DEVELOPMENT OF ANALYTICAL, PHYTO- AND MYCO-REMEDIATION TECHNIQUES TO MANAGE PETROLEUM-CONTAMINATED SOILS

BY

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Declaration of originality

This thesis is the original work carried out by Udeme John Dickson. Contributions from others and literatures used have been properly acknowledged.

…….. 06/03/2020

Dickson, Udeme John. **Date**

Copyright declaration

Dedication

This thesis is dedicated to my supervisors namely **Dr Marcello Di Bonito, Dr Nicholas Ray, Dr Michael Coffey, Professor Barry Smith and Professor Rob Mortimer** for their guidance and support during the research period.

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ABSTRACT

Practical solutions to soil pollution by the petroleum industry are still to be fully realised. With new, unresolved and recurring cases, remediation options that are readily available, cost-effective and environmentally friendly are required. Analytical methods for quick and easy monitoring are also crucial. To find appropriate solutions to petroleum-contaminated soils particularly for the Niger Delta, Nigeria; options, which satisfy the above principles, were investigated. Thus, the aims of this research were to identify readily available and sustainable techniques for remediation of petroleum-contaminated soils; evaluate ways to overcoming associated limitations, thereby enhancing these techniques; and investigate for readily available methods of monitoring the petroleum-contaminated and remediated soils. After a systematic and critical literature review, phyto- and myco-remediation were identified as viable options for this research, their limitations were evaluated. The actual study involved sampling of petroleum-contaminated soils, treatment with phyto- and myco-remediation agents and investigation of methods for analysis and monitoring of the soils. Agents used for the remediation (evaluated in terms of reduction in Total Petroleum Hydrocarbons-TPHs in the soil samples) were: 3 species of sunflowers (*Helianthus annus-pacino gold*, *Helianthus sunsation* & *Helianthus annus-sunny dwarf*), the fern-*Dryopteris affinis,* fermented palm wine (from 2 species of palm trees -*Elaeis guineensis & Raffia africana*), and oyster mushroom (*Pleurotus ostreatus*). Supplementing the agents with Tween 80 and the use of alternative substrates and methods for application of *P. ostreatu*s enabled the investigation of possible enhancement of their remediation efficiency. The investigation revealed up to 525 g of TPHs per Kg dry weight of soils. The remediation treatments produced as much as 69% reduction in TPHs by the sunflower species, 70% by fermented palm wine, 74% by *D. affinis* and 85% by *P*. *ostreatus*; with up to 100% enhancement on the addition of Tween-80. It was also found that substrates type and method of application has a significant effect on the remediation efficiency of *P. ostreatus.* The study further revealed that available nitrate, electrical conductivity, standardised crude oil and the biomarkers-dodecane and benzene-1,3-bis(1,1-dimethylethyl) can be used to broadly monitor the concentration of TPHs and remediation progress in soils. This research thus demonstrated that, phyto-and myco-remediation can provide readily available and sustainable techniques for remediation of TPHs in soils. Further studies are required to evaluate the application of these techniques for individual petroleum contaminant components such as the polycyclic aromatic hydrocarbon compounds and Asphaltenes.

List of publications

A). Published articles

1. Dickson, Udeme John; Coffey, Michael; Mortimer, Robert John George; Di Bonito, Marcello and Ray, Nicholas (2019). **Mycoremediation of Petroleum-contaminated soils: Progress, Prospects & Perspectives.** *Environmental Science: Processes & Impacts,* 21: 1446–1458.
2. Dickson, Udeme John; Coffey, Michael; Mortimer, Robert John George; Smith, Barry; Di Bonito, Marcello and Ray, Nicholas (2020). **Investigating the Potential of Sunflower Species, Fermented Palm Wine and *P. ostreatu*s for Treatment of Petroleum-contaminated soils.** *Chemosphere*, 240 (124881): 1-10.

B). Other publication plans

3. Dickson, Udeme John; Coffey, Michael; Mortimer, Robert John George; Di Bonito, Marcello and Ray, Nicholas (2019). **Phytoremediation Could Provide Solutions for Petroleum-contaminated soils.** *Nature Sustainability* (Submitted-awaiting decision).

4. Dickson, Udeme John; Giadom, Ferdinand; Coffey, Michael; Mortimer, Robert John George; Smith, Barry; Di Bonito, Marcello and Ray, Nicholas (2019). **Effect of Tween 80 on Some Phyto- and Myco-remediation Agents on Petroleum-contaminated Silty Loam Soil from Ogoniland, Niger Delta, Nigeria.** *Chemosphere* (Submitted-awaiting decision).

5. Dickson, Udeme John; Giadom, Ferdinand; Coffey, Michael; Mortimer, Robert John George; Smith, Barry; Di Bonito, Marcello and Ray, Nicholas (2019). **Utilising Mycoremediation for Treatment of Petroleum-contaminated Soils and Sediments from Ogoniland, Niger Delta, Nigeria.** *Land Degradation and Development* (Submitted-awaiting decision).

6. Dickson, Udeme John; Giadom, Ferdinand; Coffey, Michael; Mortimer, Robert John George; Smith, Barry; Di Bonito, Marcello and Ray, Nicholas (2019). **Assessment of Petroleum-contaminated Soils Using Crude Oil Standard and Biomarker Compounds-Dodecane, and Benzene-1,3-bis(1,1-dimethylethyl).** Target Journals: *Trends in Analytical Chemistry, Analytica Chimica Acta, Analytical Methods.*

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List of Abbreviations

AOAC- Association of Official Analytical Chemist

BSI- British International Standard

EC- Electrical Conductivity

EU- European Union

FID- Flame Ionisation Detector

FTIR- Fourier-Transform Infrared spectroscopy

GC- Gas Chromatography

HPLC- High Performance Liquid Chromatography

ISO – International Standard Organisation

MAE-Microwave Assisted Extraction

MS-Mass Spectrometer

PAHs- Polycyclic Aromatic Hydrocarbons

PFE- Pressurised Fluid Extraction

S/N- Serial number

TPHCWG- Total Petroleum Hydrocarbon Working Group

TPHs- Total Petroleum Hydrocarbons

UNEP-United Nations Environmental Protection Agency

USEPA- United States Environmental Protection Agency

UTM- Universal Transverse Mercator

CHAPTER ONE

General introduction and study background

1.1 Introduction

The petroleum industry has a substantial multiplier effect on the world's economy because almost every sector depends directly or indirectly on its products and services (He *et al.,* 2010; Gatfaoui 2016; Wei and Guo, 2016). However, issues associated with environmental degradation, especially from oil spills tend to cast a negative light on the industry (Prasad and Kumari, 1987; Pezeshki *et al.,* 2000; Lee *et al.,* 2015). While many petroleum-contaminated sites remain untreated, new and recurring cases are frequently reported both in developed and developing countries (Jenssen, 1994; Zakaria *et al.,* 2000; Aghalino & Eyinla, 2009; Eliopoulou *et al.,* 2012). The problem with management of petroleum-contaminated sites is in two ways. These are finding a suitable, cost effective and readily available method of remediation; and that, of monitoring both the contaminated and remediated sites.

Techniques for remediation of petroleum-contaminated soils include incineration, soil washing, soil flushing, soil vapour extraction, bioventing, air biosparging, soil attenuation, bio-augmentation, bio-stimulation, composting, phytoremediation and mycoremediation (Vanderlelie *et al.,* 2003; Singh, 2006; Doni *et al.,* 2012; Wiszniewska *et al.,* 2016).

Most of these techniques are rather expensive and require significant technical inputs and expertise (Cole, 2018). These factors restrict their deployment in developing countries because of inadequate funds and lower level of technology. The consequence is undue delays in the clean-up of petroleum-contaminated sites. Numerous cases of petroleum-contaminated sites which have remained for decades without remediation are known (Usman *et al.,* 2018; French, 2019). One example is that of Ogoniland, Nigeria, where over 7000 oil spill incidents involving more than 2300 m^3 of crude oil have been reported (Allison *et al.,* 2018; Bodo, 2018; Ite, *et al.,* 2018; Mogaji, *et al.,* 2018).

Apart from the cost and technical restrictions associated with many of the remediation techniques, some of these also disrupt the physical, biological and chemical functions of soil, making it unfit for practical use (Brusseau, 2019; Xia *et al.,* 2019). Adenipekun and

Lawal (2012) and Fernández-Luqueño *et al.,* (2019) stated that for sustainable remediation of contaminated soils, it is important to utilise techniques that clean up the contaminants and preserve soil structure and ecological functions. Therefore, workable solutions to petroleum-contaminated sites require methods, which are readily available, cost-effective and environmentally friendly (Song *et al.,* 2019).

Phyto- and myco-remediation are some of the techniques that could provide sustainable remediation of petroleum-contaminated soils (Rhodes, 2014; Wiszniewska *et al.,* 2016). When compared to other techniques, phyto- and myco-remediation are cost-effective, environmentally friendly and require less technological inputs and expertise (Cunningham *et al.,* 1995; Gerhardt *et al.,* 2009; Dubchak & Bondar, 2019). These techniques have previously been employed commercially for the management of pollution episodes in developed countries in North America and Europe (Vanderlelie *et al.,* 2001; Doni *et al.,* 2012). However, the techniques are yet to be utilised in developing countries, particularly in the tropics, despite the existence of several ex-situ studies (Adenipekun and Lawal, 2012; Oti, 2015).

One difficulty with phyto- and myco-remediation is the long duration of the process (Dubchak & Bondar, 2019; Iqbal *et al.,* 2019). Other limitations include problem of plants survival and adaptability, and limited root length which confines its application to the upper soil profile (Ali *et al.,* 2013; Fasani *et al.,* 2018). Over the years, several innovations have been explored to enhance phyto- and myco-remediation processes for the clean-up of contaminated environments (Zhang *et al.,* 2010; Fatima *et al.,* 2015; Cai *et al.,* 2016; Liaoa *et al.,* 2016). Although many of the outcomes are promising, adapting these to commercial applications remains problematic. Mendez and Mair (2008) observed that most of the plant species used in phytoremediation studies are not native to contamination sites, thus promising glasshouse outcomes may not produce the expected results during in situ application, due to issues of adaptation. These challenges necessitate investigating of phyto- and myco-remediation agents that are either generally available in many parts of the world or native to contaminated sites and exploring ways to enhance their remediation potential for the management of petroleum-contaminated soil.

Generally, monitoring of petroleum-contaminated soils require sampling, extraction and analysis to establish the extent of contamination or remediation. The analysis typically employs TPHs mix as the analytical standard in Gas Chromatography coupled with detectors such as FID or MS (EPA SW-846, Methods 8015, 8015A; ISO/TS 16558-2, 2015). Nevertheless, as crude oil is a mixture of many organics other than hydrocarbons, the use of TPHs standards can overlook other organics especially those of halogenated aromatics (Blaisdell & Smallwood, 1993). There are also instances where these standards are not readily available (Blaisdell and Smallwood, 1993; Anderson and McCarthy, 1994; McKenna *et al.,* 1995). Therefore, it is also imperative to explore other options for the quick assessment of petroleum contaminants, and remediation processes in soils.

Petroleum biomarkers are utilised in the oil industry for several purposes and consist of individual compounds representative of TPHs aliphatic, alicyclic, aromatic, fused-ring and hetero-substituted classes usually found in petroleum or source rocks (Van *et al.,* 2011; Madu and Ugwu, 2017; Krajewski *et al.,* 2018). Biomarker analysis can provide reliable evidence for spilled crude oils and petroleum products and can be used to identify sources of oil spills (Han and Clement, 2018; Walters *et al.,* 2018). However, there is very limited information on the application of petroleum biomarkers for quantitative assessment of petroleum contaminants in soils and sediments.

Ultimately, there is a need to investigate phyto- and myco-remediation agents that are available in many parts of the world or native to sites of petroleum contamination and in addition, examine ways of enhancing their remediation potentials. There is also an opportunity to explore readily available options for the assessment of petroleum-contaminated and remediated soils.

1.2 Aims of the Study

The overall aim of this study was to develop analytical, phyto- and myco-remediation techniques to manage petroleum-contaminated soils. These include:

(i) identification of readily available phyto- and myco-remediation agents for the reduction of Total petroleum hydrocarbons (TPHs) in petroleum-contaminated soils;

(ii) evaluating ways to overcoming associated limitations, thus enhancing these techniques; and

(iii) investigation for readily available methods of monitoring the petroleum-contaminated and remediated soils.

1.3 Scope of the study

The soil samples used in this study were from historic contamination sites in Tibshelf, Derbyshire (United Kingdom) and Ogoniland, Niger Delta (Nigeria). The research was targeted towards achieving cost effective, time efficient and readily available options for remediation and monitoring of TPHs in petroleum-contaminated soils.

The first phase of the study involved the use of petroleum-contaminated soils from Tibshelf (359414 N, 444927 E) in Derbyshire, United Kingdom. The outcomes were applied on different soil types (sand, clay, loam) and sediments from the Niger Delta, Nigeria. Petroleum-contaminated soils and sediments from Tibshelf, Derbyshire, United Kingdom and Ogoniland (-4997083 N, 1468956 E) in the Niger Delta region of Nigeria were used as case studies for this research.

The reduction in concentrations of TPHs in the soils was used as a measure of the remediation efficiency of the phyto- and myco-remediation agents.

1.4 Conceptual plan and approach to the study

1.4.1 Approach to the study

1. Having identified the problem of interest (petroleum-contaminated soils in the Niger Delta, Nigeria), an appraisal of the techniques for remediation of petroleum-contaminated soils was carried out. Based on readily availability, ease of application and environmental sustainability; phytoremediation and mycoremediation were chosen for this study.

2. A systematic and critical literature review to appraise the concept of phyto remediation, mycoremediation, and the methods for monitoring and analysis of petroleum-contaminated soils was then carried. These reviews were used to identify the progress, limitations, and prospects of these methods for management of petroleum-contaminated soil. Gaps in knowledge were also identified and recommendations for enhancement of these techniques proffered. The methods used for the study were therefore built up from the knowledge from these literature reviews.

3. Thus, the study methodologies were chosen from standard and recommended procedures. The methods were at certain times investigated for optimisation based on identified limitations (from literature), and the optimised methods used for the study.

4. Petroleum-contaminated soils from Tibshelf, Debyshire, United Kingdom were used for the first part of the investigation. This allowed for immediate investigation of TPHs remediation properties of the identified phyto-and myco-remediation agents before application to soils from the Niger Delta, Nigeria. The use of petroleum-contaminated soil samples from Tibshelf, Derbyshire (temperate region), and the Niger Delta, Nigeria, also allowed for evaluation of the techniques on both temperate and tropical soils.

5. Phyto- and mycoremediation agents that are found in many parts of the world, and especially the Niger Delta, Nigeria were used for the study. This was to investigate the prospect of such readily available options for the clean-up of TPHs in petroleum-contaminated soil. The phytoremediation agents used for the study were three species of sunflower plants namely, *Helianthus annus* (Pacino gold), *Helianthus sunsation*, and *Helianthus annus* (Sunny dwarf), and the Fern-*Dryopteris affinis*. The mycoremediation agents were (1) fermented palm wine

from two species of palm trees (*Elaeis guineensis and Raffia africana*); and (2) white rot fungus- *Pleurotus ostreatus.*

These agents were also specifically chosen due to the following reasons:

(a). The sunflower, *Helianthus annus* has been used for phytoremediation of soils in temperate regions (Park *et al.,* 2011). Its use can provide aesthetic relief to objectionable sites of petroleum contamination. However, from extensive literature review, no published studies have been carried out using the sunflower on tropical soils. Also, sunflowers species with different biomass are known (CalamaiValkova, *et al.,* 2018). There are also no reports relating phytoremediation of sunflowers to their different species or biomass. Thus, sunflowers were chosen for the phytoremediation study to assess its efficiency on petroleum-contaminated soil from the tropical region of the Niger Delta, Nigeria, and to investigate the variation of the remediation efficiency of the different species. This will help in the choice of the plant type for use in future remediation projects.

(b). Palm wine is a juice obtained from Palm trees which are abundant in many tropical regions such as the Niger Delta, Nigeria, and consists of a consortium of microorganisms principally the yeast- Saccharomyces species (Nwaiwu *et al.,* 2016). Mycoremediation potential of Saccharomyces (yeast) on crude oil has been reported (Abioye *et al.,* 2013). Since palm wine consist principally of Saccharomyces, it was needful to investigate the potential of palm wine in remediation of petroleum-contaminated soil. This could also provide readily available method for remediation of such soils.

(c). The white rot fungus, *Pleurotus ostreatus* is found in many parts of the world including the Niger Delta, and is known for degradation of TPHs in soils (Stamet, 2005; Ferdeş *et al.,* 2018). Current methods of its application requires substrates sterilisation, which is energy consuming. Adapting this fungi for remediation of petroleum-contaminated soils in the Niger Delta, Nigeria, will require substrates,

which are abundant in the region. It will also require appropriate techniques for successful in situ applications. Thus, various substrates and methods for optimal application of the fungus on petroleum-contaminated soils were also investigated.

(d). During the sampling and collection of petroleum-contaminated soils in the Niger Delta, Nigeria, for the study, the fern - *Dryopteris affinis* AGM was observed as one of the prominent resistant plants growing on the petroleum-contaminated soils. It was also necessary to investigate the phytoremediation potential of such a specie. Thus, *Dryopteris affinis* was investigated for its potential phytoremediation properties on petroleum-contaminated soils of the Niger Delta.

6. One significant limitation of phyto- and mycoremediation is the long duration of its application. Therefore, this study at certain points sought to enhance the remediation activities of these agents by the addition of Tween 80 to the soils.

7. Finally, the need for readily available methods to assess the concentration of TPHs in petroleum-contaminated and remediated soils led to investigation of readily available options for evaluating TPHs in soils. This was investigated using physicochemical parameters, contaminated crude oil as standard against the conventional TPHs standards, and the use of biomarker compounds.

1.4.2 Thesis Chapters

This thesis consists of eight chapters arranged in progressing order of activities. It starts with a general introduction in chapter one; chapter two, the literature review; and chapter three, the general methodology while chapters four, five, six and seven deal with the main experiments, results and discussions. The final chapter (eight) provides a summary of the thesis and the main conclusions.

Chapter one

This chapter provides a general introduction and background of the study, the problem statement as well as the aims and scope. The conceptual and thesis plan is also provided in this chapter.

Chapter Two

This presents a critical review on phytoremediation, mycoremediation and techniques for analysis and monitoring of petroleum-contaminated soils. The progress, application trends and methods, limitations and advancement of the techniques have been identified in this chapter. Gaps in study in these techniques are identified, and recommendations for improvement also proffered.

The specific objectives of the study are also outlined in this chapter.

Chapter three

Chapter three provides a general survey of the methodology used for soil sampling, glasshouse preparations, and sample collection after glasshouse treatments, sample preparations and the analysis carried out in the research. The methods specific to certain sections of the thesis are discussed in their relevant chapters. The general approach to the research was identification and sampling of petroleum-contaminated soils, followed by glasshouse remediation treatments with the phyto- and myco-remediation agents.

The knowledge acquired during the literature review in chapter two was used to initiate and at certain times modify the study methodologies.

For instance, conventional petroleum-contaminated soils were used for the study because such provided typical contamination situations. The agents used for remediation were also those with high tolerance to petroleum contamination and locally available in the Niger Delta, Nigeria.

Chapter four

Chapter four investigated the remediation potential of sunflower species, fermented palm wine and *P. ostreatus* on petroleum-contaminated soils.

The soil used for the study in this chapter were petroleum-contaminated soils obtained from Tibshelf, Derbyshire, United Kingdom. The outcomes were to be applied to soils from the Niger Delta, Nigeria to assess the application of the methods in both temperate and tropical soils.

Chapter five

The results obtained in chapter four were applied to soils from the Niger Delta, Nigeria, in this chapter.

In addition to the agents used in chapter four, another agent, the fern-*Drypoteris affinis* which was observed as the dominant resistant specie growing in petroleum-contaminated soils and swarms in the Niger Delta, Nigeria was added to the investigation.

This chapter further investigated for possible enhancement of the remediation efficiency of the agents. One of the ways of enhancing phyto- and myco-remediation (from the literature review) was the used of surface-active agents. Thus, Tween 80 which is readily available, cheap and biodegradable was added to the phyto- and myco-remediation to investigate for possible enhancements of TPHs remediation.

Hence, chapter five investigated the effect of Tween 80 on phyto- and myco-remediation agents applied on petroleum contaminated silty loam soil from the Niger Delta, Nigeria.

Chapter 6

Results obtained from chapters four and five revealed that optimal remediation was obtained with the mycoremediation agents palm wine, and *P.* ostreatus. Thus, it was now expedient to evaluate the application of the mycoremediation technique on different soil types of sandy, clay, and loamy; as well as sediments from the Niger Delta, Nigeria.

Therefore, chapter six deals with utilization of mycoremediation for the treatment of petroleum-contaminated soils and sediments from the Niger Delta, Nigeria.

Chapter 7

In chapter seven, the investigation is carried out to identify readily available methods for assessment of the petroleum-contaminated and remediated soils.

Two approaches are investigated in this chapter. Firstly, the used of the contaminating crude oil as analytical standard, and secondly the use of certain biomarkers as indicators of TPHs concentrations in soils.

Chapter 8

Chapter eight sums up the main finding of the thesis, limitations, practical considerations for application of the techniques and recommendations for further studies.

CHAPTER TWO

Literature review

2.1 Phytoremediation of petroleum-contaminated soils

Phytoremediation, the use of plants to clean up contaminated environments, has been a topic of interest for many years because of its anticipated benefits (Abou-Shanab *et al.,* 2019; Song *et al.,* 2019). The technique is environmentally friendly, cost-effective, and easier to operate and monitor (Salt *et al*., 1995; Burges *et al.,* 2018; Han *et al.,* 2018). This makes it preferable to other physical, chemical and biological techniques of soil remediation (Yavari *et al.,* 2015). However, there are certain factors which tend to limit the maximisation of this technique. These include long duration of the remediation process, problem of plants adaptability, and limited root length which confines its application to the upper soil profile (Ali *et al.,* 2013; Fasani *et al.,* 2018; Raman and Gnansounou, 2018).

Phytoremediation proceeds via different mechanisms (Siciliano & Germida, 1998; Huang *et al.,* 2005). Different categories of phytoremediation processes have also been identified (Figure 2.1). Pilon-Smits (2005) and Rascio and Navari-Izzo (2010) categorised phytoremediation of contaminated soils into phytoextraction, phytodegradation, phytostimulation, phytostabilisation, phytovolatilization, and phytodesalination. The entire process of phytoremediation in soils may be a combination of two or more of these mechanisms (Pilon-Smits, 2005).

One of the most important factors in phytoremediation is the ability of plants to survive and grow comfortably in the target-contaminated environment (Bernabé-Antonio *et al.,* 2018; Fatima *et al.,* 2018; and Feng *et al.,* 2018). Thus, identification and development of tolerant plant species and appropriate conditions for the plants' growth is essential for effective application of phytoremediation (Arthur *et al.,* 2000; Glick, 2003).

The application of phytoremediation to petroleum-contaminated soils is necessitated by the need to explore cheaper, locally available and environmentally friendly options for management of environmental issues arising from the petroleum sub-sector (Aisien *et al.,* 2015; Asghar *et al.,* 2015). Several investigations have been carried out to identify

ideal plant species and conditions for phytoremediation of petroleum-contaminated soils and will be discussed in section 2.1.1

Several factors that influence phytoremediation are also identified. These include type of contaminating crude oil, the concentration of the oil in the soil, plants type and adaptability to growing in the soil, climatic factors as well as edaphic variables such as physicochemical and nutrient contents of the soil (Aisien *et al.,* 2015; Sheoran *et al.,* 2016).

Many innovations have been introduced over time to improve the process of phytoremediation (Han *et al.,* 2018; Lin *et al.,* 2018; Muthusaravanan *et al.,* 2018; Nayak *et al.,* 2018). However, translating these advances to commercial applications especially in crude oil contaminated soils is yet to be realised. Most petroleum producing areas are associated with vast forest and abundant phyto- resources (Looney *et al.,* 1993; Ige, 2011). There is ultimately a need to explore the prospect of phytoremediation for the management of petroleum-contaminated soils.

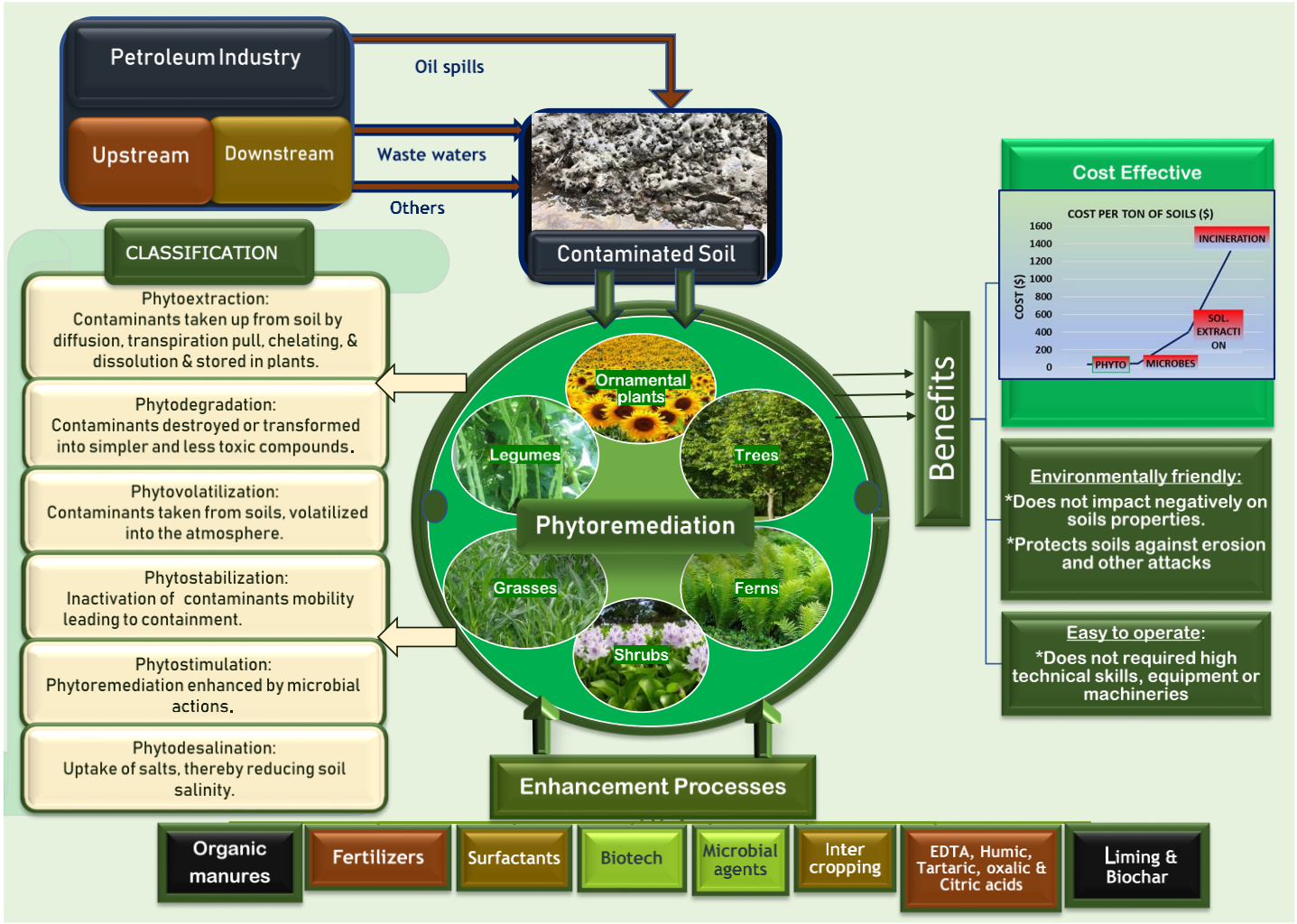


Figure 2.1: Phytoremediation processes on petroleum-contaminated soil

2.1.1 Plant types and families with phytoremediation potential on petroleum-contaminated soils

Several plant types and families including grasses, ornamental plants, legumes, shrubs, and trees have been utilised for phytoremediation of contaminated soils (Appendix V-1). Some commercial applications of phytoremediation on petroleum-contaminated soils have also been reported (Table 2.1).

Phytoremediation of petroleum-contaminated soils have been reported with grasses such as *Panicum virgatum, Festuca arundinacea, Eleusine indica, Brachiaria brizantha, Cyperus aggregatus, Lolium multiflorum, Cynodon dactylon, Lolium perenne, Spartina patens, Cyperus rotundus, Sorghum bicolor, Hordeum vulgare, Axonopus compressus, Leptochloa fusca, Brachiaria mutica, Triticum repens, Linum Usitatissumum, Zea mays,* and *Panicum maximum* (Saadawi *et al.*, 2015; Fatima *et al.*, 2018).

Wang *et al.* (2008) demonstrated that up to 3-4 times degradation of petroleum hydrocarbons can be achieved on petroleum-contaminated soil using grass plants such as *Panicum virgatum, Festuca arundinacea,* and *Eleusine indica* compared to controls. Glasshouse experiments were carried out using the agents for a period of 150 days on conventional petroleum-contaminated soils. The contaminated soils were mixed with pollutant-free soil to reduce the oil content from an initial concentration of 9,175 to 5,000 $mg \cdot kg^{-1}$. Merlk *et al.* (2005), reported that the remediation properties of certain grasses such as *Brachiaria brizantha, Eleusine indica* and *Cyperus aggregatus* extend beyond the treatment of total petroleum hydrocarbons to specific fractions such as saturates and aromatics. In a study, soil samples were artificially contaminated with 5% (w/w) of a heavy crude oil, and treated with the agents *Brachiaria brizantha, Eleusine indica* and *Cyperus aggregatus* for a period of 190 days in a glasshouse. Results obtained revealed up to 70% degradation of saturates fraction and approximately 15% higher reduction in aromatics than controls. This outcome tends to illustrate that phytoremediation with these grasses favours aliphatic, rather than the aromatics. White *et al.* (2006), however demonstrated that alkylated two-ring naphthalene were successfully degraded in insitu treatment of petroleum-contaminated sites with up to 9,175 mg/kg (TPHs) concentration over a period of one year using the grass plants- *Lolium multiflorum* and *Cynodon dactylon.* Also, an

increased degradation of the larger three-ring alkylated phenanthrenes-anthracenes and dibenzothiophenes were also observed compared to controls. These outcomes illustrated that phytoremediation of petroleum hydrocarbon groups may be plant related, and that grass plants have the potential to remediate either saturates, aromatic and polyaromatic fractions of petroleum contaminants in soils.

Lin and Mendelssohn (2008) investigated the effect of crude oil concentration on phytoremediation potential of grass plants using soils artificially contaminated with crude oil at concentrations of 0, 40, 80, 160, 320, 640 and 800 mg/g (w/w) of a crude oil per soil for a period of 8 months. The result revealed that phytoremediation efficiency of grasses depends on its tolerance limits, and that these plants can only operate successfully within the tolerance limits. For *the grass Spartina patens,* its tolerance limits was found to be 320 mg/g dry soil which is over 30% of crude oil contamination in the soil.

Shirazia *et al.* (2015) stated that the use of ornamental plants for phytoremediation of petroleum-contaminated soils could provide aesthetic appeals to the objectionable scenes associated with petroleum contamination. Ornamental plants that have been used for remediation of petroleum-contaminated soils include *Mirabilis Jalapa, Crotalaria pallida Aiton, Dracaena reflexa, Melampodium Paludosum, Echinacea purpurea, Gaillardia aristate, Matricaria chamomilla, Mimosa, Zinnia elegans, Gazania linearis, Ipomoea quamoclit, Bassia scoparia, Iris pseudacorus, Impatiens balsamina,* and *Canna generalis* (Boonsaner *et al.*, 2011; Ikeura *et al.*, 2016).

Ornamental plants have also been used for removal of petroleum organics including saturated hydrocarbons, aromatic hydrocarbons, asphaltene, and polar compounds in soils. Peng *et al.* (2009) demonstrated up to 63% TPHs in soils by the ornamental plant *Mirabilis Jalapa* in a glasshouse. In the study, highly contaminated conventional soils were first diluted with uncontaminated soils to a concentration (Woil/Wsoil) of 0.5% (5000 mg/kg), 1.0% (10,000 mg/kg), and 2.0% (20,000 mg/kg) before application of the agents. Baruah *et al.* (2016) however reported up to 78 % removal of TPHs at 60,000 ppm concentration of crude oil in soil by *Dracaena reflexa.* Dadrasnia and Agamuthu (2013), Shirazia *et al.* (2015) and Wang *et al.* (2016) all demonstrated that the removal rate of TPHs in soils by ornamental plants was dependent on the initial concentrations, with

higher efficiencies obtained in soils with lower crude oil contamination. Izinyon and Seghosime (2013) also established that the remediation efficiency of ornamental plants on contaminated soils is also a function of the duration of application. About 34 % reduction of TPHs were obtained by treatment of contaminated soils with *Melampodium Paludosum* after 2 weeks, compared to over 60 % obtained after 16 weeks. Boonsaner *et al.* (2011) further reported that the ornamental plant *Canna generalis* can removed up to 80% of BTEX and that these substances were concentrated in the root and rhizome of the plant.

Among the ferns, phytoremediation potential of water ferns on crude oil contaminated soil has been reported. Kösesakal *et al.* (2016) demonstrated that the degradation rate of total aliphatic and aromatic (phenathrene) hydrocarbons was up 94% and 81%, respectively, by the water fern *Azolla filiculoides*. The study also illustrated that the degradation rate is favoured at lower concentrations of the contaminants, and contaminants were degraded rather than extracted. There however seems to be no report on phytoremediation of terrestrial ferns on petroleum-contaminated soils.

Legumes such as *Glycine max, Calapoigonium mucunoides, Ricinus communis, Stylosanthes capitate, Centrosema brasilianum, Aeschynomene americana, Vicia faba, Arachis hypogea, Cajanus Cajan Lablab purpureus* have been employed for the remediation of petroleum-contaminated soils (Ibrahim *et al.*, 2013; Saadawi *et al.,* 2015). Ibrahim *et al.* (2013) demonstrated that legumes can effect up to 99.8% remediation of TPHs in soils in an insitu application, and like other plants, the phytoremediation efficiency of leguminous plants is also dependent on contaminants concentrations, decreasing with increasing contamination. Although leguminous plants especially cover crops offers additional advantages of nutrient replenishment and soils protection in addition to remediation efficiency (White *et al*., 2002), Njoku *et al.* (2009) established that phytoremediation potential of legumes can be inhibited at certain high concentration of crude oil in soils.

Shrubs and trees have also been used for remediation of TPHs, saturated, aromatics and polycyclic aromatic compounds in soils (Mathur *et al.,* 2010; Saadawi *et al.*, 2015). Saadawi *et al.* (2015) described up to 76% and 89% of TPHs removal by the shrubs *Ricinus communis* and *Malva parviflora,* respectively in soils. Shirdam *et al.* (2009) and Kitamura and Maranho (2016) demonstrated up to 65% and 67% reduction of TPHs in contaminated soils by the shrubs *Linum usitatissimum* and *Desmodium incanum*, respectively during a 90-days glasshouse study. Unlike other plants, trees have the advantage of reaching down to lower soil profile. El-Gendy *et al*. (2009) reported that the Poplar tree (*Populus nigra*) exhibited up to 81%, 90%, 67%, 78%, and 82%, decrease of toluene, ethylbenzene, xylene, and gasoline range organics respectively in lower soil profile during an insitu application. Dadrasnia and Agamuthu (2013) further established that the remediation efficiency of trees can be enhanced with addition of soil amendments. The study revealed up to 90% and 99% degradation of crude oil in contaminated soils amended with soy bean cake compared to 52% and 62%, observed in unamended soil using the tree *Dracaena reflexa*.

Table 2.1: Some in situ/large scale phytoremediation of crude oil contaminated soils.

Plants	**Sites**	**Sources**
Polar plants	Cabin Creek, West Virginia, USA	El-Gendy *et al.* (2009)
Rye grass (*Lolium perenne*) and White clover (*Trifolium repens*)	An industrial site in the Republic of Ireland	Germaine *et al.* (2015)
Rye grass	An oil-sludge pit on the Saratov Petroleum Refinery grounds, Russia.	Muratova *et al.*(2008),
Rye grass, Augustine grasses; Sorghum	Gulf Coast, USA	Flathman and Lanza (1998).
White clover, tall fescue, and *bermuda* grass)	U.S. Navy's Craney Island Fuel Terminal near Norfolk, Virginia, USA	Flathman and Lanza (1998).
Willow trees	An industrial site in Wisconsin, USA	Carman *et al.* (1998).
Tall wheat grass; Altai wild rye; Alfalfa	A weathered hydrocarbon flare-pit site in southeastern Saskatchewan, Canada.	Phillips *et al.* (2009)
Ryegrass (*Lolium perenne*); Tall fescue (*Festuca arundinacea, var. Inferno*); Barley (*Hordeum vulgare*)	A Southern Ontario site (~130 g kg^{-1} TPH) used for land farming of refinery hydrocarbon waste for many years.	Gurska *et al.* (2009)
Cyperus rotundus (Linn.) and *Cyperus Brevifolius* (*Rottb*.) Hassk. Fertilized and unfertilized treatments	hydrocarbon sludge contaminated soil in Duliajan, Assam (India) (initial TPH concentration of 65,000–75,000 mg.kg–1)	Basumatary *et al.* (2013)
Poplars	Limon, Colorado, USA with TPH - 1000 milligrams per liter (mg/L)	www.epareachit.org
Poplars	Tacoma, WA, USA.	www.epareachit.org
Hybrid poplar	Warren, OH, USA	Van Epps (2006)
Willows and poplars	Abandoned Gasoline Station; Axelved, Ronnede, Denmark with TPH - More than 20,000 mg/kg	Trapp *et al.* (2001)
Bermuda grass, Rye grass, White clover, Tall fescue	Craney Island Fuel Terminal, Portsmouth, Virginia, USA	Hutchinson *et al.* (2003)
Sorghum; Rye grass; St. Augustine grass	Crude Oil Spill Site, Southeast Texas, USA	Nedunuri *et al.* (2000)
Hybrid Poplar	New Gretna, New Jersey	Van Epps (2006)
Willows	Menen, Belgium	Lust (2003)

Hybrid willows	Stratford, Wisconsin, USA	Carman *et al.* (2000).
White and black willows, Wooly bull rush, Rush, Native sedge, Cattail	Georgia, United States	O'Niell and Nzengung (2004)
Poplars, Willows	Indiana Harbors Canal, Near Gary, Indiana, USA with TPH - 20,000 to 430,000 mg/kg (mean of 250,000 mg/kg) Total PAHs - mean of 4,100 mg/kg	Zalesny *et al.* (2005)
Annual Rye (Rye), Black Willow (Willow), Lake Sedge (Carex), Bull Rush (Scirpus), Natural Attenuation (NA), Prairie Cord Grass (Spartina)	Jones Island Confined Disposal Facility - Milwaukee, Wisconsin, USA	Van Epps (2006)
Hybrid poplar trees	Oneida Tie Yard Site, Oneida, Tennessee, USA	Widdowson *et al.* (2005)
Grasses: Big Bluestem, Little Bluestem, Bottlebrush Grass, Prairie Cordgrass Sedges: Sprengel Sedge, Bulrush Herbaceous: Leadplant, New England Aster, Pasture Thistle, Boneset, JoePyeWeed, Prairie Smoke, Cardinal Flower, Prairie-dock Shrubs: New Jersey Tea, Common Ninebark, Meadowsweet, Arrowhead Viburnum	Allen Park, Michigan, USA	Rugh *et al.* (2005)
Red mulberry trees, Bermuda grass	Privately Owned Scrap Yard, South-eastern United States. TPH - 10 to 14,800 mg/kg (average of 4,010 mg/kg); PCBs - 0.77 to 222 mg/kg (average of 65 mg/kg)	Hurt (2005)
White clover, boreal red fescue, Kentucky Bluegrass, annual rye, perennial Rye, willow, poplar, volunteer revegetation	Utica, New York, USA	Kulakow (2000).
Western wheatgrass, sweet clover, tall fescue, switch grass	RTDF Site G, Fort Riley, North Central Kansas, USA.	Kulakow (2006).
Rye, legume, fescue, Bermuda grass	RTDF Site J, El Dorado, Arkansas, USA with TPH - 3,000 to 24,000 mg/kg	Kulakow (2006).
Prairie buffalo grass and twelve warm season grasses	Union Carbide Seadrift Plant, Seadrift, Texas, USA.	Olson *et al.* (2003).

2.1.2 Application practices and trends in phytoremediation of petroleum-contaminated soils

Some of the practices involved in phytoremediation of petroleum-contaminated soils include trial and identification of plants with phytoremediation potential, monocropping, intercropping with two or more plant species and phytoremediation with microbial agents (An *et al.,* 2011; Sun *et al.,* 2011; Agnello *et al.,* 2016[a]). Others include use of enhancement agents such as humic acids, biochar, biofertilisers and surface-active agents (Caille *et al.,* 2004; Máthé-Gáspár, and Anton, 2005; Houben *et al.,* 2013; Paz-Ferreiro *et al.,* 2014; Agnello *et al.,* 2016[b]). The influence of compost and other soil amendments, liming, the use of organic and inorganic fertilisers as well as biotechnology on phytoremediation have also been reported (Mendez and Maier, 2008; Liu *et al.,* 2013).

2.1.2.1 Application of intercropping in phytoremediation of petroleum-contaminated soils

Both intra and inter-species intercropping have been utilised for phytoremediation of petroleum-contaminated soils (Li *et al.,* 2009; Ma *et al.,* 2012; Yanqun, *et al.,* 2017). These include using legumes-grasses, different ornamental species, grasses with ornamental plants, hyperaccumulators, and economic crops as well as trees and shrubs (Ma *et al.,* 2013; Ma *et al.,* 2016, Wiche *et al.,* 2016). Mohebi and Dialami (2011) demonstrated that intercropping date palms with alfalfa, corn and sunflower significantly increased the remediation of TPHs in soils compared to using individual plants. Other reports have shown that intercropping can further be enhanced with chemical agents, soil amendments, integration of other agronomic practices such as crop rotation as well as other biological agents (Marchiol and Fellet, 2011; Zhao *et al.,* 2011; Tan *et al.,* 2015; Ju *et al.*, 2015).

Several advantages of intercropping in the remediation of petroleum-contaminated soils have been postulated (Mohebi and Dialami, 2011; Ma *et al.,* 2012). This include improved nutrient balance, reduction in oxidative damage, increased enzymatic and antioxidant activity as well as increased chances of plants' survival (Fuksová *et al.,* 2009; Cui *et al.,* 2018; Luo and Tu, 2018). Although most studies tend to support the enhancing effects of intercropping in phytoremediation, Wieshammer *et al.* (2007) demonstrated that intercropping did not enhance total cadmium (Cd) extraction. It is therefore,

necessary to identify phytoremediation agents and the specific contaminants which they exhibit the remediation potentials.

2.1.2.2 Phytoremediation practices involving combinations with microorganisms

The integration of phytoremediation with microbial agents has been demonstrated to enhance efficiency. Asghar *et al.* (2017) established increased phytodegradation of petroleum hydrocarbons by bio-augmentation of *Zea mays* with the bacteria PM32Y. Agnello *et al.,* (2016b) reported an enhancement of the remediation potential of Alfalfa on petroleum-contaminated soils by bioaugmentation with *Pseudomonas aeruginosa.* Zhang *et al.,* (2010) demonstrated that plant-microbe remediation processes could further be enhanced by the addition of biosurfactants. Fungal species such as arbuscular mycorrhizal fungi and yeast have also been combined to enhance phytoremediation of petroleum-contaminated soils (Hassan *et al.,* 2013; Salam *et al.,* 2017). Schmidt *et al.* (2018) further demonstrated that the use of a consortium of bacteria and fungi on on petroleum-contaminated soils could result in higher decrease in TPHs than phytoremediation alone.

2.1.2.3 Phytoremediation practices involving combinations with enhancements agents e.g humic acids, bio surfactants, biochar, liming, organic fertilisers, inorganic fertilisers

Several soil amendments are known to improve phytoremediation efficiencies. This includes additions of EDTA, oxalic acid, humic acid, citric acid and tartaric acid (Fiorentino *et al.,* 2017; Yan *et al.,* 2017), liming and biochar (Lu *et al.,* 2015; Wu *et al.,* 2012), organic and inorganic manures (Park *et al.,* 2011) and surfactants (Liao *et al.,* 2016; Liduino *et al.,* 2018; Wei *et al.,* 2018). Chemically synthesised surfactants and biosurfactants improve phytoremediation by increasing the solubility and bioavailability of the hydrophobic petroleum contaminants (Pacwa-Płociniczak *et al.,* 2011; Liao *et al.,* 2016; Cheng *et al.,* 2018). Organic and inorganic fertilisers enrich the soil with the required nutrients (Adewole & Bulu, 2012; Xiu-Zhen *et al.,* 2011). According to Park *et al.* (2011), organic amendments also act as a conditioner, helping to improve the physical properties and fertility of soils and enhance contaminant bioavailability. Organic amendments also help to dilute highly contaminated soils to concentrations that sustains the growth of phytoremediation plants (Muratova *et al.,* 2018).

2.1.2.4 Application of biotechnology and engineering on phytoremediation of petroleum-contaminated soils.

Biotechnological and engineering strategies are also combined with phytoremediation for optimisation. Examples inlcude the use of transgenic plants and modifications of the molecular mechanism of phytoremediation agents resulting in enhancements of transportation and degradation of contaminants (Agnihotri & Seth, 2019; Ahmed *et al.,* 2019; Kaur *et al.,* 2019). Doty (2000) reported the development of transgenic poplars (*Populus spp*.) by overexpressing a mammalian cytochrome P450, a set of enzymes commonly involved in the metabolism of toxic compounds. This boosted the plant's ability to metabolise trichloroethylene and clean up other organics such as benzene, vinyl chloride, chloroform and carbon tetrachloride. Stepanova *et al.* (2016) demonstrated an improved phytoremediation of petroleum-contaminated soils using transgenic alfalfa plants (*Medicago sativa*). Higher degradation rates were further obtained when the plant was integrated with the fungi *Candida maltose*. Other reports on enhancing phytoremediation using transgenic plants include Ruiz *et al.* (2011), Song *et al.* (2003) and Bennett *et al.* (2003).

2.2 Mycoremediation of petroleum-contaminated soil

Mycoremediation offers an alternative environmentally friendly technique for remediation of contamination in environmental matrices (Baldrian *et al.,* 2000; Stamets, 2005; Acevedo *et al.,* 2011; Thakur, 2014). It entails the use of fungi and has been applied to both soil and water (Kulshreshtha *et al.*, 2014; Anderson and Juday, 2016). The technique has several advantages over other methods of bioremediation. Apart from cost and technical ease, fungi are found in many parts of the world, which could afford a wide-spread application in different regions (Loske *et al.,* 1990; Khan *et al.,* 2004). Leonardi *et al.* (2007) and Rahman *et al.* (2013) stated that mycoremediation seems to be the safest means of soil remediation in terms of ecological impact and human health. This is because most organic contaminants are degraded rather than extracted which reduces the risk of bioaccumulation and transfer of pollutants into the food chain (Hammel, 1989; Flouri *et al.,* 1995; Eggen and Majcherczyk, 1998; Haritash and Kaushik, 2009). According to Asamudo *et al.* (2005) and Adenipekun and Lawal (2012), mycoremediation is unique even among other biological techniques such as bacterial, because there is no requirement for pre-conditioning to a particular pollutant. Asamudo

et al. (2005) further stated that in mycoremediation, the efficiency is not also limited to specific pollutant concentrations. It is, therefore, necessary to examine why the technique of mycoremediation has not been maximised for clean-up of petroleum-contaminated soils, and possible solutions to the challenges that may arise.

2.2.1 Factors affecting mycoremediation efficiency

The efficiency of mycoremediation is affected by factors such as temperature, sunlight, oxygen level, nutrients and moisture content (Bhattacharya *et al.,* 2012). It has been demonstrated that mycoremediation is optimal at temperatures of 25-30^{0}C (Hoa *et al.,* 2015). Aguilarivera *et al.* (2012) reported that 70% relative humidity is ideal for mycoremediation with *P. ostreatus*. According to Brady and Weil (2007) a carbon-nitrogen ratio of 10 in soil is optimal. Nutrient requirement is usually maintained using both organic and inorganic manures (Hoa *et al.,* 2015). Gueren (2000) demonstrated that a combination of mycoremediation with compost resulted in up to 50% increase in the remediation efficiency of PAHs. The addition of compost also aids in temperature optimization (Anderson and Juday, 2016). Amjad *et al.* (2017) further listed factors affecting the efficiency of mycoremediation to include environmental and genetic factors, e.g. pH, ecology, type of substrate, enzyme type and mycelium age. Das and Chandran (2011) reported that nutrients such as nitrogen and phosphorus could be the limiting factors. The fungal biomass content, length of remediation process and type of substrates as well as mobilizing agents are also known to affect the efficiency of mycoremediation (Kapahi and Sachdeva, 2017). Other factors include life cycle of fungi agents, fungal species, soil geochemistry as well as surface active and chelating agents (Bamforth and Singleton, 2005; D'Annibale *et al.,* 2013).

2.2.2 Fungal types used for mycoremediation of petroleum-contaminated soils

Different species of fungi have been demonstrated for the remediation of petroleum-contaminated soils (Table 2.2). These include microfungi such as arbuscular mycorrhiza, yeast (Sood *et al.,* 2010; Kumari and Abraham, 2011; Abioye *et al.,* 2013; Xie, and Qin, 2014), as well as penicillium and *Aspergillus species* (Al-Nasrawi, 2012; El Hanafy *et al.,* 2015). Mycoremediation with macro fungi (mushrooms) is also known (Adenipekun and Lawal, 2013; Rhodes, 2014).

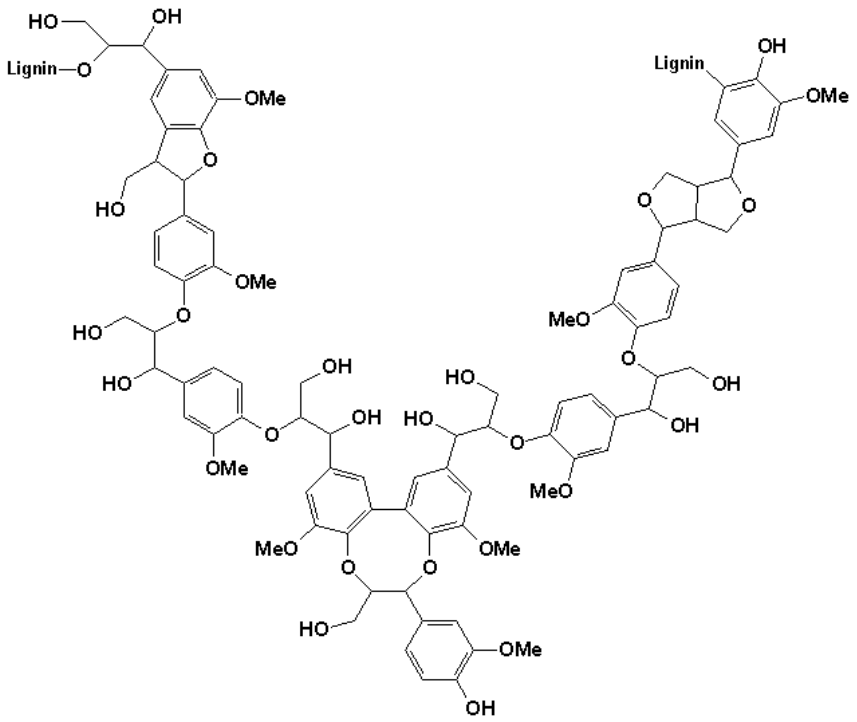
One significant class of fungi with demonstrated mycoremediation potential on petroleum-contaminated soils are the ligninolytic fungi such as white rot fungi (Isikhuemhen *et al.,* 2003; Gao *et al.,* 2010; Fan *et al.,* 2013). Lebo *et al.* (1991), Fetzer (2000) and Gargulak and McNally (2001) stated that the ability of white rot fungi to degrade most recalcitrant organic pollutants stems from the fact that these mushrooms naturally feed on and degrade lignin, a substance with similar monomeric unit to most recalcitrant organic contaminants (Figure 2.2).

Mycoremediation potential in fungi other than white rot have also been reported. This includes brown rot fungi such as *Ganoderma species*; edible (button) mushroom such as *Agaricus species* (which grows naturally on soils) (Cerniglia, and Perry, 1973; Davies and Westlake, 1979; Prenafeta-Boldu *et al.,* 2000; D'Annibale, *et al.,* 2006).

2.2.3 Mechanism of mycoremediation

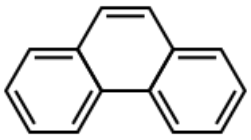
The mechanism of fungal degradation of organic contaminants in soils is presently thought to follow a similar mechanism for degradation of lignin (Barr and Aust, 1994; Novotny *et al.,* 2004; Das, and Chandran, 2011). Several mechanisms have been proposed including both direct and indirect oxidation of the organic molecule by the fungal enzymes namely Lignin-peroxidase (LiP), Manganese peroxidase (MnP) (Figure 2.3), Versatile peroxidase (VP) and Lacasses (Have and Teunissen, 2001; Christian *et al.,* 2005; Górska *et al.,* 2014). Hatakka (1994) suggested a possible combination of two or more enzyme mechanisms in the degradation process. Hofrichter (2002) proposed a radical-mediated reaction initiated by manganese peroxidase (MnP). This involves indirect oxidation of aromatic (phenolic) rings (ether peroxide formation), spontaneous ring opening to produce muconic acid derivatives and decarboxylation of the formed carboxyl groups to carbon dioxide (Figure 2.3). This mechanism does not necessarily produce small fragments since the aromatic rings are gradually degraded extracellularly (Das and Chandran, 2011).

A small segment of lignin polymer

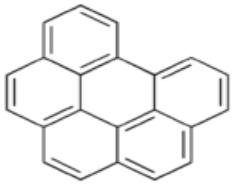


Some Polyaromatic Hydrocarbons contaminants in soils

Phenanthrene



Benzo[*ghi*]perylene



Triphenylene

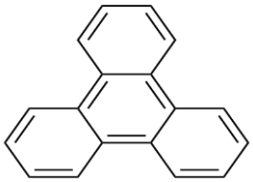


Figure 2.2: Structures of lignin polymer fragment and some petroleum contaminants. This illustrate the similarities in the structure of lignin and other recalcitrant contaminants in petroleum Thus, the ability of lignin degrading fungi to also degrade them (Barr and Aust, 1994; Novotny *et al.,* 2004)

Table 2.2: Mycoremediation of petroleum-contaminated soils

S/N	STUDIES	FUNGI SPECIES	EXPERIMENT DESIGN	CLIMATIC REGION	OUTCOME
1.	Adenipekun *et al.* (2015).	1. *Pleurotus pulmonarius* 2. *Pleurotus ostreatus* (All macroscopic)	1. Laboratory /glasshouse Scale: 2 months 2. Artificially contaminated soils (composition: 0, 10, 20 or 30%) with spent cutting fluid-SCF and fresh cutting fluids-FCF 3. Target contaminants: 16 priority PAHs 4. Solvent for extraction: Hexane, Dichloromethane (3:1). 5. Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ Overall range of PAHs degradation by *P. pulmonarius* inoculated on FCF contaminated soil was 17.3 to 27.3%, while for *P. ostreatus* inoculated soil was 69.0 to 99.07% at different contamination levels. ➢ Overall PAHs degradation for *P. pulmonarius* and *P. ostreatus* inoculated on SCF ranged from 27.4 to 57.4% and from 37.8 to 45.2%, respectively.
2.	Nicholas (2015)	1. *Heterobasidion annosum* 2. *Resinicium bicolor* (All macroscopic)	1. Laboratory /glasshouse Scale: 36 days 2. Artificially contaminated soils with diesel (3.5 and 7.0%) 3. Target contaminants: TPHs 4. Solvent for extraction: Dichloromethane 5. Method of application: prepared mushroom spawn (rice grain colonised with fungi- substrates) were transferred to and mixed with soil	Temperate Climate	➢ ANOVA showed significant decrease in TPHs over time.
3.	Young *et al.* (2015)	1. *Irpex lacteus* 2. *Trichaptum biforme* 3. *Phlebia radiata* 4. *Trametes versicolor* 5. *Pleurotus ostreatus* (All macroscopic)	1. Laboratory /glasshouse Scale: 180 days 2. Artificially contaminated soil samples were used 3. Principal substrate: white pine (*Pinus strobus*) sawdust was used for *Irpex lacteus* while others were grown on wheat bran and sawdust, which was properly moistened. 4. 20 g of Bunker C oil was added to each substrate, which were then maintained at 27^0C for 180 days 5. Target contaminants: TPHs and PAHs 6. Solvent for extraction: Methylene Chloride	Temperate Climate	➢ Average degradation efficiency between C10 and C14 alkanes was observed to be 98.1% and 48.6%, respectively after 180 days. ➢ Highest efficiency was obtained for P. ostreatus against Phenanthrene (94.9%) after 180 days
4.	El Hanafy *et al.* (2015)	1. *Aspergillus niger* 2. *Penicillium commune* (all microscopic)	1. Laboratory /glasshouse Scale: 2 weeks 2. Crude oil samples were used 3. Germinating fungi pre-cultured for one week were transferred to 100ml of Bushnell Haas media then to 500ml conical flask containing 1% crude oil, 0.1% V/V Tween 80 and 0.016 mg/ml of redox oxidation. The flask was incubated for 2 weeks before assessment.	Temperate climate	➢ *Aspergillus niger* degraded 54% of crude oil, while ➢ *Penicillium commune* degraded 48%
5.	Flayyih and Al-Jawhari (2014).	1. *Aspergillus niger,* 2. *Aspergillus fumigatus,* 3. *Fusarium solani* 4. *PenicIllium funiculosum* (all microscopic)	1. Laboratory /glasshouse Scale: 28 days 2. Artificially contaminated soil samples with crude oil (2% w/w) were used 3. Target contaminant: TPHs 4. Extracting solvent for TPHs: Dichloromethane	Temperate climate	➢ Highlighted that time is factor on remediation efficiency ➢ Highest remediation efficiency was 95% with *A. niger* after 28 days of treatment. ➢ Highest remediation efficiency by mixed cultures of fungi were 90% with A. niger and A. fumigatus.

S/N	STUDIES	FUNGI SPECIES	EXPERIMENT DESIGN	CLIMATIC REGION	OUTCOME
6.	Fana *et al.* (2014).	Yeast- *Candida tropicalis SK2* (all microscopic)	1. Laboratory /glasshouse Scale:180days 2. Naturally contaminated soil samples with crude oil 3. The Candida tropicalis SK21 was inoculated into the soil to reach a density of 1.0×106 CFU/g. 4. Target contaminant: TPHs and PAHs Extracting solvent for TPHs: Dichloromethane	Temperate climate	➢ Microbial enumeration showed that the yeast SK21 could grow well in the contaminated soil ➢ Yeast removed 83% of TPH in 180 days ➢ 815 of PAHs were removed by the fungi during the period of 180days
7.	Rahman, *et al.* (2013)	Oyster mushroom (macroscopic)	1. Laboratory /glasshouse Scale: 31 days 2. Artificially contaminated soil samples with crude oil were used 3. Layer of oyster mushrooms substrate were equally distributed on top of the soil and gently compacted	Temperate climate	➢ Fruit bodies of mushroom was found 7 days after inoculation ➢ 35% of TPH was removed
8.	Abioye *et al.* (2013)	Yeast-*Saccharomyces cerevisiae* (microscopic)	1. Laboratory /glasshouse Scale: 28 days 2. crude oil samples (3.5 and 7.0%) 3. Yeast was isolated from Zobo drink and developed on Sabauroud dextrose agar by spread plate method incubated at 25^0C for 48 hours. 4. Cultured yeasts were then inoculated on a 50ml Mineral salt medium containing 1g of crude oil and maintained at 30^0C for 28 days 5. Target contaminants: TPHs 6. Solvent for extraction: Dimethyl ether	Tropical climate	➢ Degradation activities increased with days ➢ 49.29% of crude oil degradation was achieved after 28 days.
9.	Al-Nasrawi (2013).	1. *Aspergillus niger* 2. *Penicillium documbens,* 3. *Cochliobolus lutanus* 4. *Fusarium solani.* (all microscopic)	1. Laboratory /glasshouse Scale: 21 days 2. Naturally contaminated soils taken from 0-15cm of contaminated sites were used 3. Prepared fungi on nutrient medium were used to inoculates soils and maintained at 4. Extracting solvent: acetone and dichloromethane (DCM)-1:1 5. Target contaminants: PAHs Method of application: Layering growing substrates and active spawn on top of soil	Temperate climate	➢ Highest degradation was recorded for *Pennicillin documbens* at 21 days.
10.	Edema *et al.* (2011)	*Basidiomycetes* (macroscopic)	1. Laboratory /glasshouse Scale: 4 weeks 2. Artificially contaminated soils (soils thoroughly mixed with crude oil 1l/5.0 Kg). 3. Extracting solvent: acetone and dichloromethane (DCM)-1:1 4. Target contaminants: PAHs Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ 98.93% PAHs reduction was achieved

S/N	STUDIES	FUNGI SPECIES	EXPERIMENT DESIGN	CLIMATIC REGION	OUTCOME
11.	Kristanti *et al.* (2011)	*Polyporus sp* (macroscopic)	1. Laboratory /glasshouse Scale: 60 days 2. Artificially contaminated soils with crude oil (3000 mg) 3. Extracting solvent: hexane, dichloromethane (DCM) and chloroform successively 4. Target contaminants: TPHs Method of application: wood meal pre-grown fungi were applied to contaminated soil surface, then mixed thoroughly	Temperate climate	➢ highest degradation rate of crude oil was 93% in the soil after 60 days
12.	Adenipekun and Lawal (2011)	*Pleurotus pulmonarius* (macroscopic)	1. Laboratory /glasshouse Scale: 2 months 2. Artificially contaminated soils with Crude oil and Palm kernel oil (0- 40%). 3. Target contaminants : Total Petroleum Hydrocarbons (TPHs) Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ Trace metal contents decreased during treatment ➢ There was a 40.80% degradation of TPHs at 1% crude oil concentration and 9.28% at 40% after 2 months.
13.	Adenipekun and Fasidi (2005).	*Lentinus subnudus* (macroscopic)	4. Laboratory /glasshouse Scale: 3-6 months 5. Artificially contaminated soils (soils thoroughly mixed with crude oil concentrations (0, 1, 2.5, 5, 10, 20 and 40%). 6. Target contaminants : Total Petroleum Hydrocarbons (TPHs) 7. Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ Total Petroleum Hydrocarbon decrease were 33.04%, 56.67%, 14.85%, 25.27%, 22.57% and 15.25% respectively for each concentration after 3months, and ➢ 60.60%, 78.25%, 85.64%, 89.54%, 95.12% and 95.12% respectively after 6months
14.	Stamets (2005)	*Pleurotus ostreatus* (macroscopic)	1. Large Scale: 8 weeks 2. Naturally contaminated soils of diesel and oils approximately 20,000 parts per million of Total Aromatic Hydrocarbons 3. 4 piles of contaminated soils where place on a large sheets of 6mm black polythene tarps. Each pile measured about 4 X 20 X 8 feet in width 4. A corresponding 30 % of sawdust spawns were mixed to contaminated soils. 5. Spawn where placed in layers between contaminated soils in a parallel sheet spawning 6.	Temperate Climate	➢ About 99% of TPHs were degraded after 8 weeks
15.	Isikhuemhen *et al.* (2003)	*Pleurotus tuberregium* (macroscopic)	1. Laboratory /glasshouse Scale: 30 days 2. Artificially contaminated soils (1, 3, 5, 10 and 15%, w/w) of crude oil 3. Target contaminants: TPHs 8. Solvent for extraction: Xylene Method of application: mixing contaminated soils with substrates, then inoculation active spawn (25% w/w)	Tropical climate	➢ There was 85% reduction in TPHs after 30days

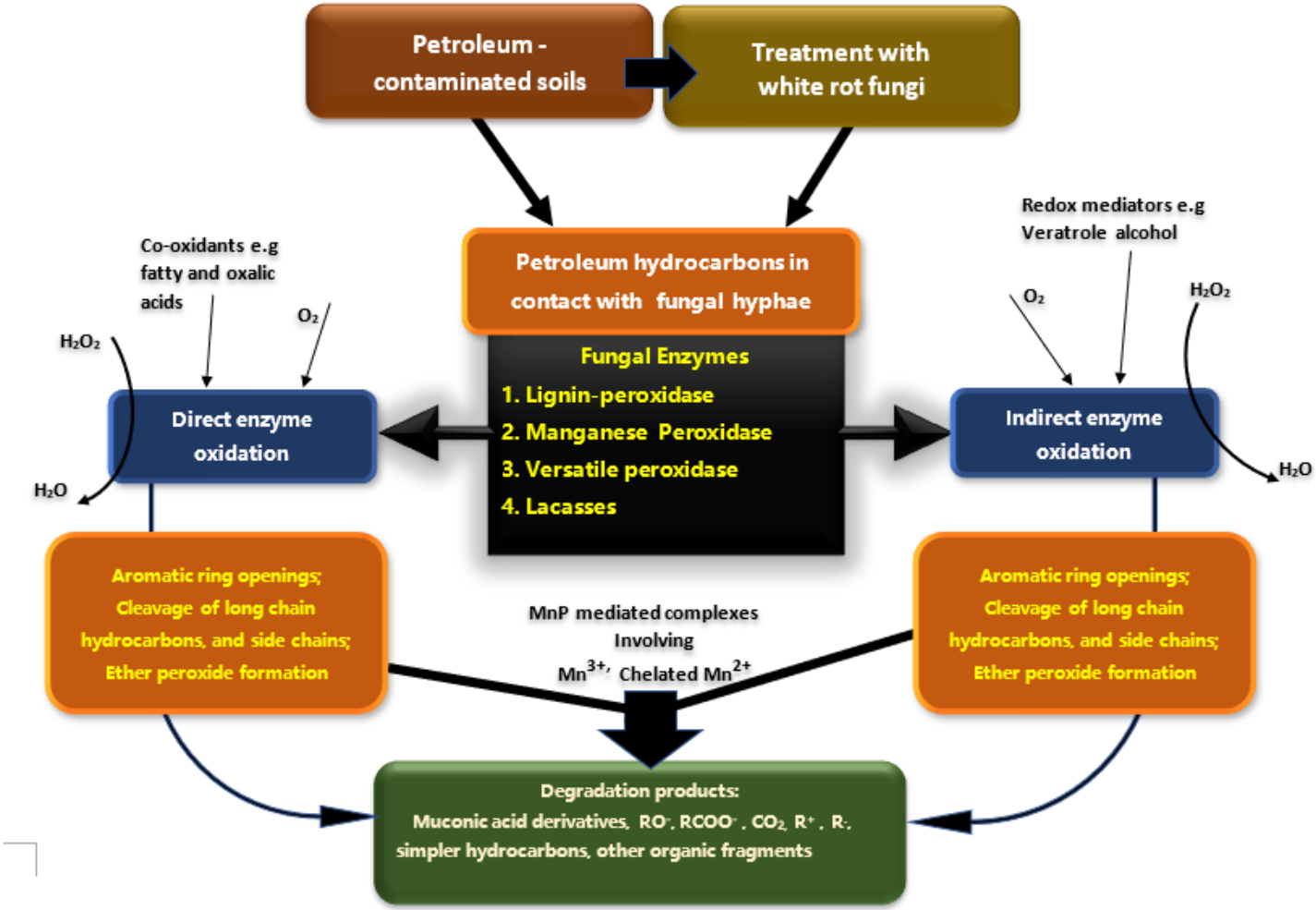


Figure 2.3: Mechanism of mycoremediation of petroleum-contaminated soil by white rot fungi

2.2.4 Application procedures for mycoremediation on contaminated soils

The general procedure for application of white rot fungi to contaminated soils is by layering (Sasek, 2003; Elisashavili *et al.,* 2008; Singh *et al.,* 2011). This has been carried out by layering actively growing substrates on topsoil or by way of vertical and horizontal sandwiching of active fungal substrates between contaminated soils (Stamets, 2005; Adenipekun *et al.,* 2015). The actively growing fungal substrates may be pre-developed to a level where mycelia are actively sprouting before inoculation of soils, or spawns may be inoculated directly on substrates layered on soils (Bhatt *et al.,* 2000; Adenipekun and Fasidi, 2005).

Adenipekun *et al*. (2015), described a procedure whereby 400 g of soil was artificially contaminated with 0-30% crude oil and placed in sterile 350 ml bottles. 80 g of moistened rice straw were then laid on these soils, and after sterilization and cooling, 10 g of actively growing mushrooms spawns were inoculated on the samples. In Bhatt *et al.* (2000), 250 g of contaminated soil was placed between two layers of rice straw colonized with fungal mycelium (i.e. 50 g of the fungal mycelium on top and 50 g at bottom of the soil). Matsubara *et al.* (2005) reported that instead of layering, contaminated soils could also be mixed with substrates followed by inoculation of fungal spawns. In a study, 450 g of sawdust was mixed with 550 g of contaminated soils, then previously prepared spawns were inoculated into the mix (Matsubara *et al.,* 2005).

For microscopic fungi, these are often prepared first on their respective growth media before inoculation on soils (Al-Nasrawi, 2013; Flayyih and Al-Jawhari, 2014).

2.2.5 Substrates for mycoremediation of contaminated soils

Substrates that have been used for growing white-rot fungi include rice straw, cotton waste, wheat bran, rice bran, shredded straw, corn cobs, soybeans flour, pasteurised/fermented/fresh cereal straw, pine barks, fragmented woods (sawdust), and straw bales, sugar beet pulps and coffee pulps (Rolz *et al.,* 1988; Zadrazil and Reiniger, 1998). These substrates could also be enriched with animal manures in different proportions for effective growth (Obire *et al.,* 2005; Nwogu *et al.,* 2015).

The substrates are often sterilised in autoclaves before used for inoculation of fungal spawns (Stamet, 2005; Adenipekun *et al.,* 2011). Adenipekun *et al*. (2015) reported that

sterilization of fungal substrates reduces competition by other microbial organisms which could inhibit the growth of the mushrooms.

2.2.6 Advances on mycoremediation of petroleum-contaminated soils

Studies on mycoremediation have evolved from the direct use of fungi to that of fungal-derived enzymes as well as spent fungal substrates (Giraud *et al.,* 2001; Sasek, 2003; Elisashavili *et al.,* 2008; Singh *et al.,* 2011). These involve both in situ and ex-situ studies, and there are some large-scale applications (Zhou, *et al.,* 2007; Thomas *et al.,* 2011; Zebulun *et al.,* 2011). There are also reports on mycoremediation in both temperate and tropical regions (Table 2.3), as well as developed and developing countries (Stamets, 2005; Edema *et al.,* 2010; Anasonye *et al.*, 2014; Rhodes, 2014; Winquist, *et al.,* 2014; Adenipekun *et al.,* 2015). There are also reports that combination of fungi and bacteria can enhance the efficiency of mycoremediation (Li *et al*., 2008; Chen *et al.,* 2009).

Studies such as Aranda *et al.* (2010), Hirantsuka (2005) and Sack *et al.* (1997) have shown that fungal enzyme extracts could degrade organic contaminants in soils. Anasonye *et al.* (2014) however, reported that MnP-enzymes extracts of the fungus *Kuehneromyces mutabilis* could not replicate the activity exhibited by the fungi itself on contaminated soils. These observations illustrate that a combination of more enzymes systems and other physiological processes are involved during mycoremediation. Winquist *et al.* (2014, demonstrated that laboratory outcomes of mycoremediation could be applied in the field. Okparanma *et al.* (2011), Zitte *et al.* (2012) and Albert and Anyanwu (2016) have also shown that spent mushroom substrates can be used for mycoremediation of petroleum contaminated soils.

2.3 Techniques for monitoring and analysis of petroleum-contaminated soils

2.3.1 Methods for sampling of contaminated soils

Several protocols, techniques and instruments are available for sampling of petroleum-contaminated soils (Table 2.3). The collection of soil samples requires several visits to site to characterise the nature of the pollution problem (ISO/DIS 18400-203, 2015E).

Table 2.3: Methods of sampling and handling of soil samples.

Criteria	Protocol	Description
Soil sampling	USEPA SESDPROC-300-R3 (2014)	Operating procedure for soil sampling,
	AWE (2009)	Sampling of contaminated land
	ISO/DIS 18400-203 (2015E)	Soil quality sampling - investigation of potentially contaminated sites
	ISO/DIS 18400-202 (2015E)	Soil quality sampling preliminary investigations
	BS EN ISO 25177 (2011)	Soil quality — field soil description, ISO/DIS
	18400-107 (2014E)	Soil quality sampling – recording and reporting,
Storage of soil samples	BS ISO 18512 (2007)	Guidance on long- and short-term storage of soil samples.
Pre-treatment of soil samples	BS ISO 11464(2006)	Pre-treatment of soil samples for physicochemical analysis,
	ISO 23909 (2008E)	Soil quality —preparation of laboratory samples from large sample,

2.3.2 Methods of extraction of petroleum-contaminated soils

Petroleum organics which include TPHs and PAHs can be extracted from soil matrices using a variety of methods. This includes soaking, soxhlet extraction, automated soxhlet, ultrasonic extraction, pressurised fluid extraction (PFE), accelerated solvent extraction, super critical fluid extraction (SCFE), and microwave assisted extraction (MAE) (USEPA, METHOD 3500C, 2007; Coulon and Wu, 2014; Vane *et al.,* 2014). According to Adeniji *et al.* (2017), each method exhibits peculiar extraction efficiency depending on the sample and solvent matrix. Typical solvents for extraction are methylene chloride, hexane, heptane or dichloromethane (Vane *et al.,* 2011). Most current procedures utilise 1:1 mixture of Dichloromethane- acetone; or acetone and either of hexane or heptane (Weisman, 1998; Richter, 2000; Al-Doury, 2019).

Soxhlet apparatus was traditionally the method of extraction of petroleum organics with reported high recovery rates (Raza *et al.,* 2018). Anyakora *et al.* (2005) demonstrated between 91% to 118% recovery of PAHs using the soxhlet method. A major limitation of the soxhlet method is that the technique is time-consuming with typical extraction time of up to 24 hours (Lau *et al.,* 2010; Raza *et al.,* 2018). Adeniji *et al.* (2017) reported the use of mechanical shaking with suitable solvents to extract petroleum hydrocarbons. Schwab *et al.* (1999) demonstrated that the shaking method could give TPHs concentrations equal to or greater than that obtained with the soxhlet method. The

shortcoming of these methods is that it is labour intensive and impractical when dealing with large number of samples. USEPA Method SW-846 3550C (2007) describes an ultrasonic extraction method for semi-volatiles including TPHs and PAHs in soil matrices. This method is faster than soxhlet extraction and uses lower volume of solvents. However, it requires multiple extractions, decanting, and filtration for every sample processed and is therefore labour-intensive (Majid *et al.,* 2015).

Supercritical fluid extraction, accelerated solvent extraction and microwave assisted extraction offer a faster and more economically efficient method of extraction of TPHs in soils in terms of solvent utilization and extraction time as compared to the alternative solvent extraction (Wang & Weller 2006; Antunes *et al.,* 2019). The issue with these methods is the cost of the equipment and associated parts in events of a break-down. According to Prevot *et al.* (2001), microwave assisted extraction offers an excellent way for the extraction of multiple samples. Typically, it utilises a solvent ratio of 1:1 acetone-hexane or heptane mixture to a homogenised soil matrix. Saari *et al.* (2007) demonstrated the efficiency of the 3 methods MAE, Soxhlet and CEN shake extraction for the extraction of TPHs in soil matrices to be in the order 99% for MAE, 80% for Soxhlet and 72% for CEN shake extraction. The reproducibility of MAE was also better when compared to the other two methods with a relative standard deviation of 3% compared to Soxhlet (5%) and CEN shake (11%).

2.3.3 Instrumental methods of analysis of petroleum-contaminated soils

Instrumental methods for analysis of TPHs, PAHs and other petroleum organic contaminants in soils include immunoassay (IMA), gravimetry, infrared spectroscopy (IR), raman spectroscopy, fluorescence spectroscopy, Iatroscan technique, HPLC techniques and gas chromatography with detectors such as FID and MS (Okparanma and Mouazen, 2013; Adeniji *et al.,* 2017). Generally, instruments and methods of choice depend on instrument availability as well as the targeted aim of the analysis. For instance, although GC-based methods are broadly useful for different kinds of petroleum organics, the methods are most suitable for analysis of nonpolar hydrocarbons. The typical ranges for GC are those with carbon numbers between C6 and C25 or C36 (TPHCWG, 1998). Gravimetric, IR or Iatroscan methods are often preferred for very heavy fractions of petroleum, such as molecules found in lube oils with more than 40 carbon atoms, which are difficult to detect by GC (TPHCWG, 1998).

