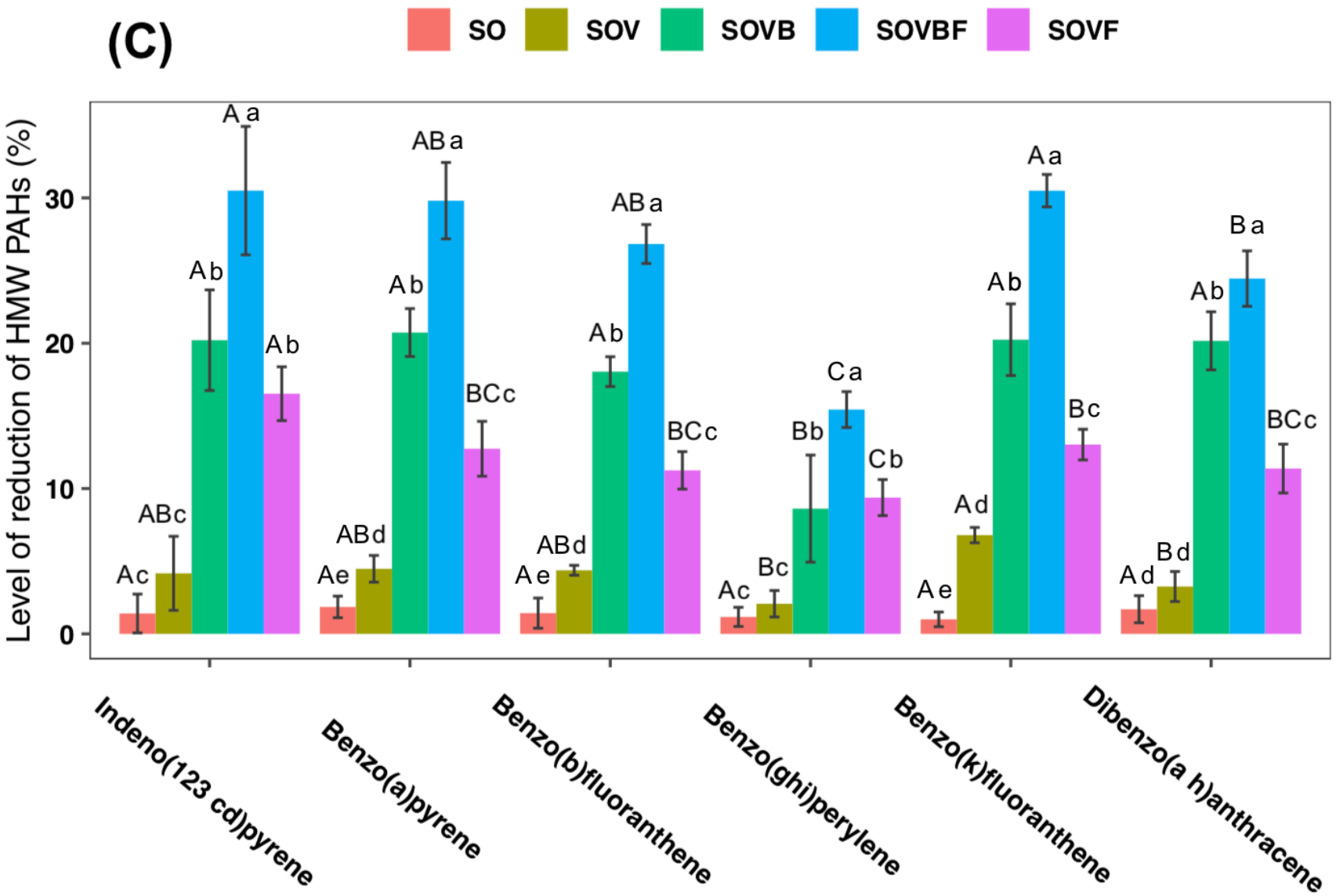

(C)
SO
SOV
SOVB
SOVBF
SOVF
Level of reduction of HMW PAHs (%)
0
10
20
30
Ac
ABc
Ab
Aa
Ab
Ae
ABd
Ab
ABa
BCc
Ae
ABd
Ab
ABa
BCc
Ac
Bc
Bb
Ca
Cb
Ae
Ad
Ab
Aa
Bc
Ad
Bd
Ab
Ba
BCc
Indeno(123 cd)pyrene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(ghi)perylene
Benzo(k)fluoranthene
Dibenzo(a h)anthracene

Figure 4.2 The percentage of reduction of the 16 PAHs in crude oil contaminated soils after a period of 32 weeks. The values are given as mean ± standard deviation SD, (n=4). The lowercase alphabetical letters indicate the significant differences between different treatments for each compound. The uppercase alphabetical letters indicate the significant differences between various compounds in different treatments ($p \leq 0.05$).

(A) The level of reduction of the LMW PAHs in crude oil contaminated soil.
(B) The level of reduction of the MMW PAHs in crude oil contaminated soil.
(C) The level of reduction of the HMW PAHs in crude oil contaminated soil.

The abbreviations are: SO = crude oil contaminated soil only, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated crude oil contaminated soil vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*.

### 4.3.4 Accumulation and distribution of PAHs in plant tissues

The accumulation of PAHs in the tissues of *C. zizanioides* was affected by various soil treatments after four weeks of phytoremediation experiment. The results revealed that most of the PAHs accumulated in the plant shoots and roots including Naphthalene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene, Benzo[b]fluoranthene (Table 4.3). However, a few compounds were not detected in the shoots including Acenaphthylene, Acenaphthene, Fluorene and Benzo[a]anthracene (Table 4.3). Benzo[k]fluoranthene was not detected in the shoots of plants grown in samples treated with fertiliser alone and the untreated control samples. Benzo[a]pyrene was also not detected in the shoots of plants grown in the untreated control samples. The findings of our research have indicated that the accumulation of total PAHs in plant tissues was proportional to their concentration in crude oil-contaminated soil (Table 4.3). The results also revealed that the total PAHs in the soil was higher than the total PAHs accumulated in the shoots and roots of *C. zizanioides* during phytoremediation process (Figure 4.3). The concentration of total PAHs in the soil, 2083.01(μg $Kg^{-1}$ DW) was statistically significant ($p > 0.05$)

when compared to that in the shoots of plants grown in samples treated with both fertiliser and biosurfactant, 693.32 (µg $Kg^{-1}$ DW), treated exclusively with fertiliser, 615.94 (µg $Kg^{-1}$ DW), treated exclusively with biosurfactant, 584.41 (µg $Kg^{-1}$ DW) and in the untreated control samples, 478.12 (µg $Kg^{-1}$ DW). The results also suggests that the concentration of total PAHs in the soil, 2083.01(µg $Kg^{-1}$ DW) was statistically significant ($p > 0.05$) when compared to that in the roots of plants grown with a combination of fertiliser and biosurfactant, 1032.22 (µg $Kg^{-1}$ DW), treated exclusively with fertiliser, 941.62 (µg $Kg^{-1}$ DW), treated exclusively with biosurfactant, 862 (µg $Kg^{-1}$ DW), and in the untreated control samples, 767.47 (µg $Kg^{-1}$ DW).

The results have also shown that the total PAHs accumulated in the shoots of plants grown under the combined effect of fertiliser and biosurfactant, 693.32 (µg $Kg^{-1}$ DW) was statistically significant ($p < 0.05$) when compared to that in the shoots of plants grown in the untreated control samples, which was 478.12 (µg $Kg^{-1}$ DW). Also, the total PAHs accumulated in the shoots of plants grown exclusively with fertiliser, 615.94 (µg $Kg^{-1}$ DW) was statistically significant ($p < 0.05$) when compared to that in the shoots of plants grown in the untreated control samples, 478.12 (µg $Kg^{-1}$ DW). Similarly, the total PAHs accumulated in the shoots of plants grown exclusively with biosurfactant, 584.41 (µg $Kg^{-1}$ DW) was statistically significant ($p < 0.05$) when compared to that in the shoot of plants grown in the untreated control samples, 478.12 (µg $Kg^{-1}$ DW). However, there was no significant difference ($p > 0.05$) between the total PAHs accumulated in the shoot of plants under the combined effect of fertiliser and biosurfactant, 693.32 (µg $Kg^{-1}$ DW), that in the shoot of plants grown exclusively with fertiliser, 615.94 (µg $Kg^{-1}$ DW), and in the shoots of plants grown exclusively with biosurfactant, 584.41 (µg $Kg^{-1}$ DW). The total PAHs accumulated in the shoot of plants grown exclusively with fertiliser, 615.94 (µg $Kg^{-1}$ DW) was also statistically not significant ($p > 0.05$) when compared to that in the shoot of plants grown exclusively with biosurfactant, 584.41 (µg $Kg^{-1}$ DW).

Our results have also shown that the total PAHs accumulated in the root of plants under the combined effect of fertiliser and biosurfactant, 1032.22 (µg $Kg^{-1}$ DW) was statistically significant ($p < 0.05$) when compared to that in the root of plants grown exclusively with biosurfactant, 862 (µg $Kg^{-1}$ DW), and that in the untreated control samples, 767.47 (µg $Kg^{-1}$ DW). Except for the total

PAHs accumulated in the root of plants grown exclusively with fertiliser, 941.62 (µg $Kg^{-1}$ DW). The total PAHs accumulated in the roots of plants grown exclusively with fertiliser, 941.62 (µg $Kg^{-1}$ DW) was also statistically significant ($p < 0.05$) when compared to that in the root of plants grown in the untreated control samples, 767.47 (µg $Kg^{-1}$ DW). Except for the total PAHs accumulated in the roots of plants grown exclusively with biosurfactant, 862 (µg $Kg^{-1}$ DW).

Our findings have also shown that the total PAHs accumulated in the root of plants grown under the combined effect of fertiliser and biosurfactant, 1032.22 (µg $Kg^{-1}$ DW) was statistically significant ($p < 0.05$) when compared to that in the shoot of plants under the combined effect of fertiliser and biosurfactant, 693.32 (µg $Kg^{-1}$ DW), treated exclusively with fertiliser, 615.94 (µg $Kg^{-1}$ DW), treated exclusively with biosurfactant, 584.41 (µg $Kg^{-1}$ DW), and in the untreated control samples, 478.12 (µg $Kg^{-1}$ DW).

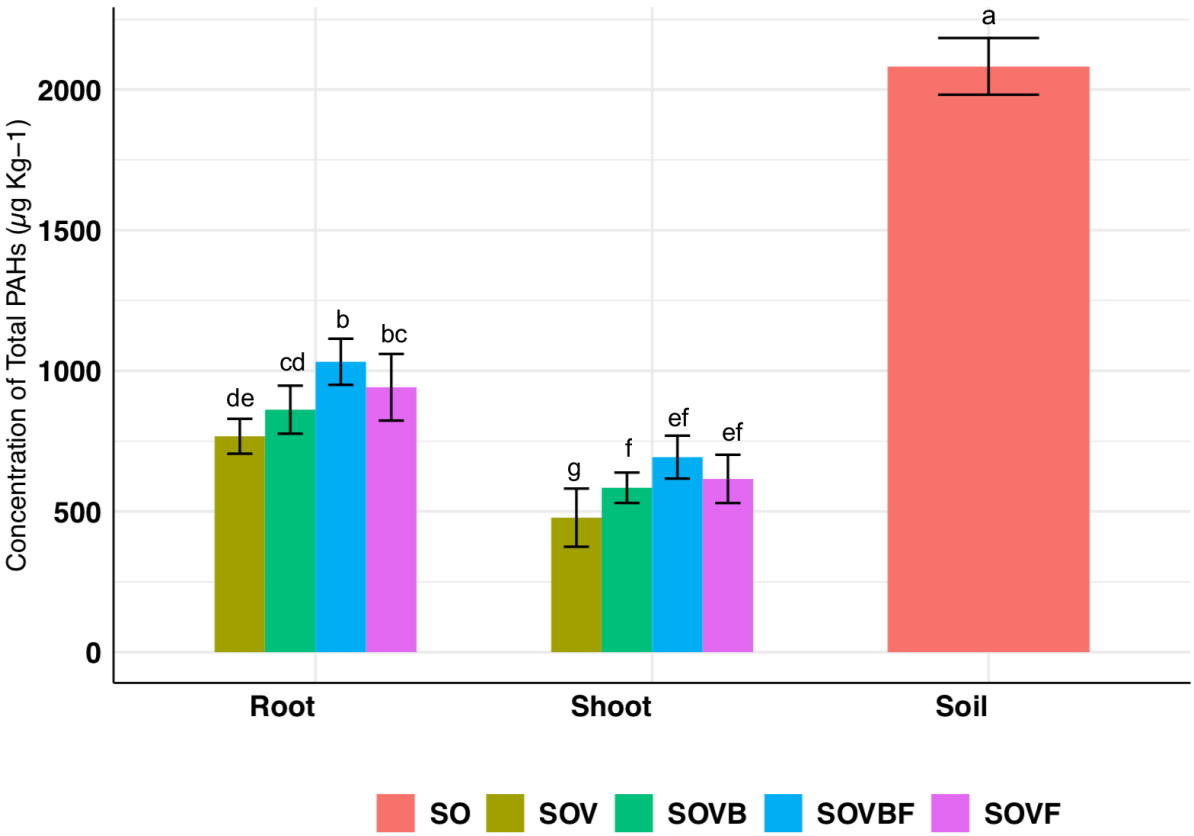



Figure 4.3 Concentration of total PAHs in crude oil contaminated soil and plant parts. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SO = crude oil contaminated soil only, SOV = crude oil contaminated soil vegetated with *C. zizanioides*, SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated crude oil contaminated soil vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*.

Table 4.3 Concentration of individual and total PAHs in crude oil contaminated soil and their bioaccumulation in the shoots and roots of *C. zizanioides* after a period of 4 weeks. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between various treatments ($p \leq 0.05$).

| | | Concentration (conc.) of PAHs (µg $Kg^{-1}$ DW) in plants grown in different samples | | | |
|---|---|---|---|---|---|
| Compound | Conc. of PAHs in soil (µg $Kg^{-1}$) | Plant part | SOVBF | SOVF | SOVB | SOV (Control) |
| Nap | $100.4 \pm 11.3^{hi}$ | Shoot | $50.2 \pm 4.3^{a}$ | $44.9 \pm 3.0^{ab}$ | $38.5 \pm 2.9^{b}$ | $40.4 \pm 3.9^{b}$ |
| | | Root | $63.1 \pm 8.7^{a}$ | $51.2 \pm 6.1^{ab}$ | $60.9 \pm 4.9^{ab}$ | $49.5 \pm 3.5^{b}$ |
| Acy | $106.9 \pm 1.9^{gh}$ | Shoot | ND | ND | ND | ND |
| | | Root | $66.6 \pm 2.7^{a}$ | $61.0 \pm 4.4^{ab}$ | $49.0 \pm 6.0^{c}$ | $53.7 \pm 6.0^{bc}$ |
| Ace | $148.9 \pm 1.8^{d}$ | Shoot | ND | ND | ND | ND |
| | | Root | $70.5 \pm 3.4^{a}$ | $67.6 \pm 8.0^{ab}$ | $58.8 \pm 5.5^{b}$ | $56.4 \pm 3.8^{b}$ |
| Flo | $183.7 \pm 3.1^{c}$ | Shoot | ND | ND | ND | ND |
| | | Root | $75.3 \pm 8.9^{a}$ | $58.0 \pm 5.2^{bc}$ | $62.7 \pm 3.8^{b}$ | $48.9 \pm 4.1^{c}$ |
| Phe | $82.8 \pm 2.1^{jkl}$ | Shoot | $40.8 \pm 3.5^{ab}$ | $49.2 \pm 8.2^{a}$ | $40.2 \pm 3.5^{ab}$ | $30.5 \pm 6.1^{b}$ |
| | | Root | $66.0 \pm 5.0^{a}$ | $64.0 \pm 5.0^{a}$ | $47.2 \pm 9.1^{b}$ | $45.6 \pm 3.0^{b}$ |
| Ant | $134.0 \pm 2.0^{def}$ | Shoot | $48.3 \pm 5.5^{a}$ | $47.4 \pm 7.7^{ab}$ | $38.4 \pm 3.7^{ab}$ | $37.2 \pm 1.9^{b}$ |
| | | Root | $70.1 \pm 5.3^{a}$ | $66.3 \pm 11.6^{ab}$ | $55.1 \pm 6.1^{ab}$ | $52.8 \pm 3.3^{b}$ |
| Flu | $126.2 \pm 1.4^{ef}$ | Shoot | $48.7 \pm 3.8^{a}$ | $41.1 \pm 8.5^{ab}$ | $44.6 \pm 2.1^{a}$ | $31.8 \pm 3.5^{b}$ |
| | | Root | $64.4 \pm 7.0^{a}$ | $61.4 \pm 11.6^{a}$ | $58.9 \pm 3.7^{ab}$ | $45.0 \pm 2.4^{b}$ |
| Pyr | $209.9 \pm 3.8^{b}$ | Shoot | $50.6 \pm 5.5^{a}$ | $40.0 \pm 4.11^{b}$ | $41.9 \pm 5.2^{ab}$ | $35.3 \pm 3.3^{b}$ |
| | | Root | $72.3 \pm 4.2^{a}$ | $51.4 \pm 3.2^{b}$ | $63.0 \pm 9.4^{ab}$ | $51.4 \pm 9.3^{b}$ |
| BaA | $98.5 \pm 1.1^{ijk}$ | Shoot | ND | ND | ND | ND |
| | | Root | $62.1 \pm 2.5^{a}$ | $62.7 \pm 3.9^{a}$ | $44.6 \pm 3.1^{b}$ | $46.8 \pm 4.1^{b}$ |
| Chr | $272.8 \pm 3.9^{a}$ | Shoot | $50.3 \pm 9.2^{a}$ | $35.4 \pm 5.5^{b}$ | $36.7 \pm 1.1^{b}$ | $31.8 \pm 5.6^{b}$ |
| | | Root | $68.4 \pm 11.2^{a}$ | $65.7 \pm 14.2^{a}$ | $64.3 \pm 3.7^{a}$ | $53.3 \pm 4.2^{a}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| BbF | 119.5 ± 2.2$^{fg}$ | Shoot | 36.9 ± 3.1$^{a}$ | 33.7 ± 5.8$^{a}$ | 34.3 ± 4.4$^{a}$ | 24.4 ± 3.2$^{b}$ |
| | | Root | 56.1 ± 1.6$^{a}$ | 54.7 ± 10.9$^{a}$ | 47.4 ± 5.1$^{a}$ | 47.5 ± 3.0$^{a}$ |
| BkF | 104.3 ± 1.7$^{gh}$ | Shoot | 38.5 ± 4.3$^{a}$ | ND | 32.7 ± 3.1$^{a}$ | ND |
| | | Root | 57.7 ± 3.1$^{ab}$ | 54.4 ± 4.2$^{ab}$ | 61.9 ± 9.5$^{a}$ | 46.2 ± 5.1$^{b}$ |
| BaP | 88.2 ± 3.2$^{hijk}$ | Shoot | 39.2 ± 4.2$^{a}$ | 35.9 ± 5.1$^{a}$ | 32.4 ± 3.8$^{a}$ | ND |
| | | Root | 69.0 ± 2.2$^{a}$ | 61.7 ± 7.0$^{a}$ | 41.2 ± 5.3$^{b}$ | 41.3 ± 3.3$^{b}$ |
| BP | 135.2 ± 2.0$^{de}$ | Shoot | 38.5 ± 4.6$^{a}$ | 32.4 ± 3.2$^{ab}$ | 29.9 ± 3.9$^{bc}$ | 24.0 ± 2.2$^{c}$ |
| | | Root | 60.4 ± 3.5$^{ab}$ | 68.5 ± 9.5$^{a}$ | 60.1 ± 2.4$^{ab}$ | 51.5 ± 0.7$^{b}$ |
| DahA | 80.3 ± 2.0$^{jklm}$ | Shoot | 34.7 ± 4.4$^{a}$ | 29.0 ± 3.8$^{ab}$ | 28.3 ± 2.5$^{ab}$ | 22.3 ± 4.1$^{b}$ |
| | | Root | 52.7 ± 7.0$^{a}$ | 44.0 ± 5.5$^{ab}$ | 42.7 ± 4.3$^{ab}$ | 36.6 ± 4.2$^{b}$ |
| IP | 91.5 ± 6.1$^{hij}$ | Shoot | 37.3 ± 4.9$^{a}$ | 34.0 ± 5.2$^{a}$ | 30.5 ± 4.0$^{ab}$ | 23.4 ± 4.1$^{b}$ |
| | | Root | 57.7 ± 5.8$^{a}$ | 49.1 ± 8.1$^{ab}$ | 44.2 ± 3.4$^{b}$ | 40.9 ± 2.3$^{b}$ |
| Σ Total PAHs | 2083.1 | Shoot | 514 | 423 | 428.4 | 301.1 |
| | | Root | 1032.4 | 941.7 | 862 | 767.4 |

The abbreviations are: SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SOV = crude oil contaminated soil vegetated with *C. zizanioides*. Nap = Naphthalene, Acy = Acenaphthylene, Ace= Acenaphthene, Flo = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Flu = Fluoranthene, Pyr = Pyrene, BaA = Benzo[a]anthracene, Chr = Chrysene, BbF = Benzo[b]fluoranthene, BkF = Benzo[k]fluoranthene, BaP = Benzo[a]pyrene, BP = Benzo[ghi]perylene, DahA = Dibenzo[a,h]anthracene, IP = Indeno[1,2,3-cd]pyrene.

### 4.3.5 Effect of soil treatments on bioconcentration factor (BCF)

The effect of different soil treatments during the process of phytoremediation has affected the BCF of total PAHs in plant tissues. The results revealed that the combined effect of fertiliser and biosurfactant led to the highest BCF in the tissues of *C. zizanioides* (Figure 4.4). The average BCF in plant roots grown in samples treated with a combination of fertiliser and biosurfactant (0.55) was statistically significant ($p < 0.05$) compared to that of the plants grown exclusively with biosurfactant (0.47) and the control plants, (0.38). Also, the average BCF in plant roots grown exclusively with fertiliser (0.5) was statistically significant ($p < 0.05$) when compared to that of the plants grown in the untreated control sample (0.38). The results also indicated that the average BCF in the root of plants grown exclusively with biosurfactant (0.47) was not statistically significant ($p > 0.05$) when compared to that of the plants grown in the untreated control samples (0.38).

The results also revealed that the average BCF in plant shoots grown in samples treated with fertiliser and biosurfactant (0.37) was statistically significant ($p < 0.05$) when compared to that of the plants grown in the untreated control samples (0.21). The results also revealed that the average BCF in the shoot of plants grown exclusively with fertiliser (0.33) was statistically significant ($p < 0.05$) when compared to that of plants grown in the untreated control samples (0.21). The average BCF in the shoot of plants grown exclusively with biosurfactant (0.31) was also found to be statistically significant ($p < 0.05$) when compared to that of the plants grown in the untreated control samples (0.21).

Our findings also suggests that the average BCF in plant roots grown in samples treated with fertiliser and biosurfactant (0.55) was statistically significant ($p < 0.05$) when compared to the average BCF in the shoot of plants grown in samples treated with fertiliser and biosurfactant (0.37), the shoot of plants grown in samples treated exclusively with biosurfactant (0.31), and that of plants grown in the untreated control samples (0.21). The results also suggests that the average BCF in the roots of plants grown exclusively with fertiliser (0.5) was statistically significant ($p < 0.05$) when compared to that in the shoot of plants grown in samples treated with fertiliser and biosurfactant

(0.37), the shoot of plants grown in samples treated exclusively with biosurfactant (0.31), and the shoot of plants grown in the untreated control samples (0.21). Similarly, the results also revealed that the average BCF in the roots of plants grown exclusively with biosurfactant (0.47) was statistically significant ($p < 0.05$) when compared to the average BCF in the shoot of plants grown in samples treated with fertiliser and biosurfactant (0.37), the shoot of plants grown exclusively with biosurfactant (0.31), and in the shoot of plants grown in the untreated control samples (0.21).

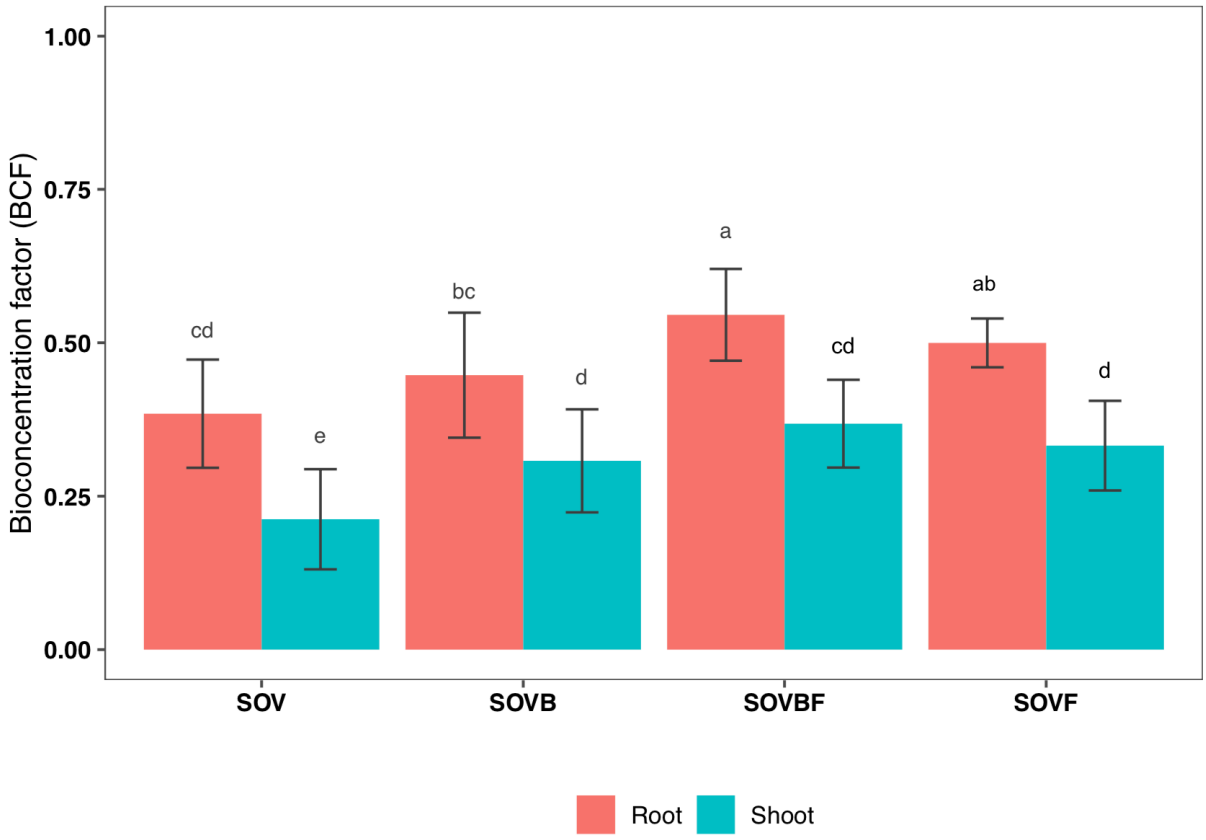



Figure 4.4 The bioconcentration factor (BCF) of total PAHs in plant tissues. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides*, SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*

### 4.3.6 Effect of soil treatments on Translocation factor (TF)

The effect of different soil treatments on the TF of PAHs in *C. zizanioides* was observed during our phytoremediation experiment. The results revealed a close merging among the TF in plants grown in samples amended with different treatments (Figure 4.5). The results suggest that the average TF in plants grown exclusively with biosurfactant (0.69) was statistically significant ($p < 0.05$) when compared to that of the untreated control samples (0.4). The results also indicated that the average TF in plants grown in samples treated with both fertiliser and biosurfactant (0.67) was statistically significant ($p < 0.05$) when compared to that of the plants grown in the untreated control samples (0.4). Similarly, there was a significant difference ($p < 0.05$) between the average TF in plants grown exclusively with fertiliser (0.66) and that of the plants grown in the untreated control samples (0.4).

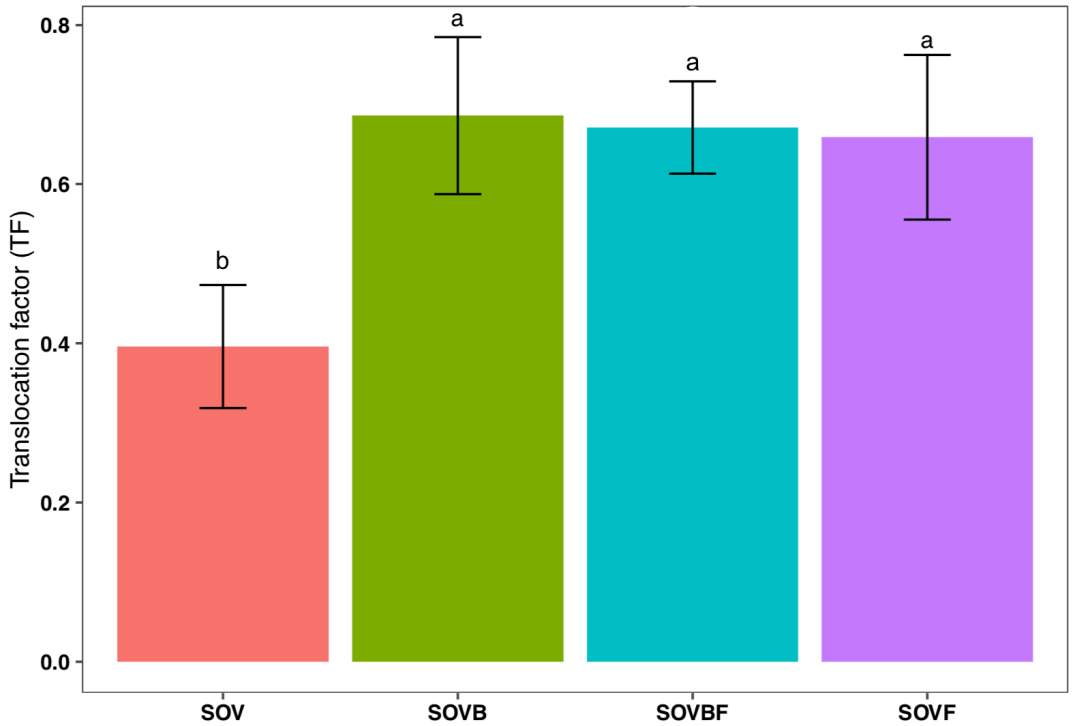



Figure 4.5 Translocation factor (TF) of total PAHs in *C. zizanioides*. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides*, SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*

## 4.4 Discussion

### 4.4.1 Efficiency of biosurfactants on TPH removal

Mild shaking was used to test the effectiveness of two biosurfactants namely 95% Di Rhamnolipid and 95% Mono Rhamnolipid in eliminating TPH from crude oil contaminated soil. Our results have indicated that both biosurfactants were effective in removing the TPH (Figure 4.1). The results suggest that there was no significant difference between the effect of 95% di Rhamnolipid, which removed 61% of TPH and that of 95% mono Rhamnolipid, which removed 53.1% of TPH. The effects of both the former and the latter were statistically significant when compared to the control samples treated exclusively with water, which removed 23.1%. This could be attributed to the high emulsifying effect of both biosurfactants. These results support the findings of Fanaei et al., (2020) on biosurfactant enhanced washing of oil contaminated soil, which removed 86% of TPH. Zhao et al., (2020), discovered that rhamnolipids produced from soya beans, which contained 9 mono- and 8 di-rhamnolipids, was effective at lowering oil viscosity. The conclusions from previous research have also revealed the positive effect of biosurfactants in treating petroleum hydrocarbon contaminated soils (Almansoory et al., 2015; Liduino et al., 2018; Wei et al., 2020). The results indicate that crude oil-contaminated soil amended with biosurfactant 95% di-rhamnolipid had the highest rate of TPH removal (61%). Thus, 95% di-Rhamnolipid was selected for this research to enhance phytoremediation of crude oil-contaminated soil using *C. zizanioides*.

### 4.4.2 Removal of PAHs from the soil

A greenhouse experiment was conducted to eliminate PAHs from a crude oil contaminated soil using *C. zizanioides* amended with various treatments. The results revealed that the LMW PAHs had the highest rate of PAH dissipation during the phytoremediation process (Figures 4.2 A). The

results suggest that the highest rate of all PAHs reductions occurred under the combined effect of fertiliser and biosurfactant (Figures 4.2 A, B, and C). This may have been caused by the effect of biosurfactant which improved the plant's utilisation of the fertiliser and the solubilisation, absorption, and dissipation of the PAHs. These results contradicts the findings of Bezza and Nkhalambayausi, (2016), who discovered a reduction in PAH breakdown after combining biosurfactant and nutrients ($NH_2NO_3$ and $KH_2PO_4$) during bioremediation of aged PAHs in contaminated soil. Our results also showed that the sole effect of biosurfactant has enhanced the removal of PAHs in the contaminated soil. This might have happened as a direct result of the biosurfactant, which helped make PAHs more soluble and easier for plants to absorb from the soil. This supports the findings of Lu et al., (2019), who discovered the efficiency of rhamnolipids on PAHs removal during the process of bioremediation. Sun et al., (2019), studied the effect of biosurfactant-producing *pseudomonas aeruginosa* S5 isolated from coking water on bioremediation of PAH and discovered the removal of the PAH was enhanced by the biosurfactant. Bezza and Nkhalambayausi-Chirwa, (2015), also discovered that application of biosurfactant produced by *Pseudomonas aeruginosa* strain increased the desorption and bioavailability for phenanthrene and pyrene, from 18% to 73% and 6% to 51%, respectively. Our findings also showed a significant reduction of PAHs in samples treated solely with fertiliser compared to untreated oil-contaminated soil vegetated with *C. zizanioides* and untreated and unvegetated oil-contaminated soil. This could be because of the effect of fertiliser, which increased the growth of the plant, allowing it to absorb contaminants from the soil. However, the level of PAHs removed from the soil treated with fertiliser alone was lower than that of the samples under the combined effect of fertiliser and biosurfactant and that of biosurfactant alone. This result is consistent with the findings of Brown et al., (2017), which revealed the lowest removal of PAHs through the addition of fertiliser alone during bioremediation of petroleum hydrocarbons. This present study has demonstrated that there is a synergy between the combined effect of fertiliser and biosurfactant and the ability of *C. zizanioides* to remove PAHs from crude oil contaminated soils during the process of phytoremediation.

### 4.4.3 Accumulation of PAHs in tissues of *C. zizanioides*

The build-up of PAHs in the tissues of *C. zizanioides* was investigated considering public concerns about the generation of toxic contamination of the food web. We investigated how different soil amendments might affect the accumulation of PAHs in plant tissues during phytoremediation of crude oil contaminated soil. Our findings showed that high levels of PAHs had accumulated within the tissues of the plants grown in samples amended with fertiliser and biosurfactant during the greenhouse experiment (Figure 4.3) and (Table 4.3). This could be attributed to the role of biosurfactant in solubilising the PAHs, which aided their absorption and accumulation by *C. zizanioides*. This result is supported by the findings of Zhu and Zhang., (2008) on the effect of rhamnolipids on PAH uptake by ryegrass, which revealed increased PAH uptake by the plant. Previous research has also drawn conclusions that adding biosurfactants to contaminated soils during phytoremediation improves PAH uptake by plants (Gao et al., 2008; Xiao et al., 2012; Liao et al., 2016). This present study has demonstrated that the combined effect of fertiliser and biosurfactant during the greenhouse experiment caused a substantial accumulation of PAHs within plant tissues. This may be related to the nutrients provided by fertiliser application, which promoted plant growth, and the impact of biosurfactants, which facilitated the solubilisation and bioaccumulation of more individual and total PAHs within the tissues of the plants. The findings of our research showed that accumulation of individual and total PAHs in the roots of *C. zizanioides* was significantly higher than in the shoots. This could be attributed to the high sorption of the PAHs in the roots of the plant during the process of phytoremediation. This supports the findings of Sushkova et al., (2020), which revealed more accumulation of PAH in the roots of *Phragmites australis* in a long term chemical contamination. This present research showed that the concentration of total PAHs in plant tissues was lower than that of the crude oil-contaminated soil. This could have resulted from the high sorption of the PAHs to SOM in the crude oil contaminated soil. This current study also found that the accumulation of total PAHs in plant tissues was proportional to their concentration in crude oil-contaminated soil. A few LMW compounds, including Acenaphthylene, Acenaphthene, and Fluorene, were not found in the shoots of plants grown in all

samples. The MMW compound Benzo[a]anthracene was not present in all samples. Benzo[k]fluoranthene, which is an HMW compound, was also absent in plant shoots grown solely with fertiliser and in untreated control samples. Furthermore, the HMW compound benzo[a]pyrene was not present in untreated control plant shoots. The absence of these LMW compounds in the plant shoots could have been caused by volatilisation or their quantity being below the instrument detection limit. While the absence of MMW and HMW compounds could have been caused by poor transpiration of the compounds within the plants due to their insolubility and low bioavailability.

### 4.4.4 Uptake and translocation of PAHs in *C. zizanioides*

The ability of *C. zizanioides* to extract PAHs from crude oil contaminated soil during phytoremediation was tested using BCF and TF. Furthermore, we investigated how different soil modifications could affect the BCF and TF in plants. The results revealed that the levels of BCF in all samples ranged from 0.21 to 0.55, which was generally low. This could have happened because of low transfer of PAHs from the roots of the plants to the shoots. This result is supported by the findings of Zheng et al., (2021), who studied the *in-situ* phytoremediation of PAH contaminated soil and discovered that PAHs remained in the roots of *Libanotis seseloides* than being transferred to the shoots. Our results also revealed higher BCF of PAHs in the roots of *C. zizanioides* than in shoots of plants grown in all treatments. This could have happened due to the high sorption of PAHs within the plant roots. This result agrees with findings of Košnář et al., (2020), who studied the phytoremediation of PAH-contaminated soil using *Salix smithiana Willd.* and discovered lower BCF of individual PAHs in the shoots of the plants than in the roots. Zheng et al., (2020), studied the microbe-plant co-remediation of PAHs- dichloro-diphenyl-trichloroethane (DDTs) contaminated agricultural soil and discovered that the PAHs in the roots was higher than in shoots of *Brassica rapa.* Fismes et al., (2002), studied root-to-shoot transfer of PAHs and discovered low concentration and BCF of PAHs in vegetables grown on industrial contaminated soils. This present research revealed the highest level of BCF was discovered within the plants grown in samples

treated with a combination of fertiliser and biosurfactant. This may be related to the effects of fertiliser, which promoted the development of roots in *C. zizanioides*, and biosurfactant, which made PAHs more soluble and enhanced the plant's ability to absorb them through its roots and transfer them to the shoots. This result suggests a synergy between the effects of fertiliser and biosurfactant, the growth of *C. zizanioides,* and the BCF of PAHs from crude oil-contaminated soil.

In terms of TF, the results revealed a close merging between the levels of TF in plants grown in all treatments. Interestingly, the TF in all samples was significantly higher when compared to the TF in plants grown in the untreated control samples. The findings from previous research have indicated the effect of biosurfactants and increased microbial quantity on increasing the solubility and absorption of organic contaminants by plants  Liao et al., 2016; Ali Khan et al., 2017; Liduino et al., 2018; Meglouli et al., 2019). This current study has demonstrated that fertiliser application can potentially increase TF in *C. zizanioides* during phytoremediation of crude oil contaminated soil. This was most likely due to increased root growth, which increased microbial quantity and potential activity in the rhizosphere, resulting in increased PAH absorption. The results on the growth of the plants are discussed in (section 6.3.3). The current study also found that BCF and TF levels in *C. zizanioides* increased after the application of biosurfactant and a fertiliser-biosurfactant mixture during phytoremediation of crude oil contaminated soil. However, BCF levels were found to be generally low in the plants, which could be attributed to the low solubility of petroleum hydrocarbons.

## 4.5 Conclusion

The findings of this research have demonstrated that phytoremediation of crude oil contaminated soil using *C. zizanioides* can be enhanced through the combined effect of fertiliser and biosurfactant. Our findings showed that the biosurfactant 95% Di Rhamnolipid was successful in eliminating 61% of TPH from the soil, which led to its selection for the phytoremediation of soil

contaminated with crude oil. Our results, together with other studies, have suggested that the application of biosurfactant was effective in aiding the dissipation of PAHs during phytoremediation of crude oil contaminated soil. This could be because of biosurfactant's effect on PAH solubility and bioavailability, which may have enhanced their absorption by *C. zizanioides* and subsequent dissipation. The high rate of dissipation observed in samples treated with a combination of fertiliser and biosurfactant could be attributed to the nutrients provided by fertiliser application, which aided plant root growth and promoted microbial development, as well as the effect of the biosurfactant, which aided PAH solubility, bioavailability, and dissipation. Our findings, along with those of other studies, show that using fertiliser exclusively improved the removal of PAHs from petroleum-contaminated soil. This could be because fertiliser increased plant growth and allowed it to absorb contaminants from the soil. It appears that using only fertiliser to facilitate the removal of PAHs from oil-contaminated soil is more cost-effective. However, the level of PAHs removed from soil treated with fertiliser alone was lower than in samples treated with a combination of fertiliser and biosurfactant alone or biosurfactant alone. Our findings have also revealed that the combined effect of fertiliser and biosurfactant have promoted PAH bioaccumulation in the tissues of *C. zizanioides* during phytoremediation. Additionally, the concentration PAH in the soil was found to be significantly higher than the PAH accumulated in the tissues of the plant. Our result suggests that the uptake of petroleum hydrocarbons by the plant could have facilitated their removal from the soil. According to our findings, the soils treated with fertiliser alone removed the least quantity of PAHs among the various soil amendments utilised. However, the application of fertiliser alone is the most cost-efficient way of aiding the removal of PAHs from the soil. Our findings suggest that amending crude oil-contaminated soil with a combination of fertiliser and biosurfactant may potentially aid the dissipation of PAHs from the soil.

# CHAPTER FIVE

# FATE OF PETROLEUM HYDROCARBONS IN THE SOIL

## 5.0. ROLE OF *C. ZIZANIOIDES* IN IMMOBILISING PETROLEUM CONTAMINANTS IN THE SOIL

**Abstract**

Polycyclic aromatic hydrocarbons form a fraction of petroleum contaminants that persist in the environment with a potential human carcinogenic effect. There is a growing concern over the possible spread and migration of these pollutants within ecosystems. We investigated the sorption of petroleum hydrocarbons by soil organic matter (SOM), particularly to immobilise the HMW PAHs in the soil during phytoremediation of soil contaminated with crude oil. We also investigated the effect of fertiliser and biosurfactant on the formation of soil organic matter (SOM) through the biomass increase of *C. zizanioides* during phytoremediation of crude oil-contaminated soil. The results suggest that applying fertiliser and biosurfactant has increased the formation of SOM through enhanced growth of *C. zizanioides*, leaf litter, and root turnover during phytoremediation of crude oil-contaminated soil. The effects of fertiliser alone and a combination of fertiliser and biosurfactant have increased the level of SOM by 23.5% and 21.8%, respectively. The increased level of SOM resulted in a significant decrease in the concentrations of HMW PAHs in samples under the combined effect of fertiliser and biosurfactant compared to the samples treated with fertiliser alone and biosurfactant alone, the untreated samples vegetated with *C. zizanioides*, and the untreated and unvegetated control samples. The effects of fertiliser application, which promoted the growth of *C. zizanioides* and root turnover, which in turn increased the level of SOM, along with the impact of biosurfactant, which solubilized the pollutants for easy plant absorption, may have facilitated the sorption, immobilisation, and degradation of the HMW PAHs in the soil. These findings point to a synergy between fertiliser and biosurfactant applications on the growth of *C. zizanioides* and root turnover that aided the sorption and immobilisation of the HMW PAHs.

## 5.1 Introduction

Environmental pollution often occurs through spills as a result of human anthropogenic activities such as the exploration and exploitation of petroleum oil that can potentially affect human health and the surrounding environment (Brown and Eyenghe, 2015). Environmental pollution is said to be the major cause of many illnesses and accounted for an estimated mortality rate of 9 million people across the globe in 2015 (Landrigan et al., 2018). The World Health Organisation (WHO) has also reported that an estimated mortality rate of 12.6 people occurred globally in the year 2012 mainly due human exposure to contaminated environments (WHO, 2016). This is as a result of the distribution of contaminants such as petroleum hydrocarbons in the environment through evaporation, dispersion and microbial degradation (CRAM et al., 2004).

The composition, toxicity, bioavailability, and distribution of petroleum in the soil normally changes due to the physiochemical and biological processes known as weathering including volatilisation, sorption, biodegradation, and dissolution. These processes are usually affected by factors such as soil texture, moisture, organic matter, temperature and rainfall (Fine et al., 1997; Reid et al., 2000). The sorption of organic contaminants in the soil is mainly controlled by soil organic matter (SOM) such as decayed plants (Pignatello, 1999; Gunasekara and Xing, 2003; Cornelissen et al., 2005). A large quantity of the non-volatile portion of petroleum spilt into the soil is absorbed by organic matter which affects their distribution, bioaccumulation, bioavailability and rate of mass transfer for biodegradation (Lai et al., 2009). The dissolution of petroleum contaminants in the soil can be described as the process of weathering where a portion of petroleum hydrocarbons dissolves and infiltrates into the groundwater (Nadim et al., 2000b). These petroleum hydrocarbons are referred to as the non-aqueous phase liquids (NAPL) that are normally not readily soluble in water (Youdeowei, 2012). Migration of NAPL in saturated zones of groundwater usually occur vertically due to the gravitational and capillary forces based on the solubility of the NAPL (Müller and Sedláčková, 2003; Sun et al., 2019). Consequently, this poses threat to human health due to exposure to toxic substances in the environment primarily through ingestion, inhalation and skin

contact often leading to respiratory/cardiopulmonary diseases, mental disorder and cancer (Rodríguez-Trigo et al., 2007; Goldstein et al., 2011).

There are a lot of public concerns over the presence of polycyclic aromatic hydrocarbons (PAHs) in soil and groundwater because of their potential carcinogenic effect (Nganje et al., 2007; Liu et al., 2015; Ololade et al., 2021). The United States Environmental Protection Agency (US EPA) has considered 16 PAHs as priority pollutants based on available information, toxicity, and human exposure (Bojes and Pope, 2007). These PAHs are characterised by low solubility, high molecular weight, low bioavailability, and several aromatic rings (Johnsen et al., 2005). Seven out of the 16 EPA PAHs, including Benzo [a] anthracene, Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]chroysene, Dibenzo[a, h]anthracene, and indeno[1,2,3,c]pyrene, are classified as potential human carcinogens (Rengarajan et al., 2015). Hence, their presence and persistence in the soil and migration to groundwater cause a threat to human health and the surrounding environment.

Soil remediation is carried out to address the problem of pollution by reducing, immobilising or removing contaminants from the environment (Fawzy, 2008; Schwitzguébel et al., 2011; Novo et al., 2018). The need for remediation is often determined by the potential risks posed by contaminants that can affect the soil and groundwater (Catherine et al., 2000; Iturbe et al., 2005). However, most of the conventional soil remediation techniques seem to be unsustainable due to their high cost and harmful effects on the environment (Batty and Dolan, 2013*;* Novo et al., 2018; Bernardino et al., 2020). Hence, scientists are exploring phytoremediation a technique that involves the use of certain plant species to restore the environment (Mench et al., 2009). This technique also serves as an alternative to the challenges mentioned above as it is inexpensive, treatment of contaminants occurs *in situ*, and there is no use of thermal energy and harmful chemicals such as strong oxidising or reducing agents.

Phytoremediation generally involves growing plants in areas affected by contamination to immobilise or remove the contaminants and restore vegetation in the affected areas. The plants

normally immobilise, bioaccumulate and degrade soil contaminants in the rhizosphere which prevents the migration of contaminants into the atmosphere and groundwater. However, the use of non-invasive plant species such as *C. zizanioides* (Vetiver grass) with promising capabilities to immobilise and extract contaminants within the soil and groundwater is inadequately elucidated. *C. zizanioides* can utilize leachates at the lower depths of the soil due to its long fibrous roots that grows deep down into the depth of the soil (Lavania, 2003). It is also capable of surviving in all soil types, tolerant to extreme temperatures, and survives under flood and drought (Lavania, 2004; Srivastava et al., 2008; Danh et al., 2009). These outstanding features makes *C. zizanioides* a suitable plant candidate for the purpose of phytoremediation of crude oil contaminated soil under the influence of fertilizer and biosurfactants.

Vetiver grass is a quick growing perennial plant that originates from India and belongs to the family Poaceae, subfamily of Panicoideae, tribe of Andropogonae and subtribe of Sorghina (Danh et al., 2009 Chou et al., 2016). The plant was recently reclassified and it is now referred to as *C. zizanioides* (L.) Roberty (Srivastava et al., 2008 Danh et al., 2009). The plant has ten different existing species, but *C. zizanioides* is the most widely recognised among other species (Maffei, 2003). It grows to a height of about 2 to 3 meters having vertically straight stems with long cluster branches (Maffei, 2003; Smitha et al., 2014; Chou et al., 2016). The plant has a tough and fibrous root system with long structures that grows from 3 meters into the depth of the soil (Tscherning et al., 1995). This robust structure of the root system allows the plant to deeply penetrate into the soil and utilize the soil moisture at great depths for survival under harsh environmental conditions such as drought (Danh et al., 2009; Suelee et al., 2017). It also enables the plant to have great contact with the soil thereby ensuring effective phytoremediation and preventing contaminants from leaching into ground water (Danh et al., 2009). By and large the unique root system of *C. zizanioides* also enables it to be suitable for water conservation and waste water treatment a technology that became prominent in the 1980s (Danh et al., 2009; Chou et al., 2016; Suelee et al., 2017). Therefore, the plant *C. zizanioides* can be considered a most suitable plant candidate for the purpose of immobilizing and removing contaminants from the soil.

Phytoremediation is limited by certain factors including the toxicity and low solubility of petroleum contaminants which affect the growth of plants and bioavailability of petroleum hydrocarbons (Xia et al., 2014; Lopes et al., 2009; Rusin et al., 2015; Chaudhary and Kim, 2019). However, the growth of plants, and the solubility and absorption of petroleum hydrocarbons can be improved through application of fertilisers and biosurfactants, respectively, during the process of phytoremediation (Das and Chandran, 2011; Xia et al., 2014; Chen et al., 2015; Kumar and Nikhil, 2016). Consequently, the improved growth of plants from the effect of fertiliser application could potentially address the above-mentioned issue of petroleum contaminants leaching into groundwater through the production of SOM from leaf litter and root turnover. Existing literature has demonstrated that greater plant growth improves the development of SOM and aggregate stability through the yield of roots and plant litter (Cotrufo et al., 2015; Cotrufo et al., 2015; Wei et al., 2016; Hudek et al., 2017). Hence, there is a need to investigate the effect of fertiliser and biosurfactant on improving the formation of SOM and soil aggregate stability through enhanced growth of *C. zizanioides* for the immobilisation of petroleum contaminants in the soil, which has yet to be fully elucidated.

The aim of this research is to investigate the role of *C. zizanioides* in immobilising petroleum hydrocarbons during phytoremediation of crude oil contaminated soil. The objectives of the research are (i) determination of the combined effect of fertiliser and biosurfactant on the growth of *C. zizanioides* and the formation of soil organic matter (SOM) during phytoremediation (ii) determination of the ability of *C. zizanioides* to immobilise petroleum hydrocarbons through sorption by SOM formed during the process of phytoremediation and (iii) determination of the ability of *C. zizanioides* contain petroleum hydrocarbons through soil compaction and aggregate stability.

## 5.2 Materials and Methods

### 5.2.1 Sample preparation and analysis

The preparation and analysis of samples including soil properties, soil spiking, preparation and transplantation of plants, experimental set-up, application of biosurfactant and irrigation are explained in detail in sections 3.2, 3.3, 3.4, 3.5 and 3.6.

### 5.2.2 Determination of SOM

The SOM was determined using the loss on ignition method described by Heiri et al (2001) as previously explained in section 3.2.4.

### 5.2.3 Determination of PAHs leaching

The leaching of PAHs from crude oil-contaminated soils was analysed by the column leaching test method adapted by Reemtsma and Mehrtens, (1997). 300g of freeze-dried soil was placed into a glass filtration funnel attached to a column of 20cm in length and 5cm in inner diameter. The column was covered with glass wool at the bottom to prevent soil particles from passing into the leachate (Zand et al., 2010). The soil was percolated at room temperature with 1000ml of deionised water with the aid of a peristaltic pump attached to the filtration funnel for 24 hours. The sample was filtered through a $Na_2SO_4$ florisil Solid Phase Extraction (SPE) tube and the concentration of PAHs in samples was analysed using Agilent GC (6890N) coupled with MS (5973) as previously described in section 3.7.

### 5.2.4 Determination of soil aggregate water stability

The water stability of soil aggregates formed by the roots of *C. zizanioides* during phytoremediation was determined using a method adapted from Graf and Frei, (2013). Soil aggregates were separately placed on 20 mm sieve and placed in deionised water for 10 mins before draining the water. Then the roots of the plants were carefully removed and the remaining soil on the sieve as well as the 20 mm sieved soil were oven dried at 105$^{o}$C for 24 hours. The aggregate stability (AS) was then calculated using the formula described by Aksakal et al., (2020) as shown below:

$$\text{AS}\ (\%) = \frac{(\text{Weight of Aggregates} + \text{Sand}) - \text{Weight of Sand})}{\text{Weight of sample} - \text{Weight of Sand}}\ \text{X}\ 100$$

Equation (10). Aggregate stability

### 5.2.5 Statistical analysis

The statistical analysis was carried out using R studio version 1.2.5033 for Mac OSX as previously explained in detail in section 3.9. ANOVA test of variance was used to examine significant differences in the mean values of SOM, concentrations of PAHs from leachate, and soil aggregate stability. A Tukey post-hoc test was used to determine if there was a significant difference between the variables ($p < 0.05$).

## 5.3 Result

### 5.3.1 Soil properties

Prior to the start of the experiment, different soil parameters were analysed, as explained in detail in section 3.2. The results revealed that the soil has electric conductivity of 0.44 ± 0.01 (mS/cm),

a moisture content of 2.74 ± 0.15 (%), an average pH level of 7.4 ± 0.06, and an organic matter content of 1.18 ± 0.04 (%).

### 5.3.2 Formation of SOM

The formation of SOM during phytoremediation of crude oil contaminated soil using *C. zizanioides* was influenced by various soil treatments. Our findings revealed that the SOM produced in samples treated solely with a fertiliser dose was significantly increased ($p < 0.05$) by 23.5% from the starting concentration of 1.18% when compared to SOM in samples treated solely with biosurfactant, which increased by 17.6%, and the untreated, unvegetated control samples, by 6.7% (Figure 5.1). However, there was no significant difference ($p > 0.05$) between the SOM in samples treated solely with fertiliser which increased by 23.5% and that of the samples treated with a combination of fertiliser and biosurfactant by 21.8%, and in the untreated control samples vegetated with *C. zizanioides* by 20.2%. Interestingly, the SOM in samples treated with a combination of fertiliser and biosurfactant which increased by 21.8% was also statistically significant ($p < 0.05$) when compared to that in the samples treated solely with biosurfactant by 17.6%, and the untreated, unvegetated control samples by 6.7%. However, there was no significant difference between the SOM in samples treated with a combination of fertiliser and biosurfactant which increased by 21.8% and that in the untreated control samples vegetated with *C. zizanioides* by 20.2%. We also observed the SOM in the untreated control samples vegetated with *C. zizanioides* which increased by 20.2% was statistically significant ($p < 0.05$) when compared to that in the untreated, unvegetated control sample, which increased by 6.7%.

