SYNERGISTIC POTENTIALS OF RHIZOSPHERE FUNGI, SPENT MUSHROOM COMPOST AND MEGATHYRSUS MAXIMUS (JACQ) SIMONS & JACOBS IN REMEDIATION OF POLLUTED SOILS

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ABSTRACT

Soil pollution is a major anthropogenic problem which affects global food security and human health. Many bioremediation mechanisms have been used to ameliorate the problem, but there is dearth of knowledge on the use of combined plant and fungal actions. This study was therefore designed to investigate the synergistic potentials of Rhizosphere Fungi (RF), Spent Mushroom Compost (SMC) and *Megathyrsus maximus* (Mm) in remediation of hydrocarbon and pesticide polluted soils.

Four sites [Hydrocarbon Polluted Sites, HPS_1 (Ugboroko), HPS_2 (Ibadan) and Pesticides Polluted Sites, PPS_1 and PPS_2 (Akure)], were investigated. On each site, 5 kg of soil were collected from 20 points and composited while rhizosphere soils were also obtained from tussocks of 10 grasses (100 g each). The RF were isolated from rhizosphere soils and identified using morphological and molecular techniques. Frequently occurring RF were selected for 90-day synergistic remediation of the composited soil with SMC and Mm; pure RF cultures were mixed with SMC (1:10 w/w) and applied to sterilised composited soil (5 kg) at synergistic concentrations of 10%, 20%, 30% and 40% with two controls (Mm only and SMC+RF). Polycyclic Aromatic Hydrocarbons (PAHs) and pesticides [2, 2-dichlorovinyldimethylphosphate (dichlorvos) and γ-hexachlorocyclohexane (lindane)] in soil samples were analysed using GC/MS and values obtained were used to calculate Degradation Efficiencies (DE), degradation rates (K_1) and half-life ($t_{1/2}$). Responses of root and leaf structures were studied using standard anatomical methods. Genes encoding the production degrading enzymes in RF were determined by PCR while their expressions were assessed by RT-PCR amplifications. Enzyme activities were monitored using standard procedures and data obtained were subjected to ANOVA at $\alpha_{0.05}$.

Out of the 200 RF identified and characterised, 16 strains were most-frequently occurring (≥50%). In HPS_1 and HPS_2, synergistic treatment at 40% best reduced total PAHs of 851.61 and 805.00 mg/kg by 95.28 DE and 93.58 DE, respectively while Mm and SMC+RF gave 45.28 and 50.23 DE, respectively. In PPS_1 and PPS_2, 40% synergistic treatment reduced dichlorvos (30.00 mg/kg) and lindane (45.00 mg/kg) by 82.70 and 88.67 DE, respectively

as compared to Mm only (62.20 DE) and SMC+RF (72.58 DE). The same treatment also gave the best K_1 and $t_{1/2}$ of 3.05 and 0.23 day^{-1} respectively in HPS_1, 2.73 and 0.252 day^{-1} in HPS_2. The 40% synergistic treatment gave the best K_1 and $t_{1/2}$ for dichlorvos (1.75 and 0.40 day^{-1}) and lindane (2.18 and 0.32 day^{-1}) in PPS_1 and PPS_2, respectively. The root and shoot structures showed increased cell size and reduced intercellular air-spaces as the synergistic treatment concentration increased. Degrading genes *lig2-lig6*, *mnp*, *lcc*, *opd-A, mpd*, *afk2-afk4* and *caM* were over-expressed in all RF while *lig1, cbh, trpC* and *cam* were moderately-expressed in some RF. The pollutants significantly increased the enzyme activities in most RF. Activities (U/mL) of Laccase (168.00±7.49), Manganese peroxidase (111.00±13.01), Lignin peroxidase (105.00±1.00) and Catalase (87.00±3.00) were obtained in most RF.

Rhizosphere fungal strains acting in synergy with spent mushroom compost and *Megathyrsus maximus* had better degradation effect on Polycyclic Aromatic Hydrocarbons, dichlorvos and lindane in polluted soils than when applied alone.

Keywords: Fungal remediation, Hydrocarbon polluted soil, Pesticide degradation, *Megathyrsus maximus*

Word count: 500

CERTIFICATION

This is to certify that this work was carried out by Mr Michael Dare ASEMOLOYE in the Department of Botany, Faculty of Science University of Ibadan, Ibadan.

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DEDICATION

I dedicate this research project to my mother, Mrs Beatrice Abiye Asemoloye and to the loving memory of my late father, High Chief E. O. Asemoloye for their words of inspiration and encouragement in pursuit of excellence which still linger on.

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CHAPTER ONE

INTRODUCTION

1.1 The study Background

Soil pollution is an intrinsic part of the environmental problems caused by excessive anthropogenic activities and presently threatening the agricultural activities, food/nutrition security and global health (Anaisell *et al*., 2014). In an already polluted environment, adequate remediation becomes imminent and several clean-up approaches such as physical, chemical, mechanical, or photo-degradation have been suggested (Ruttens *et al*., 2011). However, many of these approaches are usually expensive as they often require the application of modern technologies and may not be applicable in small scale. Many others if not well implemented, may lead to formation of other toxic compounds that can also be toxic to the environment (Cerniglia and Sutherand, 2010).

'Bioremediation' which is one of the suggested approaches has been gaining public interests and acceptance for soil remediation due to its applicability; environmental friendly and cost effectiveness approach (Cerniglia and Sutherand, 2010). It utilizes the capability of the living organisms, their part or products in soil remediation. Bioremediation technology is promising for soil remediation but it still has some challenges. It may not be easy to establish the growth of biological entity in heavily polluted soil and some of its mechanism may as well require more time to complete (Ruttens *et al*., 2011).

These challenges triggered interests in developing a concept of bioremediation which relies on remediation of pollutanted soils at less cost, less time/period and as well applicable in large or small scale (Ruttens *et al*., 2011). It was thought that the possibilities of combining different bioremediation mechanisms could enhance the remediation of polluted soils; this is possible using the rhizhosphere (soil area around the root) as a medium through which microorganisms and plant can exchange beneficial materials for enhance their survival,

growth and performances in such environment (Vaajasari *et al*., 2002) hence, the reason for investigating the concept of synergistic rhizosphere mechanism.

The synergistic rhizosphere mechanism focuses on the exploitation of rhizosphere microorganisms and plants' root and many results have proven that it is effective and speeds up soil remediation. This technology has also been reported to be cheap and applicable both in large and small scale (Beazely *et al*., 2012). The biostimulatory efficacy of Spent Mushroom Compost (SMC) was employed in some other studies such as Marco-Urrea *et al*. (2009) to fuel the synergistic fungi-plant mechanism and affirmed that it enhanced the co-action of rhizosphere fungi and plant's root.

1.2 Clean-Technology as a Tool for Soil Remediation

Generally, 'Clean-Technology' is often used to describe biotechnological method of removing different contaminants from the human environment (Beazely *et al*., 2012). The clean-up of polluted soils especially those generated from industrial processes has been a great concern (Adedokun, 2015). Environmental pollution is an unavoidable evil, it still percist today despite several regulations imposed by many government agencies like the Environmntal Protection Agencies for the disposal of wastes (Beazely *et al*., 2012). It was mentioned in the reports of Tang *et al*. (2009) as well as Alloway and Trevors (2013) that above 30 % of the total area of land covered by human beings is already contaminated due to excessive anthropogenic activities and this is increasing yearly with concurrent health threat (Valentin *et al*., 2013). Therefore, clean-up strategies for the removal of environmental contaminations have been the focus of many researchers for decades.

Fortunately, human beings have been able to develop different clean technologies for removing contaminants from their environments (Tang *et al*, 2009; Dadrasnia and Agamuthu, 2013a, b; Arezoo and Salma, 2015); These strategies involve biological, physical, chemical and thermal processes (Rubilar *et al*., 2011; Dadrasnia and Agamutu, 2013a): Presently, there are five (5) most adopted clean-up techniques, viz:

i. **Chemical technique**: This involves reactions of chemical compounds for soil decontamination. Here, toxic pollutants are converted to none or less toxic compounds which are easily biodegraded.
ii. **Physical technique**: Here, the contaminated part of the soil is excavated or disposed.
iii. **Verification or Solidification methods**: These methods employ both the physical and chemical means to reduce/remediate soil contaminants.
iv. **Thermal technique**: This method entails the use of heat treatment for soil clean-up examples of this treatment are incineration, volatilization or pyrolysis process of soil treatment. This technique destroys contaminants effectively but also destroys the biological components in the soil and also burns out the organic nutrients in the soil.
v. **Biological technique**: This method is also known as 'Bioremediation technology', it involves biological mechanisms using the living organisms either through their whole body, body parts or products for degradation, mineralization or removal of soil contaminant.

1.3 Bioremediation Technology

Bioremediation of polluted soil can be in-situ (right in its original location) and/or ex-situ (away from its original location) remediation of polluted soils. Many reports like those of Scow and Hicks (2005) and Adams *et al*. (2015) had earliar demonstrated different bioremediatory mechanisms using different organisms. Bioremediation is however a broad term in 'Biotechnology', with several mechanisms based on the type of the organism involved, for example: Bacterial remediation (the use of bacteria), Mycoremediation (the use of fungi), Phytoremediation (plants), Phycoremediation (algae), Vermiremediation (earthworms), Zooremediation (animals) (Vidalli *et al*., 2014).

Furthermore, both insitu and exsitu bioremediation mechanisms can both be enhanced by 'Bio-stimulation' which involves the process of nutrient enhancement for the growth of bio degraders in the soil and 'Bioaugmentation' which involves the addition/introduction of biological component into the polluted sites for faster remediation time (Vcao *et al*., 2015;

Gentry *et al*., 2015). Recently it was suggested that some genetically modified organisms (GMOs) that have been fortified with special abilities capable of degrading specific contaminant can be employed as bioaugmentation agent to enhance soil bioremediation (Vidalli, 2014 and Adams *et al*., 2014; 2015).

1.4 Synergistic Bioremediation

Synergistic bioremediation is the combination of two or more different biological mechanisms to hasten/speed-up the remediation time (Vidalli, 2014; Huang *et al*. 2004a). Bioremediation is a less energy-demanding method, it is environmentally friendly, low cost and effective (Segura and Ramos, 2013), it has higher public recognition and acceptance over other conventional clean-up methods. However, bioremediation processes are still being improved upon through the combination of multiple bioremediation techniques (Huang *et al*. 2004a); microorganisms for example can be employed to enhance the plant's remediation through the rhizosphere supplementation (Liu, 2007; Guo *et al*., 2014). Also, the microorganisms which are readily available in the root rhizospheres can enhance plant's response through different mutualistic actions with the plant's roots (Weyens *et al*., 2009).

The microorganisms benefit from the absorption of essential exudates such as sucrose, malic acid, and some other essential substances from the root while the plant in turn benefits from the microorganisms by obsorbing nutrients released by microorganisms in soils. Novel example is the rhizobium and the legumes; Esalante-Espinosa *et al*. (2005) suggested that the combin mechanism between plant and microorganisms can enhance soil remediation. The synergistic rhizosphere interaction can be greatly influenced by the following factors (Segura and Ramos, 2013):

i. Prolific microbial growth in the rhizosphere
ii. Repression or induction by enzymes in catabolic reactions
iii. Co-oxidation of contaminants by combine actions of the organisms
iv. Changes in bioavailability as the organisms co-exist
v. Selection of biodegraders and chemotaxis of competent strains

The synergistic bioremediatory approach have been reported to reduce bioaccumulation in plant tissues as the contaminants are more degraded and mineralised through combined actions and mineralisation (Huang *et al*., 2004b). It has been established that the symbiotic association between the plant and microorganism promotes effective degradation/clean-up of different soil pollutants (Guo *et al*., 2014): This mechanism, if well implemented can also enhance effective and ecological stability according to Segura *et al*. (2009) and Zhang *et al*. (2010), they identified many factors which are to be considered while setting up such mechanisms:

i. Physical and chemical characteristics of the soil such as the soil's Cation Exchange Capacity (CEC), nutrients, surface properties/profile, the pH, the texture and bulk density which usually influence the water, plant and soil relationships.
ii. Chemical characteristics of the contaminant in the soil, its toxicity or bioavailability.
iii. Biology of the plant, species/type and its biomass characteristics.
iv. The degradative potential of the associated microorganism.
v. The microbial diversity, their degradative potentials, genetic make-up and their abundance in the soil.

1.5 Importance of Guinea Grass in Phytoremediation

Guinea grass (*Megathyrsus maximus*) is also known as Buffalograss, Green panic grass, or Tanganyika grass. It is formally called *Panicum maximus* Jacq having different varieties such as the common *P. coloratum*, *P. hirsutissimum*, *P. publiglume*, and *P. trichogume* (Tang *et al*., 2015). In 2003, the subgeneric name *Megathyrsus maximus* (Jacq.) B. K. Simon and W. L. Jacobs was anoted to Guinea grass (Simon and Jacobs, 2003) and classified as follows:

Family:	Plantae
Phylum:	Angiosperms
Sub Phylum:	Monocot
Unranked:	Commelinids
Order:	Poales
Family :	Poaceae
Genus:	Megathyrsus
Species:	*M. maximus*

Binomial name: *Megathyrsus maximus* (Jacq.) B. K. Simon and W. L. Jacobs

It is a pantropical and perennial large bunch grass with varying morphological characteristics from species to species for example; the height generally ranges from 0.5-3.5 m with diameter of 5-10 mm.

M. maximus is of two major types viz; the tall/medium tussock type and short tussock type but generally they have fibrous root system with creeping rhizome, erect culms, nodes hirsutate with blade shaped leaves (Cook *et al*., 2005). It is a native plant to Africa, Yemen and Palestine and commonly useful across the tropics as pasture, sillage and hay. It is fast in growing and commonly used as protein supplement for livestock. It is commonly seen in open grasslands, shady places but could tolerate varying atmospheric conditions. It is a fire harzard plant and could tolerate light frost, low pH, heavy metal saturations, or drought (Kim *et al*., 2007) and these characteristics have made it a good phytoremediating agent.

Phytoremediation also known as Green technology is a technology that involves the use of green plants for soil remediation often regarded as an environmentally safe and cost effective technology (Kim *et al*., 2007; Tang *et al*., 2015). *M. maximus* root can ramify the polluted soils than many taproot systems as it covers more area of the top soil where contaminats. It is commonly regarded as a phytoextractor for heavy-metals. Orgi *et al*. (2015) reported *M. maximus* in removal of crude oil hydrocarbons in tropical soil

1.6 Status of Soil Fertility and Conservation in Nigeria

Nigeria as a country is blessed with different natural resources such as fertile arable land and fresh water (Kadiri and Mustapha, 2010). Nigeria was estimated to encompass approximately 61 million cultivable hectares of land mass with about 280 km^3/yr of the total renewable water resource (FAO, 1995). However, Nigeria has been found to exceed the carrying capacity as its population tends to exceed the cultivated land resources (FAO, 1995). Nigeria as country cultivates at low level (FAO, 1987) presently, the Nigerian population is above 150 million people as at the last 2006 population census and this as estimated by Musa (2001) may increase up to 220 million by the year 2025. This exponential increase in the Nigerian population necessitates increasing the food production from 0.26 million hectares as recorded in 1995 to 0.90 million/hectares by the year 2025. To achieve this, Nigeria needs to expand her cultivated land area by 350 % (Musa, 2001) and it is also expected that Nigeria should correspond the food production rate with its population growth rate to avoid problems associated with severe competition, inflation, starvation, malnutrition etc.

Unfortunately, ecological problems such as deforestation, inappropriate mechanical tillage, oil spillage, wrong use of pesticides and the likes have subjected the Nigerian farm-land to devastating situations such as erosion and soil pollution (Fetters, 2008). Many of the Nigeria lands are been over-utilised and degraded (Jonathan *et al.,* 2011; Ahaneku, 2010). However, adequate agricultural practices under a reduced polluted environment are needed with optimal and proper management of land resources. Improved management of natural resources such as land and water is a prerequisite for sustainable agriculture needed for the

production food adequate for the increasing Nigeria population, economic growth, food/nutrition security and poverty reduction (Ahaneku, 2010).

1.7 Status and the Impact of Hydrocarbon Pollution in Nigeria

Many factors such as; oil spills due to equipment failures, blowouts and leakages from weak or corroded pipeline networks, faulty operational systems and pipeline vandalization by the local militant groups and leakages from oil bunkering by people who hack into the pipeline for selfish desires, fire and explosion hazards are responsible for pollution in Nigeria (Stephen *et al*., 2011). It was reported in 2006 that the contamination caused by petroleum in the Niger Delta, Nigeria has caused the environment to be listed as one of the most impacted ecosystem in the world (Stephen *et al*., 2011).

However, oil spills have created huge awareness on associated ecological effects over some few decades (Benka-Coker and Ekundayo, 1995, 1999; Daniel-Kalio and Solomon, 2002). Oil spill contamination of agricultural soils in Niger Delta for example was first reported in 1971 (Odu, 1972; Snowden and Ekweozor, 1987), they reported that this led to increase in incidences of acute or chronic toxicities in the host community (UNEP, 2011); Many other incidences of spills where reported by Blumer (1972) and Odu (1972) till UNEP (2011) and Nyeche *etal*. (2015). They all reported different hydrocarbon contaminations in Nigerian soil from different locations across the country and the regions of Nigeria where oil exploratory/exploitation activities have resulted in spillages which always result to environmental degradation.

About 3 million petroleum barrels were reported to have been spilled in six thousand (6, 000) cases around year 1976 to 2000 and only about five hundred and fifty thousand barrels (550, 000) were recovered (Edoho, 2008). More than 700 people died in 1998 when a sabotaged petroleum pipeline exploded around Jesse town; more than 200 people died in another huge gasoline explosion in mid-2000 around Adeje village, in Niger Delta in 1999, other examples includes Shell Bomu II blowout due to oil leakages in 1970, SAFRAP (now known as Elf) blowout in 1972, and both the Texaco Faniwa and Agip Oyakama blowouts in 1980 (Daniel-Kalio and Solomon, 2002). More than 500 cases of fuel pipelines sabotage

were reported by Nigeria State Petroleum Company and only five percent (5 %) were actually recovered while others escaped into the environment without adequate remediation process, this in turn increased the resultant environmental health risks in the host communities. These incidences are increasing yearly in Nigeria which can be also associated to community conflicts, insincere and instability in the Nigerian political process (Blumer, 1972; Nwilo and Badejo, 2006).

Oil contamination in soil can repel oxygen proliferation and cause death of soil fauna due to asphyxiation (Osuji and Onojake, 2004). The United Nation (UN) team were shocked at some of their findings from a study conducted in Ejama Ebubu, River State Nigeria; they found out that many soils that were claimed to have been cleaned are still containing some concentration of crude oil of over 40 years' pollution. Researchers also found refined oil floating with about 8cm deep into the underground water in wells located at Nisisoken Ogale, Eleme community around the pipeline of the Nigerian National Petroleum Company (Edoho, 2008). It has also been reported that natural remediation of oil spill contamination may require more than forty (40) years to remediate, a studied oil contaminated site in Niger Delta area found more than five metres deep contamination (Ukaegbu-Obi and Mbakwem-Aniebo, 2014), most of oil contaminated sites that were claimed to be remediated are still containing oil and different hydrocarbon contaminants, many oil firms sill dumpcontaminants in unlined pits and many crude oil contaminated water are several times dangerous than the level allowed safe standards (Ukaegbu-Obi and Mbakwem-Aniebo, 2015). Considering the importance of crude oil to Nigerian economy with the damage done over time, there is need for implementation of many approaches with adequate management strategies that would the less expensive and effective for soil remediation and restoration of already polluted sites.

1.8 Statement of the Problem

There are about 3 million polluted sites across the 15 EU countries (which includes Nigeria) and it was estimated that 250, 000 of these sites still require immediate clean-up (Kim *et al*., 2007). Uncontrolled discharge or spillage of petrochemicals, heavy metals, chemicals such as pesticides and other contaminants into the environment are increasing and these are due to the actions of different kinds of automobiles, machineries, engines, vehicles and industries. These are causes of soil pollution which threatens the production and the food security; many pollutants are well implicated to be carcinogenic, mutagenic and neurotoxic posing several health hazards and threatening the food security (Adams *et al*., 2015). They contaminate natural environment damaging/altering the natural ecosystem, many of them are too difficult or require prolonged time for natural remediation due to their complex structures (Agarry *et al*., 2013).

Accumulation of several pollutants in soils can be transferred across the food web of the ecosystem (Biogenic precursors) and may eventually get into human (Rodriguez-Trigo *et al*., 2010). The organism that accumulates and transfers these toxic hydrocarbon compounds from a source (a carrier host) in to another organism that feeds on them; for example, they can be transferred from biogenic precursors such as plants to human or another organism that ingest them (Rodriguez-Trigo *et al*., 2010). This interaction in most cases is as a result of accumulation of pollutants in living cells or tissues, plants for example are primary producer in any ecosystem, or phytoplankton and other primary producers in the aquatic habitat may bioaccumulate these pollutant and transfer them into secondary producers then to tertiary producers and so on (Joner *et al*., 2012).

1.9 Justification

Already polluted sites are needed to be cleaned in order to again become useful. The most important factor for soil remediation in this industrialised/mechanised age with increasing generation of wastes year by year is development of a clean technology that emphasizes on conversion of waste generation into useful form under an environmentally safe condition, of which biological methodology plays crucial role.

Many plants in grass family (Poaceae) have been well documented for phytoremediation due to their root structure structure and functions. Guinea grass (*Megathyrsus maximus*) for example was regarded as hyper-accumulator by many researchers (Mager, 2002; Waraporn *et al.,* 2013) due to its importance in phytoremediation. It is a perennial grass with ranging morphology (usually grows to about 0.5-3.5 m tall with 5-10 mm girth diameter), it is fast growing, tufted, has short creeping root rhizome with erect culms (Waraporn *et al*. 2013) and has morphological and physiological abilities suitable for phytoremediation (Valencia and Mager 2003).

However, plant's growth and establishment in heavily polluted soil need to be improved upon during phytoremediation with adequate biomass production and stress tolerance. To enhance the use of plants in soil remediation, strategic combination of plants and fungal community holds promising landmark in the remediation of contaminated soils and waterways. This unfortunately, has not been well developed and adopted for large-scale remediation.

This research presents a synergistic bioremediation mechanism which focuses on the combination of spent mushroom compost, the rhizosphere fungi and the plant root for speedy and effective soil clean-up. It is an easy to set-up mechanism; it is cheap, fast and achievable in small or large scale.

1.10 Aim and Research Objectives

1.10.1 Aim

The aim of this study was to investigate the synergistic potentials of Spent Mushroom Compost (SMC), rhizosphere fungi and the *Megathyrsus maximus* for effective and faster soil remediation.

1.10.2 Objectives

The objectives of this study are to:

i. isolate and characterize dominant fungal strains available in the rhizosphere of grasses growing on selected polluted soils using morphological and molecular methods.
ii. study the presence and expression of some hydrocarbon and pesticide degrading genes in the selected rhizosphere fungi using RT-PCR method.
iii. study the activities (U/mL) of the enzymes produced by the fungi in aliquots.
iv. analyse the Degradation Efficiency (DE) of fungi-SMC-plant treatments on the hydrocarbon and pesticide concentrations in polluted soils as well as their degradation constant (K_1) and half life ($t_{1/2}$).
v. analyse the effects of fungi-SMC-plant synergistic treatments on physical and chemical characteristic of selected polluted soil samples in relation to the growth, physiological and anatomical responses of the test plant (*M. maximus*).

CHAPTER TWO
LITERATURE REVIEW

2.1 Soil Pollution and Pollutants

Generally, pollution can be defined as the uncontrolled discharge/disposal of waste substances either in form of solid, liquid, gases and or any other forms such as heat, sound and/or radioactive materials in human environment especially in quantity that affects the human health while 'Pollutants' are the particular substances which are discharged (Shammas *et al*., 2015). The pollutants that are human made or produced by human activities for examples are often refer to as xenobiotic (Holliger *et al*., 2012).

The common soil contaminants include heavy metals, hydrocarbons, pesticides and some other chemicals which are grouped as the Persistent Organic Pollutants some of which are Polybrominated Diphenylethers (PBDEs), Polynuclear Aromatic Hydrocarbons (PAHs), Polychlorinated Naptalenes (PCNs) and Perfluorooctanoic Acids (PFOAs) (Lohman *et al.,* 2007). Several of such pollutants usually cause an alteration in the natural soil environment, some hydrocarbon pollution in soil for example are typically caused by industrial activity of refinery and the use of petrochemicals, excessive use of some agricultural chemical such as inorganic fertilizers and improper disposal of hydrocarbon wastes.

The effect of soil contamination has raised concerns globally; several health hazards have been associated with concurrent soil pollution due to their interaction with direct contact in form of vapour, water supply or secondary contaminations during crop cultivation, animal husbandry or food production (Olawoyin *et al*., 2012). Execution of soil treatment in polluted sites with adequate clean-up technologies is required and most of the suggested methods require much time to complete, expensive, can only be applied by expertise and can not be implemented in small scale remediation. To develop an adequate soil remediation mechanism, adequate knowledge of Chemistry, Physics, Biology, Geology, Computer modelling skills and Biochemistry is required and combining these expertise

may result in development of adequate clean up technology that would be environment friendly, cost effective and applicable in small scale (Li *et al*., 2014).

2.1.1 Hydrocarbon pollution

Hydrocarbons are the chemical compounds which contain only hydrogen and carbon elements, they usually exist in form of solids or waxes like naphthalene, in form of liquid like benzene, in form of gasses like methane or in form of polymers such as polythene (Luo *et al*., 2013). Decomposing organic matter for example contains abundant carbon and hydrogen elements and forms the parent material from which the crude oil is formed; this makes hydrocarbon the major component of crude oil (from 70 to 98 %) depending on the nature and the extraction methods (Luo *et al*., 2013). Crude oil however may also contain some other elements such as nitrogen, oxygen and sulphur.

Based on the structural characteristics, hydrocarbons are usually grouped in the classes viz:

i. Aliphatic hydrocarbon
ii. Aromatic hydrocarbon

The aliphatic hydrocarbon includes the group of straight chains compounds which are further grouped as saturated or unsaturated hydrocarbons in chemistry. The examples of saturated hydrocarbon are alkane and the unsaturated hydrocarbons are alkenes and alkynes (Tang *et al*., 2009). Aromatic hydrocarbons are mainly the cyclic hydrocarbons (for example the cycloalkanes), the cycloalkanes also known as arenes of which benzene (one ringed hydrocarbon) is an example. The aromatic hydrocabons are generally more water soluble than the aliphatic hydrocarbons even if they are of equal numbers of carbon (Palmroth, 2006).

2.1.2 Sources of hydrocarbon pollutants and their health implications

Generally, hydrocarbon pollution of soils has been well reported to cause different aspect of crude oil exploration such as accidental oil spillages, uncontrolled discharge or dumping of oil and fuel and other incidences such as mining, uncontrolled industrial waste disposal. Unfortunately, prevalence in excessive disposal of hydrocarbons in human environments calls for attention and immediate remediative measures (Dadrasnia *et al*., 2013a).

Heavy dependence on petroleum for energy, industrialisation and excessive use of petrochemicals has been reported to negatively affect the growth of human population and human/environmental health. The quantity of crude oil of about 600,000.00 metric tons was reported to have spilled with an about 200,000.00 lost in the environment (Kvenvolden and Cooper, 2013). This uncontrol discharge f crude oil is still increasing til date and still causing recurrent soil and water pollution which has been reported with different health hazards (Holliger *et al*., 2012).

The oil pollution usually causes disruptions in natural population diversity and natural equilibrium between the living species in the host environment. As reported by Das and Chandran (2010 and 2011), hydrocarbon pollutants are well known to be associated with different human health problems such as cancer, mutations, different kinds of neurological ailments, skin irritations, kidney and lung problems and so on.

2.1.3 Group of hydrocarbon contaminants

Hydrocarbons in organic chemistry are the organic compounds mainly compost of carbon and hydrogen elements (Luo *et al*., 2013), are devoid of one hydrogen atom in their functional groups are refer to ashydrocarbyls. Hydrocarbons majorly exist as natural rock oil or crude oil which is usually formed through the decomposition of some organic matters (which are very rich in carbon and hydrogen elements) under certain environmental conditions. Based on IUPAC nomenclature, hydrocarbons are classified (Luo *et al*., 2013) as itemised below:

i. **The saturated hydrocarbons:**

This are the group of the simplest hydrocarbons, they are usually formed with single hydrogen bond and their general chemical formula is C_nH_{2n+2} (hydrocarbon is non-cyclic). Example of this group is alkanes which are the hydrocarbons that form the basis of petroleum fuels; they form linear or branched chains and usually undergo the substitution reactions. Example of such substitution reaction is the chlorination reaction which results in the formation of chloroform. They also from isomers which are hydrocarbons that both having the same molecular formular with different structural appearances. They can appear branched or chiral (chirals include the side chains biomolecules such as chlorophyll and tocopherol).

ii. **The unsaturated hydrocarbons:**

They are group of hydrocarbons which are distinguished with bonds which are double or triple. These bonds are usualy formed between their carbon-atoms those with double bond for example are called 'alkenes'; they are usually identified with general formula C_nH_{2n} (i.e. those which form non-cyclic structures in appearance) while those which posses triple bonds are generally refers to as 'alkynes', they are generally identified with formular C_nH_{2n-2}.

iii. **The Cycloalkanes:**

These are group of hydrocarbons which azumes ring shapes. They usually have one or more rings and they have general general formula, C_nH_{2n} for example is a formular to denote a one-ringed saturated hydrocarbon.

iv. **Aromatic hydrocarbons:**

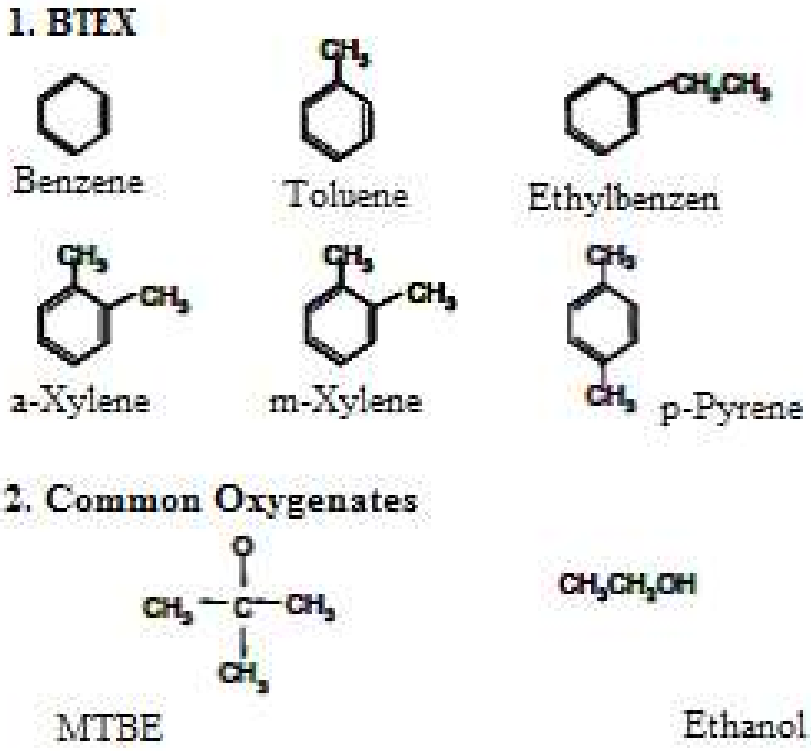
These include the hydrocarbons such as arenes that are made up of at least one aromatic ring.

All the above hydrocabons usually occur as gass (e.g. propane and methane), liquid (e.g. benzene and hexane), wax (also known as melting solids such as paraffin and naphthalene waxes) or in form of polymer such as polystyrene, polypropylene and polyethylene). Hydrocarbon which exists in liquid form in the earth crust are usually refers to as

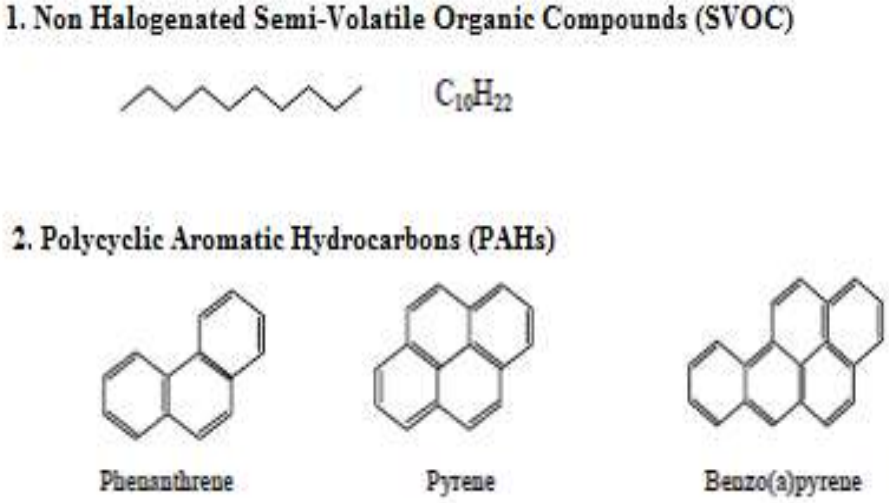
petroleum, rock oil or mineral oil while the hydrocarbons that exists as gasses in the earth crust are generally refers to as natural gasses. The petroleum and natural gasses are usually detected in the earth subsurfaces by the petroleum geologists during oil exploration; they form and serve as significant raw materials for production of organic chemicals.

Discovery and liquid hydrocarbon extraction from the sedimentary basins form an integral development in advanced modern energy generation. A hydrocarbon mining from oil sands and shale has increased many nations' wealth and income. The oil reserve is usually distilled and upgraded to produce petroleum which is generally used as oil to support energy, transportation and petrochemical industries. Economic importance of hydrocarbons can not be overemphasised; they are basis for today fuels, plastics, waxes, paraffins, solvents and so on. However, soil contaminations associated to these processes have raised another health threathning issues. Generally, the hydrocarbon pollutants are grouped into two different types (Ujowundu *et al*., 2011).

i. **The gasoline range organics:** they include the small chain alkanes which consist about 6-10 carbon atoms (C6-C10), they generally posses low boiling point (69-170 ^{o}C). The common examples of hydrocarbons in this group are xylene, toluene, n-butane and so on. (Figure 1.1).
ii. **The diesel range organics:** there group of hydrocarbon pollutants which includes longer chains alkanes, they contain more carbon atoms of about C10-C40, they are usually hydrophobic and posses higher boiling point. Examples are the polycyclic aromatic hydrocarbons (PAHs) as shown in Figure 1.1.



a.



b.

Figure 1.1: Examples of (a) Gasoline Range Organics (GRO) and (b) Diesel Range Organics. Source: Ujowundu *et al*. (2011).

PAHs are the major contaminants in many oil producing countries due to extraction and processing of the oil, these hydrocarbons are known to be toxigenic and mutagenic as they affect both mammals and aquatic organisms. PAHs are also abundant in petroleum spill soil and they are produced as residues in combustion of materials. Harms *et al*. (2003) identified many PAHs in the soil and water; they associated their accumulations in the body with cancer, mutations, skin irritations, lung, kidney damages and several other heath hazards. PAHs as also reported by Li *et al.* (2014) exist in different forms, thaey are primarily formed or produced from pyrolysis or when organic materials are not completely burnt off, heating of residential fuels, incineration or emissions from faulty engines and industrial mining of coke, oil aluminum or metals. They generally include single to multi ringed hydrocarbons such as the 16 listed USEPA polycyclic aromatic hydrocarbon including Napthalene, Acenaphthylene, Phenanthrene, Pyrene, Anthracene, Phenanthrene, Flourene (Dendoven *et al*., 2011)

There are increasing rise in PAHs contamination throughout the whole world due to advancement, industrialisation, continuous oil exploration, and the excessive use of petrochemicals (Bugress *et al*., 2003). Crude oil hydrocarbon can has been reported to cause sevel health hazards and this have raisen public concerns due to their health implications and different control measures have been suggested.

2.1.4 Fate of hydrocarbons in soil

Hydrocarbon contaminants are of different characteristics and act in different ways, there are two main factors determining the action of a hydrocarbon in soil:

1. Hydrocarbon chemical nature
2. The kind of environment it contaminates (Taylor, 2007).

The extent to which a hydrocarbon percolates in soil profile also depends on its viscosity, hydrophobicity, and insolubility, and the soil's moisture content, temperature, texture and soil structure. The hydrocarbon sorption in soil depends on the size and its complexity (Jayashree and Vasudevan, 2007). Hydrocarbons generally percolate horizontally in the wet

soil with low temperature and vertically in dry soil with high temperature and they may spread in lateral flow in water saturated areas, those that enter into the soil may become bind and form complexes with humus or nutrients in the soil and cause blockages of soil pores and sometimes create an aerobic condition against some bio degraders. Soil retention of hydrocarbons may also depend on the present solutes and concentrations in the surrounding solution. They also depend on CEC and the soil pH partly.

Soils which are contaminated with hydrocarbons are usually seen different from normal soils due to different physicochemical properties and biological content (Robertson *et al*., 2007) 'biota'. Hydrocarbons usually cause reduction in soil microorganism's component in soil, and after some times the surviving microorganism will develop ability to tolerate and utilize the pollutant, leading to increasing number of biodegrading microbes (Bollag *et al*., 2014). However, this contamination may disrupt the plant and microbial relationship as the attack the root hair and exudation (Kirk *et al*., 2005).

Many of these pollutants are hydrophobic in nature, they by this affects the soil's water holding capacity and moisture content. Polluted soils unlike unpolluted ones are usually associated with low water holding capacity, low water content, low conductivity, reduction in soil pH and unstable soil structure (Osuji and Opiah, 2007a; Osuji and Nwoye, 2007b; Ujowundu *et al*., 2011). Hydrophobic natures of hydrocarbon contaminated soils affect seed germination, reduction in leaf area and root development due to water deficit and oxygen reduction (Rahbar *et al*., 2012).

2.2 Heavy Metal Pollutants

2.2.1 Implications of elevated heavy metal concentrations in soils

Heavy metals are formed when the normal concentration of metals (micronutrients) becomes elevated in quantity that is injurious to living cells or tissues. There are several metal elements identified today examples are Arsenic (As), Cadmiuum (Cd), Copper (Cu), Chromium (Cr), Iron (Fe), Lead (Pb), Mercury (Hg), Zinc (Zn) and so on. They known to be micronutrients that are naturally available in the enivironment but indiscriminate human

activities have increased their natural concentration and geochemical; cycles hence having an altered in biochemical status. Heavy metal accumulation in soils and water was reported to be assocated with several health hazards both to man and animals, especially when accumulated in body tissues.

Many heavy metals usually occur as natural elements after pedogenetic weathering of parental materials, flooding, erosion, and vocanicity and/or through excessive anthropogenic activities. The anthropogenic activities such as mining, oil exploration, excesive application of inorganic fertilizers and pesticides, uncontrolled disposal of wastes e.t.c as identified by Modaish *et al*. (2007) as well as Wuana and Okieimen (2011) have contributed emensely to the elevated pollution levels. Indiscriminate rise in heavy metals concentrations in human environment has raised global health concerns and these heavy metals have been associated with several heath hazards such as cancer, malformations in cells, mutations and so on (Salem *et al*., 2000).

2.2.2 Health implications associated with heavy metal

Elevated concentrations of metallic ellements in soils have been identified as pollution and this has been associated with many health hazards due to their toxicity, and this started since a long time ago but the methods and procedures for heavy metal detection and their toxicity studies started from 1868 and becoming advancing year by year up till date (Shammers *et al*., 2015). Degraeve (1981) reported the adverse effects many heavy metals such as As, Cu, Fe, Mn, Pd, Zn and so on in dinking water while Alloway and Trevors (2013) also reported the relatedness between toxicity of an element and its atomic weight.

Evidences of heavy metal toxicity are also available from several scientific reports; this is presented in Table 2.1 below. Living cell accumulates heavy metals and this usually results in reactions between the cell and heavy metals binding with vital cellular components such as proteins, nucleic acids and or enzymes forming harzadous complexes (Shammas *et al*., 2015). Heavy metals effects on living tissue/cell vary from
one another, symptoms of heavy metal accumulation in plant tissues for example, toxicity of one heavy metal may vary from nother based their concentrations and the affected tissue.

Excessive exposure to a particular heavy metal usually increases the potency of such heavy metal and its concurrent health hazards. In human, symptoms such as improper or disfunctioning of central and pheripheral nervous system, defects in activities of circulatory, cellular malformations are general symptoms associated with excessive accumulation of heavy metals (Table 2.1).

2.2.3. Bioremediation of heavy metal polluted sites

Bioremediation is an efficient technonology for heavy metal removal as compared to other methods and this requires the exploitation of many organisms depending on their abilities to survive and detoxify varying concentration of heavy metals. Generally, they exhibit different mechanisms such as bio-sorption, bio-transformation bio-accumulation or bio-mineralization for removal of heavy metals in-situ or ex-situ (Gad, 2000; Lin and Lin, (2005).

Several reports had been made on the success of bioremediation in laboratory scale but there is dearth of knowledge and limited information on the massive application of bioremediation for removing heavy metals from contaminated soils (Elekwachi *et al*., 2014). Elekwachi *et al.* (2014) reported a survey on the adoption of bioremediation for the remediation of heavy metal polluted soils; they however reported that many countries aspire to apply bioremediation techniques, but the developed countries are still focusing on other technologies which are more expensive as compared to bioremediation (Satinder *et al*., 2006) while the undeveloped economies have not been involved in massive implementation of bioremediation despite the fact that it is a low cost technology (Lim *et al*., 2003).

Recently, the use of plant and fungi have been reported for the bioremediation of heavy metal polluted soils as presented in Tables 2.2 and 2.3

Table 2.1: Influence of heavy metal accumulation on the human's health

Elements (Heavy metals)	EPA Regulatory Limit (ppm) (USEPA, 2009)	Toxic Effects	References
Argon	0.10	Skin burnt with gray or blue-gray coloration, difficulty in breathing, stomach cramps, throat ache and irritations in lung	ATSDR, 1990
Astatine	0.01	Alterations and malfunctions in some key cellular activities (e.g. ATP synthesis and oxidative phosphorylation)	Tripathi *et al*. (2008)
Beryllium	2.0	Arrhythmias of cardiac region, gastrointestinal dysfunction, failures in respiration, elevated blood pressure and muscle twitching	Acobs *et al*. (2002)
Cadmium	5.0	It is carcinogenic and mutagenic, causes endocrine disruption, damage lung and makes bones fragile, affects regulation of calciumin biosystems	Dagraeve (1981) ; Acobs *et al*. (2000)
Chromium	0.1	Causes air removal	Salem *et al*. (2000)
Cupper	1.3	Damages brain, kidneys and lung and increases the severity of liver cirrhosis and chronic anemia. It is also causes the stomach and intestinal irritations	Wuana and Okieimen (2011); Ainza *et al*. (2010)
Mercury	2.0	Causes lung and kidney failure, immune diseases, drowsiness, depression, fatigue, insomnia, tremors, loss of hair and memory, restlessness, blurred vision, hypertension and brain damage	Neustadt and Pieczeni (2007); Ainza *et al*. (2010)
Nickel	0.2	Concentration above WHO permissible limit causes cancer of the lungs, hair loss, and allergic skin diseases like itching of nose, sinuses and throat. It is toxic to immune system, neurons, genes and affects fertility	Salem *et al*. (2000); Khan *et al*., (2007); Duda-Chodak and Baszozyk (2008); Das *et al*. (2008)
Lead	15	Causes impaired development, disabilities in learning, short-term memory loss, reduced	Wuana and Okieimen (1981); Salem *et al*.

		intelligenceas well as coordination problems in infants, higher dose may result in cardiovascular disease	(2000); Padmayathiammma and Li (2007)
Serilium	50	Concentration above 300 µg/day may causedysfunction in endocrine system and disrupt cells activity, it is hepatotoxicity and may cause gastrointestinal disturbances	Vinceti *et al*. (2010
Zinc	0.5	High dose causes dizziness, fatigue and so on.	Hess an Schmid (2002)

Table 2.2: Plants that have been documented for phytoaccumulation of heavy metals

Elements (Heavy metals)	**The Plant Used**	**References**
Pb, Zn, Cd, and Cu	*Salix viminalis* and other plant species like *fragillis*	Pulford and Watson, 2003; Volk *et al*., 2006;Ruttens *et al*., 2011
Castor containing Cd	*Ricinus* sp e.g. *communis*	Huang *et al*., 2011
Corn containing Cd, Zn nd Pb	Zea mays (Maize plant)	Meers *et al*., 2010
Zn, Cu, Cd and Pd	*Populus deltoides, P. nigra, P. trichocarpa* and other *Populus* sp	Ruttens *et al*., 2011
Cd, Ni, Cu and	*Jatropha curcas* L. (Jatropha plant)	Abhilash *et al.,* 2009; Jamil *et al*., 2009
Hg	*Populus* sp e.g *deltoides*	Che *et al*., 2003
Se	*Brassica juncea, Astragalus bisulcatus*	Bitther *et al*., 2012
Zn	*Populus canescens*	Bittsanszkya *et al*., 2005

Table 2.3: Mushrooms that are commonly exploited in soil remediation of heavy metals

Organism (Mushrooms)	**Heavy metals**	**Mechanisms**	**References**
Lactarius piperatus and *Agaricus bisporu*	Cd^{2+}	Bioacummulation	Nagy *et al*., 2013
Formes spp e.g *Formes fasciatus*	Cu^{2+}	Biosorbent and this increase in hot-alkali treatment	Sutherland and Venkobachaar, 2013
Oyster mushrooms such as *Pleurotus platypus*, *Agaricus bisporus* as well as *Calocybe indica*	Cu, Cd, Fe, Ni, Pb and Zn	Functions as biosorbent fo trapping heavy metals in aqueous state solution	Lamrood and Raleganker, 2013
Flammulina velutipes	Cu	Biosorbent (using their SMC)	Luong *et al*., 2014
Oyster mushrooms *(Pleurotus tuber-regium and P. ostreatus*	Several heavy metals	Bio-extraction and act as Biosorbent	Oyetayo *et al*., 2012
P. ostreatus	Cd	Biosorption	Tay *et al*., 2011
P. pulmonarius	Zn	Biosequestration	Jibran and Milsee, 2011

2.2.4 Plant and fungi as agents for removal of heavy metal in soils

Many methods have been suggesd for clean up of heavy-metal contaminated soils, examples are the photocatalytic, chemical oxidation, precipitation, filteration, exaporation, membrane technologies and so on. Unfortunately, most of this mechanism may be ineffective on sites which are polluted with less than 100 mg/L heavy metal concentrations (Haritash and Kaushik, 2009). However, the use plants and fungi in bioremediation have also been well studied (Tables 2.2 and 2.3) and these are forms of bioremediation that should be encouraged in massive on-site (in-situ) remediation in polluted soils (Onwubuya *et al*., 2009). Many people prefer bioremediation as an environment friendly method in treating the heavy metal contaminated soils (USEPA, 2007). The use of living organisms in heavy metal removal usually involves bioaccumulation and/or adsorption mechanisms (Hussain *et al.,* 2007).

Microorganisms are generally made up of polysaccharide, lipoprotein rich cell wall which enhances their diverse functional roles in binding heavy metals (Scott and Karanjkar, 1992). Fungi for example are well known for heavy metal extraction, many species of *Penicillium*, *Aspergillus* and *Rhizopus* groups are well reported as heavy metal chaleting organisms in aqueous solutions as reported by Volesky and Hokan (1995), Xiao *et al*. (2010) as well as Huang *et al.* (2012). These researchers reported the biosorbent abilities of different fungi in removal of heavy metals, many other fungi are reported as endophytes, hyper-accumulators, or metal extractors according to Xiao *et al*. (2010). Also, Sun *et al*. (2010) reported faster cupper remediation (ranging from 63% to 125% percentage removal) by inoculating the root of *Elshotzia apliendens* and *Commelina communis* with some endophytic bacteria, they reported an increase in root dry weights and shoot tissues than the uninoculated plants, similar observations were also made by Ceregani and Malayeri (2007), Modaish *et al*. (2007).

2.3 Pesticides as soil Pollutants

2.3.1 Pesticides as biocides, classification and their mode of actions

Pesticides are substances which are synthesised or are naturally available to be used for controlling undesired pests such as insects, rodents, aves, plants and microorganisms. Generally, pesticides act by attracting and destroying the pathogen and they are commonly refers to as 'biocide'. They are commonly grouped into two main classes:

i. Class of chemical biocide
ii. Class of biological biocides such as the use of virus, bacteria or fungi

They are also grouped based on the kind of pathogen acted upon, examples include insect growth regulator, herbicide, insecticide, nematicide, rodenticide, bactericide, fungicide, molluscicide, termiticide, avicide, piscicide, predacide, animal repellent, insect repellent, and disinfectant (antimicrobial). Biocides as well can also be grouped according to the way they are degraded after application; examples of this are the biodegradable biocides (when they are degradable/ broken down by microorganisms or other living organisms into less toxic compounds and they can also be grouped as persistent pesticides when they are not easily degraded. Accumulation of this persistent pesticides for example dichlorodiphenyltrichloroethane (DDT), are more implicated inthe environmental pollution (Jayashree and Vasudevan, 2007).

Moreover, chemical biocides can be further grouped into chemical families such as the organophosphate group, the organochlorine group and cabamates (cabamates can be further grouped into thiocarbamate and dithiocarbamates). DDT which is an example of organochlorinated pesticide s which are often degraded to simpler, less or non-toxic compounds such cyclodienes dichlorodiphenylethane. Chemical biocides generally act by attacking the nerve fibers and alter the sodium and potassium balance, the organophosphate and carbamate biocides act by inhibiting the enzyme acetylcholinesterase (which is a neurotransmitter) in targeted pests and affect the nervous system by allowing an indefinitely acetylcoline transfer to the nerve impulses causing symptoms such as weakness or paralysis. Most organophosphates can be used as insecticides, they were first produced

in the early 19th century but in 1932, it was discovered that they exert similar effects on insects and humans. Also, organochlorine insecticides, like chlordane and toxaphene are very injurious to human health, though they were the most commonly used pesticides in the past but most of them are now banned in the market today.

Pyrethroid are synthetically modified from naturally occurring chemicals; pyrethrin in chrysanthemums have been modified for better action on pests, many of the pyrethroid pesticides are found to be very toxic to the nervous system. Sulfonylurea herbicides includes bensulfuron-methyl, oxasulfuron, primisulfuron-methyl, amidosulfuron, chlorimuron-ethyl, flazasulfuron, ethoxysulfuron, nicosulfuron, flupyrsulfuron-methyl-sodium, azimsulfuron, rimsulfuron, imazosulfuron, pyrithiobac-sodium, the halosulfuron-methyl pesticide, the pyrazosulfuron-ethyl, sulfometuron-methyl, sulfosulfuron, terbacils, bispyribact-sodiumand, cyclosulfamuron and so on (Arnold *et al*., 2002), while others such as nicoulfuran, triflusulfuron-methyl and chlorosulfuron were identified by EFSA (2008) to affect the enzyme acetolactate synthase in plants and they are used as broad-spectrum herbicides. They are very effective only 1 kg of this pesticide is required for protecting crop cover in one hectare of land which is an equivalent of 0.89 lb/acre in the 1960s, but only 1% sulfonylureates can achieve the same effect (Lamberth *et al*., 2013).

Biocides can also be classified based on mechanism of action or methods of application, for example a systemic pesticide attack from within inside a pest following absorption. Paldoxins are recent fungicides (announced in 2009), they work by compromising the plant's natural defense mechanisms which involves the production of phytoalexins need for detoxification of the fungal enzymes, and these fungicidesare believed to be more safe and greener.

2.3.2 Bio-pesticide/biocides

Biopesticides arespecial types of natural biocides which are living organisms or products derived from the in controlling pests due to their pesticidal effects examples of such biopesticides are canola oil and baking soda. The microorganisms (e.g virus, bacteria, fungi etc) that posses pesticidal effects are known as micropesticides, some of them are regarded

as entomopathogenic organisms (e.g. nematodes) based on their mode of action. More also, there are biochemical or herbal biopesticides which are derived from botanical extracts or biological secretions, they include plants extracts, phytohormones, pherohormones ad so on. Many of these plant incorporated protectants (PIPs) have been developed today; the advancement of science has also led to development of different genetically modified organisms (GMOs) which are also employed as biopesticides.

2.3.3 History of pesticide usage

Pesticides have been used by humans for protections of self, environment, food and/or their crops either during cultivation or storage. This pesticide's usage started as far back as 2000 BC during the ancient Mesopotamia era which is over 4,500.00 years. Commonly used pesticide then was the elementary sulfur which is most commonly used during the summer and the use of some poisonous plants for controlling pests, this was mentioned in Rig Veda which recorded as far back as 4,000 years ago (Rao *et al*., 2007). In the 15th century more chemicals were used as pesticides examples are arsenic, mercury and lead compounds while this advanced to the use of nicotine sulfate extracted from tobacco leaves in the 17th century. 19th century marked the introduction of many more biopesticides; pyrethrum for example was extracted from *chrysanthemums*plant while rotenone was estracted from some tropical plant's roots which are mostly vegetable (Miller, 2002).

Miller (2002), revealled that the arsenic containing compounds was the dominantly used pesticides in the 17th century; DDT however was discovered in early 19th century by Paul Müller as a very effective insecticide. There were increased usage of DDT and other organochlorines during this period; DDT was replaced in the US with organophosphate pesticides and carbamates around 1975 while the pyrethrine later on gained its popularity (Daly *et al*., 1998). Herbicides usage as reported by Daly *et al*. (1998), became very commonly used for controlling weeds in 1960s, the first most commonly used herbicide was triazine and some other nitrogenous based chemicals such as carboxylic acids like 2, 4-dichloroacetic acid and glyphosphate (Daly *et al*., 1998).

Federal authority legislation on the use and regulation of pesticides was first enacted in 1910 and after some decades during the 1940s many synthetic pesticide manufacturers sprang up for mass production of synthetic pesticides, the use of these synthetic pesticides became popular at this period (Daly *et al*., 1998). Some other sources however believe that the start of the pesticide era began around 1940s and 1950s. USEPA was created in 1970 and amended into law for the control of pesticides use in 1972 (Miller, 2002), but their have been manifold increment in pesticide use has been experienced since then (Miller, 2002). 75 % of the world's pesticides are used by the developing countries such as the Africans, as reported in the Rachel Carson's best-selling book in 1960 called 'Silent Spring' (written about biological magnification) reported that DDT can be used to control birds from eating fishes as it can prevent them from reproducing and their later on caused serious threat to biodiversity of many fish eating bird species.

The use of DDT in agricultural practices was later banned under the Stockholm Convention, This Stockholm Convention was an international treaty for environmental protection which was enacted in 2001 as the governing council by the United Nations Environmental Programme (UNEP), the convention was created to resist or eliminate production and spread of persistent organic pollutants (POPs) and became effective in May 2004, but DDT is still used in many developing nations for prevention of malaria by killing mosquitoes and use to control some other tropical diseases.

2.3.4 Effect of chemical biocides on the human's health

Chemical biocides are very importantindustrial by-products in the world today but they may as well threaten both wild-life and mankind when the residues accumulated indiscriminately in their tissue due to environmental pollution. Pesticides may become accumulated in tissues through direct or indirect means; generally, pesticides can into body tissues through oral route, inhalation of pesticide residue in air, eye contact, and/or through skin contacts (Lorenz, 2007). Toxic pesticide accumulation in cell or body tissues has been reported to cause fertility problems, birth complications, symptomatic defects in foetus, tumours, nerve disorders, genetic mutations, problems with the circulatory system, destruction of endocrine system and so on (Lorenz, 2007). The pesticides poisoning has

also been observed with symptoms such as wild skin irritations, convulsions, foaming in the mouth, blindness, and in some cases death. The symptoms however depend on the nature, type or toxicity of a particular pesticide and the susceptibility of the victim.

