EVALUATION OF HYDROCARBON REMEDIATING POTENTIALS OF AUTOCHTHONOUS MICROORGANISMS AND SOME TROPICAL PLANTS GROWING IN THE VICINITY OF AUTOMOBILE WORKSHOPS IN NIGER STATE

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ABSTRACT

This study was conducted to evaluate the hydrocarbon remediating potentials of some tropical plants growing in the vicinity of automobile workshops (AW) in parts of Niger State, Nigeria. This involved a preliminary survey to major AW in Minna, Bida, Suleja and Tegina in Niger State to identify spent engine oil (SEO) tolerant plants growing in the vicinity of the AW. The microorganisms in the rhizosphere and non- rhizhosphere were enumerated using the spread-plate method on standard media and were identified using morphological, biochemical and molecular techniques. Besides, the microorganisms were screened for SEO utilization using spectrophotometric analysis. The physicochemical properties of the SEO polluted soil were determined using standard methods. The bioremediation potential of *Melissia officinalis* (Lemon balm) and *Urena lobata* (Caesar weed) were assessed by monitoring the Total Petroleum Hydrocarbon, heavy metal content, GCMS analysis of residual oil and the generation of plant exudates during the phytoremediation process. The experimental setup was a complete randomized design (CRD) conducted at the biological garden of the Federal University of Technology, Minna. The results of the total aerobic heterotrophic bacterial counts in SEO contaminated soil within the various workshops showed that the counts were generally low when compared to the counts from both rhizosphere and non-rhizosphere soils in the AW vicinity. The mean bacterial counts of the AW were more in Tegina ($2.37\times10^6\pm4.01\times10^6$ cfu/g), while Bida had the lowest bacterial counts ($1.56\times10^5\pm2.13\times10^5$cfu/g) when compared to Minna ($2.03\times10^6\pm3.44\times10^6$ cfu/g) and Suleja ($1.36\times10^6\pm2.28\times10^6$cfu/g). However, statistical analysis revealed that there were no significant differences ($p>0.05$) in bacterial counts among the stations. The total fungal counts of soil within the AW were low as compared to the rhizosphere and the non-rhizosphere soils, with Bida having the highest counts ($1.36\times10^6\pm2.26\times10^6$ cfu/g) while Tegina and Minna had the lowest counts ($3.41\times10^5\pm5.71\times10^5$ cfu/g). Statistical analysis showed that there were no significant differences ($p>0.05$) across the locations. The SEO utilizing bacterial counts were low. The mean counts ranged from $2.32\times10^3\pm5.17\times10^3$ cfu/g to $1.44\times10^3\pm5.17\times10^3$ cfu/g while the SEO utilizing fungal counts ranged from $9.01\times10^2\pm5.17\times10^2$ cfu/g to $1.02\times10^1\pm5.17\times10^1$ cfu/g. Statistical analysis showed significant differences ($p<0.05$) among the locations. Microorganisms isolated in the study were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Penicillium notatum* and *Fusarium oxysporium.* Species of *Bacillus, Staphylococcus* and *Aspergillus* were more consistently isolated and constituted 9.16-9.83%. *Bacillus subtilis* strain EE681738 and *Aspergillus niger* strain HQ659967 were the most efficient spent engine oil utilizers. The residual total petroleum hydrocarbon in *M. officinalis* treated soil was 56.61%, while it was 50.55% in *U. lobata* treated soil after seven months. The physicochemical properties of SEO polluted soil remediated with *M. officinalis* and *U. lobata* revealed that after seven months of bioremediation, nitrogen, organic matter, potassium and phosporus contents gradually increased. GCMS analysis of the hydrocarbons revealed that 9H-Fluorene, Diphenylacetylene, Tritriacontane, Nonahexacontane, Hentriacontantane, Octatetracontane, IH-Pyrrolo[2,3-b] quinoxalin, Ethanone, Eicosylisobutyl ether, Inolin-2-yl, Carbonic acid were completely degraded while the following compounds, Phenyleamine, Pyrimidine, Carbonic acid, Coumarin, Acetic acid, Naphthalene, 4-Chlorophenoxy were found in the exudates secreted by *M. officinalis* and *U. lobata.* The results obtained suggest that plants found within the vicinity of AW particularly, *M. officinalis* and *U. lobata* are good candidates for phytoremediation of SEO polluted soil in the tropics.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Spent engine oil (SEO) is a common and toxic environmental contaminant not naturally found in the environment but large amounts are disposed into the environment when motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by automobile and generator mechanics (Akpabio*et al.*, 2017). The presence of oil and refined petroleum products in the soil can lead to toxic effects on plants and soil microorganisms and acts as a source of ground water contamination (Zand*et al.*, 2016, Aransiola*et al.*, 2021).Spent engine oil creates an unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH (Ugoh and Moneke, 2011, Oyewole*et al.*, 2021). It has been shown that marked changes occur in soil contaminated with hydrocarbons and these changes affect the physical, chemical and microbiological properties of the soil (Otobong and Victoria, 2017, Oyewole*et al.*, 2021).