Targeted analysis may be in terms of Total Petroleum Hydrocarbon (TPHs), Petroleum Group Type (PGT), or Petroleum Constituent (PC) measurement (TPHCWG, 1998). TPHs measurements are carried out for determination of the overall amount of petroleum hydrocarbons present in an environmental matrix. Such determinations can be performed with gas chromatography (GC), infrared spectrometry (IR), gravimetric analysis, Iatroscan and immunoassay (Paíga *et al.,* 2012). Petroleum group type measurement is carried out for determination of the quantity of the various classes of petroleum compounds such as saturates, aromatics, and polar or resins that are present in a contaminated sample (Shi *et al.,* 2010). Techniques used for the petroleum group type test include high-performance liquid chromatography (HPLC), multidimensional gas chromatography, and thin layer chromatography (TLC) (TPHCWG, 1998; Barman *et al.*, 2000).

Petroleum constituent measurement is usually used to analyse for individual compounds. This helps for the detection of individual components and their concentrations in a sample. This is particularly very useful for human health risk assessment (TPHCWG, 1998). Techniques for measurement of petroleum constituent include gas chromatography with second column confirmation, gas chromatography with multiple selective detectors and gas chromatography with mass spectrometry detection (GC/MS). (TPHCWG, 1998; Frysinger *et al.,* 1999).

Gravimetric methods involve the use of a weight difference technique after extraction of the petroleum contaminants from the soils (Kawahara, 1994). Though the gravimetric method would seem quick, easy and inexpensive method, the long-time involved for complete solvent evaporation, increases the costs of the process. The method is therefore not readily available for quick analysis and could be laborious and unsuitable for large-scale samples. Gravimetric methods for the determination of TPHs in soils are also non-specific and do not indicate the types of compounds present. This limits any assessment on the toxicity of the polluting compounds present (Mathew, 2009). Stenstrom and Silverman (1986) stated that gravimetric methods are more applicable to samples with a high concentration of analytes and are impracticable for measurement of low concentrations especially of the very toxic components such as PAHs.

IR analysis involves measurement of absorbance of the extracts at a specific wave number and comparing such with that of a calibration standard with a known TPHs concentration. This method is very swift, simple and inexpensive and was previously listed as an official TPHs screening method by the USEPA. The method also has the advantage to distinguish different classes of hydrocarbon in the gasoline range organics (GRO), oil and grease (OG), or total petroleum hydrocarbons (TPHs) (Stenstrom and Silverman, 1986). Falkova *et al.* (2016) demonstrated that IR methods could be automated for onsite determination of petroleum contaminants. The method is also applied to identifying the sources of petroleum (Lynch and Brown, 1973). Abdulkadir *et al.* (2016) reported that the use of Fourier Transform Infrared (FTIR) in the determination of petroleum contaminants could give results comparable to those obtained in GC analysis.

However, the limitations of the IR method include non-specificity to petroleum hydrocarbons and inadequate information on the type of hydrocarbons present (Lambert *et al.,* 2001; Strother *et al.,* 2013). The frequency of use of IR methods had decreased massively recently due to the ban on the commonly used eluting solvent Freon (1, 1, 2-trichlorotrifluoroethane), due to its harmful effects on the ozone layer (Strother *et al.,* 2013). The availability of portable spectrophotometers, recent innovations such as application of near- and mid-infrared (NIR and MIR) spectroscopy and the introduction of systems with direct sample applications has helped to bring back the popularity of the IR method (Forrester *et al.,* 2010).

The Iatroscan instrument is a rapid and inexpensive way to determine the saturates, aromatics, resins, and asphaltenes fractions in crude oils and bitumen extracts. It combines the thin-layer chromatography (TLC) resolution efficiency with the quantification capacity of detectors such as the flame ionisation detector or flame photometric detector (Rezaee *et al.,* 2019). The instrument uses chromorods (Quartz rods coated with Silica), to perform like columns/TLC plates and uses flame to burn the separated samples for final analysis on detectors (FID and FPD). The system was developed for the analysis of organic compounds, which show no UV-absorption and no fluorescence. Using the Iatroscan, a direct detection can be made for a large variety of organic compounds especially those with higher boiling point which are difficult to

analyse by GC, and those that are problematic to detect with LC (Karlsen & Larter, 1991). Data obtained with the Iatroscan, can be more reproducible because the volatile components are not separated and are measured as a part of saturates or aromatic fractions, and the process automation significantly reduces human error. Associating Iatroscan with FPD allows for simultaneous determination of inorganic compounds such as Phosphorous and Sulphur on the system (Jiang *et al.,* 2008). The analysis with Iatroscan is also cost effective in terms of equipment maintenance and price, because of the low consumption of solvents and reusability of the Chromarods. The equipment is also easy to set up and maintain in the laboratory. There are several limitation on the use of the Iatroscan in petroleum analysis. For instances, relatively high amounts of polar compounds are retained near the spot location of the TLC rods, potentially causing both separation and quantitation problems during the analysis of heavy oils and tar sands. Also, heteroatoms in polar fractions cause different FID response factors, further complicating the quantitation of the saturated and aromatic fractions (Bissada *et al.,* 2016).

Gas Chromatographic (GC)-based methods provide the best option for the determination of TPHs and other petroleum organic compounds in environmental matrices. The methods provide sensitivity and selectivity and can be used for the detection of a wide range of petroleum hydrocarbons. GC-methods are suitable both for the identification and quantification of petroleum hydrocarbons and can measure as low as 0.5 mg/L of TPHs in water or 1.0 mg/kg in soil (TPHCWG, 1998). There are several protocols by the USEPA for GC determination of petroleum hydrocarbons. These include EPA SW-846, Methods 8015, 8015A as well as the modified Method 8015. Other procedures are the ISO/BSI methods such as BS EN ISO 16703(2011), ISO 18287(2006), ISO/TR 11046(1994), ISO/DIS 13859 (2012), ISO/DIS 11504 (2015), ISO/TS 16558-2 (2015) and ISO 18287 (2006-01 E).

A number of detectors are available for GC analysis of petroleum contaminants. These include Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), Photoionisation Detector (PID) and Mass Spectrometer Detector (MSD) (Lough and Wainer, 1995; Andersson, 2014). Each detector has its advantages and limitations. The MSD has a sensitivity of between 1-10 ng (full scan) or 1-10 pg (SIM) with a linear range

of 10^5-10^6. It operates a temperature: range of 250-300°C (transfer line) and 150-250°C (source). The selectivity of MSD covers any compound that fragments within the selected mass range. It may also include range of masses (full scan) or only selected ions (SIM) (Gregg *et al.*, 2006; Andersson, 2014). The FID has great selectivity for compounds with C-H bonds. It is however associated with poor response for some non-hydrogen organics such as hexachlorobenzene. The sensitivity of the FID is very high and range from 0.1-10 ng with a linear range of 10^5-10^7. The temperature range for the detector is 250-300°C, and 400-450°C for high temperature analyses (Dal and Juvet, 1962; Sarafraz-Yazdi *et al.,* 2009).

For most petroleum analysis, FID is often used for the measurement of total hydrocarbons, while the aromatic fraction can be determined with a photoionisation detector (PID). Estimation of the aliphatic fraction is done by subtracting the result of the aromatics from the total hydrocarbon. One drawback with PID is that analytical results could be overestimated because the detector is not entirely specific for aromatics (Langhorst, 1981; Soo, 2018). MSD, used in the selected ion monitoring (SIM) mode, is ideal for aromatics because of its ability to detect and quantify compounds. The MSD also has the added advantage of offering more detailed information on the identity of individual components of the petroleum matrix (Cortes, *et al.,* 2012). An MSD is described as a universal detector because it has no discrimination between compounds, and can measure TPH, PAHs, or individual compounds.

The main drawback of GC methods is the cost of the instrument. This makes its application practically unavailable for ultimate end users (Cheng *et al.,* 2018). There is a possibility of TPHs concentration overestimation arising from the detection of non-petroleum compounds (e.g. chlorinated compounds, plant oils and waxes). This can be eliminated by a silica gel clean-up, which may also remove some polar hydrocarbons (TPHCWG, 1998; Muijs & Jonker 2009). A baseline-to-baseline integration mode can also be used for quantification of the unresolved complex mixture (UCM) (Bai *et al.,* 2018).

Immunoassay methods involve a biochemical test, which measures the concentration of a macromolecule in a solution using an antibody or sometimes an antigen. For TPHs measurement, the method correlates the response of antibodies to specific petroleum components (Patnaik, 2010). USEPA method 4030 provides a procedure on the use of

immunoassay for the screening of petroleum hydrocarbons in soils. Several portable test kits designed for online field measurement of TPHs are also available. Immunoassay methods are presently designed for measurements of aromatic fractions of petroleum hydrocarbons such as BTEX and PAHs and are mostly used as a screening technique (Okparanma and Mouazen, 2013; Yu Goryacheva *et al.,* 2017). A correction factor can then be used to estimate the concentration of TPHs. Based on product type, this correction factor could vary because it attempts to correlate TPHs with the measured surrogates.

Typically, the range of TPHs detection limits with Immunoassay tests is from 10-500 mg/kg in soil and 200 to 500 µg/L in water. Fillmann *et al.* (2007) demonstrated that the results of analysis of petroleum hydrocarbons using Immunoassay tests could compare well with those obtained by GC-methods and stated that the method could serve as a useful screening protocol. One limitation of the Immunoassay method is that it does not identify specific fuel types, thus it is best used as a screening tool. When used for clay and other cohesive soils, its low capacity to extract hydrocarbons from such samples tends to limit its application. Thus, soil type and homogeneity tend to affect results obtained with the method (TPHCWG, 1998).

High Performance Liquid Chromatography (HPLC) can be used to separate, identify, and quantified petroleum fractions (Robards, 1994; Taylor, 2005). Different detectors such as UV-Vis absorbance, Diode Array Detectors (DAD), Charged Aerosol Detector (CAD), and multiple detectors such as a combination of UV-Vis with a mass spectrometer on an LCMS can be integrated for its routine analysis (Vanini *et al.,* 2018). Individual aromatics and polyaromatic compounds can also be evaluated (USEPA Method 8310). Suatoni and Swab (1975) demonstrated that HPLC can be used to quantitatively fractionate crude oils into saturates, resins, aromatics, and asphaltenes. Assadi and Mathur (1991) reported that the technique can provide good separations and quantitation of saturates, aromatics, polars (resins) and asphaltenes crude oil. Mao *et al.* (2009) utilised HPLC–GCXGC/FID to achieve a detailed chemical compositional analysis of crude oil contamination with better predictions of the leaching potentials and ecotoxicological risk of petroleum and hydrocarbons in soils. Typical concentration ranges of target

analytes that can be determined in sample matrices with HPLC techniques are from 0.013-2.3 µg/L. The technique of HPLC is particularly useful for risk analysis and ecotoxicological assessment of petroleum-contaminated matrices.

2.4 Critical evaluation of phytoremediation, mycoremediation and methods of monitoring and analysis of petroleum-contaminated soils

Phytoremediation is often applied as a post contamination measure after oil spills (Li *et al.,* 2009; Lu *et al.,* 2015; Asghar *et al.,* 2017). Thus, the contaminants have already moved down the soil profiles beyond plant root zones. Obviously, avoiding oil spills altogether is preferable, however adequate provisions should be put in place in events of spills. Thus, integrating phytoremediation into the petroleum industry environmental management strategy would help overcome the limitation of plant roots not reaching the lower soil profile. This is because the plants would be already available to pick up these contaminants while they are still within the root zones (Cunningham & Ow, 1996; Tangahu *et al.,* 2011). The process can further be improved by using plants which have high tolerance for petroleum contamination and are either ubiquitous or native species to avert issues of plant adaptability (Mendez and Maier, 2008b). Identification of nutrient requirements, as well as appropriate nutrient sources, is essential for the successful implementation of phytoremediation on petroleum-contaminated soils (Dineshkumar *et al.,* 2019; Kumar *et al.,* 2019). This is because, as stated by Feng *et al.,* (2018), Bernabé-Antonio *et al.,* (2018), and Fatima *et al.,* (2018), the plants need to grow well, before effecting their remediation potentials.

An understanding of the phytoremediation mechanism associated with each plant would aid the effective selection of plant species for intercropping (Khandare and Govindwar, 2015). Intercropping would also need to be carried out in such a way to incorporate different plant types and their associated functions such as hyperaccumulators and hyperdegraders (Kumar, 2019), plants with high tolerance to petroleum contaminants (Kulakow, Schwab & Banks, 2000; Bidhendi & Mehrdadi, 2010), deep and shallow rooted plants (Chaudhary *et al.*, 2019; Martínez-Oró *et al.,*2019), ornamentals, legumes and plants with extensive root systems (Bandowe *et al.,* 2019; Dubchak & Bondar, 2019). Yavari *et al.* (2015) reported that careful selection

of plant types could further reduce the cost of phytoremediation resulting in overall cost efficiency. As stated by Reichenauer and Germida (2008) and Das (2018) these would all need to be executed with appropriate agronomy practices such as crop rotation and effective nutrient supply.

Biotechnology and engineering with transgenic species offer great prospects for phytoremediation (Cherian & Oliveira, 2005; Doty, 2007). Since there are many plants with promising potential for phytoremediation of petroleum contaminated soil (Table 2.1 & 2.2), integrating biotechnology into these species could help enhance their potential. There is a need for more biotechnological inputs in the development of enhanced species of plants and associated microbes to aid phytoremediation of crude oil contaminated soils. Certain plant enzymes have been identified to aid phytodegradation of petroleum contaminants (Schnoor, 2002; Schwitzguebel, *et al.,* 2009; Lew, 2018). Other organic exudates by plants that stimulate the activities of petroleum-degrading microbes in soils have also been identified (Li *et al.,* 2019a; Li *et al.,* 2019b). Efforts on developments of biotechnology techniques such as isolation and commercial preparation of such enzymes and incorporating such with phytoremediation would result in enhancements. Biotechnology would also have to incorporate elucidation of metabolic pathways for transport and degradation of petroleum contaminants as well as developments of more transgenic species for phytoremediation of petroleum contaminants.

Certain fungi such as the white rot fungi like *Pleurotus ostreatus* have been identified as good degraders of petroleum hydrocarbons (Yateem *et al.,* 1999; Isikhuemhen *et al.,* 2003; Kristanti *et al.,* 2011). Combining these agents with plants could be advantageous. Huang *et al.* (2000, 2004) demonstrated that a combination of physical, photochemical, microbial and phytoremediation could be synergistic, resulting in a more efficient removal of PAHs. Thus, the concept of coupling phyto- and myco-remediation agents to increase remediation efficiency is plausible and worth researching.

Surface active agents such as Tween-80, and biosurfactants like Rhamnolipid have shown potential to enhance phytoremediation and would be highly relevant in the remediation of organics like crude oil (Yan-Zheng *et al.,* 2007; Liao *et al.,* 2015). Research seems centred around the use of synthetic and biosurfactants. Several plants can

produce natural surfactants (Tmáková *et al.,* 2016; Kregiel *et al.,* 2017). However, little investigations have been carried out on the use of biosurfactants from plants in soil remediation. Plants could also be bioengineered to produce bio-surfactants (Stepanova *et al.,* 2016). It is therefore worth investigating the potential of using such plants or their biosurfactants to aid phytoremediation of petroleum-contaminated soils. Enhanced phytoremediation of petroleum-contaminated soils with surfactants is itself very promising due to increased solubility and bioavailability of the contaminants (Gao *et al.,* 2006; Yan-Zheng *et al.,* 2007).

The limitations of phytoremediation on petroleum-contaminated soils have been identified (Naees *et al.,* 2011 Ramamurthy & Memarian, 2012; Mahar *et al.,* 2016); but potential solutions are available (Table 2.4).

Table 2.4: Solutions to challenges on application of phytoremediation on petroleum contaminated soils.

Challenges	Possible solutions
Long duration of the remediation process	➢ Enhancement of phytoremediation using several agents/methods. ➢ More research to identify ideal enhancement agents with respect to soil types, crude oil type and concentration levels. ➢ Used of hyperaccumulators or hyperdegraders of petroleum contaminants.
Low plants biomass and slow growth rate	➢ Integration of Biotechnology to developed high biomass plants with enhanced efficiency. ➢ Biotechnology can also be used to developed plants with faster growth rate.
Problem of disposal of extracted contaminants in plants system	➢ Solvent extraction system could be used to recover petroleum substances accumulated in plants system. ➢ Plants could be prune or uprooted and the biomass treated in Bioremediation piles for degradation of petroleum substances accumulated in plants systems. ➢ Plants biomass could also be used for generation of biofuels.
Bio-transfer of contaminants into food chain	➢ Proper handling of plants biomass through adequate monitoring, timely pruning, uprooting and treatments of biomass. ➢ Digging up and treatment in biopiles.
Non-bioavailability of contaminants	➢ Integration of surfactants, compost and other organic manure would aid for bioavailability of contaminants.
Introduction of invasive species	➢ Used of ubiquitous and plants species locally available to contaminated sites.
Climatic factors	➢ Identification of ideal climatic conditions for application of phytoremediation programmes through research
Limits of plants root to upper soil profile	➢ Integration of phytoremediation with onset of petroleum activities ➢ Use of deep-rooted plants and Intercropping

Most studies on mycoremediation of petroleum-contaminated soils are carried out using artificially contaminated soils in glasshouses, under sterile conditions (Abioye *et al.,* 2013; Rahman, *et al*., 2013). There could be several issues in translating results from such studies in glasshouse to field scale.

Firstly, the varying concentrations added to soils to create the artificial contamination cannot be compared to ideal crude oil spills with massive quantities of petroleum contaminants in soils. Liu *et al.* (2012) reported up to 50% Total Petroleum Hydrocarbons in petroleum contaminated sites in Shengli Oil Field, China. Thus, it would be ideal to use conventional petroleum contaminated soils for mycoremediation studies.

Secondly, substrate sterilisation and incubation at room temperatures creates an artificial environment which is different from those of the field during in situ applications. Using unsterilised substrates and conditions identical to field conditions would aid for replication of laboratory outcomes during field applications.

Thirdly, climatic and edaphic factors are not usually incorporated into laboratory or glasshouse studies and this will certainly be encountered in the natural environment. Ideal research in mycoremediation of petroleum-contaminated soils should be tailored towards real-life situations using typical petroleum-contaminated soils and unsterilised conditions. This would involve investigations into various substrates and conditions which can be easily replicated during in situ applications. Using substrates and fungi species which are ubiquitous or native to sites of contamination would also help mitigate potential adaptation problems.

There seem to be limited reports on application of enhancement agents such as the use of surfactants, combination with other microbial communities, as well as combinations of mycoremediation with phytoremediation for treatment of petroleum contaminated soils. Surfactants can increase the bioavailability of organic contaminants (Pacwa-Płociniczak *et al.,* 2011; Liao *et al.,* 2016). Therefore, integrating surfactants with mycoremediation, and combination of mycoremediation with other biological agents could lead to increase efficiency on petroleum-contaminated soils.

Finally, much of biotechnology and engineering have not been incorporated into mycoremediation for the management of petroleum-contaminated soils. Bamforth and Singleton (2005) and D'Annibale *et al.* (2013) reported that factors such as life cycle, size of the fruity body and mycelium biomass influence the efficiency of mycoremediation. Biotechnology can be integrated to developed mushrooms with optimal fruit body size,

mycelium biomass and improved enzymes yield (Ohga and Kitamoto, 1997; Tautorus and Townsley, 1984), which will result in increased mycoremediation efficiency.

2.5 Conclusions from chapter two

Phytoremediation of petroleum-contaminated soils is very promising because most of the regions associated with petroleum have vast flora resources (Looney *et al.,* 1993; Ige, 2011). The technique of phytoremediation can be used to modify petroleum-contaminated soils. However, physical and biological techniques could be integrated to improve the effectiveness of the remediation (Figure 2.1). This review has identified plants with reported phytoremediation potential on petroleum-contaminated soil. With vast flora resources, more research is required to identify potential phytoremediation agents especially those that are ubiquitous or local to petroleum-contaminated sites. Some techniques with promising potential for enhancement of phytoremediation of petroleum-contaminated soils have also been identified. Most of these techniques are also cheap and locally available (Doty, 2008). Thus, the concept of phytoremediation if enhanced and properly integrated into the petroleum industry environmental plans could offer a reliable, cost-effective and environmentally friendly approach for remediation of petroleum contaminated soil.

Mycoremediation is also capable of providing reliable options for the treatment of petroleum-contaminated soils. This is because fungi afford cheaper and safer means for the simultaneous degradation of organic contaminants and extraction of inorganic species (Adenipekun *et al.,* 2015). In addition, most fungi are found in many parts of the world, which ensures their widespread applications. The typical warm temperatures for growth of macro fungi makes the technique ideal for tropical regions with varieties of fungi and locally available substrates. There are a number of innovations on mycoremediation of contaminated soils, notably, the use of fungal enzymes, and spent mushroom substrates. These provide options in situations where the fungi cannot be cultivated. Most of the macro fungi species are hyperaccumulators of trace metals. It is therefore worth not only studying their degradation/extraction efficiencies but possible speciation and transformation of the inorganic species. The macro fungi have short life cycles, which is somewhat an advantage, because a remediation cycle can be achieved within a short time. However, care must be taken such that the mushrooms are not

consumed as food, and that substances already taken up are not returned to soils via putrefaction. The end use and treatment of the harvested mushrooms should also be integrated into remediation programs.

The challenge in the development of mycoremediation from laboratory studies to large-scale field applications on petroleum-contaminated soils lies in incorporating ideal environmental, edaphic and climatic factors of a typical contaminated site into the process from first principles. There is still much to be done to maximize the potential of mycoremediation on petroleum-contaminated soils. Areas for further development include integrating processes that could enhance mycoremediation on petroleum-contaminated soils. Identification of ideal environmental and edaphic conditions and methods of application of fungi species to petroleum-contaminated soils is essential to translate glasshouse outcome to field success.

Determination of petroleum hydrocarbons is necessary for assessment, planning and evaluation of remediation programs on contaminated soils. However, the choice of techniques for monitoring and analysis of petroleum contaminants depends on the target aim of such programs. There is also an added element of function of instrument availability and expertise. Most times advance instruments such as GC may not be readily available for routine investigation of petroleum-contaminated soils especially in remote communities, therefore other methods such as gravimetric, immunoassay and use of FTIR, which are more readily available, could be employed for a rapid assessment. Risk assessment, which would require separation and quantification of petroleum group types such as aromatics, would also require LC methods, while biomarker analysis would require a GC method (TPHCWG, 1998). When using a method, the analyst must be aware of the various limitations associated with such and the implications in the assessment.

For adequate assessment of petroleum-contaminated sites, recommended procedures for sampling, sample preparations, storage and analysis must be followed for data reliability and adequate reflection of the environmental situation. Methods, which are easy to operate and are quick and readily available, would help for timely evaluation of contaminants level, risk assessments and planning for remediation.

2.6 Objectives of the study

The specific objectives of the study were to:

- Evaluate soil quality parameters for typical petroleum-contaminated sites at Tibshelf, Derbyshire, United Kingdom, and Ogoniland, Niger Delta, Nigeria.
- Assess the use of sunflower species (*Helianthus annus-pacino gold, Helianthus sunsation and Helianthus annus-sunny dwarf)* and ferns (*Dryopteris affinis)* for remediation of petroleum-contaminated soils in the study areas.
- Assess the use of mycoremediation agents such as white rot fungus (*Pleurotus ostreatus)* and palm wine (from *Raphia africana* and *Elias guineensis*) for treatment of petroleum-contaminated soils.
- Investigate ways of enhancing the phyto- and myco-remediation efficiency of these agents using surface-active agents.
- Evaluate the use of crude oil from the contaminating source for monitoring TPHs levels and the overall remediation efficiency of crude oil contaminated soils.
- Evaluate the use of biomarker compounds for assessment of petroleum-contaminated soils and remediation progress.
- Investigate other options for the quick assessment of petroleum-contaminated and remediated soils.

CHAPTER THREE

General methodology

3.1 Study areas

Two main study areas were selected namely Tibshelf in Alfreton, Derbyshire, United Kingdom and Ogoniland in the Niger Delta region of Nigeria. Soils from Tibshelf, United Kingdom were used for method development, which was later applied to those from Ogoniland, Nigeria. A third site at Brackenhurst, Nottingham, United Kingdom was used for verification of the techniques involving the use of the biomarkers dodecane and benzene-1,3-bis (1,1-dimethylethyl).

3.1.1 Study site at Derbyshire, United Kingdom

The sampling site at Derby, United Kingdom, was located at Oilwell Nursery, Tibshelf, Derbyshire, United Kingdom. Tibshelf is a community of 3,787 inhabitants at an elevation of 154 meters above sea level, with coordinates 359414 N and 444927 E (Figure 3.1). The village is in the Bolsover district of Derbyshire, United Kingdom and is surrounded by other villages such as Hardstoft, Morton, Pilsley, Teversal and Newton (HS2, 2018). Tibshelf is home to Hardstoft No. 1, Britain's first mainland oil well. Hardstoft No.1 was the first successful oil exploration well ever drilled in the UK with oil struck on the 27th May 1919. The oil well produced light oil from a depth of 934 metres. The initial production output was about 1 metric ton/day for many years but later doubled to about 2 metric tons /day (Brentnall, 1995). According to Craig *et al.* (2013), two additional wells were drilled on the Hardstoft Anticline in the 1920s. However, although some gas was found, no additional oil production was gained. The gas was used to power the site for several years. Before decommissioning of the site, the total oil production from the Hardstoft No. 1 well between 1920 and 1946 was about 11 metric tons. The site is currently used for Oilwell Nursery, a small garden centre. Although the site has been decommissioned for years, crude oil still seeps out of the pump head and spills around the surrounding soils (Boothroyd *et al.,* 2016).

The topography of Tibshelf varies with locations and is characterised by the River Doe Lea valley with protruding points of higher land to the east (184m Above Ordnance Datum-AOD) and west (194m AOD), and even more undulating high ground in the north (HS2, 2018). At certain points, the topography is levelled with some moderate slopes.

Prominent topography features includes the undulating lowlands comprising woodlands intermingled with diverse arable and pastoral farming (Creighton, 2002; LDBP, 2011). The main drainage consists of a series of tributaries and smaller waterways, which drain the land toward the Doe Lea River (DCC, 2014). Soils in Tibshelf are categorised as disturbed soils (Avery, 1980). The most predominant soil types comprise fine-textured soils derived from carboniferous mudstone with the top consisting of silty-clay, clay-loam, clay or sandy-clay-loam (MAF&F, 1998). Sandy-silt-loam and sandy-loam topsoil are also predominant. A variable soil profile is expected for the area (SSE&W, 1984; HS2, 2018).

In terms of geology, Tibshelf is underlain by Triassic sandstones consisting of the Peak Limestone Group (formerly 'Carboniferous Limestone' in the Peak District). This predominantly consists of commonly thin bedded, cherty limestones with reef knolls in the uppermost part of the sequence with more massive, cherty and often porcellaneous limestone below the uppermost 60 m (Banks, 2017). The limestones are capped by up to 15 m of mudstones and are interbedded with considerable thicknesses of basic volcanic strata, which has two discrete basalt lava horizons. The upper horizon is up to 37 m thick, while the lower ranges up to 45 m. Major parts of these limestones have been intensively dolomitised, with a commonly sharp contact between limestone and dolomite which cuts across bedding at most locations (Boothroyd *et al.*, 2016). There are also widespread silicification of the limestones, and most have been subject to intense mineralisation, resulting in the presence of ore bodies and mineral veins. Thus, minerals such as galena, calcite, sphalerite, barites and fluorspar which are of economic importance are also found in the area. Mining for lead, zinc and silver have also been reported (Sorkhabi, 2018).

The crude oil obtained from Tibshelf is classified as light oil found in a fractured sandy limestone unit at the top of the lower carboniferous limestone succession at a depth of 3,070 ft. The oil is further described as exceedingly mature and likened to Pennsylvania Grade Crude oil (Craig *et al.,* 2013). Pennsylvania Grade Crude oil is a type of sweet crude oil having superior qualities such as trace quantities of nitrogen and sulphur, and absence of asphaltic components (Patil *et al.,* 2019). Such oils are thermally stable with high viscosity index and are very high in paraffin and other waxes which makes it highly desirable for refinement into petroleum lubricants such as motor

oil, and for use in certain hydraulic applications (Riehm *et al.,* 2015). Pennsylvania Grade Crude oils possess excellent characteristics for refining into lubricants; and have been used for medicinal purposes and as a source of lamp fuel and machinery lubrication. The oils have green or fluorescent colour when reflected in sunlight or ultraviolet lights, respectively. Thus, crude oil from Tibshelf, like the Pennsylvania grade crude oils can be broken down into gasoline, kerosene, fuel oil, gas oil, wax distillate, cylinder stock (or bottoms) and other products like white oil and paraffin (Riehm *et al.,* 2015, Patil *et al.,* 2019).

The weather and climate in Tibshelf are typical of Derbyshire. The area has a temperate maritime climate with cool to cold winters and warm summers and the characteristics four distinct seasons of winter, spring, summer and autumn (Corden *et al.,* 2003; Hollins *et al.,* 2004). The warmest period of the year is usually in July and the coldest- January with average temperatures and precipitation values at 4-11^{0}C and 108mm, respectively. The area has an annual average rainfall of 700-800 (1961-2000 long-term average), with May and December as the driest and wettest months (Corden, Stach & Millington, 2002).

The vegetation at Tibshelf consists of semi-natural calcareous grassland mixed with temperate woodland (Anderson & Radford, 1994; Kotilínek *et al.,* 2018). The area is mainly semi-rural with the land principally used for agriculture (Boothroyd *et al.,* 2016). It is associated with earlier industrial sites and includes important zones restored from previous open cast coal mines to agriculture. Major natural resources associated with Tibshelf include crude oil, coal and timber (Boothroyd *et al.,* 2016).

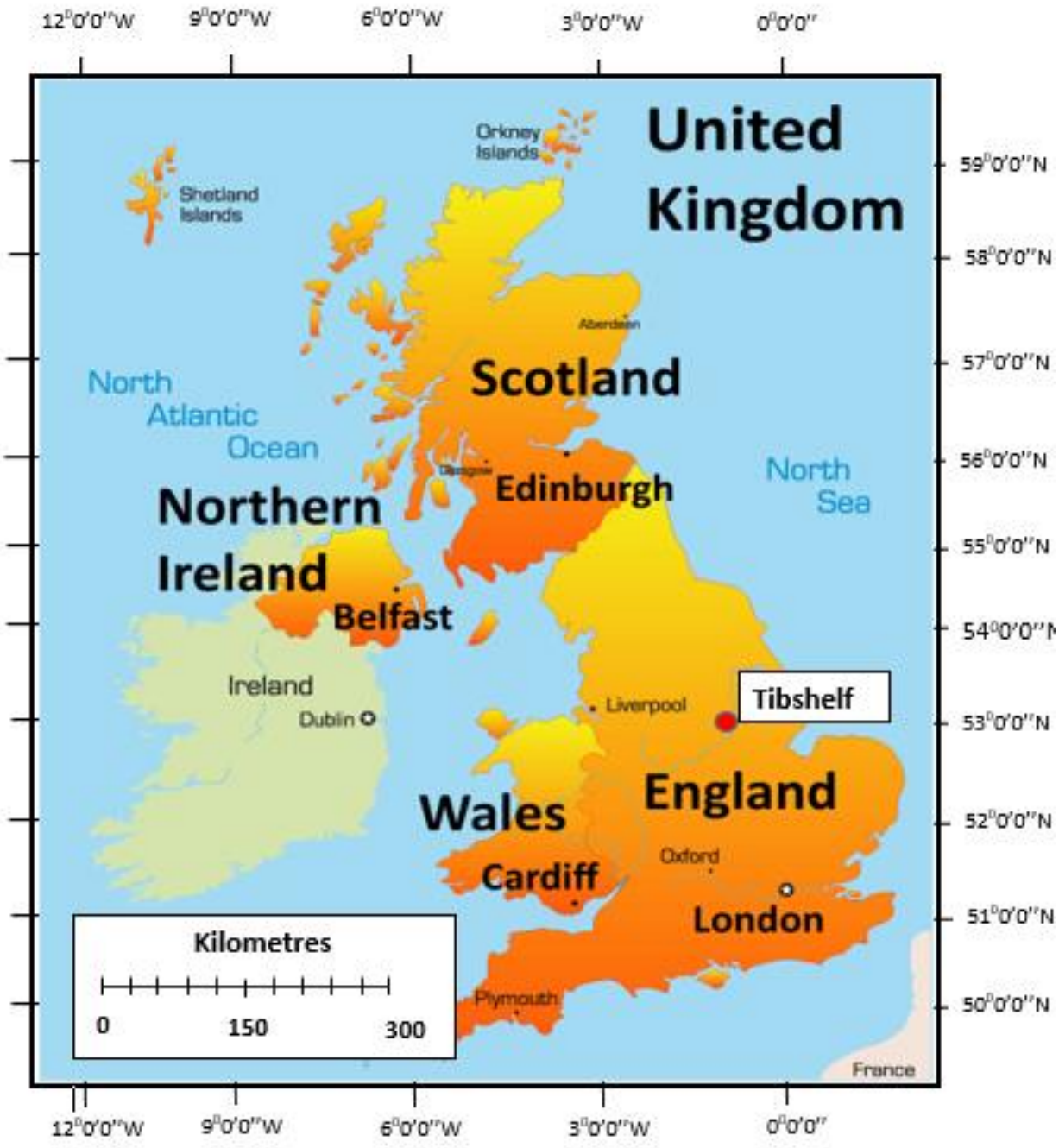


Figure 3.1a: Map of United Kingdom showing the location of Tibshelf, Derbyshire, United Kingdom (map drawn with coordinates obtained with GPS Garmin GPSMAP 64 Handheld Navigator using ArcGIS 10.x)

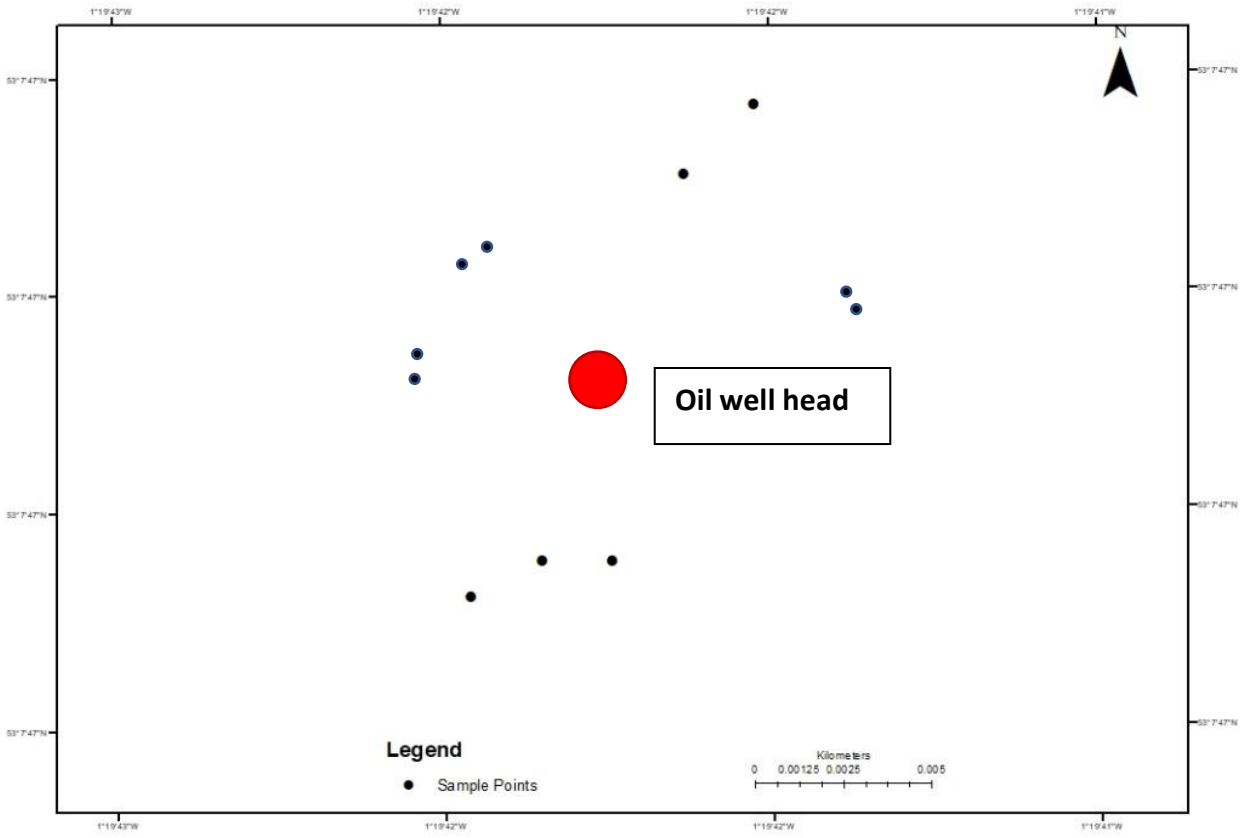


Figure 3.1b: Location of the sampling point around the oil well head at Tibshelf, United Kingdom (map drawn with coordinates obtained with GPS Garmin GPSMAP 64 Handheld Navigator using ArcGIS 10.x)

3.1.2 Study sites at Ogoniland, Niger Delta, Nigeria

Ogoniland is part of the coastal plain of the Niger Delta region of Nigeria. The area is home to approximately 2 million people and covers about 1,050 km^2. It is located within the geographical coordinates 4° 53' 57" N, 4° 28' 48" N, 6° 52' 30" E and 7° 35' 37" E (Figure 3.2) (UNEP, 2011). The climate of the area is tropical, with distinct rainy and dry seasons. Rainfall occurs throughout the year, with an average of 4,700 mm/year (Ite, 2013). The rainy season starts from February or March and ends in October or November. Even during the dry season, the area receives up to 150 mm of rainfall. Relative humidity fluctuates between 90% and 100% for most of the year, while the temperature range is 28 to 33^0C (UNEP, 2011; Brown and Tari, 2015).

The topography of Ogoniland is characterised by a combination of swamps, lakes, lagoons, creeks, and rivers (UNEP, 2011). The land surface can be categorized into the freshwater

zone, mangrove swamps and coastal sand ridge zone. The mainland consists of the riverine area, whose land surface is between 2 and 5 metres above sea level, and the drier uplands 10 to 45 metres above mean sea level. The majority of water channels in the freshwater zone are surrounded by natural embankments. These support settlements and agriculture, which is the main occupation of the local communities (Ite, 2018). These water channels are also intermingled with small ridges and shallow swamp basins, as well as gently sloping terraces intersected by deep valleys that carry water intermittently (UNEP, 2011). Soil type consists principally of silt and clay foundation, which are more susceptible to perennial inundation by river floods. The soils of the area are also mostly silty-loam, with sandy and sandy-loam around the coastal sand ridges (Nrior & Jirigwa, 2017).

Ogoniland has a tropical rain forest vegetation. The riverine part is characterised by three hydro-vegetation zones. These are beach ridge, salt water and fresh water, each with its characteristics and composition (Ozigis, 2018). Dominant vegetation consists of the palm tree -*Elaeisis guineensis*. Other dominants crops include coconut (*Cocos nucifera*), raffia palm (*Raffia africana*) and cocoyam (*Xanthosoma spp*). Two distinct storeys can be identified within the forest strata. Emergents include *Symphonia globulifera, Cleistopholis patens, Uapaca spp., Musanga cecropioides, Hallea ledermannii, Terminalia spp., Anthostema aubryanum, Tectona grandis and Elaeis guineensis.* The understory is characterised by species such as *Calamus deeratus, Alchornea cordifolia, Monodora tenuifolia, Harungana madagascariensis, Strophanthus preussii, Rauvolfia vomitoria* and *Raphia spp* (Fentiman and Zabbey, 2015).

The main mineral resources found in Ogoniland is petroleum, which includes crude oil and associated natural gas. Production and exploration of petroleum commenced in the 1950s in Ogoniland. This was followed by extensive production facilities established over three decades. The sole oil exploration company operating in Ogoniland is the Royal - Dutch company, Shell Petroleum Development Company (SPDC). Table 3.1 summarises crude oil facilities available in Ogoniland (UNEP, 2011).

Table 3.1: Crude oil facilities available in Ogoniland (UNEP, 2011).

Oil facility	Number
Oilfields	12
Wells drilled	116
Wells completed	89
Flow stations	5

Ogoniland has a history of crude oil contamination and environmental degradation associated with petroleum activities. The first major oil spill was reported in 1970 with thousands of cubic metres of crude oil spilled on farmland and rivers. By the year 2000, over 7,000 spills have been reported (Ite, *et al.,* 2013). Many of the spill sites have been left untreated for decades. Although oil production operations have been suspended, oil facilities are still widespread within the region. While some of these facilities have deteriorated, others are frequently vandalised giving rise to recurring episodes of spills. These spill sites are spread around the three local government areas of Gokana, Tai and Eleme. Several protests and campaigns have been carried out by the Ogoni people against the environmental degradation caused by the petroleum industry in their area. A notable episode is that which culminated in the killing of nine environmental rights activists in the region, including Ken Sarowia (Oviasuyi and Uwadiae, 2010; Yakubu, 2017).

According to UNEP (2011), about 1,000 km^2 area of Ogoniland has been contaminated with crude oil. This will take up to 25-30 years for environmental restoration (Ite, 2018). The the oil pollution has spread into drinking water, which contains dangerous levels of benzene and other pollutants with hydrocarbons levels in water reaching more than 1,000 times the allowable level of drinking water standards (UNEP, 2011). Levels of oil contamination in soils were found to have reached a depth of greater 5 meters (UNEP, 2011). The lands are still highly contaminated even in areas where remediation has been reportedly carried out. There are also indications that oil firms have been dumping contaminated soil in unlined pits (Ugochukwu and Ertel, 2008; Mmom and Igbuku, 2015).

Sampling points for this research were located to reflect contaminated sites across the oil-producing local government areas of Ogoniland. The samples were taken to reflect spatial

and profile variations as well as different soil types. Decription and distribution of the sampling sites in the study area are given in Figure 3.4 and Table 3.2.

Table 3.2: Sampling sites and description of soils samples from Ogoniland, Nigeria

Sampling locations	**Sampling Sites**	**GPS Coordinates (UTM)-** *Cassotto et al.,* 2019.		**Profile depth (meters)**	**Soil Characteristics**	**Visible geology**	**Land use**
Ogale		**N**	**E**				
	1	0294996	0532999	0-0.15	Light brown, silty sand	Dry land, level surface topography	Farming, Residential
	2	0294965	0532977	0-0.15	Light brown, silty sand	Dry land, level surface topography	Farming, Residential
	3	0295428	0533596	0-0.15	dark brown, silty sand	Dry land, level surface topography	Farming, Residential
Gio	**4**	0304418	0519421	0-0.15	dark brown, silty sand	Dry land, hilly slope topography	Farming, Residential, oil bunker reservoir, Illegal refinery point
	5	0304409	0519399	0-0.15	Yellowish brown, silty sand	Dry land, hilly slope topography	Farming, Residential, oil bunker reservoir, Illegal refinery point
	6	0304429	0519401	0-0.15	dark brown, silty sand	Dry land, hilly slope topography	Farming, Residential, oil bunker reservoir, Illegal refinery point
K-dere	**7**	0308842	0515267	0-0.15	Light brown, silty sand	Dry land, level surface topography	Farming, Residential
	8	0308690	0515438	0-0.15	dark brown, silty sand	Dry land, level surface topography	Farming, Residential
Okwale	**9**	0321707	0529849	0-0.15	dark brown, silty sand	Dry land, level surface topography	Farming, Residential
Bodo	**9**	0305473	0510286	0-0.15	dark grey, clay	Marshy area surrounded by rivers	Fishing
	10	0305325	0510090	0-0.15	dark grey, clay	Marshy area surrounded by rivers	Fishing
	11	0307283	0509572	0-0.15	dark grey, sandy clay	Coastal plan, bank of a river	Fishing

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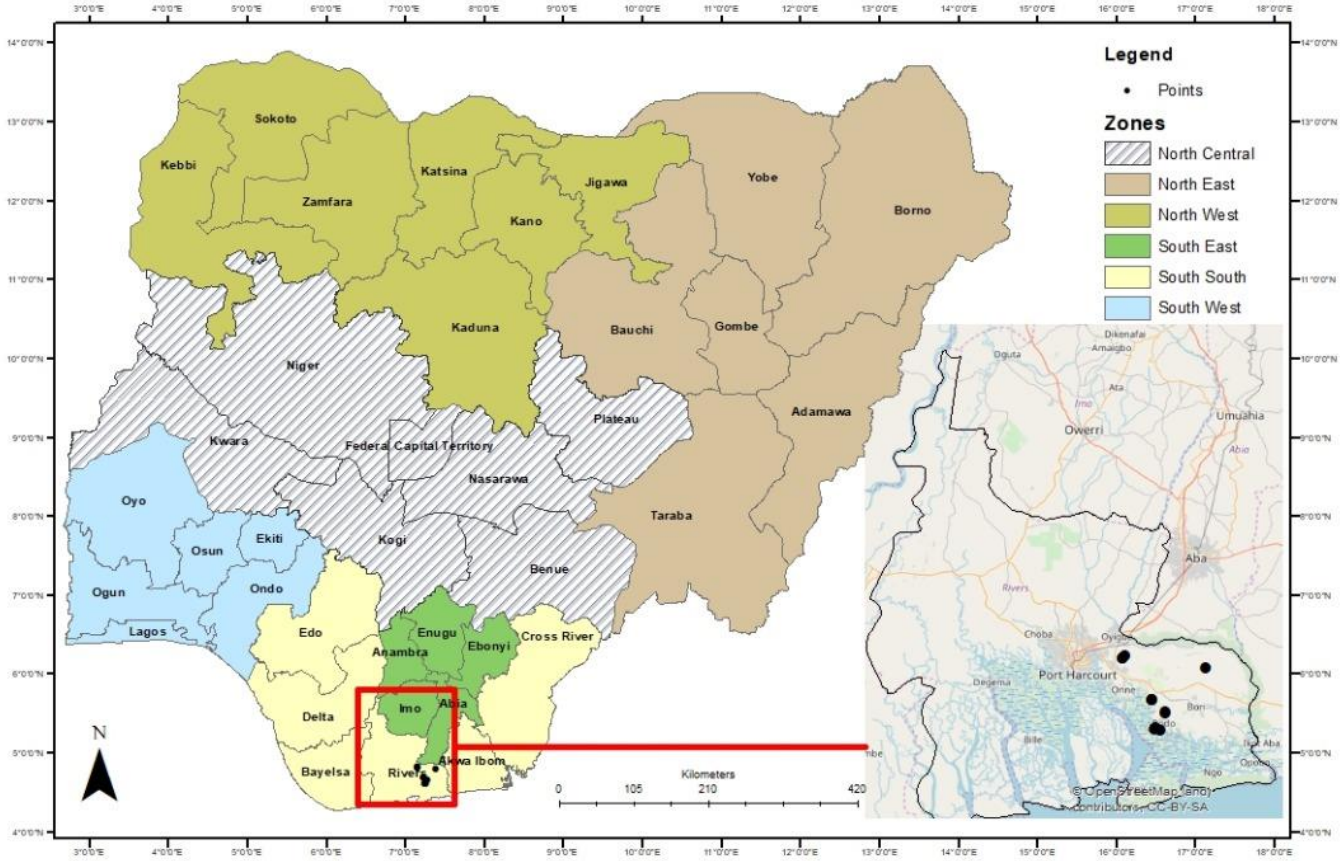

Figure 3.2a: Location of Ogoniland in Niger Delta, Nigeria, showing the sampling points (map drawn with coordinates obtained with GPS Garmin GPSMAP 64 Handheld Navigator using ArcGIS 10.x)

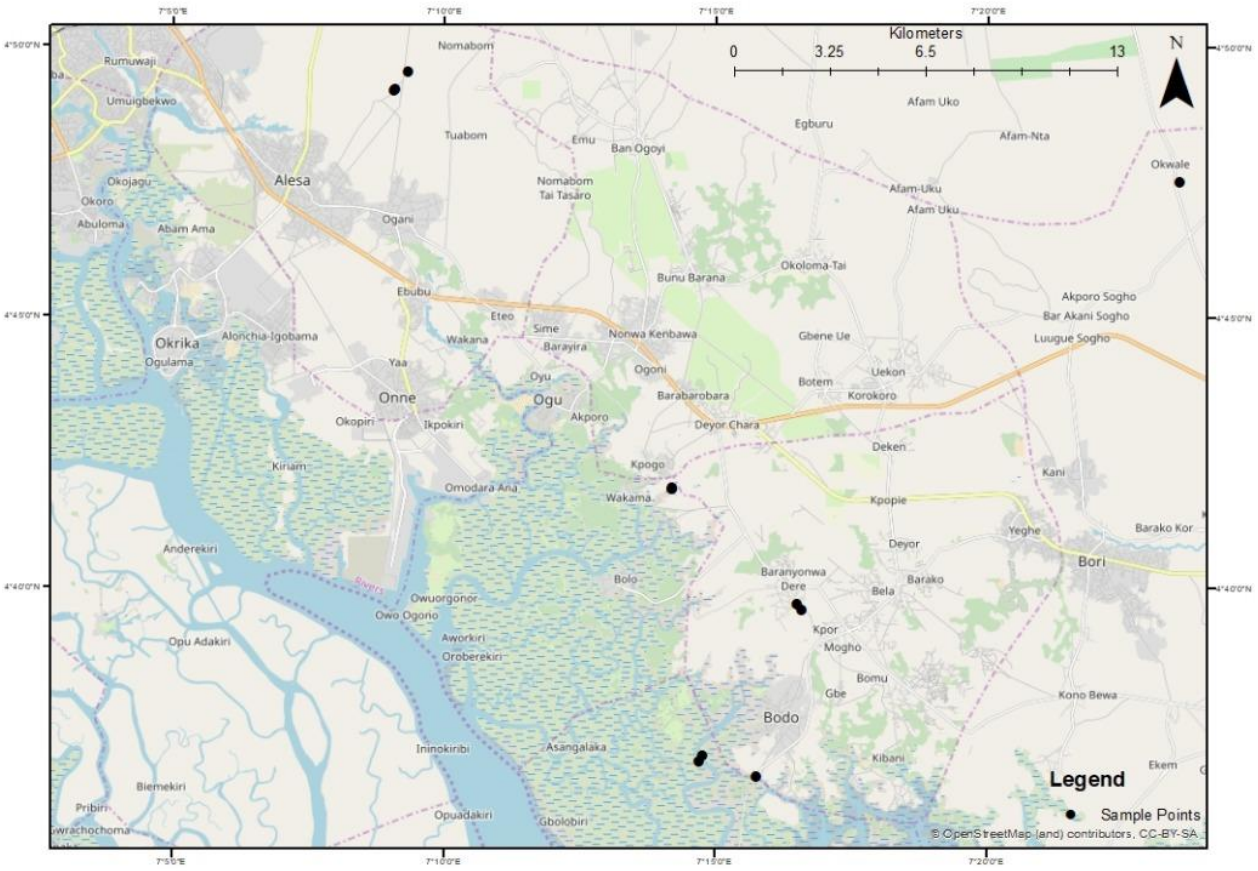

Figure 3.2b: Ogoniland showing the sampling points (map drawn with coordinates obtained with GPS Garmin GPSMAP 64 Handheld Navigator using ArcGIS 10.x)

3.2 Soil sampling for the study

3.2.1 Sampling at Tibshelf, Derbyshire Site

A preliminary visit to the site was carried out on the 26th November 2016; when possible sampling and control sites were identified. Sampling of the contaminated soil was carried out on the 27th of March 2017. Site selection, sampling, transportation and preservation of soil samples were carried out according to Methods BSI ISO/DIS 18400-203 (2016) on the sampling of potentially contaminated soil, and reported in the following sections.

3.2.2 Sample collection at Tibshelf, Derbyshire Site

Contaminated soil samples were taken directly from the spill site while control soil samples were collected from about 200 m uphill. After clearing vegetation and leaf litter, the soil was dug to the depth of the shovel blade (30 cm) around the vicinity of the oil well. Up to 100 kg of crude oil contaminated soils, 50 kg of uncontaminated soils and 1L of crude oil samples were collected. Samples were collected in thick black plastic bags then placed in dark plastic boxes with lids and transported to the glasshouse at NTU Brackenhurst campus, where they were preserved under airtight conditions during the same day of sampling.

3.2.3 Glasshouse set up/activities with soils from Tibshelf, Derbyshire Site

Glasshouse activities were carried out according to the methods of Yadav *et al.* (2009) and Ciurli *et al.* (2014) using the dedicated glasshouse facility at Nottingham Trent University. Soil samples were spread out, extraneous materials removed, air-dried, ground, sieved through a 2mm sieve, homogenised and stored. The samples < 2 mm soil fraction were then weighed out and placed in pots for the remediation studies.

3.2.4 Glasshouse pots, preparations and designations with soils from Tibshelf, Derbyshire

1.5-litre plant pots were used for the study. The pots were placed in plant trays (Grow bag standard 100 x 40 x 5 cm) to avoid seepages from the pots into the environment. 300 g of the homogenised soil was then weighed out and placed in each 1.5-litre plant pot. The pots were labelled according to individual constituents (Table 3.3). Each plant pot for the glasshouse study was prepared by the addition of cow manure to the soils

in a ratio 1:6 (50 g of cow compost added to 300 g of soils) (Marques *et al.,* 2000). The cow manure was mixed with soils for uniformity. This is referred to as the amended soil samples. A subset of the amended soil samples was selected and given treatments for the growing of *P. ostreatus*.

Pots for growing *P. ostreatus* were prepared as follows: 10 g of the dried and grounded stumps of the palm tree (substrates) were added to amended soils. 5 g spawn of *P. ostreatus* were then added by uniformly spreading into the soils. This was then followed by layering of another 10 g of the substrates on top of these soils. The layered palm substrates were also inoculated with 5 g of the fungal spawn. The arrangement allowed *P. ostreatus* to be applied by mixing the substrates with the soil and also by layering it on top of the soil. This approach was a modification of usual practices of layering substrate and *P. ostreatus* for remediation (Adenipekun *et al.,* 2015).

Further subsets of the amended soils were treated with fermented palm wine in a ratio detailed in Table 3.3.

Table 3.3: Composition and designation of glasshouse pots using soils from Derby, UK.

Sample Groups	No. of pots	Soil	Cow manure	Sunflower	Mushroom substrate	Mushroom Spawn	Palm wine
S1: (control 1) uncontaminated soil	9 {3 (triplicates) X 3 months)	**300.00g**	-	-	-	-	-
S2: (control 2) contaminated soil without amendment	9 {3 (triplicates) X 3 months)	**300.00g**	-	-	-	-	-
S3: (control 3) contaminated soil with amendment	9 {3 (triplicates) X 3 months)	**300.00g**	**50.00g**	-	-	-	-
S4: contaminated soil + amendment + Sunflowers	9 {3 (triplicates) X 3 months)	**300.00g**	**50.00g**	**1 seedling**	-	-	-
S5: contaminated soil + amendment + mushrooms	9 {3 (triplicates) X 3 months)	**300.00g**	**50.00g**	-	**20.00g**	**10.00g**	-
S6: contaminated soil + amendment + Palm wine	9 {3 (triplicates) X 3 months)	**300.00g**	**50.00g**	-	-	-	**0.25 litres**

3.2.5 Sampling of soils from glasshouse with Derby soils for laboratory analysis

The glasshouse study was carried out for a duration of 3 months in the months of July to October 2017. Composite soil samples were collected at the start of the study (time = 0 days) and after a 3-month treatment period (90 days) (Adenipekun *et al.,* 2015). Soil samples were homogenised prior to laboratory treatment and analysis. Methods BS ISO 11464 (2016) was used for sample preparation prior to determination of other parameters. The soil samples were air-dried, homogenised, ground and sieved through a 2 mm mesh before extraction of TPH was carried out (Vane *et al.,* 2014).

3.3 Sampling at Ogoniland, Niger Delta, Nigeria

Method BSI ISO/DIS 18400-203 (2016) on the sampling of potentially contaminated soil was used for site selection, sampling, transportation and preservation of soil samples. The soil samples from Ogoniland, Nigeria, were carefully taken to reflect 3 different textural soil classes of sand, loam and clay. Prior to arrival, the contact person in Nigeria Dr Ferdinand Giadom had arranged for a team consisting of experienced professionals and indigenes of the study area. The team members had previously been involved in environmental sampling of Ogoniland by the United Nations environmental program (UNEP) and included a soil scientist, a community Chief, a youth leader and postgraduate student at the Department of Geology, University of Port Harcourt, Nigeria, where Dr Giadom works as a lecturer (Appendix 5). On arrival, an initial meeting was held to discuss the situation in the study area. This ranged from political, socio-economic, geographical as well as environmental issues. Also, a plan of action for the sampling program was discussed and agreed. This included approach to local communities, sampling sites and dates, as well as preservation and storage of samples.

3.3.1 Sample collection at Ogoniland, Niger Delta, Nigeria

The sampling sites were in remote communities and required a significant amount of travel time with an adequate vehicle (at least 1 hour from camp base). On arrival at site, consultations were made with interest groups and site guides were allocated to take the team on a survey of the area. After these surveys, actual sampling sites were discussed and agreed upon, based on history of contamination, approximation to oil facilities such

as pipe lines, well heads, tank farms, bunkering activity zones, physical and geographical barriers as well as avoiding restricted areas.

Once an ideal sampling point was established, the geographical coordinates of the site was determined using a GPS meter model NF-178. Soil samples were then taken at 0-15 cm with the help of a soil auger. The next two sampling points were often taken 500 m both sides of the first. Control sites samples were taken at areas far away from the contaminated zones based on history. Each of the soil samples was collected into sealable plastic bags, then placed in a black plastic bag which is sealed again before been placed in a cooler for onward transportation to the University of Port Harcourt geochemistry research laboratory for preservation.

At each sampling point (Tables 3.2), a method of hand feeling and ribbon (Whiting *et al.,* 2014; Salley *et al.,* 2018) was used to determine approximate soil texture. Following this, 3 bulk samples corresponding to sandy, silty and clayey soils were collected. These samples bulked according to soil texture were to be used for glasshouse remediation study. After collection, soil samples were placed in sealable plastics containers and bagged in thick black plastic bags, then placed in thick dark travelling bags for transportation to the United Kingdom.

The packaged soil samples were taken to the Port Harcourt international Airport, after customs checks and clearances, the bags containing the samples were transported via air cargo to the United Kingdom under the acquired licence for transportation soil samples by the Nottingham Trent University. On arrival at Birmingham International Airport, UK, the samples were cleared then transported to Nottingham and subsequently to the storage facilities at NTU Brackenhurst campus, for further research studies. The overall time taken for sampling and transportation of samples from sampling sites to the glasshouse facilities at Brackenhurst campus, NTU, was one week.

3.3.2 Glasshouse set up with soils from Ogoniland, Niger Delta, Nigeria

The same methods used for setting up glasshouse remediation treatments with the soils from Tibshelf, UK were also employed for soils of the Niger Delta. However, some modifications were introduced to the glasshouse pots and remediation plans as follows:

1. The introduction of a new plant namely a fern (*D. affinis*) that was found growing naturally on crude oil contaminated soils and is typical flora of the Niger Delta region of Nigeria
2. Introduction of the surface-active agents Tween-80 -added subset, to each set of the glasshouse pots sets.

Table 3.4: Glasshouse set up for investigation of effect of Tween 80 on petroleum contaminated silty loamy soils from Ogale, Ogoniland, Nigeria**.**

Sample Groups	**Sample number Total no. of pots**	**Soil**	**Cow Manure**	**Sunflower**	**Mushroom substrate**	**Mushroom Spawn**	**Palm wine**
	Control Soil's set						
S7: (Control 1) uncontaminated soil	3 pots	300.00g	-	-	-	-	-
S8: (control 2) contaminated soil without amendment	3 pots	300.00g	-	-	-	-	-
S9: (control 2) contaminated soil without amendment with **TWEEN 80**	3 pots	300.00g					
S10: (control 3) contaminated soil with amendment	3 pots	300.00g	50.00g	-	-	-	-
S11: (control 3) contaminated soil with amendment with **TWEEN 80**	3 pots	300.00g	50.00g	-	-	-	-
Sunflower							
S12: contaminated soil + Sunflower only	3 pots	300.00g	50.00g	1 seedling	-	-	-
S13: contaminated soil + Sunflower only with **TWEEN 80**	3 pots	300.00g	50.00g	1 seedling			
Ferns							
S14: Contaminated soil + ferns alone	3 pots	300.00g	50.00g	1 seedling of ferns	-	-	-
S15: Contaminated soil + ferns alone with **TWEEN 80**	3 pots	300.00g	50.00g	1 seedling of ferns	-	-	-
P. ostreatus							
S16: Contaminated soil + *P. ostreatus* alone	3 pots	300.00g	50.00g		20.00g	10.00g	-
S17: Contaminated soil + *P. ostreatus* + Tween-80	3 pots	300.00g	50.00g		20.00g	10.00g	-
Palm wine							
S18: Contaminated soil + *Palm wine*	-	-	-	-	-	-	0.25l
S19: Contaminated soil + *Palm wine* + Tween-80	-	-	-	-	-	-	0.25l

3.3.3 Collection of samples from glasshouse using soils from Ogoniland, Niger Delta, Nigeria for laboratory analysis

Glasshouse studies using the Niger Delta soils were also carried out for the same duration of 3 months. Composite soil samples were collected at the start of the study (time = 0 days) and every 30 days for the 3 months. Thus, samples were collected at T=0, 30, 60 and 90 days. The frequency of collection of glasshouse soil samples during treatment with soils from Ogoniland was increased to monthly basis from the previous three months used for the soils from Tibshelf. This was to allow for increased periodic monitoring of the remediation after the results with the soils from Tibshelf, UK showed a remarkable decrease in soil's TPHs at three months. The soil samples were homogenised prior to laboratory treatment and analysis. Methods BS ISO 11464 (2016) was used for sample preparation prior to determination of other parameters. Soil samples were air-dried, homogenised, ground and sieved through a 2mm mesh.

3.3.4 Choice of soils samples from Ogoniland, Nigeria

Different soils types and sediments from Ogoniland were used for different aspects of the study (refer to 6.1 and 6.2). First, for enhancement of the remediation efficiency of the agents, silty loamy soils from Ogoniland was used. This was because silty loam is the predominant soil in the study area. Loamy soil is also predominantly used for farming of food crops in the study area (Venturini *et al.,* 2008). Thus, remediation of this soil type would aid for food sustainability, job creation and prevents bio-transfer of contaminants into food chain. Different soil types of sandy, clayey and loamy from Ogoniland were also used for the study. The choice of the different soil types was to evaluate the adaptability of the methods developed in this research to different soil types that are found in the Niger Delta region of Nigeria. Petroleum-contaminated sediments from the study area were also treated for remediation. This was further carried out to evaluate the application of fermented palm wine for the treatment of such environmental matrices where the growth of plants or mushrooms may be difficult.

3.4 Physicochemical parameters of soils

3.4.1 Determination of moisture content of soil samples

Soil moisture content was determined as a volume fraction according to methods BS EN ISO 11461(2014). 30 g of field-wet soil was measured out and placed in a clean and dry weighing aluminium coring sleeve of known volume and the lid properly capped. The container was carefully filled to volume to eliminate spaces and allow for correct volume of soil. This volume was noted and recorded. The capped weighing aluminium coring sleeve with the field wet soil was weighed and recorded (W1). These were then placed in an oven and dried between 105^0C and 110^0C for 16 hours. After 16 hours, the container and its contents were removed from the oven and place in the dedicator to cool and weighed again. The process was repeated by placing back the container and its contents in the oven and drying for another 4 hours at same temperatures, then removal and cooling in a dedicator followed by weighing, until a constant (Final) weight (W2) was obtained.

Moisture content (M_c) was calculated as follows:

W = Mass of the weighing tin and lids in Kilograms
W_1 = Total mass of field weight soil, weighing tin and lids in Kilograms
Then mass of field wet soil only (W_2) = $W_1 - W$
W_3 = Total mass of oven dried soil, weighing tin and lids in Kilograms
Then mass of oven dried soil only (W_4) = $W_3 - W$
Thus % Moisture content (M_c) as volume fraction was calculated as:

$$M_c = \frac{W_2 - W_4}{\rho_W \cdot V} \times 100\%$$

Where W_2 = Total mass of field weight soil
W_4 = Total mass of oven dried soil
ρ_W = density of water at soil temperature, in Kilograms per cubic meter
V = volume of tin container or coring sleeve used.

3.4.2 Determination of temperature of soil samples

Temperature was determined in situ on plants' pots by methods of World Meteorological Organisation (WMO) – No 8 (2008) using a standard soil thermometer in cooperated in a HI-98129 Pocket EC/TDS and pH Tester. A screwdriver (Pozi #2 Phillips

Screw Driver) was used to create a pilot hole to a depth of 5-6 cm in the soil. The hole was made a little wider by gentle twisting of the screwdriver. The standard soil thermometer was then introduced to the hole and its bulb allowed to firmly make contact with the soil for about 2 minutes for the temperature to register and a reading taken.

3.4.3 Determination of pH of soil samples

Soil pH was determined by methods of BS ISO 10390 (2005). 10 g of soil was measured into a 50 ml beaker, which was then made up to 50 ml mark with distilled water. The sample was placed in a mechanical shaker and shaken for 60 minutes, then allow to rest for about 1 hour. The pH of the suspension was then measured using a pH meter (HI-98129 Pocket EC/TDS and pH Tester).

3.4.4 Determination of electrical conductivity of the soil

Electrical conductivity of soil was measured by methods BS ISO 11265 (2016). 10 g of soil was measured into a 50 ml beaker, which was then made up to 50 ml mark with distilled water. The sample was placed in a mechanical shaker and shaken for 30 minutes, then allow to rest for 1 hour. Electrical conductivity of the suspension was then measured using a conductivity meter (a HI-98129 Pocket EC/TDS and pH Tester). The temperature was maintained at 20^0C by carrying out the measurement with the sample in a thermostatic controlled water bath model SWBR17 SHEL LAB.

3.4.5 Determination of particle size distribution and soil texture

Particle size distribution of soil was determined using laser diffraction systems - Beckman LS 13 320 laser diffraction particle size analyzer (Seagal *et al.*, 2009; Wanogho, and Gettinby and Caddy, 1987). 5 g of air-dried and sieved (<2mm) soil samples were weighed out into 50 ml beakers. For crude oil contaminated soils, samples were pre-washed with 10 ml hexane solution (99% v/v) to remove hydrocarbon contaminants. 5 ml of 30% H_2O_2 solution as added to the samples for oxidation of organic matter. Finally, 10 ml of Calgon (sodium polymetaphosphate) solution was added and the sample left overnight. The sample was then dried in a desiccator.

400 mg of the soil sample was weighed out and prepared in 10 ml of distilled water using ultrasonic bath (SWBR17 SHEL LAB). The sample holder of the granulometer was filled

with 380 ml of reagent water. The water was sonicated, scanned and the background number of particles determined. The soil sample was thoroughly shaken and transferred into the sample holder. All soil particles were transferred by repeated washing with reagent water. Finally, the volume of suspension in the sample holder was adjusted to 400 ml using the reagent water. The suspension was stirred, sonicated and introduced into the sample handler of the Laser diffraction system for particle size measurement, from where the particle size distribution was determined.

3.4.6 Determination of soil texture

Soil texture was determined from the % composition of the particle sizes using a textural triangle and confirmation with the online tool found on the US Department of Agriculture website https://www.nrcs.usda.gov/ wps/portal/nrcs/detail/soils/survey/

?cid=nrcs142p2_054167

3.5 Laboratory treatment and analysis of soil samples

Treatments carried out on the prepared samples include extraction of Total Petroleum Hydrocarbons (TPHs), GC-MS analysis for biomarker compounds, and determination of TPHs using both hydrocarbons standards and crude oil standards.

3.5.1 Extraction

TPHs were extracted by microwave-assisted extraction with a Milestone MA182-001 ETHOS UP Microwave system, using a 1:1 acetone - heptane solvent mixture (USEPA 3546:2007). 10 g of air-dried and sieved (<2mm) soil samples were weighed into the glass vials of the extraction vessels of the microwave. 25 ml of extracting solvent (1:1 acetone- heptane) was added to the soil samples. Both Teflon heating pads and magnetic stirrer were inserted into the extracting vessel, which was then sealed, placed into the microwave instrument and extracted for 15 minutes. Conditions of the microwave are as listed in Table 3.5. Method blanks, as well as matrix spikes with the surrogates, 2-flurobiphenyl and 4-terphenyl-d14, were also prepared similarly to the samples and placed along for extraction (ISO13859, 2014) for determination of extraction efficiency.

After extraction, extracts were allowed to cool in the extractor for 15 minutes, then removed and filtered into a centrifuge tube. These extracts now contained the TPHs, as well as the acetone and heptane solvents. To remove the acetone, deionised water was added to the extracts, the extracts were centrifuged at 4500 rpm for 10 minutes and allowed to settle. The supernatant (heptane containing the TPHs) was carefully pipetted out into a falcon tube and stored prior to analysis.

Table 3.5: Operating conditions of microwave assisted extraction instrument

Temperature:	100 - 115 0C
Pressure:	50 - 150 psi
Time at Temperature:	15 min
Cooling:	To room temperature

3.5.2 Analysis in GC-MS

Semi-quantitative standards suitable for quantification of TPHs (USEPA 8270E; ISO/16558-2, 2015; Weber *et al.,* 2018; Dahl *et al*., 2019) were used in the study. This includes commercial TPHs gasoline diesel range and TPHs C10-C40, dodecane and benzene-1,3-bis(1,1-diemthylethyl) standards (Section 3.6).

3.5.2.1 Identification of marker compounds in the crude oil and crude oil contaminated soil

First, several concentrations of the contaminating crude oil collected at site were prepared as follows. 1 g of crude was weighed out and dissolved in 10 ml of n-heptane to give a concentration of 0.1 g/ml (100,000 mg/l). From these concentrations of 1, 10, 50, 100, 300, 700, 1000, 1500, 2500, 5000, and 8000 mg/l of the crude oil samples were prepared via serial dilutions (Appendix II). Extracts from the crude contaminated soils (at T=0) were also diluted with heptane by a factor of 5. The solutions were all analysed in a GC-MS according to methods of ISO 13859 (2014). GC-MS conditions are listed in Table 3.6.

Table 3.6: GC-MS conditions for TPHs

Column	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
Oven	45 °C (3 min), 20 °C/min to 360 °C (10 min)
Carrier gas	helium, 1.3 mL/min. constant
Injection	1.0 µL, splitless
Liner	2 mm I.D. straight
Injector temp.	250 °C
Detector	MSD, 300°C

From the chromatograms obtained, searches were conducted using peak-by-peak analysis for compounds that fits into the classes of hydrocarbon compounds in crude oil namely: saturated straight chain, substituted aromatic and substituted cyclic (non-aromatic). The compounds identified in this sample were determined by a NIST library search of the mass spectrum using the AMDIS GC-MS program, and the best match percentage (those with highest probability) chosen as possible biomarkers. The common occurrence of the peaks on the chromatogram was also considered in making this decision. The compounds identified within the various classes are listed in Table 3.7 and Appendix I.

Table 3.7: Compounds identified within the various classes of organics in the contaminated soils

Peak no	**Compound**	**Retention time (mins)**	**Match %**	**Class of organics**
1	Dodecane	10.315	40	Aliphatic
2	Benzene,1,3-bis(1,1dimethylethyl)	11.064	81	Substituted aromatic
3	Tridecane	11.774	41	Aliphatic
4	Dodecane 2,6,10-trimethyl	12.789	28	Substituted aliphatic
5	Tetradecane	13.147	32	Aliphatic
6	Pentadecane	14.436	27	Aliphatic
7	2,4-ditertbutylphenol	14.532	42	Substituted aromatic
8	Hexadecane	15.650	26	Aliphatic
9	tert-hexadecanethiol	16.009	10	Substituted aliphatic
10	Octadecane	17.897	19	Aliphatic
11	Eicosane	19.929	27	Aliphatic
12	17-pentatriacontene	21.625	28	Aliphatic (unsaturated)

Only one compound was selected as a representative biomarker for each of the classes of compounds expected in crude oil. The marker compounds were selected so that their abundance could be monitored by simply observing the relevant peak size in the chromatogram. These compounds were then monitored for consistency in retention time among the various concentrations of the crude oil prepared; and in the extract of the crude oil contaminated soils.

From this analysis, a number of marker compounds were identified (Table 3.8). Only two of these compounds could be chosen. The choice of the compounds was because they were consistently present at different concentrations of the crude oil and soil samples. Another reason was because their standards were also readily available. The compounds were dodecane (aliphatic) and benzene 1,3 -bis(1,1-dimethylethyl) (aromatic). The biomarker compounds were confirmed by running standard solutions of the compounds independently, mixed standards (of the markers compounds) and as spikes on the crude oil concentrations of 100, 700 and 1000 mg/l. Mass spectra and retention times of the biomarker compounds in the standards, crude oil and soil extracts were compared and confirmed according to methods of USEPA 8270E (Appendix I).

Table 3.8: Potential representative marker compounds: only dodecane and benzene 1,3-bis(1,1dimethylethyl) were selected.

Retention time (mins)	Compound	Match %
14.912	Dodecane	42
16.255	Benzene 1,3-bis(1,1-dimethylethyl)	78
18.892	Tridecane, 4-cyclohexyl	3
21.042	tert-hexadecanethiol	7
23.090	2,4-ditertbutylphenol	38
42.190	17-pentatriacontene	34

3.5.2.2 Quantification of biomarker compounds in the crude oil and crude oil contaminated soils

Methods ISO 13859(2014) and USEPA 8270E were used for quantification of the marker compounds in the crude oil samples, contaminated soils and treated soils. Three basic steps were involved in the quantification. First was the initial calibration of the

instruments, followed by the evaluation of the concentration of the marker compounds, then carrying out of calibration verification.

The initial calibration was carried out to establish the working range of the calibration. According to methods of ISO 13859 (2014), a 10 µg/ml stock solution of the native standard of dodecane and benzene 1, 3 -bis (1,1-dimethylethyl) were prepared by measuring out 25 µl of 4000 µg/ml of the stock standards of these compounds and dissolving the same amount in 10 ml of heptane. In addition, a 10 µg/ml stock solution containing deuterated internal standard of the dodecane-d26 was also prepared by measuring out 25 µl of 4000 µg/ml certified stock of deuterated internal standard (dodecane d-26) and dissolving it in 10 ml of heptane. These two stock solutions were then used to prepare the calibration standards.

The calibration standard was prepared over a range of 1 µg/ml to 10 µg/ml (1, 3, 5, 8, and 10 µg/ml) by transferring 0.5 to 5.0 ml (0.5, 1.5, 2.5, 4.0, and 5.0 µg/ml) of the stock solution containing the native markers compounds- dodecane and benzene 1,3 -bis(1,1-dimethylethyl); and a constant volume of 0.5 ml of the internal standard (dodecane-d26) to a 10 ml volumetric flask and addition of appropriate volume of heptane to complete a 5 ml volume. Each of these calibration standards contained 1.0 µg/ml of the internal standard.

These solutions were then run in the GC-MS for the initial calibration of the instrument. The relative response ratio for the native biomarkers and the internal standards were calculated by plotting the ratios of the mass concentration against the ratio of the peak areas according to methods of ISO 13859(2014) and USEPA 8270E using the equation:

$$\frac{A_n}{A_d} = s.\frac{P_n}{P_d} + \mathrm{b}$$

Where:

A_n = measured response (Peak Area) of the native biomarker
A_d = measured response (Peak Area) of the deuterated biomarker
S = slope of the calibration function
P_n = mass concentration of the native biomarker in the calibration solution, expressed in microgram per litre (µg/l)
P_d = mass concentration of the deuterated biomarker in the calibration solution, expressed in microgram per litre (µg/l)

b = intercept of the calibration curve with the ordinate

For evaluation of the concentration of the marker compounds, 1 µg/ml (or 0.1 ml of the stock) of internal standard were added to test samples (extracts) as in the case of the calibration standard. The samples were run in the GC-MS. Calibration verification standards of 2.0, 6.0 and 9.0 µg/ml representing 20%, 60% and 90% of calibration range were also included in each batch of the analysis according to methods of ISO 13859(2014).

Concentrations of individual marker compounds of dodecane and benzene 1,3 -bis (1,1-dimethylethyl) from the multipoint calibration of the total method was calculated according to methods of ISO 13859(2014), using the equation

$$w_n = \frac{C_s}{m.d_s} \ .\mathrm{V.f}$$

With $C_s = \frac{(A_n/A_d)-b}{s} \ . \ p_d$

Where:

w_n = content of the individual marker compound in the sample, expressed in milligram per kilogram (mg/kg) on the basis of the dry matter;
A_d = measured response (Peak Area) of the deuterated compound in the sample extract;
A_n = measured response (Peak Area) of the native marker compound in the sample extract;
S = slope of the calibration function;
b = intercept of the calibration curve with the ordinate;
P_d = mass concentration of the deuterated marker compound in the sample extract, expressed in microgram per litre (ug/l);
m = mass of the test sample used for extraction, expressed in grams (g);
d_s = dry matter fraction in the field moist sample, determined according to ISO 11465, expressed in percent (%);
V = volume of the final solution, expressed in millilitres (ml).