Pesticides may cause toxic injury to human heath(acute and delayed health effects) when accumulated in tissues (USEPA, 2009) and affect skin, eyes or nervous system, sometimes they mimic human hormones and cause reproductive problems, and or cancer (USEPA, 2009). In a systematic review Bassil *et al*. (2007) reported that human exposures to many synthethic pesticides can cause Hodgkin lymphoma and leukemia diseases and therefore recommended the use of cosmetic pesticides. There are many evidencesthat many organophosphate insecticide results in neurobehavioral alterations in humans (Weselak *et al*., 2007; Mink *et al*., 2011). Sandborn (2007) explained that there are some other evidencesfor other negative impact of pesticide exposure like fetal deaths, birth defects, neurological problems,

The WHO and UNEPA during a sumit estimated more than three million infected workers yearly due to pesticide poisoning during agricultural practices in developing countries, they also estimated that about 18, 000.00 people die due to pesticide poisoning in developing countries yearly as reported by Miller (2004). Many death cases in developing countries have been reported to have occurred through pesticide misuse. Kalkbremer *et al*. (2014) also estimated that, about 25, 000 000.00 workers suffer on mild pesticide poisoning every year in developing countries. Pesticide exposure may also put some individuals in other several careers like pet groomers, groundskeepers, and fumigators aside from agriculture at risk of health effects (Gunnel *et al*., 2007; Kalkbremer *et al*., 2014).

2.3.5 Effect of chemical biocides on the human environment

This have been reported to be a great concern globally in the past 20 years as they greatly affect the agricultural soils, surface water and groundwater quality. Many pesticides are very toxic to man and animals as well as other organisms. Generally, the pesticide's toxicity can be defined s. The toxicity of a pesticide can be defined as its capacity or ability to insight injury or negatively affect the normal health status of living organism, pesticides

that are grouped as POPs can retain about 50-95 % of their concentration in the environment and their accumulations are usually not easily degraded by natural means, many of such POPs usually affect non-target species in air, water and soil and this had raised several environmental issues (Kalkbremer *et al*., 2014). Pesticide drift, transport or spread in the air as particles from the point of usage to contaminate other areas, these causesair, water or soil pollution.

Also, pesticide contamination can affect the biodiversity, of an area (Kalkbremer *et al*., 2014), or destroys habitat. It has been reported that continual exposure of some pests to some pesticides can cause them to develop resistance to the pesticide and the use on higher dose/concentration of such pesticide to combact the resistance may in turn tend to worsen the pollution problems. The loss of pesticides in the environment is usually due to some environmental processes and conditions (Sims and Sommers, 1986; Sims and Cupples, 1999).

2.3.6 Remediation of pesticide pollution in the human environment

Many pesticides' residue have been detected in tissues of living organisms and this has been linked with different health hazards therefore several technologies have been reported for the removal pesticides from the environment, the use of living organism is however currently attracting interests due to its environmental friendly, simplicity and cost effective approach (Fushiwaki and Urano, 2001). Some living organisms can metabolize or mineralize pesticides as a result of their capacityies to secret several c assettes of degrading enzymes. Generally, bioremediation of pesticides usually includes; biodegradation, mineralization, volatilization, sorption or desorption, adsorption and so on (Clausen *et al*., 2001). The mineralization and degradation mechanisms are usuallyinvolvng the breakdown of more complex pesticides to less complex or less toxic compounds, in some cases the pesticides are broken-down to an utilizable carbon and nitrogen sources. Microbial sorption mechanisms are usually employed in pesticide removal from sediments or water environments while the adsorption mechanism is usually applicable to the soil environment especially around the root rhizospheres.

2.4 Bioremediation and its Kinetics

Most of other methods of remediation other than bioremediation have some drawbacks because they may leave behind residual compounds which may also be toxic. Biological treatments is guided by the concept of being environmental friendly, save and cost effective, it creates the best environment for remediating contaminated soil. Bioremediation process involves two major mechanisms based on oxygen requirement, viz:

1. Aerobic bioremediation mechanism and
2. Anaerobic bioremediation mechanism.

However, most of the bio-remediating organisms employ the aerobic remediation and it is in the equation below summarised for hydrocarbon compound:

Aerobic biodegradation:

Biota + Hydrocarbon compound + Oxygen (O_2) ± Enzyme(s) = Biota + Water (H_2O) + Residue(s) (1)

Anaerobic biodegradation:

Biota + Hydrocarbon compound + Carbon dioxide (CO_2) ± Enzyme(s) = Biota + methane (CH_4) + Water H_2O) + Residue(s) (2)

Example of bacteria that exhibit aerobic degradation (in the presence of oxygen) of pollutants are *Escherichia*, *bacillus*, *Pseudomonas*, *Gordonia*, *Micrococcos*, *Rhodococcus*, *Moraxella*, *Sphingobium, Panderaea* and many others which usually exhibit anaerobic degradation (without oxygen) such as *Desulphobrio, Pelatomaculum, Methanospirillum, Desulfotomaculum, Methanosaeta, Syntrophobacter, Syntrophus* and so on (Shimao, 2001; Jayasekara *et al*., 2005) while *Aromatoleum aromaticum* a facultative bacteria that uses toluene or ethylbenzene as substrate has been identified as anaerobic degrading species, other are *Geobacter metallireducens*, *Dechloromonas aromatic*, different species of

Dehalococcoides, *Desulfitobacterium, Hafniens* and so on (Cao *et al*., 2009). These microorganisms can utilize the contaminants after complete mineralization by absorbing them into their cell; converting them into intermediate products after secretion of enzymes and many organic acids like maic, citric, acetic, alpha-ketoglconic acids e.t.c. (Monica *et al*., 2011).

Mineralization is the complete transformation or degradation of persistent hydrocarbons such as PAHs by some capable microorganisms into organic acids, inorganic CO_2 and water as presented in Figure 2.1, mineralization process makes use of an appropriate electron acceptor for completed oxidation of hydrocarbon pollutants into less toxic compound that can be mobilised into the cell (Volkering and Beure, 2003), under aerobic environments, oxygen is utilised as a preferred terminal electron by the dehydrogenase enzyme. Plants degrades PAH by secretion of metabolites through the roots during the process called 'Phytoremediation' (as shown in Figure 2.2) and they sometimes employs different mechanisms such as Phytoaccummulation, Phytovolatization, Phytostabilization and so on.

An example of plant action is the production of the enzyme called cis-dehydrodiol or or other dehydrogenase enzyme through the root into the rhizosphere for degradation or hydrocarbon pollutants such as PAHs. Hydrocarbon degrading microbes like fungi and bacteria usually degrade PAHs to lesser or more water solube compounds through complex metabolism to facilitate their subsequent excretion (Figure 2.1 and 2.3) and some degrading bacteria can utilize hydrocarbon pollutants produce energy (Figure 2.1 and 2.4). These organisms have different metabolic pathways as presented Figure 2.1, 2.3 and 2.4.

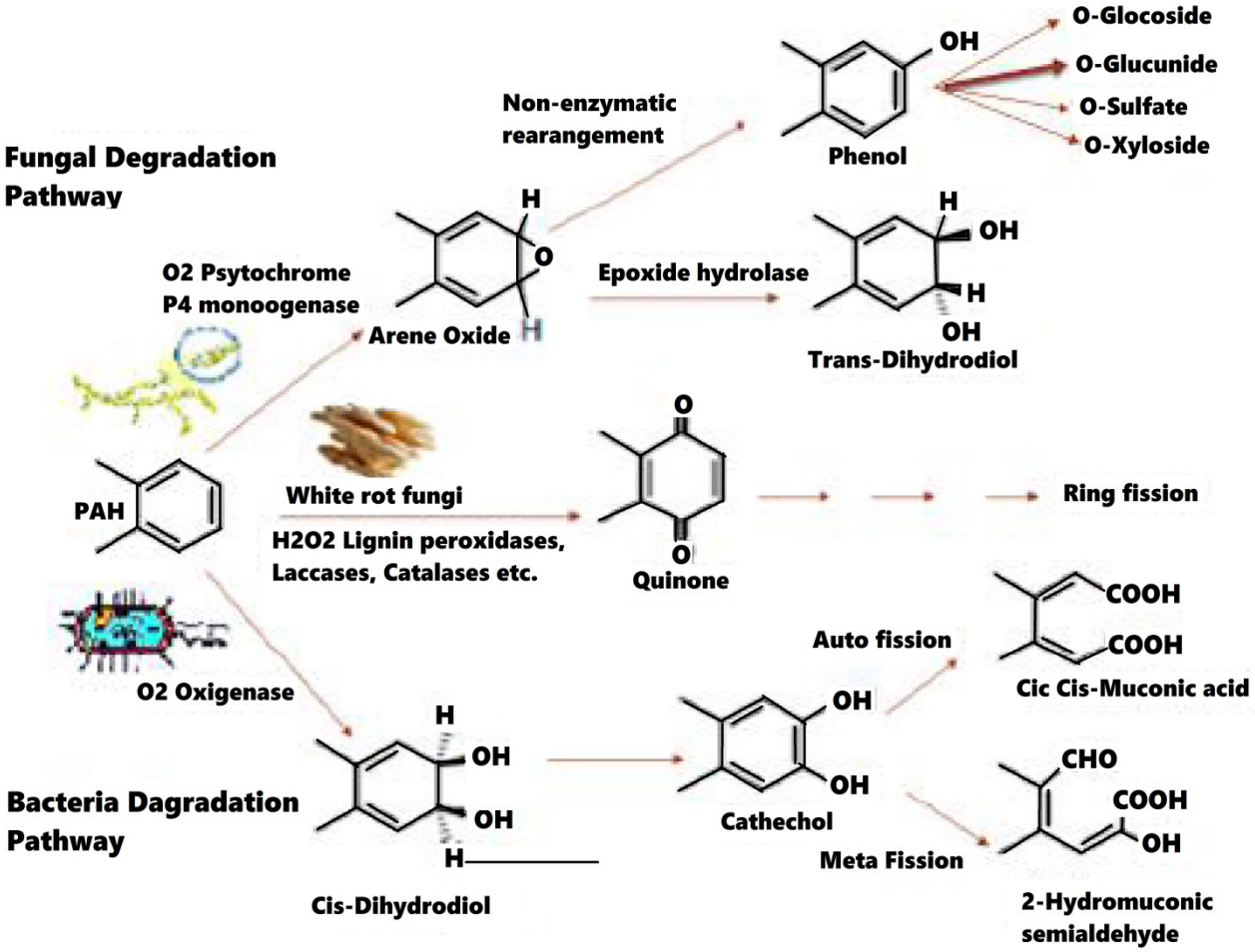


Figure 2.1: General pathways showing different microbial mechanisms for degradation of PAHs (Cerniglia, 1989)

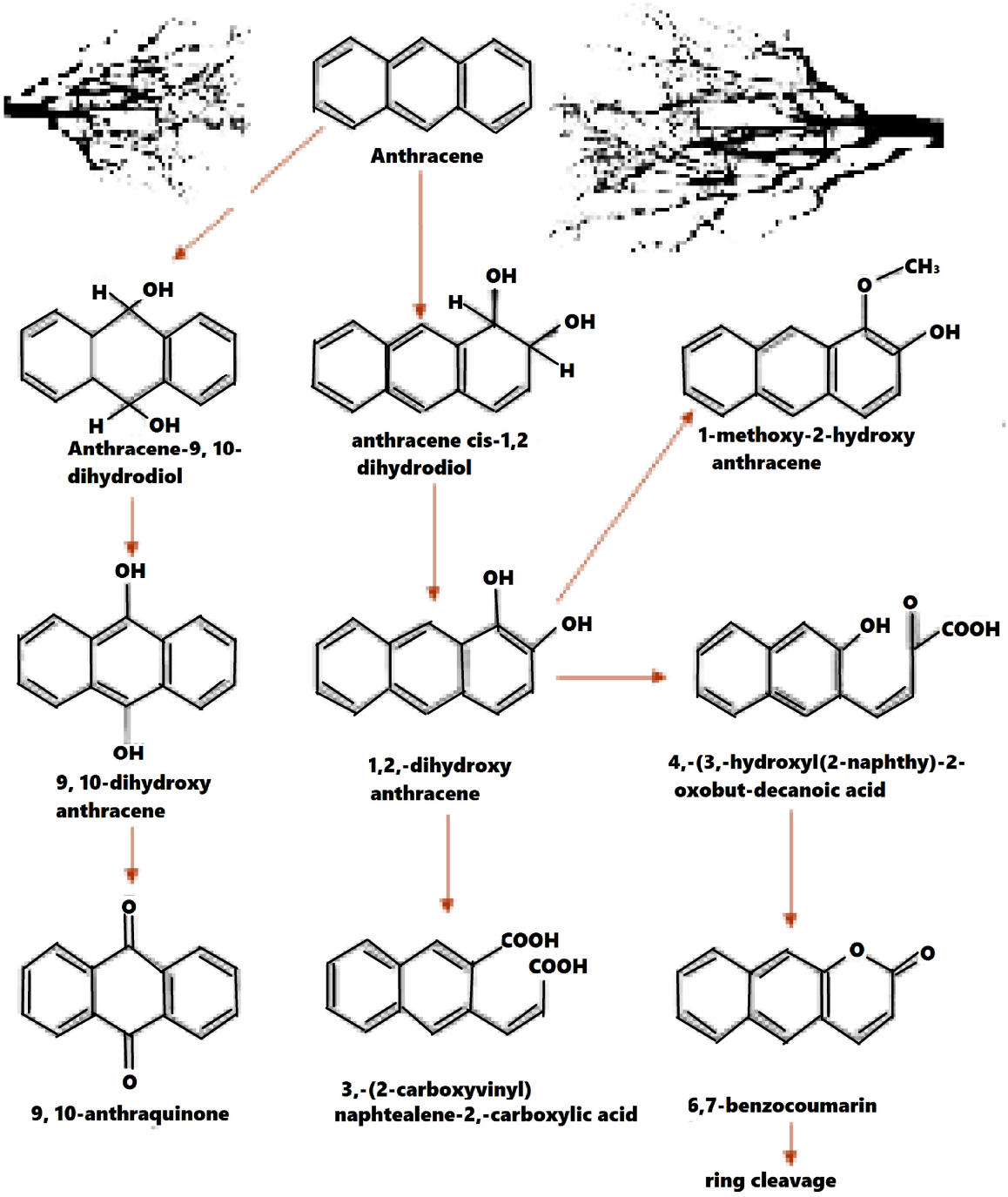


Figure 2.2: Plants transformation of anthracene during phytoremediation as adapted from Arey and Atkinson, (2003) and Kodjo-wayo, (2006).

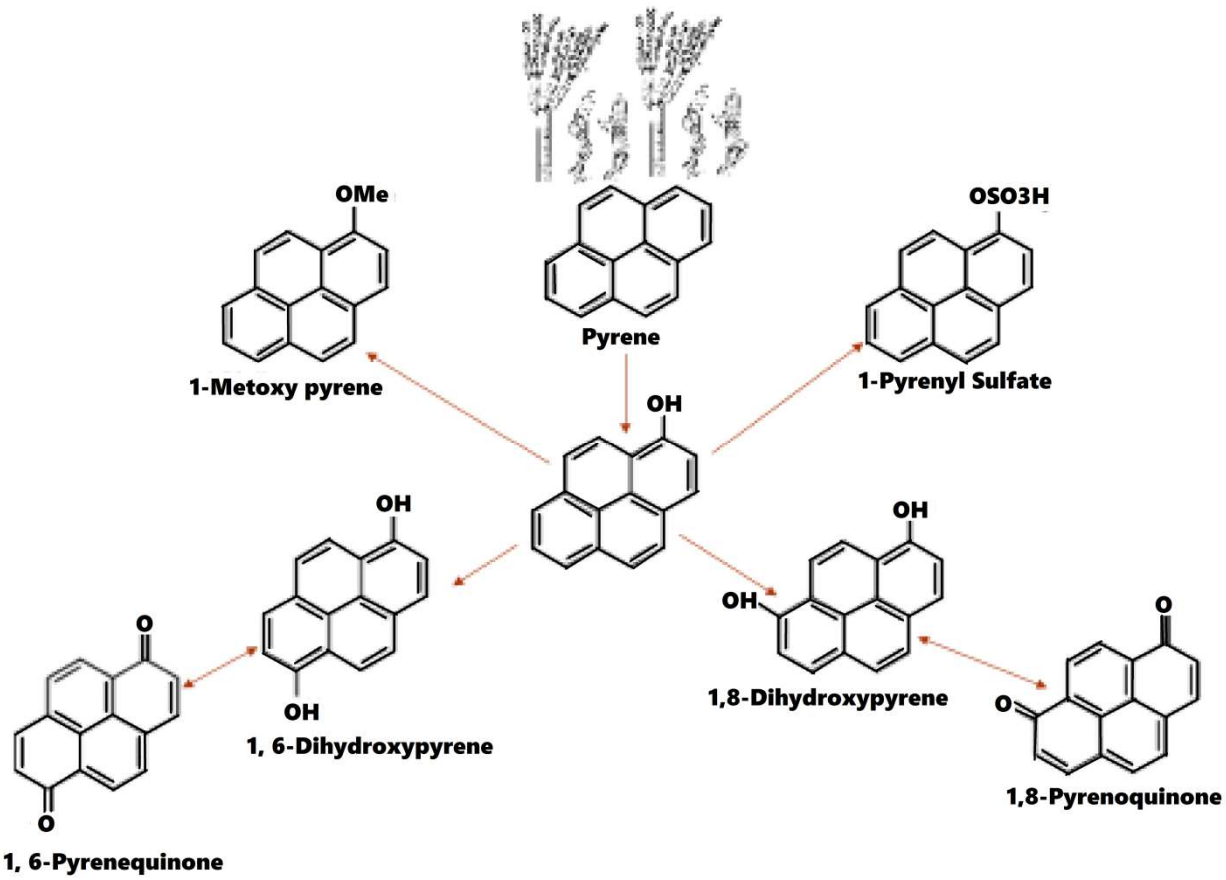


Figure 2.3: Proposed fungal (*Penicillium glabrum* strain TW 9424) pathways for metabolism of pyrene (Wunder *et al*., 1997)

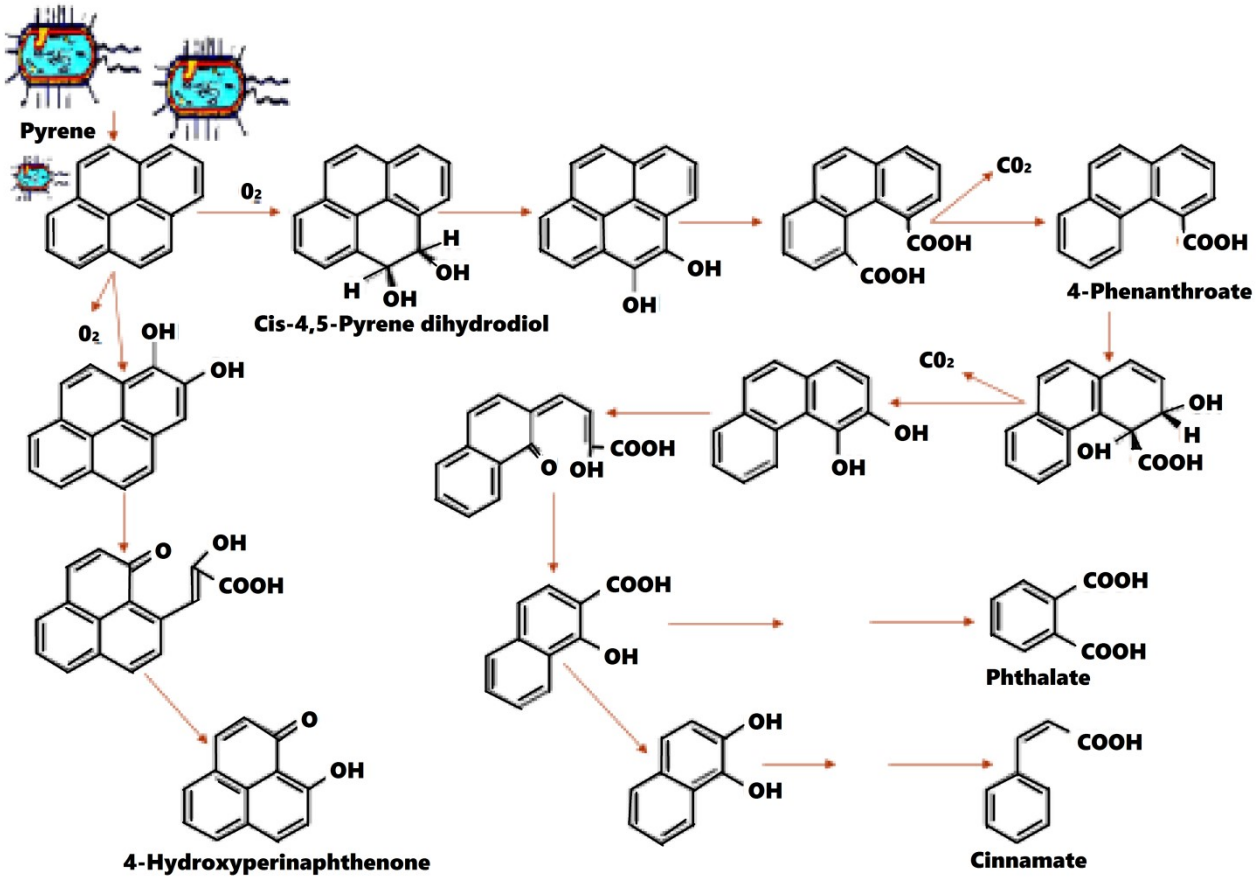


Figure 2.4: Proposed bacterial (*Mycobacterium* sp PYR-1) pathways for metabolism of pyrene

The fungal hydrocarbon degrading pathway usually resembles those by humans and other mammals as shown in Figure 2.1 and 2.4). Many fungi can oxidize many poly-aromatic hydrocarbons (PAHs) by production of different extracellular enzymes such as extracellular enzymes like the peroxidases (LiPs and MnPs), catalases, and laccasses or generally through the cytochrome P450 mechanisms. This is usualy oxidised to form phenol or in many cases trans-dihydrodiol. Moreover, bacteria usually degrade PAHs starting from the enzyme secretions which enhance the ring cleavage leading to the formation of cis-hydrodiol compound and further degraded to form cathechol (Figure 2.3). During the bacterial degradation of hydrocarbons, there usually an indication that ring cleavage has occurred and this usually occurs in the two hydroxyl groups of hydrocarbons; the *ortho fission* which usually occur at the hydroxyl group and the *meta fission* which usually occur at the ring. The hydrocarbon ring degradation usually becomes successful after ring cleavage and then the molecule can be mineralised.

Generally metabolic pathways for many microorganisms especially for different bacteria and fungi are already verified and reported, some experiments are demonstrated in pure cultures for degradation of a different PAHs, this was previously reviewed by Cerniglia and Suderland (2010), Sutherland *et al.* (2013; 1995), Wunder *et al*. (1997) and Kanaly *et al.* (2002). Figures 2.3 and 2.4 portray the pathway for bacteria and fungal degradation of PAH called pyrene, despite other cometabolic actions which may be takin place pyrenoquinones and 4-hydroxyperinaphthenone are the alternative pathway depending on the microbe involved (Cerniglia 1989).

Biological mechanismsattack Polycyclic aromatic hydrocarbons by free hydroxyl radicals ($OH\cdot$) using some enzymes catalysts as shown in Figure 2.3. Pathway (1) in Figure 2.3 is the fungal pathway where a fungus may use the enzyme cis-dihydrodiol or dehydrogenase enzyme to facilitate the degradation of naphthalene which is a two ringed polycyclic aromatic hydrocarbons. More also, the pathway (2) shows the fungal pathway where a fungus uses the cytochrome enzyme called Cyt P-450 monooxygenase with phenol backbone or lignin peroxidase with quinone backbone to degrade naphthalene which is a two ringed polycyclic aromatic hydrocarbons.

The fungi work in the presence of (nitrate (NO_2) shown by pathway (2a) or in the presence of oxygen O_2 shown by pathway (2b) to facilitate the degradation of naphthalene a polycyclic aromatic hydrocarbon.

2.5 Enzymes and Genetic Inferences of Bioremediation

Microbial degradation of different pollutants such PAHs is usually associated with secretion of different cassettes of enzymes, this is known to be possible by microbes that had developed genetic and metabolic mechanisms to do so. It was reported that enzymes secretion is often controlled by some specific genes which have been well studied in microorganisms, examples of such enzymes are oxygenase, dehydrogenase, hydrolase and so on. Lignolytic enzymes in fungi for example are extracellularly secreted as peroxidases such as LiP and the MnP, catalases, laccases etc. They are involved in catalyzing the oxidation of different xenobiotic pollutants by bond cleavage in the pollutants molecule as explaine by Hofrichter *et al*. (1999). Laccase and peroxidase are usually produced extracellularly by fungi, in an experiment conducted by Bogan and Lamar (1995); it was observed that there was a strong relationship between PAH disappearance and the ionization potentials of forming phenols or trans-dihydrodiols.

Enzyme actions rely on the chain length and complexity, but the mechanism of action is basically the ability to introduce oxygen (oxidation) in the substrate. Many higher fungi and some bacteria contain many individual Cytocrome of P450 families partake in conversion of hydrocarbons to isoform substrates as shown in Table 2.4. Some yeast fungi were documented with capality of mineralizing n-alkane hydrocarbon (hydrocarbon) and some other aliphatic hydrocarbons as sole carbon source in other to generate energy, this they do by using different microsomal P-450 and these enzymes as reported by Sutherland *et al*. (1995) were isolated from the fungi *Candida albicans, C. tropicalis, C. maltosa* as well as *C. apicola*. P450 system have been shown to consist of alkaneoxygenase enzyme actively able to oxidize alkanes under aerobic conditions, others are hydrolase enzyme with di-iron alkane group that is controlled by gene *alkB*, enzyme monooxegenase with di-iron methane and some other methane monooxygenases containing cupper (Van Beilen and Funhoff, 2005). These enzyme actions form the major platform/drivers of bioremediation process

which is heightened during synergist bioremediation. The synergistic bioremediation employs many organisms and their enzyme activities for soil remediation.

Ability of bioremediation organisms to degrade hydrocarbon pollutants has been linked to some genes that code for specific protein enzymes. Generally, the diversity of organisms found in hydrocarbon polluted soils and their ability of bioremediating some hydrocarbon pollutant has been associated with genes encoding degradative enzymes and pathways, these are essential novel catalytic activity and can be employed in modern biotechnology, with this new microorganism can be clone with specific degrading genes for effective cleanup, soil microbes that are inhabiting an oil spill soil can also be selected for studies after a short term exposure (Vandermeer *et al*., 1992; 1994) and these organisms are able to undergo genetic modifications and adaptations that might have led to new hydrocarbon tolerating strains

In oil spill environment, microbial communities in this habitat develop genes even on the plasmids and this can be exchanged between the species, with these different catabolic/oxidative pathways are synergised for efficient (Rabus *et al*., 2005). However, it may be difficult to characterize natural microbial communities in hydrocarbon polluted sites, but genetic tools are already being developed to solve this in modern day biotechnology. Metagenomic method can now be employed to study environmental samples from oil degraded soils, this enables us to study the abundance and distribution of oil degrading microbes in polluted sites, and with this we can construct family structure with particular function, analyse the role of each microorganism over time, evaluate the effect of the contaminant and develop a good strategy for effective clean-up.

Development in genetics also enables us to detect specific microorganism and specific gene involved in bioremediation through the general molecular amplification called Polymerase Chain Reaction (PCR) however, the emergence of modern DNA microarray methods has improved rapid detection and tracking of many genes at one time. Development in the use of the PCR, the reverse transcriptomic PCR (RT-PCR), the Quantitave polymerase chain reaction (q-PCR) have made it possible to quantify special sequence of the nucleic acid responsible for a particular expression and trough this we can easily check the amount of

DNA, CDNA, as well as RNA in a particular sample. Moreover, the recent discovery of reporter system facilitates real-time monitoring of bioremediation processes, the genes and pathways involved can be assembled for developing GMOs specifically for biodegradation. Many GMOs has been successfully cloned and used as bioaugmentation or biocatalyst entities for adequate degradation (Paul *et al*., 2005).

2.6 Mycoremediation

This therminology is commonly used to describe a form of bioremdiation technology which utilizes fungi or their products (introduced or native) to remove and degrade contaminants (Kulshreshtha *et al*., 2014; Emuh, 2010; Adenipekun and Omoruyi, 2008). It involves the action of fungi in degrading/mineralize persistent chemicals/pollutants to form lesser or nontoxic compounds, the toxic pollutants are usually converted into simpler forms. Fungi were first discovered to be capable of degrading different plant polymers such as cellulose, hemicelluloses lignin and different arrays of other molecules such as waxes, wood, animal flesh, feathers, insect cuticles, rubbers and so on, they are known to be well associated with waste and biodeterioration of crops and food stuffs (dirty works). Today several fungi are being reported for degrading/mineralizing hydrocarbons and pesticides and as well man, fungi are commonly exploited in soil remediation of heavy-metal polluted soils. There mechanism or action generally vary from different species of fungi and the specific pollutants; they are structurally and biologically unique organism. They abound in various habitats (i.e. they are ubiquitous or cosmopolitan).

Most fungi are more tolerant and respond faster to pollutants compared to bacteria, their capabilitie have been studied since 1980s and many fungi have been documented in many bioremediation mechanisms. Fungal degradative activities and survival in polluted environments have been linked with their developed ability to produce different cassettes of enzymes which enhances their actions (Agrawal and Sushil, 2015). Mushrooms for example were reported for their abilities in accumulation of several pollutants and their secretions of novel catalytic enzmes like lignin peroxidase (LiP), manganese peroxidase (MnP), laccases (LCC), catalases (Cat) and so on (Adenipekun and Lawal, 2012).

2.6.1 Mushrooms as fungi and their roles in Bioremediation

Fungi are cosmopolitan; they are commmonly found in severy environments such as air, water, soil decaying wood, on plant, dung hill, even on the leaf or decomposing materials anywhere with conducive environmental condition (Jonathan *et al*., 2008). Mushrooms are the fruit bodies of fungi which usually assume an umbrella shape and can be seen with the naked eyes. Mushrooms are groups of fungi that possess conspicuous umbrella-shaped fruiting body (sporophore), they are as well called Macrofungi because they can be seen with the naked eyes. Mushrooms are cultivated commercially and many other grow freely in the wild whenever environmental condition becomes favorable these are picked eaten or sold in the markets because they have been reported to have high nutritive and nourishing value.

Efficiency of different species of mushrooms in producing protaineous food through their fruit bodies/biomass depend on their abilities to grow on different wastes or substrates according to Kuforiji and Fasidi (2009) as well as Zhu *et al*. (2013). Mushrooms arewell known to be capabe of degrading lignin and woody materials in other to absorb nutrient from them; as saprophytic organisms, they developed abilities to produce different enzymes which enhances them in pre-softening or degrading complex substrates before they can absorb their nutrients (Jonathan *et al*., 2008). This actually led to more research inmushroom cultivation for waste remediation, for example Kim and Nicell (2006) as well as Nguyen *et al*. (2013) discovered that enzyme-catalysis by many fungi were reported to be improved upon by mediator compound which usually speed up the transfer of electrons between the targeted compounds and the enzyme.

Today, many scientists are reporting the use of mushrooms for remediation purposes; they suggested that the mushroom employ processes of biodegradation, biosorption and bioconversion of soil pollutants (Novotny *et al*., 2004; Okerentugba *et al.,* 2015). The mushrooms are not only useful as bioremediation tool but also as nutritive food as a source of protein. Mushroom with white colored spores are refers to as White-Rot Fungi (WRF), the ‘rot’ in the name attribute their ability to decompose wood and they are the most common known to efficiently degrade pollutants. Hofrichter (2002), Pozdnyakova (2012)

and Adedokun *et al.* (2015) explained their attribute of '*Total mineralization'* because the mushrooms would also be free of any petroleum products. Employing mushrooms for bioremediation will not only regenerate soils for farming, but also serve as source of food and income (Ogbo and Okhuaya, 2009; Emuh, 2010).

They exhibit low specificity of the enzymes produced and do not require preconditioning to the particular pollutants (Adenipekun and Lawal, 2012; Kulshreshtha *et al.*, 2013a, b). It was also revealed by Luong *et al.* (2014) that biosorption and biodegradation abilities of *Trametes versicolor* enhanced the remediation of soils polluted with different industrial chemicals, pesticides, UV filters, and so on. Also, certain species of fungi were also reported to be able to degrade carbamazepine as reported by Rodarte-Morales *et al*. (2010 and 2011). Several researchers like Patricia *et al.* (2009) as well as Hata *et al.* (2010) established some roles played by the extracellular enzyme systems of mushrooms such as those called white rot fungi, they reported that those mushrooms are able to produce novel enzymes such as peroxiases, laccases and catalases can enhance the fungal ability to degrade several organic and hydrocarbon compounds such as TrOC which deficult for bacterial degradation while some other scientists had also reported the roles of several intracellular enzymes such as P450 system in fungal bioremediation of different persistent chemicals/pollutants (Hata *et al*., 2010).

Recently, interests of many mycologists have been towards using different fungi for soil remediation. Tran *et al*. (2010) for example reported the breakdown of chlorofibril acid using some white rot fungal trains; other researcher also made similar findinngs, Marco-Urrea *et al.* (2009), Rodarte-oralles *et al*. (2010 and 2011) who reported fungal degradation pollutants such as naproxene, Tamagwa *et al*. (2005) reported estrone degradation, Marco-Urrea *et al*. (2009) and Cajthamal *et al*. (2009) had also affirmed the use of fungi for ibrufen and bisphenol degradation. Likewise, Yang *et al*. (2013a, 2013b and 2014) established the phytoextraction capabilities of several muchrooms on heavy metals from polluted soils through the use of fungal whole cell treatments on the polluted soils.

In addition, Marco-Urrea *et al.* (2009) observed up to 97 % degradation of chlorofibric acid using several strains of a mushroom called *Trametes versicolor* strains while Tran *et al*.

(2010) 30-100 % degradation a pharmaceutical pollutant called naproxen, 10-40 % degradation of ibruprofen as well as 25-50 % degradation of ketoprofen, they also observed that the laccase activity increased from 2 to 6 (UmL^{-1}) in response to the concentrations of the pharmaceuticals in aliquots. Many other researchers also analysed detoxification of heavy metals by some fungi (Jelic *et al*., 2012; Da-Luz *et al*., 2013; Yang *et al*., 2013b; Daasi *et al*., 2013). Many other works on some tropical mushrooms are presented in Table 2.4.

2.7 Phytoremediation (Phytotechnology)

This technology is also known as Green technology; it is a technology which involves the exploitation of green plants in soil remediation. It is regarded as an environmentally safe and cost effective technology (Kim *et al*., 2007; Kim *et al*., 2010; Tang *et al*., 2015). Phytotechnology is easy to apply and it is applicable in small quantity (Couto, 2012). Some scientists have also suggested the exploitation of phytoremediation mechanism to degrade, extracts, sequester or detoxify different pollutants; others have used field trials to demonstrate phytomediation of polluted sites (Stephen and Ijah, 2011 and Ukaegbuand Mbakwem, 2015). *In-situ* phytoremediation strategy makes use of plants naturally living or genetically engineered for phytoaccumulation or phytoremediation of toxic substances like heavy metal, radioactive compound, hydrocarbon and some other organic compounds from the soil (Zhou *et al*., 2011).

The plant's mechanism of bioremediation usually employs the root system, this is because it is the plant part that is in contact with the soil, the root mechanisms includes rhizodgrdation, hyperaccumulation, phystabilization or hydraulic control. Many plant's roots have been well documented for special soil remediation mechanism (Gleba, 1999; Bano *et al*., 2015 and Ukaegbu and Mbakwem, 2015). The areas around plant root in the soil are refers to as rhizosphere (Bano *et al.,* 2015).

Table 2.4: Bioconversion of different wastes by mushrooms and other fungi species

Fungal species	Waste Converted	Results	References
Pleurotus citrinopileatus; *P. pulmonarius*	Wood paper and cardboard Industrial waste	Good Basidiocarps growth on the waste and no genotoxicity	Kulshreshtha *et al*. (2013 a and b); Akinyele *et al*. (2012)
P. ostreatus, Volvariella volvacea	Many oxobiodegradable plastics, sawdusts and other ignin riched materials	Planstics degraded, while sawdusts were useds substrates to produce biomass	DaLuz *et al*. (2013); Akinyele *et al*. (2011 and 2012)
Lentinula edodes	2,4-dichlorophenol (DCP)	DCP degradedusing vanillin as an activator	Tsujiyama *et al*. (2013)
Pleurotus pulmonarius	The mushroom remediated radioactive cellulosic based	Mushroom mycellium degraded waste solidifiedwith portland cement acting as barrier against the relese of radioactive	Eskander *et al*.(2012)
	Waste crude oil	The crude oil were mineralised	Olusola and Anslem (2010)
Jelly sp., *Schizophyllum commune* and *Polyporous* sp.	malachite green	*Jelly* sp degraded 99.75%, *Schizophyllum commune* 97.5% and*Polyporous* sp 68.5%of dye in 10 days	Rajput *et al*. (2011)
Coriolus versicolor	PAH	PAHs was degraded using enzymes Poly-R 478, Laccase (lcc), MnP and LiP	Jang *et al*. (2009)
Acremonium	Oil waste	It possesses mechanism to degrade hydrocarbons	Llanos and Kjoller (1976)
Aspergillus	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	Bartha and Atlas (1977)
Aureobasidium	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	Bartha and Atlas (1977); Obire *et al*. (2008)
Candida	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	Bartha and Atlas (1977); Obire *et al*. (2008)
Cephalosporium sp.	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	

Cladosporium	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	Walkerettl. (1973); Bartha and Atlas (1977
Cunninghamella	Hydrocarbons associated with oil spill	It possesses mechanism to degrade hydrocarbons	Bartha and Atlas (1977);
Fusarium sp.	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976); Obire *et al*. (2008)
Geotricum sp.	Oil waste	Crude oil was degraded	Obire *et al*. (2008)
Gliocladium	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976)
Graphium sp.	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976)
Hansenula	Oil spill	It possesses ability to degrade and remediate organic compounds in soil	Bartha and Atlas (1977; 1997); Llanos and Kjoller (1976)
Mortierella	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976)
Mucor mucedo	Oil waste	Crude oil was degraded	Obire *et al*. (2008)
Paecilomyces sp.	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976)
Penicillium sp.	Oil waste	Crude oil was degraded	Llanos and Kjoller (1976); Bartha and Atlas (1977)
Rhodosporidium	Hydrocarbon in aquatic environment	Hydrocarbon contaminants were degraded	Ahearn and Mayers (1976); Bartha and Atlas (1977)
Rohodotorula	petroleum hydrocarbon	Crude oil was degraded	Bartha and Atlas (1977); Obire *et al*. (2008)
Saccharomyces	Oil spill	It possesses ability to degrade and remediate organic compounds in soil	Bartha and Atlas (1977)
Sphaeropsidale	Oil spill	It possesses ability to degrade and remediate organic compounds in soil	Llanos and Kjoller (1976)
Torulopsis	Oil spill	It possesses ability to degrade and remediate organic compounds in soil	Bartha and Atlas (1977)
Trichoderma	clofibric acid, pharmaceuticals such as naproxen, ibuprofen and ketoprofen	97% removal of clofibric acid, naproxen, ibuprofen and ketoprofen removal of plastics, ketoprofen	Llanos and Kjoller (1976); Obire *et al*. (2008); Marco-Urrea *et al*. (2009); Tran *et al*. (2010); Jalic *et al*. (2012); Daasi *et al*. (2013)
Trichosporon	Crude oil	Degraded crude oil in the marine habitat	Ahearn and Mayers (1976); Bartha and Atlas (1977)

2.7.1 Phytoremediation mechanisms

Phytoremediation have been well documented using many plants and it was reported that many plant have different mechanism of actions depending on their type, nature, character or biology of the organism used. Plants are generally ionvolved in several mechanisms as highlighted below;

i. *Phytodregradation or Phytotransformation:* the process which plants break down pollutants through the secretion of enzymes or other metabolites.

ii. *Phytostimulation or Rhizodegradation*: the process in which plants degrade organic pollutants in the rhizosphere with the help of soil microorganisms.

iii. *Phytostabilization or Phytoimmobuilization*: the process throug which plants use to immobilize some soil contaminants and improve their bioavailability by developing specialised cells/tissues for ther to prevent them from re-entring or translocated to another part or into the environment.

iv. *Phytovolatilization*: in this mecaqnism, the plants absorb the pollutants reduce the toxicity and then transpire/release them from their tissues in modified forms into the the atmosphere. This in most times passes through the leaf o plants.

v. *Phytoextration or Phytoaccumulaton*: this mechanism is the use of plant for remediation heavy-metal pollution. The plants known as hyperaccumulators usually absorb/uptake the soil contaminants and degrade/detoxify or translocate along their cells/tissues with less damage; many of such plants usually develop special tissues or some specialised cellular structures for this purpose.

vi. *Rhizofiltraton*: this process is similar to phytoextraction, the heavy metals are extracted from contaminated water mostly through the roots and translocated through the plant tissues.

Table 2.5: Summary of Phytoremediation Mechanisms

Phytoremediation method	**Mechanism of action**	**Medium of reaction**
Phytoextraction	Uptake of metal concentration into the plant tissue through direct uptake	From the soil
Phytotransformation	Uptake of compounds by plants and degradation through the tissue	From the surface water ground or underground water
Phytostabiliation	Precipitation and and deconcentration of metals through the root root exudates	Hydrocarbon contaminated soils, mine tailing, or ground water
Rhizofilteration	Plant root functions in uptake of metals	Soils or surface water
Rhizodegradation	Reactions by plant roots with microbes within the rhizosphere to enhance degradation of contaminants	Rhizosphere
Rhyzovolatization	Uptake and evaporation of compounds like volatile hydrocarbons (e.g BTEX), selenium or mercury	Soils or groundwater

2.7.2 Limitations and selection of plant for phytoremediation

Phytoremedation is a very important as it uses the plant mechanisms, but selection of the plant may be affected by climate conditions and seasonal cycles. It may be very difficult to establish seed germination/plant's cover in an already polluted envonment and phytoremediation is limited to areas covered by the root (rhizosphere) or the depth at which the root can cover. Generally, many factors can affect the use of plants for soil remediation these facors are given below according to Sharma (2011):

i. The tolerance level of plant in reation to the type of pollutant present on the site
ii. The biology and the plants attribute (growth rate, biomass, root system etc.)
iii. Level of ontamination (i.e. concentration of the pollutants)
iv. The plant's tolerance and response (ability to adapt)
v. Habitat preference (terretial, arid, semiarid, aquatic plants)
vi. The soil physicochemical roperties (pH, salt level, CEC, nutrients etc.)
vii. Root characteristics and so on

There are so many situations where phytoremediation may be generally impossible or slowed down. For examples metalsthat are not inbioavailable form to a plant might not be possibly/easily remediated by the plant. More also, it may be difficult for plant to act if soil contaminants or metals bind or form complexes with the organic and as well the hydrophobic contaminants are much more difficult to pytoremediate. Some contaminants can affect the plant's responses by inducing the production of some metabolites such as ethylene thereby inhibiting the roots and plants' growth.

2.8 Rhizoremediation

Rhizoremediation is the breakdown or extraction of soil pollutants by the plant root system, the concept focuses more on the root interaction with the soil other than the whole plant. Literarily the plant root is also called rhizome and area surrounding the root is called 'Rhizosphere'. Many pollutants around the rhizosphere react with the root system and breakdown through the process of rhizodegradation this is often made possible by microorganisms inhabiting the rhizosphere. The root, soil and microbial interaction are major driver of rhizodegradation (DenHerder *et al*., 2010).

Researchers are now interested in developing improvements in phytoremedition using the root technology, the initiation of root hairs, apex/cap and cellular chracteristics such as the casparian bands of the endodermal tissue and so on are very important factors to be improved in plants to be used for phytomediation (Hinsinger *et al*., 2011). The green technology considers root as mediators for nutrient uptake, remediation efficiency, plant respose and tolerance (deDorlodot *et al*., 2007; Lynch, 2007; Ghanem *et al*., 2011). The roots of plant are usually the main determinants of soil uptakes (usually water and nutrients); they determine the plants sensing of chemicals in the soils, this is also essential in detecting interactions between the soil pollutant and the root's chemotropism. Many plants have been documented as hyperaccumulators for metal extractions in soils (Alford *et al*., 2016), this was also confirmed by Liu *et al*. (2010 and Jaak *et al*. (2015). Unfortunatey, there is dearth of knowledge on the mechanism which determines the action of hydrocarbons, pestiocides and or heavy-metals on the root system/initiation (Lequeux *et al*., 2010).

Also, Remans *et al*. (2012) investigated effect cadmium (Cd) and copper (Cu) ions on root in combination with some selected bacteria, they observed that the plant produced more root in cultures with bacteria and so do reduction of heavy-metal concentrations. Graham *et al*. (2012) observed increase in heterotrophic respiration by plants' through the roots in relation to rhizosphere interactions with the soil nicroorganisms which are very important factors in root response. In addition, Valentine *et al*. (2012) demonstrated extenstive

interaction of soil and root elongation, the revealed how Scotland soil types, their physical and chemical properties affected root elongation.

2.9 Myco-rhizodegradation

This is a compound word which we coined from two words 'Mycoremediation and Rhizodegradation'. The word explains the combination of fungi and plant cohabiting together to bioremediate contaminants and this set off a new insight to a biological clean technology. Several fungi are in habiting the soil and playing different roles in their ecological niches, as saprophic organisms, fungi require nutrients (macro or micro nutrients) for survival and with this regard many of them produce enzymes and some other produce metabolites that predigest or pre-soften organic compounds before they can be absorbed or utilised as carbon source with this, fungi enzymes have become very important tool in decompositions of different soil contaminants. Many soil fungi have developed mechanisms through wish they degrade soil pollutants in other to utilize them as nutrient. It has also been revealed that many fungi degrade soil pollutants as an adaptive mechanism in other to survive thbe polluted environment. In addition, the plant root is the part of plant which is in contact with the soil, and this is the plant's part thbat majorly drives phytoremediation.

Adequate symbiotic association between the plants's root and soil microorganisms as been established to promote rhizoremediation. Establishment of synmbiotic co-habitation of plants and fungi as well enhances adequate mycorhizodegradation/ mycorhizoremmediation of polluted soils. Many soil contaminants such as heawvy metals can affect the root initiation in plants thereby affecting the plant biomass and inducing stresses in plants, this is also a major factor affecting the phytoremediation however, it has been reported to be using biaugmentation methods (using plant growth promoting microorganisms) in plants rhizospheres as reported in the study of Abhilash *et al*. (2012). Phytoremediation mechanisms by plants in heavy-metal removal can be enhanced by supplentation of heavy metal-resistant microbes for enhanced extraction and ranslocation, and this in addition will reduce heavy metal phytotoxicity (Li *et al*., 2013; Ukaegbu and Mbakwenm, 2014). Plants and fungi in symbiotic association has been established to promote phytodegradation of

many POPs, root of alfalfa and rye grass for example were shown to increased the rhizobacterial population around the root and this enhanced the crude oil and hydrocarbon degradation (Nie, 2011; Dadrasnia and Agamutu, 2013; 2014).

Plant growth promoting fungi usually enhance nutrient uptake in plants and the fungi in turn usually benefit from root exudates. Plant-fungal symbiotic association has been employed for faster soil remediation and better ecological sustainability (Rhodes *et al*., 2013; Bano *et al*., 2015). This association also enhances the bioavailability of soil contaminants for example; the hydrophobic pollutants can be desorbed and converted from solid to liquid form thereby reducing their concentrations (Semple *et al*., 2003; Alexander, 2005).

2.10 Spent Mushroom Compost (SMC) and its Potential as a Source of Fertilizer and Bio-remediating Agent

Spent Mushroom Compost (SMC) is the waste leftover from the substrate used after harvest of the cultivated mushroom crop. It is also described by Fasidi *et al.* (2008) as the waste leftover after mushroom cultivation and harvest. SMC is a byproduct substrate from mushroom cultivation; it is a compost which can be used as manure to enhance plants growth according to Chang *et al*. (2000) and Danny (2002), mushroom growing is environment friendly as it makes use of wastes generated from agriculture, horticulture, poultry, and brewery and so on. The use of SMC in bioremediation is also an added advantage. There are several research and trials which prove SMC as valuable product for effective bioremediation and nutrient enhancement for soils. This therefore presents a cheap method of bioremediation (Orji *et al.*, 2012; 2013) and recently many scientists have reported SMC to be a good organic fertilizer for soil supplementation. The advantages of using SMC as soi amendment onver synthetic fertilizers is that slowly releases it nutrients as it decomposes with no residual effect, it also consists and attracts many beneficiall microorganisms for crop plant's growth (Beyer, 1999; Zebulun *et al*., 2011; Monica *et al*., 2012; Okerentugba *et al*., 2015).

Spent mushroom substrate usually acts as compost in soils to improve the soil quality and nutrients (Kadiri amd Mustapha, 2000; Chang *et al*., 2010; Jonathan *et al*., 2012a and b;

Garcia *et al*., 2014). This compost is easily biodegradable and stabilizes soils against erosion, dampens emperature fluctuation, poor water infilteration/retention, pathogens and provides nutrients to plants (Westerman and Bicudo, 2005; Adhikari *et al*., 2009; Ennis *et al*., 2014). Generally, composts usually en hance the soil nutrients and enhance the soil's microbial populations (Adieze, 2012a), some other researches have also reported the potentials of SMC in plant pest and disease managements (Adieze *et al*., 2012b), they also observed that SMC enhaced the microbial population and enhances active actions of other soil orgnisms like worms, centipedes, sow bugs, and some other critters in soil and balance the soil ecology. It enhances the soil pH, CEC and nutrients (Jonathan *et al*., 2012a, b and c; Lopez *et al*., 2012 and Garcia *et al*., 2014). It can therefore be summarided for general characteristics of SMC as a good bioremediating agent are that it is capable of:

i. Improving the soil aeration and enhance diffusion
ii. Nutrient supplementation (Humus)
iii. Solutions of Enzymes
iv. Water supplementation
v. pH balancing
vi. Substrate for soil microorganisms

However, soil type and the quantity of nutrients supplemented may affect the bioremediation of such soil. Degradation of soil pollutants is usually enhanced by nutrient addition ad availability of oxygen due to the fact that mostbiodeggraders are aerobic oganisms. Addition of nutrients in bioremediation is termed 'Biostimulation' which focusses on nutent enhancement for activating the microbial activitis duri boremedation (Agarry *et al*., 2012). Okerentugba *et al.* (2015) discovered a decrease in heavy metal concentration in SMC treated crude oil spilled soil and reported that it served as bioaugmenter and acted as ion exchanger. Soil treatements with SMC has also been shown to reduce odours and reduced some organic compounds either in form of volatile or semivolatile, heating fuel, some explosives and PAHs (Monica *et al*., 2012). It was reveaed that SMC binds and forms complexes with them and reduced their concentrations as also observed for some heavy-metals; this was also affirmed by Beyer *et al.* (1999) and Okerentugba *et al*. (2015).

Supplementation/amendment of polluted soils with SMC as compost provides a save and less cost remediation method for polluted soils, Chang *et al*. (2010) established that the SMC supplemention enhanced higher degradation of PAHs as compared with only bacterial degradation. Aparna *et al*. (2010) also observed that biostimulation of polluted soil using a compost increased the activity of enzyme dehydrogenase from 5.8 µgINTF/gdw under a period of 36 days while Lee *et al*. (2011) reported significant decrease in tota petroleum hydrocarbon (TPH) of a polluted soil after biostimulation and bioaugmentation of the soil using SMC and some fungi strains for 23 days. Lin *et al*. (2011) observed increase in bacterial population (2.0 x 106 with increase of 3.2 x 106 CFU/mL) of a diesel oil polluted soil after one week of treatment wih an oganic compost. In addition, Bento *et al*. (2005) and Dadrasnia and Agamutu (2013c) observed increase in microbial population of apolluted soil after treatement with crop residues. Addition of SMC in remediation crude oil spilled soil was observed by Li *et al*. (2014) to reduce it's PAHs.

Fisseha and Evans (2015) earlier observed by Yang *et al.* (2009) established the action of SMC in improving desorpion efficiency, degradation rate as well as the bioavailabilities of some hydrophobic compounds. Mushroom compost (SMC) usually ehances the hydrophobicity of many oganic componds by forming aqueous system with the pollutant as it decomposes. This mechanism includes the reduction in capillary force, surface and interfacial tension as well as the contact angle; the soluiloization usually takes place through the incorporation of pollutant molecules into the micelle of the SMC (Urum *et al*., 2003; Pacwa *et al*., 2011).

Spent Mushroom Compost (SMC) is today becoming very popular in bioremediation. This have again been confirmed in the work of Pei *et al*. (2010) and Wang *et al*. (2014), it was estabshed that SMC enhance soil microbial population and hence promotes bioremediation, Chen (2005) affirmed that SMC contains abundance of enzymes which are secreted by the fungi extracellulary and can still be useful in bioremediation, biosorbent attributes of SMC for heavy metals were also affirmed in a laboratory bactch system. Fermor *et al*. (2000) observed rapid phase II decomposition of some xenobiotics pollutants such as PAHs, chlorophenols and some aromatic monomers in soils supplemented with SMC while

Hanson (2002) and Ahlawat *et al*. (2006a, b) established dyes and inks degradation extracts from SMC in paper waste management. SMC was also found to enhance the populations and catabolic capabilities of *Streptomyces* sp and *Thermonospora* sp in amended soil (Ahlawat *et al*., 2007c; Monica *et al.*, 2012; Zebulum *et al*., 2011), they also confirmed that SMC that were produced from cultivations of edible mushrooms *Lentinus edodes* and *Agaricus bisporus* enhanced PAH degradation. Ting *et al*. (2011) and Ahlawat *et al*. (2007a and b) also reported that degradative abilities of SMC from *Ganoderma lucidium* in degradation of several pesticides respectively. In addition, reports from the works of Chang *et al*. (2000), those of Rubilar *et al*. (2011) and Carlos *et al*. (2014) who used SMC to enhance the remediation of acide mines and PAHs respectively in contaminated soils.

2.11 Importance of Guinea Grass (*Megathyrsus maximus*) in Bioremediation

Grasses have been widely used for phytoremediation based on their root architecture and function. Grass called *Megathursus maximum* (guinea grass) has been well documented as a hyperaccumulator, phytoremediator or phytoaccumulator (Mager, 2002; Hernandez-Valencia and Mager, 2003; Merkl *et al*., 2006; Waraporn *et al.,* 2013). Jiamjitrrpanich *et al.* (2012) affirmed that *M. maximus* acted as a Phytoextractor for removal of TNT/ZVI in soil at ratio 1:10 while Waraporn *et al*. (2013) reported this plant in phytoremediation of soil polluted with trinitoluene pesticide. Hernandez-Valencia and Mager (2003) reported that their was enhanced germination and biomass of six different plants after phytoremediation of 3 % crude oil polluted soil using *M. maximus*.

Many other grasses have also been reportedly capable to remediate contaminants in soils, little examples are the reports of Nichols *et al*. (1997) and Njoku *et al*. (2009) which affirmed that *M. maximus* can significant remediate of TPH in the vegetated soils. Merkl *et al*. (2005) also reported that grass called *Calogonium mucunoides*, *Stylosanthes captata* and *Centrosema brasilium* which are all leguminous plants and other other grasses such as *Brachiaria brizantha*, *Eleusine indica* and *Cyperus aggregatus* in phytoremediation of contaminated soils, they reported that many grasses can tolerate and reduced the heavy-metal concentrations in soils. Merkl *et al*. (2005) also established the aabbility of *B. brizantha* and *C. aggregatus* grasses in the remediation hydrocarbon polluted area.

2.11.1 Guinea grass (*Megathyrsus maximus*) and Phytoremediation

The root of *Megathyrsus maximus* formally known as *Panicum maximum* ramifies the polluted soils more than the taproot system; it covers more area of the top soils where contaminats are more aboundant. Notable grass with extensive fibrous root system is *M. maximus*, it is a very useful grass as it is becoming known as phytoextractor for heavy-metals Pradhan *et al.* (1998) for example reported higher removal of PAHs in soils planted with *Panicum virgatum* (switchgrass) and *Schizachyrium scoparium* (Bluestm grass) were grown. Yusuf and Fawole (2006) established that guinea grass enhances the Phosphorus level in soil and also enhance the maize yield.

Guinea grass was reported by Orgi *et al*. (2015) to be a good bioremediation agent for the cleaning of crude oil hydrocarbons in tropical soils, they observed that the plant with some inoculated microorganisms (bioaugmentation agents) yielded better phytoremediation. This plant was also suggested to be use in stoping soil erosion by planting it in slopes as a result of its extensive root and it has been used as a fodder plant. This plant is hghly resistant to drought and can survive fire attacks better than some other plants; it provides a seed for birds such as Mmunials from its seeds and its leang leaves provides shielter for small animals and some nesting birds such as Bbaya Weaver (*Ploceus philippinu*s).