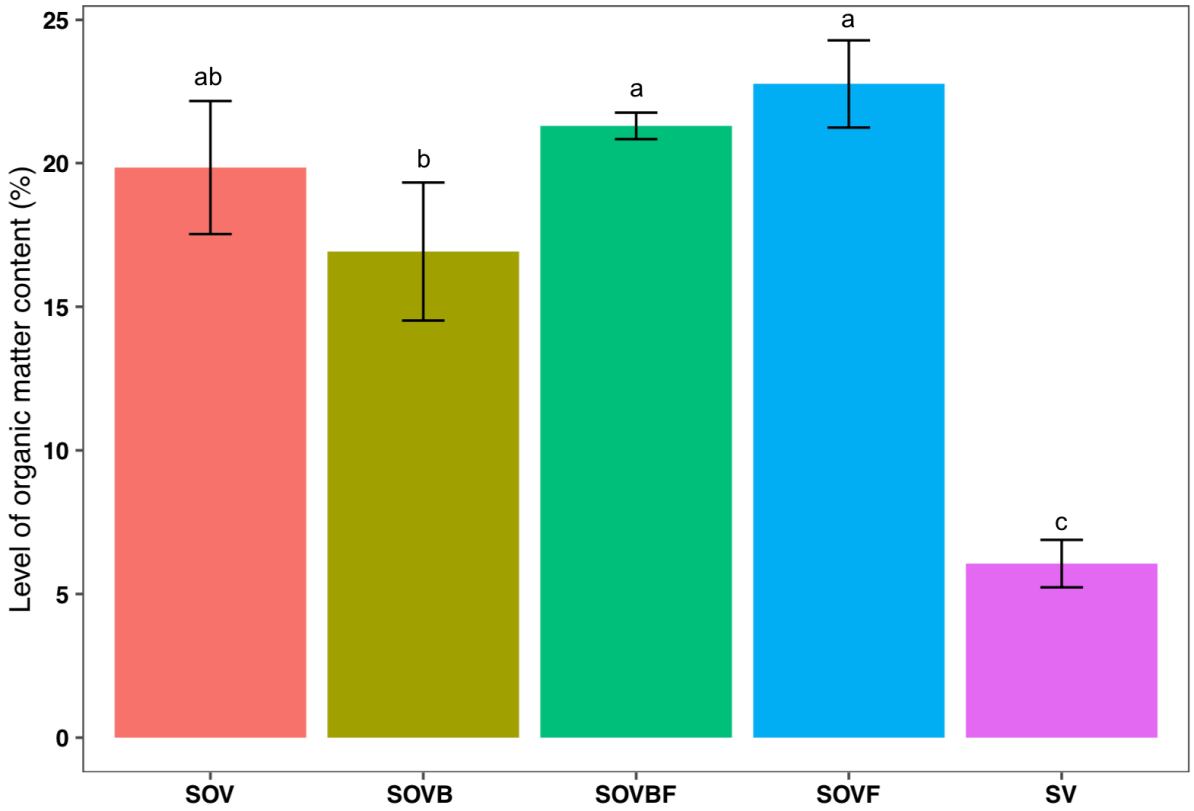



Figure 5.1 Effect of various soil amendments on the level of organic matter produced by *C. zizanioides* during phytoremediation of crude oil contaminated after 72 days. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil *C. zizanioides* only.

### 5.3.3 Concentration of PAHs from leachates

The concentrations of the 7 HMW PAHs namely Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene and Dibenzo[a,h]anthracene from the leachate of crude oil contaminated soils were generally affected

by different soil treatments on SOM formation. According to our findings, the concentration of Benzo[a]anthracene under the combined effect of fertiliser and biosurfactant, which was 55.2 µg/L was significantly lower ($p < 0.05$) than that in samples treated solely with fertiliser 80.7 µg/L, samples treated solely with biosurfactant 85.5 µg/L, the untreated control samples vegetated with *C. zizanioides* 89.5 µg/L, and 96 µg/L in the untreated and unvegetated control samples (Table 5.1). Also, the concentration of Benzo[a]anthracene in samples treated exclusively with fertiliser 80.7 µg/L was significantly lower ($p < 0.05$) than that in the samples treated solely with biosurfactant 85.5 µg/L, the untreated control samples vegetated with *C. zizanioides* 89.5 µg/L, and 96 µg/L in untreated and unvegetated control samples. The results also showed that the concentration of Benzo[a]anthracene 85.5 µg/L in samples treated solely with biosurfactant was significantly lower ($p < 0.05$) than that in the untreated and unvegetated control samples 96 µg/L.

Our findings also revealed that the concentration of Chrysene under the combined effect of fertiliser and biosurfactant, which was 228.3 µg/L was significantly lower ($p < 0.05$) than that in samples treated solely with fertiliser 267.7 µg/L, the untreated control samples vegetated with *C. zizanioides* 284.3 µg/L, and 407.5 µg/L in untreated and unvegetated control samples (Table 5.1). The concentration of Chrysene, which was 267.7 µg/L in samples treated solely with fertiliser was also significantly lower ($p < 0.05$) when compared to that in the untreated control samples vegetated with *C. zizanioides* 284.3 µg/L, and the untreated and unvegetated control samples, which was 407.5 µg/L. Interestingly, the concentration of Chrysene in samples treated solely with biosurfactant, which was 226.2 µg/L was significantly lower ($p < 0.05$) than that in samples treated solely with fertiliser, which was 267.7 µg/L, the untreated control samples vegetated with *C. zizanioides* 284.3 µg/L, and the untreated and unvegetated control samples, which was 407.5 µg/L. Also, the concentration of Chrysene in the untreated control samples vegetated with *C. zizanioides*, which was 284.3 µg/L was found to be significantly lower ($p < 0.05$) when compared to that in the untreated and unvegetated control samples 407.5 µg/L.

The results also indicated that the concentration of Benzo[b]fluoranthene under the combined effect of fertiliser and biosurfactant 82.2 µg/L was significantly lower ($p < 0.05$) than that of the samples treated solely with fertiliser 115.4 µg/L, the samples treated solely with biosurfactant 103.9

µg/L, the untreated control samples vegetated with *C. zizanioides* 119.7 µg/L, and 142.1 µg/L in the untreated and unvegetated control samples (Table 5.1). The concentration of Benzo[b]fluoranthene, which was 103.9 µg/L in samples treated solely with biosurfactant was also found to be significantly lower ($p < 0.05$) than that of the samples treated solely with fertiliser 115.4 µg/L, the untreated control samples vegetated with *C. zizanioides* 119.7 µg/L, and the untreated and unvegetated control samples 142.1 µg/L. Furthermore, the concentration of Benzo[b]fluoranthene 115.4 µg/L in samples treated solely with fertiliser was found to be significantly lower ($p < 0.05$) than that of the untreated and unvegetated control samples 142.1 µg/L. Likewise, the concentration of Benzo[b]fluoranthene 119.7 µg/L in the untreated control samples vegetated with *C. zizanioides* was significantly lower ($p < 0.05$) than that of the untreated and unvegetated control samples 142.1 µg/L.

Our findings also revealed that the concentration of Benzo[a]pyrene in samples treated with a combination of effect of fertiliser and biosurfactant, which was 58.1 µg/L was significantly lower ($p < 0.05$) when compared to that of the samples treated exclusively with fertiliser 67.9 µg/L, the samples treated exclusively with biosurfactant 68.5 µg/L, the untreated control samples vegetated with *C. zizanioides* 81.1 µg/L, and 87.5 µg/L in the untreated and unvegetated control samples (Table 5.1). The results also showed that the concentration of Benzo[a]pyrene 67.9 µg/L in samples treated exclusively with fertiliser was significantly lower ($p < 0.05$) compared to that of the untreated control samples vegetated with *C. zizanioides* 81.1 µg/L, and that of the untreated and unvegetated control samples 87.5 µg/L. Furthermore, the concentration of Benzo[a]pyren in samples treated exclusively with biosurfactant, which was 68.5 µg/L was significantly lower ($p < 0.05$) compared to that in the untreated control samples vegetated with *C. zizanioides* 81.1 µg/L, and the untreated and unvegetated control samples 87.5 µg/L. The results also showed that the concentration of Benzo[a]pyrene, which was 81.1 µg/L in the untreated control samples vegetated with *C. zizanioides* was significantly lower ($p < 0.05$) when compared to that of the untreated and unvegetated control samples 87.5 µg/L.

Our findings also revealed that the concentration of Indeno[1,2,3-cd]pyrene, which was 50.7 µg/L in samples treated exclusively with biosurfactant was significantly lower ($p < 0.05$) compared to

that in samples under the combined effect of fertiliser and biosurfactant 61.8 µg/L, the samples treated exclusively with fertiliser 66.7 µg/L, the untreated control samples vegetated with *C. zizanioides* 74.6 µg/L and the untreated and unvegetated control samples 104.5 µg/L. The results also showed that the concentration of Indeno[1,2,3-cd]pyrene 61.8 µg/L in samples under the combined effect of fertiliser and biosurfactant was significantly lower ($p < 0.05$) compared to that in the untreated control samples vegetated with *C. zizanioides* 74.6 µg/L and the untreated and unvegetated control samples 104.5 µg/L. Interestingly, the concentration of Indeno[1,2,3-cd]pyrene, which was 66.7 µg/L in samples treated exclusively with fertiliser was also found to be significantly lower ($p < 0.05$) compared to that in the untreated control samples vegetated with *C. zizanioides* 74.6 µg/L and the untreated and unvegetated control samples 104.5 µg/L. The result also showed that concentration of Indeno[1,2,3-cd]pyrene 74.6 µg/L in the untreated control samples vegetated with *C. zizanioides* was significantly lower ($p < 0.05$) than that of the untreated and unvegetated control samples 104.5 µg/L.

Our results also revealed that the concentration of Dibenzo[a,h]anthracene, which was 54.3 µg/L in samples under the combined effect of fertiliser and biosurfactant was significantly lower ($p < 0.05$) compared to that of the samples under the exclusive effect of biosurfactant 63.1 µg/L, the samples treated exclusively with fertiliser 77.4 µg/L, the untreated and unvegetated control samples 77.8 µg/L and the untreated control samples vegetated with *C. zizanioides* 85.6 µg/L. The results also indicated that the concentration of Dibenzo[a,h]anthracene 63.1 µg/L in samples treated exclusively with biosurfactant was significantly lower compared to that of the samples treated exclusively with fertiliser 77.4 µg/L, the untreated and unvegetated control samples 77.8 and the untreated control samples vegetated with *C. zizanioides* 85.6 µg/L. Furthermore, the results revealed that the concentration of Dibenzo[a,h]anthracene, which was 77.4 µg/L under the exclusive influence of fertiliser was significantly lower compared to that of the untreated control samples vegetated with *C. zizanioides* 85.6 µg/L.

Table 5.1 Level of concentration of PAHs from leachate test after 32 weeks of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters represent the significant difference between variables, ($p \leq 0.05$). The (+) sign in the content of treatments indicates presence while the (–) sign indicates absence.

| Compound | Concentration (µg/L) | Treatment | Crude oil | *C. zizanioides* | Biosurfactant | Fertiliser |
|---|---|---|---|---|---|---|
| Benzo[a]anthracene | 55.2 ± 1.4$^{d}$ | SOVBF | + | + | + | + |
| | 80.7 ± 2.2$^{c}$ | SOVF | + | + | - | + |
| | 85.5 ± 2.9$^{b}$ | SOVB | + | + | + | - |
| | 89.5 ± 1.6$^{b}$ | SOV | + | + | - | - |
| | 96.0 ± 2.5$^{a}$ | SO (control) | + | - | - | - |
| Chrysene | 228.3 ± 4.1$^{d}$ | SOVBF | + | + | + | + |
| | 267.7 ± 2.1$^{c}$ | SOVF | + | + | - | + |
| | 226.2 ± 1.6$^{d}$ | SOVB | + | + | + | - |
| | 284.3 ± 1.7$^{b}$ | SOV | + | + | - | - |
| | 407.5 ± 1.6$^{a}$ | SO (control) | + | - | - | - |
| Benzo[b]fluoranthene | 82.2 ± 4.5$^{d}$ | SOVBF | + | + | + | + |
| | 115.4 ± 4.0$^{b}$ | SOVF | + | + | - | + |
| | 103.9 ± 4.2$^{c}$ | SOVB | + | + | + | - |
| | 119.7 ± 2.9$^{b}$ | SOV | + | + | - | - |
| | 142.1 ± 1.6$^{a}$ | SO (control) | + | - | - | - |
| Benzo[k]fluoranthene | 67.7 ±2.2$^{ab}$ | SOVBF | + | + | + | + |
| | 64.1 ± 1.0$^{b}$ | SOVF | + | + | - | + |
| | 64.6 ± 3.8$^{b}$ | SOVB | + | + | + | - |
| | 71.5 ± 4.2$^{a}$ | SOV | + | + | - | - |
| | 73.5 ± 2.9$^{a}$ | SO (control) | + | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Benzo[a]pyrene | 58.1 ± 2.1[d] | SOVBF | + | + | + | + |
| | 67.9 ± 2.4[c] | SOVF | + | + | - | + |
| | 68.5 ± 4.2[c] | SOVB | + | + | + | - |
| | 81.1 ± 1.4[b] | SOV | + | + | - | - |
| | 87.5 ± 3.1[a] | SO (control) | + | - | - | - |
| Indeno[1,2,3-cd]pyrene | 61.8 ± 2.0[c] | SOVBF | + | + | + | + |
| | 66.7 ± 2.6[c] | SOVF | + | + | - | + |
| | 50.7 ± 2.2[d] | SOVB | + | + | + | - |
| | 74.6 ± 2.1[b] | SOV | + | + | - | - |
| | 104.5 ± 4.9[a] | SO (control) | + | - | - | - |
| Dibenzo[a,h]anthracene | 54.3 ± 1.8[d] | SOVBF | + | + | + | + |
| | 77.4 ± 1.2[b] | SOVF | + | + | - | + |
| | 63.1 ± 2.9[c] | SOVB | + | + | + | - |
| | 85.6 ± 3.5[a] | SOV | + | + | - | - |
| | 77.8 ± 2.8[b] | SO (control) | + | - | - | - |

### 5.3.4 Growth of roots and soil aggregate formation

The growth of roots of *C. zizanioides* under various soil amendments have impacted the formation of soil aggregates after 32 weeks of phytoremediation. The findings from our research have shown that the formation of the soil aggregates was aided by the growth of the roots of the plants (Figure 5.2). The highest amount of soil aggregates formed were observed in samples treated exclusively with fertiliser and the samples treated with a combination of fertiliser and biosurfactants. The results revealed that the soil aggregates formed in samples treated exclusively with fertiliser (A) was significantly higher than the soil aggregates formed in the untreated contaminated soil (C), samples treated exclusively with biosurfactant (D), and the untreated and uncontaminated samples (E). However, the soil aggregates formed in samples treated exclusively with fertiliser (A) was not greatly higher than the treated with a combination of fertiliser and biosurfactant (B). The results also revealed that the aggregates of soil formed under the combined effect of fertiliser and biosurfactant (B) was significantly higher than the soil aggregates formed in the untreated contaminated soil (C), samples treated exclusively with biosurfactant (D), and the untreated and uncontaminated samples (E). Furthermore, the results showed that the aggregates of soil formed in the untreated contaminated soil (C) was more than the soil aggregates in samples treated exclusively with biosurfactant (D), and the untreated and uncontaminated samples (E). The results also indicated that the aggregates of soil formed in samples treated exclusively with biosurfactant (D), was more than the soil aggregates in the untreated and uncontaminated samples (E).




Figure 5.2 Soil aggregates compacted by roots of *C. zizanioides* in different soil treatments after 32 weeks of phytoremediation. The arrows in (a,b,c and d) show aggregates of soil compacted by the roots of the plant. The arrow in (e, control), indicates reduced growth of roots with no soil aggregate formed.

### 5.3.5 Soil aggregate stability

The effect of various soil treatments on aggregate formation has affected soil aggregate stability. Our results have shown that the highest aggregate stability occurred in samples treated exclusively with fertiliser (Figure 5.3). The findings have indicated that the aggregate stability in samples treated solely with fertiliser (64.5 %) was significantly higher ($p < 0.05$) when compared to that of the samples treated with a combination of fertiliser and biosurfactant (60.3 %), the untreated samples vegetated with *C. zizanioides* (24.9 %), and the samples treated exclusively with biosurfactant (6.5 %). The results also showed that the aggregate stability (60.3 %) in samples

treated with a combination of fertiliser and biosurfactant was statistically significant ($p < 0.05$) compared to that of the untreated samples vegetated with *C. zizanioides,* (24.9 %), and the samples treated exclusively with biosurfactant, (6.5 %). The results also indicated that the aggregate stability, (24.9 %) in untreated samples vegetated with *C. zizanioides* was significantly higher ($p < 0.05$) that that of the samples treated exclusively with biosurfactant (6.5 %).

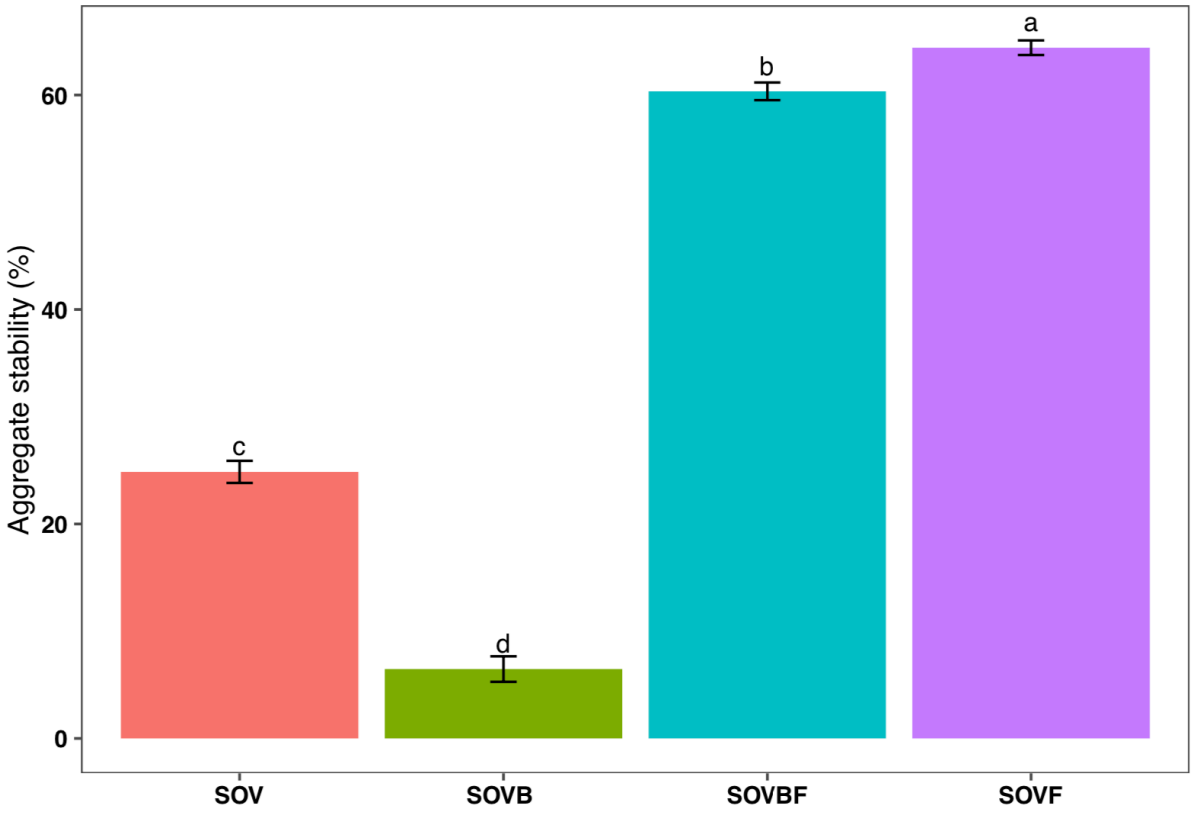



Figure 5.3 Effect of various soil amendments on soil aggregate stability. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides.*

## 5.4 Discussion

### 5.4.1 Effect of soil treatments on SOM formation

The impacts of various soil treatments on the production of SOM were investigated in a 72-day greenhouse study on the phytoremediation of crude oil-contaminated soil. Our results showed that the most SOM was formed in samples treated solely with fertiliser (Figure 5.1). The results have revealed that the level of SOM formed in samples treated solely with fertiliser was more than the level of SOM in samples treated solely with biosurfactant, and the untreated, unvegetated control samples. The elevated level of SOM could be attributed to the impact of fertilizer, which increased the growth of *C. zizanioides* and potentially increased root turnover as well as leaf litter from the plant. For instance, Wei et al., (2016) suggested in their research that fertiliser amendment of soil had caused increased yield of three crops namely wheat, maize and rice. They also observed increased SOM content through the application of fertiliser. Cotrufo et al., (2015) discovered the formation of SOM through the decomposition of plant litter. The findings of this current research have demonstrated that the combined effect of fertiliser and biosurfactant has led to increased growth of *C. zizanioides* and root turnover, which potentially increased the level of SOM. This could be attributed to the effect of the nutrients supplied by the fertiliser, which increased the growth of *C. zizanioides* and the formation of SOM through root turnover, as well as the effect of the biosurfactant, which likely facilitated the solubilisation of the oil contaminants and aided the absorption of nutrients by the plants. This also suggests that the application of biosurfactant during phytoremediation of oil-contaminated soil does not impede the growth of *C. zizanioides* or root turnover. Existing literatures have also revealed the positive effects of biosurfactants on encouraging plant development (Bhuyan-Pawar et al., 2015; Mishra et al., 2020; Khare and Arora, 2021; El-Sheshtawy et al., 2022). This present research also suggests that the soil moisture in the control sample increased SOM. This may have happened because of irrigation utilised during the experiment to maintain the soil's moisture content, which encouraged the flourishing of the soil

biota and consequently raised the SOM. This supports the findings of Xue et al., (2017), which revealed that soil water content influences nutrient availability and microbial biomass in both planted and unplanted soils.

### 5.4.2 Role of SOM on the sorption of 7 HMW PAHs

A leaching test was performed using different soil treatments to better understand how SOM affects the sorption of the 7 HMW PAHs and to address concerns about possible pollutant migration. The findings of our research have revealed that the concentration of the HMW PAHs from the leachates in samples treated with a combination of fertiliser and biosurfactant was generally lower than the concentration of HMW PAHs in other treatments (Table 5.1). The sorption of the contaminants to SOM may be responsible for immobilising the HMW PAHs by reducing the concentration of contaminants observed in the leachate test under the combined effect of fertiliser and biosurfactant. The findings from previous research have also indicated that plant root turnover and SOM are capable of immobilising and degrading contaminants in the soil (Zand and Grathwohl, 2016; Xu et al., 2018; Kwiatkowska-Malina, 2018). The findings of this current research suggest that the growth of *C. zizanioides* and root turnover, that resulted in increased level of SOM, and the effect of the biosurfactant, which solubilised the contaminants for easy absorption by plants might have aided the sorption, degradation, and immobilisation of the HMW PAHs in the soil. The results also suggest that samples that were solely exposed to biosurfactant had lower amounts of HMW PAHs than other samples (Table 5.1). This could be attributed to the effect of the biosurfactant, which may have helped dissolve the petroleum hydrocarbons in the soil to make them easily absorbable by the plants during phytoremediation and possibly aided the HMW PAHs to sorb and immobilise. Previous studies have also shown that the application of biosurfactants enhances the solubilisation of PAHs in the soil (Li et al., 2015; Cazals et al., 2020; Zang et al., 2021). The results showed that biosurfactant and fertilizer-treated samples had lower

concentrations of HMW PAHs including Benzo[a]anthracene, Dibenzo[a,h]anthracene, Benzo[a]pyrene, Benzo[k]fluoranthene, and Indeno[1,2,3-cd]pyrene than other treatments. This might be the result of the application of fertiliser, which aided in the sorption, degradation, and immobilisation of the HMW PAHs in the soil by promoting the growth of *C. zizanioides* and root turnover, thereby increasing the amount of SOM, and the effect of the biosurfactant, which solubilized the contaminants for simple absorption by plants during the process of phytoremediation.

### 5.4.3 Impact of root growth on soil aggregate formation

The impact of the root growth of *C. zizanioides* on soil aggregate formation was investigated during 32 weeks of phytoremediation with various soil amendments. The results have demonstrated that the formation of soil aggregate was aided by the plant roots development during the greenhouse experiment. The highest soil aggregates formed were observed in samples treated exclusively with fertiliser, followed by samples with a combination of fertiliser and biosurfactant (Figure 5.2). The soil aggregates formed in samples treated exclusively with fertiliser (A) was significantly higher than the soil aggregates formed in the untreated contaminated soil (C), samples treated exclusively with biosurfactant (D), and the untreated and uncontaminated samples (E). However, the soil aggregates formed in samples treated exclusively with fertiliser (A) was similar in quantity with the soil aggregates formed in samples treated with a combination of fertiliser and biosurfactant (B). The increased amount of soil aggregates formed in samples treated solely with fertiliser could be attributed to fertilizer's effect on plant growth and root development, which aided in the formation of soil aggregates during the greenhouse experiment on phytoremediation of crude oil-contaminated soil. This finding is similar to the finding of Chen et al., (2017), which revealed that soil aggregation was improved by the broad root system in the rubber-based agroforestry systems. Wang et al., (2020), studied the effect of rhizosphere priming and discovered that it is strongly

linked to root-driven aggregate turnover. Additionally, previous studies have shown that plant roots promote the formation of soil aggregates (Stumpf et al., 2016; Kalhoro et al., 2017; Schomburg et al., 2018). This present study has shown that the interaction between fertiliser and biosurfactant may have helped soil aggregate formation during phytoremediation of crude oil-contaminated soils. This suggests that the application of biosurfactant during *C. zizanioides*-based phytoremediation of oil-contaminated soil is safe for plant growth, root development, and soil aggregate formation.

### 5.4.4 Impact of root development on soil aggregate water stability

The impact of root development on soil aggregate stability was investigated after 72 weeks of phytoremediation with various soil amendments on crude oil-contaminated soil using *C. zizanioides.* The findings revealed the highest water stable soil aggregates in samples treated exclusively with fertiliser (Figure 5.3). This might be related to the impact of fertiliser, which promoted plant growth and root development and hence most likely facilitated the production of water-stable aggregates. This finding is in support of the research conducted by Hudek et al., (2017), which discovered that soil aggregate stability was increased by the presence of roots in an alpine vegetation in the Aosta Valley (NW-Italy). Li et al., (2020), discovered that plant roots promote aggregate stability after studying the effect of fibrous and tap root plants including *Saccharum arundinaceum* Retz., *Miscanthus sinensis, Zea mays* Linn. Sp, *Arthraxon prionodes, Dioscorea zingiberensis*, *Imperata cylindrica* (L.) Beauv, *Artemisia argyi*, *Sesamum indicum* L, *Setaria viridis* (L.) Beauv, *Juncus effusus, Triarrhena sacchariflora* (Maxim.) Nakai, *Xanthium sibiricum* Patrin ex Widder, *Chenopodium album* L., *Pterocypsela indica* (L.) Shih, *Sesamum indicum* L, *Arachis hypogaea* Linn., *Erigeron annuus* (L.) Pers., *Commelina communis* Linn., *Solanum pseudocapsicum* L., *Chenopodium serotinum* L., *Chenopodium serotinum* L., *Digitaria sanguinalis* (L.) Scop, *Themeda triandra and Bothriochloa ischaemum* (L.) Keng. Furthermore, Xiao et al., (2020), studied an abandoned cropland in China and discovered that increased soil

aggregate stability was influenced by plant roots. Previous studies have also discovered the influence of plant roots on aggregate stability Kalhoro et al., 2017; Kumar et al., 2017; Le Bissonnais et al., 2018; Demenois et al., 2018). The findings of this present research have revealed that both the exclusive application of fertiliser as well as a combination of fertiliser and biosurfactant during the phytoremediation of crude oil-contaminated soils using *C. zizanioides* can potentially increase the growth of the plant and root development, thus improving the stability of the soil aggregate. This current research also suggests that the exclusive addition of biosurfactant has reduced the soil aggregate stability. However, the application of fertiliser will counteract this issue by improving the aggregate stability. These results imply that there is a synergy between the effects of fertiliser and biosurfactant on the growth of *C. zizanioides* and root turnover, as well as the formation of water-stable soil aggregates that facilitate the sorption and immobilisation of the HMW PAHs during phytoremediation of petroleum hydrocarbon-contaminated soil.

## 5.5 Conclusion

This study has discovered that using fertiliser and biosurfactant during phytoremediation of crude oil-contaminated soil increased the formation of SOM through enhanced growth of *C. zizanioides*, leaf litter, and root turnover. This could have potentially led to the sorption and immobilisation of petroleum hydrocarbons in the soil. This current study has revealed that the effects of fertiliser application during phytoremediation, which promoted the growth of *C. zizanioides* and root turnover, which in turn increased the level of SOM, along with the impact of biosurfactant, which solubilized the pollutants for easy plant absorption, may have facilitated the sorption, immobilisation, and degradation of the HMW PAHs in the soil. Additionally, the findings of this present study also demonstrate that samples that were solely treated with biosurfactant had decreased HMW PAHs concentrations in the leachate from oil-contaminated soils compared to the other samples. This may be linked to the impact of biosurfactant, which solubilised the petroleum hydrocarbons during phytoremediation, thus reducing the concentration of the HMW PAHs in the

soil. Our findings suggest that the establishment of a wide root system in plants promotes the creation of water-stable aggregates in soil. Overall, the results of this present study point to a synergy between fertiliser and biosurfactant applications on the growth of *C. zizanioides* and root turnover, as well as the formation of water-stable soil aggregates that facilitate the sorption and immobilisation of the HMW PAHs during phytoremediation. Hence, it can be concluded that the application of biosurfactant during phytoremediation does not affect the nutrients supplied by the fertiliser that are required to promote the growth of the plants. Rather, it aids in the immobilisation of the HMW PAHs through solubilisation for easy uptake by plants.

# CHAPTER SIX

## 6.0. BIOSURFACTANT ASSISTED PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL USING *C. ZIZANIOIDES*.

**Abstract**

Environmental pollution from oil spills has a significant impact on the ecosystem. The toxicity of petroleum hydrocarbons affects plant growth, which in turn affects phytoremediation efficiency. We investigated how petroleum hydrocarbons affect the growth, physiology, and morphology of *C. zizanioides*, as well as the quantity of soil microbial population, during phytoremediation. The effect of fertiliser, biosurfactant, and soil minerals on enhancing the growth and tolerance of the plant to crude oil contamination was also studied. The root-shoot biomass ratio, proline content, chlorophyll content, and cell damage were used to assess the plant's stress response and tolerance to petroleum hydrocarbons in the soil. The results have shown that the growth of the plants in soils treated with fertiliser alone, which was 63.4 cm, was slightly higher than that of plants grown in soils treated with a combination of fertiliser and biosurfactant, which was 56.9 cm. However, the combined effect of fertiliser and biosurfactant was most effective in removing 86% of TPH from the oil-contaminated soil. The results also revealed that the plants grown in soils treated with a combination of fertiliser and biosurfactant were more tolerant to petroleum hydrocarbon-induced stress. The results indicated that the abundance of the silicate mineral quartz in all samples did not enhance the plant's tolerance to petroleum hydrocarbon-induced stress. It is suggested that the combined effect of fertiliser and biosurfactant has strong potential for enhancing the growth and tolerance of *C. zizanioides* for effective treatment of petroleum hydrocarbon-contaminated soil.

## 6.1 Introduction

Global population growth and accompanying high rate of industrialisation has led to extensive soil pollution due to high pressure on natural resources such as petroleum (Singh and Jain, 2003; Gupta, 2006; Kang, 2014; Ashraf and Hanfiah, 2017). The spills and leaks that occur during the extraction, processing and transport of petroleum normally affects the soil and groundwater (Brown and Eyenghe, 2015). Consequently, the presence of spilt oil in the environment exposes humans to a wide range of toxic substances such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals that can potentially lead to respiratory diseases, cancer, mental disorder and cardiopulmonary diseases (Al-Hashem et al., 2007; Sonibare et al., 2010; Briggs and Briggs, 2018).

Crude oil comprises of various types of hydrocarbon molecules, which vary in their level of toxicity and potential for negative environmental impacts (Jorgensen, 2010; Foroozanfar, 2017). It can also contain high amount of elements such as sulphur, nitrogen, and oxygen, as well as heavy metals such as copper, lead, iron, zinc, cadmium, and nickel (Jorgensen, 2010; Adesina and Adelasoye, 2014).

When crude oil is introduced into the soil, it increases the levels of potential toxic elements like iron and zinc, which can reduce the amount of nutrients available to support plant growth (Omosun et al., 2009; Kolesnikov et al., 2014; Adesina and Adelasoye, 2014). Plants generally respond to stress factors including the toxic effect of petroleum in the soil in different ways. The literature has shown that high level of proline concentration in plants indicates their response to stress factors Ábrahám et al., 2010; Long et al., 2012; Ma et al., 2017). The root/shoot biomass ratio of plants is also considered as an important parameter that indicates the level of stress induced in plants (Xu et al., 2018). Another factor that determines the level of stress induced in plants is decreased level of chlorophyll pigment (Arellano et al., 2017; Ma et al., 2018).

The traditional methods for soil remediation, such as mechanical, thermal, and chemical treatments, often cause damage to the environment through soil excavation, heating, and application of strong and toxic chemicals (Batty and Dolan, 2013a). As a result, there has been a growing interest in phytoremediation as a cost effective, environmentally friendly and sustainable approach for soil remediation (Mench et al., 2009; Haq et al., 2020; Shah and Daverey, 2020). However, the effectiveness of phytoremediation of petroleum hydrocarbons is limited by their low solubility which affects their bioavailability and thus, affects their removal from the environment (Lai et al., 2009a; Liao et al., 2016b; Brown et al., 2017). Nevertheless, biosurfactants are known to increase the bioavailability and mobility of organic contaminants through solubilisation and emulsification and subsequent removal from the soil (Banat et al., 2010; Das and Chandran, 2011; Almansoory et al., 2015; Brown et al., 2017). As mentioned in section 1.2 of chapter one (introduction), previous research on the use of biosurfactants to enhance phytoremediation of petroleum contaminated soils have involved the use of plants such as *Medicago sativa*, *Zea mays* and *Ludwigia octovalvis* However, the use of invasive plant species and plants with short roots affects the environment in a negative way and treats contaminants that are only present in a shallow depth of the soil (Ansari et al., 2014; Zand and Hoveidi, 2016). Therefore, it is important to investigate the phytoremediation potentials of *C. zizanioides* which is a fast growing, tolerant, deep rooted, non-invasive, and perennial plant to address the issue of the abovementioned limitations. According to recent research on *C. zizanioides*, the use of compost to amend petroleum contaminated soils was the primary driving force for effective treatment of petroleum contaminants (Nisa and Rashid, 2015; Nero, 2021). However, the use of compost to amend petroleum contaminated soil during phytoremediation may enhance microbial degradation than plant-based remediation. Furthermore, there is concern that the use of compost may pose a risk to human health because pathogenic antibiotic-resistant bacteria and genes may be introduced into the environment (Soobhany, 2018; Cunningham et al., 2020).

The distinct morphology and physiology of *C. zizanioides* make it a good candidate for phytoremediation of crude oil-contaminated soil. These include high biomass content, high

photosynthetic efficiency, extensive root system, ability to survive in various soil types and ability to withstand harsh environmental conditions such as frost, drought and flood (Lavania, 2004; Brandt et al., 2006; Srivastava et al., 2008; Banerjee et al., 2016). These unique features enable the plant to grow in all the geographical regions of the globe. Thus, the plant has successfully been used to remediate various types of contaminants such as heavy metals, petroleum hydrocarbons, waste water and antibiotics (Brandt et al., 2006; Roongtanakiat et al., 2007; Danh et al., 2010; Ng et al., 2016; Seroja et al., 2018; Panja et al., 2020).

The toxicity of petroleum hydrocarbons also affects microbial quantity and activity in the root region of plants which plays a major role in the degradation of petroleum contaminants (Nie et al., 2011; Adesina and Adelasoye, 2014). Thus, there is concern over potential stress being induced on *C. zizanioides,* and the quantity of soil microorganisms may also be affected by the toxicity of petroleum contaminants during the process of phytoremediation of crude oil contaminated soil. However, there is a paucity of information on the level of stress tolerance of *C. zizanioides* in crude oil-contaminated soil. Hence, the need to investigate the effect of stress induced on *C. zizanioides* and microbial quantity during the phytoremediation process under the influence of fertiliser and biosurfactant. According to published research, the presence of quartz silica in the soil promotes plant growth and stress tolerance (Dresler et al., 2015; Abdel-Haliem et al., 2017; Puspitasari and Indradewa, 2019; Rashad and Hussien, 2020). However, there is a lack of research on the effect of silica on enhancing the growth and stress tolerance of *C. zizanioides* in crude oil-contaminated soil. As a result, there is a need to investigate the effect of soil silica on the stress tolerance of *C. zizanioides* exposed to crude oil-contaminated soil. The literature has also shown that sufficient nutrients in the soil such as Nitrogen, Phosphorus and Potassium (NPK) tend to improve the growth of plants and microbial quantity in the rhizosphere, which brings about improvement in the breakdown of organic contaminants in the soil (Basumatary et al., 2012; Chang et al., 2013; Bramley-Alves et al., 2014; Atma et al., 2017). Thus, the need to investigate the impact of NPK fertiliser on the growth of *C. zizanioides* and the associated microbial population during

phytoremediation of crude oil contaminated soil under the influence of biosurfactant that is yet to be fully elucidated.

As mentioned above, previous studies have demonstrated the ability of biosurfactants in enhancing the solubility and bioavailability of organic contaminants during phytoremediation of petroleum hydrocarbons (Bordoloi and Konwar, 2009; Adrion et al., 2016; Brown et al., 2017). However, there is no available information on the impact of NPK fertiliser on the growth of plants and associated soil microbial population under the influence of biosurfactants during phytoremediation of crude oil contaminated soil. There is also lack of information on the effect of biosurfactants, NPK fertiliser and crude oil on root elongation of non invasive and deep rooted plants such as *C. zizanioides* that can absorb leachate in the depth of the soil.

Also, there is concern that the presence of elements such as Al in crude oil contaminated soils could inhibit plant absorption and utilisation of nutrients (Mariano and Keltjens, 2005). Therefore, there is the need to investigate the potential effect of the elements and minerals already existing in the soil on the growth of *C. zizanioides* during the process of phytoremediation.

This research is aimed at determining the ability of *C. zizanioides* in removing petroleum contaminants from a crude oil contaminated soil. The main objectives include (i) determination of the ability of *C. zizanioides* to dissipate petroleum hydrocarbons under the influence of NPK fertiliser and biosurfactant (ii) determination of the effect of NPK fertiliser on the growth of *C. zizanioides* and microbial quantity during phytoremediation of crude oil contaminated soil under the influence of biosurfactant (iii) determination of the effect of elements and minerals already existing in the soil on the growth of plants and microbial population (iv) determination of the effect of crude oil and biosurfactant on the growth of *C. zizanioides* and microbial population during the process of phytoremediation (v) determination of the effect of NPK fertiliser and biosurfactant in enhancing the dissipation of total petroleum and hydrocarbons in the soil (vi) determination of the combined effect of fertiliser and biosurfactant in enhancing the tolerance of *C. zizanioides* to crude oil contaminated soil (vii) determination of the ability of soil minerals such as silica in

enhancing the tolerance of *C. zizanioides* to crude oil contaminants during phytoremediation and (viii) determination of the effect of crude oil on the physiology and morphology of *C. zizanioides* during the process of phytoremediation.

## 6.2 Materials and methods

### 6.2.1 Soil analysis

The analysis of sandy loam soil used in this research was previously described in detail in sections 3.4 of chapter 3 (materials and method). The soil was air-dried at $20^{O}C$ for 72 hours and then homogenised to get the desired texture (Zornoza et al., 2006). The soil was tested for pH, EC, moisture, and organic matter (OM).

### 6.2.2 Sampling and analysis

As previously described in detail in sections 3.5, 3.6 and 3.8 of Chapter 3 (materials and method), using a method adapted from Brown et al., (2017), 1 kg of crude oil was added to 70 kg of homogenised soil and allowed to weather for 100 days. 1kg of the weathered soil was transferred into terracotta pots and vegetated with seedlings of *C. zizanioides* of similar heights at a depth of 5cm (Wato, 2019). Soils were irrigated with deionized water and relevant samples were treated with biosurfactant. According to the supplier's instructions, fertiliser treatment of samples was carried out to improve plant growth.

### 6.2.3 Elemental analysis of soil using X-ray fluorescence (XRF)

The concentrations of elements in the soil were determined using Bruker S8 Tiger X-ray fluorescence (WDXRF) spectrometer with touch control. The samples were dried at room temperature for 72 hours and sieved at 250 μm in order to homogenise the particle sizes (Ene et al., 2010). The samples were placed into labelled sample cups and were analysed using the XRF spectrometer. Results were interpreted using a Spectra plus software prior to statistical analysis using R studio.

### 6.2.4 Mineralogical analysis of soil

#### 6.2.4.1 FTIR spectroscopic technique

The analysis of soil minerals was carried out according to the method described by Gbadamosi et al., (2018) using Fourier transform infrared spectroscopy (FTIR). 30 mg of soil was ground using an agate mortar. The finely pulverised sample was dried at room temperature for 72 hours to ensure that moisture was removed. Following this the sample was mixed with KBr where the maximum transmittance observable peak was obtained at a ratio of 1:30 (Ramasamy et al., 2009). Subsequently, the resultant mixture was pressed into a transparent disc in evacuable die with sufficient pressure. The infrared spectra of samples were recorded using a Perkin Elmer FTIR Spectrometer within the region of 4000-400 $cm^{-1}$.

#### 6.2.4.2 X-ray diffraction (XRD) technique

The minerology of the finely pulverised samples as described above were evaluated at room temperature using a Bruker D8 X-ray diffractometer equipped with Cu $K_{\alpha 1}$ radiation source ($\lambda$ =

1.5406 Å). The diffractogram of the samples were obtained in the 2θ region between 10° and 90° with a step size of 0.01°. Thereafter, the phase matching of the XRD patterns was processed using Powder Diffraction File (PDF) database to identify the minerals present.

### 6.2.5 Determination of Total Petroleum and Hydrocarbons

The quantification of TPH in soil was determined using a modified gravimetric method adapted from Tang et al., (2010) as explained in detail in section 3.9 of chapter 3 (methodology).

### 6.2.6 Determination of plant growth

The growth of *C. zizanioides* was analysed using a method adopted from Novo et al., (2013). Plant samples were harvested after 72-days of greenhouse experiment, and washed with tap water, followed by deionized water to remove any soil that had accumulated. The plant shoots and roots were separated, and the weight of fresh biomass was determined by weighing the plant on a scale Mettler Toledo (SB24001) Delta Range. The dry biomass of the plant was determined after drying the plant in an oven at 50 $^{O}$C for 24 hours after which the plant was transferred into sealable plastic bags and the weight was measured. The length of plant shoots was also recorded every week during the greenhouse experiment from the first week of transplanting. The length of roots was also measured at the end of the experiment. The percentage of water content (WC) of the plant was calculated using the formula below.

$$\mathrm{WC}\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{FW}} \times 100$$

Equation (11). percentage of water content

Where FW is the fresh weight of the plant and DW is the dry weight of the plant.

### 6.2.7 Determination of root-shoot biomass ratio

The determination of root/shoot ratio of *C. zizanioides* was carried out using a method adopted from Xu et al., (2015). Plant samples were collected and the root and shoots were divided and washed with tap water before being rinsed with deionised water to remove any accumulated soil (Novo et al., 2013a). The samples were then oven dried at 72°C to a constant weight and the root/shoot ratio was calculated using the formular described by Xu et al., (2018) as shown below.

$$\text{Root/Shoot (R/S) biomass ratio } = \frac{\text{root}_b}{\text{shoot}_b}$$

Equation (12). Root-shoot biomass ratio

Where $\text{root}_b$ is the biomass of dry root (g) and $\text{shoot}_b$ is the biomass of dry shoot (g).

### 6.2.8 Determination of proline content

The level of accumulation of proline in plants was determined by colorimetric assay method adopted from Mosa et al., (2017). 100 mg of fresh plant material was collected from each sample in the early hours of morning (7:30 am) and preserved in ice. Then 3% sulfosalicylic acid (5 µL/mg) was immediately added to the plant material and ground using a pestle and mortar and centrifuged at 3500 rpm for 5 mins under a room temperature. Thereafter, 100 µL of the supernatant was transferred into 1.5 ml microcentrifuge tube and added with 100 µL 3% sulfosalicylic acid, 200 µL glacial acetic acid and 200 µL acidic ninhydrin and mixed on a vortex for 10 secs after which the lid of tube was punctured with a needle to avoid opening due to pressure from heat. The mixture was then incubated at 96°C for 1 hour and then placed on ice. Following this, 1 mL of toluene was added to the mixture and vortexed for 20 secs and left to settle for 5 mins. The chromophore containing toluene was transferred into a new tube and the absorbance was measured at 520 nm using a JENWAY (model 6505) spectrophotometer using toluene as reference material.

### 6.2.9 Determination of Chlorophyll

The determination of chlorophyll in *C. zizanioides* was carried out by using a modified spectrophotometric analysis according to the method described by Ali et al., (2012). One leaf was collected from each of the 4 replicates of *C. zizanioides* and 250 mg/0.25 g of each leaf sample was weighed. 5 ml of aqueous 80% acetone was added, and the leaf was ground using a pestle and mortar to homogenise the sample. Another 20 ml aqueous 80% acetone was added, and the sample was filtered through a Whatman syringe filter (0.22 µm) into a 25 ml flask. 100 µL of the extract was transferred into tubes and centrifuged at 3000 rpm for 5 minutes. Following this, the absorbance was determined at wavelengths of 647 nm and 664 nm using a JENWAY (model 6505) spectrophotometer. Subsequently, the total chlorophyll in the leaves of *C. zizanioides* were calculated using the formula described by Sumanta et al., (2014) as shown below.

$$Chl\ a = 12.25A_{664} - 2.79A_{647}$$

$$Chl\ b = 21.5A_{647} - 5.1A_{664}$$

$$Chl\ a + b = 12.25A_{664} - 2.79A_{647}$$

Equation (13). total chlorophyll.

### 6.2.10 Determination of plant morphological changes

Morphological changes in *C. zizanioides* during the process of phytoremediation was carried out using a method adopted from Balliana et al., (2017). Fresh samples of the plant cultivated in different treatments were randomly selected for microscopic analysis after 72 days period of greenhouse experiment. The mid region of plant shoots was cut and embedded in paraffin to form a wax to facilitate precision in dissection of plant tissues. Then the transverse sections of the embedded plants were dissected into slices using a rotary microtome cutting instrument. The

slices were submerged in 1ppm of Propidium Iodide (PI) for 10 mins, placed on cover slides and examined at magnification of 50x using fluorescence microscope (Leica DMRE). The images of the slides were captured with the use of Hamamatsu OrcaR2 CCD camera and analysed with RC-Image acquisition software.

### 6.2.11 Determination of soil microbial quantity

The soil microbial count was analysed by the serial dilution method as described by Foght and Aislabie (2005). 20g of each soil sample was placed in a conical flask containing 180 ml sterile water and placed on a shaker at 150 rpm for 1 hour for thorough mixing. 1 ml of the resulting liquid mixture was transferred into a test tube with 9 ml sterile water for subsequent serial dilution to get dilutions from $10^{-2}$ to $10^{-7}$. Following that, 0.1 ml of three dilutions ($10^{-3}$, $10^{-4}$, and $10^{-5}$) were spread on nutrient petri plates and incubated at $30^{O}C$ inverted for 48 hours in inverted manner to prevent water vapour from falling on the nutrient plates. Thereafter, the bacterial colonies were counted and expressed as the colony forming units per gramme ($CFU^{-1}$).

### 6.2.12 Statistical analysis

The statistical analysis was carried out using R studio version 1.2.5033 for Mac OSX as previously described in section 3.9.

## 6.3 Result

### 6.3.1 Soil characterisation

The analysis of soil carried out before the start of the experiment revealed that the soil has moisture

content of 2.74±0.15 (%), organic matter (OM) content of 1.18±0.04 (%), electric conductivity (EC) of 0.44±0.01 (mS/cm), and pH level of 7.4±0.06. All values are average (SD, n=4)

### 6.3.2 Concentration of soil elements

The results in Table 6.2, below, show the concentration of various elements, including phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), chlorine (Cl), aluminium (Al), titanium (Ti), and silicon (Si), that are present in all soil samples after 72 days of the greenhouse experiment.

Table 6.1 Concentration of soil elements. The alphabetical letters represent the significant difference between variables, ($p \leq 0.05$). The abbreviations are: S = soil only, SO = soil and oil only, SV = soil and *C. zizanioides* only, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated crude oil contaminated soil vegetated with *C. zizanioides*, and SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*.

| Treatment | S | SO | SV | SOV | SOVF | SOVBF | SOVB |
|---|---|---|---|---|---|---|---|
| Element | wt.% | | | | | | |
| P | $1.2^{ab}$ | $0.8^{ab}$ | $1^{bc}$ | $0.8^{abc}$ | $1.3^{abc}$ | $1.9^{a}$ | $1.4^{c}$ |
| K | $6.1^{a}$ | $6.9^{a}$ | $7^{a}$ | $7.3^{a}$ | $6.8^{a}$ | $7^{b}$ | $5.6^{b}$ |
| Ca | $8.4^{bc}$ | $4.8^{d}$ | $7.8^{bc}$ | $6.5^{cd}$ | $5.4^{ab}$ | $13.1^{a}$ | $8.1^{abc}$ |
| Mg | $1.1^{a}$ | $1.1^{a}$ | $0.8^{a}$ | $0.9^{a}$ | $0.8^{a}$ | $1.2^{a}$ | $1^{a}$ |
| S | $0.5^{ab}$ | $0.5^{ab}$ | $0.4^{b}$ | $0.6^{ab}$ | $0.5^{a}$ | $0.9^{b}$ | $0.8^{ab}$ |
| Fe | $4.3^{bcd}$ | $3.5^{cd}$ | $2.9^{d}$ | $3.8^{cd}$ | $4.2^{ab}$ | $7.1^{a}$ | $5.1^{abc}$ |
| Cl | ND | $0.2^{bc}$ | ND | $0.2^{bc}$ | $0.2^{ab}$ | $0.3^{a}$ | $0.2^{ab}$ |
| Al | $7.9^{c}$ | $8.2^{bc}$ | $7.5^{b}$ | $8.4^{bc}$ | $7.6^{d}$ | $8.1^{a}$ | $7.4^{e}$ |
| Ti | $0.5^{a}$ | $0.5^{a}$ | $0.5^{a}$ | $0.5^{a}$ | $0.5^{a}$ | $0.6^{a}$ | $0.5^{a}$ |
| Si | $70.3^{ab}$ | $72.7^{a}$ | $71.9^{ab}$ | $70.4^{a}$ | $72.9^{bc}$ | $58.5^{c}$ | $69.6^{a}$ |

### 6.3.3 Soil minerals analysis

#### 6.3.3.1 Characterisation of soil minerals

The observed wave numbers ($cm^{-1}$) recorded from the FTIR spectra of all samples are tabulated, along with the corresponding minerals (Table 6.2). The FTIR spectrum of soil in the sample (SOV), selected as a representative, is shown in (Fig 6.1). The results revealed the presence of quartz silica in all samples, which is explained by Si–O symmetrical bending vibrations in the range of 690–695 $cm^{-1}$ and Si–O symmetrical stretching vibrations ranging from 776–780 and 795–800 $cm^{-1}$(Table 6.2). Quartz is a non-clay silicate mineral and the strongest bonds in the silicate structure are the Si–O bonds, which are easily identifiable in the infrared spectra of such minerals (Ramasamy et al., 2009). The existence of quartz in the samples can be confirmed by characteristic peaks in the range of 460–1874 $cm^{-1}$ (Hlavay et al., 1978; Ramasamy et al., 2014; Gbadamosi et al., 2018). This present study has revealed that only two peaks were observed at 3620 and 3690 $cm^{-1}$ thus, suggesting that the kaolinite present in the samples is in disordered state (Table 6.2). Kaolinite is a clay silicate mineral with chemical composition $Al_2Si_2O_5(OH)_4$ that has one tetrahedral sheet linked to one octahedral sheet of alumina octahedral via oxygen molecules (Ramasamy et al., 2014). The presence of absorption band around 3615, 3620 and 3690 $cm^{-1}$ signifies the presence of Kaolinite as a component of clay mineral (Ramasamy et al., 2009; Ramasamy et al., 2011; Ramasamy et al., 2014). Kaolinite is said to be in ordered state when four peaks are observed in the range of 3620–3697 $cm^{-1}$ (Ramasamy et al., 2009). The results also revealed that calcite was present in all samples (Table 6.2). Calcite is the most common carbonate mineral found in soils The presence of calcite is recognised by the appearance of peaks in the range of 1420–1438 $cm^{-1}$(Ramasamy et al., 2010; Ramasamy et al., 2011). Gibbsite is an aluminium hydroxide mineral that forms when several aluminous and alumino-silicate minerals are subjected to extreme weathering (Ramasamy et al., 2014). The existence of a peak in the range of 666–670 $cm^{-1}$ indicates the presence of the mineral gibbsite (Ramasamy et al.,

2010), and our results have shown the presence of gibbsite in all samples (Table 6.2). The presence of organic carbon in all the samples is indicated by the appearance of peaks in the region of 2850–2853 and 2923–2926 $cm^{-1}$ (Song et al., 2001).

Table 6.2 Characterisation of soil minerals using FTIR. The abbreviations of treatments include: SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SV = soil and *C. zizanioides* only, SO = crude oil contaminated soil only and S = uncontaminated soil only.

| **Sample name** | SOVBF | SOVF | SOVB | SOV | SV | SO | S |
|---|---|---|---|---|---|---|---|
| **Mineral name** | **Observed wave numbers ($cm^{-1}$)** | | | | | | |
| 1. Quartz | 690 – 695 | 690 – 695 | 690 – 695 | 690 –695 | 690 – 695 | 690 – 695 | 690 – 695 |
| | 776 – 780 | 776 – 780 | 776 – 780 | 776 – 780 | 776 – 780 | 776 – 780 | 776 – 780 |
| | 795 – 800 | 795 – 800 | 795 – 800 | 795 – 800 | 795 – 800 | 795 – 800 | 795 – 800 |
| | 1080 – 1085 | 108 – 1085 | 1080 – 1085 | 1080 – 1085 | 1080 – 1085 | 1080 – 1085 | 1080– 1085 |
| | 1169– 1174 | 1169– 1174 | 1169 – 1174 | 1169 – 1174 | 1169 – 1174 | 1169 – 1174 | 1169 – 1174 |
| | 1616 – 1619 | 1616 –1619 | 161 6– 1619 | 161 6– 1619 | 1616 – 1619 | 161 6– 1619 | 1616 – 1619 |
| | 1871– 1876 | 1871– 1876 | 1871 – 1876 | 1871 – 1876 | 1871 – 1876 | 1871 – 1876 | 1871 – 1876 |
| 2. Kaolinite | 1015 – 1019 | 1015– 1019 | 1015 – 1019 | 1015 – 1019 | 1015 – 1019 | 1015 – 1019 | 1015 – 1019 |
| | 3618 – 3622 | 3618 –3622 | 3618 – 3622 | 3618 – 3622 | 3618 – 3622 | 3618 – 3622 | 3618 – 3622 |
| | 3690 – 3691 | 3690– 3691 | 3690 – 3691 | 3690 – 3691 | 3690 – 3691 | 3690 – 3691 | 3690 – 3691 |
| 3. Calcite | 1420 – 1438 | 1420 –1438 | 1420 – 1438 | 1420 – 1438 | 1420 – 1438 | 1420 – 1438 | 1420 – 1438 |
| 4. Gibbsite | 666 – 670 | 666 – 670 | 666 – 670 | 666 – 670 | 666 – 670 | 666 – 670 | 666 – 670 |
| 5. Organic carbon | 2852 – 2854 | 2852– 2854 | 2852 – 2854 | 2852 – 2854 | 2852 – 2854 | 2852 – 2854 | 2852 – 2854 |
| | 2923 – 2927 | 2923– 2927 | 2923 – 2927 | 2923 – 2927 | 2923 – 2927 | 2923 – 2927 | 2923 – 2927 |

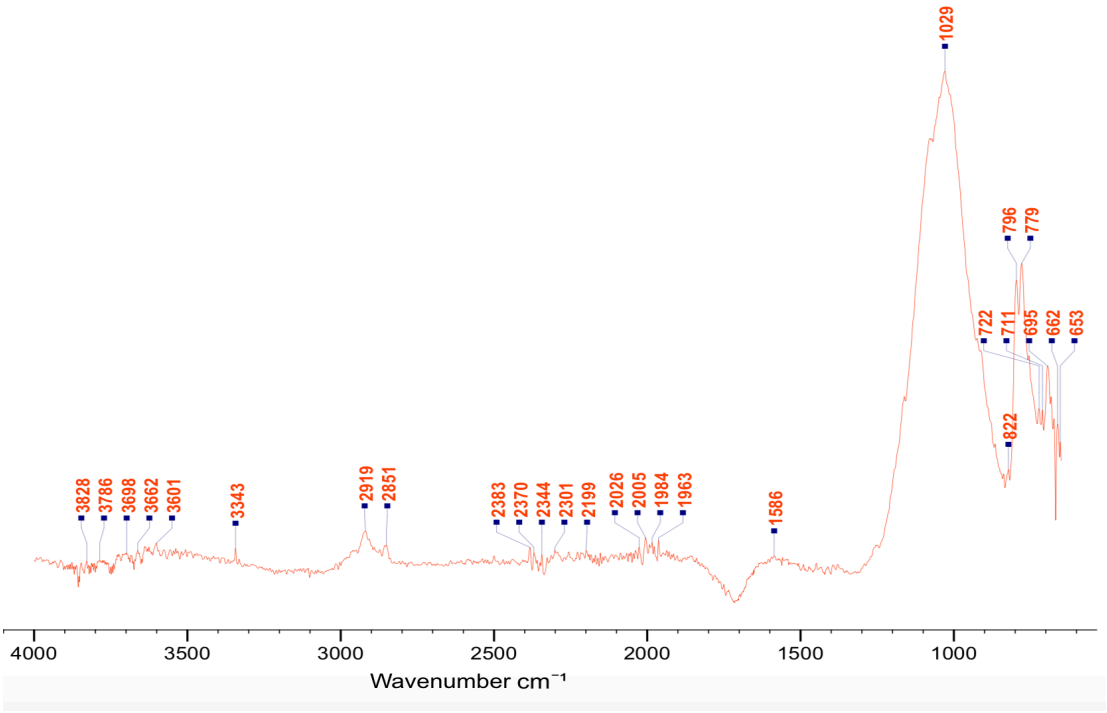



Figure 6.1 FTIR spectrum of sandy loam soil, sample (SOV)

#### 6.3.3.2 X-ray diffraction analysis of soil minerals

The XRD analysis was carried out to determine the mineralogical composition and crystalline nature of the minerals. The X-ray diffractogram is depicted along with the names of minerals present (Figure 6.2). The minerals contained in the samples were identified using the Powder Diffraction File (PDF) database. The minerals identified in the XRD patterns include quartz, microcline feldspar, orthoclase feldspar, calcite, hematite, albite, aragonite, and monazite. However, the most abundant mineral identified in all samples was found to be silica (quartz).