The result was expressed in milligram per kilogram (mg/kg) dry matter and rounded to two significant figures.

3.5.2.3 Determination of Total Petroleum Hydrocarbons using hydrocarbon standards

Total Petroleum Hydrocarbon mix standard was purchased from Sigma-Aldrich, UK. Retention time window (RTW) standard solution was prepared by weighing 30 mg of n-

tetracontane into a 1 L volumetric flask and dissolving it completely in an appropriate volume of n-heptane. 30 µL of n-decane was then added; the solutions were mixed by shaking and sonication and then made up to the 1 L. Calibration standard solution of the TPHs was then prepared according to methods of ISO/16558-2(2015) by diluting the TPH mixed standard solution with appropriate aliquots of the RTW solution to give concentrations of 10, 50, 100, 500, 1000, 1500, and 2000 mg/l of TPH mix which was used for calibration of the instrument. The LOD, LOQ, the linear range and the working range of the instrument were established prior to running of samples.

Samples analysed in the GC-MS include blank (n-heptane), sample extracts, calibration standards, control solutions and retention time standard solution. Three control solutions of 300, 700 and 1300 mg/l were used in each run, for checking calibrations did not shift during the run. The total area between the n-decane (C10) and n-tetracontane (C40) peaks of the chromatogram was integrated. The integration started at the retention time just after the end of n-decane peak and the signal level in front of the solvent peaks and ended at the retention time just before the beginning of the n-tetracontane at the same signal level. N-tetracontane was integrated separately for the recovery check.

Calculation of the TPH was carried according to ISO/TS 16558-2(2015) out using the formula

$$c = c_s \cdot \frac{V_h}{M} \cdot f \cdot \frac{100}{d_m} \cdot \frac{1}{p}$$

With $c_s = \frac{A_s - b}{a}$

Where:

c = hydrocarbon content of the sample, expressed in milligram per kilogram dry matter (mg/kg dm);

c_s = hydrocarbon content of the extract calculated from the calibration function in milligram per litre (mg/l);

V_h = volume of the n-heptane extract, expressed in millilitres (ml);

f = dilution factor (when the extract is diluted);

p = fraction of soil extract used for the analysis;
M= mass of the sample taken for analysis, in grams (g);
d_m = dry matter content, determined according to ISO 11465, expressed in %;
A_s = integrated peak area of the sample, expressed in instrument dependent units;
a = slope of the calibration function, expressed in litres per milligram (l/mg);
b = intercept of the calibration curve with the ordinate, expressed in instrument dependent units;

3.5.2.4 Determination of Total Petroleum Hydrocarbons using the standardised crude oil

Several dilution concentrations of the contaminating crude oil samples obtained from the sampling points were prepared. 1 g of the crude oil sample was weighed out and dissolved to 10 ml of n-heptane in a 50 ml measuring flask. Crude oil concentration of these solutions was evaluated as 100,000 mg/l. From this several dilutions, first of 1 – 10 mg/l, then 10 to 100 mg/l, then 100, 300, 700, 1000, 1500 and 2500 mg/l were prepared.

The first lower dilution series were prepared to ascertain the LOQ and LOD of the crude oil samples, while the later higher series were created for calibrations of the GC instrument with respect to the crude oil calibrations.

The above-prepared crude oil concentrations were used to assess the consistency in the relationship between the concentrations of the biomarkers and the TPHs contents in varying concentration of the crude oil. This was also used to determine the remediation efficiencies of the phyto- and myco-remediation agents on the crude oil contaminated soils using the crude oil as analytical standard.

3.6 Data treatment, validity and reliability

Values for Total petroleum hydrocarbons (TPHs) were obtained in mg/Kg but converted to g/Kg dry weight of soils due to large values. Each sample was extracted and analysed in triplicate and the values were reported as mean values ± standard deviation (2σ) (Bao *et al.,* 2018; Hanley, 2019). Consistencies in replicate analysis were evaluated by precision (Cumming, 2014). Accuracy in analysis of TPHs was evaluated by running calibration verification in each set of analysis and evaluating the accuracy.

Paired-samples T-test was used to evaluate differences in TPHs at different treatment times (Time=0 days and Time = 90 day) with same sample treatment to determine if such a difference was significant (Zheng *et al.,* 2019). Analysis of variance (ANOVA) were carried out to evaluate variability in TPHs content among soils samples from different sampling points, glasshouse pot and the different remediation treatment sets. The result data and standard deviations are presented to four significant figures while p-values and are presented to three decimal places. The paired-sample T-test and analysis of variances were carried out using Microsoft office excel spread sheet (Donatelli & Lee, 2013).

For collection of soils from the glasshouse pots, samples were taken from at least 10 different parts of the pots reflecting each section of the soil profile such as the top, the middle and the bottom strata. This was also carried out with the aid of a small hand auger, which can sample at different pot depths. The samples were always homogenised, and composite samples used for extraction and analysis.

Internal consistency

Internal consistency was evaluated in terms of instrumental variability, analytical accuracy, reproducibility reliability and sampling variability. Internal consistency returned $0.8 \leq \alpha < 0.9$ for the analytical results obtained in the research (Krall, *et al.,* 2009; Yang *et al.,* 2009).

Reproducibility reliability

Certain sets of samples were selected and analysed monthly for 3 months. Variability was evaluated by Two-way ANOVA without replications.

Analytical accuracy

Analytical accuracy was > 95%. This was verified by running calibration verification standards at regular intervals of sample runs and evaluating accuracy and precision. Calibration verification returned $0.8 \leq \alpha < 0.9$.

Instrumental validity

This was determined by repeat analysis of an adequate subset of samples repeatedly carried out monthly for 3 months along with calibration verification. Results obtained were treated to 2-way ANOVA test.

Extraction validity

Extraction validity was evaluated by spiking soil samples with known concentrations of surrogate compounds. The spiked soils were extracted with same conditions of microwave and extracts analysed in GC-MS in triplicates. Extraction validity returned $\alpha > 0.95$.

Sampling validity

For sampling validity in glasshouse pots, three different composite samples were prepared from 3 representative pots analysed in triplicates. These were often carried out at each point of the sampling for TPHs analysis (that is at Time= 30 days, 60 days & 90 days). Sampling validity returned $0.8 \leq \alpha < 0.9$.

CHAPTER FOUR

Investigating the potential of sunflower species, fermented palm wine and *P. ostreatus* for remediation of petroleum-contaminated soils

4.1 Background information

Environmental issues arising from the petroleum sector are well known (Al-Nasrawi, 2012; Adenipekun *et al.,* 2013; Lee *et al*., 2015). These can be particularly serious in remote communities and developing countries where resources for effective management are not readily available (Sagrera, 2014; Schweitzer *et al.,* 2015; Albert *et al.*, 2018). Even with the current drive for greener energy sources, it will be difficult to completely obviate the need for petroleum (Gatfaoui, 2016; Wei and Guo, 2016). The scale of environmental pollution by hydrocarbons requires concerted efforts to develop techniques for its effective management. This is necessary to create a balance between resource utilization and environmental sustainability (Rhodes, 2014; Wiszniewska *et al*., 2016).

There are several reports on remediation potential of sunflowers (Dominguez-Rosado *et al.,* 2004; Diab, 2008; Liduino *et al.,* 2018). Sunflowers species with different biomass are also known (CalamaiValkova, *et al.,* 2018; Rigi, 2018; dos Santos Rocha *et al.,* 2019). However, there are no reports relating phytoremediation of sunflowers to species or biomass. To maximise the potential of sunflower plants for clean-up of petroleum-contaminated soils, it is also necessary to investigate the variation of the remediation efficiency of different species. This will help in the choice of the plant type for use in future remediation projects.

Palm trees are abundant in many tropical regions such as the Niger Delta, Nigeria (Cheng *et al.,* 2018; Izah & Seiyaboh, 2018). The juice of palm trees (palm wine) is used as food and ceremonial drink (Okwu & Nnamdi, 2008). The drink becomes sour and unfit for drinking if left overnight due to fermentation and is often discarded (Santiago-Urbin & Ruiz-Teran, 2014). Palm wine consists of a consortium of microorganisms principally the yeast- *Saccharomyces* species (Chandrasekhar *et al.,* 2012; Nwaiwu *et al.,* 2016). Consortiums of microorganisms have been used for the effective treatment of petroleum-contaminated soils (Robichaud *et al.,* 2019). Consequently, it is needful to

investigate the potential of fermented palm wine in remediation of petroleum-contaminated soil.

P. ostreatus are found in both temperate and tropical regions of the world (Ferdeş *et al.,* 2018; Familoni, *et al.,* 2018; Zhang *et al.,* 2018). These fungi are known agents for treatment of petroleum-contaminated soils (Stamet, 2005; Gao *et al.,* 2010). Adapting these fungi for remediation of petroleum-contaminated soils in the Niger Delta, Nigeria, will require substrates, which are abundant in the region. It will also require appropriate techniques for successful in situ applications.

The aim of this chapter was to investigate the potential of some locally available sunflower species, fermented palm wine and *P. ostreatus* for treatment of petroleum-contaminated soils that can be adapted to the Niger Delta, Nigeria. The chapter specifically evaluates the variation of remediation efficiency of the agents with respect to their species, and application methods. This information is important in the choice of the agents among their numerous species. Adapting *P. ostreatus* for use in the clean-up of petroleum-contaminated soils has been problematic (Stamet, 2005; Dickson *et al.,* 2019). The present study also seeks to develop a realistic approach and investigate a novel substrate (Palm tree which is abundant in tropical regions) for application of *P. ostreatus* in the clean-up of petroleum-contaminated soils.

The phytoremediation agents used for the study were 3 species of sunflower plants namely, *Helianthus annus* (Pacino gold), *Helianthus sunsation*, and *Helianthus annus* (Sunny dwarf). The mycoremediation agents were (1) fermented palm wine from 2 species of palm trees (*Elaeis guineensis* and *Raffia africana*); and (2) white rot fungi-*Pleurotus ostreatus* grown on palm tree substrates. These agents are found in many parts of the world and are particulary abundant in the Niger Delta region of Nigeria. Soils from a petroleum-contaminated site at Tibshelf, Derbyshire, United Kingdom (temperate soils) were used for the initial pilot study. The outcomes would be applied to petroleum-contaminated soils from a tropical region (the Niger Delta, Nigeria), to evaluate the adaptability of the methods for both temperate and tropical soils.

4.2 Materials and Method

Soil samples were collected from an oil spill site near a decommissioned British oil well (Figure 4.1) at Tibshelf, Derbyshire, UK (359414N, 444927E). The soils samples include petroleum-contaminated soils collected at the immediate vicinity of the oil well, and uncontaminated (control) soil samples collected at 200 m uphill from the oil well. These samples were packaged, transported to the glasshouse and stored under airtight conditions (BSI ISO/DIS 18400-203; 2016). Sunflower seedlings were purchased from Nicky's Nursery Ltd, Broadstairs, Kent, UK and pre-grown on Ericaceous compost for a period of 2 weeks. The Ericaceous compost was purchased from Amenity Land Solutions, Allscott Park, Shropshire, UK. Palm wine was purchased from African grocery shops in Nottingham, United Kingdom, while grain spawns of *Pleurotus ostreatus* were purchased from Ann Muller's Mushrooms Ltd, Aberdeenshire, UK. Palms stump of *Trachycarpus fortunei* was purchased from Brookfields garden centre, Mapperley, Nottingham, UK.

Figure 4.1a: *Sampling site at Tibshelf, Derbyshire, United Kingdom*

Figure 4.1b: Sampling team to Tibshelf, Derbyshire, UK

4.2.1 Glasshouse experiments with petroleum-contaminated soils from Tibshelf, UK

Glasshouse experiments were carried out using the methods of Ciurli *et al.* (2014) and Yadav *et al.* (2009) in the glasshouse facility at Nottingham Trent University, Brackenhurst campus, UK. 1.5-litre terracotta plant pots (Grow bag standard 100 x 40 x 5 cm) were used for the study. The pots were placed in plant trays to avoid seepage. The soil samples were air dried, ground, extraneous materials removed, sieved through a 2 mm sieve and homogenised. 300 g of the homogenised soil was weighed out and placed in each 1.5-litre plant pots. Three sets of glasshouse pots were prepared to consist of uncontaminated soils sets, contaminated soils without amendments and contaminated soils with amendments. Cow manure was used as soil amendment and was added to the contaminated soils to provide nutrients and as a diluent. Each Pot with the amendment was prepared by the addition of cow manure to the soils in a ratio of 1:6 (50 g of cow manure compost added to 300 g of soils). The amendment was properly mixed with soils for homogeneity. The amended petroleum-contaminated soils were used for growing sunflowers, treatment with fermented palm wine as well as the white rot fungus, *P. ostreatus* (Table 4.1). All glasshouse setups were carried out in triplicates for a period of 3 months (90 days).

Table 4.1: Glasshouse setups for remediation of petroleum-contaminated soils from Tibshelf, UK using sunflower species, palm wine and *P. ostreatus*

Samples	Soil quantity	Cow manure	Sunflower	Mushroom substrate	Mushroom Spawn	Palm wine
Set1: (Control 1) uncontaminated soil	300.00g	-	-	-	-	-
Set 2: (control 2) contaminated soil without amendment	300.00g	-	-	-	-	-
Set3: (control 3) contaminated soil with amendment	300.00g	50.00g	-	-	-	-
Set 4: contaminated soil + Sunflower	300.00g	50.00g	1 seedling	-	-	-
Set 5: contaminated soil + Palm wine	300.00g	50.00g	-	-	-	0.20l
Set 6: contaminated soil + *P. ostreatus*	300.00g	50.00g	-	20.00g	10.00g	-

4.2.2 Physicochemical properties of soil from Tibshelf, UK

Physicochemical properties of the soils such as particle size analysis, temperature, pH, electrical conductivity and available nitrate were monitored at the start of the glasshouse experiments and the end of the 90 days treatment period. Soil temperature, pH and electrical conductivity were determined insitu with soil conductivity meter model HI-98129 (Liebig *et al.,* 1996; Scoggins & van Iersel, 2006). Available nitrate was measured with a Horiba - LAQUAtwin NO3-11 - NO3-11C - NO3-11S Compact portable nitrate meter (Kubota *et al.,* 1996; Hampton *et al.,* 2019).

4.2.3 Glasshouse experiments with sunflowers

The aim of this setup was to investigate phytoremediation efficiency of sunflower species on petroleum-contaminated soils (Figure 4.2). Sunflower seedlings of *Helianthus annus* (Pacino gold), *Helianthus sunsation* and *Helianthus annus* (sunny dwarf) were first pre-grown on the Ericaceous compost for a period of 2 weeks. The 2 weeks old seedlings were then transplanted to the experimental pots containing the amended petroleum-contaminated soils in the glasshouse (Section 4.2.1). A control set of sunflower plants were also grown on compost. Soil moisture content was maintained by watering of the containing trays and uptake by capillary rise, and by vertical spraying every 4 days.

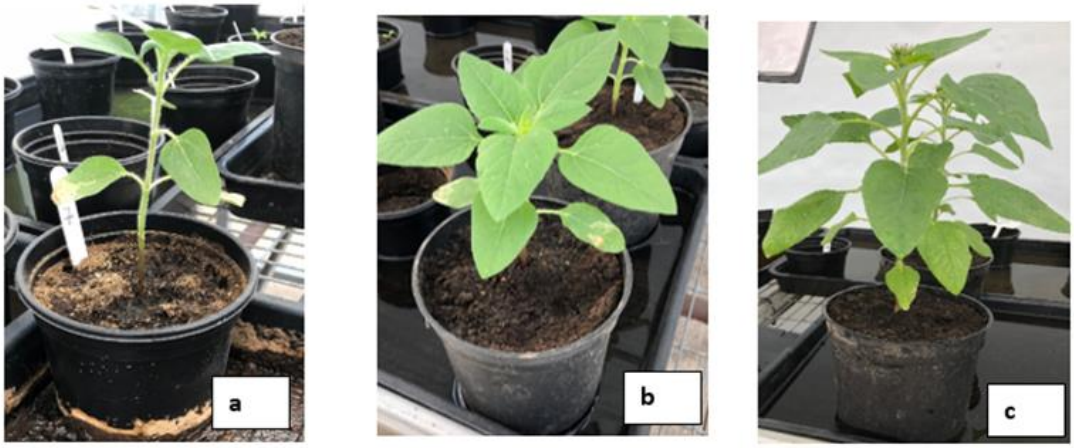

***Figure 4.2**: Sunflower plants growing on petroleum contaminated soils*
(a). Helianthus annus sensation, (b). Helianthus annus- sunny dwarf (c). Helianthus annus pacino gold
(d). sunflowers grown on uncontaminated soils

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4.2.3 Glasshouse experiments with fermented palm wines

Two of the dominant species of palm trees in the Niger Delta, Nigeria from which palm wine is tapped are *Elaeis guineensis* and *Raffia africana.* The remediation efficiency of palm wine from these two species of palm trees on petroleum contaminated soils was therefore investigated. The palm wines were left in the open overnight (12-18 hours) to ferment (Santiago-Urbina & Ruíz-Terán, 2014). 200 ml of each of the fermented palm wines were measured out and added to glasshouse pots containing amended petroleum-contaminated soils (Table 4.1). A further 200 ml of each freshly prepared fermented palm wines were added to the pots each week (Figure 4.3). The use of the 2 species of palm wine was to evaluate any variability in their remediation efficiency.

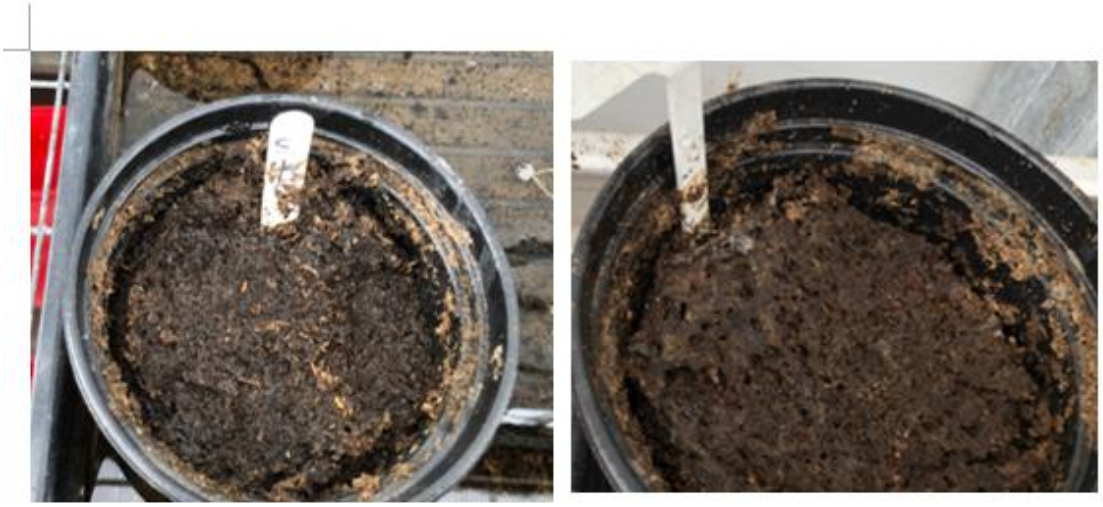

Figure 4.3: Glasshouse pots for remediation of petroleum contaminated soil with fermented palm wine

4.2.4 Glasshouse set with *P. ostreatus*

Two set of experiments were carried out with *P. ostreatus.* First, was an investigation of alternative substrates for growing the white rot fungus under unsterilized conditions. This was followed by assessing the effect of different application methods on the remediation efficiency of *P. ostreatus* on petroleum-contaminated soils.

For the first part, six substrates (Table 4.7) were investigated for growing *P. ostreatus* under sterilised and unsterilized conditions. Based on outcomes, Palms substrates of *Trachycarpus fortunei* was selected for the study. The palm substrates were prepared by maceration of plant parts (stems, roots, branches) and air-drying for one week. These parts were further pulverized to sawdust form after air-drying and used as substrate for growing *P. ostreatus*.

For assessing the effects of application procedures, three subsets were created. Firstly, the fungal spawns (10 g) were applied directly on petroleum-contaminated soils by mixing without the substrates. Secondly, the fungal spawns (10 g) were applied to the substrates (20 g) layered on top of the contaminated soils (Adenikpekun and Fasisdi, 2005; Ekundayo, 2014). Lastly, part of the substrates and spawns was mixed with the contaminated soils, with some parts also layered on top of the soils. This last set was prepared as follows: 10 g of substrates were mixed with amended soils. 5 g the fungal spawn were then added by uniformly spreading into the soils. This was then followed by

layering of another 10 g of substrates on top of these soils. The layered substrates were also inoculated with 5 g of the fungal spawns.

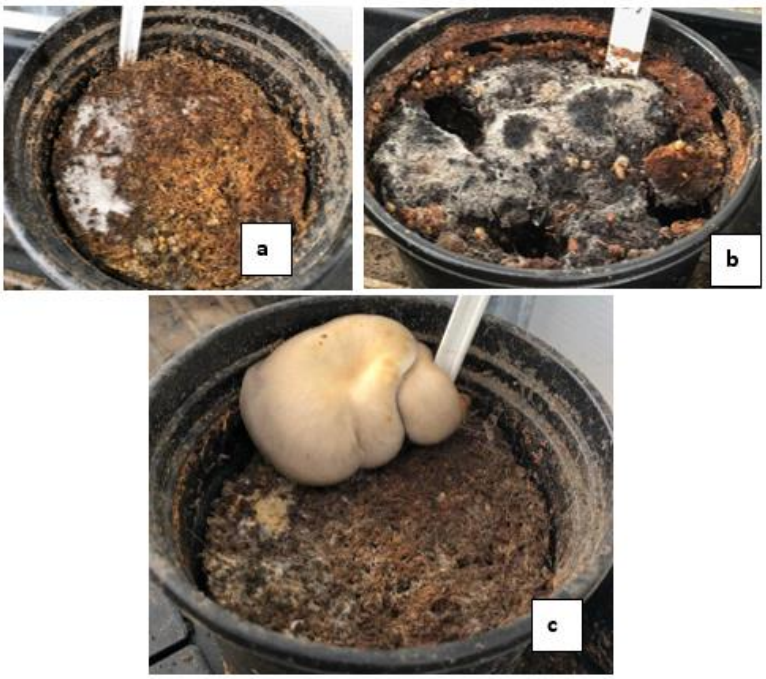

Figure 4.4: Glasshouse pots for remediation of petroleum contaminated soil with *P. ostreatus* using palm tree substrates.

(a). mushroom mycelia starting to germinate (b) mycelia fully germinated (c). fruity body of mushroom formed

4.2.5 Sample collection, preparation and analysis

After glasshouse set up, composite soil samples were collected at the start of the study (Time= 0 days) and after a 3-month treatment period (Time = 90 days). Samples were air dried, sieved through a 2mm mesh and extraneous materials removed, ground and homogenised prior to laboratory treatment and analysis (BS ISO 11464, 2014; Vane *et al*., 2014). Extraction of TPHs in samples was carried out using a microwave-assisted extraction with a Milestone MA182-001 ETHOS UP Microwave system, using a 1:1 acetone – heptane mixture (USEPA METHOD 3546; Punt *et al*., 1999). Commercial TPH diesel range standard was used for quantification of TPHs as described in section 3.5.2.3. Sample extracts and TPH standards were all analysed in a GC-MS (model Agilent Technologies 7000 GC/MS Triple Quad with 7890 GC and 7693 Autosampler (USEPA 8270E). GC-MS conditions are listed in Table 4.2.

Quantification of TPHs in the soils was carried out using the Methods of BS EN ISO 16703 (2011). Initial calibration of the instruments and evaluation of the concentration of TPHs, were carried out. Calibration verification was also carried out.

Table 4.2: GC-MS conditions for TPHs analysis

Column	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
Oven	45 °C (3 min), 20 °C/min to 360 °C (10 min)
Carrier gas	helium, 1.3 mL/min. constant
Injection	1.0 µL, splitless
Liner	2 mm I.D. straight
Injector temp.	250 °C
Detector	MSD, 300°C

4.3 Results

4.3.1. Physicochemical properties of the soils

Particle size analysis of the soil samples (Table 4.3), revealed higher clay contents (50%), followed by silt (30%) and sand particles (20%) in control soils from Tibshelf, Derbyshire. A similar trend was observed in the petroleum-contaminated soils from the area. Levels of clay particles were higher in petroleum-contaminated soils than those of controls (Table 4.3).

Table 4.3: Particle size analysis of soils from Tibshelf, UK. (particle size analysis was carried out after the removal of the crude oil from the soil. 5g of the soil samples were pre-washed with 10 ml hexane solution (99% v/v) to remove hydrocarbon contaminants (Taubner *et al.,* 2009).

		Particle size composition %			
Soil sample	**Description**	**Sand**	**Clay**	**Silt**	**Classification** (Wentworth, 1922).
1	Uncontaminated soil	20	50	30	Clayey loam
2	Petroleum-Contaminated soil	10	65	25	Clayey loam

Soil pH was significantly affected ($p<0.05$) by petroleum contamination (Table 4.4). pH of control soil samples was near neutral (7.35-7.55) and were not significantly different

during the experiments. The pH of untreated petroleum-contaminated soil samples was acidic (pH 6.53 – 6.58) and were significantly different (p= 0.000 at T=0, 0.002 at T=3) from those of uncontaminated soils. The pH of cow manure was in the range of 9.42-9.49. The addition of cow manure to the petroleum-contaminated soils increased soil pH from acid to alkaline (Table 4.4). The pH values were maintained at slightly alkaline levels (8.50 – 8.90) throughout the duration of the remediation. pH in soils treated with cow manure were not statistically different ($p>0.05$) during the treatment period from those of controls.

A sharp decrease in concentration of available nitrate was observed in untreated petroleum-contaminated soils compared to controls (Table 4.4). The addition of cow manure to the petroleum-contaminated soils significantly increased ($p< 0.05$) the concentration of available nitrate in the soils (Table 4.4). The concentration of available nitrate also increased as remediation progressed and correlated positively with % decrease in the soils' TPHs (Figure 4.5). A Similar trend was also observed for electrical conductivity. Electrical conductivity values increased as remediation progressed and correlated positively with % decrease in TPHs (Figure 4.6). Variation in temperature values was also observed during the treatment but no particular trend could be deduced for temperature. Soil temperature range during the treatment was 19- 23^0C.

Table 4.4: Variation in the physicochemical properties of petroleum-contaminated soils and controls from Tibshelf, UK during glasshouse remediation treatment with sunflower species, fermented palm wine and *P. ostreatus.* Values are given as average of triplicates with standard error (S.E). Sample size, n=36

Samples/Treatment	**Temperature °C**				**Ph**				**Electrical conductivity**				**Available Nitrate (mg/l)**			
	T=0	**S.E**	**T=3**	**SE**	**T=0**	**S.E**	**T=3**	**SE**	**T=0**	**S.E**	**T=3**	**SE**	**T=0**	**S.E**	**T=3**	**SE**
Uncontaminated soil samples	20.43	0.17	22.80	0.14	7.35	0.04	7.55	0.05	7.35	0.03	1.03	0.03	621.3	5.21	610.0	4.96
Cow manure	18.83	0.18	21.20	0.47	9.42	0.04	9.49	0.08	9.42	0.02	3.24	0.02	700.3	4.95	700.0	3.77
Untreated petroleum-contaminated soil without amendment (Control 1)	21.75	0.13	21.74	0.30	6.40	0.02	6.44	0.11	6.40	0.01	0.23	0.01	33.33	0.27	36.00	0.47
Untreated petroleum-contaminated soil + amendment (Control 2)	21.17	0.01	22.43	0.44	8.54	0.06	8.70	0.05	8.54	0.05	2.85	0.08	436.7	2.72	469.0	4.78
Petroleum-contaminated soil + *Helianthus annus*	20.60	0.25	22.67	0.28	8.61	0.11	8.53	0.03	8.61	0.04	2.68	0.01	486.7	2.72	793.3	2.72
Petroleum-contaminated soil + *Helianthus sunsation*	20.85	0.12	21.96	0.04	8.63	0.11	8.70	0.07	8.63	0.05	2.81	0.03	486.0	2.49	756.7	15.15
Petroleum-contaminated soil + *Helianthus annus (sunny dwarf)*	19.50	0.25	20.70	0.74	8.65	0.19	8.90	0.00	8.65	0.04	3.07	0.05	465.0	4.08	910.0	4.71
Petroleum-contaminated soil + fermented palm wine from *Elaeis guineensis*	21.00	0.00	20.80	0.66	8.82	0.02	8.90	0.02	8.82	0.08	3.47	0.05	416.7	17.84	903.3	2.72
Petroleum-contaminated soil + fermented palm wine from *Raffia Africana*	20.57	0.46	20.84	0.36	8.55	0.15	8.89	0.01	8.55	0.05	3.80	0.08	393.3	5.44	873.3	5.44
Petroleum-contaminated soil + *P. ostreatus* without substrates	20.60	0.17	21.00	0.47	8.57	0.01	8.60	0.00	8.57	0.07	2.77	0.03	440.0	4.71	483.3	2.72
Petroleum-contaminated soil + *P. ostreatus +* substrates layered on soil	21.03	0.03	20.30	0.09	8.55	0.13	8.90	0.00	8.55	0.00	3.00	0.00	430.0	0.00	720.0	0.00
Petroleum-contaminated soil + *P. ostreatus +* substrates mixed with soils and layered	21.01	0.08	20.60	0.33	8.79	0.04	8.73	0.12	8.79	0.02	3.83	0.03	483.3	11.86	973.3	5.44

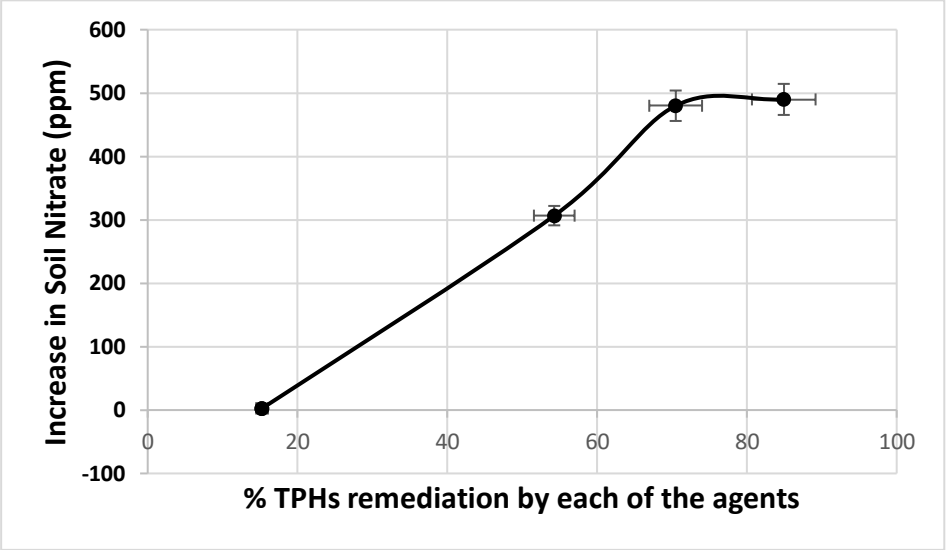

Figure 4.5: Variation in soil nitrate with % remediation (reduction) in TPHs during remediation of petroleum-contaminated soils from Tibshelf, UK (Error bars represents standard deviation δ). The figure is a plot of the % reduction in TPHs by each of the agents against the corresponding increase in soil nitrate associated with each agent during such remediation. For instance, % remediation was obtained my subtracting the concentration of TPHs at time=0, and time=90 days for a particular agent. The increase in concentration of nitrate was also similarly evaluated (at time=0, time=90 days for each of the agents). The curve shows a steady rise of available nitrate with increase remediation and peaks at remediation efficiency of 70%. These points correspond to TPHs concentrations in uncontaminated soils. Sample size, n=12

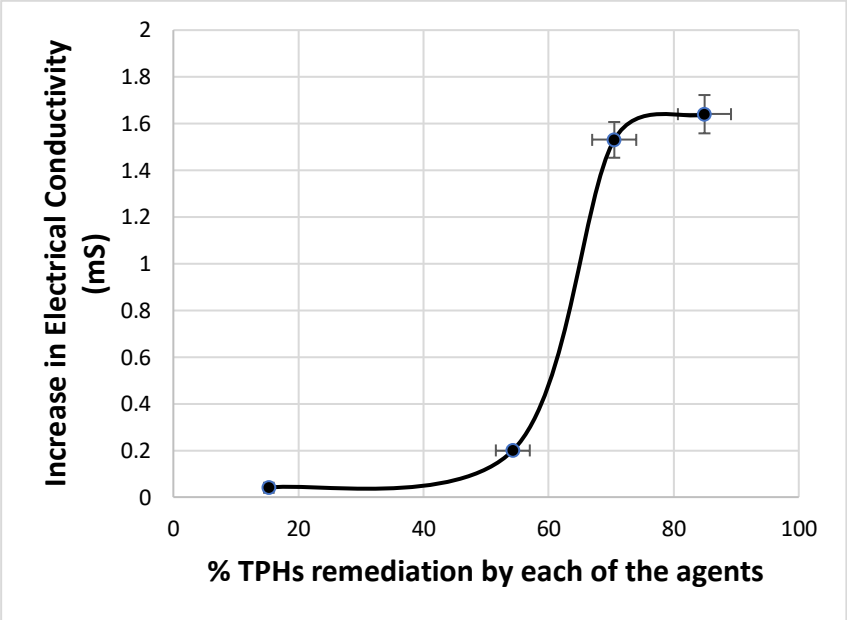

Figure 4.6: Variation of soil's EC with % remediation (reduction) in TPHs during remediation of petroleum-contaminated soils from Tibshelf, UK (Error bars represents standard deviation δ). The figure is a plot of the % reduction in TPHs by each of the agents against the corresponding increase in soil electrical conductivity associated with each agent during such remediation. For instance, % remediation was obtained my subtracting the concentration of TPHs at time=0, and time=90 days for a particular agent. The increase in electrical conductivity values was also similarly evaluated (at time=0, time=90 days for each of the agents). The curve shows a steady rise of electrical conductivity with increase remediation and peaks at remediation efficiency of 70%. These points correspond to TPHs concentrations in uncontaminated soils. Sample size, n=12.

4.3.2 Concentrations of TPHs in soils of Tibshelf, UK

Initial concentrations of TPHs in glasshouse pots varied from 130 to 340 g of TPHs/Kg dry weight of soil with the highest (340 g/Kg) observed in pots of untreated soils. Variations were also observed among the treatment sets such as those of sunflower species, palm wine and those treated with *P. ostreatus* (Figure 4.7). For the set involving the three-sunflower species, initial TPHs concentrations were 200 g/Kg for *H. annus-sunny dwarf,* 250 g/Kg for *H. sunsation a*nd 150 g/kg for *H. annus-pacino gold.* Glasshouse pots treated with fermented palm wine from *E. guineensis* had initial TPHs concentration of 340 g/Kg while that of *R. africana* was 280 g/Kg dry weight of soil. Concentration of TPHs at time = 0 days were 290, 130, and 210 g/Kg dry soils for *P. ostreatus* applied without substrates, applied *by* layering *substrates* on topsoil and that applied by a combination of mixing substrate with soil and layering.

After the 90 days remediation treatment, the concentration of TPHs decreased to 290, 90, 120 and 50 g/Kg dry soil for untreated soils and those treated with *H. annus-sunny dwarf*, *H. sunsation* and *H. annus-pacino gold*. For the set treated with palm wine the concentration of TPHs were 100 for *E. guineensis* and 90 for *R. africana*. Treatments with *P. ostreatus* resulted in 210, 60 and 30 g of TPHs per Kg dry soil for the applications without substrates, layering substrates on topsoil and those by a combination of mixing substrate with soil and layering, respectively.

4.3.3 Remediation efficiencies of sunflower species, fermented palm wine and *P. ostreatus* on the petroleum-contaminated soils

All the agents used in the treatment of the petroleum-contaminated soils demonstrated noticeable remediation efficiency after 90 days (Figure 4.7). A comparison of remediation efficiencies of the agents revealed the following order *P. ostreatus* > palm wine > sunflower species (Table 4.5). This was however based on enhanced application method of mixing substrates and the fungus with soils followed by layering. With respect to the typical method of layering *P. ostreatus* and substrates on soils, the remediation efficiency of the agents was observed in the following order Palm wine > *P. ostreatus* > sunflower species.

For the sunflower species, highest percentage reduction in TPHs was obtained with *H. annus* (Pacino gold) and was 69% (Table 4.5). This was followed by *H. annus* (Sunny dwarf) (54%) and lastly by *H. sunsation* (53%). Phytoremediation efficiencies of the sunflower species were significant ($p<0.05$) compared to those of control soils. Similar remediation efficiency was observed in the remediation potential of *H. sunsation* and *H. annus (*Sunny dwarf) ($p>0.05$). Remediation efficiency of *H. annus (*pacino gold) was observed to be significantly higher ($p<0.05$) than those of both *H. sunsation* and *H. annus (*Sunny dwarf) (Table 4.5).

Table 4.5: % reductions in TPHs levels in petroleum-contaminated soils treated with sunflower species, fermented palm wine and *P. ostreatus.* Sample size, n=18.

Samples/Treatment	**% reduction In TPHs contents of soils between T=0 days and T=90 days**	**p-values (@ 95% CI) of T=90 days values against T=0 days**
Untreated Petroleum-contaminated soil (Control)	15	0.294
Petroleum-contaminated soil + *H. annus (Sunny dwarf)*	54	0.001
Petroleum-contaminated soil + *H. sunsation*	53	0.003
Petroleum-contaminated soil + *H. annus (*Pacino gold*)*	69	0.011
Petroleum-contaminated soil + fermented palm wine from *E. guineensis*	70	0.001
Petroleum-contaminated soil + fermented palm wine from *R. africana*	69	0.000
Petroleum-contaminated soil + *P. ostreatus* without substrates	29	0.164
Petroleum-contaminated soil + *P. ostreatus +* substrates layered on soil	60	0.000
Petroleum-contaminated soil + *P. ostreatus +* substrates mixed with soils and layered	84	0.000

p-values are for T-test on TPHs concentrations at T=0 days against Time = 90 days, to see if TPHs concentrations at Time = 90 days (after remediation) are significantly different from T=0 days (before remediation). Raw data are in Appendix III-2. P-values highlighted in red signify results where there is no significant differences in TPHs concentration at Time =0 days and 90 days ($p>0.05$). Those highlighted yellow signify results where there is significant differences between in TPHs concentration at time= 0 days and 90 days.

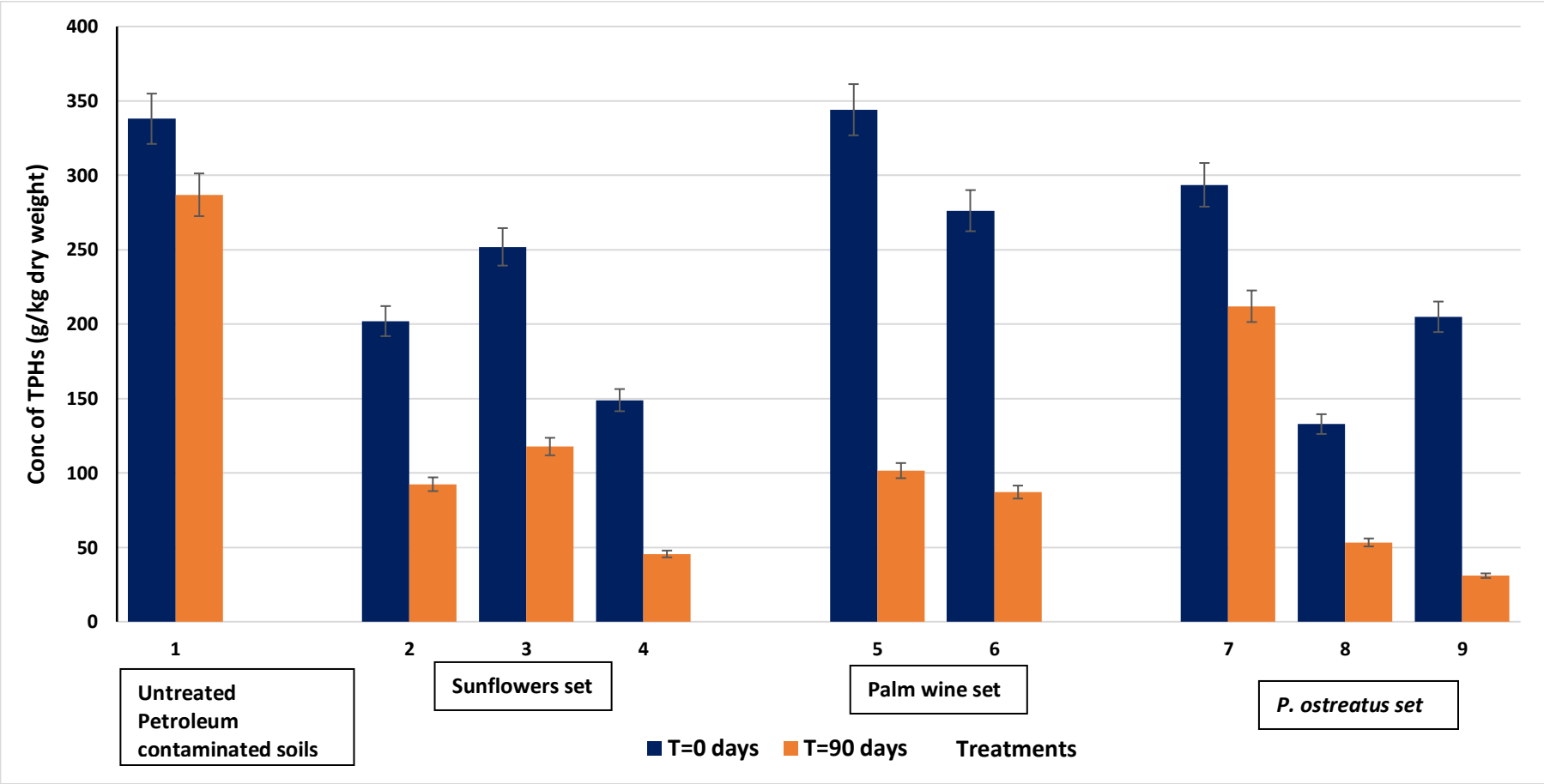

Figure 4.7: Concentration of TPHs in soils of Tibshelf, UK, at start of remediation (T= 0 days) and after T= 90 days of treatments with the different agents. Errors bars represents standard deviation from the mean of triplicate analysis. Raw values are available in Appendix III-2: Treatment numbers are as follows:
(1). Untreated Petroleum-contaminated soil (Control); **(2).** Contaminated soil + Helianthus annus (Sunny dwarf); **(3).** Contaminated soil + Helianthus sunsation **(4).** Contaminated soil + Helianthus annus (Pacino gold); **(5).** Contaminated soil + fermented palm wine from Elaeis guineensis **(6).** Contaminated soil + fermented palm wine from Raffia africana **(7).** Contaminated soil + *P. ostreatus* without substrates **(8).** Contaminated soil + *P. ostreatus* + substrates layered on soil **(9).** Contaminated soil + *P. ostreatus* + substrates mixed with soils and also layered. . The soils used for the study were amended with cow manure. Sample size, n= 54. Soil used for the study were amended with cow manure.

Physiological parameters such as height and biomass of sunflower plants were negatively affected by presence of petroleum contaminants in soils (Table 4.6). Plants growing in contaminated soils had lower height and biomass. A positive correlation was observed between phytoremediation efficiency of sunflower species and their biomass (Figure 4.8). *H. annus (Pacino gold)* had both the highest biomass and remediation efficiency with respect to TPHs on petroleum-contaminated soil (Table 4.6).

Table 4.6: Physiological properties of 3 species of sunflower plants grown on petroleum-contaminated soils and controls from Tibshelf, UK. Values are mean of triplicates measurements with standard deviation. Sample size, n= 18.

Samples	**Height of plants (cm)**		**Total dry biomass of plants (g)**	
Sunflower species	Grown on uncontaminated soil	Grown on petroleum contaminated soil	Grown on uncontaminated soil	Grown on petroleum contaminated soil
H. annus (pacino gold)	75.11 ± 2.00	45.80 ± 0.95	49.49 ± 0.53	23.75 ± 0.46
H. sunsation	29.40 ± 0.32	19.28 ± 0.41	29.30 ± 0.43	16.75 ± 0.93
H. annus (sunny dwarf)	32.84 ± 1.19	20.67 ± 0.70	30.17 ± 0.82	18.29 ± 0.59

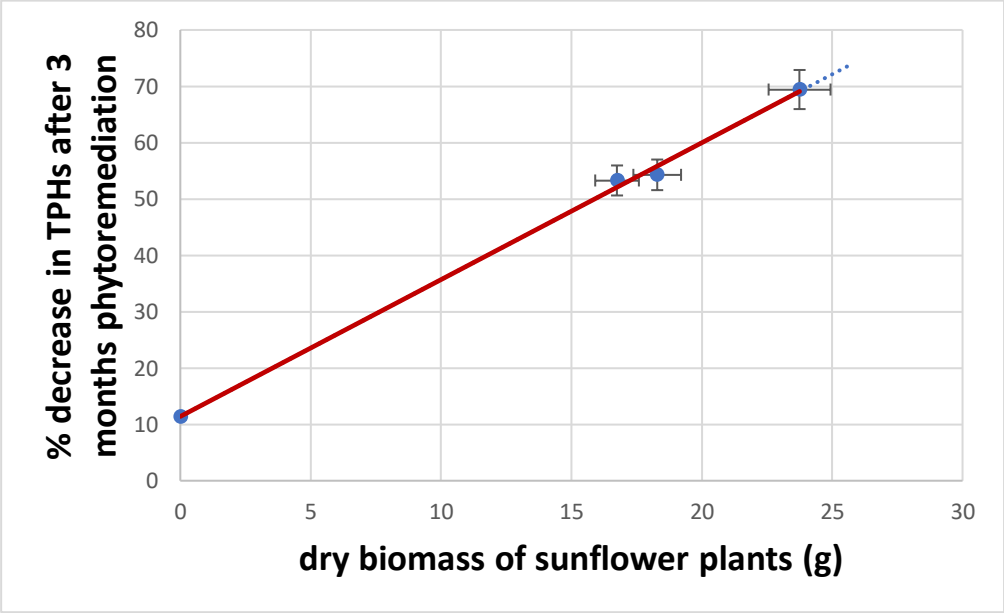

Figure 4.8: Correlations between remediation efficiencies (% decrease in TPH) and dry biomass of sunflower plants. Errors bars represents standard deviation of the mean. Sample size, n=12.

Fermented palm wine from the two species of palm trees (*E. guineensis* and *R. africana*) each demonstrated significant remediation potential ($p<0.05$), on petroleum-contaminated soils compared to controls (Figure 4.7). Palm wine from *E. guineensis* effected up to 71% reduction of TPHs while that from *R. africana* was 69% (Table 4.5). There was no significant difference ($p=0.000$) in remediation efficiencies of fermented palm wine obtained from *E. guineensis* or *R. africana.*

Investigation of alternative substrates for growing *P. ostreatus* revealed that the fungus germinated and grew faster under sterilised conditions (Table 4.7). Growth of *P. ostreatus* was still achieved under unsterilized conditions at temperatures of 10-15^{0}C. Shorter timeframe was observed for germination and fruiting of *P. ostreatus* with substrates such as palm tree and pine bark compared to others.

Table 4.7: Growth of *P. ostreatus* under sterilised and unsterilized conditions using different substrates. Estimated lignin content of substrates is also given. Sample size, n=18

		Time Taken for mushrooms to germinate and produce fruity body (weeks)		**Temperature of growth = 10 - 15^{0}C**	
S/N	**Substrate Type**	**Without sterilisation**	**With sterilisation**	**Extent of germination of mushroom mycelia**	**Estimated lignin content of substrates**
1	Cassava peels	**6**	**3-4**	Very extensive	7.52 % (Daud *et al.,* 2014)
2	Pine barks	**3-4**	**2**	Very extensive	53.36% (OLÁR *et al.,* 1998)
3	Palm tree	**3-4**	**2**	Very extensive	32.8% (Abdul *et al.,* 2006)
4	Maize cob	**8**	**3-4**	Very extensive	16–18% (Wang *et al.,*2011)
5	Saw dust	**8**	**4**	Extensive	Varies according to wood type (Joshua, 2016).
6	Hay	**8**	**4**	Extensive	7.1 and 7.8 (Whitehead & Quicke, 1964).

The effectiveness of these substrates to support the growth of the fungus can also be related to their lignin contents (Table 4.8). *P. ostreatus* was able to grow faster on substrates with higher lignin contents. Pine bark and palm tree substrates exhibited better growth even in unsterilised conditions.

The highest remediation efficiency of 85% for TPHs in soils treated with *P. ostreatus* was obtained by the method of mixing both the fungus and substrates with soils combined with layering. This was followed by that of layering substrates and fungus on soil (60%), while the method of mixing the fungsl spawn with soils without substrates resulted in 28% TPHs reduction (Table 4.5). Application of *P. ostreatus* using the combined method of mixing the fungus and substrates with soils and layering resulted in 25% increase in remediation efficiency compared to the usual method of layering. There was also a significant difference in remediation efficiency between contaminated soils treated by layering *P. ostreatus* with substrates and the control (Table 4.5). Although there were reductions in TPHs in untreated soils and those treated with *P.* ostreatus without substrates (Table 4.5), the difference was not significant (p=0.294) when compared to the TPHs values in soils at Time =0 and Time =90 days.

4.3.2 Discussion

4.3.2.1 Physicochemical properties of the soil from Tibshelf, UK

From particle size analysis, the soil from Tibshelf, Derbyshire can be categorised as clayey loam. This finding agrees with data from the UK soil maps (Soilscapes, 2017). Higher levels of clay particles in petroleum-contaminated soils obtained from the study area suggest a particle degradability by crude oil on soil. Studies such as Okoro *et al.* (2011) and Abosede (2013) demonstrated that crude oil pollution could increase clay and silt particulates in soils.

The constituents of the contaminating crude oil and ease of abrasion are two possible factors that could boost the weathering of soil particles during contamination. Crude oil consists of trace metals, and acidic compounds such as sulphur, halides and nitrogenous compounds (Dickson & Udoessien, 2012; Ribeiro *et al.,* 2019; Vane *et al.,* 2019). Organic acids have also been reported in crude oil samples (Meredith *et al.,* 2000; Robichaud *et al.,* 2019). These compounds can interact with soil chemicals resulting in solutions, which could accelerate the breakdown of soil particles (Blattmann *et al.,* 2019). In terms of abrasion, the presence of oil in soils can lead to more frequent drifting and rubbing of particles, which could enhance break down, by mechanical actions. There is also a

possible combined effect of chemical and mechanical weathering of soil particles induced by the presence of petroleum contaminants (Holbrook *et al.,* 2019).

Gogoi *et al.* (2003) reported a pH of 4.5 in soils at a petroleum-spilled site. Osuji & Nwoye (2007), reported a pH of 4.9 – 5.1 in petroleum-contaminated soils. The soil acidification by petroleum contaminants observed in this study thus corroborated those of Gogoi *et al.* (2003) and Osuji & Nwoye (2007). The observed decrease in soil pH by petroleum contaminants is due to the constituents of the contaminating petroleum. For instance, petroleum with high contents of acids and acid anhydrides will reduce soil pH. Interactions of these constituents with soil chemicals could further results in acidic substances, which can further reduce the pH of petroleum-contaminated soils (Sarkar *et al.,* 2005).

As observed in this study, Whalen *et al.* (2000) reported that cow manure can be used to amend acidic soil to near neutrality. The result of the study further revealed that the cow manure acted as a buffer, which provided appropriate pH during the remediation treatments (Table 4.5, 4.7, and 4.9). Gogoi *et al.* (2003) stated that favourable pH range is required for optimal performance of remediation agents in soils. The suitable pH aids in the release of soil nutrients (Tisdale & Nelson, 1958). Degradation of organic contaminants in soils is also known to proceed faster at slightly alkaline pH (Owen *et al.* 1977; Xu *et al.,* 1994). Therefore, the pH condition (8.50-8.9) induced by the addition of cow manure in the present study provided suitable conditions for the remediation to proceed.

Solubility and bioavailability of the hydrophobic petroleum contaminants are possible reasons for the observed increase in remediation activity at the relatively high pH (Xu *et al.,* 1994). The solubility of organic matter is known to be relatively low around pH 4.6 and 6.4 but increases markedly beyond this range to a maximum of around 7.7 (Ashworth & Alloway, 2008). The high solubility of organic matter at slightly alkaline pH leads to more organic matter in soil solutions (Jardine *et al.,* 1989). The solutions become more hydrophobic and more petroleum contaminant molecules are drawn into it, thereby increasing their bioavailability.

John *et al.* (2016) stated that the presence of petroleum hydrocarbons in soils immobilises available nitrate. From the present investigations, it was observed that high concentrations of petroleum contaminants resulted in low values of nitrate in soils and vice versa. When these contaminants were taken out of the soils during remediation, more nitrate were realised (Table 4. 4). This illustrated the release of nitrate that were immobilised by petroleum contamination as remediation progressed. At high levels of remediation (Table 4.4, Figure 4.4), values of the available nitrate were greater than those of contaminated soil, contaminated soils with amendment and even those of the cow manure alone. It can therefore be deduced that in addition to the nitrate supplied from the cow manure (Tarkalson *et al.,* 2006), certain nitrate that were immobilised by petroleum contamination were then available due to reduction in the concentration of TPHs as remediation progressed.

The observed reduction in available nitrate with high petroleum contaminations in soils is related to the great affinity for organic compounds such as organic matter by soil nitrate (Taylor & Townsend, 2010). Therefore, some of the nitrate is sequestered by the high content of petroleum contaminants making them unavailable in soil solutions. In addition, the petroleum contaminants also take up much of the soil pores thereby reducing nitrate availability. The addition of cow manure increased organic matter and moisture contents (Raviv *et al.,* 2004) and consequently diluted the concentration of the petroleum contaminants. Thus, more nitrates were available in soil solutions. The presence of certain functional groups (such as carboxylic acids and hydroxyl groups) in the organic matter of cow manure further aided the preferential attraction of nitrate away from the petroleum contaminants into soil solutions, making them more available.

Tejada *et al.* (2006) and Khamehchiyan *et al.* (2007) described increase in physicochemical properties of petroleum-contaminated soils on addition of organic manure. In this study, the addition of cow manure to the petroleum-contaminated soils increased soil pH, electrical conductivity and available nitrate. The cow manure served as diluents reducing the concentration of TPHs in soils and provided nutrients for the plants' growth. As remediation progressed, the values of soil nitrate and electrical

conductivity increased and was proportional to the remediation efficiency of the agents (Table 4.4). Thus, available nitrate and electrical conductivity can serve as possible indicators of TPHs' remediation progress in petroleum-contaminated soils.

4.3.2.2 Remediation efficiency of the agents

Variation in the concentration of TPHs in the soil samples used for the glasshouse study was due to the inhomogeneity of the petroleum contaminants in the soil matrix. Variable concentration of TPHs is expected in soils due to factors such as proximity to contaminants source, duration of impact, constituent soils particles, soil structure and chemistry (Bu *et al.,* 2009; Yang *et al.,* 2015). Soil samples collected at proximity to contaminant sources are expected to have higher concentrations of contaminants than others (Dudhagara *et al.,* 2016). The soil samples for the present study, soils were collected at various locations around the point of contamination, then mixed as composite. Some points at the sampling points had large objects such as dead leaves, large roots, gravels and wood chips. These substances can obstruct penetration and distribution of the oil in the soil matrices resulting in uneven spread, hence variation. The soils from Tibshelf, used in the study had predominant clay particles (Table 4.3) which are crumby and difficult to break. These crumbs each accumulate peculiar levels of the petroleum contaminants and there may even be sub-aggregates within the soil crumbs. In this study, the soil samples were mixed by hand, there is also the chances of poor homogeneity. These factors all resulted in the observed variability in TPHs levels at T= 0 days in the soil samples (Puri *et al.*,1994; Kristensen *et al.*, 2010; Xing *et al.*, 2011).

Variations in contaminant levels is expected even during in-situ applications due to the indicated factors. This has been a major challenge in the remediation of soils (Zalesny *et al.,* 2005). The applicability of a remediation method under varying concentrations of the target contaminants is therefore important. Adenipekun *et al.* (2015) stated that mycoremediation methods are not specific to contaminant concentrations, thus variation in the initial levels of concentrations should not affect the applicability of the results. However, it would be ideal to further investigate the effect of initial concentrations on remediation potential of the phyto-and myco-remediation agents.

The general observation in this study was that concentrations of TPHs at Time = 90 days was proportional to their respectively concentrations at Time = 0 days. For instance, for the sunflower species, TPHs concentrations at time = 0 was 200 and 90 g/Kg at time = 90 days for *H. annus-sunny dwarf* and 250 and 120 g/Kg for *H. sunsation*. This trend also holds for the palm wine set (340 and 100 g/Kg for *E. guineensis;* 280 and 90 g/Kg dry soil for *R. africana* at time = 0 and 90 days, respectively). Winquist *et al.* (2014) demonstrated a 96% degradation of PAHs with an initial concentration of 3500 mg/kg in a glasshouse, and 94% during a field study with an initial concentration of 1400 mg/kg of soil, after three months. Thus, it is reasonably assumed that the initial concentration of TPHs at the start of the remediation does not affect the remediation potential of the agents. Hence, the variability in initial concentrations of TPHs among sample treatment sets can be accommodated.

Although the samples labelled 1 (figure 3) were not treated, natural attenuation plays a part in reduction of TPHs in soils, however, the progress is very slow, and most times insignificant (O'Brien *et al.,* 2019).

4.3.2.2.1 Sunflower species

Plants can phyto-extract, phyto-degrade or phytostabilise organic contaminants in soils (Pilon-Smits, 2005). Thus, the sunflowers could utilise any of these mechanisms. Hassan *et al.,* (2018) demonstrated a phytoremediation efficiency of up to 56% with the sunflower (*Helianthus annus)* in the remediation of crude oil contaminated soils supplemented with inorganic fertilisers. Liduino *et al.* (2018) demonstrated up to 58% and 48% reduction of TPHs by sunflowers (*Helianthus annus L.)* supplanted by biosurfactants on petroleum-contaminated soils after three months. A similar range of phytoremediation efficiencies (53%, 54%) was observed for 2 of the sunflower species used in the present study, substantiating the potential of sunflowers for remediation of petroleum-contaminated soils. The sunflower plants could not grow in the high concentrations of crude oil in the soils of the present study without amendment. The plants only grew in the amended soils. The use of cow manure as a source of amendment in this study demonstrated that organic manures can be used to successfully initiate phytoremediation of petroleum-contaminated soils.

The reduction in height and biomass of the sunflower plants observed in the present investigation are comparable to that of Brandt *et al.* (2006). These reductions can be attributed to difficulties in adapting to the stress environment, insufficient nutrients or toxicity of the petroleum contaminants (Merkl *et al.,* 2005). Jong (1980) reported that soil contamination by crude oil leads to a reduction in nutrient parameters such as available nitrate with a marked reduction in water uptake. These are essential factors for plant growth. Thus, any constrain which negatively affects nutrients and water availability would invariably result in poor growth and biomass yield.

Robinson *et al.* (1998) and Chekol *et al.* (2004) stated that phytoremediation efficiency is influenced by biomass of plants. Plant species with higher biomass are known to exhibit better phytoremediation potential (Kayser *et al.,* 2000; Mejáre & Bülow, 2001; Chekol *et al.*, 2004). Both *H. annus (sunny dwarf)* and *H. sunsation* exhibited similar heights and biomass (Table 4.6). The measured height and biomass of *H. annus (Pacino gold)* was observed to be significantly higher than those of *H. annus (sunny dwarf)* and *H. sunsation* (Table 4.6). Therefore, the remediation efficiency of the sunflower species with respect to TPHs is related to their biomass (Figure 4.2). This finding is important for in-situ application. It implies that using sunflower species with high biomass would produce better results in the treatment of petroleum-contaminated soils. In addition to the TPHs remediation effectiveness, the use of sunflowers for treatment of petroleum-contaminated soils will provide the additional benefits of removing trace metals and other pollutants, with aesthetic appeal (Hull *et al.,* 2000; Duncan *et al.,* 2004; Simus, 2008; Barrett, 2011; Chauhan & Mathur, 2018).

4.3.2.2.2 Fermented palm wine

Chandrasekhar *et al.* (2012) stated that fermented palm wine consists principally of yeast of the *Saccharomyces* species. A consortium of microbial species such as yeast, *candida*, *pichia*, *lactobacillus* and *acetobacter* are also found in palm wine (Santiago-Urbina & Ruíz-Terán, 2014; Nwaiwu *et al.,* 2016). Therefore, the remediation potential of palm wine may be a synergy among the different species of microorganisms present in the fermentation product (Santiago-Urbina & Ruíz-Terán, 2014). Enhanced remediation of petroleum-contaminated soils by synergistic microbial relationships is

well known (Chhatre *et al.,* 1996; Rahman *et al.,* 2003; Gallego *et al.,* 2007; Fan & Qin, 2014). Chandrasekhar *et al. (*2012) and Nwaiwu *et al.* (2016) also reported that fermented palm wine chemically consists of mixtures of alcohols such as ethanol, propanol and methanol; esters like ethyl propanoate; and organic acids such as ethanoic, methanolic and propanoic acids. These compounds are organic solvents and can act as surface-active agents (Mahmood *et al.,* 2019; Stjerndahl *et al.,* 2019). Therefore, the observed remediation potential of fermented palm wine would be a combined action of a consortium of microorganism and that of surfactants organic compounds.

The observed similarity in mycoremediation potential of fermented palm wine on petroleum-contaminated soils from the two species of palms trees indicated that palm wine obtained from these sources may be of similar microbial or chemical constituents. This implies that palm wine from other palm trees can also be used for the treatment of petroleum-contaminated soils. The use of fermented palm wine for remediation of petroleum-contaminated soils is very promising because there are varieties of palm trees for the supply of palm wine in tropical climates like the Niger Delta, Nigeria (Svenning, 1999; Kwon-Ndung *et al.,* 2016; Asuk *et al.,* 2018). Application of fermented palm wine for remediation of petroleum contaminated soils as observed in this study did not required much expertise and preconditioning, substrates or nursery activities. The fermented Palm wine can easily be applied directly to the soil. This makes it a method of choice compared to the other agents used in the study.

4.3.2.2.3 P. ostreatus

4.3.2.2.3.1 Investigating of substrate for growing P. ostreatus

Adenipekun and Lawal (2012) stated that substrates sterilisation helped in the decontamination of other microflora, which can compete and slow down the growth of white rot fungi. Thus, these fungi could grow faster in sterilised substrates due to absence of competition from other microflora. However, the destruction of these natural microflora by sterilisation can eliminate their activities which may be useful in the remediation processes. It was, therefore, necessary to investigate the feasibility of growing the fungus- *P. ostreatus* without sterilisation. This investigation found that *P. ostreatus* can still grow in substrates without sterilisation (Table 4.8). The outcome is

useful for potential in situ application since it saves energy and removes the need for sterilisation equipment. It would also allow for the contribution of natural microflora towards remediation.

White rot fungi such as *P. ostreatus* naturally feed on lignin (Crawford & Crawford, 1976; Rabinovich *et al.,* 2004); therefore, substrates with higher lignin contents should favour its growth. *P. ostreatus* germinated and fruited faster in substrates with higher lignin contents (Table 4.7). A further study to also investigate the types of lignin present in these substrates and possible application of biotechnology for extraction is required. This would aid their commercial preparations for prospective use in the treatment of petroleum-contaminated soil (Gottlieb *et al.,* 1950). The cultivation of white rot fungi on palm substrates is yet to be reported; therefore, this investigation has added palm trees as promising substrates for the cultivation of white rot fungi. Palm substrates will also serve as valuable substrates for the application of the mycoremediation on petroleum-contaminated soil, particularly in tropical regions like the Niger Delta, Nigeria.

4.3.2.2.3.2 Effects of application procedures on mycoremediation efficiency of *P. ostreatus*

The typical method of application of *P. ostreatus* on petroleum-contaminated soils is by layering (Stamet, 2005; Adenipekun *et al*., 2015). The results in this study demonstrated that mixing palm tree substrates and the fungus with soil combined with layering on topsoil resulted in significant enhancement (p=0.000) of mycoremediation efficiency of *P. ostreatus* on petroleum-contaminated soils even under unsterilized conditions (Table 4.5). The observations can be attributed to an increase in surface area of contact between the fungus and the hydrocarbons contaminants in the soil matrix (Wincele *et al.,* 2004; Singh & Gauba, 2014). Since these substrates were not sterilised, activities of other microorganisms may also contribute to the observed results.

Adenipekun and Fasidi (2005) obtained a biodegradation of 20% after 3 months and 40% after 6 months on petroleum-contaminated soils. Mehrasbi *et al.* (2003) reported remediation efficiencies of 36%, 55% and 60% after 6 months. The results obtained by layering in this study under unsterilised conditions are comparable to those of Adenipekun and Fasidi (2005), Mehrasbi *et al.,* (2003), Chiu *et al.,* (2009) and

Adenipekun *et al*., (2015) using *P. ostreatus* under sterilised conditions. However, the remediation efficiency obtained by mixing the substrates and the fungus with soils combined with layering is much higher than those reported in the literature. The soils used in this study had very high initial levels of TPHs and were conventional petroleum-contaminated soils taken from a site in close proximity to an exuding oil well at Tibshelf, UK. Thus, the remediation outcome of *P*. ostr*eatus* (85%) under these conditions is unique.

Although fungi can grow straight from spawn without substrates, most times this is not ideal (Royse & Beelman, 2007). Meysami and Baheri (2003) reported that substrates are required for action of white rot fungi on petroleum-contaminated soils. Mamiro & Royse (2008) stated that a small quantity of fungal spawn can inoculate a much greater amount of substrate resulting in better fungal growth and yield compared to using the spawn alone. The low remediation efficiency in TPHs contents obtained in untreated soils and those treated with *P.* ostreatus without substrates (Table 4.5) are comparable to those of Meysami and Baheri (2003). The present investigation verified that a suitable substrate is required for the application of mycoremediation on petroleum-contaminated soils. The use of palm substrates resulted in up to 60% reduction in the concentration of TPHs with layering and 85% when the substrates are both mixed with the soils and also layered under unsterilised conditions. A comparison of remediation efficiency for the methods of layering of substrates, with that of mixing the fungus and substrate with soils combined with layering, revealed an increase of about 25% for the later. Thus, substrates type, and method of application can influence mycoremediation efficiency of white rot fungi on petroleum-contaminated soils.

The application procedure of mixing the substrates and the fungus with soils combined with layering is advantageous for in situ applications of mycoremediation on petroleum-contaminated soils. Palm substrates can be ploughed into contaminated soils followed by the inoculation of the fungal spawns for remediation of petroleum-contaminated soils. The combination of the mixing procedures with layering would also allow for protection of the remediation process against heavy rains, which are common in tropical climates (Larsen & Simon, 1993; Yabi & Afouda, 2012). Thus, the spawns

underneath the layered samples could continue the remediation almost undisturbed even during rainy season.

Commercial fungal spawns are usually available as grain or sawdust spawns (Leatham, 1981; Bonnen *et al.,* 1994; Royse, 2002; Chang & Hayes, 2013). There is therefore, the possibility for the development of sawdust spawn of *P. ostreatus* using palm tree substrates, which could be used for remediation of petroleum-contaminated soils. Overall, mixing the substrates and mushroom with soils followed by layering resulted in optimisation of the mycoremediation efficiency of *P. ostreatus* compared to the usual method of layering.

4.3.2.3 Comparative remediation efficiencies of the agents

A comparison of remediation efficiency of the phyto- and mycoremediation agents used in this study demonstrated that any of these agents can be used for remediation of petroleum-contaminated soils. The efficiency of these agents is a function of pH, nutrients supply, substrates type as well as the method of application. For remediation of the petroleum-contaminated soil, fermented palm wine was the most effective. This is because it does not require substrates for application like the white rot fungi, or pre-growing like the sunflowers. Fermented palm wine can simply be applied directly to the petroleum-contaminated soils for remediation.

4.4 Conclusions from chapter four

This study investigated the potential of sunflower species, fermented palm wine and *P. ostreatus* for treatment of petroleum-contaminated soils. The main conclusions are as follows:

- Cow manure can be used to increase soil nutrients, decrease acidity in soils and provide buffer for soil during remediation of petroleum-contaminated soils.
- Soils available nitrate and electrical conductivity increases with remediation efficiency and can be used to monitor remediation progress of petroleum-contaminated soils.

- Phytoremediation efficiency of sunflower species could be related to their biomass, with those having higher biomass exhibiting better remediation potentials.
- Fermented palm wine can be used for the remediation of petroleum-contaminated soils.
- Palm tree substrates can be used for growing white rot fungi under unsterilized conditions. The substrate can also be used for the application of white rot fungi on petroleum-contaminated soils.
- Mycoremediation potential of *P. ostreatus* can be enhanced by mixing the substrates and mushrooms with the contaminated soil combined with layering.
- Mycoremediation efficiency of white rot fungi on petroleum-contaminated soil depends on the application method and type of substrates.

The concept of phyto-and myco-remediation has been developed overtime with many challenges and short-comings. The present study goes beyond the remediation of petroleum-contaminated soils by sunflowers to the assessment of the remediation efficiency with species; and has established that this varies with biomass. This information is important in the choice of sunflowers for remediation. The use of fermented palm wine for treatment of petroleum-contaminated soils is novel and is useful for tropical regions where palm trees are abundant. Adapting *P. ostreatus* for use in the clean-up of petroleum-contaminated soils has been problematic. The present study has developed a realistic approach and with a novel substrate (Palm tree which is abundant in tropical regions) for application *of P. ostreatus* in the clean-up of petroleum-contaminated soils.

The applications of these phyto- and myco-remediation techniques can provide environmentally friendly options for treatment of petroleum-contaminated soils. These techniques would be beneficial to tropical regions like the Niger Delta, Nigeria because of the abundance of the phyto – and mycoremediation resources such as palm trees and palm wine. The methods would also provide readily available and cost-effective alternatives for the management of petroleum-contaminated soil.

CHAPTER FIVE

Effect of Tween 80 on some phyto- and myco-remediation agents on petroleum-contaminated silty loam soil from Ogoniland, Niger Delta, Nigeria

5.1 Background information

Surface active agents have the ability to enhance the efficiency of bioremediation substances on petroleum-contaminated soils (Zhang *et al.,* 2002; Rulli *et al.,* 2019). Enhancement with surfactants is considered a viable option because it helps solubilise the contaminating crude oil, making the contaminants readily available for actions of the applied agents. Both synthetic and biosurfactants are known, but biosurfactants are preferred because of their biodegradability. Biosurfactants often exhibit specificity towards soil contaminants which limits their widespread application. Thus, synthetic surfactants still enjoy extensive patronage (Noordman & Janssen, 2002; Peng *et al.,* 2007). The advantage of Tween 80 over other surfactants is that it is cheap, readily available and environmentally friendly (Cheng *et al.,* 2018). Tween- 80 also has the advantage of non-specificity often not encountered with other biosurfactants (Zheng & Obbard, 2001; Fonseca, 2011; Sánchez-Vázquez *et al.,* 2017).

The research in this chapter was therefore initiated to investigate the effect of Tween 80 on the identified remediation efficiency of the phyto- and myco-remediation agents used in the previous chapter. The target soils were petroleum-contaminated soils from Ogoniland, Niger Delta, Nigeria. From the sunflower species, sunflower-*Helianthus annus-pacino gold* was selected because it exhibited the highest efficiency among the three species used. Based on the same criteria, palm wine from *Elias guineensis* was also selected from palm wine. The white rot fungi- *Pleurotus ostreatus* was also used while a new phytoremediation agent, the ferns - *Dryopteris affinis AGM* was added. The choice of the fern was based on the observation that it was one of the prominent resistant plants found on petroleum-contaminated soils in the Niger Delta, Nigeria (Fagbami *et al.,* 1988; Ige, 2009).

5.2 Materials and Methods

Methods for soil sampling, glasshouse remediation, sample preparation and analysis are as discussed in Chapter 3 and chapter 4. Silty loamy soils contaminated with petroleum and controls (uncontaminated silty loam soils) were collected from Ogale, Ogoniland,

Nigeria (0295428 N, 0533596 E). The choice of the loamy soil from Ogoniland, Nigeria for the study was because it is the dominant soils in the study area and the soil type used by farmers for the cultivation of crops (Venturini *et al.,* 2008).

5.2.1 Application of Tween-80 on glasshouse pots

5 % aqueous solution of Tween 80 was used for the study (Cheng *et al.,* 2019; Meng *et al.,* 2019). The Tween 80 solution was prepared as follows. 25 ml of Tween 80 was measured out into a 500 ml flask and dissolved in deionised water with gentle swirling. The solution was then made up the mark and homogenised with a sonicator. The prepared solution was applied on subsets of each of the glasshouse remediation involving *Helianthus annus- pacino gold*, *Dryopteris affinis* (Figure 5.1), palm wine and *P. ostreatus* (Table 3.9) as follows. 10 ml of the solution was added to the soils in sample pots by uniformly spreading the liquid around the soil samples. This application was repeated every 2 weeks for a period of 3 months.

Overall, two sets of experiments were set up for each agent, one without Tween-80, and the other with Tween- 80.

Figure 5.1**:** *Dryopteris affinis* growing on petroleum-contaminated silty loam soils from Ogale, Nigeria in glasshouse pots.

5.2.2 Determination of remediation efficiency of *H. annus- pacino gold*, *D. affinis,* palm wine and *P. ostreatus* on silty loam soil from Ogale, Nigeria

For evaluation of remediation efficiency of the agents, composite soils samples were collected from glasshouse pots for a period of 3 months at time=0, 30, 60 and 90 days. Assessment for TPHs contents was carried out and percentage remediation evaluated as reduction in TPHs concentration between the periods t= =0, 30, 60 and 90 days. Determination of soil texture and analysis of the concentration of TPHs was carried out as reported in 3.4.2.5 and 3.5.2.2, respectively.

5.2.3 Kinetic studies on the remediation efficiency of *H. annus- pacino gold, D. affinis*, palm wine and *P. ostreatus*

Kinetics studies were carried out on the data obtained from 5.2.2. Concentrations of TPHs at the start of the experiment (A_0) and those at different points of the remediation (Time =30, 60, 90 days) were inserted in the rate equations of zero, first and second order reactions. These were carried out for each treatment at the different options of using the agents with or without Tween 80. The uniqueness of each data to fit into any of the rate laws as indicated by the shape of the linear plot, slope and intercept was used to determine the order of reaction at which the remediation progressed (Espenson, 1995). Thus, the order of reaction at which the remediation progressed were determined for each subset of the treatment.

5.3 Results

5.3.1 Particle size analysis of soils from Ogale, Nigeria

Particle size analysis revealed the soils from Ogale as silty loam (Table 5.1). There were no significant differences (p=1.00) in soil textural properties between contaminated soils and control (Table 5.1).

Table 5.1: Particle size analysis of soils from Ogale, Ogoniland, Nigeria. Sample size, n=6

		Particle size composition %			
Soil sample location	**Description**	**Sand**	**Clay**	**Silt**	**Classification** (Wentworth, 1922).
0294996 N, 0532999 E	Uncontaminated soil (Control)	45.33	20.83	33.83	Silty loam
0295428 N, 0533596 E	Petroleum-Contaminated soil	42.67	23.83	33.50	Silty loam
p-value (for T-test of mean of uncontaminated soils against petroleum contaminated soil				1.00	

5.3.2 Remediation efficiency *H. annus-pacino gold*, *D. affinis,* fermented palm wine and *P. ostreatus* on petroleum-contaminated silty loam soil from Ogale, Ogoniland, Niger delta, Nigeria.

The concentration range of TPHs observed for the petroleum-contaminated loamy soils at the start of the glasshouse study was 227 to 576 g/Kg dry weight of soil.

Remediation efficiency of the agents increased proportionally with time (Figure 5.2). *H. annus-pacino gold r*educed the concentration of TPHs in the contaminated soils at the rate of 31, 39 and 60% after a time of 30, 60- and 90-days respectively. For *D. affinis*, its remediation efficiency was 58, 72 and 74%, respectively. Fermented palm wine from *Elias guineensis* exhibited 53%, 81% and 87%; while for *P. ostreatus,* the observed efficiency was 53, 61 and 88 %. A decrease in the concentration of TPHs was also observed in untreated contaminated soils. This also increased proportionally with time. Percentage decrease in the concentration of TPHs for untreated soils was 9, 10 and 20% at 30, 60 and 90 days, respectively.