2.12 Status of spent mushroom composts in Nigeria

Nigerian government has not been involved in mobilizing the farmers to use the SMC as organic fertilizer and much work on the use of SMC has not been carried in Nigeria. However, some scientists in Nigeria have reported the importance of this substrate. Jonathan *et al*. (2011, 2012a, b and c) investigated the ability of SMC of *pleurotus ostreatus* in proving the growth of some vegetables such as *Abelmoschus esculentus*, *Lycopersicum esculentum*, *Capsicumannum* and *Capsicum chinense,* as soil conditioner and their results revealed positive response of the vegetables to SMC amendments in soil. Also, Kadiri and Mustapha (2010) reported positive response of cowpea and vegetables to both fresh and weathered SMC amendment in terms of growth and yielding.

However, modern cultivation of mushrooms is still growing in Nigeria, there are few companies involved in mushroom growing which could not meet the demands from the populace (Jonathan *et al*., 2011) therefore, the SMC produced annually from these mushroom industries are also not large enough to meet the inorganic farm yard manure demand of the farmers. However, considering the importance of vegetables and application of SMS in enhancing their yield in Nigeria will be of a great importance. Spent mushroom compost which is produced from many mushrooms is well documented by many Nigerian scientists to biotreat some oil contaminated soils and some PAHs associated with drill cuttings under laboratory conditions (Reuben, 2011).

2.13 Nigeria as an Oil Producing Nation

Nigeria which is a West African country shares borderline together with Niger republic in noth side, Chad and Cameroon along the eastern part and the Republic of Benin in the west and on the south part laid its Gulf of Guinea coast along Atlantic Ocean. It is rich in fertile land and natural resources, it was reported that Nigeria has about 61 million hectares of land mass that can be used for cultivation which consists about 280 km3/yr of total renewable water resource (FAO, 1995). Nigeria agricultural system was self-sufficient before the civil war (known as Biafran War) that occurred in July 1967 to January 1970, but unfortunately today, the country's increasing population has gone above the land resource carrying capacity due to inadequate utilization of soils and low level of modernised food cultivation technology (FAO, 1987).

Crude oil resource exploration in Nigeria started as far back as decades ago and since then, series of events has happened, the country's crude deposition was first noticed 1908 when some German surveyors of the Nigeria Bitumen Corporation noticed their might be oil deposits in some areas in southern region of the country while they were prospecting for tar sand deposits, they proposed their views then but it was obstructed by the World war 1 that broke out in 1914, no oil resource exploration during this period until 1938 but the country gave the Royal Dutch Shell (former British Petroleum and Shell D'Arcy) right to prospect

the country's oil after the war. This again was obstructed by Second World War in 1939-1945 and it thereafter resumed after 1946 in Niger Delta regions.

The Niger Delta are the states which are having crude oil deposits in Nigeria, these states comprise about 185 local government areas across the states with over 800 communities. More than 900 oil wells have been dug in these areas (Osuji and Onojake, 2004). This region is located at the tip of Guinea Golf Western Coast of Africa and on the South/South zone of Nigeria containing about 31 million and 75, 000 km2 total land area that makes up about 7.5 % total land mass of Nigeria (Young, 2012), located (Haack *et al.,* 2000; Doust, 1990). In 1951 many oil exploration wells were made, Shell D'Arcy enjoyed complete monopoly over this during this period (1938-1955) but Mobil producing Nigeria Limited got licence in 16th of June, 1969. The famous Bomu Oilfield was discovered in 1958 with an estimated recovery of about 0.311 billion Euro barrels and over 0.608 billion oil barrels' equivalent including gas and Oloibiri and Afam oil field started in 1970 as well (Vassiliou, 2009). Nigeria as the number 12 of the wold's largest petroleum and it is the 8th largest oil exporter, Nigeria joined OPEC in July 1971 and since then crude oil has been substantially improving the nation's economy for over ten years. An estimate of $600 billion has been gotten from oil since 1960's which the nation (Wurthmann, 2006) is above 40 % of the total gross domestic production of this country and takes more than 80 % total income.

Niger Delta basin (Niger Delta province) is complex with about 300,000 km^2 total area and sediment fill of about 500, 000 km3, it is known to among the world's most studied delta systems (Obaje, 2009). Obaje (2009) reported that, more than 1,182 oil/gas wells have been drilled in over 400 documented fields, about 2 million barrels is produced in Niger Delta basin on daily basis and it is suspected that the entire system may have oil of over 34.5 billion barrels and 94 trillion ft^3 of natural gas. However, according to UNDP oil exploration has high influence on environmental quality and sustainability in this region, larger percentage of the populace depend on exploitation of naturally available materials agriculture, fishing and forest products to source for food and improve their livelihood, but today the fundamental development and wellbeing of people in the area have been highly affected due to pollution caused by crude oil exploration.

2.13.1 Petroleum contamination in Nigeria and remediation

Oil spills, gas flaring and waste dumping has affected the Niger Delta populace in many aspects, the soil, water and air quality has been greatly damaged by the pollution. In 2006, a team of Nigerian and inteatonal experts carried out a search and reported the Nigerian Niger Delt areas have become among the word's most impacted ecosystems as a result of repeated environmental contaminations associated with oil exploration. Oil exploloration has severelly affected the livelihood and health of native communities (Stephen *et al*., 2011). Oil pollution in Nigeria has been increasing, over 2, 567, 966 crude oil barrels had spilled between the years 1976 to 2000 which occurred in about 5,733 incidences while unfortunately only 549,060.00 barrels were recovered, this means that the remaining barrels of about 1,820,411 were lost (Edoho, 2008). Other examples of incidents that have increased oil pollution in this area includes blowouts and leakages in pipelines, there was incidence of pipeline blowout from the Bomu Shell-BP II well in 1970, another one is the blowout around SARAP (which is now known as ELF) at Obagi in 1972, others includes Texaco blowout at Faniwa community in 1980 and the Agip pipeline leakage a Oyakama community in 1980 according to the report of Daniel-Kalio and Solomon (2002).

Oil spill usually happens as a result of equipment failure, oil blowouts, and leakages from weak or corroded pipelines network, it also happens as a result of sabotage, operational mistakes, and pipline vandalization by the militants. However, the pollution by vandalization increasing yearly in Nigeria due to civil instability (Nwilo and Badejo, 2006), and this increase has potential threats to health of the populace in these communities. Many effect of massive oil pollution on plants of the coastal areas have been documented (Blumer, 1972), most effect are defoliation and intense motility of mangroves in swamps (Linden and Jermelov, 1980). Oil spillage contamination of agricultural soils in the Niger Delta was first reported in 1971 (Odu, 1972; Snowden and Ekweozor, 1987) Hydrocarbons associated with crude oil pollution usually exert acute or chronic toxicity effects of soil and microfloral perties and microflora (Odu, 1972; Snowden and Ekweozor, 1987; Benka-Coker and Ekundayo, 1995; 1999 and 2000; UNEP, 2011). Presence of petroleum hydrocarbons in high quantities in soils may result in anaerobic condition in soils and hence affects soil fauna and floral (Osuji and Onojake, 2006).

Many researches have been conducted to investigate negative health and ecological (such as the soil profile, plants, animals and microorganisms) implications of excessive crude-oil pollutions. These are usually done based on responses of biotic component to crude oil through growth and population; in palnts for example, growth, yield, germination or root proliferation are usually studied (Agbogidi, 2010). Agbogidi (2010) affirms that hydrocarbons can result in reduced crop yielding as also observed by Osuji and Onojake (2006) in the Niger Delta areas. Also, impacts of oil exploration process and associated pollution on humans have not been well reported and understood in Niger Delta, although many researchers have linked this with some respiratory disorders (Lyons *et al*., 1999; Rodriguez-Trigo *et al*., 2007). Polycyclic aoromatic hydrocarbon were are also of significance concern in crude oil pollution due to their carcinogenic, mutagenic, pulmonary and circulatory toxicity, they are known to be lipophilic in human tissues and can be easily bio-accummulated (Anderson and Lovely, 1999).

Oil spill has also been associated with chronic respiratory problems and damages on genetic materials in exposed humans (Perez-Cadahia, 2008; Rodriguez-Trigo *et al*., 2010). These researchers reported that crude oil can cause bronchial infection in children, skin irritation, chromosomal disorders, cancer and tumours (Perez-Cadahia, 2008). Ogoni people, has been complaining repeatedly has the oil pollution impacted directly upon their lives, their environment and social lives (Boele *et al*., 2001; Baird, 2010). The oil explorating companies in Nigeria have failed to adequately implement remedial actions to restore many polluted soils in Niger Delta area of Nigeria over the past 50 years. Unfortunately, there are no adequate government statutory rules and regulations to adequately protect the environment.

Nigeria government have not been involved in mobilizing the workers or encourage the use of living organisms (plants or animals) or SMC as means of remediating soil contamination and much work has not been carried out with SMS in Nigeria. However, there still need for adequate soil remediation mechanism/technology for adequate removal/remediaton of hydrocarbon contaminants in soils. Many Nigerian scientists had previously suggested the use of so many organisms in remediation of contaminated soils, while many others

suggested the use of orgnic wastes in form of composts like poultry wastes, cow dung, pig dung, goat and horse dungs. Many researchers such as Orji *et al.* (2012 and 2015), Adams *et al*. (2014) as well as Choron *et al*. (2012) and Adams *et al*. (2014 Choron *et al.* (2012), Adams *et al*. (2014) and Hamzah *et al*. (2014 Hamzah *et al*. (2014) and so on had reported several bioremediative mechanisms for removal of hydrocarbons in Nigeria soils. Biostimulation (the use organic manres/fertilizers) and Bioaugmentation (introduction of biodegrading microorganisms) were reorted as the best environment friendly and cost effective soil remediation. Ukaegbu and Mbakwem (2015) some presented a significant reduction in petroleum hydrocarbon in Nigerian polluted soil treated withn plant and rhizobacteria.

2.13.2 Environmental regulations for oil and gass in Nigeria

In Nigeria, Niger Delta is seriously combating environmental pollutions as a result of oil exploraton, larger percentage of the land area of land is degraded with oil spill and soil and underground water in many cases are also affected. However, the government has developed different strategies to enhance environmental safety with oil producing activities in the nation, several regulations to guide the oil/gas sectors were created in the country, these laws started in 1956 with the Oil Pipeline Act and amended in June, 1965, others are Mineral Oil (Safety) Regulation prescribing roles on drilling, storage and handlings by companies it was first created in 1963, another one is Oil in Navigable Waters to guide against oil spill injection into water (1963), Petroleum Acts to guide the storage, transport, import, production, blending, or reclaiming of crude oil (1969) and Associated Gas Reinjection Acts for gas (1979). Some other regulations were also enacted in the 1990's.

The establishment of FEPA in Nigeria act of 1988 impose penalties and enforce regulation mechanisms on the petroleum industries and different oil companies in the nation, this a bit improved the Nigeria legal environmental law as at 1991, the pollution Abatement in Industries and Facilities Generating Wastes (PAIFGWA) was later enacted as rregulation in Nigeria under section 37 FEPA act for the control of industrial discharge, another one is the National Effluent Limitation Regulation which was first created under S.I. 8, 1991 but later presented un der the section 40 of Environmental Protection act and the Environmental

Impact Assessment degree No 86, 1992. Moreover, other laws were later created such as National Policy on Environment now under the ministry of environment earlier created 1989, revised in 1999, Environmental and Standard for Petroleum Industries in Nnigeria (EGASPIN) was enacted in 2002 to control pollutions by various petroleum industries and creation of Department of Petroleum Resources.

Establishment of these laws and regulations aimed at impact of different oil exploration activities in the country and the populace quality of life but these are grossly inadequate, ineffective and are poorly implemented (Ogri, 2001) explained that there are different factors that prevent effective management of these regulations in Nigeria's, leading to failure and adequate management of oil multinational company's activities. These regulations are often sabotaged by inadequate or limited technology and little consideration of public opinions (Doyle *et al*., 2008). There is need for host community's involvement in environmental decision making with adequate sustainable development goals.

Generally, we can say that the oil companies in Nigeria have failed to implement adequate oil production measures with good control of resultant pollution in connection with the operations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and sites of polluted soil samples

Four different polluted sites; Ugboroko in Delta State, Owo in Ondo and Ibadan in Oyo States, Nigeria were investigated in this study. These sites were selected based on their history of repeated exposure to pollution over many years (Figure 3.1). The sites were located using the Geographical Information System (GIS) and sampling spots were determined using purposive sampling technique. The site and soil locations are as follows:

3.1.1 Crude oil polluted site (S1)

Soil samples were gotten around the crude oil well at Ugborodo community ($5^{o}34^{‟}60$N, $5^{o}10^{‟}0$E). This community consists of three quarters; Orgonoko, Kana and Arunton, situated in between the River Escavos and Atlantic Ocean at Delta State in Nigeria called Warri community. The sample site has been exposed to continual oil-spillage, situated around the oil processing facility where there is more runoff opening.

3.1.2 Black oil and engine oil polluted site (S2):

The second contaminated site is the mechanic village, at Bodija Ibadan, Oyo State of Nigeria ($7.44^{0}12$ N, $9.06^{0}13$ E), this site represents the site that is contaninated with black oil. It is an area which has been in continuous operation as repair site for several automobile over 20 years.

3.1.3 Organophoshate pesticide polluted Site (S3):

This site is an area around effluent run-off sewage pipe 'a' from a local pesticides producing and marketing company in Owo Nigeria (7^{o} 11'0" N, 5^{0} 35'0" E). Analysis of soils sampled from this area revealed more concentration of 2, 2-Dichlorovinyl dimethyl phosphate commonly called Dichlorvos, DDVP or Sniper which is an organophosphate pesticide, and the soil was chosen as S3 for this experiment.

3.1.4 Organochlorine pesticide polluted Site (S4):

This site is an area around the effluent run-off sewage pipe 'b' from a local pesticide (Lindane) producing and marketing company at Owo Nigeria (7^{o} 11'0" N, 5^{0} 35'0" E). The soil analysis reveled that the sample contained more of Gamma-hexachlorocyclohexane popularly known as Lindane which is an organochlorine pesticide, it is packaged by the company as 'cyperazine' because lindane used has been barned. The soil was chosen as S4 for this experiment.

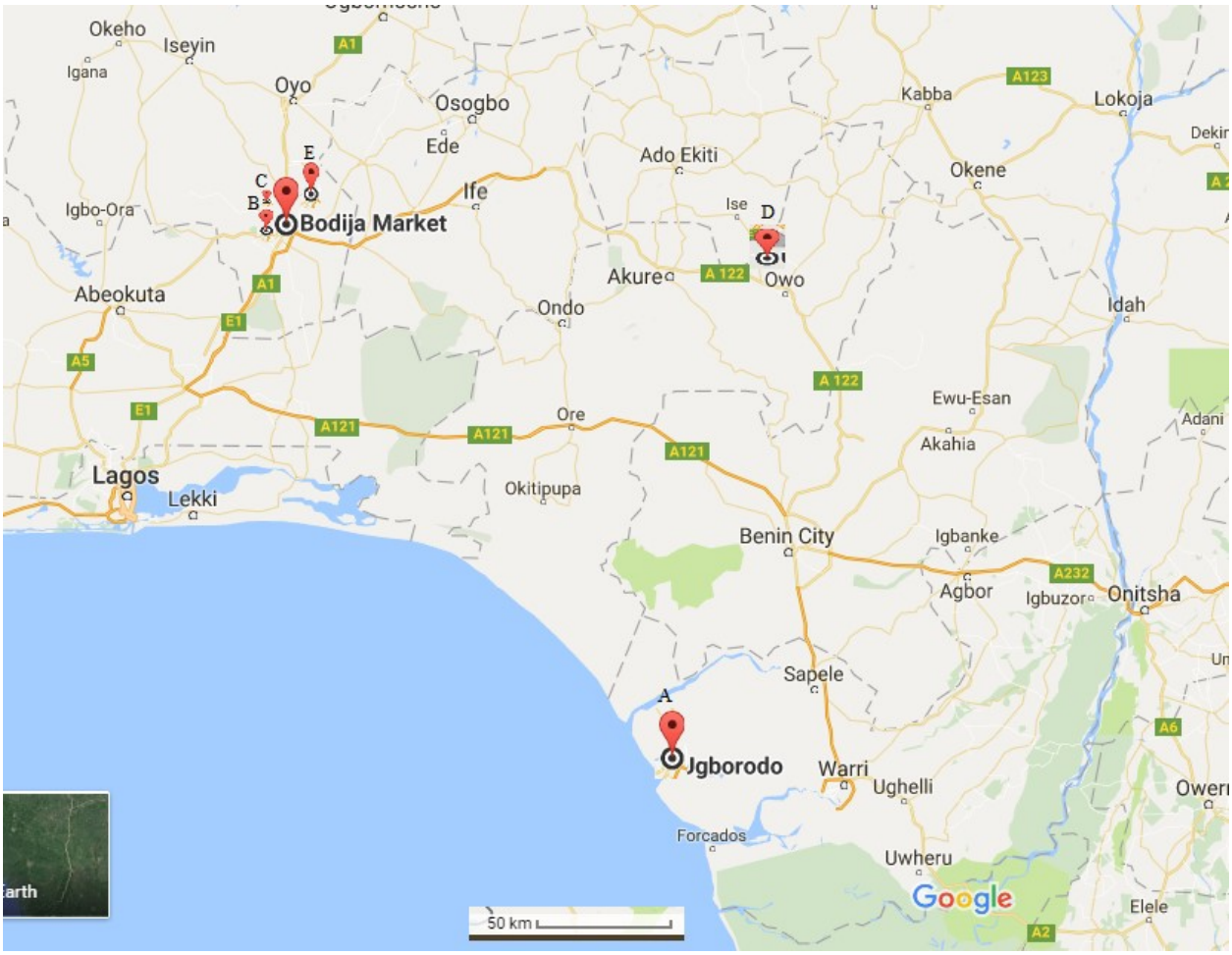


Figure 3.1: Locations of soil samples and study site; Location A: Ugborodo community, Nigeria (5°34‟ 60 N; 5°10‟ 0 E); Location B: Mechanic village, Bodija Ibadan, Nigeria (7.44902° 12” N, 3.887906° 13” E), Location C: Bodija Ibadan Nigeria (7.440775 °4” N, 3.916457 °8” E), Location D: Location C: Bodija Ibadan, Nigeria (7.440775 ^{0}4” N, 3.916457 ^{0}8” E). Location D: Pesticide producing and marketing company at Owo, Ondo State, Nigeria (7^{0}11 0” N, 50 35’ 0” E); Location E: University of Ibadan, Nigeria. (Source: Google Earth map).

3.1.5 Collection of soil samples

Polluted soil samples from the sites included the bulk soil sample and rhizosphere soil sample. Five kilograms of the bulk soil were taken from 20 different samping points on each site at the depths of 2-4 cm, the samples were mixed together to have a composite sample and packed inside a sterile bag. Soil samples (rhizosphere soils) were also obtained from tussocks of 10 grasses (100 g each); this was done by uprooting the grasses growing on the site and the soils that are attached to the root was collected in sterile cryoval bottles and labelled based on the location of collection. The collected soil samples were then transferred to laboratory for sterilization with hot steam.

3.1.6 Chemicals and standards used for fungal tolerance test

The hydrocarbon grade used for fungal tolerance was the Nigerian Bonny Light crude oil, spent black oil was gotten from a mechanic shop while the pesticides; 2, 2-Dichlorovinyl dimethyl phosphate and Gamma-hexachlorocyclohexane were of analytical grades ordered from Sigma-Aldrich Canada, Ltd. 2149 Winston Park.

3.2 Production of Spent Mushroom Compost

Spent Mushroom Compost was used as a biostimulatory agent in this study; it was produced through cultivation of an edible mushroom called *Pleurotus ostreatus* using the method of Fasidi *et al*. (2008). This started with the tissue culture from a young basidiocarp (mushroom), followed by mother spawn production, substrate preparation, and inoculation.

3.2.1 Production of *Pleurotus ostreatus* mycelium through Tissue culture

Fungal tissue culture was done by excising a very small tissue aseptically from the pileus of a young freshly harvested mushroom fruithbody using a sterile scapel, and this was aseptically inoculated in a prepared sterile Potato Dextrose Agar (PDA) plate used as growth medium. The plate was then transferred in incubator set at 30±2 ^{0}C for 5 days after which the fungal pure culture was used to produce the mother spawn.

3.2.2 Production of mother spawn

The mother spawn was produced from wheat grains; the wheat grains were briefly rinsed in distilled water, packed in four (4) 500 mL glass bottles, filled up with distilled water, covered up and kept overnight. Excess water was drained off the bottles, the grains were mixed with lime (2 % w/w) and all together sterilised in authoclave at 121ºC for 30mins. The bottles and their contents were allowed to cool down and inoculated with pure mycelial culture of the mushroom. All bottles were placed in incubator (30 ºC) until the grains were fully ramified (mycelia run over the whole surface and also permeate the entire wheat grains).

3.2.3 Substrate preparation (Composting), sterilization and inoculation

The substrate was prepared using *Gmelina arborea* (gmelina) sawdust; the sawdust has been previously composted. The composting was done according to the method of Diaz *et al*. (1996) by mixing the sawdust with 10 % poultry waste; this was kept in pit, and covered up with polythene nylon for one month. Thereafter, the composted saw dust were mixed with little water and additives (1 % lime and 10 % rice bran), this was packed in autoclavable polythene bags (500g per bag). The mouth of each polythene bag was tied with rubber band and pasteurized in drums for 6hrs, the bags were aseptically inoculated with 250 mg of mother spawn and arranged in the mushroom house at room temperature (25-30 ^{o}C) until they were fully ramified, the tip were opened, until fruit body (mushroom) emerged. The mushroom was harvested and the compost left was the SMC.

3.3 The test plant

The test plant (a grass) used for this experiment was *Megathyrsus maximus* Jacq. (Jacob and Simon), it is commonly called Guinea grass. This was selected as test plant based on its popularity and common in Nigeria, it is usually seen growing on polluted environment and it has been well reported as a phytoextractor due to its massive extensive root system. Treated commercially available guinea grass seeds (GuineaClean®) with germination index 98 % used in this study were bought from an agro care company at Ibadan Nigeria.

3.4 Isolation of fungi associated with the rhizosphere of plants growing on the polluted sites

This was carried out using Potato Dextrose Agar (PDA) in the Mycology Laboratory at the Botany Department, University of Ibadan, Ibadan, Nigeria (7°26'6.4531" N, 3°54'51.5254" E). Ten grams of rhizosphere soil samples were weighed aseptically into 90 mL of sterile distilled water. The mixture were homogenised and serially diluted subsequently up to 10^{-3} fold after which 1 mL of the diluents were inoculated on sterile PDA plates (in which has been prepared with streptomycin sulphate). The inoculated plates were incubated at 25 ^{0}C for 4-7 days. After incubation, distinct colonies were subcultured to get pure culture of the isolates.

3.4.1 Characterisation of the rhizosphere fungi isolated from the samples

The isolated rhizosphere fungi were characterised and further utilized synergitic fungi-SMC-plant degradation experiment in this study; they identified based on morphological and genetical methods.

3.4.1.1 Morphological characterisation of the fungal strains

This was done by studying the growth, microscopic and macroscopic characteristics of the isolated fungal strains. They were grown on PDA plate and their mycelial growth morphology was recorded based on the colour of mycelium on plate, plate underside colour, colony diameter, colony serration, exudate, sclerotium size and shape. In addition, the miscroscopic features such as seriation, vesicle, stipe, conidia, ascocarp, ascospore, and cleistothecial wall of the strains were studied by mounting strand of fungal hyphae on slide and observed in an Olympus microscope (BX51) with sigma scan.

3.4.1.2 Molecular identification of the rhizosphere fungal strains

3.4.1.2.1 Extraction of total genomic DNA

This was done according to the methods of Raeder and Broda (1985) and Fan *et al*. (2014) using ITS-Amplification. The Internal Transcribed Spacer gene was amplified in each strain by first extracting the fungal DNA using the conventional cTAB method with some modifications. The prepared extraction cTAB buffer contains 50 mM tris HCl at pH 8.0, 100 mM of EDTA and 150 mM of NaCl. The buffer was heated in heat block 600 µL and was added to fungal mycelium inside crucible grinded briefly with pestle and recollected in 1.5 mL tube. The tube was briefly vortexed followed by repeated spinning at 6,000 rpm for 10 min until all cell debris were removed. The supernatant was then decanted in Eppendouf tube containting 400 µL of Isopropanol to make 1000 µL and kept in -20 ^{o}C freezers for 12 hrs. The mixture was thereafter spinned for 20 minutes at 12,000.00 rpm under 4 ^{o}C to crytallize the DNA content across the wall of the tube while the other fluid was discarded. The DNA pellets were washed (using 70 % ethanol), respinned, air dried and then diluted with 40-60 µL TE to preserve the DNA, the quantity and quality of DNA extracted were assessed using NANO drop spectrophotometer and Gel electrophoresis respectively and then kept in -80 ^{o}C for further use (Fan *et al*., 2014) .

3.4.1.2.2 PCR-Amplification of ITS gene

The gene amplification was done on rRNA region of 18S (1609-1627) and 28S (287-266) using ITS1 and ITS4 combination, the gene was amplified in all the selected fungal strains according to the protocols of Al-Nasrawi (2012). The primers used were made up of the following sequence:

i. pITS1_F (5'-TCC GTAGGTGAACCT GCCG-3')
ii. pITS4_R (5'-TCCTCCGCTTATTGATATGC-3').

Isolated genomic DNA from each fungus was used to prepare a 40 µL reaction volume. The total composition of the 40 µL reaction volume in PCR tube contained 17.2 µL Enzynomic REDE master mix, 1.5 µL each for both primers (20 ng/µL), 2 µL fungal genomic DNA and 14.8 µL DEPC water. The mixture was then transferred to the chamber of thermal cycler (Peltier MS, USA model PTC 200) with preset amplification conditions given as follows:

Initil extention temperature of 94 ˚C
Initial denaturation temperature of of 94 ˚C/30s
Anneal temperature of 55˚C [1min], } 35 Cycles
Extention 72 ˚C/1.5 min
Final extention temperature of 72 ˚C/7 min
4˚C [infinity]; End

Quality of the amplified PCR product was assessed on gel-electrophoresis while its quantity was measured by loading 1 µL of the amplified product on Nanorop spectrophotometer at light absorbance of 260 and 280 nm respectively (Al-Nasrawi, 2012). Amplified products of 300 ng above were sent to MDX, Republic of Korea for sequencing.

The sequence result from each fungus was analysed on the NCBI genbank using the sequence BLAST tool which compares each sequence with other sequences already documented in NCBI. Phylogenetic relationship between the rhizosphere fungi and other

related organisms was contructed on dendrogram using MABL (Phylogeny.fr) tool and the strains were also documented in NCBI with generated accession numbers (Al-Nasrawi, 2012).

3.5 Percentage Occurence of the Rhizosphere Fungi

Isolated fungal strains on each site were subjected to the percentage incidence based on the number of occurrence of each fungal strain in the collected rhizosphere soil samples. It was hypothesised that the fungi whith higest percentage incidence are the most dominant ones on the site. The % incident was then calculated according to Jonathan *et al*. (2016a) as presented in equation 3.

$$\% \text{ Occurence} = \frac{\text{Number of incident time for a fungus}}{\text{Total incident number of all fungi isolated}} \text{ X } 100 \qquad (3)$$

Four (4) fungi with most % occurrence above 50 % were selected from each site in this study for further experient/analysis.

3.6 Hydrocarbon and Pesticide Tolerating Potential of the selected Fungi

This test was done to study the levels at which each of the isolated rhizosphere fungi can tolerate and survival hydrocarbon and pesticides. They were exposed to crude oil and two commonly used pesticides; 2, 2-Dichlorovinyl dimethyl phosphate commonly called DDVP or Sniper (an organophosphate pesticide) and Gamma-hexachlorocyclohexane commonly known as Lindane (an organochlorine pesticide).

Response and tolerance of the isolated rhizosphere fungal strains were studied on PDA plate cultures set-up according to the method of Al-nasrawi *et al*. (2012) and Anaisell *et al*. (2014) with some modifications. The growth medium (PDA) was prepared according to the manufacturer's prescription, sterilized and supplied with different concentrations of each of the pollutant as follows:

PDA (15 mL) + no pollutant = control

PDA (15 mL) + pollutant (0.75 mL) = 5 %

PDA (15 mL) + pollutant (1.50 mL) = 10 %

PDA (15 mL) + pollutant (2.25 mL) = 15 %

PDA (15 mL) + pollutant (3.00 mL) = 20 %

This was prepared in six (6) replications, they were mixed properly and poured in Petri-plate with perpendicular line at the underside, and each of the fungi was inculated at the center of the plate where the perpendicular lines meet after solidification in six replications. The fungal inoculant contain 5 µL of solution containg 1 x 10^4 spores /mL, the plates were sealed, labeled and tranfered to incubator set at 30 ^{0}C. To establish the fungal response and tolerance, their radial growth was measured at 12 hrs interval for 7 days.

Data obtained were used to calculate the Dose Inhibition Response Percentage (DIRP) by the fungi in response to the concentration of pollutants in the medium. The DIRP was calculated according equation 4 below

$$\text{DIRP} = \frac{\text{Initial radial growth rate} - \text{Final radial growth rate (Reduction)}}{\text{Initial growth rate}} \text{ X } 100\ \% \quad (4)$$

3.7 Detection of Putative Degrading Genes in the Rhizosphere Fungi

To affirm the degradative abilities of the selected fungi, genes that encode Peroxidases, Laccases, Catalases, α-Galacturoxidases, Phosphosterases and Catechol 1, 2-dioxygenases were studied in each of the rhizosphere fungi. These fungal genes are known with wide coverage for production of several cassettes of enzymes which catalyses the degradation of several xenobiotics, most of these genes have earlier been reported in *Phanerochaete chrysosporium* by Tempelaars *et al*. (1994) as well as Brooks *et al*. (1993).

Presence these genes were tested in each fungus through DNA amplification (Paul *et al*., 2005) using the primer set listed in Table 3.1. To achieve this, reaction volume of 25 µL was prepared was preapared from 12.88 µL sterile distilled water, 5 µL buffer (5X), 05 µL dNTPs (10 µM), 1.5 µL $MgCl_2$ (25 mM), 0.5 µL Primer-Upstream (10 µM), 0.5 µL Primer-Downstrea (10 µM), 0.12 µL Taq (5 U/µl), 4 µL RT (200 U/µl), and 4 µL

Template. The mixture was then tranfered into thermal cycler machine (model Peltier MS PTC-200, USA) and amplified at temperatures designated for each of the primers (Table 3.1).

The quality of the PCR products were checked on gel-electrophoresis while the amplicons were quantified in NANODROP UV Spectrophotometer, light absorbance in the machine was set at 260 and 280 nm respectively and analysed by loading 1 μL of the amplified product.

Table 3.1: Specific primers for the amplification of xenobiotic degrading genes in rhizosphere fungi

Enzyme type	Primer name	Published Gene	Sequence	Annealing Temp. (^{0}C)
Catalase	CBH1.1	*cbhI.1-u*	ACA ATG TTC CGC ACT GCT ACT T	61
		cbhI.1-d	AGG GTG CCC GCG GAG GTG CC	61
Catalase	CBH1.2	*cbhI.2-u*	CAC TCC TCG CAT TCA CTT GTC T	61
		cbhI.2-d	CTG CCG GTC TCG GTC CAG TTG C	61
Catalase	CBH11	*cbhII-u*	CCT CAG CCC TTA CTA CGC	55
		cbhII-d	CCA ATC TAC CTC TAC AGC	55
Lignin peroxidase	LIG1	*lig1-u*	GCC GCA ATT TCT CTT GCT CTT TCC A	68
		lig1-d	TAC ATC GAA CCA CGC GCA CGA TGA TT	68
Lignin peroxidase	LIG2	*lig2-u*	CAT CGC AAT TTC GCC CGC CAT GGA GGC A	70
		lig2-d	ACC TTC TGA ACG AAT GGC TTC TGG AGC	70
Lignin peroxidase	LIG4	*lig4-u*	GTG CGC CTG GTT CCC CAT TCT GCA G	63
		lig4-d	AAT TGG TCT CGA TAG TAT CGA AGA C	63
Lignin peroxidase	LIG6	*lig6-u*	GAC CTG CTC GAA CGG CAA GGT CGT CC	68
		lig6-d	CAT GAT AGA ACC ATC GGC GCC TCG C	68
Manganese peroxidase	MnP	*mnp1-u*	TCC GGT CAA CGG CTT GGT ATT CCA G	64
		mnp1-d	GCG ATC GTC TTG TTC GGG CGG CCA G	64
α-Galactosidase	TRPC	*trpc-u*	CAC GGG CAT CGT GAC GGA TAC	63
		trpc-d	TGG GTC TTG AGT GTG TAG TGG	63
Laccase	LCC	*lcc1-u*	TGGTA(T/C)CA(T/C)AGTCATTATTC	60
		lcc1-d	ATGTG(A/G)CA(A/G)TG(A/G)AA(C/G)(A/G)GCCA	60
Phosphoesterase	Opd	*A-opd-u*	GATCGTGGATCCCAATCGGTACAGGCGATCTG	48
		A-opd-d	GATCGTAAGCTTTTCATCGTTCGTTCGGTATCTT GACGGGGAAT	48
Phosphoesterase	MPD	*mpd-u*	AGCAGGTCGACGAGATCTAC	52
		mpd-d	TTGACGACCGAGTAGTTCAC	52
Catechol 1,2-dioxygenase	AFK1	*afk3-u*	TCATGCACGGCCGGGTGATC	95
		afkr3-d	GGGTGTCGGTCCATGAGCTC	55
Catechol 1,2-dioxygenase	AFK2	*afk4-u*	TCATGCACGGCCGGGTGATC	55
		afkr4-d	CTACGCCTGGTCCGCCACCA	55
Calmodulin (CaM)	CaM	*CaM-u*	CGGATCCAGGACATGATCAAC	55
		CaM-d	CGGGATCCGCCTCGCGGATCATCT	55

3.8 Expression of the Putative Degrading Genes in the Rhizosphere Fungi

Expresions of the putative genes presented in Table 3.1 above defined the ability of the selected fungi in producing degrading enzymes hence, their bioremediatory potentials and this was done through the use of Reverse Transcriptase-PCR method. The fungal RNA was first extracted, quantified, and used to synthesise complementary DNA (cDNA) to be used for gene expression. To compare the gene expression strength among the fungi, equal volume (2 ng) of RNA was used to synthesise cDNA in each fugus as explained below.

3.8.1 Fungal RNA extraction

The RNA extraction was done based on modified Al-Nasrawi (2012) and Li *et al*. (2014) protocols using cTAB extraction buffer. Normal DNA isolation steps were followed as explained earlier however; the use of isopropanol was replaced with 3 M lithium chloride solution ($LiCl_2$). $LiCl_2$ was added to cTAB supernatant in other to precipitate the RNA as pellets. The tubes were kept in -20 ^{0}C freezer for 12 hrs and thereafter, spinned in centrifuge for 20 minutes at the rate of 12,000.00 rpm under the temperature of 4 ^{o}C. The pellets of extracted RNA were formed on the walls of tubes, the supernatants were discarded and pellets by the RNA were washed with ethanol (70 %), it was air dried and desolved in 30-50 µL TE buffer depending on the extracted RNA quantity. The of RNA extracted was assessed by loading 6 µL of RNA mixed with 2 µL loading dye (2X Enzynomics) on the prepred gel-eletrophoresis to assess the band formed while the RNA quantity was assessed using in Nanodrop spectrophotometer.

For the sake of gene expression, all the RNA of the the rhizosphere fungi were quantified on nanodrop and equal volume of RNA (2 ng/µL) was picked for each fungal RNA and used to synthesise complimentary DNA for reverse transcriptase (RT)-PCR gene expression.

3.8.2 Sythesis of complementary DNA

The complementary DNA commonly called cDNA was synthesised from the same amount of RNA for each rhizosphere fungi that were isolated from all polluted soils and used for the reverse RT-PCR expression study on putative fungal degrading genes in the fungal isolates. The cDNA synthesis kit (Enzynomic) was used, following manufactural procedures; Equal RNA from each fungus (2 ng/mL) was reacted with 1 µL oligo dT_{18} of 50-100 µM and water (RNAase free), this was incubated by placing the tube in water bath heating at 70 ^{0}C for 5 min after which it was quickly removed and placed in ice transfered placed in ice for 15min. Two µL of reaction buffer (Top script), dNTP (2µL) where added together with the mixture in ice and then RNA inhibitor (0.5 µL) and Reverse transcriptase enzyme (1 µL) were also added to the mixture, these were all vortexed in PCR tube and transferred to thermal cycler and incubated at reaction conditions for 15 mins of 60 ^{0}C and 5 min of 95 ^{o}C to stop the process.

3.8.3. Gene expression

This was carried out on each of the rhizosphere fungi after successful cDNA synthesis, the genes were expressed by reacting 1 µl of cDNA of each fungal strain with each of the gene specific primer sets listed in Table 3.1. The primers for each gene was reacted with cDNA in 20 µL reaction volume consisting 1 µL cDNA, 7.4 µL of DEPC water, 1.5 µL each for each primer and 8.6 µL master-mix containing TAG polymerase, tris HCL (10mM, pH 8.3) and KCl_2 (50mM). The mixture was reacted in the machine at the listed annealing temperature in 35 cycles, the quantity and quality of the expressed product was examined and compared within the selected rhizosphere fungi.

3.9 Assay for Fungal Enzymes Activities in Response to the pollutants

3.9.1 Fungal incubation for enzyme production

Ability of the selected rhizosphere fungi to produce key degrading enzymes; catalase, peroxidases and laccase in response to the pollutants (Crude oil, Black oil, Dichlorvos and Lindane) was studied in aliquot. These enzymes were selected based on their verse importance in fungal degradation of soil pollutants.

Bushnell Haas (BH) medium containing magnesium sulfate (0.2 g/L), calcium chloride (0.02 g/L), potassium dihydrogen orthophosphate (0.1 g/L), dipotassium phosphate (1.0 g/L), ammonium nitrate (1.0 g/L) and ferric chloride (0.05 g/L) was used for this experiment (Ameen *et al*., 2016). The BH broth (30 ml) was was poured in beakers and supplemented with 5, 10, 15 and 25 % of each of the pollutants (crude oil, black oil, dichlorvos and lindane) with a control having no additive. These were sterilised at 121 ^{o}C for 20 min, inoculated with the fungi, and kept in rotating incubator at 30 ^{o}C for sixteen (16) days.

The medium with the fungal mycelium was spin in centrifuge at 5,000 rpm for 10 min in other to separate the fungal mycelium from the medium; the supernatant medium was then dicanted into a clean tube. This was tested for peroxidase, catalase and laccase activities using the standard protocol of Ameen *et al*. (2016).

3.9.2 Assay for catalase activities

This was analysed in the BH medium extract based on the evolution of hydrogen peroxide according to the methods of Aebi (1983) and Ameen *et al.* (2016). 300 µL of the extracted BH medium extract was reacted with 100 µL H_2O_2 (15mM) and 300 µL phosphate buffer (50 mM, pH 7.0), this mixture was read on UV-Visible spectrophotometer and absorbance was taking in 5 min interval under 240 nm wavelenght.

3.9.3 Assay for laccase activities

The laccase activity was an analysed in the BH medium extract based on the oxidation of 2, 2-azino-bis-3-benzthiazoline-6-sulfonic acid (ABTS) using the method of Novotny *et al.* (1999) and Ameen *et al*. (2016). Extracted BH medium (300 µL) was mixed with 300 µL sodium tartarate solution (100 mM, 4.5) and 300 µL ABTS (1 mM). This mixture was read UV spectrophotometer at 420 nm and decline in absorbance was checked at 5 min interal.

3.9.4 Assay for peroxidase activities

The peroxidase activities were detected in the BH medium extract according to the protocols described by both Paszczynski and Crawford (2000) and Ameen *et al*. (2016). The activity of manganese peroxidase (MnP) was assayed based on the evolution of hydrogen peroxide using phenol as indicator. Extracted BH medium (300 µL) was mixed with 300 µL magnesium sulphate (1 M), 300 µL sodium tartarate (100mM pH 4.5) and 100 µL hydrogen peroxide. The decline in absorbance was checked at 5 min interval on UV spectrophotometer at 460 nm.

The lignin peroxides (LiP) activity was detected in the BH medium extract using sodium tartarate as substrate. The extracted BH medium (300 µL) was mixed with 100 mM of 300 µL sodium tartarate (100mM pH 4.5), 300 µL vetrytryl alcohol (2 mM) and 100 µL of hydrogen peroxide. The decline in absorbance was checked at 5 min interval on UV spectrophotometer at 460 nm.

3.10 Soil Analysis (Pre and Post-Experiment)

3.10.1 Determination of Soil Characteristics and Heavy Metals

The physical and chemical characteristics of the polluted soil samples were were analysed before and after the experiment using Atomic absorption Spectrophotometer (AAS) Perkin Elmer 800B (Wellesly, MA) according to the methods of Wilson and Pryatt (2007), Adewuyi and Olowu (2012).

The collected soil samples were first air dried, sieved and 10 g of each of the samples were digested by adding 100 mL conc. nitric acid (HNO_3) and Tris (3:1 v/v) in aqua regia for 6 hours and then filtered using Wattman filter paper 1. One militre of the filterate was re-desolved in 100 mL distilled deionised water and 10 mL of this was re-dilluted with 90 mL of distilled deionised water. The values of micro and macro-nutrients such as Manganese, Molebdinum, Soldium, Calcium, Magnesium, Phosphorus, Potassium and Nitrogen and heavy metals such as Cadmium, Cupper, Lead, Iron, Nickel, Chromium, Mercury, Cobalt, Arsenic and Zinc were quantified in the diluted solution using AAS.

The soil pH was taken on the site with the use of a pH meter (Eco Eutester) while other parameters such as % Nitrogen, organic Carbon, available Phoshorus, cation exchangeable capacity (CEC) were also analysed according to the method of (Adewuyi and Olowu, 2012).

3.10.2 Analysis of Polycyclic Aromatic Hydrocarbon's content in soils 1 and 2

Values of all the sixteen (16) USEPA listed PAHs were analysed in the hydrocarbons polluted soil samples 1 and 2 before and after the experiment due to their toxic health effects. This was done using Gas Chromatograph/Mass Spectrophotometer (GC/MS) clarus model 6890 equiped with FID-ECD detector according to the method of Nguyen *et al*. (2013). The PAHs analysed included Naphthalene, Acenaphthylene, Acenaphtene, Flourene, Phenanthrene, Anthracene, Flouranthene, Pyrene, Ben(a)anthracene, Chrysene,

Benzo(b)flouranthene, Benzo(k)flouranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Indeno(1,2,3-cd)pyrene, and Benzo(ghi)perylene.

The soil samples (10 g each) were first digested with 100 mL of acetone and hexane (60:50 v/v) for 6 hrs and filtered using Wattman filter paper 1. The filterate was spiked with 1 µL of PAH standard which contains all the selected 16 EPA PAHs for better reading and the mixture was thereafter placed in altrasonic bath (Elmsonic S40H model) for for another 6 hrs. This was spinned at 400 rpm in centrifuge for 6 min and 1 µL the supernatant was loaded on the GC/MS machine which is already programmed as follows:

Oven temperature = 40 ^{o}C for 3 minutes
Ramp to 270 ^{o}C for 3 minutes for 1 and 2
Final hold time = 5 minutes
MSD TI scan = 40.00-550.00 amu

This machine was fortified with tiplus AS autosampler and helium gas carrier; it also has quadruple mass spectrophotometer (MS) Agilent 5975 MSD series for better reading.
Generated chromatograms were compare with those obtained for PAHs standard and as well analysed using the NIST library (2014). The values obtained were recorded in mg/kg of PAH concentration.

3.10.3 Analysis of pesticides contents (dichlorvos and lindane) in soils 3 and 4

The pesticide's concentrations (dichlorvos and lindane) were quatified in the soil samples 3 and 4 respectively, according to the method of Beazley *et al.* (2012) using GC/MS clarus (PerkinElma 8085). The soil samples (10 g each) were first digested by adding 100 mL dichroromethane (DCM) and placed in shaker for 6 hrs, there were thereafter spinned in centrifuge at 6,000.00 rpm for 6 min. The supernatant formed were collected and 1 µL of these were loaded in GC/MS for each sample.

The machine was programmed with split-less injector, with oven temperature of 80 ^{o}C with no hold, ovum condition temperature programme at 275 ^{o}C of injector port temperature

(isothermal), then ramped up till 290 ^{o}C at 20 ^{o}C/minute, 4.5 min hild and transfer line mass spectrometer, the machine was programmed at oven run time of about 15 min and injection time of 20 min less. Generated chromatograms were compare with those obtained for dichlorvos and lindane standards and as well analysed using the NIST library (2014). The values obtained were recorded in mg/kg concentration.

3.11 Synergistic Fungi-SMC-Plant Remediation Experiment (Experimental Design and Layout)

The remediation of the polluted soils 1-4 was carried out using the synergistic fungi-SMC-Plant process. This included the use of all the selected rhizosphere fungi strains, SMC, and test plant guinea grass (*Megathyrsus maximus*) in remediation of the polluted soil. The bulked soil was first sieved at ≤1 mm to remove particles and sterilised at 121 ^{o}C using soil sterilizer, this was done to remove interference of other microorganisms. The soil was then potted 5 kg each, in other to establish synergistic treatment, pure fungal cultures were harvested and mixed with composted SMC in 1:10 (v/v), the mixture was then used to treat the bulk soils by mixing them together using a modified method of Jonathan *et al*. (2012a-c) as given below:

Treatment 1 (T_1): SMC-Fungal mixture at 0.5 kg + Potted soil (5 kg) + *M. maximus* = Synergistic treatment

Treatment 2 (T_2): SMC-Fungal mixture at 1.0 kg + Potted soil (5 kg) + *M. maximus* = Synergistic treatment

Treatment 3 (T_3): SMC-Fungal mixture at 1.5 kg + Potted soil (5 kg) + *M. maximus* = Synergistic treatment

Treatment 4 (T_4): SMC-Fungal mixture at 2.5 kg + Potted soil (5 kg) + *M. maximus* = Synergistic treatment

Control 1 (0%1): Plant + Soil (5 kg) = Phytoremediation

Control 2 (0%2): (SMC + Fungi v/v) + Soil (5 kg) = Mycoremediation

The seeds of the test plant were first raised in tray and young plant of equal heights and weights were transplanted on the prepared pots. This set-up was prepared for each of the

four polluted and laid out in Completely Randomised Designed (CRD), the pots were spaced in 25 cm away from each other and the young test plants (2 weeks old) of equal 10 cm tall were arefully uprooted and replanted in the prepared pots. They were watered on daily basis with 100 ml of distilled water to maintain equal field capacity, plant growth analysis started after two weeks of transplanting and repeated on weekly basis while and the anatomical response of the test plant was studied at four weeks interval. The experiment was allowed to stay on the field for 90 days (three months) and samples were taken for post analysis to reveal effect of the treatments.

3.12 Estimation of Degradation kinetics of the Treatments

3.12.1 Degradation Efficiency

Degradation efficiency (DE) was deduced for each of the set-up treatments based on the percentage loss of total hydrocarbon, dichlorvos and lindane in the experimental soils. This was calculated from the intial and final concentrations of the pollutant (Total PAHs, Dichlorvos and Lindne) as analysed in the experiment soils before and after the experimment. The degradation efficiency was determined using equation 6 according to Lagergren (1898) and Reuben *et al*. (2011).

$$\mathrm{DE} = \frac{\mathrm{C0 - Ct\ (Lost\ mg/kg)}}{\mathrm{C0\ (mg/kg)}}\ X\ 100 \qquad (6)$$

Where C_0 = Initial pollutant's concentration in soil before the experiment (mg/kg); the C_t = Final or residual pollutant's concentration in the treated polluted soil after the experiment (mg/kg).

3.12.2 Degradation rate constant (K_1)

The degradation kinetic in terms of degradation rate constant (K_1) and half life ($t_{1/2}$) were calculated in each of the experimental pots using the initial and final concentrations of total PAHs (in soils 1 and 2), dichlorvos (soil 3) and lindane (soil 4) before and after the experiment.

The K_1 was determined based on the chemical first order reaction as given in equation 7 below according to Pala *et al*. (2006), Agarry *et al*. (2010) and Zahed *et al*. (2011). The values were calculated using LINEST function in Microsoft excel 2016.

$$Log\left(Co - Ct\right) = Log\ Co - Log\frac{K1}{2.303}t \qquad (7)$$

Where Where C_0 = Initial pollutant's concentration in soil before the experiment (mg/kg); the C_t = Final or residual pollutant's concentration in the treated polluted soil after the experiment (mg/kg); t = time (/day) and K_1 = degradation rate of each treatments (/day).

3.12.3 Estimation of the half life of the pollutants in each treatment

This is the time required to degrade half of the pollutants (PAH, dichlorvos and lindane) in each of the treated soil and control. Half life was calculated from the calculated K_1 as given in equation 8 below by Yeung *et al*. (1997) and Dimitrov *et al*. (2007), Mathies and Klasmier (2008) as well as Agarry *et al*. (2013).

$$t_{1/2} = \frac{\ln 2}{K1} \qquad (8)$$

Where $t_{1/2}$ is the half life (/day) while K_1 = degradation rate of each treatments (/day).

3.13 Growth Analysis of the Test Plant (Guinea grass)

The plant's growth analysis started after two weeks of transplant, it was conducted on each of the plant stand on each treatment and this was done according to the methods of Jonnathan *et al*. (2012). The plant growth parameters were measured on weekly basis for 90 days after which the plant was harvested and subjected to phytomass analysis. The growth parameters taken include the following:

i. plant length using a measuring tape in cm
ii. stem girth using an automated vaneer caliper in cm
iii. leaf area using an automated machine called LI-COOR in cm^2 and
iv. the total leaf numbers through counting and recorded with the dead leaves

3.14 Physiological and anatomical responses of *M. maximus* (Guinea Grass)

3.14.1 Chlorophyll contents

Chlorophyll a, chorophyll b and the total chlorophyll were detected in actively growing plants during the experiment using the method described by Garcia (2008). A fully expanded leaf (2 weeks old) was plucked from each of the plant, it was then immediately covered with an aluminium foil and and brought to the lab immediately, sample discs of 200 mm diamter were cut from the leaf and placed into 95 % ethanol, they were grounded in crucible, and filtered using Wattman filter papr no 1. The chlorophyll contents was quantified in the filterate extract by loading 1 mL on the altraspec II spectrophotometer model LKB at absorbance of 665 and 649 nm respectively and this as done in four replications for each treatment.

3.14.2 Potential photosynthetic rate (Amax)

Young leaf (two weeks old) was plucked from a growning plant on each treatment and quantified for potential photosynthetic rate; this was measured in terms of Amax using a Clark electrode meter, the meter was used to monitor the emitted oxygen level from the leaf. Discs of equal sizes were cut out from the leaf and they were placed on the photon chamber of the meter under a direct linear light source. The electrode was set at light flow (photon) density (about 1.500 $\mu mol^{-2}S^{-2}$ and temperature of 35±4 ^{o}C) with 1 M $NaHCO_3$ on the ground level to serve as carbon (iv) oxide source, the oxygen eluted from the leaf disc was measured by the electrode and recorded in m mol $O_2^{-2}S^{-2}$.

3.14.3 Phytomass efficiency

Phytomass efficiency was determined by harvesting each of the plant in different treatments after the 3 month experiment, the root and shoots were separated based on each treatment as below and off ground biomass. They were rinsed in sterile water to remove all the adhering soils or particles and the fresh biomass was taken in three replicates using weighing balance. To obtain the dried biomass, harvested shoots and roots were oven dried under the drying temperature of 60 ^{o}C during which the plant's parts were weighed constantly in 6 hrs intervals until a constant weight was obtained and the phytomass efficiency of each treatment was determined using the equation 5 below and this was calculated for above and below ground biomass.

$$\text{Phytomass Efficiency (PE)} = \frac{\text{FDP}}{\text{TFDP}} \div 100\% \qquad (5)$$

The FDW is Field Dried Weight of a particular plant while the TFDP is the Total Field Dried Weight of all the plant stands harvested

3.14.4 Root and leaf anatomical responses

To acert the effects of the polluted soil and amendment potential of the synergistic treatments on the test plant, the root and leaf of this plant from each treatment were sectioned and studied for their anatomical variations. Roots and leaves were freshly excised from plants at six week of growth and preserved in solution of formaldehyde, acetic acid and ethyl acetate (5:5:90 v/v) according to the methods of Johnson (1940) as well as Marcelo *et al*. (2011).

Transverse tissue section was made from root apexes and leaves (at 6.0±0.5 cm from leaf petiole) using a sledge microtome while paradermal cuts were made along the leaf midrib. The tissues were stained with 5 % methylene blue counter stained with safranine while glycerine was used to mount the slides according to procedure of Kraus and Arduin (1997). The slides were studied in an Olympus microscope with sima scan having a filter band pass of 420 nm wavelength for photomicrographic measurements. The parameters studied included the root exodermis and endodermal layers; number of protoxylem and metaxylem elements, thickness of walls of tracheary elements a cortical cell wall. Other parameters measured for the root response are the root diameter, root hairs, and the root dimensions. The leaf anatomical responses was measured based on the thickness of mid rib, thickness of the lower epidermis, sclerenchymatous cell, and vascular bundles, the numbers of rows formed by the sclerenchyma, parenchymer,and xylem vessels, diameter of xylem and phloem and so on.

3.15 Germination Supporting Ability of the Remediated Soils

This was estimated based on the ability of each of the soil (treated and control) in supporting germination to rating the toxicity level according to Vaajasaari *et al.* (2002) as well as Plaza *et al*. (2005). Viable seeds of *M. maximum* (guinea grass) were used due to its sensitivity, 30 viable seeds were placed evenly on the plated 150 g soil. All the plates were prepared in three replicates and were equally flooded with equal volume of 50 ml of sterile water plates were thereafter, incubated at room temperature for 8 days. The number of seeds that germinated from the plates were counted and recorded while the root length was

also recorded. Germination index was then calculated based on the number of viable seeds that germinated in each soil compared with the length of root formed using equation 9:

$$\text{Germination Index } = \frac{(\%\ NSG)\ X\ (\%\ RL)}{100} \quad (9)$$

Where NSG = No of Seed Germinated, RL = Root Length.

3.16 Statistical Data Analysis

Data obtained in this study were recorded as mean of three replicates based on statistical test, Analysis of Varance (ANOVA) using Minitab statistical application (version 17). The means of the treatments and controls were also compared and separated using the statistical test called Duncan Multiple Range Test (DMRT) at significance level set at $\alpha_{0.05}$.

CHAPTER FOUR

RESULTS

Th synergistic potentials of rhizosphere fungi, Spent mushroom compost and *Megathyrsus maximus* in the remediation of polluted soils was established in this study.

4.1 Characterisation of the Isolated Rhizosphere Fungi Strains

4.1.1 Morphological charactersation

The macro and microscopic studies on the isolated rhizosphere fungal strains based on the colony diameter on PDA plate, surface and underside color, vesicle shape, conidial head, colony serration, and conidiophore characteristics are presented in Table 4.1 and Plate 4.1 below.

The characteristics of the sixteen selected rhizosphere fungi revealed that they possess varying characteristics and distinctly belonging to class Ascomycetes and Zygomycetes including different groups such as *Aspergillus*, *Cunninghamella*, *Candida*, *Talaromyces, Penicillium*, *Trichoderma*, *Fusarium* and *Yarrowia*.

Specifically, they were coded and identified as *Aspergillus niger* (asemoA), *Cunninghamella elegans* (AsemoB), *A. niger* (asemoC), *Candida albican* asemoD, *Aspergillus awamori* (asemoE), *Talaromyces purpurogenus* (asemoF), *Talaromyces atroroseus* (asemoG), *Penicillium sp* (asemoH), *Fusarium solani* (asemoI), *Trichoderma harzianum* (asemoJ), *Aspergillus oryzae* (asemoK), *A. clavatus* (asemoL), *Aspergillus flavus* (asemoM), *T. purpurogenus* (asemoN), *Yarrowia lipolytica* (asemoO) as well as *A. flavus* (asemoP) as presented in Table 4.2 as well as Plate 4.1.