Spent engine oil (SEO) is typically referred to as used motor oil that has been collected from mechanical workshops, garages, and industry sources such as hydraulic oil, turbine oils, process oil and metal working fluids. Spent oil can also originate at seaports from ocean-going vessels which can contain salty sea water, heavy and intermediate fuel oil along with various heavy metals common to such fuel oil (Akpabio*et al.*, 2017, Abioye*et al.*, 2021a).

Contamination of existing and potential agricultural lands is a major problem associated with the processing and distribution of crude and refined petroleum products in many oil producing countries (Isah, 2013; Musa *et al.,* 2019). The problems of pollution have led to the exploration of many remedial approaches to effect the cleanup of the polluted soils. Pollution control strategies involving physicochemical methods have often aggravated the problem rather than eliminate it. Bioremediation is being favored as a good option for the remediation of polluted sites mainly because it uses inexpensive equipment, environmentally friendly and simple. Phytoremediation is one of the forms of bioremediation (Oyewole*et al.,* 2019).

Phytoremediation (Greek: phyton = plant; Latin: remediare = remedy) is emerging 'green bioengineering technology' for environmental cleanup that uses plants to remove pollutants from the soil or to render them harmless. It takes advantage of the natural abilities of plants to take up, bioaccumulate, store or degrade organic or inorganic substances. They are cost- effective, aesthetically pleasing, passive, solar-energy driven and pollution abating nature's biotechnology meeting the same objectives of fossil-fuel driven and polluting conventional technology (Osuagwu*et al.,* 2017; Abioye*et al.,* 2020). Plants involved in phytoremediation are adapted to thrive in very harsh environmental conditions of soil and water; absorb, tolerate, transfer, assimilate, degrade and stabilize highly toxic materials (heavy metals, radionuclides and organics such as solvents, crude oil, pesticides, explosives, chlorinated compounds and polyaromatic hydrocarbons) from the polluted soil and water. The organic pollutants may ideally be degraded to simpler compounds like carbon dioxide (CO_2) and water (H_2O), thus reducing the environmental toxicity significantly (Shahida *et al.,* 2015; Tripathi *et al.,* 2016; Akpabio *et al.,* 2017; Xu *et al.,* 2018; Aransiola*et al.,* 2021).

Several plants have also been described to have phytoremediation potentials to clean up petroleum pollutants. Some plants aid in degradation indirectly by supporting microbial population, other plants take up inorganic contaminants from soil and concentrate them in plant tissues or roots to become hyperaccumulators. Therefore phytoremediation employs human initiative to enhance the natural attenuation of contaminated sites and is a process that is intermediate between engineering and natural attenuation. Pollution effects of automobile village activities in Nigeria have received limited attention even though these activities have been shown to produce petroleum based wastes. Therefore, this research focused on the evaluation of hydrocarbon remediating potential of tropical plants thriving in the vicinity of automobile workshops in parts of Niger State, Nigeria.

1.2 Statement of the Research Problem

Spent engine oil can enter the environment through improper disposal by automobile repairers when servicing cars. The increase in the number of vehicles in Nigeria has necessitated a higher production and use of engine oil (EO). This has subsequently given rise to the generation of large quantities of spent engine oil (SEO) at the time of servicing the vehicles. This SEO is considered as ordinary waste by majority of workers of the automobile workshops in Nigeria, who dispose this off on soil surface (Otobong and Victoria, 2017). Spent engine oil management in Nigeria is not well supervised hence the indiscriminate discharge into the soil drains and sometimes open water. This has attendant implications on soil and water quality, contamination on soil ecosystem alters soil biochemistry, immobilizes nutrients and creates oxygen tension (Akpabio*et al.*, 2017). Spent engine oil contains impurities in the course of usage and handling; toxic and harmful substances such as benzene, lead, cadmium, polycyclic aromatic hydrocarbons (PAHs), zinc, arsenic, polychlorinated biphenyls (PCBs) etc. which are

hazardous and detrimental to the soil and the surrounding environment (Nwachukwu *et al.*, 2010; Adu*et al.*, 2015).

With increasing number of automobile workshops in Niger State, and many more under construction, and the increasing number of vehicles being serviced or repaired at the automobile workshops, it can therefore be established that the amount of SEO from vehicles in Niger State is on a steady increase (Badamasi*et al*., 2020). In recent time government of some North Central States, including Niger State is evacuating automobile workshops from some areas to make room for the sighting of sporting centers and more lucrative ventures. The problem is the restoration of such sites and thiscall for immediate action.

1.3 Aim and Objectives of the Study

The aim of this study was to evaluate hydrocarbon remediating potentials of some tropical plants thriving in the vicinity of automobile workshops in parts of Niger State.