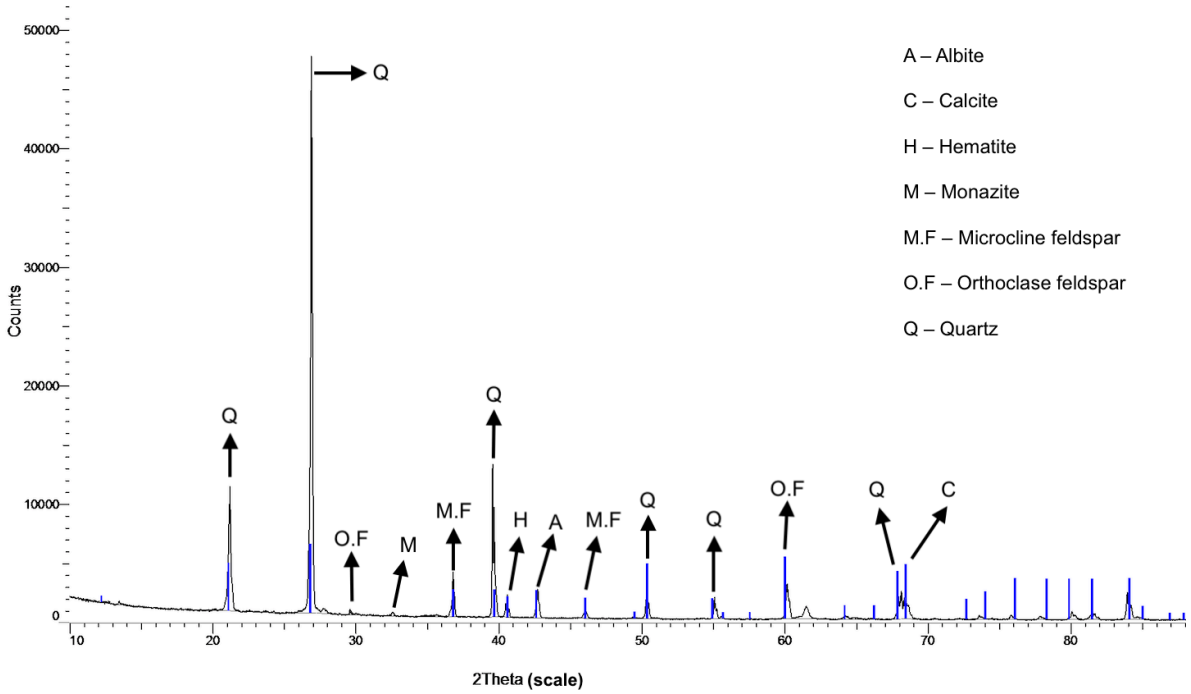



Figure 6.2 XRD pattern of sandy loam soil, sample (SOV)

### 6.3.4 Removal of Total Petroleum and Hydrocarbons

The various soil treatments used during the phytoremediation process have influenced the removal of TPH from crude oil-contaminated soils. Our findings have revealed that the highest rate of TPH removal, 86.0%, was influenced by the combined effect of fertiliser and biosurfactant (Figure 6.3). The results revealed that the quantity of TPH removed under the combined effect of fertiliser and biosurfactant, 86.0%, was significantly higher ($p < 0.05$) than that of the samples treated solely with biosurfactant, 47.2%, the samples treated solely with fertiliser, 28.1%, the untreated oil contaminated samples, 14.4%, and the untreated, unvegetated oil contaminated samples, 4.8%. The TPH removed from the samples treated solely with biosurfactant, 47.2%, was also found to be significantly higher ($p < 0.05$) than that of the samples treated solely with fertiliser, 28.1%, the untreated oil contaminated samples, 14.4%, and the untreated unvegetated oil contaminated samples, 4.8%. Furthermore, the TPH removed from the samples treated solely with fertiliser, 28.1%, was statistically significant ($P < 0.05$) when compared to that of the untreated and

unvegetated oil contaminated samples, 4.8%. Except for the untreated oil contaminated samples vegetated with *C. zizanioides*, which was 14.4%. Furthermore, the TPH removed from the untreated oil contaminated samples vegetated with *C. zizanioides*, 14.4%, was significantly higher ($p < 0.05$) than that of the untreated, unvegetated oil contaminated samples, 4.8%.

Our results also showed that the samples treated with a fertiliser and biosurfactant mixture had the highest removal rates of the aromatic, saturated, and polar fractions of TPH, with removal rates of 71.1%, 67.4%, and 51.2%, respectively (Figure 6.4). The results suggest that the quantity of aromatic fraction removed from samples under the combined effect of fertiliser and biosurfactant, 71.1%, was significantly higher ($p < 0.05$), than that of the samples treated solely with biosurfactant, 52.2%, the samples treated solely with fertiliser, 30.5%, the untreated oil-contaminated samples vegetated with *C. zizanioides*, 21.7%, and the untreated, unvegetated oil contaminated samples, 8.2%. The results also showed that the aromatic fraction removed from samples treated solely with biosurfactant, 52.2%, was significantly higher ($p < 0.05$) than that of the samples treated solely with fertiliser, 30.5%, the untreated oil-contaminated samples, 21.7%, and the untreated, unvegetated oil contaminated samples, 8.2%. Similarly, the aromatic fraction removed from samples treated solely with fertiliser, 30.5%, was significantly higher ($p < 0.05$), than that of the untreated oil-contaminated samples, 21.7%, and the untreated and unvegetated oil contaminated samples, 8.2%. Furthermore, the aromatic fraction removed from the untreated oil-contaminated samples, 21.7% was significantly higher ($p < 0.05$) than that of the untreated and unvegetated oil contaminated samples, 8.2%.

The results also showed that the saturated fraction removed from samples treated with a mixture of fertiliser and biosurfactant, 67.4%, was significantly higher ($p < 0.05$) compared to that of the samples treated solely with biosurfactant, 41.2%, the samples treated solely with fertiliser, 34.0%, the untreated oil-contaminated samples vegetated with *C. zizanioides*, 16.3%, and the untreated and the untreated, unvegetated oil contaminated samples, 10.7% (Figure 6.4). The results also showed that the saturated fraction removed from the samples treated solely with biosurfactant, 41.2% was significantly higher ($p < 0.05$) than that of the samples treated solely with fertiliser,

34.0%, the untreated oil-contaminated samples vegetated with *C. zizanioides*, 16.3% and the untreated and unvegetated oil contaminated samples, 10.7%. Furthermore, the saturated fraction removed from the samples treated solely with fertiliser, 34.0%, was significantly higher ($p < 0.05$) than that of the untreated oil-contaminated samples, 16.3%, and the untreated and unvegetated oil contaminated samples, 10.7%. Similarly, the saturated fraction removed in the untreated oil-contaminated, 16.3%, was statistically significant ($p < 0.05$), when compared with the untreated, unvegetated oil contaminated samples, 10.7%.

In terms of the polar fraction, the results revealed that the quantity of the polar fraction removed in samples treated with a mixture of fertiliser and biosurfactant, 51.2%, was significantly higher ($p < 0.05$), than that of the samples treated solely with biosurfactant, 28.8%, the samples treated solely with fertiliser, 18.1%, the untreated oil-contaminated samples, 12.5%, and the untreated, and unvegetated oil contaminated samples, 4.2%. The results also showed that the polar fraction removed from samples treated solely with biosurfactant, 28.8%, was statistically significant ($p < 0.05$) when compared to that of the samples treated solely with fertiliser, 18.1%, the untreated oil-contaminated samples, 12.5% and the untreated and unvegetated oil contaminated samples, 4.2%. Similarly, the polar fraction removed from the samples treated solely with fertiliser, 18.1%, was significantly higher ($p < 0.05$) than that of the untreated oil-contaminated samples, 12.5%, and the untreated and unvegetated oil contaminated samples, 4.2%. Furthermore, the polar fraction removed from the untreated oil-contaminated samples, 12.5%, was significantly higher ($p < 0.05$), than that of the untreated and unvegetated oil contaminated samples, 4.2% (Figure 6.4).

Our results have also revealed that the quantity of the aromatic fraction removed from the samples treated with a mixture of fertiliser and biosurfactant, 71.1%, was significantly higher ($p < 0.05$), than that of the polar fraction removed from samples treated with a mixture of fertiliser and biosurfactant, 51.2%, the saturated and polar fractions removed from samples treated solely with biosurfactant, 41.2% and 28.8%, respectively, the saturated and polar fractions removed from samples treated solely with fertiliser, 34.0% and 18.1%, the saturated and polar fractions removed from the untreated oil-contaminated samples, 16.3% and 12.5%, and the saturated and polar fractions

removed from the untreated and unvegetated oil contaminated samples, 10.7% and 4.2%, respectively. Except for the saturated fraction removed from the samples treated with a mixture of fertiliser and biosurfactant, 67.4%. The results have also showed that the aromatic fraction removed from samples treated solely with biosurfactant, 52.2%, was significantly higher ($p < 0.05$), than that of the saturated and polar fractions removed from samples treated solely with biosurfactant, 41.2% and 28.8%, the saturated and polar fractions removed from the samples treated solely with fertiliser, which were 34.0% and 18.1%, the saturated and polar fractions removed from the untreated oil-contaminated samples, 16.3% and 12.5%, and the saturated and polar fractions removed from the untreated and unvegetated oil contaminated samples, 10.7% and 4.2%, respectively. Furthermore, the results also suggest that the aromatic fraction removed from samples treated solely with fertiliser, 30.5%, was significantly higher ($p < 0.05$), than that of the polar fraction removed from samples treated solely with fertiliser, 18.1%, the saturated and polar fractions removed from the untreated oil-contaminated samples, 16.3% and 12.5% respectively and the untreated and unvegetated oil contaminated samples, 10.7% and 4.2%, respectively. Except, for the saturated fraction removed from samples treated solely with fertiliser which was 34.0%. The results also showed that the aromatic fraction removed from the untreated oil contaminated samples, 21.7%, was significantly higher than that of the saturated and polar fractions removed from the untreated oil-contaminated samples, 16.3% and 12.5% and the untreated and unvegetated oil contaminated samples, 10.7% and 4.2%, respectively.

Our findings also revealed that the quantity of saturated fraction removed from samples treated with a mixture of fertiliser and biosurfactant, 67.4%, was significantly higher ($p < 0.05$) than that of the polar fraction removed from samples treated with a mixture of fertiliser and biosurfactant, 51.2%, the polar fractions removed from samples treated solely with biosurfactant, 28.8%, the polar fraction removed from samples treated solely with fertiliser, 18.1%, the polar fraction removed from the untreated oil-contaminated samples vegetated with *C. zizanioides*, 12.5% and the polar fraction removed from the untreated and unvegetated oil contaminated samples, 4.2%.

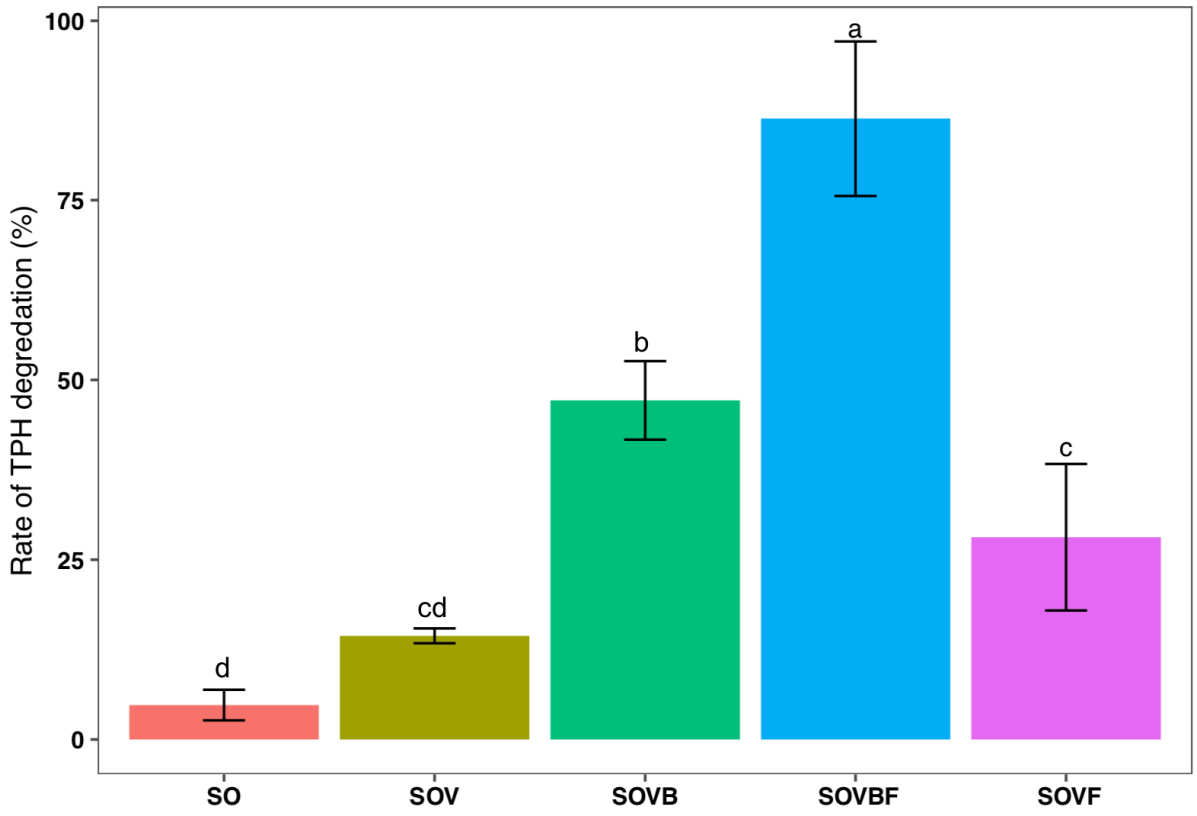



Figure 6.3 TPH removal (%) from crude oil contaminated soils after 32 weeks. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SO = crude oil contaminated soil only, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*.

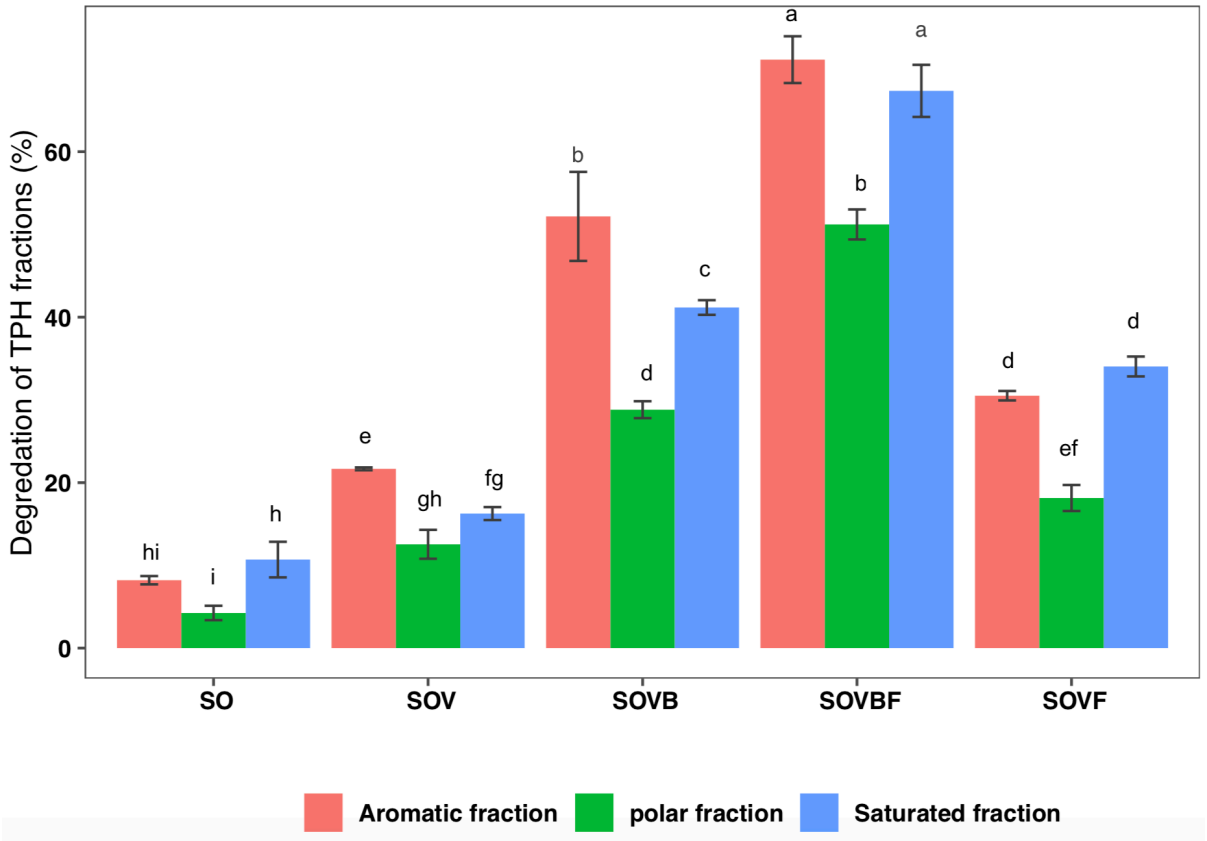



Figure 6.4 Rate of removal of TPH fractions from crude oil contaminated soils after 32 weeks period of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SO = crude oil contaminated soil only, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, and SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*.

### 6.3.5 Growth of plants

The growth of *C. zizanioides* was observed in a greenhouse experiment over a 72-day period of phytoremediation of crude oil-contaminated soil to determine the impact of various soil amendments on the plant's growth. Our results have revealed that the plants grown in samples amended solely with fertiliser exhibited the highest growth of shoots (Figures 6.5). The shoot of plants grown in samples treated with fertiliser alone (63.4 cm), was significantly higher ($p < 0.05$) than that of plants grown in samples grown under the combined effect of fertiliser and biosurfactant

(56.9 cm), the untreated oil-contaminated samples (24.8 cm), the samples treated exclusively with biosurfactant (23.2 cm), and plants in the untreated uncontaminated samples (18.5 cm). The results also showed that the shoot of plants grown in samples under the combined effect of fertiliser and biosurfactant (56.9 cm), was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil-contaminated samples (24.8 cm), the samples treated exclusively with biosurfactant (23.2 cm), and the untreated, uncontaminated samples (18.5 cm). Furthermore, the results also indicated that the shoot of plants grown in the untreated oil-contaminated samples (24.8 cm,) was significantly higher ($p < 0.05$) than that of plants grown in the untreated and uncontaminated samples (18.5 cm).

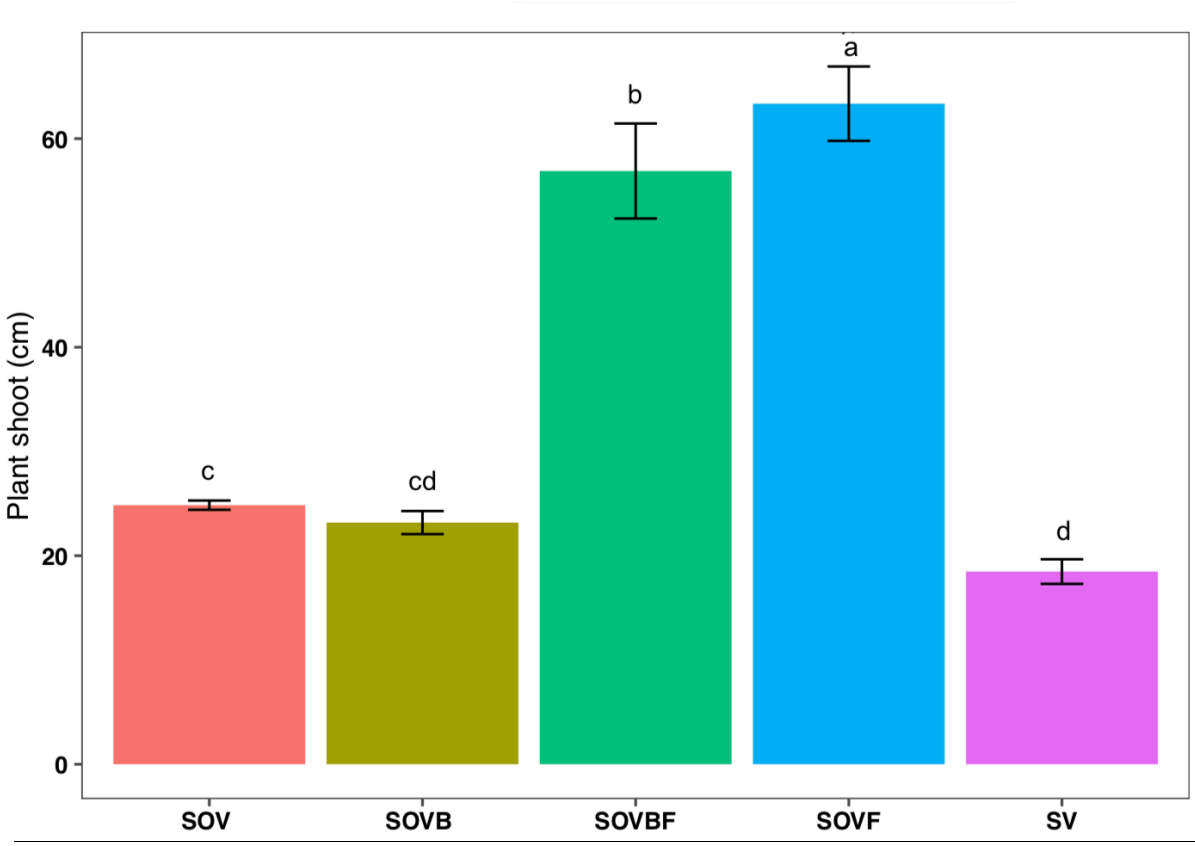



Figure 6.5 Growth of plant shoots under different soil treatments after 72 days. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

Our results (Figure 6.6) discovered that the root of plants grown in samples treated with fertiliser alone (30.4 cm), was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil-contaminated samples, (21.4 cm), the samples treated exclusively with biosurfactant (17.0 cm), and the untreated and uncontaminated samples (10.1 cm). Except for the plants grown in samples under the combined effect of fertiliser and biosurfactant (29.2 cm). The results also showed that the root of plants in samples under the combined effect of fertiliser and biosurfactant (29.2 cm) was significantly higher ($p < 0.05$) than that of the plants in the untreated oil-contaminated samples (21.4 cm), the samples treated exclusively with biosurfactant (17 cm), and the plants in the untreated uncontaminated samples (10.1 cm). Furthermore, the results also indicated that the root of plants grown in the untreated oil-contaminated samples (21.4 cm), was significantly higher ($p < 0.05$) than that of the plants grown in samples treated exclusively with biosurfactant (17 cm) and the plants grown in the untreated and uncontaminated samples (10.1 cm). The root of plants grown in samples treated exclusively with biosurfactant (17 cm) was also found to be significantly higher ($p < 0.05$) than that of the plants grown in the untreated, uncontaminated samples (10.1 cm).

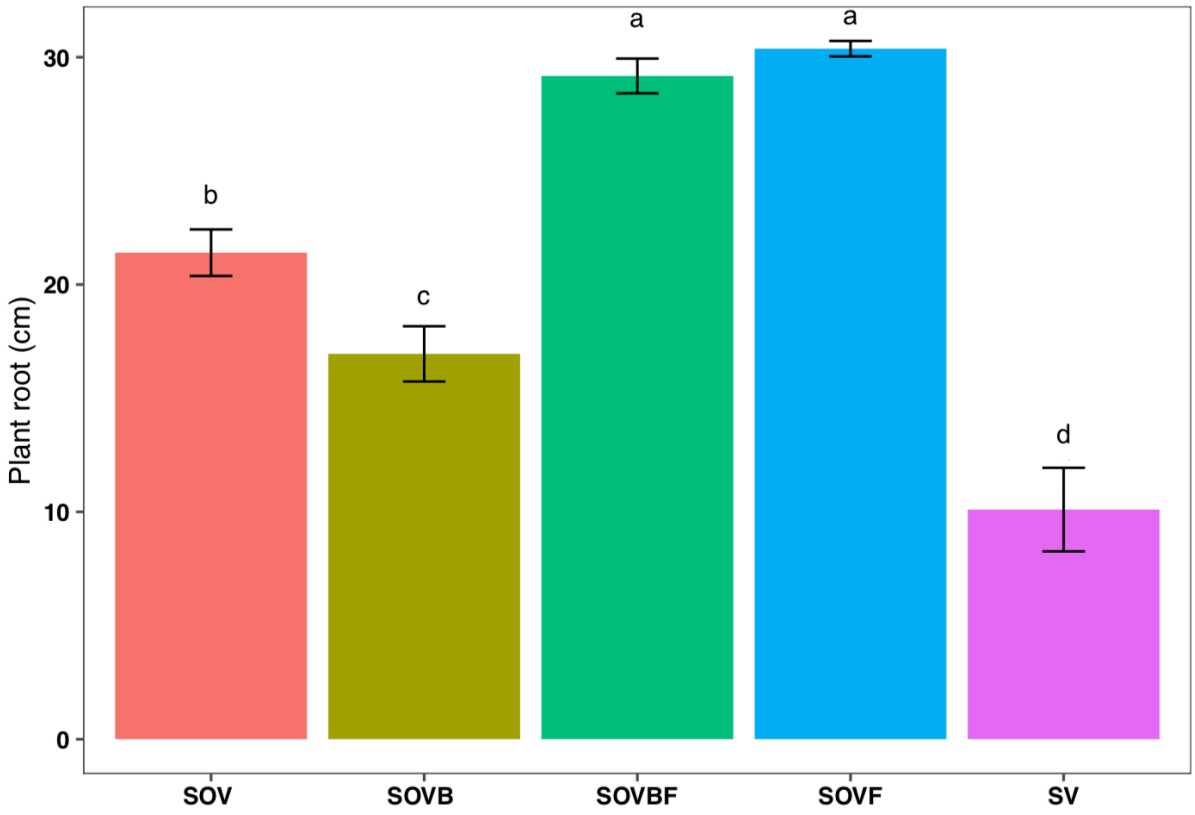



Figure 6.6 Growth of plants roots under different soil treatments after 72 days. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

Our results (Figure 6.7), have also shown that the shoot of plants grown in samples treated with fertiliser alone (63.4 cm), was statistically significant ($p < 0.05$) when compared to the root of plants grown in samples treated with fertiliser alone (30.4 cm). The results also showed that the shoot of plants grown in samples under the combined effect of fertiliser and biosurfactant (56.9 cm), was significantly higher ($p < 0.05$) than that of the root of plants grown under the combined effect of fertiliser and biosurfactant (29.2 cm). The shoot of plants grown in the untreated oil-contaminated sample (24.8 cm), was also significantly higher ($p < 0.05$) than the root of plants grown in the

untreated oil-contaminated samples (21.4 cm). Furthermore, the shoot of plants grown in samples treated exclusively with biosurfactant (23.2 cm) was significantly higher than the root of plants grown in the samples treated exclusively with biosurfactant (17 cm). The shoot of plants grown in the untreated and uncontaminated samples (18.5 cm) was also found to be significantly higher ($p < 0.05$) than that of the root of plants grown in the untreated and uncontaminated samples (10.1 cm).

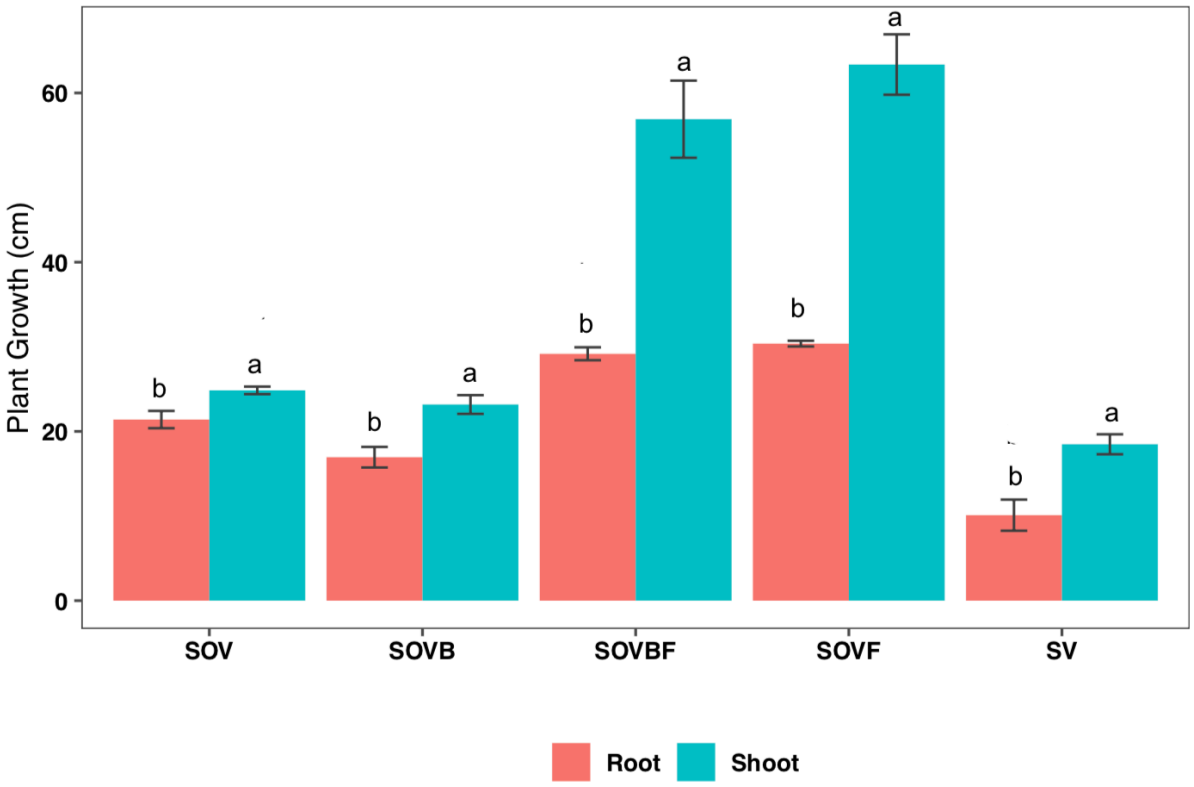



Figure 6.7 Growth of plant shoots and roots under different soil treatments after 72 days. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

Our results (Figure 6.8) have also shown that the DW biomass of the plants grown under the combined effect of fertiliser and biosurfactant (6.4 $g/m^2$), was significantly higher ($p < 0.05$) than

that of the plants grown in samples treated exclusively with fertilizer (4.0 g/m$^2$), the plants grown in samples treated exclusively with biosurfactant (2.8 g/m$^2$), the untreated oil-contaminated sample (2.2 g/m$^2$), and the untreated and uncontaminated samples (1.9 g/m$^2$). The results have also shown that the FW biomass of the plants grown in samples treated exclusively with fertilizer (4.0 g/m$^2$) was significantly higher ($p < 0.05$) than that of the untreated oil-contaminated sample (2.2 g/m$^2$), and the untreated and uncontaminated samples (1.9 g/m$^2$). Except for the FW biomass of plants grown in samples treated exclusively with biosurfactant (2.8 g/m$^2$).

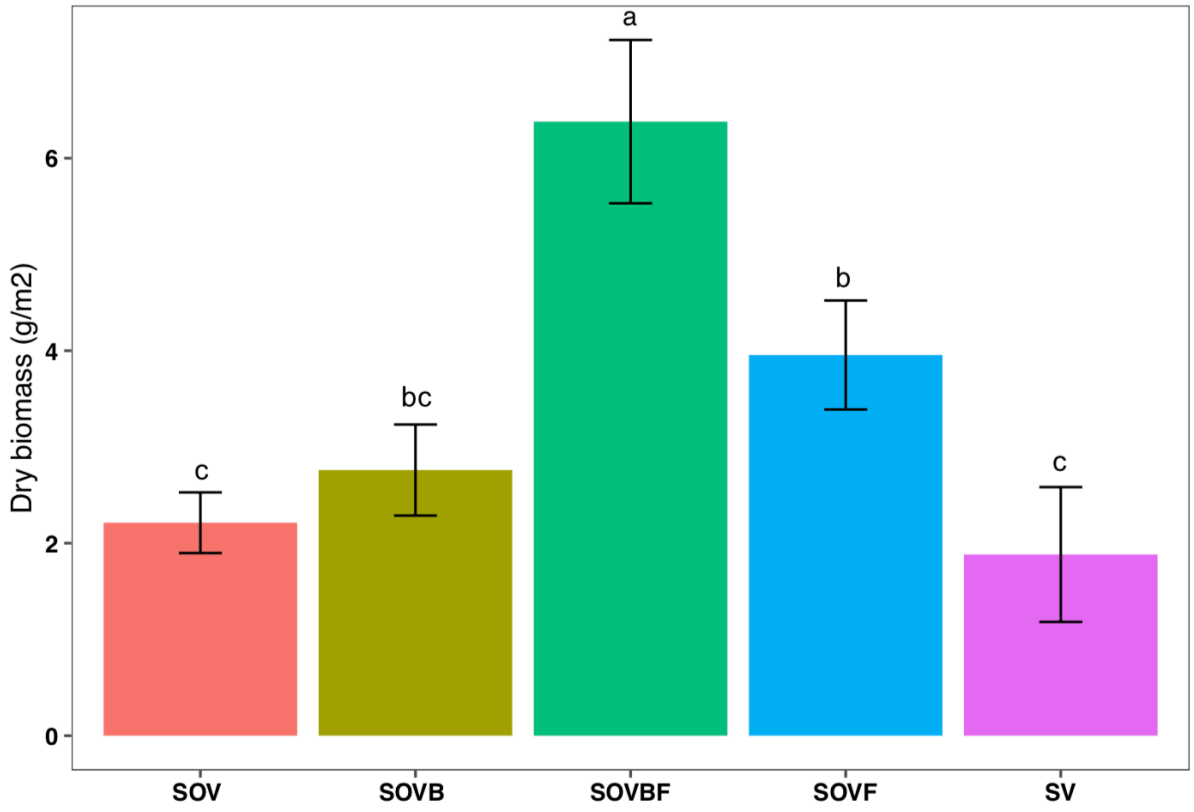



Figure 6.8 Dry biomass of plant after 72 days of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$).The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

In terms of the FW biomass, the results (Figure 6.9) have shown that the FW biomass of plants grown under the combined effect of fertiliser and biosurfactant (31.8 g/m$^2$), was significantly higher ($p < 0.05$) than that of plants grown in samples treated exclusively with fertilizer (26.0 g/m$^2$), the samples treated exclusively with biosurfactant (15.9 g/m$^2$), the untreated oil-contaminated samples (14.8 g/m$^2$), and the untreated, uncontaminated samples (13.8 g/m$^2$). The FW biomass of plants grown in samples treated exclusively with fertilizer (26.0 g/m$^2$) was also significantly higher ($p < 0.05$) than that of the samples treated exclusively with biosurfactant (15.9 g/m$^2$), the untreated oil-contaminated sample (14.8 g/m$^2$), and the untreated, uncontaminated samples (13.8 g/m$^2$).

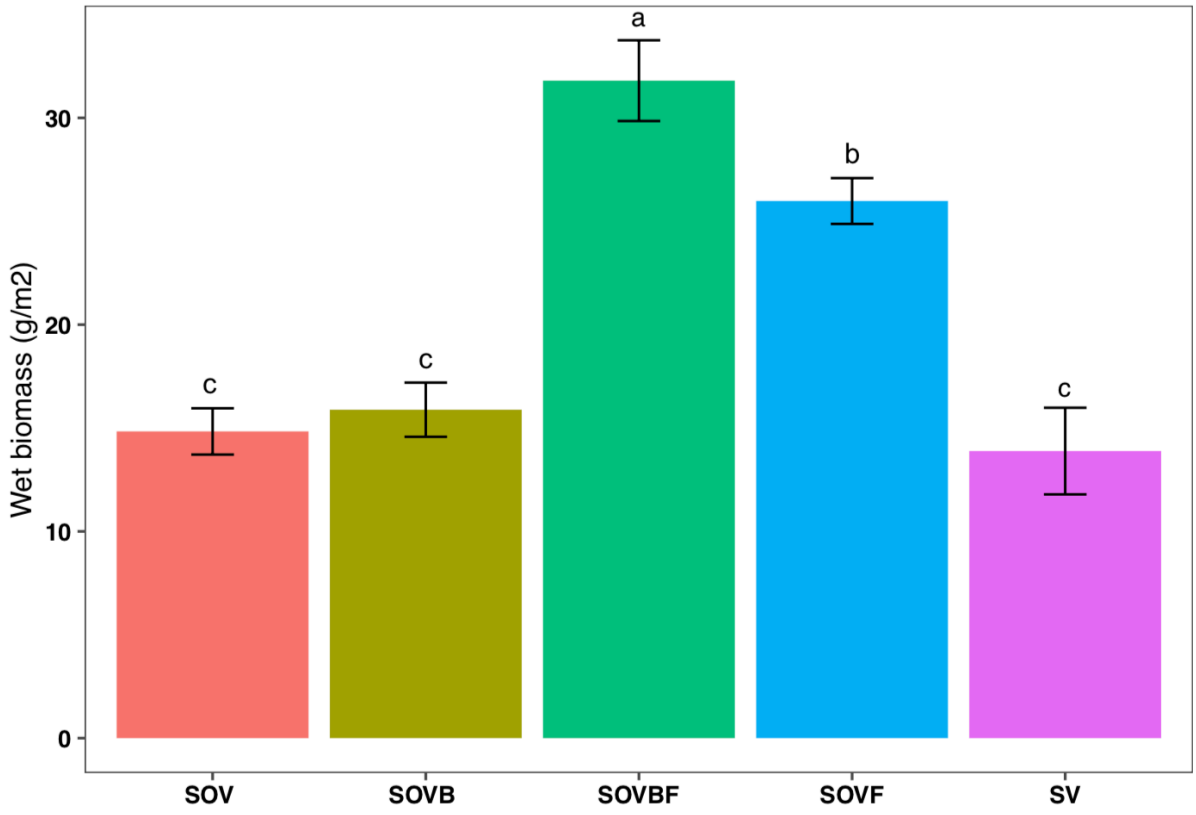



Figure 6.9 Wet biomass of plant after 72 days of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$).The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

Our results (Figure 6.10) have also shown that the FW biomass of plants grown under the combined effect of fertiliser and biosurfactant (31.8 g/m$^2$) was significantly higher ($p < 0.05$) than the DW biomass of the plants grown in samples under the combined effect of fertiliser and biosurfactant (6.4 g/m$^2$). The results also showed that the FW biomass of plants grown in samples treated exclusively with fertilizer (26.0 g/m$^2$) was significantly higher ($p < 0.05$) than the DW biomass of plants grown in samples treated exclusively with fertilizer (4.0 g/m$^2$). The FW of plants grown in samples treated exclusively with biosurfactant (15.9 g/m$^2$) was also significantly higher ($p < 0.05$) than the DW biomass of the plants grown in samples treated exclusively with biosurfactant (2.8 g/m$^2$). Furthermore, the FW biomass of samples in the untreated oil-contaminated sample (14.8 g/m$^2$) was significantly higher ($p < 0.05$) than the DW biomass of plants grown in the untreated oil-contaminated sample (2.2 g/m$^2$). The FW biomass of plants grown in the untreated, uncontaminated samples (13.8 g/m$^2$) was also significantly higher ($p < 0.05$) than the DW biomass of the plants grown in the untreated and uncontaminated samples (1.9 g/m$^2$).

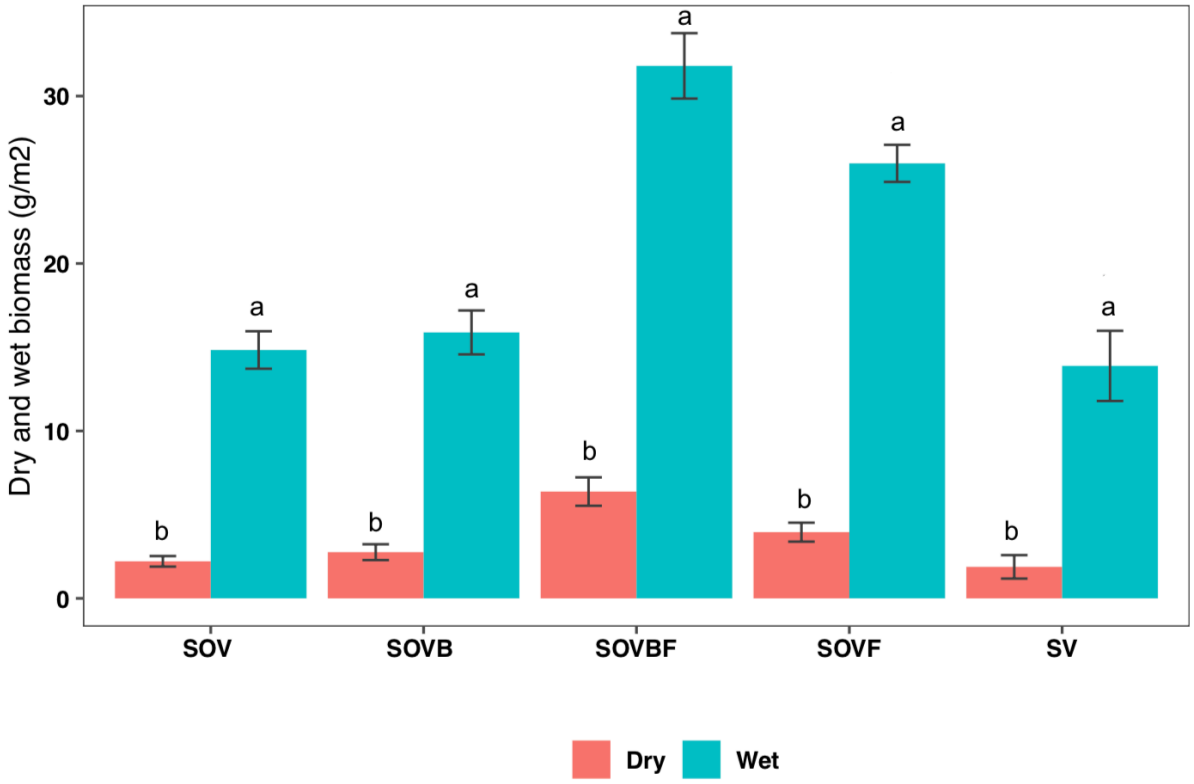



Figure 6.10 Wet and dry biomass of plant after 72 days of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$).The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil

Interestingly, the lowest water content was observed in plants grown in samples treated with a combination of fertiliser and biosurfactants. According to our findings (Figure 6.11), the water content in plants grown under the combined effect of fertiliser and biosurfactant (80.0%), was significantly lower ($p < 0.05$) than that of the plants grown in samples treated exclusively with fertiliser (84.8%), the untreated oil-contaminated samples (85.1%), and the untreated and uncontaminated samples (86.8%). Except for the plants grown in samples treated exclusively with biosurfactant, which was 82.7%.

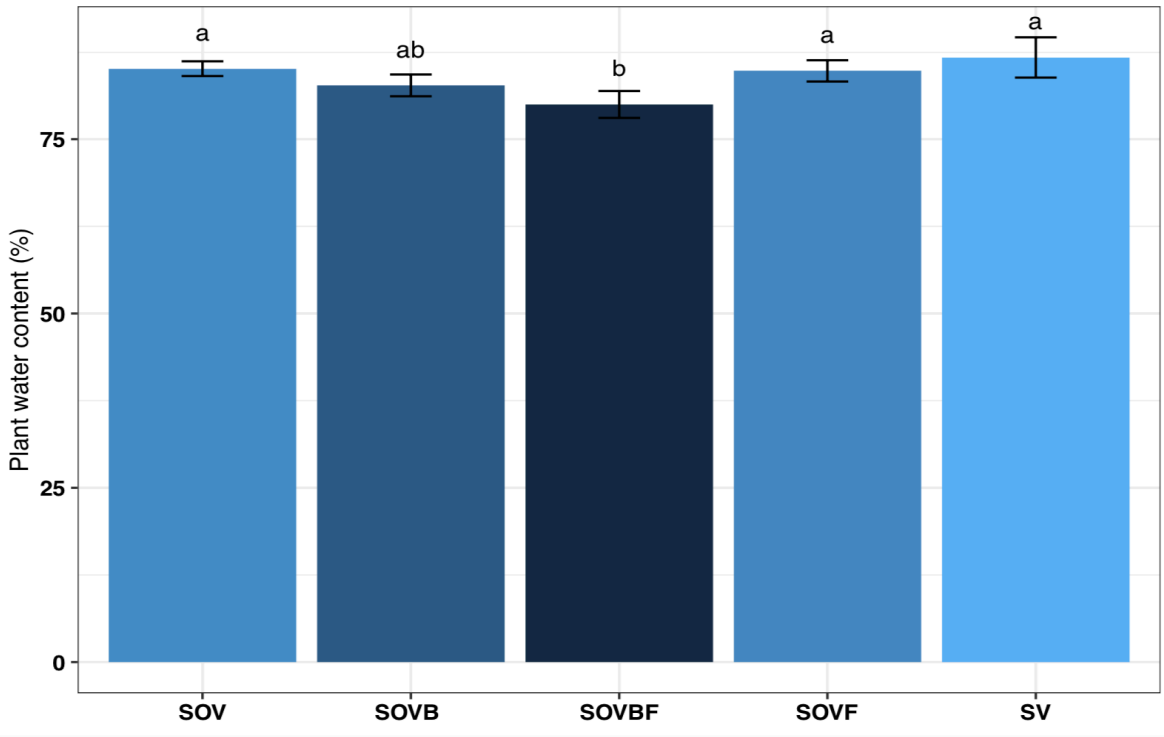



Figure 6.11 Water content in plants after 72 days of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$).The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

### 6.3.6 Root-shoot (R/S) biomass ratio

The several soil amendments applied during the process of phytoremediation of crude oil-contaminated soil have influenced the root-shoot biomass ratio of *C. zizanioides*. Our results have revealed that the highest root-shoot biomass ratio with an average of 1.72, was produced by the plants grown in samples treated exclusively with biosurfactant (Figure 6.8). The results revealed that the root-shoot biomass ratio of plants grown in samples treated solely with biosurfactant (1.72), was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil-contaminated samples (1.35), the samples treated solely with fertiliser (0.81), the samples treated with a mixture of fertiliser and biosurfactant (0.75), and the untreated and uncontaminated samples (0.18). The results also showed that the root-shoot biomass ratio of plants grown in the untreated oil-contaminated samples (1.35) was significantly higher ($p < 0.05$) than that of the samples treated solely with fertiliser (0.81), the samples treated with a mixture of fertiliser and biosurfactant (0.75), and the untreated and uncontaminated samples (0.18). Furthermore, the results also revealed that the root-shoot biomass ratio of plants grown in the samples treated solely with fertiliser (0.81), was significantly higher ($p < 0.05$) than that of the untreated uncontaminated samples (0.18). Except for the plants grown in samples treated with a mixture of fertiliser and biosurfactant (0.75). Furthermore, the root-shoot biomass ratio of plants grown in the samples treated with a mixture of fertiliser and biosurfactant (0.75), was significantly higher ($p < 0.05$) than that of the plants grown in the untreated, uncontaminated samples (0.18).

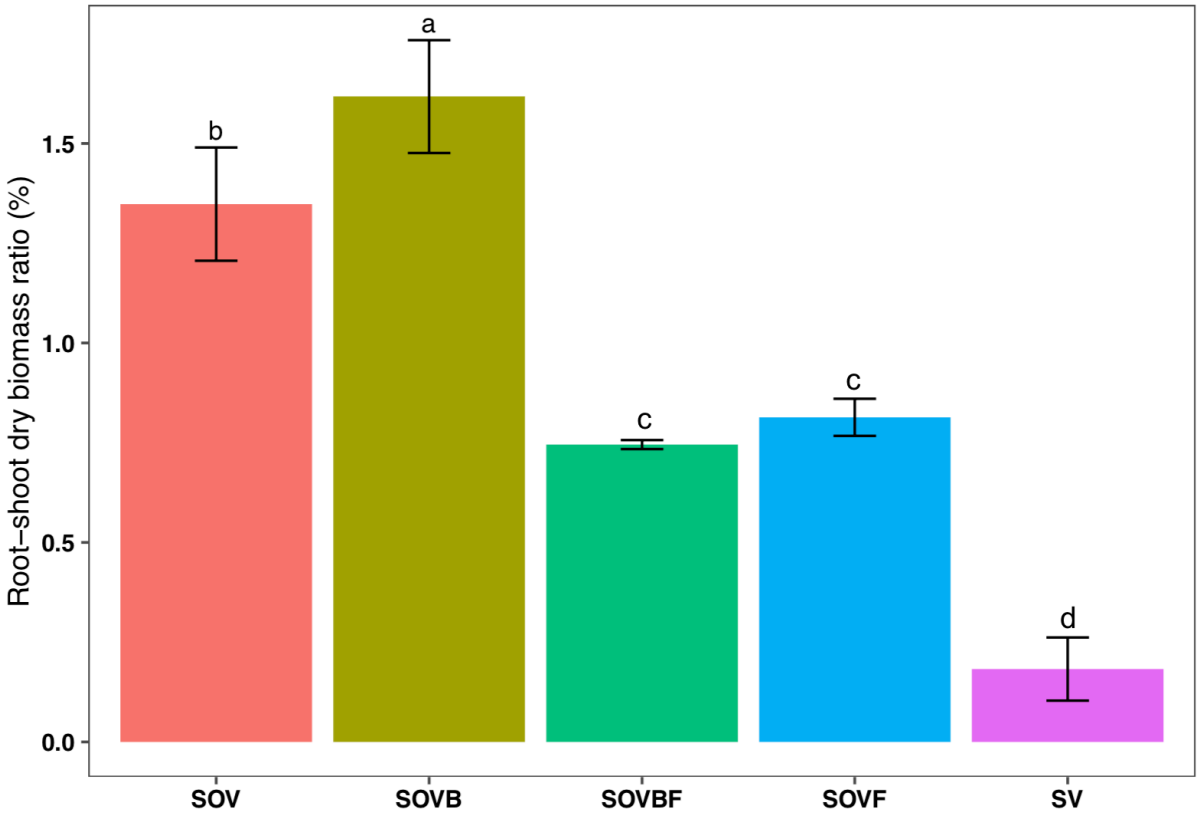



Figure 6.8 Root-shoot dry biomass of *C. zizanioides* after 32 weeks of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

### 6.3.7 Proline accumulation in plants

After 72 days, the level of proline accumulated in *C. zizanioides* during the phytoremediation of crude oil-contaminated soil was discovered to be affected by different soil treatments. Our findings have shown the highest level of proline accumulation in the plants (0.32 µg/g) in plants cultivated in the samples treated solely with biosurfactant (Figure 6.9). The results suggest that the level of proline accumulated in the plants grown in samples treated solely with biosurfactant (0.32 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown in the untreated and

uncontaminated samples (0.28 µg/g), the samples treated solely with fertiliser (0.24 µg/g) and the samples treated with a combination of fertiliser and biosurfactant, (0.19 µg/g). Except for the plants grown in the untreated oil contaminated samples (0.31 µg/g). The results also revealed that the level of proline accumulated in the plants grown in the untreated oil contaminated samples (0.31 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown in the untreated and uncontaminated samples (0.28 µg/g), the samples treated solely with fertiliser (0.24 µg/g) and the samples treated with a combination of fertiliser and biosurfactant (0.19 µg/g). Furthermore, the level of proline accumulated in plants grown in the untreated and uncontaminated samples (0.28 µg/g) was found to be significantly higher ($p < 0.05$) than that of the plants grown in samples treated solely with fertiliser (0.24 µg/g) and the samples treated with a combination of fertiliser and biosurfactant (0.19 µg/g). Furthermore, the level of proline accumulated in plants grown in samples treated solely with fertiliser (0.24 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown in samples treated with a combination of fertiliser and biosurfactant (0.19 µg/g).

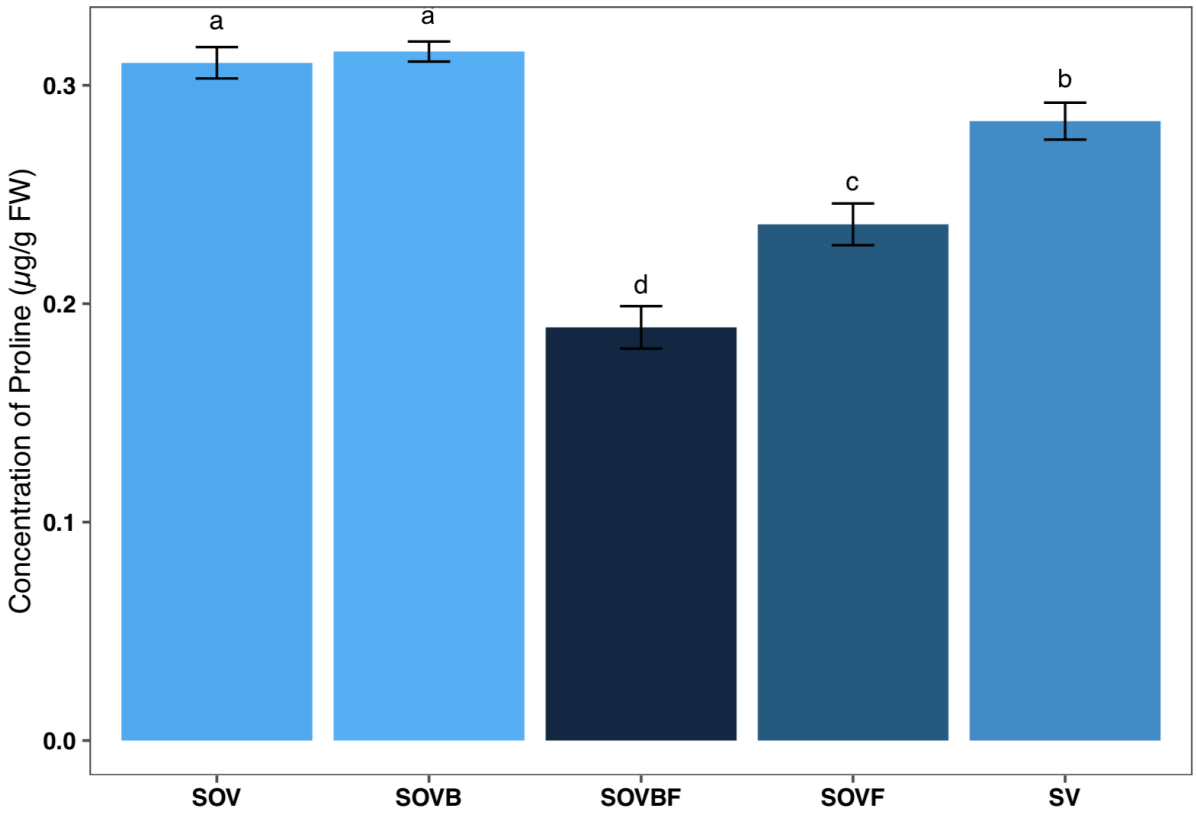



Figure 6.9 Concentration of proline in plants after 72 days of phytoremediation experiment. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

### 6.3.8 Spectrophotometric analysis of chlorophyll

The photosynthetic ability of *C. zizanioides* was impacted by different soil treatments used during the 72-day period of phytoremediation. The results (Figure 6.10), suggest that the quantity of chlorophyll A (2.5 µg/g) in plants grown under the combined effect of fertiliser and biosurfactant was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil contaminated samples (1.0 µg/g). Interestingly, the results also revealed that the quantity of chlorophyll A in

plants grown under the exclusive influence of fertiliser (2.4 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil contaminated samples (1.0 µg/g).