Table 4.1: Morphological characterization of the selected rhizosphere fungal strains

	Morphological/cultural features on plates			Microscopic features			Fungi species
Strain code	**Colony diameter (cm)**	**Colour on plate surfaces**	**Colour on the reverse plate surfaces**	**Conidial head**	**Colony serration**	**Conidiophores**	
asemoA	5.8±0.2	Dark brown to black densely matted conidia, rare white mycelia with cream margin	Yellow to dull brown reverse plate, mycelia had wrinkled texture	Conidial is radiate head	Seration biseriate	Brownish, relatively long with smooh conidiophores	*A. niger*
asemoB	3.4±0.4	White mycelium with black spots	Milky white	Conidia is uniseriate	Seriate	Conidiophore is rough, short and whitish	*Cunninghamella elegans*
asemoC	8.5±0.2	Densely matted conidia with dark brownish to desnsly black mycelia having cream margins	Dull black with wrinkled texture	Radiate	Biseriate	Conidiophore is smooth, long and brownish	*A. niger*
asemoD	2.5±0.7	Whitish transparent colony with round formation	Deep dul whitish formation				*Candida albican*
asemoE	6.2±1.8	Densely matted conidia, with white tip and brown sprores	It appears yellowish to dull brown in underside with wrinkled texture	Radiate	Biseriate	Brownish, relatively long conidiophores with smooth surfaces	*Aspergillus awamori*
asemoF	4.2±0.3	Light brown/darker green with thin white tip	Redish	Ovoid	Serrated	symmetrical, biverticillate *conidiophores* terminated by a whorl of branches	*Talaromyces purpurogenus*
asemoG	5.6±0.6	Dark brown with thick white tip	Redish	Ovoid	Serrated	symmetrical, biverticillate *conidiophores* terminated by a whorl of branches	*Talaromyces atroroseus*
asemoH	4.3±1.1	Green with white tip	Redish	Round to ovoid and in chains	Serrated	Multiverticillate coniodophore with long chained branching at the tip	*Penicillium sp*

asemoI		Whitish to orange reverse with alternating whitish and brown concentric rings	Globose	Uniseriate	Colourless short and finely roughened		*Fusarium solani*
asemoJ	4.9±0.6						*Trichoderma harzianum*
AsemoK	Almost full plate	Coniferous green conidia with white mycelia white margin	Cream reverse with slightly wrinkled centre	Columnar	Uniseriate	Colourless short and finely roughened	*Aspergillus oryzae*
asemoL	7.4±0.6	Conidia is faint green having tin whitish margin	Reverse side is wrinkled darkish	Columnar	Uniseriate	Colourless long and roughened	*Aspergillus clavatus*
asemoM	Almost full plate	Conidia is coniferous green having tin whitish margin	Reverse side is creamy with wrinkled center	Columnar	Uniseriate	Colourless short and finely roughened	*Aspergillus flavus*
asemoN	3.6±0.1	Beadlike sporangiospores broomlike	Creamy Underside	Broomlike	Seriate	Greenish	*Talaromyces purpurogenus*
asemoO	3.6±0.1	Light green	Creamy Underside	Broomlike	Seriate	Greenish	*Yarrowia lipolytica*
asemoP	3.4±0.2	The conidia appears bluish green with a white tip mycelium	Center appears brownish and concentric rings of alternation yellow and brown color	It appear radiate	It is uniseriate in nature	Brownish, short and finely roughened	*Aspergillus flavus*

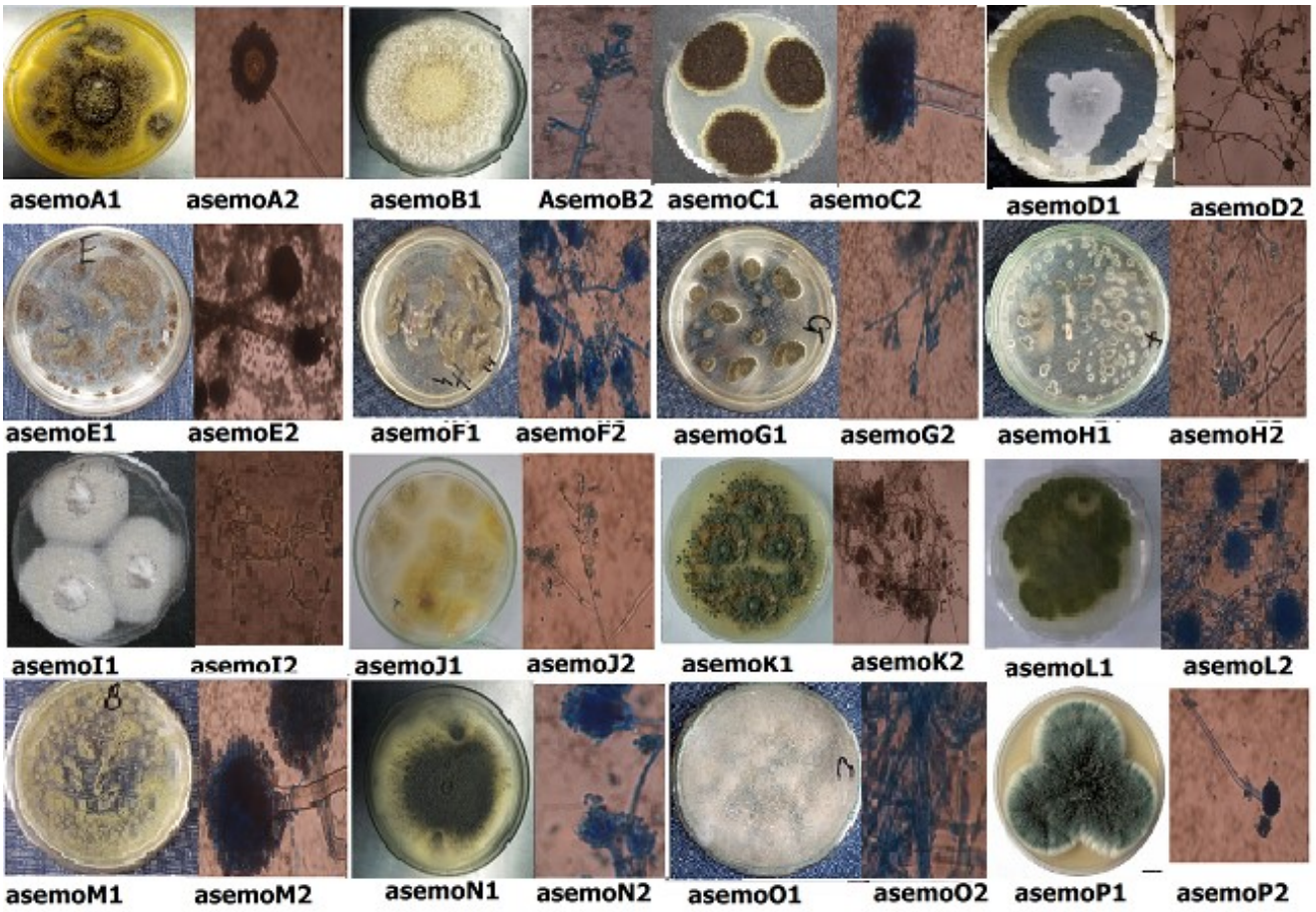


Plate 4.1: Rhizosphere fungi morphological and microscopic characteristics (x40)
asemoA (1&2) *Aspergillus niger*; asemoB (1&2) *Cunninghamella elegans;* asemoC (1&2) *A. niger*; asemoD (1&2) *Candida albican*; asemoE (1&2) *A. awamori*; asemoF (1&2) *T. purpurogenus*; asemoG (1&2) *T. astroroseus*; asemoH (1&2) *Penicillium spp*.; asemoI (1&2) *Fusarium solani*; asemoJ (1&2) *Trichoderma harzianum*; asemoK (1&2) *A. oryzae*; asemoL (1&2) *A. clavatus*; asemoM (1&2) *A. flavus*; asemoN (1&2) *T. purpurogenus*; asemoO (1&2) *Yarrowia lipolitica* and asemoP (1&2) *A. flavus* asemoP.

4.1.2 Molecular charactersation

Generated sequenced product of the amplified ITS regions of 5.8-28S rRNA through BLAST seach on the NCBI shows the alignments of the fungal strains with the other most similar documented strains. The phylogenetic relationships between five other most similar strains were further revealed using MABL software as presented in Figure (4.1a-g).

Phylogenetic analysis of the fungal strains showed that many of the strains are directly related to other strains which are already been documented in the NCBI with enoumous degrading capacities.

The isolated *Aspergillus* groups are mostly related with each other but share similar ancestral lineage with other strains which were already documented in NCBI (Figure 4.1a). *A. flavus* asemoM (KY488467) is mostly related with *A. oryzae* asemoK (KY693972) which together share similar ancestral lineage with *A. niger* asemoC (KY693970), *A. awamori* asemoE (KY488462), *A. niger* asemoA (KY47956) and *A. flavus* asemoP (KY488467). They all share common ancestral lineage wth different *Aspergillus* species as shown in Figure 4.1a.

Cunninghamella elegans asemoB (KY693969) showed a close phylogenetic relationship with *C. elegans* FSU4755, they both shared similar ancestral lineage with *C. elegans* HA009069 (Figure 4.1b). The three fungi share common ancestor with other species *C. elegans* KJ467777, *C. elegans* AF113421 and *C. elegans* AF11342. Also, *Fusarium solani* asemoI (KY693971) is dinstinct but shares direct ancestral lineage with *Necteria haematococca* JX868661, *Fusarium solani* (JF323003), *Fusarium solani* (JF323004), *Fusarium solani* (JF323998) and KR080846.

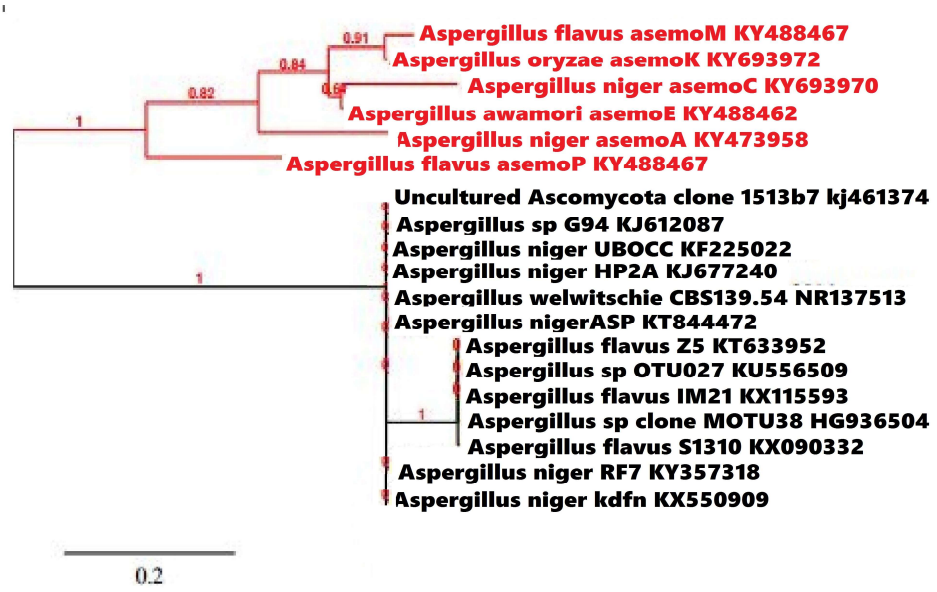
Penicillium sp asemoH (KY488465) is also dinstinct but share direct ancestral lineage with other fungal strains JQ663996, KJ775716, K909185, KM036429 and JF910286 (Figure 4.1d). Likewise, asemoJ (KY48846) shares common ancestral lineage with *Trichoderm harzianum* strains JF831483, KR868237, KR868284, KR868336 and KR868342 (Figure 4.1e) while *Yarrowia lipolitica* asemoO (KY488469) shares common ancestral lineage

with other *Y. lipolitica* strains KY105968, KP132914, KY105967, KP132907 and K132933.

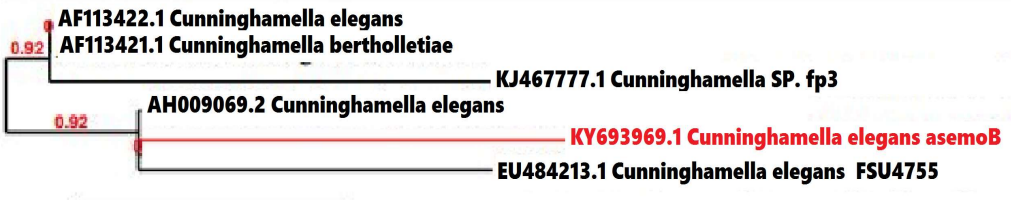
It was also discovered that, *Talaromyces purpurogenus* asemoN (KY488468) and asemoF (KY48863) share close phylogenetic similarities with other strains CBS133442 and asmoG (KY488464) while they all share common ancestral lineage with strains AB872828, AB872819, KC692214, KJ775716, KF3305747 AN KC344974 (Figure 4.1g).

Table 4.2: ICBN Submitted barcodes and the accession numbers for the isolated rhizosphere fungal strains

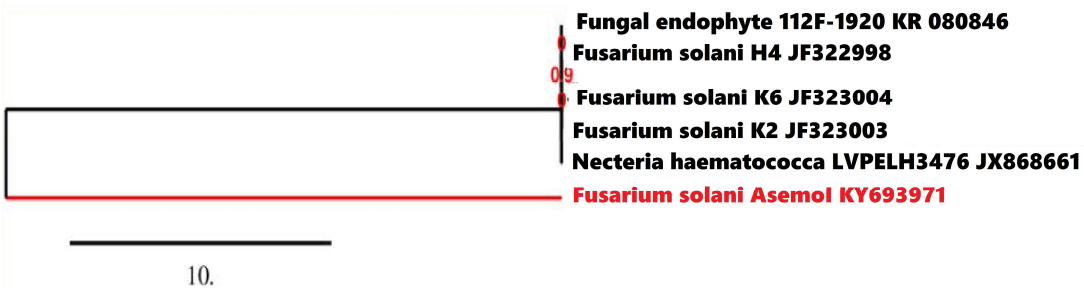
Strain	ID (NCBI Submission)	Accession number	Identified name	Blast search similarity
asemoA	1982332	KY473958	*Aspergillus niger*	98%
asemoB	1997057	KY693969	*Cunninghamella elegans*	99%
asemoC	1997057	KY693970	*Aspergillus niger*	97%
asemoD	2014587	MF065983	*Candida albican*	96%
asemoE	1984212	KY488462	*Aspergillus awamori*	99%
asemoF	1984212	KY488463	*Talaromyces purpurogenus*	98%
asemoG	1984212	KY488464	*Talaromyces atroroseus*	99%
asemoH	1984212	KY488465	*Penicillium sp*	99%
asemoI	1997057	KY693971	*Fusarium solani*	99%
asemoJ	1984212	KY488466	*Trichoderma harzianum*	99%
asemoK	1997057	KY693972	*Aspergillus oryzae*	99%
asemoL	2014587	MF96984	*Aspergillus clavatus*	98%
asemoM	1984212	KY488467	*Aspergillus flavus*	97%
asemoN	1984212	KY488468	*Talaromyces purpurogenus*	98%
asemoO	1984212	KY488469	*Yarrowia lipolytica*	99%
asemoP	1997057	KY693973	*Aspergillus flavus*	98%


Aspergillus flavus asemoM KY488467
Aspergillus oryzae asemoK KY693972
Aspergillus niger asemoC KY693970
Aspergillus awamori asemoE KY488462
Aspergillus niger asemoA KY473958
Aspergillus flavus asemoP KY488467
Uncultured Ascomycota clone 1513b7 kj461374
Aspergillus sp G94 KJ612087
Aspergillus niger UBOCC KF225022
Aspergillus niger HP2A KJ677240
Aspergillus welwitschie CBS139.54 NR137513
Aspergillus nigerASP KT844472
Aspergillus flavus Z5 KT633952
Aspergillus sp OTU027 KU556509
Aspergillus flavus IM21 KX115593
Aspergillus sp clone MOTU38 HG936504
Aspergillus flavus S1310 KX090332
Aspergillus niger RF7 KY357318
Aspergillus niger kdfn KX550909
0.2

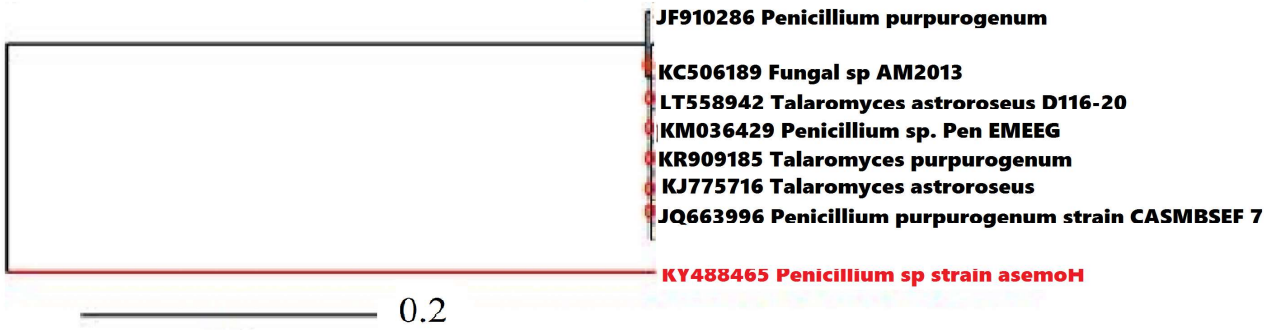
a.


AF113422.1 Cunninghamella elegans
AF113421.1 Cunninghamella bertholletiae
KJ467777.1 Cunninghamella SP. fp3
AH009069.2 Cunninghamella elegans
KY693969.1 Cunninghamella elegans asemoB
EU484213.1 Cunninghamella elegans FSU4755

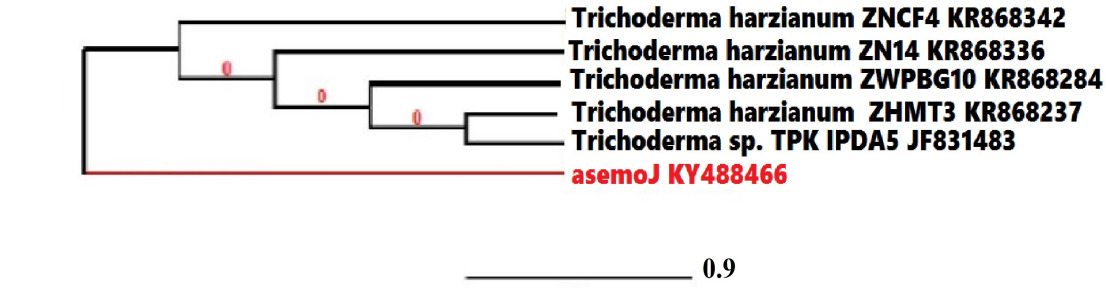
b.


Fungal endophyte 112F-1920 KR 080846
Fusarium solani H4 JF322998
Fusarium solani K6 JF323004
Fusarium solani K2 JF323003
Necteria haematococca LVPELH3476 JX868661
Fusarium solani AsemoI KY693971
10.

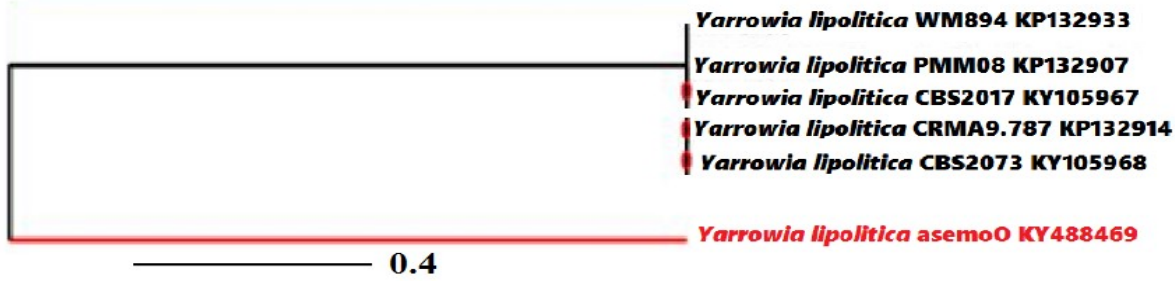
c.


JF910286 Penicillium purpurogenum
KC506189 Fungal sp AM2013
LT558942 Talaromyces astroroseus D116-20
KM036429 Penicillium sp. Pen EMEEG
KR909185 Talaromyces purpurogenum
KJ775716 Talaromyces astroroseus
JQ663996 Penicillium purpurogenum strain CASMBSEF 7
KY488465 Penicillium sp strain asemoH
0.2

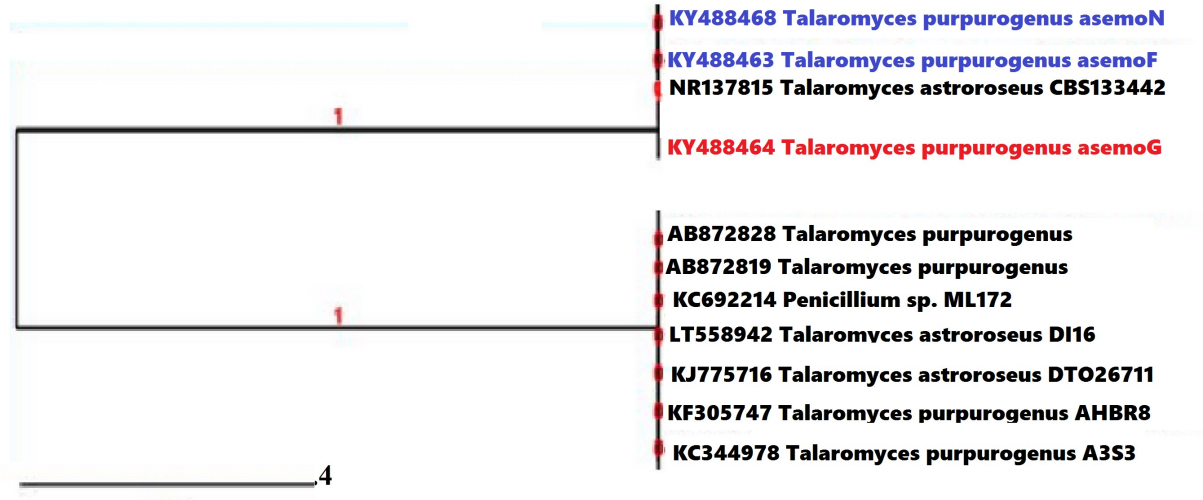
d.



e.



f.



g.

Figure 4.1a-g: Phylogenetic relationship between the isolated rhizosphere fungi strains and some other similar fungal strains already documented in NCBI

4.2 Dominant rhizosphere fungal strains in different polluted soils (S1-S4)

A total of 200 strains of the rhizosphere fungal were isolated from the polluted soil samples (50 obtained from each polluted soil). The strains with high incidence of 50 % above (Table 4.3) were selected for synergistic remediation.

The result shows that *T. harzianum* asemoJ had the highest percentage incidence of 86 % in soil 1 collected from Ugboroko community, Niger State Nigeria, followed by *A. niger* asemoA (84 %) and then *A. awamori* asemoF and *A. flavus* asemoM with 80 % respectively while *C. elegans* asemoB, *A. clavatus* asemoL, *T. purpurogenus* asemoN and *Y. lipolitica* asemoO had the least at 8 % respectively (Table 4.3). In soil 2 (which was collected from Bodija, Ibadan, Nigeria), *A. niger* asemoA and *T. harzianum* asemoJ had the highest incidence (80 %) followed by *A. flavus* asemoM (78 %), *A. clavatus* asemoL (56 %), *A. awamori* asemoE (52 %) and *C. elegans* asemoB (52 %) while *A. niger* asemoC (02 %), *T. purpurogenus* asemoF (06 %), *C. albicans* asemoD (10 %) and *T. purpurogenus* asemoN (16 %) had the least occurence.

However, in pesticides polluted soils 3 and 4 (from Owo, Ondo State, Nigeria), *C. elegans* asemoB had the highest occurence (74 %) in soil 3, followed by *T. astroroseus* asemoG and *A. flavus* asemoP (66 % respectively) and *A oryzae* asemoK (64 %) while *A. awamori* asemoF (18 %), *A. niger* asemoC and *A. clavatus* asemoL (24 % respectively) had the least incidence. In soil 4, *A. niger* asemoC had the highest incidence (82 %) followed by *Y. lipollitica* asemoO and *A. flavus* asemoP (68 % respectively), *C. albicans* asemoD had 62 % occurence while *T. harzianum* asemoJ (04 %), *C. elegans* asemoB (08 %) and *A. flavus* asemoM (14 %) had the least incidence.

In all, Soil 1 had the highest total number of incidence time (TNIT) of 308, followed by soil 3 (284) and soil 4 (271) while soil 2 had the least (268).

Table 4.3: Incidence of rhizosphere fungal strains isolated from four polluted soil samples

Strain Code	Fungal Species	SOIL 1 (N=50)	% Incidence	SOIL 2 (N=50)	% Incidence	SOIL 3 (N=50)	% Incidence	SOIL 4 (N=50)	% Incidence
AsemoA	*Aspergillus niger*	42	84	40	80	00	00	03	06
AsemoB	*Cunninghamella elegans*	04	08	26	52	37	74	04	08
AsemoC	*Aspergillus niger*	11	22	01	02	12	24	41	82
AsemoD	*Candida albican*	22	44	05	10	13	26	31	62
AsemoE	*Aspergillus awamori*	34	68	26	52	00	00	00	00
AsemoF	*Talaromyces purpurogenus*	40	80	03	06	09	18	00	00
AsemoG	*Talaromyces atroroseus*	12	24	00	00	33	66	28	56
AsemoH	*Penicillium sp*	08	16	00	00	26	52	12	24
AsemoI	*Fusarium solani*	07	14	12	24	26	52	23	46
AsemoJ	*Trichoderma harzianum*	43	86	40	80	00	00	02	04
AsemoK	*Aspergillus oryzae*	28	56	11	22	32	64	12	24
AsemoL	*Aspergillus clavatus*	04	08	28	56	12	24	12	24
AsemoM	*Aspergillus flavus*	40	80	39	78	00	00	07	14
AsemoN	*Talaromyces purpurogenus*	04	08	23	16	24	48	28	56
AsemoO	*Yarrowia lipolytica*	04	08	00	00	27	54	34	68
AsemoP	*Aspergillus flavus*	05	10	14	28	33	66	34	68
	TNIT	308		268		284		271	

TNIT = Total number of Incidence Time; Soil 1 = soil polluted with crude oil; soil 2 = soil polluted with black and engine oil, soil 3 = dichlorvos polluted soil; soil 4 = lindane polluted soil; only isolate with more than 50% percentage incidence were selected for further studie

4.3 Tolerance and response of the selected rhizosphere fungal strains to different concentrations of hydrocarbons and pesticides

All the rhizosphere fungal strains were able to survive crude oil, black oil, dichlorvos and lindane up to 20 % concentrations (v/v) as shown in Tables 4.4-4.7, but their growths decrease as the concentration increased.

Aspergillus niger asemoA, *A. awamori* asemoE, *Trichoderma harzianum* asemoJ and *Aspergillus flavus* asemoM were isolated from soil S1 (Crude oil polluted soil), they had lesser radial extention rate in medium supplemented with different concentrations of crude oil as compared to the control. *Trichoderma harzianum* asemoJ (3.20 cm), *Aspergillus oryzae* asemoK (2.90 cm), *Aspergillus awamori* asemoE (2.55 cm) had the highest radial growth on control medium (PDA) respectively while *Aspergillus niger* asemoA had the least (2.40 cm). Dose inhibition (DIRP) of the fungi against crude oil is presented in Table 4.4, it was observed that fungus *Trichoderma harzianum* asemoJ had the highest crude oil tolerance (43.75 % DIRP) followed by *Talaromyces purpurogenus* asemoF (50.00 %), asemoK (62.41) and *Aspergillus flavus* asemoM (62.41) while *Aspergillus niger* asemoA and *Aspergillus flavus* asemoM had the least tolerance of 90.32 and 86.42 % DIRP as shown in Table 4.4.

In addition, the rhizosphere fungi that were isolated from the black-oil polluted soil (Soil 2) *Aspergillus niger* asemoA, *Trichoderma harzianum* asemoJ, *Aspergillus clavatus* asemoL, *Aspergillus awamori* asemoE and *Aspergillus flavus* asemoM showed significantly higher radial extention rate in control than treatments ($P \leq 0.05$). The fungal strains *C. elegans* asemoB (3.10 cm), *A. awamori* asemoE (2.90 cm) and *A. flavus* asemoM (2.40 cm) had the highest radial growth on solid medium (PDA) respectively in control medium while *T. harzianum* asemoJ had the least (0.55 cm). However, the inhibition of toxic pollutant DIRP by the rhizosphere fungi shown in Table 4.5, fungus *T. harzianum* asemoJ had the highest tolerance (54.54 % DIRP) followed by *C. elegans* asemoB (61.29 %), asemoE (65.52) and asemoA (69.13) while asemoL and *A. awamori* asemoMhad the least tolerance of 74.44 and 71.25 % DIRP (Table 4.5).

Table 4.4: Fungal growth (cm day^{-1}) in response to crude oil mixed wih PDA at different concentrations

		Growh rate (cm/day)					
Fungal strains	**Strain code**	**0 %**	**5 %**	**10 %**	**15 %**	**20%**	**DIRP**
Aspergillus niger	AsemoA	1.55±0.02^{a}	0.49±0.04^{b}	0.31±0.01^{c}	027±0.01^{d}	0.15±0.01^{e}	90.32
A. awamori	AsemoE	2.55±0.06^{a}	1.51±0.02^{b}	1.54±0.08^{b}	0.69±0.05^{bc}	0.54±0.08^{d}	78.82
Talaromyces purpurogenus	AsemoF	1.20±0.02^{a}	1.02±0.01^{a}	0.89±0.01^{b}	0.80±0.01^{b}	0.60±0.008^{c}	50.00
Trichoderma harzianum	AsemoJ	3.20±0.56^{a}	2.51±0.42^{b}	2.40±0.08^{b}	1.90±0.02^{c}	1.80±0.011^{d}	43.75
A. oryzae	AsemoK	2.90±0.12^{a}	2.67±0.10^{a}	1.99±0.07^{b}	1.87±0.03^{b}	1.09±0.02^{c}	62.41
A. flavus	AsemoM	1.40±004^{a}	0.34±0.06^{b}	0.27±0.03^{c}	0.24±0.01^{c}	0.19±0.07^{d}	86.42

Each value is a mean of six replicated determinations±Standard deviation (SD); Means in rows having the same superscript letter are not significantly different (α0.05); DIRP is the Dose Inhibition Resistance Percentage.

Table 4.5: Fungal growth (cm day^{-1}) in response to black oil mixed wih PDA at different concentrations

	Strain	Radial extension rate (cm/day)					
Fungal strains	**code**	**0 %**	**5 %**	**10 %**	**15 %**	**20%**	**DIRP**
Aspergillus niger	asemoA	2.30±0.14^{a}	1.84±0.05^{b}	1.29±0.13^{b}	0.60 ± 0.08^{c}	0.71±0.04^{c}	69.13
Cunninghamella. elegans	asemoB	3.10±0.59^{a}	2.60±0.51^{a}	1.50±0.21^{b}	1.8±0.12b^{c}	1.20±0.09b^{c}	61.29
A. awamori	asemoE	2.90±0.11^{a}	2.90±0.08^{a}	2.50±0.07^{a}	1.20±0.02^{b}	1.00±0.02^{b}	65.52
Trichoderma harzianum	asemoJ	0.55±0.022^{a}	0.49±0.04^{b}	0.31±0.01^{c}	0.27±0.01^{c}	0.25± 0.01^{c}	54.54
A.clavatus	asemoL	0.90±0.01^{a}	0.60±0.01^{b}	0.45±0.004^{c}	0.40±0.003^{c}	0.23±0.001^{d}	74.44
A. flavus	asemoM	2.40±0.04^{a}	1.34±0.06^{b}	1.24±0.028^{c}	0.82±0.013^{d}	0.69±0.07^{d}	71.25

Each value is a mean of six replicated determinations±Standard deviation (SD); Means in rows having the same superscript letter are not significantly different (α0.05); DIRP is the Dose Inhibition Resistance Percentage.

Rhizosphere fungi which were gotten from pesticide contaminated soils 3 and 4 showed radial extention rates (Tables 4.6 and 4.7) which are significantly less in pesticides supplemented media as compared to the control ($P \leq 0.05$). Strains isolated from dichlorvos polluted soil performed better in control compared to the dichlorvos treated media, *A. oryzae* asemoK had the highest radial growth of 7.10 cm on the control solid medium (PDA) followed by *A. niger* asemoA (5.40 cm) and *T. harzianum* asemoJ (3.55 cm) while *Penicillium* sp. asemoH had the least (1.20 cm). However, the DIRP assessment showed that strains *A. niger* asemoA had best pesticide tolerance (55.00 % DIRP) followed by *A. oryzae* asemoK (56.67 %), *Fusarium solani* asemoI (59.32) and *A. flavus* asemoP (70.00 %) while *Y. lipolytica* asemoO and *T. harzianum* asemoJ had the least tolerance of 87.14 and 70.70 % DIRP (Table 4.6).

Morever, strains that were isolated from lindane polluted soil (soil 4) also showed improved growth rate in control over the treatment (Table 4.6), *Y. lipolytica* asemoO (4.23 cm) gave the highest radial growth on solid medium (PDA) in the control followed by *C. albican* asemoD (3.55 cm) and *A. niger* asemoC (3.40 cm) while *T. purpurogenus* asemoN gave the least (0.55 cm). However, based on the Dose Inhibition Resistant Percentage (DIRP) as shown in Table 4.7, *C. albican* asemoD, *T. purpurogenus* asemoN and *Y. lipolytica* asemoO had the highest tolerance (55.00, 54.54 and 55.31 % DIRP respectively) followed by asemoC (76.18 %) while *T. astrorous* asemoG and *A. flavus* asemoP had the least tolerance of 86.88 and 86.43 % DIRP (Table 4.7).

Table 4.6: Fungal growth (cm day^{-1}) in response to dichlorvos mixed wih PDA at different concentrations

		Radial extension rate (cm/day)					
Fungal strains	**Strain code**	**0 %**	**5 %**	**10 %**	**15 %**	**20%**	**DIRP**
Aspergillus niger	asemoA	5.40±0.04^{a}	4.34±0.02^{b}	4.23±0.02^{b}	2.92±0.008^{c}	2.43±0.003^{c}	55.00
Trichoderma harzianum	asemoJ	3.55±0.016^{a}	3.531±0.07^{b}	2.35±0.016^{b}	1.69±0.025^{bc}	1.04±0.018^{d}	70.70
Penicillium sp.	asemoH	1.20±0.07^{a}	0.97±0.04^{b}	0.61±0.03^{c}	0.49±0.01^{c}	0.38±0.01^{c}	68.33
Fusarium solani	asemoI	0.59±0.02^{a}	0.38±0.04^{b}	0.28±0.02^{c}	0.27±0.02^{c}	0.24±0.01^{c}	59.32
A. oryzae	asemoK	7.10±0.23^{a}	4.20±0.21^{b}	3.33±0.12^{c}	2.89±0.11^{d}	2.45±0.10^{e}	56.67
Yarrowia lipolitica	asemoO	1.40±0.04^{a}	0.36±0.05^{b}	0.25±0.028^{c}	0.23±0.013^{c}	0.18±0.07^{d}	87.14
A. flavus	asemoP	1.50±0.07^{a}	0.98±0.05^{b}	0.68±0.05^{b}	0.60±0.04^{c}	0.45±0.01^{c}	70.00

Each value is a mean of six replicated determinations±Standard deviation (SD); Means in rows having the same superscript letter are not significantly different (α0.05); DIRP is the Dose Inhibition Resistance Percentage.

Table 4.7: Fungal growth (cm day^{-1}) in response to lindane mixed wih PDA at different concentrations

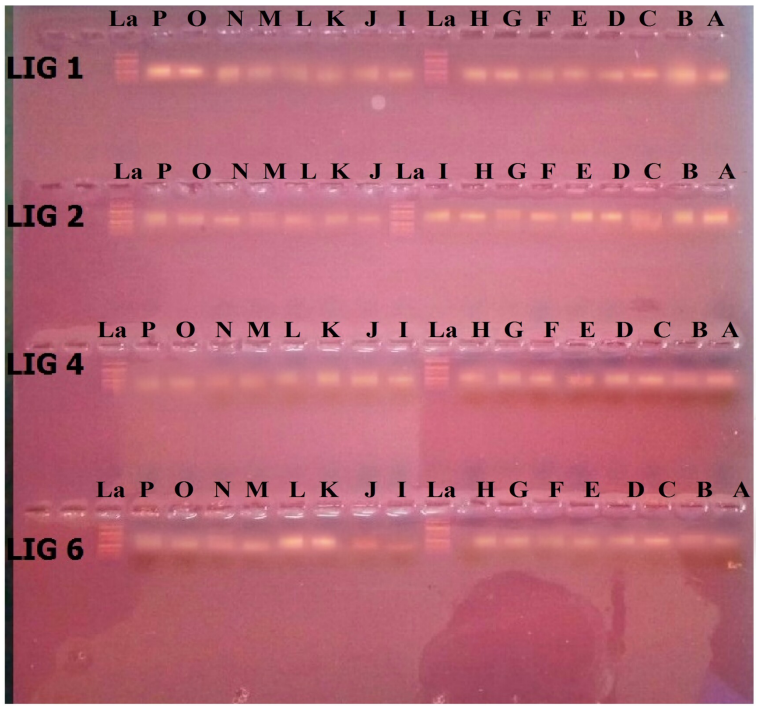
	Strain	Radial extension rate (cm/day)					
Fungal strains	**code**	**0 %**	**5 %**	**10 %**	**15 %**	**20%**	**DIRP**
Aspergillus niger	asemoC	3.40±0.04^{a}	2.48±0.07^{b}	1.59±0.03^{c}	1.40±0.01^{c}	0.81±0.01^{c}	76.18
Candida albicans	asemoD	3.55±0.016^{a}	2.81±0.07^{b}	2.55±0.016^{b}	1.93±0.025^{cd}	1.59±0.018^{d}	55.21
Talaromyces astroroseus	asemoG	2.21±0.07^{a}	1.99±0.06^{b}	1.23±0.04^{b}	0.65±0.01^{c}	0.29±0.005^{c}	86.88
Talaromyces purpurogenus	asemoN	0.55±0.022^{a}	0.49±0.04^{b}	0.31±0.01^{c}	0.27±0.01^{c}	0.25±0.01^{c}	54.54
Yarrowia lipolitica	asemoO	4.23±0.67^{a}	3.23±0.59^{a}	2.28±0.23^{b}	2.05±0.08^{b}	1.89±0.03^{b}	55.31
Aspergillus flavus	asemoP	1.4 ±0 0.04^{a}	0.34±0.06^{b}	0.24±0.028^{c}	0.22±0.013^{c}	0.19±0.07^{d}	86.43

Each value is a mean of six replicated determinations±Standard deviation (SD); Means in rows having the same superscript letter are not significantly different (α0.05); DIRP is the Dose Inhibition Resistance Percentage.

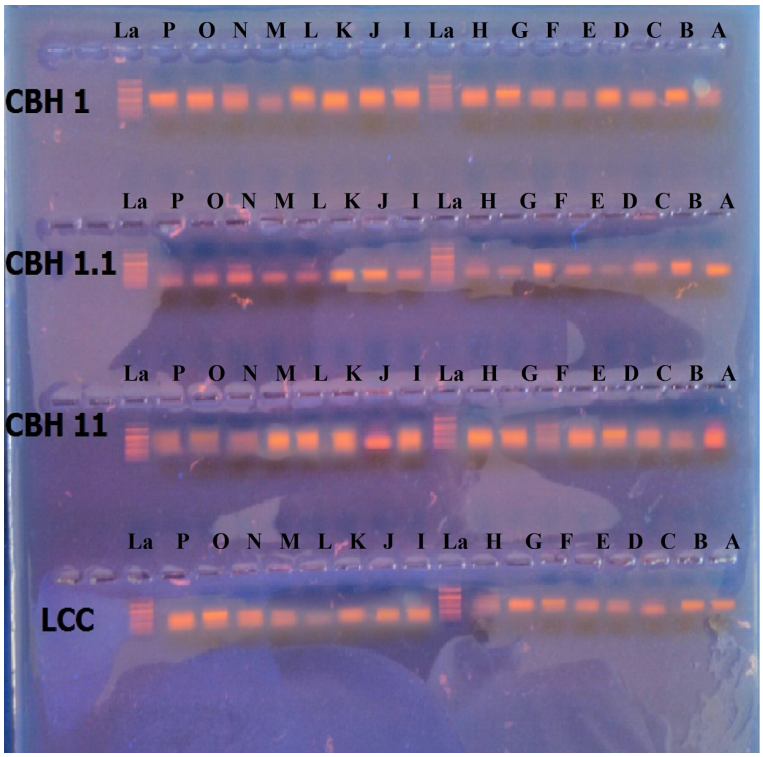
4.4 Presence of different degrading genes in the selected rhizosphere fungi

All the fifteen (15) tested degrading genes were detected to be present in each of the fungal strains after the PCR reaction; the primers used for the amplification of these catalytic enzyme coding genes are reported in Table 4.1.

The tested genes are novel lignin peroxidases (*lig1-lig6*), and manganese peroxidase (*mnp*), which are earlier detected in *Phanaerochaete chrysosporium* (a basidiomycete higher fungi), others are Catalases (*cbh1-cbh3*), Laccase (*lcc*), α-Galactosidase (*trpc*), Phosphoesterases (*opd* and *mpd*), Catechol 1,2- dioxygenases (*afk2-efk4*) and Calmodulin (*cam*) as shown in Plates 4.2a-d.

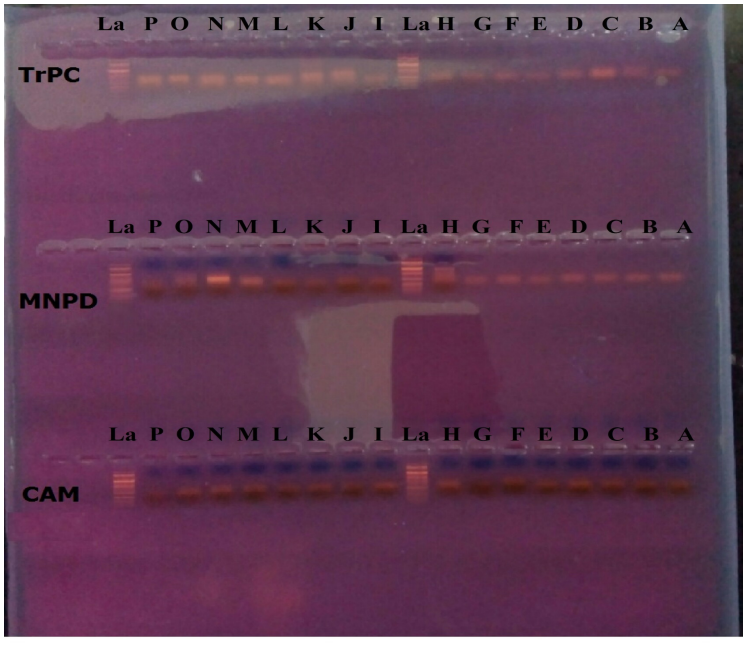

La P O N M L K J I La H G F E D C B A
LIG 1
La P O N M L K J La I H G F E D C B A
LIG 2
La P O N M L K J I La H G F E D C B A
LIG 4
La P O N M L K J I La H G F E D C B A
LIG 6

a


La P O N M L K J I La H G F E D C B A
CBH 1
La P O N M L K J I La H G F E D C B A
CBH 1.1
La P O N M L K J I La H G F E D C B A
CBH 11
La P O N M L K J I La H G F E D C B A
LCC

b

c



d

Plate 4.2: PCR-detection of xenobiotics degradin genes in selected rhizosphere fungi **(a)** LIG 1, LIG 2, LIG 4 and LIG 6 = lignin peroxidase genes; **(b)** CBH 1.1, CBH 1.2 and CBH 11= catalases genes while LCC = laccase gene; **(c)** AFG1 and CBH 2 = catechol 1, 2,-dioxygenate genes, MNP = manganesse peroxidase gene while OPD = phosphoesterase gene **(d)** TRPC = alpha-galactoxidate gene, MPD = phosphoesterase gene while CAM = calmodulin gene; A-P = asemoA to P; La = DNA ladder.

4.5 Gene expression in the rhizosphere fungi

Expression of all the dectected genes through the use of reverse transcripase PCR method which commonly known as RT-PCR method, the genes were amplified on synthesised complementary DNA (cDNA) and this showed that many of the genes were over-expressed or moderately expressed in the fungal strains with some little number of the fungal strains showing an underexpression of some genes.

Quality of the extracted fungal RNA is shown in Plate 4.3; the expression studies were done after the mRNA have been converted in complementrary DNA. Plate 4.4 shows the result of the gene expression in all the sixteen (16) fungal strains asemoA-P. It was observed that lignin peroxidase genes (*lig1, lig2, lig4 and lig6*) were overexpressed in *C. albicans* asemoD, *T. purpurogenus* asemoF, *T. astroroseus* asemoG, *Penicillium* sp. asemoH, *A. flavus* asemoM, and *T. purpurogenus* asemoN; moderately expressed in *C. elegans* asemoB and *F. solani* asemoI while they were underxpressed in *A. niger* asemoA and *Y. lipolitica* asemoO. Also, *lig*4 and *lig*6genes were also overexpressed in *A. niger* asemoC, *T. harzianum* asemoJ and *A. clavatus* asemoL but underexpressed in *lig*1 while *A. awamori* asemoE, *F. solani* asemoI and *A. flavus* asemoP showed moderate expression of *lig*4 and *lig*6 (Plate 4.4a).

Also, catalase (*cbh*1.1, *cbh*1.2 and *cbh*11) were overexpressed in *A. niger* asemoC, *C. albicans* asemoD, *F. solani* asemoI, *A. flavus* asemoM and *T. purpurogenus* asemoN; moderately expressed in strains *Y. lipolitica* asemoO but *A. flavus* asemoA, *A. awamori* asemoE, *T. astroroseus* asemoG, *A. oryzae* asemoK and *A. clavatus* asemoL show an underexpression of *cbh* 1.1 (Plate 4.4b), laccase (*lcc*) was overexpressed in *A. niger* asemoC, *C. albicans* asemoD, *A. awamori* asemoE, *T. astroroseus* asemoG, *Penisilium* sp. asemoH, *F. solani* asemoI, *T. harzianum* asemoJ, *A. clavatus* asemoL, *A. flavus* asemoM and *T. purpurogenus* asemoN but moderately expressed in *A. niger* asemoA, *C. elegans* asemoB, *A. oryzae* asemoK, *Y. lipolitica* asemoO and *A. flavus* asemoP (Plate 4.4a), *afk*3 and *afk*4 genes were overexpressed in *A. niger* asemoC, *T. purpurogenus* asemoF, *Penisilium* sp. asemoH, *F. solani* asemoI, *T. harzianum* asemoJ, *A. oryzae* asemoK, *A. clavatus* asemoL, *A. flavus* asemoM and *A. flavus* asemoP; moderately expressed in *A.*

niger asemoA and *T. purpurogenus* asemoN while *C. elegans* asemoB and *Y. lipolitica* asemoO shows an overexpression of *afk*4 but moderately expression of afk 3 but in general *afk*4 is more expressed in the fungal strain compared to *afk*3 (Plate 4.5a).

The manganese peroxidase gene (*mnp*) was overexpressed in all the strains except *A. niger* asemoA that showed a moderate expression of *mnp*, this expression in far more that the lignin peroxidase genes shown in Plate 4.5b, the fungal strains can therefore be said to exibited more expression of manganese peroxidase over the lignin peroxidase. Moreover, their was also robust expression of *opd-A* in the fungal strains, this gene catalyses the degradation of pesticides and it was overexpressed generally in all the fungal trains except in *T. pupurogenus* asemoF and *A. flavus* asemoP which showed moderate expressions of *opd-A*, *trpc*gene was only over expressed in asemoC and *F. solani* asemoI but moderately expressed in *A. niger* asemoA, *C. albicans* asemoD, *A. awamori* asemoE, *T. harzianum* asemoJ, *A. clavatus* asemoL, *A. flavus* asemoM, *Y. lipolitica* asemoO and *A. flavus* asemoP. The *mpd* gene was overexpressed in *A. niger* asemoC, *T. pupurogenus* asemoF and *F. solani* asemoI, but moderately expressed in every other fungal strains. Calmodulin (*cam*) gene was moderately expressed in all the fungal strains but overexpressed in *A. niger* asemoC, *T. harzianum* asemoJ and *A. oryzae* asemoK (Plate 4.5b).

Furthermore, on the basis of each strain, the genes were more expressed in some fungal isolates than others (Plates 4.6a-b). More expression of the genes was observed in *C. albicans* asemoD, *T. astroroeus* asemoG, *Penicillium* sp. asemoH, *F. solani* asemoI, *T. pupurogenus* asemoF, *T. harzianum* asemoJ, *A. oryzae* asemoK, *A. flavus* asemoM and *T. purpurogenum* asemoN followed by *C. elegans* asemoB, *A. niger* asemoC, *A. awamori* asemoE, *A. clavatus* asemoL, *Y. lipolitica* asemoO and *A. flavus* asemoP while the least expression was observed in *A. niger* asemoA.

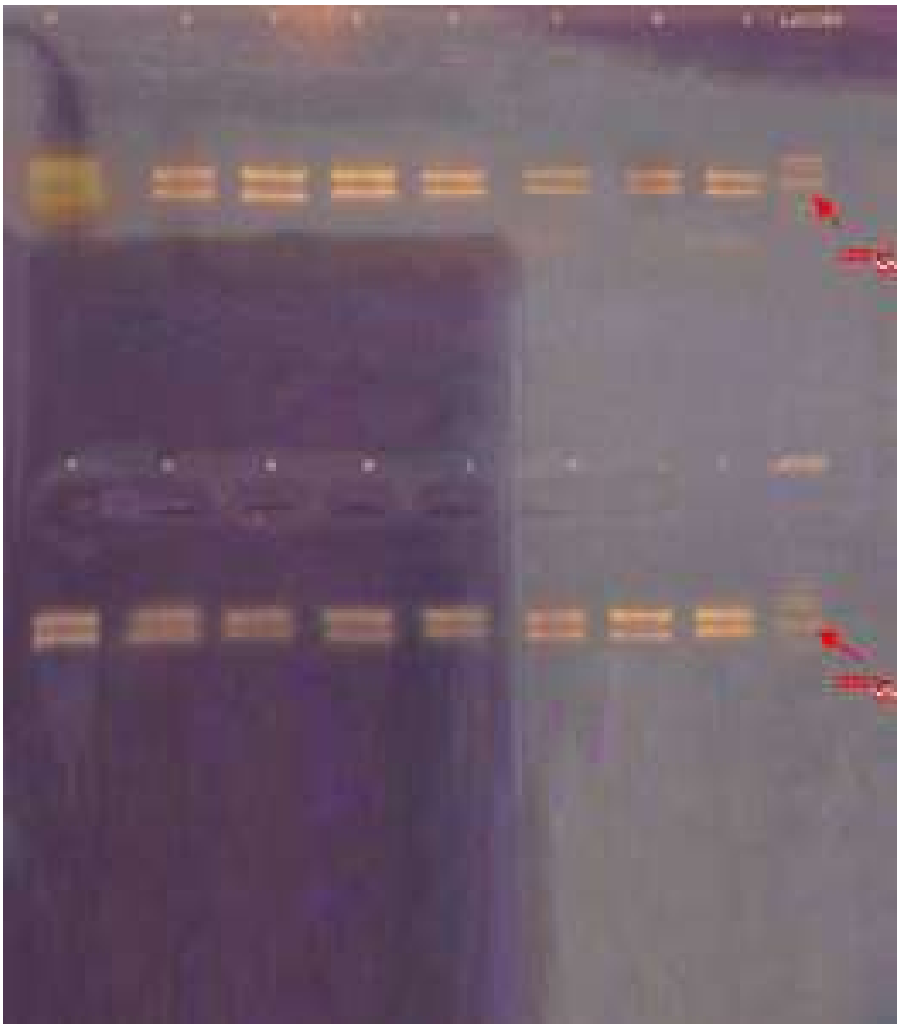


Plate 4.3: Quality of RNA used for RT-PCR as viewed on gel electrophoresis; A-P = asemoA to P; La = DNA ladder.

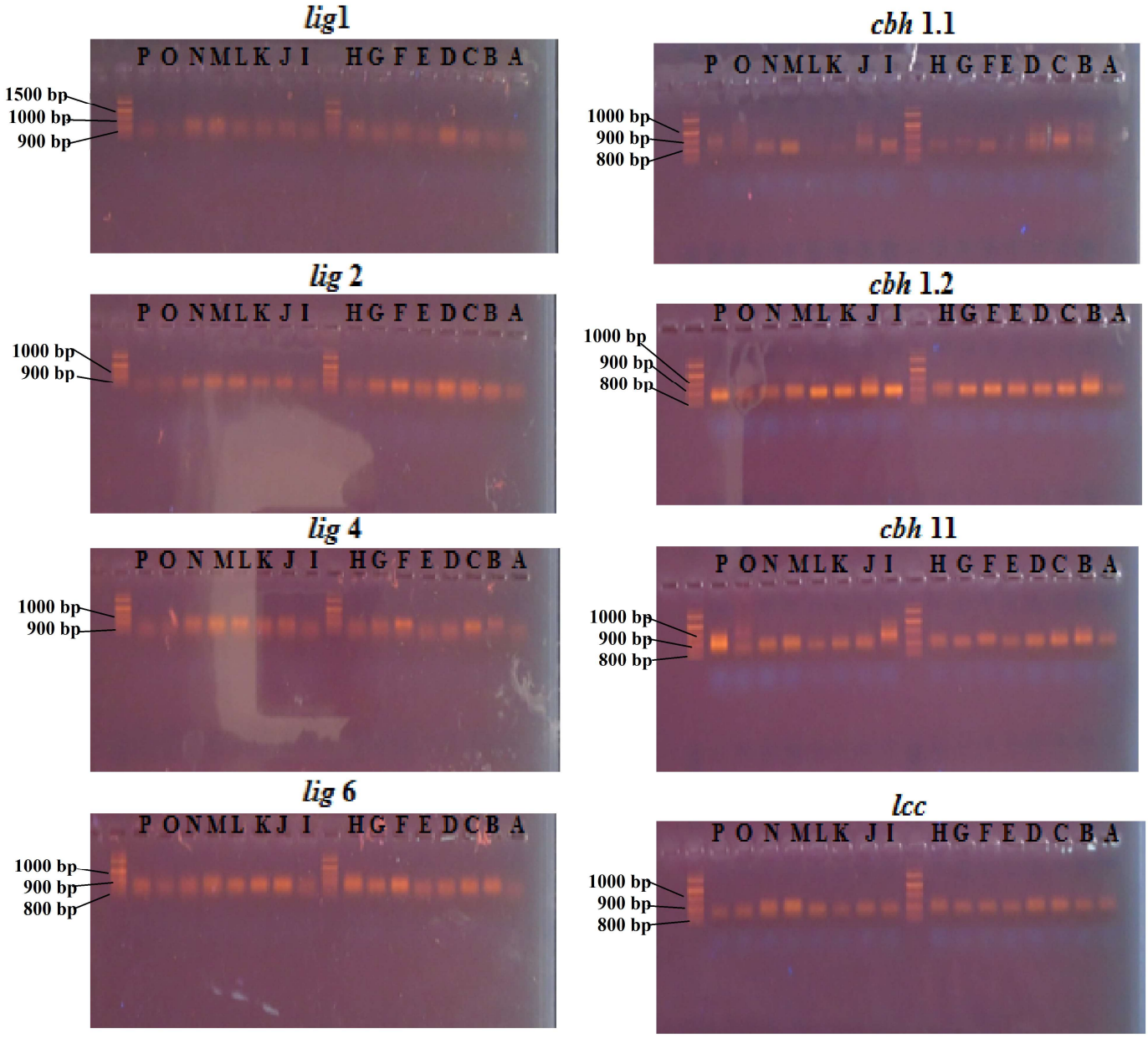


Plate 4.4a: RT-PCR expression of peroxidase genes (*lig*1, *lig*2, *lig*4, *lig*6), catalase genes (*cbh*1, *cbh*1.1, *cbh*11) and laccase genes (*lcc*) in rhizosphere fungal strains; A-P = asemoA to P.