The highest remediation efficiency after 30 days was observed in *D. affinis* followed by *P. ostreatus, palm wine* then *H. annus* (Figure 5.2). During the second month (60 days), remediation efficiency was in the order palm wine > *D. affinis* > *P. ostreatus* > *H. annus*. At the end of the 90 days, the highest remediation efficiency of the agents was observed for *P. ostreatus* followed by palm wine, *D. affinis* then *H. annus-pacino gold.*

A general increase in remediation efficiency was observed for each of the agents on addition of Tween 80 (Figure 5.2). The increase was more noticeable with *H. annus-pacino gold* (Table 5.2). Increase in remediation efficiency of the agents was also time dependent. This was more from 30 to 60 days than from 60 to 90 days (Figure 5.2). The

highest increment in the remediation efficiency with addition of Tween 80 for the agents was observed for *H. annus-pacino gold* (103%) after 60 days, while the lowest was for palm wine (8%) after 60 days (Table 5.2). For *H. annus-pacino gold* and *P. ostreatus,* addition of Tween 80 resulted in increased remediation efficiency from 0 to 30 days which peaked at 60 days and decreased after 90 days. For *D. affinis* and fermented palm wine, the increase began at 30 days, lowered at 60 days but increased again between 60 and 90 days. Overall, the highest remediation efficiency for all the agents on addition of Tween 80 was observed for fermented palm wine (98%). This was followed by *P ostreatus* (96%), *D. affinis* (92%) and *H. annus-pacino gold* (92%) after a period of 3 months treatment.

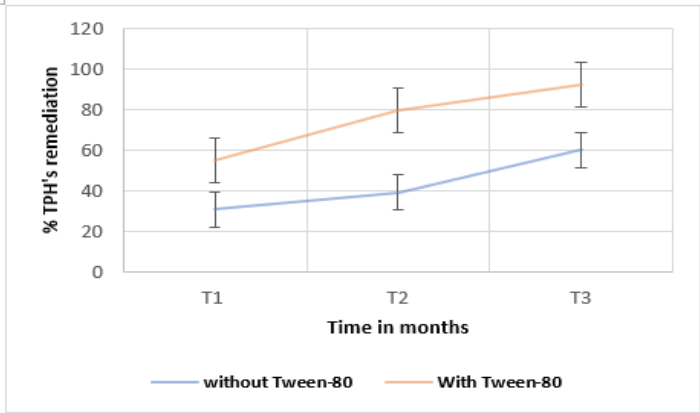

Figure 5.2a: Effect of Tween 80 on remediation efficiency of Helianthus annus

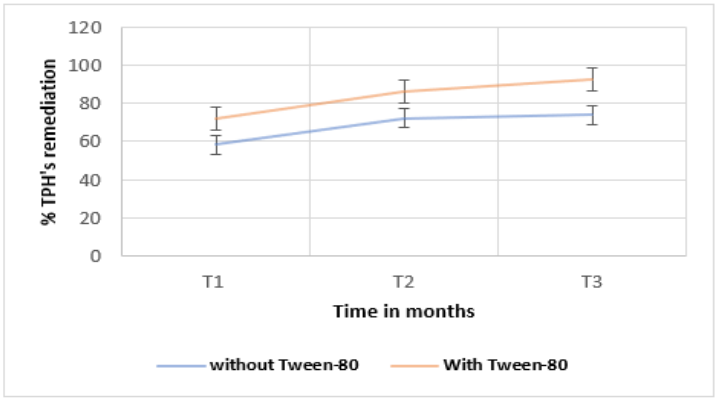

Figure 5.2b:Effect of Tween 80 on remediation efficiency of Dryopteris affinis

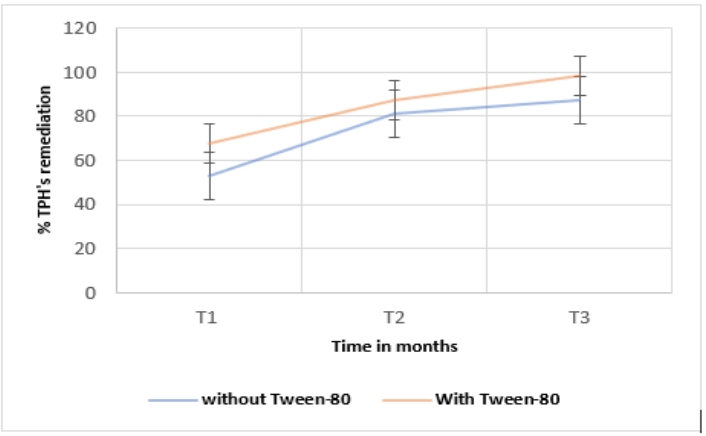

Figure 5.2c:Effect of Tween 80 on remediation efficiency of fermented palm wine from Elias guineensis

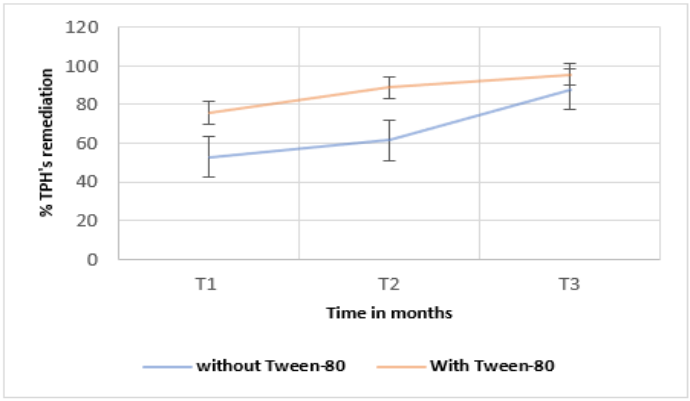

Figure 5.2c:Effect of Tween 80 on remediation efficiency of P. ostreatus

Figure 5.2: *Effect of Tween 80 on remediation efficiency of H. annus, D. affinis, palm wine and P. ostreatus on petroleum-contaminated silty loam soils from Ogale, Nigeria*. Error bars represent standard deviation of the mean (σ). Raw result data is available at AP III-3

Table 5.2: Increment in remediation efficiency on addition of Tween-80

	% Increase in remediation efficiency by addition of Tween-80		
Agents	**Time =30 days (1 month)**	**Time = 60 days (2 months)**	**Time = 90 days (3 months)**
Helianthus annus-pacino gold	78	100	53
Dryopteris affinis	24	20	25
Palm wine from *Elias guineensis*	29	8	12
P. ostreatus	43	45	9

5.3.3 Kinetic studies on remediation efficiency of the agents

A plot of lnA against time (Time =0, 30, 60, 90 days) for remediation with *H. annus-pacino gold* without Tween 80 resulted in a straight-line graph with slope =-K and intercept lnA_0. Where A is the concentration of TPHs at any time (Time = 30, 60 or 90 days) and A_0 is the initial concentration of TPHs at the start of the remediation (Figure 5.3). A plot of 1/A against time (Time = 30, 60 or 90 days) for remediation with *H. annus-pacino gold* with Tween 80 produced a straight-line graph with slope = K and intercept $1/A_0$. Where A is the concentration of TPHs at a given time (Time = 30, 60 or 90 days) and A_0 is the initial concentration of TPHs. Similar kinetic plots were obtained for *D. affinis* and *P. ostreatus* except for palm wine (Figure 5.3). For palm wine, a plot of a plot of lnA against time (Time = 30, 60 or 90 days) for remediation without Tween 80 produced a straight-line graph with slope =-K and intercept lnA_0. This was also the case with the addition of Tween 80.

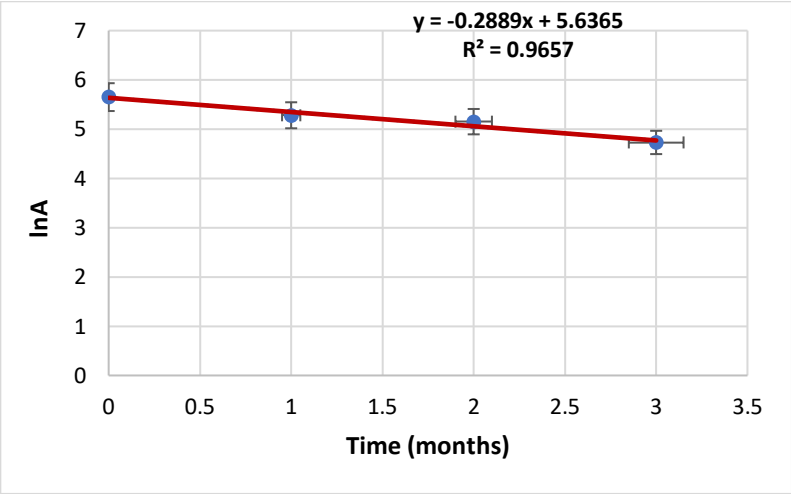

Figure 5.3a: First order rate plot for degradation of TPHS by *Helianthus annus* without Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively

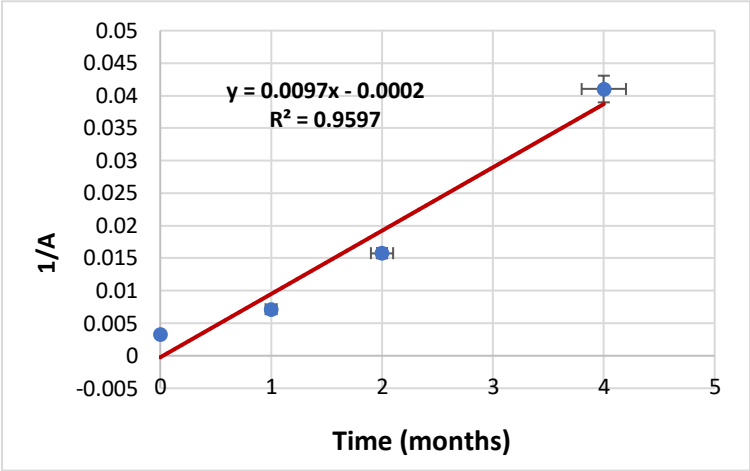

Figure 5.3b: Second order rate plot for degradation of TPH'S by *Helianthus annus* with Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively

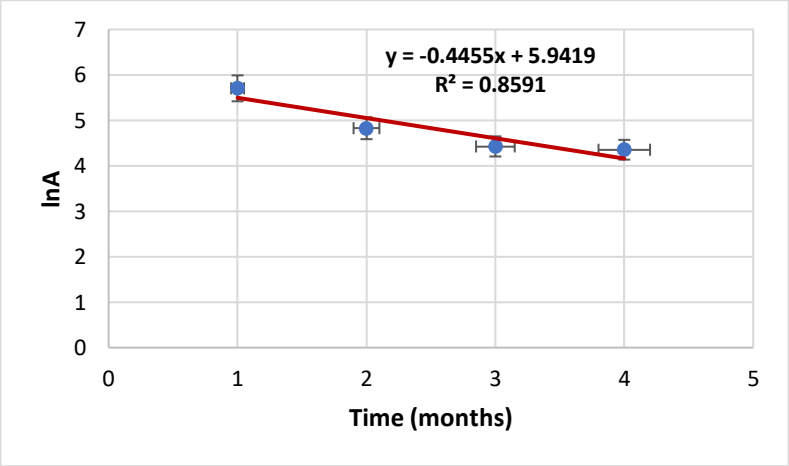

Figure 5.3c: First order rate plot for degradation of TPHS by *Dryopteris affinis* without Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

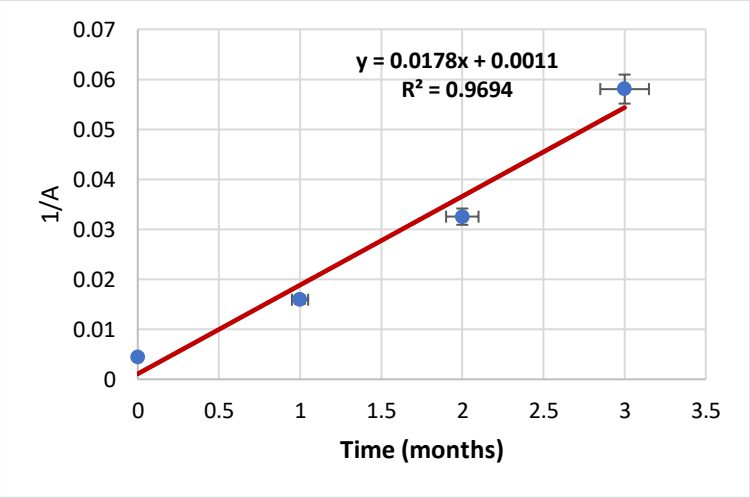

Figure 5.3d: Second order rate plot for degradation of TPH'S by *D. affinis* with Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

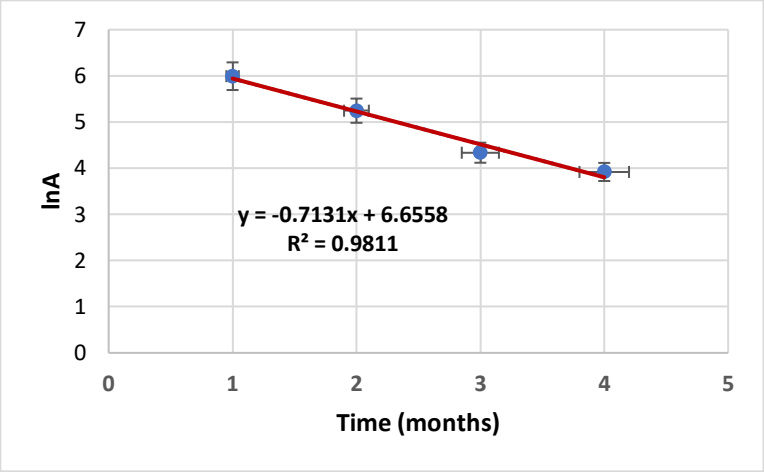

Figure 5.3e: First order rate plot for degradation of TPHS by fermented Palm wine without Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

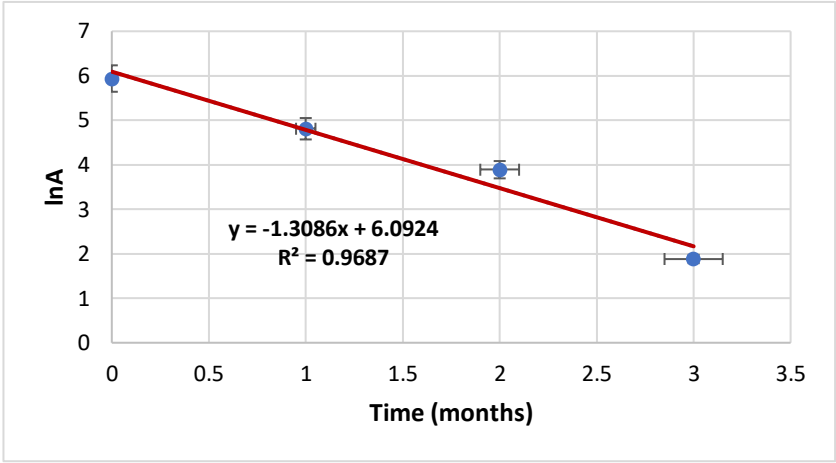

Figure 5.3f: First order rate plot for degradation of TPH'S by fermented Palm wine with Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

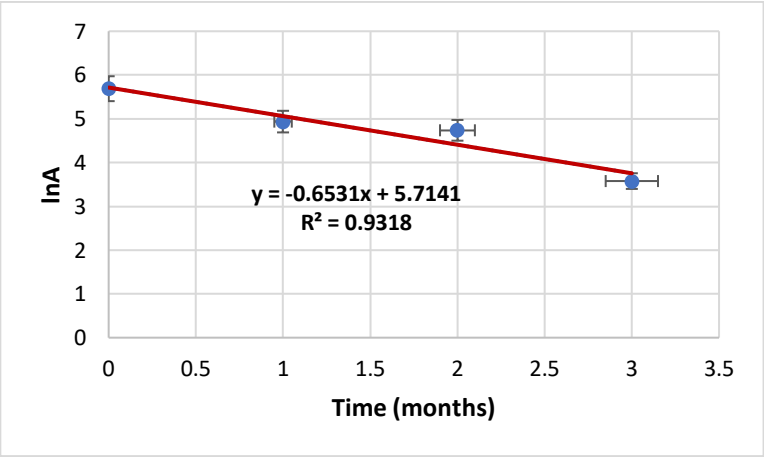

Figure 5.3g: First order rate plot for degradation of TPHS by *P. ostreatus* without Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

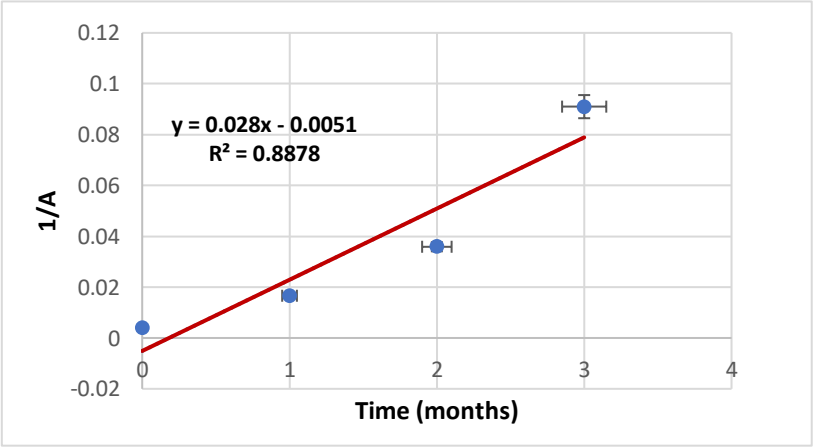

Figure 5.3h: Second order rate plot for degradation of TPH'S by *P. ostreatus* with Tween 80. Time 1, 2, 3, represents 30, 60 and 90 days, respectively.

5.4 Discussion

5.4.1 Particle size analysis of soils from Ogale, Ogoniland, Nigeria

The similarity in textural properties between the petroleum-contaminated soils and controls from Ogale, Ogoniland, indicated that the soils are within proximate location (Chang & Islam, 2000). During sampling, controls soils samples were obtained some 500m from the petroleum-contaminated sites. Unlike the soils from Tibshelf, UK (chapter 4), there were no observable effects on soil textural properties by petroleum contaminants (Table 5.1). Thus, it would seem petroleum contamination may not necessarily lead to degradation on soil textural properties as reported by *Okoro et al.* (2011) and Abosede (2013). The ability of petroleum contaminants to degrade soil textural properties towards clay particles may however, be related to the type of crude oil as well as volume and duration of impact. The site where the contaminated soils were collected was reported as those of fresh spills (F. Giadom, personal communications, March 2017). This explains why the textural properties of both the contaminated and control sites are similar.

5.4.2 Concentration of TPHs in soils from Ogale, Ogoniland, Nigeria

The relatively high concentration of TPHs in petroleum-impacted soils from Ogale compared to those at control sites demonstrated that the major source of TPHs contamination in soils of the area is oil spillage. Ogoniland and particularly the Ogale area has been subjected to oil spills over the past 2 decades without clear programs for clean-up (Ite *et al.*, 2013; Lindén & Palsson, 2013). The ability of soils from the study area to hold up to 50% of TPHs contamination per dry weight is alarming because of potential health hazards and ecological impact. The highest concentration of TPHs (576 g/Kg dry soil) observed in soils from Ogale, Nigeria are comparable to 420 g/Kg obtained by Kim *et al.* (2019) from petroleum-contaminated soils in China. Soils like these could act as reservoirs for petroleum contaminants releasing such into other environmental components such as air and water (Ünlü & Demirekler, 2000). Thus, there is an associated high risk to cultivated crops, aquatic organisms, animal and ultimately human lives (Venturini, *et al.,* 2008). Ogale is a renowned farming settlement and has close links with major cities in the south and eastern Nigeria. The consequence is that food crops cultivated in the area are sold to cities and processed into food. Thus, toxic contaminants

can be transferred across cities and via food exchange into biological systems. The situation requires frequent monitoring of petroleum facilities and soils within the area to ascertain the risk level. It also requires clear and well-planned programs for rapid response to issues of petroleum contamination in the area.

5.4.3 Remediation efficiency H. annus-pacino gold, D. affinis, fermented palm wine and P. ostreatus on petroleum contaminated silty loam soils from Ogoniland, Niger Delta, Nigeria**.**

The remediation efficiency of each of the agents revealed that time is a factor in utilising these agents for the treatment of contaminated soils (Figure 5.2). Therefore, it is important to identify the ideal time frame at which optimal remediation occurs so that these agents can be uprooted and disposed to avoid the contaminants returning to the soil. This is particularly to the phytoremediation agents, where extraction is the main mechanism (Hutchinson *et al.,* 2001). For instance, at 60 days, the remediation efficiency of *D.* affinis was at 72% and 74% at 90 days. That of *Helianthus annus* was 60% after 90 days. Thus, it would be ideal to uproot, disposed and replace *D.* affinis after 60 days of usage in the remediation of petroleum-contaminated soil. For fermented palm wine, the remediation efficiency was 81% at 60 days and 87% at 90 days. Remediation efficiency of fermented palm wine has been linked to the presence of consortium of microorganisms and certain chemicals in the fermentation product (Chapter 4). Thus, at 60 days most of the components of palm wine are either exhausted or the organisms would have developed insensitivity to the petroleum contaminants at this time. Therefore, soil treated with palm wine may require reconditioning for a more productive outcome.

Bernabé-Antonio *et al.* (2018), Fatima *et al.* (2018) and Feng *et al.* (2018) stated that one unique quality of an ideal phytoremediation agent is its ability to survive in a stressful environment. The ability of *H. Annus* and *D. affinis* to act on soils with high levels of petroleum contaminants in this study is significant. *D. affinis* is a major inhabitant of petroleum-contaminated soils and swamps in the Niger Delta, Nigeria (Fagbami *et al.,* 1988; Ige, 2009). Therefore, its potential use for remediation of petroleum-contaminated soils will be beneficial to the region. The plant is not used for food; this

eliminates the danger of bio-transfer of contaminants via consumption; or shortage of its foodstuff.

The remediation efficiency of these agents in chapter four at 90 days were 69% for *H. annus (*Pacino gold*)*, 70% for fermented palm wine from *E. guineensis* and 84% for *P. ostreatus*. These were grown in glasshouse pots of silty loam contaminated soils from temperate maritime climate (Tibshelf, UK). The initial concentrations of the TPHs in the soils at which these agents were grown were 150 g/kg for *H. annus-pacino gold*, 340 g/Kg for *E. guineensis*, and 210 g/Kg dry soils for *P. ostreatus.* In the present study (Chapter five), the initial concentrations of TPHs in the silty loam soil from Ogale, Ogoniland, Nigeria in the glasshouse pots where these agents were applied were 220 g/kg for *H. annus-pacino gold*, 310 g/Kg for *E. guineensis*, and 220 g/Kg dry soils for *P. ostreatus* (Appendix III-3). The observed remediation efficiencies in soils from Ogale, at 90 days were 60, 87 and 88% for *H. annus-pacino gold*, *E. guineensis* and *P. ostreatus*, respectively (Table 5.3).

Table 5.3: Comparison of remediation efficiencies of *H. annus-pacino gold*, Fermented palm wine from *E. guineensis* and *P. ostreatus* on petroleum contaminated soils from Ogale, Niger Delta, Nigeria and Tibshelf UK.

Agents	**Soil Types**			
	Silty loamy soils from Ogale, Niger Delta, Nigeria		Silty clay soils from Tibshelf, UK	
	Starting TPH conc. @time = 0 (g/Kg dry soil)	% Remediation efficiency at 90 days	Starting TPH conc. @time = 0 (g/Kg dry soil)	% Remediation efficiency at 90 days
H. annus-pacino gold	220	60	150	69
Fermented palm wine from *E. guineensis*	310	87	340	70
P. ostreatus	220	88	210	84

The ability of the agents *H. annus-pacino gold,* fermented palm wine and *P. ostreatus* to replicated similar remediation efficiency from soils of Tibshelf, UK (Chapter 4) to that of the Niger Delta, Nigeria, demonstrated their potential for use in remediation of

petroleum-contaminated soils both in temperate and tropical regions. This is also significant as these species are ubiquitous.

5.4.4 Effect of Tween-80 on remediation efficiency of *H. annus, D. affinis,* palm wine and *P. ostreatus* on a silty loamy soil from Ogale, Niger Delta, Nigeria

Tween 80 is known to promote the remediation efficiency of agents on contaminated soils (Sun *et al.,* 2013; Cheng *et al.,* 2018). Hence, the observed general increase in remediation efficiency of the agents on the application of Tween 80 was expected. The ability of Tween 80 to enhance remediation efficiency of *P. ostreatus and D. affinis* within a period of 30 days by reducing the concentration of TPHs in affected soils to that lower than the residual concentration in control (Table 5.2) is remarkable. This is because the method can be used to clean-up sites, which requires urgent actions. Addition of Tween 80 can be particularly used in the treatment of petroleum-contaminated soils with *H. annus-pacino gold* (Figure 5.2). For this agent, the remediation efficiency increased at over 70% at 30 days and doubled at 60 days. This implied that the method can be utilised where sunflower is the most readily available option.

Ramamurthy & Memarian (2012) reported a remediation efficiency of 75% in soils contaminated with 500 mg/Kg of TPHs by addition of Tween 80 to *Brassica juncea* compared to the 38% by the phytoremediation alone, after 50 days. This illustrated an increase of 37%. Agnello *et al.* (2016b) demonstrated a 2-fold increase in the remediation efficiency of *Medicago sativa* on soils co-contaminated with metals and petroleum hydrocarbons on the addition of Tween 80, after 90 days. The result of this study demonstrated an increase of 24-78% in the remediation efficiency of the agents after 30 days, and up to 100%, after 90 days (Table 5.2).

It was further observed that the application of Tween 80 to all the agents in this study provided a remediation efficiency of over 90% after 90 days (Figure 5.2). The results demonstrated that at this point, the TPHs contaminants from crude oil were completely cleaned up. This is because values for the remaining TPHs were all below that of residual concentrations of TPHs in control soils. A comparison of the result obtained by addition of Tween 80 to the agents to that without (Figure 5.2), demonstrated that Tween 80 has an enhancing effect on remediation efficiency of *H. annus-pacino gold, D. affinis*,

fermented palm wine and *P. ostreatus* applied on petroleum-contaminated silty loam soils from Ogoniland, Nigeria.

The ability of Tween 80 to enhance remediation of petroleum-contaminated soils stem from its ability to reduce the surface tension between the hydrophobic petroleum contaminants and soil solution, thereby increasing their solubility and making the contaminants more readily available in soils solutions (Pacwa-Płociniczak *et al.,* 2011; Liao *et al.,* 2016; Cheng *et al.,* 2018). The increased bioavailability is utilised differently by the different agents and is also a function of soil chemistry, and the associated remediation mechanisms (Brown *et al.,*1994; Sun *et al.,* 2013). Agnello *et al.* (2016) reported a general increase in translocation and bioconcentration factors of *Medicago sativa* on treatment of Tween-80 which aided in the enhancement of its remediation potential.

The highest effect of the addition of Tween 80 was always observed for sunflower (30, 60, 90 days). This illustrates that the rhizosphere of the roots hairs of the sunflower plant has better mechanisms to draw out the dissolved organics in soil solutions. The observation also illustrates that the possible mechanism of the sunflower plant is phytoextraction than degradation (White *et al.,* 2006; Martins *et al.,* 2014). This seems plausible because the remediation efficiency of the mycoremediation agents like *P. ostreatus* on the addition of the Tween 80 tend to be constant at time = 30 and 60 days. The same trend is also observed for fermented palm wine at 60 and 90 days.

P. ostreatus is a known degrader of petroleum contaminants (Márquez-Rocha *et al.,* 2000, Sukor *et al.,* 2012), thus, its behaviour under this condition can offer useful insight to possible mechanisms of the agents. As observed for *D. affinis* the increase in its remediation efficiency on addition of Tween 80 also remained constant during the treatment periods. This possibly points to some elements of phytodegradation mechanism by the plants. Kösesakal *et al.* (2016) reported that the water fern- *Azolla filiculoides* has the ability to degrade both aliphatic and aromatic (phenathrene) hydrocarbons in crude oil. Therefore, the possible mechanism of remediation by the fern- *D. affinis* in this study is phytoderation, and collaborates with the findings of Kösesakal *et al.* (2016). The exponential differences in enhanced efficiencies of the sunflower with time, distinctly points to the fact that its mechanism of remediation is

different from those of the other agents. Evaluation of the mechanisms associated with the activities of these agents requires further investigation.

5.4.5 Kinetic studies on remediation potential of the phyto- and myco-remediation agents

Remediation efficiency of all the agents without addition of Tween 80 proceeded by first order kinetics. Thus, the rate equation ln[A]=−kt+ln[A]o can be applied for decision making.

Where K is the slope of the rate plot (Figure 5.3) and $In[A]_0$ the intercept.

The result of this study revealed residual concentration of TPHs in soils of the controls from the study area at the range of 45 to 46 g/Kg dry soils. This amounts to 5% of TPHs concentration in the control soils. Using this information and setting target remediation TPHs concentration to 46g/Kg dry soils, the time required for each agent to effect complete removal of TPHs from the petroleum-contaminated soils can be estimated.

Using the integrated first order rate law ln[A]=−kt+ln[A]o

Where [A] is the targeted concentration of TPHs (46 g/Kg dry soil) and [A] = initial concentration of TPHs in contaminated soils, K the slope for each of the agents (Figure 5.2), and t, the time in days; the time for each of the agents to effect complete remediation of TPHs in the soils has been evaluated (Table 5.4).

Table 5.4: Estimated time for complete removal of TPHs in soils by each of the agents without the addition of Tween 80

Glasshouse Treatment	Initial TPH conc $[A_0]$ (g/Kg dry soil)	Target reduction level-[A] (g/Kg dry soil)-	$ln(A_0/A)$	K	1/k	**Time (days)**
Contaminated soils + *Helianthus annus-pacino gold*	284.3	45.67	1.820	0.2889	3.460	**190**
Contaminated soils + *Dryopteris affinis*	300.3	45.67	1.880	0.4455	2.240	**127**
Contaminated soils + Fermented Palm wine from *Elias guineensis*	400.4	45.67	2.170	0.7131	1.400	**91**
Contaminated soils + *P. ostreatus*	295.2	45.67	1.860	0.6531	1.530	**86**

Remediation efficiency on addition of Tween 80 to fermented palm wine still followed first order kinetics (Figure 5.2). Again, using the first order rate equation, the evaluated time to reach the remediation target for fermented palm wine with Tween 80 is given in Table 5.5

Table 5.5: Estimated time for complete removal of TPHs in soils by fermented palm wine on addition of Tween 80

Glasshouse Treatment	Initial TPH conc **[A_0]** g/Kg dry soil)	Target reduction level [**A]** (g/Kg dry soil)-	$\ln(A_0/A)$	K	1/k	**T (days)**
Contaminated soils + Fermented Palm wine from *Elias guineensis* + tween 80	379.0	45.67	2.110	1.309	0.7641	**48**

Addition of Tween 80 to all the agents except fermented palm wine followed second order kinetics. For second order kinetics, the rate law is

$1/[A]=1/[A_0]+kt$. All variables retaining same meanings.

Again, putting the variables into the second order rate equation, time required to reach the target remediation concentration with addition of Tween 80 can also be evaluated (Table 5.6).

Table 5.6: Estimated time for complete removal of TPHs in soils by each of the agents with the addition of Tween 80

Glasshouse Treatment	Initial TPH conc $[A_0]$ (g/Kg dry soil)	Target reduction level [**A]** (g/Kg dry soil)-	1/A	1/A0	$(1/A)-(1/A_0)$	K	**T (days)**
Contaminated soils + *Helianthus annus-pacino gold*	309.5	45.67	0.0219	0.0032	0.0187	0.0100	**58**
Contaminated soils + *Dryopteris affinis*	227.0	45.67	0.0219	0.0044	0.0175	0.0178	**29**
Contaminated soils + *P. ostreatus*	246.0	45.67	0.0219	0.0041	0.0178	0.028	**19**

The above kinetic studies revealed a reduction of estimated time for complete removal of TPHs in the soils by *H. annus* from 190 to 58 days on addition of Tween 80. That of *D. affinis* was from 127 to 29 days, fermented palm wine from 91 to 48 days, and *P. ostreatus* from 86 to 19 days.

Thus, the kinetic studies confirmed that addition of Tween 80 to the phyto and mycoremediation agents enhanced their remediation efficiency on the petroleum-contaminated silty loam soils.

5.5 Conclusions from chapter five

This study has demonstrated that soils of Ogoniland have been impacted with high concentrations of TPHs from crude oil sources. It has also revealed that *H. annus-pacino gold*, *D. affinis*, fermented palm wine and *P. ostreatus* can be used for the remediation of TPHs in petroleum-contaminated loamy soil from the Niger Delta, Nigeria. The study has further established that Tween 80 has an enhancing effect on the remediation efficiency of *Helianthus annus-pacino gold*, *Dryopteris affinis*, fermented palm wine and *P. ostreatus,* when used to treat petroleum-contaminated soils.

The comparative rate of success for complete removal of TPHs in the silty loamy soil from the Niger Delta, Nigeria without the addition of Tween-80 was Fermented Palm wine > *P. ostreatus* > *D. affinis* > *H. annus-pacino gold*. On addition of Tween 80, the rate of success was *P. ostreatus*> *D. affinis* > Fermented Palm wine > *H. annus-pacino gold.*

Overall, the study has demonstrated that the results obtained with soils of Tibshelf, UK (chapter four) can be applied to petroleum-contaminated soils from the Niger Delta, Nigeria. It also demonstrates that kinetic studies can be used to evaluate the mechanism of phyto- and myco-remediation.

Chapter Six

Utilising mycoremediation for treatment of petroleum-contaminated soils and sediments from Ogoniland, Niger Delta, Nigeria

6.1 Background information

The results obtained with soils from Tibshelf and the Niger Delta in chapters four and five revealed that treatment with the mycoremediation agents produced better outcomes compared to the phytoremediation agents. Therefore, mycoremediation options were further chosen to treat different soil types and sediments contaminated with petroleum in the Niger Delta, Nigeria. *P.* ostreatus was applied to 3 different textural soil types (clay, sand and loam) from Ogoniland, Nigeria, while palm wine supplemented with Tween 80, was used to treat petroleum-contaminated sediments.

The choice of *P. ostreatus* for the treatment of the different soil types was because it produced the highest remediation efficiency among the agents. Furthermore, palm tree substrates and the white rot fungus -*P. ostreatus* are abundant in the Niger Delta. This makes its ultimate use significant. For the treatment of sediments, fermented palm wine supplemented with Tween 80 was used. This was because it offers a more feasible option in the treatment of sediments due to difficulties that would be encountered in growing the fungus -*P. ostreatus* in aquatic conditions.

The aim of the study in this chapter was to assess the application of mycoremediation for the treatment of different soil types and sediments contaminated by petroleum in the Niger Delta, Nigeria.

6.2 Methodology

Three textural soil types of loamy, sandy and clay, contaminated by petroleum were sampled at 0-0.15m soil profile depth from three different locations in Ogoniland, Niger Delta, Nigeria (BSI ISO/DIS 18400-203, 2016). The locations were Ogale (0294996 N, 0532999 E), Gio (0304418 N, 0519421 E) and Bodo (0305325N, 0510090 E). Textural properties of the soils were first assessed onsite by hand feeling and ribbon method (Whiting *et al.,* 2014; Salley *et al.,* 2018) and later determined with laser density particle size analyser LS 13 (Yang *et al.,* 2015; Yang *et al.,* 2019). Petroleum-contaminated river

sediments were also collected from Gio (0304429 N, 0519401 E) and Bodo (0307283 N, 0509572 E).

The soils were separately treated with *P. ostreatus* using the application method of mixing the substrates and the fungus combined with layering the substrates and mushroom on top soil described in section 4.2.4. Treatment of the sediments was carried out using a combination of Tween 80 and fermented palm wine from *Elias guineensis*. All treatments were carried out in glasshouse for a period of 90 days.

Samples were collected at the beginning of the study (Time = 0 days) and after 3 months (90 days) and analysed for TPHs and remediation efficiency evaluated.

6.3 Results

High concentrations of TPHs were observed in the soil samples from Ogoniland, Nigeria (Table 6.1). The highest TPHs contamination of 525 g/kg dry weight of soil was found in clay soil. This was followed by loamy soil (213 g/kg dry weight) then sandy soils (121 g/kg dry weight of soil). *P. ostreatus* exhibited significant remediation efficiency (p=0.000) on each of the contaminated soils types of loamy, clay and sandy compared to controls. Remediation efficiency of the fungus was found to be 100% on loamy soils and clay soils and 81% on sandy soils after the 3 months treatment period. A comparison of the remediation efficiency of *P. ostreatus* on the Niger Delta soils with those of Tibshelf, UK (Chapter 4) revealed an increase of over 16% for loamy soil, 15% for clay soil and a decreased of 4% for sandy soil.

Table 6.1: Remediation efficiency *P. ostreatus* on petroleum-contaminated loamy, sandy and clay soils from Ogoniland, Niger Delta. Results are given as mean ± standard deviation (σ). The soils used for this study were amended with cow manure. Sample size, n= 18

Samples/ Treatment	Coordinates			Levels of TPHs (g/kg dry weight of soil)			
	N	E	Sample locations	T=0 (Months)	T=3 Months	% Reduction	P-VALUES (@ 95% CI) of T3 Values against T0
P. ostreatus on loamy soil	0294996	0532999	Ogale	212.8 ±4.09	Below quantification limit (BQL)	100.0	0.0000
P. ostreatus on Sandy soil	0304418	0519421	Gio	120.6± 2.96	23.20 ± 0.95	80.76	0.0000
P. ostreatus on Clay soil	0305325	0510090	Bodo	525.0 ± 11.75	2.47 ± 0.25	99.53	0.0000

For the sediments, the concentration range of TPHs from the 2 locations was 297 to 346 g/Kg dry weight. Sediments from Bodo had higher concentration of TPHs compared to those of Gio. A combination of Tween-80 and fermented palm wine yielded a remediation efficiency of 96% after 90 days for river sediments from Gio. For sediments collected from Bodo, a remediation efficiency of 98% was obtained. There was no significant difference between the remediation efficiency of the method for removal of TPHs from the 2 locations.

Table 6.2: Remediation efficiency of the combination of Tween-80 and fermented palm wine on petroleum-contaminated river sediments from Gio and Bodo communities in Ogoniland, Niger Delta, Nigeria. Results are given as mean ± standard deviation (σ). The sediments were amended with cow manure. Sample size, n= 12.

Sampling point	**Sample description**	**TPH (g/Kg dry soil)**		
		T0	**T3**	**% remediation**
0307283 N, 0509572 E	Bodo sediment	243.4 ± 56.64	Below quantification limit (BQL)	100.0
0304429 N, 0519401 E	Gio sediment	369.1 ± 14.16	BQL	100.0

6.3 Discussion

6.3.1 Remediation efficiency *P. ostreatus* on petroleum-contaminated loamy, sandy and clay soils from Ogoniland, Nigeria.

The concentration of TPHs in the soil types and sediments observed in this chapter varied from 120 to 525 g/Kg dry soil. The variations in the TPHs concentration at the different sampling points and soils types from Ogoniland is largely due to their respective distances from contamination source. Some of the samples were collected from points of direct impact while others were obtained some distances away from contaminant sources. The range of values in this chapter (120-525 g/Kg dry soil) are comparable to those in chapter four (150-450), chapter five (280-400) and the 420 g/kg reported by Kim *et al.* (2019). The close range in the concentrations of TPHs in chapter five is due to the fact these samples came from one bulk of silty loam obtained from the same location.

Issues of crude oil contamination are frequent in Ogoniland (Ite *et al.,* 2013; Lindén & Pålsson, 2013). Most oil spill sites have remained untreated for decades and new cases are also reported (Emoyan, 2008). The high concentrations of TPHs obtained in the soils from Ogoniland in these studies may be due to recurring episodes on same locations with cumulative effects. Timely treatment of contaminated sites would help reduced cumulative effects of these contaminants, prevent leaching, and transport of contaminants to other locations and biological systems. The highest concentrations of TPHs were observed on clayey soils, followed by loamy, then sandy soils. Clay soils have smaller particles (Schapel *et al.,* 2019). The cohesive and adhesion properties of clay soils are also higher compared to other soils (Khamehchiyan *et al.,* 2007). Thus, crude oil contaminants are more tightly bound to the clay particles than in other soil types (Ren *et al.,* 2019).

The observed remediation efficiency of *P. ostreatus* on the different soil types of loamy, clay and sandy from Ogoniland, Nigeria was in the order loamy > clay > sandy. Loamy soil is a mixture of sand, clay and silt particles in equal or nearly equal proportion (Melero *et al.,* 2006). This allows for easy distribution of soil particles, contaminants and fungal mycelia. The ease of contact of the fungal mycelia with contaminants accounts for increased efficiency of remediation in the loamy soil. Clay soils are very sticky and dense (Mitchell & Soga, 2005). The addition of organic manure loosens the clay particles and allows for penetration of fungal mycelia for remediation (Aggelides & Londra, 2000). The method of mixing the substrates with the fungal spawns with soils combined with layering further loosens the aggregated particles and creates more room for contact of the mushroom mycelia with petroleum contaminants resulting in increased remediation efficiency. Soil particles are loosely held in sandy soils. Sandy soils also have larger pore spaces. The addition of organic manure binds the sandy particles (Yu *et al.,* 2012). Further mixing of the substrates and the fungus-*P. ostreatus* with the soil can result in sandy particles and the contaminant molecules being further part from contact with the mushroom mycelia. Thus, the observed trend in the remediation of efficiency of *P. ostreatus* on the different soil types.

A comparison of the remediation efficiency of *P. ostreatus* on petroleum-contaminated soils from Tibshelf, UK with those of Ogoniland, Nigeria revealed that methods developed with the soils from Tibshelf, can be reliably applied to soils in the Niger Delta, Nigeria. This finding is beneficial for future research. During the study, conditions were replicated to represent those typical of the Niger Delta, Nigeria with temperatures at 15-25^{0}C and watering conditions. The study also utilised conventional petroleum-contaminated soils, soils amendment with cow manure and was carried out under unsterilized conditions. These would allow for easy applications of the methods either in situ or in bioremediation plants.

Application of *P. ostreatus* by method of mixing palm substrates and the fungal spawn combined with layering the substrates and spawn on topsoil yielded significant efficiency on all the soil types from Ogoniland. These demonstrated that *P. ostreatus* can be used for treatment of petroleum-contaminated soils in the Niger Delta region of Nigeria. The soils in this study were conventional petroleum-contaminated soils from sites within the Niger Delta. Therefore, this technique would be ideal for treatment of petroleum-contaminated soils in the region.

6.3.2 Remediation efficiency of fermented palm wine supplemented with Tween-80 on petroleum-contaminated sediments from Gio and Bodo communities in Ogoniland, Niger Delta, Nigeria.

The concentration of TPHs in sediments of Gio and Bodo, further confirmed the precarious situation of Ogoniland environments curtesy of petroleum contamination. UNEP (2011), reported that soil, air and water as well as underground water in Ogoniland, Nigeria are impacted by petroleum contamination. The result of this study has also revealed that river sediments in the area are equally impacted. Possible sources of river contamination in the area include oil bunkering and illegal refining of petroleum products and transportation of such along the Ogoniland waterways (Ite *et al.,* 2013; Lindén & Pålsson, 2013). Others include failed oil facilities of petroleum industry, which had operated in the area. Such facilities include oil well heads, flow stations and

pipelines (Emoyan, 2008). The high concentrations of TPHs in river sediments of the area implied possible bio transfer by seafood which is a major product of the area.

The problem of contaminated river sediments affects the growth and metabolic activities in aquatic lives. Carman *et al.* (1995) stated that marine organisms have several mechanisms of adapting to high concentrations of contaminants. Thus, toxic substances in petroleum-contaminated sediments can be accumulated over time and bio-transferred in high amounts into biological systems. The effects could be pathological for both plants and animals.

Combining fermented palm wine with Tween 80 significantly reduced the concentration of TPHs in contaminated sediments to a level below the limit of quantification. This is significant for the study because palm wine is one of the abundant resources of the area (Williamson, 1970). The technique of using fermented palm wine is also easy to apply. The potential of utilising fermented palm wine for remediation of petroleum-contaminated soils and sediments could also boast the economic lives of the rural people in the area who would engage more in the cultivation of palm trees and production of the juice. Because fermented palm wine is a plant juice, the result may also apply to other fermented plant juice. There is, therefore the potential of using other fermented plant juice such as fruit juice for treatment of petroleum-contaminated soils. This requires further investigation.

Overall, combining fermented palm wine and Tween 80 will offer a good option for remediation of petroleum-contaminated sediments with respect to cost, resource availability and time taken to achieve remediation.

6.5 Conclusions from chapter six

The investigation has demonstrated that both soils and sediments of Ogoniland have been impacted with high concentration of TPHs from crude oil sources. The range of TPHs values in soils and sediment of the area was from 5% in controls soils to over 50% of TPHs per Kg dry weight of soils in contaminated sites. The study has also revealed that *P. ostreatus* can be used for remediation on petroleum-contaminated soils in both temperate and tropical climates. This chapter has further demonstrated that

remediation options developed using petroleum-contaminated soils from Tibshelf, UK can be applied to different soil types and sediments in the Niger Delta, Nigeria. Overall, mycoremediation can provide a reliable technique for the clean-up of contaminated soils and sediments. The findings of this chapter further indicate the prospect of fermented plant juice in the treatment of petroleum-contaminated soils.

Chapter Seven

Assessment of petroleum-contaminated soils using crude oil standards and the biomarker compounds-dodecane, and benzene-1,3-bis(1,1-dimethylethyl)

7.1 Background information

Analysis of petroleum-contaminated soils is achieved by techniques which involve extraction of Total Petroleum Hydrocarbons (TPHs) prior to instrumental analysis. The instrumental analysis itself employs analytical standards for the calibration and quantification of TPHs (Abbasi & Keshavarzi, 2019). A number of commercial TPHs standards are available. However, these standards are not 'over the counter' laboratory reagents and must be ordered when required. Analytical standards are relatively expensive and sometimes times difficult to come by. The acquisition and delivery process of these standards can cause delays in the analysis and evaluation of TPHs, even when the analytical equipment is available. Harmsen *et al., (*2005) reported that with current TPH standards as reference points, further developments in analytical standards can be achieved for monitoring of petroleum contaminants in environmental matrices. Therefore, investigation for alternatives standards for TPHs analysis is highly desirable.

Petroleum biomarkers are utilised in the oil industry for oil-oil and oil-source correlation, identification of organic matter type, depositional environment, and degree of thermal maturation and extent of biodegradation of crude oils (Peters & Moldowan, 1993; Peters *et al.,* 2007). Biomarker analysis can provide reliable evidence for spilled crude oils and petroleum products and can correlate to suspected sources (Han and Clement, 2018; Walters *et al.,* 2018). Biomarkers classes used in petroleum analysis include n-alkanes, aromatics, isoprenoids, porphyrins, hopanes, and steranes (Ian *et al.,* 2003). Because these compounds persist in oil spills, refinery products and archaeological artefacts, they can be used to identify stratigraphic origin, migration pathways and associated environmental conditions prevalent during the formation and alteration of existing petroleum deposits (Frysinger & Gaines, 2001; Wang, Stout and Fingas, 2006; Vane *et al.,* 2011). Lerch *et al.* (2018) stated that petroleum formed under different geological conditions and ages may exhibit different biomarker fingerprints. Thus, biomarker analysis can effectively discriminate petroleum substances from different sources.

Information on concentrations of biomarkers can relate to a quantitative measure on oil spill investigations (Yang *et al.,* 2010; Wang, Stout and Fingas, 2006; Stout *et al.,* (2000). Bouchard *et al.* (2018) reported that the use of biomarkers for monitoring remediation progress offers a process-specific and often compound-specific information on contaminant removal. Hence, using petroleum biomarkers for oil spills quantification and evaluation of remediation progress can afford alternatives for evaluation of both contamination levels and risk factors.

However, there seems to be limited number of studies relating petroleum biomarkers to the quantification of oil spills or the evaluation of remediation programs. Development of methods, which can correlate concentrations of biomarkers with that of petroleum contaminants in soils can provide options for quick and easy monitoring of petroleum contaminants and remediation processes.

Thus, the study in this chapter was carried out to explore alternatives for the quick assessment of petroleum-contaminated soils that can complement those involving the use of commercial TPHs standards. The outcome can provide readily available options for assessment of the concentrations of TPHs in soils particularly in the monitoring of remediation programs. The investigations here evaluated the prospects of using crude oil from contaminating sources; and biomarker compounds found in both the contaminating crude oil, and the contaminated soil for assessment of TPHs concentrations in soils.

Hence, the study in this chapter can be summarised into two parts. First, the contaminating crude oil was used as analytical standard for evaluating TPHs concentration in soil in comparison to commercial TPHs standards. This was followed by another investigation for possible biomarker compounds that can be used to evaluate TPHs concentrations in soils.

7.2 Methodology

Petroleum contaminated soils were collected from a site at Tibshelf, Derbyshire; and 7 other sites in Ogoniland, Niger Delta, Nigeria. Uncontaminated soils (controls) were also collected from three sites at Brackenhurst, the site at Tibshelf, United Kingdom and 2 sites in Ogoniland, Nigeria. Glasshouse remediation treatments were carried out on

petroleum-contaminated soils from Tibshelf as discussed in Chapter 4. Phyto- and myco-remediation agents used for the remediation are also as reported in chapter 4.

7.2.1 Sample preparation and analysis

Soil samples were prepared for analysis as discussed in Chapter 4. Extraction of TPHs in samples was carried out using a microwave-assisted extraction with a Milestone MA182-001 ETHOS UP Microwave system, using a 1:1 acetone – heptane mixture (USEPA METHOD 3546; Punt *et al*., 1999). TPHs and biomarker standards were prepared as stated in ISO/TS 16558-2:2015(E) and ISO 18287:2006(E), and reported in sections 3.5.2.1, and 3.5.2.3, respectively.

Sample extracts, TPHs, crude oil and biomarker standards were all analysed in a GC-MS according to ISO 13859 (2014). GC-MS conditions are listed in Table 4.2.

7.2.2 Utilising contaminating crude oil as standard for evaluating concentrations of TPHs in soils

This section was set to evaluate the possibility using the contaminating crude oil as an analytical standard in comparison to commercially available TPHs standards. This would help provide alternatives in events of unavailability of commercial TPHs standards.

7.2.2.1 Preparation of the crude oil standard

Crude oil samples were collected from the 2 locations associated with the soil sampling points: (1) Tibshelf, Derby, UK and (2) Gio, Ogoniland, Nigeria. The crude oil standards were prepared as follows: a given mass of oil was weighed out and dissolved in 10 ml of n-heptane (Table 7.1). The solution obtained was filtered to remove undissolved solids. Mass of the residue was determined and subtracted from the initial mass of the crude oil to determine the actual mass of the oil in solution. From this, the concentration of this stock solution of crude oil in heptane was determined in milligram per liter (mg/l) (Table 7.1). Calibration standards of 8000, 5000, 2500, 1000, 500 and 100 mg/l of the crude oil concentrations were prepared by serial dilutions of the stock solution (Table 7.2).

Table 7.1: Preparation of stock solutions of crude oil standard

	Derby (UK) Crude oil	**Nigeria Crude oil**
Initial mass of crude oil	1.850 g	1.875 g
Mass of residue after dissolution and filtration	0.709 g	0.651 g
Actual mass of crude oil in 10ml of n-heptane solution	1.152 g	1.225 g
Initial concentrations of crude stock solution in mg/l	**115,000 mg/l**	**123,000 mg/l**

Table 7.2: Preparation of calibration solutions of crude oil standard from stock solutions in mg/l

	Derby (UK) Crude oil			**Nigeria Crude oil**		
Calibration solutions (mg/l) using $C_1V_1 = C_2V_2$	Volume of stock (µl)	Volume of solvent(ml)	Total volume (ml)	Volume of stock (µl)	Volume of solvent(ml)	Total volume (ml)
8000	695.7	9.300	10.00	650.4	9.350	10.00
5000	3125	1.880	5.000	3125	1.880	5.000
2500	1563	3.440	5.000	1563	3.440	5.000
1500	837.5	4.160	5.000	837.5	4.160	5.000
1000	625.0	4.380	5.000	625.0	4.380	5.000
700	437.5	4.560	5.000	437.0	4.560	5.000
500	312.5	4.690	5.000	312.5	4.690	5.000
300	187.5	4.810	5.000	187.5	4.810	5.000
100	62.50	4.940	5.000	62.50	4.940	5.000

7.2.2.2 Quantification of TPHs using the crude oil and TPHs standards

The method ISO/TS 16558-2 (2015) was used for the quantification of TPHs using both the crude oils and commercial TPHs standards (TPHs C10-C40 and TPHs-gasoline diesel range). Both the TPHs and crude oil standards were used for the estimation of TPHs concentrations in samples. The respective crude oils were also used to assess TPHs concentrations in associated soils (i.e. crude oil from Tibshelf was used for soil samples from Tibshelf while those from Nigeria were used for soil samples from Nigeria). Initial

calibration of the instruments, followed by evaluation of the concentration of the TPHs, along with calibration verification was carried out. Chromatograms of the crude oil and commercial TPHs standards are given in Figure 7.1, Figure 7.2, Figure 7.3, Figure 7.4 and Figure 7.5. Calibration functions for each of the standards are given in Figure 7.6 - 7.10.

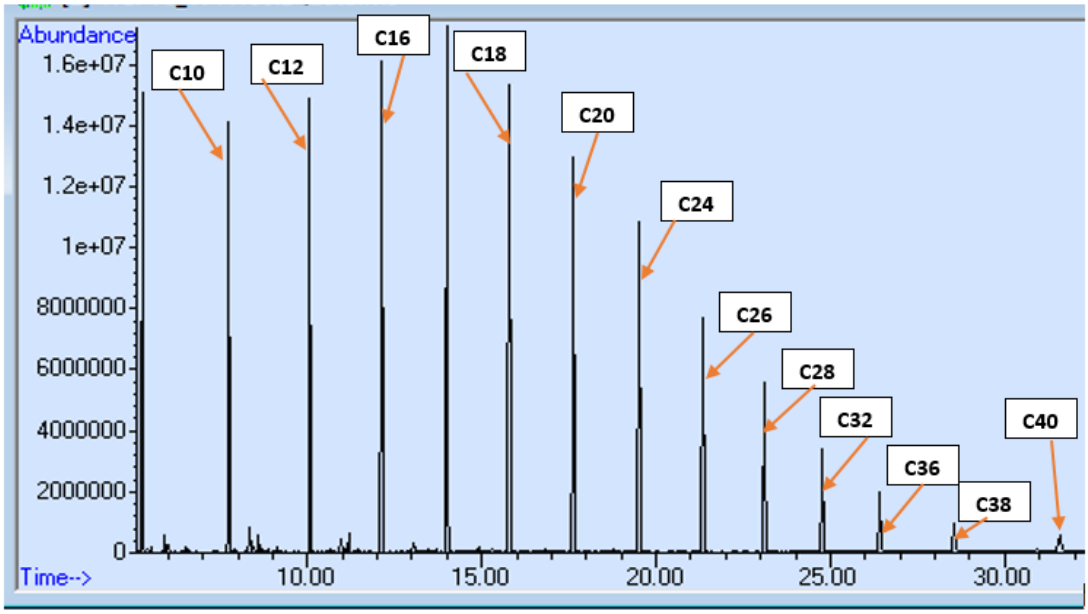

Figure 7.1: Chromatogram of commercial TPHs standard over a range of C10-C40. The names of individual peaks are given in table 7.2b

Table 7.2b: Names of individual peaks in Figure 7.1

No of carbon atoms	Names of petroleum hudrocarbons in Figure 7.1
C10	Decane
C12	Dodecane
C16	Hexadecane
C18	Octadecane
C20	Eicosane
C24	Tetracosane
C26	Hexacosane
C28	Octacosane
C32	Dotricontane
C36	Hexatricontane
C38	Octatricontane
C40	Tetracontane

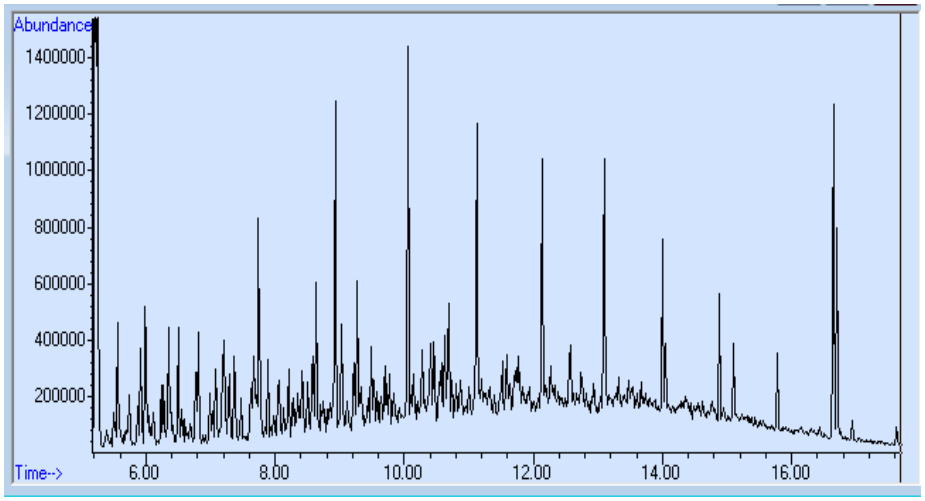

Figure 7.2: Chromatogram of commercial TPHs Gasoline-diesel standard

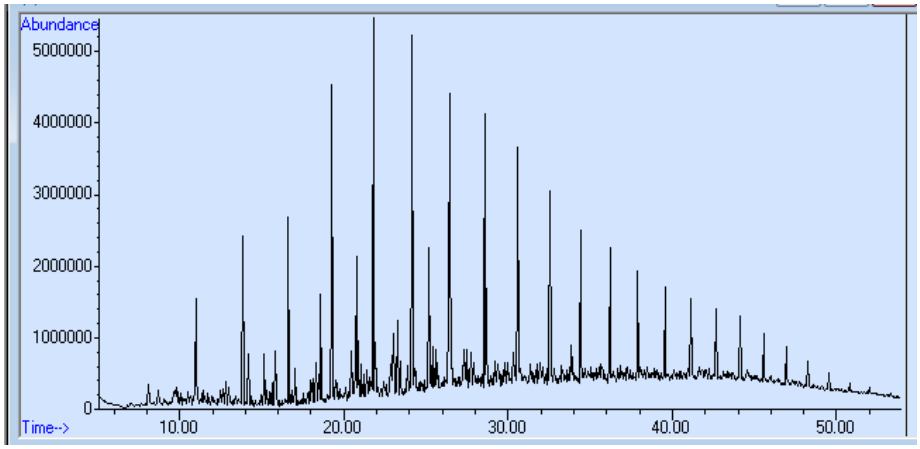

Figure 7 .3: Chromatogram of crude oil standard-Derby crude oil

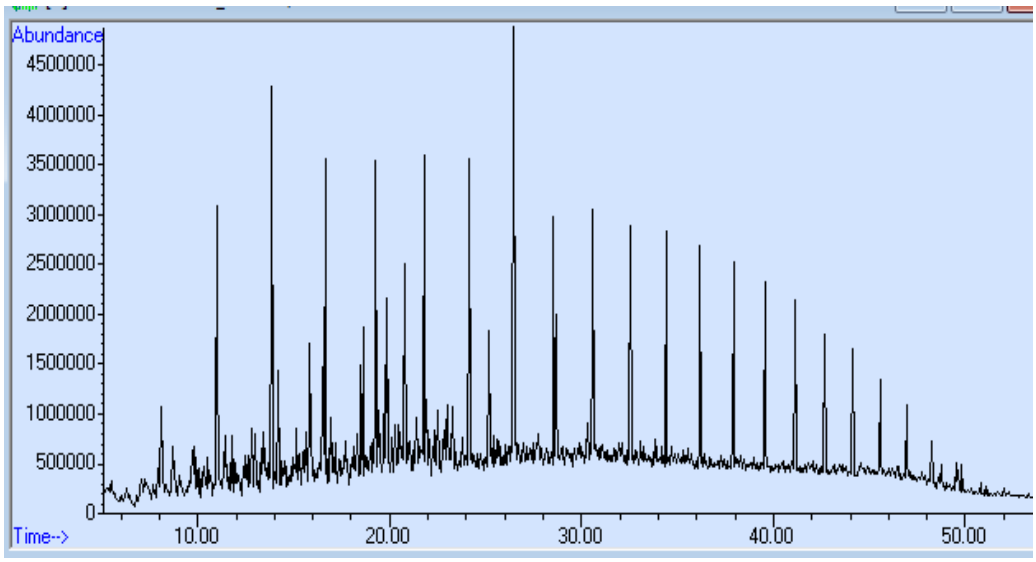

Figure 7.4: Chromatogram of Nigerian crude oil standard

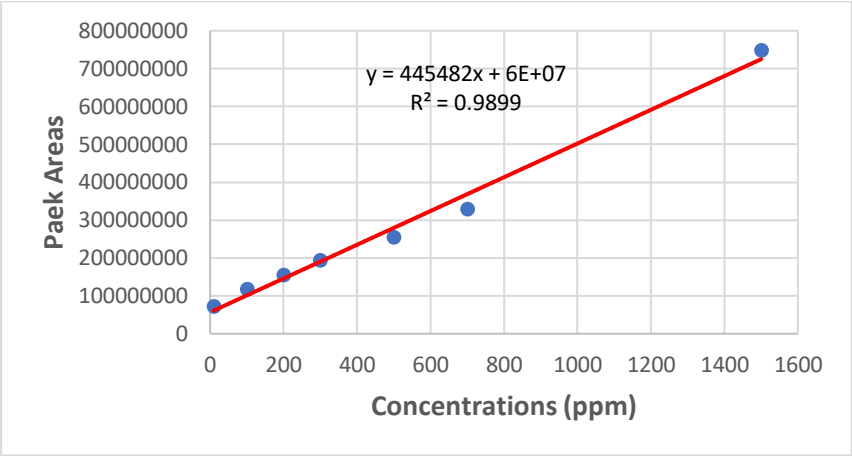

Figure 7.5: Calibration curves for TPHs-gasoline-diesel standard

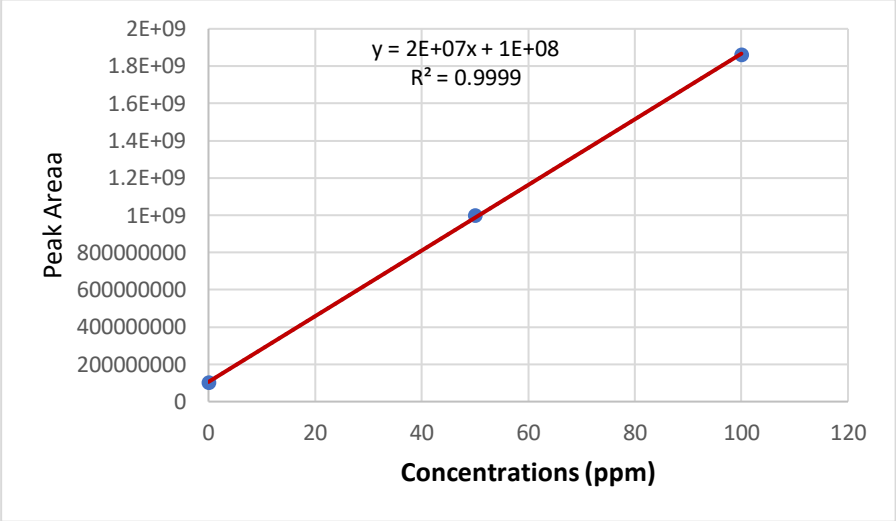

Figure 7.6: Calibration curves for TPHs-C10-C40 standard

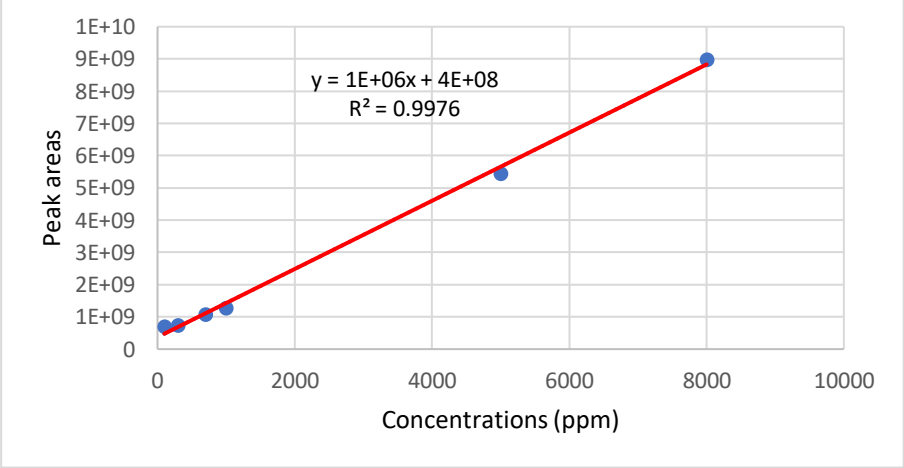

Figure 7.7: Calibration curves for Derby Crude oil standard

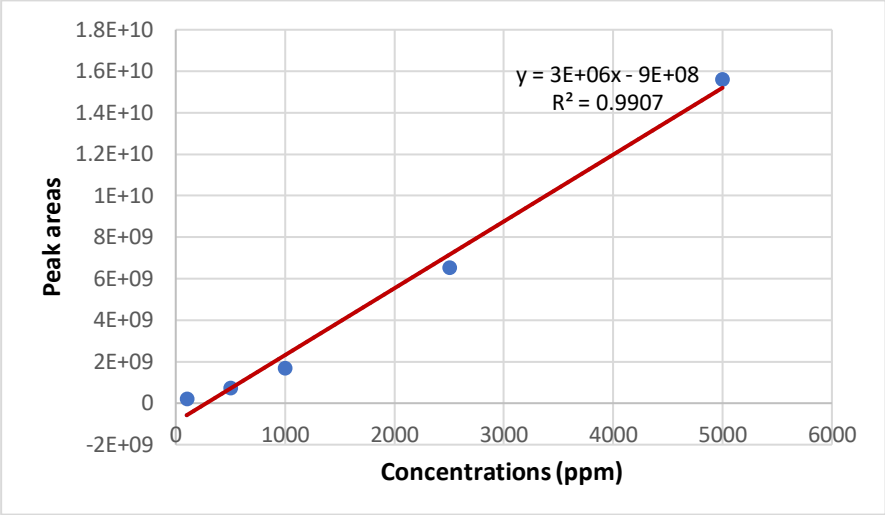

Figure 7.8: Calibration curves for Nigerian Crude oil standard

7.2.3 Utilising petroleum biomarker compounds for evaluating TPHs concentration in soils

This section was carried out to further investigate possible relationship between concentration of certain biomarker compounds present in the contaminating crude oil, and the contaminated soil, and their TPHs concentrations. This could also provide alternatives for a quick evaluation of TPHs concentration in soil samples.

Thus, in the following subsections, the crude oil and soil samples were investigated to identify biomarker compounds common to the contaminating crude oil, and the contaminated soil samples. This was followed by statistical analysis to assess possible relation between the identified biomarker compounds and TPH's concentrations in the crude oil and the soil samples.

7.2.3.1 Identification of the biomarker compounds

From the chromatograms of the sample extracts and crude oil samples obtained from section 7.2.1, searches were conducted peak by peak for different classes of hydrocarbon biomarker compounds expected in petroleum namely, saturated straight chain, substituted aromatic and substituted cyclic (non-aromatic) (Wang, Stout and Fingas, 2006; *Peters et al.,* 2005). Common occurrence of the peaks on the chromatogram of sample extracts and crude oil samples was also considered in making this decision. Compounds identified within the various classes are listed in Table 7.3. One representative compound was later selected from each of the classes based on

structure and toxicity references (ISO 11504:2017). These compounds were then monitored for consistency in retention time among sample extracts (at T=0), and the standardised crude oil. From this, certain marker compounds were finally selected (Table 7.4). Only 2 of these compounds could be chosen as marker candidates. The choice of the compounds was because they were consistently present at different concentrations of the crude oil and soil samples. Another reason was because their standards were also readily available. The compounds were dodecane (aliphatic) and benzene 1,3 -bis(1,1-dimethylethyl) (aromatic). The marker compounds were confirmed by running standard solutions of the compounds independently, mixed standards (of the markers compounds) (Figure 7.11, Figure 7.12 & Figure 7.13) and as spikes on the crude oil concentrations of 100, 700 and 1000 mg/l. The mass spectra and retention times of the marker compounds in the standards, crude oil and soil extracts were compared and confirmed according to methods of USEPA 8270E.

Table 7.3: Compounds identified within the various classes of hydrocarbon in the crude oil

Retention time (mins)	Compound	Match %
10.315	Dodecane	40
11.064	Benzene, 1,3-bis(1,1dimethylethyl)	81
11.774	Tridecane	41
12.789	Dodecane 2,6,10-trimethyl	27.5
13.147	Tetradecane	32
14.436	Pentadecane	26.6
14.532	2,4-ditertbutylphenol	42.2
15.650	Hexadecane	26.4
16.009	tert-hexadecanethiol	10.4
17.897	Octadecane	19.3
19.929	Eicosane	26.9
21.625	17-pentatriacontene	28.3

Table 7.4: selected representative marker compounds in the crude oil

Marker	Structure of compound	Compound identified
1	Saturated straight chain	Dodecane ($C_{12}H_{26}$)
2	Unsaturated straight chain	17-pentatriacontene ($C_{35}H_{70}$)
3	Substituted Aromatic	Benzene, 1,3-bis(1,1-dimethylethyl) ($C_{14}H_{22}$)
4	Substituted Cyclic (non-aromatic)	Tridecane, 4-cyclohexyl- ($C_{19}H_{38}$)

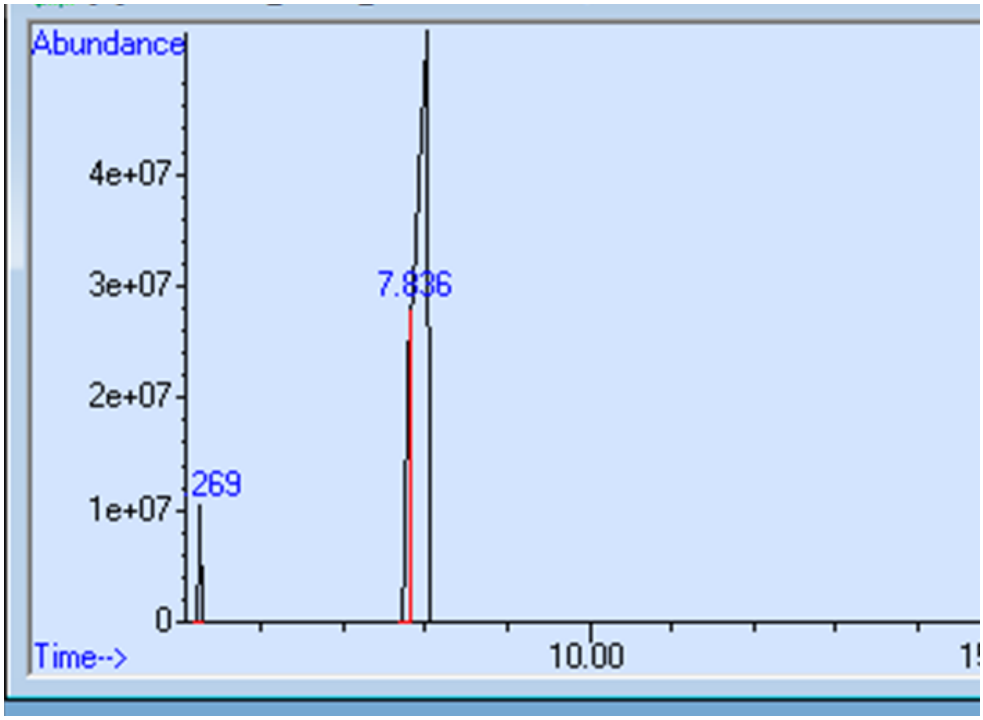

Figure 7.9: Chromatogram of dodecane standard

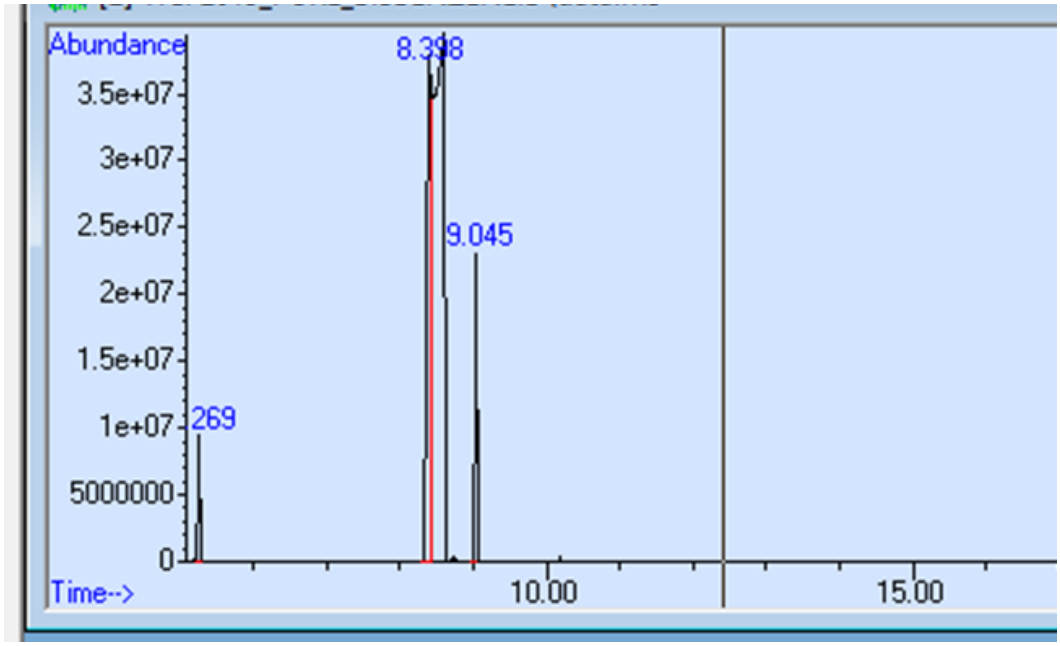

Figure 7.10: Chromatogram of benzene-1,3-bis(1,1-dimethylethyl) standard

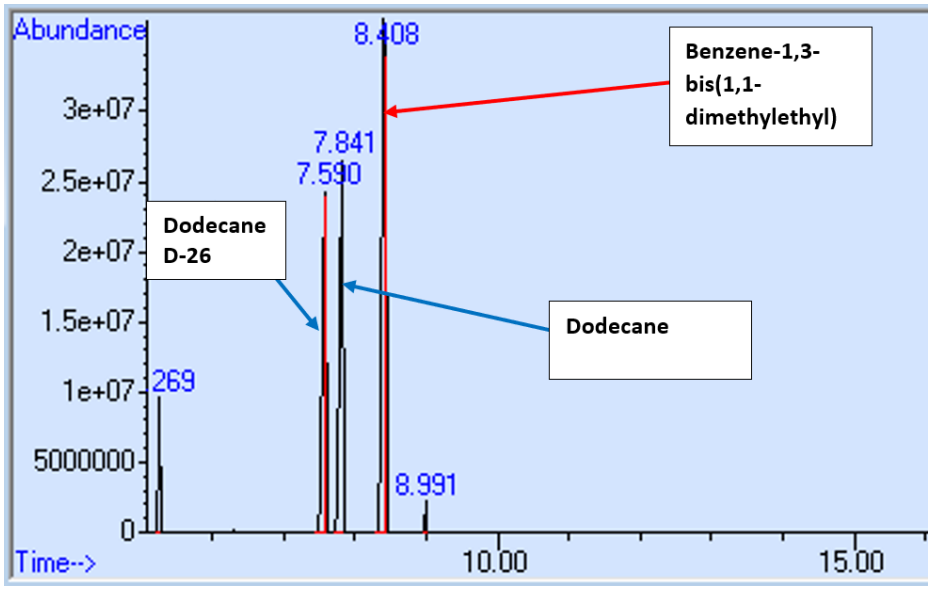

Figure 7.11: Chromatogram of mixed standard of dodecane, benzene-1,3-bis(1,1-dimethylethyl) standard and internal standard (Dodecane-d26)

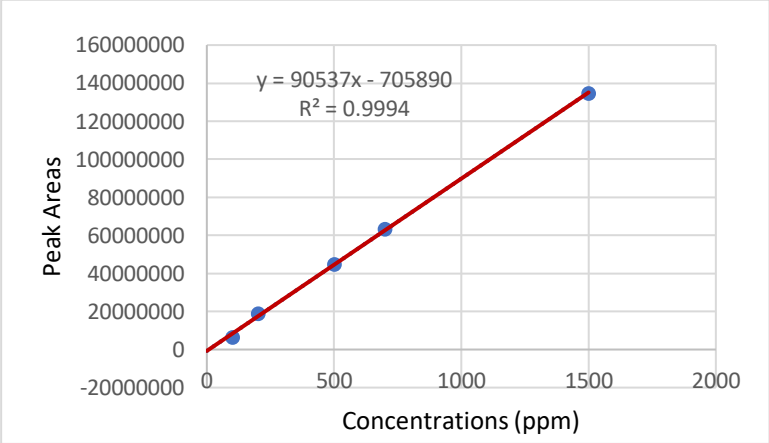

Figure 7.12: Calibration curves for dodecane

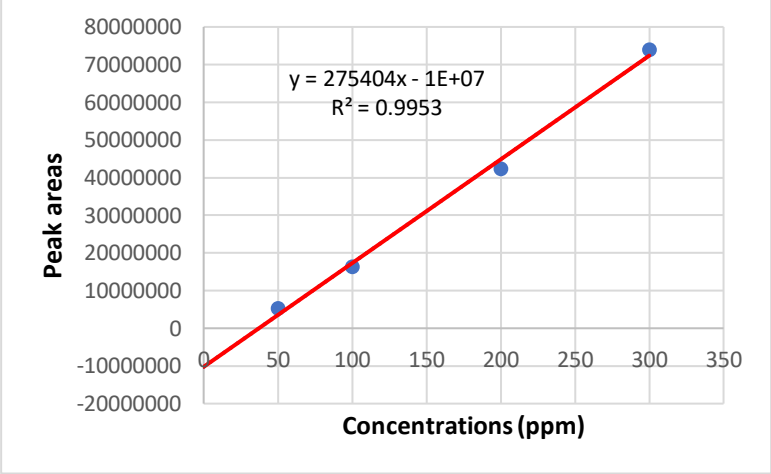

Figure 7.13: Calibration curves for benzene-1,3-bis (1,1dimethylethyl)

7.2.3.2 Quantification of marker compounds- dodecane and benzene-1,3-bis(1,1-dimethylethyl)

Methods of ISO 13859(2014) and USEPA 8270E were used for quantification of the marker compounds. Initial calibration of the instruments followed by evaluation of the concentration of the marker compounds along with calibration verification were carried out. Calibration curves for dodecane and benzene-1,3-bis(1,1dimethylethyl) are shown in Figure 7.12 and 7.13.