In term of chlorophyll B, the results revealed that the quantity of chlorophyll B in plants grown in samples treated solely with fertiliser (8.4 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown under the combined effect of fertiliser and biosurfactant (6.3 µg/g), the untreated and uncontaminated samples (4.3 µg/g), the samples treated solely with biosurfactant (4.2 µg/g), and the untreated oil contaminated samples (3.1 µg/g). Furthermore, the results showed that the quantity of chlorophyll B in plants grown under the combined effect of fertiliser and biosurfactant (6.3 µg/g) was significantly higher ($P < 0.05$) than that of the plants grown in the untreated and uncontaminated samples (4.3 µg/g), the samples treated solely with biosurfactant (4.2 µg/g), and the untreated oil contaminated samples (3.1 µg/g). Likewise, the quantity of chlorophyll B in plants grown in the untreated and uncontaminated samples (4.3 µg/g), was also found to be significantly higher ($p < 0.05$) than that of the plants cultivated in the untreated oil contaminated samples (3.1 µg/g). Except for the plants grown in samples treated solely with biosurfactant (4.2 µg/g). Interestingly, the results indicated that the quantity of chlorophyll B in plants cultivated in the samples treated solely with biosurfactant (4.2 µg/g) was significantly higher ($p < 0.05$) than that of the plants grown in the untreated oil contaminated samples (3.1 µg/g).

Our findings have also shown that the quantity of chlorophyll B in plants grown in samples treated solely with fertiliser (8.4 µg/g) was significantly higher ($p < 0.05$) than that of chlorophyll A in plants grown under the combined effect of fertiliser and biosurfactant (2.5 µg/g) and the plants cultivated in samples treated exclusively with fertiliser (2.4 µg/g), the untreated and uncontaminated samples (1.7 µg/g), the samples treated exclusively with biosurfactant (1.7 µg/g) and the untreated oil contaminated samples (1.0 µg/g). The quantity of chlorophyll B in plants grown under the combined effect of fertiliser and biosurfactant (6.3 µg/g), was also significantly higher ($p < 0.05$) than that of chlorophyll A in plants grown under the combined effect of fertiliser and biosurfactant (2.5 µg/g) the plants cultivated in samples treated exclusively with fertiliser (2.4 µg/g), the untreated and

uncontaminated samples (1.7 µg/g), the samples treated exclusively with biosurfactant, (1.7 µg/g) and the untreated oil contaminated samples (1.0 µg/g). Furthermore, the results also indicated that the quantity of chlorophyll B in the untreated and uncontaminated samples (4.3 µg/g) was significantly higher ($p < 0.05$) than that of chlorophyll A in plants grown under the combined effect of fertiliser and biosurfactant ( 2.5 µg/g) the samples treated exclusively with fertiliser (2.4 µg/g), the untreated and uncontaminated samples (1.7 µg/g), the samples treated exclusively with biosurfactant (1.7 µg/g) and the untreated oil contaminated samples (1.0 µg/g). Furthermore, the results indicated that the quantity of chlorophyll B in samples treated solely with biosurfactant (4.2 µg/g) was significantly higher ($p < 0.05$) than that of chlorophyll A in plants grown in samples under the combined effect of fertiliser and biosurfactant ( 2.5 µg/g) the samples treated exclusively with fertiliser ( 2.4 µg/g), the untreated and uncontaminated samples (1.7 µg/g), the samples treated exclusively with biosurfactant (1.7 µg/g) and the untreated oil contaminated samples (1.0 µg/g). The quantity of chlorophyll B in plants grown in the untreated oil contaminated samples (3.1 mg/g) was also found to be significantly higher ($p < 0.05$) than that of chlorophyll A in plants cultivated in the untreated and uncontaminated samples (1.7 µg/g), the samples treated exclusively with biosurfactant (1.7 µg/g) and the untreated oil contaminated samples (1.0 µg/g). Except for chlorophyll A in plants cultivated in samples grown under the combined effect of fertiliser and biosurfactant (2.5 µg/g) and the samples treated exclusively with fertiliser (2.4 µg/g).

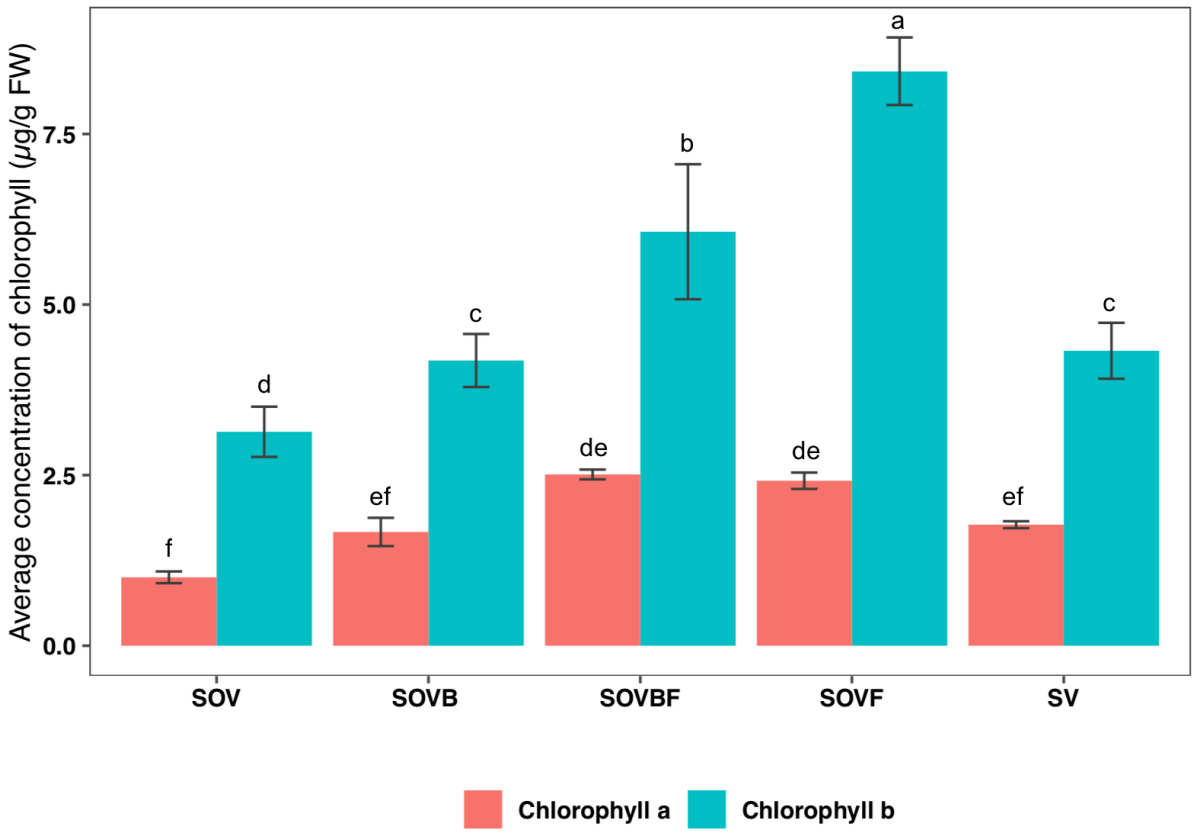



Figure 6.10 Average concentration of chlorophyll in plants after 72 days of phytoremediation. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

### 6.3.9 Morphological changes in plants

After 72 days of phytoremediation, microscopic analysis of *C. zizanioides* revealed that various soil treatments had impacted the plant's morphological structure. The results suggest that the highest rupture of parenchymatous cells occurred in plants grown in oil-contaminated samples in the absence of fertiliser (Figures 6.11 C and E). The results revealed a significant rupture of cells in plants grown in samples treated solely with biosurfactant (C) when compared to those grown in samples treated with a mixture of fertiliser and biosurfactant (A), the samples treated solely with

fertiliser (B), and the untreated and uncontaminated samples (D). The results also revealed that the cell rapture in plants grown in the untreated oil-contaminated samples (E) was significantly higher than that of the plants grown in samples treated with a mixture of fertiliser and biosurfactant (A), samples treated solely with fertiliser (B), and the untreated and uncontaminated control samples (D). Furthermore, the results showed that cell rapture was significantly higher in plants grown in samples treated solely with fertiliser (B) than in untreated and uncontaminated control samples (D). The results also suggest that the cell rapture that occurred in plants grown in samples treated with a mixture of fertiliser and biosurfactant (A), was significantly higher than that of plants grown in the untreated and uncontaminated samples (D). However, there was not much difference observed in the level of cell rapture that occurred between the plants grown in samples treated with a mixture of fertiliser and biosurfactant (A) and the samples treated solely with fertiliser (B).

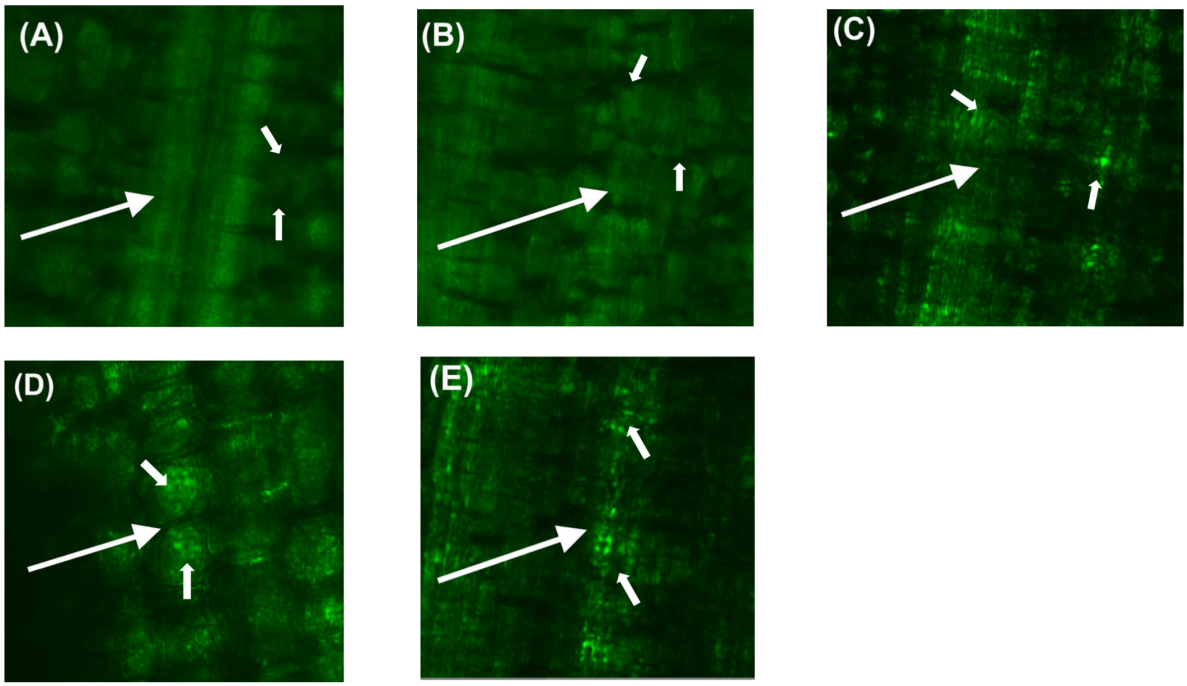



Figure 6.11 Transverse section of the mid region of the cells of shoots of *C. zizanioides* grown in different soil treatments after 72 days of phytoremediation. Arrows in (a,b,c and e) indicate areas of damaged plant cells. Arrows in (d control), indicates areas of fused plant cells (Magnification x40)

### 6.3.10 Soil microbial quantity

The quantity of soil microorganisms was affected by different soil amendments during the process of phytoremediation of crude oil contaminated soil. Our results have revealed that the combined effect of fertiliser and biosurfactant, as well as the solitary effect of fertiliser, have both promoted microbial quantities after a period of four weeks (Figures 6.12 A and B). The results suggest that the microbial growth in samples treated with fertiliser and biosurfactant mixture (596 cfu/g) was significantly higher ($p < 0.05$) than that of the samples treated solely with biosurfactant (467 cfu/g), the untreated oil contaminated samples vegetated with *C. zizanioides* (409 cfu/g), the unvegetated and untreated oil contaminated samples (361 cfu/g), the untreated and uncontaminated sample vegetated with *C. zizanioides*, (282 cfu/g), and the unvegetated, untreated and uncontaminated control sample (236 cfu/g). Except for the samples treated solely with fertiliser (522 cfu/g). The results also indicated that the microbial growth in samples treated solely with fertiliser (522 cfu/g), was significantly higher ($p < 0.05$) than that of the untreated oil contaminated samples (409 cfu/g), the unvegetated and untreated oil contaminated samples (361 cfu/g), the untreated and uncontaminated samples (282 cfu/g), and the unvegetated, untreated and uncontaminated control sample (236 cfu/g). Except for the samples treated solely with biosurfactant (467 cfu/g). Furthermore, the results revealed that the microbial growth in samples treated solely with biosurfactant (467 cfu/g) was statistically significant ($p < 0.05$) when compared with the unvegetated and untreated oil contaminated samples (361 cfu/g), the untreated and uncontaminated samples vegetated with *C. zizanioides* (282 cfu/g), and the unvegetated, untreated and uncontaminated samples (236 cfu/g). Except for the untreated oil contaminated samples (409 cfu/g). Furthermore, the results showed that the microbial growth in the untreated oil contaminated samples (409 cfu/g), was statistically significant ($p < 0.05$) when compared with the untreated and uncontaminated samples (282 cfu/g), and the unvegetated, untreated and uncontaminated samples (236 cfu/g). Except for the unvegetated and untreated oil contaminated samples (361 cfu/g). The microbial growth in the unvegetated and untreated oil contaminated samples (361 cfu/g) was also found to be statistically significant ($p < 0.05$) when compared with

the unvegetated, untreated and uncontaminated samples (236 cfu/g). Except for the untreated and uncontaminated samples vegetated with *C. zizanioides* (282 cfu/g). The microbial growth in the unvegetated and untreated oil contaminated sample (361 (cfu/g) was also found to be significantly higher than that of the untreated and uncontaminated samples (282 (cfu/g).

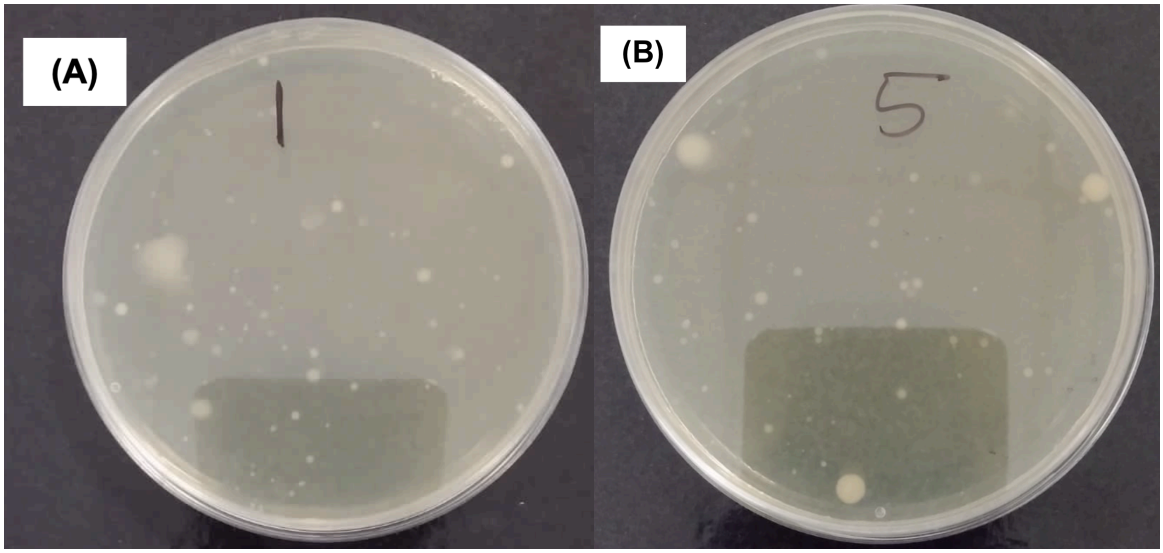



Figure 6.12 Colony-forming unit (cfu) in samples treated with fertiliser alone (A) and fertiliser/biosurfactant combination (B) after 72 days of phytoremediation experiment.

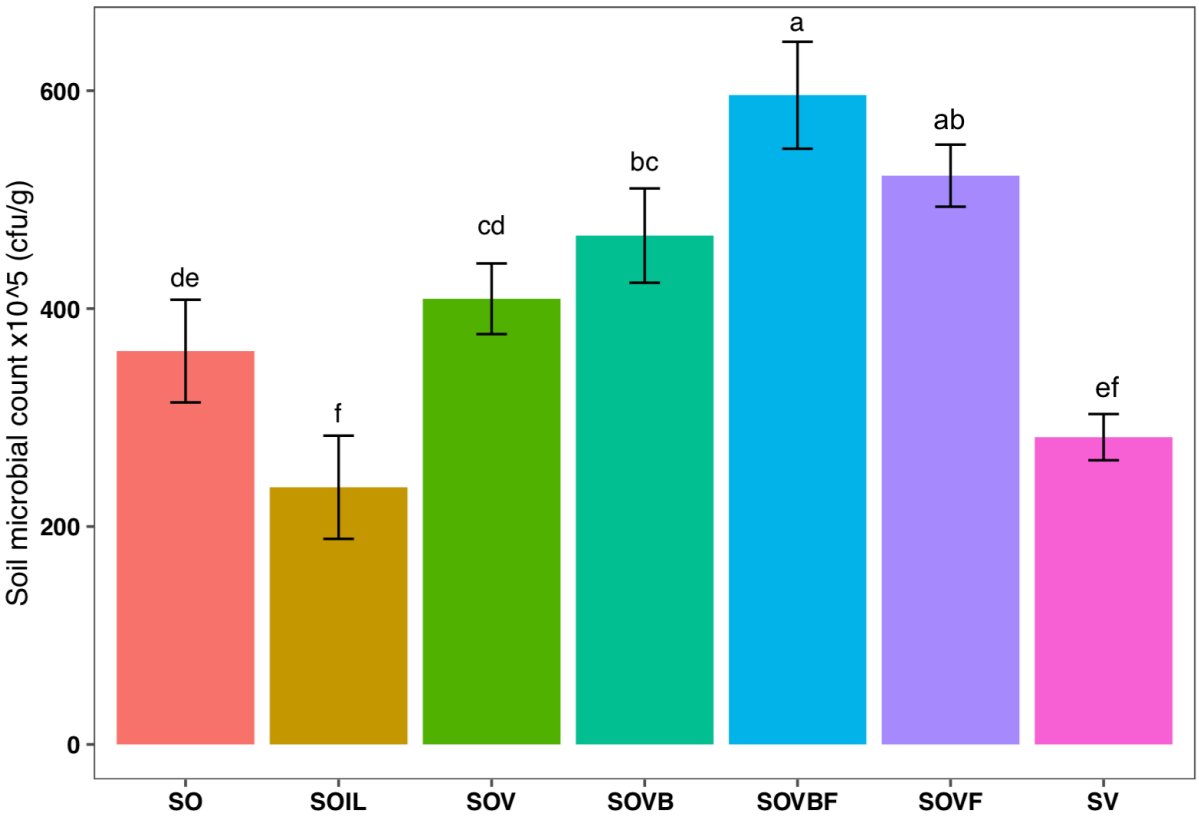



Figure 6.13 Soil microbial count after 72 days of greenhouse experiment. The values are given as mean ± standard deviation SD, (n=4). The alphabetical letters indicate the significant differences between variables, ($p \leq 0.05$). The abbreviations are: SO = crude oil contaminated soil only, SOV = crude oil contaminated soil vegetated with *C. zizanioides,* SOVB = biosurfactant only treated crude oil contaminated soil vegetated with *C. zizanioides*, SOVBF = biosurfactant and fertiliser treated, crude oil contaminated soil, vegetated with *C. zizanioides*, SOVF = fertiliser only treated crude oil contaminated soil vegetated with *C. zizanioides*, and SV = soil and *C. zizanioides* only.

### 6.3.11 Pearson's correlation coefficient analysis

The linear Pearson product moment correlation coefficient (r) was utilized to examine the presence and direction of a linear relationship between two sets of variables (Figure 6.14). The analysis demonstrated a strong positive correlation coefficient ($p < 0.05$) between the element Fe and TPH reduction. Similarly, a significant positive correlation coefficient ($p < 0.05$) was observed between the element Fe and dry biomass (Figure 6.14). Additionally, positive correlations ($p < 0.05$) were

found between the element Fe and chlorophylls a and b. Furthermore, the element P showed a high positive correlation coefficient ($p < 0.05$) with TPH reduction and displayed positive correlations ($p < 0.05$) with both wet and dry biomass. The results also indicated significant positive correlations ($p < 0.05$) between the element P and chlorophylls a and b. Moreover, positive correlations ($p < 0.05$) were observed between the element P and the shoot and root lengths. The element Si also showed a high positive correlation coefficient ($p < 0.05$) with proline. Furthermore, positive correlations ($p < 0.05$) were observed among the elements Al, Ti, Cl, and K. Additionally, positive correlations ($p < 0.05$) were found between the element Cl and TPH reduction, as well as between the element Cl and wet biomass. Moreover, the element Cl demonstrated positive correlations ($p < 0.05$) with both wet and dry biomass and with the root and shoot lengths. A positive correlation ($p < 0.05$) was also observed between the element Cl and chlorophyll A. Furthermore, a positive correlation ($p < 0.05$) was observed between the element Ti and chlorophyll a, as well as with the root and shoot lengths. Furthermore, the element Ti exhibited positive correlations ($p < 0.05$) with the elements Mg, S, and Ca. Additionally, the element Ca displayed a strong positive correlation ($p < 0.05$) with both wet and dry biomass and with chlorophyll a. Moreover, the element Ca showed positive correlations ($p < 0.05$) with the elements S and Mg. A positive correlation ($p < 0.05$) was also observed between the element Mg and chlorophyll a. The element Mg also showed a positive correlation ($p < 0.05$) with root length. Regarding plant growth parameters, there were high positive correlations ($p < 0.05$) between root length and chlorophyll a, as well as between root length and chlorophyll b. Similarly, strong positive correlations ($p < 0.05$) were found between wet biomass and chlorophyll a, and between dry biomass and chlorophyll b. Additionally, positive correlations ($p < 0.05$) were observed between TPH reduction and chlorophyll a, and between TPH reduction and chlorophyll b. The wet biomass also exhibited high positive correlations ($p < 0.05$) with shoot length and root length. On the other hand, proline content showed high negative correlations ($p < 0.05$) with chlorophylls a and b. Additionally, proline content was negatively correlated with both shoot and root lengths, and with both wet and dry biomass. Furthermore, proline content exhibited high negative correlations ($p < 0.05$) with TPH reduction. The results also revealed negative correlations between proline and the

elements Al, K, Fe, P, Cl, Ti, Ca, S, and Mg. Additionally, there were negative correlations between the element Al and chlorophylls a and b, as well as between the element Al and root length. Moreover, a negative correlation ($p < 0.05$) was observed between the element Al and the element Si. Furthermore, the element Si demonstrated strong negative correlations ($p < 0.05$) with the elements Mg, S, Ca, Ti, Cl, P, Fe, and K. Additionally, the element Si was found to have a significant negative correlation with TPH reduction, and it also showed a negative correlation with chlorophyll A. Furthermore, the element Si was negatively correlated with both shoot and root lengths. The element Ti also exhibited a negative correlation with the element Si. Similarly, negative correlations were found between the element Ca and the element Si, and between the element Mg and the element Si. Finally, the element Al was negatively correlated with chlorophyll b and with the element Si.

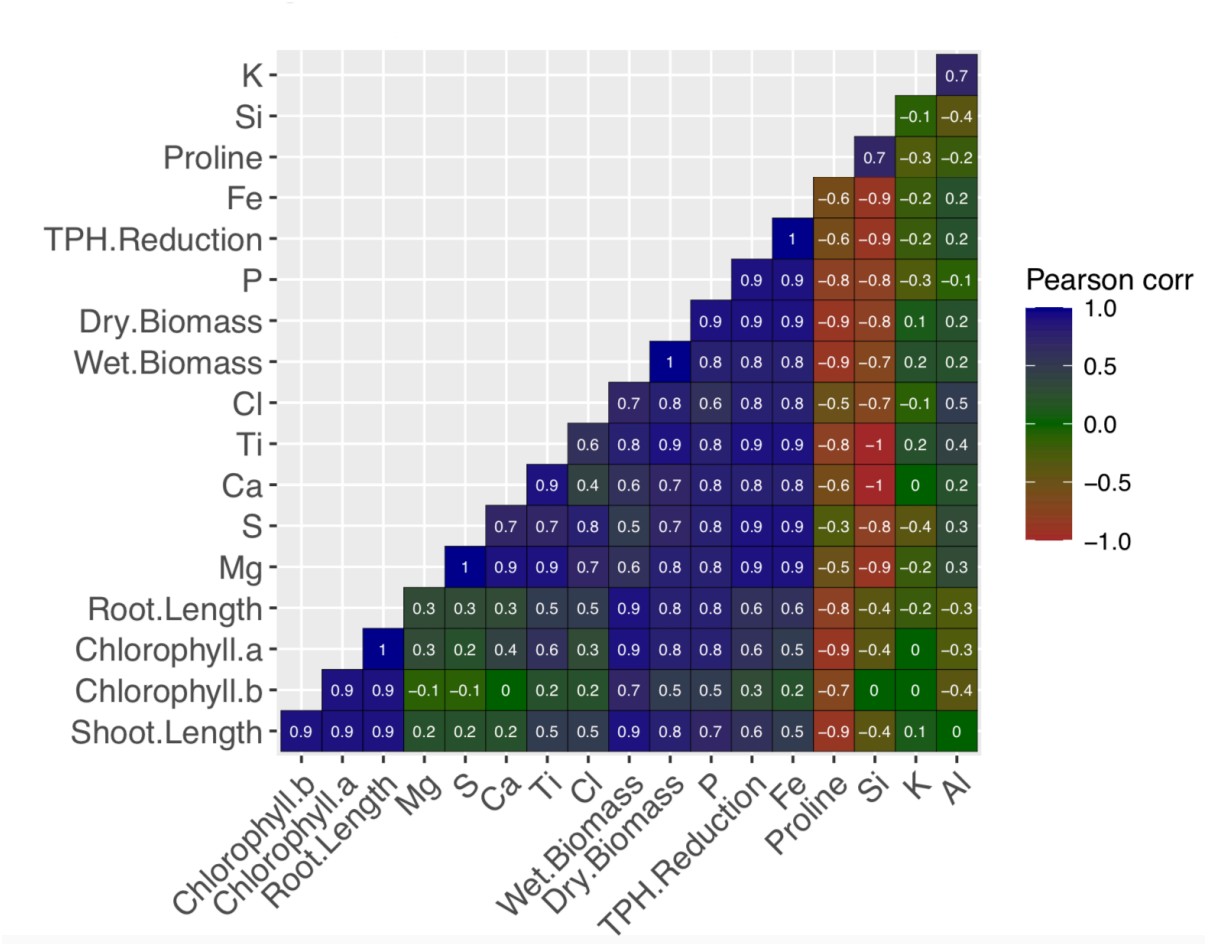



Figure 6.14 Pearson's correlation matrix for soil elements and their relationship with variables. The abbreviations are Fe = Iron, P = Phosphorous, Ti = Titanium, Mg = Magnesium, S = Sulphur, Ca = Calcium, Cl = Chlorine, Al = Aluminium, K = Potassium, and Si = Silicon, ($p \leq 0.05$).

## 6.4 Discussion

### 6.4.4 Effect of soil minerals on the growth and tolerance of *C. zizanioides*

The analysis of soil minerals was carried out FTIR and XRD techniques to determine the minerals present in the soil using. This present research revealed the presence of quartz silica in all samples. The other minerals including quartz, kaolinite, calcite, gibbsite and organic carbons have been identified from the FTIR spectrum by comparing the observed wave numbers with available literature (Ramasamy et al., 2009; Ramasamy et al., 2011; Ramasamy et al., 2014). However, the main minerals found in the samples were quartz and kaolinite, with trace amounts of calcite, gibbsite, and organic carbon (Table 6.2). The findings of this research also showed that the minerals identified in the XRD patterns include quartz, microcline feldspar, orthoclase feldspar, calcite, hematite, albite, aragonite, and monazite. However, the most abundant mineral identified in all samples was found to be silica (quartz). The absence of some minerals such as kaolinite, gibbsite, and organic carbon in the XRD analysis that were identified through FTIR may be linked to their disordered state due to loss of crystalline nature (Ramasamy et al., 2014). Also, the presence of small amounts of minerals such as monazite, zircon, and magnetite in the XRD analysis but their absence in the FTIR indicates their crystalline nature. Therefore, the minerals identified in the FTIR analysis have been verified by the results obtained in the XRD analysis. Our results revealed that the plants grown in all samples had survived throughout the 32 weeks of the phytoremediation experiment. This could be linked to the effect of quartz silica in the soil, which promoted the survival of the plants throughout the period of the research. This findings support the findings of Rashad and Hussien, (2020), who studied the agronomic efficiency of quartz silica, feldspar, zeolite on *Glycine max* L. and discovered that quartz silica was more efficient in improving

the growth and yield of the plant. Puspitasari and Indradewa, (2019), studied the effect of silica on the growth and yield of Sheena, and Snow-White varieties of *Dendranthema sp.,* and discovered an improvement in the growth and yield of the Snow-White variety. Abdel-Haliem et al., (2017), studied the effect of silica ion and nano silica on rice plants under salinity stress and discovered the efficiency of silica ion in reducing the physiological and biochemical impact of NaCl on the plant. Previous studies have also shown the beneficial effects of quartz silica on plant growth and stress tolerance. The current study found that all plants (*C. zizanioides*) exposed to crude oil-contaminated soil suffered from the effects of petroleum contaminants (Figures 6.11 and 6.13). This could be attributed to the phytotoxic effect of petroleum contaminants, which had a negative physiological and biochemical impact on the plants. This implies that the abundance of quartz silica in the soil has promoted the survival of *C. zizanioides* in crude oil-contaminated soil during phytoremediation and enhanced the growth and stress tolerance of the plant.

### 6.4.3 Effect of soil treatments on removal of total petroleum and hydrocarbons

The influence of different soil amendments on the removal of TPH during phytoremediation of crude oil-contaminated soil using *C. zizanioides* was investigated. The findings of this present research have indicated that the highest removal of TPH and their fractions from the soil was influenced by the combined effect of fertiliser and biosurfactant (Figures. 6.6 and 6.7). Perhaps the effect of biosurfactant may have aided nutrient absorption and growth of *C. zizanioides*, which in turn increased the absorption to SOM and degradation of TPH solubilized by biosurfactants. The results have also revealed that the exclusive application of biosurfactant resulted in reducing the concentration of TPH and their fractions in the soil (Figures. 6.6 and 6.7). The direct effect of biosurfactant may have solubilised the TPH for easy absorption by the plant thereby aiding the removal of TPH in the soil. Almansoory et al., (2015), also found 93.5% reduction of TPH, that was facilitated by biosurfactant in their research on the potential of biosurfactant on the treatment of gasoline contaminated soil. Previous studies have also determined the potential of fertiliser and

biosurfactants in enhancing the removal of TPH from the soil (Liao et al., 2016; Brown et al., 2017; Lu et al., 2019). Furthermore, the results have also shown that the samples treated solely with fertiliser had the lowest reduction in TPH. This could have been caused by the insolubility of TPH, which may have affected their bioavailability and subsequent removal through absorption by the plants. This result agrees with the findings of Brown et al., (2017), which revealed the lowest TPH reduction in samples treated with fertiliser alone compared to other treatments including biosurfactant, bulking and sorption agents, enzyme extract, as well as soil neutralisation. Accordingly, the findings of this study suggest that the combined effect of fertiliser and biosurfactant is most efficient when utilising *C. zizanioides* to remove TPH from soil contaminated with crude oil.

### 6.4.1 Effect of soil treatments on the growth of *C. zizanioides*

The effects of various soil treatments on the growth of *C. zizanioides* were investigated in a 72-day greenhouse experiment on the phytoremediation of crude oil-contaminated soil. The findings have revealed the highest growth and biomass of plants in samples under the exclusive effect of fertiliser (Figures. 6.1 and 6.2). This may be attributed to the controlled application of fertiliser, which prevented nutrient deficiencies caused by petroleum toxicity and thus increased plant growth. This is supported by the findings of the research conducted by Cartmill et al., (2014) that showed increased growth of *Lolium multiflorum lam* by preventing the effect of petroleum contaminants through fertiliser application. It has previously been reported that the presence of Al in the soil inhibits plants' ability to absorb and use essential nutrient elements like phosphorus, calcium, magnesium, and sulphate in the soil (Mariano and Keltjens, 2005; Silva et al., 2010; Yang et al., 2013; Roshani et al., 2014). The findings of this present research revealed that the combined effect of fertiliser and biosurfactant has enhanced the growth and biomass production of *C. zizanioides* despite the presence of Al in the soil (Figures 6.1, 6.2 and Table 6.1). This may have been caused by the effect of the nutrients supplied by the fertiliser, which attenuated the toxicity

of Al in the crude oil contaminated soil promoted plant growth. Our findings show a significant increase in plant growth under the sole influence of biosurfactant and in untreated crude oil-contaminated soil compared to untreated and uncontaminated control soils. This could have happened due to the plant's absorption and utilisation of petroleum as a carbon source, which increased the growth of the plants. This result agrees with the finding of Das and Kumar, (2016), which revealed the effect of biosurfactants in improving the growth of *Withania somnifera* through the use of petroleum contaminants as a carbon source. Furthermore, the results have also shown that the effect of petroleum contaminants has inhibited the growth of *C. zizanioides* in the absence of fertiliser (Figure 6.1 and 6.2). This may have been caused by the toxicity of petroleum contaminants, which reduced the growth of shoots and the biomass yield of the plant during the process of phytoremediation in the absence of fertiliser. Previous research has also found that the toxicity of petroleum contaminants has a negative impact on plant growth (Meudec et al., 2007; Razmjoo and Adavi, 2012; Zarinkamar et al., 2013; Adesina and Adelasoye, 2014). The findings of our research imply that NPK fertilisers play a major role in enhancing the growth of *C. zizanioides* in crude oil-contaminated soil, which also facilitates the phytoremediation process of petroleum contaminants. It also suggests that the application of biosurfactants during phytoremediation of crude oil-contaminated soil does not affect plant growth.

### 6.4.5 Effect of soil treatments on root-shoot biomass ratio

The impact of different soil treatments on root-shoot biomass ratio of *C. zizanioides* during phytoremediation of crude oil-contaminated soil was investigated. The results showed a higher root-shoot biomass ratio in plants grown in untreated, oil-contaminated samples than the fertilizer-treated and the uncontaminated control samples. This could have happened because of the accumulation of petroleum contaminants in the plants due to the high concentration of petroleum contaminants in the untreated soil, which was absorbed by the plants. This result agrees with the

findings of Kiamarsi et al., (2022), who studied the bio-removal of crude oil by *C. zizanioides* and bacterial consortium and discovered increased root-shoot biomass ratio in plant caused by petroleum contaminants. Duan et al., (2023), studied the effect of petroleum contaminated soil on the growth of *Hippophae rhamnoides* and discovered increased root-shoot biomass ratio as a result of increased concentration of petroleum contaminants. Hussain et al., (2019), studied the physiological and growth responses of *Lolium multiflorum* and *Lotus corniculatus* to a diesel and vegetable oil amended soil and discovered the negative impact of the contaminants on the root-shoot biomass. Nie et al., (2010), studied plants response to petroleum pollution and discovered increased root-shoot biomass ratio of *Phragmites australis* due to high concentration of petroleum contamination. This present research revealed the highest root-shoot biomass ratio in plants grown in samples treated with biosurfactant alone (Figure 6.10). This could have happened because of the accumulation of petroleum contaminants in the plants, which was aided by the effect of the biosurfactant making them more bioavailable to be absorbed by the plants. Our results revealed a low root-shoot biomass ratio in plants grown in samples treated with fertiliser alone. This may have been caused by the nutrients supplied by fertiliser applications, which enhanced the plants' tolerance to petroleum contaminants and thus promoted their growth. This result contradicts the findings of Poeplau and Kätterer, (2017), who studied the impact of soil texture (clay loam and sandy soils) on root-shoot ratio of *Hordeum vulgare*, L. and discovered that the long-term history of NPK fertiliser application had no effect on the root-shoot ration of the plant. Our result is supported by the findings of Hoang et al., (2022), who studied the effect of phosphorous application on three wild plant species including *Banksia seminuda*, *Chloris truncata*, a grass, and *Hakea prostrata*, a shrub grown in petroleum contaminated soil. The results revealed lower root-shoot biomass ratio in samples treated with phosphorous than the untreated petroleum contaminated samples. Monsuru and Daud, (2016), discovered that the root-shoot ratio of *Hevea brasiliensis* was positively impacted by fertiliser application. Chen et al., (2020), studied the impact of nitrogen application on cotton in Yellow River Basin, China and discovered that application of moderate rate of nitrogen had a positive impact on the plant. This present research revealed a low root-shoot biomass ratio in plants grown in samples treated with a combination of fertiliser and biosurfactant.

This could be attributed to the positive impact of fertiliser on the plant, which enhanced the plant's tolerance to the toxicity of petroleum contaminants and enhanced plant growth.

### 6.4.6 Effect of petroleum induced stress on proline accumulation

The accumulation of proline in *C. zizanioides* in response to petroleum-induced stress during phytoremediation was investigated after a period of 72 days. The results have revealed that there were significant levels proline concentrations of 0.32 µg/g and 0.31 µg/g in plants grown in soils treated with biosurfactant alone and in the untreated crude oil contaminated soil (Figure 6.11). This may be linked to the stress induced by the toxicity of petroleum contaminants on the plants. This result supports the findings of Shahzad et al., (2016) that revealed that the toxicity of petroleum contaminants can induce stress on plants. The conclusions drawn from previous research have also shown that high proline accumulates in plants when subjected stress (Khedr et al., 2003; Cartmill et al., 2014; Ahmad et al., 2015). The results also revealed that proline accumulation was significantly lower in plants grown in samples treated solely with fertiliser (Figure 6.11). This could be due to the direct effect of fertiliser application, which alleviated the toxicity of petroleum contaminants and aided the growth of *C. zizanioides*. This result is supported by the findings of Zhang et al., (2022), who discovered that nitrogen and phosphorous fertiliser reduced the toxic effect petroleum and heavy metals on the growth of ryegrass. Saleem et al., (2021), also discovered that the use of NPK fertiliser and gibberellic acid helped to reduce the boron toxicity on *Coriandrum sativum* L. and promoted the growth of the plant. Dawood et al., (2014), studied the effect of potassium fertiliser on *Phaseolus vulgaris* and discovered that it enhanced the plant's salt tolerance. This current study discovered that plants grown with a fertiliser and biosurfactant combination accumulated the least amount of proline. This could be attributed to the use of fertiliser, which was able to reduce the toxicity of petroleum contaminants while also preventing plant growth inhibition despite the presence of biosurfactant that could enhance the absorption of

petroleum contaminants. The results of this current research have also indicated a positive correlation ($p < 0.05$) between the element Si and proline (Figure 6.14). Overall, these findings provide valuable insights into the relationships between the element Si and proline accumulation, helping to understand the complex dynamics within the plant when subjected to stress.

### 6.4.7 Effect of petroleum induced stress on plant pigment

The effect of petroleum contaminants on chlorophyll concentrations in during phytoremediation was studied using various soil amendments. The results revealed that plants grown in samples treated solely with fertiliser had the highest concentration of chlorophyll b (Figure 6.12). The concentration of chlorophyll a in plants grown in samples treated exclusively with fertiliser was also found to be higher than that of the untreated, oil-contaminated samples. This could be linked to the effect of fertiliser application, which prevented the toxicity of petroleum contaminants from inhibiting the growth and chlorophyll content of *C. zizanioides*. This finding supports the findings of Wilkinson et al., (2019) which revealed increased chlorophyll content in Casablanca leaf by the effect of stabilised urea nitrogen (SAN). The use of fertilisers for soil amendment is generally known to improve the yield of plants. The results also revealed that the effect of petroleum contaminants on plants in the absence of fertiliser and biosurfactant (untreated samples) resulted in a decrease in concentration of chlorophylls a and b (Figure 6.12). This negative effect could be directly related to crude oil toxicity on plants, which could have affected their physiology. This result is supported by the findings of Abdollahzadeh et al., (2019), who studied the phytoremediation of petroleum-contaminated soil by Salicornia and discovered a decrease in chlorophylls a and b due to the toxicity of the petroleum contaminants in the plant. Atta et al., (2020), M investigated green technology for crude oil-contaminated water remediation using *Eichornia crassipes* (Mart.) Solms and discovered that the toxicity of crude oil contamination reduced the chlorophyll and carotenoid content in plant tissues. Cartmill et al., (2014) also discovered decreased chlorophyll content of

*Lolium multiflorum Lam* grown in petroleum contaminated soil. This current study has discovered a significantly higher concentration of chlorophyll b in plants grown in samples under the combined effects of fertiliser and biosurfactant than in the other samples without fertiliser treatment. This most likely occurred because of the effect of the fertiliser, which alleviated petroleum toxicity, improved plant growth, and thus increased the chlorophyll concentration in the plant despite the influence of the biosurfactant that could promote petroleum absorption by the plants. The findings of this research have also showed a strong positive correlation ($p < 0.05$) between the element P and chlorophylls a and b. These findings provide significant practical implications for enhancing plant productivity and developing effective environmental remediation strategies, especially in areas contaminated with petroleum.

### 6.4.8 Phytotoxic effect of petroleum on plant morphology

The potential phytotoxic effect induced by petroleum contaminants on the cells of *C. zizanioides* was investigated after 72 days of phytoremediation. The current study's findings revealed that the cellular morphology of plants grown in oil-contaminated samples treated exclusively with biosurfactant suffered significant damage (Figure 6.13C). Most likely, this occurred due to increased solubility and bioavailability of the petroleum contaminants which aided their bioaccumulation within the tissues of the plant. Furthermore, significant cell damage was discovered in plants grown in untreated crude oil-contaminated samples (Figure 6.13E). This could be due to the direct phytotoxic effect of petroleum contaminants absorbed by plants grown in crude oil-contaminated soil during phytoremediation. This result is similar to the findings of Akapo et al., (2011), which revealed that the presence of crude oil in the environment caused cell shape disruptions, changes in mitotic indices, and apical cellular morphology and structure distortions of *P. stratiotes.* This current study found a minor change in the cellular morphology of plants grown in soil treated with a fertiliser and biosurfactant mixture (Figure 6.13A). This could be due to

biosurfactants increasing the solubility of petroleum contaminants while also assisting plant nutrient absorption, thereby reducing the toxic effects of petroleum contaminants on plants. The present study also discovered a minor change in the cellular morphology of plants grown in soil treated solely with fertiliser (Figure 6.13B). This could be due to the nutrients provided by the fertilizer, which may have reduced the toxicity of petroleum contaminants on the plants, resulting in the minor effect on the plants.

### 6.4.2 Effect of soil treatments on microbial quantity

A serial dilution was carried out to determine the effect of various soil treatments on the microbial quantity in the soil during phytoremediation of crude oil contaminated soil. The results have shown an increase in soil microbial quantity through the exclusive application of fertiliser. This could have occurred because of the direct effect of fertiliser, which encouraged both microbial and plant growth and may have positively impacted the proliferation of soil microorganisms in the rhizosphere. This result support the findings of Okwonna and Otaraku, (2022), who discovered increased microbial quantity through the application of NPK fertiliser during the *in situ* treatment of petroleum hydrocarbon contaminated soil. Aghalibe et al., (2017), studied bioremediation of petroleum hydrocarbons from crude oil contaminated soil and discovered that NPK fertiliser was effective at promoting microbial growth. Margesin et al., (2007), also discovered the positive effect of inorganic fertiliser on increasing soil microbial biomass. Previous research have also drawn conclusions on the importance of fertilisers and biosurfactant in increasing soil microbial colony (Unterbrunner et al., 2007; M et al., 2010; Liang et al., 2011; Chen et al., 2015). This present study has revealed that the combined effect of fertiliser and biosurfactant has produced the highest soil microbial counts (Figures. 6.4B and 6.5). This could be linked to the previously mentioned synergistic effect of fertiliser and biosurfactant, which promoted plant growth through facilitated nutrient uptake and thus favoured more microbial growth in the root region of the plant. Our results also revealed

increased microbial quantity, which resulted from the sole effect of the biosurfactant during the phytoremediation process. This may be linked to the effect of the biosurfactant, which solubilised the petroleum contaminants, making them more bioavailable, which in turn promoted microbial growth. This result backs up the findings of Guo and Wen, (2021), who investigated the effect of a biosurfactant extracted from swine waste water on the bioremediation of benzo(a)pyrene-contaminated soil and water and discovered an increase in microbial quantity as a result of biosurfactant application. Gao et al., (2022), studied how different petroleum hydrocarbon pollution levels affected microecosystems and discovered that heavy contamination increased the abundance of the *Proteobacteria phylum* by 3.91%–57.01% and medium contamination increased the *Actinobacteria phylum* by 1.69%–0.26%. Our findings suggest that using a biosurfactant during phytoremediation of crude oil-contaminated soil by *C. zizanioides* helps to solubilize petroleum contaminants, making them more bioavailable, which in turn encourages microbial growth.

### 6.4.9 Relationship between soil elements, plant growth and phytoremediation

A correlation analysis was performed to strengthen the relationship between pairs of variables. There was a high positive correlation between the element P and dry biomass (Figure 6.14). This indicates that the effect of P in the soil has promoted the growth of *C. zizanioides* during phytoremediation of crude oil contaminated soil. The results also showed that the presence of P in the soil also produced a positive, non-significant correlation with *C. zizanioides* shoot length, root length, and wet biomass. This suggests that the effect of the essential nutrient P, has improved the growth of *C. zizanioides* during the phytoremediation process. Zhu et al., (2011), studied growth characteristics and nutrient uptake in 7 plants *Oenanthe javanica*, *Gypsophila* sp., *Rohdea japonica*, *Dracaena sanderiana*, *Gardenia jasminoides* Var. *grandiflora*, *Gardenia jasminoides* Var. *prostrata*, and *Salix babylonica.* The results showed a positive correlation

between the accumulation of the element P and the amount of biomass in all the 7 plants. Previous research has also found a positive correlation between the element the essential element P and enhanced plant development in petroleum contaminated soils (Hou et al., 2015; Wei et al., 2019; Saeed et al., 2021; Ilyas et al., 2021; Ali et al., 2020). This present study revealed that the element P was positively correlated with TPH reduction. This might be explained by how the element P affects plant development, which in turn makes it easier to remove TPH from the soil. Our results also showed a strong positive correlation between the element Fe and dry biomass. This suggests that the effect of Fe present in the soil has promoted the growth of *C. zizanioides* during phytoremediation process. The availability of Fe in tropical soil was studied by Mielki et al., (2016), who discovered a positive correlation between the availability of Fe and dry biomass in *Zea mays*. Our results also showed that the presence of Fe in the soil also produced a positive, non-significant correlation with *C. zizanioides* shoot length, root length, and wet biomass. This was most likely because of Fe as a micronutrient, which promoted plant growth. Previous studies have also found similar correlation between micronutrients such as Fe and plant yield (Liu et al., 2008; Garau et al., 2014; Zaheer et al., 2020; Prematuri et al., 2020; Hoang et al., 2021; Lin et al., 2022). In this study we observed a high positive correlation between the element Fe and TPH reduction. This suggests that the growth of *C. zizanioides* during phytoremediation process facilitated TPH degradation. These findings suggest that applying NPK fertiliser, particularly element P, during phytoremediation of crude oil-contaminated soil could improve *C. zizanioides* growth and removal of petroleum hydrocarbons from the soil. Additionally, plant development and the breakdown of petroleum pollutants may be aided by the element Fe acting as a micronutrient in the soil.

## 6.5 Conclusion

This study found that applying fertiliser to a crude oil-contaminated soil during phytoremediation boosted *C. zizanioides* growth and biomass, as well as microbial quantity. The results have

revealed the highest growth of plants in samples treated exclusively with fertiliser. There was a strong positive correlation between the presence of the element P and the dry biomass of the plant. This could have occurred as a direct result of the fertiliser treatment, which prevented nutrient deficiency and aided plant growth, increasing microbial quantity during phytoremediation. This present research discovered that the combined effect of fertiliser and biosurfactant has specifically improved the growth and biomass of *C. zizanioides,* which may have in turn increased the soil microbial quantity and reduced petroleum hydrocarbons. Precisely, the results have shown the ability of biosurfactants to solubilize petroleum contaminants for easy absorption and utilisation by *C. zizanioides*. It has also demonstrated the effect of fertiliser on the growth of *C. zizanioides*, which in turn absorbs more contaminants, thus reducing the quantity of TPH. There was a strong positive correlation between the presence of the element P and TPH reduction. The results also indicate that fertiliser application helped reduce the level of stress induced on *C. zizanioides* by petroleum contaminants. This is most likely because the presence of essential elements in NPK fertiliser improved plant physiology, resulting in a low root-shoot biomass ratio, low proline accumulation, a high chlorophyll concentration, and low cell damage. The breakdown of petroleum pollutants and plant development may both be aided by the element Fe, which acts as a micronutrient in soil. This current study's findings also suggest that the combined effect of fertiliser and biosurfactant played a critical role in increasing the tolerance of *C. zizanioides* to crude oil-contaminated soil. According to the findings, plants grown in samples containing both fertiliser and biosurfactant had lesser amount of root-shoot biomass ratio, the least amount of proline and the highest concentration of chlorophyll. This current study's findings also suggest that the combined effect of fertiliser and biosurfactant played a critical role in increasing the tolerance of *C. zizanioides* to crude oil-contaminated soil. According to the findings, plants grown in samples containing both fertiliser and biosurfactant had a lesser root-shoot biomass ratio, the least amount of proline, and the highest concentration of chlorophyll. This was most likely because of the biosurfactant, which improved the solubility of petroleum contaminants and aided plant nutrient absorption in the soil, increasing the chlorophyll concentration, decreasing the root-shoot biomass ratio, and reducing proline accumulation and cell damage. The findings of this present research have indicated that

silica (quartz) is the most abundant mineral found in samples based on the FTIR and XRD analyses. However, the presence of quartz silica in the soil had a little or no effect on promoting the growth and tolerance of *C. zizanioides* exposed to crude oil-contaminated soil. The presence of quartz silica in the soil, on the other hand, promotes plant survival in crude oil-contaminated soil.

# CHAPTER SEVEN

# SUMMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

## 7.1 Summary

Phytoremediation has gradually emerged and is now recognised as a cost-effective and environmentally friendly approach for cleaning contaminated soils (Szczygłowska et al., 2011; Guarino and Sciarrillo, 2017; Liu et al., 2018). Research has explored the immense potential of phytoremediation and there have been notable improvements in phytoremediation achieved through a better understanding of the mechanisms of absorption, metabolism, and enzymatic activities in plants, as well as the interaction between plants and soil microorganisms such as the use of plant growth-promoting bacteria (PGPB) and bio-degradative bacteria to increase the effectiveness of phytoremediation (Dietz and Schnoor, 2001; Macek et al., 2009; Glick, 2010; (Agostini et al., 2013; Saleem, 2016; Adetunji and Adejumo, 2018; Orozco-Mosqueda et al., 2020). However, there are concerns over the use of plants with short roots, invasive plant species and the phytotoxic effect of organic compounds on the plants that may affect the efficiency of phytoremediation (Germaine et al., 2009; Malar et al., 2014; Zand and Hoveidi, 2016; Oyuela Leguizamo et al., 2017; Keeran et al., 2019). Therefore, it is best to choose robust plants that can both grow well on low-nutrient soils and can remove pollutants from the soil Nouri et al., 2009). Previous research on *C. zizanioides* phytoremediation of petroleum-contaminated soils discovered that only compost amendments effectively reduced petroleum contaminants (Nisa and Rashid, 2015; Nero, 2021). However, the ability of *C. zizanioides* to extract PAHs through bioaccumulation and phytodegradation of the contaminants is yet to be elucidated.

The general aim of this thesis was to determine the effectiveness of *C. zizanioides* in the phytoremediation of crude oil contaminated soil, with particular emphasis on PAHs due to their toxicity and persistence in the environment. This was achieved by evaluating the ability of the plant to bioaccumulate and phytodegrade petroleum hydrocarbons in contaminated soil while being influenced by biosurfactant and fertiliser, the role of the plant in immobilizing petroleum hydrocarbons in the soil, the effect of petroleum contaminants on plant growth and microbial quantity during phytoremediation.

- A mild shaking experiment conducted to determine the efficacy of biosurfactants in removing TPH from crude oil-contaminated soil showed that 61.3% of TPH was removed by 95% di-rhamnolipid dominant and 53.1% was removed by 95% mono-rhamnolipid dominant. As a result, the 95% di-rhamnolipid dominant biosurfactant was used to improve petroleum hydrocarbon solubility during phytoremediation. However, there was no statistically significant difference between the level of TPH removed by the 95% di-rhamnolipid dominant and by the 95% mono-rhamnolipid dominant. It was discovered that both surfactants outperformed the control (water). These findings agree with the finding of Fanaei et al., (2020) on biosurfactant enhanced washing of oil contaminated soil which removed 86% of TPH from the soil.

- An experiment was conducted using GC-MS to determine the level of dissipation of PAHs from the soil under the influence of fertiliser and biosurfactant. This revealed that the plants grown in samples treated with a combination of fertiliser and biosurfactant resulted in the highest rate of PAH dissipation. The concentration of the LMW PAHs, including naphthalene, was reduced by 89.6%; acenaphthylene was reduced by 68%; phenanthrene by 58.3%; acenaphthene by 44.6%; anthracene by 38.6%; and fluorene by 32.1% (Figure 4.2A). The concentrations of the MMW PAHs, including fluoranthene, were reduced by 36.1%; benzo[a]anthracene was reduced by 32.8%; pyrene by 21.2%; and chrysene by 13.7% (Figure 4.2B). The concentrations of HMW PAHs, including indeno[1,2,3-cd]pyrene, were reduced by 30.5%, 29.8% for benzo[a]pyrene, 26.8% for benzo[k]fluoranthene, 24.5% for dibenzo[a,h]anthracene, and 15.4% for benzo[g,h,i]perylene (Figure 4.2 C). This clearly demonstrate that there is a synergy between the combined effect of fertiliser and biosurfactant and the ability of *C. zizanioides* to remove PAHs from a crude oil contaminated soils during the process of phytoremediation. The exclusive use of biosurfactant has also led to high reduction of

PAHs in the oil contaminated soil (Figures 4.2 A, B and C). These findings agree with the findings of Lu et al., (2019), which also revealed the efficiency of rhamnolipids on PAHs removal during the process of bioremediation.

- The concentration of PAHs accumulated in the roots and shoots of *C. zizanioides* was also determined using the GC-MS experiment. This revealed that plants grown in samples treated with a combination of fertiliser and biosurfactant had the highest accumulation of PAHs in their shoots and roots, including naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[b]fluoranthene, Acenaphthylene, acenaphthene, fluorene, and benzo[a]anthracene, on the other hand, were not found in the plant shoots. The exclusive effect of biosurfactant has also led to high accumulation of PAHs in the tissues of the plants. This agrees with the finding of Zhu and Zhang., (2008) on the effect of rhamnolipids on PAH uptake by ryegrass, which revealed increased PAH uptake by the plant. The total PAHs in the soil was found to be higher than the total PAHs accumulated in the shoots and roots of *C. zizanioides.* However, the total PAHs accumulated in the roots of plants grown under the combined effect of fertiliser and biosurfactant, which was 1032.22 (µg $Kg^{-1}$ DW) was more than the total PAHs accumulated in the shoots of plants grown under the combined effect of fertiliser and biosurfactant of 693.32 (µg $Kg^{-1}$ DW). It was discovered that the combined effect of fertiliser and biosurfactant led to the highest BCF, which was 0.55 in the roots of the plant than the shoot, which was 0.37. This contradicts the findings of Li et al. (2014), who discovered higher BCF levels in the leaves than in the roots of plants from three different contaminated mangrove swamps in Shenzhen, China. Also, there was a close merging among the TF in plants grown with various treatments, except for the untreated control samples.