The objectives of the study were to:

i) enumerate and identify some major tropical plants found in the vicinity of automobile workshops in parts of Niger State.
ii) enumerate and identify the microorganisms found in the rhizosphere and soil in the vicinity of automobile workshops.
iii) screen the microbial isolates for potential to utilize spent engine oil.
iv) determine the total petroleum hydrocarbon (TPH), microbiological, physical and chemical properties of spent engine oil contaminated soil.
v) determine the bioremediation ability of the plants in spent engine oil (SEO) polluted soil.

vi) identify the exudates in the rhizosphere of the plants during the phytoremediation process.

1.4 Justification for the Study

Most mechanical methods, such as incineration or burial in secured landfills to reduce hydrocarbon pollution are expensive and time consuming. These are effective treatments but after burning, the soil gets depleted of nutritents and structure. These methods do not remove the contamination but only relocate the problem, therefore, it is of importance to find efficient, affordable and more environment- friendly method of spent engine oil treatment especially in developing countries (Nooraldeen, 2018). The use of chemicals is a very fast method of soil remediation but, the costs needed for the implementation of this method are usually too high and the chemicals may react with other beneficial soil components (Ijah and Antai, 2003). Phytoremediation is therefore, quite suitable as a remedial action.

Phytoremediation is cost effective, environmental friendly, beautifies the environment and it often does not leave residual after clean up. Several plants have phytoremediation ability, but for this study, tropical plants from the vicinity of the automobile workshop were used. For the plants (*Urena lobata* and *Melissa officinalis*) to have survived in the vicinity of the automobile workshops suggest that they have the ability to carry-out phytoremediation. At the end of this research,tropical plants with remediation ability would have been identifiedthat can be used for phytoremediation of SEO polluted soil.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1 Definition and Composition of Spent Engine Oil

Engine oil can simply be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine (Shahida *et al.*, 2015). Spent engine oil as the name implies represents oil that has undergone destructive changes in the property when subjected to oxygen, combustion gases, and high temperature. The spent engine oil also undergoes viscosity changes as well as additive depletion and oxidation (Shahida *et al.*, 2015).

Engine oil is the very stable, non-volatile and smallest fraction of crude petroleum. As petroleum products are essentially composed of hydrocarbons, engine oils have hydrocarbon structures, containing from 20 to 70 carbon atoms per molecule. The engine oil molecules can be divided into three broad groupings, that is, paraffinic, naphthenic and aromatic. Paraffinic molecules are predominantly straight chains, tend to be waxy, have a high pour point, good viscosity and better temperature stability (Nooraldeen, 2018). Naphthenic molecules are straight chains with a high proportion of five and to a lesser extent six membered ring structures. They tend to have a low pour point. For this reason, they are used as refrigeration oils. Aromatics are straight chains with six membered ring benzene structures. In practice, no sharp distinction exists between these various groupings as many engine oil molecules are a combination, to varying degrees, of the different types of hydrocarbons (Motshumi*et al.*, 2013). Spent engine oils contain high percentage of aromatic and aliphatic hydrocarbons, nitrogen,

sulphur compounds, and metals (Zn, Pb, Cr and Fe) than fresh oils, some of these metals in used engine oil can dissolve in water and move through the soil easily and

may be found in surface water and groundwater (Mohd *et al.*, 2011; Abdulsalam *et al.*, 2012).

2.2 Sources of Spent Engine Oil to the Soil

The disposal of spent engine oil (SEO) into gutters, water drains, open vacant plots, and farms is a common practice in Nigeria especially by automobile repairers. This oil also called spent lubricant or waste engine oil is usually obtained after servicing and subsequently draining from automobile and generator engines (Abdulsalam *et al.*, 2012) and much of this oil is poured into the soil. It is a common and toxic environmental contaminant not naturally found in the environment (Adu*et al.*, 2015). Spent oil is produced when new mineral-based crankcase is subjected to high temperature and high mechanical strain (Godheja*et al.*, 2016). It is a mixture of different chemicals (Abioye, 2011) including petroleum hydrocarbons, chlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products and heavy metals that come from engine parts as they wear away (Agbogidi, 2010). After undergoing several production processes, additives are usually incorporated to boost some of the oil properties like viscosity, thermal and oxidation stability, etc (Dauda and Obi, 2000).

The spent engine oil gets to the environment due to discharge by motor and generator mechanics (Dike*et al.*, 2013) and from the exhaust system used and due to engine leaks (Agbogidi, 2010). The contamination of the natural environment by petroleum- derived substances contributes to the degradation of land (Das*et al.*, 2011; Panda*et.al.*,2017). Oxidation of lubricating oil hydrocarbons at the point of application is accompanied by release of free radicals that transform to peroxides, subsequent condensation and polymerization of which produce per acids, naphthenic acids, etc (Ladke and

Choudhari,2016). Nevertheless, this is dependent on the local environmental conditions and on the kind of soil constituents present in the soil-water system.