7.2.3.3 Utilising the biomarkers -dodecane and benzene-1,3-bis(1,1-dimethylethyl) for monitoring of petroleum-contaminated and remediated soils.

Concentrations of the biomarkers- dodecane and benzene-1,3-bis(1,1-dimethylethyl) were monitored in the soils sampled in the UK along with their TPHs contents. The soils were:

- Petroleum contaminated soils from Tibshelf, Derbyshire, UK
- Remediation treatments on petroleum soils from Tibshelf using the agents described in chapter 4
- Control soils from Tibshelf
- Uncontaminated soils from 3 sites at Brackenhurst, UK.

Concentrations of the biomarkers were also monitored at different concentrations of the crude oil prepared. These concentrations were related to TPHs contents of the soil. The ratio of dodecane to benzene-1,3-bis(1,1-dimethylethyl) was also evaluated and related with TPHs contents of contaminated, controls and remediated soils.

7.2.3.4 Assessment of petroleum-contaminated soils in Ogoniland, Nigeria using crude oil standard and the biomarkers-dodecane and benzene-1,3-bis (1,1-dimethylethyl)

TPHs concentrations were determined in petroleum-contaminated soils, controls soils and crude oil samples from different locations in Ogoniland, Nigeria using both TPHs gasoline-diesel range standards and the standardised crude oil.

Concentrations of the biomarkers-dodecane and benzene-1,3-bis (1,1-dimethylethyl) were also determined in these petroleum-contaminated soils, controls soils and crude oil samples from the Niger Delta, Nigeria. The relationship between ratio of dodecane

to benzene-1,3-bis (1,1-dimethylethyl), and the TPHs in the Nigerians sample was also evaluated.

Results of the commercial TPHs standard, crude oil standard and the biomarkers were used to comparatively assess concentrations of TPHs in soil samples from contaminated hotspots in Ogoniland, Nigeria.

Results of the concentrations of the petroleum biomarker compounds- dodecane and benzene-1,3-bis (1,1-dimethylethyl), was also further used to evaluate TPHs concentration in different soil samples.

7.3 Results

A general observation revealed lower values of TPHs obtained with TPHs C10-C40 standard when compared to TPH gasoline-diesel and the crude oil standard (Table 7.5). TPHs values obtained by TPH gasoline-diesel range standard were also observed to be generally higher than those by the crude oil standard. Positive correlation was observed for TPHs values obtained by both TPH gasoline-diesel and the crude oil standard (Figure 7.14).

For monitoring of remediation efficiency in treated soils, similar percentage reduction in TPHs concentrations were observed with all the standards (Table 7.5, Figure 6.16). There was no significant difference in % reduction of TPHs obtained by used of either TPHs C10-C40, TPHs Gasoline-diesel range or the crude oil standard (Table 7.6).

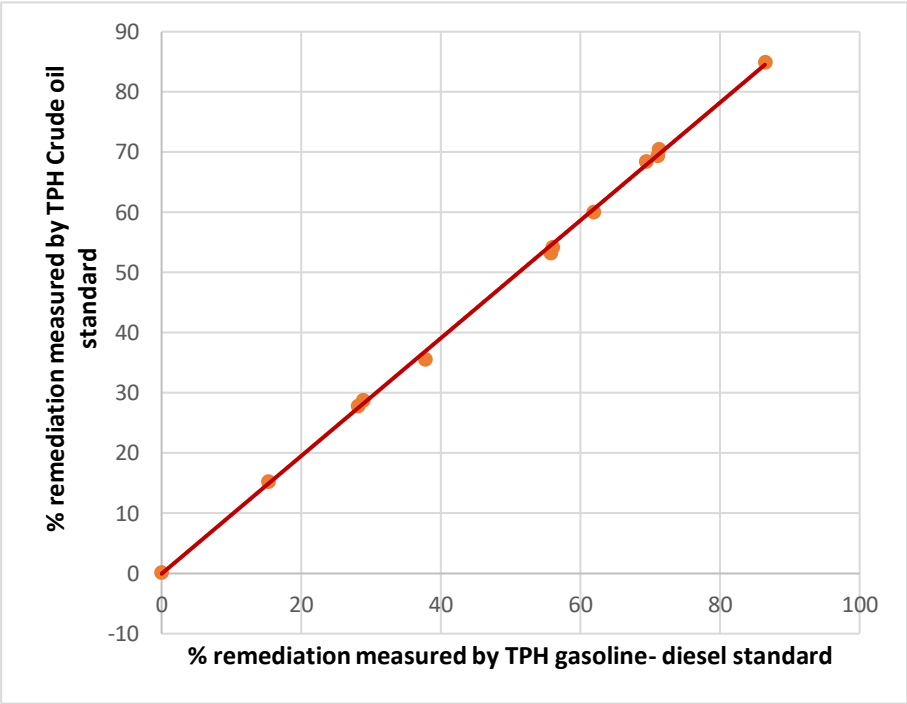

Figure 7.14: Correlations analysis for measurement of % TPHs reduction in soils using TPHs Gasoline-diesel range standards and crude oil as standard

Table 7.5: Comparative analysis of TPHs in soils of Tibshelf, UK using crude oil standard from Tibshelf, Derbyshire, UK and commercial TPHs standard (gasoline-diesel range & C10-C40). Values are given as mean and standard deviation (σ). Sample size (n=198). Yellow-coloured columns are used to indicate similarity in reduction of TPHs measurement for all the standards.

	Levels of TPHs (g/kg dry weight of soil)								
	Conecentrations of TPHs (mg/kg dry weight of soil) using TPHs standard (Gasoline- Diesel range)			Conecentrations of TPHs (mg/kg dry weight of soil) using TPHs standard (C10-C40)			Conecentrations of TPHs (mg/kg dry weight of soil) using crude oil as standard		
Samples/Treatment	**T=0 (MONTHS)**	**T=3 MONTHS**	**% REDUCTION**	**T=0 (MONTHS)**	**T=3 MONTHS**	**% REDUCTION**	**T=0 (MONTHS)**	**T=3 MONTHS**	**% REDUCTION**
UNCONTAMINATED SOIL SAMPLES	18.18 ± 1.63	11.71 ± 0.46	35.60	2.200 ± 0.20	1.420 ± 056	35.84	7.630 ± 0.73	4.750 ± 0.20	37.78
Untreated petroleum-contaminated soil without amendment (Control 1)	338.6 ± 19.12	286.9 ± 29.34	15.27	41.47 ± 2.34	35.13 ± 3.59	15.28	150.4 ± 8.52	127.48 ± 13.07	15.32
Untreated petroleum-contaminated soil + amendment (Control 2)	334.59 ± 5.39	238.2 ± 19.19	28.80	40.96 ± 0.66	29.16 ± 2.35	28.80	148.5 ± 2.40	105.67 ± 8.55	28.89
Petroleum-contaminated soil + *Helianthus annus*	202.0 ± 9.86	92.38 ± 1.52	54.28	13.43± 0.293	5.61 ± 0.09	58.20	87.29 ± 4.39	38.43 ± 0.682	55.97
Petroleum-contaminated soil + *Helianthus sensation*	251.9 ± 84.14	117.7 ± 3.85	53.29	14.93 ± 0.22	7.160 ± 0.23	52.04	86.36 ± 4.81	38.21 ± 3.79	55.75
Petroleum-contaminated soil + *Helianthus annus (sunny dwarf)*	148.9 ± 18.70	45.53 ± 1.19	69.43	18.20 ± 2.29	5.530 ± 0.14	72.10	64.98 ± 8.33	18.79 ± 0.48	71.08
Petroleum-contaminated soil + fermented palm wine from *Elaeis guineensis*	276.2± 1.60	87.12 ± 7.76	68.46	33.79 ± 0.19	10.62 ± 0.95	68.57	121.4 ± 0.69	37.14 ± 3.46	69.40
Petroleum-contaminated soil + fermented palm wine from *Raffia Africana*	344.09 ± 24.94	10.15 ± 1.92	70.49	42.10 ± 3.06	12.39 ± 0.24	70.58	151.62 ± 11.11	43.57 ± 0.86	71.26
Petroleum-contaminated soil + *P. ostreatus* without substrates	293.6 ± 28.92	212.0 ± 26.52	27.80	35.92 ± 3.54	25.92 ± 3.25	27.84	129.1 ± 12.88	92.78± 11.82	28.15
Petroleum-contaminated soil + *P. ostreatus* + substrates layered on soil	132.8 ± 38.14	53.28 ± 8.061	60.13	16.21 ± 4.67	6.46 ± 0.99	64.71	78.32 ± 0.65	29.87 ± 1.12	61.85
Petroleum-contaminated soil + *P. ostreatus* + substrates mixed with soils and layered	204.9 ± 6.08	30.90 ± 1.60	84.92	25.05 ± 0.75	3.73 ± 0.20	85.18	89.70 ± 2.71	12.16 ± 0.72	86.44

Table 7.6: Test of significance and Correlations Analysis in measurement of TPHs using TPHs gasoline-Diesel range Standard and crude oil as standard. P-values are for T-test of significance values obtained by commercial Gasoline-diesel standard against the standardised crude oil standard. Correlation values are also from similar comparison. Samples size, n=33.

Samples/Treatment	**p-values (@ 95% CI) @T=0**	**Correlation Coefficient**	**p-values (@ 95% CI) @T=90 days**	**Correlation Coefficient**
UNCONTAMINATED SOIL SAMPLES	0.0086	1	0.0003	1
Untreated petroleum-contaminated soil without amendment (Control 1)	0.0018	1	0.0154	1
Untreated petroleum-contaminated soil + amendment (Control 2)	0.0000	1	0.0067	1
Petroleum-contaminated soil + *Helianthus annus*	0.0010	1	0.0000	1
Petroleum-contaminated soil + *Helianthus sensation*	0.0014	1	0.0092	1
Petroleum-contaminated soil + *Helianthus annus (sunny dwarf)*	0.0286	1	0.0000	1
Petroleum-contaminated soil + fermented palm wine from *Elaeis guineensis*	0.0000	1	0.0000	1
Petroleum-contaminated soil + fermented palm wine from *Raffia Africana*	0.0045	1	0.0000	1
Petroleum-contaminated soil + *P. ostreatus* without substrates	0.0132	1	0.0285	1
Petroleum-contaminated soil + *P. ostreatus +* substrates layered on soil	0.0000	1	0.0003	1
Petroleum-contaminated soil + *P. ostreatus +* substrates mixed with soils and layered	0.0001	1	0.0009	1

For the biomarkers, while the ratio of benzene-1,3-bis (1,1-dimethylethyl) to dodecane decreased as remediation progressed, the reverse was observed for that of dodecane to benzene-1,3-Bis(1,1-dimethylethyl). Same observation was obtained in the varying concentrations of the crude oil samples (Table 7.7). Ratios of dodecane to benzene-1,3-Bis(1,1-dimethylethyl) in different concentrations of the crude oil samples decreased with increasing concentrations while that of benzene-1,3-bis(1,1-dimethylethyl) to dodecane increased.

Statistical analysis using paired T-test for test of significance revealed a significant difference in the ratios of benzene-1,3-bis(1,1-dimethylethyl) to dodecane obtained in crude oil samples from Tibshelf, UK and those from Ogoniland, Nigeria (Table 7.8).

Tables 7.7: Ratios of the biomarkers -dodecane and benzene-1,3-bis(1,1-dimethylethyl) in petroleum contaminated soils from Tibshelf, UK during remediation treatments with some phyto- and myco-remediation agents. Sample size, n= 33.

	Ratio of dodecane to benzene-1,3-bis(1,1-dimethylethyl)		**Ratio of benzene-1,3-bis(1,1-dimethylethyl) to dodecane to**	
Soils samples	**T=0**	**T=3 Months**	**T=0**	**T=3 Months**
Uncontaminated soils	1.16	1.26	0.86	0.79
Untreated petroleum-contaminated soil without amendment (Control 1)	0.47	0.53	2.13	1.89
Untreated petroleum-contaminated soil + amendment (Control 2)	0.61	0.72	1.64	1.39
Petroleum-contaminated soil + *Helianthus annus*	0.59	1.60	1.69	0.63
Petroleum-contaminated soil + *Helianthus sensation*	0.48	1.35	2.08	0.74
Petroleum-contaminated soil + *Helianthus annus (suuny dwarf)*	0.48	2.32	2.08	0.43
Petroleum-contaminated soil + fermented palm wine from *Elaeis guineensis*	0.34	1.92	2.94	0.52
Petroleum-contaminated soil + fermented palm wine from *Raffia Africana*	0.61	2.07	1.64	0.48
Petroleum-contaminated soil + *P. ostreatus* without substrates	0.68	0.80	1.47	1.25
Petroleum-contaminated soil + *P. ostreatus* + substrates layered on soil	0.54	2.01	1.85	0.50
Petroleum-contaminated soil + *P. ostreatus* + substrates mixed with soils and layered	0.34	2.54	2.94	0.39

Table 7.8: Comparison of ratios of benzene-1,3-bis (1,1-dimethylethyl) to dodecane in crude oil samples from Tibshelf, UK and Ogoniland, Nigeria. Sample size, n=30.

Crude oil concentrations (ppm)	Ratio of benzene-1,3-bis(1,1-dimethylethyl) to dodecane		
	Crude oil from Tibshelf, UK	Crude oil from Ogoniland, Nigeria	p-values for T-test of means of ratio of benzene-1,3-bis(1,1-dimethylethyl) to dodecane in Crude oil from Tibshelf, UK against Crude oil from Ogoniland, Nigeria
500	504.56×10^{-3}	1000×10^{-3}	0.021
1000	536.58×10^{-3}	870.0×10^{-3}	
2500	552.20×10^{-3}	1240×10^{-3}	
5000	622.24×10^{-3}	1838×10^{-3}	
8000	756.34×10^{-3}	2378×10^{-3}	

7.4 Discussion

7.4.1 Assessment of petroleum-contaminated soils and remediation progress using the crude oil standard

Similarity in the concentrations of TPHs in contaminated soils obtained by both commercial TPHs and the crude oil standards demonstrated that the crude oil standards

can be used to estimate TPHs levels in soils. This is further supported by similar percentage remediation efficiency obtained by using any of the standards. A look at the GC chromatogram of the commercial TPHs gasoline-diesel range standard and that of the crude oil standard (Figure 7.2, Figure 7.3) revealed similar distribution patterns of the components. Therefore, the crude oil standard can pick up signals of TPHs components within same range in which the commercial TPHs standard does. The commercial TPH-gasoline diesel range standard is prepared by a 1:1 w:w mixture of neat diesel and mineral oil in 95 % n-hexane (limzhanova *et al*., 2016). These are typical components of crude oil, hence the observed similarities in TPHs measurements obtained by both the crude oil standard and the TPHs gasoline-diesel range standard.

Michelsen & Boyce (1993) reported that commercial TPHs gasoline-diesel range standard has the advantage of accounting for a wide range of petroleum hydrocarbons in environmental matrices. He however stated that many of the commercial TPHs standards were developed for targeted contaminants at particular contaminated sites. Therefore, these standards may not be very suitable for other sites. The use of crude oil from contaminated sources for evaluation of the concentrations of TPHs in soils would offer the advantage of specificity in addition to availability. Yang *et al.* (2015) demonstrated that the use of TPHs standards can account for risk assessment of petroleum contaminated sites. Similarity in remediation efficiency obtained by both standards indicated that the crude oil standard can also give a measure of toxicity index in the remediation process.

TPHs C10-C40 standard consists more of individual hydrocarbon components in the range of C10-C40 (Figure 7.2). Therefore, many components contaminants of crude oil mix (Eganhouse *et al.,* 1993) may be overlooked using this standard. This accounts for the relatively lower quantification values of TPHs observed with the TPHs C10-C40 standard compared to the crude oil and TPHs gasoline-diesel standard.

Although TPHs values obtained by the crude oil standard in this study where slightly lower than those with the conventional TPH-gasoline-diesel standard, the values are comparable to those reported by Salanitro *et al.* (1997) using TPHs standard. The crude oil standard yielded values for % reduction in TPHs during remediation study which were in agreement with those obtained by both the TPH C10-C40 and gasoline-diesel

standard. Thus, using the crude oil standard is reliable. This finding revealed that the crude oil standard could provide a better measure of TPHs contaminants emanating from contamination crude oil compared to TPHs C10-C40 standard but less than those of the gasoline diesel standard, thereby discriminating residual soils hydrocarbons.

Positive correlation both in the values of TPHs and percentage reductions of TPH for all the standards (Table 7.5, Table 7.6, Figure 7.14), indicated that the three standards can be used either qualitatively or semi-quantitatively for evaluation of soils TPHs concentrations, especially during remediation programs. Overall, these findings demonstrated that crude oil from a contaminating source can be used as analytical standard for evaluation of contamination levels and remediation progress of TPHs in petroleum contaminated soils. The outcome would aid for a quick evaluation of TPHs contents in soils in events of unavailability of commercial TPHs standards.

7.4.2 Assessment of petroleum-contaminated soils using the biomarkers- dodecane and benzene-1,3-bis(1,1-dimethylethyl)

Benzene-1,3-bis(1,1-dimethylethyl) has a molar mass of 190 g/mol, polarizability of $24.98\times10^{-24}cm^3$ and heat of vaporization of 83 kJ/mol and is practically insoluble in water (IVerschueren, 2001). Dodecane, on the other, hand has a relatively lower molar mass of 170 g/mol, and a solubility of less than 1 mg/mL at 77°F. It also has a lower heat of volatilisation of 62 kJ/mol at 25^0C (Kertes, 1989). The high ratios of benzene 1,3-bis(1,1-dimethylethyl) to dodecane in the contaminated soils illustrated a preferential retention of the less volatile component in the soils. This is probably because when soil pores are saturated with petroleum contaminants, the more hydrophobic molecules are sequestered within soil pores and become less available or exposed for removal. Cousins *et al.* (1999) reported that during soil contamination, the less hydrophobic components are easily moved downward. Cotrufo *et al.* (2003) stated that aromatic compounds and their derivatives have better tendencies to form associations in soils and become matrix stabilized by bonding with mineral. Thus, in this case, dodecane is comparatively more available to be lost to air and other agents. In the course of remediation, many of the side chains of benzene-1,3-bis(1,1-dimethylethyl) are broken off, and one of these could recombine to form straight chain compounds under favourable conditions thus the increased concentration of dodecane.

Furthermore, microbial degradation of benzene ring can occur in soil by aerobic or anaerobic processes (Coates *et al.,* 2001; Vogt *et al.,* 2011). This can be achieved by bacteria, fungi or yeasts (Evans, 1963; Evans & Fuchs, 1988). Oxidative microbial degradation of benzene occurs with different electron acceptors (Table 7.9). Anaerobic benzene oxidation by *Decholoromonas* and other soil microbes have also been reported (Boyd *et al.,* 1983; Cerniglia, 1984). The products of such degradations include smaller aliphatic fragments and radicals, which can initiate additive reactions with other fragments to increase dodecane concentrations (Vogt *et al.,* 2011).

Table 7.9: Stoichiometric equations for benzene oxidation with different electron acceptors. Adapted from Vogt *et al.,* (2011).

Electrons acceptors oxidised/reduced	**Stoichiometric equations**
CO_2/CH_4	$C_6H_6 + 6.75\ H_2O \rightarrow 2.25\ HCO_3^- + 3.75\ CH_4 + 2.25\ H^+$
SO_4^{2-}/H_2S	$C_6H_6 + 3\ H_2O + 3.75\ SO_4^{2-} \rightarrow 6\ HCO_3^- + 1.875\ H_2S + 1.875\ HS^- + 0.375\ H^+$
Fe^{3+}/Fe^{2+}	$C_6H_6 + 18\ H_2O + 30\ Fe^{3+} \rightarrow 6\ HCO_3^- + 30\ Fe^{2+} + 36\ H^+$
NO_3^-/N_2	$C_6H_6 + 6\ NO_3^- \rightarrow 6\ HCO_3^- + 3\ N_2$
NO_3^-/NO_2^-	$C_6H_6 + 15\ NO_3^- + 3\ H_2O \rightarrow 6\ HCO_3^- + 15\ NO_2^- + 6\ H^+$
ClO_3^-/Cl^-	$C_6H_6 + 5\ ClO_3^- + 3\ H_2O \rightarrow 6\ HCO_3^- + 5\ Cl^- + 6\ H^+$
O_2/H_2O	$C_6H_6 + 7.5\ O_2 + 3\ H_2O \rightarrow 6\ HCO_3^- + 6\ H^+$

The observed correlation in percentage reduction of TPHs and the ratios of the biomarker compounds demonstrated that these ratios can be used for qualitative evaluations of the extent of TPHs contamination and remediation progress. Aromatic compounds in petroleum-contaminated soils also relate to the toxicity index of petroleum (Schreiner *et al.,* 1996). Therefore, the observed reduction in benzene-1,3-bis(1,1-dimethylethyl) to dodecane ratio during the remediation treatments further demonstrated the potential of the agents to reduce toxicity associated with petroleum contaminants in soils.

Peters & Moldowan (1993) and Peters *et al.* (2007) stated that biomarker analysis can be used to discriminate crude oil sources. Lerch *et al.* (2018) reported that petroleum

formed under different geological conditions and ages may exhibit different biomarker fingerprints. The uniqueness of benzene-1,3-bis(1,1-dimethylethyl) to dodecane ratios for each crude oil samples (Table 6.10), established that benzene-1,3-bis(1,1-dimethylethyl)- dodecane ratios can also be used to discriminate crude oil samples from different sources. This is further confirmed by the p-values (0.021) which revealed that the crude oils are significantly different. This outcome further signified that the biomarkers, dodecane and benzene-1,3-bis(1,1-dimethylethyl) can be used to discriminate crude oils samples.

Variation in ratios of dodecane and benzene-1,3-bis(1,1-dimethylethyl) observed for petroleum contaminated and remediated soils can be used to create a pattern for evaluating petroleum contaminated soils. It was generally observed that the ratios of dodecane to benzene-1,3-bis(1,1-dimethylethyl) were generally higher in soils with lower concentration of TPHs (Table 6.8). This ratio also increased during remediation treatment of the petroleum contaminated soils. Hence, dodecane-benzene-1,3-bis(1,1-dimethylethyl) ratios can also be used to evaluate soils contaminated with crude oil, with higher ratios indicating soils with lower TPHs concentration. The converse is also true. Higher ratios of benzene-1,3-bis(1,1-dimethylethyl) to dodecane indicated soils with comparative higher concentrations of TPHs. Thus, the biomarkers dodecane and benzne-1,3-bis(1,1-dimethylethyl) can also be used to evaluate the extent of TPHs contamination and remediation in soils.

It can therefore be generalised that, the ratio of dodecane and benzene -1,3-bis(1,1-dimethylethyl) can be used to evaluate extent of TPHs in soils. Higher ratios of benzene -1,3-bis(1,1-dimethylethyl) to dodecane indicates sites with higher TPHs contents in soils.

Also, the ratios of benzene -1,3-bis(1,1-dimethylethyl) to dodecane can be used to discriminate crude oils from different sources. The p-values for ratios of benzene -1,3-bis(1,1-dimethylethyl) to dodecane for various concentrations of crude oils from different sources is statistically significant.

These two findings requires further investigations which are carried out in the subsections that follows.

7.4.3 **Verification of dodecane - benzene-1,3-bis(1,1-dimethylethyl) ratios for evaluation of TPHs concentrations in soil**

To verify the findings from section 7.2.2, the concentrations of TPHs and the biomarkers-dodecane and benzene-1,3-bis(1,1-dimethylethyl) were determined in soils samples collected from 3 locations at Nottingham Trent University, Brackenhurst, UK (Table 7.10) and 6 locations in Ogoniland, Niger Delta, Nigeria (Table 7.11). Four of the locations in Ogoniland were renowned sites of petroleum contamination while 2 were controls. The ratios of benzene-1,3-bis(1,1-dimethylethyl) to dodecane were evaluated in the soil samples and related to their TPHs contents (Table 7.10, Table 7.11).

Table 7.10: Relationship between TPHs concentration and benzene-1,3-bis(1,1-dimethylethyl)-dodecane ratios in soils of Brackenhurst, United Kingdom. Samples size, n=9.

Sampling points	Coordinate	TPHs level (g/Kg dry soil)	benzene-1,3-bis(1,1-dimethylethyl) (g/Kg dry soil)	Dodecane (g/Kg dry soil)	benzene-1,3-bis(1,1-dimethylethyl)-dodecane ratio	Dodecane-benzene-1,3-bis(1,1-dimethylethyl) ratio
BRACK1	53.063624N, 0.962283W	18.84	27.01	16.79	1.610	0.6200
BRACK2	53.063594N, 0.962282W	5.96	38.93	45.07	0.8900	1.140
BRACK3	53.063624N, 0.962283W	14.25	21.92	12.52	1.750	0.5700

Table 7.11: Relationship between TPHs concentrations and benzene-1,3-bis(1,1-dimethylethyl)-dodecane ratios in soils of Ogoniland, Nigeria. Sample size, n= 18.

LOCATIONS	Coordinates	TPH by diesel-gasoline (g/Kg dry weight)		TPH BY Crude oil standard (g/Kg dry weight)		Benzene-1,3-bis (1,1) - dodecane ratio	
			SE		SE		X 10^{-3}
GIO	0304409N, 0519399E	17.11	0.52	13.94	0.36	0.5704	570.39
OKWALE	0321707N, 0529849E	6.990	1.57	5.790	1.02	0.6783	678.37
BODO	0305473N, 0510286E	416.8	5.90	504.2	7.04	2.8014	2801.43
K-DERE	0308842N, 0515267E	161.1	7.60	115.1	4.95	1.3039	1303.97
K-DERE CONTROL	0308690N, 0515438E	4.950	0.24	12.27	0.43	0.4609	460.86
OGALE	0294965N, 0532977 E	575.3	12.08	346.7	7.21	2.086	2086.91

The investigation revealed that just like the contaminated soils from Tibshelf, UK, ratios of benzene-1,3-bis (1,1) to dodecane increased with increasing concentrations of TPHs in soils from NTU Brackenhurst, UK. The Brackenhurst sites with relatively higher concentrations of TPHs were associated with vehicular parking or machinery, while the site with concentration of TPHs was an isolated tree shed.

For soils from Ogoniland, Nigeria, the ratios of benzene-1,3-bis(1,1-dimethylethyl) to dodecane again increased with increasing TPHs concentration. Benzene-1,3-bis(1,1-dimethylethyl)- dodecane ratios were however relatively higher in the petroleum-contaminated soils from Ogoniland, Nigeria when compared to those from the UK soils. These observations may be due to high aromatic contents associated with Nigerian crude oils (Jones *et al.,* 1986).

From the results (Table 7.11), locations in the Niger Delta soils with high TPHs contents corresponded to those with high benzene-1,3-bis(1,1-dimethylethyl)- dodecane- ratios. Same correlation was also observed for TPHs contents of soils using both TPHs gasoline-diesel range standard and the crude oil standard. Sites at Ogale, Bodo and K-dere are historic petroleum contaminated sites. There are reports that the site at K-dere has been treated for remediation, yet high concentrations of TPHs (161 g/Kg dry weight of soil) were obtained for this location during this investigation. Therefore, remediation programs initiated in the area must be frequently monitored to ensure complete removal of targeted contaminants.

7.5 Conclusions from chapter seven

The present investigation has demonstrated that crude oil standard and petroleum biomarker compounds such as dodecane and benzene-1,3-bis(1,1-dimethylethyl), can be used to evaluate concentrations of TPHs in soils. These biomarkers can also be used to distinguish crude oil samples from the different sources from Tibshelf, UK and Gio, Nigeria. The development of these analytical approaches can provide alternatives for monitoring petroleum-contaminated soils as well as the remediation process. The methods are also quick, easier and readily available.

CHAPTER EIGHT

Summary, overall conclusions and suggestions for further studies

8.1 Summary

The focus of this research was to identify readily available options for monitoring and remediation of petroleum-contaminated soils that can be applied to the Niger Delta, Nigeria. The research began with a literature search to explore the various options for the remediation of petroleum-contaminated soils. Based on environmental-friendliness, cost-effectiveness and resource availability; phyto- and myco-remediation techniques were chosen for the study.

This thesis consists of eight chapters arranged in progressing order of activities. It started with a general introduction in chapter one; chapter two, the literature review; and chapter three, the general methodology. Chapters four, five, six and seven dealt with the main experiments, results and discussions. This final chapter provides a summary of the thesis and the main conclusions.

After the general introduction and literature review in chapters one and two, chapter three provided a general survey of the methodology employed for soil sampling, glasshouse preparations, sample collection after glasshouse treatments, sample preparations and the analyses carried out in the research. The methods specific to certain sections of the thesis were discussed in their relevant chapters. The general approach to the research was identification and sampling of petroleum-contaminated soils, followed by glasshouse remediation treatments with phyto- and myco-remediation agents. Soil samples from Tibshelf, UK were used for the initial phase of the study and the outcomes applied to different soil types (sand, clay, loam) and sediments from the Niger Delta, Nigeria.

The knowledge compiled during the literature review in chapter two was used to initiate and modify the various methodologies used for the research. For instance, conventional petroleum-contaminated soils were used for the study because such provided typical contamination situations. The agents used for remediation were also those with high tolerance to petroleum contamination and locally available in the Niger Delta, Nigeria. The phytoremediation agents used for the study include 3 species of sunflower namely

Helianthus sensation, Helianthus sunny dwarf and Helianthus annus- pacino gold; and the f*ern-Dryopteris affinis.* Mycoremediation agents were fermented palm wine from two species of palm trees namely, *Elias guineensis* and *Raffia africana*, as well as *Pleurotus ostreatus.* Each of these agents was used for the treatment of petroleum-contaminated soils for remediation (reduction) of TPHs.

The research itself consisted of four main parts reported separately in chapters four, five, six and seven. The first part involved investigating the remediation potential of sunflower species, fermented palm wine and *P. ostreatus* on petroleum-contaminated soils. Next, the ability of Tween 80 to enhance the remediation efficiency of the identified phyto- and myco-remediation agents was investigated. This was followed by utilization of mycoremediation for the treatment of petroleum-contaminated soils and sediments from the Niger Delta, Nigeria. The final part involved method development for assessment of the petroleum-contaminated and remediated soils.

Results from chapter four demonstrated up to 340 g/Kg dry weight of TPHs in the contaminated soils from Tibshelf, UK. The highest remediation efficiency among the agents (84%) was achieved by *P. ostreatus*. The remediation efficiency of the sunflower species varied according to their biomass with the highest (69%) demonstrated by *Helianthus annus* (Pacino gold). Although fermented palm wine recorded a remediation efficiency of 70%, the ease of its application makes it the most ideal among all the agents.

Based on the results in chapter four, chapter five investigated the possibility of enhancing the identified remediation efficiency of the agents. Silty loamy soil from Ogoniland, Niger Delta, Nigeria was used. The results revealed an increase in the remediation efficiency of *Helianthus annus-pacino gold* at 78% after 30 days, 100% after 60 days and 53% after 90 days on addition of Tween 80. Kinetic studies were further applied to the results, from which timeframes for complete remediation of the contaminated soils with or without the addition of the surface-active agent were estimated. Thus, from chapter five, it was established that phyto-and myco-remediation of the petroleum contaminated soils can be enhanced by the addition of Tween 80. This chapter also demonstrated that kenetic studies can be used to evaluate the mechanisms of of remediation as either extraction or degration.

The study in chapter six was carried out to assess the applicability of the results obtained in chapter four and five on different soil and sediments from the Niger Delta, Nigeria. Petroleum-contaminated sandy, loamy and clay soils; and sediments from different locations in Ogoniland, Nigeria were treated with the mycoremediation agents- *P. ostreatus and fermented* palm wine. The choice of mycoremediation was because optimal remediation efficiencies in chapters four and five were obtained from the mycoremediation agents. After a 90-day treatment on the soil types, the highest remediation efficiency of 100% was obtained by *P. ostreatus* on loamy and clay soils, then sandy soils (81%). Fermented palm wine supplemented by Tween 80 also demonstrated up to 100% remediation efficiency on the petroleum-contaminated sediments. This chapter, therefore demonstrated that the results obtained with soils from Tibshelf, UK, can be applied to soils in the Niger Delta, Nigeria. It illustrated that the phyto- and myco-remediation techniques can be adapted to different soils types and sediments as well as temperate and tropical soils.

One difficulty in the assessment of remediation efficiency during the treatment of petroleum-contaminated soils is the absence of readily available monitoring techniques. Usually, TPHs concentrations are analysed in GC-MS or GC-FID and quantification determined using commercial TPHs standards. Hence, the concentrations of TPHs in chapters four, five and six of this Thesis were determined using commercial TPHs gasoline-diesel range standard. However commercial TPHs standards are not 'over the counter' reagents. Thus, acquisition and delivery processes of these standards can lead to delays in quantification of TPHs in environmental matrices. During this research, it took an average of 5 months for acquisition of the commercial TPHs standard. It was, therefore, necessary to investigate other options for monitoring TPHs concentrations in soils, especially during remediation programs.

In chapter seven, the research was therefore designed to investigate alternative methods for the assessment of the petroleum-contaminated and remediated soils. A two-stage process was investigated. First, the use of crude oil as analytical standard for the quantification of TPHs. The second stage was, the use of petroleum biomarker compounds-dodecane and benzene-1,3-bis(1,1-dimethylethyl) for the monitoring concentration of TPHs in the soils. These two methods were used to monitor and

confirm the concentrations of TPHs and remediation efficiencies in chapters four and five. The results from this chapter established that standardised crude can be used as analytical standard for quantification of TPHs. It also revealed that the ratios of the biomarkers, dodecane and benzene-1,3-bis(1,1-dimethylethyl) can be used to discriminate crude oil from different sources, and for assessment of TPHs in soils.

Other options that have been identified during this research for monitoring the remediation progress of petroleum hydrocarbons in soils include monitoring of available nitrate and electrical conductivity levels.

8.2 Overall conclusions

Although there are several conventional techniques for remediation and assessment of TPHs in petroleum-contaminated soils, these are often not readily available for a quick application, due to the associated high cost, technology, and other logistics. Therefore, cases of crude oil pollution tend to linger for a long time, particularly in remote areas and particularly in developing countries like Nigeria. Petroleum-contaminated soils are objectionable sites resulting in land degradation and serves as reservoirs where contaminants are released to other environmental matrices such the atmosphere, underground water, and even food chains. The case of the Ogoniland, Niger Delta, Nigeria, is alarming. The region is associated with large oil spill sites, most of which have remained for decades without feasible remediation options. The problem of crude oil soil contamination is also common to other regions of the world which are associated with petroleum activities, and is worse in developing countries. Even when a remediation has been carried out, there are often questions of if such is effective enough, due to the absence of readily available techniques for assessment of both the contaminated and remediated sites.

This study therefore aimed to investigate readily available and sustainable techniques for remediation of petroleum-contaminated soils, and to evaluate ways of overcoming any limitations associated with the identified methods, thereby enhancing these techniques. It was also designed to investigate readily available methods of monitoring the petroleum-contaminated and remediated soils.

The main research questions in this study were:

1. Can readily available and sustainable techniques be found for remediation of petroleum-contaminated soils that can be utilised in the Niger Delta, Nigeria and other regions of the world?
2. What techniques could be available for a quick assessment of TPHs in petroleum-contaminated and remediated soils, in addition to the already established standards?
3. If there are limitations to these identified options, in what ways can these limitations be overcome?
4. Can the identified options be practically applied to solving the problems of petroleum-contaminated soils, particularly in the Niger Delta, Nigeria, and what could be the possible limitations of these techniques?

This study has identified that although associated with several limitations, phyto- and mycoremediation techniques are sustainable. The techniques are readily available, cost effective and can be enhanced for remediation of petroleum-contaminated soils. The agents that have been identified and can be sustainably utilised for phyto- and myco-remediation of TPHs in petroleum-contaminated soils in this study are the sunflowers species (*Helianthus pacino gold, Helianthus sunsation* and *Helianthus annus*), the fern (*Dryopteris affinis*), palm wine (from *Elais guineensis* and *Raffia africana*), and the white rot fungus- *Pleurotus ostreatus*.

For the sunflower plants, the remediation efficiency of TPHs in soils is related to their biomass. Thus, using sunflower plants with higher biomass will result in better remediation efficiency. The use of the sunflowers can in addition to the remediation offer aesthetic appeal to the obnoxious petroleum-contaminated sites.

The application of palm wine to petroleum-contaminated soils, requires first, the palm wine to be fermented, then this substance can be applied directly to the soil.

P. ostreatus requires suitable substrates for its application for the remediation of TPHs in soils. The method of application is also important. The substrate used for the application of *P. ostreatus* for remediation of TPHs in this study was palm tree substrates which is also in vast quantities in the Tropics. Thus, substrate type and method of application can greatly affect the remediation outcome of *P. ostreatus.* Mixing the substrate with soils, followed by layering is very effective for application of *P. ostreatus* during the treatment of TPHs in soils.

In terms of the mechanisms of remediation, kinetic studies revealed that, the sunflower plants exhibited phytoextraction while the other agents (*D. Affinis*, palm wine, and *P. ostreatus*) exhibited phyto-degradation. The potential of these agents to phyto-degrade TPHs is important because the contaminants are degraded thereby reducing the risk of biotransfer into food chain. However, care must be taken to ensure that substances take up by these agents are not bio-transferred into food chain.

The phyto- and myco-remediation agents namely sunflowers (*Helianthus spp*), fern (*D.* affinis), palm wine and *P. ostreatus*, are readily available and in vast abundances in many regions of the world especially in the Tropics and are also cheaper to obtain, and easy to apply. Thus, with respect to the first research question, these methods are readily available and sustainable and can be used for the remediation of petroleum-contaminated soils, particularly for the Niger Delta, Nigeria and other regions of the world.

Soil available nitrate and electrical conductivity are directly co-related with TPHs concentration in petroleum-contaminated soil. Highly TPHs contaminated soils is associated with low available nitrate and low electrical conductivity. The available nitrate and electrical conductivity increase with decreasing TPHs in soil during remediation. Therefore, these physicochemical parameters have been identified as readily available techniques for a quick assessment of TPHs concentration during

remediation of petroleum-contaminated soils. These parameters are also quick and easy to be assessed using portable instruments.

In addition to the commercially available TPHs standards, crude from the contaminating source can be standardised and used as standard for analysis of TPHs concentration in soil using the GC-MS, with outcomes comparable to those of commercially available TPHs standards. The petroleum biomarker compounds, benzene-1,3- (dimethyl ethyl), and dodecane have also been identified as common components in crude oil and their associated contaminated soil. The ratio of benzene-1,3- (dimethyl ethyl) to dodecane correlates positively with concentrations of TPHs in soil samples. This ratio is also distinct for crude oil samples from different sources. Therefore, the biomarker compounds can also be used to evaluate TPHs concentration in soil, and to discriminate crude oil from different sources.

Hence, in addition to the already established techniques, physicochemical parameters such available nitrate and electrical conductivity have been identified for the quick assessment of TPHs in soils. Crude oil standard and the biomarker compounds benzene-1,3- (dimethyl ethyl) can also be used for a quick assessment of TPHs, in event of unavailability of the commercially available TPHs standards.

One of the limitations of phyto- and myco-remediation is that these methods take a longer time to achieve remediation of TPHs in soils. This study was able to enhance the remediation efficiency of the identified phyto- and myco-remediation agents by the addition of the surface-active agents Tween 80 to the soil samples during the remediation treatments. The addition of Tween 80 significantly increased TPHs remediation efficiency of the agents, and significantly reduced the length of time taken for the remediation (reduction in concentration) of TPHs in the contaminated soils by the agents. For instance, for the sunflower plant (*Helianthus annus*), the TPHs remediation efficiency increased from 50 to 100%, and the time taken for complete removal was reduced from 190 days to 58 days. For *D. affinis*, the efficiency increased from 68 to 100% and remediation time reduced from 127 to 48 days. Palm wine exhibited an increase from 70 to 100%, with a reduction in

remediation time from 91 to 29 days, while for *P. ostreatus*, the efficiency was improved from 70 to 100% and the remediation time reduced from 86 to 19 days.

Most of the issues raised in the literature such as the absence of readily available techniques, limitations of phyto- and myco-remediation, and challenges in assessment of TPHs have been addressed in this study. In the course of this study, new agents for phyto- and myco-remediation of petroleum-contaminated soils have been identified, along with new substrates and approach to application to application of *P. ostreatus*. New techniques for assessment of TPHs concentration in petroleum-contaminated and remediated soils have also been identified. An enhancement of the remediation efficiency of the identified phyto- and myo-remediation agents, to overcome the known limitations of these techniques have also been carried out. The identified methods have also been applied to different soil types of sandy, clay, and loam as well as river sediments from the Niger, Delta, Nigeria, and a remediation of up to 100% reduction in TPHs achieved. The schematic representation of the research carried out, and outcomes in this study is illustrated in Figure 8.1.

Overall, this research was able to:

- Identify phyto- and myco-remediation agents that are found in many parts of the world, which can be used for remediation of petroleum-contaminated soils, in both temperate and tropical climates like the Niger Delta, Nigeria.
- Demonstrate an enhancement of the phyto- and myco-remediation of petroleum-contaminated soils using a surface-active agent.
- Develop readily available options for analysis and monitoring of petroleum-contaminated soils.

The research specifically achieved the following:

- Identification of variability in textural properties and TPHs concentrations in the soils of Ogoniland, Nigeria. The soil types include sandy, clayey and loamy soils.

- Remediation of typical petroleum-contaminated soils and sediments using different species of sunflower (*Helianthus annus-pacino gold, Helianthus sunsation.;n, and Helianthus annus-sunny dwarf*), *Dryopteris affinis, Pleurotus ostreatus* and fermented Palm wine from *Raffia affricana* and *Elais guineensis*.
- Enhancement of remediation efficiency of *H. annus-pacino gold, D. affinis,* P. *ostreatus,* and fermented Palm wine on petroleum-contaminated soils using the surface-active agents-Tween 80.
- Assessment of petroleum-contaminated soils using available nitrate and electrical conductivity, crude oil standard and the biomarkers-dodecane and benzene 1,3 -bis(1,1-dimethylethyl).

Interesting novel outputs from this research include:

- Use of fermented palm wine for remediation of petroleum-contaminated soils.
- Use of palm substrates for the cultivation of the white rot fungi-*Pleurotus ostreatus* under unsterilized conditions.
- Use of the fern-*Dryopteris affinis* for remediation of petroleum-contaminated soils.
- Identification of variability in TPHs remediation efficiency of sunflower species, and that the remediation efficiency is related to biomass.
- Enhanced methods for application of phyto-and myco-remediation agents on petroleum-contaminated soils using Tween 80.
- Assessment of TPHs remediation progress on petroleum-contaminated soils using available nitrate and electrical conductivity.
- Evaluation of TPHs concentration in petroleum-contaminated soils and sediments using the contaminated crude oil as analytical standard in GC-MS analysis.
- Identification of the biomarker compounds- dodecane and benzene 1,3-bis(1,1-dimethylethyl) as common components of contaminating crude oil and the associated contaminated soils.
- Evaluation of TPHs concentration in soils using the biomarker compounds dodecane and benzene 1,3-bis(1,1-dimethylethyl).

- Discrimination of crude oil from different sources using the biomarkers- dodecane and benzene 1,3-bis(1,1-dimethylethyl).

From this study, it is concluded that phyto- and myco-remediation can provide viable and environmentally friendly options for the management of petroleum-contaminated soils and sediments. Some of the agents that can be used in the remediation of soils and sediments in the Niger Delta, Nigeria include *Helianthus annus, Dryopteris affinis, Pleurotus ostreatus* and fermented Palm wine. It is also concluded that the contaminating crude oil can be prepared and used as an analytical standard for GC-MS analysis of TPHs. Furthermore, the biomarkers- dodecane and benzene 1,3 -bis(1,1-dimethylethyl), available nitrate and electrical conductivity can also be used to broadly monitor the concentrations of total petroleum hydrocarbons in soils. The remediation potential of fermented palm wine from this study further illustrate the potential of fermented plant juice for the treatment of petroleum-contaminated soils.

8.3 Limitations of the study

This study was carried out in a glasshouse under experimental conditions. Thus, in situ application of these techniques on petroleum-contaminated soils is further required.

The soil samples used in this study were manually homogenised. There, is therefore a possibility of variability in preparations of the soil samples for glasshouse treatments and during sampling of the soils for analysis. Analytical variability resulting from analytical standards and reagents, and instrumental errors, is also possible. However, these limitations have been reasonably contained through the use of composite samples, replicate sampling and analysis, and evaluation of data validity by assessment of accuracy, precision, analytical, instrumental and sampling variability.

Adequate care must however be taken when applying these techniques, first to prevent the transfer of contaminants into the food chain and secondly, to prevent the introduction of invasive species of plants and saprophytic fungi capable of destroying other components of the ecosystem. Plants like the ferns are ecologically very resistant species and are difficult to uproot from the soil. Ferns also reproduce by spores which implies their capacity for invasion of the ecosystem if introduced. The white rot fungus

– *P. ostreatus* requires substrate for its propagation, and thus could utilise any suitable substrate in the ecosystem thereby creating an ecological imbalance, by destroying useful economic and food crops. Therefore application of these techniques requires adequate demarcation of treated areas in a away that the invasive effect of these agents, and biotransfer of the contaminants is properly contained.

The present study investigated the reduction of TPHs in the soils by the agents as remediation. There are several other contaminant components from crude oil in soils. Such include trace metals, individual polycyclic aromatic compounds, and heavy fractions of crude that cannot be analysed using GC-MS. The use of GC-MS for the analysis and quantification of the TPHs in the present study limits the assessment of higher molecular compounds present in crude oil such as the asphaltenes and resins. Therefore, an evaluation of the remediation efficiency of the techniques on trace metals, polar and higher molecular compounds using methods such as ICP-MS, LC-MS and Iatroscan respectively, requires further investigation.

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8.4 Practical considerations for application of the phyto- and myco-remediation techniques in the Niger Delta, Nigeria.

Practical considerations for utilisation of these techniques in real pollution scenarios involving petroleum contamination in soils require consideration of factors such as relative costs of each approach and geography, careful considerations of the following:

- The practicality of utilising cow manures for large scale pollution episodes
- The practicality of utilising palm wine for large scale pollution episodes
- The adaptability of the results obtained with the soils from Tibshelf, UK, in a glasshouse to Nigerian soils.

In the present study, 50 g of cow manure was used per 300 g of soil. This amounts to 17% of cow manure addition to soils. The actual quantity of cow manure required for a remediation would then depend on the extent and quantity of soils to be treated. One kilogram of petroleum-contaminated soil at the levels used in this study would require 170 g of cow manure for amendment.

According to the United State Department of Agriculture (1995), a single cow on the average produces 27 Kg of manure per day and 200 cows can produce as much manure as a community of 5000-10,000 people. This however, varies with the body weight of the animal (Table 8.1). Thus, a single cow can produce manure for the amendment of 160 kg of petroleum-contaminated soils.

Lawal-Adebowale (2012), reported that the documented population of cattle in Nigeria is over 13.9 million. This number is capable of producing over 4 X 10^7 Kg of manure per day. From this study, the quantity of cow manure produced per day is enough to amend 2 X 10^6 Kg of soils. Although the actual quantity of soils contaminated by crude oil in Nigeria is yet to be documented, considering the population in the Niger Delta, Nigeria (20 million people), with an area of 70, 000 square kilometres and quantity of oil spills (1,400,000 and 2,100,000 m^3) (Baird, 2010; Dare, 2013). The total quantity of cow manure produced in Nigeria is more than enough for utilization of the method in the region.

Table 8.1: Dairy Cattle Manure Production and Characteristics (Fischer, 1998).

Cow Size (Kg)	**Quantity of manure Kg/day**	**Nutrient content, Kg/day**		
		N	**P_2O_5**	**K_2O**
60	5	0.03	0.01	0.02
114	9	0.05	0.02	0.04
230	19	0.09	0.04	0.08
450	37	0.19	0.08	0.15
640	52	0.26	0.10	0.21

A daily production of 5-10 litres and up to 150 litres monthly of palm wine is obtained from a single palm tree (Simonart and Laudelot, 1951; Okafor, 1978). In this study, 0.25 litres of fermented palm wine were used on 300 g of soils. This implies 1000 Kg of soils will require 800 litres of fermented palm wine for remediation. This equates to the quantity of palm wine produced from 80 palm trees in a day and 5 in a month. Over 3000 species of palm trees are known with over 3 million hectares of formal oil palm plantations in Nigeria (Dimelu & Anyaiwe, 2011; Rivas *et al.,* 2012; Ini-mfon *et al.,* 2013). A hectare of oil palm plantation is estimated to have as much as 150 individual trees (Sheil *et al.,* 2009). Thus, over 450 million populations of oil palm trees can be estimated

for Nigeria, and these plantations are majorly found in the Niger Delta (Kajisa *et al.* 1997). This is in addition to other populations of palms such as raffia palms, coconuts and date palms (Okafor, 1978; Chandrasekhar *et al.,* 2012).

From the estimated 150 litres of palm wine by a single palm tree, 100,000 population of palm trees can produce 15 million litres of palm wine which can be used to treat 20,000 tons (2×10^6 Kg) of petroleum-contaminated soils. Chandrasekhar *et al.* (2012) reported that controlled tapping of palm wine from oil palm trees can be carried without interfering with the primary purpose of palm oil production. Other species such as raffia palms are principally used for the production of palm wine (Eze & Ogan, 1988; Mbuagbaw & Noorduyn, 2012). Therefore, considering the population and varieties of palm trees in the Niger Delta, Nigeria, the use of fermented palm wine for remediation of petroleum-contaminated soil is viable.

The present study was carried during the summer with temperatures between 18-25^{0}C and up to 10-14 hours daylight (Küller *et al.,* 2006). This replicate typical conditions of the Niger Delta, Nigeria (Ani & Okpara, 2019). Therefore, the results obtained in this study would apply to soils of the Niger, Delta Nigeria. However, other factors such as the soil type, chemistry and microbiology; contaminants type, and levels of contamination will influence the application of these methods in soils of the Niger Delta.

The Niger Delta, and particularly Ogoniland, is bedevilled with the problem of petroleum-contaminated sites, many of which are yet to be remediated. This study has demonstrated that resources, which are locally available in the Niger Delta, can be used for the clean-up. For practical application of these techniques in the Niger Delta, the following is proposed:

- Adequate evaluation of the contamination situation in the area. This include comprehensive auditing and mapping of existing contaminated areas and identifying areas with high possibility of oil spill incidences.
- Evaluation of the extent of petroleum contamination in the affected area. This would involve spatial and profile extents as well as concentration levels.

- Identification of the remediation agents that are locally available in each of the contaminated sites and the feasibility of acquiring other agents in terms of cost, particularly transportation.
- Setting out plans for remediation and courses of action. For instance, in areas where contaminants are far down in soil profiles, bioremediation plants can be constructed. This would allow soils to be dug out, treated and returned.
- Pre-planning remediation programs in areas with the potential impact of oil spills. This includes areas where oil facilities such as pump head flow stations and tank farms are located.
- Education and training of people on the techniques for awareness.
- Adequate plans to overcome unforeseen challenges such as physical and geographical barriers, and resistance by hostile groups in the communities.

For the overall success of such a program, all stakeholders in environmental management in the Nigerian oil sector must be involved. This includes the government, government agencies, multinational and indigenous oil companies and most importantly the host communities. The host communities must be properly enlightened and carried along with the remediation programmes. These people must be aware of the methods and resources, and where they can easily provide services such as supply of the remediation materials like palm tree substrates and palm wine. The overall benefits of the scheme should be properly communicated to all stakeholders.

Ideal application of the technique for the clean-up of contaminated soils in the Niger Delta, Nigeria would involve a combined or sequential pattern where the contaminated soils are treated with each of the agents in rotations.

8.5 Suggestions for Further Studies

To achieve more available options for remediation of petroleum-contaminated soils, particularly in the Niger Delta, Nigeria, the following recommendations for further studies are made.

- Assessment of profile and spatial variation of TPHs contaminants in the soils of Ogoniland, Niger Delta, Nigeria. This will aid the evaluation of the extent of

contamination down the soil profile and possible application of the identified phyto and myco-remediation techniques.

- Investigation on the application of the identified phyto and mycoremediation techniques towards remediation of individual petroleum contaminants such as trace metals, polycyclic aromatic compounds, and high molecular mass organic compounds that could be present in the petroleum contaminated soils. This would aid specific risk evaluation of the remediation process.
- Investigation into other methods of enhancement of remediation efficiency of phyto- and myco-remediation agents. This includes coupling phyto-and myco-remediation agents, use of locally available bio-surfactants, biotechnology and nanotechnology.
- Investigation of the distribution of the contaminants in various parts of each of the phyto- and myco-remediation agents as well as the investigation of the mechanism of remediation as this is important to prevent undue accumulation and bio-transfer of these contaminants.
- Investigation of remediation potentials of other fermented plant juice for the treatment of petroleum-contaminated soils.
- Effect of Initial TPHs concentrations in soils on the remediation efficiency of phyto- and myco-remediation agents.
- Utilising the biomarkers- dodecane and benzene 1,3-bis (1,1-dimethyl ethyl) to discriminate crude oil from different sources.

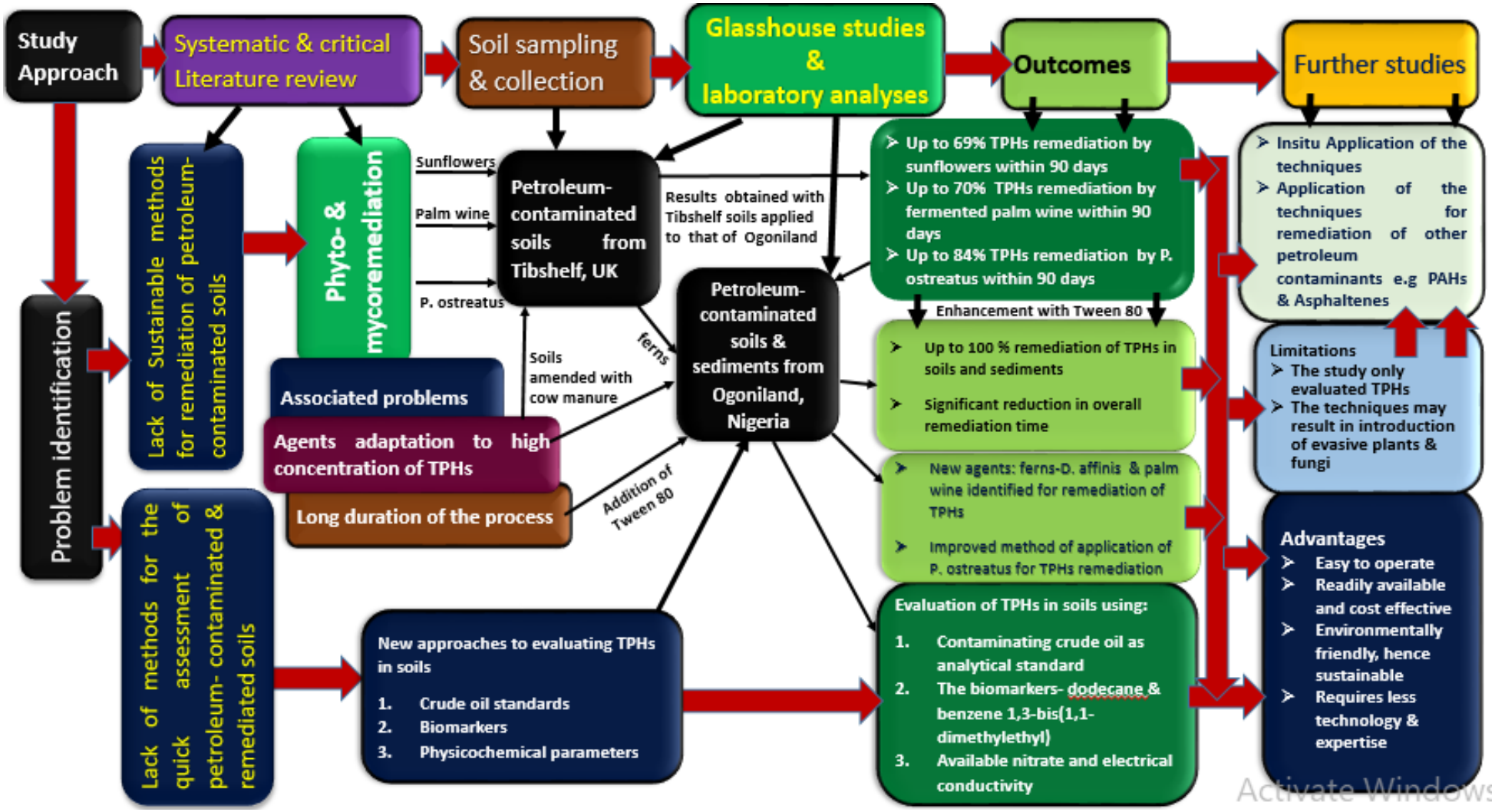


Figure 8.1: Summary of the main research activities and findings in this study

References

Abdul-Khalil, H. P. S., Siti Alwani, M., Ridzuan, R., Kamarudin, H., & Khairul, A. (2008). Chemical composition, morphological characteristics, and cell wall structure of Malaysian oil palm fibers. *Polymer-Plastics Technology and Engineering*, 47(3): 273-280.

Abioye, O.P., Akinsola, R.O., Aransiola, S.A., & Damisa, D. (2013). Biodegradation of crude oil by Saccharomyces cerevisiae isolated from fermented zobo (locally fermented beverage in Nigeria). *Pakistani Journal of Biological Sciences*, 16(24): 2058-61.

Abosede, E. E. (2013). Effect of Crude Oil Pollution on some Soil Physical Properties. *Journal of Agriculture and Veterinary Science*, 6 (93): 14-17.

Abou-Shanab, R. A., El-Sheekh, M. M., & Sadowsky, M. J. (2019). Role of Rhizobacteria in Phytoremediation of Metal-Impacted Sites. In Emerging and Eco-Friendly Approaches for Waste Management (pp. 299-328). Springer, Singapore.

Acevedo, F., Pizzul, L., Castillo, M., del P., Cuevas, R., & Diez, M. C. (2011). Degradation of polycyclic aromatic hydrocarbons by the Chilean white-rot fungus Anthracophyllum discolor. *Journal of Hazardous Materials*, *185*(1): 212–219.

Adedokun, O., & Ataga, A. (2016). Degradation of Crude Oil by Indigenous Edible Mushrooms. *American Journal of Experimental Agriculture*, *11*(1): 1–8.

Adenipekun, C. O. (2008). Bioremediation of engine-oil polluted soil by Pleurotus tuber-regium Singer, a Nigerian white-rot fungus. *African Journal of Biotechnology,* 7 (1): 055-05.

Adenipekun, C. O., & Fasidi, I. O. (2005). Bioremediation of oil-polluted soil by *Lentinus subnudus,* a Nigerian white-rot fungus. *African Journal of Biotechnology,* 4(8):796-798.

Adenipekun, C. O., & Lawal, Y. (2011). Mycoremediation of Crude Oil and Palm Kernel Contaminated Soils by *Pleurotus pulmonarius* Fries (Quelet). *Nature and Science,* 9(9):125-13.

Adenipekun, C. O., & Lawal, R. (2012). Uses of mushrooms in bioremediation: A review. *Biotechnology and Molecular Biology Reviews,* 7(3): 62-68.

Adenipekun, C. O., Ipeaiyeda, A. R., Olayonwa, A. J., & Egbewale, S. O. (2015). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in spent and fresh cutting fluids contaminated soils by *Pleurotus pulmonarius* (Fries). Quelet and *Pleurotus ostreatus* (Jacq.) Fr. P. Kumm. *African Journal of Biotechnology,* 14(8): 661-66.

Adewole, M. B., & Bulu, Y. I. (2012). Influence of different organic-based fertilizers on the phytoremediating potential of Calopogonium mucunoides Desv. from crude oil polluted soils. *Journal of Bioremediation and Biodegradation*, 3(4): 1000144.

Adjei, M., & Overå, R. (2019). Opposing discourses on the offshore coexistence of the petroleum industry and small-scale fisheries in Ghana. *The Extractive Industries and Society*, *6*(1): 190-197.

Agbogidi, O. M., Eruotor, P. G., Akparobi, S. O., & Nnaji, G. U. (2007). Evaluation of crude oil contaminated soil on the mineral nutrient elements of maize (Zea mays L.). *Journal of Agronomy*, *6*(1): 188.

Aggelides, S. M., & Londra, P. A. (2000). Effects of compost produced from town wastes and sewage sludge on the physical properties of a loamy and a clay soil. *Bioresource technology*, 71(3): 253-259.

Aghalino, S. O., & Eyinla, B. (2009). Oil exploitation and marine pollution: Evidence from the Niger Delta, Nigeria. *Journal of Human Ecology*, *28*(3): 177-182.

Agnello, A. C., Bagard, M., Van Hullebusch, E. D., Esposito, G., & Huguenot, D. (2016a). 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: 693-703.

Agnello, A. C., Huguenot, D., Van Hullebusch, E. D., & Esposito, G. (2016b). Citric acid- and Tween® 80-assisted phytoremediation of a co-contaminated soil: alfalfa (Medicago sativa L.) performance and remediation potential. *Environmental Science and Pollution Research*, 23(9): 9215-9226.

Agnello, A. C., Huguenot, D., Van Hullebusch, E. D., & Esposito, G. (2014). Enhanced phytoremediation: a review of low molecular weight organic acids and surfactants used as amendments. *Critical Reviews in Environmental Science and Technology*, 44(22): 2531-2576.

Agnihotri, A., & Seth, C. S. (2019). Transgenic Brassicaceae: A Promising Approach for Phytoremediation of Heavy Metals. In Transgenic Plant Technology for Remediation of Toxic Metals and Metalloids (pp. 239-255). Academic Press.

Aguilar-Rivera, N., Moran, A. C., Lagunes, A. D. R., & Gonzalez, J. M. (2012). Production of pleurotus ostreatus (oyster mushroom) grown on sugar cane biomass (trash, bagasse and pith). *Mushrooms: types, properties and nutrition:* 77-103.

Ahmed, I., Sebastain, A., Prasad, M. N. V., & Kirti, P. B. (2019). Emerging Trends in Transgenic Technology for Phytoremediation of Toxic Metals and Metalloids. In Transgenic Plant Technology for Remediation of Toxic Metals and Metalloids (pp. 43-62). Academic Press.

Aisien, F. A., Aisien, E. T., & Oboh, I. O. (2015). Phytoremediation of Petroleum-Polluted Soils. In Phytoremediation (Pp 243-252). Springer, Cham.

Aislabie, J., Mcleod, N., & Fraser, R. (1998). Potential for biodegradation of hydrocarbons in soil from the Ross dependency; Antartica. *Applied Microbiolology & Biotechnology*, 49: 210 – 214.

Akpokodje, E. G. (1987). The engineering-geological characteristics and classification of the major superficial soils of the Niger Delta. *Engineering Geology,* 23(3-4): 193- 211.

Albert, E., Anyanwu, D. I., & Kim, K.-H. (2016). Evaluation of Composting with “Spent” Mushroom Substrate and Sawdust for Remediation of Petroleum Hydrocarbon-Polluted Soil. *Journal of Agriculture and Ecology Research International Philippines. 9*(91), 1–9.

Albert, O. N., Amaratunga, D., & Haigh, R. P. (2018). Evaluation of the impacts of oil spill disaster on communities and its influence on restiveness in Niger Delta, Nigeria. *Procedia engineering,* 212: 1054-1061.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—Concepts and applications". *Chemosphere*. 91 (7): 869–881.

Allison, C., Oriabure, G., Ndimele, P. E., & Shittu, J. A. (2018). Dealing with oil spill scenarios in the Niger Delta: Lessons from the past. In *The political ecology of oil and gas activities in the Nigerian aquatic ecosystem* (pp. 351-368). Academic Press.

Al-Doury, M. M. I. (2019). Treatment of oily sludge using solvent extraction. *Petroleum Science and Technology*, *37*(2), 190-196.

Al-Jarri, A. S., & Startzman, R. A. (1997). Worldwide Petroleum-Liquid Supply and Demand (includes associated papers 52597 and 52598). *Journal of Petroleum Technology*, *49*(12), 1-329.

Allen, M. F., Swenson, W., Querejeta, J. I., Egerton-Warburton, L. M., & Treseder, K. K. (2003). Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology,* 41(1): 271-303.

Al-Nasrawi, H. (2012). Bioremediation & Biodegradation. *Journal of Bioremediation and Biodegradation, 3*(4):4.

Al-Nasrawi, H. (2013). Biodegradation of Crude Oil by Fungi Isolated from Gulf of Mexico. *Journal of Bioremediation & Biodegradation*, 3(4):1-6.

Amjad, A. L. I., Di, G. U. O., Mahar, A., Ping, W. A. N. G., Feng, S. H. E. N., Ronghua, L. I., Zhang, Z. (2017). Mycoremediation of potentially toxic trace elements— a biological tool for soil clean-up: A review. *Pedosphere*, 27(2): 205-222.

An, L., Pan, Y., Wang, Z., & Zhu, C. (2011). Heavy metal absorption status of five plant species in monoculture and intercropping. *Plant and soil,* 345(1-2):237-245.

Anasonye, F., Winquist, E., Kluczek-Turpeinen, B., Räsänen, M., Salonen, K., Steffen, K.T., Tuomela, M. (2014). Fungal enzyme production and biodegradation of polychlorinated dibenzo-p-dioxins and dibenzofurans in contaminated soil. *Chemosphere*, 110: 85–90.

Anderson, P., & Radford, E. (1994). Changes in vegetation following reduction in grazing pressure on the National Trust's Kinder Estate, Peak District, Derbyshire, England. *Biological Conservatio*n, 69(1): 55-63.

Anderson, C. and Juday, G. (2016). Mycoremediation of Petroleum: A Literature Review. *Journal of Environmental Science and Engineering A 5:* 397-405.

Anderson, G. K., & McCarthy, J. E. (1994). Use of risk-based standards for clean-up of petroleum contaminated soil. Air force center for environmental excellence brooks afb tx.

Ani, A. O., & Okpara, M. O. (2019). Effect of Ambient Temperature on the Performance of Shaver Brown Hens in Hot Humid Environment. *Asian Journal of Advances in Agricultural Research:* 1-10.

Antunes, P., Rodrigues, A., Neto, A., Cardoso, V. V., Benoliel, M. J., & Almeida, C. M. M. (2019). In-house Validation of Accelerated Solvent Extraction-High Performance Liquid Chromatographic Method for Determination of Polycyclic Aromatic Hydrocarbons in Portuguese Sewage Sludge. *Polycyclic Aromatic Compounds:* 1-16.

Aranda, E., Ullrich, R., Hofrichter, M., (2010). Conversion of polycyclic aromatic hydrocarbons, methyl naphthalenes and dibenzofuran by two fungal peroxygenases. *Biodegradation,* 21: 267–281.

Armstrong, R. D., Perris, R., Munn, M., Dunsford, K., Robertson, F., Hollaway, G. J., & O'Leary, G. J. (2019). Effects of long-term rotation and tillage practice on grain yield and protein of wheat and soil fertility on a Vertosol in a medium-rainfall temperate environment. *Crop and Pasture Science,* 70(1): 1-15.

Arthur, E., Crews, H., & Morgan, C. (2000). Optimizing plant genetic strategies for minimizing environmental contamination in the food chain: report on the MAFF funded joint JIC/CSL Workshop held at the John Innes Centre, October 21-23, 1998. *International journal of phytoremediation,* 2(1): 1-21

Asghar, H. N., Rafique, H. M., Khan, M. Y., & Zahir, Z. A. (2017). Phytoremediation of Light Crude Oil by Maize (Zea mays L.) Bio-Augmented with Plant Growth Promoting Bacteria. Soil and Sediment Contamination: *An International Journal,* 26(7-8): 749-763.

Asamudo, N. U., Daba, A.S., & Ezeronye, O. U. (2005). Bioremediation of textile effluent using Phanerochaete chrysosporium. *African Journal of Biotechnology,* 4: 1548–1553.

Asiabadi, F. I., Mirbagheri, S. A., Najafi, P., & Moatar, F. (2014). Phytoremediation of petroleum-contaminated soils around Isfahan Oil Refinery (Iran) by sorghum and barley. *Current World Environment,* 9(1): 65.

Asuk, S. A., Offiong, E. E., & Ifebueme, N. M. (2018). Species composition and diversity of mangrove swamp forest in southern Nigeria. *International Journal of Avian & Wildlife Biology*, 3(2): 166-171.

Aust, S. D. (1995). Mechanisms of Degradation by White Rot Fung. *Environmental Health Perspectives,* 103(5): 59-63.

Avery, B. W. (1980). Soil Classification for England and Wales (higher categories), Soil Survey Technical Monograph No. 14, Rothamsted Experimental Station, Harpenden, UK.

Bahraminia, M., Zarei, Mehdi, Ronaghi, A., & Ghasemi-Fasaei, R. (2016). Effectiveness of Arbuscular Mycorrhizal Fungi in Phytoremediation of Lead-contaminated Soil by Veriver grass. *International Journal of Phytoremediation,* 18(7):730-737.

Baird, J. (2010). Oil's shame in Africa. Newsweek, Lagos, Nigeria, July 26, pp: 27.

Baldrian, P. (2008). Wood-inhabiting ligninolytic basidiomycetes in soils: ecology and constraints for applicability in bioremediation. *Fungal Ecology,* 1(1): 4-12.

Baldrian, P., Inderwiesche, C., Gabriel, J., Nerud, F. and Zadrazil, F. (2000). Influence of cadmium and mercury on activities of Ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *pleurotus ostreatus* in soil. *Applied Environmental Microbiology,* 66(6): 2471-2478.

Bamforth, S. M., & Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology & Biotechnology*, 80(7): 723-736.

Bandowe, B. A. M., Leimer, S., Meusel, H., Velescu, A., Dassen, S., Eisenhauer, N., ... & Wilcke, W. (2019). Plant diversity enhances the natural attenuation of polycyclic aromatic compounds (PAHs and oxygenated PAHs) in grassland soils. *Soil Biology and Biochemistry,* 129: 60-70.

Banks, V. (2017). Hydrogeology of the Peak District and its River Basin Management planning. *Mercian Geologist*, *19*(2): 94.

Bao, W., Zhu, S., Guo, S., Wang, L., Huang, S., Fu, J., & Ye, Z. (2018). Particle size distribution mathematical models and properties of suspended solids in a typical freshwater pond. *Environmental pollution,* 241: 164-171.

Barr, D. P., & Aust, D. (1994). Mechanisms white rot fungi use to degrade pollutants. *Environmental Science & Technology,* 28: 78 – 87.

Barrett, N. (2011). Allen Carlson and Sheila Lintott (eds): Nature, Aesthetics, and Environmentalism: From Beauty to Duty. *Journal of Agricultural and Environmental Ethics,* 24 (*6*): 659–668.

Baruah, P., Deka, S., & Baruah, P. P. (2016). Phytoremediation of crude oil-contaminated soil employing Crotalaria pallida Aiton. *Environmental Science and Pollution Research,* 23(11): 10595-10603.

Basumatary, B., Saikia, R., & Bordoloi, S. (2012). Phytoremediation of crude oil contaminated soil using nut grass, Cyperus rotundus. *Journal of environmental biology*, 33(5): 891.

Bennet, J. W., Connick, W. J., Daigle, D., & Wunch, K. (2001*). Formulation of fungi for in situ bioremediation*. In: Fungi in bioremediation, ed. Gadd, G.M. pp. 97 – 108. USA: Cambridge University Press.

Bennett, L. E., Burkhead, J. L., Hale, K. L., Terry, N., Pilon, M., & Pilon-Smits, E. A. (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *Journal of environmental quality,* 32(2): 432-440.

Bernabé-Antonio, A., Maldonado-Magaña, A., Estrada-Zúñiga, M. E., Buendía-González, L., & Cruz-Sosa, F. (2018). Procedure for Estimating the Tolerance and Accumulation of Heavy Metals Using Plant Cell Cultures. In Plant Cell Culture Protocols Humana Press (pp. 333-337.). New York, NY.

Bhatt, M., Cajthaml, T., & Sasek, V. (2002). Mycoremediation of PAH-contaminated soil. *Folia Microbiologica; Dordrecht:* 255-8.

Bhattacharya, S., Angayarkanni, J., Das, A., & Palaniswamy, M. (2012). Mycoremediation of Benzo[a]Pyrene by Pleuirotus *ostreatus* isolated from Wayanad district in Kerala, India. *International Journal of Pharmacy & Biological Science,* 2 (2): 84-93.

Bissada, K. A., Tan, J., Szymczyk, E., Darnell, M., & Mei, M. (2016). Group-type characterization of crude oil and bitumen. Part I: Enhanced separation and quantification of saturates, aromatics, resins and asphaltenes (SARA). Organic Geochemistry, 95: 21-28.

Blaisdell, R. A., & Smallwood, M. E. (1993). Evaluation of the total petroleum hydrocarbon standard for clean-up of petroleum contaminated sites (No. AFIT/GEE/ENV/93S-1). MSc Thesis, Air Force Inst of Tech Wright- Patterson Afboh School of Engineering.

Bodo, T. (2018). The petroleum exploitation and pollution in Ogoni, Rivers State, Nigeria: The community perspective. *European Scientific Journal, ESJ,* 14(32): 197.

Bojan, B. W., Lamar, R. T., Burjus, W. D., & Tien, M. (1999). Extent of humification of anthrecene, fluoranthene adbenzo (a) pyrene by *Pleurotus ostreatus* during growth in PAH-contaminated soils. *Letters Applied Microbiology,*28(4): 250-254.

Bonnen, A. M., Anton, L. H., & Orth, A. B. (1994). Lignin-degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. *Applied Environmental Microbiology*, 60(3): 960-965.

Boonsaner, M., Borrirukwisitsak, S., & Boonsaner, A. (2011). Phytoremediation of BTEX contaminated soil by Canna× generalis. *Ecotoxicology and environmental safety,* 74(6): 1700-1707.

Boothroyd, I. M., Almond, S., Qassim, S. M., Worrall, F., & Davies, R. J. (2016). Fugitive emissions of methane from abandoned, decommissioned oil and gas wells. *Science of the Total Environment*, 547: 461-469.

Boyd, S. A., Shelton, D. R., Berry, D., & Tiedje, J. M. (1983). Anaerobic biodegradation of phenolic compounds in digested sludge. *Applied Environmental Microbiology,* 46(1): 50-54.

Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C., & Broll, G. (2006). Potential of vetiver (Vetiveria zizanioides (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. *International journal of phytoremediation*, 8(4): 273-284.

Brentnall, I. (1995). Britain's first mainland oil well at Tibshelf/Hardstoft. *Ashfield Historian* 3(3):1.

Brown, S. L., Chaney, R. L., Angle, J. S., & Baker, A. J. M. (1994). Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc-and cadmium-contaminated soil. *Journal of Environmental Quality,* 23(6): 1151-1157.

Brown, I., & Tari, E. (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,* 4(4): 273-282.

Bruns, T. (2006). Evolutionary biology: A kingdom revised. *Nature* 443: 758-761.

Brusseau, M. L. (2019). Soil and groundwater remediation. In Environmental and Pollution Science (pp. 329-354). Academic Press.

BS EN ISO 11461 (2014). Soil quality — Determination of soil water content as a volume fraction using coring sleeves — Gravimetric method. BSI group, London, United Kingdom.

BS EN ISO 14254 (2017). Soil quality — Determination of exchangeable acidity in barium chloride extracts. BSI group, London, United Kingdom.

BS EN ISO 16703 (2011). Soil Quality—Determination of Content of Hydrocarbon in the Range C10 to C40 by Gas Chromatography. BSI group, London, United Kingdom.