- An experiment was conducted using the loss on ignition method described by Heiri et al., (2001) to determine the effect of various soil treatments on the formation of SOM during phytoremediation. This revealed that the highest levels of SOM were formed in samples treated exclusively with fertilizer, which increased by 23.5%. This agrees with the findings of Wei et al., (2016), which revealed increased SOM content through the application of fertiliser. This is followed by high level of SOM in samples treated with a combination of fertiliser and biosurfactant, which was 21.8% and then the untreated sample vegetated with *C. zizanioides,* which was 20.2%.

- An experiment using column leaching test method adapted by Reemtsma and Mehrtens, (1997), was conducted to determine the level of sorption of PAHs to SOM during phytoremediation. This revealed that the combined effect of fertiliser and biosurfactant resulted in the less concentration of most of the 7 HMW PAHs including Benzo[a]anthracene, which was 55.2 µg/L, Chrysene 228.3 µg/L, Benzo[b]fluoranthene 82.2 µg/L, Benzo[k]fluoranthene 67.7 µg/L, Benzo[a]pyrene 58.1 µg/L, Indeno[1,2,3-cd]pyrene 61.8 µg/L, and Dibenzo[a,h]anthracene 54.3 µg/L.

- The growth of roots and soil aggregate stability was determined using a method adapted from Graf and Frei, (2013). This revealed the highest growth of roots, aggregate formation, and stable aggregate of 64.45 % in samples treated exclusively with fertiliser (Figure 5.2 and 5.3). This agrees with the finding of Chen et al., (2017), which revealed that soil aggregation was improved by the broad root system in the rubber-based agroforestry systems. This is followed by high growth of roots, aggregate formation, and stable aggregate of 60.34 % in samples treated with a combination of fertiliser and biosurfactant.

- The effect of various soil treatments on the growth of *C. zizanioides* and microbial quantity was determined. This revealed that the plants grown in samples amended solely with fertiliser exhibited the highest growth of shoots, which was 63.4 cm and

roots, which was 30.4 cm. This agrees with the findings of Cartmill et al., (2014) that showed that fertiliser application increased the growth of *Lolium multiflorum lam* by preventing the effect of petroleum contaminants. Also, there was a strong positive correlation between the presence of phosphorous and the dry biomass of the plant (Figure 6.14). It was also discovered that the samples under the combined effect of fertiliser and biosurfactant had the highest FW (31.8 g/$m^2$) and DW biomass (6.4 g/$m^2$), as well as microbial quantity, which was 596 (cfu/g) (Figures 6.1, 6.2, 6.4 and 6.5).

- An experiment using a modified gravimetric method adapted from Tang et al., (2010) was conducted to determine the level of removal of TPH in soil during phytoremediation. This revealed that the highest rate of TPH removal, which was 86.0% occurred under the combined influenced of fertiliser and biosurfactant (Figures 6.6 and 6.7). The combined effect of fertiliser and biosurfactant also led to highest removal of the aromatic (71.1%), saturated (67.4%), and polar (51.2%) fractions of TPH during phytoremediation. The exclusive application of biosurfactant has also resulted in reducing 47.2 % of TPH in the soil, where the aromatic fraction was removed by 52.2%, saturated fraction was removed by 41.2 % and the polar fraction, which was removed by 28.8%. This agrees with the findings of Almansoory et al., (2015), which also found 93.5% reduction of TPH, that was facilitated by biosurfactant.

- The analysis of soil minerals was conducted using Fourier transform infrared spectroscopy (FTIR), using the method described by Gbadamosi et al., (2018). This revealed that the main minerals found in all samples were quartz and kaolinite, with trace amounts of calcite, gibbsite, and organic carbon (Table 6.2). Furthermore, XRD analysis was conducted to determine the mineralogical composition and crystalline nature of the minerals. This revealed that the most abundant mineral identified in all samples was found to be silica (quartz). The abundance of silica (quartz) in the soil

might have aided the plant's response to stress induced by petroleum contaminants during phytoremediation. In general, these discoveries provide valuable perspectives on the interconnections between Si and proline levels, contributing to a better understanding of the plant's response to stress.

- The response of *C. zizanioides* to the stress induced by petroleum contaminants was analysed using the root-shoot biomass ratio, proline content, chlorophyll content, and the morphological changes in the plants. These revealed that the plants grown in samples treated exclusively with biosurfactant produced the highest root-shoot biomass ratio, with an average of 1.72 and proline concentration, which was 0.32 µg/g (Figures 6.9 and 6.10). The plants grown in the untreated oil contaminated samples, also produced high root-shoot biomass ratio, with an average of 1.35. This agrees with the findings of Nie et al., (2010) that revealed an increase in root-shoot ratio of *Phragmites autralis* due to high concentration of petroleum contamination. The untreated oil contaminated samples also produced a high proline concentration, which was 0.32 µg/g. This agrees with the findings of Shahzad et al., (2016) that revealed that the toxicity of petroleum contaminants can induce stress on plants. The highest concentration of chlorophyll A, which was 2.5 µg/g was discovered in plants cultivated in samples under the combined effect of fertiliser and biosurfactant (Figure 6.11). However, the highest concentration of chlorophylls B, which was 8.4 µg/g was observed in plants grown in samples under the solitary effect of fertiliser. This finding supports the finding of Wilkinson et al., (2019) which revealed increased chlorophyll content in Casablanca leaf by the effect of stabilised urea nitrogen (SAN). The microscopic analysis of the plant also revealed the impact of petroleum contaminant under various soil treatments. The highest rupture of parenchymatous cells was observed in plants grown in samples treated solely with biosurfactant (Figure 6.13 C). This is followed by the level of cell damaged in observed in plants grown in the untreated oil contaminated samples (Figure 6.13 E).

## 7.2 Conclusions

High levels of PAHs found in petroleum-contaminated soils endanger both public health and the ecosystem. The phytotoxicity of petroleum hydrocarbons and their persistence in the environment due to their low bioavailability impede the efficiency of phytoremediation. Hence, the use of *C. zizanioides*, which is a robust, non-invasive plant species because it possesses exceptional morphological, physiological, and ecological features like large roots that grow deeply into the soil and the ability to withstand acidic, alkaline, and saline soils as well as extreme environmental conditions has ideally facilitated the phytodegradation of petroleum hydrocarbons in the soil. The application of fertiliser and biosurfactant has helped to improve the growth of plants and the solubility of petroleum hydrocarbons for easy absorption by the plants. This also demonstrate the synergy that exist between fertiliser and biosurfactant application, the growth of *C. zizanioides* and the extraction of petroleum hydrocarbons from the contaminated soil. These findings clearly demonstrate the potential of *C. zizanioides* for phytoremediation of crude oil-contaminated soil when influenced by fertiliser and biosurfactant. However, the use of fertiliser alone is more cost saving as the cost-effectiveness of this technique may be affected by the cost of biosurfactant.

## 7.3 Research gaps and opportunities of phytoremediation

The potential aspects for further research are suggested in the following areas:

- The future research should investigate the ability of *C. zizanioides* to extract petroleum hydrocarbons and toxic metals that may be present in oil-contaminated soils.
- It will be useful to study the interaction between plant growth-promoting bacteria (PGPB), earthworms and biosurfactants and their ability to facilitate the extraction of petroleum

contaminants during phytoremediation. It is more cost-efficient to culture PGPB and *Pseudomonas aeruginosa* to produce rhamnolipid biosurfactant.

- It will be interesting to study the link between soil moisture and an increase in SOM.
- Further research is needed to determine the efficacy of combining different tolerant and non-invasive plant species with *C. zizanioides* in removing petroleum contaminants from soil under the influence of PGPB and biosurfactants.
- It will be interesting for future research to study the synergy between biochar, PGPB, and biosurfactant on the growth of various plants and their efficacy in removing contaminants during phytoremediation of crude-oil contaminated soil.
- It will be useful to study the pests and pathogens that could affect *C. zizanioides* and other tolerant non-invasive plant species during phytoremediation of crude oil contaminated soil.
- Future research should also study the production of biogas as a safe way to dispose of plants used for phytoremediation of crude oil-contaminated soil. This will help address the issue of adding harm to the environment through the disposal of the by-products of phytoremediation.

**References:**


Abbasian, F., Lockington, R., Megharaj, M., Naidu, R., 2016. The Biodiversity Changes in the Microbial Population of Soils Contaminated with Crude Oil. Curr Microbiol 72, 663–670. https://doi.org/10.1007/s00284-016-1001-4

Abdel-Haliem, M.E.F., Hegazy, H.S., Hassan, N.S., Naguib, D.M., 2017. Effect of silica ions and nano silica on rice plants under salinity stress. Ecological Engineering 99, 282–289. https://doi.org/10.1016/j.ecoleng.2016.11.060

Abdel-Shafy, H.I., Mansour, M.S.M., 2018. Phytoremediation for the Elimination of Metals, Pesticides, PAHs, and Other Pollutants from Wastewater and Soil, in: Kumar, V., Kumar, M., Prasad, R. (Eds.), Phytobiont and Ecosystem Restitution. Springer, Singapore, pp. 101–136. https://doi.org/10.1007/978-981-13-1187-1_5

Abdollahzadeh, T., Niazi, A., Moghadam, A., Heydarian, Z., Ghasemi-Fasaei, R., Kaviani, E., Pourdad, N., 2019. Phytoremediation of petroleum-contaminated soil by Salicornia: from PSY activity to physiological and morphological communications. Environmental Technology 40, 2789–2801. https://doi.org/10.1080/09593330.2018.1453551

Abdullah, S.R.S., Al-Baldawi, I.A., Almansoory, A.F., Purwanti, I.F., Al-Sbani, N.H., Sharuddin, S.S.N., 2020. Plant-assisted remediation of hydrocarbons in water and soil: Application, mechanisms, challenges and opportunities. Chemosphere 247, 125932. https://doi.org/10.1016/j.chemosphere.2020.125932

Abdullahi, M., Li, X., Abdallah, M.A.-E., Stubbings, W., Yan, N., Barnard, M., Guo, L.-H., Colbourne, J.K., Orsini, L., 2022. Daphnia as a Sentinel Species for Environmental Health Protection: A Perspective on Biomonitoring and Bioremediation of Chemical Pollution. Environ. Sci. Technol. 56, 14237–14248. https://doi.org/10.1021/acs.est.2c01799

Abioye, P.O., 2011. Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil.

Adams, RP., M, Z., Y, T., MR, D., 1998. DNA fingerprinting reveals clonal nature of Vetiveria zizanioides (L.) Nash gramineae and sources of potential newgermplasm. Mol Ecol 7:813–818.

Adesina, G.O., Adelasoye, K.A., 2014a. Effect of crude oil pollution on heavy metal contents, microbial population in soil, and maize and cowpea growth. Agricultural Sciences 2014. https://doi.org/10.4236/as.2014.51004

Adesina, G.O., Adelasoye, K.A., 2014b. Effect of crude oil pollution on heavy metal contents, microbial population in soil, and maize and cowpea growth. Agricultural Sciences 2014. https://doi.org/10.4236/as.2014.51004

Adetunji, C.O., Adejumo, I.O., 2018. Efficacy of crude and immobilizedenzymes from Bacillus licheniformis for production of biodegraded feather meal and their assessment on chickens. Environmental Technology & Innovation 11, 116–124. https://doi.org/10.1016/j.eti.2018.05.002

Adipah, S., 2019. Introduction of Petroleum Hydrocarbons Contaminants and its Human Effects. Journal of Environmental Science and Public Health 3, 1–9.

Adnan, M., Xiao, B., Xiao, P., Zhao, P., Li, R., Bibi, S., 2022. Research Progress on Heavy Metals Pollution in the Soil of Smelting Sites in China. Toxics 10, 231. https://doi.org/10.3390/toxics10050231

Adrion, A.C., Nakamura, J., Shea, D., Aitken, M.D., 2016. Screening Nonionic Surfactants for Enhanced Biodegradation of Polycyclic Aromatic Hydrocarbons Remaining in Soil After Conventional Biological Treatment. Environ. Sci. Technol. 50, 3838–3845. https://doi.org/10.1021/acs.est.5b05243

Afshar-Mohajer, N., Fox, M.A., Koehler, K., 2019. The human health risk estimation of inhaled oil spill emissions with and without adding dispersant. Science of The Total Environment 654, 924–932. https://doi.org/10.1016/j.scitotenv.2018.11.110

Afzal, M., Yousaf, S., Reichenauer, T.G., Kuffner, M., Sessitsch, A., 2011. Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel. Journal of Hazardous Materials 186, 1568–1575. https://doi.org/10.1016/j.jhazmat.2010.12.040

Aghalibe, C.U., Igwe, J.C., Obike, A.I., 2017. Studies on the Removal of Petroleum Hydrocarbons (PHCs) from a Crude Oil Impacted Soil Amended with Cow Dung, Poultry Manure and NPK Fertilizer.

AGI, 2010. Gulf oil spill threatens subsurface biodiversity | EARTH Magazine [WWW Document]. URL https://www.earthmagazine.org/article/gulf-oil-spill-threatens-subsurface-biodiversity (accessed 1.26.17).

Agnello, A.C., Bagard, M., van Hullebusch, E.D., Esposito, G., Huguenot, D., 2016. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Science of The Total Environment 563–564, 693–703. https://doi.org/10.1016/j.scitotenv.2015.10.061

Agostini, E., Talano, M.A., González, P.S., Oller, A.L.W., Medina, M.I., 2013. Application of hairy roots for phytoremediation: what makes them an interesting tool for this purpose? Appl Microbiol Biotechnol 97, 1017–1030. https://doi.org/10.1007/s00253-012-4658-z

Ahmad, A., 2022. Phytoremediation of heavy metals and total petroleum hydrocarbon and nutrients enhancement of Typha latifolia in petroleum secondary effluent for biomass growth. Environ Sci Pollut Res 29, 5777–5786. https://doi.org/10.1007/s11356-021-16016-5

Ahmad, A., Hadi, F., Ali, N., 2015. Effective Phytoextraction of Cadmium (Cd) with Increasing Concentration of Total Phenolics and Free Proline in Cannabis sativa (L) Plant Under Various Treatments of Fertilizers, Plant Growth Regulators and Sodium Salt. International Journal of Phytoremediation 17, 56–65. https://doi.org/10.1080/15226514.2013.828018

Aishatu, S., Chukwudi, O.J., Hauwa'u, I., 2016. The Environmental Impact of Pipeline Vandalism - A Challenge to Biodiversity in Portharcourt Area of Rivers State, Nigeria. IJACEBS 3. https://doi.org/10.15242/IJACEBS.A0516206

Akapo, A.A.-R., Omidiji, S.O., Otitoloju, A.A., 2011. Morphological and anatomical effects of crude oil on Pistia stratiotes. Environmentalist 31, 288–298. https://doi.org/10.1007/s10669-011-9333-x

Akpona, O.N., 2013. PRACTICAL USE OF THE VETIVER SYSTEM AS AN EFFECTIVE MULTI-APPLICATION BIO-ENGINEERING TOOL FOR SOIL AND WATER MANAGEMENT: IMPERATIVES FOR NIGERIA. World 5, 95–103.

Akram, R., Turan, V., Hammad, H.M., Ahmad, S., Hussain, S., Hasnain, A., Maqbool, M.M., Rehmani, M.I.A., Rasool, A., Masood, N., Mahmood, F., Mubeen, M., Sultana, S.R., Fahad, S., Amanet, K., Saleem, M., Abbas, Y., Akhtar, H.M., Hussain, S., Waseem, F., Murtaza, R., Amin, A., Zahoor, S.A., Sami ul Din, M., Nasim, W., 2018. Fate of Organic and Inorganic Pollutants in Paddy Soils, in: Hashmi, M.Z., Varma, A. (Eds.), Environmental Pollution of Paddy Soils, Soil Biology. Springer International Publishing, Cham, pp. 197–214. https://doi.org/10.1007/978-3-319-93671-0_13

Aksakal, E.L., Angin, I., Sari, S., 2020. A new approach for calculating aggregate stability: Mean weight aggregate stability (MWAS). CATENA 194, 104708. https://doi.org/10.1016/j.catena.2020.104708

Aladesanmi, O.T., Oroboade, J.G., Osisiogu, C.P., Osewole, A.O., 2019. Bioaccumulation Factor of Selected Heavy Metals in Zea mays. Journal of Health and Pollution 9. https://doi.org/10.5696/2156-9614-9.24.191207

Al-Baldawi, I.A., Abdullah, S.R.S., Anuar, N., Suja, F., Mushrifah, I., 2015. Phytodegradation of total petroleum hydrocarbon (TPH) in diesel-contaminated water using Scirpus

grossus. Ecological Engineering 74, 463–473. https://doi.org/10.1016/j.ecoleng.2014.11.007
Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., Wang, M.-Q., 2021. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics 9, 42. https://doi.org/10.3390/toxics9030042
Alexander, M., 2000. Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants. Environ. Sci. Technol. 34, 4259–4265. https://doi.org/10.1021/es001069+
Al-Hashem, M.A., Brain, P.F., Omar, S.A., 2007. Effects of oil pollution at Kuwait's greater Al-Burgan oil field on polycyclic aromatic hydrocarbon concentrations in the tissues of the desert lizard Acanthodactylus scutellatus and their ant prey. Ecotoxicology 16, 551–555. https://doi.org/10.1007/s10646-007-0161-1
Ali, E.A.M., A. Sayed, M., A. Abdel-Rahman, T.M., Hussein, R., 2021. Fungal remediation of Cd( ii ) from wastewater using immobilization techniques. RSC Advances 11, 4853–4863. https://doi.org/10.1039/D0RA08578B
Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals—Concepts and applications. Chemosphere 91, 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075
Ali Khan, A.H., Tanveer, S., Alia, S., Anees, M., Sultan, A., Iqbal, M., Yousaf, S., 2017. Role of nutrients in bacterial biosurfactant production and effect of biosurfactant production on petroleum hydrocarbon biodegradation. Ecological Engineering 104, 158–164. https://doi.org/10.1016/j.ecoleng.2017.04.023
Ali, M.H., Sattar, M.T., Khan, M.I., Naveed, M., Rafique, M., Alamri, S., Siddiqui, M.H., 2020. Enhanced Growth of Mungbean and Remediation of Petroleum Hydrocarbons by Enterobacter sp. MN17 and Biochar Addition in Diesel Contaminated Soil. Applied Sciences 10, 8548. https://doi.org/10.3390/app10238548
Ali, M.M., Al-Ani, A., Eamus, D., Tan, D.K., 2012. A new image processing based technique to determine chlorophyll in plants. American-Eurasian Journal of Agricultural and Environmental Sciences 12, 1323–1328.
Alinnor, I.J., Ogukwe, C.E., Nwagbo, N.C., 2014. Characteristic level of total petroleum hydrocarbon in soil and groundwater of oil impacted area in the Niger Delta region, Nigeria. Journal of Environment and Earth Science 4, 188–194.
Allard, A.-S., Remberger, M., Neilson, A.H., 2000. The negative impact of aging on the loss of PAH components in a creosote-contaminated soil. International biodeterioration & biodegradation 46, 43–49.
Al-Majed, A.A., Adebayo, A.R., Hossain, M.E., 2012. A sustainable approach to controlling oil spills. Journal of Environmental Management 113, 213–227. https://doi.org/10.1016/j.jenvman.2012.07.034
Almansoory, A.F., Abu Hasan, H., Idris, M., Sheikh Abdullah, S.R., Anuar, N., 2015. Potential application of a biosurfactant in phytoremediation technology for treatment of gasoline-contaminated soil. Ecological Engineering 84, 113–120. https://doi.org/10.1016/j.ecoleng.2015.08.001
Al-Mansoory, A.F., Idris, M., Abdullah, S.R.S., Anuar, N., 2017. Phytoremediation of contaminated soils containing gasoline using Ludwigia octovalvis (Jacq.) in greenhouse pots. Environ Sci Pollut Res 24, 11998–12008. https://doi.org/10.1007/s11356-015-5261-5

Alsghayer, R., Salmiaton, A., Mohammad, T., Idris, A., Ishak, C.F., 2020. Removal Efficiencies of Constructed Wetland Planted with Phragmites and Vetiver in Treating Synthetic Wastewater Contaminated with High Concentration of PAHs. Sustainability 12, 3357. https://doi.org/10.3390/su12083357

Anae, J., Ahmad, N., Kumar, V., Thakur, V.K., Gutierrez, T., Yang, X.J., Cai, C., Yang, Z., Coulon, F., 2021. Recent advances in biochar engineering for soil contaminated with complex chemical mixtures: Remediation strategies and future perspectives. Science of The Total Environment 767, 144351. https://doi.org/10.1016/j.scitotenv.2020.144351

Anejionu, O.C.D., Ahiarammunnah, P.-Ann.N., Nri-ezedi, C.J., 2015. Hydrocarbon pollution in the Niger Delta: Geographies of impacts and appraisal of lapses in extant legal framework. Resources Policy 45, 65–77. https://doi.org/10.1016/j.resourpol.2015.03.012

Antoniadis, V., Shaheen, S.M., Stärk, H.-J., Wennrich, R., Levizou, E., Merbach, I., Rinklebe, J., 2021. Phytoremediation potential of twelve wild plant species for toxic elements in a contaminated soil. Environment International 146, 106233. https://doi.org/10.1016/j.envint.2020.106233

Arellano, P., Tansey, K., Balzter, H., Tellkamp, M., 2017. Plant Family-Specific Impacts of Petroleum Pollution on Biodiversity and Leaf Chlorophyll Content in the Amazon Rainforest of Ecuador. PLOS ONE 12, e0169867. https://doi.org/10.1371/journal.pone.0169867

Arya, S.S., Devi, S., Angrish, R., Singal, I., Rani, K., 2017. Soil Reclamation Through Phytoextraction and Phytovolatilization, in: Choudhary, D.K., Sharma, A.K., Agarwal, P., Varma, A., Tuteja, N. (Eds.), Volatiles and Food Security: Role of Volatiles in Agro-Ecosystems. Springer, Singapore, pp. 25–43. https://doi.org/10.1007/978-981-10-5553-9_3

Ashraf, M.A., Hanfiah, M.M., 2017. Recent advances in assessment on clear water, soil and air. Environ Sci Pollut Res 24, 22753–22754. https://doi.org/10.1007/s11356-017-9267-z

Asif, M., Muneer, T., 2007. Energy supply, its demand and security issues for developed and emerging economies. Renewable and Sustainable Energy Reviews 11, 1388–1413. https://doi.org/10.1016/j.rser.2005.12.004

ATSDR, 1999. Toxicological Profile for Total Petroleum Hydrocarbons (TPH). no. September.

Atta, A.M., Mohamed, N.H., Hegazy, A.K., Moustafa, Y.M., Mohamed, R.R., Safwat, G., Diab, A.A., 2020. Green Technology for Remediation of Water Polluted with Petroleum Crude Oil: Using of Eichhornia crassipes (Mart.) Solms Combined with Magnetic Nanoparticles Capped with Myrrh Resources of Saudi Arabia. Nanomaterials 10, 262. https://doi.org/10.3390/nano10020262

Awa, S.H., Hadibarata, T., 2020. Removal of Heavy Metals in Contaminated Soil by Phytoremediation Mechanism: a Review. Water Air Soil Pollut 231, 47. https://doi.org/10.1007/s11270-020-4426-0

Azubuike, C.C., Chikere, C.B., Okpokwasili, G.C., 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32, 180. https://doi.org/10.1007/s11274-016-2137-x

Badri, D.V., Weir, T.L., van der Lelie, D., Vivanco, J.M., 2009. Rhizosphere chemical dialogues: plant–microbe interactions. Current Opinion in Biotechnology, Chemical biotechnology ● Pharmaceutical biotechnology 20, 642–650. https://doi.org/10.1016/j.copbio.2009.09.014

Balasubramaniyam, A., 2015. The Influence of Plants in the Remediation of Petroleum Hydrocarbon-Contaminated Sites. Pharmaceutical Analytical Chemistry 1, 1–11. https://doi.org/10.4172/paco.1000105

Balliana, A.G., Moura, B.B., Inckot, R.C., Bona, C., 2017. Development of Canavalia ensiformis in soil contaminated with diesel oil. Environ Sci Pollut Res 24, 979–986. https://doi.org/10.1007/s11356-016-7674-1

Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J., Marchant, R., 2010a. Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87, 427–444. https://doi.org/10.1007/s00253-010-2589-0

Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J., Marchant, R., 2010b. Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87, 427–444. https://doi.org/10.1007/s00253-010-2589-0

Banerjee, R., Goswami, P., Pathak, K., Mukherjee, A., 2016. Vetiver grass: An environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecological Engineering 90, 25–34. https://doi.org/10.1016/j.ecoleng.2016.01.027

Banks, M.K., Schultz, K.E., 2005. Comparison of Plants for Germination Toxicity Tests in Petroleum-Contaminated Soils. Water Air Soil Pollut 167, 211–219. https://doi.org/10.1007/s11270-005-8553-4

Banuelos, G.S., Lin, Z.-Q., Moraes, M.F., Guilherme, L.R.G., Reis, A.R. dos, 2015. Global Advances in Selenium Research from Theory to Application: Proceedings of the 4th International Conference on Selenium in the Environment and Human Health 2015. CRC Press.

Barbosa, B., Fernando, A.L., 2018. Chapter 9 - Aided Phytostabilization of Mine Waste, in: Prasad, M.N.V., Favas, P.J. de C., Maiti, S.K. (Eds.), Bio-Geotechnologies for Mine Site Rehabilitation. Elsevier, pp. 147–157. https://doi.org/10.1016/B978-0-12-812986-9.00009-9

Basu, A., Basu, S., Bandyopadhyay, S., Chowdhury, R., 2015. Optimization of evaporative extraction of natural emulsifier cum surfactant from Sapindus mukorossi—Characterization and cost analysis. Industrial Crops and Products 77, 920–931. https://doi.org/10.1016/j.indcrop.2015.10.006

Basumatary, B., Bordoloi, S., Sarma, H.P., 2012. Crude Oil-Contaminated Soil Phytoremediation by Using Cyperus brevifolius (Rottb.) Hassk. Water Air Soil Pollut 223, 3373–3383. https://doi.org/10.1007/s11270-012-1116-6

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060

Batty, L.C., Dolan, C., 2013a. The Potential Use of Phytoremediation for Sites With Mixed Organic and Inorganic Contamination. Critical Reviews in Environmental Science and Technology 43, 217–259. https://doi.org/10.1080/10643389.2011.604254

Batty, L.C., Dolan, C., 2013b. The Potential Use of Phytoremediation for Sites With Mixed Organic and Inorganic Contamination. Critical Reviews in Environmental Science and Technology 43, 217–259. https://doi.org/10.1080/10643389.2011.604254
Beans, C., 2017. Phytoremediation advances in the lab but lags in the field. Proc. Natl. Acad. Sci. U.S.A. 114, 7475–7477. https://doi.org/10.1073/pnas.1707883114
Bell, J.W., 2018. Proceedings of the 45th Industrial Waste Conference May 1990, Purdue University. CRC Press.
Benson, A., Ram, G., John, A., Melvin Joe, M., 2017. Inoculation of 1-aminocyclopropane-1-carboxylate deaminase–producing bacteria along with biosurfactant application enhances the phytoremediation efficiency of *Medicago sativa* in hydrocarbon-contaminated soils. Bioremediation Journal 21, 20–29. https://doi.org/10.1080/10889868.2017.1282934
Bernardino, C.A.R., Mahler, C.F., Alvarenga, P., Castro, P.M.L., da Silva, E.F., Novo, L.A.B., 2020. Recent Advances in Phytoremediation of Soil Contaminated by Industrial Waste: A Road Map to a Safer Environment, in: Saxena, G., Bharagava, R.N. (Eds.), Bioremediation of Industrial Waste for Environmental Safety: Volume I: Industrial Waste and Its Management. Springer, Singapore, pp. 207–221. https://doi.org/10.1007/978-981-13-1891-7_10
Besalatpour, A., Khoshgoftarmanesh, A.H., Hajabbasi, M.A., Afyuni, M., 2008. Germination and Growth of Selected Plants in a Petroleum Contaminated Calcareous Soil. Soil and Sediment Contamination: An International Journal 17, 665–676. https://doi.org/10.1080/15320380802425113
Besha, A.T., Bekele, D.N., Naidu, R., Chadalavada, S., 2018. Recent advances in surfactant-enhanced In-Situ Chemical Oxidation for the remediation of non-aqueous phase liquid contaminated soils and aquifers. Environmental Technology & Innovation 9, 303–322. https://doi.org/10.1016/j.eti.2017.08.004
Bezza, F.A., Chirwa, E.M.N., 2017. The role of lipopeptide biosurfactant on microbial remediation of aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil. Chemical Engineering Journal 309, 563–576. https://doi.org/10.1016/j.cej.2016.10.055
Bezza, F.A., Nkhalambayausi, C.E.M., 2016. Biosurfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated soil. Chemosphere 144, 635–644. https://doi.org/10.1016/j.chemosphere.2015.08.027
Bezza, F.A., Nkhalambayausi-Chirwa, E.M., 2015. Desorption kinetics of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil and the effect of biosurfactant supplementation on the rapidly desorbing fractions. Biotechnology & Biotechnological Equipment 29, 680–688. https://doi.org/10.1080/13102818.2015.1028444
Bhandari, A., Surampalli, R.Y., Champagne, P., Ong, S.K., Tyagi, R.D., Lo, I.M.C., 2007. Remediation Technologies for Soils and Groundwater. American Society of Civil Engineers.
Bharti, P., Singh, B., Bauddh, K., Dey, R.K., Korstad, J., 2017. Efficiency of Bioenergy Plant in Phytoremediation of Saline and Sodic Soil, in: Bauddh, K., Singh, B., Korstad, J. (Eds.), Phytoremediation Potential of Bioenergy Plants. Springer, Singapore, pp. 353–369. https://doi.org/10.1007/978-981-10-3084-0_14

Bhattacharya, B., Ghosh, T., Das, N., 2017. Application of Bio-Surfactants in Cosmetics and Pharmaceutical Industry.
Bhuyan-Pawar, S., Yeole, R.P., Sanam, V.M., Bashetti, S.P., Mujumdar, S.S., 2015. Biosurfactant mediated plant growth promotion in soils amended with polyaromatic hydrocarbons. Int J Curr Microbiol App Sci 2, 343–356.
Bognolo, G., 1999. Biosurfactants as emulsifying agents for hydrocarbons. Colloids and Surfaces A: Physicochemical and Engineering Aspects 152, 41–52. https://doi.org/10.1016/S0927-7757(98)00684-0
Bojes, H.K., Pope, P.G., 2007a. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. Regulatory Toxicology and Pharmacology 47, 288–295. https://doi.org/10.1016/j.yrtph.2006.11.007
Bojes, H.K., Pope, P.G., 2007b. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. Regulatory Toxicology and Pharmacology 47, 288–295. https://doi.org/10.1016/j.yrtph.2006.11.007
Bolade, O.P., Adeniyi, K.O., Williams, A.B., Benson, N.U., 2021. Remediation and optimization of petroleum hydrocarbons degradation in contaminated water using alkaline activated persulphate. Journal of Environmental Chemical Engineering 9, 105801. https://doi.org/10.1016/j.jece.2021.105801
Bolan, N.S., Park, J.H., Robinson, B., Naidu, R., Huh, K.Y., 2011. Chapter four - Phytostabilization: A Green Approach to Contaminant Containment, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 145–204. https://doi.org/10.1016/B978-0-12-385538-1.00004-4
Boopathy, R., 2000. Factors limiting bioremediation technologies. Bioresource Technology 74, 63–67. https://doi.org/10.1016/S0960-8524(99)00144-3
Borah, D., Chaubey, A., Sonowal, A., Gogoi, B., Kumar, R., 2021. Microbial Biosurfactants and Their Potential Applications: An Overview, in: Inamuddin, Ahamed, M.I., Prasad, R. (Eds.), Microbial Biosurfactants: Preparation, Properties and Applications, Environmental and Microbial Biotechnology. Springer, Singapore, pp. 91–116. https://doi.org/10.1007/978-981-15-6607-3_5
Bordoloi, N.K., Konwar, B.K., 2009. Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. Journal of Hazardous Materials 170, 495–505. https://doi.org/10.1016/j.jhazmat.2009.04.136
Borin, M., Florio, G., Barbera, A.C., Cirelli, G.L., Albergo, R., Palazzo, S., 2011. Preliminary evaluation of macrophyte wetland biomasses to obtain second generation ethanol, in: Proceedings of 19th European Biomass Conference and Exhibition. pp. 6–10.
BP p.l.c, 2022. Statistical Review of World Energy 2022.
Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C., Broll, G., 2006a. Potential of vetiver (Vetiveria zizanioides (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. International Journal of Phytoremediation 8, 273–284. https://doi.org/10.1080/15226510600992808
Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C., Broll, G., 2006b. Potential of vetiver (Vetiveria zizanioides (L.) Nash) for phytoremediation of petroleum hydrocarbon-

contaminated soils in Venezuela. International Journal of Phytoremediation 8, 273–284. https://doi.org/10.1080/15226510600992808
Brewer, R., Nagashima, J., Kelley, M., Heskett, M., Rigby, M., 2013. Risk-Based Evaluation of Total Petroleum Hydrocarbons in Vapor Intrusion Studies. International Journal of Environmental Research and Public Health 10, 2441–2467. https://doi.org/10.3390/ijerph10062441
Briggs, D., 2003. Environmental pollution and the global burden of disease. British Medical Bulletin 68, 1–24. https://doi.org/10.1093/bmb/ldg019
Brittingham, M.C., Maloney, K.O., Farag, A.M., Harper, D.D., Bowen, Z.H., 2014. Ecological Risks of Shale Oil and Gas Development to Wildlife, Aquatic Resources and their Habitats. Environ. Sci. Technol. 48, 11034–11047. https://doi.org/10.1021/es5020482
Brown, D.M., Okoro, S., van Gils, J., van Spanning, R., Bonte, M., Hutchings, T., Linden, O., Egbuche, U., Bruun, K.B., Smith, J.W.N., 2017a. Comparison of landfarming amendments to improve bioremediation of petroleum hydrocarbons in Niger Delta soils. Science of The Total Environment 596–597, 284–292. https://doi.org/10.1016/j.scitotenv.2017.04.072
Brown, D.M., Okoro, S., van Gils, J., van Spanning, R., Bonte, M., Hutchings, T., Linden, O., Egbuche, U., Bruun, K.B., Smith, J.W.N., 2017b. Comparison of landfarming amendments to improve bioremediation of petroleum hydrocarbons in Niger Delta soils. Science of The Total Environment 596–597, 284–292. https://doi.org/10.1016/j.scitotenv.2017.04.072
Brown, I., Eyenghe, T., 2015. An Evaluation Of The Effects Of Petroleum Exploration And Production Activities On The Social Environment In Ogoni Land Nigeria. International Journal of Scientific & Technology Research VOLUME 4, 273–282.
Burken, J.G., 2003a. Uptake and Metabolism of Organic Compounds: Green-Liver Model, in: Phytoremediation. John Wiley & Sons, Ltd, pp. 59–84. https://doi.org/10.1002/047127304X.ch2
Burken, J.G., 2003b. Uptake and Metabolism of Organic Compounds: Green-Liver Model, in: McCutcheon, S.C., Schnoor, J.L. (Eds.), Phytoremediation. John Wiley & Sons, Inc., pp. 59–84. https://doi.org/10.1002/047127304X.ch2
Bwire, K.M., Njau, K.N., Minja, R.J.A., 2011. Use of vetiver grass constructed wetland for treatment of leachate. Water Science and Technology 63, 924–930. https://doi.org/10.2166/wst.2011.271
Bykova, M.V., Alekseenko, A.V., Pashkevich, M.A., Drebenstedt, C., 2021. Thermal desorption treatment of petroleum hydrocarbon-contaminated soils of tundra, taiga, and forest steppe landscapes. Environ Geochem Health 43, 2331–2346. https://doi.org/10.1007/s10653-020-00802-0
Calabrese, E.J., 2017. Safe Drinking Water Act (1989). CRC Press.
Caliman, F.A., Robu, B.M., Smaranda, C., Pavel, V.L., Gavrilescu, M., 2011. Soil and groundwater cleanup: benefits and limits of emerging technologies. Clean Techn Environ Policy 13, 241–268. https://doi.org/10.1007/s10098-010-0319-z
Cao, W., Zhang, L., Miao, Y., Qiu, L., 2021. Research progress in the enhancement technology of soil vapor extraction of volatile petroleum hydrocarbon pollutants.

Environmental Science: Processes & Impacts 23, 1650–1662. https://doi.org/10.1039/D1EM00170A
Carlon, C., Pizzol, L., Critto, A., Marcomini, A., 2008. A spatial risk assessment methodology to support the remediation of contaminated land. Environment International, Proceedings of the 1st Conference of the UK Network on Persistent Organic Pollutants (POPs) 29th and 30th March 2006, University of Birmingham, UK 34, 397–411. https://doi.org/10.1016/j.envint.2007.09.009
Cartmill, A.D., Cartmill, D.L., Alarcón, A., 2014. Controlled Release Fertilizer Increased Phytoremediation of Petroleum-Contaminated Sandy Soil. International Journal of Phytoremediation 16, 285–301. https://doi.org/10.1080/15226514.2013.773280
Catherine, F., Henrietta, W., Bates, N., Murray, V., 2000a. The Chemical Incident Management Handbook.
Catherine, F., Henrietta, W., Nicola, B., Virginia, M., 2000b. The Chemical Incident Management Handbook. ResearchGate.
Cazals, F., Huguenot, D., Crampon, M., Colombano, S., Betelu, S., Galopin, N., Perrault, A., Simonnot, M.-O., Ignatiadis, I., Rossano, S., 2020. Production of biosurfactant using the endemic bacterial community of a PAHs contaminated soil, and its potential use for PAHs remobilization. Science of The Total Environment 709, 136143. https://doi.org/10.1016/j.scitotenv.2019.136143
Chandra, R., Dubey, N.K., Kumar, V., 2017. Phytoremediation of Environmental Pollutants. CRC Press.
Chaudhary, D.K., Kim, J., 2019. New insights into bioremediation strategies for oil-contaminated soil in cold environments. International Biodeterioration & Biodegradation 142, 58–72. https://doi.org/10.1016/j.ibiod.2019.05.001
Chaudhry, Q., Schröder, P., Werck-Reichhart, D., Grajek, W., Marecik, R., 2002. Prospects and limitations of phytoremediation for the removal of persistent pesticides in the environment. Environ. Sci. & Pollut. Res 9, 4. https://doi.org/10.1007/BF02987313
Chen, C., Liu, W., Jiang, X., Wu, J., 2017. Effects of rubber-based agroforestry systems on soil aggregation and associated soil organic carbon: Implications for land use. Geoderma 299, 13–24. https://doi.org/10.1016/j.geoderma.2017.03.021
Chen, C.-H., Liu, P.-W.G., Whang, L.-M., 2019. Effects of natural organic matters on bioavailability of petroleum hydrocarbons in soil-water environments. Chemosphere 233, 843–851. https://doi.org/10.1016/j.chemosphere.2019.05.202
Chen, C.S., Tien, C.-J., 2020. Factors affecting in situ analysis of total petroleum hydrocarbons in contaminated soils by using a mid-infrared diffuse reflectance spectroscopy. Chemosphere 261, 127751. https://doi.org/10.1016/j.chemosphere.2020.127751
Chen, J., Liu, L., Wang, Z., Zhang, Y., Sun, H., Song, S., Bai, Z., Lu, Z., Li, C., 2020. Nitrogen Fertilization Increases Root Growth and Coordinates the Root–Shoot Relationship in Cotton. Frontiers in Plant Science 11.
Chen, J., Yang, Z., Su, Y., Han, F., Monts, D.L., 2011. Phytoremediation of heavy metal/metalloid-contaminated soils. Contaminated Soils: Environmental Impact, Disposal and Treatment 181–206.
Chen, L., Yang, J., Wang, D., 2020. Phytoremediation of uranium and cadmium contaminated soils by sunflower (Helianthus annuus L.) enhanced with

biodegradable chelating agents. Journal of Cleaner Production 263, 121491. https://doi.org/10.1016/j.jclepro.2020.121491
Chen, X., Li, Z., Liu, M., Jiang, C., Che, Y., 2015. Microbial community and functional diversity associated with different aggregate fractions of a paddy soil fertilized with organic manure and/or NPK fertilizer for 20 years. Journal of Soils and Sediments 15, 292–301.
Chen, Y., Shen, Z., Li, X., 2004. The use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. Applied Geochemistry 19, 1553–1565. https://doi.org/10.1016/j.apgeochem.2004.02.003
Cherian, S., Oliveira, M.M., 2005. Transgenic Plants in Phytoremediation: Recent Advances and New Possibilities. Environ. Sci. Technol. 39, 9377–9390. https://doi.org/10.1021/es051134l
Chilvers, B.L., Morgan, K.J., White, B.J., 2021. Sources and reporting of oil spills and impacts on wildlife 1970–2018. Environ Sci Pollut Res 28, 754–762. https://doi.org/10.1007/s11356-020-10538-0
Chorom, M., Sharifi, H.S., Motamedi, H., 2010. Bioremediation of a crude oil-polluted soil by application of fertilizers.
Chou, S.-T., Shih, Y., Lin, C.-C., 2016. Chapter 96 - Vetiver Grass (Vetiveria zizanioides) Oils, in: Preedy, V.R. (Ed.), Essential Oils in Food Preservation, Flavor and Safety. Academic Press, San Diego, pp. 843–848. https://doi.org/10.1016/B978-0-12-416641-7.00096-1
Christou, A., Papadavid, G., Dalias, P., Fotopoulos, V., Michael, C., Bayona, J.M., Piña, B., Fatta-Kassinos, D., 2019. Ranking of crop plants according to their potential to uptake and accumulate contaminants of emerging concern. Environmental Research 170, 422–432. https://doi.org/10.1016/j.envres.2018.12.048
Cocarta, D., Stoian, M., Badea, A., 2015. Assessment of human health risk from crude oil contaminated soils : pollution with PAHs [WWW Document]. URL https://www.semanticscholar.org/paper/Assessment-of-human-health-risk-from-crude-oil-%3A-Cocarta-Stoian/394de3441ff416724a0aab7bfe324444ad5f3b82 (accessed 5.28.22).
Coker, A.K., 2018. Petroleum Refining Design and Applications Handbook, Volume 1. John Wiley & Sons.
Concetta Bruzzoniti, M., Fungi, M., Sarzanini, C., 2010a. Determination of EPA's priority pollutant polycyclic aromatic hydrocarbons in drinking waters by solid phase extraction-HPLC. Analytical Methods 2, 739–745. https://doi.org/10.1039/B9AY00203K
Concetta Bruzzoniti, M., Fungi, M., Sarzanini, C., 2010b. Determination of EPA's priority pollutant polycyclic aromatic hydrocarbons in drinking waters by solid phase extraction-HPLC. Analytical Methods 2, 739–745. https://doi.org/10.1039/B9AY00203K
Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., van Noort, P.C.M., 2005. Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation. Environ. Sci. Technol. 39, 6881–6895. https://doi.org/10.1021/es050191b

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geosci 8, 776–779. https://doi.org/10.1038/ngeo2520

Coulon, F., Whelan, M.J., Paton, G.I., Semple, K.T., Villa, R., Pollard, S.J.T., 2010. Multimedia fate of petroleum hydrocarbons in the soil: Oil matrix of constructed biopiles. Chemosphere 81, 1454–1462. https://doi.org/10.1016/j.chemosphere.2010.08.057

CRAM, S., SIEBE, C., ORTÍZ-SALINAS, R., HERRE, A., 2004. Mobility and Persistence of Petroleum Hydrocarbons in Peat Soils of Southeastern Mexico. Soil and Sediment Contamination: An International Journal 13, 341–360. https://doi.org/10.1080/10588330490500392

Cristaldi, A., Copat, C., Conti, G.O., Zuccarello, P., Grasso, A., Ferrante, M., 2020. Chapter 10:Phytoremediation, in: The Handbook of Environmental Remediation. pp. 268–298. https://doi.org/10.1039/9781788016261-00268

Cui, X., Mao, P., Sun, S., Huang, R., Fan, Y., Li, Yongxing, Li, Yingwen, Zhuang, P., Li, Z., 2021. Phytoremediation of cadmium contaminated soils by Amaranthus Hypochondriacus L.: The effects of soil properties highlighting cation exchange capacity. Chemosphere 283, 131067. https://doi.org/10.1016/j.chemosphere.2021.131067

Cunningham, C.J., S. Kuyukina, M., B. Ivshina, I., I. Konev, A., A. Peshkur, T., W. Knapp, C., 2020. Potential risks of antibiotic resistant bacteria and genes in bioremediation of petroleum hydrocarbon contaminated soils. Environmental Science: Processes & Impacts 22, 1110–1124. https://doi.org/10.1039/C9EM00606K

Dalton, P.A., RJ, S., PNV, T., 1996. Vetiver grass hedges for erosion control on a cropped flood plain: hedge hydraulics. Agric Water Manage 31:91–104.

Dalton, P. A., Smith, R.J., Truong, P.N.V., 1996. Vetiver grass hedges for erosion control on a cropped flood plain: hedge hydraulics. Agricultural Water Management 31, 91–104.

Damian, G.E., Micle, V., Sur, I.M., Chirilă Băbău, A.M., 2019. From Environmental Ethics to Sustainable Decision-Making: Assessment of Potential Ecological Risk in Soils Around Abandoned Mining Areas-Case Study “Larga de Sus mine” (Romania). J Agric Environ Ethics 32, 27–49. https://doi.org/10.1007/s10806-019-09767-2

Danh, Luu Thai, Truong, P., Mammucari, R., Tran, T., Foster, N., 2009a. Vetiver Grass, Vetiveria Zizanioides: A Choice Plant for Phytoremediation of Heavy Metals and Organic Wastes. International Journal of Phytoremediation 11, 664–691. https://doi.org/10.1080/15226510902787302

Danh, Luu Thai, Truong, P., Mammucari, R., Tran, T., Foster, N., 2009. Vetiver Grass, Vetiveria Zizanioides: A Choice Plant for Phytoremediation of Heavy Metals and Organic Wastes. International Journal of Phytoremediation 11, 664–691. https://doi.org/10.1080/15226510902787302

Danh, Luu Thai, Truong, P., Mammucari, R., Tran, T., Foster, N., 2009b. Vetiver Grass, Vetiveria Zizanioides: A Choice Plant for Phytoremediation of Heavy Metals and Organic Wastes. International Journal of Phytoremediation 11, 664–691. https://doi.org/10.1080/15226510902787302

Das, A.J., Kumar, R., 2016. Bioremediation of petroleum contaminated soil to combat toxicity on Withania somnifera through seed priming with biosurfactant producing plant growth promoting rhizobacteria. Journal of Environmental Management 174, 79–86. https://doi.org/10.1016/j.jenvman.2016.01.031

Das, K., Mukherjee, A.K., 2007. Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology 98, 1339–1345. https://doi.org/10.1016/j.biortech.2006.05.032

Das, N., Chandran, P., 2011. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview [WWW Document]. Biotechnology Research International. https://doi.org/10.4061/2011/941810

Das, N., Chandran, P., 2010. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International 2011, e941810. https://doi.org/10.4061/2011/941810

Dawood, M.G., Abdelhamid, M.T., Schmidhalter, U., 2014. Potassium fertiliser enhances the salt-tolerance of common bean (Phaseolus vulgaris L.). The Journal of Horticultural Science and Biotechnology 89, 185–192. https://doi.org/10.1080/14620316.2014.11513067

defeatingpain, 2014. Vetiver, the Root that is Dug. Defeating Pain. URL https://defeatingpain.wordpress.com/2014/05/16/vetiver-the-root-that-is-dug/ (accessed 11.30.17).

Delin, G.N., Herkelrath, W.N., 2012. Ground Water Contamination By Crude Oil 2.

Demenois, J., Carriconde, F., Bonaventure, P., Maeght, J.-L., Stokes, A., Rey, F., 2018. Impact of plant root functional traits and associated mycorrhizas on the aggregate stability of a tropical Ferralsol. Geoderma 312, 6–16. https://doi.org/10.1016/j.geoderma.2017.09.033

Dessaux, Y., Grandclément, C., Faure, D., 2016. Engineering the Rhizosphere. Trends in Plant Science, Special Issue: Unravelling the Secrets of the Rhizosphere 21, 266–278. https://doi.org/10.1016/j.tplants.2016.01.002

Dhanwal, P., Kumar, A., Dudeja, S., Chhokar, V., Beniwal, V., 2017. Recent Advances in Phytoremediation Technology, in: Kumar, R., Sharma, A.K., Ahluwalia, S.S. (Eds.), Advances in Environmental Biotechnology. Springer, Singapore, pp. 227–241. https://doi.org/10.1007/978-981-10-4041-2_14

Diagboya, P.N., Mtunzi, F.M., Adebowale, K.O., Olu-Owolabi, B.I., 2021. Assessment of the effects of soil organic matter and iron oxides on the individual sorption of two polycyclic aromatic hydrocarbons. Environ Earth Sci 80, 227. https://doi.org/10.1007/s12665-021-09530-9

Dietz, A.C., Schnoor, J.L., 2001. Advances in phytoremediation. Environmental Health Perspectives 109, 163–168. https://doi.org/10.1289/ehp.01109s1163

Ding, W., Zhang, J., Wu, S.-C., Zhang, S., Christie, P., Liang, P., 2019. Responses of the grass Paspalum distichum L. to Hg stress: A proteomic study. Ecotoxicology and Environmental Safety 183, 109549. https://doi.org/10.1016/j.ecoenv.2019.109549

Dixit, A., 2018. A development of a Decision Support System for Remediation Option Selection for Contaminated Sites 57, 18–22.

Dlamini, L.X., 2018. The competitive effect of vetiver grass on native grasses and implications for grassland rehabilitation in South Africa. (PhD Thesis).

Doherty, V.F., Otitoloju, A.A., 2013. Monitoring of soil and groundwater contamination following a pipeline explosion and petroleum product spillage in Ijegun, Lagos

Nigeria. Environ Monit Assess 185, 4159–4170. https://doi.org/10.1007/s10661-012-2858-8
Dong, T.T.T., Lee, B.-K., 2009. Characteristics, toxicity, and source apportionment of polycylic aromatic hydrocarbons (PAHs) in road dust of Ulsan, Korea. Chemosphere 74, 1245–1253. https://doi.org/10.1016/j.chemosphere.2008.11.035
D'Orazio, V., Ghanem, A., Senesi, N., 2013. Phytoremediation of Pyrene Contaminated Soils by Different Plant Species. CLEAN – Soil, Air, Water 41, 377–382. https://doi.org/10.1002/clen.201100653
dos Santos, J.J., Maranho, L.T., 2018. Rhizospheric microorganisms as a solution for the recovery of soils contaminated by petroleum: A review. Journal of Environmental Management 210, 104–113. https://doi.org/10.1016/j.jenvman.2018.01.015
Doty, S.L., Freeman, J.L., Cohu, C.M., Burken, J.G., Firrincieli, A., Simon, A., Khan, Z., Isebrands, J.G., Lukas, J., Blaylock, M.J., 2017. Enhanced Degradation of TCE on a Superfund Site Using Endophyte-Assisted Poplar Tree Phytoremediation. Environ. Sci. Technol. 51, 10050–10058. https://doi.org/10.1021/acs.est.7b01504
Dresler, S., Wójcik, M., Bednarek, W., Hanaka, A., Tukiendorf, A., 2015. The effect of silicon on maize growth under cadmium stress. Russ J Plant Physiol 62, 86–92. https://doi.org/10.1134/S1021443715010057
Dror, I., Gerstl, Z., Prost, R., Yaron, B., 2002. Abiotic behavior of entrapped petroleum products in the subsurface during leaching. Chemosphere 49, 1375–1388. https://doi.org/10.1016/S0045-6535(02)00529-5
Du, W., Wan, Y., Zhong, N., Fei, J., Zhang, Z., Chen, L., Hao, J., 2011. Status quo of soil petroleum contamination and evolution of bioremediation. Pet. Sci. 8, 502–514. https://doi.org/10.1007/s12182-011-0168-3
Duan, L., Naidu, R., Thavamani, P., Meaklim, J., Megharaj, M., 2015. Managing long-term polycyclic aromatic hydrocarbon contaminated soils: a risk-based approach. Environ Sci Pollut Res 22, 8927–8941. https://doi.org/10.1007/s11356-013-2270-0
Duan, Q., Han, G., Cui, B., Rennenberg, H., 2023. The effects of petroleum contaminated soils on the growth, gas exchange and antioxidative level of sea-buckthorn. Plant Soil. https://doi.org/10.1007/s11104-023-05888-w
Dudai, N., Tsion, I., Shamir, S.Z., Nitzan, N., Chaimovitsh, D., Shachter, A., Haim, A., 2018. Agronomic and economic evaluation of Vetiver grass (Vetiveria zizanioides L.) as means for phytoremediation of diesel polluted soils in Israel. Journal of Environmental Management 211, 247–255. https://doi.org/10.1016/j.jenvman.2018.01.013
Dzantor, E.K., 2007. Phytoremediation: the state of rhizosphere 'engineering' for accelerated rhizodegradation of xenobiotic contaminants. Journal of Chemical Technology & Biotechnology 82, 228–232. https://doi.org/10.1002/jctb.1662
Effendi, H., Widyatmoko, Utomo, B.A., Pratiwi, N.T.M., 2020. Ammonia and orthophosphate removal of tilapia cultivation wastewater with Vetiveria zizanioides. Journal of King Saud University - Science 32, 207–212. https://doi.org/10.1016/j.jksus.2018.04.018
Eissa, M.A., 2017. Phytoextraction mechanism of Cd by Atriplex lentiformis using some mobilizing agents. Ecological Engineering 108, 220–226. https://doi.org/10.1016/j.ecoleng.2017.08.025

Elliott, E.G., Trinh, P., Ma, X., Leaderer, B.P., Ward, M.H., Deziel, N.C., 2017. Unconventional oil and gas development and risk of childhood leukemia: Assessing the evidence. Science of The Total Environment 576, 138–147. https://doi.org/10.1016/j.scitotenv.2016.10.072

Elsamani, Z.A.E., Abdel Aziz, M.E.M., 2016. Environmental Study of Khartoum Refinery Liquid Effluents (Thesis). Sudan University of Science and Technology.

El-Sheshtawy, H.S., Mahdy, H.M., Sofy, A.R., Sofy, M.R., 2022. Production of biosurfactant by Bacillus megaterium and its correlation with lipid peroxidation of Lactuca sativa. Egyptian Journal of Petroleum 31, 1–6. https://doi.org/10.1016/j.ejpe.2022.03.001

Ene, A., Bosneaga Sion, A., Georgescu, L., 2010. Determination of heavy metals in soils using XRF technique. Romanian Journal of Physics 55, 815–820.

Entwistle, J.A., Hursthouse, A.S., Marinho Reis, P.A., Stewart, A.G., 2019. Metalliferous Mine Dust: Human Health Impacts and the Potential Determinants of Disease in Mining Communities. Curr Pollution Rep 5, 67–83. https://doi.org/10.1007/s40726-019-00108-5

EPA, 2001. Document Display | NEPIS | US EPA [WWW Document]. URL https://nepis.epa.gov/Exe/ZyNET.exe/10002SQD.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000003%5C10002SQD.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL (accessed 5.30.22).

Esteve-Turrillas, F.A., Scott, W.C., Pastor, A., Dean, J.R., 2005. Uptake and bioavailability of persistent organic pollutants by plants grown in contaminated soil. J. Environ. Monit. 7, 1093–1098. https://doi.org/10.1039/B507414B

Etemire, U., Muzan, M.A., 2017. Governance and regulatory strategies beyond the state: stakeholder participation and the ecological restoration of Ogoniland. Griffith Law Review 26, 275–298. https://doi.org/10.1080/10383441.2017.1366253

Etim, E.E., 2012. Phytoremediation and its mechanisms: a review. Int J Environ Bioenergy 2, 120–136.

Fanaei, F., Moussavi, G., Shekoohiyan, S., 2020. Enhanced treatment of the oil-contaminated soil using biosurfactant-assisted washing operation combined with H2O2-stimulated biotreatment of the effluent. Journal of Environmental Management 271, 110941. https://doi.org/10.1016/j.jenvman.2020.110941

Fawzy, E.M., 2008. Soil remediation using in situ immobilisation techniques. Chemistry and Ecology 24, 147–156. https://doi.org/10.1080/02757540801920154

Fenibo, E.O., Ijoma, G.N., Selvarajan, R., Chikere, C.B., 2019. Microbial Surfactants: The Next Generation Multifunctional Biomolecules for Applications in the Petroleum Industry and Its Associated Environmental Remediation. Microorganisms 7, 581. https://doi.org/10.3390/microorganisms7110581

Ferguson, A., Solo-Gabriele, H., Mena, K., 2020. Assessment for oil spill chemicals: Current knowledge, data gaps, and uncertainties addressing human physical health risk. Marine Pollution Bulletin 150, 110746. https://doi.org/10.1016/j.marpolbul.2019.110746

Fine, P., Graber, E.R., Yaron, B., 1997. Soil Interactions With Petroleum Hydrocarbons | Clay [WWW Document]. Scribd. URL https://www.scribd.com/document/257148371/Soil-Interactions-With-Petroleum-Hydrocarbons (accessed 12.27.16).