2.3 Uses of Engine Oil

Lubricating oils such as engine oils, gear, hydraulic oils, turbine oils, etc., are used to reduce friction between moving surfaces. They also serve to remove heat from working parts in machinery created by moving surfaces and provide a protective layer on the metal surfaces to avoid corrosion (Nooraldeen, 2018). They act as a sealant to fill the microscopic ridges and valleys in any metal surfaces to increase the machinery efficiency. In addition, they serve as a cleaning agent to carry away dirt or other debris that may damage the bearings or other parts that are operated in tight tolerance. Debris is removed through the engine oil filter or the transmission filter (Songita*et al.*, 2018). Engine oils are usually blended with a number of chemical additives to provide products that last longer and allow the machinery to work better under severe operating conditions.

2.4Effects ofSpent Engine Oil

Due to the differences in the composition of the different petroleum products, the petroleum products affect the environment in different manners. For instance, Wyszkowski and Ziolkowska(2008) reported that petrol and diesel oil affected the organic carbon and mineral components in soils at different rates. This means the growth and development of organisms depending on such soils can be affected at different rates by petroleum products. Germination of *Amaranthus hybridus* seeds were significantly affected in spent engine oil polluted soil (Odjegba and Sadiq, 2002). Agbogidi and Nweke (2005) and Agbogidi*et al.* (2006) reported that crude oil application to soil significantly reduced growth of Okro and five cultivars of Soy beans

respectively. Daniel-Kalio and Pepple (2006) reported a significant higher plant height, leaf area and dry weight of *Comelinabegalensis* (day flower) at 0 mg g^{-1} oil pollution than at 50 mg g^{-1} pollution level. Similarly, Ibmesin (2010) reported that vegetative cutting of Paspalum (Sour grass) grew well in the absence of oil and salinity and that 75% of the test plants survived in low oiling but heavy oiling resulted in mortality.

Hydrocarbon contamination of the air, soil, and freshwater especially by PAHs attracts public attention because many PAHs are toxic, mutagenic, and carcinogenic (Van Hamme,*et al*., 2003). Prolonged exposure to high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow, and an increased risk of cancer (Panda *et al.*, 2017). In addition, PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment (Van Hamme, 2003). The illegal dumping of used motor oil is an environmental hazard with global ramifications (Abioye*et al.*, 2012). Spent egine oil contains heavy metals and polycyclic aromatic hydrocarbons (PAHs) that could contribute to chronic hazards including mutagenicity and carcinogenicity (Owolabi *et al.*, 2013).

The contaminants in spent engine oil have adverse environmental and health impacts. The presence of degraded additives, contaminants, and by-products of degradation render waste oils more toxic and harmful to health and environment than virgin base oils. If put into storm water drains or sewers, they can affect waterways and coastal waters. When dumped in soil or sent to landfill, they can migrate into ground and surface waters through numerous land treatment processes. In addition, uncontrolled used oils are a threat to plant and animal life, which can further result in economic losses(i.e. recreation and fishing industries). For example, used oil from internal

combustion engines generally carries a variety of contaminants which increase the oil's toxicity (Yu-Lung and Chun-Chu, 2010).

Improper application of spent engine oil for multiple customary purposes also leads to various environmental degradation and health effects. When spent engine oil enters surface water, oil films will block sunlight, impair photosynthesis, and prevent the replenishment of dissolved oxygen, which lead to the death of aquatic plants and animals (Jadhav *et al.,* 2017). When spent engine oil is dumped down the drain and enters a sewage treatment plant, very small concentrations of oil in the wastewater (50 to 100 ppm) can foul sewage treatment processes (Dalla *et al.*, 2012).

Spent engine oil that is dumped onto soil can be washed into surface water by rain, or it can seep through the soil into groundwater to contaminate water sources. Spent engine oil in the soil can also evaporate into the air,or cause great damage to soil and soil microflora. It creates an unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH (Ugoh and Moneke, 2011).

The effect of spent engine oil on soil mainly includes the following aspects: Firstly, because of the small density, higher viscosity and lower emulsifying ability of spent engine oil, it is easy to be absorbed in soil surface, affecting the permeability and porosity of soil (Wang*et al*., 2008 and Jadhav *et al.*, 2017). Petroleum is rich in carbon and a small amount of nitrogen compounds, so it can change the composition and structure of soil organic matter and impact the C/N, C/P, salinity, pH and conductivity of soil (Shan*et al.*, 2014 and Efsun*et al.*, 2015). Secondly, microorganisms in natural environment are quite abundant in healthy and clean soil. In normal situation the microorganisms which can resist the oil pollution stress are not developed, while in contaminated soil, in order to adapt to this kind of environment, they can produce