BS EN ISO/DIS 18400: 101-107(2016). Soil Quality Sampling; Packaging, Transport, Storage and Preservation of Samples. BSI group, London, United Kingdom.

BS ISO 11265 (2016). Soil quality -- Determination of the specific electrical conductivity. BSI group, London, United Kingdom.

BS ISO 11464 (2016). Soil quality — Pre-treatment of samples for physico-chemical analysis. BSI group, London, United Kingdom.

BS ISO 12914 (2012). Soil quality -- Microwave-assisted Extraction of the Aqua Regia Soluble Fraction for the Determination of Elements. BSI group, London, United Kingdom.

BS ISO 14869-3 (2017). Soil quality. Dissolution for the determination of total element content. Dissolution with hydrofluoric, hydrochloric and nitric acids using pressurised microwave technique. BSI group, London, United Kingdom.

BS ISO/TC 134 N 353(2017). Determination of Soil Cation Exchange Capacity at pH7 buffered by 1 M ammonium acetate. BSI group, London, United Kingdom. BSI group, London, United Kingdom

BS ISO/TS 16558-2 (2015). Soil quality — Risk-based petroleum hydrocarbons Part 2: Determination of aliphatic and aromatic fractions of semi-volatile petroleum hydrocarbons using gas chromatography with flame ionization detection (GC/FID). . BSI group, London, United Kingdom.

BS PD ISO/TS 16965(2013). Soil quality — Determination of Trace Elements using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). BSI group, London, United Kingdom.

BSI ISO 10390 (2005): Soil quality —Determination of pH. BSI group, London, United Kingdom.

BSI ISO/DIS 18400-203 (2016): Soil quality — Sampling — Part 203: Investigation of potentially contaminated sites. BSI group, London, United Kingdom.

Bu, Q. W., Zhang, Z. H., Lu, S., & He, F. P. (2009). Vertical distribution and environmental significance of PAHs in soil profiles in Beijing, China. *Environmental geochemistry and health*, *31*(1): 119-131

Bumpus, J. A., Tien, M., Wright, D., & Aust, S. D. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. *Science,* 228(4706): 1434-1436.

Burges, A., Alkorta, I., Epelde, L., & Garbisu, C. (2018). From phytoremediation of soil contaminants to phytomanagement of ecosystem services in metal contaminated sites. *International journal of phytoremediation,* 20(4): 384-397.

Cai, B., Ma, J., Guangxu, Y., Xiaoli, D., Min, L., & Shaohui, G. (2016). Comparison of Phytoremediation, Bio augmentation and Natural Attenuation for Remediating Saline soil Contaminated by Heavy Crude Oil. *Biochemical Engineering Journal,* 112: 170-177.

Cai, Z., Zhou, Q., Peng, S., & Li, K. (2010). Promoted biodegradation and microbiological effects of petroleum hydrocarbons by Impatiens balsamina L. with strong endurance. *Journal of Hazardous Materials,* 183(1-3): 731-737.

Caille, N., Swanwick, S., Zhao, F. J., & McGrath, S. P. (2004). Arsenic hyperaccumulation by Pteris vittata from arsenic contaminated soils and the effect of liming and phosphate fertilisation. *Environmental Pollution*, 132(1): 113-120.

Calamai, A., Masoni, A., Palchetti, E., Grassi, C., & Brilli, L. (2018). Evaluation of Agronomic Performance and Seed Oil Composition of 15 Sunflower Genotypes in South Madagascar. *Agricultural Sciences*, 9: 1337-1353

Cambardella, C. A., & Karlen, D. L. (1999). Spatial analysis of soil fertility parameters. *Precision Agriculture,* 1(1): 5-14.

Carman, K. R., Fleeger, J. W., Means, J. C., Pomarico, S. M., & McMillin, D. J. (1995). Experimental investigation of the effects of polynuclear aromatic hydrocarbons on an estuarine sediment food web. *Marine Environmental Research,* 40(3): 289-318.

Cassotto, R., Fahnestock, M., Amundson, J. M., Truffer, M., Boettcher, M. S., DE LA PEÑA, S. A. & Howat, I. (2019). Non-linear glacier response to calving events, Jakobshavn Isbræ, Greenland. *Journal of Glaciology,* 65(249): 39-54.

Cerniglia, C. E., & Perry, J. J. (1973). Crude Oil Degradation by Microorganisms Isolated from the Marine Environment. *The Journal of Basic Microbiology*, (4): 299-306.

Cerniglia, C. E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbons. *Advances in applied microbiology*, 30: 31-71.

Chandrasekhar, K., Sreevani, S., Seshapani, P., & Pramodhakumari, J. (2012). A review on palm wine. *International Journal of Research in Biological Sciences*, 2(1): 33-38.

Chang, D. H., & Islam, S. (2000). Estimation of soil physical properties using remote sensing and artificial neural network. Remote Sensing of Environment, 74(3): 534-544.

Chang, S. T., & Hayes, W. A. (Eds.). (2013). The biology and cultivation of edible mushrooms. Academic press.

Chaudhary, K., Saraswat, P. K., & Khan, S. (2019). Improvement in fluoride remediation technology using GIS based mapping of fluoride contaminated groundwater and microbe assisted phytoremediation. *Ecotoxicology and environmental safety*, 168: 164-176.

Chauhan, P., & Mathur, J. (2018). Potential of Helianthus annuus for phytoremediation of multiple pollutants in the environment: A Review. *Journal of Biological Sciences and Medicine*, 4(3): 5-16.

Chekol, T., Vough, L. R., & Chaney, R. L. (2004). Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. *Environment international*, 30(6): 799-804.

Chen, L., Chen, W., Chen, L., & Chen, Y. (2009). Study on Fungi-Bacteria Augmented Remediation of Petroleum Contaminated Soil from Northwest of China. *Journal of Food, Agriculture and Environment,* 7 (3-4. pt. 2):750-753.

Cheng, M., Zeng, G., Huang, D., Yang, C., Lai, C., Zhang, C., & Liu, Y. (2018). Tween 80 surfactant-enhanced bioremediation: toward a solution to the soil contamination by hydrophobic organic compounds. *Critical reviews in biotechnology*, 38(1): 17-30.

Cheng, Y., Yu, L., Xu, Y., Liu, X., Lu, H., Cracknell, A. P., ... & Gong, P. (2018). Towards global oil palm plantation mapping using remote-sensing data. *International Journal of remote sensing,* 39(18): 5891-5906.

Cherian, S., & Oliveira, M. M. (2005). Transgenic plants in phytoremediation: recent advances and new possibilities. *Environmental science & technology*, 39(24): 9377-9390.

Chhatre, S., Purohit, H., Shanker, R., & Khanna, P. (1996). Bacterial consortia for crude oil spill remediation. *Water Science and Technology,* 34(10): 187-193.

Chikere, C. B., & Ekwuabu, C. B. (2014). Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. *African Journal of Environmental Science and Technology*, 8(6): 401-406.

Chiu, S. W., Gao, T., Chan, C. S. S., & Ho, C. K. M. (2009). Removal of spilled petroleum in industrial soils by spent compost of mushroom *Pleurotus pulmonarius. Chemosphere*, 75(6): 837-842.

Christian, V., Shrivastava, R., Shukla, D., Modi, H. A., & Vyas, B. R. M. (2005). Degradation of Xenobiotic Compounds by Lignin-degrading White-rot Fungi: Enzymology and Mechanisms involved. *Indian Journal of Experimental Biology, 43:* 301-312.

Ciurli, A., Lenzi, L., Alpi, A. and Pardossi, A. (2014). Arsenic Uptake and Translocation by Plants in Pot and Field Experiments. *International Journal of Phytoremediation*, 16:804–823.

Cole, G. M. (2018). Assessment and remediation of petroleum contaminated sites. New York, (pp1-338), Routledge.

Coates, J. D., Chakraborty, R., Lack, J. G., O'Connor, S. M., Cole, K. A., Bender, K. S., & Achenbach, L. A. (2001). Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of Dechloromonas. *Nature*, 411(6841): 1039.

Cohen, R., Persky, L., & Hadar, Y. (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus Pleurotus. *Applied Microbiology and Biotechnology,* 58 (5): 582–94.

Contreras-Ramos, S. M., Hernandez-Carballo, J., Perales-Garcia, J., Gomez-Guzman, L. A., Martinez-Rabelo, J., Rodriguez-Campos, J., Baroi, J., and Hernandez- Castellanos, B. (2017). Removal of oil hydrocarbons using the grass Panicum maximum and a bacterial consortium in contaminated soil. Joint Event: 5th International Conference on Green Chemistry and Technology & 6th International Conference on Environmental Chemistry and Engineering.

Corden, J. M., Millington, W. M., & Mullins, J. (2003). Long-term trends and regional variation in the aeroallergen Alternaria in Cardiff and Derby UK–are differences in climate and cereal production having an effect?. *Aerobiologia*, 19(3-4): 191-199.

Corden, J. M., Stach, A., & Millington, W. M. (2002). A comparison of Betula pollen seasons at two European sites; Derby, United Kingdom and Poznan, Poland (1995–1999). *Aerobiologia*, 18(1): 45-53.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-M atrix S tabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter*? Global Change Biology,* 19(4): 988-995.

Cousins, I. T., Gevao, B., & Jones, K. C. (1999). Measuring and modelling the vertical distribution of semi-volatile organic compounds in soils. I: PCB and PAH soil core data. *Chemosphere*, 39(14): 2507-2518.

CPP-Parish population 2011. Neighbourhood Statistics. Office for National Statistics. Retrieved 18 March 2016.

Craig, J., Gluyas, J., Laing, C., and Schofield, P. (2013). Hardstoft–Britain's first oil field. *Oil Industry History, 14*(1): 97-116.

Crawford, D. L., & Crawford, R. L. (1976). Microbial degradation of lignocellulose: the lignin component. *Applied Environmental Microbiology*, 31(5): 714-717.

Creighton, O, (2002), Castles and Landscapes: Power, Community and Fortification in Medieval England.

Croan, S. C. (2000). Conversion of wood waste into value-added products by edible and medicinal (Fr.) P. Karst. Species. *Agaricales S. I., Basidiomycetes*: 73-80.

Cui, T., Fang, L., Wang, M., Jiang, M., & Shen, G. (2018). Intercropping of Gramineous Pasture Ryegrass (Lolium perenne L.) and Leguminous Forage Alfalfa (Medicago sativa L.) Increases the Resistance of Plants to Heavy Metals. *Journal of Chemistry:* 1-11.

Cumming, G. (2014). The new statistics: Why and how. Psychological science, 25(1): 7-29.

Cunningham, S. D., Berti, W. R., & Huang, J. W. (1995). Phytoremediation of contaminated soils. *Trends in biotechnology*, *13*(9): 393-397.

Cunningham, S. D., & Ow, D. W. (1996). Promises and prospects of Phytoremediation. *Plant physiology*, *110*(3): 715.

D'Annibale, A., Rosetto, F., Leonardi, V., Federici, F., & Petruccioli, M. (2006). Role of autochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons. *American Society of Microbiology,* 72(1): 28–36.

Dadrasnia, A., & Agamuthu, P. (2013). Diesel fuel degradation from contaminated soil by Dracaena reflexa using organic waste supplementation*. Journal of the Japan Petroleum Institute,* 56(4): 236-243.

Dadrasnia, A., & Agamuthu, P. (2013b). Organic wastes to enhance phyto-treatment of diesel- contaminated soil. *Waste Management & Research,* 31(11): 1133-1139.

Dahl, M., Survo, S., Välitalo, P., Kabiersch, G., Alitalo, O. S., Penttinen, O. P., & Rantalainen, A. L. (2019). Identification of toxicants from a highly C10–C40-contaminated sediment influenced by the wood industry: Petroleum hydrocarbons or biogenic organic compounds? *Environmental Toxicology and Chemistry,* 38(5): 936–946.

Daou, L., & Shipley, B. (2019). The measurement and quantification of generalized gradients of soil fertility relevant to plant community ecology. *Ecology*, 100(1): e02549.

Dare, E. A. (2013). Environmental education for sustainable human and resource development in Nigeria. *Mediterranean Journal of Social Sciences,* 4(12): 97.

Das, N., & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International, 1-13.*

Daud, Z., Awang, H., Kassim, A. S. M., Hatta, M.Z.M., & Arinpin, A. M. (2014). Comparison of Pineapple Leaf and Cassava Peel by Chemical Properties and Morphology Characterization. *Advanced Materials Research, 974:* 384-388.

Davies, J. S., & Westlake, D. W. (1979). "Crude Oil Utilization by Fungi." *Canadian Journal of Microbiology,* 25 (2): 146-156.

DCC-Derbyshire County Council, (2014), The Landscape Character of Derbyshire (4th Edition, March 2014) Available online at: https://www.derby.

Diab, E. A. (2008). Phytoremediation of oil contaminated desert soil using the rhizosphere effects. *Global Journal of Environmental Research,* 2(2): 66-73.

Dickson, U. J., & Udoessien, E. I. (2012). Physicochemical Studies of Nigeria's Crude Oil Blends. *Petroleum & Coal*, 54(3): 244-251.

Dimelu, M. U., & Anyaiwe, V. (2011). Priorities in smallholder oil palm producers in Ika local government area of Delta state: Implication for agricultural extension service in Nigeria. *World Journal of Agricultural Sciences,* 7(2): 117-123.

Dineshkumar, M., Seenuvasan, M., & Sarojini, G. (2019). Phytoremediation Strategies on Heavy Metal Removal. In Water and Wastewater Treatment Technologies (pp. 81-101). Springer, Singapore.

Dominguez-Rosado, E., Pichtel, J., & Coughlin, M. (2004). Phytoremediation of soil contaminated with used motor oil: I. Enhanced microbial activities from laboratory and growth chamber studies. *Environmental Engineering Science,* 21(2): 157-168.

Donatelli, R. E., & Lee, S. J. (2013). How to report reliability in orthodontic research: Part 1. *American Journal of Orthodontics and Dentofacial Orthopedics,* 144 (1): 156-161.

Doni, C., Macci, E., Peruzzi, M., Arenella, B., Ceccantia, B. and Masciandaro, G. (2012). In situ Phytoremediation of a Soil Historically Contaminated by Metals, Hydrocarbons and Polychlorobiphenyls. *Scientific Journal of Environmental Monitoring,* 14:1383.

dos Santos Rocha, A., Entringer, G. C., Daher, R. F., de Amaral Gravina, G., & dos Santos, Z. M. (2019). Agronomic Evaluation of Sunflower Cultivars Adapted to Northern Fluminense Conditions and Grown for Biomass Production. *Functional Plant Breeding Journal*, 1(1): 2176-81.

Doty, S.L. (2000). Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1, Procedure of National Academy of Science U.S.A., (97): 6287-629.

Doty, S.L. (2007). Enhanced phytoremediation of volatile environmental pollutants with transgenic trees, Procedure of National Academy of Science, U.S.A. (04): 16816-16821.

Dubchak, S., & Bondar, O. (2019). Bioremediation and Phytoremediation: Best Approach for Rehabilitation of Soils for Future Use. In Remediation Measures for Radioactively Contaminated Areas (pp. 201-221). Springer, Cham.

Dudhagara, D. R., Rajpara, R. K., Bhatt, J. K., Gosai, H. B., Sachaniya, B. K., & Dave, B. P. (2016). Distribution, sources and ecological risk assessment of PAHs in historically contaminated surface sediments at Bhavnagar coast, Gujarat, India. *Environmental pollution*, 213: 338-346.

Duncan, C. A., Jachetta, J. J., Brown, M. L., Carrithers, V. F., Clark, J. K., Ditomaso, J. M., ... & Rice, P. M. (2004). Assessing the Economic, Environmental, and Societal Losses from Invasive Plants on Rangeland and Wildlands1. *Weed Technology*, *18*(sp1): 1411-1417.

Dzantor, E. K., Chekol, T., & Vough, L. R. (2000). Feasibility of using forage grasses and legumes for phytoremediation of organic pollutants. *Journal of Environmental Science & Health Part A,* 35(9): 1645-1661.

Ebegbulem, J.C., Ekpe,D., & Adejumo, T.O. (2013). Oil Exploration and Poverty in the Niger Delta Region of Nigeria: A Critical Analysis. *International Journal of Bussiness and Social Science,* 4(3): 265-279.

Edema, C. U., Idu, T. E., & Edema, M. O. (2011). Remediation of soil contaminated with polycyclic aromatic hydrocarbons from crude oil. *African Journal of Biotechnology,* 10(7): 1146-1149.

Efe, S. I., & Okpali, A. E. (2012). Management of petroleum impacted soil with phytoremediation and soil amendments in Ekpan Delta State, Nigeria. *Journal of Environmental Protection,* 3(05): 386.

Eggen, T., & Majcherczyk, A. (1998). Removal of polycyclic aromatic hydrocarbons (PAH) in contaminated soil by white-rot fungus, Pleurotus ostreatus. *International Journal of Biodeterioration & Biodegradation,* 41 (2): 111-117.

Ekundayo, F. (2014) Comparative Studies on Biodegradative Abilities of Pleurotus ostreatus and P. pulmonarius in Soils Contaminated with Crude and Used Engine Oils. *Advances in Microbiology,* 4: 849-855.

El Hanafy, A. A., Anwar, Y., Mohamed, S. A., Al-Garni, M.S., Sabir, J.S.M., Osama, A. H., & Zinadah, M. M. (2015). Isolation and Molecular Identification of Two Fungal trains Capable of Degrading Hydrocarbon Contaminants on Saudi Arabian Environment. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 9(12):1075-78.

Elekes, C. C., & Busuioc, G. (2010). The Mycoremediation of Metals Polluted Soils Using Wild Growing Species of Mushrooms. *Latest Trends on Engineering Education:* 36-39.

El-Gendy, A. S., Svingos, S., Brice, D., Garretson, J. H., & Schnoor, J. (2009). Assessments of the efficacy of a long-term application of a phytoremediation system using hybrid poplar trees at former oil tank farm sites. *Water Environment Research,* 81(5): 486-498.

Eliopoulou, E., Papanikolaou, A., Diamantis, P., & Hamann, R. (2012). Analysis of tanker casualties after the Oil Pollution Act (USA, 1990). *Proceedings of the Institution of Mechanical Engineers, Part M: Journal of Engineering for the Maritime Environment*, *226*(4): 301-312.

Elisashavili, V., Kachlishvili, E., & Penninckx, M. J. (2008). "Lignocellulolytic Enzymes Profile During Growth and Fruiting of *Pleurotus ostreatus* on Wheat Straw and Tree Leaves." *Acta Microbiologica et Immunologica Hungarica*, 55 (2): 157-168.

Emmanuel, A. O., & Babatunde, A. W. (2009). Poverty, oil exploration and Niger Delta crisis: The response of the youth. *African Journal of Political Science and International Relations,* 3(5): 224-232.

Emoyan, O. O. (2008). The oil and gas industry and the Niger Delta: Implications for the environment. *Journal of Applied Sciences and Environmental Management*, 12(3):17-29.

Espenson, J. H. (1995). Chemical kinetics and reaction mechanisms (Vol. 102). New York: McGraw-Hill.

Evans, W. C. (1963). The Microbiological Degradation of Aromatic Compounds. *Journal of general Microbiology,* 32: 177-184.

Evans, W. C., & Fuchs, G. (1988). Anaerobic degradation of aromatic compounds. *Annual Reviews in Microbiology*, 42(1): 289-317.

Eze, M. O., & Ogan, A. U. (1988). Sugars of the unfermented sap and the wine from the oil palm, Elaeis guinensis, tree. *Plant Foods for Human Nutrition,* 38(2): 121-126.

Familoni, T. V., Ogidi, C. O., Akinyele, B. J., & Onifade, A. K. (2018). Genetic diversity, microbiological study and composition of soil associated with wild Pleurotus ostreatus from different locations in Ondo and Ekiti States, Nigeria. *Chemical and Biological Technologies in Agriculture*, 5(1): 7.

Fan, B., Zhao, Y., Mo, G., Ma, W., & Wu, J. (2013). Co-remediation of DDT-contaminated soil using white rot fungi and laccase extract from white rot fungi. *Journal of Soils & Sediments,* 13: 1232-1245.

Fan, M. Y., Xie, R. J., & Qin, G. (2014). Bioremediation of petroleum-contaminated soil by a combined system of biostimulation–bioaugmentation with yeast. *Environmental technology*, 35(4): 391-399.

Fernández-Luqueño, F., Medina-Pérez, G., López-Valdez, F., Gutiérrez-Ramírez, R., Campos-Montiel, R. G., Vázquez-Núñez, E., ... & Madariaga-Navarrete, A. (2018). Use of Agronanobiotechnology in the Agro-Food Industry to Preserve Environmental Health and Improve the Welfare of Farmers. In Agricultural Nanobiotechnology (pp. 3-16). Springer, Cham.

Faraco, V., Pezzella, C., Miele, A., Giardina, P., & Sannia, G. (2009). Bio-remediation of colored industrial wastewaters by the white-rot fungi Phanerochaete chrysosporium and Pleurotus ostreatus and their enzymes. *Biodegradation*, *20*(2): 209-220.

Fasani, E., Manara, A., Martini, F., Furini, A., & DalCorso, G. (2018). The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. *Plant, cell & environment,* 41(5): 1201-1232.

Fatima, K., Imran, A., Amin, I., Khan, Q. M., & Afzal, M. (2018). Successful phytoremediation of crude-oil contaminated soil at an oil exploration and production company by plants-bacterial synergism. *International journal of phytoremediation,* 20(7): 675-681.

Fatima, K., Muhammad A., Asma, I., &Khan, Q. (2015). Bacterial Rhizosphere and Endosphere Populations Associated with Grasses and Trees to be Used for Phytoremediation of Crude Oil Contaminated Soil*. Bulletin of Environmental Contamination and Toxicology,* 94(3):314–320.

Feng, J., Lin, Y., Yang, Y., Shen, Q., Huang, J., Wang, S., ... & Li, Z. (2018). Tolerance and bioaccumulation of Cd and Cu in Sesuvium portulacastrum. *Ecotoxicology and environmental safety,* 147: 306-312.

Fentiman, A., & Zabbey, N. (2015). Environmental degradation and cultural erosion in Ogoniland: A case study of the oil spills in Bodo. *The Extractive Industries and Society,* 2(4): 615-624.

Ferdeş, M., Dincă, M., Zăbavă, B., Paraschiv, G., Munteanu, M., & Ionescu, M. (2018, February). Laccase enzyme production and biomass growth in liquid cultures of wood-degrading fungal strains. In 46th International Conference "Actual Tasks on Agricultural Engineering (pp. 341-388).

Fetzer, J. C. (2000). "The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons". *Polycyclic Aromatic Compounds*. New York: Wiley. 27 (2): 143–162.

Fiorentino, N., Ventorino, V., Rocco, C., Cenvinzo, V., Agrelli, D., Gioia, L., ... & Fagnano, M. (2017). Giant reed growth and effects on soil biological fertility in assisted phytoremediation of an industrial polluted soil. *Science of the Total Environment,* 575: 1375-1383.

Fischer, D. B. (1998). Energy Aspects of Manure Management. Illini Dairy Net Papers. The Board of Trustees. University Of Illinois.

Flayyih, I., & Al-Jawhari, H. (2014). Ability of some Soil Fungi in Biodegradation of Petroleum Hydrocarbon. *Journal of Applied & Environmental Microbiology*, 2014 2 (2): 46-52.

Flouri, F., Soterchos, D., Loannidou, S., & Ballis, C. (1995). Decolourization of olive oil mill liquid wastes by chemical and biological means. *In: proceedings of the international Symposium on olive oil processes and By- products recycling, Granada*.

Fonseca, B., Pazos, M., Figueiredo, H., Tavares, T., & Sanromán, M. A. (2011). Desorption kinetics of phenanthrene and lead from historically contaminated soil. *Chemical engineering journal,* 167(1): 84-90.

French, N. (2019). Not All Black and White: The Environmental Dimension of Arctic Exploration. In Arctic Triumph (pp. 129-146). Springer, Cham.

Frezza, C., Venditti, A., Serafini, M., & Bianco, A. (2019). Phytochemistry, Chemotaxonomy, Ethnopharmacology, and Nutraceutics of Lamiaceae. In Studies in Natural Products Chemistry (Vol. 62, pp. 125-178). Elsevier.

Fuksová, Z., Száková, J., & Tlustoš, P. (2009). Effects of co-cropping on bioaccumulation of trace elements in Thlaspi caerulescens and Salix dasyclados. *Plant, soil & environment,* 55(11): 461-467.

Gagnon, N., Hall, C., & Brinker, L. (2009). A preliminary investigation of energy return on energy investment for global oil and gas production. *Energies*, 2(3): 490-503.

Gallego, J. L. R., García-Martínez, M. J., Llamas, J. F., Belloch, C., Peláez, A. I., & Sánchez, J. (2007). Biodegradation of oil tank bottom sludge using microbial consortia. *Biodegradation*, 18(3): 269-281.

Galliano, D., Magrini, M. B., Tardy, C., & Triboulet, P. (2018). Eco-innovation in plant breeding: Insights from the sunflower industry. *Journal of Cleaner Production,* 172: 2225-2233.

Gao, D., Du, L., Yang, J., Wu, W. and Liang, H. (2010). A critical review of the application of white rot fungus to environmental pollution control. *Critical Reviews in Biotechnology,* 30(1): 70–77.

Gao, Y., Dai, X., Chen, G., Ye, J. and Zhou, S. (2003). A randomized, placebo-controlled, multicenter study of Ganoderma lucidum (W. Curt.:Fr.) Lloyd (Aphylloromycetidae) polysaccharides (Ganopoly R) in patients with advanced lung cancer. *International Journal of Medicinal Mushrooms* 5:369–381.

Gao, Y., Ling, W., & Wong, M. H. (2006). Plant-accelerated dissipation of phenanthrene and pyrene from water in the presence of a nonionic-surfactant. *Chemosphere*, 63(9): 1560-1567.

Gatfaoui, H. (2016). Linking the gas and oil markets with the stock market: Investigating the U.S. relationship. *Energy Economics 53:* 5–16.

Gerhardt, K. E., Huang, X. D., Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant science*, *176*(1): 20-30.

Giacomino, A., Malandrino, M., Abollino, O., Velayutham, M., Chinnathangavel, T., & Mentast, E. (2010). An approach for arsenic in a contaminated soil: Speciation, fractionation, extraction and effluent decontamination. *Environmental Pollution:* 158 416–423.

Giraud, F., Guiraud, P., Kadri, M., Blake, G., and Steiman, R. (2001). "Biodegradation of Anthracene and Fluoranthene by Fungi Isolated from an Experimental Constructed Wetland for Wastewater Treatment." *Water Research,* 35 (17): 4126-46.

Glick, B. R. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology advances,* 21(5): 383-393.

Gogoi, B. K., Dutta, N. N., Goswami, P., & Mohan, T. K. (2003). A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. *Advances in Environmental Research*, 7(4): 767-782.

Górska, E. B., Urszula, J., Dobrzyński, J., J. D., Gałązka, A., Sitarek, M., Gozdowski, D., Russel, S. and Kowalczyk, P. (2014). Production of Ligninolytic Enzymes by Cultures of White Rot Fungi. *Polish Journal of Microbiology,* 3(4): 461–465.

Gottlieb, S., Day, W. C., & Pelczar, M. J. (1950). The biological degradation of lignin. II. The adaptation of white-rot fungi to growth on lignin media. *Phytopathology*, 40(10):926-935.

Graham, J. M. (2006). Congeneric and (essentially) tau-equivalent estimates of score reliability: What they are and how to use them. *Educational and Psychological Measurements,* 66(6): 930-944.

Grimberg, S. J., Stringfellow, W. T., & Aitken, M. D. (1996) Quantifying the biodegradation of phenanthrene by Pseudomonas stutzeri P16 in the presence of a nonionic surfactant. *Applied Environmental Microbiology,* 62(7): 2387–2392.

Guerin, T. F. (2000). The Differential Removal of Aged Polycyclic Aromatic Hydrocarbons from Soil during Bioremediation. *Environmental Science and Pollution Research*, 7 (1): 19-26.

Hall, J., Soole, K., & Bentham, R. (2011). Hydrocarbon phytoremediation in the family Fabacea—a review. *International journal of phytoremediation,* 13(4): 317-332.

Hammel, K. E. (1989). Organo-pollutants degradation by ligninolytic fungi Enzyme. *Microbial Technonology,* 11: 776-777.

Hampton, C., Leowenthal, M., & Smith, R. (2019). Real time monitoring of nitrate at farm wells in the Cotswold Oolite. *Quarterly Journal of Engineering Geology and Hydrogeology*, qjegh 2018-113.

Han, R., Dai, H., Yang, C., Wei, S., Xu, L., Yang, W., & Dou, X. (2018). Enhanced phytoremediation of cadmium and/or benzo (a) pyrene contaminated soil by hyperaccumlator Solanum nigrum L. *International journal of phytoremediation*, 20(9): 862-868.

Hanley, Q. S. (2019). The Distribution of standard Deviations Applied to High throughput screening. *Scientific reports,* 9(1): 1268.

Haritash, A. K., & Kaushik, C. P. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *Journal of Hazardous Materials,* 169 (1-3): 1-15.

Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology,* 9(3): 177.

Hassan, M., Abdelhamid, M., Nassef, O. A., & Abdel Harith, M. (2018). Spectrochemical Analytical Follow up of Phytoremediation of Oil-Contaminated Soil. Soil and Sediment *Contamination: An International Journal,* 27(6): 485-500.

Hassan, S. E., Hijri, M., & St-Arnaud, M. (2013). Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. *New biotechnology*, 30(6): 780-787.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi – production and role in lignin degradation. *FEMS Microbiology Reviews,* 13:125–13.

Have, R. T., & Teunissen, P. J. M. (2001). Oxidative Mechanisms Involved in Lignin Degradation by White-Rot Fungi. *Chemical Society Review, 101: 3397−3413.*

Hawksworth, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. *Mycological Research,* 95: 641-655.

Harwood, C. S., & Gibson, J. (1997). Shedding light on anaerobic benzene ring degradation: a process unique to prokaryotes? *Journal of bacteriology*, *179*(2): 301.

He, Y., Wang, S., & Lai, K. K. (2010). Global Economic Activity and Crude Oil Prices: A Cointegration Analysis. *Energy Economics,* 32 (4): 868–876.

Heidari, S., Fotouhi Ghazvini, R., Zavareh, M., & Kafi, M. (2018). Physiological responses and phytoremediation ability of Eastern Coneflower (Echinacea purpurea) for crude oil contaminated soil. *Caspian Journal of Environmental Sciences,* 16(2): 149-164.

Heiri, O., Lotter, A., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content. *Journal of Paleolimnology*, 25: 101-110.

Hiratsuka, N., Oyadomari, M., Shinohara, H., Tanaka, H., & Wariishi, H., (2005). Metabolic mechanisms involved in hydroxylation reactions of diphenyl compounds by the lignin-degrading basidiomycete Phanerochaete chrysosporium. *Biochemical Engineering Journal*, 23: 241–246.

Hoa, H.T., Wang, C. L., & Wang, C.H. (2015). The Effects of Temperature and Nutritional Conditions on Mycelium Growth of Two Oyster Mushrooms *(Pleurotus Ostreatus and Pleurotus Cystidiosus). Mycobiology* 43 (4): 423–34.

Hofrichter, M. (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzyme. *Microbiology Technology,* 30:454–466.

Hollins, P. D., Kettlewell, P. S., Atkinson, M. D., Stephenson, D. B., Corden, J. M., Millington, W. M., & Mullins, J. (2004). Relationships between airborne fungal spore concentration of Cladosporium and the summer climate at two sites in Britain. *International Journal of Biometeorology*, *48*(3): 137-141.

Houben, D., Evrard, L., & Sonnet, P. (2013). Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (Brassica napus L.). *Biomass and Bioenergy,* 57: 196-204.

HS2 (2018). High Speed Rail (Crewe to Manchester and West Midlands to Leeds). Working Draft Environmental Statement Volume 2: Community Area report LA10: Tibshelf to Shuttlewood. Department of Transport, United Kingdom.

Huang, X. D., El-Alawi, Y., Penrose, D. M., Glick, B. R., & Greenberg, B. M. (2004). A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. *Environmental pollution,* 130(3):465-476.

Huang, X. D., Glick, B. R., & Greenberg, B. M. (2000). Combining remediation techniques increases kinetics for removal of persistent organic contaminants from soil. In Environmental Toxicology and Risk Assessment: Science, Policy, and Standardization—Implications for Environmental Decisions: Tenth Volume. ASTM International.

Huang, Y., Fu, Y. G., & Zhao, J. F. (2005). Research Progress on Mechanisms of Phytoremediation for Eutrophic Water [J]. *Journal of Agro-environmental Science* S: 1.

Hull, R. B., Robertson, D. P., Buhyoff, G. J., & Kendra, A. (2000). What are we hiding behind the visual buffer strip? Forest aesthetics reconsidered. *Journal of Forestry*, *98*(7), 34-38.

Ibrahim, M. L., Ijah, U. J. J., Bilbis, L. S., & Manga, S. B. (2013). Phytoremediation of Escravos (Nigeria) Light Crude Oil Contaminated Soil Using Legumes. Proceedings of 23rd Annual International Conference on Soil, Water, Energy & Air, (pp 37-41). San Diego, CA.

IEA-International Energy Agency (2014). Key World Energy Statistics. Lea.org.IEA pp: 6,8.

Ige, O. E. (2011). Vegetation and climatic history of the Late Tertiary Niger Delta, Nigeria, based on pollen record. *Research Journal of Botany*, 6(1): 21-30.

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(5): 460-466.

Ini-mfon, V. P., Sunday, B. A., Samuel, J. U., Daniel, E. J., & Ubong, E. E. (2013). Factors affecting performance of palm oil processors in the South-South Region of Nigeria. *International Journal of Agricultural Economics and Extension*, 1(4): 17-23.

Iqbal, N., Hayat, M. T., Zeb, B. S., Abbas, Z., & Ahmed, T. (2019). Phytoremediation of Cd-Contaminated Soil and Water. In Cadmium Toxicity and Tolerance in Plants (pp. 531-543). Academic Press.

Isikhuemhen, O. S., Anoliefo, G. O., & Oghale, O. I. (2003). Bioremediation of crude oil polluted soil by the white rot fungus, Pleurotus tuberregium (Fr.) Sing. *Environmental Science and Pollution Research, 1*0(2): 108-112.

Isikhuemhen, O. S., Anoliefo, G.O., & Oghale, O. I. (2003). Bioremediation of crude oil polluted soil by the white rot fungus, *Pleurotus tuberregium* (Fr.) Sing. *Environmental Science & Pollution Research International,* 10(2):108-12.

ISO 11504 (2017). Soil quality - Assessment of impact from soil contaminated with petroleum hydrocarbon. The British standard Institutions.

ISO 18287 (2006- E). Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) — Gas chromatographic method with mass spectrometric detection (GC- MS). The British standard Institutions.

Ite, A. E., Ibok, U. J., Ite, M. U., & Petters, S. W. (2013). Petroleum exploration and production: past and present environmental issues in the Nigeria's Niger Delta. *American Journal of Environmental Protection,* 1(4):78-90.

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. *Journal of Environment Pollution and Human Health,* 6(2): 51-61.

IVerschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. p. 1017.

Ivshina, I. B., Kuyukina, M. S., Krivoruchko, A. V., Elkin, A. A. Makarov, S. O., Cunningham, C. J., Peshkur, T. A. Atlasd, R. M., & Philp, J. C. (2015). Oil spill problems and sustainable response strategies through new technologies. *Environmental Sciences, Processes Impacts,* 17: 1201–1219.

Iwegbue, C. M. A., Egobueze, F. E., & Opuene, K. (2006). Preliminary assessment of heavy metals levels of soils of an oil field in the Niger Delta, Nigeria. *International Journal of Environmental Science & Technology,* 3(2): 167-172.

Izah, S. C., & Seiyaboh, E. I. (2018). Changes in the Protected Areas of Bayelsa State, Nigeria. *International Journal of Molecular Evolution and Biodiversity*, 8(1): 1-11.

Izinyon, O. C., & Seghosime, A. (2013). Assessment of show star grass (Melampodium paludosum) for phytoremediation of motor oil contaminated soil. Civil *Environmental Resources*, 3(3):55-61.

Jansen, R. G., Wiertz, L. F., Meyer, E. S., & Noldus, L. P. J. J. (2003). Reliability analysis of observational data: Problems, solutions, and software implementation. *Behavior Research Methods, Instruments & Computers*, 35(3):1-399.

Jenssen, B. M. (1994). Effects of oil pollution, chemically treated oil, and cleaning on thermal balance of birds. *Environmental Pollution*, *86*(2): 207-215.

Jiang, C., Larter, S. R., Noke, K. J., & Snowdon, L. R. (2008). TLC–FID (Iatroscan) analysis of heavy oil and tar sand samples. *Organic Geochemistry*, *39*(8): 1210-1214.

John, R. C., Ntino, E. S., and Itah, A. Y. (2016). Impact of crude oil on soil nitrogen dynamics and uptake by legumes grown in wetland ultisol of the Niger Delta, Nigerian *Journal of Environmental Protection*, *7*(4): 507.

Joshua, J. A., Ahiekpor, J. C., and Kuye, A. (2016). Nigerian hardwood (Nesogordonia papaverifera) sawdust characterization: Proximate analysis, cellulose and lignin contents. *Lignocellulose* 5(1): 50-58.

Ju, S. Y., Wang, J., Sehn, L. B., Li, Z., Chen, Y. H., Wu, L. H., & Luo, Y. M. (2015). Phytoremediation of heavy metal contaminated soils by intercropping with Sedum

plumbizincicola and Triticum aestivum and rotation with Solanum melongena. *Chinese Journal of Ecology*, 34(8):2159-2181.

Kajisa, K., Maredia, M. K., & Boughton, D. (1997). Transformation versus stagnation in the oil palm industry: A comparison between Malaysia and Nigeria (No. 1099- 2016-89087).

Kalac, P., Niznanska, M., Berilaqua, D., & Staskova, I. (1996). Concentration of Mercury, Copper, Cadmium and Lead in fruiting bodies of edible Mushrooms in the vicinity of a mercury smelter and a copper smelter. *Science of Total Environment*: 177:251.

Kalae, P., Burda., J., & Satskova, I. (1991). Concentration of Lead, Cadmium, Mercury and Copper in Mushroom in the vicinity of a Lead Smelter. *The Science of the Total Environment, 105:109-119.*

Kapahi, M., & Sachdeva, S. (2017). Mycoremediation potential of Pleurotus species for heavy metals: a review. *Bioresources and bioprocessing,* 4(1): 32.

Karlsen, D. A., & Larter, S. R. (1991). Analysis of petroleum fractions by TLC-FID: applications to petroleum reservoir description. Organic geochemistry, 17(5): 603-617.

Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H. R., Gupta, S. K., & Schulin, R. (2000). Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environmental Science & Technology,* 34(9): 1778-1783.

Kertes, A. S. (1989). Hydrocarbons with Water and Seawater Part II. Hydrocarbons C8 to C31. Solubility Data Series Vol 38; Shaw PC ed; Pergamon Press, UK: pp 553

Khamehchiyan, M., Charkhabi, A. H., & Tajik, M. (2007). Effects of crude oil contamination on geotechnical properties of clayey and sandy soils. *Engineering geology*, *89*(3-4): 220-229.

Khan, F. I., Husain, T., & Hejazi, R. (2004). An Overview and Analysis of Site Remediation Technologies. *Journal of Environmental Management,* 71: 95-122.

Khandare, R. V., & Govindwar, S. P. (2015). Phytoremediation of textile dyes and effluents: Current scenario and future prospects. *Biotechnology Advances*, 33(8): 1697-1714.

Kim, N., Kwon, K., Park, J., Kim, J., & Choi, J. W. (2019). Ex situ soil washing of highly contaminated silt loam soil using core-crosslinked amphiphilic polymer nanoparticles. *Chemosphere*, 224: 212-219.

Kohlmeier, S., Smits, T. H., Ford, R. M., Keel, C., Harms, H., & Wick, L. Y. (2005). Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. *Environmental science & technology*, 39(12): 4640-4646.

Konne, B. R. (2014). Inadequate monitoring and enforcement in the Nigerian oil industry: the case of shell and Ogoniland. *Cornell International LJ,* 47: 181.

Korcan, S. E., Ciğerci, H. I. and Konuk, M. (2012). White-Rot Fungi in Bioremediation. *Soil Biology,* 32: 371-390.

Kösesakal, T., Ünal, M., Kulen, O., Memon, A., & Yüksel, B. (2016). Phytoremediation of petroleum hydrocarbons by using a freshwater fern species Azolla filiculoides Lam. *International journal of phytoremediation,* 18(5): 467-476.

Kotilínek, M., Tatarenko, I., & Jersáková, J. (2018). Biological Flora of the British Isles: Neottia cordata. *Journal of Ecology*, 106(1): 444-460.

Krall, L., Huege, J., Catchpole, G., Steinhauser, D., & Willmitzer, L. (2009). Assessment of sampling strategies for gas chromatography–mass spectrometry (GC–MS) based metabolomics of cyanobacteria. *Journal of Chromatography B,* 877(27): 2952-2960.

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., ... & Zhang, B. (2017). Saponin-based, biological-active surfactants from plants. In Application and Characterization of Surfactants. IntechOpen, DOI: 10.5772/68062.

Kristanti, R. A., Hadibarata, T. , Toyama, T., Tanaka, Y., & Mori, K . (2011). Bioremediation of Crude Oil by White Rot Fungi Polyporus sp. *Journal of Microbiology & Biotechnology*, 21(9): 995–1000.

Kristensen, A. H., Poulsen, T. G., Mortensen, L., & Moldrup, P. (2010). Variability of soil potential for biodegradation of petroleum hydrocarbons in a heterogeneous subsurface. *Journal of hazardous materials*, *179*(1-3): 573-580.

Kubota, A., Thompson, T. L., Doerge, T. A., & Godin, R. E. (1996). A petiole sap nitrate test for cauliflower. *HortScience*, 31(6): 934-937.

Kulakow, P. A., Schwab, A. P., & Banks, M. K. (2000). Screening plant species for growth on weathered, petroleum hydrocarbon-contaminated sediments. *International Journal of Phytoremediation,* 2(4), 297-317.

Küller, R., Ballal, S., Laike, T., Mikellides, B., & Tonello, G. (2006). The impact of light and colour on psychological mood: a cross-cultural study of indoor work environments. *Ergonomics*, 49(14): 1496-1507.

Kulshreshtha, S., Mathur, N., & Bhatnagar, P. (2014). Mushroom as a product and their role in mycoremediation. *AMB Express*. 4: 29.

Kumar, D., Anand, S., Tiwari, J., Kisku, G. C., & Kumar, N. (2019). Removal of Inorganic and Organic Contaminants from Terrestrial and Aquatic Ecosystems through Phytoremediation and Biosorption. In Environmental Biotechnology: For Sustainable Future (pp. 45-71). Springer, Singapore.

Kumar, P. S. (2019). Soil Bioremediation Techniques. In Advanced Treatment Techniques for Industrial Wastewater (pp. 35-50). IGI Global.

Kumari, M., & Abraham, J. (2011). Biodegradation of Diesel Oil using Yeast *Rhodosporidium toruloides*. *Research Journal of Environmental Toxicology, 5: 369-377.*

Kwon-Ndung, E. H., Akomolafe, G. F., Goler, E. E., Terna, T. P., Ittah, M. A., Umar, I. D., ... & Markus, M. (2016). Diversity complex of plant species spread in Nasarawa State, Nigeria. *International Journal of Biodiversity and Conservation*, 8(12): 334-350.

Lawal-Adebowale, O. A. (2012). Dynamics of ruminant livestock management in the context of the Nigerian agricultural system. In Livestock production. *IntechOpen* 4: 62-80.

Larsen, M. C., & Simon, A. (1993). A rainfall intensity-duration threshold for landslides in a humid-tropical environment, Puerto Rico. *Geografiska Annaler: Series A, Physical Geography,* 75(1-2): 13-23.

Lau, E. V., Gan, S., & Ng, H. K. (2010). Extraction techniques for polycyclic aromatic hydrocarbons in soils. International journal of analytical chemistry: 1-10.

LDBP-Lowland Derbyshire Biodiversity Partnership, (2011), Lowland Derbyshire Biodiversity Action Plan 2011-2020.

Lebo, S. E., Gargulak, J. D. and McNally, T. J. (2001). "Lignin" *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons, Inc.

Lee, K., Boufadel, M., Chen, B., Foght, J., Hodson, P., Swanson, S., & Venosa, A. (2015). Expert Panel Report on the Behaviour and Environmental Impacts of Crude Oil Released into Aqueous Environments. Royal Society of Canada, Ottawa, ON. ISBN: 978-1- 928140-02-3. RSC: EPR 15-1.

Leonardi, V., Vaclav Sasek, V., Petruccioli, M., D'Annibale, A., Erbanova, P.M & Cajthaml, T. (2007). Bioavailability modification and fungal biodegradation of PAHs in aged industrial soils. *International Journal of Biodeteriotion & Biodegradation,* 60 (3) : 165-170.

Leštan, D., Luo, C. L., & Li, X. D. (2008). The use of chelating agents in the remediation of metal-contaminated soils: a review. *Environmental pollution*, *153*(1): 3-13.

Lew, D. (2018). Organic Compounds: Mechanisms Of Organic Contaminant Phytoremediation, published 17th February, 2017. https://www.drdarrinlew.us/organic-compounds. Retrieved 22/08/2018.

Li, J., Luo, C., Zhang, D., Cai, X., Jiang, L., Zhao, X., & Zhang, G. (2019a). Diversity of the active phenanthrene degraders in PAH-polluted soil is shaped by ryegrass rhizosphere and root exudates. *Soil Biology and Biochemistry*, 128:100-110.

Li, N. Y., Li, Z. A., Zhuang, P., Zou, B., & McBride, M. (2009). Cadmium uptake from soil by maize with intercrops. *Water, air, and soil pollution,* 199(1-4): 45-56.

Li, X., Song, Y., Wang, F., Bian, Y., & Jiang, X. (2019). Combined effects of maize straw biochar and oxalic acid on the dissipation of polycyclic aromatic hydrocarbons and microbial community structures in soil: A mechanistic study. *Journal of hazardous materials*, 364: 325-331.

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.

Liao, C., Xu, W., Lu, G., Deng, F., Liang, X., Guo, C., & Dang, Z. (2016). Biosurfactant-enhanced phytoremediation of soils contaminated by crude oil using maize (Zea mays. L). *Ecological Engineering,* 92: 10-17.

Liaoa, C., Wending, X., Lua, G., Denga, F., Lianga, X., Guo, C., & Danga, Z. (2016). Biosurfactant- enhanced Phytoremediation of Soils Contaminated by Crude oil using Maize (Zea mays. L). *Ecological Engineering,* 92: 10–17.

Liduino, V. S., Servulo, E. F., & Oliveira, F. J. (2018). Biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using sunflower (*Helianthus annuus L*.). *Journal of Environmental Science and Health, Part A*, 53(7): 609-616.

Liduino, V. S., Servulo, E. F., & Oliveira, F. J. (2018). Biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using sunflower (Helianthus annuus L.). *Journal of Environmental Science and Health, Part A*, 53(7): 609-616.

Liebig, M. A., Doran, J. W., & Gardner, J. C. (1996). Evaluation of a field test kit for measuring selected soil quality indicators. *Agronomy Journal*, 88(4): 683-686.

Lin, H., Zhang, X. H., Chen, J., Liang, L., & Liu, L. H. (2018). Phytoremediation potential of Leersia hexandra Swartz of copper contaminated soil and its enhancement by using agronomic management practices. *Ecological Engineering.* In press.

Lin, Q., & Mendelssohn, I. A. (2008). Determining tolerance limits for restoration and phytoremediation with Spartina patens in crude oil-contaminated sediment in greenhouse. *Archives of Agronomy and Soil Science,* 54(6): 681-690.

Lindén, O., & Pålsson, J. (2013). Oil contamination in Ogoniland, Niger delta. *Ambio*, 42(6), 685-701.

Liu, R., Jadeja, R. N., Zhou, Q., & Liu, Z. (2012). Treatment and remediation of petroleum-contaminated soils using selective ornamental plants. *Environmental engineering science*, 29(6): 494-501.

Liu, Y., Zhuang, P., Li, Z., Zou, B., Wang, G., Li, N., & Qiu, J. (2013). Effects of fertiliser and intercropping on cadmium uptake by maize. *Chemistry and Ecology,* 29(6): 489-500.

Looney, P. B., Gibson, D. J., Blyth, A., & Cousens, M. I. (1993). Flora of the Gulf Islands National Seashore, Perdido Key, Florida. *Bulletin of the Torrey Botanical Club*: 327-341.

Loske, D., Huttermann, A., Majerczk, A., Zadrazil, F., Lorsen, H. and Waldinger, P. (1990*). Use of white rot fungi for the clean-up of contaminated sites. In: Advances in biological treatment of lignocellulosic materials* (Ed.). M. P. Coughlan and Collaco, pp.311 -321. Elsevier, London.

Lu, H., Li, Z., Fu, S., Méndez, A., Gascó, G., & Paz-Ferreiro, J. (2015). Combining phytoextraction and biochar addition improves soil biochemical properties in a soil contaminated with Cd. *Chemosphere*, 119: 209-216.

Luo, Y., & Tu, C. (Eds.). (2018). Twenty years of research and development on soil pollution and remediation in China. Springer Singapore. Pp 56-63.

Ma, T. T., Teng, Y., Luo, Y. M., & Christie, P. (2013). Legume-grass intercropping phytoremediation of phthalic acid esters in soil near an electronic waste recycling site: a field study. *International journal of phytoremediation,* 15(2): 154-167.

Ma, T., Luo, Y., Christie, P., Teng, Y., & Liu, W. (2012). Removal of phthalic esters from contaminated soil using different cropping systems: A field study. *European Journal of soil biology,* 50: 76-82.

Ma, T., Zhou, L., Chen, L. K., Li, Z., Wu, L., Christie, P., & Luo, Y. (2016). Oxytetracycline toxicity and its effect on phytoremediation by Sedum plumbizincicola and Medicago sativa in metal-contaminated soil. *Journal of agricultural and food chemistry,* 64(42): 8045-8053.

MAF&F-Ministry of Agriculture, Fisheries and Food, (1988), Agricultural Land Classification of England and Wales – Revised guidelines and criteria for grading the quality of agricultural land.

Mahar, A., Wang, P., Ali, A., Awasthi, M. K., Lahori, A. H., Wang, Q., ... & Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. *Ecotoxicology and environmental safety,* 126: 111- 121.

Mahmood, K., Kamilah, H., Sudesh, K., Karim, A. A., & Ariffin, F. (2019). Study of electrospun fish gelatin nanofilms from benign organic acids as solvents. *Food Packaging and Shelf Life*, 19: 66-75.

Marchiol, L., & Fellet, G. (2011). Agronomy towards the Green Economy. Optimization of metal phytoextraction. *Italian Journal of Agronomy,* 6(3): 30-36.

Márquez-Rocha, F. J., Hernández-Rodríguez, V. Z., & Vázquez-Duhalt, R. (2000). Biodegradation of soil-adsorbed polycyclic aromatic hydrocarbons by the white rot fungus Pleurotus ostreatus. *Biotechnology Letters,* 22(6): 469-472.

Marques, A. P., Oliveira, R. S., Rangel, A. O., & Castro, P. M. (2008). Application of manure and compost to contaminated soils and its effect on zinc accumulation by Solanum nigrum inoculated with arbuscular mycorrhizal fungi. *Environmental Pollution,* 151(3): 608-620.

Martínez-Oró, D., Párraga-Aguado, I., Querejeta, J. I., Álvarez-Rogel, J., & Conesa, H. M. (2019). Nutrient limitation determines the suitability of a municipal organic waste for phytomanaging metal (loid) enriched mine tailings with a pine-grass co-culture. *Chemosphere*, 214: 436-444.

Martins, D. C., Liduini, V. S., Olivera, F. J., Servulo, E. F. C. (2014). Phytoremediation of Soils Multi-contaminated with Hydrocarbons and Heavy Metals using Sunflowers. *International Journal of Engineering & Technology,* 14(5):1-10.

Mâsu, S., Morariu, F., & Dragomir, N. (2013). Using different tolerant plant for phytoremediation of contaminated soils with total petroleum hydrocarbons. Scientific Papers *Animal Science and Biotechnologies,* 46(2): 175-179.

Máthé-Gáspár, G., & Anton, A. (2005). Phytoremediation study: Factors influencing heavy metal uptake of plants. *Acta Biologica Szegediensis*, 49(1-2): 69-70.

Mathur, N., Singh, J., Bohra, S., Bohra, A., Chauhan, M., Vyas, M., & Vyas, A. (2010). Phytoremediation of oil contaminated soil by some arid legume tree species. *Advances in Environmental Sciences,* 2(1): 25-32.

Mbuagbaw, L., & Noorduyn, S. G. (2012). The palm wine trade: occupational and health hazards. *International Journal of Occupational Environmental Medicine* (The IJOEM), 3(4): 67-79.

Mehrasbi, M.R., Haghighi, B., Shariat, M., Naseri, S. & Naddafi, K. (2003). Biodegradation of Petroleum Hydrocarbons in Soil. *Iranian Journal of Public Health,* 32(3): 28-32.

Mejáre, M., & Bülow, L. (2001). Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *TRENDS in Biotechnology,* 19(2): 67-73.

Melero, S., Porras, J. C. R., Herencia, J. F., & Madejon, E. (2006). Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil and Tillage Research,* 90(1-2): 162-170.

Mendez, M. O., & Maier, R. M. (2008). Phytoremediation of mine tailings in temperate and arid environments. *Reviews in Environmental Science and Bio/Technology,* 7(1): 47-59.

Merkl, N., Schultze-Kraft, R., & Arias, M. (2005). Influence of fertilizer levels on phytoremediation of crude oil-contaminated soils with the tropical pasture grass Brachiaria brizantha (Hochst. ex a. rich.) stapf. *International Journal of Phytoremediation,* 7(3): 217-230.

Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. *Water, Air, and Soil Pollution,* 165(1-4): 195-209.

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(1): 86-91.

Meysami, P., & Baheri, H. (2003). Pre-screening of fungi and bulking agents for contaminated soil bioremediation. *Advances in Environmental Research,* 7(4): 881-887.

Microbiology online (2016). http://microbiologyonline.org/about-microbiology/introducing- microbes/fungi. Retrived 21/01/2017, 19.59 Hours.

Mitchell, J. K., & Soga, K. (2005). Fundamentals of soil behavior (Vol. 3). Hoboken, NJ: John Wiley & Sons.

Mmom, P., & Igbuku, A. (2015). Challenges and prospect of environmental remediation/Restoration in Niger Delta of Nigeria: The case of Ogoniland. *Journal of Energy Technology and Polymers,* 5: 5-5.

Mogaji, O. Y., Sotolu, A. O., Wilfred-Ekprikpo, P. C., & Green, B. M. (2018). The Effects of crude oil exploration on fish and fisheries of Nigerian aquatic ecosystems. In The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem (pp. 111-124). Academic Press.

Moore, D., Robson, G. D. and Trinci, A. P. J. (2011). 21st *Century guide to fungi. Cambridge University Press.*

Moore, R. T. (1980). "Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts". *Botanica Marina*. 23: 361–373.

Morgan, P., Lewis, S. T. and Watkinson, R. J. (1991). Comparison of abilities of white-rot fungus to mineralize selective xenobiotic compounds. Applied *Microbiology & Biotechnology,* 34: 693-696.

Moubasher, H. A., Hegazy, A. K., Mohamed, N. H., Moustafa, Y. M., Kabiel, H. F., & Hamad, A. A. (2015). Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. *International Biodeterioration & Biodegradation,* 98, 113-120.

Muijs, B., & Jonker, M. T. (2009). Evaluation of clean-up agents for total petroleum hydrocarbon analysis in biota and sediments. *Journal of Chromatography A*, *1216*(27): 5182-5189.

Munsell Colour Company (2000). 'Munsell soil color charts.' Munsell Colour Co. Baltimore, MD.

Muratova, A. Y., Turkovskaya, O. V., Hübner, T., & Kuschk, P. (2003). Studies of the efficacy of alfalfa and reed in the phytoremediation of hydrocarbon-polluted soil. *Applied Biochemistry and Microbiology,* 39(6): 599-605.

Muthusaravanan, S., Sivarajasekar, N., Vivek, J. S., Paramasivan, T., Naushad, M., Prakashmaran, J., ... & Al-Duaij, O. K. (2018). Phytoremediation of heavy metals: mechanisms, methods and enhancements. *Environmental Chemistry Letters:* 1-21.

Myers, A, (1998-2000a), An Archaeological Resource Assessment of the Mesolithic in Derbyshire. East Midlands Archaeological Research Framework.

Naees, M., Ali, Q., Shahbaz, M. & Ali, F. (2011). Role of rhizobacteria in phytoremediation of heavy metals: an overview. *International Research Journal of Plant Sciences,* 2: 220–232.

Naluba, N. G. (2011). Relationship between Local Government Headquarters and Rural Hinterland Settlements in Rivers South East Senatorial District of Rivers State, Nigeria. *Journal of Agriculture and Social Research (JASR*), 11(2), 95-102.

National Petroleum Corporation, NNPC (2016). Business, Upstream, Oil Production.http://www.nnpcgroup.com/nnpcbusiness/upstreamventures/oilproduction.aspx. Retrieved 29/06/2016, 9.30 pm.

Nayak, A. K., Panda, S. S., Basu, A., & Dhal, N. K. (2018). Enhancement of toxic Cr (VI), Fe, and other heavy metals phytoremediation by the synergistic combination of native Bacillus cereus strain and Vetiveria zizanioides L. *International Journal of phytoremediation,* 20(7): 682-691.

Nicholas, O. I. (2015), Bioremediation Potentials of *Heterobasidion annosum* 13.12B and *Resinicium bicolor* in Diesel Oil Contaminated Soil Microcosms. *Journal of Applied Science & Environmental Management,* 19(3):513-519.

Nigam, P., Banat, I.M., McMullan, G., Dalel, S., & Marchant, R. (1995). Microbial degradation of textile effluent containing Azo, Diazo and reactive dyes by aerobic and anaerobic bacterial and fungal cultures, pp.37-38. Paper presented in 36th Annual Conference AMI, Hisar (HARYANA) INDIA.

Njoku, K. L., Akinola, M. O., & Oboh, B. O. (2009). Phytoremediation of crude oil contaminated soil: the effect of growth of Glycine max on the physico-chemistry and crude oil contents of soil. *Nature and Science,* 7(10): 79-87.

Noordman, W. H., & Janssen, D. B. (2002). Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. Applied. Environmental Microbiology, 68(9): 4502-4508.

Novotny, C., Erbanova, P., Sasek, V., Kubatova, A., Cajtham, l. T., Lang, E., Krahl, J. and Zadrazil, F. (1999) Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi. *Biodegradation*, 10(3):159–168.

Novotny, C., Svobodova, K., Erbanova, P., Cajthaml, T., Kasinath, A., Lange, E., & Sasek, V. (2004). Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. *Soil Biology & Biochemistry,* 36(10): 1545-1551.

Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N., & Anyanwu, B. N. (2006). Characterisation of palm wine yeast isolates for industrial utilisation. *African Journal of biotechnology*, *5*(19:109-123.

Nwaiwu, O., Ibekwe, V., Amadi, E., Udebuani, A., Nwanebu, F., Oguoma, O., & Nnokwe, J. (2016). Evaluation of fermentation products of palm wine yeasts and role of Sacoglottis gabonensis supplement on products abundance. *Beverages,* 2(2): 9-21.

Nwogu, T. P., Azubuike, C. C., & Ogugbue, C. J. (2015). Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons after Amendment with Capra aegagrus hircus (Goat) Manure. *Biotechnology Research International,* 7:1-7.

Nrior, R. R., & Jirigwa, C. C. (2017). Comparative bioremediation potential of Mucor racemosus and Paecilomyces variotii on crude oil spill site in Gio Tai, Ogoni land. *Journal of Environmental Science, Toxicology and Food Technology* 5(2): 56-67.

O'Brien, P. L., DeSutter, T. M., & Casey, F. X. (2019). Natural degradation of low-level petroleum hydrocarbon contamination under crop management. Journal of Soils and Sediments, 19(3): 1367-1373.

Obire, O., Anyanwu, E., & Okigbo, R. 2008. Saprophytic and Crude Oil Degradation Fungi from Caw Dung Dropping as Bioremediation Agents. *International Journal of Agriculture Technology,* 4(2), 81-89.

Ohga, S., & Kitamoto, Y. (1997). XVI. Future of mushroom production and biotechnology. *Food Reviews International,* 13(3): 461-469.

Okafor, N. (1978). Microbiology and biochemistry of oil-palm wine. In Advances in Applied Microbiology (Vol. 24, pp. 237-256). Academic Press.

Okeke-Ogbuafor, N., Gray, T., & Stead, S. M. (2016). A comparative analysis of the role of traditional and modern community-based organizations in promoting community development in Ogoniland, Nigeria. *Community Development Journal,* 53(1)*:* 173-189.

Okoro, D., Oviasogie, P. O., & Oviasogie, F.E. (2011). Soil quality assessment 33 months after crude oil spillage and clean-up, *Chemical Speciation & Bioavailability,* 23:1-6.

Okparanma, R. N., Ayotamuno, J. M., Davis, D. D., & Allagoa, M. (2011). Mycoremediation of polycyclic aromatic hydrocarbons (PAH)- contaminated oil-based drill-cuttings. *African Journal of Biotechnology,* 10(26):5149- 5156.

Okwu, D. E., & Nnamdi, F. U. (2008). Evaluation of the chemical composition of Dacryodes edulis and Raphia hookeri Mann and Wendl exudates used in herbal medicine in south eastern Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines,* 5(2), 194-200.

OLÁR, R., Melcer, I., & Kačík, F.(1988). Comparative study of pine wood and pine bark (Pinus silvestris L.) dioxan lignins. *Cellulose Chemistry and Technology,* 22: 39-52.

Osuji, L. C., & Nwoye, I. (2007). An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Owaza experience. *African journal of agricultural research,* 2(7): 318-324.

Osuji, L. C., Egbuson, E. J., & Ojinnaka, C. M. (2005). Chemical reclamation of crude-oil-inundated soils from Niger Delta, Nigeria. Chemistry and Ecology, 21(1), 1-10.

Oteh, C. O., & Eze, R. C. (2012). Vandalization of oil pipelines in the Niger Delta region of Nigeria and poverty: An overview. *Studies in Sociology of Science, 3(2):* 13-21.

Oti, W. J. (2015). Phytoremediation Study of Oil Spill Site Using Common Nigerian vegetables. *International Journal of Resources in Sciences*, 1(3):12-16.

Oviasuyi, P. O., & Uwadiae, J. (2010). The Dilemma of Niger-Delta Region as Oil Producing States of Nigerian *Journal of Peace, Conflict and Development,*16:111-126.

Ozigis, M. S., Kaduk, J. D., & Jarvis, C. H. (2019). Mapping terrestrial oil spill impact using machine learning random forest and Landsat 8 OLI imagery: a case site within the Niger Delta region of Nigeria. *Environmental Science and Pollution Research,* 26(4): 3621-3635.

Oztan, S., & During, R. (2012). Microwave assisted EDTA extraction—determination of pseudo total contents of distinct trace elements in solid environmental matrices. *Talanta* 99: 594–602.

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(1): 633-654.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., & Chung, J. W. (2011). Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Journal of hazardous materials,* 185(2-3): 549-574.

Park, S., Kim, K. S., Kim, J. T., Kang, D., & Sung, K. (2011). Effects of humic acid on phytodegradation of petroleum hydrocarbons in soil simultaneously contaminated with heavy metals. *Journal of Environmental Sciences,* 23:1-19.

Parrish, Z. D., Banks, M. K., & Schwab, A. P. (2004). Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil. *International Journal of Phytoremediation,* 6(2): 119-137.

Patil, P. D., Kozminski, M., & Peterson, J. (2019). Pilot Plant Study on Continuous Propane Deresining of Atmospheric Crude Cylinder Stock. *Fuel*, *235:* 17-22.

Paz-Ferreiro, J., Lu, H., Fu, S., Méndez, A., & Gascó, G. (2014). Use of phytoremediation and biochar to remediate heavy metal polluted soils: a review. *Solid Earth,* 5(1): 65-75.

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(2-3): 1490-1496.

Pezeshki, S. R., Hester, M. W., Lin, Q., & Nyman, J. A. (2000). The Effects of Oil Spill and Clean-up on Dominant US Gulf Coast Marsh Macrophytes: a review. *Environmental Pollution,* 108 (2):129-39.

Pilon-Smits, E. (2005), Phytoremediation. *Annual Reviews of Plant Biology* 56: 15-39.

Pinedo-Rivilla, C., Aleu, J., & Collado, I. G. (2009). "Pollutants Biodegradation by Fungi." *Current Organic Chemistry*, 13: 1194-1214.

Prasad, M. S. and Kumari, K. (1987). Toxicity of Crude Oil to the Survival of the Fresh Water Fish Puntius sophore (HAM.)". *Acta Hydrochimica et Hydrobiologica*. 15: 29.

Prenafeta-Boldu, F. X., Kuhn, A., Luykx, D. M. A. M., Anke, H., van Groenestijn, J. W., & de Bont, J. A. M. (2001). "Isolation and Characterisation of Fungi Growing on Volatile Aromatics Hydrocarbons as their Sole Carbon and Energy Source." *Mycological Research,* 105 (4): 477-484.

Pulford, I. D., & Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International,* 29(4): 529-540.

Punt, M. M., Raghavan, G. S. V., Bélanger, J. M. R.., & Paré, J. R. J. (1999). Microwave-Assisted Process (MAP™) for the Extraction of Contaminants from Soil. *Journal of Soil Contamination,* 8(5):577–592.

Puri, V. K., Das, B. M., Cook, E. E., & Shin, E. C. (1994). Geotechnical properties of crude oil contaminated sand. In Analysis of soils contaminated with petroleum constituents. ASTM International.

Purnomo, A. S., Nawfa, R., Martak, F., Shimizu, K., and Kamei, I. (2017). Biodegradation of aldrin and dieldrin by the white-rot fungus *Pleurotus ostreatus*. *Current microbiology*, *74*(3): 320-324.

Rabie, G. H. (2005). Role of arbuscular mycorrhizal fungi in phytoremediation of soil rhizosphere spiked with poly aromatic hydrocarbons. *Mycobiology,* 33(1): 41-50.

Rabinovich, M. L., Bolobova, A. V. & Vasil'chenko, L. G. (2004). Decomposition of natural aromatic structures and xenobiotics by fungi. *Prikladnaia biokhimiia i mikrobiologiia,* 40(1): 5-23.

Rahman, F. A., Rafati, R., Affandi, N. M., Jamiah, W., Hamid, H., & Zulkifili, M. (2013). The application of wild mushrooms in controlling the amount of crude oil in polluted environments. *Proceedings of SPE Asia pacific oil and gas conference and exhibition, APOGCE: Maximising the mature, elevating the Young: 1359-1363.*

Rahman, K. S., Rahman, T. J., Kourkoutas, Y., Petsas, I., Marchant, R., & Banat, I. M. (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. *Bioresource Technology,* 90(2):159-168.

Raj, D. D., Mohan, B., & Vidya, S. B. M. (2011). Mushrooms in the Remediation of Heavy Metals from Soil. *International Journal of Environmental Pollution Control and Management, 3(1):89-101*

Ramamurthy, A.S. & Memarian, R. (2012). Phytoremediation of mixed soil contaminants. *Water, Air, Soil Pollution,* 223: 511–518.

Raman, J. K., & Gnansounou, E. (2018). A review on bioremediation potential of vetiver grass. In Waste Bioremediation, (pp. 127-140). Springer, Singapore.

Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?. *Plant science,* 180(2), 169-181.

Reichenauer, T. G., & Germida, J. J. (2008). Phytoremediation of organic contaminants in soil and groundwater. *ChemSusChem*: *Chemistry & Sustainability Energy & Materials,* 1(8-9): 708-717.

Ren, X., Zeng, G., Tang, L., Wang, J., Wan, J., Liu, Y., ... & Deng, R. (2018). Sorption, transport and biodegradation–an insight into bioavailability of persistent organic pollutants in soil. *Science of the Total Environment,* 610: 1154-1163.

Rezaee, S., Doherty, R., Tavakkoli, M., & Vargas, F. M. (2019). Improved Chromatographic Technique for Crude Oil Maltene Fractionation. *Energy & fuels,* 33(2): 708-713.

Rhodes, C. J. (2014). Mycoremediation (bioremediation with fungi) – growing mushrooms to clean the earth*. Chemical Speciation & Biology* 26(3): 196- 198.

Riehm, D. A., Neilsen, J. E., Bothun, G. D., John, V. T., Raghavan, S. R., & McCormick, A. V. (2015). Efficient Dispersion of Crude Oil by Blends of Food-grade Surfactants: Toward Greener oil-spill Treatments. *Marine pollution bulletin*, *101*(1): 92-97.

Rigi, K. (2018). Effect of seed priming on some physiological and agronomic characteristics of sunflower varieties (Doctoral dissertation, University of Zabol).

Rivas, M., Barbieri, R. L., & Maia, L. C. D. (2012). Plant breeding and in situ utilization of palm trees. *Ciência Rural,* 42(2): 261-269.

Robinson, B. H., Leblanc, M., Petit, D., Brooks, R. R., Kirkman, J. H., & Gregg, P. E. (1998). The potential of Thlaspi caerulescens for phytoremediation of contaminated soils. *Plant and Soil*, 203(1): 47-56.

Rolz, C., De Leon, R. and De Arriola, M. C. (1988). Solid substrate growth of white rot fungi on coffee pulp. *Acta Biotechnologica,* 8(3):211 – 223.

Royse, D. (2002). Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size, and time to production. *Applied Microbiology and Biotechnology,* 58(4): 527-531.

Ruiz, O. N., Alvarez, D., Torres, C., Roman, L., & Daniell, H. (2011). Metallothionein expression in chloroplasts enhances mercury accumulation and phytoremediation capability. *Plant biotechnology Journal,* 9(5): 609-617.

Ruiz-Aguilar, G. M. L., Fernandez-Sanchez, J. M., Rodriguez-Vazquez, R. and Poggi-Varaldo, H. (2002) Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. *Advanced Environmental Resources,* 6(4):559–568.

Ruiz-Lozano, J. M., & Azcón, R. (1995). Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. *Physiologia plantarum*, 95(3): 472-478.

Russell, J. R., Huang, J., Anand, P., Kucera, K., Sandoval, A. G., & Dantzler, K. W. (2011). "Biodegradation of Polyester Polyurethane by Endophytic Fungi." *Applied and Environmental Microbiology,* 77 (17): 6076.

Saadawi, S., Algadi, M., Ammar, A., Mohamed, S., & Alennabi, K. (2015). Phytoremediation effect of Ricinus communis, Malva parviflora and Triticum repens on

crude oil contaminated soil. *Journal of Chemical and Pharmaceutical Research,* 7(9): 782-786.

Sack, U., Hofrichter, M., & Fritsche, W., (1997). Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of Nematoloma frowardii. *FEMS Microbiological Letters,* (152): 227–234.

Sagrera, C. (2014). New Challenge in Latin American and Caribbean Oil Spill Control: Offshore Prevention and Response after the DWH Milestone. In International Oil Spill Conference Proceedings (Vol. 2014, No. 1, pp. 1-13). American Petroleum Institute.

Salam, J. A., Hatha, M. A., & Das, N. (2017). Microbial-enhanced lindane removal by sugarcane (Saccharum officinarum) in doped soil-applications in phytoremediation and bioaugmentation. *Journal of environmental management,* 193: 394-399.

Salanitro, J. P., Dorn, P. B., Huesemann, M. H., Moore, K. O., Rhodes, I. A., Rice Jackson, L. M., ... & Wisniewski, H. L. (1997). Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environmental Science & Technology,* 31(6): 1769-1776.

Salley, S. W., Herrick, J. E., Holmes, C. V., Karl, J. W., Levi, M. R., McCord, S. E., ... & Van Zee, J. W. (2018). A comparison of soil texture-by-feel estimates: implications for the citizen soil scientist. *Soil Science Society of America Journal,* 22*:*99-101*.*

Salt, D. E., Blaylock, M., Kumar, N. P., Dushenkov, V., Ensley, B. D., Chet, I., & Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Nature biotechnology,* 13(5): 468.

Sánchez-Vázquez, V., Shirai, K., González, I., & Gutiérrez-Rojas, M. (2017). Fungal biocatalyst activated by an electric field: Improved mass transfer and non-specificity for hydrocarbon degradation in an airlift bioreactor. Journal of hazardous materials, 337: 62-71.

Santiago-Urbina, J. A., & Ruíz-Terán, F. (2014). Microbiology and biochemistry of traditional palm wine produced around the world. *International Food Research Journal,* 21(4):13-27.

Sarkar, D., Ferguson, M., Datta, R., & Birnbaum, S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. *Environmental pollution*, *136*(1), 187-195.

Sasek, V. (2003). Why mycoremediation have not yet come into practice. In: The utilization of bioremediation to reduce soil contamination: problems and solution, pp.247–266. The Netherlands: Kluwer Academic Publishers, Amsterdam, The Netherlands.

Sato, J. H., de Figueiredo, C. C., Marchão, R. L., Madari, B. E., Benedito, L. E. C., Busato, J. G., & de Souza, D. M. (2014). Methods of Soil Organic Carbon Determination in *Brazilian Savannah Soils. Sci. Agric.,* 71(4): 302-308.

Schapel, A., Marschner, P., & Churchman, J. (2019). Influence of clay clod size and number for organic carbon distribution in sandy soil with clay addition. *Geoderma*, 335: 123-132.

Schmidt, C. S., Mrnka, L., Frantík, T., Lovecká, P., & Vosátka, M. (2018). Plant growth promotion of Miscanthus× giganteus by endophytic bacteria and fungi on non-polluted and polluted soils. *World Journal of Microbiology and Biotechnology*, 34(3): 48.

Schnoor, J. L. (2002), Phytoremediation of Soil and Groundwater, Technical Evaluation Report 02-01, Ground Water Remediation Technologies Analysis Center, Pittsburgh, PA.

Schnoor, J. L. Light, L. A., McCutchen, S. C., Wolfe, N. L., & Carrie, L. H. (1995), Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology* 29: 318A-323A.

Schweitzer, P., Stammler, F., Ebsen, C., Ivanova, A., & Litvina, I. (2015). Social Impacts of Non-Renewable Resource Development on Indigenous communities in Alaska, Greenland and Russia. ReSDA Gap Analysis Report #2 (2016). Obtainable at http://yukonresearch.yukoncollege.yk.ca/wpmu/wpcontent/uploads/sites/2/2013/09/2-Schweitzer-Gap-analysis-final.pdf. Retrieved on 30/03/2019, 16:34

Schwitzguebel, J.-P., Kumpiene, J., Comino, E., & Vanek, T. (2009), From green to clean: A promising and sustainable approach towards environmental remediation and human health for the 21st century, *Agrochimica* 53: 209-237.

Scoggins, H. L., & van Iersel, M. W. (2006). In situ probes for measurement of electrical conductivity of soilless substrates: Effects of temperature and substrate moisture content. *HortScience*, 41(1): 210-214.

Sheil, D., Casson, A., Meijaard, E., Van Noordwijk, M., Gaskell, J., Sunderland-Groves, J., ... & Kanninen, M. (2009). The impacts and opportunities of oil palm in Southeast Asia: What do we know and what do we need to know? (Vol. 51). Bogor, Indonesia: Center for International Forestry Research.

Sheoran, V., Sheoran, A. S., & Poonia, P. (2016). Factors affecting phytoextraction: a review. *Pedosphere*, 26(2): 148-166.

Shirazia, A. R., Karimib, L., Baneshia, M. M., Rezaeia, S., Zadeha, A. M., & Jamshidia, A. (2015). Phytoremediation of Petroleum Contaminated Soil Using Matricaria Chamomilla. *Acta Medica,* 31: 1387.

Shirdam, R., Daryabeigi Zand, A., & Mehrdadi, N. (2009). Removal of total petroleum hydrocarbons (TPHs) from oil-polluted soil in Iran*. Iranian Journal of Chemistry and Chemical Engineering (*IJCCE), 28(4): 105-113.

Shirdam, R., Zand, A., Bidhendi, G., & Mehrdadi, N. (2008). Phytoremediation of hydrocarbon- contaminated soils with emphasis on the effect of petroleum hydrocarbons on the growth of plant species. *Phytoprotection*, 89(1): 21-29.

Siciliano, S. D., & Germida, J. J. (1998). Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. *Environmental reviews,* 6(1): 65-79.

Siegel, S. A., Galun, M., & Siegel, B. Z.(1990). Filamentous fungi as metal bioadsorbents. A review. *Water, Air &Soil Pollution,* 53: 335-334.

Simonart, P., & Laudelot, H. (1951). Etude microbiologique et biochimique du vin de palme. *Bulletin de l'Institut Royal Colonial Beige,* 22: 385-401.

Simus, J. (2008). Environmental art and ecological citizenship. *Environmental Ethics*, *30*(1): 21-36.