Fingas, M., 2016. Oil Spill Science and Technology. Gulf Professional Publishing.

Fingas, M., 2014. Handbook of Oil Spill Science and Technology. John Wiley & Sons.

Fingas, M., 2010. Oil Spill Science and Technology - 1st Edition [WWW Document]. URL https://www.elsevier.com/books/oil-spill-science-and-technology/fingas/978-1-85617-943-0 (accessed 5.29.22).

Fismes, J., Perrin-Ganier, C., Empereur-Bissonnet, P., Morel, J.L., 2002. Soil-to-Root Transfer and Translocation of Polycyclic Aromatic Hydrocarbons by Vegetables Grown on Industrial Contaminated Soils. Journal of Environmental Quality 31, 1649–1656. https://doi.org/10.2134/jeq2002.1649

Foroozanfar, M., 2017. Environmental control in petroleum operations. J. CleanWAS 1, 18–22. https://doi.org/10.26480/jcleanwas.02.2017.18.22

Francis, A.J., Nancharaiah, Y.V., 2015. 9 - In situ and ex situ bioremediation of radionuclide-contaminated soils at nuclear and norm sites, in: van Velzen, L. (Ed.), Environmental Remediation and Restoration of Contaminated Nuclear and Norm Sites, Woodhead Publishing Series in Energy. Woodhead Publishing, pp. 185–236. https://doi.org/10.1016/B978-1-78242-231-0.00009-0

Fryer, M., Collins, C.D., Ferrier, H., Colvile, R.N., Nieuwenhuijsen, M.J., 2006. Human exposure modelling for chemical risk assessment: a review of current approaches and research and policy implications. Environmental Science & Policy 9, 261–274. https://doi.org/10.1016/j.envsci.2005.11.011

Futughe, A.E., Purchase, D., Jones, H., 2020. Phytoremediation Using Native Plants, in: Shmaefsky, B.R. (Ed.), Phytoremediation: In-Situ Applications, Concepts and Strategies in Plant Sciences. Springer International Publishing, Cham, pp. 285–327. https://doi.org/10.1007/978-3-030-00099-8_9

Gadagi, R.S., Krishnaraj, P.U., Kulkarni, J.H., Sa, T., 2004. The effect of combined Azospirillum inoculation and nitrogen fertilizer on plant growth promotion and yield response of the blanket flower Gaillardia pulchella. Scientia Horticulturae 100, 323–332. https://doi.org/10.1016/j.scienta.2003.10.002

Gajić, G., Mitrović, M., Pavlović, P., 2020. 6 - Feasibility of Festuca rubra L. native grass in phytoremediation, in: Pandey, V.C., Singh, D.P. (Eds.), Phytoremediation Potential of Perennial Grasses. Elsevier, pp. 115–164. https://doi.org/10.1016/B978-0-12-817732-7.00006-7

Galuzzi-Silva, M.C., Mata-Lima, H., 2021. Risk assessment of accidental oil release in a coastal region: the são paulo case study. Maritime Policy & Management 0, 1–15. https://doi.org/10.1080/03088839.2021.1968057

Gao, H., Wu, M., Liu, H., Xu, Y., Liu, Z., 2022. Effect of petroleum hydrocarbon pollution levels on the soil microecosystem and ecological function. Environmental Pollution 293, 118511.
Gao, H., Zhang, J., Lai, H., Xue, Q., 2017. Degradation of asphaltenes by two Pseudomonas aeruginosa strains and their effects on physicochemical properties of crude oil. International Biodeterioration & Biodegradation 122, 12–22. https://doi.org/10.1016/j.ibiod.2017.04.010
Gao, Y., Shen, Q., Ling, W., Ren, L., 2008. Uptake of polycyclic aromatic hydrocarbons by Trifolium pretense L. from water in the presence of a nonionic surfactant. Chemosphere 72, 636–643. https://doi.org/10.1016/j.chemosphere.2008.02.032
Garcia-Vaquero, M., Rajauria, G., Tiwari, B., 2020. Conventional extraction techniques: Solvent extraction, in: Sustainable Seaweed Technologies. Elsevier, pp. 171–189. https://doi.org/10.1016/B978-0-12-817943-7.00006-8
Gaspard, S., Ncibi, M.C., 2013. Biomass for Sustainable Applications: Pollution Remediation and Energy. Royal Society of Chemistry.
Gavrilescu, M., 2022. Enhancing phytoremediation of soils polluted with heavy metals. Current Opinion in Biotechnology 74, 21–31. https://doi.org/10.1016/j.copbio.2021.10.024
Gay, J., Shepherd, O., Thyden, M., Whitman, M., 2010. The health effects of oil contamination: A compilation of research. Worcester: Worcester Polytechnic Institute.
Gay, J.R., Korre, A., 2006. A spatially-evaluated methodology for assessing risk to a population from contaminated land. Environmental Pollution 142, 227–234. https://doi.org/10.1016/j.envpol.2005.10.035
Gbadamosi, M.R., Afolabi, T.A., Banjoko, O.O., Ogunneye, A.L., Abudu, K.A., Ogunbanjo, O.O., Jegede, D.O., 2018. Spatial distribution and lifetime cancer risk due to naturally occurring radionuclides in soils around tar-sand deposit area of Ogun State, southwest Nigeria. Chemosphere 193, 1036–1048. https://doi.org/10.1016/j.chemosphere.2017.11.132
Georgees, R.N., Hassan, R., 2022. Performance-related properties of low-volume roads when stabilised with a sustainable anionic polyacrylamide: particle and specimen-levels study. Road Materials and Pavement Design 23, 565–582. https://doi.org/10.1080/14680629.2020.1831945
Germaine, K.J., Keogh, E., Ryan, D., Dowling, D.N., 2009. Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiology Letters 296, 226–234. https://doi.org/10.1111/j.1574-6968.2009.01637.x
Ghavzan, N.J., Trivedy, R.K., 2005. Environmental pollution control by using phytoremediation technology. Pollution Research 24, 875.
Ghazaryan, K., Movsesyan, H., Ghazaryan, N., Watts, B.A., 2019. Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia. Environmental Pollution 249, 491–501. https://doi.org/10.1016/j.envpol.2019.03.070
Ghosh, I., Ghosh, M., Mukherjee, A., 2017. Remediation of Mine Tailings and Fly Ash Dumpsites: Role of Poaceae Family Members and Aromatic Grasses, in: Anjum, N.A., Gill, S.S., Tuteja, N. (Eds.), Enhancing Cleanup of Environmental Pollutants: Volume 1:

Biological Approaches. Springer International Publishing, Cham, pp. 117–167. https://doi.org/10.1007/978-3-319-55426-6_7
Gil-Martínez, M., López-García, Á., Domínguez, M.T., Kjøller, R., Navarro-Fernández, C.M., Rosendahl, S., Marañón, T., 2021. Soil fungal diversity and functionality are driven by plant species used in phytoremediation. Soil Biology and Biochemistry 153, 108102. https://doi.org/10.1016/j.soilbio.2020.108102
Gingade, S., Varghese, T., Manivel, P., 2015. Cultivation of Vetiver [Vetiveria zizanioides (Linn)].
Gkorezis, P., Daghio, M., Franzetti, A., Van Hamme, J.D., Sillen, W., Vangronsveld, J., 2016a. The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. Frontiers in Microbiology 7.
Gkorezis, P., Daghio, M., Franzetti, A., Van Hamme, J.D., Sillen, W., Vangronsveld, J., 2016b. The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01836
Glick, B.R., 2010. Using soil bacteria to facilitate phytoremediation. Biotechnology Advances 28, 367–374. https://doi.org/10.1016/j.biotechadv.2010.02.001
Gnansounou, E., Alves, C.M., Raman, J.K., 2017. Multiple applications of vetiver grass – a review. International Journal of Education and Learning Systems 02.
Gojgic-Cvijovic, G.D., Milic, J.S., Solevic, T.M., Beskoski, V.P., Ilic, M.V., Djokic, L.S., Narancic, T.M., Vrvic, M.M., 2012. Biodegradation of petroleum sludge and petroleum polluted soil by a bacterial consortium: a laboratory study. Biodegradation 23, 1–14. https://doi.org/10.1007/s10532-011-9481-1
Goldstein, B.D., Osofsky, H.J., Lichtveld, M.Y., 2011. The Gulf Oil Spill. New England Journal of Medicine 364, 1334–1348. https://doi.org/10.1056/NEJMra1007197
Gómez, L., Contreras, A., Bolonio, D., Quintana, J., Oñate-Sánchez, L., Merino, I., 2019. Chapter Ten - Phytoremediation with trees, in: Cánovas, F.M. (Ed.), Advances in Botanical Research, Molecular Physiology and Biotechnology of Trees. Academic Press, pp. 281–321. https://doi.org/10.1016/bs.abr.2018.11.010
Graf, F., Frei, M., 2013. Soil aggregate stability related to soil density, root length, and mycorrhiza using site-specific Alnus incana and Melanogaster variegatus s.l. Ecological Engineering 57, 314–323. https://doi.org/10.1016/j.ecoleng.2013.04.037
Greenfield, J.C., 1995. Vetiver grass (Vetiveria spp.), the ideal plant for metalliferous wastes and land after metal mining. In: Grimshaw RG, Helfer L (eds) Vetiver grass for soil and water conservation, land rehabilitation, and embankment stabilization. The World Bank, Washington DC, pp 3–38.
Greipsson, S., 2011. Phytoremediation. Nature Education Knowledge 3, 7.
Gross, S.A., Avens, H.J., Banducci, A.M., Sahmel, J., Panko, J.M., Tvermoes, B.E., 2013. Analysis of BTEX groundwater concentrations from surface spills associated with hydraulic fracturing operations. Journal of the Air & Waste Management Association 63, 424–432. https://doi.org/10.1080/10962247.2012.759166
Groysman, A., 2014. History of Crude Oil and Petroleum Products, in: Groysman, A. (Ed.), Corrosion in Systems for Storage and Transportation of Petroleum Products and Biofuels: Identification, Monitoring and Solutions. Springer Netherlands, Dordrecht, pp. 221–226. https://doi.org/10.1007/978-94-007-7884-9_10

Guarino, C., Sciarrillo, R., 2017. Effectiveness of in situ application of an Integrated Phytoremediation System (IPS) by adding a selected blend of rhizosphere microbes to heavily multi-contaminated soils. Ecological Engineering 99, 70–82. https://doi.org/10.1016/j.ecoleng.2016.11.051

Guerriero, E., Rotatori, M., Mosca, S., Rossetti, G., Pomponio, S., 2009. Comparison of different methods of purification of environmental samples for analysis of polycyclic aromatic hydrocarbons. Organohalogen Compd 71, 2167–2171.

Guidi Nissim, W., Castiglione, S., Guarino, F., Pastore, M.C., Labra, M., 2023. Beyond Cleansing: Ecosystem Services Related to Phytoremediation. Plants 12, 1031. https://doi.org/10.3390/plants12051031

Gunasekara, A.S., Xing, B., 2003. Sorption and Desorption of Naphthalene by Soil Organic Matter. Journal of Environmental Quality 32, 240–246. https://doi.org/10.2134/jeq2003.2400

Gunathilakae, N., Yapa, N., Hettiarachchi, R., 2018. Effect of arbuscular mycorrhizal fungi on the cadmium phytoremediation potential of Eichhornia crassipes (Mart.) Solms. Groundwater for Sustainable Development 7, 477–482. https://doi.org/10.1016/j.gsd.2018.03.008

Guo, J., Luo, Y., Ge, F., Wang, D., Diao, M., Yang, Q., Liu, Y., Qu, Z., Zhang, T., 2019. Study on Crude Oil and its Emulsification Characteristics, in: IGARSS 2019 - 2019 IEEE International Geoscience and Remote Sensing Symposium. Presented at the IGARSS 2019 - 2019 IEEE International Geoscience and Remote Sensing Symposium, pp. 8257–8259. https://doi.org/10.1109/IGARSS.2019.8898844

Guo, J., Wen, X., 2021. Performance and kinetics of benzo(a)pyrene biodegradation in contaminated water and soil and improvement of soil properties by biosurfactant amendment. Ecotoxicology and Environmental Safety 207, 111292. https://doi.org/10.1016/j.ecoenv.2020.111292

Guo, S., Xiao, C., Zhou, N., Chi, R., 2021. Speciation, toxicity, microbial remediation and phytoremediation of soil chromium contamination. Environ Chem Lett 19, 1413–1431. https://doi.org/10.1007/s10311-020-01114-6

Gupta, A., Yadav, S.S., Solanki, R.B., 2017. Phytoremediation of Agrochemicals: A sustainable strategy. INDiAN FARmER 276.

Gupta, O.P., 2019. Elements of Petroleum Refinery Engineering. Khanna Publishing House.

Gupta, R.D., 2006. Environment Pollution: Hazards And Control. Concept Publishing Company.

Guterres, J., Rossato, L., Doley, D., Pudmenzky, A., Bee, C., Cobena, V., 2019. Assessing germination characteristics of Australian native plant species in metal/metalloid solution. Journal of Hazardous Materials 364, 173–181. https://doi.org/10.1016/j.jhazmat.2018.10.019

Hakeem, K., Sabir, M., Ozturk, M., Mermut, A., 2014. Soil remediation and plants: prospects and challenges. Academic Press.

Hakeem, K.R., Bhat, R.A., Qadri, H. (Eds.), 2020. Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-35691-0

Haleyur, N., Shahsavari, E., Mansur, A.A., Koshlaf, E., Morrison, P.D., Osborn, A.M., Ball, A.S., 2016. Comparison of rapid solvent extraction systems for the GC–MS/MS

characterization of polycyclic aromatic hydrocarbons in aged, contaminated soil. MethodsX 3, 364–370. https://doi.org/10.1016/j.mex.2016.04.007
Halmemies, S., Gröndahl, S., Nenonen, K., Tuhkanen, T., 2003. Estimation of the Time Periods and Processes for Penetration of Selected Spilled Oils and Fuels in Different Soils in the Laboratory. Spill Science & Technology Bulletin 8, 451–465. https://doi.org/10.1016/S1353-2561(03)00002-1
Han, G., Cui, B.X., Zhang, X.X., Li, K.R., 2016. The effects of petroleum-contaminated soil on photosynthesis of Amorpha fruticosa seedlings. Int. J. Environ. Sci. Technol. 13, 2383–2392. https://doi.org/10.1007/s13762-016-1071-7
Han, Y., Nambi, I.M., Prabhakar Clement, T., 2018. Environmental impacts of the Chennai oil spill accident – A case study. Science of The Total Environment 626, 795–806. https://doi.org/10.1016/j.scitotenv.2018.01.128
Hano, C., 2022. Plant Hormones: Recent Advances, New Perspectives and Applications. BoD – Books on Demand.
Hao, X., Taghavi, S., Xie, P., Orbach, M.J., Alwathnani, H.A., Rensing, C., Wei, G., 2014. Phytoremediation of Heavy and Transition Metals Aided by Legume-Rhizobia Symbiosis. International Journal of Phytoremediation 16, 179–202. https://doi.org/10.1080/15226514.2013.773273
Haokip, I.C., Devi, M.H., Mishra, R., Kumar, D., Dey, P., 2023. Remediation of Polluted Soils for Managing Toxicity Stress in Crops of Dryland Ecosystems, in: Naorem, A., Machiwal, D. (Eds.), Enhancing Resilience of Dryland Agriculture Under Changing Climate: Interdisciplinary and Convergence Approaches. Springer Nature, Singapore, pp. 259–303. https://doi.org/10.1007/978-981-19-9159-2_15
Haq, S., Bhatti, A.A., Dar, Z.A., Bhat, S.A., 2020. Phytoremediation of Heavy Metals: An Eco-Friendly and Sustainable Approach, in: Hakeem, K.R., Bhat, R.A., Qadri, H. (Eds.), Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation. Springer International Publishing, Cham, pp. 215–231. https://doi.org/10.1007/978-3-030-35691-0_10
Harmsen, J., Naidu, R., 2013. Bioavailability as a tool in site management. Journal of Hazardous Materials 261, 840–846. https://doi.org/10.1016/j.jhazmat.2012.12.044
Harrad, S., 2005a. SUMMARY OF ANALYTICAL METHODS AND ASSOCIATED QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) PROCEDURES FOR SEMI-VOLATILE ORGANIC COMPOUNDS.
Harrad, S., 2005b. SUMMARY OF ANALYTICAL METHODS AND ASSOCIATED QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) PROCEDURES FOR SEMI-VOLATILE ORGANIC COMPOUNDS.
Hasnaoui, S.E., Fahr, M., Keller, C., Levard, C., Angeletti, B., Chaurand, P., Triqui, Z.E.A., Guedira, A., Rhazi, L., Colin, F., Smouni, A., 2020. Screening of Native Plants Growing on a Pb/Zn Mining Area in Eastern Morocco: Perspectives for Phytoremediation. Plants 9, 1458. https://doi.org/10.3390/plants9111458
Hazarika, A., Saikia, S., Devi, B., Yadav, M., Yadav, H.S., 2022. 6 - Oxidoreductase metalloenzymes as green catalyst for phytoremediation of environmental pollutants, in: Kumar, V., Shah, M.P., Shahi, S.K. (Eds.), Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water. Elsevier, pp. 141–172. https://doi.org/10.1016/B978-0-323-85763-5.00021-0

Hazen, T.C., 2018. Bioremediation, The Microbiology of the Terrestrial Deep Subsurface. CRC Press. https://doi.org/10.1201/9781351074568-14

He, Y., Langenhoff, A.A.M., Sutton, N.B., Rijnaarts, H.H.M., Blokland, M.H., Chen, F., Huber, C., Schröder, P., 2017. Metabolism of Ibuprofen by Phragmites australis: Uptake and Phytodegradation. Environ. Sci. Technol. 51, 4576–4584. https://doi.org/10.1021/acs.est.7b00458

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25, 101–110. https://doi.org/10.1023/A:1008119611481

Hlavay, J., Jonas, K., Elek, S., Inczedy, J., 1978. Characterization of the Particle Size and the Crystallinity of Certain Minerals by IR Spectrophotometry and other Instrumental Methods—II. Investigations on Quartz and Feldspar. Clays Clay Miner. 26, 139–143. https://doi.org/10.1346/CCMN.1978.0260209

Hoang, S.A., Lamb, D., Sarkar, B., Seshadri, B., Kit Yu, R.M., Anh Tran, T.K., O'Connor, J., Rinklebe, J., Kirkham, M.B., Vo, H.T., Bolan, N.S., 2022. Phosphorus application enhances alkane hydroxylase gene abundance in the rhizosphere of wild plants grown in petroleum-hydrocarbon-contaminated soil. Environmental Research 204, 111924. https://doi.org/10.1016/j.envres.2021.111924

Hoang, S.A., Lamb, D., Seshadri, B., Sarkar, B., Cheng, Y., Wang, L., Bolan, N.S., 2021. Petroleum hydrocarbon rhizoremediation and soil microbial activity improvement via cluster root formation by wild proteaceae plant species. Chemosphere 275, 130135. https://doi.org/10.1016/j.chemosphere.2021.130135

Horne, A.J., 2020. Phytoremediation by Constructed Wetlands, in: Phytoremediation of Contaminated Soil and Water. CRC Press.

Hou, J., Liu, W., Wang, B., Wang, Q., Luo, Y., Franks, A.E., 2015. PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. Chemosphere 138, 592–598. https://doi.org/10.1016/j.chemosphere.2015.07.025

Houshani, M., Salehi-Lisar, S.Y., Motafakkerazad, R., Movafeghi, A., 2021. Proposed Pathways for Phytodegradation of Phenanthrene and Pyrene in Maize (Zea Mays L.) Using GC-Ms Analysis (preprint). In Review. https://doi.org/10.21203/rs.3.rs-1110084/v1

Huang, W., Peng, P., Yu, Z., Fu, J., 2003. Effects of organic matter heterogeneity on sorption and desorption of organic contaminants by soils and sediments. Applied Geochemistry 18, 955–972. https://doi.org/10.1016/S0883-2927(02)00205-6

Huang, X.-D., El-Alawi, Y., Penrose, D.M., Glick, B.R., Greenberg, B.M., 2004a. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution 130, 465–476. https://doi.org/10.1016/j.envpol.2003.09.031

Huang, X.-D., El-Alawi, Y., Penrose, D.M., Glick, B.R., Greenberg, B.M., 2004b. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Pollut. 130, 465–476. https://doi.org/10.1016/j.envpol.2003.09.031

Hudek, C., Stanchi, S., D'Amico, M., Freppaz, M., 2017. Quantifying the contribution of the root system of alpine vegetation in the soil aggregate stability of moraine.

International Soil and Water Conservation Research 5, 36–42. https://doi.org/10.1016/j.iswcr.2017.02.001
Huesemann, M.H., Hausmann, T.S., Fortman, T.J., 2005. Leaching of BTEX from Aged Crude Oil Contaminated Model Soils: Experimental and Modeling Results. Soil and Sediment Contamination: An International Journal 14, 545–558. https://doi.org/10.1080/15320380500263733
Hunt, J., 2010. Mission Without a Map: The Politics and Policies of Restoration Following the Exxon Valdez Oil Spill, 1989-2002. EVOS Trustee Council.
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. Environ Sci Pollut Res 26, 18451–18464. https://doi.org/10.1007/s11356-019-04819-6
Hussain, K., Hoque, R.R., Balachandran, S., Medhi, S., Idris, M.G., Rahman, M., Hussain, F.L., 2018. Monitoring and Risk Analysis of PAHs in the Environment, in: Hussain, C.M. (Ed.), Handbook of Environmental Materials Management. Springer International Publishing, Cham, pp. 1–35. https://doi.org/10.1007/978-3-319-58538-3_29-2
Hutchinson, S. Lewis, Banks, M.K., Schwab, A.P., 2001. Phytoremediation of aged petroleum sludge: effect of inorganic fertilizer. Journal of environmental quality 30, 395–403.
Hutchinson, S. L., Banks, M.K., Schwab, A.P., 2001. Phytoremediation of aged petroleum sludge: effect of inorganic fertilizer. J. Environ. Qual. 30, 395–403.
Hyland, K.C., Blaine, A.C., Higgins, C.P., 2015. Accumulation of contaminants of emerging concern in food crops—part 2: Plant distribution. Environmental Toxicology and Chemistry 34, 2222–2230. https://doi.org/10.1002/etc.3068
Ikeura, H., Kawasaki, Y., Kaimi, E., Nishiwaki, J., Noborio, K., Tamaki, M., 2016. Screening of plants for phytoremediation of oil-contaminated soil. International Journal of Phytoremediation 18, 460–466. https://doi.org/10.1080/15226514.2015.1115957
Ilyas, N., Shoukat, U., Saeed, M., Akhtar, N., Yasmin, H., Khan, W., Iqbal, S., 2021. Comparison of plant growth and remediation potential of pyrochar and thermal desorption for crude oil-contaminated soils. Sci Rep 11, 2817. https://doi.org/10.1038/s41598-021-82243-y
Islam, B., 2015. Petroleum sludge, its treatment and disposal: A review. Int. J. Chem. Sci 13, 1584–1602.
Islam, M.A., Islam, M.S., Elahi, T.E., 2020. Effectiveness of Vetiver Grass on Stabilizing Hill Slopes: A Numerical Approach 106–115. https://doi.org/10.1061/9780784482797.011
Ite, A.E., Harry, T.A., Obadimu, C.O., Asuaiko, E.R., Inim, I.J., 2018. Petroleum Hydrocarbons Contamination of Surface Water and Groundwater in the Niger Delta Region of Nigeria. JEPHH 6, 51–61. https://doi.org/10.12691/jephh-6-2-2
Iturbe, R., Flores, C., Flores, R.Ma., Torres, L.G., 2005. Subsoil TPH and other petroleum fractions-contamination levels in an oil storage and distribution station in north-central Mexico. Chemosphere 61, 1618–1631. https://doi.org/10.1016/j.chemosphere.2005.04.047
Jadia, C.D., Fulekar, M.H., 2009. Phytoremediation of heavy metals: Recent techniques. African Journal of Biotechnology 8. https://doi.org/10.4314/ajb.v8i6.59987

Jafarinejad, S., 2016. Petroleum waste treatment and pollution control. Butterworth-Heinemann.
Jain, P.K., Soloman, P.E., Gaur, R.K., 2022. Chapter 18 - Higher plant remediation to control pollutants, in: Kumar, S., Hashmi, M.Z. (Eds.), Biological Approaches to Controlling Pollutants, Advances in Pollution Research. Woodhead Publishing, pp. 321–363. https://doi.org/10.1016/B978-0-12-824316-9.00005-7
Jameson, C.W., 2019. Polycyclic aromatic hydrocarbons and associated occupational exposure.
Jayampathi, T., Atugoda, T., Jayasinghe, C., 2019. 4 - Uptake and accumulation of pharmaceuticals and personal care products in leafy vegetables, in: Prasad, M.N.V., Vithanage, M., Kapley, A. (Eds.), Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology. Butterworth-Heinemann, pp. 87–113. https://doi.org/10.1016/B978-0-12-816189-0.00004-4
Jeffries, J., Martin, I. (Eds.), 2008. Updated technical background to the CLEA model: using science to create a better place. Environment Agency, Bristol.
Jernelöv, A., 2010. The Threats from Oil Spills: Now, Then, and in the Future. AMBIO 39, 353–366. https://doi.org/10.1007/s13280-010-0085-5
Jia, L., Zhou, J., Cao, J., Wu, Z., Liu, W., Yang, C., 2020. Foam fractionation for promoting rhamnolipids production by Pseudomonas aeruginosa D1 using animal fat hydrolysate as carbon source and its application in intensifying phytoremediation. Chemical Engineering and Processing - Process Intensification 158, 108177. https://doi.org/10.1016/j.cep.2020.108177
Jiang, S., Dai, G., Zhou, J., Zhong, J., Liu, J., Shu, Y., 2022. An assessment of integrated amendments of biochar and soil replacement on the phytotoxicity of metal(loid)s in rotated radish-soya bean-amaranth in a mining acidy soil. Chemosphere 287, 132082. https://doi.org/10.1016/j.chemosphere.2021.132082
John, G.F., Han, Y., Clement, T.P., 2016. Weathering patterns of polycyclic aromatic hydrocarbons contained in submerged Deepwater Horizon oil spill residues when re-exposed to sunlight. Science of The Total Environment 573, 189–202. https://doi.org/10.1016/j.scitotenv.2016.08.059
Johnsen, A.R., Wick, L.Y., Harms, H., 2005. Principles of microbial PAH-degradation in soil. Environmental Pollution 133, 71–84. https://doi.org/10.1016/j.envpol.2004.04.015
Jorgensen, E., 2010. Ecotoxicology. Academic Press.
Kalhoro, S.A., Xu, X., Chen, W., Hua, R., Raza, S., Ding, K., 2017. Effects of Different Land-Use Systems on Soil Aggregates: A Case Study of the Loess Plateau (Northern China). Sustainability 9, 1349. https://doi.org/10.3390/su9081349
Kamath, R., Rentz, J.A., Schinoor, J.L., Alvarez, P.J.J., 2004. Phytoremediation of hydrocarbon contaminated soils: Principles and applications. In: Petroleum biotechnology development and perspectives. Vazquez-Duhalt, R and Quintero-Ramirez, R. (eds). Elsevi er, Amsterdam, pp 447 – 478.
Kang, J.W., 2014. Removing environmental organic pollutants with bioremediation and phytoremediation. Biotechnol Lett 36, 1129–1139. https://doi.org/10.1007/s10529-014-1466-9

Kang, S.-W., Kim, Y.-B., Shin, J.-D., Kim, E.-K., 2010. Enhanced Biodegradation of Hydrocarbons in Soil by Microbial Biosurfactant, Sophorolipid. Appl Biochem Biotechnol 160, 780–790. https://doi.org/10.1007/s12010-009-8580-5

Kanwal, Q., Shad, S., 2018. Aspects of xenobiotics phytoremediation and role of endophytic bacteria and arbuscular mycorrhizal association.

Kar, K., Last, T., Haywood, C., Raine, R., 2009. Measurement of Vapor Pressures and Enthalpies of Vaporization of Gasoline and Ethanol Blends and Their Effects on Mixture Preparation in an SI Engine. SAE International Journal of Fuels and Lubricants 1, 132–144.

Karakurt, I., Aydin, G., 2013. Fossil fuel use and related carbon dioxide emissions: A global perspective. 23rd International Mining Congress and Exhibition of Turkey, IMCET 2013 3, 2137–2141.

Karlapudi, A.P., Venkateswarulu, T.C., Tammineedi, J., Kanumuri, L., Ravuru, B.K., Dirisala, V. ramu, Kodali, V.P., 2018. Role of biosurfactants in bioremediation of oil pollution-a review. Petroleum 4, 241–249. https://doi.org/10.1016/j.petlm.2018.03.007

Kassaye, G., Gabbiye, N., Alemu, A., 2017. Phytoremediation of chromium from tannery wastewater using local plant species. Water Practice and Technology 12, 894–901. https://doi.org/10.2166/wpt.2017.094

Keeran, N.S., Balasundaram, U., Govindan, G., Parida, A.K., 2019. Chapter 18 - Prosopis juliflora: A Potential Plant for Mining of Genes for Genetic Engineering to Enhance Phytoremediation of Metals, in: Prasad, M.N.V. (Ed.), Transgenic Plant Technology for Remediation of Toxic Metals and Metalloids. Academic Press, pp. 381–393. https://doi.org/10.1016/B978-0-12-814389-6.00018-3

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., Dumat, C., 2017. A comparison of technologies for remediation of heavy metal contaminated soils. Journal of Geochemical Exploration, Remediation of Polluted Soils - Part 2 182, 247–268. https://doi.org/10.1016/j.gexplo.2016.11.021

Khan, F.I., Husain, T., Hejazi, R., 2004. An overview and analysis of site remediation technologies. Journal of Environmental Management 71, 95–122. https://doi.org/10.1016/j.jenvman.2004.02.003

Khare, E., Arora, N.K., 2021. Biosurfactant based formulation of Pseudomonas guariconensis LE3 with multifarious plant growth promoting traits controls charcoal rot disease in Helianthus annus. World J Microbiol Biotechnol 37, 55. https://doi.org/10.1007/s11274-021-03015-4

Khedr, A.H.A., Abbas, M.A., Wahid, A.A.A., Quick, W.P., Abogadallah, G.M., 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of Pancratium maritimum L. to salt-stress. Journal of Experimental Botany 54, 2553–2562. https://doi.org/10.1093/jxb/erg277

Kiamarsi, Z., Kafi, M., Soleimani, M., Nezami, A., Lutts, S., 2022. Evaluating the bio-removal of crude oil by vetiver grass (Vetiveria zizanioides L.) in interaction with bacterial consortium exposed to contaminated artificial soils. International Journal of Phytoremediation 24, 483–492. https://doi.org/10.1080/15226514.2021.1954876

Kiiskila, J.D., Sarkar, D., Panja, S., Sahi, S.V., Datta, R., 2019. Remediation of acid mine drainage-impacted water by vetiver grass (Chrysopogon zizanioides): A multiscale

long-term study. Ecological Engineering 129, 97–108. https://doi.org/10.1016/j.ecoleng.2019.01.018
Kirk, J.L., Klironomos, J.N., Lee, H., Trevors, J.T., 2005. The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environmental Pollution 133, 455–465. https://doi.org/10.1016/j.envpol.2004.06.002
Kolesnikov, S.I., Zharkova, M.G., Kazeev, K.Sh., Kutuzova, I.V., Samokhvalova, L.S., Naleta, E.V., Zubkov, D.A., 2014. Ecotoxicity assessment of heavy metals and crude oil based on biological characteristics of chernozem. Russ J Ecol 45, 157–166. https://doi.org/10.1134/S1067413614030059
Košnář, Z., Mercl, F., Tlustoš, P., 2020. Long-term willows phytoremediation treatment of soil contaminated by fly ash polycyclic aromatic hydrocarbons from straw combustion. Environmental Pollution 264, 114787. https://doi.org/10.1016/j.envpol.2020.114787
Kostecki, P., 1999. Assessments And Remediation Of Oil Contaminated Soils. Taylor & Francis.
Koukoulakis, K.G., Kanellopoulos, P.G., Chrysochou, E., Koukoulas, V., Minaidis, M., Maropoulos, G., Nikoleli, G.-P., Bakeas, E., 2020. Leukemia and PAHs levels in human blood serum: Preliminary results from an adult cohort in Greece. Atmospheric Pollution Research 11, 1552–1565. https://doi.org/10.1016/j.apr.2020.06.018
Koul, B., Taak, P., 2018. Chemical Methods of Soil Remediation, in: Biotechnological Strategies for Effective Remediation of Polluted Soils. Springer Singapore, Singapore, pp. 77–84. https://doi.org/10.1007/978-981-13-2420-8_4
Kozdrój, J., van Elsas, J.D., 2001. Structural diversity of microbial communities in arable soils of a heavily industrialised area determined by PCR-DGGE fingerprinting and FAME profiling. Applied Soil Ecology 17, 31–42.
Kristanti, R.A., Hadibarata, T., 2023. Phytoremediation of contaminated water using aquatic plants, its mechanism and enhancement. Current Opinion in Environmental Science & Health 32, 100451. https://doi.org/10.1016/j.coesh.2023.100451
Kumar, A., Dorodnikov, M., Splettstößer, T., Kuzyakov, Y., Pausch, J., 2017. Effects of maize roots on aggregate stability and enzyme activities in soil. Geoderma 306, 50–57. https://doi.org/10.1016/j.geoderma.2017.07.007
Kumar, D., Nikhil, K., 2016a. Vetiver grass for manifold uses: a critical review. International Journal of Engineering & Technical Research (IJETR) 4, 146–1152.
Kumar, D., Nikhil, K., 2016b. Effect of FYM, NPK and Algal fertilizers on the Growth and Biomass of Vetiver Grass [Vetiveria zizanioides L.Nass] 3, 5.
Kumar, V., Shahi, S.K., Singh, S., 2018. Bioremediation: An Eco-sustainable Approach for Restoration of Contaminated Sites, in: Singh, J., Sharma, D., Kumar, G., Sharma, N.R. (Eds.), Microbial Bioprospecting for Sustainable Development. Springer, Singapore, pp. 115–136. https://doi.org/10.1007/978-981-13-0053-0_6
Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K., 2020a. Fate of Total Petroleum Hydrocarbons in the Environment, in: Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K. (Eds.), Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation. Springer International Publishing, Cham, pp. 57–77. https://doi.org/10.1007/978-3-030-24035-6_3

Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K., 2020b. Impact of Total Petroleum Hydrocarbons on Human Health, in: Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K. (Eds.), Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation. Springer International Publishing, Cham, pp. 139–165. https://doi.org/10.1007/978-3-030-24035-6_6

Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K., 2020c. Bioavailability of Total Petroleum Hydrocarbons, in: Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K. (Eds.), Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation. Springer International Publishing, Cham, pp. 79–94. https://doi.org/10.1007/978-3-030-24035-6_4

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

Kwiatkowska-Malina, J., 2018. Functions of organic matter in polluted soils: The effect of organic amendments on phytoavailability of heavy metals. Applied Soil Ecology, HUMUSICA 3 - Reviews, Applications, Tools 123, 542–545. https://doi.org/10.1016/j.apsoil.2017.06.021

Lai, C.-C., Huang, Y.-C., Wei, Y.-H., Chang, J.-S., 2009a. Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials 167, 609–614. https://doi.org/10.1016/j.jhazmat.2009.01.017

Lai, C.-C., Huang, Y.-C., Wei, Y.-H., Chang, J.-S., 2009b. Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials 167, 609–614. https://doi.org/10.1016/j.jhazmat.2009.01.017

Lai, H.-Y., Hseu, Z.-Y., Chen, T.-C., Chen, B.-C., Guo, H.-Y., Chen, Z.-S., 2010. Health Risk-Based Assessment and Management of Heavy Metals-Contaminated Soil Sites in Taiwan. International Journal of Environmental Research and Public Health 7, 3595–3614. https://doi.org/10.3390/ijerph7103596

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Basu, N. (Nil), Baldé, A.B., Bertollini, R., Bose-O'Reilly, S., Boufford, J.I., Breysse, P.N., Chiles, T., Mahidol, C., Coll-Seck, A.M., Cropper, M.L., Fobil, J., Fuster, V., Greenstone, M., Haines, A., Hanrahan, D., Hunter, D., Khare, M., Krupnick, A., Lanphear, B., Lohani, B., Martin, K., Mathiasen, K.V., McTeer, M.A., Murray, C.J.L., Ndahimananjara, J.D., Perera, F., Potočnik, J., Preker, A.S., Ramesh, J., Rockström, J., Salinas, C., Samson, L.D., Sandilya, K., Sly, P.D., Smith, K.R., Steiner, A., Stewart, R.B., Suk, W.A., van Schayck, O.C.P., Yadama, G.N., Yumkella, K., Zhong, M., 2018. The Lancet Commission on pollution and health. The Lancet 391, 462–512. https://doi.org/10.1016/S0140-6736(17)32345-0

Lane, D.J., Jokiniemi, J., Heimonen, M., Peräniemi, S., Kinnunen, N.M., Koponen, H., Lähde, A., Karhunen, T., Nivajärvi, T., Shurpali, N., Sippula, O., 2020. Thermal treatment of municipal solid waste incineration fly ash: Impact of gas atmosphere on the volatility of major, minor, and trace elements. Waste Management 114, 1–16. https://doi.org/10.1016/j.wasman.2020.06.035

Larramendy, M., Soloneski, S., 2015. Emerging Pollutants in the Environment: Current and Further Implications. BoD – Books on Demand.

Lau, E.V., Gan, S., Ng, H.K., 2010. Extraction Techniques for Polycyclic Aromatic Hydrocarbons in Soils. International Journal of Analytical Chemistry 2010, e398381. https://doi.org/10.1155/2010/398381

Lavania, U., 2004. Vetiver system ecotechnology for water qualityimprovement and environmental enhancement. Curr Sci86(1):11–14.

Lavania, U.C., 2004. MEETING REPORT: Vetiver system ecotechnology for water quality improvement and environmental enhancement. Current Science 86, 11–14.

Lavania, U.C., 2000. Vetiver grass technology for environmental protection and sustainable development. Current Science 78, 944–946.

Le Bissonnais, Y., Prieto, I., Roumet, C., Nespoulous, J., Metayer, J., Huon, S., Villatoro, M., Stokes, A., 2018. Soil aggregate stability in Mediterranean and tropical agro-ecosystems: effect of plant roots and soil characteristics. Plant Soil 424, 303–317. https://doi.org/10.1007/s11104-017-3423-6

Lee, B.-K., 2010. Sources, Distribution and Toxicity of Polyaromatic Hydrocarbons (PAHs) in Particulate Matter, Air Pollution. IntechOpen. https://doi.org/10.5772/10045

Lee, B.X.Y., Hadibarata, T., Yuniarto, A., 2020. Phytoremediation Mechanisms in Air Pollution Control: a Review. Water Air Soil Pollut 231, 437. https://doi.org/10.1007/s11270-020-04813-6

Lee, H., 2011. Impacts of oil spills: Ecological, human health and economic.

Lee, J.H., 2013a. An overview of phytoremediation as a potentially promising technology for environmental pollution control. Biotechnol Bioproc E 18, 431–439. https://doi.org/10.1007/s12257-013-0193-8

Lee, J.H., 2013b. An overview of phytoremediation as a potentially promising technology for environmental pollution control. Biotechnol Bioproc E 18, 431–439. https://doi.org/10.1007/s12257-013-0193-8

Lee, Y.-C., Kim, E.J., Ko, D.A., Yang, J.-W., 2011. Water-soluble organo-building blocks of aminoclay as a soil-flushing agent for heavy metal contaminated soil. Journal of Hazardous Materials 196, 101–108. https://doi.org/10.1016/j.jhazmat.2011.08.077

Leifert, H., 2012. Gulf oil spill threatens subsurface biodiversity [WWW Document]. URL https://www.earthmagazine.org/article/gulf-oil-spill-threatens-subsurface-biodiversity (accessed 5.29.22).

Li, C., Zhou, K., Qin, W., Tian, C., Qi, M., Yan, X., Han, W., 2019. A Review on Heavy Metals Contamination in Soil: Effects, Sources, and Remediation Techniques. null 28, 380–394. https://doi.org/10.1080/15320383.2019.1592108

Li, D., Quan, X., Zhang, Y., Zhao, Y., 2008. Microwave-Induced Thermal Treatment of Petroleum Hydrocarbon-Contaminated Soil. Soil and Sediment Contamination: An International Journal 17, 486–496. https://doi.org/10.1080/15320380802304375

Li, J., Peng, Q., Liang, D., Liang, S., Chen, J., Sun, H., Li, S., Lei, P., 2016. Effects of aging on the fraction distribution and bioavailability of selenium in three different soils. Chemosphere 144, 2351–2359. https://doi.org/10.1016/j.chemosphere.2015.11.011

Li, J., Yuan, X., Ge, L., Li, Q., Li, Z., Wang, L., Liu, Y., 2020. Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification. Agriculture, Ecosystems & Environment 304, 107126. https://doi.org/10.1016/j.agee.2020.107126

Li, R.-L., Liu, B.-B., Zhu, Y.-X., Zhang, Y., 2016. Effects of flooding and aging on phytoremediation of typical polycyclic aromatic hydrocarbons in mangrove sediments by Kandelia obovata seedlings. Ecotoxicology and Environmental Safety 128, 118–125. https://doi.org/10.1016/j.ecoenv.2016.02.002
Li, S., Pi, Y., Bao, M., Zhang, C., Zhao, D., Li, Y., Sun, P., Lu, J., 2015. Effect of rhamnolipid biosurfactant on solubilization of polycyclic aromatic hydrocarbons. Marine Pollution Bulletin 101, 219–225. https://doi.org/10.1016/j.marpolbul.2015.09.059
Li, S.-N., Zhang, C., Li, F., Ren, N.-Q., Ho, S.-H., 2023. Recent advances of algae-bacteria consortia in aquatic remediation. Critical Reviews in Environmental Science and Technology 53, 315–339. https://doi.org/10.1080/10643389.2022.2052704
Li, X., Wang, X., Chen, Y., Yang, X., Cui, Z., 2019. Optimization of combined phytoremediation for heavy metal contaminated mine tailings by a field-scale orthogonal experiment. Ecotoxicology and Environmental Safety 168, 1–8. https://doi.org/10.1016/j.ecoenv.2018.10.012
Liang, B., Yang, X., He, X., Zhou, J., 2011. Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. Biol Fertil Soils 47, 121–128. https://doi.org/10.1007/s00374-010-0511-7
Liao, C., Liang, X., Lu, G., Thai, T., Xu, W., Dang, Z., 2015. Effect of surfactant amendment to PAHs-contaminated soil for phytoremediation by maize (Zea mays L.). Ecotoxicology and Environmental Safety 112, 1–6. https://doi.org/10.1016/j.ecoenv.2014.10.025
Liao, C., Xu, W., Lu, G., Deng, F., Liang, X., Guo, C., Dang, Z., 2016a. Biosurfactant-enhanced phytoremediation of soils contaminated by crude oil using maize (Zea mays. L). Ecological Engineering 92, 10–17. https://doi.org/10.1016/j.ecoleng.2016.03.041
Liao, C., Xu, W., Lu, G., Deng, F., Liang, X., Guo, C., Dang, Z., 2016b. Biosurfactant-enhanced phytoremediation of soils contaminated by crude oil using maize (Zea mays. L). Ecological Engineering 92, 10–17. https://doi.org/10.1016/j.ecoleng.2016.03.041
Liduino, V.S., Servulo, E.F.C., Oliveira, F.J.S., 2018. Biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using sunflower (Helianthus annuus L.). Journal of Environmental Science and Health, Part A 53, 609–616. https://doi.org/10.1080/10934529.2018.1429726
Limmer, M., Burken, J., 2016. Phytovolatilization of Organic Contaminants. Environ. Sci. Technol. 50, 6632–6643. https://doi.org/10.1021/acs.est.5b04113
Lin, L., Li, Z., Wu, C., Wang, J., Liang, D., Xia, H., Lv, X., Tang, Y., Wang, X., Deng, Q., Liao, M., 2022. Melatonin promotes iron uptake and accumulation in peach. Scientia Horticulturae 306, 111481. https://doi.org/10.1016/j.scienta.2022.111481
Lindén, O., Pålsson, J., 2013. Oil Contamination in Ogoniland, Niger Delta. AMBIO 42, 685–701. https://doi.org/10.1007/s13280-013-0412-8
Ling, N., Wang, T., Kuzyakov, Y., 2022. Rhizosphere bacteriome structure and functions. Nat Commun 13, 836. https://doi.org/10.1038/s41467-022-28448-9
Liu, J., Xin, X., Zhou, Q., 2018. Phytoremediation of contaminated soils using ornamental plants. Environ. Rev. 26, 43–54. https://doi.org/10.1139/er-2017-0022
Liu, L., Li, W., Song, W., Guo, M., 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. Science of The Total Environment 633, 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161

Liu, P.-W.G., Chang, T.C., Chen, C.-H., Wang, M.-Z., Hsu, H.-W., 2013. Effects of soil organic matter and bacterial community shift on bioremediation of diesel-contaminated soil. International Biodeterioration & Biodegradation 85, 661–670. https://doi.org/10.1016/j.ibiod.2013.01.010

Liu, Q., Tang, J., Bai, Z., Hecker, M., Giesy, J.P., 2015. Distribution of petroleum degrading genes and factor analysis of petroleum contaminated soil from the Dagang Oilfield, China. Sci Rep 5, 11068. https://doi.org/10.1038/srep11068

Liu, W., Sun, J., Ding, L., Luo, Y., Chen, M., Tang, C., 2013. Rhizobacteria (Pseudomonas sp. SB) assist phytoremediation of oily-sludge-contaminated soil by tall fescue (Testuca arundinacea L.). Plant Soil 371, 533–542. https://doi.org/10.1007/s11104-013-1717-x

Liu, Z.-Y., Wang, G.-C., Zhou, B.-C., 2008. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresource Technology, Exploring Horizons in Biotechnology: A Global Venture 99, 4717–4722. https://doi.org/10.1016/j.biortech.2007.09.073

Longman, J., Veres, D., Finsinger, W., Ersek, V., 2018. Exceptionally high levels of lead pollution in the Balkans from the Early Bronze Age to the Industrial Revolution. Proceedings of the National Academy of Sciences 115, E5661–E5668. https://doi.org/10.1073/pnas.1721546115

Lopes, A., da Rosa-Osman, S.M., Piedade, M.T.F., 2009. Effects of crude oil on survival, morphology, and anatomy of two aquatic macrophytes from the Amazon floodplains. Hydrobiologia 636, 295–305. https://doi.org/10.1007/s10750-009-9959-6

Lorestani, B., Yousefi, N., Cheraghi, M., Farmany, A., 2013. Phytoextraction and phytostabilization potential of plants grown in the vicinity of heavy metal-contaminated soils: a case study at an industrial town site. Environ Monit Assess 185, 10217–10223. https://doi.org/10.1007/s10661-013-3326-9

Lu, H., Sun, J., Zhu, L., 2017. The role of artificial root exudate components in facilitating the degradation of pyrene in soil. Sci Rep 7, 7130. https://doi.org/10.1038/s41598-017-07413-3

Lu, H., Wang, W., Li, F., Zhu, L., 2019. Mixed-surfactant-enhanced phytoremediation of PAHs in soil: Bioavailability of PAHs and responses of microbial community structure. Science of The Total Environment 653, 658–666. https://doi.org/10.1016/j.scitotenv.2018.10.385

Lu, L., Zhang, J., Peng, C., 2019. Shift of Soil Polycyclic Aromatic Hydrocarbons (PAHs) Dissipation Pattern and Microbial Community Composition due to Rhamnolipid Supplementation. Water Air Soil Pollut 230, 107. https://doi.org/10.1007/s11270-019-4118-9

M, C., H.s, S., H, M., 2010. BIOREMEDIATION OF A CRUDE OIL - POLLUTED SOIL BY APPLICATION OF FERTILIZERS 7, 319–326.

Ma, B., He, Y., Chen, H., Xu, J., Rengel, Z., 2010. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: Synthesis through meta-analysis. Environmental Pollution 158, 855–861. https://doi.org/10.1016/j.envpol.2009.09.024

Ma, C., Naidu, R., Liu, F., Lin, C., Ming, H., 2012. Influence of hybrid giant Napier grass on salt and nutrient distributions with depth in a saline soil. Biodegradation 23, 907–916. https://doi.org/10.1007/s10532-012-9583-4

Ma, H., Wang, A., Zhang, M., Li, H., Du, S., Bai, L., Zhong, M., Chen, S., 2018. Compared the physiological response of two petroleum-tolerant contrasting plants to petroleum stress. International Journal of Phytoremediation 20, 1043–1048. https://doi.org/10.1080/15226514.2018.1460303

Ma, Y., Rajkumar, M., Moreno, A., Zhang, C., Freitas, H., 2017. Serpentine endophytic bacterium Pseudomonas azotoformans ASS1 accelerates phytoremediation of soil metals under drought stress. Chemosphere 185, 75–85. https://doi.org/10.1016/j.chemosphere.2017.06.135

Macek, T., Uhlik, O., Jecna, K., Novakova, M., Lovecka, P., Rezek, J., Dudkova, V., Stursa, P., Vrchotova, B., Pavlikova, D., Demnerova, K., Mackova, M., 2009. Advances in Phytoremediation and Rhizoremediation, in: Singh, A., Kuhad, R.C., Ward, O.P. (Eds.), Advances in Applied Bioremediation, Soil Biology. Springer, Berlin, Heidelberg, pp. 257–277. https://doi.org/10.1007/978-3-540-89621-0_14

Mackay, D., van Wesenbeeck, I., 2014. Correlation of Chemical Evaporation Rate with Vapor Pressure. Environ. Sci. Technol. 48, 10259–10263. https://doi.org/10.1021/es5029074

Maffei, M., 2003. Vetiveria: The Genus Vetiveria. CRC Press.

Maffei, M., 2002. Vetiveria: The Genus Vetiveria. CRC Press.

Magangana, T.P., Makunga, N.P., Amos Fawole, O., Opara, U.L., 2021. Effect of Solvent Extraction and Blanching Pre-Treatment on Phytochemical, Antioxidant Properties, Enzyme Inactivation and Antibacterial Activities of 'Wonderful' Pomegranate Peel Extracts. Processes 9, 1012. https://doi.org/10.3390/pr9061012

Magris, R.A., Giarrizzo, T., 2020. Mysterious oil spill in the Atlantic Ocean threatens marine biodiversity and local people in Brazil. Marine Pollution Bulletin 153, 110961. https://doi.org/10.1016/j.marpolbul.2020.110961

Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R., Zhang, Z., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotoxicology and Environmental Safety 126, 111–121. https://doi.org/10.1016/j.ecoenv.2015.12.023

Majid, S.N., Khwakaram, A.I., Rasul, G.A.M., Ahmed, Z.H., 2014. Bioaccumulation, enrichment and translocation factors of some heavy metals in Typha angustifolia and Phragmites australis Species growing ALONG Qalyasan stream in Sulaimani City/IKR. Journal of Zankoy Sulaimani-Part A 16, 93–109.

Malar, S., Manikandan, R., Favas, P.J.C., Vikram Sahi, S., Venkatachalam, P., 2014. Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in Sesbania grandiflora: A potential plant for phytoremediation. Ecotoxicology and Environmental Safety 108, 249–257. https://doi.org/10.1016/j.ecoenv.2014.05.018

Malik, B., Pirzadah, T.B., Hakeem, K.R., 2022. Chapter 20 - Phytoremediation of persistent organic pollutants (POPs), in: Bhat, R.A., Tonelli, F.M.P., Dar, G.H., Hakeem, K. (Eds.), Phytoremediation. Academic Press, pp. 415–436. https://doi.org/10.1016/B978-0-323-89874-4.00010-8

Mao, D., Lookman, R., Weghe, H.V.D., Weltens, R., Vanermen, G., Brucker, N.D., Diels, L., 2009. Combining HPLC-GCXGC, GCXGC/ToF-MS, and Selected Ecotoxicity Assays for Detailed Monitoring of Petroleum Hydrocarbon Degradation in Soil and Leaching Water. Environ. Sci. Technol. 43, 7651–7657. https://doi.org/10.1021/es9015603

Margesin, R., Hämmerle, M., Tscherko, D., 2007. Microbial Activity and Community Composition during Bioremediation of Diesel-Oil-Contaminated Soil: Effects of Hydrocarbon Concentration, Fertilizers, and Incubation Time. Microb Ecol 53, 259–269. https://doi.org/10.1007/s00248-006-9136-7

Mariano, E.D., Keltjens, W.G., 2005. Long-Term Effects of Aluminum Exposure on Nutrient Uptake by Maize Genotypes Differing in Aluminum Resistance. Journal of Plant Nutrition 28, 323–333. https://doi.org/10.1081/PLN-200047625

Martens, D., Maguhn, J., Spitzauer, P., Kettrup, A., 1997. Occurrence and distribution of polycyclic aromatic hydrocarbons (PAHs) in an agricultural ecosystem. Fresenius J Anal Chem 359, 546–554. https://doi.org/10.1007/s002160050630

Martín, C.M., González Becerra, A., Blanco Santos, M.J., 2004. [Biological treatments for contaminated soils: hydrocarbon contamination. Fungal applications in bioremediation treatment]. Rev Iberoam Micol 21, 103–120.

Martinez, E., Gros, M., Lacorte, S., Barceló, D., 2004. Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels. Journal of Chromatography A 1047, 181–188. https://doi.org/10.1016/j.chroma.2004.07.003

Masekameni, M.D., Moolla, R., Gulumian, M., Brouwer, D., 2019. Risk Assessment of Benzene, Toluene, Ethyl Benzene, and Xylene Concentrations from the Combustion of Coal in a Controlled Laboratory Environment. International Journal of Environmental Research and Public Health 16, 95. https://doi.org/10.3390/ijerph16010095

Mathur, S., Kalaji, H.M., Jajoo, A., 2016. Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. Photosynthetica 54, 185–192. https://doi.org/10.1007/s11099-016-0198-6

McCutcheon, S.C., Schnoor, J.L., 2004. Phytoremediation: transformation and control of contaminants. John Wiley & Sons.

McWhirter, C., 2015. Study Finds Link Between Dolphin Deaths, Oil Exposure [WWW Document]. WSJ. URL http://www.wsj.com/articles/study-finds-link-between-dolphin-deaths-oil-exposure-1432158102 (accessed 5.29.22).

MDH, 2005. Environmental Protection Agency Safe Drinking Water Act Standards: Volatile and Synthetic Organic Chemicals.

Meeinkuirt, W., Kruatrachue, M., Pichtel, J., Phusantisampan, T., Saengwilai, P., 2016. Influence of organic amendments on phytostabilization of Cd-contaminated soil by Eucalyptus camaldulensis. ScienceAsia 42, 83. https://doi.org/10.2306/scienceasia1513-1874.2016.42.083

Megharaj, M., Naidu, R., 2017. Soil and brownfield bioremediation. Microbial Biotechnology 10, 1244–1249. https://doi.org/10.1111/1751-7915.12840

Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., Naidu, R., 2011. Bioremediation approaches for organic pollutants: A critical perspective. Environment International 37, 1362–1375. https://doi.org/10.1016/j.envint.2011.06.003

Meglouli, H., Fontaine, J., Verdin, A., Magnin-Robert, M., Tisserant, B., Hijri, M., Lounès-Hadj Sahraoui, A., 2019. Aided Phytoremediation to Clean Up Dioxins/Furans-Aged Contaminated Soil: Correlation between Microbial Communities and Pollutant Dissipation. Microorganisms 7, 523. https://doi.org/10.3390/microorganisms7110523

Mench, M., Schwitzguébel, J.-P., Schroeder, P., Bert, V., Gawronski, S., Gupta, S., 2009. Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ Sci Pollut Res 16, 876. https://doi.org/10.1007/s11356-009-0252-z

Menendez-Vega, D., Gallego, J.L.R., Pelaez, A.I., Fernandez de Cordoba, G., Moreno, J., Muñoz, D., Sanchez, J., 2007. Engineered in situ bioremediation of soil and groundwater polluted with weathered hydrocarbons. European Journal of Soil Biology, In situ Bioremediation 43, 310–321. https://doi.org/10.1016/j.ejsobi.2007.03.005

Merkl, N., Schultze-Kraft, R., Infante, C., 2005. Phytoremediation in the tropics – influence of heavy crude oil on root morphological characteristics of graminoids. Environmental Pollution 138, 86–91. https://doi.org/10.1016/j.envpol.2005.02.023

Meudec, A., Poupart, N., Dussauze, J., Deslandes, E., 2007. Relationship between heavy fuel oil phytotoxicity and polycyclic aromatic hydrocarbon contamination in Salicornia fragilis. Science of The Total Environment 381, 146–156. https://doi.org/10.1016/j.scitotenv.2007.04.005

Mielki, G.F., Novais, R.F., Ker, J.C., Vergütz, L., Castro, G.F. de, 2016. Iron Availability in Tropical Soils and Iron Uptake by Plants. Rev. Bras. Ciênc. Solo 40. https://doi.org/10.1590/18069657rbcs20150174

Millioli, V.S., Servulo, E.L.C., Sobral, L.G.S., Carvalho, D.D. de, 2009. Bioremediation of crude oil-bearing soil: evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil bioregradation efficiency. Global Nest Journal 11, 181–188.