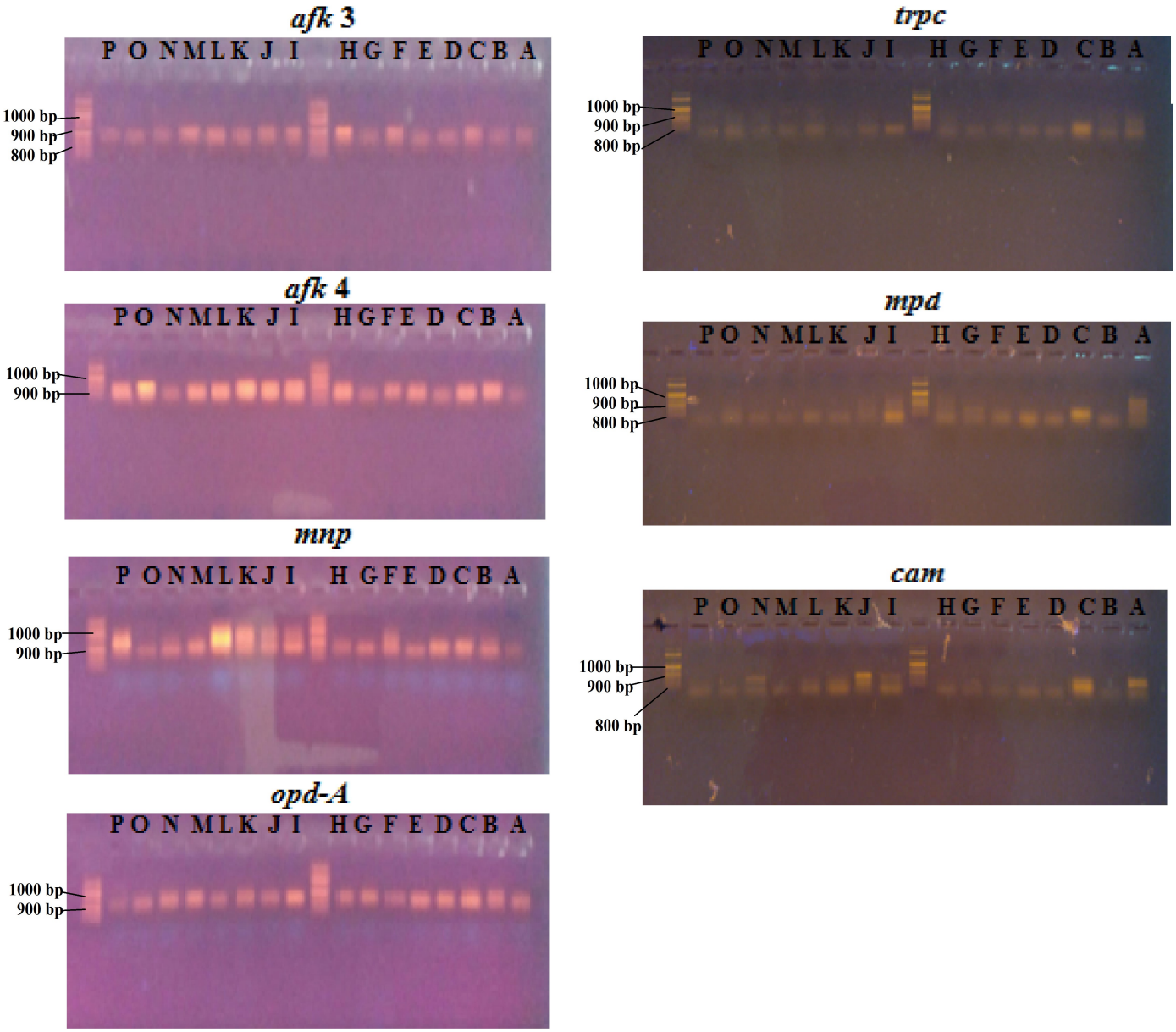


Plate 4.4b: RT-PCR expression of catechol 1, 2-dioxygenase genes (*afk3, afk4*), mangenese peroxidase gene (*mnp*), phosphoesterase genes (*opd-A and mpd*) and camodulin gene (*cam*) in rhizosphere fungal strains; A-P = asemoA to P.

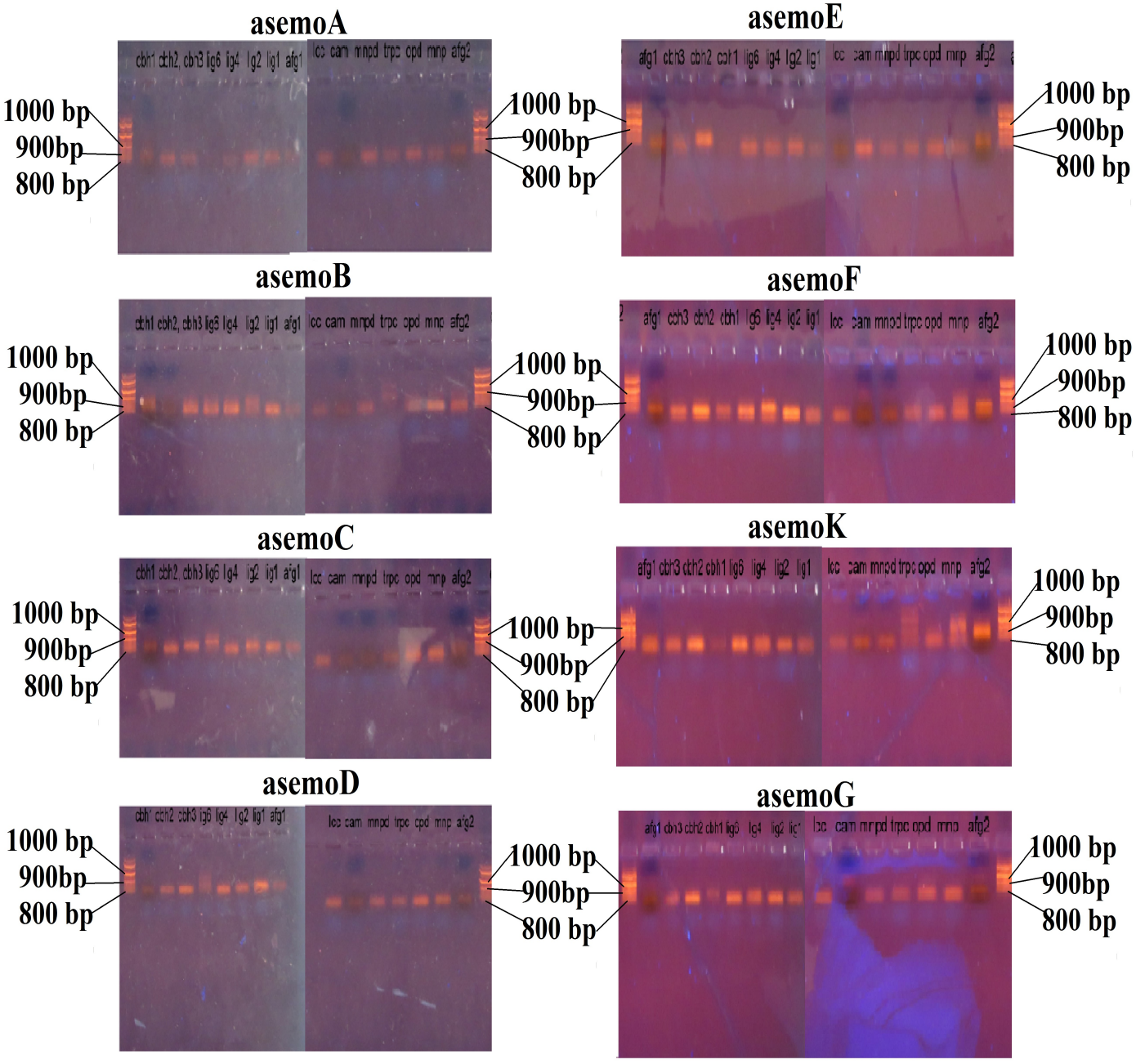


Plate 4.5a: RT-PCR expression of different degrading enzyme genes in rhizosphere fungi asemoA, asemoB, asemoC, asemoD, asemoE, asemoF, asemoK and asemoG; *lig*1, *lig*2, *lig*4, *lig*6 (peroxidase genes), *cbh*1, *cbh*1.1, *cbh*11 (catalase genes), *lcc* (laccase gene), *efk*1 and *efk*2 (catechol 1, 2,-dioxygenate genes), *mnp* (manganesse peroxidase gene), *opd, mpd* (phosphoesterase genes), *trpc* (alpha-galactoxidate gene) and calmodulin gene (*cam*).

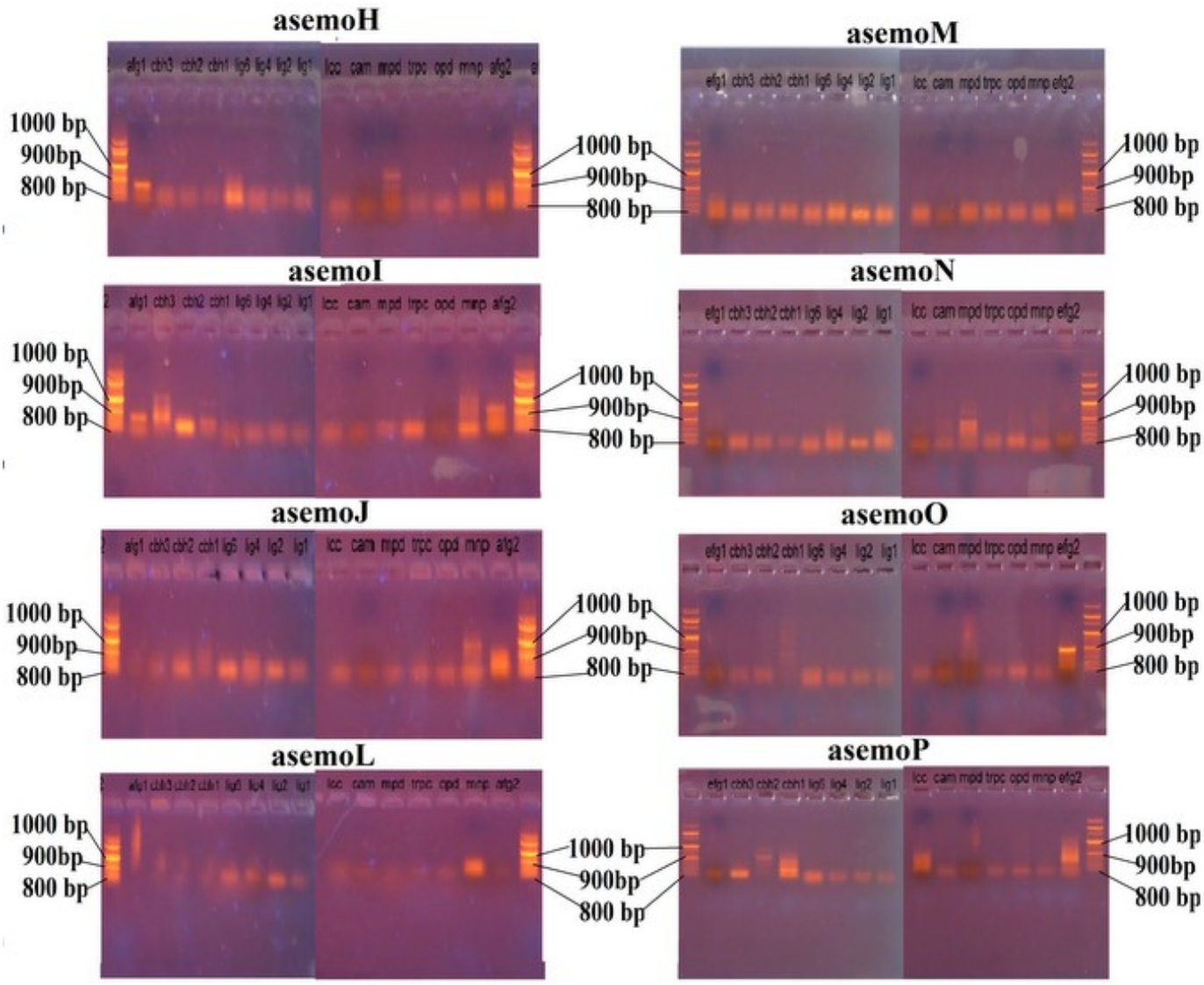


Plate 4.5b: RT-PCR expression of different degrading enzyme genes in rhizosphere fungi asemoH, asemoI, asemoJ, asemoL, asemoM, asemoN, asemoO and asemoP; *lig*1, *lig*2, *lig*4, *lig*6 (peroxidase genes), *cbh*1, *cbh*1.1, *cbh*11 (catalase genes), *lcc* (laccase gene), *efk*1 and *efk* 2 (catechol 1, 2,-dioxygenate genes), *mnp* (manganesse peroxidase gene), *opd, mpd* (phosphoesterase genes), *trpc* (alpha-galactoxidate gene) and calmodulin gene (*cam*).

4.6 Fungal Extracellular Enzyme Activities

Results on the enzyme activities which reproduced the selected rhizosphere are present on the Figure 4.2a-d below. Enzyme activities were observed to be influenced by crude oil concntrations. In most of the strains, the lignin peroxidase activity was more observed in *T. purpurogenus* asemoF, *Penicillium* sp. asemoH, *T. harzianum* asemoJ and *A. flavus* asemoM respectively (Figure 4.2a), this as well correspond its geneoverexpression as earlier reported. However, *A. awamori* asemoE and *A. flavus* asemoP showed reverse response to crude oil concentration as their lignin peroxidase activities decrease with oil concentration.

There was higher manganesse peroxidase activity produced by *T. astroroseus* asemoG, *T. harzianum* asemoJ, and *A. clavatus* asemoL with direct response to crude oil concentration (Figure 4.2b) The manganesse peroxidase activity showed that, the activity increases as the oil concentration increases. While *T. purpurogenus* asemoF and *A. flavus* asemoP also decrease the manganese peroxidase production as the oil concentration increases, *T. purpurogenus* asemoF and *A. flavus* asemoP had less tolerance to oil contaminationas their peroxidase production reduced with oil concentration increase.

Furthermore, catalase production (Figure 4.2c) was more observed in *A. niger* asemoC, *T. purpurogenus* asemoF, *T. harzianum* asemoJ, *F. solani* asemoI, *A. oryzae* asemoK and *A. flavus* asemoM followed by *Y. lipolitica* asemoO, *A. flavus* asemoP, *C. elegans* asemoB, *A. awamori* asemoE, *T. astroroseus* asemoG, and *Penicillium* sp. asemoH while the highest laccase production (Figure 4.2d) were observed in *T. purpurogenus* asemoF, *A. flavus* asemoM and *T. purpurogenus* asemoN it would be recalled that more expression of these genes were observed in *T. purpurogenus* asemoF, *A. flavus* asemoM and *T. purpurogenus* asemoN.

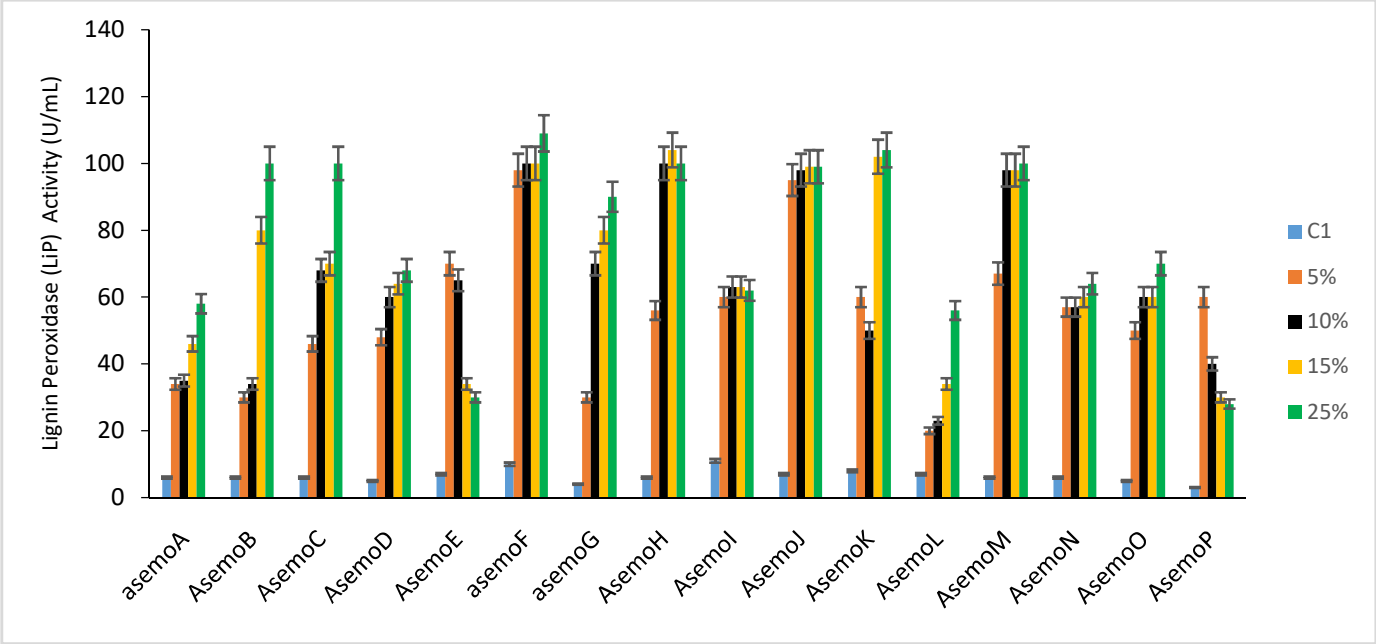


Figure 4.2a: Activities of Lignin peroxidase (LiP) produced by the rhizosphere fungi (U/mL) at different crude oil concentrations (%)

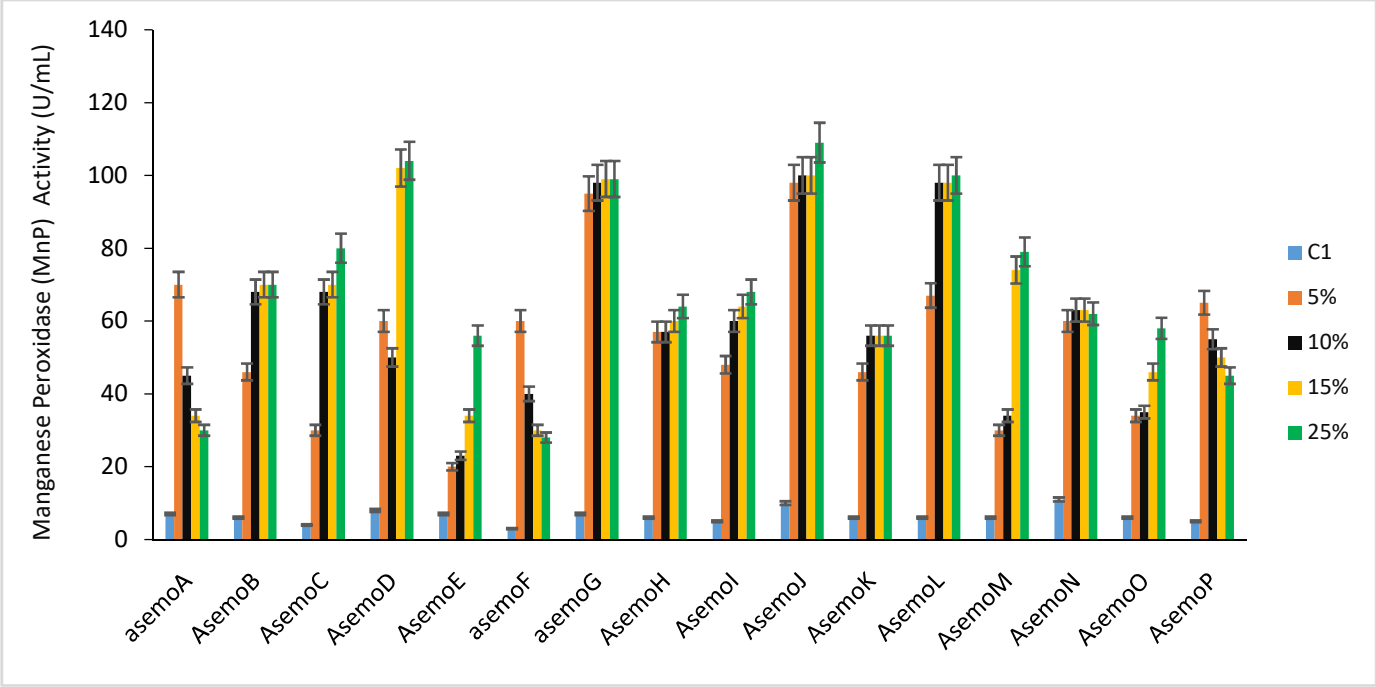


Figure 4.2b: Activities of Manganese peroxidase (MnP) produced by the rhizosphere fungi (U/mL) at different crude oil concentrations (%)

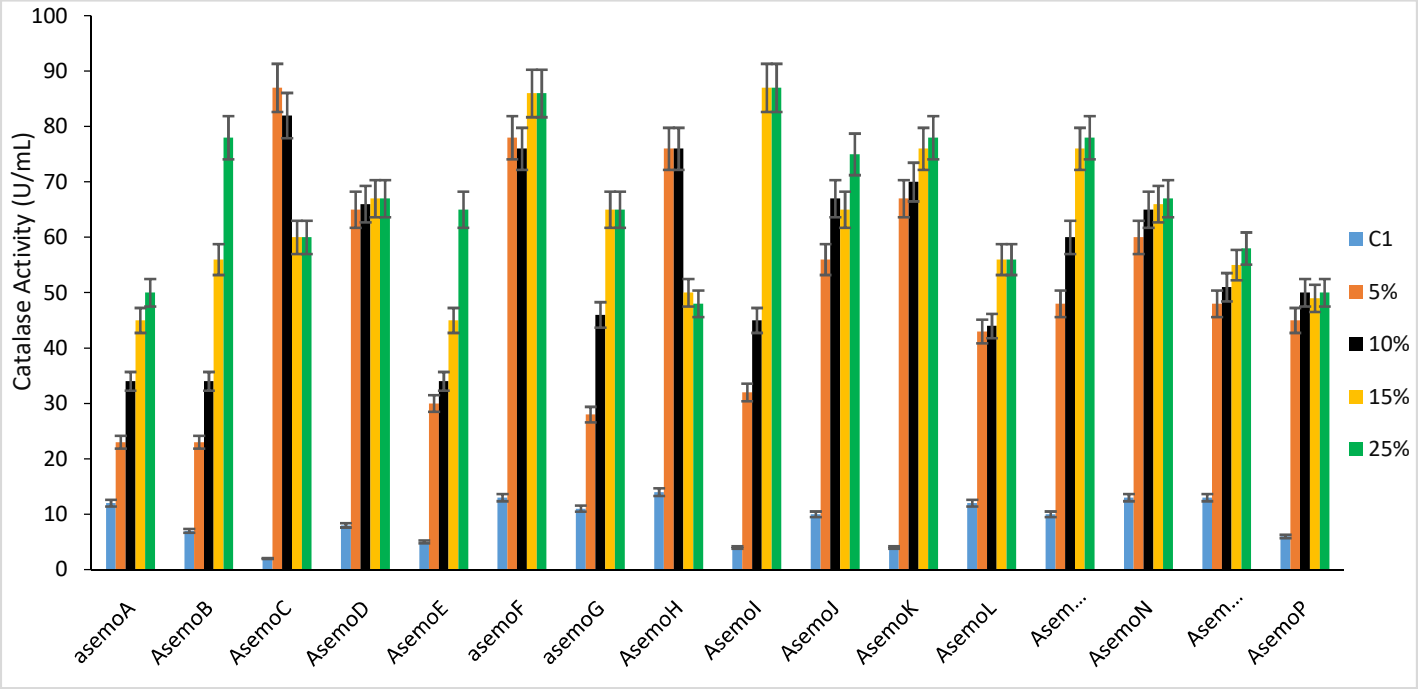


Figure 4.2c: Activities of Catalase (CAT) produced by the rhizosphere fungi (U/mL) at the presence of different cooncentrations of crude oil (%)

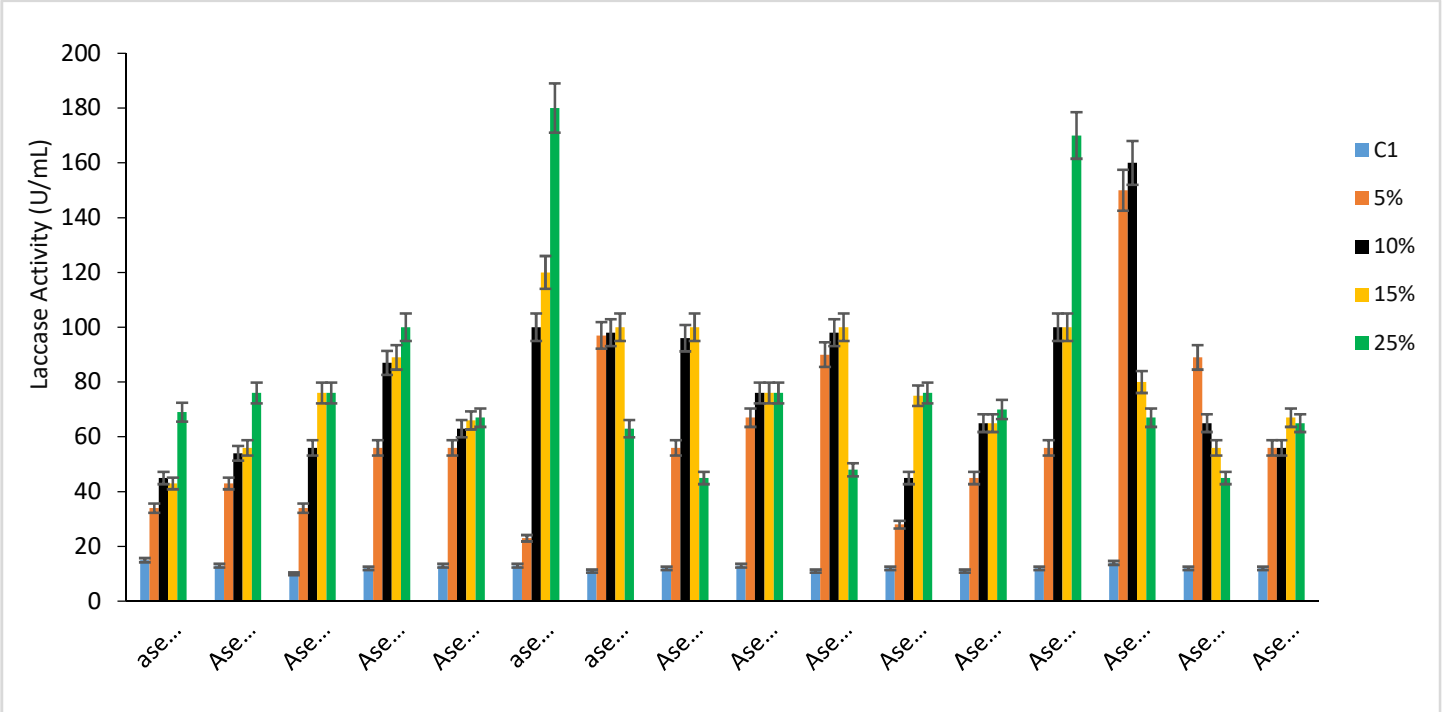


Figure 4.2d: Activities of Laccase (LCC) produced by the rhizosphere fungi (U/mL) at the presence of different cooncentrations of crude oil (%)

4.7 Synergistic Bioremediation

4.7.1 Effect of synergistic remediation on the soil nutrient

The data obtained from the pre and post experimental annalysis showed that the synergistic fungi-SMC-plant treatments enhanced both the nutrient and remediation of poluted soils (Table 4.8).

In soil 1, the soil pH increased from an initial acidic condition (4.7) to normal at 6.9 (almost neutral) before and after the synergistic experiment, the N, P and Kcontent in this soil also increased with more improvement as the treatment increases. There were however sharp reductions in organic carbon, N, Ca, and Mg in the controls $0\%_1$(plant alone without SMC-Fungi treatment) and $0\%_2$ (SMC-Fungi mixture alone) and these may be as a result of phyto and myco-accummulation of these nutrients from the soil in both controls. Na however increased in the treatments as 0.238 and 0.411 mg/L were recorded at 20 and 30 % treatments respectively after the experiment (Table 4.8) with other important macronutrients Mg, K, and P which were recorded higher in the tretated soils than in the controls. Notably here is the enhancement of the soil's cation exchanngeable capacity the control, initial CEC of 7.68 Me/100g recorded control (plant alone) and this rose to 8.62 after the experiment however, further increaments of 31.1 and 30.9 Me/100g were observed in 40 and 30 % treatments respectively.

Similar results were observed in other soils 2, 3 and 4. The pH was brough to normal in treatments to enhance effective functioning of the soil organisms. All the tested soils 1, 2, 3 and 4 were very acidic within the range of 4.3-5.2 but they were all enhanced after the experiments up till 6.9 in most soil. Initial organic carbon in contaminated soils (1-4) range from 1.0 in soil 2 to 5.0 in soil 4 but these increased to 6.1 and 5.8 in the two soils respectively in 40 % treatment, Total nitrogen, average potassium and magnesium were also increased in the treatment (Table 4.8). The soil CEC, NPK values and some other recorded macro and micronutrientswere positively enhanced as the treatment increases,

some initially absent nutrients such as Na and Mg were detected in 30 and 40 % treatments in soils 1 and 2 after the experiment.

This affirms the importance of SMC as biostimulant and manure. Mixing it with rhizosphere fungi in polluted soil treatment is of emense importance as it helps the soil remediation at the same time enhances the soil nutrients bringing about perfect restoration of the impacted soil

Table 4.8: The effects of fungi-SMC-root interaction on the characteristic of the studied polluted soils (1-4) after 3 months

Soil	Treatments	pH (H_2O)	Org. C (%)	Org N (%)	Av. P (ppm)	CEC Me/100g	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Mo (mg/L)	Mn (mg/L)	K (mg/L)	P (mg/L)
	(m)	4.7^{c}	2.2^{c}	2.5^{b}	20.7^{d}	7.7^{c}	3.17^{c}	0.00^{d}	0.00^{b}	17.03^{b}	7.846^{c}	21.62^{a}	20.66^{c}
	0%	4.9^{c}	2.3^{c}	2.2^{b}	20.7^{d}	8.6^{c}	0.61^{d}	0.12^{d}	0.00^{b}	18.57^{a}	17.85^{a}	36.6^{a}	23.68^{c}
	$0\%_2$	5.1^{b}	2.3^{c}	2.4^{ab}	20.0^{d}	7.9^{c}	0.82^{d}	0.23^{d}	0.01^{b}	18.34^{a}	18.11^{a}	33.23^{a}	23.11^{c}
	T_1	5.7^{b}	3.6^{b}	2.7^{a}	31.2^{c}	20.8^{b}	3.39^{c}	3.14^{c}	0.12^{a}	18.75^{a}	19.61^{a}	41.68^{a}	34.78^{b}
1	T_2	6.6^{a}	3.7^{b}	2.6^{a}	61.4^{a}	20.8^{b}	6.22^{b}	5.87^{b}	0.238^{a}	18.43^{a}	14.93^{b}	41.68^{a}	40.80^{a}
	T_3	6.8^{a}	3.7^{b}	2.9^{a}	61.4^{a}	30.9^{a}	9.76^{a}	9.08^{a}	0.411^{a}	19.01^{a}	14.07^{b}	41.68^{a}	40.86^{a}
	T_4	6.9^{a}	5.9^{a}	2.9^{a}	41.5^{b}	31.1^{a}	3.80^{c}	1.59^{d}	0.00^{b}	18.01^{a}	11.73^{c}	41.69^{a}	40.88^{a}
	(m)	5.2^{b}	1.0^{a}	2.6^{b}	20.9^{c}	10.7^{d}	3.33^{c}	0.00^{c}	0.00^{b}	19.95^{a}	11.73^{c}	31.66^{a}	20.63^{c}
	0%	6.6^{a}	2.1^{a}	2.8^{a}	41.3^{b}	30.7^{c}	2.80^{c}	3.65^{b}	0.24^{b}	20.94^{a}	20.92^{b}	41.67^{b}	20.64^{c}
	$0\%_2$	6.8^{a}	1.9^{a}	2.9^{a}	43.23^{b}	31.1^{c}	3.00^{c}	3.24^{b}	0.28^{b}	21.34^{a}	42.32^{a}	45.23^{b}	22.45^{a}
	T_1	6.6^{a}	4.1^{a}	2.8^{a}	51.4^{a}	40.7^{b}	8.50^{b}	5.173^{a}	0.56^{b}	20.75^{a}	25.8^{b}	51.68^{a}	24.80^{a}
2	T_2	6.8^{a}	6.1^{a}	2.7^{b}	51.4^{a}	51.1^{a}	11.15^{a}	5.726^{a}	0.57^{b}	20.87^{a}	32.2^{a}	41.68^{b}	23.84^{a}
	T_3	6.7^{a}	6.2^{a}	2.9^{a}	$51.^{5a}$	51.1^{a}	12.17^{a}	3.16^{b}	0.083^{b}	21.4^{a}	20.92^{b}	48.69^{a}	30.85^{a}
	T_4	6.8^{a}	6.1^{a}	$2.^{9a}$	51.4^{a}	51.1^{a}	7.90^{b}	4.492^{a}	1.09^{a}	23.15^{a}	28.85^{b}	41.68^{b}	30.87^{a}
	(m)	4.3^{c}	3.1^{b}	2.9a	$24.^{8c}$	10.7^{e}	4.22^{b}	0.22^{c}	0.12^{c}	16.23^{b}	8.9^{c}	21.63^{c}	21.59^{c}
	0%	5.7^{b}	3.7^{b}	2.8a	30.9^{c}	21.1^{d}	3.21^{c}	4.55^{b}	0.56^{b}	19.22^{a}	18.22^{b}	31.65^{a}	30.64^{c}
	$0\%_2$	6.0^{a}	3.9^{b}	3.1a	56.8^{a}	62.2^{a}	7.8^{a}	8.22^{a}	1.82^{a}	22.67^{a}	16.33^{b}	35.45^{a}	42.13^{b}
	T_1	6.3^{a}	5.7^{a}	2.9a	51.4^{b}	61.1^{a}	7.3^{a}	6.8^{a}	1.22^{a}	23.1^{a}	16.78^{b}	31.65^{b}	40.65^{c}
3	T_2	6.7^{a}	5.7^{a}	2.9a	51.5^{b}	51.1^{b}	7.8^{a}	6.9^{a}	1.31^{a}	22.1^{a}	40.2^{a}	39.68^{a}	40.77^{b}
	T_3	6.8^{a}	5.5^{a}	3.1a	51.5^{b}	41.1^{c}	6.2^{a}	8.8^{a}	1.12^{a}	23^{a}	40.2^{a}	34.68^{a}	40.81^{a}
	T_4	6.8^{a}	5.6^{a}	3.1a	51.5^{b}	41.1^{c}	5.99^{b}	8.2^{a}	0.99^{a}	23.11^{a}	39.2^{b}	41.69^{a}	40.88^{a}
	(m)	4.9^{b}	5.0^{a}	2.8b	22.7^{d}	21.7^{c}	3.2^{c}	2.11^{c}	0.01^{b}	19.23^{a}	7.2^{c}	31.7^{a}	14.67^{c}
	0%	4.9^{b}	2.6^{b}	2.8b	30.9^{c}	20.9^{c}	4.5^{c}	4.5^{b}	0.34^{a}	23.11^{a}	7.9^{c}	41.7^{a}	20.76^{b}
	$0\%_2$	6.3^{a}	5.2^{a}	3.3a	45.23^{b}	34.2^{b}	5.2^{b}	4.82^{b}	0.56^{a}	23.89^{a}	10.34^{c}	43.34^{a}	28.98^{a}
	T_1	6.5^{a}	5.5^{a}	2.9b	40.9^{b}	20.9^{c}	6.7^{b}	5.6^{a}	0.45^{a}	22.12^{a}	23.45^{b}	41.7^{a}	30.82^{a}
4	T_2	6.5^{a}	5.7^{a}	3.1a	41.2^{b}	20.9^{c}	5.60^{b}	5.55^{a}	0.46^{a}	21.3^{a}	34.22^{b}	41.66^{a}	40.83^{a}
	T_3	6.6^{a}	5.7^{a}	3.1a	41.4^{b}	61.2^{a}	8.00^{a}	4.2^{b}	0.52^{a}	22.1^{a}	40.3^{a}	41.68^{a}	40.85^{a}
	T_4	6.7^{a}	5.8^{a}	3.2a	61.5^{a}	61.1^{a}	8.23^{a}	4.5^{b}	0.51^{a}	22.1^{a}	45.2^{a}	41.68^{a}	40.86^{a}

Each value is a mean of six replicated determinations; Means in columns having the same superscript letter are not significantly different (α0.05); T1-T4 are the synergistic fungi-SM C-plant treatments at 10-40% concentrations respectively.

4.7.2 Effects of the synergistic fungi-SMC-plant treatments on heavy metals

The analysed heavy metal content of the soils 1-4 revealed that the soils initially contained high heavy metal concentrations as shown in Table 4.9. Soils 1 and 2 which are hydrocarbon contaminated soils had higher heavy metal contents compared to pesticides polluted soils 2 and 3.

In soil 1, there were high initial concentrations of heavy metals many of which became reduced after 3 month synergistic experiment. Notably, As with initial contentration of 6.33 mg/L became reduced to about 2.49 mg/L in 20 and 30 % treatemts expecially in 20 and 30 %, Hg reduced from 5.654 to 1.72 mg/L in 30 % treatment, Fe reduced from 4.288 to mg/L to 2.2 in 40 %, initia concentration of 3.83 mg/L Zn was reduced to 0.484 mg/L while Cu reduced from 1.17 mg/L0.202 at 40 % treatment. Cr and Ni were completely removed in 40 and 30 % treatments respectively (Table 4.9).

Soil 2 had higher initial concentrations of Fe 9.34 mg/L but became reduced to 8.40 at 10 %, Hg reduced from 6.311 to 3.40 mg/L in 40 %, Ar reduced from 5.41 to 4.00 mg/L in 40 %, Zn from 1.65 to 0.48 mg/L in 40 % and Cu from 1.35 to 0.06 mg/L in 40% (Table 4.9). Here there was total extraction of Fe and Cr in 0 (control).

The same trend was observed in pesticides polluted soils 3 and 4 (Tables 4.9); initial concentrations of all the heavy metals are reduced in control but became more reduced in the treatments. There was complete removal of Fe, Cd, Cr and Co at 30 and 40 % treatments in soil 3 while Cr, Ni, Pb, Cd and Cu were completely removed in soil 4.

Table 4.9: Effect of synergistic fungi-SMC-plant treatments on heavy metal content in polluted soils after 3 months

Soil	Treatment	Heavy metals (mg/L)									
		Cd	**Cu**	**Pb**	**Fe**	**Ni**	**Cr**	**Hg**	**Co**	**As**	**Zn**
	(m)	0.064^{a}	1.170^{9a}	0.296^{a}	4.288^{a}	0.632^{a}	0.019^{a}	5.654^{a}	0.130^{a}	6.330^{a}	3.830^{a}
	$0\%_1$	0.020^{d}	1.094^{b}	0.220^{a}	2.156^{b}	0.403^{b}	0.00^{b}	3.902^{b}	0.060^{b}	3.112^{b}	3.320^{a}
	$0\%_2$	0.054^{a}	1.050^{b}	0.220^{a}	3.670^{a}	0.40^{b}	0.00^{b}	3.230^{b}	0.100^{a}	3.020^{b}	3.110^{b}
	T_1	0.046^{b}	1.113^{b}	0.179^{b}	3.279^{a}	0.00^{d}	0.00^{b}	3.202^{b}	0.090^{b}	3.220^{b}	1.310^{b}
S1	T_2	0.037^{b}	0.259^{c}	0.171^{b}	3.804^{b}	0.29^{c}	0.00^{b}	2.816^{c}	0.068^{b}	2.890^{b}	1.490^{b}
	T_3	0.021^{d}	0.219^{c}	0.022^{c}	3.124^{b}	0.00^{d}	0.00^{b}	1.726^{d}	0.077^{b}	2.990^{b}	1.972^{b}
	T_4	0.015^{c}	0.202^{c}	0.190^{c}	2.20^{c}	0.00^{d}	0.00^{b}	2.498^{c}	0.072^{b}	3.110^{b}	0.484^{c}
	(m)	0.073^{a}	1.350^{a}	0.396^{a}	9.846^{a}	0.26^{a}	0.046^{a}	6.811^{a}	0.161^{b}	5.410^{a}	1.643^{a}
	$0\%_1$	0.036^{c}	0.156^{b}	0.383^{a}	0.00^{b}	0.00^{d}	0.022^{b}	5.946^{b}	0.150^{b}	4.200^{b}	1.644^{a}
	$0\%_2$	0.000^{e}	0.100^{b}	0.300^{a}	0.00^{b}	0.00^{d}	0.040^{a}	5.210^{b}	0.160^{b}	3.320^{c}	1.020^{a}
	T_1	0.037^{c}	0.296^{c}	0.331^{a}	8.400^{a}	0.127^{b}	0.021^{b}	2.549^{c}	0.150^{b}	3.330^{c}	0.633^{b}
S2	T_2	0.043^{b}	0.134^{b}	0.205^{b}	9.070^{a}	0.00^{d}	0.007^{c}	5.729^{b}	0.170^{a}	4.220^{b}	0.627^{b}
	T_3	0.018^{d}	0.085^{d}	0.326^{b}	8.702^{a}	0.003^{c}	0.041^{b}	3.593^{c}	0.150^{b}	4.120^{b}	0.859^{b}
	T_4	0.025^{e}	0.081^{d}	0.207^{c}	7.935^{a}	0.00^{d}	0.007^{b}	3.484^{c}	0.137^{c}	4.000^{b}	0.484^{b}
	(m)	0.020^{a}	1.110^{a}	0.330^{a}	7.100^{a}	0.32^{a}	0.020^{a}	6.200^{a}	0.230^{a}	5.800^{a}	1.650^{a}
	$0\%_1$	0.000^{b}	0.660^{b}	0.240^{b}	6.700^{b}	0.05^{b}	0.000^{b}	5.900^{b}	0.020^{b}	4.200^{b}	1.220^{b}
	$0\%_2$	0.000^{b}	0.120^{c}	0.220^{b}	6.520^{b}	0.000^{c}	0.000^{b}	3.000^{c}	0.000^{c}	4.300^{b}	0.450^{c}
	T_1	0.000^{b}	0.650^{b}	0.230^{b}	6.300^{b}	0.04^{b}	0.000^{b}	5.200^{b}	0.030^{b}	4.300^{b}	0.600^{c}
S3	T_2	0.000^{b}	0.570^{b}	0.000^{d}	5.200^{c}	0.000^{c}	0.000^{b}	5.100^{b}	0.020^{b}	4.200^{b}	0.490^{c}
	T_3	0.000^{b}	0.620^{b}	0.230^{b}	6.300^{b}	0.000^{c}	0.000^{b}	3.200^{c}	0.000^{c}	4.200^{b}	0.450^{c}
	T_4	0.000^{b}	0.560^{b}	0.060^{c}	5.600^{c}	0.000^{c}	0.000^{b}	3.500^{c}	0.000^{c}	3.900^{b}	0.390^{c}
	(m)	0.000	0.880^{a}	0.450^{a}	4.500^{a}	0.23^{a}	0.000	5.200^{a}	0.170^{a}	3.900^{a}	0.900^{a}
	$0\%_1$	0.000	0.860^{a}	0.230^{b}	2.600^{b}	0.000^{b}	0.000	4.900^{b}	0.120^{b}	1.400^{b}	0.270^{b}
	$0\%_2$	0.000	0.300^{b}	0.220^{b}	2.280^{b}	0.000^{b}	0.000	2.600^{c}	0.110^{b}	1.120^{b}	0.230^{b}
	T_1	0.000	0.320^{b}	0.030^{c}	2.200^{b}	0.000^{b}	0.000	2.900^{c}	0.130^{b}	2.400^{a}	0.130^{b}
S4	T_2	0.000	0.000^{d}	0.020^{c}	2.100^{b}	0.000^{b}	0.000	2.200^{c}	0.110^{b}	2.100^{a}	0.140^{b}
	T_3	0.000	0.000^{d}	0.000^{d}	2.020^{b}	0.000^{b}	0.000	2.000^{c}	0.090^{c}	1.900^{b}	0.190^{b}
	T_4	0.000	0.02^{c}	0.000^{d}	1.600^{c}	0.000^{b}	0.000	2.100^{c}	0.010^{c}	1.600^{b}	0.200^{b}

Each value is a mean of six replicated determinations; Means in columns having the same superscript letter are not significantly different (α0.05); m is the initial concentration; T1-T4 are the synergistic fungi-SM C-plant treatments at 10-40% concentrations respectively.

4.7.3 Effects of the synergistic fungi-SMC-plant treatments on the PAHs in Soil 1

Data obtained from the pre-experiment and post-expriment of the PAHs in soil 1 revealed that the soils had higher concentrations of 4 rings or more ringed, complex and recalcitrant PAHs as presented in Table 4.10 below. In soil 1, Benzo(k)flouranthene (110.02 mg/kg) followed by Benzo(a)pyrene (140.24 mg/kg), Dibenzo(a,h)anthracene (40.30 mg/kg) while others detected more ringed hydrocarbons include Indeno(1,2,3-cd)pyrene (96.24 mg/kg) and Benzo(ghi)perylene (80.37), while the less ringed PAHs are Naphthalene (1.61 mg/kg), Acenaphthylene (1.12 mg/kg), Acenaphtene (0.91 mg/kg), Flourene (3.61 mg/kg), Phenanthrene (23.51) and Anthracene (22.12 mg/kg), others were Flouranthene (88.01 mg/kg), Pyrene (70.32 mg/kg) and Ben(a)anthracene (79.45) and so on Table 4.10**.**

This same trend was observed in the Total polycyclic aromatic hydrocarbons, (Table 4.10). It was also polluted with more concentrations of many rings hydrocarbons like soil 1. Initial concentration of the total 5 and 6 ringed hydrocarbon recorded in soil 1 was 467.17 mg/kg compared to the total 4 and 3 ringed hydrocarbon that was 318.17 and 64.27 mg/kg respectively while the total of all the detected PAH concentrations (TPAH) in soil 2 made a total of 851.61 mg/kg as presented in Table 4.10 below.

Table 4.10: Initial concentration of the 16 EPA PAHs in soil 1

PAHs	Rings	Mean concentration (mg/kg)	Percentage of the total in soil
Naphthalene	2	1.610	0.1895
Acenaphthylene	3	1.120	0.1300
Acenaphtene	3	0.910	0.1318
Flourene	3	3.610	0.4249
Phenanthrene	3	36.51	4.2973
Anthracene	3	22.12	2.6035
Flouranthene	4	88.01	10.359
Pyrene	4	70.32	8.2767
Ben(a)anthracene	4	79.45	9.3513
Chrysene	4	80.36	9.4584
Benzo(b)flouranthene	5	-	-
Benzo(k)flouranthene	5	110.02	12.949
Benzo(a)pyrene	5	140.24	16.506
Dibenzo(a,h)anthracene	5	40.300	4.7434
Indeno(1,2,3-cd)pyrene	6	96.240	11.480
Benzo(ghi)perylene	6	80.370	9.4596
Total of all 3 ringed hydrocarbons	-	64.270	
Total of all 4 ringed hydrocarbons	-	318.17	-
Total of all 5 and 6 ringed hydrocarbons	-	467.17	-
Total PAHs analysed	-	851.61	

- *Values are mean of three replicate determinations*
- *Benzo(b)flouranthene and Benzo(k)flouranthene are detected on the same peak.*

4.7.4. Loss (mg/kg) and percentage loss (%) of polycyclic aromatic hydrocarbon in soil 1

The loss and percentage loss (Degradation efficiency)was recorded for each treatment based on initial and final concentrations before and after the experiment(Table 4.11), from the available data, the most highly concentrated PAH in soil 1 was Indeno(1,2,3-cd)pyrene having initial concentration of 96.24 mg/kg, 47.8 mg/kg of it was loss in the control 1 after 3 month making 49.68 % loss while 87.55 and 94.35 mg/kg were loss in 20 and 30 % respectively and same trend was observed for other PAHs. However, in 0 % treatment highest percentage losses were observed for acenaphthene (91.96 %), naphthalene (86.96 %), Acephtene (70.32 %) and Ben(a)anthracene (61.67 %) most of which are smaller molecular weight PAH with smaller number of rings while the ighest percentage loss in 40 % treatment were recorded in naphthalene with highest percentage loss (98.74 %), crysene (95.77 %), Benzo(a)pyrene (94.87), and anthracene (92.18 %). 20 and 30 % treatments recorded Flourene (97.51 %), Naphtalene (98.14%), anthracene (95.57 %), acenaphtene (95.98 %), Benzo(k)flouranthene (96.07 %), Benzo(a)pyrene (95.72 %) and so on.

Table 4.11: Effect of synergistic fungi-SMC-plant treatments on the 16 EPA PAH contents in crude oil polluted soil (Soil 1) after 3 months

PAHs	No Rings	Control 1				Control 2			T_1			T_2			T_3			T_4		
		C_0	C_t	Loss	% Loss (BE)	C_t	Loss	% Loss (BE)	C_t	Loss	% Loss (BE)	C_t	Loss	% Loss (BE)	C_t	Loss	% Loss (BE)	C_t	Loss	% Loss (BDE)
Naphthalene	2	1.610	0.200	1.410	87.58	0.210	1.400	86.95	0.110	1.500	93.17	0.240	1.370	85.09	1.030	1.580	98.13	0.020	1.590	98.76
Acenaphthylene	3	1.120	0.080	1.040	92.86	0.090	1.030	91.96	0.120	1.000	89.29	0.340	0.780	69.64	0.075	1.080	95.98	0.223	0.897	80.09
Acenaphtene	3	0.910	0.200	0.710	78.02	0.270	0.640	70.33	0.120	0.790	86.81	0.104	0.806	88.57	0.030	0.887	97.47	0.080	0.830	91.21
Flourene	3	3.610	1.44	2.170	60.11	1.030	2.580	71.47	1.00	2.610	72.30	0.090	3.520	97.51	0.120	3.490	96.68	0.470	3.14	86.98
Phenanthrene	3	36.51	18.60	17.93	49.11	17.05	19.46	53.30	15.00	21.51	58.92	14.20	22.31	61.11	2.450	34.06	93.29	2.340	34.17	93.59
Anthracene	3	22.12	10.26	11.86	53.62	9.210	12.91	58.36	6.23	15.89	71.84	3.230	18.89	85.40	0.980	21.14	95.57	1.730	20.39	92.18
Flouranthene	4	88.01	60.32	27.69	31.46	60.34	27.67	31.44	45.23	42.78	48.61	12.45	75.56	85.85	4.670	83.34	94.69	9.210	78.80	89.54
Pyrene	4	70.32	51.40	18.92	26.91	49.40	20.92	29.75	36.34	33.98	48.32	11.23	59.09	84.03	5.200	65.12	92.61	8.450	61.87	87.98
Ben(a)anthracene	4	79.45	25.41	54.04	68.02	30.45	49.00	61.67	23.45	56.00	70.48	12.32	67.13	84.49	3.400	76.05	95.72	10.32	69.13	87.01
Chrysene	4	80.36	50.11	30.25	37.64	49.78	30.58	38.05	32.20	48.16	59.93	11.56	68.80	85.61	4.200	76.16	94.77	3.400	76.96	95.77
Benzo(b)flouranthene	5	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-		
Benzo(k)flouranthene	5	110.0	57.82	52.20	47.45	67.32	42.70	38.81	28.30	81.72	74.28	22.34	87.68	79.69	4.320	105.7	96.07	11.34	98.68	89.69
Benzo(a)pyrene	5	140.2	54.69	85.55	61.00	64.65	75.59	53.90	22.30	117.94	84.10	20.80	119.44	85.17	6.000	134.2	95.72	7.200	133.04	94.87
Dibenzo(a,h)anthracene	5	40.3	30.45	9.850	24.44	27.30	13.00	32.26	12.10	28.20	69.98	11.40	28.9	71.71	2.400	37.90	94.04	7.650	32.65	81.02
Indeno(1,2,3-cd)pyrene	6	96.24	58.43	37.81	39.29	48.43	47.81	49.67	12.23	84.01	87.29	11.98	84.26	87.55	1.890	94.35	98.04	14.98	81.26	84.43
Benzo(ghi)perylene	6	80.37	50.22	30.15	37.51	48.23	32.14	39.99	23.10	57.27	71.26	12.43	67.94	84.53	3.40	76.97	95.77	11.00	69.37	86.31

Values are means of three replicated determinations; Co = Intial concentration of PAH in soils (mg/g); Ct = final/residual concentration of PAH in soil (mg//kg); Loss= Co-Ct (mg/kg); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively

4.7.5 Effects of synergistic treatments on the polycyclic aromatic hydrocarbon content in soil 2

Pre and Post-analysis of the 16 EPA polycyclic aromatic hydrocarbons (PAH) in all soil 2 also revealed that soil had higher concentrations of the 4 or more ringed and more complex recalcitrant PAHs; this is presented in Tables 4.12 below. In soil 2, hydrocarbons which had the highest concentrations in the soil 2 are Benzo(a)pyrene which had 150 mg/kg concentration, Benzo(k)flouranthene was 100.23 mg/kg others are crysene (80.36 mg/kg), Benzo(ghi)perylene (74.38 mg/kg) and flouranthene (76.34 mg/kg) followed by pyrene which gave 69.34 mg/kg , followed by Indeno(1,2,3-c)pyrene which gave 63.20 mmg/kg, phenanthrene was 40.01 mg/kg while Dibenz(a,h)anthracene and Anthracene gave 39.2 mg/kg and 20.34 respectively. The less ringed PAHs included Acenaphthylene (0.96 mg/kg) , Acenaphtene (1.23 mg/kg), Naphthalene (1.22 mg/kg), Flourene (4.34 mg/kg) and so on Table (4.12).

Soil 2 was also more polluted with more concentrations of many ringed hydrocarbons, initial concentration of the total 5 and 6 ringed hydrocarbon recorded in soil 2 was 467.17 mg/kg compared to the total 4 and 3 ringed hydrocarbon that were 318.14 and 51.27 mg/kg respectively. The gross concentration of the sum total of all the PAHs in soil 2 (TPAH) gave 805.00 mg/kg as presented in Table 4.12.

Table 4.12: PAHs concentrations in the contaminated soil (Soil 2)

PAHs	Rings	Mean concentration (mg/kg)	Percentage of the total in soil
Naphthalene	2	1.220	0.190
Acenaphthylene	3	0.960	0.130
Acenaphtene	3	1.230	0.110
Flourene	3	4.340	0.430
Phenanthrene	3	40.01	2.800
Anthracene	3	20.34	2.640
Flouranthene	4	76.34	10.50
Pyrene	4	69.34	8.390
Ben(a)anthracene	4	80.32	9.480
Chrysene	4	80.36	9.590
Benzo(b)flouranthene	5	-	-
Benzo(k)flouranthene	5	100.23	13.13
Benzo(a)pyrene	5	150.23	16.73
Dibenzo(a,h)anthracene	5	39.200	4.810
Indeno(1,2,3-cd)pyrene	6	63.200	11.48
Benzo(ghi)perylene	6	78.340	9.590
Total of all 3 ringed hydrocarbons	-	51.270	-
Total of all 4 ringed hydrocarbons	-	318.14	-
Total of all 5 and 6 ringed hydrocarbons	-	467.17	-
Total PAHs analysed	-	805.00	

- *Values are mean of three replicated determinations*
- *Benzo(b)flouranthene and Benzo(k)flouranthene were analysed to have the same peak*

4.7.6 Loss (mg/kg) and percentage loss (%) of polycyclic aromatic hydrocarbon in soil 2

The most highly concentrated PAHs in soil 2 included the more ringed hydrocarbons like Benzo(a)pyrene which was 150.2 mg/kg as well as Benzo(k)flouranthene with 100.2 mg/kg, these concentrations were however brougth down to 64.6 and 67.32 in control respectively after the experiment to 4.20 and 3.34 mg/kg in 40% and 30% treatment respectively (Table 4.13). More also, % loss of Benzo(a)pyrene in the control gave 56.7 % while 89.48 and 97.20 % loss were both recorded in 30 and 40 % treatments respectively and same trend was observed for other PAHs. Percent loss of Benzo(k)flouranthene (96.67 %), crysene (90.79 %), Benzo(a)anthracene (90.88 %), Benzo(ghi)perylne (96.17 %) and Ideno(1,2,3-cd)pyrene (98.44 %) were recorded in 40% treatment but 30% and 20% treatment had the highest percentage loss of 97.01 % was recorded for Ideno(1,2,3-cd)pyrene followed by 96.01 % for Benzo(a)pyrene, 95.67 % for Benzo(k)flouranthene and 95.66 % for Benzo(ghi)perylene while the control had the least percentage loss of 23.37, 62.09, 28.3, and 38.44 in 10, 20 30 and 40 % treatments respectively.