Singh, A. D., Vikineswary, S., Abdullah, N.M & Sekaran, M. (2011). Enzymes from spent mushroom substrate of Pleurotus sajor-caju for the decolourisation and detoxification of textile dyes. *World Journal of Microbiology & Biotechnology,* 27(3):535–545.

Singh, A., & Gauba, P. (2014). Mycoremediation: a treatment for heavy metal pollution of soil. *Journal of Civil Engineering and Environmental Technology*, 1: 59-61.

Singh, H. (2006). Mycoremediation: Fungal Bioremediation. John Wiley & Sons, Inc., (Pp1151-147). New Jesey.

Soilscapes (2017). Cranfield soil and Agric Institute obtained at http://www.landis.org.uk/soilscapes

Song, B., Xu, P., Chen, M., Tang, W., Zeng, G., Gong, J., ... & Ye, S. (2019). Using nanomaterials to facilitate the phytoremediation of contaminated soil. *Critical Reviews in Environmental Science and Technology,* 1-34.

Song, W. Y., Sohn, E. J., Martinoia, E., Lee, Y. J., Yang, Y. Y., Jasinski, M., ... & Lee, Y. (2003). Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nature biotechnology*, 21(8):914.

Song, Y., Kirkwood, N., Maksimović, Č., Zhen, X., O'Connor, D., Jin, Y., & Hou, D. (2019). Nature based solutions for contaminated land remediation and brownfield redevelopment in cities: A review. *Science of the Total Environment,* 663: 568-579.

Sood, N., & Lal, B. (2009). Isolation of a novel yeast strain Candida digboiensis TERI ASN6 capable of degrading petroleum hydrocarbons in acidic conditions. *Journal of Environmental Management.* 90: 1728-1736.

Sood, N., Patle, S. and Lal, B. (2010). Bioremediation of acidic oily sludge-contaminated soil by the novel yeast strain Candida digboiensis TERI ASN6. *Environmental Science & Pollution Research,* 17:603–610.

Sorkhabi, R. (2018). Sir Thomas Boverton Redwood (1846–1919): A Watershed in the British Oil Industry. *Geological Society, London, Special Publications*, *465*(1): 423-451.

Stamets, P. *(2005). Mycelium running: how mushrooms can help save the world. Random House, Inc. pp. 83–84.*

Stanišić, S. M., Ignjatović, L. M., Stević, M. C., & Đorđević, A. R. (2011). A Comparison of sample extraction procedures for the determination of inorganic anions in soil by ion chromatography. *Journal of Serbian Chemical Society* 76 (5): 769–780.

Stanley, H. O., Umolo, E. A., &Stanley, C. N. (2011). Cultivation of Oyster Musroom (Pleurotus pulmonarius) on amended corncob substrate. *Agriculture and Biology Journal of North America,* 2(10): 1336-1339.

Stefan F, Zoltán G, Borut P, Vekoslava S, Radmila M, Miklós P, Peter R., & Martin, B.(2005). Theoxidativestress response of the yeast Candida intermedia to copper, zinc, and selenium exposure. *Journal of Basic Microbiology,* 45(2):125–135.

Stella, T., Covino, S., Køesinová, Z., D'Annibale, A., Petruccioli, M. and Cajthaml, T. (2012). Mycoremidiation of PCBs dead – end metabolites: In vivo and in vitro degradation of chlorobenzoic acids by the white rot fungus, Lentinus tigrinus. *Environmental Engineering & Management Journal,* 11:9.

Stepanova, A. Y., Orlova, E. V., Teteshonok, D. V., & Dolgikh, Y. I. (2016). Obtaining transgenic alfalfa plants for improved phytoremediation of petroleum-contaminated soils. *Russian Journal of Genetics: Applied Research,* 6(6): 705-711.

Stjerndahl, M., Lundberg, D., Chauhan, V., Bordes, R., & Holmberg, K. (2019). Cleavable Surfactants: A Comparison between Ester, Amide, and Carbonate as the Weak Bond. *Journal of Surfactants and Detergents,* 40(23):21-29.

Stringini, M., Comitini, F., Taccari, M., and Ciani, M. (2009). Yeast diversity during tapping and fermentation of palm wine from Cameroon. *Food microbiology*, *26*(4): 415-420.

Sudarat, B., Britz, M.L., & Stanley, G. A. (1998) Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by stenotrophomonas maltophilia. *Biotechnology Bioengineering,* 59(4): 482 & 494.

Sukor, M. Z., Yin, C. Y., Savory, R. M., & Abdul-Talib, S. (2012). Biodegradation kinetics of naphthalene in soil medium using Pleurotus ostreatus in batch mode with addition of fibrous biomass as a nutrient. *Bioremediation Journal,* 16(3): 177- 184.

Sun, M., Fu, D., Teng, Y., Shen, Y., Luo, Y., Li, Z., & Christie, P. (2011). In situ phytoremediation of PAH-contaminated soil by intercropping alfalfa (Medicago sativa L.) with tall fescue (*Festuca arundinacea Schreb.)* and associated soil microbial activity. *Journal of Soils and Sediments*, 11(6): 980-989.

Sun, Y., Xu, Y., Zhou, Q., Wang, L., Lin, D., & Liang, X. (2013). The potential of gibberellic acid 3 (GA3) and Tween-80 induced phytoremediation of co-contamination of Cd and Benzo [a] pyrene (B [a] P) using *Tagetes patula*. *Journal of environmental management,* 114: 202-208.

Svenning, J. C. (1999). Microhabitat specialization in a species-rich palm community in Amazonian Ecuador. *Journal of Ecology,* 87(1): 55-65.

Tan, J. B., Chen, X., Guo, X. H., Li, Y., & Zu, Y. Q. (2015). Distribution characteristics of Pb and Cd in different parts of *Sonchus asper* and *Zea mays* in an intercropping system. *Ecology and Environmental Sciences,* 24(4):700-707.

Tangahu, B. V., Abdullah, S., Rozaimah, S., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering:* 1-31.

Tarkalson, D. D., Payero, J. O., Ensley, S. M., & Shapiro, C. A. (2006). Nitrate accumulation and movement under deficit irrigation in soil receiving cattle manure and commercial fertilizer. *Agricultural water management*, *85*(1-2): 201-210.

Tautorus, T. E., & Townsley, P. M. (1984). Biotechnology in commercial mushroom fermentation. *Biotechnology*, 2(8): 696.

Tejada, M., Garcia, C., Gonzalez, J. L., & Hernandez, M. T. (2006). Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. *Soil Biology and Biochemistry*, *38*(6): 1413-1421.

Tewari, S. & Sirvaiya, A. (2015). Oil Spill Remediation And Its Regulation Oil Spill Remediation And Its Regulation. *International Journal of Research In Science & Engineering*, 1(6): 71-77.

Thakur, Monika (2014). Mycoremediation-a potential tool to control soil pollution. *Asian Journal of Environmental Sciences* 9(1): 21-31.

Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu university journal of science, engineering and technology, 8(1): 164-170.

Thomas, S. A., Aston, L. M., Woodruff, D. L., & Cullinan, V. I. (2009). Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the Dungeness Watershed, Washington. Pacific Northwest National Laboratory, Richland, Washington 99352.

Thomas, S., Becker, P., Pinza, M. R., & Word, J. Q. (1998). Mycoremediation of Aged Petroleum Hydrocarbon Contamination in Soil. Research Report, Washington State Department of Transportation, Olypia, Washington, 98504.

Tisdale, S. L., & Nelson, W. L. (1958). Soil fertility and fertilizers. Macmillan Company.; New York.

Tisma, M., Zelic, B., & Vasic-Racki, D. (2010). White-rot fungi in phenols, dyes and other xenobiotics treatment – a brief review. *Croatian Journal of Food Science & Tech*nology, 2 (2): 34 - 47.

Tmáková, L., Sekretár, S., & Schmidt, Š. (2016). Plant-derived surfactants as an alternative to synthetic surfactants: surface and antioxidant activities. *Chemical Papers,* 70(2): 188-196.

Taubner, H., Roth, B., & Tippkötter, R. (2009). Determination of soil texture: Comparison of the sedimentation method and the laser-diffraction analysis. *Journal of Plant Nutrition and Soil Science*, *172*(2): 161-171.

Ugochukwu, C. N., & Ertel, J. (2008). Negative impacts of oil exploration on biodiversity management in the Niger Delta area of Nigeria. *Impact assessment and project appraisal,* 26(2): 139-147.

United States Department of Agriculture (1995). Animal Manure Management. RCA Issue Brief #7 December 1995.

UNEP. (2011). Environmental assessment of Ogoniland. Background to Environmental Degradation in Ogoni Land.

US EPA Method 8270 (PAH only): GC Analysis of PAHs on SLB®-5ms. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA 3015A (2007). Microwave Assisted Acid Digestion of Aqueous Samples And Extracts. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA 350M/3550 (2014). Microwave assisted extraction. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA 350M/3550 (2014). Microwave assisted extraction. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA 3546. Microwave assisted extraction. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA 6010D (2014). Inductively Coupled Plasma-Atomic Emission Spectrometry. .S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA METHOD 300.0 (1993). Determination of Inorganic Anions by Ion Chromatography: John D. Pfaff Inorganic Chemistry Branch Chemistry Research Division, Revision 2.1. Environmental Monitoring Systems` Laboratory Office of Research and Development, U.S. Environmental Protection Agency Cincinnati, Ohio 45268.

USEPA METHOD 3051A (2007). Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. Revision-1, Washington D.C., USA.

USEPA METHOD 3052 (1998). Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. 3rd Revision, Washington D.C., USA.

USEPA METHOD 3546 (2007). Microwave Assisted Extraction of Total Petroleum Hydrocarbons from Soils and Sediments. Revision-0, Washington D.C., USA.

USEPA METHOD -846 Test Method 8270E: Semi volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA SSOP (2014). Soil Sampling Operating Procedures. SESDPROC-300-R3:1-24. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA SSOP (2014). Soil Sampling Operating Procedures. SESDPROC-300-R3:1-24. .S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

Usman, M., Hanna, K., & Faure, P. (2018). Remediation of oil-contaminated harbor sediments by chemical oxidation. *Science of the Total Environment,* 634: 1100-1107.

Valkova, D., Nenova, N., Penchev, E., Encheva, V., & Georgiev, G. (2018). Hybridization Between Cultivated Sunflower and Wild Species Helianthus Bolanderi A. Gray. *Journal of Crop Science,* 54(4): 9-13.

Vane, C. H., Chenery, S. R., Harrison, I., Kim, A. W., Moss-Hayes, V., & Jones, D. G. (2011). Chemical signatures of the Anthropocene in the Clyde estuary, UK: sediment-hosted Pb, 207/206Pb, total petroleum hydrocarbon, polyaromatic hydrocarbon and polychlorinated biphenyl pollution records. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences,* 369(1938): 1085-1111.

Vane, C. H., Kim, Alexander W., Beriro, D. J., Cave, M. R., Knights, K., Moss-Hayes, V., & Nathanail, P. C. (2014). Polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) in urban soils of Greater London, UK. *Applied Geochemistry,* 51: 303-314.

Vanderlelie, D., Chwitzguebel, J., Glass, D.J., Vangronsve, D. J., & Baker, A.(2001). Assessing Phytoremediation Progress in the United States and Europe. *Env. Sc. & Tech:* 447A.

Vogt, C., Kleinsteuber, S., & Richnow, H. H. (2011). Anaerobic benzene degradation by bacteria. *Microbial biotechnology*, 4(6): 710-724.

Waller, N. G. (2008). Commingled samples: A neglected source of bias in reliability analysis. *Applied Psychological Measurement*, 32(3): 211-223.

Walter, S. D., Eliasziw, M., & Donner, A. (1998). Sample size and optimal designs for reliability studies. *Statistics in Medicine,* 17(1): 101-110.

Wang, J., Zhang, Z., Su, Y., He, W., He, F., & Song, H. (2008). Phytoremediation of petroleum polluted soil. *Petroleum Science,* 5(2): 167-171.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, 17(6), 300-312.

Wang, L., Yang, M., Fan, X., Zhu, X., Xu, T., & Yuan, Q. (2011). An environmentally friendly and efficient method for xylitol bioconversion with high-temperature-steaming corncob hydrolysate by adapted Candida tropicalis. *Process Biochemistry,* 46(8): 1619–1626.

Wang, Y. N., Cheng, L. J., & Zhou, Q. X. (2016). Phytoremediation of Petroleum Contaminated Soils with Iris pseudacorus L. and the Metabolic Analysis in Roots. Huan jing ke xue= Huanjing kexue, 37(4): 1531-1538.

Wanogho, S.., Gettinby, G., & Caddy, B. (1987). Particle-size distribution analysis of soils using laser diffraction. *Forensic Science International,* 33 (2): 117-128.

Weber, F., & Passon, M. (2019). Characterization and quantification of polyphenols in fruits. In Polyphenols in Plants (pp. 111-121). Academic Press.

Weber, S., Schrag, K., Mildau, G., Kuballa, T., Walch, S. G., & Lachenmeier, D. W. (2018). Analytical methods for the determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH)—A short review. *Analytical chemistry insights,* 13:1-16.

Wei, S., Xu, L., Dai, H., & Hu, Y. (2018). Ornamental hyperaccumulator Mirabilis jalapa L. phytoremediating combine contaminated soil enhanced by some chelators and surfactants. *Environmental Science and Pollution Research,* 1-6.

Wei, T., & Guo, X. (2016). An empirical analysis of the relationship between oil prices and the Chinese macro-economy. *Energy Economics,* 53:88–100.

Wentworth, C. K. (1922). A scale of grade and class terms for clastic sediments. The Journal of geology, 30(5): 377-392.

Whalen, J. K., Chang, C., Clayton, G. W., & Carefoot, J. P. (2000). Cattle manure amendments can increase the pH of acid soils. *Soil Science Society of America Journal*, *64*(3): 962-966.

White, P. M., Kirkpatrick, W. D., Wolf, D. C., & Thoma, G. J. (2002). Phytoremediation of Crude Oil-contaminated Soil (Doctoral dissertation, University of Arkansas, Fayetteville).

White, P. M., Wolf, D. C., Thoma, G. J., & Reynolds, C. M. (2006). Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. *Water, Air, and Soil Pollution*, 169(1-4): 207-220.

White, J. C., Ross, D. W., Gent, M. P., Eitzer, B. D., & Mattina, M. I. (2006). Effect of mycorrhizal fungi on the phytoextraction of weathered p, p-DDE by Cucurbita pepo. *Journal of hazardous materials*, *137*(3): 1750-1757.

Whitehead, D. L., & Quicke, G. V. (1964). A comparison of six methods of estimating lignin in grass hay. *Journal of the Science of Food and Agriculture*, *15*(6): 417-422.

Whiting, D., Card, A., Wilson, C., & Reeder, J. (2014). Estimating soil texture. Colorado State University Extension Publication, 214.

Wiche, O., Székely, B., Kummer, N. A., Moschner, C., & Heilmeier, H. (2016). Effects of intercropping of oat (Avena sativa L.) with white lupin (Lupinus albus L.) on the mobility of target elements for phytoremediation and phytomining in soil solution. *International Journal of phytoremediation,* 18(9): 900-907.

Wieshammer, G., Unterbrunner, R., García, T. B., Zivkovic, M. F., Puschenreiter, M., & Wenzel, W. W. (2007). Phytoextraction of Cd and Zn from agricultural soils by Salix ssp. and intercropping of Salix caprea and Arabidopsis halleri. *Plant and Soil,* 298(1-2): 255-264.

Williamson, K. (1970). Some food plant names in the Niger Delta. *International Journal of American Linguistics,* 36(2): 156-167.

Wincele, D. E., Wrenn, B. A., & Venosa, A. D. (2004). Sedimentation of oil-mineral aggregates for remediation of vegetable oil spills. *Journal of environmental engineering,* 130(1): 50-58.

Winquist, E., Björklöf, K., Schultz, E., Räsänen, M., Salonen, K., Anasonye, F., Cajthaml, T., Steffen, K.T., Jørgensen, K.S., & Tuomela, M. (2014). Bioremediation of PAH-contaminated soil with fungi – from laboratory to field scale. *International Biodeterioration & Biodegradation,* 86: 238- 247.

Wiszniewska, A., Ewa Hanus-Fajerska, E. M., & Ciarkowska, K. (2016). Natural Organic Amendments for Improved Phytoremediation of Polluted Soils: A Review of Recent Progress. *Pedosphere,* 26(1): 1–12.

World Meteorological Organisation (WMO) – No 8 (2008). Measuring soil temperatures: Guide to Meteorological Instruments and Methods of Observation. WMO, Geneva 2, Switzerland. p1.2-6.

Wu, L., Li, Z., Han, C., Liu, L., Teng, Y., Sun, X., & Christie, P. (2012). Phytoremediation of soil contaminated with cadmium, copper and polychlorinated biphenyls. *International Journal of Phytoremediation,* 14(6): 570-584.

Xia, S., Song, Z., Jeyakumar, P., Shaheen, S. M., Rinklebe, J., Ok, Y. S., ... & Wang, H. (2019). A critical review on bioremediation technologies for Cr (VI)-contaminated soils and wastewater. *Critical Reviews in Environmental Science and Technology*, 1-52.

Xing, X., Qi, S., Zhang, J., Wu, C., Zhang, Y., Yang, D., & Odhiambo, J. O. (2011). Spatial distribution and source diagnosis of polycyclic aromatic hydrocarbons in soils from Chengdu Economic Region, Sichuan Province, western China. *Journal of Geochemical Exploration*, *110*(2): 146-154.

Xiu-Zhen, H. A. O., Dong-Mei, Z. H. O. U., Dan-Dan, L. I., & JIANG, P. (2012). Growth, cadmium and zinc accumulation of ornamental sunflower (Helianthus annuus L.) in contaminated soil with different amendments. *Pedosphere*, 22(5): 631-639.

Yabi, I., & Afouda, F. (2012). Extreme rainfall years in Benin (West Africa). *Quaternary International,* 262: 39-43.

Yadav, S. K., Juwarkar, A. A., Kumar, G. P., Thawale, P. R., Singh, S. K., & Chakrabarti, T. (2009). Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by Jatropha curcas L.: impact of dairy sludge and biofertilizer. *Bioresource Technology,* 100(20): 4616–4622.

Yadav, S. K., Juwarkar, A. A., Kumar, G. P., Thawale, P. R., Singh, S. K., & Chakrabarti, T. (2009). Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by *Jatropha curcas L*.: impact of dairy sludge and biofertilizer. *Bioresource Technology,* 100(20): 4616-4622.

Yakubu, O. H. (2017). Addressing Environmental Health Problems in Ogoniland through Implementation of United Nations Environment Program Recommendations: Environmental Management Strategies. E*nvironments*, 4(2): 28.

Yan, L., Li, C., Zhang, J., Moodley, O., Liu, S., Lan, C., ... & Zhang, W. (2017). Enhanced Phytoextraction of Lead from Artificially Contaminated Soil by Mirabilis jalapa with Chelating Agents. *Bulletin of environmental contamination and toxicology*, 99(2): 208-212.

Yang, T. H., Bolten, C. J., Coppi, M. V., Sun, J., & Heinzle, E. (2009). Numerical bias estimation for mass spectrometric mass isotopomer analysis. *Analytical biochemistry,* 388(2): 192-203.

Yang, X., Yuan, X., Zhang, A., Mao, Y., Li, Q., Zong, H., ... & Li, X. (2015). Spatial distribution and sources of heavy metals and petroleum hydrocarbon in the sand flats of Shuangtaizi Estuary, Bohai Sea of China. *Marine Pollution Bulletin*, *95*(1): 503- 512.

Yang, X., Zhang, Q., Li, X., Jia, X., Wei, X., & Shao, M. A. (2015). Determination of soil texture by laser diffraction method. *Soil Science Society of America Journal,* 79(6): 1556-1566.

Yang, Y., Wang, L., Wendroth, O., Liu, B., Cheng, C., Huang, T., & Shi, Y. (2019). Is the Laser Diffraction Method Reliable for Soil Particle Size Distribution Analysis? *Soil Science Society of America Journal,* 83(2): 276-287.

Yanqun, Z. U., Li, Q. I. N., Fangdong, Z. H. A. N., Jiong, W. U., Yuan, L. I., Jianjun, C. H. E. N., ... & Wenyou, H. U. (2017). Effects of Intercropping of Sonchus asper and Vicia faba on Plant Cadmium Accumulation and Root Responses. *Pedosphere*: 1-19.

Yan-Zheng, G. A. O., Wan-Ting, L. I. N. G., Li-Zhong, Z. H. U., Bao-Wei, Z. H. A. O., & Zheng, Q. S. (2007). Surfactant-enhanced phytoremediation of soils contaminated with hydrophobic organic contaminants: potential and assessment. *Pedosphere*, 17(4): 409-418.

Yarnold, P. R., & Soltysik, R. C. (2005). Reliability analysis. In P. R. Yarnold & R. C. Soltysik (Eds.), Optimal data analysis: A guidebook with software for windows (pp. 121-140). Washington, DC: American Psychological Association.

Yateem, A., Balba, M. T., Al-Awadhi, N., & El-Nawawy, A. S. (1999). White rot fungi and their role in remediating oil-contaminated soil. *Environment International,* 24(1-2): 181-187.

Yavari, S., Malakahmad, A., & Sapari, N. B. (2015). A review on phytoremediation of crude oil spills. *Water, Air, & Soil Pollution,* 226(8): 279.

Young, D., Rice, J., Martin, R., Windquist, E., Lipzen, A., Grigoriev, A., & Hibbett, D. (2015). Degradation of Bunker C Fuel Oil by White-Rot Fungi in Sawdust Cultures Suggests Potential Applications in Bioremediation. *PLoS ONE*, 10(6): 1-15.

Yu, H., Ding, W., Luo, J., Geng, R., & Cai, Z. (2012). Long-term application of organic manure and mineral fertilizers on aggregation and aggregate-associated carbon in a sandy loam soil. *Soil and Tillage Research,* 124: 170-177.

Zadrazil, F., & Reiniger, P (1998). *Treatment of Lignocellulosics with White Rot fungi*. Elsevier Applied Science publisher Ltd, Essex, United Kingdom. Pp1-14.

Zakaria, M. P., Horinouchi, A. I., Tsutsumi, S., Takada, H., Tanabe, S., & Ismail, A. (2000). Oil pollution in the Straits of Malacca, Malaysia: Application of molecular markers for source identification. *Environmental science & technology*, 34(7): 1189-1196.

Zalesny Jr, R. S., Bauer, E. O., Hall, R. B., Zalesny, J. A., Kunzman, J., Rog, C. J., & Riemenschneider, D. E. (2005). Clonal variation in survival and growth of hybrid poplar and willow in an in situ trial on soils heavily contaminated with petroleum hydrocarbons. *International journal of phytoremediation*, 7(3): 177-197.

Zand, A. D., Bidhendi, G. N., & Mehrdadi, N. (2010). Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species in Iran. *Turkish Journal of Agriculture and Forestry*, 34(5): 429-438.

Zawawi, D., Halizah, A., Angzzas, S., Mohd, K., Mohd, Z., Mohd, H., A. & Mohd, A. (2014). Comparison of Pineapple Leaf and Cassava Peel by Chemical Properties and Morphology Characterization", *Advanced Materials Research,* 974:384-388.

Zebulun, O. H., Isikhuemhen, O.S., & Hilary, I. (2011). Decontamination of anthracene-polluted soil through white rot fungus- induced biodegradation. *Environmentalist*, 31: 11-19.

Zhang, J., Yin, R., Lin, X., Liu, W., Chen, R., & Li, X. (2010). Interactive effect of biosurfactant and microorganism to enhance phytoremediation for removal of aged polycyclic aromatic hydrocarbons from contaminated soils. *Journal of Health Science,* 56(3): 257-266.

Zhang, Z., Qixing Z., Shengwei P., & Cai, Z. (2010). Remediation of Petroleum Contaminated Soils by joint Action of Pharbitis nil L. and its Microbial Community. *Science of the Total Environment,* 408: 5600–5605.

Zhang, Z., Wen, J., Li, J., Ma, X., Yu, Y., Tan, X., & Gong, L. (2018). The evolution of genomic and epigenomic features in two Pleurotus fungi. *Scientific reports,* 8(1): 8313.

Zhang, Z., Zhou, Q., Peng, S., & Cai, Z. (2010). Remediation of petroleum contaminated soils by joint action of Pharbitis nil L. and its microbial community. *Science of the total environment,* 408(22), 5600-5605.

Zhao, B., Shen, L. B., Cheng, M. M., Wang, S. F., Wu, L. H., Zhou, S. B., & Luo, Y. M. (2011). Effects of intercropping Sedum plumbizincicola in wheat growth season under wheat-

rice rotation on the crops growth and their heavy metals uptake from different soil types. Ying yong sheng tai xue bao. *The journal of applied ecology,* 22(10): 2725-2731.

Zheng, Y., Li, J., Cao, W., Liu, X., Jiang, F., Ding, J., ... & Sun, C. (2019). Distribution characteristics of microplastics in the seawater and sediment: A case study in Jiaozhou Bay, China. *Science of The Total Environment*. 674: 27-35.

Zheng, Z., & Obbard, J. P. (2001). Effect of non-ionic surfactants on elimination of polycyclic aromatic hydrocarbons (PAHs) in soil-slurry by Phanerochaete chrysosporium. *International Research in Process, Environmental & Clean Technology,* 76(4): 423-429.

Zhou, J., Jiang, W., Ding, J., Zhang, X., & Gao, S. (2007). Effect of Tween 80 and betacyclodextrin on degradation of decabromodiphenyl ether (BDE-209) by white rot fungi. *Chemosphere,* 70(2):172–177.

Zitte, L.F., Awi-Waadu, G.D.B., & John, A. U. (2012). Effect of Oyster Mushroom (*Pleurotus Ostreatus*) Mycelia on Petroleum Hydrocarbon Contaminated Substrate. *Journal of Agriculture and Social Research (JASR),* 12(2): 115.

Appendix I- Images

AP1.1: Supervisory team

AP 1.2: sampling of petroleum contaminated sites at Tibshelf, Derbyshire, United kingdom

AP1.3: Petroleum contaminated sites at Tibshelf, Derbyshire, United kingdom

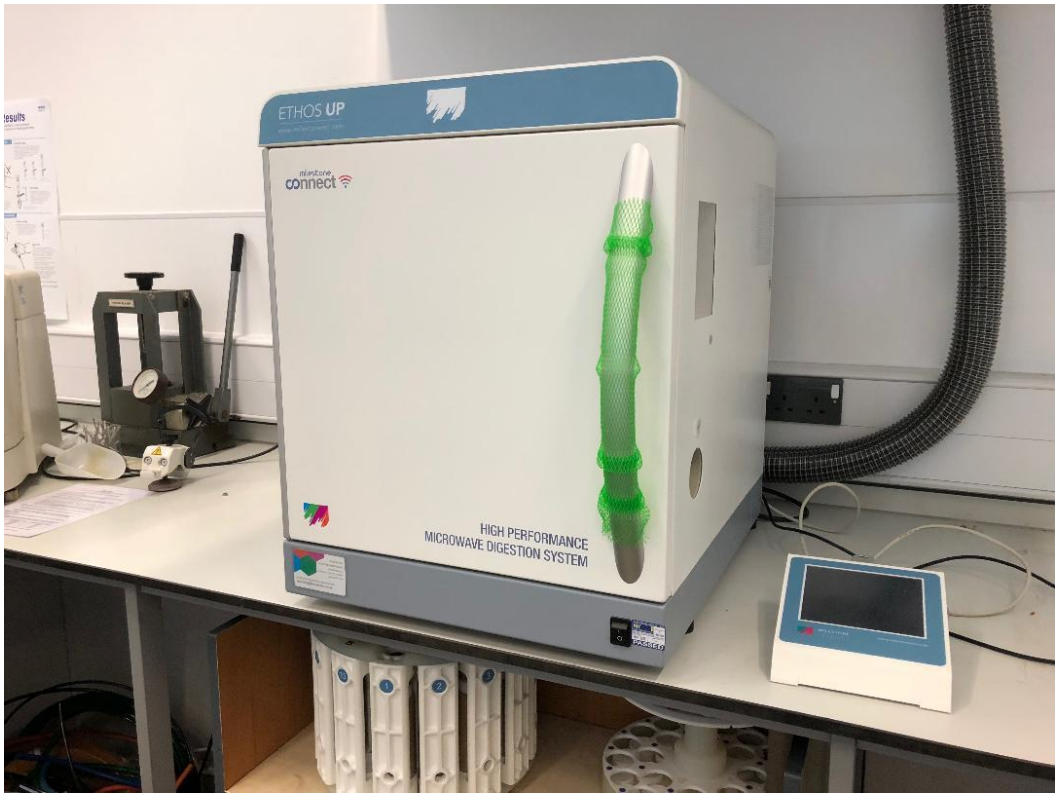
AP 1.4: Sampling team for Ogoniland, Niger Delta, Nigeria: 1ST right-Dr Giadom Ferdinand

AP 1.5: Sampling of petroleum contaminated sites at Bodo, Ogoniland, Nigeria

AP 1.6: Contaminated sediments from Bodo, Ogoniland, Nigeria.

AP 1.7: Effect of petroleum contamination on Bodo river at Bodo, Ogoniland, Nigeria. A dead fish, an effect of the observable visible contamination of Bodo river by crude oil

AP 1.8: Petroleum contaminated sites at Gio, Ogoniland, Nigeria.



AP 1.9: High performance microwave extraction system used for extraction of TPHs from soils

AP 1.10: GC-MS system used for the analysis of TPHs

AP 1.11: Centrifuge system

AP 1.12: Glasshouse pots for of control sunflowers

AP 1.13a: Glasshouse pots for phytoremediation of petroleum contaminated sites from Ogale Ogoniland, Nigeria, using D. *affinis*.

AP 1.13b: Glasshouse pots for phytoremediation of petroleum contaminated sites from Ogale Ogoniland, Nigeria, using *H. annus-pacino gold*.

Appendix II-GC-chromatogram of samples

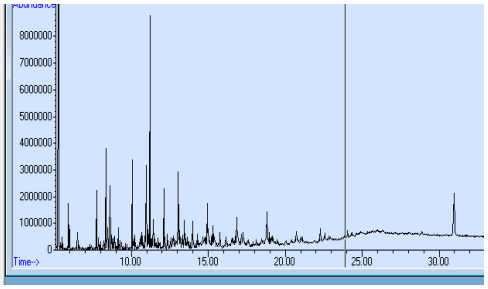


Figure AP2.1a: Chromatogram of *Uncontaminated soils @T=0*

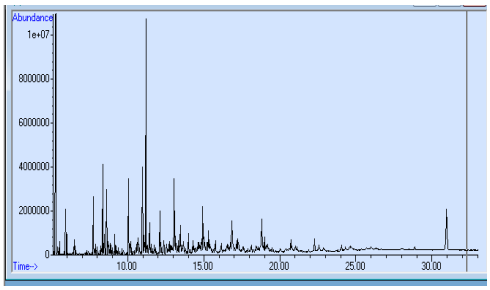


Figure AP2.1b: Chromatogram of *Uncontaminated soils @T=3*

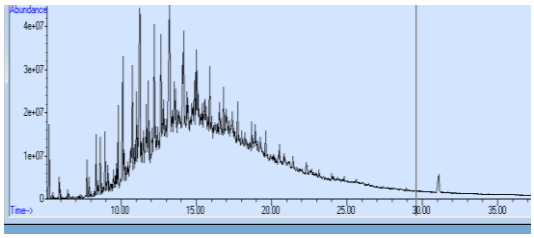


Figure AP2.2a: *Contaminated soils without amendment @T=0*

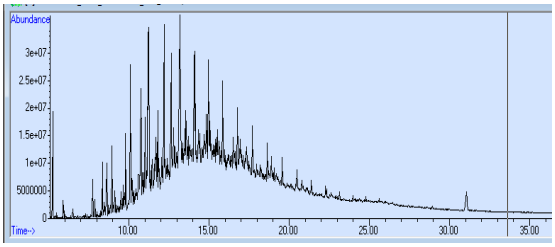


Figure AP2.2b: *Contaminated soils without amendment @T=3*

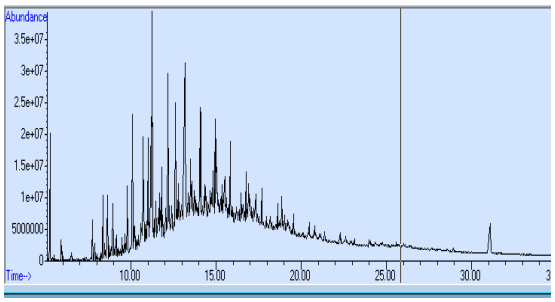


Figure AP2.3a: *Contaminated soils + amendments @ T= 0*

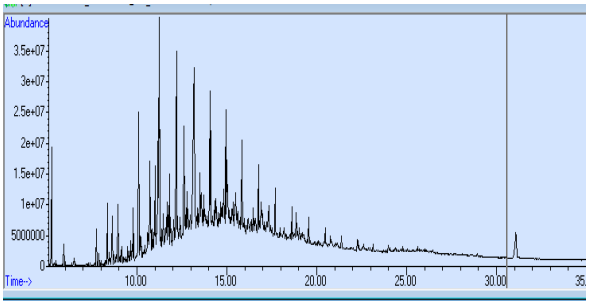


Figure AP2.3b: *Contaminated soils + amendments @ T= 3*

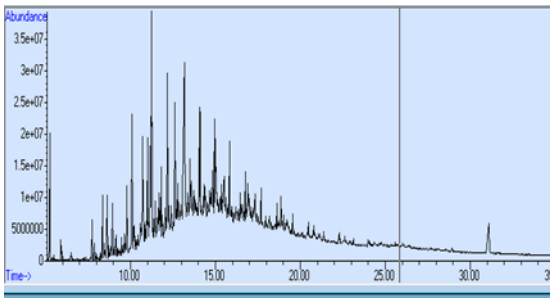


Figure AP2.4a*: Mushrooms applied without substrates T=3*

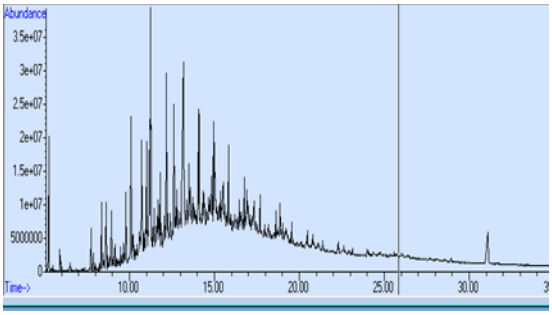


Figure AP2.4b: *Mushrooms applied without substrates T=3*

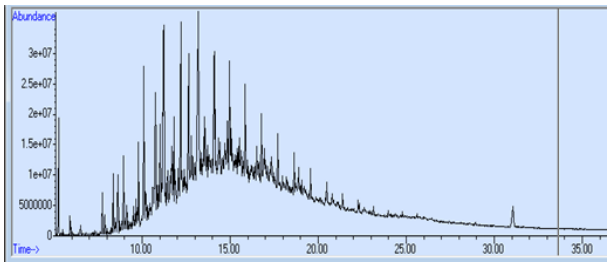


Figure AP2.5a: *Mushrooms + substrates layered on top T= 0*

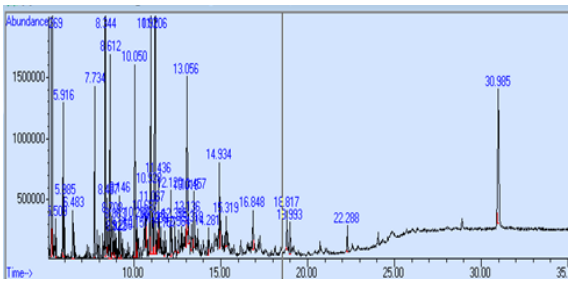


Figure AP2.5b: *Mushrooms + substrates layered on top T= 3*

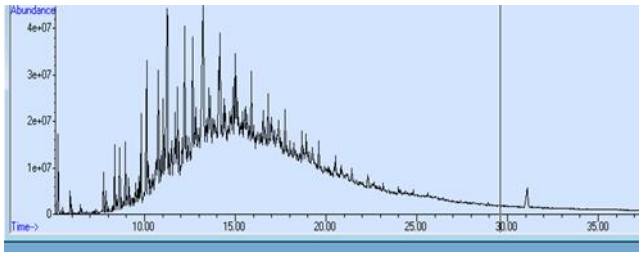


Figure AP 2.6a: *Mushrooms + substrates mixed and layered @T=0*

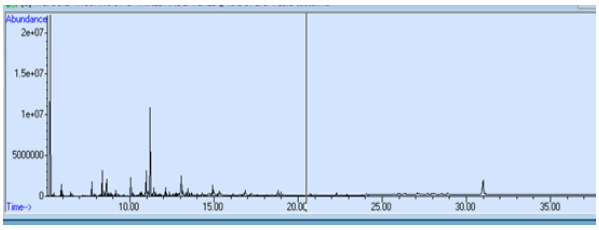


Figure AP 2.6b: *Mushrooms + substrates mixed and layered @T=3*

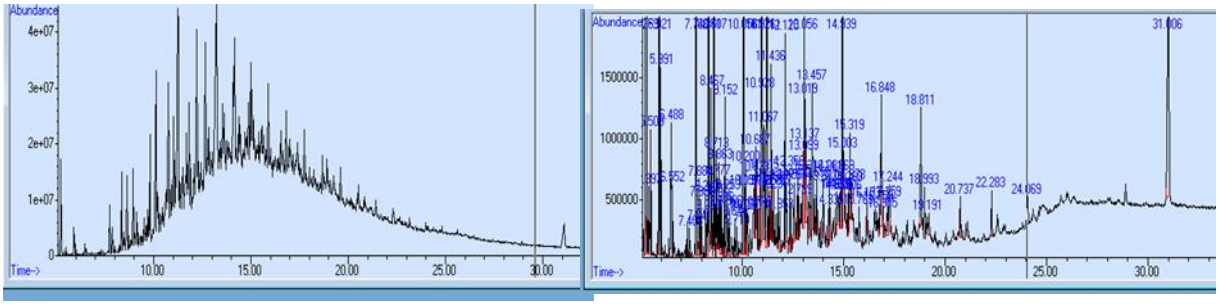


Figure AP 2.7a*: Soil treated with Helianthus annus pacino gold @T=0*

Figure AP 2.7b*: Soil treated with Helianthus annus pacino gold @T=3*

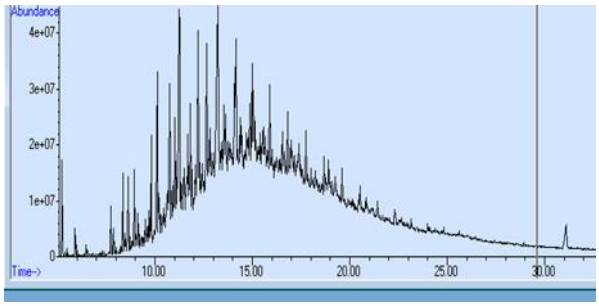


Figure AP 2.8a: *Soil treated with Helianthus annus sunsation @T=0*

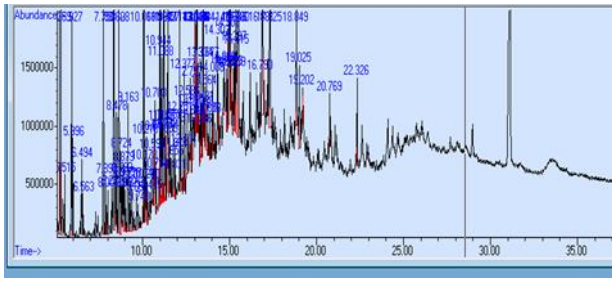


Figure AP 2.8b: *Soil treated with Helianthus annus pacino gold @T=3*

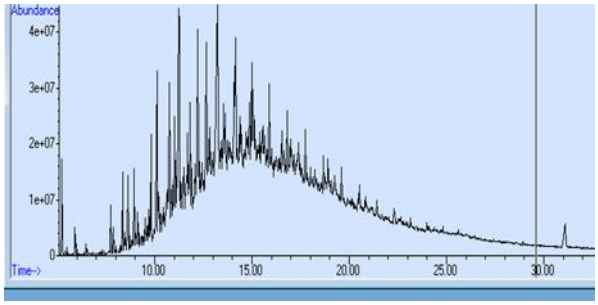


Figure AP 2.9a: *Soil treated with Helianthus annus-sunny dwarf @T=0*

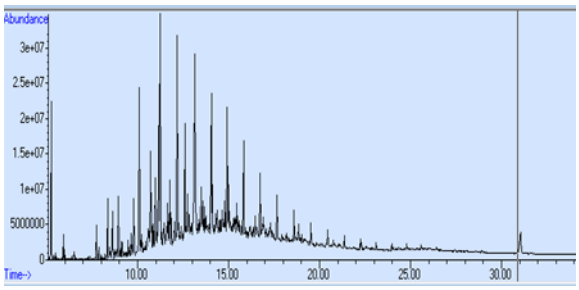


Figure AP 2.9b*: Soil treated with Helianthus annus-sunny dwarf @T=3*

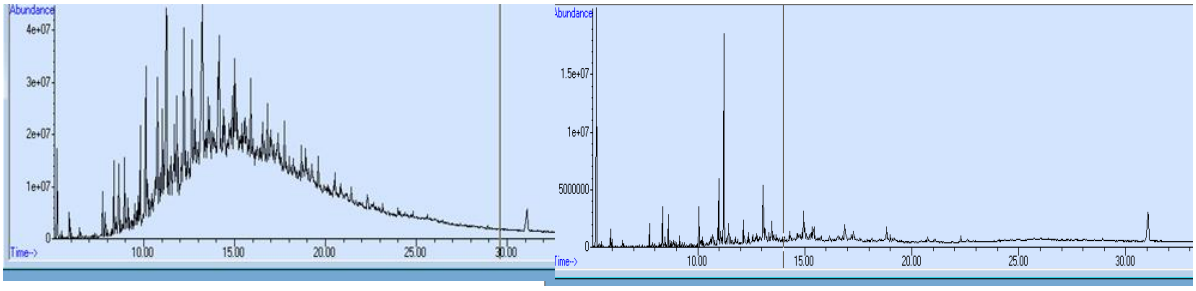


Figure AP2.10a*: Soil treated with fermented palm wine (Elaies guneasis) @T=0*

Figure AP2.10b: *Soil treated with fermented palm wine (Elaies guneasis) @T=3*

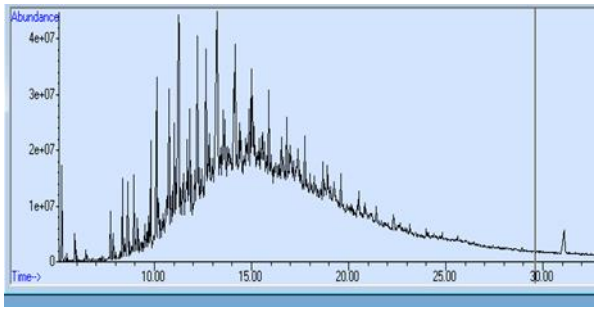


Figure AP2.11a*: Soil treated with fermented palm wine (Raffia africana) @T=0*

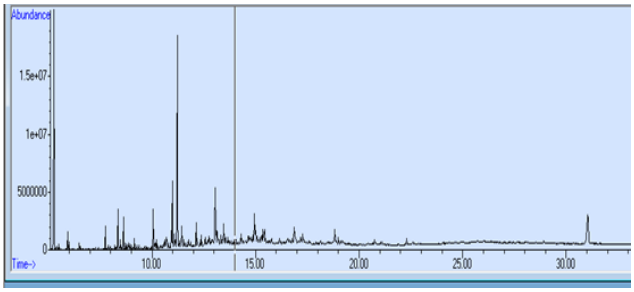


Figure AP2.11b*: Soil treated with fermented palm wine (Raffia africana) @T=3*

Appendix III-Raw data for results

Raw data for chapter 4

Appendix III-1: Raw data for Physicochemical properties of petroleum contaminated soils and controls from Tibshelf, UK.

		Temp (°C)		pH		Electrical conductivity		Nitrates	
TREATMENTS		T=0	T=3	T=0	T=3	T=0	T=3	T=0	T=3
UNCONTAMINATED SOIL @T=0	1	20.80	23.10	7.36	7.66	1.03	0.99	620.0	600.0
	2	20.10	22.50	7.44	7.56	0.99	1.10	611.0	609.0
	3	20.40	22.80	7.26	7.44	1.10	1.00	633.0	621.0
COW MANURE	1	18.40	21.20	9.47	9.34	3.24	3.22	690.0	692.0
	2	19.10	20.20	9.31	9.48	3.24	3.21	711.0	700.0
	3	19.00	22.20	9.47	9.66	3.17	3.30	700.0	708.0
CONT_SOIL_WITHOUT_AD@T=0	1	21.80	21.05	6.41	6.47	0.18	0.22	34.00	36.00
	2	22.00	21.87	6.35	6.66	0.20	0.25	33.00	35.00
	3	21.44	22.30	6.44	6.20	0.20	0.22	33.00	37.00
CONT_SOIL +_AD@T=0	1	21.10	23.50	8.41	8.59	3.00	2.67	440.00	460.00
	2	21.40	22.00	8.55	8.70	2.80	3.00	430.0	467.0
	3	21.00	21.80	8.67	8.80	2.98	2.87	440.0	480.0
SOIL TREATED WITH SUNFLOWER 1 @T=0	1	20.8	23.10	8.43	8.49	2.55	2.65	490.0	800.0
	2	19.99	22.00	8.53	8.50	2.40	2.70	480.0	790.0
	3	21.00	22.90	8.86	8.61	2.50	2.70	490.0	790.0
SOIL TREATED WITH SUNFLOWER 2 @T=0	1	21.00	22.00	8.88	8.61	2.66	2.80	480.0	720.0
	2	21.00	22	8.55	8.88	2.70	2.88	490.0	770.0
	3	20.55	21.87	8.45	8.60	2.50	2.74	488.0	780.0
SOIL TREATED WITH SUNFLOWER 3 @T=0	1	19.00	19.00	8.88	8.89	2.50	3.00	460.0	920.0
	2	19.50	22.10	8.88	8.90	22.50	3.00	460.0	900.0
	3	20.00	21.00	8.20	8.90	2.60	3.20	475.0	910.0
SOIL TREATED WITH PALMWINE 1 @T=0	1	21.00	22.40	8.80	8.85	2.23	3.40	390.0	900.0
	2	21.00	20.00	8.86	8.90	2.03	3.40	460	900
	3	21.00	20.00	8.80	8.95	2.35	3.60	400	910
SOIL TREATED WITH PALMWINE 2 @T=0	1	21.70	20.00	8.80	8.87	2.16	3.60	380	860
	2	20.00	21.00	8.66	8.90	2.30	3.90	400	880
	3	20.00	21.50	8.20	8.90	2.35	3.90	400	880
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=0	1	21.30	20.00	8.56	8.60	2.60	2.80	440	480
	2	21.00	22.00	8.60	8.60	2.40	2.70	450	490
	3	20.60	21.00	8.56	8.60	2.30	2.80	430	480
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=0	1	21.10	20.30	8.86	8.89	2.29	3.00	420	720
	2	21.00	20.50	8.40	8.90	2.30	3.00	430	725
	3	21.00	20.10	8.40	8.90	2.30	3.00	410	730
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=0	1	21.20	19.80	8.79	8.68	2.14	3.9	510	980
	2	20.85	21.00	8.88	8.50	2.22	3.80	460	960
	3	20.99	21.00	8.70	9.00	2.22	3.80	480	980

Appendix III-2: Raw data for Investigation of remediation potential of sunflower species, fermented palm wine and *P. ostreatus on* petroleum contaminated soils from Tibshelf, UK, using TPHs-Gasoline diesel standard

	Calibration function: y = 445482x + 6E+07 R^2 = 0.9899 calculation of concentration per dry weight of soil C=(Cs.Vn.f.100)/(M.Dm.p), parameters as defined in section 3.5.2.3 of chapter 3																	
Treatments		Peak area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc /Dry weight (mg/kg)	Mean (mg/kg dry soil)	SE	SD	(2σ)	% decrease
UNCONTAMINATED SOIL @T=0	1	7232393207	60000000	4454482	16100.30	3	10	1	100	66.22	0.33	1.373	22103.04	18180.02	2829.57	2829.57	5659.13	35.60
	2	5544647138	60000000	4454482	12311.71	3	10	1	100	66.22	0.33	1.373	16901.94					
	3	5101099202	60000000	4454482	11316.06	3	10	1	100	66.22	0.33	1.373	15535.07					
UNCONTAMINATED SOIL @T=3	1	3998650891	60000000	4454482	8841.32	3	10	1	100	66.22	0.33	1.373	12137.67	11708.07	459.15	795.26	1590.53	
	2	4081640922	60000000	4454482	9027.62	3	10	1	100	66.22	0.33	1.373	12393.42					
	3	3497452990	60000000	4454482	7716.26	3	10	1	100	66.22	0.33	1.373	10593.14					
CONT_SOIL_WITHOUT_AD@T=0	1	1.04581E+11	60000000	4454482	234623.96	3	10	1	100	71.89	0.33	1.265	296695.66	338631.66	19118.48	33114.18	66228.36	15.27
	2	1.331E+11	60000000	4454482	298642.66	3	10	1	100	71.89	0.33	1.265	377651.04					
	3	1.20382E+11	60000000	4454482	270092.97	3	10	1	100	71.89	0.33	1.265	341548.29					
CONT_SOIL_WITHOUT_AD@T=3	1	9064871866 2	60000000	4454482	203349.90	3	10	1	100	71.89	0.33	1.265	257147.78	286909.52	29336.83	50812.87	101625.75	
	2	1.2633E+11	60000000	4454482	283446.75	3	10	1	100	71.89	0.33	1.265	358434.92					
	3	8642065479 5	60000000	4454482	193858.91	3	10	1	100	71.89	0.33	1.265	245145.88					
CONT_SOIL+_AD@T=0	1	9259085548 0	60000000	4454482	207709.53	3	10	1	100	57.4	0.33	1.584	328966.62	334490.34	5393.69	9342.14	18684.28	28.79
	2	9199787834 6	60000000	4454482	206378.44	3	10	1	100	57.4	0.33	1.584	326858.47					
	3	9784492261 4	60000000	4454482	219503.64	3	10	1	100	57.4	0.33	1.584	347645.94					
CONT_SOIL_+_AD@T=3	1	5670407030 8	60000000	4454482	127152.32	3	10	1	100	57.4	0.33	1.584	201381.57	238189.92	19186.69	33232.32	66464.64	
	2	7935236297 6	60000000	4454482	177992.29	3	10	1	100	57.4	0.33	1.584	281901.00					
	3	6511584736 4	60000000	4454482	146034.74	3	10	1	100	57.4	0.33	1.584	231287.20					
SOIL TREATED WITH SUNFLOWER 1 @T=0	1	9971095377	60000000	4454482	22248.03	3	5	3	100	68.22	0.33	7.996	177884.60	202037.08	9861.96	17081.41	34162.82	54.28
	2	1196770919 9	60000000	4454482	26729.94	3	5	3	100	68.22	0.33	7.996	213719.87					
	3	1201155344 4	60000000	4454482	26828.36	3	5	3	100	68.22	0.33	7.996	214506.79					
SOIL TREATED WITH SUNFLOWER 1 @T=3	1	5321071881	60000000	4454482	11809.84	3	5	3	100	68.22	0.33	7.996	94425.85	92377.54	1519.74	2632.26	5264.53	
	2	5299869967	60000000	4454482	11762.25	3	5	3	100	68.22	0.33	7.996	94045.32					
	3	4999899678	60000000	4454482	11088.89	3	5	3	100	68.22	0.33	7.996	88661.45					
SOIL TREATED WITH SUNFLOWER 2 @T=0	1	1364802561 7	60000000	4454482	30501.85	3	5	1	100	68.22	0.33	2.665	81292.75	251881.76	84136.61	145728.88	291457.76	53.29
	2	1345987699 2	60000000	4454482	30079.50	3	5	3	100	69.22	0.33	7.880	237026.89					
	3	2371188351 1	60000000	4454482	53092.79	3	5	3	100	66.22	0.33	8.237	437325.66					
SOIL TREATED WITH SUNFLOWER 2 @T=3	1	6034114621	60000000	4454482	13410.45	3	5	3	100	67.22	0.33	8.114	108818.68	117664.30	3850.31	6668.94	13337.88	
	2	5999879911	60000000	4454482	13333.60	3	5	3	100	58.22	0.33	9.369	124920.54					

	Calibration function: y = 445482x + 6E+07 R^2 = 0.9899 calculation of concentration per dry weight of soil C=(Cs.Vn.f.100)/(M.Dm.p), parameters as defined in section 3.5.2.3 of chapter 3																	
Treatments		Peak area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc /Dry weight (mg/kg)	Mean (mg/kg dry soil)	SE	SD	(2σ)	% decrease
	3	6120011445	60000000	4454482	13603.27	3	5	3	100	62.22	0.33	8.767	119253.69					
SOIL TREATED WITH SUNFLOWER 3 @T=0	1	2175424284848	60000000	4454482	48698.36	3	5	3	100	68.22	0.33	7.996	389368.83	297850.16	37396.49	64772.62	129545.23	69.43
	2	1411987699 6	60000000	4454482	31561.04	3	5	3	100	69.22	0.33	7.880	248701.46					
	3	1387713589 6	60000000	4454482	31016.15	3	5	3	100	66.22	0.33	8.237	255480.21					
SOIL TREATED WITH SUNFLOWER 3 @T=3	1	4754284737	60000000	4454482	10537.54	3	5	3	100	67.22	0.33	8.114	85506.54	91050.30	2384.50	4130.07	8260.14	
	2	4596899785	60000000	4454482	10184.25	3	5	3	100	58.22	0.33	9.369	95414.72					
	3	4746754821	60000000	4454482	10520.64	3	5	3	100	62.22	0.33	8.767	92229.67					
SOIL TREATED WITH PALMWINE 1 @T=0	1	2551205575 3	60000000	4454482	57133.75	3	10	3	100	55.64	0.33	4.902	280049.08	276231.06	1559.64	2701.38	5402.75	68.46
	2	2498112194 7	60000000	4454482	55941.93	3	10	3	100	55.64	0.33	4.902	274207.21					
	3	2500199563 9	60000000	4454482	55988.78	3	10	3	100	55.64	0.33	4.902	274436.89					
SOIL TREATED WITH PALMWINE 1 @T=3	1	7128540224	60000000	4454482	15867.17	3	10	3	100	55.64	0.33	4.902	77775.18	87122.53	7762.59	13445.20	26890.39	
	2	9706094643	60000000	4454482	21653.16	3	10	3	100	55.64	0.33	4.902	106136.02					
	3	7099567891	60000000	4454482	15802.14	3	10	3	100	55.64	0.33	4.902	77456.40					
SOIL TREATED WITH PALMWINE 2 @T=0	1	3309535257 7	60000000	4454482	74156.43	3	10	3	100	55.64	0.33	4.902	363488.13	344087.06	24940.36	43197.97	86395.94	70.49
	2	2589102195 1	60000000	4454482	57984.43	3	10	3	100	55.64	0.33	4.902	284218.85					
	3	3500992689 2	60000000	4454482	78454.18	3	10	3	100	55.64	0.33	4.902	384554.20					
SOIL TREATED WITH PALMWINE 2 @T=3	1	9706094643	60000000	4454482	21653.16	3	10	3	100	55.64	0.33	4.902	106136.02	101542.09	1920.94	3327.16	6654.32	
	2	8999714642	60000000	4454482	20067.51	3	10	3	100	55.64	0.33	4.902	98363.72					
	3	9159927989	60000000	4454482	20427.15	3	10	3	100	55.64	0.33	4.902	100126.55					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=0	1	2030932218 3	60000000	4454482	45454.86	3	10	3	100	55.64	0.33	4.902	222803.38	293619.22	28921.93	50094.25	100188.50	27.80
	2	2980628996 2	60000000	4454482	66773.27	3	10	3	100	55.64	0.33	4.902	327298.56					
	3	3012049228 0	60000000	4454482	67478.58	3	10	3	100	55.64	0.33	4.902	330755.73					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=3	1	1508724899 6	60000000	4454482	33732.56	3	10	3	100	55.64	0.33	4.902	165344.89	212005.49	26524.35	45941.53	91883.06	
	2	2500679443 2	60000000	4454482	55999.56	3	10	3	100	55.64	0.33	4.902	274489.69					
	3	1788984958 9	60000000	4454482	40023.73	3	10	3	100	55.64	0.33	4.902	196181.91					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=0	1	3905002743	60000000	4454482	8631.11	3	10	3	100	51.64	0.33	5.281	45583.62	132797.38	38141.18	66062.45	132124.91	59.88
	2	1746547933 2	60000000	4454482	39071.12	3	10	3	100	51.874	0.33	5.257	205416.18					
	3	1254799557 5	60000000	4454482	28032.55	3	10	3	100	51.87	0.33	5.258	147392.33					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=3	1	5482075689	60000000	4454482	12171.26	3	10	3	100	47.24	0.33	5.773	70267.43	53281.42	8061.67	13963.21	27926.43	

	Calibration function: y = 445482x + 6E+07 R^2 = 0.9899 calculation of concentration per dry weight of soil C=(Cs.Vn.f.100)/(M.Dm.p), parameters as defined in section 3.5.2.3 of chapter 3																	
Treatments		Peak area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc /Dry weight (mg/kg)	Mean (mg/kg dry soil)	SE	SD	(2σ)	% decrease
	2	4014194692	60000000	44548	8876.22	3	10	3	1000	45.24	0.33	6.028	53509.87					
	3	2666312067	60000000	44548	5850.54	3	10	3	1000	44.24	0.33	6.165	36066.97					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=0	1	2075380482 4	60000000	44548	46452.62	3	10	3	1000	57.64	0.33	4.732	219793.47	204931.40	6084.19	10538.12	21076.24	84.92
	2	1910164206 3	60000000	44548	42743.91	3	10	3	1000	58.74	0.33	4.643	198458.12					
	3	1863855164 4	60000000	44548	41704.38	3	10	3	1000	57.87	0.33	4.713	196542.64					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=3	1	3220075169	60000000	44548	7093.61	3	10	3	1000	59.24	0.33	4.604	32657.34	30901.95	1597.62	2767.15	5534.30	
	2	3125027432	60000000	44548	6880.25	3	10	3	1000	56.77	0.33	4.804	33053.23					
	3	2578707775	60000000	44548	5653.89	3	10	3	1000	57.12	0.33	4.775	26995.29					

Raw data for chapter 5 and 6

Appendix III-3: Raw data for effects of Tween 80 on some phyto- and myco-remediation agents on petroleum contaminated soils and sediments from Ogoniland, Niger Delta, Nigeria

		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc/ Dry Weight (mg/kg)	MEAN (mg/kg dry soil)	S.E	SD	2*SD
CONT_NG_LOAM @T0	1	20271746819	60000000	445482	45370.51	8	10	1	100	66.3	0.125	9.65	437965.74	445188.69	5897.52	10214.80	20429.61
	2	20271746819	60000000	445482	45370.51	8	10	1	100	66.3	0.125	9.65	437965.74				
	3	21271746819	60000000	445482	47615.27	8	10	1	100	66.3	0.125	9.65	459634.61				
CONT_NG_LOAM @T1	1	18726780188	60000000	445482	41902.43	8	10	1	100	66.3	0.125	9.65	404488.05	404594.33	512.58	887.81	1775.63
	2	18684137333	60000000	445482	41806.71	8	10	1	100	66.3	0.125	9.65	403564.03				
	3	18784137333	60000000	445482	42031.19	8	10	1	100	66.3	0.125	9.65	405730.92				
CONT_NG_LOAM @T2	1	16066934256	60000000	445482	35931.72	8	10	1	100	66.3	0.125	9.65	346852.19	398892.25	34029.70	58941.18	117882.35
	2	22271746819	60000000	445482	49860.03	8	10	1	100	66.3	0.125	9.65	481303.48				
	3	17066934256	60000000	445482	38176.48	8	10	1	100	66.3	0.125	9.65	368521.07				
CONT_NG_LOAM @T3	1	13644457550	60000000	445482	30493.84	9	10	1	100	66.3	0.111	12.23	372922.12	359185.48	10325.13	17883.65	35767.30
	2	13563796708	60000000	445482	30312.78	9	10	1	100	66.3	0.111	12.23	370707.81				
	3	13744457550	60000000	445482	30718.32	8	10	1	100	66.3	0.111	10.87	333926.52				
CONT_NG_LOAM +TWEEN80@T0	1	18684137333	60000000	445482	41806.71	8	10	1	100	66.3	0.125	9.65	403564.03	403564.03	10214.80	17692.56	35385.12
	2	19684137333	60000000	445482	44051.47	8	10	1	100	66.3	0.125	9.65	425232.90				
	3	17684137333	60000000	445482	39561.95	8	10	1	100	66.3	0.125	9.65	381895.16				
CONT_NG_LOAM +TWEEN80@T1	1	16606174397	60000000	445482	37142.18	8	10	1	100	66.3	0.125	9.65	358536.92	372982.83	5897.52	10214.80	20429.61
	2	17606174397	60000000	445482	39386.94	8	10	1	100	66.3	0.125	9.65	380205.79				
	3	17606174397	60000000	445482	39386.94	8	10	1	100	66.3	0.125	9.65	380205.79				
CONT_NG_LOAM +TWEEN80@T2	1	15606174397	60000000	445482	34897.42	8	10	1	100	66.3	0.125	9.65	336868.05	346667.45	35312.05	61162.27	122324.53
	2	19719217160	60000000	445482	44130.22	8	10	1	100	66.3	0.125	9.65	425993.04				
	3	12849833822	60000000	445482	28710.10	8	10	1	100	66.3	0.125	9.65	277141.26				
CONT_NG_LOAM +TWEEN80@T3	1	21433674914	60000000	445482	47978.76	7	10	1	100	66.3	0.142	7.44	356734.58	277202.10	32471.38	56242.08	112484.16
	2	14235918235	60000000	445482	31821.53	7	10	1	100	66.3	0.142	7.44	236601.34				
	3	14335918235	60000000	445482	32046.00	7	10	1	100	66.3	0.142	7.44	238270.38				
CONT_NGLOAM+SUNFLOWERS@T0	1	9002885118	60000000	445482	20074.63	8	10	1	100	68.22	0.125	9.38	188328.37	221277.99	14338.21	24834.50	49669.00
	2	11849833822	60000000	445482	26465.34	8	10	1	100	68.22	0.125	9.38	248282.31				
	3	10849833822	60000000	445482	24220.58	8	10	1	100	68.22	0.125	9.38	227223.30				
CONT_NGLOAM+SUNFLOWERS@T1	1	7634699507	60000000	445482	17003.38	8	10	1	100	68.22	0.125	9.38	159515.73	153955.69	2513.06	4352.75	8705.49
	2	7233664383	60000000	445482	16103.15	8	10	1	100	69.22	0.125	9.25	148887.85				
	3	7133664383	60000000	445482	15878.68	8	10	1	100	66.22	0.125	9.66	153463.50				
CONT_NGLOAM+SUNFLOWERS @T2	1	6016078283	60000000	445482	13369.96	8	10	1	100	67.22	0.125	9.52	127295.10	135878.41	4458.58	7722.48	15444.96
	2	5977376999	60000000	445482	13283.09	8	10	1	100	58.22	0.125	10.99	146018.15				
	3	5877376999	60000000	445482	13058.61	8	10	1	100	62.22	0.125	10.29	134321.96				
CONT_NGLOAM+SUNFLOWERS @T3	1	5144894741	60000000	445482	11414.37	8	10	1	100	68.22	0.125	9.38	107082.89	89984.07	6989.35	12105.90	24211.81
	2	3947855572	60000000	445482	8727.30	8	10	1	100	69.22	0.125	9.25	80691.60				
	3	3847855572	60000000	445482	8502.83	8	10	1	100	66.22	0.125	9.66	82177.71				
CONT_NGLOAM+SUNFLOWERS + TW80@T0	1	8961611081	60000000	445482	19981.98	8	10	1	100	55.64	0.125	11.50	229843.01	241322.16	4725.69	8185.14	16370.28
	2	9578476159	60000000	445482	21366.69	8	10	1	100	55.64	0.125	11.50	245770.71				
	3	9678476159	60000000	445482	21591.17	8	10	1	100	55.64	0.125	11.50	248352.75				

		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc/ Dry Weight (mg/kg)	MEAN (mg/kg dry soil)	S.E	SD	2*SD
CONT_NGLOAM+SUNFLOWERS + TW80@T1	1	4497185328	60000000	445482	9960.41	8	10	1	100	55.64	0.125	11.50	114569.83	110992.08	1582.33	2740.67	5481.34
	2	4339341222	60000000	445482	9606.09	8	10	1	100	55.64	0.125	11.50	110494.23				
	3	4239341222	60000000	445482	9381.62	8	10	1	100	55.64	0.125	11.50	107912.19				
CONT_NGLOAM+SUNFLOWERS + TW80@T2	1	4755796463	60000000	445482	10540.93	5	10	1	100	55.64	0.2	4.49	47362.21	50105.89	2662.60	4611.75	9223.50
	2	5671872815	60000000	445482	12597.31	5	10	1	100	55.64	0.2	4.49	56601.84				
	3	4655796463	60000000	445482	10316.46	5	10	1	100	55.64	0.2	4.49	46353.60				
CONT_NGLOAM+SUNFLOWERS + TW80@T3	1	5071872815	60000000	445482	11250.45	3	10	1	100	55.64	0.33	1.63	18381.89	19237.68	555.78	962.64	1925.28
	2	5171872815	60000000	445482	11474.93	3	10	1	100	55.64	0.33	1.63	18748.65				
	3	5671872815	60000000	445482	12597.31	3	10	1	100	55.64	0.33	1.63	20582.49				
CONT_NGLOAM+FERNS @T0	1	12252947110	60000000	445482	27370.24	7	10	1	100	55.64	0.1429	8.80	240966.80	233436.27	14960.45	25912.27	51824.53
	2	10109809933	60000000	445482	22559.41	7	10	1	100	55.64	0.1429	8.80	198612.41				
	3	13252947110	60000000	445482	29614.99	7	10	1	100	55.64	0.1429	8.80	260729.61				
CONT_NGLOAM+FERNS @T1	1	5371663952	60000000	445482	11923.41	7	10	1	100	55.64	0.1429	8.80	104973.37	98711.72	2598.40	4500.56	9001.12
	2	4946404070	60000000	445482	10968.80	7	10	1	100	55.64	0.1429	8.80	96569.04				
	3	4846404070	60000000	445482	10744.33	7	10	1	100	55.64	0.1429	8.80	94592.76				
CONT_NGLOAM+FERNS @T2	1	9753053269	60000000	445482	21758.57	4	10	1	100	51.64	0.25	3.10	67416.19	65227.43	908.13	1572.93	3145.86
	2	9372433780	60000000	445482	20904.18	4	10	1	100	51.874	0.25	3.08	64476.77				
	3	9272433780	60000000	445482	20679.70	4	10	1	100	51.87	0.25	3.08	63789.32				
CONT_NGLOAM+FERNS @T3	1	7772433780	60000000	445482	17312.56	4	10	1	100	47.24	0.25	3.39	58636.95	60826.47	951.53	1648.10	3296.21
	2	7772433780	60000000	445482	17312.56	4	10	1	100	45.24	0.25	3.54	61229.21				
	3	7772433780	60000000	445482	17312.56	4	10	1	100	44.24	0.25	3.62	62613.24				
CONT_NGLOAM+FERNS + TW80@T0	1	8900601970	60000000	445482	19845.03	7	10	1	100	57.64	0.1429	8.50	168652.78	176968.94	3563.17	6171.59	12343.17
	2	9613119619	60000000	445482	21444.46	7	10	1	100	58.74	0.1429	8.34	178832.70				
	3	9713119619	60000000	445482	21668.93	7	10	1	100	57.87	0.1429	8.46	183421.34				
CONT_NGLOAM+FERNS + TW80@T1	1	5357103242	60000000	445482	11890.72	5	10	1	100	59.24	0.2	4.22	50180.30	49636.58	241.35	418.02	836.05
	2	5074068934	60000000	445482	11255.38	5	10	1	100	56.77	0.2	4.40	49565.70				
	3	5064068934	60000000	445482	11232.93	5	10	1	100	57.12	0.2	4.38	49163.74				
CONT_NGLOAM+FERNS + TW80@T2	1	6671729575	60000000	445482	14841.74	3	10	1	100	57.12	0.33	1.59	23621.31	24113.74	554.35	960.16	1920.32
	2	7185227419	60000000	445482	15994.42	3	10	1	100	57.12	0.33	1.59	25455.85				
	3	6571729575	60000000	445482	14617.27	3	10	1	100	57.12	0.33	1.59	23264.05				
CONT_NGLOAM+FERNS + TW80@T3	1	4571729575	60000000	445482	10127.75	3	10	1	100	57.12	0.33	1.59	16118.77	13737.02	972.35	1684.16	3368.31
	2	3571729575	60000000	445482	7882.99	3	10	1	100	57.12	0.33	1.59	12546.14				
	3	3571729575	60000000	445482	7882.99	3	10	1	100	57.12	0.33	1.59	12546.14				
CONT_NGLOAM+PALM WINE@T0	1	9786456033	60000000	445482	21833.56	9	10	1	100	57.12	0.1111	14.18	309645.46	312022.81	1226.79	2124.87	4249.73
	2	9848470611	60000000	445482	21972.76	9	10	1	100	57.12	0.1111	14.18	311619.72				
	3	9948470611	60000000	445482	22197.24	9	10	1	100	57.12	0.1111	14.18	314803.26				
CONT_NGLOAM+PALM WINE@T1	1	4813336703	60000000	445482	10670.10	9	10	1	100	57.12	0.1111	14.18	151324.30	150124.93	895.99	1551.90	3103.80
	2	4806825408	60000000	445482	10655.48	9	10	1	100	57.12	0.1111	14.18	151117.01				
	3	4706825408	60000000	445482	10431.01	9	10	1	100	57.12	0.1111	14.18	147933.47				
CONT_NGLOAM+PALM WINE @T2	1	3211699012	60000000	445482	7074.81	7	10	1	100	57.12	0.1429	8.58	60672.54	61329.76	460.27	797.20	1594.41
	2	3304118256	60000000	445482	7282.27	7	10	1	100	57.12	0.1429	8.58	62451.68				
	3	3221699012	60000000	445482	7097.25	7	10	1	100	57.12	0.1429	8.58	60865.05				

		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	TPH Conc/ Dry Weight (mg/kg)	MEAN (mg/kg dry soil)	S.E	SD	2*SD
CONT_NGLOAM+PALM WINE @T3	1	2211699012	60000000	445482	4830.05	7	10	1	100	57.12	0.1429	8.58	41421.80	41357.63	52.39	90.75	181.50
	2	2211699012	60000000	445482	4830.05	7	10	1	100	57.12	0.1429	8.58	41421.80				
	3	2201699012	60000000	445482	4807.60	7	10	1	100	57.12	0.1429	8.58	41229.29				
CONT_NGLOAM+PALM WINE + TW80@T0	1	15140055677	60000000	445482	33851.10	7	10	1	100	57.12	0.1429	8.58	290302.25	293793.79	6758.03	11705.25	23410.50
	2	14684170823	60000000	445482	32827.75	7	10	1	100	57.12	0.1429	8.58	281526.13				
	3	16140055677	60000000	445482	36095.86	7	10	1	100	57.12	0.1429	8.58	309552.99				
CONT_NGLOAM+PALM WINE+ TW80@T1	1	22503311445	60000000	445482	50379.84	4	10	1	100	57.12	0.125	5.60	282239.99	95964.06	76046.84	131716.98	263433.97
	2	289727290	60000000	445482	515.68	4	10	1	100	57.12	0.125	5.60	2888.98				
	3	279727290	60000000	445482	493.23	4	10	1	100	57.12	0.125	5.60	2763.22				
CONT_NGLOAM+PALM WINE + TW80@T2	1	6686241717	60000000	445482	14874.32	5	10	1	100	57.12	0.33	2.65	39455.27	38356.08	470.18	814.37	1628.74
	2	6459340838	60000000	445482	14364.98	5	10	1	100	57.12	0.33	2.65	38104.21				
	3	6359340838	60000000	445482	14140.51	5	10	1	100	57.12	0.33	2.65	37508.77				
CONT_NGLOAM+PALM WINE + TW80@T3	1	1359340838	60000000	445482	2916.71	4	10	1	100	57.12	0.33	2.12	6189.43	5713.08	224.55	388.94	777.88
	2	1159340838	60000000	445482	2467.76	4	10	1	100	57.12	0.33	2.12	5236.73				
	3	1259340838	60000000	445482	2692.23	4	10	1	100	57.12	0.33	2.12	5713.08				
CONT_NGLOAM+P. OSTREATUS @T0	1	12066641620	60000000	445482	26952.02	7	10	1	100	57.12	0.1429	8.58	231136.75	229460.90	4588.73	7947.92	15895.84
	2	11436061043	60000000	445482	25536.52	7	10	1	100	57.12	0.1429	8.58	218997.61				
	3	12436061043	60000000	445482	27781.28	7	10	1	100	57.12	0.1429	8.58	238248.35				
CONT_NGLOAM+P. OSTREATUS@T1	1	5740684102	60000000	445482	12751.77	7	10	1	100	57.12	0.1429	8.58	109357.38	109611.97	1092.47	1892.21	3784.42
	2	5880359090	60000000	445482	13065.31	7	10	1	100	57.12	0.1429	8.58	112046.23				
	3	5640684102	60000000	445482	12527.29	7	10	1	100	57.12	0.1429	8.58	107432.30				
CONT_NGLOAM+P. OSTREATUS@T2	1	9102137289	60000000	445482	20297.42	5	10	1	100	57.12	0.2	4.38	88836.77	89006.11	241.67	418.59	837.17
	2	9177991047	60000000	445482	20467.70	5	10	1	100	57.12	0.2	4.38	89582.01				
	3	9077991047	60000000	445482	20243.22	5	10	1	100	57.12	0.2	4.38	88599.54				
CONT_NGLOAM+P. OSTREATUS @T3	1	4880359090	60000000	445482	10820.55	5	10	1	100	57.12	0.33	2.65	28702.33	28305.37	162.06	280.69	561.39
	2	4780359090	60000000	445482	10596.07	5	10	1	100	57.12	0.33	2.65	28106.89				
	3	4780359090	60000000	445482	10596.07	5	10	1	100	57.12	0.33	2.65	28106.89				
CONT_NGLOAM+ P. OSTREATUS + TW80@T0	1	10586575695	60000000	445482	23629.63	7	10	1	100	57.12	0.1429	8.58	202644.38	191336.40	8459.20	14651.76	29303.53
	2	8924360419	60000000	445482	19898.36	7	10	1	100	57.12	0.1429	8.58	170645.51				
	3	10486575695	60000000	445482	23405.16	7	10	1	100	57.12	0.1429	8.58	200719.31				
CONT_NGLOAM+P. OSTREATUS + TW80@T1	1	5310287108	60000000	445482	11785.63	5	10	1	100	57.12	0.2	4.38	51582.78	47596.21	1643.90	2847.32	5694.64
	2	4751636273	60000000	445482	10531.60	5	10	1	100	57.12	0.2	4.38	46094.17				
	3	4651636273	60000000	445482	10307.12	5	10	1	100	57.12	0.2	4.38	45111.69				
CONT_NGLOAM+P. OSTREATUS + TW80@T2	1	6623188971	60000000	445482	14732.78	3	10	1	100	57.12	0.333	1.58	23236.65	21900.17	545.68	945.14	1890.29
	2	6061956665	60000000	445482	13472.95	3	10	1	100	57.12	0.333	1.58	21249.64				
	3	6051956665	60000000	445482	13450.50	3	10	1	100	57.12	0.333	1.58	21214.23				
CONT_NGLOAM+P. OSTREATUS + TW80@T3	1	2751636273	60000000	445482	6042.08	3	10	1	100	57.12	0.333	1.58	9529.61	8939.53	254.94	441.57	883.14
	2	2551636273	60000000	445482	5593.12	3	10	1	100	57.12	0.333	1.58	8821.52				
	3	2451636273	60000000	445482	5368.65	3	10	1	100	57.12	0.333	1.58	8467.47				

Raw data for chapter 7

Appendix III-4: Raw data for exploration for phyto and myco-remediation options for management of petroleum contaminated soils using standardised crude oil standard