Mirzaee, M.M., ZakeriNia, M., Farasati, M., 2021. The effects of phytoremediation of treated urban wastewater on the discharge of surface and subsurface drippers (Case study: Gorgan wastewater treatment plant in northern Iran). Cleaner Engineering and Technology 4, 100210. https://doi.org/10.1016/j.clet.2021.100210

Mishra, A.K., Kumar, G.S., 2015. Weathering of Oil Spill: Modeling and Analysis. Aquatic Procedia, INTERNATIONAL CONFERENCE ON WATER RESOURCES, COASTAL AND OCEAN ENGINEERING (ICWRCOE'15) 4, 435–442. https://doi.org/10.1016/j.aqpro.2015.02.058

Mishra, I., Fatima, T., Egamberdieva, D., Arora, N.K., 2020. Novel Bioformulations Developed from Pseudomonas putida BSP9 and Its Biosurfactant for Growth Promotion of Brassica juncea (L.). Plants 9, 1349. https://doi.org/10.3390/plants9101349

Mohan, I., Goria, K., Dhar, S., Kothari, R., Bhau, B. s., Pathania, D., 2021. Phytoremediation of Heavy Metals from the Biosphere Perspective and Solutions, in: Pollutants and Water Management. John Wiley & Sons, Ltd, pp. 95–127. https://doi.org/10.1002/9781119693635.ch5

Molins, S., Mayer, K.U., Amos, R.T., Bekins, B.A., 2010. Vadose zone attenuation of organic compounds at a crude oil spill site — Interactions between biogeochemical reactions and multicomponent gas transport. Journal of Contaminant Hydrology, Frontiers in

Reactive Transport: Microbial Dynamics nad Redox Zonation in the Subsurface 112, 15–29. https://doi.org/10.1016/j.jconhyd.2009.09.002
Mondal, S., Patel, P.P., 2020. Implementing Vetiver grass-based riverbank protection programmes in rural West Bengal, India. Nat Hazards. https://doi.org/10.1007/s11069-020-04025-5
Monsuru, S.A., daud, N. wan, 2016. Effect of Fertilizer rates and Soil Series on Root Morphological Traits and Root: Shoot Ratio of Immature Natural Rubber (Hevea brasiliensis). International Journal of Scientific and Engineering Research 7.
Morakinyo, O.M., Mukhola, M.S., Mokgobu, M.I., 2020. Concentration levels and carcinogenic and mutagenic risks of PM2.5-bound polycyclic aromatic hydrocarbons in an urban–industrial area in South Africa. Environ Geochem Health 42, 2163–2178. https://doi.org/10.1007/s10653-019-00493-2
Morikawa, H., Erkin, Ö.C., 2003. Basic processes in phytoremediation and some applications to air pollution control. Chemosphere, Environmental and Public Health Management 52, 1553–1558. https://doi.org/10.1016/S0045-6535(03)00495-8
Moritsuka, N., Kawamura, K., Tsujimoto, Y., Rabenarivo, M., Andriamananjara, A., Rakotoson, T., Razafimbelo, T., 2019. Comparison of visual and instrumental measurements of soil color with different low-cost colorimeters. Soil Science and Plant Nutrition 65, 605–615. https://doi.org/10.1080/00380768.2019.1676624
Mosa, K.A., Ismail, A., Helmy, M., 2017. Introduction to Plant Stresses, in: Mosa, K.A., Ismail, A., Helmy, M. (Eds.), Plant Stress Tolerance: An Integrated Omics Approach, SpringerBriefs in Systems Biology. Springer International Publishing, Cham, pp. 1–19. https://doi.org/10.1007/978-3-319-59379-1_1
Moussavi, G., Rezaei, M., 2017. Exploring the advanced oxidation/reduction processes in the VUV photoreactor for dechlorination and mineralization of trichloroacetic acid: Parametric experiments, degradation pathway and bioassessment. Chemical Engineering Journal 328, 331–342. https://doi.org/10.1016/j.cej.2017.07.006
Moustafa, Y.M.M., 2014. Petroleum in View of its Classification, Assay and Analysis.
Mucciarelli, M., Bertea, C.M., Cozzo, M., Scannerini, S., Gallino, M., 1998. VETIVERIA ZIZANIOIDES AS A TOOL FOR ENVIRONMENTAL ENGINEERING | International Society for Horticultural Science [WWW Document]. URL http://www.actahort.org/books/457/457_33.htm (accessed 2.26.17).
Müller, F.K.-Z.B., Sedláčková, M.K.-I., 2003. Contamination of soils and groundwater by petroleum hydrocarbons and volatile organic compounds–Case study: ELSLAV BRNO. Bulletin of Geosciences 78, 225–239.
Mulligan, C.N., 2005a. Environmental applications for biosurfactants. Environmental Pollution 133, 183–198. https://doi.org/10.1016/j.envpol.2004.06.009
Mulligan, C.N., 2005b. Environmental applications for biosurfactants. Environmental Pollution 133, 183–198. https://doi.org/10.1016/j.envpol.2004.06.009
Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering Geology, Geoenvironmental Engineering 60, 193–207. https://doi.org/10.1016/S0013-7952(00)00101-0
Muthusaravanan, S., Sivarajasekar, N., Vivek, J.S., Paramasivan, T., Naushad, Mu., Prakashmaran, J., Gayathri, V., Al-Duaij, O.K., 2018. Phytoremediation of heavy

metals: mechanisms, methods and enhancements. Environ Chem Lett 16, 1339–1359. https://doi.org/10.1007/s10311-018-0762-3
Nadim, F., Hoag, G.E., Liu, S., Carley, R.J., Zack, P., 2000a. Detection and remediation of soil and aquifer systems contaminated with petroleum products: an overview. Journal of Petroleum Science and Engineering 26, 169–178. https://doi.org/10.1016/S0920-4105(00)00031-0
Nadim, F., Hoag, G.E., Liu, S., Carley, R.J., Zack, P., 2000b. Detection and remediation of soil and aquifer systems contaminated with petroleum products: an overview. Journal of Petroleum Science and Engineering 26, 169–178. https://doi.org/10.1016/S0920-4105(00)00031-0
Nasirpouri, F., Mostafaei, A., Fathyunes, L., Jafari, R., 2014. Assessment of localized corrosion in carbon steel tube-grade AISI 1045 used in output oil–gas separator vessel of desalination unit in oil refinery industry. Engineering Failure Analysis 40, 75–88. https://doi.org/10.1016/j.engfailanal.2014.02.012
Nathanail, C.P., Bardos, R.P., 2005. Reclamation of contaminated land. John Wiley & Sons.
Ndimele, Prince Emeka, Saba, A.O., Ojo, D.O., Ndimele, C.C., Anetekhai, M.A., Erondu, E.S., 2018. Chapter 24 - Remediation of Crude Oil Spillage, in: Ndimele, Prince E. (Ed.), The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem. Academic Press, pp. 369–384. https://doi.org/10.1016/B978-0-12-809399-3.00024-0
Nero, B.F., 2021. Phytoremediation of petroleum hydrocarbon-contaminated soils with two plant species: Jatropha curcas and Vetiveria zizanioides at Ghana Manganese Company Ltd. International Journal of Phytoremediation 23, 171–180. https://doi.org/10.1080/15226514.2020.1803204
Newman, L.A., Reynolds, C.M., 2004. Phytodegradation of organic compounds. Current opinion in Biotechnology 15, 225–230.
Ng, C.C., Boyce, A.N., Rahman, M.M., Abas, M.R., 2016. Effects of Different Soil Amendments on Mixed Heavy Metals Contamination in Vetiver Grass. Bull Environ Contam Toxicol 97, 695–701. https://doi.org/10.1007/s00128-016-1921-5
Nganje, T.N., Edet, A.E., Ekwere, S.J., 2007. Distribution of PAHs in Surface Soils from Petroleum Handling Facilities in Calabar. Environ Monit Assess 130, 27–34. https://doi.org/10.1007/s10661-006-9453-9
Nie, M., Wang, Y., Yu, J., Xiao, M., Jiang, L., Yang, J., Fang, C., Chen, J., Li, B., 2011. Understanding Plant-Microbe Interactions for Phytoremediation of Petroleum-Polluted Soil. PLoS One 6. https://doi.org/10.1371/journal.pone.0017961
Nie, M., Yang, Q., Jiang, L.-F., Fang, C.-M., Chen, J.-K., Li, B., 2010. Do plants modulate biomass allocation in response to petroleum pollution? Biol. Lett. 6, 811–814. https://doi.org/10.1098/rsbl.2010.0261
Nisa, W.U., Rashid, A., 2015. POTENTIAL OF VETIVER (VETIVERIA ZIZANIOIDES L.) GRASS IN REMOVING SELECTED PAHS FROM DIESEL CONTAMINATED SOIL 6.
Njobuenwu, D.O., Amadi, S.A., Ukpaka, P.C., 2005. Dissolution Rate of BTEX Contaminants in Water. The Canadian Journal of Chemical Engineering 83, 985–989. https://doi.org/10.1002/cjce.5450830608
NNPC, 2015. Industry History.
Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A.H., Yousefi, N., 2009. Accumulation of heavy metals in soil and uptake by plant species with

phytoremediation potential. Environ Earth Sci 59, 315–323. https://doi.org/10.1007/s12665-009-0028-2
Novo, L.A.B., Castro, P.M.L., Alvarenga, P., da Silva, E.F., 2018. Chapter 16 - Plant Growth–Promoting Rhizobacteria-Assisted Phytoremediation of Mine Soils, in: Prasad, M.N.V., Favas, P.J. de C., Maiti, S.K. (Eds.), Bio-Geotechnologies for Mine Site Rehabilitation. Elsevier, pp. 281–295. https://doi.org/10.1016/B978-0-12-812986-9.00016-6
Novo, L.A.B., Covelo, E.F., González, L., 2013a. Phytoremediation of amended copper mine tailings with Brassica juncea. International Journal of Mining, Reclamation and Environment 27, 215–226. https://doi.org/10.1080/17480930.2013.779061
Novo, L.A.B., Covelo, E.F., González, L., 2013b. The Potential of Salvia verbenaca for Phytoremediation of Copper Mine Tailings Amended with Technosol and Compost. Water Air Soil Pollut 224, 1513. https://doi.org/10.1007/s11270-013-1513-5
Nwaichi, E.O., Chuku, L.C., Ighoavwogan, E., 2016. Polycyclic Aromatic Hydrocarbons and Selected Heavy Metals in Some Oil Polluted Sites in Delta State Nigeria. Journal of Environmental Protection 7, 1389–1410. https://doi.org/10.4236/jep.2016.710120
Nwozor, A., 2020. Depoliticizing environmental degradation: revisiting the UNEP environmental assessment of Ogoniland in Nigeria's Niger Delta region. GeoJournal 85, 883–900. https://doi.org/10.1007/s10708-019-09997-x
Obayori, O.S., Ilori, M.O., Adebusoye, S.A., Oyetibo, G.O., Omotayo, A.E., Amund, O.O., 2009. Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. World J Microbiol Biotechnol 25, 1615–1623. https://doi.org/10.1007/s11274-009-0053-z
Öberg, T., Bergbäck, B., 2005. A Review of Probabilistic Risk Assessment of Contaminated Land (12 pp). J Soils Sediments 5, 213–224. https://doi.org/10.1065/jss2005.08.143
Octaviani, M., Tost, H., Lammel, G., 2017. Polycyclic aromatic hydrocarbons - fate and long-range atmospheric transport studied using a global model, EMAC-SVOC 17626.
Odoh, C.K., Zabbey, N., Sam, K., Eze, C.N., 2019. Status, progress and challenges of phytoremediation - An African scenario. Journal of Environmental Management 237, 365–378. https://doi.org/10.1016/j.jenvman.2019.02.090
Ojewumi, M.E., Anenih, E.V., Taiwo, O.S., Adekeye, B.T., Awolu, O.O., Ojewumi, E.O., 2018. A Bioremediation Study of Raw and Treated Crude Petroleum Oil Polluted Soil with Aspergillus niger and Pseudomonas aeruginosa. Journal of Ecological Engineering 19, 226–235.
Ojuederie, O.B., Babalola, O.O., 2017. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. International Journal of Environmental Research and Public Health 14, 1504. https://doi.org/10.3390/ijerph14121504
Okop, I.J., Okorie, F.S., 2013. Characteristic assessment of the levels of hydrocarbon contamination of soil after massive crude oil spill. J Biol Chem Research 30, 1–12.
Okwonna, O.O., Otaraku, I.J., 2022. Kinetic modelling of in situ treatment of petroleum hydrocarbon contaminated soil using bone char and NPK fertilizers. Sustain Environ Res 32, 14. https://doi.org/10.1186/s42834-022-00124-z
Olowomofe, T.O., Oluyege, J.O., Aderiye, B.I., Oluwole, O.A., 2019. Degradation of poly aromatic fractions of crude oil and detection of catabolic genes in hydrocarbon-

degrading bacteria isolated from Agbabu bitumen sediments in Ondo State. AIMS Microbiol 5, 308–323. https://doi.org/10.3934/microbiol.2019.4.308
Omosun, G., Edeoga, H., Markson, A.-A., 2009. Anatomical changes due to crude oil pollution and its heavy metals component in three Mucuna species. Recent Research in Science and Technology, 1(6): 264 – 269. 1.
Omwoma, S., Mbithi, B.M., Pandelova, M., Ssebugere, P., Lalah, J.O., Wang, Y., Bi, Y., Henkelmann, B., Schramm, K.-W., 2019. Comparative exposomics of persistent organic pollutants (PCBs, OCPs, MCCPs and SCCPs) and polycyclic aromatic hydrocarbons (PAHs) in Lake Victoria (Africa) and Three Gorges Reservoir (China). Science of The Total Environment 695, 133789. https://doi.org/10.1016/j.scitotenv.2019.133789
Onwurah, I.N.E., Ogugua, V.N., Onyike, N.B., Ochonogor, A.E., Otitoju, O.F., 2007. Crude oil spills in the environment, effects and some innovative clean-up biotechnologies.
Ordinioha, B., Brisibe, S., 2013. The human health implications of crude oil spills in the Niger delta, Nigeria: An interpretation of published studies. Niger Med J 54, 10–16. https://doi.org/10.4103/0300-1652.108887
Orozco-Mosqueda, Ma. del C., Glick, B.R., Santoyo, G., 2020. ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. Microbiological Research 235, 126439. https://doi.org/10.1016/j.micres.2020.126439
Ortega, H.E., Torres-Mendoza, D., Cubilla-Rios, L., 2020. Patents on Endophytic Fungi for Agriculture and Bio- and Phytoremediation Applications. Microorganisms 8, 1237. https://doi.org/10.3390/microorganisms8081237
Ossai, I.C., Ahmed, A., Hassan, A., Hamid, F.S., 2020. Remediation of soil and water contaminated with petroleum hydrocarbon: A review. Environmental Technology & Innovation 17, 100526. https://doi.org/10.1016/j.eti.2019.100526
Osuji, L.C., Nwoye, I., 2007. An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Owaza experience. AJAR 2, 318–324. https://doi.org/10.5897/AJAR.9000036
Overton, E.B., Wade, T.L., Radović, J.R., Meyer, B.M., Miles, M.S., Larter, S.R., 2016. Chemical Composition of Macondo and Other Crude Oils and Compositional Alterations During Oil Spills. Oceanography 29, 50–63.
Oyuela Leguizamo, M.A., Fernández Gómez, W.D., Sarmiento, M.C.G., 2017. Native herbaceous plant species with potential use in phytoremediation of heavy metals, spotlight on wetlands — A review. Chemosphere 168, 1230–1247. https://doi.org/10.1016/j.chemosphere.2016.10.075
Pachura, P., Ociepa-Kubicka, A., Skowron-Grabowska, B., 2016. Assessment of the availability of heavy metals to plants based on the translocation index and the bioaccumulation factor. Desalination and Water Treatment 57, 1469–1477. https://doi.org/10.1080/19443994.2015.1017330
Pacwa-Płociniczak, M., Płaza, G.A., Piotrowska-Seget, Z., Cameotra, S.S., 2011. Environmental Applications of Biosurfactants: Recent Advances. International Journal of Molecular Sciences 12, 633–654. https://doi.org/10.3390/ijms12010633
Pacwa-Płociniczak, M., Płociniczak, T., Iwan, J., Żarska, M., Chorążewski, M., Dzida, M., Piotrowska-Seget, Z., 2016. Isolation of hydrocarbon-degrading and biosurfactant-

producing bacteria and assessment their plant growth-promoting traits. Journal of Environmental Management 168, 175–184. https://doi.org/10.1016/j.jenvman.2015.11.058

Padmanabhan, G., Suresh, G., Kannappan, V., Jayakumar, S., Balasubramanian, R., 2014. Original Research Article Estimation of Bio-Surfactant produced using Bacillus Subtilis CS14 in emulsion using Ultrasonic Probing technique. Int.J.Curr.Microbiol.App.Sci 3, 737–744.

Pande, Veni, Pandey, S.C., Sati, D., Pande, Veena, Samant, M., 2020. Bioremediation: an emerging effective approach towards environment restoration. Environmental Sustainability 3, 91–103. https://doi.org/10.1007/s42398-020-00099-w

Pandotra, P., Raina, M., Salgotra, R.K., Ali, S., Mir, Z.A., Bhat, J.A., Tyagi, A., Upadhahy, D., 2018. Plant-Bacterial Partnership: A Major Pollutants Remediation Approach, in: Oves, M., Zain Khan, M., M.I. Ismail, I. (Eds.), Modern Age Environmental Problems and Their Remediation. Springer International Publishing, Cham, pp. 169–200. https://doi.org/10.1007/978-3-319-64501-8_10

Panja, S., Sarkar, D., Datta, R., 2020. Removal of antibiotics and nutrients by Vetiver grass (Chrysopogon zizanioides) from secondary wastewater effluent. International Journal of Phytoremediation 22, 764–773. https://doi.org/10.1080/15226514.2019.1710813

Pansu, M., Gautheyrou, J., 2007. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. Springer Science & Business Media.

Paria, S., 2008. Surfactant-enhanced remediation of organic contaminated soil and water. Advances in Colloid and Interface Science 138, 24–58. https://doi.org/10.1016/j.cis.2007.11.001

Parihar, J.K., Parihar, P.K., Pakade, Y.B., Katnoria, J.K., 2021. Bioaccumulation potential of indigenous plants for heavy metal phytoremediation in rural areas of Shaheed Bhagat Singh Nagar, Punjab (India). Environ Sci Pollut Res 28, 2426–2442. https://doi.org/10.1007/s11356-020-10454-3

Patel, A., Patra, D.D., 2017. A Sustainable Approach to Clean Contaminated Land Using Terrestrial Grasses, in: Bauddh, K., Singh, B., Korstad, J. (Eds.), Phytoremediation Potential of Bioenergy Plants. Springer Singapore, Singapore, pp. 305–331. https://doi.org/10.1007/978-981-10-3084-0_12

Patel, S., Homaei, A., Patil, S., Daverey, A., 2019. Microbial biosurfactants for oil spill remediation: pitfalls and potentials. Appl Microbiol Biotechnol 103, 27–37. https://doi.org/10.1007/s00253-018-9434-2

Patnaik, P., 2007. A Comprehensive Guide to the Hazardous Properties of Chemical Substances. John Wiley & Sons.

Patowary, K., Patowary, R., Kalita, M.C., Deka, S., 2017. Characterization of Biosurfactant Produced during Degradation of Hydrocarbons Using Crude Oil As Sole Source of Carbon. Frontiers in Microbiology 8.

Patra, D.K., Pradhan, C., Patra, H.K., 2020. Toxic metal decontamination by phytoremediation approach: Concept, challenges, opportunities and future perspectives. Environmental Technology & Innovation 18, 100672. https://doi.org/10.1016/j.eti.2020.100672

Paul, O., Jasu, A., Lahiri, D., Nag, M., Ray, R.R., 2021. In situ and ex situ bioremediation of heavy metals: the present scenario. Journal of Environmental Engineering and Landscape Management 29, 454–469. https://doi.org/10.3846/jeelm.2021.15447

Paz-Alberto, A.M., Sigua, G.C., 2013. Phytoremediation: A Green Technology to Remove Environmental Pollutants 2013. https://doi.org/10.4236/ajcc.2013.21008

Pei, S.-L., Chen, T.-L., Pan, S.-Y., Yang, Y.-L., Sun, Z.-H., Li, Y.-J., 2020. Addressing environmental sustainability of plasma vitrification technology for stabilization of municipal solid waste incineration fly ash. Journal of Hazardous Materials 398, 122959. https://doi.org/10.1016/j.jhazmat.2020.122959

Peng, S., Zhou, Q., Cai, Z., Zhang, Z., 2009. Phytoremediation of petroleum contaminated soils by Mirabilis Jalapa L. in a greenhouse plot experiment. Journal of Hazardous Materials 168, 1490–1496. https://doi.org/10.1016/j.jhazmat.2009.03.036

Pérez-Hernández, I., Ochoa-Gaona, S., Adams Schroeder, R.H., Rivera-Cruz, M.C., Geissen, V., 2013. Tolerance of Four Tropical Tree Species to Heavy Petroleum Contamination. Water Air Soil Pollut 224, 1637. https://doi.org/10.1007/s11270-013-1637-7

Petrovic, M., Sremacki, M., Radonic, J., Mihajlovic, I., Obrovski, B., Vojinovic Miloradov, M., 2018. Health risk assessment of PAHs, PCBs and OCPs in atmospheric air of municipal solid waste landfill in Novi Sad, Serbia. Science of The Total Environment 644, 1201–1206. https://doi.org/10.1016/j.scitotenv.2018.07.008

Petruzzelli, G., Pedron, F., Rosellini, I., Barbafieri, M., 2015. The Bioavailability Processes as a Key to Evaluate Phytoremediation Efficiency, in: Ansari, A.A., Gill, S.S., Gill, R., Lanza, G.R., Newman, L. (Eds.), Phytoremediation: Management of Environmental Contaminants, Volume 1. Springer International Publishing, Cham, pp. 31–43. https://doi.org/10.1007/978-3-319-10395-2_3

Pflugmacher, S., Kühn, S., Lee, S.-H., Choi, J.-W., Baik, S., Kwon, K.-S., Contardo-Jara, V., 2015a. Green Liver Systems® for Water Purification: Using the Phytoremediation Potential of Aquatic Macrophytes for the Removal of Different Cyanobacterial Toxins from Water. American Journal of Plant Sciences 6, 1607–1618. https://doi.org/10.4236/ajps.2015.69161

Pflugmacher, S., Kühn, S., Lee, S.-H., Choi, J.-W., Baik, S., Kwon, K.-S., Contardo-Jara, V., 2015b. Green Liver Systems® for Water Purification: Using the Phytoremediation Potential of Aquatic Macrophytes for the Removal of Different Cyanobacterial Toxins from Water. American Journal of Plant Sciences 06, 1607. https://doi.org/10.4236/ajps.2015.69161

Phillips, B., 2017. Oil Pipelines and Spills - Climate, Energy, and Society - College of Liberal Arts - Auburn University [WWW Document]. URL https://cla.auburn.edu/ces/energy/oil-pipelines-and-spills/ (accessed 5.29.22).

Phillips, L.A., Greer, C.W., Farrell, R.E., Germida, J.J., 2012. Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil. Applied Soil Ecology 52, 56–64. https://doi.org/10.1016/j.apsoil.2011.10.009

Pidlisnyuk, V., Stefanovska, T., Lewis, E.E., Erickson, L.E., Davis, L.C., 2014. Miscanthus as a Productive Biofuel Crop for Phytoremediation. Critical Reviews in Plant Sciences 33, 1–19. https://doi.org/10.1080/07352689.2014.847616

Pierzynski, G.M., Vance, G.F., Sims, J.T., 2005. Soils and Environmental Quality. CRC Press.

Pignatello, J.J., 1999. The Measurement and Interpretation of Sorption and Desorption Rates for Organic Compounds in Soil Media. Advances in Agronomy 69, 1–73. https://doi.org/10.1016/S0065-2113(08)60946-3
Pilon-Smits, E., 2005. Phytoremediation. Annual Review of Plant Biology 56, 15–39. https://doi.org/10.1146/annurev.arplant.56.032604.144214
Pinedo, J., Ibáñez, R., Lijzen, J.P.A., Irabien, Á., 2013. Assessment of soil pollution based on total petroleum hydrocarbons and individual oil substances. Journal of Environmental Management 130, 72–79. https://doi.org/10.1016/j.jenvman.2013.08.048
Plant, J.A., Voulvoulis, N., Ragnarsdottir, K.V., 2012. Pollutants, Human Health and the Environment: A Risk Based Approach. John Wiley & Sons.
Poeplau, C., Kätterer, T., 2017. Is soil texture a major controlling factor of root:shoot ratio in cereals? European Journal of Soil Science 68, 964–970. https://doi.org/10.1111/ejss.12466
Pouresmaieli, M., Ataei, M., Forouzandeh, P., Azizollahi, P., Mahmoudifard, M., 2022. Recent progress on sustainable phytoremediation of heavy metals from soil. Journal of Environmental Chemical Engineering 10, 108482. https://doi.org/10.1016/j.jece.2022.108482
Prakash, M., Arivudainambi, S., Rameshkumar, S., Babu, S., 2021. Coastal Agriculture and Climate Change. CRC Press.
Prematuri, R., Mardatin, N.F., Irdiastuti, R., Turjaman, M., Wagatsuma, T., Tawaraya, K., 2020. Petroleum hydrocarbons degradation in contaminated soil using the plants of the Aster family. Environ Sci Pollut Res 27, 4460–4467. https://doi.org/10.1007/s11356-019-07097-4
Prokop, Z., Nečasová, A., Klánová, J., Čupr, P., 2016. Bioavailability and mobility of organic contaminants in soil: new three-step ecotoxicological evaluation. Environ Sci Pollut Res 23, 4312–4319. https://doi.org/10.1007/s11356-015-5555-7
Pruss-Ustun, A., Corvalán, C.F., Organization, W.H., 2006. Preventing disease through healthy environments: towards an estimate of the environmental burden of disease. World Health Organization.
Prusty, B.A.K., Chandra, R., Azeez, P.A., 2017. Wetland Science: Perspectives From South Asia. Springer.
Puspitasari, S.A., Indradewa, D., 2019. The Effects of Silica on Growth and Yield of Chrysanthemum Plants (Dendranthema Sp.) Cultivar Sheena and Snow White. ipas 4, 98. https://doi.org/10.22146/ipas.46129
Qayyum, S., Khan, I., Meng, K., Zhao, Y., Peng, C., 2020. A review on remediation technologies for heavy metals contaminated soil. Cent. Asian J. Environ. Sci. Technol. Innov. 1. https://doi.org/10.22034/CAJESTI.2020.01.03
Qiao, M., Wang, C., Huang, S., Wang, D., Wang, Z., 2006. Composition, sources, and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. Environment International 32, 28–33. https://doi.org/10.1016/j.envint.2005.04.005
Qu, Y., Gong, Y., Ma, J., Wei, H., Liu, Q., Liu, L., Wu, H., Yang, S., Chen, Y., 2020. Potential sources, influencing factors, and health risks of polycyclic aromatic hydrocarbons

(PAHs) in the surface soil of urban parks in Beijing, China. Environmental Pollution 260, 114016. https://doi.org/10.1016/j.envpol.2020.114016
Radziemska, M., Bęś, A., Gusiatin, Z.M., Cerdà, A., Mazur, Z., Jeznach, J., Kowal, P., Brtnický, M., 2019. The combined effect of phytostabilization and different amendments on remediation of soils from post-military areas. Science of The Total Environment 688, 37–45. https://doi.org/10.1016/j.scitotenv.2019.06.190
Rahman, M. Azizur, Reichman, S.M., De Filippis, L., Tavakoly Sany, S.B., Hasegawa, H., 2016. Phytoremediation of Toxic Metals in Soils and Wetlands: Concepts and Applications, in: Hasegawa, H., Rahman, I.Md.M., Rahman, Mohammad Azizur (Eds.), Environmental Remediation Technologies for Metal-Contaminated Soils. Springer Japan, Tokyo, pp. 161–195. https://doi.org/10.1007/978-4-431-55759-3_8
Rajaei, S., Seyedi, S.M., 2018. Phytoremediation of Petroleum-Contaminated Soils by Vetiveria zizanioides (L.) Nash. CLEAN – Soil, Air, Water 46, 1800244. https://doi.org/10.1002/clen.201800244
Rajendran, P., Jayakumar, T., Nishigaki, I., Ekambaram, G., Nishigaki, Y., Vetriselvi, J., Sakthisekaran, D., 2013. Immunomodulatory Effect of Mangiferin in Experimental Animals with Benzo(a)Pyrene-induced Lung Carcinogenesis. Int J Biomed Sci 9, 68–74.
Rajendran, S., Priya, T.A.K., Khoo, K.S., Hoang, T.K.A., Ng, H.-S., Munawaroh, H.S.H., Karaman, C., Orooji, Y., Show, P.L., 2022. A critical review on various remediation approaches for heavy metal contaminants removal from contaminated soils. Chemosphere 287, 132369. https://doi.org/10.1016/j.chemosphere.2021.132369
Rajkhowa, S., Sarma, J., 2022. Biosurfactant: An Alternative Towards Sustainability, Innovative Bio-Based Technologies for Environmental Remediation. CRC Press. https://doi.org/10.1201/9781003004684-20
Rakshit, A., Parihar, M., Sarkar, B., Singh, H.B., Fraceto, L.F., 2021. Bioremediation Science: From Theory to Practice. CRC Press.
Ram, G., Melvin Joe, M., Devraj, S., Benson, A., 2019. Rhamnolipid production using Shewanella seohaensis BS18 and evaluation of its efficiency along with phytoremediation and bioaugmentation for bioremediation of hydrocarbon contaminated soils. International Journal of Phytoremediation 21, 1375–1383. https://doi.org/10.1080/15226514.2019.1633254
Ramadass, K., Smith, E., Palanisami, T., Mathieson, G., Srivastava, P., Megharaj, M., Naidu, R., 2015. Evaluation of constraints in bioremediation of weathered hydrocarbon-contaminated arid soils through microcosm biopile study. Int. J. Environ. Sci. Technol. 12, 3597–3612. https://doi.org/10.1007/s13762-015-0793-2
Ramasamy, V., Rajkumar, P., Ponnusamy, V., 2009. Depth wise analysis of recently excavated Vellar river sediments through FTIR and XRD studies. Indian J Phys 83, 1295–1308. https://doi.org/10.1007/s12648-009-0110-3
Ramasamy, V., Sundarrajan, M., Suresh, G., Paramasivam, K., Meenakshisundaram, V., 2014. Role of light and heavy minerals on natural radioactivity level of high background radiation area, Kerala, India. Applied Radiation and Isotopes 85, 1–10. https://doi.org/10.1016/j.apradiso.2013.11.119

Ramasamy, V., Suresh, G., Meenakshisundaram, V., Ponnusamy, V., 2011. Horizontal and vertical characterization of radionuclides and minerals in river sediments. Applied Radiation and Isotopes 69, 184–195. https://doi.org/10.1016/j.apradiso.2010.07.020

Ramirez, M.I., Arevalo, A.P., Sotomayor, S., Bailon-Moscoso, N., 2017. Contamination by oil crude extraction – Refinement and their effects on human health. Environmental Pollution 231, 415–425. https://doi.org/10.1016/j.envpol.2017.08.017

Ramos-Arcos, S.A., González-Mondragón, E.G., López-Hernández, E.S., Rodríguez-Luna, A.R., Morales-Bautista, C.M., Lagunas-Rivera, S., López-Martínez, S., 2021. Phytoremediation Potential of Chrysopogon zizanioides for Toxic Elements in Contaminated Matrices, Biodegradation Technology of Organic and Inorganic Pollutants. IntechOpen. https://doi.org/10.5772/intechopen.98235

Rashad, R.T., Hussien, R.A., 2020. Agronomic Efficiency of Feldspar, Quartz Silica, and Zeolite as Silicon (Si) Fertilizers in Sandy Soil. Communications in Soil Science and Plant Analysis 51, 1078–1088. https://doi.org/10.1080/00103624.2020.1751184

Rauckyte, T., Żak, S., Pawlak, Z., Oloyede, A., 2010. Determination of oil and grease, total petroleum hydrocarbons and volatile aromatic compounds in soil and sediment samples. Journal of Environmental Engineering and Landscape Management 18, 163–169. https://doi.org/10.3846/jeelm.2010.19

Razmjoo, K., Adavi, Z., 2012. Assessment of Bermudagrass Cultivars for Phytoremediation of Petroleum Contaminated Soils. International Journal of Phytoremediation 14, 14–23. https://doi.org/10.1080/15226514.2011.560212

Reátegui-Zirena, E.G., Stewart, P.M., Whatley, A., Chu-Koo, F., Sotero-Solis, V.E., Merino-Zegarra, C., Vela-Paima, E., 2014. Polycyclic aromatic hydrocarbon concentrations, mutagenicity, and Microtox® acute toxicity testing of Peruvian crude oil and oil-contaminated water and sediment. Environ Monit Assess 186, 2171–2184. https://doi.org/10.1007/s10661-013-3527-2

Reemtsma, T., Mehrtens, J., 1997. Determination of polycyclic aromatic hydrocarbon (PAH) leaching from contaminated soil by a column test with on-line solid phase extraction. Chemosphere 35, 2491–2501. https://doi.org/10.1016/S0045-6535(97)00317-2

Rego, E.C.P. do, Netto, A.D.P., 2007. PAHs and BTEX in Groundwater of Gasoline Stations from Rio de Janeiro City, Brazil. Bull Environ Contam Toxicol 79, 660–664. https://doi.org/10.1007/s00128-007-9300-x

Rehman, R., Ali, M.I., Ali, N., Badshah, M., Iqbal, M., Jamal, A., Huang, Z., 2021. Crude oil biodegradation potential of biosurfactant-producing Pseudomonas aeruginosa and Meyerozyma sp. Journal of Hazardous Materials 418, 126276. https://doi.org/10.1016/j.jhazmat.2021.126276

Reid, B.J., Jones, K.C., Semple, K.T., 2000. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environmental Pollution 108, 103–112. https://doi.org/10.1016/S0269-7491(99)00206-7

Rengarajan, T., Rajendran, Peramaiyan, Nandakumar, N., Lokeshkumar, B., Rajendran, Palaniswami, Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific Journal of Tropical Biomedicine 5, 182–189.

Renuka, N., Prasanna, R., Sood, A., Bansal, R., Bidyarani, N., Singh, R., Shivay, Y.S., Nain, L., Ahluwalia, A.S., 2017. Wastewater grown microalgal biomass as inoculants for

improving micronutrient availability in wheat. Rhizosphere 3, 150–159. https://doi.org/10.1016/j.rhisph.2017.04.005
Ribeiro, M.H.L., Carvalho, P., Martins, T.S., Faustino, C.M.C., 2019. Lipoaminoacids Enzyme-Based Production and Application as Gene Delivery Vectors. Catalysts 9, 977. https://doi.org/10.3390/catal9120977
Riser-Roberts, E., 2020. Remediation of Petroleum Contaminated Soils: Biological, Physical, and Chemical Processes. CRC Press.
Rizvi, A., Zaidi, A., Ameen, F., Ahmed, B., F. AlKahtani, M.D., Saghir Khan, M., 2020. Heavy metal induced stress on wheat: phytotoxicity and microbiological management. RSC Advances 10, 38379–38403. https://doi.org/10.1039/D0RA05610C
Rodríguez-Trigo, G., Zock, J.P., Montes, I.I., 2007. Health Effects of Exposure to Oil Spills. Archivos de Bronconeumología ((English Edition)) 43, 628–635. https://doi.org/10.1016/S1579-2129(07)60141-4
Ron, E.Z., Rosenberg, E., 2002. Biosurfactants and oil bioremediation. Current Opinion in Biotechnology 13, 249–252. https://doi.org/10.1016/S0958-1669(02)00316-6
Roshani, M., Abbaspour, H., Saeidi-sar, S., 2014. Effect of Aluminium Stress on Germination and Mineral Nutrition of Kidney Bean Cultivars with Different Sensitivity to Aluminium. Biosci., Biotechnol. Res. Asia 11, 545–553. https://doi.org/10.13005/bbra/1305
Rusin, M., Gospodarek, J., Nadgórska-Socha, A., 2015. The Effect of Petroleum-Derived Substances on the Growth and Chemical Composition of Vicia faba L. https://doi.org/10.15244/PJOES/41378
Russell, K., 2005. The use and effectiveness of phytoremediation to treat persistent organic pollutants. US Environmental Protection Agency Office of Solid Waste and Emergency Response Technology Innovation and Field Services Division.
Ryerson, T.B., Aikin, K.C., Angevine, W.M., Atlas, E.L., Blake, D.R., Brock, C.A., Fehsenfeld, F.C., Gao, R.-S., de Gouw, J.A., Fahey, D.W., Holloway, J.S., Lack, D.A., Lueb, R.A., Meinardi, S., Middlebrook, A.M., Murphy, D.M., Neuman, J.A., Nowak, J.B., Parrish, D.D., Peischl, J., Perring, A.E., Pollack, I.B., Ravishankara, A.R., Roberts, J.M., Schwarz, J.P., Spackman, J.R., Stark, H., Warneke, C., Watts, L.A., 2011. Atmospheric emissions from the Deepwater Horizon spill constrain air-water partitioning, hydrocarbon fate, and leak rate. Geophysical Research Letters 38. https://doi.org/10.1029/2011GL046726
Saeed, M., Ilyas, N., Jayachandran, K., Gaffar, S., Arshad, M., Sheeraz Ahmad, M., Bibi, F., Jeddi, K., Hessini, K., 2021. Biostimulation potential of biochar for remediating the crude oil contaminated soil and plant growth. Saudi Journal of Biological Sciences 28, 2667–2676. https://doi.org/10.1016/j.sjbs.2021.03.044
Saier, M.H., Trevors, J.T., 2010. Phytoremediation. Water Air Soil Pollut 205, 61–63. https://doi.org/10.1007/s11270-008-9673-4
Sakib, S.M.N., 2021. The Impact of Oil and Gas Development on the Landscape and Surface in Nigeria (SSRN Scholarly Paper No. 3947462). Social Science Research Network, Rochester, NY.
Saleem, H., 2016. Plant-Bacteria Partnership: Phytoremediation of Hydrocarbons Contaminated Soil and Expression of Catabolic Genes. Bullet of Environmental Studies 1, 17–27.

Saleem, M.H., Wang, X., Ali, Sajjad, Zafar, S., Nawaz, M., Adnan, M., Fahad, S., Shah, A., Alyemeni, M.N., Hefft, D.I., Ali, Shafaqat, 2021. Interactive effects of gibberellic acid and NPK on morpho-physio-biochemical traits and organic acid exudation pattern in coriander (Coriandrum sativum L.) grown in soil artificially spiked with boron. Plant Physiology and Biochemistry 167, 884–900. https://doi.org/10.1016/j.plaphy.2021.09.015

Samolada, M.C., Zabaniotou, A.A., 2014. Comparative assessment of municipal sewage sludge incineration, gasification and pyrolysis for a sustainable sludge-to-energy management in Greece. Waste Management 34, 411–420. https://doi.org/10.1016/j.wasman.2013.11.003

Sanchez-Hernandez, J.C., 2021. A toxicological perspective of plastic biodegradation by insect larvae. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 248, 109117. https://doi.org/10.1016/j.cbpc.2021.109117

Sandermann, H., 1994. Higher plant metabolism of xenobiotics: the “green liver” concept. Pharmacogenetics 4, 225–241.

Sandermann, J.H., 1999. Plant Metabolism of Organic Xenobiotics. Status and Prospects of the ‘Green Liver’ Concept, in: Altman, A., Ziv, M., Izhar, S. (Eds.), Plant Biotechnology and In Vitro Biology in the 21st Century, Current Plant Science and Biotechnology in Agriculture. Springer Netherlands, pp. 321–328. https://doi.org/10.1007/978-94-011-4661-6_74

Sarıkoç, S., 2020. Fuels of the Diesel-Gasoline Engines and Their Properties. Diesel and Gasoline Engines 31.

Sarubbo, L.A., Silva, M. da G.C., Durval, I.J.B., Bezerra, K.G.O., Ribeiro, B.G., Silva, I.A., Twigg, M.S., Banat, I.M., 2022. Biosurfactants: Production, properties, applications, trends, and general perspectives. Biochemical Engineering Journal 181, 108377. https://doi.org/10.1016/j.bej.2022.108377

Scheffran, J., Felkers, M., Froese, R., 2020. Economic Growth and the Global Energy Demand, in: Vertès, A.A., Qureshi, N., Blaschek, H.P., Yukawa, H. (Eds.), Green Energy to Sustainability. Wiley, pp. 1–44. https://doi.org/10.1002/9781119152057.ch1

Schmidt-Etkin, D., 2011. Chapter 2 - Spill Occurrences: A World Overview, in: Fingas, M. (Ed.), Oil Spill Science and Technology. Gulf Professional Publishing, Boston, pp. 7–48. https://doi.org/10.1016/B978-1-85617-943-0.10002-4

Schomburg, A., Verrecchia, E.P., Guenat, C., Brunner, P., Sebag, D., Le Bayon, R.C., 2018. Rock-Eval pyrolysis discriminates soil macro-aggregates formed by plants and earthworms. Soil Biology and Biochemistry 117, 117–124. https://doi.org/10.1016/j.soilbio.2017.11.010

Schwitzguébel, J.-P., Comino, E., Plata, N., Khalvati, M., 2011. Is phytoremediation a sustainable and reliable approach to clean-up contaminated water and soil in Alpine areas? Environ Sci Pollut Res 18, 842–856. https://doi.org/10.1007/s11356-011-0498-0

Scullion, J., 2006a. Remediating polluted soils. Naturwissenschaften 93, 51–65. https://doi.org/10.1007/s00114-005-0079-5

Scullion, J., 2006b. Remediating polluted soils. Naturwissenschaften 93, 51–65. https://doi.org/10.1007/s00114-005-0079-5

Sebastian, M.S., Armstrong, B., Córdoba, J.A., Stephens, C., 2001. Exposures and cancer incidence near oil fields in the Amazon basin of Ecuador. Occupational and Environmental Medicine 58, 517–522. https://doi.org/10.1136/oem.58.8.517
Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science 54, 809–818. https://doi.org/10.1046/j.1351-0754.2003.0564.x
Sen, R., 2008. Biotechnology in petroleum recovery: The microbial EOR. Progress in Energy and Combustion Science 34, 714–724. https://doi.org/10.1016/j.pecs.2008.05.001
Senevirathna, S.T.M.L.D., Mahinroosta, R., Li, M., KrishnaPillai, K., 2021. In situ soil flushing to remediate confined soil contaminated with PFOS- an innovative solution for emerging environmental issue. Chemosphere 262, 127606. https://doi.org/10.1016/j.chemosphere.2020.127606
Seroja, R., Effendi, H., Hariyadi, S., 2018. Tofu wastewater treatment using vetiver grass (Vetiveria zizanioides) and zeliac. Appl Water Sci 8, 2. https://doi.org/10.1007/s13201-018-0640-y
Shackira, A.M., Puthur, J.T., 2019. Phytostabilization of Heavy Metals: Understanding of Principles and Practices, in: Srivastava, S., Srivastava, A.K., Suprasanna, P. (Eds.), Plant-Metal Interactions. Springer International Publishing, Cham, pp. 263–282. https://doi.org/10.1007/978-3-030-20732-8_13
Shah, V., Daverey, A., 2020. Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. Environmental Technology & Innovation 18, 100774. https://doi.org/10.1016/j.eti.2020.100774
Shahzad, A., Saddiqui, S., Bano, A., 2016. The response of maize (Zea mays L.) plant assisted with bacterial consortium and fertilizer under oily sludge. International Journal of Phytoremediation 18, 521–526. https://doi.org/10.1080/15226514.2015.1115964
Sharifikolouei, E., Ferraris, M., 2021. Potential Role of Vitrification and Waste Vitrification in the Circular Economy, in: Baino, F., Tomalino, M., Tulyaganov, D. (Eds.), Ceramics, Glass and Glass-Ceramics: From Early Manufacturing Steps Towards Modern Frontiers, PoliTO Springer Series. Springer International Publishing, Cham, pp. 325–343. https://doi.org/10.1007/978-3-030-85776-9_10
Sharma, P., Pandey, S., 2014. Status of Phytoremediation in World Scenario. International Journal of Environmental Bioremediation & Biodegradation 2, 178–191. https://doi.org/10.12691/ijebb-2-4-5
Sharma, S., Tiwari, S., Hasan, A., Saxena, V., Pandey, L.M., 2018. Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. 3 Biotech 8, 216. https://doi.org/10.1007/s13205-018-1237-8
Shekhar, S., Sundaramanickam, A., Balasubramanian, T., 2015. Biosurfactant Producing Microbes and their Potential Applications: A Review. Critical Reviews in Environmental Science and Technology 45, 1522–1554. https://doi.org/10.1080/10643389.2014.955631
Sheoran, V., Sheoran, A.S., Poonia, P., 2016. Factors Affecting Phytoextraction: A Review. Pedosphere 26, 148–166. https://doi.org/10.1016/S1002-0160(15)60032-7
Shu, X., Huang, W., Shi, K., Chen, S., Zhang, S., Li, B., Wang, X., Xie, Y., Lu, X., 2021. Microwave vitrification of simulated radioactively contaminated soil: Mechanism

and performance. Journal of Solid State Chemistry 293, 121757. https://doi.org/10.1016/j.jssc.2020.121757
Sidhu, G.P.S., 2016. Heavy Metal Toxicity in Soils: Sources, Remediation Technologies and Challenges Submit Manuscript |. Advances in Plants & Agriculture Research 5, 00166. https://doi.org/10.15406/apar.2016.05.00166
Silva, S., Pinto-Carnide, O., Martins-Lopes, P., Matos, M., Guedes-Pinto, H., Santos, C., 2010. Differential aluminium changes on nutrient accumulation and root differentiation in an Al sensitive vs. tolerant wheat. Environmental and Experimental Botany 68, 91–98. https://doi.org/10.1016/j.envexpbot.2009.10.005
Singh, C., Lin, J., 2008. Isolation and characterization of diesel oil degrading indigenous microrganisms in Kwazulu-Natal, South Africa. African Journal of Biotechnology 7. https://doi.org/10.4314/ajb.v7i12.58860
Singh, O.V., Jain, R.K., 2003. Phytoremediation of toxic aromatic pollutants from soil. Appl Microbiol Biotechnol 63, 128–135. https://doi.org/10.1007/s00253-003-1425-1
Sinha, S., Mishra, R.K., Sinam, G., Mallick, S., Gupta, A.K., 2013. Comparative Evaluation of Metal Phytoremediation Potential of Trees, Grasses, and Flowering Plants from Tannery-Wastewater-Contaminated Soil in Relation with Physicochemical Properties. Soil and Sediment Contamination: An International Journal 22, 958–983. https://doi.org/10.1080/15320383.2013.770437
Skoczynska, E., Leonards, P., Boer, J. de, 2013. Identification and quantification of methylated PAHs in sediment by two-dimensional gas chromatography /mass spectrometry. Analytical Methods 5, 213–218. https://doi.org/10.1039/C2AY25746G
Smith, K.A., 2000. Soil and Environmental Analysis: Physical Methods, Revised, and Expanded. CRC Press.
Smith, M.T., Guyton, K.Z., Gibbons, C.F., Fritz, J.M., Portier, C.J., Rusyn, I., DeMarini, D.M., Caldwell, J.C., Kavlock, R.J., Lambert, P.F., Hecht, S.S., Bucher, J.R., Stewart, B.W., Baan, R.A., Cogliano, V.J., Straif, K., 2016. Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environmental Health Perspectives 124, 713–721. https://doi.org/10.1289/ehp.1509912
Smitha, G.R., Varghese, T.S., Manivel, P., 2014. Cultivation of Vetiver [Vetiveria zizanioides (Linn)-Directorate of Medical and Aromatic Plants Research.
Sobiecka, E., Szymanski, L., 2014. Thermal plasma vitrification process as an effective technology for fly ash and chromium-rich sewage sludge utilization. Journal of Chemical Technology & Biotechnology 89, 1115–1117. https://doi.org/10.1002/jctb.4221
Song, Z., Chouparova, E., Jones, K.W., Feng, H., Marinkovic, N.S., 2001. FTIR Investigation of Sediments from NY/NJ Harbor, San Diego Bay, and the Venetian Lagoon 5.
Soobhany, N., 2018. Preliminary evaluation of pathogenic bacteria loading on organic Municipal Solid Waste compost and vermicompost. Journal of Environmental Management 206, 763–767. https://doi.org/10.1016/j.jenvman.2017.11.029
Spaczynski, M., Seta-Koselska, A., Patrzylas, P., Betlej, A., Skorzynska-Polit, E., 2012. Phytodegrdation and biodegradation in rhizosphere as efficient methods of reclamation of soil, contaminated by organic chemicals (a review). Acta Agrophysica 19.
Speight, J.G., 2020. The Refinery of the Future. Gulf Professional Publishing.

Speight, J.G., 2016. Introduction to Enhanced Recovery Methods for Heavy Oil and Tar Sands. Gulf Professional Publishing.
Speight, J.G., 2014. The Chemistry and Technology of Petroleum, Fifth Edition. CRC Press.
Speight, J.G., Ozum, B., 2001. Petroleum Refining Processes. CRC Press.
Srivastava, J., Kayastha, S., Jamil, S., Srivastava, V., 2008a. Environmental perspectives of Vetiveria zizanioides (L.) Nash. Acta Physiol Plant 30, 413–417. https://doi.org/10.1007/s11738-008-0137-7
Srivastava, J., Kayastha, S., Jamil, S., Srivastava, V., 2008b. Environmental perspectives of Vetiveria zizanioides (L.) Nash. Acta Physiol Plant 30, 413–417. https://doi.org/10.1007/s11738-008-0137-7
Stumpf, L., Pauletto, E.A., Pinto, L.F.S., 2016. Soil aggregation and root growth of perennial grasses in a constructed clay minesoil. Soil and Tillage Research 161, 71–78. https://doi.org/10.1016/j.still.2016.03.005
Su, J.-Y., Syu, C.-H., Lee, D.-Y., 2018. Growth inhibition of rice (Oryza sativa L.) seedlings in Ga- and In-contaminated acidic soils is respectively caused by Al and Al+In toxicity. Journal of Hazardous Materials 344, 274–282. https://doi.org/10.1016/j.jhazmat.2017.10.023
Suelee, A.L., Hasan, S.N.M.S., Kusin, F.M., Yusuff, F.M., Ibrahim, Z.Z., 2017a. Phytoremediation Potential of Vetiver Grass (Vetiveria zizanioides) for Treatment of Metal-Contaminated Water. Water Air Soil Pollut 228, 158. https://doi.org/10.1007/s11270-017-3349-x
Suelee, A.L., Hasan, S.N.M.S., Kusin, F.M., Yusuff, F.M., Ibrahim, Z.Z., 2017b. Phytoremediation Potential of Vetiver Grass (Vetiveria zizanioides) for Treatment of Metal-Contaminated Water. Water Air Soil Pollut 228, 158. https://doi.org/10.1007/s11270-017-3349-x
Suman, S., Sinha, A., Tarafdar, A., 2016. Polycyclic aromatic hydrocarbons (PAHs) concentration levels, pattern, source identification and soil toxicity assessment in urban traffic soil of Dhanbad, India. Science of The Total Environment 545–546, 353–360. https://doi.org/10.1016/j.scitotenv.2015.12.061
Sumanta, N., Haque, C.I., Nishika, J., Suprakash, R., 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Res J Chem Sci 2231, 606X.
Sun, S., Wang, Y., Zang, T., Wei, J., Wu, H., Wei, C., Qiu, G., Li, F., 2019. A biosurfactant-producing Pseudomonas aeruginosa S5 isolated from coking wastewater and its application for bioremediation of polycyclic aromatic hydrocarbons. Bioresource Technology 281, 421–428. https://doi.org/10.1016/j.biortech.2019.02.087
Sun, T.-R., Cang, L., Wang, Q.-Y., Zhou, D.-M., Cheng, J.-M., Xu, H., 2010. Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. Journal of Hazardous Materials 176, 919–925. https://doi.org/10.1016/j.jhazmat.2009.11.124
Sun, Y., Zhang, S., Lan, J., Xie, Z., Pu, J., Yuan, D., Yang, H., Xing, B., 2019. Vertical migration from surface soils to groundwater and source appointment of polycyclic aromatic hydrocarbons in epikarst spring systems, southwest China. Chemosphere 230, 616–627. https://doi.org/10.1016/j.chemosphere.2019.05.007

Susarla, S., Medina, V.F., McCutcheon, S.C., 2002. Phytoremediation: An ecological solution to organic chemical contamination. Ecological Engineering 18, 647–658. https://doi.org/10.1016/S0925-8574(02)00026-5

Susarla, S., Medina, V.F., McCutcheon, S.C., 2002. Phytoremediation: an ecological solution to organic chemical contamination. Ecological Engineering 18:647–658.

Sushkova, S., MiNkiNa, T., TariGholiZadeh, S., Antonenko, E., KonstantiNova, E., Gülser, C., DudniKova, T., Barbashev, A., Kizilkaya, R., 2020. PAHs accumulation in soil-plant system of Phragmites australis Cav. in soil under long-term chemical contamination. EURASIAN JOURNAL OF SOIL SCIENCE (EJSS) 9, 242–253. https://doi.org/10.18393/ejss.734607

Swartjes, F.A., 2011. Dealing with Contaminated Sites: From Theory towards Practical Application. Springer Science & Business Media.

Szczygłowska, M., Piekarska, A., Konieczka, P., Namieśnik, J., 2011. Use of Brassica Plants in the Phytoremediation and Biofumigation Processes. International Journal of Molecular Sciences 12, 7760–7771. https://doi.org/10.3390/ijms12117760

Tang, J., Lu, X., Sun, Q., Zhu, W., 2012. Aging effect of petroleum hydrocarbons in soil under different attenuation conditions. Agriculture, Ecosystems & Environment 149, 109–117. https://doi.org/10.1016/j.agee.2011.12.020

Tang, J., Wang, M., Wang, F., Sun, Q., Zhou, Q., 2011a. Eco-toxicity of petroleum hydrocarbon contaminated soil. Journal of Environmental Sciences 23, 845–851. https://doi.org/10.1016/S1001-0742(10)60517-7

Tang, J., Wang, M., Wang, F., Sun, Q., Zhou, Q., 2011b. Eco-toxicity of petroleum hydrocarbon contaminated soil. Journal of Environmental Sciences 23, 845–851. https://doi.org/10.1016/S1001-0742(10)60517-7

Tang, J., Wang, R., Niu, X., Zhou, Q., 2010. Enhancement of soil petroleum remediation by using a combination of ryegrass (Lolium perenne) and different microorganisms. Soil and Tillage Research 110, 87–93. https://doi.org/10.1016/j.still.2010.06.010

Teng, D., Mao, K., Ali, W., Xu, G., Huang, G., Khan Niazi, N., Feng, X., Zhang, H., 2020. Describing the toxicity and sources and the remediation technologies for mercury-contaminated soil. RSC Advances 10, 23221–23232. https://doi.org/10.1039/D0RA01507E

Teng, Y., Feng, D., Song, L., Wang, J., Li, J., 2013. Total petroleum hydrocarbon distribution in soils and groundwater in Songyuan oilfield, Northeast China. Environ Monit Assess 185, 9559–9569. https://doi.org/10.1007/s10661-013-3274-4

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond B Biol Sci 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thijs, S., Sillen, W., Weyens, N., Vangronsveld, J., 2017. Phytoremediation: State-of-the-art and a key role for the plant microbiome in future trends and research prospects. International Journal of Phytoremediation 19, 23–38. https://doi.org/10.1080/15226514.2016.1216076

Thornton, I., Farago, M.E., Thums, C.R., Parrish, R.R., McGill, R.A.R., Breward, N., Fortey, N.J., Simpson, P., Young, S.D., Tye, A.M., Crout, N.M.J., Hough, R.L., Watt, J., 2008. Urban geochemistry: research strategies to assist risk assessment and remediation of brownfield sites in urban areas. Environ Geochem Health 30, 565–576. https://doi.org/10.1007/s10653-008-9182-9

Titah, H.S., Abdullah, S.R.S., Mushrifah, I., Anuar, N., Basri, H., Mukhlisin, M., 2013. Effect of applying rhizobacteria and fertilizer on the growth of Ludwigia octovalvis for arsenic uptake and accumulation in phytoremediation. Ecological Engineering 58, 303–313. https://doi.org/10.1016/j.ecoleng.2013.07.018

Tiwari, M., Sahu, S.K., Bhangare, R.C., Ajmal, P.Y., Pandit, G.G., 2013. Estimation of polycyclic aromatic hydrocarbons associated with size segregated combustion aerosols generated from household fuels. Microchemical Journal 106, 79–86. https://doi.org/10.1016/j.microc.2012.05.008

Todd, G.D., Chessin, R.L., Colman, J., 1999. Toxicological profile for total petroleum hydrocarbons (TPH).