certain enzyme system and gradually form a dominant population with symbiotic or synergy effect (Chiara *et al.*, 2009 and Yaqoob *et al.*, 2019). A number of studies have shown that the hydrocarbon pollution can change the microbial population, the composition of the community structure and the enzyme system in soil, given priority to the inhibitory action (Du*et al.*, 2011). Thirdly, it can impede the normal growth of crops such as reduce the germination rate and fertility and decline the resistance to pests and diseases (Shan *et al.*, 2014). In addition, the oil compounds could react with inorganic nitrogen and phosphorus, limiting the nitrification andremoval of phosphoric acid, so the effective nitrogen and phosphorus in the soil would decrease and the absorption of crops will be affected (Liao *et al*, 2015; Musa *et al.*, 2019). Moreover, the polycyclic aromatic hydrocarbons in petroleum chemicals have carcinogenic, mutagenic, teratogenic and other toxic effects. It can enter into the bodies of people and animals through breathing, skin contact and diet, degrading the normal function of livers and kidney etc, therefore causing great threat to human's health. At last, the oil pollutants in the soil not only impact the pedosphere, but also the atmosphere and water sphere. To be specific, the low boiling point and light weight hydrocarbons can enter into the atmosphere by evaporation easily; then through runoff and infiltration into the surface water and osmosis into the groundwater system; and finally through the food chain enter into the human's bodies (Shan *et al.*, 2014).

2.5 Petroleum Hydrocarbons

Since the beginning of the last century, crude oil and gas had become indispensable resources for modern life as fuels and raw materials. Petroleum refining yield over 2500 products, including the common ones like LPG, gasoline, kerosene, aviation fuel, diesel fuel, fuel oils, lubricating oils, and raw materials for petrochemical industry (United States Environmental Protection Agency,USEPA, 2011). The abundance

and multipurpose nature of oil and gas facilitated the unprecedented economic growth around the world and improvement in human health (Yaqoob *et al*., 2019).The majority of the compounds present in crude oils are hydrocarbons which exist as gases, liquids, and solids. Hydrocarbons present in crude petroleum could reach up to 97% by weight (e.g., in the lighter paraffinic crude oils) or ≤ 50% by weight as in heavy asphaltic crude oils (Speight, 2006). However, crude oils containing as little as 50% of hydrocarbon components still retain most of the essential characteristics of the hydrocarbons. The hydrocarbon present in crude oil is grouped into saturated hydrocarbons, unsaturated hydrocarbons, and aromatics (Pandey and Bajpai, 2019). In general, crude oils contain the classes of hydrocarbons shown in Table 2.1. Other organic compounds containing Sulphur (hydrogen sulfide, mercaptans, etc.), Nitrogen (quinotine, pyridine, pyrrole, indole, carbazole), and Oxygen (naphthenic acids, phenols, some other organic acids) are found in varying proportions among petroleum from different sources. Their presence in most instances is undesirable due to problems associated with refining, storage, and consumption of the products. For example, compounds of Sulphur, Nitrogen, and Oxygen cause foul odor, color alteration of refined products, and corrosion of oil facility respectively (Speight, 2006). Trace petroleum constituents are metallic derivatives and porphyrins.

2.5.1 Microbial degradation of petroleum hydrocarbons

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) (Chakravarty *et al*., 2017). Different factors influencing hydrocarbon degradation have been reported by Oyewole*et al*., 2021. One of the

important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded (Ameh*et al*., 2019). Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes (Correa-Garcia *et al*., 2016). Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all (Bertrand, 2020). Microbial degradation is the major and ultimate natural mechanism by which one can cleanup the petroleum hydrocarbon pollutants from the environment (Bertrand, 2020). The recognition of biodegraded petroleum-derived aromatic hydrocarbons in marine sediments was reported by Correa-Garcia *et al*., 2016. They studied the extensive biodegradation of alkyl aromatics in marine sediments which occurred prior to detectable biodegradation of n-alkane profile of the crude oil and the microorganisms, namely, *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas,* and*Rhodococcus*were found to be involved for alkylaromatic degradation. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria was reported by Ameh*et al*. (2019). Nine bacterial strains, namely, *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus,* and *Corynebacterium sp*. were isolated from the polluted stream which could degrade crude oil. Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. The reported efficiency of biodegradation ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria, and 0.003% to 100% (Asiabadi*et al*., 2018) for marine bacteria. Many scientists reported that mixed populations with overall broad enzymatic capacities are

required to degrade complex mixtures of hydrocarbons such as crude oil in soil (Ismaila*et al*., 2019), fresh water, and marine environments. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment (Ladoja*et al*., 2019). Several bacteria are even known to feed exclusively on hydrocarbons (Asiabadi*et al*., 2018). Bacterial genera, namely, Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia, and Mycobacterium isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation (Asiabadi*et al*., 2018). The degradation of polyaromatic hydrocarbons by *Sphingomonas* was reported by Ali and Khadijeh, 2019.