Treatments		Peak Area (Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	Multiplier	Conc/Dry Weight (mg/kg)	Mean (mg/kg dry soil)	S.E	SD	2SD	% decrease
UNCONTAMINATED SOIL @T=0	1	7232393207	100000000	20000000	356.6197	5	5	1	100	66.22	0.2	7.550589	2692.688	2,212.09	200.13	346.64	693.29	35.84
	2	5544647138	100000000	20000000	272.2324	5	5	1	100	66.22	0.2	7.550589	2055.515					
	3	5101099202	100000000	20000000	250.055	5	5	1	100	66.22	0.2	7.550589	1888.062					
UNCONTAMINATED SOIL @T=3	1	3998650891	100000000	20000000	194.9325	5	5	1	100	66.22	0.2	7.550589	1471.856	1,419.23	56.25	97.43	194.85	
	2	4081640922	100000000	20000000	199.082	5	5	1	100	66.22	0.2	7.550589	1503.187					
	3	3497452990	100000000	20000000	169.8726	5	5	1	100	66.22	0.2	7.550589	1282.639					
Derby Crude Oil	1	1.6578E+11	100000000	20000000	8283.977	5	5	1	100	66.22	0.2	7.550589	62548.91	67,743.30	2,121.08	3,673.82	7,347.65	
	2	1.866728E+11	100000000	20000000	9328.577	5	5	1	100	66.22	0.2	7.550589	70436.25					
	3	1.866164E+11	100000000	20000000	9303.214	5	5	1	100	66.22	0.2	7.550589	70244.74					
CONT_SOIL_WITHOUT_AD@T=0	1	1.045811E+11	100000000	20000000	5224.038	5	5	1	100	71.89	0.2	6.955507	36333.55	41,471.03	2,342.16	4,056.74	8,113.47	15.28
	2	1.331E+11	100000000	20000000	6649.997	5	5	1	100	71.89	0.2	6.955507	46251.19					
	3	1.203828E+11	100000000	20000000	6014.078	5	5	1	100	71.89	0.2	6.955507	41828.33					
CONT_SOIL_WITHOUT_AD@T=3	1	90648718662	100000000	20000000	4527.436	5	5	1	100	71.89	0.2	6.955507	31488.63	35,134.67	3,593.98	6,224.96	12,449.92	
	2	1.2633E+11	100000000	20000000	6311.521	5	5	1	100	71.89	0.2	6.955507	43897.07					
	3	8642065795	100000000	20000000	4316.033	5	5	1	100	71.89	0.2	6.955507	30018.31					
CONT_SOIL+_AD@T=0	1	9259085480	100000000	20000000	4624.543	5	5	1	100	57.4	0.2	8.710801	40283.47	40,960.17	660.77	1,144.48	2,288.96	28.80
	2	9199787346	100000000	20000000	4594.894	5	5	1	100	57.4	0.2	8.710801	40025.21					
	3	9784492614	100000000	20000000	4887.246	5	5	1	100	57.4	0.2	8.710801	42571.83					
CONT_SOIL_+_AD@T=3	1	5670407030 8	100000000	20000000	2830.204	5	5	1	100	57.4	0.2	8.710801	24653.34	29,162.64	2,350.51	4,071.21	8,142.42	
	2	7935236 2976	100000000	20000000	3962.618	5	5	1	100	57.4	0.2	8.710801	34517.58					
	3	6511584 7364	100000000	20000000	3250.792	5	5	1	100	57.4	0.2	8.710801	28317.01					
SOIL TREATED WITH SUNFLOWER 1 @T=0	1	1297109 5377	100000000	20000000	643.5548	5	5	3	100	68.22	0.2	21.98769	14150.28	13,430.94	293.89	509.03	1,018.06	58.20
	2	1196770 9199	100000000	20000000	593.3855	5	5	3	100	68.22	0.2	21.98769	13047.17					
	3	1201155 3444	100000000	20000000	595.5777	5	5	3	100	68.22	0.2	21.98769	13095.38					
SOIL TREATED WITH SUNFLOWER 1 @T=3	1	5321071881	100000000	20000000	261.0536	5	5	3	100	68.22	0.2	21.98769	5739.965	5,614.50	93.09	161.24	322.47	
	2	5299869967	100000000	20000000	259.9935	5	5	3	100	68.22	0.2	21.98769	5716.656					
	3	4999899678	100000000	20000000	244.995	5	5	3	100	68.22	0.2	21.98769	5386.873					
SOIL TREATED WITH SUNFLOWER 2 @T=0	1	1364802 5617	100000000	20000000	677.4013	5	5	3	100	68.22	0.2	21.98769	14894.49	14,928.87	222.29	385.02	770.03	52.04
	2	1345987 6992	100000000	20000000	667.9938	5	5	3	100	69.22	0.2	21.67004	14475.45					
	3	1371188 3511	100000000	20000000	680.5942	5	5	3	100	66.22	0.2	22.65177	15416.66					
SOIL TREATED WITH SUNFLOWER 2 @T=3	1	6034114621	100000000	20000000	296.7057	5	5	3	100	67.22	0.2	22.31479	6620.925	7,159.26	234.24	405.71	811.42	
	2	5999879911	100000000	20000000	294.994	5	5	3	100	58.22	0.2	25.76434	7600.326					
	3	6120011445	100000000	20000000	301.0006	5	5	3	100	62.22	0.2	24.108	7256.523					
SOIL TREATED WITH SUNFLOWER 3 @T=0	1	2175424 2848	100000000	20000000	1082.712	5	5	3	100	68.22	0.2	21.98769	23806.34	18,200.24	2,290.75	3,967.70	7,935.39	69.62
	2	1411987 6996	100000000	20000000	700.9938	5	5	3	100	69.22	0.2	21.67004	15190.56					

	3	13877135896	100000000	20000000	688.8568	5	5	3	100	66.22	0.2	22.65177	15603.82					
SOIL TREATED WITH SUNFLOWER 3 @T=3	1	4754284737	100000000	20000000	232.7142	5	5	3	100	67.22	0.2	22.31479	5192.969	5,529.05	144.46	250.21	500.42	
	2	4596899785	100000000	20000000	224.845	5	5	3	100	58.22	0.2	25.76434	5792.983					
	3	4746754821	100000000	20000000	232.3377	5	5	3	100	62.22	0.2	24.108	5601.199					
SOIL TREATED WITH PALMWINE 1 @T=0	1	25512055753	100000000	20000000	1270.603	5	5	3	100	55.64	0.2	26.95902	34254.21	33,786.47	191.07	330.94	661.88	68.57
	2	24981121947	100000000	20000000	1244.056	5	5	3	100	55.64	0.2	26.95902	33538.54					
	3	25001995639	100000000	20000000	1245.1	5	5	3	100	55.64	0.2	26.95902	33566.67					
SOIL TREATED WITH PALMWINE 1 @T=3	1	7128540224	100000000	20000000	351.427	5	5	3	100	55.64	0.2	26.95902	9474.129	10,619.25	950.98	1,647.14	3,294.28	
	2	9706094643	100000000	20000000	480.3047	5	5	3	100	55.64	0.2	26.95902	12948.55					
	3	7099567891	100000000	20000000	349.9784	5	5	3	100	55.64	0.2	26.95902	9435.075					
SOIL TREATED WITH PALMWINE 2 @T=0	1	33095352577	100000000	20000000	1649.768	5	5	3	100	55.64	0.2	26.95902	44476.12	42,099.34	3,055.38	5,292.08	10,584.15	70.58
	2	25891021951	100000000	20000000	1289.551	5	5	3	100	55.64	0.2	26.95902	34765.04					
	3	35009926892	100000000	20000000	1745.496	5	5	3	100	55.64	0.2	26.95902	47056.87					
SOIL TREATED WITH PALMWINE 2 @T=3	1	9706094643	100000000	20000000	480.3047	5	5	3	100	55.64	0.2	26.95902	12948.55	12,385.76	235.33	407.60	815.20	
	2	8999714642	100000000	20000000	444.9857	5	5	3	100	55.64	0.2	26.95902	11996.38					
	3	9159927989	100000000	20000000	452.9964	5	5	3	100	55.64	0.2	26.95902	12212.34					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=0	1	20309322183	100000000	20000000	1010.466	5	5	3	100	55.64	0.2	26.95902	27241.18	35,916.65	3,543.15	6,136.92	12,273.85	27.84
	2	29806289962	100000000	20000000	1485.314	5	5	3	100	55.64	0.2	26.95902	40042.63					
	3	30120492280	100000000	20000000	1501.025	5	5	3	100	55.64	0.2	26.95902	40466.16					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=3	1	15087248996	100000000	20000000	749.3624	5	5	3	100	55.64	0.2	26.95902	20202.08	25,918.36	3,249.43	5,628.18	11,256.37	
	2	25006794432	100000000	20000000	1245.34	5	5	3	100	55.64	0.2	26.95902	33573.14					
	3	17889849589	100000000	20000000	889.4925	5	5	3	100	55.64	0.2	26.95902	23979.85					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=0	1	3905002743	100000000	20000000	190.2501	5	5	3	100	51.64	0.2	29.04725	5526.243	16,210.76	4,672.65	8,093.26	16,186.53	60.14
	2	17465479332	100000000	20000000	868.274	5	5	3	100	51.874	0.2	28.91622	25107.2					
	3	12547995575	100000000	20000000	622.3998	5	5	3	100	51.87	0.2	28.91845	17998.84					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=3	1	5482075689	100000000	20000000	269.1038	5	5	3	100	47.24	0.2	31.75275	8544.786	6,461.50	988.63	1,712.35	3,424.71	
	2	4014194692	100000000	20000000	195.7097	5	5	3	100	45.24	0.2	33.1565	6489.05					
	3	2666312067	100000000	20000000	128.3156	5	5	3	100	44.24	0.2	33.90597	4350.665					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=0	1	20753804824	100000000	20000000	1032.69	5	5	3	100	57.64	0.2	26.02359	26874.31	25,053.99	745.21	1,290.74	2,581.49	85.10
	2	19101642063	100000000	20000000	950.0821	5	5	3	100	58.74	0.2	25.53626	24261.55					
	3	18638551644	100000000	20000000	926.9276	5	5	3	100	57.87	0.2	25.92017	24026.12					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=3	1	3220075169	100000000	20000000	156.0038	5	5	3	100	59.24	0.2	25.32073	3950.129	3,733.72	195.90	339.31	678.62	
	2	3125027432	100000000	20000000	151.2514	5	5	3	100	56.77	0.2	26.42241	3996.425					
	3	2578707775	100000000	20000000	123.9354	5	5	3	100	57.12	0.2	26.2605	3254.606					

Appendix III-5: Raw data for quantification of Dodecane during for Phyto and Myco-remediation remediation of petroleum contaminated soils from Tibshelf

Treatments		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	Vt	M	Dm	Va	Multiplier	Conc (mg/Kg)	Mean	S.E	SD	2SD	% Decrease
UNCONTAMINATED SOIL @T=0	1	26247377	70589037		36.79	3	30	10	66.22	1	0.14	5.00	6.06	0.56	0.96	1.93	-7.14
	2	31838853	70589037		42.96	3	30	10	66.22	1	0.14	5.84					
	3	41773755	70589037		53.94	3	30	10	66.22	1	0.14	7.33					
UNCONTAMINATED SOIL @T=3	1	34524241	70589037		45.93	3	30	10	66.22	1	0.14	6.24	6.49	0.43	0.75	1.49	
	2	31063188	70589037		42.11	3	30	10	66.22	1	0.14	5.72					
	3	42914883	70589037		55.20	3	30	10	66.22	1	0.14	7.50					
CONT_SOIL_WITHOUT_AD@T=0	1	41322655	70589037		53.44	10	30	10	71.89	1	0.42	22.30	23.84	0.74	1.27	2.55	-0.20
	2	44596733	70589037		57.05	10	30	10	71.89	1	0.42	23.81					
	3	48093477	70589037		60.92	10	30	10	71.89	1	0.42	25.42					
CONT_SOIL_WITHOUT_AD@T=3	1	44966333	70589037		57.46	10	30	10	71.89	1	0.42	23.98	23.89	0.57	0.99	1.99	
	2	42044425	70589037		54.24	10	30	10	71.89	1	0.42	22.63					
	3	47316955	70589037		60.06	10	30	10	71.89	1	0.42	25.06					
CONT_SOIL+_AD@T=0	1	55183089	70589037		68.75	10	30	10	57.4	1	0.52	35.93	30.16	2.36	4.08	8.17	8.76
	2	40356811	70589037		52.37	10	30	10	57.4	1	0.52	27.37					
	3	40008022	70589037		51.99	10	30	10	57.4	1	0.52	27.17					
CONT_SOIL_+_AD@T=3	1	33372220	70589037		44.66	10	30	10	57.4	1	0.52	23.34	27.52	5.12	8.87	17.74	
	2	26481044	70589037		37.05	10	30	10	57.4	1	0.52	19.36					
	3	27451699	70589037		38.12	10	30	5	57.4	1	1.05	39.84					
SOIL TREATED WITH SUNFLOWER 1 @T=0	1	38061922	70589037		49.84	10	30	5	57.4	1	1.05	52.09	51.07	1.78	3.09	6.17	-59.02
	2	39917111	70589037		51.89	10	30	5	57.4	1	1.05	54.24					
	3	33551711	70589037		44.86	10	30	5	57.4	1	1.05	46.89					
SOIL TREATED WITH SUNFLOWER 1 @T=3	1	64959299	70589037		79.55	10	30	5	57.4	1	1.05	83.15	81.22	1.07	1.85	3.70	
	2	63776511	70589037		78.24	10	30	5	57.4	1	1.05	81.78					
	3	61123455	70589037		75.31	10	30	5	57.4	1	1.05	78.72					
SOIL TREATED WITH SUNFLOWER 2 @T=0	1	30996755	70589037		42.03	10	30	5	57.4	1	1.05	43.94	50.62	2.73	4.73	9.46	-66.88
	2	39599555	70589037		51.54	10	30	5	57.4	1	1.05	53.87					
	3	39767811	70589037		51.72	10	30	5	57.4	1	1.05	54.06					
SOIL TREATED WITH SUNFLOWER 2 @T=3	1	65678111	70589037		80.34	10	30	5	57.4	1	1.05	83.98	84.48	0.92	1.6	3.19	
	2	64679889	70589037		79.24	10	30	5	57.4	1	1.05	82.83					
	3	67981122	70589037		82.88	10	30	5	57.4	1	1.05	86.64					
SOIL TREATED WITH SUNFLOWER 3 @T=0	1	49174133	70589037		62.11	10	30	5	57.4	1	1.05	64.92	54.7	5.05	8.74	17.48	-112.67

Treatments		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	Vt	M	Dm	Va	Multiplier	Conc (mg/Kg)	Mean	S.E	SD	2SD	% Decrease
	2	3067796	705890	90537	41.68	10	30	5	57.4	1	1.05	43.57					
	3	4110098	705890	90537	53.19	10	30	5	57.4	1	1.05	55.60					
SOIL TREATED WITH SUNFLOWER 3 @T=3	1	12986865	705890	90537	151.24	10	30	5	57.4	1	1.05	158.09	116.33	21.71	37.6	75.20	
	2	10030045	705890	90537	118.58	10	30	5	57.4	1	1.05	123.95					
	3	10889924	705890	90537	128.08	10	30	10	57.4	1	0.52	66.94					
SOIL TREATED WITH PALMWINE 1 @T=0	1	3027276	705890	90537	41.23	10	30	10	57.4	1	0.52	21.55	18.99	1.11	1.93	3.86	-173.15
	2	2504590	705890	90537	35.46	10	30	10	57.4	1	0.52	18.53					
	3	2219961	705890	90537	32.32	10	30	10	57.4	1	0.52	16.89					
SOIL TREATED WITH PALMWINE 1 @T=3	1	8484265	705890	90537	101.51	10	30	10	57.4	1	0.52	53.05	51.87	0.69	1.19	2.39	
	2	8359616	705890	90537	100.13	10	30	10	57.4	1	0.52	52.33					
	3	7996711	705890	90537	96.12	10	30	10	57.4	1	0.52	50.24					
SOIL TREATED WITH PALMWINE 2 @T=0	1	3647428	705890	90537	48.08	10	30	10	57.4	1	0.52	25.13	32.66	5.53	9.57	19.15	-65.44
	2	3914576	705890	90537	51.03	10	30	10	57.4	1	0.52	26.67					
	3	7291647	705890	90537	88.33	10	30	10	57.4	1	0.52	46.17					
SOIL TREATED WITH PALMWINE 2 @T=3	1	7796167	705890	90537	93.91	10	30	10	57.4	1	0.52	49.08	54.03	2.74	4.75	9.50	
	2	8400011	705890	90537	100.58	10	30	10	57.4	1	0.52	52.57					
	3	9762954	705890	90537	115.63	10	30	10	57.4	1	0.52	60.43					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=0	1	3362773	705890	90537	44.94	10	30	10	57.4	1	0.52	23.49	23.12	0.45	0.78	1.56	1.75
	2	3111007	705890	90537	42.16	10	30	10	57.4	1	0.52	22.03					
	3	3422175	705890	90537	45.60	10	30	10	57.4	1	0.52	23.83					
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=3	1	3265694	705890	90537	43.87	10	30	10	57.4	1	0.52	22.93	22.71	0.26	0.45	0.90	
	2	3300167	705890	90537	44.25	10	30	10	57.4	1	0.52	23.13					
	3	3119585	705890	90537	42.25	10	30	10	57.4	1	0.52	22.08					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=0	1	3905052	705890	90537	50.93	10	30	10	57.4	1	0.52	26.62	27.15	0.45	0.78	1.55	-88.32
	2	4187620	705890	90537	54.05	10	30	10	57.4	1	0.52	28.25					
	3	3900467	705890	90537	50.88	10	30	10	57.4	1	0.52	26.59					
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=3	1	8374752	705890	90537	100.30	10	30	10	57.4	1	0.52	52.42	51.13	2.46	4.25	8.51	
	2	7158739	705890	90537	86.87	10	30	10	57.4	1	0.52	45.40					
	3	8922117	705890	90537	106.34	10	30	10	57.4	1	0.52	55.58					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=0	1	2923467	705890	90537	40.09	10	30	10	57.4	1	0.52	20.95	18.66	1.09	1.88	3.77	-216.22
	2	2533654	705890	90537	35.78	10	30	10	57.4	1	0.52	18.70					

Treatments		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	Vt	M	Dm	Va	Multiplier	Conc (mg/Kg)	Mean	S.E	SD	2SD	% Decrease
	3	2123879	705890	90537	31.26	10	30	10	57.4	1	0.52	16.34					
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=3	1	9703401	705890	90537	114.97	10	30	10	57.4	1	0.52	60.09	59.01	0.88	1.52	3.04	
	2	9144412	705890	90537	108.80	10	30	10	57.4	1	0.52	56.86					
	3	9703401	705890	90537	114.97	10	30	10	57.4	1	0.52	60.09					

Appendix III-6: Raw data for quantification of benzene-1,3-bis(1,1-dimethylethyl) during for Phyto and Myco-remediation remediation of petroleum contaminated soils from Tibshelf

Treatments		Peak Area (Y)	Intercept	SLOPE	X (Cs)	Vn	Vt	M	Dm	Va	multiplier	Conc (mg/Kg)	MEAN	S.E	SD	2SD	% DECREASE	PVALUES
UNCONTAMINATED SOIL @T=0	1	600640	10000000	275404	38.49	3	30	10	66.22	1	0.14	5.23	5.22	0.06	0.11	0.22	0.86	0.75
	2	295895	10000000	275404	37.38	3	30	10	66.22	1	0.14	5.08			0.12	0.25		
	3	844260	10000000	275404	39.38	3	30	10	66.22	1	0.14	5.35			0.13	0.26		
UNCONTAMINATED SOIL @T=3	1	321669	10000000	275404	37.48	3	30	10	66.22	1	0.14	5.09	5.18	0.09	0.15	0.30		
	2	911857	10000000	275404	39.62	3	30	10	66.22	1	0.14	5.38			0.17	0.33		
	3	235208	10000000	275404	37.16	3	30	10	66.22	1	0.14	5.05			0.00	0.00		
CONT_SOIL_WITHOUT_AD@T=0	1	29555068	10000000	275404	143.63	10	30	10	71.89	1	0.42	59.94	51.98	3.35	5.81	11.62	12.16	0.34
	2	228448247	10000000	275404	119.27	10	30	10	71.89	1	0.42	49.77			5.28	10.55		
	3	20506968	10000000	275404	110.77	10	30	10	71.89	1	0.42	46.23			5.30	10.61		
CONT_SOIL_WITHOUT_AD@T=3	1	14575858	10000000	275404	89.24	10	30	10	71.89	1	0.42	37.24	45.66	3.44	5.95	11.91		
	2	22904719	10000000	275404	119.48	10	30	10	71.89	1	0.42	49.86			2.82	5.65		
	3	22917017	10000000	275404	119.52	10	30	10	71.89	1	0.42	49.88			2.84	5.68		
CONT_SOIL +_AD@T=0	1	19432550	10000000	275404	106.87	10	30	10	57.4	1	0.52	55.86	50.20	4.65	8.06	16.13	17.61	0.57
	2	19478987	10000000	275404	107.04	10	30	10	57.4	1	0.52	55.94			8.43	16.87		
	3	10443389	10000000	275404	74.23	10	30	10	57.4	1	0.52	38.80			8.14	16.28		
CONT_SOIL_+_AD@T=3	1	9701413	10000000	275404	71.54	10	30	10	57.4	1	0.52	37.39	41.36	10.72	18.57	37.13		
	2	994356	10000000	275404	39.92	10	30	10	57.4	1	0.52	20.86			27.17	54.33		
	3	7340880	10000000	275404	62.97	10	30	5	57.4	1	1.05	65.82			9.80	19.60		
SOIL TREATED WITH SUNFLOWER 1 @T=0	1	12613721	10000000	275404	82.11	10	30	5	57.4	1	1.05	85.83	86.93	0.46	0.79	1.59	41.62	0.00
	2	13000211	10000000	275404	83.51	10	30	5	57.4	1	1.05	87.30			16.91	33.82		
	3	13097670	10000000	275404	83.87	10	30	5	57.4	1	1.05	87.67			17.47	34.94		
SOIL TREATED WITH SUNFLOWER 1 @T=3	1	3597663	10000000	275404	49.37	10	30	5	57.4	1	1.05	51.61	50.75	0.46	0.80	1.61		
	2	3089056	10000000	275404	47.53	10	30	5	57.4	1	1.05	49.68			22.75	45.50		
	3	3428331	10000000	275404	48.76	10	30	5	57.4	1	1.05	50.97			25.14	50.27		

Treatments		Peak Area (Y)	Intercept	SLOPE	X (Cs)	Vn	Vt	M	Dm	Va	multiplier	Conc (mg/Kg)	MEAN	S.E	SD	2SD	% DECREASE	PVALUES
SOIL TREATED WITH SUNFLOWER 2 @T=0	1	15970773	10000000	275404	94.30	10	30	5	57.4	1	1.05	98.57	105.70	2.93	5.07	10.14	40.84	0.00
	2	18608672	10000000	275404	103.88	10	30	5	57.4	1	1.05	108.58			21.28	42.55		
	3	18967112	10000000	275404	105.18	10	30	5	57.4	1	1.05	109.94			21.95	43.89		
SOIL TREATED WITH SUNFLOWER 2 @T=3	1	6900912	10000000	275404	61.37	10	30	5	57.4	1	1.05	64.15	62.53	0.80	1.38	2.76		
	2	6511238	10000000	275404	59.95	10	30	5	57.4	1	1.05	62.67			24.52	49.04		
	3	6012345	10000000	275404	58.14	10	30	5	57.4	1	1.05	60.77			24.99	49.98		
SOIL TREATED WITH SUNFLOWER 3 @T=0	1	19959675	10000000	275404	108.78	10	30	5	57.4	1	1.05	113.71	113.46	0.27	0.46	0.93	56.35	0.00
	2	19997896	10000000	275404	108.92	10	30	5	57.4	1	1.05	113.86			24.80	49.59		
	3	19722345	10000000	275404	107.92	10	30	5	57.4	1	1.05	112.81			25.39	50.78		
SOIL TREATED WITH SUNFLOWER 3 @T=3	1	6003663	10000000	275404	58.11	10	30	5	57.4	1	1.05	60.74	49.53	7.80	13.51	27.01		
	2	5100678	10000000	275404	54.83	10	30	5	57.4	1	1.05	57.31			12.19	24.39		
	3	6089234	10000000	275404	58.42	10	30	10	57.4	1	0.52	30.53			11.70	23.41		
SOIL TREATED WITH PALMWINE 1 @T=0	1	19179301	10000000	275404	105.95	10	30	10	57.4	1	0.52	55.38	55.12	0.20	0.34	0.68	50.76	0.00
	2	19163185	10000000	275404	105.89	10	30	10	57.4	1	0.52	55.34			11.86	23.72		
	3	18788996	10000000	275404	104.53	10	30	10	57.4	1	0.52	54.63			12.32	24.63		
SOIL TREATED WITH PALMWINE 1 @T=3	1	5722206	10000000	275404	57.09	10	30	10	57.4	1	0.52	29.84	27.14	1.32	2.29	4.59		
	2	4412603	10000000	275404	52.33	10	30	10	57.4	1	0.52	27.35			12.71	25.41		
	3	2766889	10000000	275404	46.36	10	30	10	57.4	1	0.52	24.23			14.14	28.28		
SOIL TREATED WITH PALMWINE 2 @T=0	1	17722585	10000000	275404	100.66	10	30	10	57.4	1	0.52	52.61	53.86	0.73	1.27	2.54	51.31	0.00
	2	19300787	10000000	275404	106.39	10	30	10	57.4	1	0.52	55.61			12.82	25.65		
	3	18122345	10000000	275404	102.11	10	30	10	57.4	1	0.52	53.37			12.46	24.93		
SOIL TREATED WITH PALMWINE 2 @T=3	1	4412603	10000000	275404	52.33	10	30	10	57.4	1	0.52	27.35	26.23	0.61	1.06	2.12		
	2	3977654	10000000	275404	50.75	10	30	10	57.4	1	0.52	26.53			2.61	5.21		
	3	3070968	10000000	275404	47.46	10	30	10	57.4	1	0.52	24.81			4.65	9.30		
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=0	1	6329769	10000000	275404	59.29	10	30	10	57.4	1	0.52	30.99	34.01	1.27	2.20	4.41	16.48	0.04
	2	9065875	10000000	275404	69.23	10	30	10	57.4	1	0.52	36.18			3.44	6.89		
	3	8372058	10000000	275404	66.71	10	30	10	57.4	1	0.52	34.87			3.57	7.13		
SOIL +MUSHROOM WITHOUT SUBSTRATES @T=3	1	4917125	10000000	275404	54.16	10	30	10	57.4	1	0.52	28.31	28.41	0.88	1.52	3.05		
	2	4011345	10000000	275404	50.88	10	30	10	57.4	1	0.52	26.59			11.09	22.17		
	3	5976211	10000000	275404	58.01	10	30	10	57.4	1	0.52	30.32			9.25	18.49		
SOIL +MUSHROOM + SUBS LAYERED ON TOP @T=0	1	17269360	10000000	275404	99.02	10	30	10	57.4	1	0.52	51.75	50.89	1.49	2.59	5.17	50.10	0.00
	2	14966178	10000000	275404	90.65	10	30	10	57.4	1	0.52	47.38			11.96	23.92		
	3	18212274	10000000	275404	102.44	10	30	10	57.4	1	0.52	53.54			13.33	26.66		
SOIL +MUSHROOM +	1	3517350	10000000	275404	49.08	10	30	10	57.4	1	0.52	25.65	25.39	0.21	0.36	0.73		

Treatments		Peak Area (Y)	Intercept	SLOPE	X (Cs)	Vn	Vt	M	Dm	Va	multiplier	Conc (mg/Kg)	MEAN	S.E	SD	2SD	% DECREASE	PVALUES
SUBS LAYERED ON TOP @T=3																		
	2	31096990	10000000	275404	47.60	10	30	10	57.4	1	0.52	24.88			14.13	28.26		
	3	35167580	10000000	275404	49.08	10	30	10	57.4	1	0.52	25.65			14.06	28.12		
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=0	1	19106615	10000000	275404	105.69	10	30	10	57.4	1	0.52	55.24	55.62	0.17	0.29	0.58	58.19	0.00
	2	19353771	10000000	275404	106.58	10	30	10	57.4	1	0.52	55.71			15.46	30.93		
	3	19472394	10000000	275404	107.02	10	30	10	57.4	1	0.52	55.93			15.56	31.12		
SOIL +MUSHROOM + MIXED & LAYERED ON TOP @T=3	1	21266647	10000000	275404	44.03	10	30	10	57.4	1	0.52	23.01	23.26	0.27	0.47	0.95		
	2	20355598	10000000	275404	43.70	10	30	10	57.4	1	0.52	22.84			0.54	1.08		
	3	26042230	10000000	275404	45.77	10	30	10	57.4	1	0.52	23.92			0.00	0.00		

Appendix III-7: Raw data for quantification of dodecane in crude oil samples

		Conc of crude oil (ppm)	Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	Vt	M	Dm	Va	Dil. factor	Multiplier	Conc (mg/Kg)
Nigerian crude oil 1	1	300	1044524	705890	90537	19.33	5	25	1.15	98.5	1	1	1.10	21.30
	2	700	1168614	705890	90537	20.70	5	25	1.15	98.5	1	1	1.10	22.81
	3	1000	1207103	705890	90537	21.13	5	25	1.15	98.5	1	1	1.10	23.28
	1	5000	2447651	705890	90537	34.83	5	25	1.15	98.5	1	1	1.10	38.38
	2	8000	3369740	705890	90537	45.02	5	25	1.15	98.5	1	1	1.10	49.61
	3			705890	90537	7.80	5	25	1.15	98.5	1	1	1.10	8.59
derby oil	1	100	1069142	705890	90537	19.61	5	25	1.15	98.5	1	1	1.10	21.60
	2	300	929243	705890	90537	18.06	5	25	1.15	98.5	1	1	1.10	19.90
	3	700	1034034	705890	90537	19.22	5	25	1.15	98.5	1	1	1.10	21.17
	1	1000	1038724	705890	90537	19.27	5	25	1.15	98.5	1	1	1.10	21.23
	2	5000	1542185	705890	90537	24.83	5	25	1.15	98.5	1	1	1.10	27.36
	3	8000	1870610	705890	90537	28.46	5	25	1.15	98.5	1	1	1.10	31.36
Nigerian crude oil 2	1			705890	90537	7.80	5	25	1.15	98.5	1	1	1.10	8.59
	2	500	223252	705890	90537	10.26	5	25	1.15	98.5	1	1	1.10	11.31
	3	1000	366938	705890	90537	11.85	5	25	1.15	98.5	1	1	1.10	13.05

Appendix III-8: Raw data for quantification of benzene-1,3-bis(1,1-dimethylethyl) in crude oil samples

			PEAK AREA(Y)	INTERCEPT	SLOPE	X (Cs)	Vn	Vt	M	Dm	Va	Dil Factor	multiplier	Conc (mg/Kg)
Nigerian crude oil 1	1	100	1635138	10000000	2754004	42.25	5	25	1.15	98.5	1	3	3.31	139.68
	2	300	1393960	10000000	2754004	41.37	5	25	1.15	98.5	1	1	1.10	45.59
	3	700	1825518	10000000	2754004	42.94	5	25	1.15	98.5	1	1	1.10	47.32
	4	1000	1480362	10000000	2754004	41.69	5	25	1.15	98.5	1	1	1.10	45.94
	5	5000	1662430	10000000	2754004	42.35	5	25	1.15	98.5	1	1	1.10	46.67
	6	8000	1458006	10000000	2754004	41.60	5	25	1.15	98.5	1	1	1.10	45.85
derby oil	1	100	1707320	10000000	2754004	42.51	5	25	1.15	98.5	1	1	1.10	46.85
	2	300	1515310	10000000	2754004	41.81	5	25	1.15	98.5	1	1	1.10	46.08
	3	700	1908410	10000000	2754004	43.24	5	25	1.15	98.5	1	1	1.10	47.65
	4	1000	1906697	10000000	2754004	43.23	5	25	1.15	98.5	1	1	1.10	47.65
	4	5000	1419393	10000000	2754004	41.46	5	25	1.15	98.5	1	1	1.10	45.70
	6	8000	1966073	10000000	2754004	43.45	5	25	1.15	98.5	1	1	1.10	47.88
Niger crude oil 2	1	500	223252	10000000	2754004	37.12	5	25	1.15	98.5	1	1	1.10	40.91
	2	1000	421722	10000000	2754004	37.84	5	25	1.15	98.5	1	1	1.10	41.70
	3	2500	499400	10000000	2754004	38.12	5	25	1.15	98.5	1	1	1.10	42.01
	4	5000	728815	10000000	2754004	38.96	5	25	1.15	98.5	1	1	1.10	42.93
	5	8000	827238	10000000	2754004	39.31	5	25	1.15	98.5	1	1	1.10	43.33

Appendix III-9: Raw for quantification of TPH in Nigerian soils using standardised crude oil standard

		Peak Area(Y)	Intercept	Slope	X (Cs)	Vn	M	f	100	Dm	p	multiplier	Conc/Dry Weight (mg/kg)	Mean(mg/kg dry soil)	S.E	SD	2*SD
GIO CONTROL	1	1.04E+09	400000000	1000000	636.59	5	5	1	100	68.22	0.2	7.33	4665.75	5118.40	211.22	365.84	731.68
	2	1.11E+09	400000000	1000000	709.88	5	5	1	100	69.22	0.2	7.22	5127.73				
	3	1.14E+09	400000000	1000000	736.59	5	5	1	100	66.22	0.2	7.55	5561.72				
OKWALE CONTAMINATED	1	1.54E+09	400000000	1000000	1136.84	3	5	1	100	55.64	0.33	3.27	3714.91	2004.04	698.50	1209.84	2419.68
	2	7.46E+08	400000000	1000000	346.50	3	5	1	100	55.64	0.33	3.27	1132.27				
	3	7.56E+08	400000000	1000000	356.50	3	5	1	100	55.64	0.33	3.27	1164.95				
BODO	1	1.87E+10	400000000	1000000	18256.07	5	5	2	100	55.64	0.2	17.97	328110.60	334281.71	4819.64	8347.86	16695.72
	2	1.87E+10	400000000	1000000	18286.16	5	5	2	100	55.64	0.2	17.97	328651.30				
	3	1.97E+10	400000000	1000000	19256.07	5	5	2	100	55.64	0.2	17.97	346083.20				
K-DERE	1	2.82E+09	400000000	1000000	2422.40	5	5	3	100	55.64	0.2	26.96	65305.40	62579.58	3386.37	5865.36	11730.73
	2	2.42E+09	400000000	1000000	2019.07	5	5	3	100	55.64	0.2	26.96	54432.04				
	3	2.92E+09	400000000	1000000	2522.40	5	5	3	100	55.64	0.2	26.96	68001.30				
K-DERE CONTROL	1	7.69E+08	400000000	1000000	369.43	5	5	1	100	55.64	0.2	8.99	3319.78	3006.42	293.31	508.03	1016.06
	2	6.55E+08	400000000	1000000	254.81	5	5	1	100	55.64	0.2	8.99	2289.84				
	3	7.79E+08	400000000	1000000	379.43	5	5	1	100	55.64	0.2	8.99	3409.65				
OGALE	1	2.63E+10	400000000	1000000	25907.53	5	5	1	100	55.64	0.2	8.99	232813.90	231852.56	4931.61	8541.79	17083.59
	2	2.50E+10	400000000	1000000	24586.60	5	5	1	100	55.64	0.2	8.99	220943.60				
	3	2.73E+10	400000000	1000000	26907.53	5	5	1	100	55.64	0.2	8.99	241800.20				
OGALE CONTROL	1	1.05E+09	400000000	1000000	654.35	5	5	1	100	55.64	0.2	8.99	5880.17	10228.18	3190.31	5525.78	11051.56
	2	2.41E+09	400000000	1000000	2005.89	5	5	1	100	55.64	0.2	8.99	18025.57				
	3	1.15E+09	400000000	1000000	754.35	5	5	1	100	55.64	0.2	8.99	6778.80				

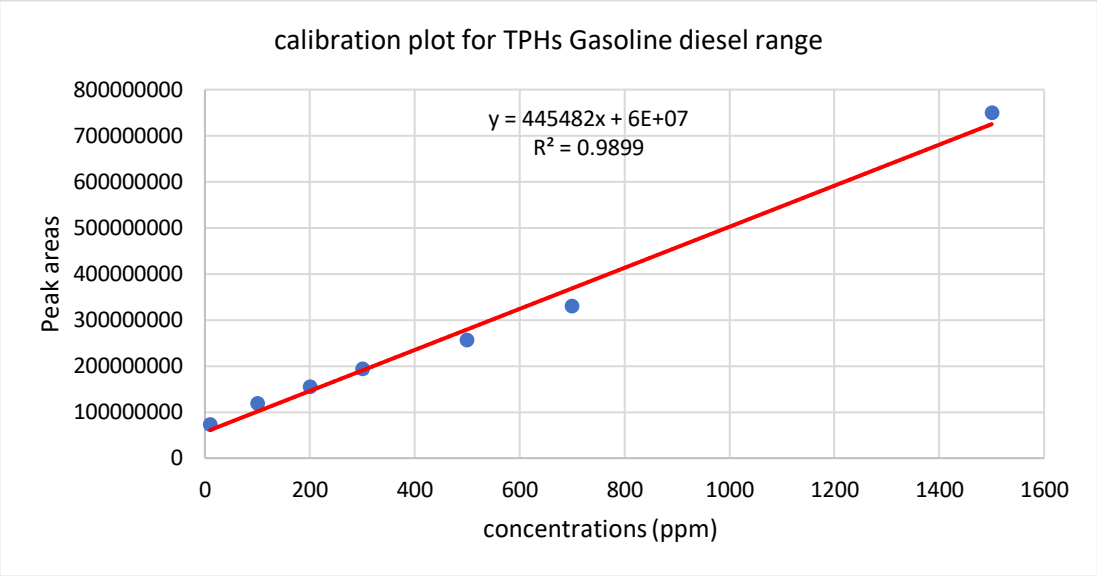
Appendix III-10: Raw for quantification of TPH in Brackenhurst soils

		Peak Area(Y)	TPH	Intercept	Slope	X (Cs)	Vn	M	F	100	Dm	p	multiplier	Conc/Dry Weight (mg/kg)	Mean(mg/kg dry soil)	S.E	SD	2SD
BRACK1	1	1145473841	TPH	60000000	4454482	2436.627835	5	5	1	100	68.22	0.2	7.329229	17858.6033	18837.8164	408.3208	707.2323	1414.465
	2	1224028145	TPH	60000000	4454482	2612.963363	5	5	1	100	68.22	0.2	7.329229	19151.00677				
	3	1245473841	TPH	60000000	4454482	2661.103795	5	5	1	100	68.22	0.2	7.329229	19503.83902				
BRACK2	1	325329427	TPH	60000000	4454482	595.6007807	5	5	1	100	68.22	0.2	7.329229	4365.294494	5961.23591	1182.197	2047.625	4095.25
	2	605911897	TPH	60000000	4454482	1225.440976	5	5	1	100	69.22	0.2	7.223346	8851.783995				
	3	335329427	TPH	60000000	4454482	618.0483768	5	5	1	100	66.22	0.2	7.550589	4666.629242				
BRACK3	1	857009710	TPH	60000000	4454482	1789.095205	5	5	1	100	67.22	0.2	7.438262	13307.75963	14245.9925	390.1658	675.7871	1351.574
	2	831473364	TPH	60000000	4454482	1731.772247	5	5	1	100	58.22	0.2	8.588114	14872.65756				
	3	867009710	TPH	60000000	4454482	1811.542801	5	5	1	100	62.22	0.2	8.036001	14557.56028				

Appendix IV

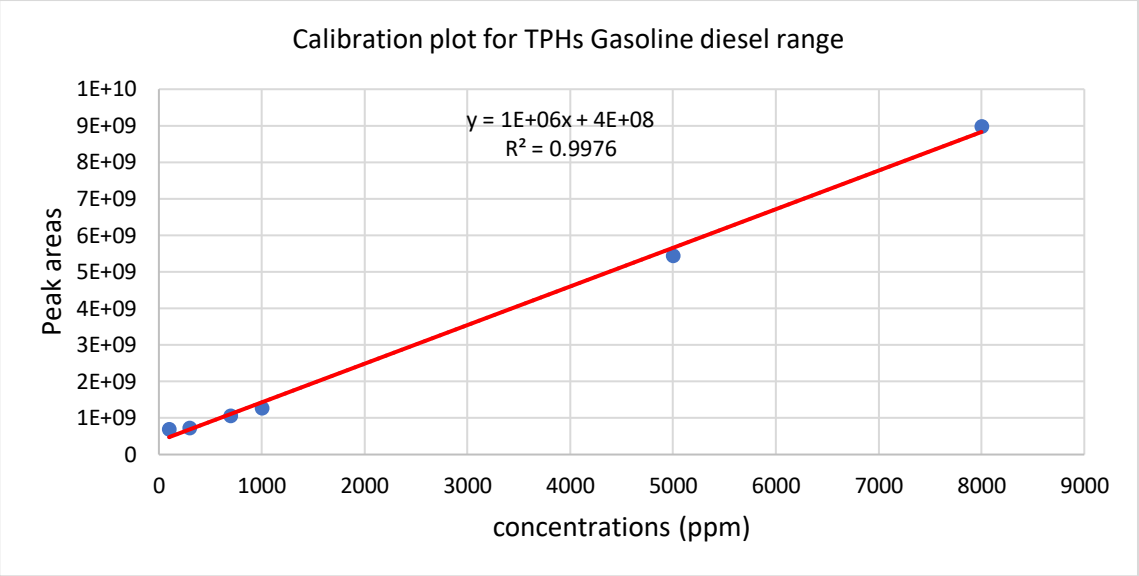
Data treatment, validity and reliability

AP IV-1: Evaluation of accuracy and precision in quantification of TPHs verification standards



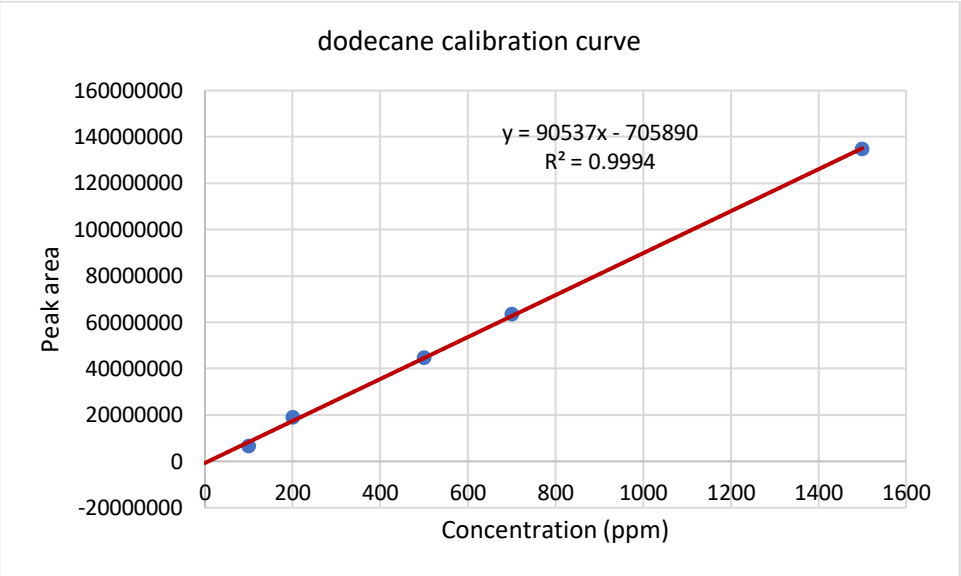
Actual Conc (ppm)	Samples	Peak areas	experimental values(ppm)	C	S	Accuracy	Mean experimental values(ppm	Standard deviation (2σ)	α
200	1	149227588	200.3	60000000	445482	100.2	207.7	10.59	1.000
200	2	154237571	211.5	60000000	445482	105.7			0.9400
200	3	154227524	211.5	60000000	445482	105.7			0.9400
300	1	193076940	298.7	60000000	445482	99.58	295.8	8.570	1.000
300	2	189076911	289.7	60000000	445482	96.58			0.9700
300	3	193176930	298.9	60000000	445482	99.65			1.000
700	1	329274932	604.4	60000000	445482	86.35	604.9	2.640	0.8600
700	2	330274988	606.7	60000000	445482	86.67			0.8700
700	3	328874938	603.5	60000000	445482	86.22			0.8600
1000	1	489495333	964.1	60000000	445482	96.41	965.0	4.230	0.9600
1000	2	491195311	967.9	60000000	445482	96.79			0.9700
1000	3	488995398	962.9	60000000	445482	96.30			0.9600
1500	1	748362580	1545	60000000	445482	103.0	1545	5.000	0.9700
1500	2	750062582	1549	60000000	445482	103.2			0.9700
1500	3	747362597	1542	60000000	445482	102.8			0.9700

AP IV-2 Evaluation of accuracy and precision in quantification of TPHs verification using standardised crude oil standard



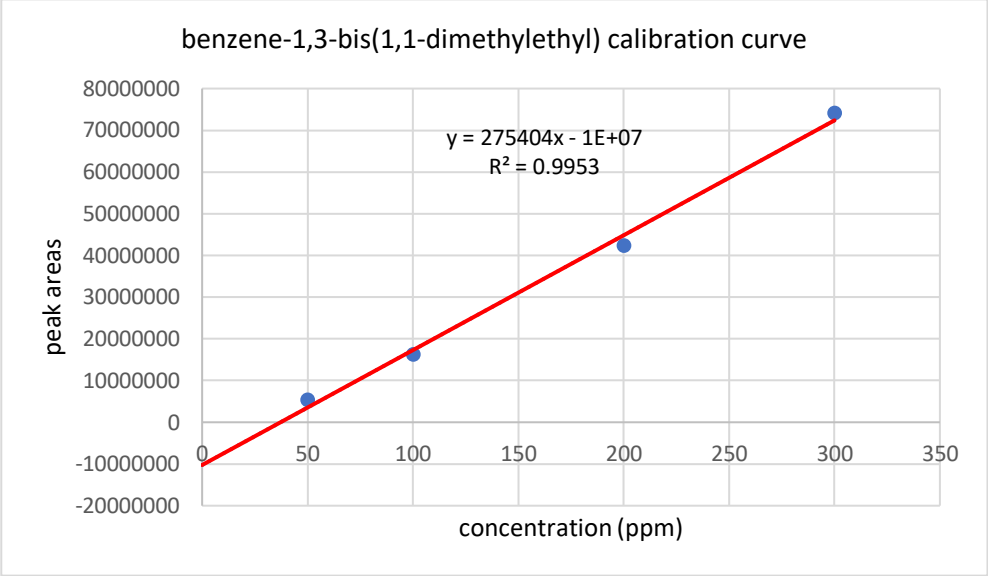
Actual Conc	Samples	Peak area	experimental values(ppm)	C	S	Mean experimental values (ppm)	Standard deviation (2σ)	Accuracy	α
300	1	729406127	329.4	400000000	1000000	326.1	8.647	91.35	0.9135
	2	728896100	328.8	400000000	1000000				
	3	719990025	319.9	400000000	1000000				
700	1	1069196960	669.1	400000000	1000000	670.1	2.898	95.60	0.9560
	2	1072122351	672.1	400000000	1000000				
	3	1068919691	668.9	400000000	1000000				
1000	1	1279255500	879.2	400000000	1000000	849.5	71.43	87.93	0.8793
	2	1269925558	869.9	400000000	1000000				
	3	1199255511	799.2	400000000	1000000				
5000	1	5441879780	5041	400000000	1000000	5061	56.21	99.16	0.9916
	2	5501179785	5101	400000000	1000000				
	3	5441234787	5041	400000000	1000000				
8000	1	8983204349	8583	400000000	1000000	8529	82.29	92.71	0.9271
	2	8883204355	8483	400000000	1000000				
	3	8922320435	8522	400000000	1000000				

AP IV-3: Estimation of accuracy and precision in quantification dodecane verification standards



Actual Conc	Samples	Peak area	experimental values(ppm)	C	S	Mean experimental values (ppm)	Standard deviation (2σ)	Accuracy	α
1500	S1	134729637	1480	705890	90537	1507	77.40	99.52	0.9952
1500	S2	134729637	1480	705890	90537	1245		98.69	0.9869
1500	S3	142111637	1561	705890	90537	982.2		95.88	0.9588
700	S1	63447466	693.0	705890	90537	691.8	1.800	99.00	0.9900
700	S2	63333463	691.7	705890	90537	622.7		98.82	0.9882
700	S3	63249341	690.8	705890	90537	555.5		98.69	0.9869
500	S1	44675852	485.7	705890	90537	483.4	13.00	97.13	0.9713
500	S2	45075822	490.1	705890	90537	388.4		98.02	0.9802
500	S3	43675800	474.6	705890	90537	291.2		94.92	0.9492
200	S1	18873049	200.7	705890	90537	196.2	9.400	99.67	0.9967
200	S2	18663049	198.3	705890	90537	194.0		99.17	0.9917
200	S3	17883049	189.7	705890	90537	189.7		94.86	0.9486

AP IV-4: Estimation of accuracy and precision in quantification dodecane verification standards



Actual Conc (ppm)	Samples	Peak area	experimental values(ppm)	C	S	Mean experimental values (ppm)	Standard deviation (2σ)	Accuracy	α
300	1	74078406	305.2	10000000	275404	305.2	0.3856	98.24	0.9824
	2	73978445	304.9	10000000	275404				
	3	74100486	305.3	10000000	275404				
200	1	42328208	190.0	10000000	275404	190.0	5.9689	95.00	0.9500
	2	44328211	197.2	10000000	275404				
	3	43128200	192.9	10000000	275404				
100	1	16322677	95.58	10000000	275404	95.58	1.9371	95.58	0.9558
	2	16877226	97.59	10000000	275404				
	3	16300698	95.50	10000000	275404				
50	1	5310099	55.59	10000000	275404	55.59	0.3440	88.82	0.8882
	2	5411100	55.96	10000000	275404				
	3	5311122	55.60	10000000	275404				

AP IV-5: Repeatability Reliability

Anova: Two-Factor without replication for repeatability reliability for uncontaminated soils @T=0 analysed at 3 different times of 30, 60 & 90 days

ANOVA: Two-Factor Without Replication for repeatability reliability for uncontaminated soils @T=0 analysed at 3 different times of 30, 60 & 90 days						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	3	299970.3	99990.11	3.63E+08		
Row 2	3	345733.3	115244.4	6.77E+08		
Row 3	3	315225	105075	1.73E+08		
Column 1	3	374871.5	124957.2	54574068		
Column 2	3	314475.5	104825.2	3.65E+08		
Column 3	3	271581.7	90527.22	77551173		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3.62E+08	2	1.81E+08	1.14553	0.404271	6.944272
Columns	1.8E+09	2	8.98E+08	5.681151	0.067797	6.944272
Error	6.32E+08	4	1.58E+08			
Total	2.79E+09	8				

*p values is larger than alpha of 0.05 hence the null hypothesis is upheld (meaning differences in analysis is not significant)
*F values is smaller than F critical hence the null hypothesis is upheld (meaning differences in analysis is not significant)

Anova: Two-Factor Without Replication for repeatability reliability for *P. ostreatus* petroleum contaminated soils from Tibshelf @T=0 analysed at 3 different times of 30, 60 & 90 days

Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	3	1127123	375707.6	5.6E+08		
Row 2	3	1134861	378286.9	7.69E+08		
Row 3	3	1114384	371461.5	7.92E+08		
Column 1	3	1215671	405223.5	17625327		
Column 2	3	1093361	364453.6	1201943		
Column 3	3	1067336	355778.7	46538523		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	71268226	2	35634113	2.397047	0.206889	6.944272
Columns	4.18E+09	2	2.09E+09	140.6656	0.000197	6.944272
Error	59463360	4	14865840			
Total	4.31E+09	8				

AP IV-6: Anova: Two-factor without replication for sampling variability for petroleum contaminated soils from Tibshelf @T=0 analysed from 3 different composite sampling preparations

Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	3	1112447	370815.8	1.99E+09		
Row 2	3	1096491	365497.1	1.89E+09		
Row 3	3	1109707	369902.2	1.88E+09		
Column 1	3	967803.1	322601	13085585		
Column 2	3	1228791	409597	24924122		
Column 3	3	1122051	374017	0.00099		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	48528507.46	2	24264254	3.530514	0.130776	6.944272
Columns	11477846905	2	5.74E+09	835.0287	5.71E-06	6.944272
Error	27490905.1	4	6872726			
Total	115538663118	8				

*p values is larger than alpha of 0.05 hence the null hypothesis is upheld (meaning differences in analysis is not significant) *F values is smaller than F critical hence the null hypothesis is upheld (meaning differences in analysis is not significant)

AP IV-7: Extraction validity

Surrogates recoveries from extractions

Surrogates 1

Spiked Conc(Ppm)	Samples	Recoveries values(ppm)	Mean experimental values (ppm)	Standard deviation (2σ)	Recoveries %
1500	S1	1480	1507	77.40	99.52
1500	S2	1480			98.69
1500	S3	1561			95.88

Surrogates 2

Spiked Conc(Ppm)	Samples	Recoveries values(ppm)	Mean experimental values (ppm)	Standard deviation (2σ)	Recoveries %
300	1	305.2	305.2	0.3856	101.67
300	2	304.9			101.63
300	3	305.3			101,77

Appendix V

AP 5.1a: Phytoremediation potentials of grasses on crude oil contaminated soil

Plant: Grasses Botanical name	Common name	Remediation efficiency	Type/duration of the study	Sources
Panicum virgatum,	Switch grass	3-4 times degradation of petroleum hydrocarbons was achieved compared to controls.	➢ Glasshouse-150 days ➢ Conventional petroleum contaminated soils mixed with pollutant-free soil to reduce the oil content to 5,000 mg·kg^{-1}	Wang *et al.* (2008)
Festuca arundinacea	Tall Fescue.	3-4 times degradation of petroleum hydrocarbons was achieved to controls; Alkylated two-ring naphthalenes were successfully degraded; Increased degradation of the larger three-ring alkylated phenanthrenes-anthracenes and dibenzothiophenes was also observed compared to controls	➢ Glasshouse-150 days ➢ Conventional petroleum contaminated soils mixed with pollutant-free soil to reduce the oil content to 5,000 mg·kg^{-1} ➢ Field scale 1 year[b] ➢ Initial onsite (TPH) concentration was 9,175 mg/kg[b].	Wang *et al.* (2008) White *et al.* (2006)[b]
Eleusine indica	Indian goose grass, yard-grass, goose grass	3-4 times degradation of petroleum hydrocarbons was achieved compared to controls.	Same as above	Wang *et al.* (2008)[a] Merlk *et al.* (2005)[b]
Brachiaria brizantha,	palisade grass	Up to 50% degradation of saturates fraction observed and a approximately 15% higher reduction in aromatics than controls	➢ Glasshouse-190 days ➢ Soil artificially contaminated with 5% (w/w) of a heavy crude oil	Merlk *et al.* (2005)
Cyperus aggregatus,	Flat sedge	Up to 70% degradation of saturates fraction was observed	Same as above	Merlk *et al.* (2005)
Lolium multiflorum	Ryegrass	Alkylated two-ring naphthalene were successfully degraded; Increased degradation of the larger three-ring alkylated phenanthrenes-anthracenes and dibenzothiophenes was also observed compared to controls.	➢ Field scale 1 year ➢ Initial onsite (TPH) concentration was 9,175 mg/kg.	White *et al.* (2006)
Cynodon dactylon	Bermuda grass	Alkylated two-ring naphthalenes were successfully degraded; Increased degradation of the larger three-ring alkylated phenanthrenes-anthracenes and dibenzothiophenes was also observed compared to controls	Same as above	White *et al.* (2006)
Lolium perenne	*winter ryegrass*	Up to 73.4% removal rate of TPH was obtained with organic fertilizer; Up t0 78.9% removal rate was obtained when mixed with volcanic eruption after eight months	➢ Glasshouse-8 months ➢ Soil artificially contaminated with 2.8% (w/w) of a crude oil	Mâsu *et al.* (2013).
Spartina patens	Salt meadow cord grass	*S. patens* tolerance limits of crude oil was at about 320 mg oil g−1 dry sediment; enhanced oil degradation in the sediment; concentrations of residual total petroleum	➢ Glasshouse-8 months ➢ Soil artificially contaminated with oil at concentrations of 0, 40, 80, 160, 320, 640 and 800 mg SLC oil g−1 (w/w) of a crude oil	Lin and Mendelssohn (2008)
Cyperus rotundus	Nut grasses	up to 50.01 % decrease in crude oil content of soil was obtained	➢ Glasshouse-180 days	Basumatary *et al.* (2012).

Plant: Grasses Botanical name	**Common name**	**Remediation efficiency**	**Type/duration of the study**	**Sources**
			➢ Soil artificially contaminated with concentrations (2.05, 4.08, 6.1, 8.15 and 10.2%) of crude oil	
Sorghum bicolor	sorghum or great millet	TPH decreased by 52%-64% in 90 days.	➢ Glasshouse-90 days ➢ Conventionally petroleum contaminated soils	Asiabadi *et al.* (2014)
Hordeum vulgare	Barley	TPH decreased by 52%-64% in 90 days.	Same as above	Asiabadi *et al.* (2014)
Axonopus compressus	carpet grass	up 59% reduction in hydrocarbon was achieved	➢ Insitu field treatment -3 months ➢ Conventionally petroleum contaminated soils	Efe & Okpali (2012).
Leptochloa fusca	Sprangle top	Up to 51% removal of crude from soil was achieved	➢ Insitu field treatment -3 months	Fatima *et al.* (2018)
Brachiaria mutica	Angola grass, buffalo grass,	61% removal of crude from soil was achieved; Maximum oil degradation (80%) was achieved with B. mutica plants augmented with the endophytes	Same as above	Fatima *et al.* (2018)
Triticum repens	couch grass	Up to 94% for 0.5 % crude oil contamination; 80% and at 1.0% crude oil contamination	➢ Glasshouse-45days ➢ Soils artificially contaminated with crude oils at 0.5 and 1%	Saadawi *et al.* (2015)
Linum *Usitatissumum*	*common flax*	TPHs reduced by 18,500 mg kg^{-1}, compared with the control treatment.	➢ Glasshouse-45days ➢ Conventionally crude oil-contaminated soils with initial concentration of TPH-50,516 mg kg^{-1} of soil	Shirdam *et al.* (2008)
Zea mays	Corn	Over 70% reduction of TPH was achieved	➢ Glasshouse-4 months ➢ Conventionally crude oil contaminated - soils	Zand *et al.* (2010).
Panicum maximum	Guinea grass	Up to (80%) TPH removal when combined with bacteria and (77%) for the grass alone	➢ Glasshouse-112 days ➢ Conventionally crude oil contaminated soils	Contreras-Ramos *et al.* (2017)

AP 5.1b: Phytoremediation potentials of ornamental plants on crude oil contaminated soil

Plant: Ornamentals Botanical name	Common name	Remediation efficiency	Type/duration of study	Sources
Mirabilis Jalapa	The marvel of Peru or four o'clock flower	up to 41.61–63.20% TPH remove, compare to 19.75–37.92% by natural attenuation was	➢ Greenhouse- 127-days ➢ Conventional crude oil contaminated soils diluted with uncontaminated soils to a concentration (Woil/Wsoil) of 0.5% (5000 mg/kg), 1.0% (10,000 mg/kg), and 2.0% (20,000 mg/kg)	Peng *et al.* (2009)
Crotalaria pallida Aiton	Assamese	Maximum dissipation of TPH was 78.66 %, at 60,000 ppm concentration of crude oil in soil.	➢ Greenhouse- 6 months ➢ Artificially contaminated soil created by mixing 3 kg of rice field soil mixed with crude oil	Baruah *et al.* (2016).
Dracaena reflexa	Song of India	Up to 90 % and 98 % of TPHs removal in soil amended with SC, at 2.5 % and 1 % fuel, respectively.	➢ Greenhouse- 270 days. ➢ Artificially contaminated soil created by mixing diesel fuel with soil to achieve concentrations levels of 1, 2.5 and 5.0 wt%) and soy cake (SC) and potato skin (PS)]	Dadrasnia and Agamuthu (2013)
Melampodium Paludosum	blackfoot daisies, Show star grass	TPH reduced from 75.46mg/g to 49.822 mg/g in two weeks after plant stabilization to 30.07 mg/g after 16 weeks.	➢ Greenhouse- 16 weeks ➢ motor oil contaminated laterite soil.	Izinyon and Seghosime (2013)
Echinacea purpurea	Purple corn flower	Up to 45.5% of TPH removal at 1% crude oil contamination	➢ Greenhouse- 90 days ➢ Artificially contaminated soil with concentrations of crude oil 0, 0.5%, 5000, 10000, and 20000 mg kg^{-1}	Heidari, *et al.* (2018)
Gaillardia aristate	blanket flower	Removal rates of TPH composition including saturated hydrocarbon, aromatic hydrocarbon, asphaltene, and polar compound reached 39.41%, higher than that in the control (only 6.90%).	➢ Greenhouse- 30 days ➢ Conventional contaminated soil obtained	Liu *et al.* (2012)
Matricaria chamomilla	*Chamomile*	Average removal percentage At the different concentrations of 0.25, 0.5, 1, 2 and 4 for light petroleum in soil was respectively 47.93, 38.73, 33.75, 25.3 and 9.4 for light crude oil; and 51.79, 45.44, 39.76, 33.91 and 9.88 for heavy crude oil.	➢ Greenhouse- 30 days ➢ Artificially contaminated soil prepared with 0.25, 0.5, 1, 2 and 4 of both light and heavy crude oil	Shirazia *et al.* (2015)
Mimosa	*bashful or shrinking; called sensitive plant, sleepy plant, or shy plant*	Up to 45–49% TPH decreased	➢ Greenhouse- 180 days ➢ Artificially contaminated soil prepared by addition of 2% of Crude oil to obtained an initial concentration of 12,916 mg diesel/kg soil	Ikeura *et al.* (2016)
Zinnia elegans,	youth-and-age, common zinnia or elegant zinnia,	T Up to 45–49% TPH decreased	Same as above	Ikeura *et al.* (2016)

Plant: Ornamentals Botanical name	Common name	Remediation efficiency	Type/duration of study	Sources
Gazania linearis	treasure flower	Up to 45–49% TPH decreased	Same as above	Ikeura *et al.* (2016)
Ipomoea quamoclit	cypress vine, cypress vine	Up to 45–49% TPH decreased	Same as above	Ikeura *et al.* (2016)
Bassia scoparia	burningbush, ragweed, summer cypress	TPH removal of 31.2 ± 1.15 to 57.7 ± 1.29%	➢ Greenhouse- 5 months ➢ Conventionally petroleum-contaminated arid land sandy soil	Moubasher *et al.* (2015)
Iris pseudacorus	yellow flag	Plants tolerance level ≤ 40,000 mg · kg^{-1} of TPHs; TPH removal rate at concentrations of 10,000 mg · kg^{-1}, 20,000 mg · kg^{-1} and 40,000 mg · kg^{-1} was 42.1%, 33.1% 31.2%	➢ Greenhouse- 5 months ➢ Conventionally contaminated soils	Wang *et al.* (2016)
Impatiens balsamina	garden balsam, garden	Up to 65.03%. TPH removal	➢ Greenhouse- 5 months ➢ Conventionally contaminated soils	Cai *et al.* (2010)
Canna generalis	Canna lilies	Removal efficiency was up to 80% of BTEX in the root and rhizome zone	➢ Greenhouse- 21 days ➢ Artificially contaminated soil prepared by addition of BTEX	Boonsaner *et al.* (2011).

AP 5.1c: Phytoremediation potentials of ferns on crude oil contaminated soil

Plant: Ferns Botanical name	Common name	Remediation efficiency	Type/duration of study	Sources
Azolla filiculoides	Water fern	Tolerance level of A. filiculoides plants to crude oil ranges between 0.1% and 0.2%. Degradation rate of total aliphatic and aromatic (phenathrene) hydrocarbons at 0.05% - 0.2% oil concentrations, was 94% - 73% and 81% - 77%, respectively	➢ Glasshouse- 15 days ➢ nitrogen-free Hoagland nutrient solution containing 0.005%, 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% crude oil	Kösesakal *et al.* (2016)

AP 5.1d: Phytoremediation potentials of legumes on crude oil contaminated soil

Plant: legumes Botanical name	**Common name**	**Remediation efficiency**	**Type/duration of study**	**Sources**
Glycine max	Soybean	Crude oil loss was enhanced in soil with 25g crude oil in the presence of G. max, but were not significant at 50g and 75g treatments.	➢ Glasshouse-110 days ➢ Soils artificially contaminated with crude oils at 25g, 50g, and 75g crude oil mixed with 4000g of soil.	Njoku *et al.* (2009).
Calapoigonium mucunoides	Wild ground nut	Highest TPH uptake (10^{-2} mg kg^{-1}) were obtained at 2.5% contamination as 1.08, 0.52 and 0.21; 1.01, 0.51 and 0.11 in the roots and shoots	➢ Glasshouse-110 days ➢ Soils artificially contaminated with crude oils at 0.0, 2.5, 5.0, 10.0 and 20.0% (v/v)crude oil mixed with 3Kg of soil.	Adewole and Bulu, (2012).
Ricinus communis	Castor bean or Castor oil plant	Up to 77% for 0.5 %; & 76% and at 1.0% crude oil contamination	➢ Glasshouse-45days ➢ Soils artificially contaminated with crude oils at 0.5 and 1%	Saadawi *et al.* (2015)
Stylosanthes capitate	Side beak;	Lower oil concentration than non-vegetated soil	➢ Glasshouse - 180days ➢ Soils artificially contaminated with heavy crude oils at 5%	Merkl *et al.* (2005)
Centrosema brasilianum,	Centrosema	Lower oil concentration than non-vegetated soil	Same as above	Merkl *et al.* (2005)
Aeschynomene americana	American joint vetch	TPH levels significantly lower in vegetated fertilized plots than in non-vegetated non-fertilized plots	➢ Field -6 months ➢ Soils contaminated with 3% by weight weathered crude oil	White *et al.* (2002)
Vicia faba	Broad bean	Up to 30% degradation of TPHs was observed	➢ Field -60 days ➢ Soils artificially contaminated 2.2-2.3% crude petroleum oil	Diab (2008).
Arachis hypogea	Peanut	Up to 55.6% to 99.8% crude reduction	➢ Glasshouse - 180days ➢ Soils artificially contaminated with (0.1% 1%, 5%, 10% and 15%) of crude oil	Ibrahim *et al.* (2013)
Cajanus cajan	Pigeon pea	Up to 55.6% to 99.8% crude reduction	Same as above	Ibrahim *et al.* (2013)
Lablab purpureus	Hyacinth bean, Lablab-bean; Egyptian kidney bean, Indian bean	Up to 55.6% to 99.8% crude reduction	Same as above	Ibrahim *et al.* (2013)

AP 5.1e: Phytoremediation potentials of trees on crude oil contaminated soil

Plant: trees Botanical name	Common name	Remediation efficiency	Type/duration of study	Sources
Prosopis cineraria	Jammi, Shami, Khejri Tree	Saturated hydrocarbons reduced by 43.0 %; aromatics reduced by 25.7 %	➢ Insitu field scale- 90 days ➢ Conventionally contaminated desert soil with 2.5-2.6% crude petroleum oil	Mathur *et al.* (2010)
Acacia Senegal	Gum acacia, Gum arabic tree, Sudan gum and Sudan gum arabic	Saturated hydrocarbons reduced by 35.2%; aromatics reduced by 7.9 %	Same as above	Mathur *et al.* (2010)
Acacia nilotica	Gum arabic tree, babul, thorn Mimosa, Egyptian acacia or Thorny acacia	Saturated hydrocarbons reduced by 31.2%; aromatics reduced by 4.1 %	Same as above	Mathur *et al.* (2010)
Populus nigra	Poplar tree	up to 81%, 90%, 67%, 78%, and 82%, decrease of toluene, ethylbenzene, xylene, and gasoline range organics respectively in lower soil.	➢ Insitu field scale- one year ➢ Conventionally contaminated soil	El-Gendy *et al.* (2009)
Dracaena reflexa	Song of India	Up to 90% and 99% degradation of oil was recorded in soil contaminated with 2.5% and 1% oil with soy cake amendment, while with 52% and 62% was observed in unamended soil	2.5% and 1% diesel fuel-contaminated soil amended individually with 5% organic wastes (tea leaf, soy cake and potato skin) for a period of 270 days	Dadrasnia and Agamuthu (2013)[b].
Podocarpus polystachyus	Sea teak	84% and 91% oil loss of TPH was observed with organic wastes in 2.5% and 1% oil, respectively.	2 Same as above	Dadrasnia and Agamuthu (2013)[b].

AP 5.1f: Phytoremediation potentials of shrubs on crude oil contaminated soil

Plant: shrubs Botanical name	Common name	Remediation efficiency	Type/duration of study	Sources
Malva parviflora	Cheeseweed, Cheeseweed mallow	Up to 89% of TPH	➢ Glasshouse-45days ➢ Soils artificially contaminated with crude oils at 0.5 and 1%	Saadawi *et al.* (2015)
Ricinus communis	Castor bean	Up to 76 % degradation of TPH	Same as above	Saadawi *et al. (*2015)
Euonymus alatus	Winged spindle, Burning bush	up to 87.63% removal of TPH With addition of peat fertilizer	➢ Glasshouse-90 days ➢ Conventional crude oil contaminated soils	Shirdam *et al.* (2009).
Linum usitatissimum	Flax, Common flax Linseed	up to 65.29% removal of TPH With addition of peat fertilizer	Same as above	Shirdam *et al.* (2009).
Desmodium incanum	Creeping beggarweed	Up to 66.9% of TPH was degraded	➢ Glasshouse-90 days Soils artificially contaminated with crude oil/soil	Kitamura and Maranho (2016).

Appendix VI: Abstract of published articles

Appendix V.1: Abstract of publised article 1 (Full article can be found at https://pubs.rsc.org/en/content/articlepdf/2019/em/c9em00101h)

CRITICAL REVIEW

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Mycoremediation of petroleum contaminated soils: progress, prospects and perspectives

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Mycoremediation, an aspect of bioremediation, has been investigated for some decades. However, there seems to be little progress on its commercial application to petroleum-contaminated soils despite some promising outcomes. In this review, mycoremediation is examined to identify development, limitations and perspectives for its optimal utilization on petroleum-contaminated soils. Mycoremediation agents and substrates that have been used for the treatment of petroleum contaminated soils have been identified, application methods discussed, recent advances highlighted and limitations for its applications accentuated. Possible solutions to the challenges in applying mycoremediation to petroleum-contaminated soils have also been discussed. From this review, we conclude that for optimal utilization of mycoremediation of petroleum-contaminated soils, ideal environmental, edaphic and climatic factors of a typical contaminated site must be incorporated into the approach from first principles. Development of application procedures that can easily translate laboratory results to field applications is also required.

Environmental significance

Mycoremediation can provide sustainable solutions for environmental remediation. Although this technique has been explored for years, it has not been maximised for practical solutions to petroleum-contaminated soils. It is therefore imperative to carry out an appraisal of mycoremediation of petroleum-contaminated soils and evaluate its progress, limitations and perspectives. This work presents direct insights into the process of mycoremediation and specifically deals with its application to petroleum-contaminated soils, which makes it distinct from other reviews. Thus, it elucidates different types of fungi and substrates used, application techniques, and mechanisms of the technique, as well as identifying progress, prospects and perspective of mycoremediation towards solving the problem of petroleum-contaminated soils. The findings offer some practical awareness on why mycoremediation has not been maximised for the treatment of petroleum-contaminated soils. The outcome also provides useful insight for greater future understanding and improvement of the technique towards solving the problem of petroleum-contaminated soils.

Appendix V.2: Abstract of publised article 2 (Fuly article can be found at https://reader.elsevier.com/reader/sd/pii/S0045653519321204?token=EAA9651900532EC88E94BD4B598662D36E4D81271FCE09E0042A39DC67808BA291A7740FC531791ECB1DD6DECF07D2AA)

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Investigating the potential of sunflower species, fermented palm wine and *Pleurotus ostreatus* for treatment of petroleum-contaminated soil

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HIGHLIGHTS

- TPHs levels in soils was as much as 339 g/kg dry weight.
- Remediation efficiency of sunflower species was up to 69%, fermented palm wine-70% and *P. ostreatus*-85%.
- Remediation efficiency of sunflower species was proportional to biomass.
- Remediation efficiency of P. ostreatus depends on substrates type and method of application.
- Available nitrate and electrical conductivity are useful indicators of TPHs concentration in soil.

GRAPHICAL ABSTRACT

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ABSTRACT

Phyto- and myco-remediation have been identified as sustainable options for treatment of petroleum-contaminated soils. To appraise the benefits thereof, the potentials of 3 sunflower species, 2 palm wine types and *P. ostreatus* to treat petroleum-contaminated soils was investigated. The study involved sampling of petroleum-contaminated soils and treatment with the phyto- and myco-remediation agents for a period of 90-days. Agents used for the remediation were 3 species of sunflowers (*Helianthus annus*-pacino gold, *Helianthus sunsation* & *Helianthus annus*-sunny dwarf), fermented palm wine (from 2 species of palm trees -*Elaeis guineensis* & *Raffia africana*), and oyster mushroom (*Pleurotus ostreatus*). The study further investigated variation in remediation efficiency among the sunflower and palm wine species, as well as different substrates and conditions for optimal application of *P. ostreatus*. The results obtained revealed up to 340 g/kg dry weight of Total petroleum hydrocarbons (TPHs) in the soils, with remediation outcomes of up to 69% by the sunflower- *Helianthus* annus (Pacino gold), 70% by fermented palm wine, and 85% by *P. ostreatus*. While the remediation efficiency of sunflower species was proportional to biomass, there was no significant difference in remediation efficiency of the palm wines. It was also found that substrates type and method of application has a significant impact on the remediation efficiency of *P. ostreatus*. The study further revealed available nitrate and electrical conductivity as possible useful indicators of TPHs concentration and remediation progress in soils.

Apendix VII

The Research Team

The Candidate

Udeme Dickson obtained his BSc in Pure Chemistry at the University of Uyo, and an MSc in Industrial Chemistry at University of Benin, both in Nigeria, before proceeding for a PhD in Analytical Chemistry and Environmental Sciences at Nottingham Trent University, UK. His expertise cuts across areas of Physical Chemistry especially Kinetics, Analytical Chemistry, Petroleum Analysis, and Environmental monitoring and remediation. Currently, his research focus is on development of analytical, phyto-and myco-remediation techniques to manage petroleum-contaminated soils. He is currently engaged as a researcher and associate lecturer in Analytical Chemistry and Forensics at Nottingham Trent University, United Kingdom.

Supervisory Team

Dr Nicholas Ray completed a BSc (Hons) in Zoology (specialising in aquatic ecology) at the University of Reading, an MRes in Science of the Environment (Lancaster University) and a CASE PhD studentship at Manchester Metropolitan University with CEH Lancaster in "Air pollution and impacts on soil and vegetation nutrient cycling". Nicholas joined Nottingham Trent University in 2005, as a lecturer in ecology and environmental science related disciplines. Formerly the Course Leader for BSc (Hons) Environmental Science, he currently serves as the Doctoral School Standards and Quality Manager. **Dr Nicholas Ray** served as the current Director of studies during the research.

Dr Marcello Di Bonito

Trained as a physical scientist at the University of Naples, Italy where he graduated as an Earth scientist, Dr Marcello specialises in Environmental Geochemistry, GIS and Spatial Analysis with various periods of study abroad (University of Granada, Spain; BGS, UK). He has also worked as a soil scientist for the Italian National Research Council, NERC Isotope Geoscience Laboratory and the Environment Agency. He studied his PhD in England, where he undertook a joint project between the University of Nottingham and the British Geological Survey. He currently works as Senior lecturer at Nottingham Trent University, UK. **Dr Marcello Di Bonito** previously served as the Director of studies at the inception of the research.

Dr Michael Coffey obtained a PhD in Marine Chemistry, University of East Anglia. He has been a Self-employed technical author (wastewater treatment) (2006 - 2013), Environmental consultant (IPPC permit drafting) (2005 - 2006), Marine chemist and analyst (Scottish Environment Protection Agency) (1993 - 1996). His research interest includes development of novel pedagogical resources in chemical science, chemical analysis of ancient human skeletal remains, fate and behaviour of illicit drugs in sewage, and fate and behaviour of pollutants in the aquatic environment (metals, nutrients, endocrine-disrupting chemicals). He currently works as a Senior lecturer in Analytical Chemistry at Nottingham Trent University, UK.

Professor Rob Mortimer is currently the Dean, School of Animal Rural and Environmental Sciences, Nottingham Trent University, UK. Before joining NTU, he was Head of the School of Earth and Environment, University of Leeds. He joined Leeds as a postdoc in 1994 and worked his way up to Lecturer (2000), Senior Lecturer (2006), and then Professor of Environmental Geochemistry (2012). Professor Mortimer holds a BSc (first-class honours) in Mining Geology from Imperial College, and a PhD in Iron Biogeochemistry from the University of Reading. He is an environmental geochemist interested in biogeochemical processes in sediments and their impact on water quality.

Professor Barry Smith obtained a BSc in Applied Chemistry from Nottingham Trent University in 1984 and a PhD in Hydrogeology and Chemistry from the University of Bath in 1987. He currently works as the Principal consultant and Director of IntelliScience Ltd, Nottingham, United Kingdom. Barry has over thirty years of experience in undertaking practical applied science in a wide range of organisations ranging from manufacturing industry, water companies, mining, applied and theoretical academic institutions and universities, international bodies (EU, WHO, IAEA, UNIDA) and foreign governments. His expertise includes Inception and management of international multidisciplinary studies, hydrochemistry, radiochemistry, geomedicine, environmental chemistry, soil science, analytical chemistry, GIS and numerical modelling.