Table 4.13: Effect of synergistic fungi-SMC-plant treatments on the 16 EPA PAH contents in crude oil polluted soil (Soil 2) after 3 months

	Ring(s)		Control 1			Control 2			T_1			T_2			T_3			T_4		
PAHs	**N**	**C_0**	**C_t**	**Loss**	**% Loss (BE)**	**C_t**	**Loss**	**% Loss (BE)**	**C_t**	**Loss**	**% Loss (BE)**	**C_t**	**Loss**	**% Loss (BE)**	**C_t**	**Loss**	**% Loss (BE)**	**C_t**	**Loss**	**% Loss (BDE)**
Naphthalene	2	1.22	0.26	0.96	78.69	0.21	1.01	82.79	0.11	1.11	90.98	0.44	0.78	63.93	0.33	0.89	72.95	0.02	1.2	98.36
Acenaphthylene	3	0.96	0.18	0.78	81.25	0.09	0.87	90.65	0.12	0.84	87.5	0.34	0.62	64.58	0.145	0.815	84.89	0.023	0.937	97.6
Acenaphtene	3	1.23	0.82	0.41	33.33	0.27	0.96	78.05	0.12	1.11	90.24	0.274	0.956	77.72	0.223	1.007	81.86	0.08	1.15	93.49
Flourene	3	4.34	2.44	1.9	43.78	1.03	3.31	76.27	1	3.34	76.96	0.83	3.51	80.88	0.52	3.82	88.01	0.41	3.93	90.55
Phenanthrene	3	40.01	22.45	17.56	43.89	17.05	22.96	57.39	15	25.01	62.51	6.23	33.78	84.43	4.45	35.56	88.87	4.34	35.67	89.15
Anthracene	3	20.34	11.28	9.06	44.54	9.21	11.13	54.72	6.23	14.11	69.37	10.24	10.1	49.66	5.98	14.36	70.59	5.23	15.11	74.29
Flouranthene	4	76.34	63.34	13	17.03	60.34	16	20.96	45.23	31.11	40.75	12.45	63.89	83.69	8.67	67.67	88.64	7.21	69.13	90.55
Pyrene	4	69.34	41.2	28.14	40.58	49.4	19.94	28.76	36.34	33	47.59	11.23	58.11	83.80	7.20	62.14	89.61	6.45	62.89	90.69
Ben(a)anthracene	4	80.32	35.49	44.83	55.81	30.45	49.87	62.09	23.45	56.87	70.8	12.32	68.00	84.66	8.40	71.92	89.54	7.32	73	90.88
Chrysene	4	80.36	56.77	23.59	29.36	49.78	30.58	38.05	32.2	48.16	59.93	11.56	68.80	85.61	9.20	71.16	88.55	7.4	72.96	90.79
Benzo(b)flouranthene	5	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Benzo(k)flouranthene	5	100.23	61.56	38.67	38.58	67.32	32.91	32.84	28.3	71.93	71.76	10.34	89.89	89.68	4.32	95.91	95.67	3.34	96.89	96.67
Benzo(a)pyrene	5	150.23	67.45	82.78	55.10	64.65	85.58	56.97	22.3	127.93	85.16	15.8	134.43	89.48	6.00	144.23	96.01	4.20	146.03	97.2
Dibenzo(a,h)anthracene	5	39.20	28.34	10.86	27.70	27.3	11.9	30.36	12.1	27.1	69.13	11.4	27.80	70.92	2.40	36.8	93.88	1.65	37.55	95.79
Indeno(1,2,3-cd)pyrene	6	63.20	52.45	10.75	17.01	18.43	14.77	23.37	12.23	50.97	80.65	8.98	54.22	85.79	1.89	61.31	97.01	0.98	62.22	98.44
Benzo(ghi)perylene	6	78.34	52.76	25.58	32.63	48.23	30.11	38.44	23.1	55.24	70.51	9.43	68.91	87.96	3.40	74.94	95.66	3.00	75.34	96.17

Values are means of three replicated determinations; Co = Intial concentration of PAH in soils (mg/g); Ct = final/residual concentration of PAH in soil (mg//kg); Loss= Co-Ct (mg/kg); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively

4.7.7 Effect of synergistic treatments on the pesticide content (dichlorvos) in soil 3

Recoded initial concentration of organophosphate pesticide dichlorvos in soil 3 gave 30.0 mg/kg, this was however reduced to 11.32 mg/kg in the control while further reductions were observed in the treatments. The initial dichlorvos concentration of 30.0 mg/kg became reduced to 11.1, 9.17, 8.33 and 5.19 mg/kgin 10, 20 30 and 40% treatments respectivelyafter the experiment.

It was then deduced that 18.66 mg/kg of DDVP was lost in the control after 3 month of remediation experiment while 18.89, 20.83 and 21.67 were lost in 10, 20 20 and 30 % treatments respectively. Highest loss 24.1 mg/kg was observed in 40% treatment. Highest DDVP percentage loss of 82.7 and 72.2 % was recorded in 40 and 30 % treatments respectively followed by 72.23, 69.43, and 62.97 in 30, 20 and 10 % treatment respectively.

Intrestinghly, some residual DDVP metabolites were detected in soil 3, these were dimethyl phosphate (11.3 mg/kg), dichloroethanol (8.32 mg/kg), glucoronide (32.4 mg/kg), glycolic acid (19.3 mg/kg), dichloroacetaldehyde (7.99 mg/kg) and so on (Table 4.14)bellow. Based on the molecular weights of the metabolites, DDVP can be said to first degrade into glucuronide, then further degraded to dichoroacetaldehyde then to dimethylphosphate and dichloroethane (Table 4.14).

Table 4.14: Effect of synergistic fungi-SMC-plant treatments on DDVP (Dichlorvos) content (mg/kg) in soil 3 after 3 months.

Pesticide	**mol. Weight (g/mol)**	**Control**				**T_1**			**T_2**			**T_3**			**T_4**		
		C_0	**C_t**	**Loss**	**% Loss**	**C_t**	**Loss**	**% Loss**	**C_t**	**Loss**	**% Loss**	**C_t**	**Loss**	**% Loss**	**C_t**	**Loss**	**% Loss**
DDVP (Dichlorvos)	220	30	11.34	18.66	62.2	11.11	18.89	62.97	9.17	20.83	69.43	8.33	21.67	72.23	5.19	24.81	82.7
Other detected compounds																	
Dimethyl phosphate	126	11.3	11.23			8.45			8.12			9.34			8.34		
Dichloroethanol	98.96	8.32	10.23			15.34			15.2			14.23			14.22		
Glucuronide	194.14	32.45	44.23			33.43			23.43			13.23			11.23		
Glycolic acid	76	19.34	22.22			26.34			32.89			36.21			28.97		
Dichloroacetaldehyde	111.9	7.99	12.34			18.36			18.11			17.34			16.74		
Bromodichloroacetaldehyde	191.8	6.34	8.98			12.32			8.34			8.11			8.34		
Oxalic acid	126	24.34	25.34			28.11			27.34			26.34			24.38		

Values are means of three replicated determinations; Co = Intial dichlorvos concentrations in soil 3 (mg/kg); Ct = final/residual dichlorvos concentration in soil 3 (mg/kg); Loss= Co-Ct (mg/kg); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively

4.7.8 Effect of synergistic treatments on the pesticide content (γ-HCH lindane) in soil 4

Recoded initial concentration of organochlorinated pesticide (lindane) in soil 4 gave 45.0 mg/kg but this became reduced to 12.34 mg/kg in the control while further reductions of 12.11, 9.11, 6.33 and 5.10 mg/kg were recorded in 10, 20 30 and 40% treatments respectively after the experiment (Table 4.15).

It was then deduced that 32.66 mg/kg of lindane was lost in the control after 3 month remediation experiment while 32.89, 35.89 and 38.67 mg/kg were lost in 10, 20 30 and 40 % treatments respectively. Highest loss of 39.9 mg/kg lindane was lost in 40% treatment while the highest percentage loss of 82.7 and 72.2 % were recorded in 40 and 30 % treatments respectively, followed by 72.23, 69.43, and 62.97 in 30, 20 and 10 % treatment respectively.

Intrestinghly, some residual lindane metabolites were detected in soil 4, these are glucuronosan (13.24 mg/kg), dichlorophenol (8.45 mg/kg), 2, 4, dichlorophenol (8.45 mg/kg) and pentachlorocylohexane (11.2 mg/kg) and so on as shown in Table 4.15 bellow. From these we can also deduce the degradation mechanism of lindane in this set up. Based on the molecular weights of the metabolites, lindane can be said to first degrade into pentachlorocyclohexane, then further degraded to 2, 4, dichlorophenol as shown in Table 4.15.

Table 4.15: Effect of synergistic fungi-SMC-plant treatments on Lindane content (mg/kg) in soil 4 after 3 months

Pesticide	mol. Weight (g/mol)	Initial (mg/kg) C_0	Control C_t	Loss	% Loss	T_1 C_t	Loss	% Loss	T_2 C_t	Loss	% Loss	T_3 C_t	Loss	% Loss	T_4 C_t	Loss	% Loss
Lindane	220.0	45.00	12.34	32.66	72.58	12.11	32.89	73.09	9.11	35.89	79.76	6.330	38.67	85.93	5.100	39.90	88.67
Other detected residual compounds																	
2,5-Dichlorobenzene (2,5-DCB)	192.0	13.24	14.23			16.23			18.34			23.32			34.32		
2,4-Dichlorophenol	162.9	8.450	10.34			12.32			11.23			18.23			18.17		
Pentachlorocyclohexene	256.4	11.26	13.32			18.94			19.32			17.23			17.11		
Chlorobenzene	112.6	9.340	23.21			19.34			23.24			30.43			45.32		
Pentachlorocyclohexanone	270.7	11.23	34.4			36.98			67.56			68.45			65.34		

Values are means of three replicated determinations; Co = Intial lindane concentrations in soil 3 (mg/kg); Ct = final/residual lindane concentration in soil 3 (mg/kg); Loss= Co-Ct (mg/kg); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively

4.8 Degradation Kinetics of Polycyclic Aromatic Hydrocarbon in SoilS 1 and 2 as Influenced by Synergistic SMC-Fungi-Plant Treatments

Tables 4.16 and 4.17 below shows the degradation pathways of total PAHs in soils 1 and 2, each treatments influenced the degradation kinetics has calculated based on the % loss, the degradation efficiency, degradation rate (K_1), and half-lfe ($t_{1/2}$).

In soil 1, the synernistic treatments perform better over the controls, control 1 (plant alone) had the lowest DE of 44.34 %whilecontrol 2 (SMC and fungi alone) had0.499. 69.71, 82.999, 95.28 and 89.61 % degradation efficiencies were recorded in 10, 20, 30 and 40 % treatments respectively (Table 4.16). In soil 2 also, the controls had the least degradation efficiencies of 44.87 and 50.23 % control 1 and 2 respectively while treatments 10, 20, 30 and 40 % had the highest efficiencies of 67.97, 84.86, 92.15 and 93.58 % respectively. In these two soils, 30 and 40 % treatments had the highest degradation efficiency (Table 4.16).

Moreover, the PAHs degradation constants (K_1) recorded in soils 1 and 2 revealled that the controls had some level of degradative capacities but not as efficient as those of synergistic treatments expecially at 30 and 40 % treatments. Concentrations of 2.265 and 3.05 mg/kg of TPAHs are constantly degraded in 40 and 30 % treatment per day (/day) while only 0.586 and 0.499 mg/kg of TPAH degrades constantly per day (/day) in controls 1 and 2 respectively (Table 4.16). Similar result was observed in soil 2, controls 1 and 2 had K1 of 0.596 and 0.977 mg/kg in one day (/day) compared to 1.138, 1.188, 2.546, and 2.746 mg/kg recorded in 10, 20 30 and 40 % treatments (Table 4.16).

Half-life recorded in soil 1 gave 1.18 and 1.39 /day in controls 1 and 2 respectively while 0.58, 0.39, 0.22 and 0.30 /day were recorded for 10, 20 30 and 40 % treatments (Table 4.15). In soil 2, controls 1 and 2 had half-life of 1.16 and 0.71 /day respectively compared to 0.61, 0.37, 0.27 and 0.25 half life /day (Table 4.16).

Table 4.16: Effect of synergistic Fungi-SMC-plant treatments on the degradation of total PAH in Soil 1

Treatments	C_o mg/kg	C_t mg/kg	Loss (C_o-C_t)	DE (%)	K_1 (/day)	$t_{1/2}$
Conntrol $0\%_1$	851.19	473.76	377.43	44.340	0.5860	1.1827
Control $0\%_2$	851.19	465.76	385.43	45.280	0.4990	1.3888
T_1	851.19	257.83	593.36	69.7095	1.1946	0.5802
T_2	851.19	144.71	706.48	82.9986	1.7730	0.3911
T_3	851.19	40.165	811.03	95.2813	3.0542	0.2269
T_4	851.19	88.410	762.78	89.6134	2.2650	0.3060

Values are means of three replicated determinations; Co = Intial concentration of total PAH in soil (mg//kg); Ct = final/residual concentration of lindane in soil (mg//kg); Loss= Co-Ct (mg/kg); DE = Degradation efficiency calculated as percentage loss; K_1 = Degradation rate constant, $t_{1/2}$ = half-life; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

Table 4.17: Effect of synergistic Fungi-SMC-plant treatments on the degradation of total PAH in Soil 2

Treatments	**C_o mg/kg**	**C_t mg/kg**	**Loss (C_o-C_t)**	**DE (%)**	**K_1 (/day)**	**$t_{1/2}$**
Conntrol $0\%_1$	805.0	443.8	361.2	44.87	0.596	1.164
Control $0\%_2$	805.0	400.3	404.7	50.23	0.977	0.709
T_1	805.0	257.8	547.2	67.97	1.139	0.609
T_2	805.0	121.9	683.1	84.86	1.888	0.367
T_3	805.0	63.13	741.9	92.16	2.546	0.272
T_4	805.0	51.65	753.3	93.58	2.747	0.252

Values are means of three replicated determinations; Co = Intial concentration of total PAH in soil (mg//kg); Ct = final/residual concentration of lindane in soil (mg//kg); Loss= Co-Ct (mg/kg); DE = Degradation efficiency calculated as percentage loss; K_1 = Degradation rate constant, $t_{1/2}$ = half-life; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

4.9 Degradation Kinetics of Pesticides (Dichlorvos and Lindane) in Soils 3 and 4 as Influenced by Synergistic SMC-Fungi-Plant Treatments

Tables 4.18 and 4.19 below shows the degradation pathways dichlorvos and lindane in soils 3 and 4 respectively, each treatments influenced the degradation kinetics has calculated based on the % loss, the degradation efficiency, degradation rate (K_1), and half-lfe ($t_{1/2}$).

In soil 3, synernistic remediation of dichlorvos in treatments performed better than the controls, controls had the lowest DE of 62.20 % in both control 1 (plant alone) and control 2 (SMC and fungi alone) while the 10, 20, 30 and 40 % treatments gave degradation efficiencies of 62.97, 69.43, 72.23 and 82.70 % respectively (Table 4.18). In soil 4 also, the controls had the least lindane degradation efficiencies of 72.57 and 73.47 % in controls 1 and 2 respectively while 10, 20, 30 and 40 % treatments had the best efficiencies of 73.09, 79.76, 85.93 and 88.67 % respectively (Table 4.19). Generally, in both soils 3 and 4, 30 and 40 % treatments performed more than the other treatment based on degradation efficiency.

Moreover, the degradation constants recorded in soils 3 and 4 revealled that the controls had some level of degradative capacities but not as efficient as those of synergistic treatments expecially at 30 and 40 % treatments. The recorded $K_1$for dichlorvos degradationin soil 3 shows that 0.973 mg/kg out of 30 mg/kg of initial dichlorvos concentration was constantly degraded in both controls 1 and 2 while 1.75 and 1.26 mg/kg are constantly dregraded in 40 and 30 % treatments per day (/day) respectively. Similar results were observed in soil 4, controls 1 and 2 degrades only 1.29 and 1.37 mg/kg of Lindale in one day (/day) compared to 1.317, 1.598, 1.96 and 2.18 K_1 /day recorded in 10, 20 30 and 40 % treatments.

Half-life recorded in soil 3 gave 0.53 and 0.51 /day in controls 1 and 2 respectively while 0.99, 1.19, 1.28 and 1.75 /day were recorded for 10, 20 30 and 40 % treatments (Table 4.18). In soil 4, controls 1 and 2 had half-life of 0.53 and 0.51 /day respectively compared to 0.53, 0.43, 0.35 and 0.318 half life /day (Table 4.19).

Table 4.18: Effect of synergistic Fungi-SMC-plant treatments on the degradation of Dichlorvos in pesticides' polluted Soil 3

Treatments	C_o mg/kg	C_t mg/kg	Loss (C_o-C_t)	DE (%)	K_1 (/day)	$t_{1/2}$
Conntrol $0\%_1$	30.00	11.34	18.66	62.20	0.973	0.712
Control $0\%_2$	30.00	11.34	18.66	62.20	0.973	0.712
T_1	30.00	11.11	18.89	62.97	0.994	0.698
T_2	30.00	9.170	20.83	69.43	1.185	0.585
T_3	30.00	8.330	21.67	72.23	1.282	0.540
T_4	30.00	5.190	24.81	82.70	1.755	0.395

Values are means of three replicated determinations; Co = Intial dichlorvos concentrations in soil 3 (mg/kg); Ct = final/residual dichlorvos concentration in soil 3 (mg/kg); DE = degradation efficiency calculated as percentage loss; K_1 = Degradation rate constant, $t_{1/2}$ = half-life; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

Table 4.19: Effect of synergistic Fungi-SMC-plant treatments on the degradation of Lindane in pesticides' polluted Soil 4

Treatments	C_o mg/kg	C_t mg/kg	Loss (C_o-C_t)	DE (%)	K_1 (/day)	$t_{1/2}$
Conntrol $0\%_1$	45.00	12.34	32.66	72.58	1.291	0.536
Control $0\%_2$	45.00	11.94	33.06	73.47	1.371	0.506
T_1	45.00	12.11	32.89	73.09	1.313	0.528
T_2	45.00	9.110	35.89	79.76	1.598	0.434
T_3	45.00	6.330	38.67	85.93	1.962	0.353
T_4	45.00	5.100	39.90	88.67	2.178	0.318

Values are means of three replicated determinations; Co = Intial lindane concentrations in soil 3 (mg/kg); Ct = final/residual lindane concentration in soil 3 (mg/kg); DE = degradation efficiency calculated as percentage loss; K_1 = Degradation rate constant, $t_{1/2}$ = half-life; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

4.10 Degradation Pathways followed by Synergistic Fungi-SMC-plant Mechanism in the Treaments of Hydrocarbon and Pesticides Polluted Soils

In this study, efforts were made to present pathways of hydrocarbon, dichlorvos and lindane degradation by the set-up synergistic fungi-SMC-plant treatments using the information gotten from GC/ MS analysis of the experimental soils. The generate spectra were ran on NIST search application, interpretation and compared with those which have been previously documented. It was discovered that, the pollutants were generally degraded in the treated soils leading to the formation of different metabolites.

The hydrocarbon degradation through the synergistic mechanism was traced to resemble the general aerobic degradation pathway that was presented by Cerniglia (1989), as presented in Figure 4.3, an hypothetical PAH degradation pathway through the synergistic fungi-SMC-plant mechanism followed three mechanisms;

(i) the cis-dihydrodiol pathway
(ii) the quinone formation pathway and
(iii) arene oxides formation pathway (Figure 4.3).

These major metabolites were further degraded to form simple glucoside, glucuronides, O-sulfates, or O-xylosides.

Depending on whatever pathway follows, faster degradation of the PAHs was observed more at 40 and 30 % treatments respectively.

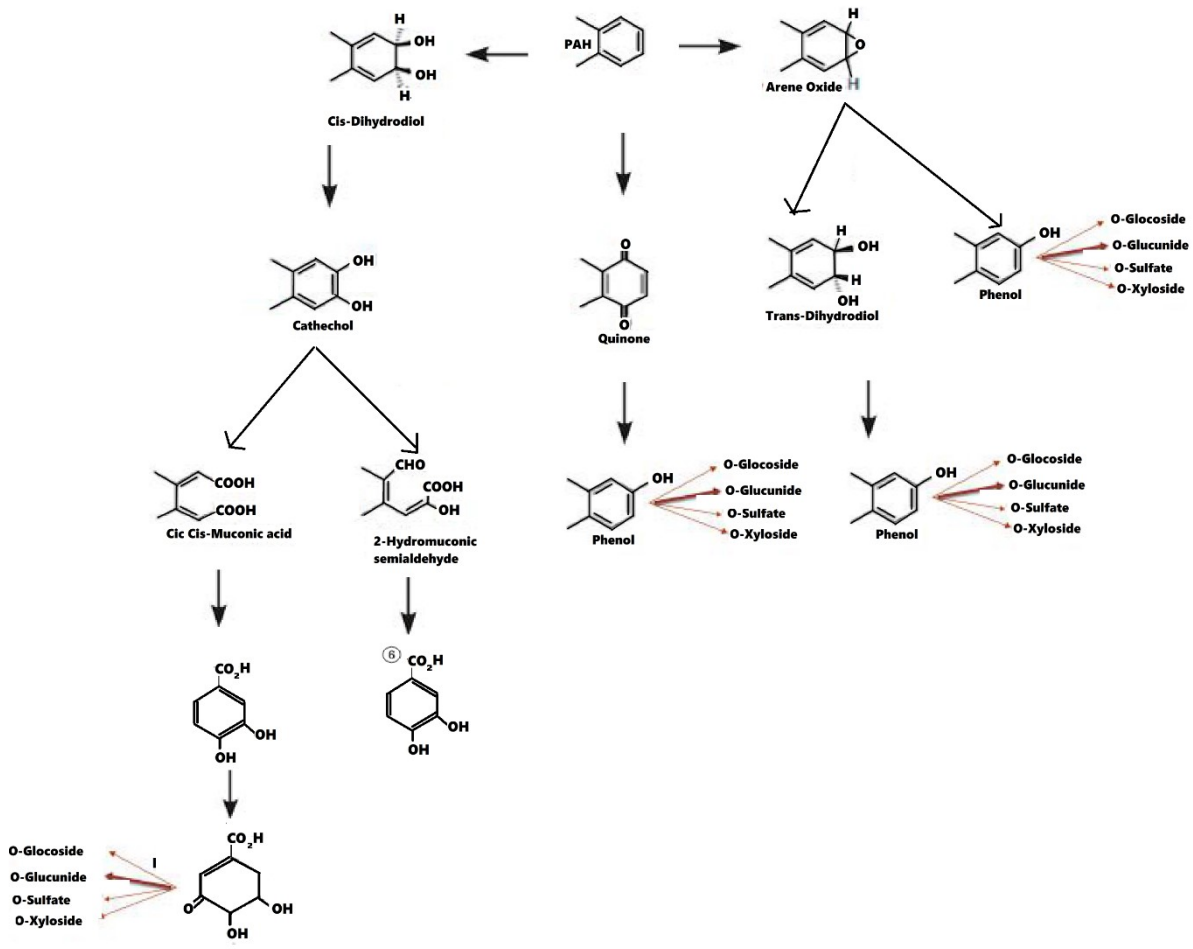


Figure 4.3 General pathway constructed for the degradation of PAHs through the set-up fungi-SMC-plant synergistic mechanisms

It was also observed that the degradation of Dichlorvos which is an organophosphate pesticide result in formations of major metabolites in polluted soil, these metabolites include *O, O*-dimethyl phosphonic ester, the Desmethyl dichlorvos which is commonly known as 2, 2-dichlorovinyl O-methylphosphate in chemistry and the *O, O, O*-trimethyl phosphoric ester which is commonly known as 2, 2-dichlorovinyl-0, 0-dimethylphosphate.

The pathway suggested for the degradation of Dichlorvos through the fungi-SMC-plant synergistic mechanism in this study is presented in Figure 4.4 below. In the mechanism (I), there was an on the hydroxyl group leading to an addition or elimination of hydrogen from Dichlorvos which causes loss of methyl group a nd formation of desmethyl dichlorvos. In another possible pathway (II), there was degradation of dichlorvos to form desmethyl dichlorvos as a result of a catalytic degradation called hydroxylation.

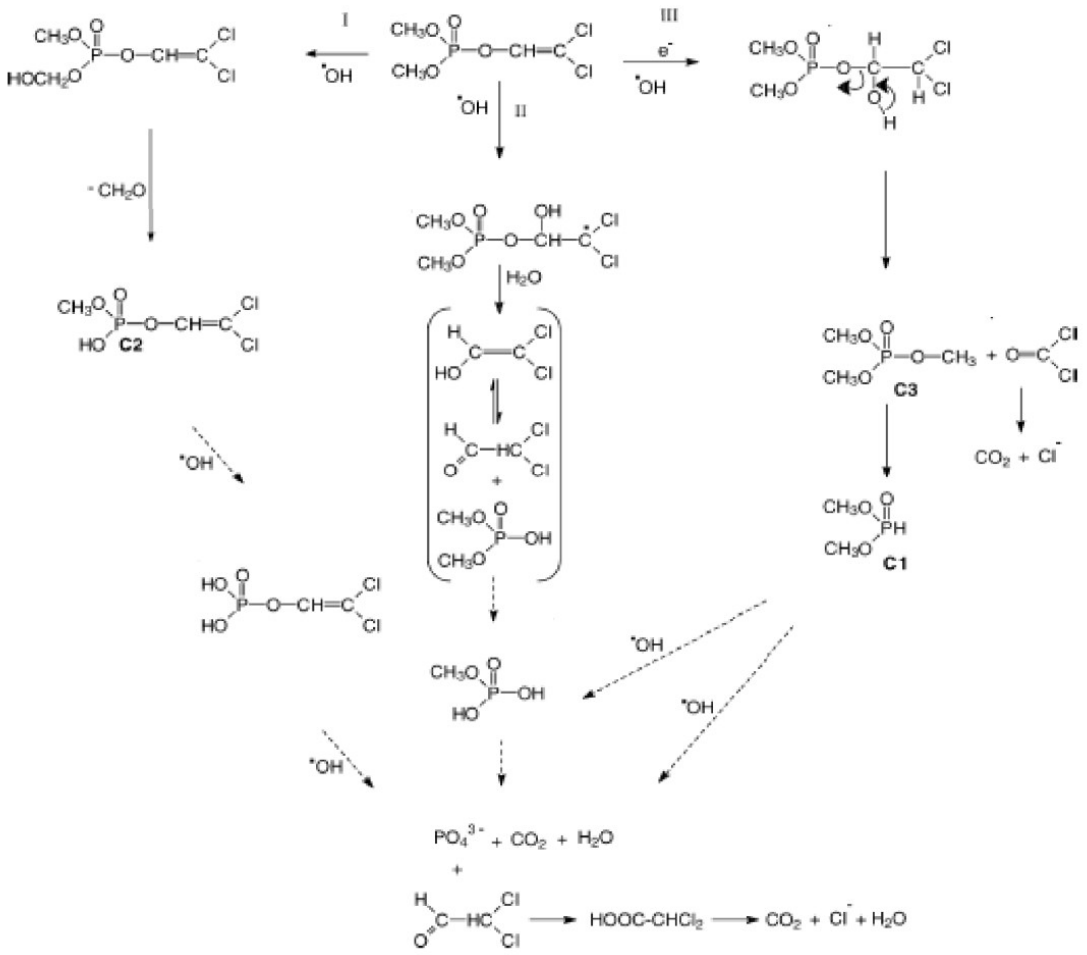


Figure 4.4 General pathway constructed for the degradation of Dichlorvos through the set-up fungi-SMC-plant synergistic mechanisms

Furthermore, the pathway for Lindane degradation through the synergistic fungi-SMC-plant mechanism set-up in this study is presented in Figure 4.5 below. The first pathway follows dichlorination of lindane to form gamma hexachlorocylcohexane which isfurther degraded to some other less toxic compounds as shown in the Figure 4.5. The second pathway also involves the enzymatic dechlorination of lindane to form pentachlorocyclohexene which as well further degraded to form glycoside acid, while the third pathway follows the formation of pentachlorocyclohexene and 1, 3, 4, 6-tetracyclohexadene.

In the third pathway, there is an enzymatic cleavage of the dichlorovinyl group in dichlorvos leading to its breakdown (Figure 4.5).

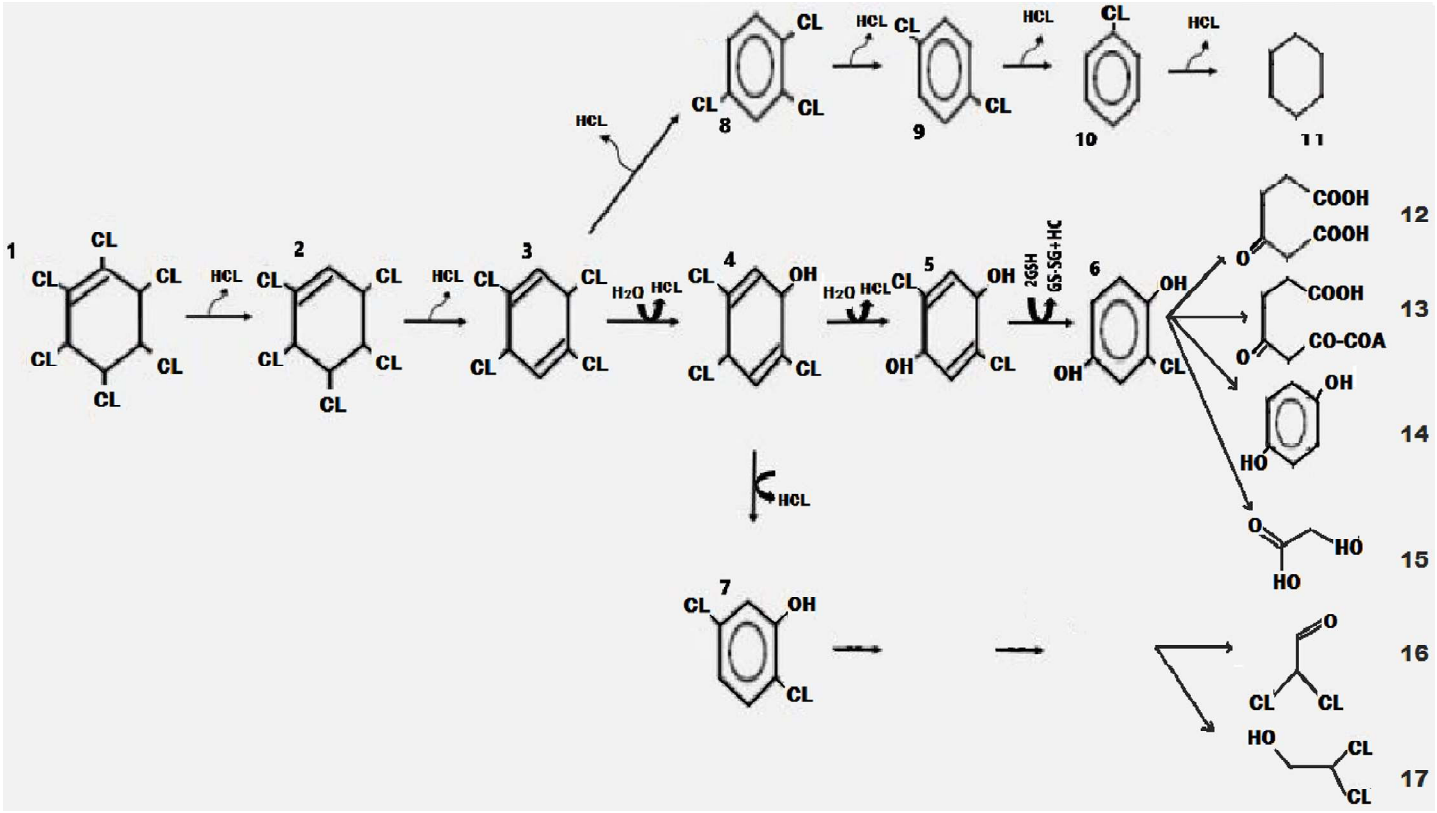


Figure 4.5 General pathway constructed for the degradation of Lindane through the set-up fungi-SMC-plant synergistic mechanisms: 1 = lindane (γ-Hexachlorocyclohexane). 2 = γ-PCCH (Pentachlorocyclohexane). 3 = 1, 4-TCDN (1, 3, 4, 6-Tetrachloro 1, 4-cychlohexadiene). 4 = 2, 4, 5-DNOL (2, 4, 5-Trichloro 2, 5-cyclohexadien-1-ol). 5 = 2, 5-DCHQ (2, 5-Dichlorohydroquinone) or 2, 5-DDOL (2, 5-Dichloro-2,5-cyclohexadiene-1,4-diol). 6 = CHQ (Chlorohydroquinone). 7 = 2, 5- DCP (2, 5-Dichlorophenol). 8 = 1, 2, 4-TCB (1, 2, 4-Trichlorobenzene). 9 = 2, 5-DCB (2, 5-Dichlorobenzene). 10 = Chlorobenzene. 11 = benzene. 12 = β-Ketoadipate (3-oxoadipate). 13 = 3-Oxoadipyl-CoA. 14 = γ-Hydroxymuconicsemialdehyde. 15 = Glycolic acid. 16 = Dichloroacethaldehide and 17 = Dichloroethanol.

4.11 Growth Response of *M. maximus* to Polluted Soils (1-4) as mediated by the synergistic SMC-Fungi-Plant Treatments

Grouth responses based on the recorded agronomic data from the test plants (Plate 4.6a and b). Leaf numbers was recorded highest in the 40 and 30 % in soils 4, 2, 1 and 3 respectively while the controls had the least. Tallest plants with broader leaves (plant height; leave area) and girth were those grown on 40, 30 and 20 % treatments (Table 4.20).

The treatments had 37.67-38.17, 27.83-35.83, 23.17-32.67 and 12.83-24.17 total leaf numbers in soils 1-4 in treatments 40, 30, 20 and 10 % while control had 6.833-11.67 (Table 4.20 and Plate 4.7). The treatments also had maximum plant height of 59.75 and 49.67 cm in treatments 40 and 30 % while the control had the least maximum height of 16.72 cm (Table 4.20 and Plate 4.7). Furthermore, treatments had maximum stem girth of 5.1000 and 5.183 cm in 40 and 30 % treatments while the control had the least maximum girth of 1.690 cm, same trend was observed for the leaf area, highest leaf area of 308.2 $cm^2$was recorded at 40 % treatment while the control had 25.75 cm^2

Plate 4.6a: seed of *M. maximus*

Plate 4.6b: Test plant (*M. maximus*) growing on treated and untreated polluted soil

Table 4.20: Effect of synergistic treatments on growth of test plant (*M. maximus*) in response to polluted soil

		Mean Plant Parameters			
PARAMETERS	**Treatments**	**Soil (1)**	**Soil (2)**	**Soil (3)**	**Soil (4)**
Leaf Number	Control	6.833±3.2^{d}	7.500±3.4^{c}	7.833±3.1^{c}	11.67±3.3^{c}
	T_1	12.83±4.3^{cd}	13.17±4.7^{ab}	15.33±6.2^{ab}	24.17±6.2^{ab}
	T_2	23.17±6.6^{abc}	23.17±6.7^{ab}	23.67±7.1^{ab}	32.67±6.9^{a}
	T_3	27.83±7.8^{ab}	29.33±7.8^{a}	31.17±8.9^{a}	35.83±8.4^{a}
	T_4	37.67±8.4^{a}	37.83±8.9^{a}	36.67±8.5^{a}	38.17±8.3^{a}
Plant Length (cm)	Control	12.63±3.6^{b}	12.37±3.7^{b}	15.38±3.7^{b}	16.72±3.8^{b}
	T_1	21.45±9.5^{a}	21.97±9.5^{a}	36.93±8.8^{a}	41.30±9.0^{a}
	T_2	27.00±9.6^{a}	27.77±9.9^{a}	43.95±10.1^{a}	45.77±9.9^{a}
	T_3	40.22±12.5^{a}	39.08±13.6^{a}	48.70±12.1^{a}	49.67±12.1^{a}
	T_4	49.85±9.3^{a}	50.15±10.3^{a}	50.22±10.0^{a}	59.75±10.2^{a}
Stem Girth (cm)	Control	1.120±0.3^{d}	1.070±0.4^{c}	1.398±0.3^{c}	1.690±0.4^{c}
	T_1	2.970±0.6^{bc}	2.820±0.5^{ab}	2.933±0.6^{ab}	3.083±0.7^{ab}
	T_2	3.750±0.7^{ab}	3.700±0.7^{a}	3.620±0.7^{a}	4.183±0.6^{a}
	T_3	4.450±0.8^{ab}	4.520±0.8^{a}	4.520±0.8^{a}	5.183±0.8^{a}
	T_4	4.830±0.8^{a}	4.700±0.8^{a}	4.950±0.8^{a}	5.100±0.8^{a}
Leaf Area (cm^2)	Control	16.78±9.6^{d}	16.03±10.0^{d}	20.55±10.7^{d}	25.75±9.6^{d}
	T_1	97.83±30.7^{cd}	97.90±30.8^{cd}	101.3±34.9^{cd}	108.9±31.9^{cd}
	T_2	159.0±52.3^{abc}	159.13±52.4^{abc}	162.7±54.2^{abc}	176.1±48.4^{abc}
	T_3	216.9±69.5^{ab}	216.98±69.5^{ab}	234.55±70.5^{ab}	251.4±63.5^{ab}
	T_4	286.1±80.3±^{a}	286.10±84.3^{a}	285.60±84.2^{a}	308.2±74.7^{a}

Values are means of three replicated determinations±Standard Deviation (SD); Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

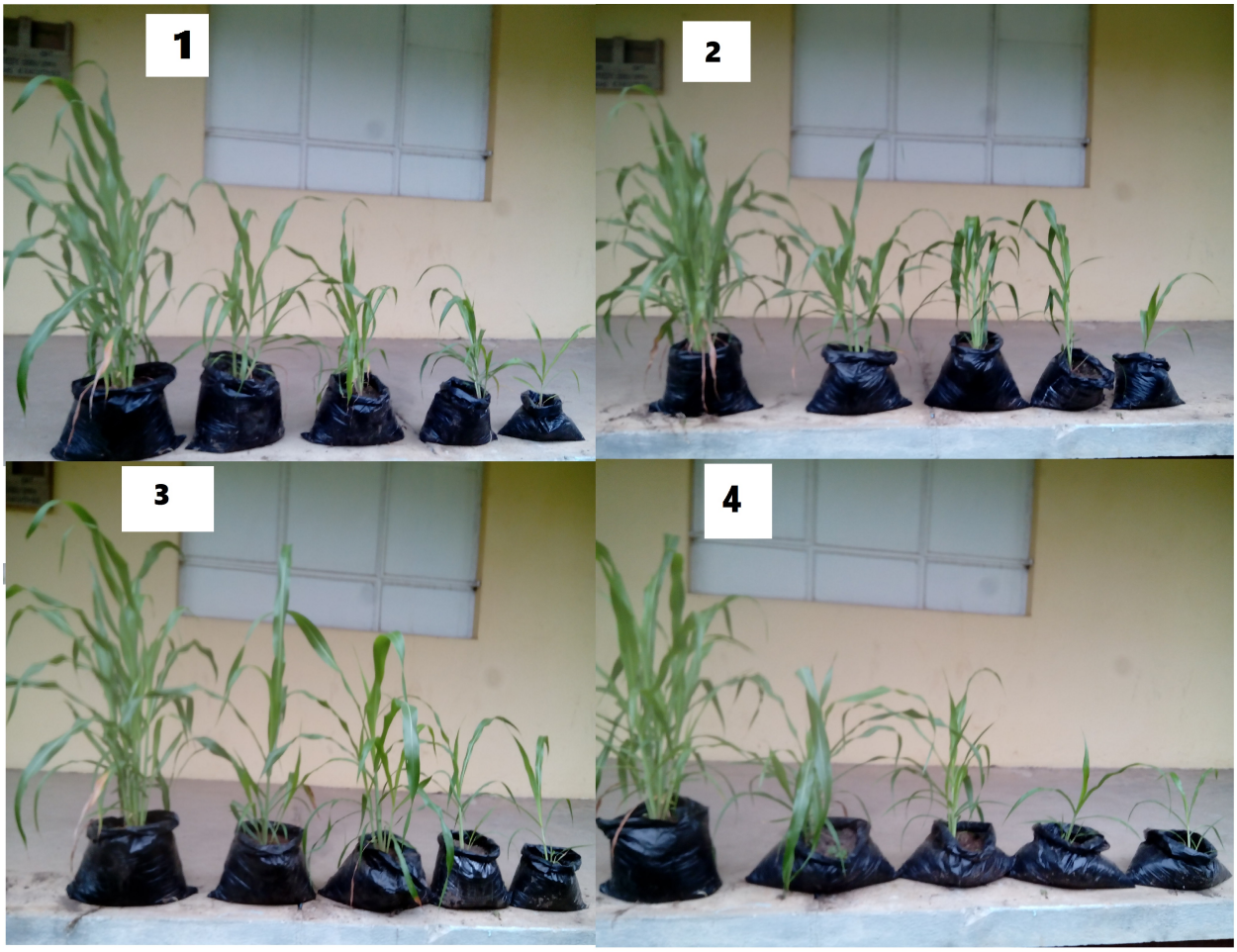


Plate 4.7: *M. maximus* planted onpolluted soils at different synergistic treatments at week 8. 1-4 are the polluted soils 1-4 respectively

4.12 Yields (Phytomass) of *M. maximus* in Polluted soils 1-4 as Mediated by Synergistic SMC-Fungal Treatments

The phytomass analysis of the plants grown on the treated and control polluted soils 1-4 shows the growth upporting effect of the synergistic treatments, the treatments had significant effect ($\alpha_{0.05}$) in enhancement of plant yield/phytomass as compared to the controls. In soil 1 for example, lowest mean root (below ground) and mean shoot (above ground) phytomass (20.17 g and 60.60 g respectively) were observed in the control compare to the treated (56.10 g and 261.00 g respectively), similar trend was also observed in olluted soils 2, 3 and 4 (Table 4.21). This is an indication that the contamination affected the root and shoot of untreated plant (control) was more than the treatment or that the treatment has reduced the toxicity of the pollutants in the soils. The calculated biological phytomass production efficiency for the test plant shows that treated plants performed higher with 22.75, 23.28, 23.78 and 24.03 % in 10, 20, 30 and 40 % treaments respectively compared to the control that had the least (6.06 %)(Table 4.21).

Table 4.21: Effect of synergistic treatments on phytomass of *M. maximus* growing on polluted soils after 3 months

SOIL	Treatment	Mean Root Phytomass (g)	Mean shoot Phytomass (g)	Total Phytomass (g)	Biological Efficiency (%)
SOIL 1	Control	20.17	60.60	80.77	6.06
	T_1	50.20	250.3	300.5	22.75
	T_2	50.60	256.9	307.5	23.28
	T_3	53.80	260.8	314.6	23.78
	T_4	56.10	261.2	317.3	24.03
	Total	230.87	1089.8	1320.67	100
SOIL 2	Control	18.60	58.40	77	6.32
	T_1	48.20	198.6	246.8	20.26
	T_2	48.40	200.3	248.7	20.42
	T_3	50.20	270.4	320.6	26.30
	T_4	50.30	274.5	324.8	26.70
	Total	215.7	1002.2	1217.9	100
SOIL 3	Control	25.30	72.30	97.6	5.930
	T_1	50.30	300.2	350.5	21.29
	T_2	55.70	304.5	360.2	21.88
	T_3	56.20	360.8	417	25.33
	T_4	60.40	360.7	421.1	25.58
	Total	247.9	1398.5	1646.4	100
SOIL 4	Control	25.10	75.30	100.4	7.970
	T_1	49.50	150.3	199.8	15.86
	T_2	49.90	200.3	250.2	19.86
	T_3	49.20	250.4	299.6	23.78
	T_4	49.30	360.5	409.8	32.53
	Total	223.00	1036.8	1259.8	100

Values are means of three replicated determinations; *T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.*

4.13. Physiological and Anatomical Responses of *M. maximus*to Polluted Soils 1-4 as Mediated by SMC-Fungal Treatments

4.13.1 Physiological responses

The synergitic treatments also enhanced the plant's chlorophyll contents as the chorophyll contents of the treated plants showed significant higher levels than the lants growing on the untreated soils (controls). However, the treated plants generally did not show significant differences ($\alpha_{0.05}$) in the chlorophylls a and b ratio (Tables 4.22-4.25). The treated plant in soil 1 for example had the highest chlorophylls a and b content (18.00 and 6.700 m/g fresh weight respectively) as compared to the control (4.600 and 1.800 m/g fresh weight respectively). Similar trends were also observed for the polluted soils 2, 3 and 4 respectively (Tables 4.22-4.25)

More also, their was an observed malformation of precocious chlorotic yellow spots which was more intense in the control at early stage of leaf development, this may be due to the stressed exerted by the soil pollutants on the plant, this justified the lower chlorophyll contents of control over the treated plants.

Table 4.22: Effect of synergistic treatments on the response of *M. maximus* to hydrocarbon pollutants in Soil 1 in terms of chlorophyll contents

Treatment	**Chll a (mg/g)**	**Chll b (mg/g)**	**Total Chll**	**Chllratio *(a/b)***
Control	4.600^{c}	1.800^{b}	6.400^{b}	2.600^{a}
T_1	17.60^{a}	6.500^{a}	23.60^{a}	2.600^{a}
T_2	16.90^{b}	6.800^{a}	23.70^{a}	2.500^{a}
T_3	17.80^{a}	6.800^{a}	24.70^{a}	2.600^{a}
T_4	18.00^{a}	6.700^{a}	24.70^{a}	2.700^{a}

Values are means of three replicated determinations; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); Chll a = Chlorophyll; Chll b = Chlorophyll b; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

Table 4.23: Effect of synergistic treatments on the response of *M. maximus* to hydrocarbon pollutants in Soil 2 in terms of chlorophyll contents

Treatment	**Chll a (mg/g)**	**Chll b (mg/g)**	**Total Chll**	**Chll ratio *(a/b)***
Control	5.400^{c}	2.100^{b}	7.500^{b}	2.570^{a}
T_1	16.90^{a}	7.200^{a}	24.10^{a}	2.350^{a}
T_2	15.60^{b}	7.100^{a}	22.70^{a}	2.200^{a}
T_3	16.80^{a}	7.400^{a}	24.10^{a}	2.270^{a}
T_4	21.70^{a}	7.700^{a}	29.40^{a}	2.820^{a}

Values are means of three replicated determinations; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); Chll a = Chlorophyll; Chll b = Chlorophyll b; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively;

Table 4.24: Effect of synergistic treatments on the response of *M. maximus* to pesticide pollutants in Soil 3 in terms of chlorophyll contents

Treatment	Chll a (mg/g)	Chll b (mg/g)	Total Chll	Chll ratio *(a/b)*
Control	5.800[c]	2.600[b]	8.400[b]	2.231[a]
T_1	27.60[a]	7.300[a]	34.90[a]	3.781[a]
T_2	26.20[b]	6.900[a]	33.10[a]	3.797[a]
T_3	26.90[a]	6.800[a]	33.70[a]	3.956[a]
T_4	28.11[a]	6.600[a]	34.71[a]	4.259[a]

Values are means of three replicated determinations; Means in the same column having the same letter aren not significantly different ($\alpha_{0.05}$); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively Chll a = Chlorophyll a in mg/g of fresh leaf weight; Chll b = Chlorophyll b in mg/g of fresh leaf weight.

Table 4.25: Effect of synergistic treatments on the response of *M. maximus* to pesticide pollutants in Soil 4 in terms of chlorophyll contents

Treatment	Chll a (mg/g)	Chll b (mg/g)	Total Chll	Chll ratio *(a/b)*
Control	5.760^{c}	2.800^{b}	8.560^{b}	2.057^{a}
T_1	26.76^{a}	6.700^{a}	33.46^{a}	3.994^{a}
T_2	26.99^{b}	6.800^{a}	33.79^{a}	3.969^{a}
T_3	28.78^{a}	6.900^{a}	35.68^{a}	4.170^{a}
T_4	28.80^{a}	6.700^{a}	35.50^{a}	4.299^{a}

Values are means of three replicated determinations; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); Chll a = Chlorophyll; Chll b = Chlorophyll b; T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

4.13.2 Potential photosynthetic rate

Potential photosynthetic rate (Amax) was measured in m mol O_2 $M^{-2}S^{-1}$, Amax was generally enhanced in the treated plants compare to the control in all the polluted soils (Figure 4.6). It is known that the soil pollutants usually stress plants that are growing on the polluted soil. The crude oil pollution (soil 1) in this study for example affected the control more than the treatments, 11.2 m mol O_2 $M^{-2}S^{-1}$ was recorded in control but highest Amax were observed in the treated plants. 40% and 30% treatments had the highest Amax of 27.9 and 27.8 m mo6l$O_2M^{-2}S^{-1}$ respectively while 25.4 and 23.4 m mol$O_2M^{-2}S^{-1}$ were recorded in 20 and 10% treatment respectively.

The synergistic SMC-Fungal treatment had good regulatory effect on the physiological photosynthetic rate on the test plant in this study, this also corroborated the observed photosynthetic pigment (chlorophyls a, b and a/b ration) which had best values in the treatments as reported earlier in 4.6.1.

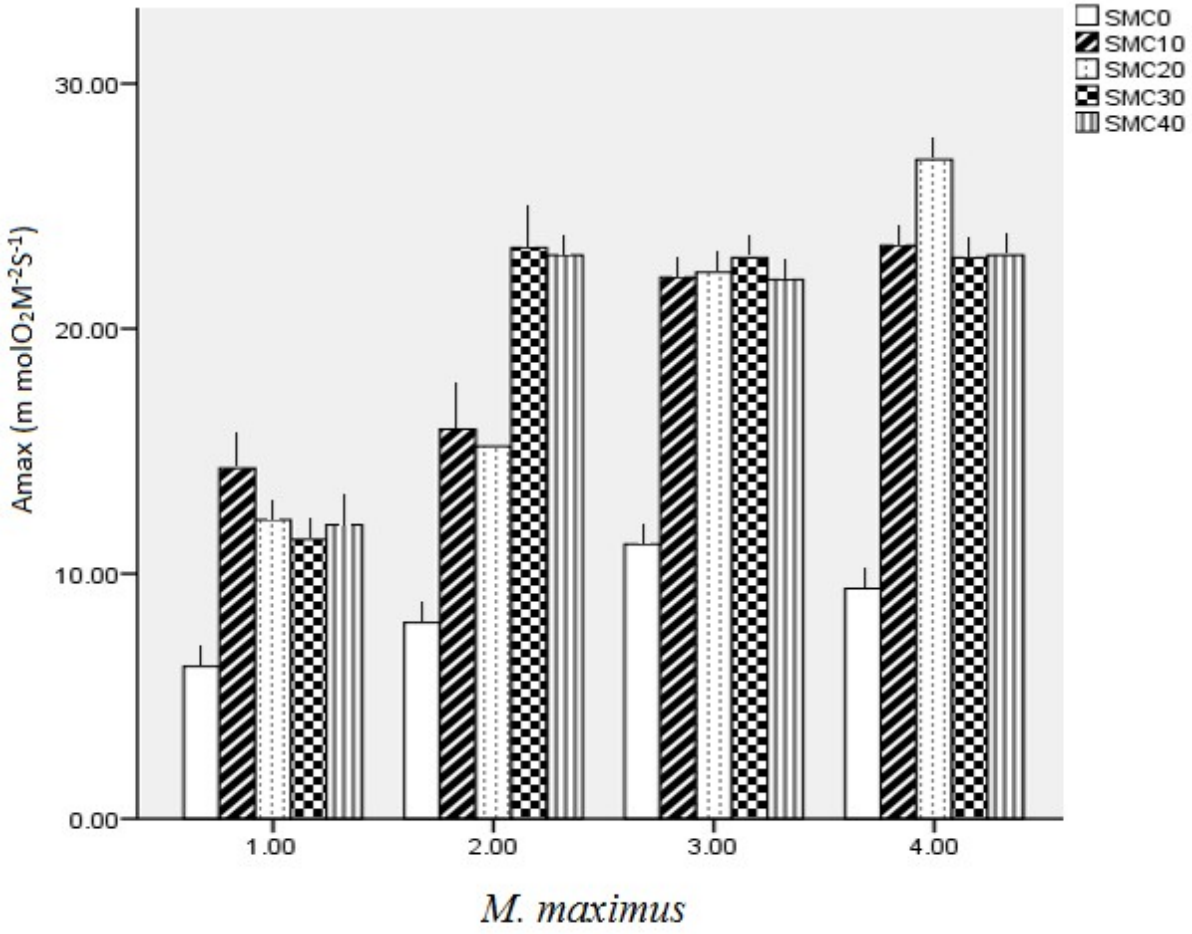


Figure 4.6: Effect of synergistic SMC-fungi and root treatments on *M. maximus* potential photosynthetic rate (Amax) measured in m $molO_2M^{-2}S^{-1}$; 1.00-4.00 = Soils 1-4

4.13.3 Root proliferation

Influence of synergistic SMC-fungal treatments on the root proliferation of plant (*M. maximus*) was established in this study. The highest root dimensions (length x width) were generally recorded in 40 and 30 % treatments in all the polluted soils, while the onntrol had the least (Table 4.26). The root area and dimentions were more enhanced in the treatments also, in soil 1 for example highest were recorded in 30 and 40% treaments with 1996.1±1.3 (25.31 x 78.87) and 1615±4.7 (23.68 x 68.24) respectively while the least was recorded in the control at 275±1.5 (13.64 x 20.13).Similar trend was also recorded for soils 2, 3 and 4 (Table 4.26), least root area and dimentions were recorded in the controls with highest recorded in 30 and 40% treatments.

The harvested roots in all the treated soils had robust and healthy root system (Plates 4.8a-d), we observed that the stresses posed by the pollutants affected the controls more but the synergistic treatments were able to suppress this effect.