Table 2.1: Hydrocarbon compounds present in petroleum mixture

Group	Hydrocarbon Family	Distinguishing Characteristics	Major Hydrocarbons	Remarks
Saturated	Paraffins (Alkanes)	They have straight carbon chain	Methane, ethane, propane, butane, pentane, hexane	General formula C_nH_{2n+2} Boiling point increases as the number of carbon atom increases. With number of carbon 25-40, paraffin becomes waxy.
	Isoparaffins (Iso alkanes)	Straight carbon chains with branches	Isobutane, Isopentane, Neopentane, Isooctane	The number of possible isomers increases in geometric progression as the number of carbon atoms increases.
	Naphthenes	5 or 6 carbon atoms in ring	Cyclopentane, Methyl cyclopentane, Dimethyl cyclopentane,	General formula $C_nH_{2n+2-2Rn}$ Rn is number of naphthenic ring The average crude oil contains about 50% by weight naphthenes. Naphthenes are modestly good.
Unsaturated	Olefins (Alkenes)	One pair of carbon atoms	Ethylene, Propylene	General formula C_nH_{2n} Olefins are not present in crude oil, but are formed during process. Undesirable in the finished product because of their high reactivity. Low molecular weight olefins have good antiknock properties.
Aromatics	Aromatics	6 carbon atom in ring with three around linkage.	Benzene, Toluene, Xylene, Ethyl Benzene, Cumene, Naphthalene	Aromatics are not desirable in kerosene and lubricating oil. Benzene is carcinogenic and hence undesirable part of gasoline.

Source: Mall(2007)

Polycyclic aromatic hydrocarbons are among the most considered compounds as a result of their toxicity, carcinogenicity, and mutagenicity (D'Souza *et al.*, 2015). The

major source of PAHs is crude petroleum, however; they are predominantly introduced to the environment through natural and anthropogenic combustion processes (Speight, 2006). The release of PAHs from natural sources is as a result of spontaneous fires from forests and grassland and also volcanic emissions. On the other hand, the anthropogenic sources are diverse ranging from simple processes of incineration of wood for cookingand heating to complex industrial activities such as refining of crude petroleum, manufacturing of chemicals, and vehicle emissions (D'Souza *et al.*, 2015). Soil and sediments are the main sinks for all the PAHs derived from pyrogenic, petrogenic, and biological activities in the environment (Abdel-Shafy and Mansour, 2016).

2.5.2 Plants used in hydrocarbon phytoremediation

One of the major focuses in phytoremediation is to identify a plant species that is resistant or tolerant to a particular contaminant with a view to maximizing its potential for remediation. Plants growing on soils with underlying contaminants or on the boundary of polluted sites are commonly resistant or tolerant (Vaziri*et al.*, 2013). There are some plant species with better remediation properties than other species; therefore, more efficient species should be selected for phytoremediation of hydrocarbons (Rodriguez *et al.*, 2005).

For a sustainable phytoremediation process, the use of plant species that are economically and ecologically valuable has been suggested (Pandey *et al.*, 2015). Additionally, some of the desirable qualities include being indigenous, ability to propagate easily and rapidly, fast growing, high biomass production, abundant root system, ability to concentrate pollutants, withstand harsh conditions, inedible, perennialand ecologically stable (Pandey and Bajpai, 2019). More so, it is advantageous

that the selected species or its product could be valorized and should also be valuable to society in terms of energy and environmental services (Pandey and Bajpai, 2019).

Currently, more than hundred plant species that have some desirable qualities and the potentials for soil and water remediation have been identified (Yaqoob *et al.*, 2019). This includes a broad range of plants such as trees (e.g. poplar trees), edible plants (e.g. rice), aquatic weeds (e.g. duckweed) and terrestrial grasses (Chakravarty *et al.*, 2017). Trees, legumes and grasses are frequently used in hydrocarbon remediation, with trees majorly selected for remediation of BTEX as against grasses which are morecommonly used for remediation of PAHs and TPH. Frequently, leguminous plants and grasses are considered most promising in hydrocarbon phytoremediation (Van Epps, 2006; Yaqoob *et al.*, 2019). This is because grasses have the largest root surface area, penetrate deep into soil, genetically diverse and easily grow under unfavorable soil conditions (Aprill and Sims, 1990; Sarma and Lee, 2018). Legumes however fix nitrogen; thus, limiting microbial competition for nitrogen which becomes limited in oil-contaminated sites (Sarma and Lee, 2018). They are also diverse with variety of propagation methods and able to grow in almost all forms of terrestrial environments due to enhanced defense and nutrient acquisition (Hall *et al.*, 2011). Like grass, legumes provide oxygen in soil environment which stimulate microbial activities and subsequent promotion of hydrocarbon biodegradation (Peer *et al.* 2006).

Table 2.2 shows a list of some often reported plant species with hydrocarbon phytoremediation potentials.