Trapp, S., Karlson, U., 2001. Aspects of phytoremediation of organic pollutants. J Soils & Sediments 1, 37. https://doi.org/10.1007/BF02986468

Trellu, C., Mousset, E., Pechaud, Y., Huguenot, D., van Hullebusch, E.D., Esposito, G., Oturan, M.A., 2016. Removal of hydrophobic organic pollutants from soil washing/flushing solutions: A critical review. Journal of Hazardous Materials 306, 149–174. https://doi.org/10.1016/j.jhazmat.2015.12.008

Tripathi, S., Sharma, P., Singh, K., Purchase, D., Chandra, R., 2021. Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste. Environmental Technology & Innovation 22, 101434. https://doi.org/10.1016/j.eti.2021.101434

Tripathi, S., Singh, V.K., Srivastava, P., Singh, R., Devi, R.S., Kumar, Arun, Bhadouria, R., 2020. Chapter 4 - Phytoremediation of organic pollutants: current status and future directions, in: Singh, P., Kumar, Ajay, Borthakur, A. (Eds.), Abatement of Environmental Pollutants. Elsevier, pp. 81–105. https://doi.org/10.1016/B978-0-12-818095-2.00004-7

Trojanowicz, M., Bojanowska-Czajka, A., Bartosiewicz, I., Kulisa, K., 2018. Advanced Oxidation/Reduction Processes treatment for aqueous perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) – A review of recent advances. Chemical Engineering Journal 336, 170–199. https://doi.org/10.1016/j.cej.2017.10.153

Truong, Paul, 2000. Application of the Vetiver system for phytoremediation of mercury pollution in the Lake and Yolo counties, Northern California. Proceedings of pollution solutions 1–13.

Truong, P., 2000. Allocation of the vetiver system for phytoremedi-ation of mercury pollution in the Lake and Yolo Counties, Northern California. In: Invited paper presented at the pollution solutions seminar, Clear Lake, pp 550–562.

Truong, P., Van, T.T., Pinners, E., 2008. Vetiver system applications technical reference manual. The Vetiver Network International 89.

Truong, P.N.V., Foong, Y.K., Guthrie, M., Hung, Y.-T., 2010. Phytoremediation of Heavy Metal Contaminated Soils and Water Using Vetiver Grass, in: Wang, L.K., Tay, J.-H.,

Tay, S.T.L., Hung, Y.-T. (Eds.), Environmental Bioengineering. Humana Press, Totowa, NJ, pp. 233–275. https://doi.org/10.1007/978-1-60327-031-1_8
Turkovskaya, O., Muratova, A., 2019. Plant–Bacterial Degradation of Polyaromatic Hydrocarbons in the Rhizosphere. Trends in Biotechnology 37, 926–930. https://doi.org/10.1016/j.tibtech.2019.04.010
Tyagi, B., Kumar, N., 2021. Chapter 1 - Bioremediation: principles and applications in environmental management, in: Saxena, G., Kumar, V., Shah, M.P. (Eds.), Bioremediation for Environmental Sustainability. Elsevier, pp. 3–28. https://doi.org/10.1016/B978-0-12-820524-2.00001-8
Ukalska-Jaruga, A., Smreczak, B., Klimkowicz-Pawlas, A., 2019. Soil organic matter composition as a factor affecting the accumulation of polycyclic aromatic hydrocarbons. J Soils Sediments 19, 1890–1900. https://doi.org/10.1007/s11368-018-2214-x
Ukaogo, P.O., Ewuzie, U., Onwuka, C.V., 2020. 21 - Environmental pollution: causes, effects, and the remedies, in: Chowdhary, P., Raj, A., Verma, D., Akhter, Y. (Eds.), Microorganisms for Sustainable Environment and Health. Elsevier, pp. 419–429. https://doi.org/10.1016/B978-0-12-819001-2.00021-8
UNEP (Ed.), 2011a. Environmental assessment of Ogoniland. United Nations Environment Programme, Nairobi, Kenya.
UNEP, 2011b. Cleaning up Nigerian oil pollution could take 30 years, cost billions – UN | UN News [WWW Document]. URL https://news.un.org/en/story/2011/08/383512 (accessed 6.23.23).
United Nations, 2019. Population [WWW Document]. URL https://www.un.org/en/global-issues/population (accessed 5.19.22).
Unterbrunner, R., Wieshammer, G., Hollender, U., Felderer, B., Wieshammer-Zivkovic, M., Puschenreiter, M., Wenzel, W.W., 2007. Plant and fertiliser effects on rhizodegradation of crude oil in two soils with different nutrient status. Plant Soil 300, 117–126. https://doi.org/10.1007/s11104-007-9394-2
US EPA, 2022. What are Total Petroleum Hydrocarbons (TPH)? | Urban Environmental Program in New England [WWW Document]. URL https://www3.epa.gov/region1/eco/uep/tph.html (accessed 5.24.22).
Uwakonye, M.N., Osho, G.S., Anucha, H., 2006. The Impact Of Oil And Gas Production On The Nigerian Economy: A Rural Sector Econometric Model. IBER 5. https://doi.org/10.19030/iber.v5i2.3458
Van Aken, B., 2008. Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. Trends in Biotechnology 26, 225–227. https://doi.org/10.1016/j.tibtech.2008.02.001
Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res Int 16, 765–794. https://doi.org/10.1007/s11356-009-0213-6
Varjani, S., Upasani, V.N., 2021. Bioaugmentation of Pseudomonas aeruginosa NCIM 5514 – A novel oily waste degrader for treatment of petroleum hydrocarbons. Bioresource Technology 319, 124240. https://doi.org/10.1016/j.biortech.2020.124240

Varjani, S.J., 2017. Microbial degradation of petroleum hydrocarbons. Bioresource Technology 223, 277–286. https://doi.org/10.1016/j.biortech.2016.10.037
Varjani, S.J., Upasani, V.N., 2016. Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Bioresource Technology 222, 195–201. https://doi.org/10.1016/j.biortech.2016.10.006
Venosa, A.D., Zhu, X., 2003. Biodegradation of Crude Oil Contaminating Marine Shorelines and Freshwater Wetlands. Spill Science & Technology Bulletin, Longer-Term Weathering Processes of Spilled Oil 8, 163–178. https://doi.org/10.1016/S1353-2561(03)00019-7
Vidonish, J.E., Zygourakis, K., Masiello, C.A., Sabadell, G., Alvarez, P.J.J., 2016. Thermal Treatment of Hydrocarbon-Impacted Soils: A Review of Technology Innovation for Sustainable Remediation. Engineering 2, 426–437. https://doi.org/10.1016/J.ENG.2016.04.005
Viesser, J.A., Sugai-Guerios, M.H., Malucelli, L.C., Pincerati, M.R., Karp, S.G., Maranho, L.T., 2020. Petroleum-Tolerant Rhizospheric Bacteria: Isolation, Characterization and Bioremediation Potential. Sci Rep 10, 2060. https://doi.org/10.1038/s41598-020-59029-9
Villeneuve, S., Cyr, D., Lynge, E., Orsi, L., Sabroe, S., Merletti, F., Gorini, G., Morales-Suarez-Varela, M., Ahrens, W., Baumgardt-Elms, C., Kaerlev, L., Eriksson, M., Hardell, L., Févotte, J., Guénel, P., 2010. Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: a case–control study in Europe. Occupational and Environmental Medicine 67, 837–844. https://doi.org/10.1136/oem.2009.052175
Vishnoi, S.R., Srivastava, P.N., 2007. Phytoremediation–green for environmental clean, in: Proceedings of Taal2007: The 12th World Lake Conference. p. 1021.
Wadgaonkar, S.L., Ferraro, A., Nancharaiah, Y.V., Dhillon, K.S., Fabbricino, M., Esposito, G., Lens, P.N.L., 2019. In situ and ex situ bioremediation of seleniferous soils from northwestern India. J Soils Sediments 19, 762–773. https://doi.org/10.1007/s11368-018-2055-7
Wan, X., Lei, M., Chen, T., 2016. Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. Science of The Total Environment 563–564, 796–802. https://doi.org/10.1016/j.scitotenv.2015.12.080
Wang, B., Wang, Q., Liu, W., Liu, X., Hou, J., Teng, Y., Luo, Y., Christie, P., 2017. Biosurfactant-producing microorganism Pseudomonas sp. SB assists the phytoremediation of DDT-contaminated soil by two grass species. Chemosphere 182, 137–142. https://doi.org/10.1016/j.chemosphere.2017.04.123
Wang, D., Ma, J., Li, H., Zhang, X., 2018. Concentration and Potential Ecological Risk of PAHs in Different Layers of Soil in the Petroleum-Contaminated Areas of the Loess Plateau, China. International Journal of Environmental Research and Public Health 15, 1785. https://doi.org/10.3390/ijerph15081785
Wang, H., Huang, W., Gong, Y., Chen, C., Zhang, T., Diao, X., 2020. Occurrence and potential health risks assessment of polycyclic aromatic hydrocarbons (PAHs) in different tissues of bivalves from Hainan Island, China. Food and Chemical Toxicology 136, 111108. https://doi.org/10.1016/j.fct.2019.111108

Wang, J., Song, X., Wang, Y., Bai, J., Li, M., Dong, G., Lin, F., Lv, Y., Yan, D., 2017. Bioenergy generation and rhizodegradation as affected by microbial community distribution in a coupled constructed wetland-microbial fuel cell system associated with three macrophytes. Science of The Total Environment 607–608, 53–62. https://doi.org/10.1016/j.scitotenv.2017.06.243

Wang, Q., Kelly, B.C., 2017. Occurrence, distribution and bioaccumulation behaviour of hydrophobic organic contaminants in a large-scale constructed wetland in Singapore. Chemosphere 183, 257–265. https://doi.org/10.1016/j.chemosphere.2017.05.113

Wang, X., Yin, L., Dijkstra, F.A., Lu, J., Wang, P., Cheng, W., 2020. Rhizosphere priming is tightly associated with root-driven aggregate turnover. Soil Biology and Biochemistry 149, 107964. https://doi.org/10.1016/j.soilbio.2020.107964

Wato, T., 2019. THE EFFECT OF PLANTING DEPTH ON GERMINATION AND SEEDLING VIGOURSITY OF MAIZE (Zea mays L.). https://doi.org/10.13140/RG.2.2.14279.24485

Wei, H., Le, Z., Shuxian, L., Dan, W., Xiaojun, L., Lan, J., Xiping, M., 2015. HEALTH RISK ASSESSMENT OF HEAVY METALS AND POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL AT COKE OVEN GAS PLANTS. Environ. Eng. Manag. J. 14, 487–496. https://doi.org/10.30638/eemj.2015.051

Wei, W., Li, Y., Li, H., Ma, K., Wang, L., Gao, K., 2019. Phytotoxic Diterpenoids from Plants and Microorganisms. Chemistry & Biodiversity 16, e1900398. https://doi.org/10.1002/cbdv.201900398

Wei, W., Yan, Y., Cao, J., Christie, P., Zhang, F., Fan, M., 2016. Effects of combined application of organic amendments and fertilizers on crop yield and soil organic matter: An integrated analysis of long-term experiments. Agriculture, Ecosystems & Environment 225, 86–92. https://doi.org/10.1016/j.agee.2016.04.004

Wei, Y., Wang, Y., Duan, M., Han, J., Li, G., 2019. Growth tolerance and remediation potential of six plants in oil-polluted soil. J Soils Sediments 19, 3773–3785. https://doi.org/10.1007/s11368-019-02348-w

Wei, Z., Wang, J.J., Gaston, L.A., Li, J., Fultz, L.M., DeLaune, R.D., Dodla, S.K., 2020. Remediation of crude oil-contaminated coastal marsh soil: Integrated effect of biochar, rhamnolipid biosurfactant and nitrogen application. Journal of Hazardous Materials 396, 122595. https://doi.org/10.1016/j.jhazmat.2020.122595

WHO, 2016. WHO | Environmental health [WWW Document]. WHO. URL http://www.who.int/topics/environmental_health/en/ (accessed 12.17.16).

WHO, 2008. Petroleum Products in Drinking-water - World Health Organization, WHO, 2008. - Google Search [WWW Document]. URL https://www.google.com/search?q=Petroleum+Products+in+Drinking-water+-+World+Health+Organization%2C+WHO%2C+2008.&rlz=1C5CHFA_enGB876GB876&oq=Petroleum+Products+in+Drinking-water+-+World+Health+Organization%2C+WHO%2C+2008.&aqs=chrome.0.69i59j69i60l2.807j0j4&sourceid=chrome&ie=UTF-8 (accessed 5.29.22).

Wijitkosum, S., 2020. Reducing Vulnerability to Desertification by Using the Spatial Measures in a Degraded Area in Thailand. Land 9, 49. https://doi.org/10.3390/land9020049

Wiśniowska, E., Włodarczyk-Makuła, M., 2018. The effect of selected acidic or alkaline chemical agents amendment on leachability of selected heavy metals from sewage

sludge. Science of The Total Environment 633, 463–469. https://doi.org/10.1016/j.scitotenv.2018.03.163
Wu, B., 2020. Chapter 6 - Human health hazards of wastewater, in: Ren, H., Zhang, X. (Eds.), High-Risk Pollutants in Wastewater. Elsevier, pp. 125–139. https://doi.org/10.1016/B978-0-12-816448-8.00006-X
Wu, C.H., Wood, T.K., Mulchandani, A., Chen, W., 2006. Engineering Plant-Microbe Symbiosis for Rhizoremediation of Heavy Metals. Applied and Environmental Microbiology 72, 1129–1134. https://doi.org/10.1128/AEM.72.2.1129-1134.2006
Wyke, S., Peña-Fernández, A., Brooke, N., Duarte-Davidson, R., 2014. The importance of evaluating the physicochemical and toxicological properties of a contaminant for remediating environments affected by chemical incidents. Environment International, Recent developments in assessing and managing serious health threats 72, 109–118. https://doi.org/10.1016/j.envint.2014.05.002
Xia, W., Du, Z., Cui, Q., Dong, H., Wang, F., He, P., Tang, Y., 2014. Biosurfactant produced by novel Pseudomonas sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons. Journal of Hazardous Materials 276, 489–498. https://doi.org/10.1016/j.jhazmat.2014.05.062
Xiang, L., Liu, S., Ye, S., Yang, H., Song, B., Qin, F., Shen, M., Tan, C., Zeng, G., Tan, X., 2021. Potential hazards of biochar: The negative environmental impacts of biochar applications. Journal of Hazardous Materials 420, 126611. https://doi.org/10.1016/j.jhazmat.2021.126611
Xiao, L., Yao, K., Li, P., Liu, Y., Chang, E., Zhang, Y., Zhu, T., 2020. Increased soil aggregate stability is strongly correlated with root and soil properties along a gradient of secondary succession on the Loess Plateau. Ecological Engineering 143, 105671. https://doi.org/10.1016/j.ecoleng.2019.105671
Xiao, X., Chen, H., Si, C., Wu, L., 2012. Influence of biosurfactant-producing strain Bacillus subtilis BS1 on the mycoremediation of soils contaminated with phenanthrene. International Biodeterioration & Biodegradation 75, 36–42. https://doi.org/10.1016/j.ibiod.2012.09.002
Xie, F., Zhang, T.A., Dreisinger, D., Doyle, F., 2014. A critical review on solvent extraction of rare earths from aqueous solutions. Minerals Engineering 56, 10–28. https://doi.org/10.1016/j.mineng.2013.10.021
Xu, L., Lu, S., Truong, P., 2003. Vetiver system for agriculture development. Paper presentation, IVC3, Guangzhou, China October.
Xu, S., Li, P., Sayer, E.J., Zhang, B., Wang, J., Qiao, C., Peng, Z., Diao, L., Chi, Y., Liu, W., Liu, L., 2018. Initial Soil Organic Matter Content Influences the Storage and Turnover of Litter, Root and Soil Carbon in Grasslands. Ecosystems 21, 1377–1389. https://doi.org/10.1007/s10021-018-0227-3
Xu, W., Cui, K., Xu, A., Nie, L., Huang, J., Peng, S., 2015. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. Acta Physiol Plant 37, 9. https://doi.org/10.1007/s11738-014-1760-0
Xu, Z.M., Mei, X.-Q., Tan, L., Li, Q.-S., Wang, L.-L., He, B.-Y., Guo, S.-H., Zhou, C., Ye, H.-J., 2018. Low root/shoot (R/S) biomass ratio can be an indicator of low cadmium accumulation in the shoot of Chinese flowering cabbage (Brassica campestris L. ssp.

chinensis var. utilis Tsen et Lee) cultivars. Environ Sci Pollut Res 25, 36328–36340. https://doi.org/10.1007/s11356-018-3566-x
Xue, R., Shen, Y., Marschner, P., 2017. Soil water content during and after plant growth influence nutrient availability and microbial biomass. Journal of soil science and plant nutrition 17, 702–715. https://doi.org/10.4067/S0718-95162017000300012
Xun, F., Xie, B., Liu, S., Guo, C., 2015. Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. Environ Sci Pollut Res 22, 598–608. https://doi.org/10.1007/s11356-014-3396-4
Yadav, A., Raj, A., Purchase, D., Ferreira, L.F.R., Saratale, G.D., Bharagava, R.N., 2019. Phytotoxicity, cytotoxicity and genotoxicity evaluation of organic and inorganic pollutants rich tannery wastewater from a Common Effluent Treatment Plant (CETP) in Unnao district, India using Vigna radiata and Allium cepa. Chemosphere 224, 324–332. https://doi.org/10.1016/j.chemosphere.2019.02.124
Yang, K.M., Poolpak, T., Pokethitiyook, P., Kruatrachue, M., 2022. Assessment of dynamic microbial community structure and rhizosphere interactions during bioaugmented phytoremediation of petroleum contaminated soil by a newly designed rhizobox system. International Journal of Phytoremediation 0, 1–13. https://doi.org/10.1080/15226514.2022.2040420
Yang, W., Lang, Y., Li, G., 2014. Cancer risk of polycyclic aromatic hydrocarbons (PAHs) in the soils from Jiaozhou Bay wetland. Chemosphere 112, 289–295. https://doi.org/10.1016/j.chemosphere.2014.04.074
Yanto, D.H.Y., Tachibana, S., 2014. Potential of fungal co-culturing for accelerated biodegradation of petroleum hydrocarbons in soil. Journal of Hazardous Materials 278, 454–463. https://doi.org/10.1016/j.jhazmat.2014.06.039
Yeboah, S.A., Allotey, A.N.M., Biney, E., 2015. Purification of industrial wastewater with vetiver grasses (vetiveria zizanioides): the case of food and beverages Wastewater in Ghana.
Youdeowei, P.O., 2012. Fate of subsurface migration of crude oil spill: a review, in: Crude Oil Exploration in the World. InTech.
Yu, F., Li, Y., Li, F., Zhou, Z., Chen, C., Liang, X., Li, C., Liu, K., 2019. Nitrogen fertilizers promote plant growth and assist in manganese (Mn) accumulation by Polygonum pubescens Blume cultured in Mn tailings soil. International Journal of Phytoremediation 21, 1225–1233. https://doi.org/10.1080/15226514.2019.1619161
Yuan, X., Xiong, T., Yao, S., Liu, C., Yin, Y., Li, H., Li, N., 2019. A real filed phytoremediation of multi-metals contaminated soils by selected hybrid sweet sorghum with high biomass and high accumulation ability. Chemosphere 237, 124536. https://doi.org/10.1016/j.chemosphere.2019.124536
Zaheer, I.E., Ali, S., Saleem, M.H., Noor, I., El-Esawi, M.A., Hayat, K., Rizwan, M., Abbas, Z., El-Sheikh, M.A., Alyemeni, M.N., Wijaya, L., 2020. Iron–Lysine Mediated Alleviation of Chromium Toxicity in Spinach (Spinacia oleracea L.) Plants in Relation to Morpho-Physiological Traits and Iron Uptake When Irrigated with Tannery Wastewater. Sustainability 12, 6690. https://doi.org/10.3390/su12166690
Zand, A.D., Grathwohl, P., 2016. Enhanced Immobilization of Polycyclic Aromatic Hydrocarbons in Contaminated Soil Using Forest Wood-Derived Biochar and

Activated Carbon under Saturated Conditions, and the Importance of Biochar Particle Size. Polish Journal of Environmental Studies 25.
Zand, A.D., Hoveidi, H., 2016. Plant-Aid Remediation of Hydrocarbon-Contaminated Sites. Pollution 2, 233–246. https://doi.org/10.7508/pj.2016.03.001
Zang, T., Wu, H., Yan, B., Zhang, Y., Wei, C., 2021a. Enhancement of PAHs biodegradation in biosurfactant/phenol system by increasing the bioavailability of PAHs. Chemosphere 266, 128941. https://doi.org/10.1016/j.chemosphere.2020.128941
Zang, T., Wu, H., Zhang, Y., Wei, C., 2021b. The response of polycyclic aromatic hydrocarbon degradation in coking wastewater treatment after bioaugmentation with biosurfactant-producing bacteria Pseudomonas aeruginosa S5. Water Science and Technology 83, 1017–1027. https://doi.org/10.2166/wst.2021.046
Zhang, B., Matchinski, E.J., Chen, B., Ye, X., Jing, L., Lee, K., 2019. Chapter 21 - Marine Oil Spills—Oil Pollution, Sources and Effects, in: Sheppard, C. (Ed.), World Seas: An Environmental Evaluation (Second Edition). Academic Press, pp. 391–406. https://doi.org/10.1016/B978-0-12-805052-1.00024-3
Zhang, C., Zhang, Z., Zhou, J., Wang, Y., Ai, Y., Li, X., Zhang, P., Zhou, S., 2022. Responses of the root morphology and photosynthetic pigments of ryegrass to fertilizer application under combined petroleum–heavy metal stress. Environ Sci Pollut Res 29, 87874–87883. https://doi.org/10.1007/s11356-022-21924-1
Zhang, X., Gao, B., Xia, H., 2014. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of bana grass and vetiver grass. Ecotoxicology and Environmental Safety 106, 102–108. https://doi.org/10.1016/j.ecoenv.2014.04.025
Zhang, X., Sarmah, A.K., Bolan, N.S., He, L., Lin, X., Che, L., Tang, C., Wang, H., 2016. Effect of aging process on adsorption of diethyl phthalate in soils amended with bamboo biochar. Chemosphere, Biochars multifunctional role as a novel technology in the agricultural, environmental, and industrial sectors 142, 28–34. https://doi.org/10.1016/j.chemosphere.2015.05.037
Zhang, Y., Cui, X., Xing, H., 2021. Recent advances in the capture and abatement of toxic gases and vapors by metal–organic frameworks. Materials Chemistry Frontiers 5, 5970–6013. https://doi.org/10.1039/D1QM00516B
Zhang, Z., Rengel, Z., Chang, H., Meney, K., Pantelic, L., Tomanovic, R., 2012. Phytoremediation potential of Juncus subsecundus in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). Geoderma 175–176, 1–8. https://doi.org/10.1016/j.geoderma.2012.01.020
Zhao, C., Dong, Yan, Feng, Y., Li, Y., Dong, Yong, 2019. Thermal desorption for remediation of contaminated soil: A review. Chemosphere 221, 841–855. https://doi.org/10.1016/j.chemosphere.2019.01.079
Zhao, F., Han, S., Zhang, Y., 2020. Comparative studies on the structural composition, surface/interface activity and application potential of rhamnolipids produced by Pseudomonas aeruginosa using hydrophobic or hydrophilic substrates. Bioresource Technology 295, 122269. https://doi.org/10.1016/j.biortech.2019.122269
Zhen, M., Chen, H., Liu, Q., Song, B., Wang, Y., Tang, J., 2019. Combination of rhamnolipid and biochar in assisting phytoremediation of petroleum hydrocarbon contaminated soil using Spartina anglica. Journal of Environmental Sciences, SI: Recent advances in Environmental Sciences 85, 107–118. https://doi.org/10.1016/j.jes.2019.05.013

Zheng, X., Aborisade, M.A., Wang, H., He, P., Lu, S., Cui, N., Wang, S., Zhang, H., Ding, H., Liu, K., 2020. Effect of lignin and plant growth-promoting bacteria (Staphylococcus pasteuri) on microbe-plant Co-remediation: A PAHs-DDTs Co-contaminated agricultural greenhouse study. Chemosphere 256, 127079. https://doi.org/10.1016/j.chemosphere.2020.127079

Zheng, X., Ding, H., Xu, X., Liang, B., Liu, X., Zhao, D., Sun, L., 2021. In situ phytoremediation of polycyclic aromatic hydrocarbon-contaminated agricultural greenhouse soil using celery. Environmental Technology 42, 3329–3337. https://doi.org/10.1080/09593330.2020.1727022

Zhou, H., Zeng, M., Zhou, X., Liao, B.-H., Peng, P.-Q., Hu, M., Zhu, W., Wu, Y.-J., Zou, Z.-J., 2015. Heavy metal translocation and accumulation in iron plaques and plant tissues for 32 hybrid rice (Oryza sativa L.) cultivars. Plant Soil 386, 317–329. https://doi.org/10.1007/s11104-014-2268-5

Zhou, Q., Hua, T., 2004. Bioremediation: A review of applications and problems to be resolved. Progress in Natural Science 14, 937–944. https://doi.org/10.1080/10020070412331344601

Zhu, L., Li, Z., Ketola, T., 2011. Biomass accumulations and nutrient uptake of plants cultivated on artificial floating beds in China's rural area. Ecological Engineering 37, 1460–1466. https://doi.org/10.1016/j.ecoleng.2011.03.010

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., García-Orenes, F., Mataix-Beneyto, J., 2006. Assessing air-drying and rewetting pre-treatment effect on some soil enzyme activities under Mediterranean conditions. Soil Biology and Biochemistry 38, 2125–2134. https://doi.org/10.1016/j.soilbio.2006.01.010

Zychowski, G.V., Godard-Codding, C.A.J., 2017. Reptilian exposure to polycyclic aromatic hydrocarbons and associated effects. Environmental Toxicology and Chemistry 36, 25–35. https://doi.org/10.1002/etc.3602

**Appendix:**

Appendix 1. QA/QC protocol adopted from Stuart's Research Group

**SUMMARY OF ANALYTICAL METHODS AND ASSOCIATED QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) PROCEDURES FOR SEMI-VOLATILE ORGANIC COMPOUNDS**

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## 1. Overview

This document describes the generically applicable methods and procedures that all researchers within the group must follow to ensure the reliability of their analytical data. Methods that apply only to a specific group of pollutants are not covered here. If you have any questions about anything relating to analysis, please ask your supervisor or an experienced member of the Research Group for advice.

## 2. Instrument Calibration

A full 5-point calibration must be conducted at the beginning of any measurement campaign. The exact concentrations and content of the calibration standard mixes will vary according to the pollutant class being measured but as a guide, the table below gives a typical example.

| *Compound* | *Standard A Concn (pg µ1$^{-1}$)* | *Standard B Concn (pg µ1$^{-1}$)* | *Standard C Concn (pg µ1$^{-1}$)* | *Standard D Concn (pg µ1$^{-1}$)* | *Standard E Concn (pg µ1$^{-1}$)* |
|---|---|---|---|---|---|
| All "native"1 standards | 20 | 50 | 200 | 500 | 1000 |
| Internal standards, Sampling Evaluation standards, recovery determination standards | 200 | 200 | 200 | 200 | 200 |

These standards are used to calculate relative response factors (RRFs) for each of the "target" compounds. The RRF is defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of the internal standard (IS). For example, if the response of a unit amount of the target compound is 1.5 times that for the same amount of the internal standard, the RRF =1.5. It is calculated as in equation 1.

$$RRF = \frac{A_{NAT}}{A_{IS}} \times \frac{C_{IS}}{C_{NAT}} \quad \text{(Equation 1)}$$

where $A_{NAT}$ is the peak area for the "native" compound in the standard; $A_{IS}$ is the peak area of the internal standard in the standard; $C_{NAT}$ is the concentration of the "native" compound in the standard; and $C_{IS}$ is the concentration of the internal standard in the standard.

1 "native" refers to the $^{12}C$ or $^{1}H$ isotope of the target compound. The term is used to distinguish it from the $^{13}C$ or $^{2}D$ (deuterated) isotope used as the internal standard.

Calculation of RRFs for each of the standard A-E, should reveal them to be essentially identical in each standard. Ideally, the relative standard deviation (*i.e.* ($\sigma_{n-1}$ /average) x 100%) of RRFs for a given target compound should not exceed 10%. If they do, consult your supervisor before proceeding.

A full 5-point calibration typically only needs to be conducted infrequently, or when an on-going accuracy check proves unsatisfactory. The average RRF for any subsequent full calibration should be within ± 10% of the average RRF obtained for the 1st 5-point calibration. If they do not, then you must consult your supervisor immediately.

Before each batch of samples are analysed on the GC/MS, one of the calibration standards (usually Standard C, but others are fine) must be run. The RRFs obtained from this analysis must be within ±25% of the RRFs obtained for that standard in the initial 5-point calibration. If they do not, please consult your supervisor before proceeding. At the end of each batch of samples, the same calibration standard must be run. The RRFs obtained from this analysis must be within ± 25% of the RRFs obtained for that standard in the initial 5-point calibration. The RRFs that must be used for calculating concentrations in samples in that batch will be an average of those obtained for the 2 standards run for that batch.

## 3. GC/MS tuning tips

At the start of each session, an autotune should be run. The results should be printed out and a record kept. Following the autotune (which should detect any major problems with the GC/MS), a manual tune must be conducted. The purpose of the manual tune is to maximise sensitivity and instrument performance for the particular group of compounds you are targeting. As a general rule, while during tuning the detector voltage should be set at 200V, you should set the detector voltage to 450V (*i.e.,* that necessary to detect compounds in the concentration range 10-1000

pg/component) in your acquisition file used when running standards and samples. You should also tune with the oven temperature at a temperature similar to that at which your target compounds will elute form the GC column. Typically for a DB-5 type column it will be 250 $^{o}$C but may be lower for other columns (DO NOT EXCEED THE MAXIMUM ALLOWABLE ISOTHERMAL OPERATING TEMPERATURE FOR THE COLUMN). You should choose the m/z values most appropriate to the mass range of the pollutants which you are targeting – your supervisor will be able to advise you on the best choice. The autotune uses m/z 69, 219, and 502 – this is not appropriate for PCBs for example, which lie in the mass range 256 to 394, and the manual tune should be based on tuning masses 219, 264, and 414.

## 4. Determination of Internal Standard Recoveries

It is important to note that use of the internal standard quantification method means that NO correction of concentrations for recovery is required. However, it is important that recoveries of internal standards are calculated for each sample as a QA/QC measure. Typically, such recoveries should be around 70%, but they may routinely fall in the range 30%-150%. If values exceed 150%, the sample extract should be re-analysed, and the recovery recalculated. If recoveries are below 30%, then the signal to noise (S: N) ratio of the internal standard must be calculated. The data are acceptable provided that the S:N ratio exceeds 20:1. If it is less than 20:1 the sample extract should be re-analysed, and the recovery recalculated. If the recovery percentage and S:N ratio is still unacceptable then data for that sample must be considered invalid.

Internal standard (IS) recoveries are calculated thus:

$$\% \text{ IS Recovery} = \left[ \left( \frac{A_{IS}}{A_{RDS}} \right)_S \times \left( \frac{A_{RDS}}{A_{IS}} \right)_{STD} \times \left( \frac{C_{IS}}{C_{RDS}} \right)_{STD} \times \left( \frac{C_{RDS}}{C_{IS}} \right)_S \right] \text{x } 100 \quad \text{(equation 2)}$$

where $(A_{IS}/A_{RDS})_S$ = ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ = ratio of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration

standards run for a batch of samples is used); $(C_{IS}/C_{RDS})_{STD}$ = ratio of concentration of internal standard to concentration of recovery determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ = ratio of concentration of recovery determination standard to concentration of internal standard in the sample (assuming 100% recovery). Note that this can be calculated as the amount of internal or recovery determination standard added to the sample divided by the volume of the sample extract used for GC/MS analysis (typically 25-50 µ1).

## 5. Determination of Sampling Evaluation Standard Recoveries

Recoveries of sampling evaluation standards (*i.e.,* those added to the PUF plug in air or aqueous sample analysis) are calculated for each sample as QA/QC measure. Note that SESs are NOT added to solid samples like soil or grass. Typically, such recoveries should be around 70%, but they may routinely fall in the range 30%-150%. Note that although SES recoveries should be recorded for every sample, they are a QA/QC check only, and are NOT used to correct concentrations for sampling losses. If values exceed 150%, the sample extract should be re-analysed, and the recovery recalculated. If it still exceeds 150%, then data for that sample must be considered invalid. If recoveries are below 30%, then the signal to noise (S: N) ratio of the sampling evaluation standard must be calculated. The data are acceptable provided that the S:N ratio exceeds 20:1. If it is less than 20:1 the sample extract should be re-analysed, and the recovery recalculated. If the recovery percentage and S:N ratio is still unacceptable then data for that sample must be considered invalid.

Sampling evaluation standard (SES) recoveries are calculated thus:

$$\% \text{ SES Recovery} = \left[ \left( \frac{A_{SES}}{A_{RDS}} \right)_S \times \left( \frac{A_{RDS}}{A_{SES}} \right)_{STD} \times \left( \frac{C_{SES}}{C_{RDS}} \right)_{STD} \times \left( \frac{C_{RDS}}{C_{SES}} \right)_S \right] \text{x } 100 \quad \text{(equation 3)}$$

where $(A_{SES}/A_{RDS})_S$ = ratio of sampling evaluation standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{SES})_{STD}$ = ratio of recovery determination standard peak area to sampling evaluation standard peak area in the calibration standard (the average of values

obtained for both calibration standards run for a batch of samples is used); $(C_{SES}/C_{RDS})_{STD}$ = ratio of concentration of sampling evaluation standard to concentration of recovery determination standard in the calibration standard and $(C_{RDS}/C_{SES})_S$ = ratio of concentration of recovery determination standard to concentration of sampling evaluation standard in the sample (assuming 100% recovery). Note that this can be calculated as the amount of sampling evaluation or recovery determination standard added to the sample divided by the volume of the sample extract used for GC/MS analysis (typically 25-50 µ1).

## 6. Determination and On-Going Monitoring of Accuracy

The principal means of determining method accuracy is *via* analysis of one or more certified or standard reference materials (CRMs or SRMs). Your supervisor will recommend a suitable CRM/SRM. Before you commence analysis of any samples as part of your research, you must conduct 5 replicate analyses of a suitable CRM or SRM and obtain satisfactory data for these analyses. Essentially a CRM or SRM is a sample that has been analysed a large number of expert laboratories worldwide and that has had agreed concentrations of target pollutants assigned to it. These values are usually cited as an average ± a standard deviation. The values you obtain will be compared with these. You must discuss your data with your supervisor and will only be allowed to proceed with analysis of your samples once acceptable accuracy of data are obtained.

As an ongoing measure of accuracy, you must analyse 1 aliquot of the same CRM/SRM for every 20 samples – *i.e.,* every 21$^{st}$ sample you analyse must be a CRM/SRM. If satisfactory data are not obtained, then you must consult your supervisor immediately.

Additional means of evaluating accuracy include participation in interlaboratory comparisons. Your supervisor will advise you as and when such comparisons are to take place.

## 7. Determination of Precision

This is defined as the relative standard deviation (*i.e.,* 100 x $\sigma_{n-1}$/average) of concentrations obtained from 5 replicate analyses of the same sample. Usually, this is a CRM/SRM. Typically, precision should be no more than 30%, but you must discuss your data with your supervisor.

## 8. Determination of Blank Concentrations

This is defined as the concentration of a target pollutant present in an analysis where the sample is omitted, but internal standards etc. are added. Note that for air analyses, a blank should consist of analysis of a clean PUF plug and filter paper. For calculation of blank sample concentrations, you should assume the sample mass or volume to be that typically used – *e.g.,* 1,000 $m^3$ for air samples, 50g for soil or grass samples. One blank analysis must be conducted for every 5 samples – *i.e.,* every $6^{th}$ analysis you perform must be a blank. Where the concentration of a target pollutant in a blank for a given batch of samples is 5-20% of the concentration in a sample from that batch, the blank concentration must be subtracted from that in the sample. Where the concentration in the blank exceeds 20% of that in a sample from that batch, data for that target pollutant in that sample must not be reported.

## 9. Determination of Detection limits

Two categories of detection limits exist.

1. instrument detection limit (IDL)
2. sample detection limit (SDL)

The IDL is defined as that amount of pollutant that gives a signal to noise ratio of 3:1. It is best determined by calculating the signal to noise ratio for the pollutant in your calibration standard A.

To illustrate, if the concentration of a target pollutant in that standard = 20 pg/µ1 and 1 µ1 is injected, then if a signal to noise ratio of 50:1 is obtained, then the IDL = 20 x (3/50)= 1.2 pg/injection.

The SDL can then be calculated as in equation 4:

$$\text{SDL} = \frac{IDL \times FEV}{VFEI \times SS} \times \frac{100}{\%IS\,\text{Recovery}} \quad \text{(equation 4)}$$

Where FEV = final extract volume (µ1), VFEI = volume of final extract injected (µ1); SS = sample size ($m^3$ or g); and %IS recovery = percentage recovery of internal standard used to quantify the target pollutant in a particular sample.

To illustrate, if the IDL = 1.2 pg/injection, the final extract volume for a sample is 50µl, 1 µ1 is injected; the sample size is 1,000 $m^3$, and the percentage internal standard recovery in that sample = 70%, then the SDL = ((1.2 x 50)/ (1 x 1000)) x 100/70 = 0.086 pg $m^{-3}$.

Where the concentration in the sample blank exceeds the SDL calculated as above, the effective SDL is the blank concentration. This is not an unusual occurrence.

## 10. Calculation of concentrations in samples

Concentrations in samples may be calculated *via* the equation below:

$$\textit{Concn.} = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS} \quad \text{(equation 5)}$$

Where $A_{IS}$ = peak area of internal standard in sample; $A_{NAT}$ = peak area of target pollutant in sample; RRF = relative response factor for the target pollutant (see equation 1); $M_{IS}$ = mass of internal standard added to sample (pg) and SS = sample size ($m^3$ or g).

To illustrate, where $A_{NAT}$ = 10,000 units; $A_{IS}$ = 20,000 units; RRF = 1.5; $M_{IS}$ = 20,000 pg; and SS = 50 g, the concentration of the target pollutant will be (10,000/20,000) x (1/1.5) x (20,000/50) = 133.33 pg $g^{-1}$.

## 11. Correct Storage of Calibration and internal standards

Once prepared in CERTAN vials, all standards should be stored in a freezer unless required for analysis. You should record the weight of the CERTAN vial and contents before and after each use. Before weighing, allow the vial and contents to reach room temperature, and wipe off any condensation before weighing. If at any time, the weight before use is less than 5% of the weight after recorded after the previous use, you must consult your supervisor immediately.

## 12. Criteria for Quantification of a Peak as a Target pollutant

For a given peak to be identified as a target pollutant in a sample, various criteria must be met. These are:

1. the signal to noise ratio of the peak must exceed 3:1
2. the relative retention time (RRT) of the peak in the sample must be within 0.2% of the average value determined for the 2 calibration standards run for the sample batch. Note RRT = retention time of target pollutant/retention time of internal standard used to quantify target pollutant.

The above criteria apply to all target pollutants. For organochlorine and organobromine pollutants, the following criterion also applies.

- the isotope ratio of the peak in the sample must be within 20% of the average value determined for the 2 calibration standards run for that sample batch. If it falls outside this range, then you must consult your supervisor, but it is likely that the peak cannot be quantified due to a co-eluting interference. For example, for trichlorinated PCBs, where 2 m/z values are monitored (*i.e.,* 255.95 and 257.95) the isotope ratio = area of peak for 255.95 trace/area of peak for 257.95 trace. Note that for calculating RRFs and concentrations, the m/z value providing the largest peak must be used.

**Appendix 2. X-ray diffraction patterns**

The XRD patterns of all samples analysed are presented in the figures below. The figures are A1 is the untreated and uncontaminated (raw) soil sample, A2 is the unvegetated and untreated crude oil contaminated soil, A3 is the is the crude oil contaminated soil vegetated with *C. zizanioides,* A4 is the crude oil contaminated soil vegetated with *C. zizanioides* and treated with biosurfactant only, A5 is the crude oil contaminated soil vegetated with *C. zizanioides* and treated with fertiliser only, and A6 is the crude oil contaminated soil vegetated with *C. zizanioides* and treated with fertiliser and biosurfactant.

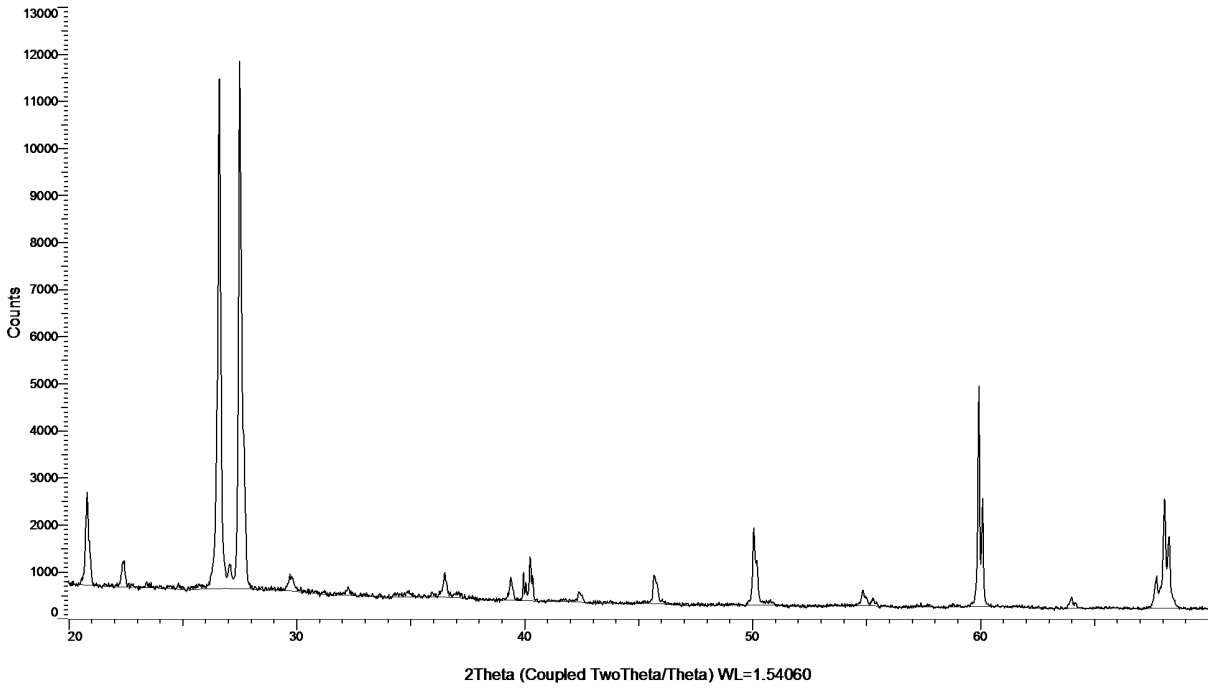



Figure A1. XRD pattern of sandy loam soil, sample (soil only)

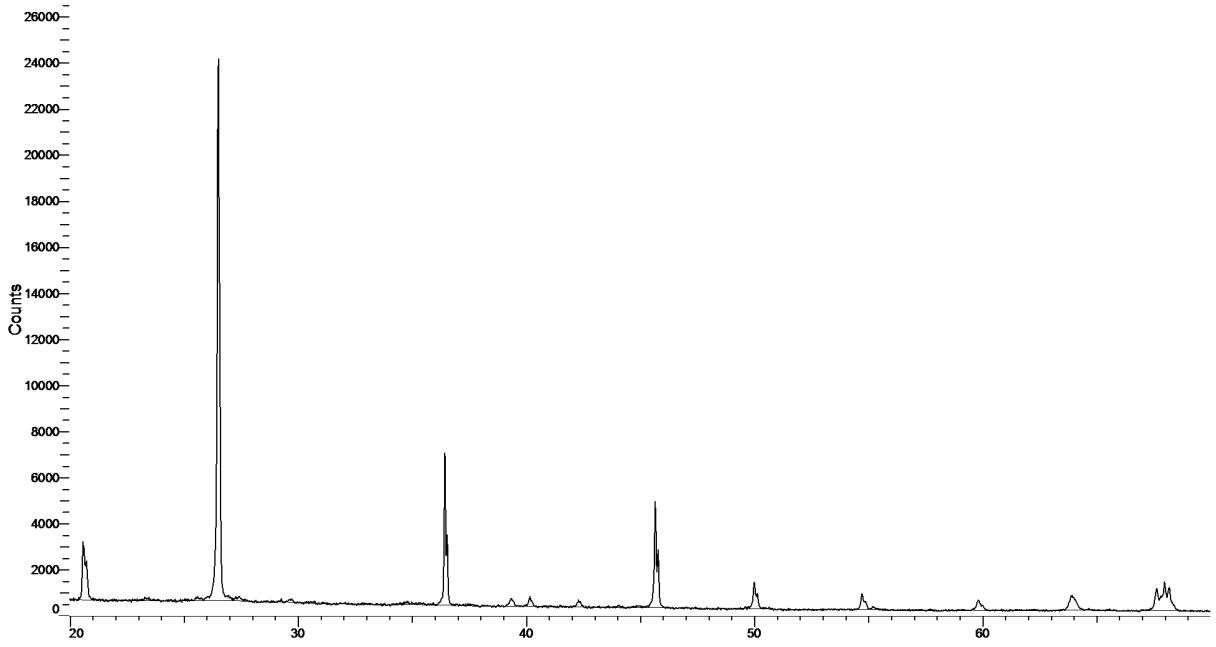



Figure A2. XRD pattern of sandy loam soil, sample (soil + oil)

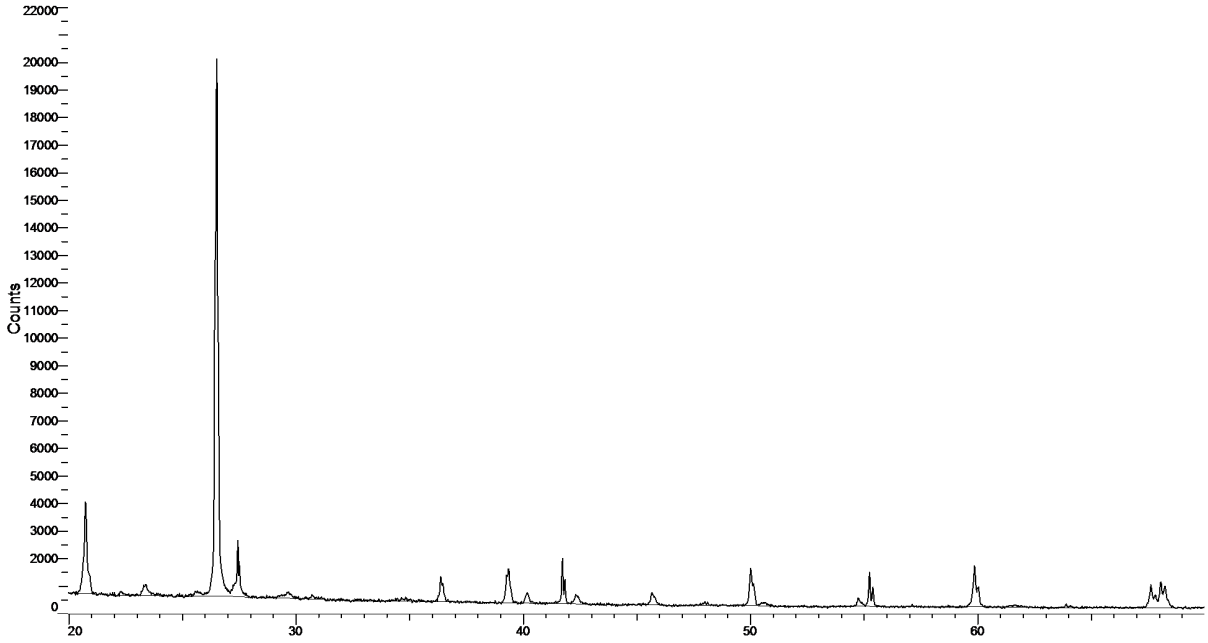



Figure A3. XRD pattern of sandy loam soil, sample (soil + oil + plant)

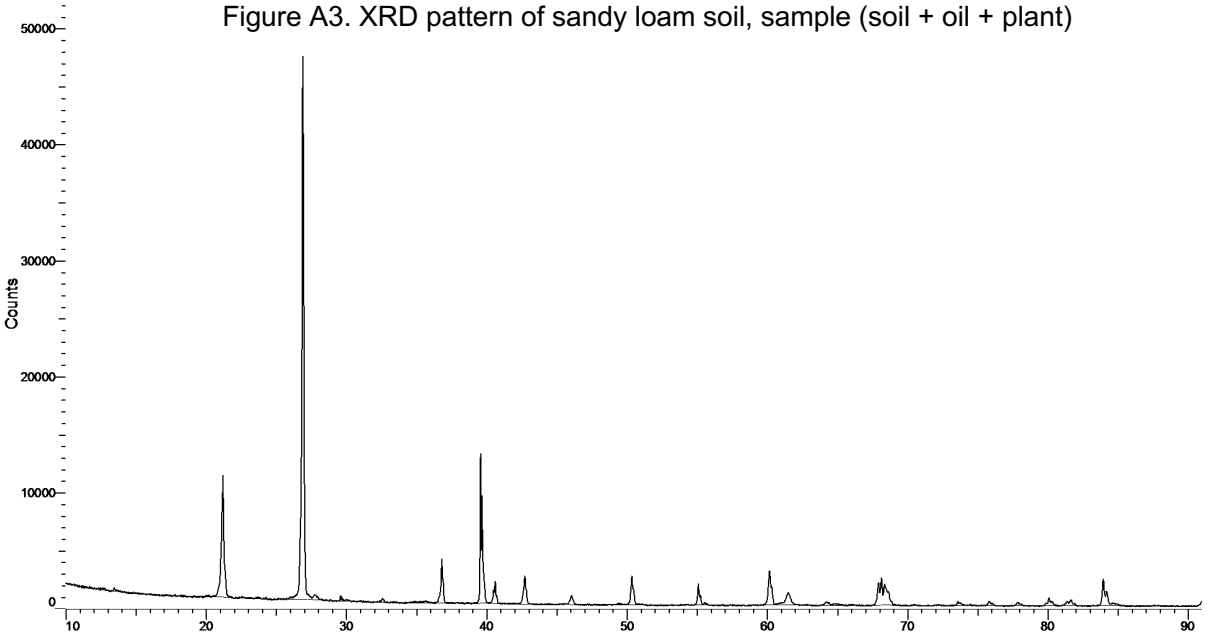



Figure A4. XRD pattern of sandy loam soil, sample (soil + oil + plant + biosurfactant)

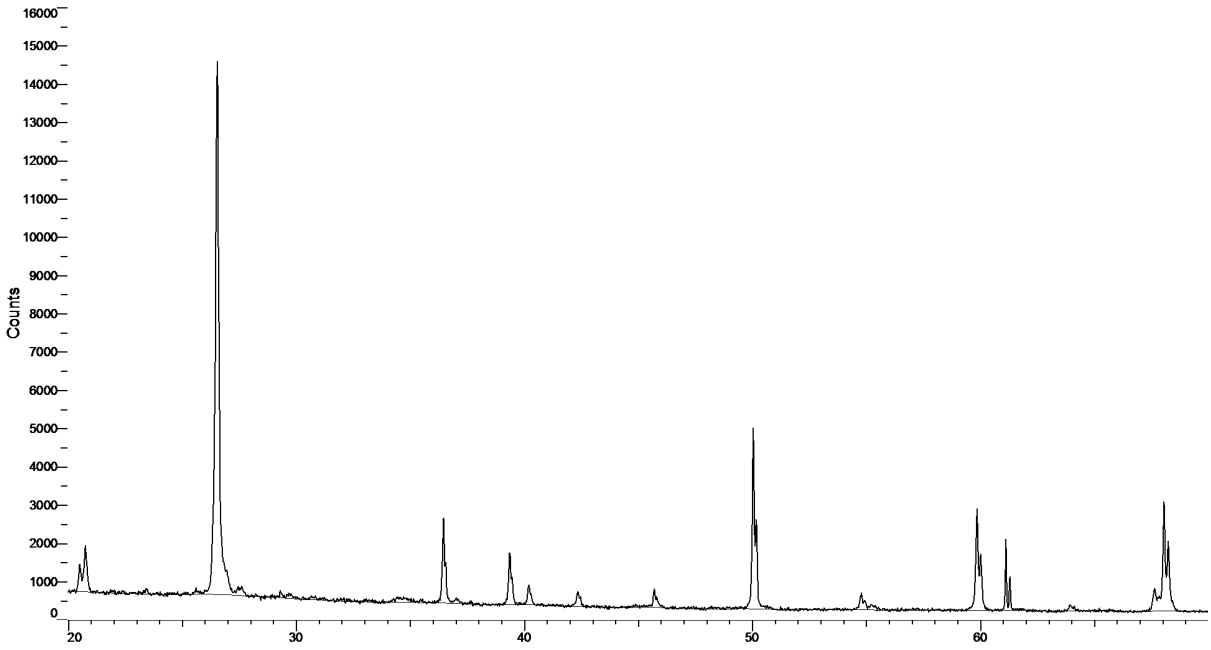



Figure A5. XRD pattern of sandy loam soil, sample (soil + oil + plant + fertiliser)

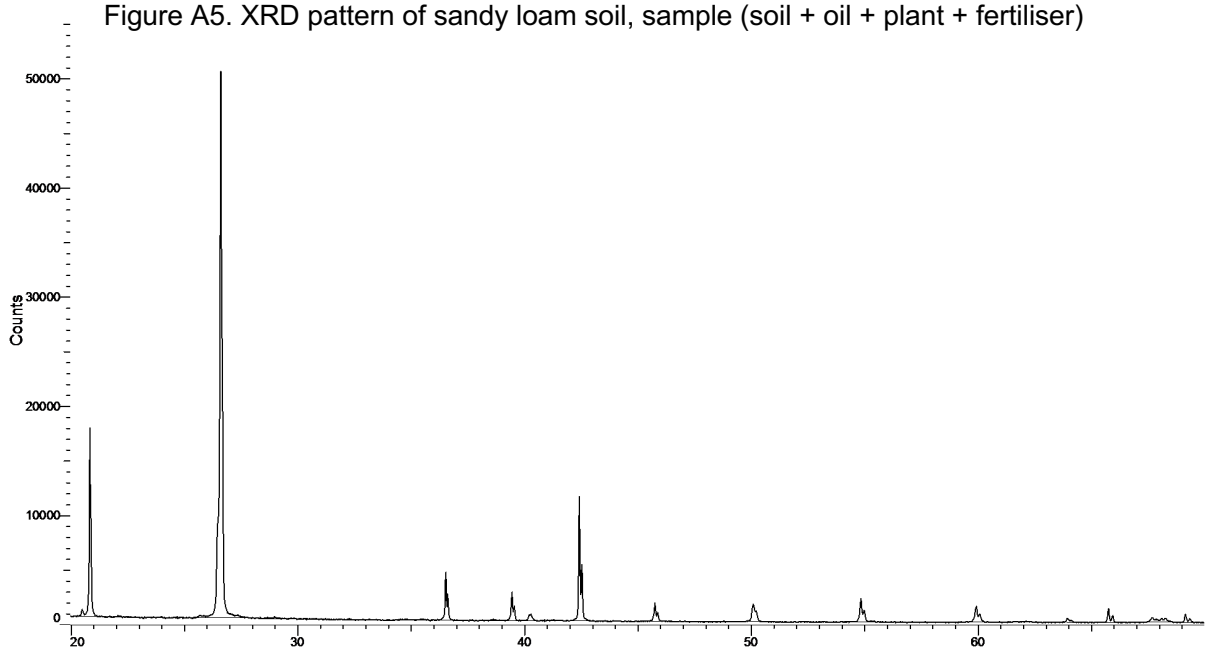



Figure A6. XRD pattern of sandy loam soil, sample (soil + oil + plant + biosurfactant + fertiliser)