Table 4.26: Effect of synergistic treatments on root proliferation of *M. maximus* after 3 months

	Treatment	Dimention	Extensive Root Area (cm^2)
Soil 1	Control	13.64 X 20.13	274.5 ± 1.5^{e}
	T_1	16.12 X 33.55	540.8 ± 3.4^{d}
	T_2	19.87 X 64.36	1278.8 ± 3.0^{c}
	T_3	23.68 X 68.24	1615 ± 4.7^{b}
	T_4	25.31 X 78.87	1996.1 ± 1.3^{a}
Soil 2	Control	12.98 X 24.93	323.64 ± 3.6^{e}
	T_1	15.68 X 42.29	663.14 ± 1.7^{d}
	T_2	20.2 X 61.66	1245.44 ± 1.3^{c}
	T_3	23.45 X 71.21	1663.74 ± 1.1^{b}
	T_4	26.24 X 80.18	2103.88 ± 0.4^{a}
Soil 3	Control	11.23 X 30.52	342.72 ± 1.2^{b}
	T_1	14.37 X 48.69	699.66 ± 0.6^{b}
	T_2	16 X46.1	737.64 ± 1.0^{b}
	T_3	20.23 X 51.01	1031.94 ± 3.0^{b}
	T_4	31.07 X 65.39	2031.63 ± 1.2^{a}
Soil 4	Control	11.26 X 29.81	335.78 ± 1.7^{c}
	T_1	13.06 X 46.20	603.24 ± 6.5^{b}
	T_2	16.52 X 33.88	559.36 ± 0.2^{b}
	T_3	27.33 X 34.89	953.768 ± 1.6^{a}
	T_4	26.99 x 34.98	954.36 ± 4.8^{a}

Values are means of three replicated determinations±Standard deviation; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

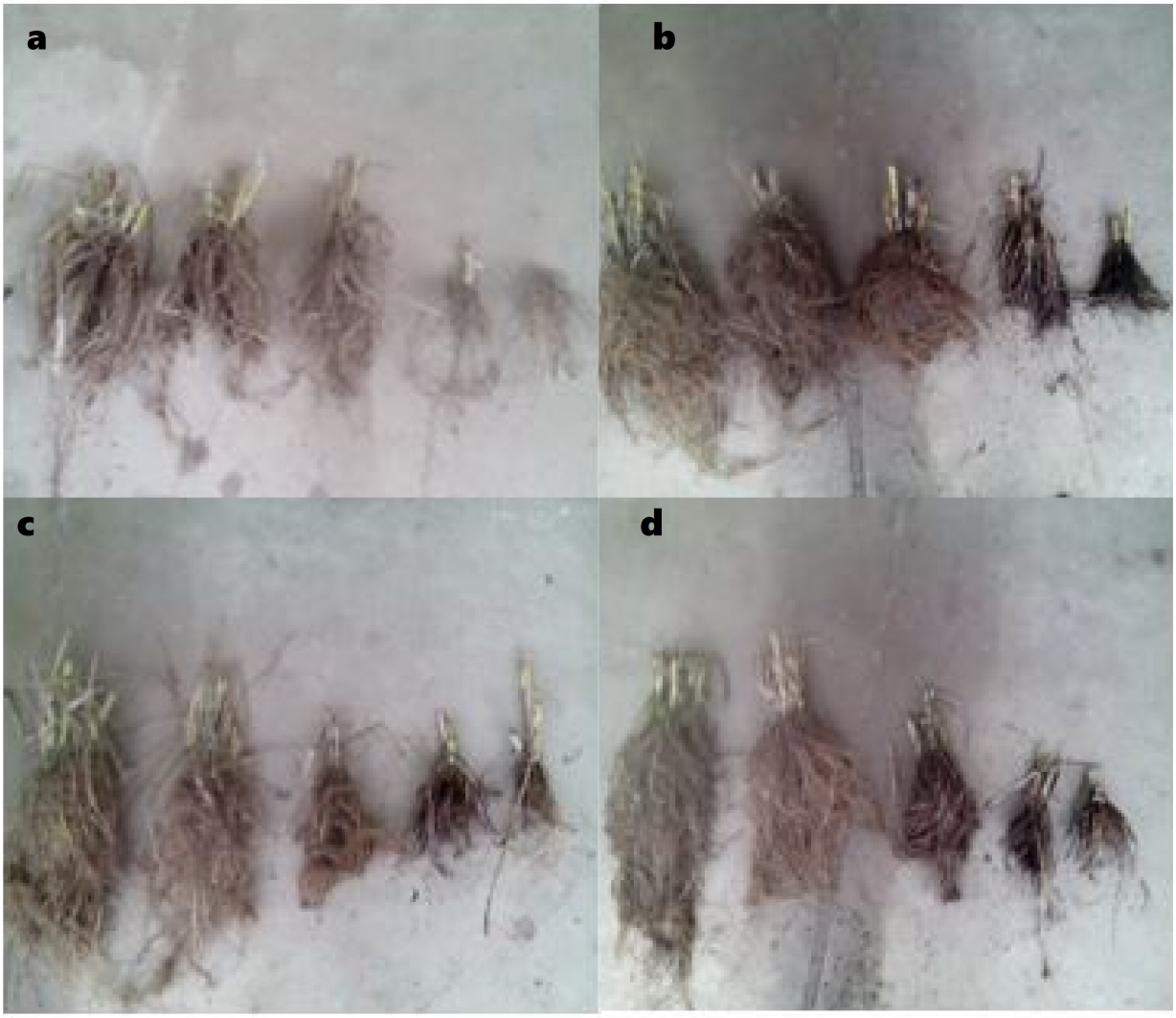


Plate 4.8: Effects of synergistic fungi-SMC-Plant treatments on root proliferation by the test plant (*M. maximus*) in polluted soils i.e a-d respectively

4.13.4 Effect of synergistic treatments on the root anatomical responses

Root anatomical response to the polluted soils 1-4 were studied in reference to the root structures such as exodermis, endodermis, area and number of the metaxylem and protoxylem and tickness of tracheary element, cortical wall, the root diameter and central cylinder diameter and cortex thickness (Table 4.27).

The control had highest exodermis (67.50 µm), endodermis (27.60 µm), number and area of metaxylem elements (9.900 and 224.6 µm respectively), number of protoxylem elements (232.0 µm) and tichness of tracheary element (5.900 µm) unlike the treated plants which are much more higher, this may be a reflective of the fact that the controls are more by the toxic soil pollutants and such affects their response to avert the effect of the toxins through the development of such adaptive feasures to protect itself.

The 30 and 40 % treated plants however had highest root diameter (1159 and 1164 µm respectively) and central cylinder diameter (490.7 and 535.1 µm respectively) and cortex thickness (312.30 and 312.9 µm respectively) due to healthier mode of life in contaminated soil over control (Table 4.27).

The treated plants also produced more root hairs and less symptomic root formation (Plate 4.9A-E).

Table 4.27: Effects of synergistic treatments on plants response (*M.maximus*) to soil pollutants in terms of root structures

	SMC-Fungal Treatments (%)				
Root characters	**$0\%_1$ (control)**	**T_1 (10%)**	**T_2 (20%)**	**T_3 (30%)**	**T_4 (40%)**
Exodermisin µm	67.50^{a}	55.80^{b}	54.30^{b}	54.10^{b}	52.90^{bc}
Endodermis in µm	27.60^{a}	19.90^{b}	19.20^{b}	16.90^{bc}	12.10^{d}
Numbers of metaxylem element	9.900^{a}	8.600^{b}	8.800^{b}	7.900^{c}	7.600^{c}
Area metaxylem elem in μm^2	224.6^{c}	256.3^{ab}	260.1^{a}	262.8^{a}	263.3^{a}
Numbers of protoxylem elements	232.0^{a}	218.6^{b}	212.8^{c}	211.6^{c}	210.4^{cd}
Thickness of traqueary elements	5.900^{a}	3.000^{b}	3.200^{b}	3.800^{b}	3.500^{b}
Thickness of the cell wall in µm					
Thickness of the cortical cell wall in µm	3.900^{a}	2.500^{b}	1.700^{c}	1.700^{c}	1.500^{c}
The root diameter	921.8^{e}	1061.1^{d}	1075.3^{c}	1159^{b}	1164^{a}
Mm					
Central cylinder	398.23^{e}	449.9^{d}	460.3^{c}	490.4^{b}	535.1^{a}
diameter µm					
Cortex	236.2^{d}	300.0^{c}	308.6^{b}	312.30^{a}	312.9^{a}
thickness µm					

Values are means of three replicated determinations; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively.

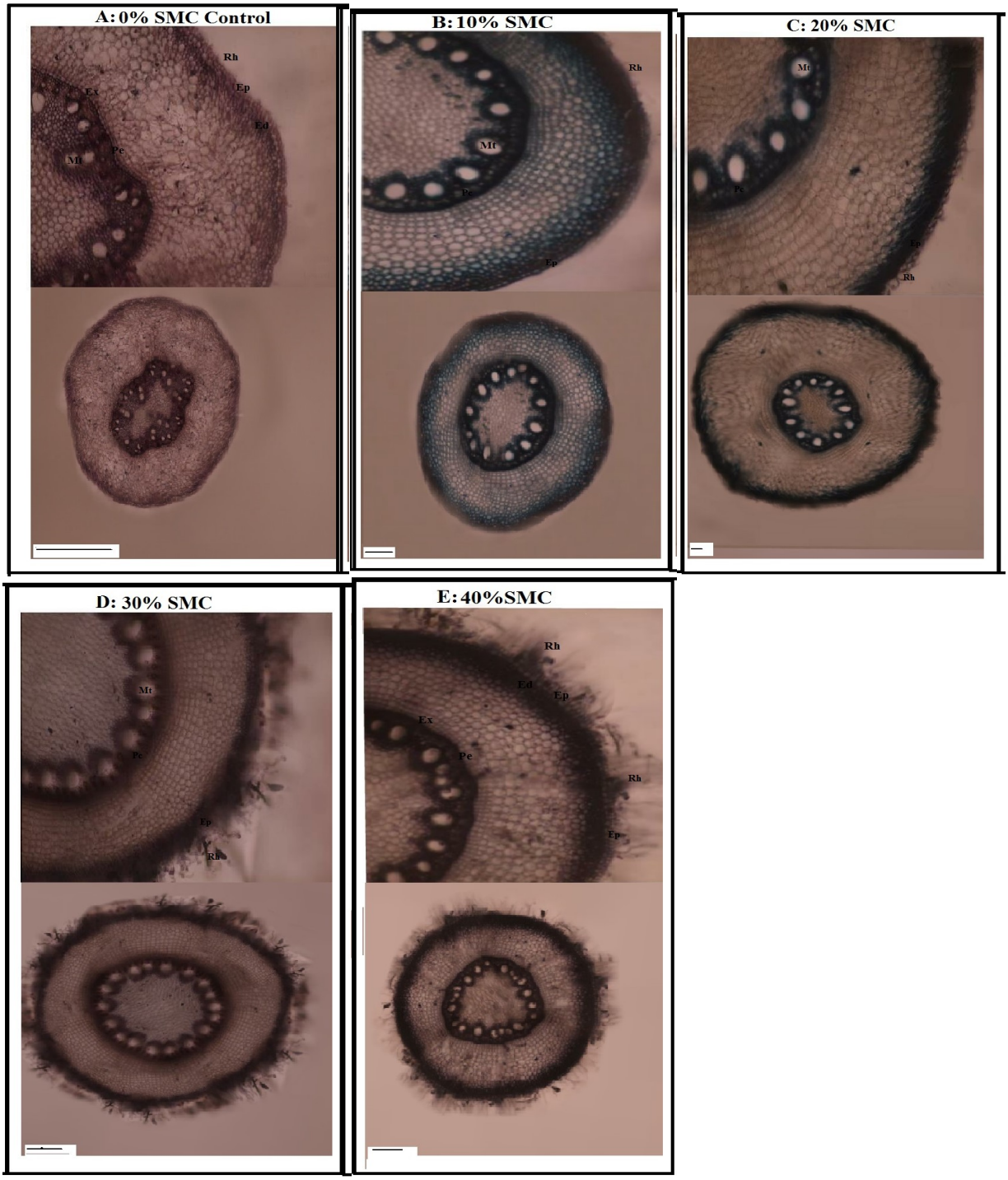


Plate 4.9: Effects of synergistic fungi-SMC-plant treatments on the root anatomical structure of *M. maximus* growing on polluted soil 1; A = control; (No synergistic treatments); B-E = 10-40% synergistic treatments respectivel; Bar scale = 50 µm.

4.13.5 Effect of synergistic treatments on the leaf anatomical responses

The effect of soil contaminats and positive effect of SMC-Fungi treatments on the plant leaf response was observed in *M. maximus* in this study (Table 4.28) and (Plate 4.10).

The 30 and 40 % treated plants however generally had higher leaf anatomical structures such as the mid rib thickness (567.9 and 723.2 μm respectively), lower epidermal and sclerenchyma thickness (61.11 and 62.70 μm respectively), and thickness of the upper epidermis (398.67 and 519.2 μm respectively) and so on due to their healthier mode of life in contaminated soil over control (Table 4.28).

In addition, Plant root structures (Plate 4.10A-E) were observed to contain both epidermises with an isodiametrical cell which are also strengthened with strong external wall which bulges with moderate thickening. Group of cells called the 'Bulliform cells' generally were also seen visible in the upper epidermis of the plants' leaves. The synergistic fungi-SMC-plant treatments also significanty ($\alpha_{0.05}$) enhanced all the plant growing on treated soils as they generally have higher thichness in the lower epidermis and sclerenchyma as compared to the controls; they have more thickened vascular bundles width as well as diameter of the xylem vessels. The results also showed an interesting observation that the controls which are the plants without treatments generally had lower thickness of the midrib and upper epidermis as well as the thichness of the parenchyma and vascular bundles as compared to the plants growing on the treated soils (Table 4.28).

These structural responses of the treated plant might be attributed with their improved physiological response in terms of chlophyl contents and potential photosynthic rate earlier reported in this study.

Table 4.28: Effects of synergistic treatments on plants response (*M.maximus*) to soil pollutants in terms of leaf structures

	SMC-fungal Treatments (%)				
Root characters	**Control**	**T_1**	**T_2**	**T_3**	**T_4**
Midrib thickness in µm	381.23^{e}	459.02^{d}	529.1^{c}	567.9^{b}	723.2^{a}
Lower epidermal and sclerenchyma hckness in µm	37.010^{e}	51.00^{cd}	55.67^{c}	61.11^{ab}	62.70^{a}
Thickness of upper epiderma and parenchyma in µm	234.55^{e}	290.98^{d}	335.3^{c}	398.67^{b}	519.2^{a}
Vascular bundle thickness in µm	105.33^{c}	134.00^{b}	134.1^{b}	133.81^{b}	146.2^{a}
Vascular bundle in µm	92.20^{c}	138.22^{ab}	142.9^{a}	145.2^{a}	143.1^{a}
Number of rows formed by sclerenchyma in lower vascular bundle	3.230^{d}	4.900^{b}	5.330^{c}	4.43^{0b}	6.000^{a}
Number of rows formed by parenchyma in upper vascular bundle	3.420^{b}	5.300^{a}	5.300^{a}	5.600^{a}	6.100^{a}
Number of xylem vessels	3.500^{a}	3.500^{a}	3.500^{a}	3.500^{a}	3.500^{a}
Diameter of xylem vesselsin µm	4.400^{c}	5.400^{b}	5.500^{b}	5.500^{b}	6.230^{a}
Thickness of phloem tissue in µm	38.81^{d}	57.80^{b}	58.20^{a}	58.50^{a}	50.20^{c}
Distance of large vascular bundle from he nearest vascular bundle in µm	43.00^{d}	52.^{1c}	52.1^{0c}	70.10^{a}	64.92^{b}

Values are means of three replicated determinations; Mean in columns having the same superscript letter aren not significantly different ($\alpha_{0.05}$); T_1-T_4 = Synergistic fungi-SMC-plant treatments at 10-40% respectively

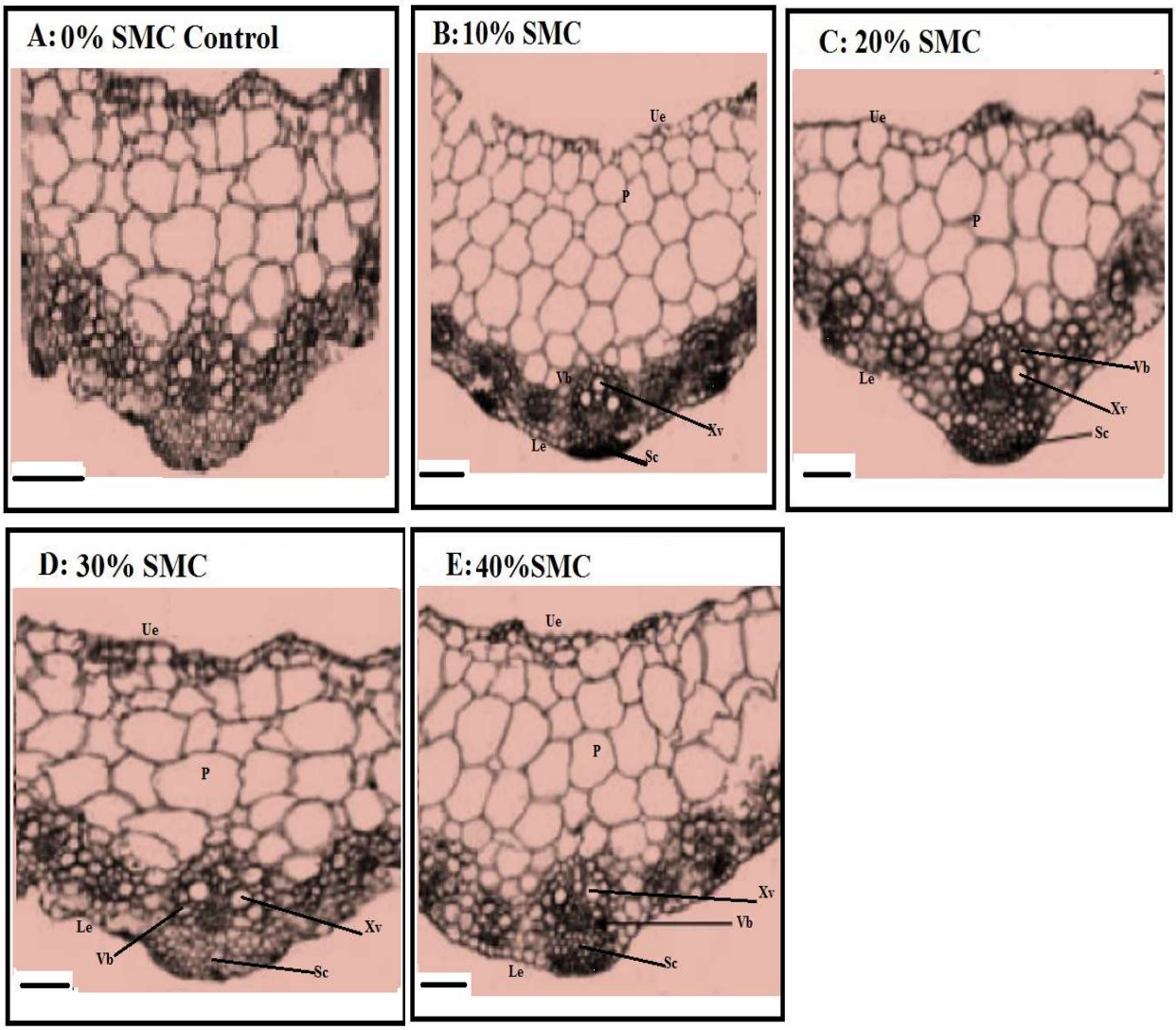


Plate 4.10: Effects of synergistic fungi-SMC-plant treatments on the leaf anatomical structure of *M. maximus* growing on polluted soils; A = control (0% No SMC treatment); B-E = 10-40% synergistic treatments respectively. Bar scale = 100 µm.

4.14 Seed Germination Supporting Ability of the Remediated Soils

It was also established that the synergistic treatments enhanced the remediation polluted soils 1-4 and thereafter, the remediated soils were subjected to seed germination supporting test. The treated soils showed improved seed germinantion as they yielded better germination index (Table 4.29) which is a reflective of the fact that the synergistic treatments enhances the soil pH and nutrients. The remediated soils 1-4 gave better seed germination index over the control soils on the sown 30 seeds of *M. maximus*over the controls (Table 4.29). In soil 1, only 1-3seedsgerminated in the controlswhile 27 seeds germinated in 30 and 40% treatments, and this gave 83-90% seed germination in the treated soils while the controlshad 3.33-10%.

Moreover, the highest % seed germination index was recorded in 40 and 30%) treatments in soil 1 as 22.71 and 21.89 % respectively compared to controls (0.09-0.811), 19.53 and 17.18in soil 2 with 2.52-2.89 % in controls. Pesticides remediated soils and 4 had 0.54-1.06 % germination index in controls compared to 21.02 and 24.39 % in 30 and 40% treatments in soil 3 respectively while controls in soil 4 had 1.05-1.92 % as compared to 21.13 and 25.23 recorded in 40 and 30% treatments in soil 4 respectively.

Table 4.29: Test for toxic level of treated polluted soils

		$0\%_1$ (control)	**$0\%_1$ (control)**	**T_1 (10%)**	**T_2 (20%)**	**T_3 (30%)**	**T_4 (40%)**	**TOTAL**
	No of seed sown	30	30	30	30	30	30	
	No of seed germinated	3	1	25	26	27	27	
	% Seed germination	10	3.33	83.33	86.67	90	90	
Soil 1	Root Growth (cm)	0.9	0.3	2.8	2.7	2.8	2.7	11.1
	% Root growth	8.11	2.7	25.23	24.32	25.23	24.32	
	Germination index (%)	0.811	0.09	21.11	21.08	22.71	21.89	
	No of seed sown	30	30	30	30	30	30	
	No of seed germinated	6	8	24	27	26	28	
	% Seed germination	20	26.67	80	90	86.67	93.33	
Soil 2	Root Growth (cm)	1.4	1.2	2.1	2.8	2.2	2.4	11.1
	% Root growth	12.61	10.81	18.92	25.23	19.82	21.62	
	Germination index (%)	2.522	2.89	15.12	22.71	17.18	19.53	
	No of seed sown	30	30	30	30	30	30	
	No of seed germinated	3	4	17	24	25	28	
	% Seed germination	10	13.33	56.67	80	83.33	93.33	
Soil 3	Root Growth (cm)	0.6	0.9	1.2	2.5	2.8	2.9	11.1
	% Root growth	5.41	8.11	10.81	22.52	25.23	26.13	
	Germination index (%)	0.541	1.08	6.13	18.02	21.02	24.39	
	No of seed sown	30	30	30	30	30	30	
	No of seed germinated	7	8	19	22	28	29	
	% Seed germination	23.33	26.67	63.33	73.33	93.33	90.33	
Soil 4	Root Growth (cm)	0.5	0.8	2.8	2.7	3	2.6	11.1
	% Root growth	4.50	7.21	25.23	24.32	27.03	23.42	
	Germination index (%)	1.05	1.92	15.98	17.83	25.23	21.13	

Values are means of three replicates determinations

CHAPTER FIVE

DISCUSSION

5.1 Rhizosphere Fungi

Oil exploration, industrial activities, production and over-utilization of synthetic pesticides, and the use different machineries have been shown to cause increase in soil pollutions unfortunately, these activities have been increasing since the mid-19th century with more complexicity due to increasing anthropogenic activities. Today, several pollutants are been discharged in human environment and this had raisen public concerns due to their toxicity and associated health hharzards.

It was earlier reported by Perelo (2010) that microorganism that exists around rhizospheres may have developed adaptic mechanisms which enable them to tolerate and mineralize the soil pollutants. In addition, many fungal strains have been discovered to be capable of degrading/mineralizing several pollutants (Hussain *et al*., 2009a, b) which often time may also lead to formation of metabolites which are less toxic or not toxic (Badawi *et al*., 2009). This study reveals the potentials of synergistic rhizosphere remediation of four different polluted soils; dominant fungal strains based percentage incidence tests were selected from the rhizosphere of grasses which are growing on the polluted soils and used for synergist remediation mechanism in conjuction with the SMC and *M. maximus* (guinea grass).

Sixteen different rhizosphere fungal strains belonging to *Aspergillus*, *Talaromyces, Yarrowia, Cunninghamella, Candida, Trichoderma, Fusarium, Penicillium* and *Mucor* genuses were identified from different polluted soils, it was observed that fungal strains *A. niger* asemoA, *A. awamori* asemoE and strains *Talaromyces purpurogenus* asemoF, *Trichoderma hazianum* asemoJ, *A. oryzae* asemoK and *A. flavus* asemoM were more dominantly occurring fungal strains in hydrocarbon polluted soil 1 and 2 while *Cunninghamella elegans* asemoB, *A. niger* asemoC, *Candida albican* asemoD, *T.*

atroroseus asemoG, *Penicillium* sp asemoH, *Fusarium solani* asemoI, *A. oryzae* asemoK, *T. purpurogenus* asemoN, *Yarrowia lipolitica* asemoO and *A. flavus* asemoP were most dominant in pesticide polluted soils 3 and 4.

These fungal strains have been well reported in degradation of several hydrocarbons and pesticides, many fungi for example *Aspergillus niger* and *Aspergillus fumigatus* have been reported to be well associated with SMC decomposition (Ikhajiagbe and Anoliefo, 2010), they were both reported to be able degrade PAHs and mineralize them (Yamazaki *et al.,* 1988). These two fungi have been previously reported in the degradation of hydrocarbons such as phenanthrene, anthracene as well as naphthalene by ring cleavage using extracellular enzymes as reported by Yogambal and Karegoudar (1997). Similarly, cytochrome P450 production was reported in. *A. fumigatus* by Venkateswarlu *et al*. (1996), it was reported that the fungus could hydrolyze benzo[a]pyrene using this enzyme. Ikhajiagbe and Anoliefo (2010) as well as Bartha and Atlas (1997) affirms that substrates such as sawdust can enhance the performance of microorganisms in degrading petroleum hydrocarbons. Likewise, substrate (SMC) enhanced the performance of rhizosphere as observed in this experiment.

The selected rhizosphere fungi were observed to tolerate and survive their source contaminants (i.e crude oil, black oil, dichlorvos and lindane) up to 20 % in the culture medium; this could be a reflective of the fact that they were able to degrade/mineralize their source contaminants which must have enhanced their survival in rhizospheres of the polluted soil. The mechanisms at which they degrade pollutant was revealed to be facilitated by the production of several cassette of enzymes, it was also reported that many fungal strains tend to produce enzymes as a result of mineralization which involves the utilization of such pollutant as sole carbon or energy sources but it was recently reported that fungal strains may also produce some enzymes as an adaptive response to toxic pollutants in their environment.

In this study, it was also observed that the fungal strains were able to produce several enzymes which were also triggered by the concentrations of hydrocarbons and pesticides

supplemented in their growth medium. This affirms that these fungi do not only produce enzymes to mineralize the polloutants but also as environmental signals or adaptation to survive polluted environments.

Fungi for many years have been able to develop different mechnisms for degradation of many complex substrates through the secretions of enzymes, they were first observed to degrade woods through the secretions of lignolytic enzymes such as psychotrome P450. However, many fungi have been identified today with capability of producing several degrading enzymes in other to utilize or degrade several pollutants. According to Hadibarata *et al*. (2009), toxic nature of soil pollutants usually create unfavorable environmental condition for fungal growth and this triggers more production of degrading enzymes to detoxify the pollutants. Also, Ramirez *et al*. (2011) explained that the production of enzymes by fungi can also be as a result of abioic stress signallings or shocks. Fungi usually produce enzyme extracellularly as secondary metabolite due to their saprophitic mode of life in which they have to predigest their substrate/food in other to absorb nutrients and as observed by Ronne (1995) can be triggered by carbon or nitrogen sources.

In this study, the rhizosphere fungi produced enzymes such as peroxidases, catalase and laccase which are of immense importance in degradation of hyrocarbons, pesticides and several POPs as reported that the capability of several fungi in bioremediation usually depends of the enzyme production capabilities (April *et al*., 2000; Atagana, 2008; Asad *et al*., 2015). Although, increase in the concentrations of hydrocarbon and pesticides pollutants in the gowth medium negatively affected their gowth, they were however able to tolerated, survive and degrade them, they were able to increase their metabolic response for more secretion of degrading enzymes as the concentrations of the pollutant increased.

Fungal life cycle has been associated to different features but little is known or documented about their genetic ability in degradation or mineralization of some pollutants, most of the reports on fungal degradation were data monitoring while little research studied the genetic inference of such capability (Sharma *et al*., 2006; Adekunle and Adebambo, 2007; Hussain

et al., 2008 and 2009a; Zhang *et al*., 2010; Janusz *et al*., 2013). Degradation of hexachlorocyclohexane (lindane) for example was reported to be usually controlled by *lin* genes which code for enzymes dehalogenase, dehydrogenase or hydrolase but there is less information on its expression. Likewise, there is dearth of knowledge on the capabities of rhizosphere fungi in expression and production of degrading enzymes in response to soil pollutants. In this study, 15 different degrading enzyme coding genes were tested for their presence and expression in the rhizosphere fungi to link their degradative abilities with their survival in hyrocarbon and pesticides polluted soils.

In this study, enzymes catalase, laccase as well as lignin and manganese peroxidase encoding genes were detected present in all the rhizosphere fungi, they also express these genes as revealed through the RT-PCR technique. Laccase is an enzyme which is a multi copper phenolic compound in nature; it has been documented to play several roles in fungal degradations of phenolics, aromatic hydrocarbons and some other POPs. Laccases are ussualy produced extracellularly by fungi, thay are known to oxidize pollutants by attacking the oxygen terminal lectron acceptors causing cleavage of bonds (Janusz *et al*., 2013). Many laccase genes which encodes protein or isoforms of about 510-550 amino acid sequences with N-terminal peptides of about 20 amio acids have been documented in fungi (Morozova *et al*., 2007). Cullen *et al.* (1997) successfully cloned the laccase genes in some fungi, they cloned and sequence 17 different laccase genes (*lcc*) in fungi and since then, the deposition of *lcc* genes has been increasing in the NCBI gene bank.

Moreover, peroxidase enzymes which are popularly known to be produced by the white rot fungi (mushrooms) for decomposition of lignin, celluloses and other organic compounds was also reported in the rhizosphere fungi in this study, little is known of this in lower fungi until 2013, only few (about 4) *mnp* genes were documented in the NCBI fungal genbank (Paszczynski and Crawford, 2000) and these were identified in some fungi like *Phlebia*sspecies such as *Phlebia radiate*, *Dichomitus squalens*, *Phanerochaete chrysosporium*, *P. Sordida*, *Trametes versicolor* strain 9522-1, *Lentinula edodes* strain MG60, *Danoderma lucidium* and some other Basidiomycete fungi.

Lignin peroxidase (LiP) is an enzyme that belongs to the class II peroxidases; it was revealed that all te rhizosphere fungi in this study possess the enzyme, the fungi produced LiP in aliquots similar to the report of Morgenstern *et al*. (2008). LiP is a monoetric protein which is produce by fungi as an extracellular enzymes, it is a haemoglobin-lignin peroxidase which also contains some few isoenzymes. LiP is known to function in diverse ways but unfortunately its expressions in degradaing fungi have been well exploited, up till 2015, only 6 *lip* genes were documented in NCBI fungal database. In this study, lignin peroxidase genes *lig1-6* and manganese peroxidase gene *mnp* were all teste present and expressed by the rhizosphere fungal strains, the fungi also showed good production these enzymes in aliquots even in reponse to hydrocarbonnn and pesticide's concentrations. In addition, other enzymes encoding genes such as phosphoesterase genes (*mpd* and *cbh*) which code for an organophosphorus hydrolase enzyme and catechol 1, 2-dioxygenases (*afk3* and *afk4*) were also detected present and expressed in the selected rhizosphere fungi in this study.

These enzymes have a wide coverage and have been well reportedly associated to catalyze the degradation of several pesticides such as parathion, fenitrothion, malathion and some other organophsphate and organochlorine pesticides. These shows that the rhizosphere fungi in this study were capable in degrading the pollutants and this as also reported by Fournier *et al.* (1996) and (Hussain *et al*., 2009a) may be synergised by group of fungi to jointly degrade the pollutants.

5.2 Biostimulatory Effect of Spent Mushroom Compost (SMC)

Spent mushroom compost (SMC) was used in this study to stimulate the synergistic fungi-plant treatment and enhance both the plant and fungal survival on the polluted soil. In this study, SMC and fungal amendments improved the soil's pH and enhanced their nutrient contents, this supports the reports of several claims that composts usually replenishe soil nutrients and pH. Application of SMC in bioremediation mechanisms has been reported to improve the soil's parameters (such as nutrient increment and PH neutralization) which in-turn enhanced the microbial growth and speed up the degradation of soil pollutants (Vidali,

2001) and sustained diverse population of microorganisms (Eggen, 1999; Trejo-Haernandez *et al*., 2001; Ikhajiagbe and Anoliefo, 2010). Lee *et al.* (2011) also reported that SMC supplementation in polluted soil reduced soil toxicity and enhanced both its nutrient and microbial enzyme activities. The SMC acted as organic manure as it gradually decomposed and slowly releases its nutrient, unlike the inorganic supplements like fertilizers that can negatively affect bioremediation rate when applied at a wrong dose into the soil.

The biostimulatory effect of SMC as observed in this study also agrees with the studies earlier reported by Laine and Jurgensen (1997) as well as Eggen and Majcherzykb (1998) who reported that composts from mushroom straw enhances degradation/minerlization of chlorophnol by the soil miroorganisms. Also, Joyce *et al.* (1998) observed that the concentrations of three to four rings PAHs like benz(a)anthracene, pyrene, phenanthrene, anthracene and flourine in solid municipal wastes supplemented with composts degrade faster that the un-supplemented ones. Soil amendment with SMC was observed to enhance the microorganism's population in polluted soil as reported by Sutherland *et al.* (1995) and Diaz *et al*. (1996), the SMC prepared from sawdust or some agricultural wastes degrades and decomposed faster in soil and this requires enzyme action which as well promotes metabolization/mineralization of hydrocarbon or pesticide. In this study however, supplementation of SMC and rhizosphere fungi to polluted soil improved the removal of heavy metals, hydrocarbons and pesticides in polluted soils, it was also observed that this enhanced the plant growth, aids positive adaptations and responses; this supported several reports by Nilanjana (2005), Hadibarata *et al*. (2009), Vimala and Das (2009), Elekes an Busoic (2009), Federici *et al.* (2011), Jonathan *et al*. (2012a), Kulshreshtha *et al*. (2014), Adedokun *et al.* (2015), Okerentugba *et al*. (2015) and so on.

5.3 Synergistic fungi-SMC-Plant Degradation and its Mechanism of Action

The combined actions of rhizosphere fungi and plant in this study enhanced speedy soil remediation as mediated by SMC ammendment. This effectively enhanced the soil physicochemical characteristics as well as the growth of plant and the rhizosphere fungi which in turn also led to speedy remediation, thus this affirms that synergistic remediation could be headway to achievable ex-situ soil remediation biotechnology.

The polluted soils (S1 and S2) had high concentration of 16 EPA PAHs, but became much reduced after the synergistic fungal-SMC-plant remediation which led to more than 85 % lost in 3 months. This percent loss is higher than the reported of Ikhajiagbe and Anoliefo, (2010) who reported less hydrocarbon degradation as they used only the sawdust substrate for soil remediation. Atagana (2008) reported a successful remediation of more than 380000 mg/kg total petroleum hydrocarbons in polluted soil with sewage sludge using fungi and compost, as well Martens (1982) and Kastner and Mahro (1996) demonstrated the use of mushroom compost in degrading hydrocarbons such as pyrene, fluoranthene, anthracene and while Eggen (1999) and Trejo-Harnandez *et al.* (2001) applied SMC as supplement in treating some polluted soils and reported PAH-degrading efficiency on immobilised SMC.

The synergistic rhizosphere interaction of fungi and plant has been reported to promote adequate enzymatic action and in turn positively influences the plant's perfomances (Boltner *et al*., 2005; Hussain *et al.,* 2009a; Marthin-Lauret *et al.,* 2006). This study also revealed that the synergistic fungi-SMC-plant mechanism promotes degradation of polyclic aromatic hydrocarbons, pesticides and heavy metal sequestration in polluted soils as their concentrations were brought down to normal utilizable form.

The synergistic mechanism also enhanced the degradation efficiency, rates, and half-life of polyclic aromatic hydrocarbons. Their concentrations reduced as the synergistic treatments increases and this showed that the symbiotic association between the rhizosphere fungi and test plant enhanced better degradation in soil as compared to when they act alone (controls

1 and 2). This means that both the fungi and plant can do less when exist singly as compared to their synergistic action. The synergistic ction of plant and rhizosphere fungi can therefore be exploited massive soil remediation as this also had positive result on the degradation rate and halflife of PAH as well as the degradation efficiency. The best performance was observed at the 40 and 30% synergistic treatments in this study. Similarly, this study revealled that the synergistic treatments enhanced the pesticide's degradation rate and half life more than the single entity (fungal or plant degradation mechanism) as observed for dichlorvos and lindane which are organophosphate and organochlorine pesticides espectivy.

The use of some persistent pesticides such as lindane was banned in 50 countries under the Stokholm Convenntion (convention against the use and dissemination of POPs) and this took effect in about 50 countries with restriction bane in some other 33 countries (Hanson, 2005; Humphreys *et al*, 2008). Unfortunately, lindane still persist today due to its continual use, it is a very active pesticide which many farmers found very useful and couldn't abandoned, it is used under different trade names or additive in different developing countries. Lindane residue was detected in India soils in higher amounts (Nawab *et al.,* 2003), John *et al.* (2014) also reported lindane residues in different foods above the tolerance limit lindane. Several reports have shown that lindane was tested present in some quantity in drinking, ground and commercialised branded water as well as soft dinks (Nawab *et al.,* 2003).

The degradation of several hydrocarbons and pesticides by soil microorganisms has been long and widely documented, and this has been presented in different pathways (Fourneir *et al*., 1975). The three metabolites detected for dichlorvos in syn ergistic fun gi-SMC-plant treatment were exactly those reported by Evgenidou *et al.* (2006) as metabolite of Dichlorvos degradation through a photo-oxidation mechanism. It is also possible that this compound was further degraded to dimethyl phosphate and then to dichloroacetaldehyde as also observed by Evgenidou *et al*. (2006). Also, the proposed pathway for lin danne degradation resembles that of Nagata *et al*. (2007) which reveal lindane degradation through the use of bacterium *Sphingobium japonicum*.

It was also revealed in this study that the set-up synergistic treatment of polluted soils speeds up the degradation rate and half life of lindane, the half life of 2.23 mg/kg less of lindane per day was observed to be metabolised to tetrachlorocyclohexane and tetracyclohexenol. This is less than the hydrolysis experiments that were performed by Mackay *et al.* (1997) who estimated the 2.3-90 mg/kg/day half life of lindane in atmosphere and 3-30 mg/kg/day days in the river and 30-300 mg/kg/day in lake. Chen *et al.* (2005) also reported that up to 12-30 % of lindane can become volatilize into the atmosphere, this claim corroborated the report by Walker *et al*. (1999) who detected up till 58 pg/m^3 of lindane in the atmosphere globally while Pereira *et al*. (2008) as well estimated some amount of lindane concentrations in the atmosphere which can be washed down by the rain.

The potentials of microorganism in degrading lindane concentration in soils was affirmed Nagata *et al*. (2007), this was buttressed by the report of Quintero *et al*. (2005) and that of Endo *et al*. (2006) while working on the aerobic degradation of lindane using a bacterium called *Sphingobium japonicum* formally known as *Sphingobium paucimobilis*, they reported that strain UT26 successfully degraded lindane in soil, they also developed an aerobic degradation pathway for lindane degradation by the bacterium. Using the same bacterium, they successfully degraded lindane to form pentachorohexane through dechlorination pathway; this was further degraded to monochlorobenzene, tetrachlorocyclohexane and the tetracyclohexenol similar lindane metabolites in the reports of Singh and Kuhad (200), Manickam *et al.* (2007) as well as Mohsenzad *et al.* (2010) while working on lindane dedradation using *Xanthomonas* sp.

Generally, pesticide degradation in soil is brought about by the metabolic action of soil microorganisms most of which are associated with their catabolic enzymatic secretions which allow the mineralization of the pesticides, microbial degradation of pesticide partly depends on the adaptic response microorganisms but mostly based on the fact that the microorganisms tend to utilize the chemical as carbon or mineral source. In this study, the best K_1 and $t_{1/2}$ of PAHs, dichlorvos and lindane were recorded at synergistic treatment 40

and 30 % which gave be best pH of 6.8, this resemble the report of Mohsenzad *et al.* (2010) who reported best mineralization of simazine a commonly used herbicide by bacteria called *Penicillium steckii* and *Moraxellaovis* sp at pH of 7 and 30 ^{0}C.

5.4 Synergistic Rhizosphere Mechanism Enhances the Plant's Response

It was noticed in this study that the control plant showed some symptoms similar to symptoms reported Fontes and Cox (1998) and Kabata-Pendas and Pendas (2001) such as reduced growth, curling leaf, chlorotic spots, weak root blade flexibility and weakened emerging leaf due to the pollutants in untreated soils. In addition, other scientists such Barcelo *et al.* (1990) and Sandalio *et al.* (2001) had all presented convincing data which explain different toxic effects of different soill polutants on the plants tissues, biomass and yield.

However, the synergistic fungi-SMC-plant interaction in this study enhanced the plant's response to the polluted soils 1-4. It is known that accumulation of toxic chemicals and heavy metals in plant tissues usually exert some alterations that are sometimes seen symptoms in the shoot or root. The test plant showed some significant structural changes in control as compared with the treatments. The synergistic treatments enable the plant to tolerate and adapt to the polluted soil with development of structural adaptive mechanism. The treated plants showed modified root and shoot structural architectures such as reduced hairs, darkened and thickened rootsin control as compared to the treated plants. This may be a reflective of the reason why the control plants produced less biomass as compared to the treatments.

Furthermore, the upper and lower tissues of sclerenchyma and pericycle in untreated plants (controls) appeared to be more thickened with less root hairs than the treated plants, this is may be due to the fact that the control plants are more stressed than those of the treated plants. It is believed that the synergistic treatment enhances the absorption of nutrients in SMC in the plant's cell walls thereby reducing the stresses caused by the soil pollutants while the control plants had to develop adaptive structures in response to the toxic pollutant

in the soils. It was then concluded that the synergistic treatment with SMC and rhizosphere fungi helped the plant in developing adaptive strategies to tolerate toxic soil pollutants. The SMC is believed to have supplied the system with the nutrients needed by both the plant and the rhizophere fungi; this in turn enhanced their co-degradation capacities.

Nutrient supplementation for enhanced plant performances during phytoremediation has been earlier reported by Atlas (1977 and 1981) and Bidlan *et al*. (2004), the list also includes several others such as Pereira *et al*. (2008) and Rodriguez *et al*. (2010) to mention a few. Total abence of epiderml tissue was also observed in controls, while the epidermal tissue of controls was observed to be completely replaced by thickened tissue in the exodermis as a result of defaults or attacks by the soil pollutants on the maturation of cell wall (Table 4.27). The contaminants also induced severe oxidative stresses on the plant as observed on the epidermal cell structure which resulted in thinner epidermal cell wall in control as compared to the treatments. This has also been similarly observed in some other plants that were also exposed to several polluted soils. Raven *et al*. (2001) observed loss of epidermal tissue in the plant called *Ranunculus* sp which was planted as a control plants while its functions was replaced by formation of an exodermal layer.

Variations observed in the epidermal tissue and bulliform cell of the control and treated plants in this study corroborated the observations of Melo *et al*. (2007). Similar observations were also made by Srighar *et al*. (2005) and Zhao *et al*. (2011) that many pollutants can reduce the mesophyll cell sizes, collapse the leaf palisade layer and whickened parenchymatous cells. The plant's cell wall usually acts as barrier which selectively regulates the passage of materials in and out of the cells thereby playing important roles in plant defense and tolerance mechanism against soil pollutants; it usually acts by protecting the protoplasm againt several external pollutants in soils as reported by Wojcik *et al*. (2005). It was also reported by some other scientist that accumulation of soil pollutants such as Cd, Pb and As in plant can cause distruptions of cell wall and in the intercellular air space (apoplast) expecially in the parenchyma of many young roots.

The upper and lower epidermis becomes more thickened as the synergistic treatments increased as well, there was improvement in the production and dimentions of the bulliform cells, other improvement such as the metaxylem area, endodermal cell numbers and area, the adaxial and abaxial areas of the sclerenchyma and the structure of the pericycle were also observed as shown in Table 4.27. Thickened epidermis and the larger bulliform cells were found to be more pronounced in threated *M. Maximus* compared to the controls, this is an adaptic mechanism aided by the synergstic treatment for the plant to be able to conserve and mnimize water loss through transpiration; this may be reason why more disease symptoms were observed in the control plants due to the toxicioty of the polluted soil.

The polluted soils 1-4 were observed to affect the leaf anatomical cell arrangements. Structurally, the plant root and leaf anatomy improved as the synergistic treatments increases, besides the induction of cell degeneration; it was observed by Barcelo *et al*. (1990) and Sandalio *et al*. (2001) that some metals cause morphologiocal changes in anatomical structures of exposed plants especially in respects of cell shape, size and organization. It was observed that some heavy metals usually interfare with the root initiation and maturation, this they do by by reacting with the hormonal balance and affect the exodermis, endodermis and apoplastic barrers which play important roles in plant defense mechanism against environmental stresses (Enstone *et al*., 2003). Research has shown that many plants has developed defense mechanisms which helps them to tolerate some heavy metals and other pollutants, some plants usually develop strategy such as thicken cell walls to minimize the traslocation of absorbed pollutants from the soil. According to Lux *et al*. (2004) observed alterations in the proportions of exodermis and endodermis tissues in the roots.

In this study the xylem, tracheary elements and the root cortical parenchyma of the controls were thicker as compared to the treated plants, the control plant also developed larger vascular cylinder however, the metaxylem elements of control plants formed larger area that those of the treated plants and this may be due to synergistic interaction of SMC-Fungal treatments in the treted soils. Similar observation were reported in many studies, Vaquez *et al*. (1992) and Wojcik *et al*. (2005) reported that SMC and soil microorgganisms

can enhance the plant's response to heavy metals in polluted soils, SMC according to them have the ability to bind many toxic substances is soils and inactivate their toxic potentials. The plants growing on the treated soil as also also observed by Enstone *et al*. (2003) formed thicker cell wall which performs protective functions; it also enhanced adaptation for adsorption of soil contaminants and ions through the formation of blockages hindering their entrance into the cell. This could be difficult for the control plant as their transpiration rate is very low due to toxic effect of the polluted soil on which they grow.

Furthermore, many soil pollutants have been reported to insight negative effects on the plant's hormonal mechanism in root initiation and proliferation and also affect the leaf morphogenesis of the and cell (Barcelo *et al*., 1990; Sandalio *et al*., 2001). Increase in heavy metal accumulation in plant roots for example usually creates an osmotic stress in plants which usually causes flow of ions in opposite direction in the vascular cylinder. The plants growing on treated oil in this study had thicker tracheary elements and more reduced area of metaxylem vessels as compared to the controls and this may be a reflective of the fact that the synergistic treatments improved the hydraulic capacity of the plant to reduce stress. It was also observed in this study that, alterations in the tracheary elements and metaxylem was initiated in plant growing on the treated soils in other to promote the root initiation and maturation.

Several researches reported shoot reduction as a result of heavy metals interfarence with the photosynthetics mechanims (Broadley *et al*., 2007; Potters *et al*., 2009; Smeets *et al*., 2009; Cuypers *et al*., 2011; Wan *et al*., 2011). They all observed and reported morphological alterances due to plant response to pollutants which are collectively refers to as Stress Induced Morphogenic Responses (SIMRs) while those of physiological alterances are refers to as induced physiogenic responses (SIPRs). The examples of SIMRs are the anatomical changes, root aberrations, symptomatic leaf malphomations etc while those of SIPRs are the hormonal aberations (auxins and ethylenes for examples), the reactive oxygen species (ROS), the phytochemical and antioxidant compositions, cell division problems such as cell differentiation, elongation and/or differentiations and so on (Potters *et al*., 2007; Ghanem *et al*., 2011). Potters *et al*. (2009) also reported altrations in auxin

gradients due to stress conditions of a polluted soil and this was linked to morphogenic response, auxin as a key phytohormone is an intrinsic factor in root development pathways according Nibau *et al.* (2008), any aberration in auxin production in plant due to stresses caused by the polluted soil or pollutants usually reflects in root architectural alterations s observed by Fukaki and Tasaka (2009) and Hodge (2009).

It wasobserved in this study that increase in concentration of SMC-Fungial treatment also result in the increase in plant's potential photosynthetic rate (Amax), this grees with the reports of Chugh and Sawhney (1999) as well as that of Prasad *et al*. (2001) that increased soil contamination reduced the photosynthetic rate. This could also be related with the observed negative influences of soil contaminants on the plant's chlorophyll contents as also observed Stobart *et al*. (1985) and it was affirmed that the soil pollutants exert some observable physiological effects on the photosynthetic rate as also reported by Churgh and and Sawhney (1999), their effect on the activites of enzymes was also reported (Singh *et al*., 2006) while Zhou and Qiu (2005) also reported their effects on the water balance in plants.

Majorly, the photosynthesis process is very important in plant' metabolism during which plants manufacture their own food, it is also need for yielding and biomass, recently photosynthetic rate in plants has been used to monitore plant response to stress which may be caused by biotic or abiotic factors. The use of photosynthetic as bioindicator of stresses was demonstrated by Sheoran *et al*. (1990a, b), this as well was being employed in thi study to detect levels at which the soil pollutants may be affecting plants and how the set up synergistic treatment can help the test plant (guinea grass) respond and survive the polluted soils 1-4 and how the synergistich fungal-SMC treatments help the plants to maintain the stress.

It was observed that the synergistic Fungal-SMC treatments enhanced the plant's photosyntentic rate as he treatment increases compared to the control which was not treated. This is a reflective of the fact that the higher degradative potentials of the synergistic treatments also reduced the toxic level of the pollutents hence resulting in

optimum photosynthetic rate. Several soil pollutants have ben reported to interfare with the photosynthetic rate in plants. In similar reports Cagno *et al*. (1999 and 2001) reported effects of metals and hydrocarbons on thhe plants chlorophyll content and enzyme activities of the calvin cycles in plants, psycotrome 2 was also found to be very sensitive to metallic ions which in turn affects the carbon assimmilation rates.

5.5 Efficacy of the Remediated Soil on Seed Germination

The remediated soils were plated with equal number of *M. maximus* viable seeds and it was observed that soils treated with synergistic mechanism produced the highest germination index over the soil with no treatment (control). Effects of soil pollutants on the plants' seed germination have been well documented (Adam and Duncan, 2002), results in this study also conform with reports of Molina-Barahona *et al*. (005) and Oleszczuk (2008) which reflected the phytoxicity of hydrocarbons in diesel oil composted sludge on germination of the plant *Lepidium sativum*. The influence of many toxic pollutants to the germinating seeds in polluted soils can however be likened to other physiological influences such as oxygen availability (Adam and Duncan, 2002; Enstone *et al*., 2003; Oleszczuk, 2008).

In polluted soils, hydrocarbons for example were reported to act by forming complexes with soil humus and make it unavailable for plant uptake. It was also reported that, some hydrocarbons and pesticides in some cases form coating covering the roots and affect the nutrient absorption process, some may get accumulated in seeds, kill the embryos and/or alter the metabolic processes necessary for the development of young seedling. Some POPs can exert different symptoms in plants based on their toxicity and type or concentration in soils; the plant may die in heavily polluted sites after penetrating the plant tissues. Adam and Duncan (2002) and Labud *et al*. (2008) reported that POPs can damage the cell membrane and reduce its physiological roles; they also reported that some can affect the metabolic transport pathways and the respiratory rate. Their properties have been shown to exert inhibitory effect on the germinating seeds due to hydrophilic nature of many hydrocarbons; they act as barriers wich prevent water from the seeds and inhibit oxygen transport (Ogboghodo *et al*., 2004).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Potential Development Impact, Innovation and Sustainability

This study on synergistic fungi-SMC-plant remediation mechanism is very promising for massive and small scale soil clean-up, it is a novel strategy that combines Mycoremediation, phytoremediation and employs the use of SMC for the restoration of soil fertility. This mechanism is also a waste to wealth approach; guinea grass (a weed) and SMC (a waste) are once again having their chances to become useful if this mechanism is well implemented.

The result of this research can be exploited in making a product which will include specially refined SMC packaged with the fungal spores and seeds of *M. maximus* to be applied on polluted soil for nutrient enhancement and removal of pollutants. Application of this product in soil (if the use in encouraged) at 40 or 30 % will enhance the soil fertility benefiting a lot of farmers as it will in-turn increase their crop output by at least 30 %. In addition, the rhizosphere fungal strains which were characterised in this study exhibit good expression of novel degrading enzymes extracellularly; these extracellular enzymes produced from the rhizosphere fungi can also be exploited in different biotechnology applications.

Therefore, a project like this should be well encouraged by the Governments, NGOs and Industries. Such research could be financed for better understanding on the applicability on field (ex-situ) for enhanced optimum results.

6.2 Conclusion

This study presents a new bioremediation mechanism which explored the synergistic influence of fungi-SMC-plant interaction for treatment of different polluted soils. The fungal strains possess and expressed putative degrading gene expression and enzymatic activities which explain their survival/tolerance and bioremediatory capacities in polluted soils while the plant showed positive response to the synergistic treatments as opposed to the soil pollutants. These fungal strains with the plant performed excellently well in rhizosphere, the resulting interaction yielded better nutrient, pH and CEC level of the polluted soil, reduced heavy metal concentrations and better degradation of PAHs, dichlorvos and lindane based on degradation efficiency, rate and half life analysis. This fungal-SMC-plant mechanism also enhanced the plant's growth and physiology. The remediated soils using this mechanim also best support the seed germination the controls.

6.3 Recommendation

The use of plant in soil remediation 'Phytoremediation' is a promising aspect in soil clean-technology but it is still faced with so many challenges. However, supporting the phytoremediation with synergistic action of rhizosphere fungi and biostimulattion using the spent mushroom compost yielded more efficient soil restoration and clean-up. It therefore recommended that application of fungi-SMC-plant treatments at 40 and 30 % is required for optimum soil remediation. However, there is need for more in-dept molecular, chemical and biochemical studies on this synergistic treatment technology for better understanding of degradation kinetics, mineralization and/or bioconversion of soil pollutants. The genes/enzymes reported in this study need to be more studied for more in-dept understanding of roles played by each particular fungal strain. Above all, synergistic mechanism of bioremediation in this study needs to be encouraged in large and small scale soil clean-up.

The following recommendations are also suggested from this study:

i. Native rhizosphere fungi can be used with SMC to enhance plant growth and survival in polluted soils
ii. Synergistic co-action of roots and rhizosphere fungal strains yield faster and more effective bioremediation.
iii. Hydrocarbons, pesticides and heavy metal polluted soils can be bio-treated with synergistic fungi-SMC-plant treatment.
iv. Spent mushroom compost and some natïve rhizosphere fungi can be utilised at 40 or 30 % in composting for dual reclamation of polluted soils and soil fertility.
v. The synergistic treatment at 40 and 30 % as set-up in this study may be employed by crude oil exploration and other chemical producing companies for treatment of waste effluents.

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APPENDICES

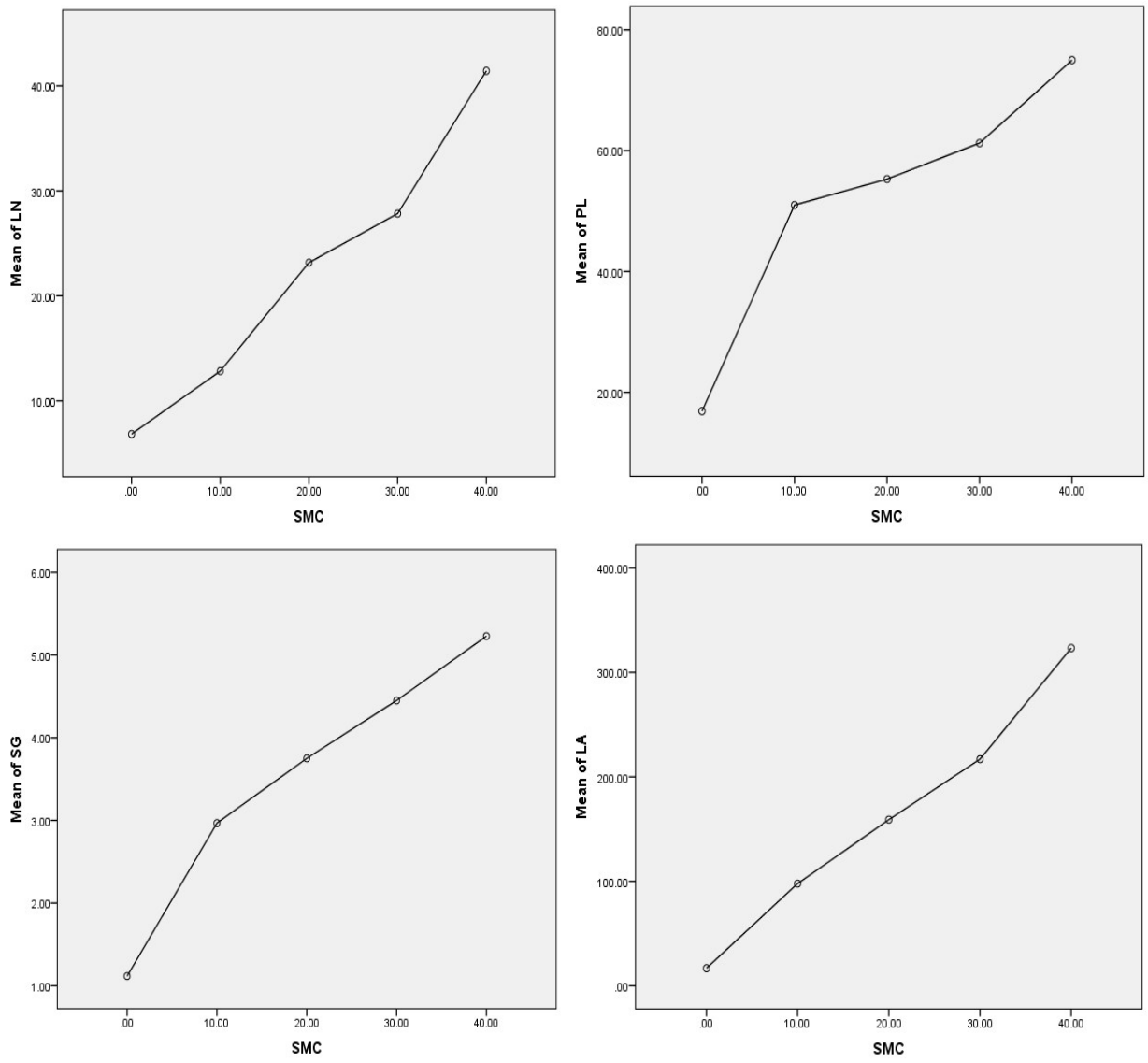
Appendix 1: Agronomic parameters recorded for *M. maximus* plant on soil 1 with or without synergistic treatments.