Table 2.2: Plants with phytoremediation ability

Plant Species	Hydrocarbons	Comment	Reference
Medicago sativa L. and *Medicago falcata* L. (Leguminosae)	Oil sludge	Stimulate microbial growth and decrease major oil fractions	Panchenko*et al.* (2017)
*Festuca arundinacea*Schreb. (Poaceae)	PAHs	The abundance of PAH degrading bacteria in the rhizosphere was substantially increased; most of 4-ring PAHs were degraded	Huang *et al.* (2004), Parrish *et al.*(2005). Sun *et al.* (2011)
Trifolium repens L. (Leguminosae) *Lolium perenne* L. (Poaceae)	PHC	Significantly reduced the hydrocarbon concentration to undetectable limits	Germaine *et al.* (2015).
Trifolium repens *Trifolium pretense* (Fabaceae)	Diesel, PAHs	Enhanced degradation of diesel; root exudates facilitated PAHs bioavailability and increased biodegradation rate	Ying *et al.* (2018), Davin *et al.* (2019)
Sorghum bicolor L. *Hordeum vulgare* L. (Poaceae)	PHC	Significant reduction in the concentration of petroleum hydrocarbons	Asiabadi*et al.* (2018)
Cynodondactylon L. (Poaceae)	PHC	About 50% reduction in PHC concentration, with amendment using organic fertilizer	Basumatary and Bordoloi (2016)
Prairie grass	PHC	Significant reduction in TPH	April and Sims (1990)
Heliamthus annus (Asteraceae)	PHC and heavy metal cocontamination	58% reduction in TPH and reduction in heavy metal concentration was observed	Vitor *et al.* (2018)
Salix smithiana L. *Salix viminalis* L. (Salicaceae)	PAHs	PAHs were removed by 50.9% after three years of soil In synergy with white rot fungi, Caused the highest PAH removal rate.	Košnář*et al.* (2020); Ma *et al.* (2020)
Triticum aestivum L. (Poaceae)	PHC	Fertilizer application enhanced the degradation	Masu*et al.* (2013)
Jatropha carcus L. (Euphorbiaceae)	PHC	caused 78.8% reduction of TPH with compost amendments	Bertrand, (2020)

2.5.3 Methods of petroleum hydrocarbon remediation

Since the time when the world's early major oil spills occurred, enormous resources have been dedicated towards oil recovery and environmental cleanup (Sebastián*et al.*, 2014; Michel and Fingas, 2016). The world has seen the evolution of different cleanup technologies in the last five decades (Strechе*et al.*, 2018; Maceiras, 2020). Popular among the treatment methods are physical, chemical, thermal and biological (Wang *et al.*, 2017; Xuezhi*et al.*, 2020). The goal of the remediation techniques is to meet any or all the following: i. elimination or alteration of contaminants, ii. extraction or separation from an environment, and/or iii. immobilization of the contaminants.

Before selection of appropriate technology for hydrocarbon clean up, feasibility study focusing on the cost implication, environmental suitability and time frame is recommended. Biological method has always been described as eco-friendly and less costly than the other techniques. In Figure 2.1, various techniques under the remediation options are outlined.

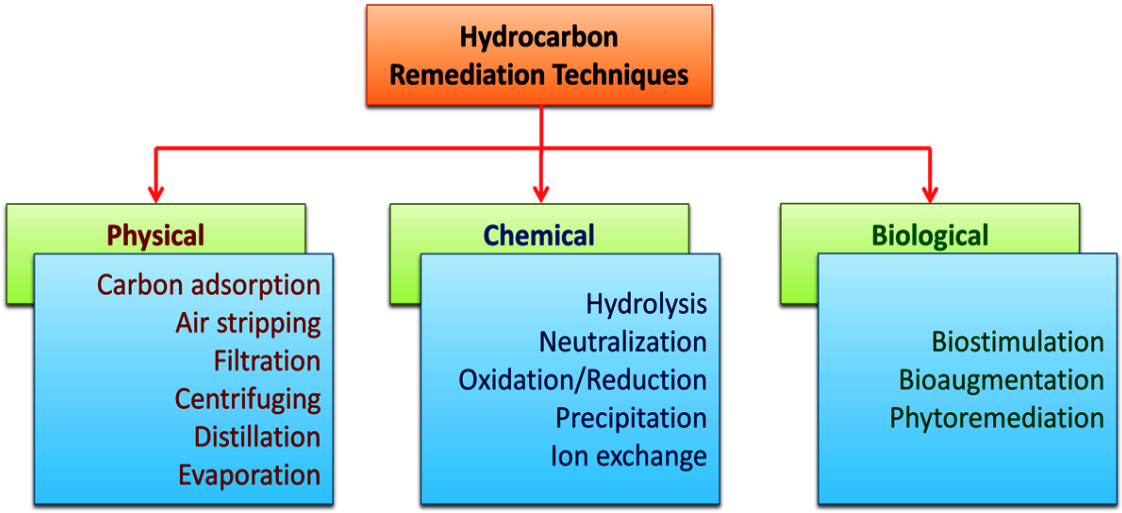

Figure 2.1: Remediation techniques of petroleum hydrocarbons

Source: Xuezhi*et al.* (2020)

2.5.4 Phytoremediation of petroleum hydrocarbons

Phytoremediation is a remediation technique that exploits plants and microorganisms to decontaminate a polluted environment. Maceiras (2020) considers phytoremediation as the use of plants and the microbial communities associated with them to sequester, degrade, or prevent the mobility of xenobiotic contaminants. In natural ecosystems, plants remove and utilize substances generated by nature. Since the inception of phytoremediation, a remarkable body of knowledge on the use of plants to remediatea wide variety of both inorganic and organic compounds has been produced (Phillips, 2008; Wang *et al.*, 2017). This may be due to the fact that the success of microbial degradation has been limited with petroleum-based constituents rather than residual organic and metal pollutants. Vegetation-based remediation however shows potential for accumulating, immobilizing and transforming complex compounds into low level of persistent contaminants (Sutar and Das 2012).