ANOVA FOR SOIL 1

				Sum of Squares	Df.	Mean Square	F.	Sig.
LN	Between Groups	(Combined)		4705.920	4	1176.480	4.367	.008
		Linear Term	Unweighted	4510.569	1	4510.569	16.741	.000
			Weighted	4598.240	1	4598.240	17.067	.000
			Deviation	107.680	3	35.893	.133	.939
	Within Groups			7005.048	26	269.425		
	Total			11710.968	30			
PL	Between Groups	(Combined)		11669.932	4	2917.483	5.355	.003
		Linear Term	Unweighted	10175.968	1	10175.968	18.678	.000
			Weighted	10106.610	1	10106.610	18.550	.000
			Deviation	1563.322	3	521.107	.956	.428
	Within Groups			14165.392	26	544.823		
	Total			25835.324	30			
SG	Between Groups	(Combined)		62.390	4	15.597	5.340	.003
		Linear Term	Unweighted	59.964	1	59.964	20.528	.000
			Weighted	59.379	1	59.379	20.328	.000
			Deviation	3.011	3	1.004	.344	.794
	Within Groups			75.946	26	2.921		
	Total			138.335	30			
LA	Between Groups	(Combined)		350479.458	4	87619.865	4.298	.008
		Linear Term	Unweighted	341179.906	1	341179.906	16.737	.000
			Weighted	346441.047	1	346441.047	16.995	.000
			Deviation	4038.411	3	1346.137	.066	.977
	Within Groups			530006.909	26	20384.881		
	Total			880486.367	30			

LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

Appendix 2: Mean gronomic parameters recorded for *M. maximus* plant on soil 1 with or without synergistic treatments.



LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

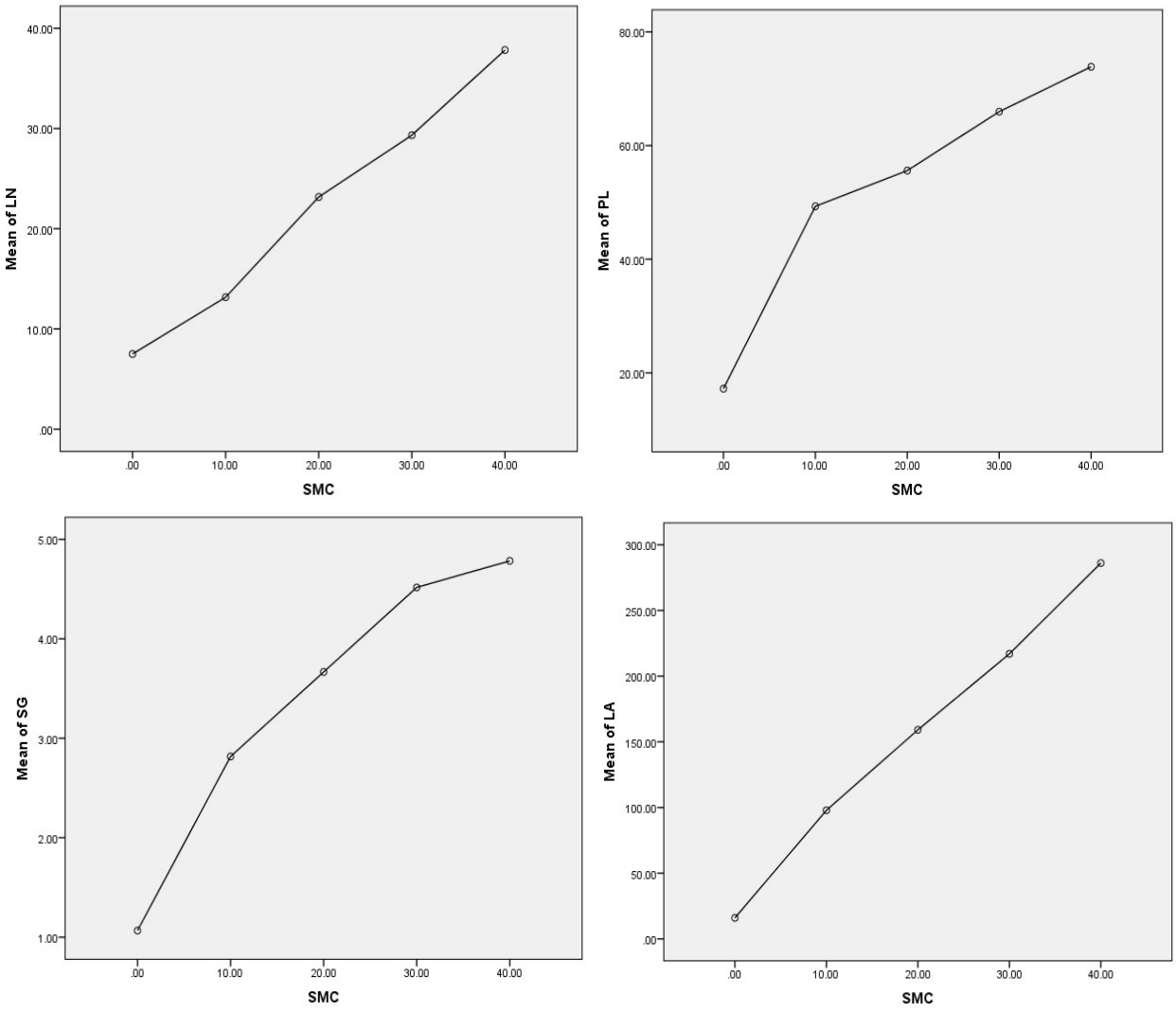
Appendix 3: Agronomic parameters recorded for *M. maximus* plant on soil 2 with or without synergistic treatments.

ANOVA FOR SOIL 2

				Sum of Squares	Df.	Mean Square	F.	Sig.
LN	Between Groups	(Combined)		4667.463	4	1166.866	4.267	.009
		Linear Term	Unweighted	4523.334	1	4523.334	16.541	.000
			Weighted	4597.693	1	4597.693	16.812	.000
			Deviation	69.770	3	23.257	.085	.968
	Within Groups			7110.214	26	273.470		
	Total			11777.677	30			
PL	Between Groups	(Combined)		12472.314	4	3118.078	5.414	.003
		Linear Term	Unweighted	11428.576	1	11428.576	19.845	.000
			Weighted	11279.866	1	11279.866	19.587	.000
			Deviation	1192.448	3	397.483	.690	.566
	Within Groups			14972.924	26	575.882		
	Total			27445.237	30			
SG	Between Groups	(Combined)		64.587	4	16.147	5.777	.002
		Linear Term	Unweighted	62.851	1	62.851	22.486	.000
			Weighted	62.115	1	62.115	22.223	.000
			Deviation	2.471	3	.824	.295	.829
	Within Groups			72.672	26	2.795		
	Total			137.259	30			
LA	Between Groups	(Combined)		351807.452	4	87951.863	4.310	.008
		Linear Term	Unweighted	342543.631	1	342543.631	16.787	.000
			Weighted	347770.842	1	347770.842	17.043	.000
			Deviation	4036.610	3	1345.537	.066	.977
	Within Groups			530547.366	26	20405.668		
	Total			882354.818	30			

LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG = is the Stem girth (cm); and LA is the Leaf area (cm^2).

Appendix 4: Mean agronomic parameters recorded for *M. maximus* plant on soil 2 with or without synergistic treatments.



LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

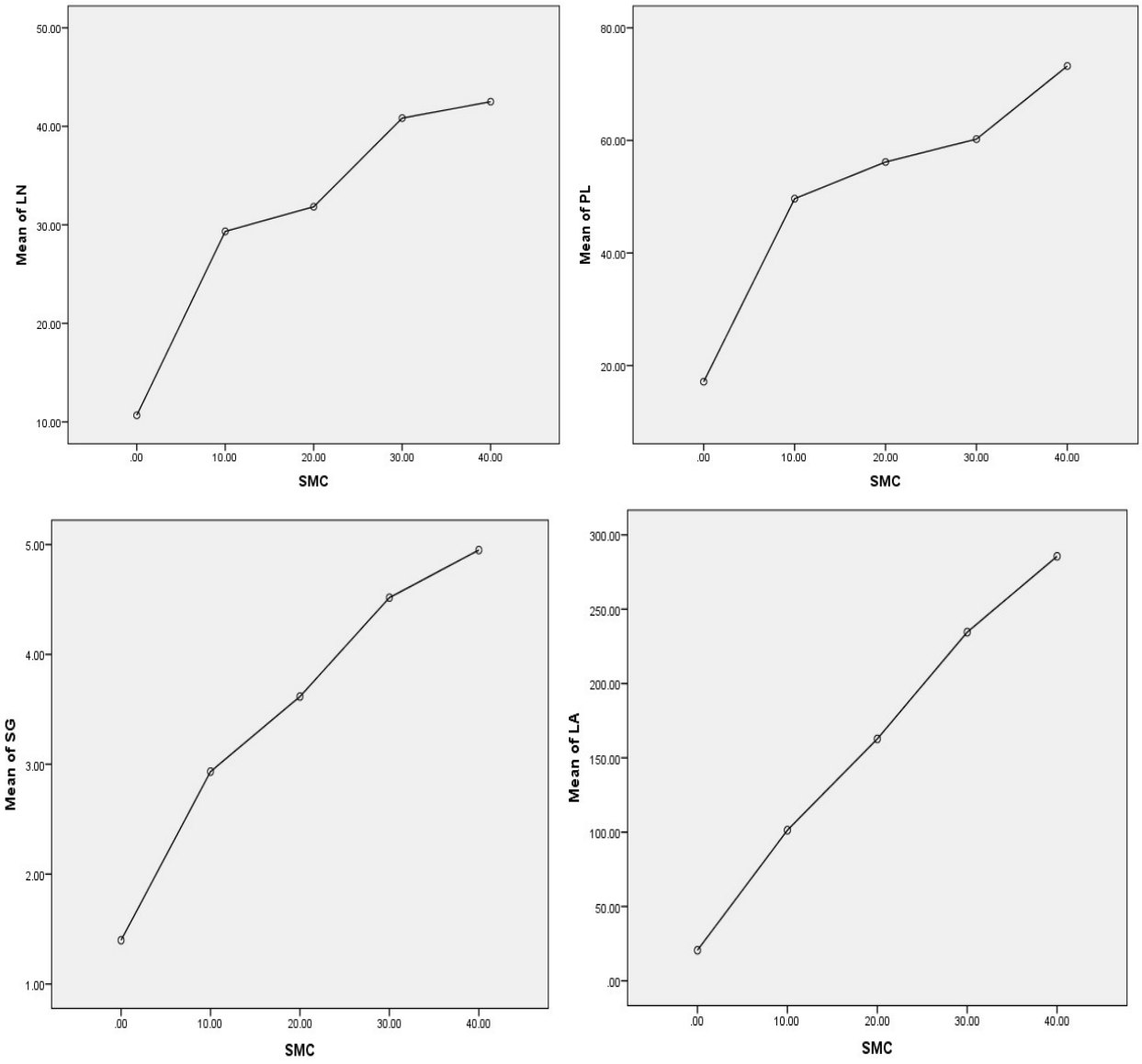
Appendix 5: Agronomic parameters recorded for *M. maximus* plant on soil 3 with or without synergistic treatments.

ANOVA FOR SOIL 3

				Sum of Squares	Df.	Mean Square	F.	Sig.
LN	Between Groups	(Combined)		3875.133	4	968.783	3.230	.029
		Linear Term	Contrast	3390.017	1	3390.017	11.303	.002
			Deviation	485.117	3	161.706	.539	.660
	Within Groups			7497.833	25	299.913		
	Total			11372.967	29			
PL	Between Groups	(Combined)		10523.465	4	2630.866	4.969	.004
		Linear Term	Contrast	9040.537	1	9040.537	17.076	.000
			Deviation	1482.927	3	494.309	.934	.439
	Within Groups			13235.963	25	529.439		
	Total			23759.428	29			
SG	Between Groups	(Combined)		47.318	4	11.830	4.159	.010
		Linear Term	Contrast	45.275	1	45.275	15.918	.001
			Deviation	2.043	3	.681	.239	.868
	Within Groups			71.107	25	2.844		
	Total			118.425	29			
LA	Between Groups	(Combined)		265344.704	4	66336.176	3.387	.024
		Linear Term	Contrast	263993.400	1	263993.400	13.480	.001
			Deviation	1351.303	3	450.434	.023	.995
	Within Groups			489586.497	25	19583.460		
	Total			754931.200	29			

LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

Appendix 6: Agronomic parameters recorded for *M. maximus* plant on soil 3 with or without synergistic treatments.



LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

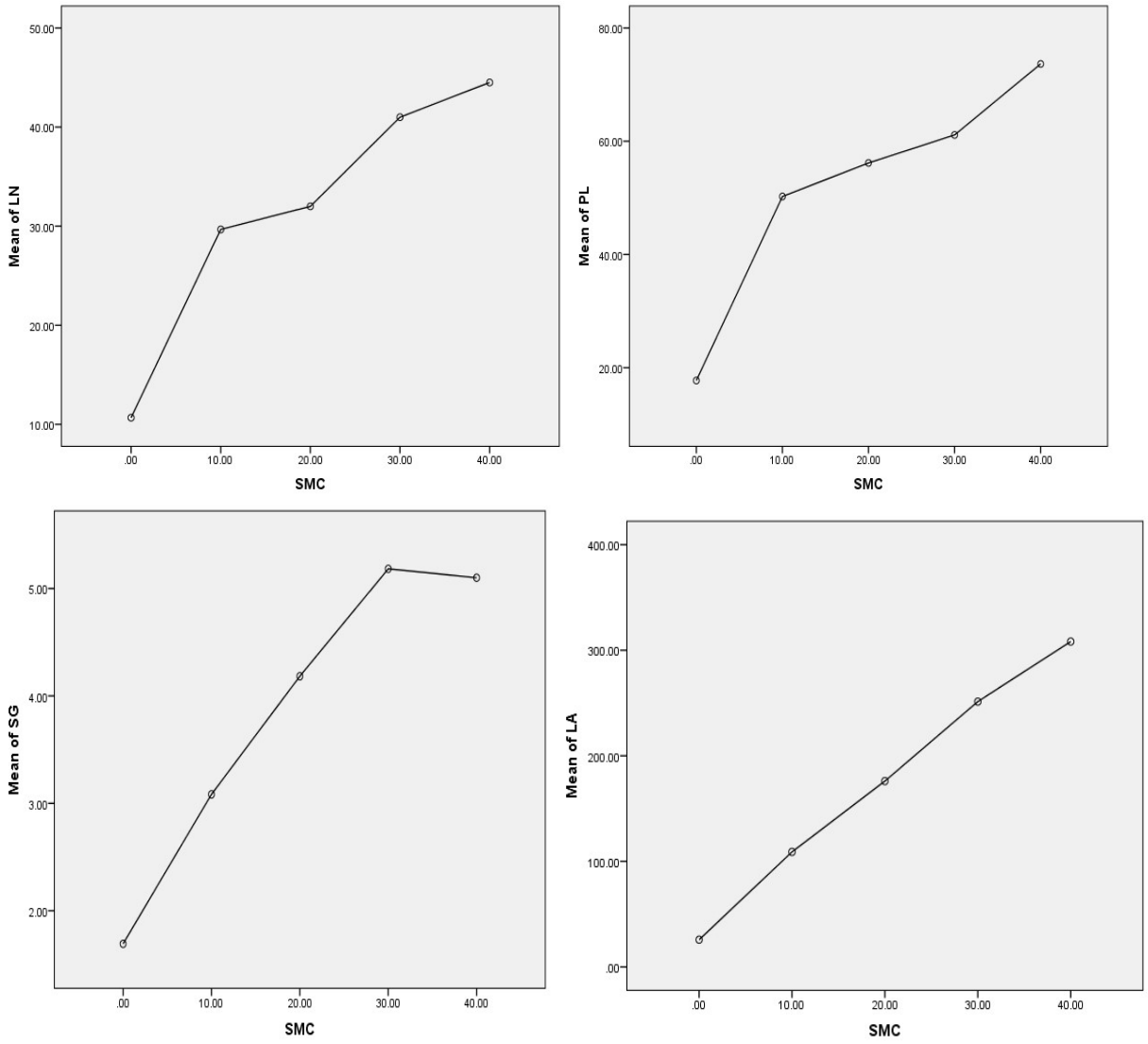
Appendix 7: Agronomic parameters recorded for *M. maximus* plant on soil 4 with or without synergistic treatments

ANOVA FOR SOIL 4

				Sum of Squares	Df.	Mean Square	F.	Sig.
LN	Between Groups	(Combined)		4181.200	4	1045.300	3.661	.018
		Linear Term	Contrast	3744.600	1	3744.600	13.115	.001
			Deviation	436.600	3	145.533	.510	.679
	Within Groups			7138.167	25	285.527		
	Total			11319.367	29			
PL	Between Groups	(Combined)		10477.685	4	2619.421	4.919	.005
		Linear Term	Contrast	9035.628	1	9035.628	16.969	.000
			Deviation	1442.056	3	480.685	.903	.454
	Within Groups			13312.223	25	532.489		
	Total			23789.908	29			
SG	Between Groups	(Combined)		52.185	4	13.046	4.635	.006
		Linear Term	Contrast	47.704	1	47.704	16.948	.000
			Deviation	4.481	3	1.494	.531	.665
	Within Groups			70.367	25	2.815		
	Total			122.552	29			
LA	Between Groups	(Combined)		301265.222	4	75316.305	4.803	.005
		Linear Term	Contrast	300192.267	1	300192.267	19.142	.000
			Deviation	1072.955	3	357.652	.023	.995
	Within Groups			392063.839	25	15682.554		
	Total			693329.061	29			

LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm); and LA is the Leaf area (cm^2).

Appendix 8: Agronomic parameters recorded for *M. maximus* plant on soil 4 with or without synergistic treatments.



LN is the Leaf number (including dead leaves); PL is the Plant length (cm); SG is the Stem girth (cm) and LA is the Leaf area (cm^2).

Appendix 9: Root proliferation measurement recorded for *M. maximus* planted on different polluted soils 1-4 with or without synergistic treatments.

ANOVA For Root Dimentions

				Sum of Squares	Df.	Mean Square	F.	Sig.
SOIL 1	Between Groups	(Combined)		6233086.349	4	1558271.587	54472.323	.000
		Linear Term	Contrast	6103473.285	1	6103473.285	213358.423	.000
			Deviation	129613.064	3	43204.355	1510.290	.000
	Within Groups			286.067	10	28.607		
	Total			6233372.416	14			
SOIL 2	Between Groups	(Combined)		6321861.601	4	1580465.400	13652.597	.000
		Linear Term	Contrast	6294825.424	1	6294825.424	54376.840	.000
			Deviation	27036.177	3	9012.059	77.849	.000
	Within Groups			1157.630	10	115.763		
	Total			6323019.231	14			
SOIL 3	Between Groups	(Combined)		5064647.150	4	1266161.788	23.286	.000
		Linear Term	Contrast	3500101.947	1	3500101.947	64.370	.000
			Deviation	1564545.203	3	521515.068	9.591	.003
	Within Groups			543745.423	10	54374.542		
	Total			5608392.573	14			
SOIL 4	Between Groups	(Combined)		1029151.410	4	257287.852	161.498	.000
		Linear Term	Contrast	922143.929	1	922143.929	578.826	.000
			Deviation	107007.481	3	35669.160	22.389	.000
	Within Groups			15931.287	10	1593.129		
	Total			1045082.697	14			

Appendix 10: Activities of Lignin Peroxidase Enzyme Produced by the Selected Rhizosphere Fungi

Conc		asemoA	asemoB	asemoC	asemoD	asemoE	asemoF	asemoG	asemoH	asemoI	asemoJ	asemoK	asemoL	asemoM	asemoN	asemoO	asemoP
0	Mean	06.00	06.00	06.00	04.67	07.00	10.00	04.00	06.00	11.00	07.00	08.00	07.00	06.00	06.00	05.00	03.00
	S.D.	01.00	01.00	01.00	00.58	01.00	05.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00
5	Mean	34.00	30.00	46.00	48.00	70.00	98.00	30.00	56.00	60.00	95.00	60.00	20.00	67.00	57.00	50.00	60.00
	S.D.	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00	10.00	01.00	10.00	10.00	01.00	01.00	10.00	01.00
10	Mean	35.00	34.00	68.00	60.00	65.00	100.00	70.00	100.0	63.33	98.00	50.00	23.00	98.00	57.00	60.00	40.00
	S.D.	10.00	01.00	01.00	10.00	01.00	50.00	10.00	50.00	00.58	01.00	10.00	01.00	01.00	01.00	01.00	10.00
15	Mean	46.00	80.00	70.00	64.00	34.00	100.0	80.00	104.0	63.00	99.00	102.0	34.00	98.00	60.00	60.00	30.00
	S.D.	10.00	01.00	01.00	10.00	10.00	50.00	10.00	01.00	01.00	01.00	01.00	10.00	10.00	10.00	01.00	01.00
20	Mean	58.00	101.0	105.0	68.00	30.00	105.0	90.00	105.0	62.00	99.00	104.0	56.00	100.0	64.00	70.00	28.00
	S.D.	10.00	10.60	05.00	04.00	10.00	01.00	05.00	50.00	01.00	01.00	04.00	02.00	01.00	10.00	10.00	01.00
Total	Mean	35.80	50.00	58.00	48.93	41.20	83.40	54.80	73.20	51.87	79.60	64.80	28.00	73.80	48.80	49.00	32.20
	S.D.	19.03	52.09	37.39	24.57	24.83	46.66	34.18	47.51	21.53	37.61	37.42	17.85	53.26	22.95	24.30	19.52

S.D. = Standard Deviation

Appendix 11: The Effect pollutants on Lignin Peroxidase Activities among the Rhizosphere Fungi

ANOVA

		Sum of Squares	Df.	Mean Square	F.	Sig.
	Between the Groups	4466.400	4	1116.600	18.487	.000
	Within the Groups	604.000	10	60.400		
asemoA * Conc	Total	5070.400	14			
	Between the Groups	17976.000	4	4494.000	12.246	.0137
	Within the Groups	20008.000	10	2000.800		
asemoB * Conc	Total	37984.000	14			
	Between the Groups	14568.000	4	3642.000	7.272	.005
	Within the Groups	5008.000	10	500.800		
asemoC * Conc	Total	19576.000	14			
	Between the Groups	8020.267	4	2005.067	46.129	.000
	Within the Groups	434.667	10	43.467		
asemoD * Conc	Total	8454.933	14			
	Between the Groups	8228.400	4	2057.100	50.667	.000
	Within the Groups	406.000	10	40.600		
asemoE * Conc	Total	8634.400	14			
	Between the Groups	20421.600	4	5105.400	5.078	.017
	Within the Groups	10054.000	10	1005.400		
asemoF * Conc	Total	30475.600	14			
	Between the Groups	15902.400	4	3975.600	87.568	.000
	Within the Groups	454.000	10	45.400		
asemoG * Conc	Total	16356.400	14			
	Between the Groups	21590.400	4	5397.600	5.394	.014
	Within the Groups	10006.000	10	1000.600		
asemoH * Conc	Total	31596.400	14			
	Between the Groups	6283.067	4	1570.767	76.005	.000
	Within the Groups	206.667	10	20.667		
asemoI * Conc	Total	6489.733	14			
	Between the Groups	19797.600	4	4949.400	4949.400	.000
	Within the Groups	10.000	10	1.000		
asemoJ * Conc	Total	19807.600	14			
	Between the Groups	19166.400	4	4791.600	109.899	.000
	Within the Groups	436.000	10	43.600		
asemoK * Conc	Total	19602.400	14			
	Between the Groups	4050.000	4	1012.500	24.575	.000
	Within the Groups	412.000	10	41.200		
asemoL * Conc	Total	4462.000	14			
	Between the Groups	19502.400	4	4875.600	12.413	.0118
	Within the Groups	20206.000	10	2020.600		
asemoM * Conc	Total	39708.400	14			
	Between the Groups	6968.400	4	1742.100	42.909	.000
	Within the Groups	406.000	10	40.600		
asemoN * Conc	Total	7374.400	14			
	Between the Groups	7860.000	4	1965.000	48.399	.000
	Within the Groups	406.000	10	40.600		
asemoO * Conc	Total	8266.000	14			
	Between the Groups	5126.400	4	1281.600	61.615	.000
	Within the Groups	208.000	10	20.800		
asemoP * Conc	Total	5334.400	14			

Appendix 12: Activities of Manganese Peroxidase Enzyme Produced by the Selected Rhizosphere Fungi

Conc		asemoA	asemoB	asemoC	asemoD	asemoE	asemoF	asemoG	asemoH	asemoI	asemoJ	asemoK	asemoL	asemoM	asemoN	asemoO	asemoP
0	Mean	06.67	06.00	04.00	07.67	07.33	04.00	08.67	06.00	05.33	10.00	06.00	06.00	06.00	10.67	06.33	05.33
	S.D.	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
5	Mean	00.58	01.00	00.00	02.52	02.52	01.00	06.66	03.00	04.51	10.00	03.00	01.00	01.00	10.50	00.58	00.58
	S.D.	27.00	46.33	30.00	61.33	20.00	60.00	93.33	57.33	48.33	98.00	46.67	67.67	31.00	60.33	34.00	60.00
10	Mean	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
	S.D.	10.00	00.58	02.00	28.02	05.00	05.00	12.58	01.53	03.51	02.00	02.08	02.08	11.53	00.58	00.00	05.00
15	Mean	44.67	68.33	68.00	50.00	23.00	41.67	87.33	55.67	60.00	100.0	55.67	95.33	34.00	63.00	33.33	56.67
	S.D.	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
20	Mean	00.58	00.58	00.00	05.00	10.00	02.89	48.88	21.03	05.00	00.00	21.50	06.43	00.00	02.00	07.64	07.64
	S.D.	35.00	70.00	70.00	105.0	34.33	30.33	99.00	60.00	64.00	100.7	56.00	98.33	75.00	63.33	46.00	50.00
Total	Mean	03.00	03.00	03.00	13.01	03.00	03.00	03.00	03.00	12.00	03.00	03.00	15.00	03.00	03.00	13.20	03.00
	S.D.	02.65	05.00	00.01	00.01	00.58	00.58	01.00	05.00	00.00	01.15	01.00	00.58	01.00	00.58	20.00	10.00

S.D. = Standard Deviation

Appendix 13: The Effect pollutants on Manganese Peroxidase Activities among the Rhizosphere Fungi

ANOVA

		Sum of Squares	Df.	Mean Square	F.	Sig.
asemoA * Conc	Between the Groups	6333.600	4	1583.400	67.667	.000
	Within the Groups	234.000	10	23.400		
	Total	6567.600	14			
asemoB * Conc	Between the Groups	9188.400	4	2297.100	430.706	.000
	Within the Groups	53.333	10	5.333		
	Total	9241.733	14			
asemoC * Conc	Between the Groups	11579.067	4	2894.767	115.176	.000
	Within the Groups	251.333	10	25.133		
	Total	11830.400	14			
asemoD * Conc	Between the Groups	19403.733	4	4850.933	29.651	.000
	Within the Groups	1636.000	10	163.600		
	Total	21039.733	14			
asemoE * Conc	Between the Groups	4132.933	4	1033.233	39.138	.000
	Within the Groups	264.000	10	26.400		
	Total	4396.933	14			
asemoF * Conc	Between the Groups	5031.067	4	1257.767	46.699	.000
	Within the Groups	269.333	10	26.933		
	Total	5300.400	14			
asemoG * Conc	Between the Groups	18161.333	4	4540.333	8.711	.003
	Within the Groups	5212.000	10	521.200		
	Total	23373.333	14			
asemoH * Conc	Between the Groups	6924.267	4	1731.067	17.497	.000
	Within the Groups	989.333	10	98.933		
	Total	7913.600	14			
asemoI * Conc	Between the Groups	7804.933	4	1951.233	51.348	.000
	Within the Groups	380.000	10	38.000		
	Total	8184.933	14			
asemoJ * Conc	Between the Groups	20489.067	4	5122.267	243.146	.000
	Within the Groups	210.667	10	21.067		
	Total	20699.733	14			
asemoK * Conc	Between the Groups	5451.733	4	1362.933	8.455	.003
	Within the Groups	1612.000	10	161.200		
	Total	7063.733	14			
asemoL * Conc	Between the Groups	19157.733	4	4789.433	498.899	.000
	Within the Groups	96.000	10	9.600		
	Total	19253.733	14			
asemoM * Conc	Between the Groups	11682.000	4	2920.500	107.371	.000
	Within the Groups	272.000	10	27.200		
	Total	11954.000	14			
asemoN * Conc	Between the Groups	6381.733	4	1595.433	68.769	.000
	Within the Groups	232.000	10	23.200		
	Total	6613.733	14			
asemoO * Conc	Between the Groups	4422.400	4	1105.600	11.948	.001
	Within the Groups	925.333	10	92.533		
	Total	5347.733	14			
asemoP * Conc	Between the Groups	5840.267	4	1460.067	39.532	.000
	Within the Groups	369.333	10	36.933		
	Total	6209.600	14			

Appendix 14: Activities of Catalase Enzyme Produced by the Selected Rhizosphere Fungi

Conc		asemoA	asemoB	asemoC	asemoD	asemoE	asemoF	asemoG	asemoH	asemoI	asemoJ	asemoK	asemoL	asemoM	asemoN	asemoO	asemoP
0	Mean	12.00	07.00	02.00	08.00	05.00	13.00	11.00	14.00	04.00	10.00	04.00	12.00	10.00	13.00	13.00	06.00
	S.D.	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
5	Mean	01.00	03.00	02.00	08.00	01.00	03.00	10.00	02.00	04.00	10.00	02.00	01.00	05.00	01.00	01.00	01.00
	S.D.	23.00	23.00	87.00	61.67	30.00	78.00	28.33	76.00	31.67	56.67	66.67	43.00	45.33	60.00	48.00	45.33
10	Mean	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
	S.D.	01.00	02.00	01.00	10.41	05.00	01.00	00.58	00.00	01.53	00.58	00.58	01.00	05.03	05.00	00.00	00.58
15	Mean	34.00	34.67	82.00	66.00	34.33	076.33	45.67	76.00	45.00	67.00	70.00	44.00	60.00	65.00	51.00	50.00
	S.D.	03.00	03.00	03.00	02.04	03.00	03.00	01.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
20	Mean	00.00	00.58	02.00	01.00	00.58	00.58	00.58	01.00	01.00	00.00	10.00	10.00	10.00	01.00	10.00	10.00
	S.D.	46.67	55.67	60.00	67.00	45.00	86.00	65.00	50.00	87.00	61.67	76.00	56.33	76.00	66.00	48.33	49.00
To	Mean	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00	03.00
tal	S.D.	07.64	01.53	10.00	10.00	01.00	01.00	10.00	05.00	00.00	10.41	01.00	00.58	00.00	02.00	14.22	01.00

S.D. = Standard Deviation

Appendix 15: The Effect pollutants on the Catalase Activities among the Rhizosphere Fungi

ANOVA

		Sum of Squares	Df.	Mean Square	F.	Sig.
	Between the Groups	3053.067	4	763.267	23.802	.000
	Within the Groups	320.667	10	32.067		
asemoA * Conc	Total	3373.733	14			
	Between the Groups	9286.000	4	2321.500	696.450	.000
	Within the Groups	33.333	10	3.333		
asemoB * Conc	Total	9319.333	14			
	Between the Groups	13682.400	4	3420.600	127.634	.000
	Within the Groups	268.000	10	26.800		
asemoC * Conc	Total	13950.400	14			
	Between the Groups	7996.667	4	1999.167	36.526	.000
	Within the Groups	547.333	10	54.733		
asemoD * Conc	Total	8544.000	14			
	Between the Groups	5823.600	4	1455.900	263.114	.000
	Within the Groups	55.333	10	5.533		
asemoE * Conc	Total	5878.933	14			
	Between the Groups	11527.067	4	2881.767	1168.284	.000
	Within the Groups	24.667	10	2.467		
asemoF * Conc	Total	11551.733	14			
	Between the Groups	6642.667	4	1660.667	36.795	.000
	Within the Groups	451.333	10	45.133		
asemoG * Conc	Total	7094.000	14			
	Between the Groups	7848.267	4	1962.067	323.418	.000
	Within the Groups	60.667	10	6.067		
asemoH * Conc	Total	7908.933	14			
	Between the Groups	15632.267	4	3908.067	961.000	.000
	Within the Groups	40.667	10	4.067		
asemoI * Conc	Total	15672.933	14			
	Between the Groups	7835.600	4	1958.900	41.917	.000
	Within the Groups	467.333	10	46.733		
asemoJ * Conc	Total	8302.933	14			
	Between the Groups	11564.267	4	2891.067	132.213	.000
	Within the Groups	218.667	10	21.867		
asemoK * Conc	Total	11782.933	14			
	Between the Groups	3946.000	4	986.500	48.044	.000
	Within the Groups	205.333	10	20.533		
asemoL * Conc	Total	4151.333	14			
	Between the Groups	9321.067	4	2330.267	77.503	.000
	Within the Groups	300.667	10	30.067		
asemoM * Conc	Total	9621.733	14			
	Between the Groups	6452.400	4	1613.100	252.047	.000
	Within the Groups	64.000	10	6.400		
asemoN * Conc	Total	6516.400	14			
	Between the Groups	3720.667	4	930.167	15.282	.000
	Within the Groups	608.667	10	60.867		
asemoO * Conc	Total	4329.333	14			
	Between the Groups	4396.267	4	1099.067	27.160	.000
	Within the Groups	404.667	10	40.467		
asemoP * Conc	Total	4800.933	14			

Appendix 16: Activities of Laccase Enzyme Produced by the Selected Rhizosphere Fungi

Conc		asemoA	asemoB	asemoC	asemoD	asemoE	asemoF	asemoG	asemoH	asemoI	asemoJ	asemoK	asemoL	asemoM	asemoN	asemoO	asemoP
0	Mean	15.33	12.00	12.33	12.00	12.67	11.33	13.00	12.33	13.33	11.00	10.67	11.67	12.67	14.00	12.00	12.33
	S.D.	00.58	01.00	02.52	01.00	01.53	01.53	01.73	01.53	00.58	01.00	01.15	02.08	00.58	01.00	01.00	01.53
5	Mean	34.33	43.00	35.00	55.00	55.67	23.00	97.33	55.67	68.00	91.00	30.00	45.00	55.67	150.0	88.33	56.00
	S.D.	00.58	03.00	01.00	01.00	00.58	01.00	01.53	00.58	01.00	01.00	03.46	05.00	01.53	0.00	02.08	01.00
10	Mean	48.00	54.67	56.00	87.33	62.67	150.0	96.33	96.00	76.00	98.00	45.33	65.33	100.3	130.0	66.00	56.00
	S.D.	06.08	02.08	03.00	01.53	01.53	50.00	03.79	03.00	01.00	01.00	08.50	00.58	00.58	30.00	22.52	01.00
15	Mean	43.00	55.67	76.00	89.00	67.00	121.0	102.7	103.33	76.00	102.0	75.33	65.33	99.67	82.67	47.33	74.00
	S.D.	01.00	00.58	01.00	01.00	01.00	01.00	04.62	04.93	01.00	03.46	01.53	00.58	01.53	03.06	17.67	11.27
20	Mean	70.00	77.67	76.33	101.3	68.00	182.3	63.33	46.33	77.00	47.00	77.00	72.67	168.0	64.00	45.00	65.00
	S.D.	01.00	02.08	04.51	01.53	01.00	02.52	02.52	01.53	01.00	02.65	01.00	03.79	01.00	02.65	02.34	03.23
Tot	Mean	42.13	48.60	51.13	68.93	53.20	97.53	74.53	62.73	62.07	69.80	47.67	52.00	87.27	88.13	52.77	50.77
al	S.D.	18.62	22.27	25.64	33.48	21.48	73.42	35.04	34.76	25.46	36.66	26.88	23.09	53.61	51.34	30.42	23.59

S.D. = Standard Deviation

Appendix 17: The Effect pollutants on the Laccase Activities among the Rhizosphere Fungi

ANOVA

		Sum of Squares	Df.	Mean Square	F.	Sig.
asemoA * Conc	Between the Groups	4772.400	4	1193.100	150.391	.000
	Within the Groups	79.333	10	7.933		
	Total	4851.733	14			
asemoB * Conc	Between the Groups	6907.600	4	1726.900	454.447	.000
	Within the Groups	38.000	10	3.800		
	Total	6945.600	14			
asemoC * Conc	Between the Groups	9128.400	4	2282.100	302.934	.000
	Within the Groups	75.333	10	7.533		
	Total	9203.733	14			
asemoD * Conc	Between the Groups	15679.600	4	3919.900	2556.457	.000
	Within the Groups	15.333	10	1.533		
	Total	15694.933	14			
asemoE * Conc	Between the Groups	6444.400	4	1611.100	1150.786	.000
	Within the Groups	14.000	10	1.400		
	Total	6458.400	14			
asemoF * Conc	Between the Groups	70440.400	4	17610.100	35.071	.000
	Within the Groups	5021.333	10	502.133		
	Total	75461.733	14			
asemoG * Conc	Between the Groups	17095.067	4	4273.767	451.454	.000
	Within the Groups	94.667	10	9.467		
	Total	17189.733	14			
asemoH * Conc	Between the Groups	16842.267	4	4210.567	549.204	.000
	Within the Groups	76.667	10	7.667		
	Total	16918.933	14			
asemoI * Conc	Between the Groups	9064.267	4	2266.067	2614.692	.000
	Within the Groups	8.667	10	.867		
	Total	9072.933	14			
asemoJ * Conc	Between the Groups	18776.400	4	4694.100	1066.841	.000
	Within the Groups	44.000	10	4.400		
	Total	18820.400	14			
asemoK * Conc	Between the Groups	9937.333	4	2484.333	139.569	.000
	Within the Groups	178.000	10	17.800		
	Total	10115.333	14			
asemoL * Conc	Between the Groups	7375.333	4	1843.833	207.951	.000
	Within the Groups	88.667	10	8.867		
	Total	7464.000	14			
asemoM * Conc	Between the Groups	40218.267	4	10054.567	7937.816	.000
	Within the Groups	12.667	10	1.267		
	Total	40230.933	14			
asemoN * Conc	Between the Groups	35065.067	4	8766.267	47.781	.000
	Within the Groups	1834.667	10	183.467		
	Total	36899.733	14			
asemoO * Conc	Between the Groups	9454.974	4	2363.744	11.465	.002
	Within the Groups	1649.333	8	206.167		
	Total	11104.308	12			
asemoP * Conc	Between the Groups	6417.641	4	1604.410	48.865	.000
	Within the Groups	262.667	8	32.833		
	Total	6680.308	12			

Appendix 18: Published Research Paper 1

Journal of Environmental Management 200 (2017) 253–262

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Mediational influence of spent mushroom compost on phytoremediation of black-oil hydrocarbon polluted soil and response of *Megathyrsus maximus* Jacq

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ABSTRACT

Ability of a plant to develop different adaptive strategies can also determine its capability for effective soil remediation. In this study, influence of spent mushroom compost (SMC) was tested on the phytoremediation of black oil hydrocarbon polluted soil and the response of *Megathyrsus maximus* (guinea grass). Studies were carried out in microcosm conditions by mixing different concentration of SMC viz., 10, 20, 30 and 40% in a 5 kg of contaminated soil along with control. Seeds of *M. maximus* was sown in tray for two weeks and allowed to grow for height of 10 cm and transplanted in to the different experimental pots. Soil nutrient, heavy metal and PAH contents were analyzed before and after the experiment. Ecophysiological and anatomical responses due to the contaminants in the soil by *M. Maximus* were analyzed after 120 days. Phytomass efficiency, potential photosynthesis (Amax) and contents of chlorophylls (a and b) as well as the total chlorophyll along with anatomical evaluations were recorded. Plant alone (control) reduced the soil heavy metal and PAH contents but further improvements were observed in SMC treatments, similar results were also observed as regards to the plant's phytoremediation efficiency (PE), phytomass and potential photosynthetic rates (m mol O_2 $M^{-2}S^{-1}$). The plant's root and shoot anatomical responses were enhanced in treatments compared to control, study infers that the treatment enhances the biostimulation and development of adaptive characteristics for *M. maximus* survival in contaminated soils and promotes its co-degradation of hydrocarbon. SMC supports remediation and as well enhances the anatomical evaluations, we therefore recommend the use of SMC on response of Megathyrsus maximus Jacq for remediation of petrochemical based phytoremediation.

1. Introduction

Plant's response in terms of physiological/anatomical structures, phytomass or yielding, nutrient uptake, and plant's response to stresses during phytoremediation are of immense importance. It is therefore very important to enhance the nutrient uptake efficiency, chemical or metallic stress, salt or drought tolerance in plants used for phytoremediation of polluted soils or in those cultivated on hydrocarbon contaminated land (Weyens et al., 2009a,b). Polluted soils are unsuitable for agricultural practice due to toxic material that can get accumulated in the plant or animal tissue, most of the soil pollutant are 'biogenetic' in nature as they may become incorporated into the food chain, such pollutant needs to be degraded, converted or transformed in any soil to be used for massive biomass production to avert any health effect on humans. More than 250 000 sites are documented to be polluted and requires immediate remedial action in the 15 EU countries and there might be up to 3 million of such sites undocumented (Bardos et al., 2011), these sites can be useful especially for crop production if well remediated. Many bioremediation methods have been suggested for soil clean part of which is the use of plants called 'Phytoremediation'.

Plant's degradation of PAHs usually follows the pathways explained by Cerniglia et al. (1984, 1992), they are oxidized

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0301-4797/

Appendix 19: Published Research Paper 2

Chemosphere 187 (2017) 1–10

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Chemosphere

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Synergistic action of rhizospheric fungi with *Megathyrsus maximus* root speeds up hydrocarbon degradation kinetics in oil polluted soil

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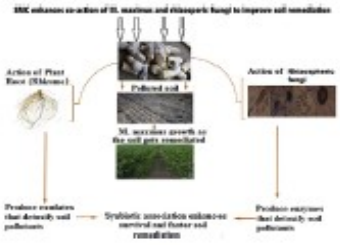
HIGHLIGHTS

- Combined action of fungi, SMC and *M. maximus* root speed-up soil remediation.
- The mechanism improved soil nutrient and reduced soil heavy metal concentrations.
- Up till 95.36% loss of total PAHs was observed at 30 and 40% fungi-SMC treatment.
- Degradation rate and half-life of PAHs in the studied polluted soil were enhanced.

GRAPHICAL ABSTRACT



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Rhizospheric fungi
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Synergistic bioremediation
Biological efficiencies
Biodegradation rate

ABSTRACT

This study was aimed at combining the potentials of plant and some rhizospheric fungal strains in remediation of crude-oil polluted soil. Four new rhizospheric fungi were identified from an aged crude-oil polluted site and used with *Megathyrsus maximus* (guinea grass) for a 90 day synergistic remediation experiment. Cultures of these strains were first mixed with spent mushroom compost (SMC), the mixture was then applied to a sterilized crude oil polluted soil at concentrations of 10%, 20%, 30% and 40% potted in three replicates. Soil with plant alone ($0\%_1$) and soil with fungi-SMC alone ($0\%_2$) served as controls. The soil's initial and final pH, nutrient, 16 EPA PAHs and heavy metal contents were determined, degradation rate, half-life and percentage loss of the total polyaromatic hydrocarbon (TPAH) were also calculated. Finally, the remediated soils were further screened for seed germination supporting index. The fungal strains were identified and registered at NCBI as *Aspergillus niger* asemoA (KY473958.1), *Talaromyces purpurogenus* asemoF (KY488463.1), *Trichoderma harzianum* asemoJ (KY488466.1) and *Aspergillus flavus* asemoM (KY488467.1). We observed for the first time that the synergistic mechanism improved the soil nutrient, reduced the heavy metal concentration and sped up hydrocarbon degradation rate. Using the initial and final concentrations of the TPAH, we recorded highest biodegradation rates (K_1) and half-life ($t_{1/2}$) in 30 and 40% treatments over controls, these treatments also had highest seed germination supporting index. This work suggests that the set-up synergistic remediation could be used to remediate crude oil polluted soil and this could be used in large scale.

1. Introduction

Soil pollution has direct effect on agricultural crops, it decreases food production both in terms of quality and quantity, it poses

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0045-6535/

Appendix 20: Published Research Paper 3

PLOS ONE

RESEARCH ARTICLE

Synergistic rhizosphere degradation of γ-hexachlorocyclohexane (lindane) through the combinatorial plant-fungal action

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OPEN ACCESS

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Data Availability Statement: All sequence and accession files are available from the NCBI database (accession number(s) KY693970, KY488464, KY488468, KY488469 and KY693973).

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Abstract

Fungi are usually involved in degradation/deterioration of many anthropogenic wastes due to their verse enzyme secretions and adaptive capabilities. In this study, five dominant fungal strains were isolated from an aged lindane polluted site, they were all mixed (100 mg each) together with pent mushroom compost (SMC) and applied to lindane polluted soil (5 kg) at 10, 20, 30, 40% and control 0% (soil with no treatment), these were used to grow *M. maximus* Jacq for 3 months. To establish lindane degradation, deductions such as Degradation rate (K_1), Half-life ($t_{1/2}$) and Degradation efficiency (DE) were made based on the analyzed lindane concentrations before and after the experiment. We also tested the presence and expressions of phosphoesterases (*mpd* and *opd-A*) and catechol 1,2-dioxygenases (*efk2* and *efk4*) genes in the strains. The stains were identified as *Aspergillus niger* (KY693970); *Talaromyces atroroseus* (KY488464), *Talaromyces purpurogenus* (KY488468), *Yarrowia lipolytica* (KY488469) and *Aspergillus flavus* (KY693973) through morphological and molecular methods. Combined rhizospheric action of *M. maximus* and fungi speed up lindane degradation rate, initially detected lindane concentration of 45 mg/kg was reduced to 11.26, 9.34 and 11.23 mg/kg in 20, 30 and 40% treatments respectively making 79.76, 85.93 and 88.67% degradation efficiencies. K_1 of 1.29 was recorded in control while higher K_1 of 1.60, 1.96 and 2.18 /day were recorded in 20, 30 and 40% treatments respectively. The best $t_{1/2}$ of 0.32 and 0.35 /day were recorded in 40 and 30% compared to control (0.54 /day). All the strains were also affirmed to possess the tested genes; *opd* was overexpressed in all the strains except KY693973 while *mpd* was overexpressed in KY693970, KY488464 but moderately expressed in KY488468, KY488469 and KY693973. However, *efk* genes were under-expressed in most of the strains except KY488469 and KY693973 which showed moderate expression of *efk4*. This work suggests that the synergistic association of the identified rhizospheric fungi and *M. maximus* roots could be used to remove lindane in soil at a limited time period and this combination could be used at large scale.

Appendix 21: Published Research Paper 4

Environmental Pollution 235 (2018) 55–64

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Transcriptomic responses of catalase, peroxidase and laccase encoding genes and enzymatic activities of oil spill inhabiting rhizospheric fungal strains☆

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ABSTRACT

Fungi are well associated with the degradation of hydrocarbons by the production of different enzymes, among which catalases (CBH), laccases (LCC) and peroxidases (LiP and MnP) are of immense importance. In this study, crude oil tolerance and enzyme secretions were demonstrated by rhizospheric fungal strains. Four most abundant strains were isolated from the rhizosphere of grasses growing in aged oil spill sites and identified through morphological characterization and molecular PCR-amplification of 5.8 –28S ribosomal rRNA using ITS1 and ITS4 primers. These strains were subjected to crude oil tolerance test at 0–20% concentrations. Presence and transcriptase responses of putative genes *lig* (*1–6*), *mnp*, *cbh* (*I.I, I.I and II*), and *lcc* encoding lignin peroxidase, manganese peroxidase, catalase, and laccase enzymes respectively were also studied in these strains using RT-PCR. In addition, activities of secreted enzymes by each strain were studied in aliquots. The strains were identified as *Aspergillus niger* asemoA (KY473958), *Talaromyces purpurogenus* asemoF (KY488463), *Trichoderma harzianum* asemoJ (KY488466), and *Aspergillus flavus* asemoM (KY488467) through sequencing and comparing the sequences' data at NCBI BLAST search software. All the isolated strains showed tolerance to crude oil at 20% concentration, but the growth rate reduced with increasing in oil concentrations. All the isolated strains possess the tested genes and *lig 1–6* gene was overexpressed in *A. niger* and *T. harzianum* while *lcc* and *mnp* genes were moderately expressed in all the four strains. Almost 145 $U.mL^{-1}$ of lignin and manganese peroxidase, 87 $U.mL^{-1}$ of catalase, and 180 $U.mL^{-1}$ of laccase enzymes were produced by these strains and it was also observed that these strain mostly produced studied enzymes in response to increasing crude oil concentrations. Considering the robust nature and diverse production of these catalytic enzymes by these strains, they can be exploited for various bioremediation technologies as well as other biotechnological applications.

1. Introduction

Crude oil usually contaminates soils with multiple aliphatic and aromatic hydrocarbons; most of these contaminants form residues that have pose-adverse effect upon human health (Prince, 1993; Wang et al., 1998; US EPA, 2012). The major crude oil fractions are the alkanes (>50%) including linear (n-alkanes), cyclic (such as cycloalkanes) or branched isoalkanes and they exist in the form of solid, liquid and gas (Asemoloye et al., 2017a). In polluted environments, crude oil pollution does not only affect plant and human alone but also the soil microbial population dynamics. However, many soil microorganisms have been reported to develop different survival strategies in oil contaminated environment through the production of several enzymes through modifications in their genetic and metabolic pathways for crude oil degradation and/or mineralization. In these two mechanisms, they convert and utilize hydrocarbons as a sole carbon sources and had been widely studied by many scientists. Microbial degradation and/or mineralization mechanisms form the bedrock for microbial 'Bioremediation'

☆ This paper has been recommended for acceptance by Baoshan Xing.
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Appendix 22: Published Research Paper 5

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Chemosphere

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Degradation of 2, 2-Dichlorovinyl dimethyl phosphate (dichlorvos) through the rhizosphere interaction between *Panicum maximum* Jacq and some selected fungi

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HIGHLIGHTS

- Five dominantly fungal strains were selected from a pesticide's contaminated soil.
- They showed presence and expressions of *opd-A*, *mpd*, *afk1* and *afk2* genes.
- Their synergistic action with SMC and root sped up degradation of dichlorvos.
- The synergistic treatment also gave the best k_1 and $t_{1/2}$ for dichlorvos in soil.
- Pesticide polluted soils can be treated with synergistic plant-fungi-SMC interaction.

GRAPHICAL ABSTRACT

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Dichlorvos
Gene expression
Degradation rate

ABSTRACT

Many fungi have been reported to enhance the plant responses and degradation of several persistent pollutants in soils. In this study, five dominant fungi strains were identified from a pesticides polluted soil in Nigeria and screened for the expression of phosphoesterase (*opd* and *mpd*) and catechol 1, 2-dioxygenase (*afk2* and *afk4*) genes using Reverse Transcriptase-PCR technique. Their rhizosphere interaction with plant (*Panicum maximum*) was further studied for the degradation of 2, 2 Dichlorovinyl dimethyl phosphate (dichlorvos). Fungal strains were mixed with Spent Mushroom Compost (SMC) of *Pleurotus ostreatus* in 1:100 w/w and then applied to a sterilized pesticide polluted soil (5 kg) at increasing concentrations of 10, 20, 30 and 40% with two controls (plant only and fungi-SMC mixture only). Degradation efficiency (DE), degradation rate (K_1) and half-life ($t_{1/2}$) of dichlorvos was calculated in each treatment after 90-day of planting. All the strains were registered at NCBI gene-bank with accession numbers KY693969, KY488464, KY488465, KY693971 and KY693972; they all possess the tested genes although *mpd* and *opd* were over-expressed in all the strains while *afk2* and *afk4* were moderately expressed. The plant-fungi-SMC interaction synergistically sped-up dichlorvos degradation rate in less time period, appreciable loss of dichlorvos at 72.23 and 82.70% DE were observed in 30 and 40% treatments respectively as compared to controls 1 and 2 having 62.20 ± 3.07 and 62.33 ± 4.69% DE respectively. In the same way, the 40% treatment gave the best k_1 and $t_{1/2}$ of 1.755 and 0.40 ± 0.02/day respectively.

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Appendix 23: Published Research Paper 6

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
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Taylor & Francis
Taylor & Francis Group

REVIEW PAPER

Synergistic plant-microbes interactions in the rhizosphere: a potential headway for the remediation of hydrocarbon polluted soils

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ABSTRACT

Soil pollution is an unavoidable evil; many crude-oil exploring communities have been identified to be the most ecologically impacted regions around the world due to hydrocarbon pollution and their concurrent health risks. Several clean-up technologies have been reported on the removal of hydrocarbons in polluted soils but most of them are either very expensive, require the integration of advanced mechanization and/or cannot be implemented in small scale. However, "Bioremediation" has been reported as an efficient, cost-effective and environment-friendly technology for clean-up of hydrocarbon"s contaminated soils. Here, we suggest the implementation of synergistic mechanism of bioremediation such as the use of rhizosphere mechanism which involves the actions of plant and microorganisms, which involves the exploitation of plant and microorganisms for effective and speedy remediation of hydrocarbon"s contaminated soils. In this mechanism, plant"s action is synergized with the soil microorganisms through the root rhizosphere to promote soil remediation. The microorganisms benefit from the root metabolites (exudates) and the plant in turn benefits from the microbial recycling/solubilizing of mineral nutrients. Harnessing the abilities of plants and microorganisms is a potential headway for cost-effective clean-up of hydrocarbon"s polluted sites; such technology could be very important in countries with great oil producing activities/records over many years but still developing.

KEYWORDS

Hydrocarbon pollution; health risks; synergistic mechanism

Introduction

The world's population is increasing yearly particularly in developing countries, and this requires an increment in agricultural produce, this means that agricultural food production must correspond with the pace at which the population is growing, adequate management of soil and water resources is therefore needed for optimal and sustainable agricultural practices. However, the need for mass crop production is greatly affected by oil contamination especially in almost all oil producing host communities, especially in the developing countries. Attention is shifted on the oil exploration and up until date pollution problems associated with oil exploration in many of these countries have not been adequately managed. Larger percentage of people living in oil-producing countries in Africa depend on exploitation of naturally available materials, agriculture, fishing and forest products for food to improve their livelihood, but today the fundamental development and wellbeing of these people have been highly affected due to pollution associated with crude oil exploration (Asemoloye et al. 2017a).

The concentration of toxic substances, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals in soils is increasing due to incidents, such as oil spillage due to pipeline sabotage, accidental discharges from oil wells/rigs, oil bunkering, and tankers, indiscriminate discharge of raw sewage from petrochemical manufacturing industries, use of different types of automobiles, machines, industrial engines contaminate soils and these are causing alterations in natural environment and ecosystem. Hydrocarbon contaminants in soil affect the soil microflora and bioavailability and alter the natural equilibrium between the living organisms and the natural environment biota (Odu 1972; Snowden and Ekweozor 1987; Amadi et al. 1996). This is also unsafe for crop production, for example, PAHs may become integrated into the food cycle/web of an ecosystem as they can become accumulated in plants during cultivation or contaminate underground water or fresh water, this threatens the food security as most of these toxic compounds are known to be carcinogenic, neurogenic and mutagenic (Asemoloye et al. 2017b).

Considering the wealth of huge income recovered from oil exploration, it cannot be advocated to be shut down but adequate control measures must be followed to avoid health risks associated with oil pollution. Several remediation methods have been suggested for clean-up of hydrocarbon pollutants, these include chemical, physical and mechanical

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bijp

Appendix 24: Published Research Paper 7

In: Soil Contamination
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Chapter 5

CLEAN-BIOTECHNOLOGY WITH ENHANCED PLANT-MICROBIAL SYNERGISTIC ACTION: A NOVEL AND FUNCTIONAL REMEDIATION MECHANISM FOR PESTICIDE POLLUTED SOILS

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