The fundamental principles governing phytoremediation include (Kathi and Khan, 2011).

i. Absorption of organic compounds from the root zone.

ii. Processing and deposition of these chemicals via lignification, volatilization, metabolization, or mineralization.

iii. Enzymatic degradation of complex organic molecules into simpler molecules (ultimately carbondioxide and water).

iv. Enrichment of the root zone with nutrients, carbon and oxygen which promotes microbial activity.

Plant and microbial synergism remediate petroleum hydrocarbons through three fundamental mechanisms in soil and groundwater. These mechanisms include degradation, containment, as well as transfer of the hydrocarbons to atmosphere (Cunningham *et al.*, 1996). In containment process, plants reduce or eliminate the bioavailable contaminants from the environment. Plants contain petroleum hydrocarbons by accumulation within the plants, adsorption on the root surface and as organic pumps that allow its isolation within the root zone, thus limiting its spread. Indirectly, humification – a process that binds contaminants into soil organic matter as a result of enzymatic activities is exercised. Humification is enhanced by increasing soil organic matter content (Cunningham *et al.*, 1996). In the case of hydrocarbon transfer

from soil to atmosphere, plants absorb and translocate the compounds and then get liberated into atmosphere by transpiration (Frick *et al.*, 1999). However, the process may lead to subsequent contamination of the atmosphere which results to breach of air quality regulatory standards. In degradation however, plants and microorganisms play a

direct or indirect role in the breakdown of hydrocarbons into simpler products that are generally considered less toxic and less recalcitrant thanthe parent compounds. There are speculations on the effectiveness of direct hydrocarbon degradation process by plants and microorganisms.Sharma *et al*. (2018) suggested the degradation pathway as follows:

n-alkane → primary alcohol → fatty acids → acetyl-CoA → various compounds.

Conversely, the indirect role of plants in degradation of hydrocarbons is well established and considerable body of information is available. The plants employ three mechanisms to accomplish degradation. These include alteration of soil's physical and chemical conditions by plants and their root systems, enhancement of rhizosphere effect through root exudation and release of rootassociated enzymes capable of transforming organic contaminants through co-metabolism. Researchers reported variation in hydrocarbon degradation from as little as 5% to greater than 50% using different plant species (Sharma *et al*. 2018). Degradation refers to breakdown or transformation of complex or toxic substances to simpler and less toxic ones and is believed to be the major mechanism for organic contaminants cleanup.

2.6 Phytoremediation

Phytoremediation is an emerging low-cost technology that utilizes plants to remove, transform, or stabilize contaminants including organic pollutants located in water, sediments, or soils. The advantages of phytoremediation over usual bioremediation by microorganisms are that plants, as autotrophic systems with large biomass, require only modest nutrient input and they prevent the spreading of contaminants through water and wind erosion (Sharma *et al.*, 2018). Plants also supply nutrients for rhizosphere bacteria, allowing the growth and maintenance of a microbial community for further contaminant

detoxification. Numerous plant species have been identified for the purpose of phytoremediation like Alfalfa and Juniper. Certain plant species, known as hyperaccumulators, are attractive candidates as they are able to accumulate potentially phytotoxic elements to concentrations 50-500 times higher than average plants (Panda *et al.*, 2017). The high bioconcentration factor and the efficient root-to-shoot transport system endowed with enhanced hydrocarbon and metal tolerance provide hyperaccumulators with a high potential detoxification capacity (Irshad *et al.*, 2015).

However, many of the hyperaccumulators are slow growing and have reduced biomass production, thus requiring several years for decontamination of the polluted sites. Trees, on the other hand, appear as an attractive alternative due to their extensive root system, high water uptake, rapid growth, and large biomass production (Shen *et al.*, 2017). The remedial capacity of plants can be significantly improved by genetic manipulation and plant transformation technologies (Songita*et al.*, 2018).

2.6.1 Mechanisms of phytoremediation

The mechanisms and efficiency of phytoremediation depend on the type of contaminant, bioavailability and soil properties (Irshad *et al.*, 2015). There are several ways by which plants clean up or remediate contaminated sites (Figure 2.2). The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing toxicity are found. The root system provides an enormous surface area that absorbs and accumulates water and nutrients essential for growth along with other non-essential contaminants (Raskin and Ensley, 2000). Although overlap or similarities can be observed between some of these mechanisms, and the nomenclature varies, each of these mechanisms will have an effect on the volume, mobility, or

toxicity of contaminants, as the application of phytoremediation is intended to do (Sarma and Lee, 2018).

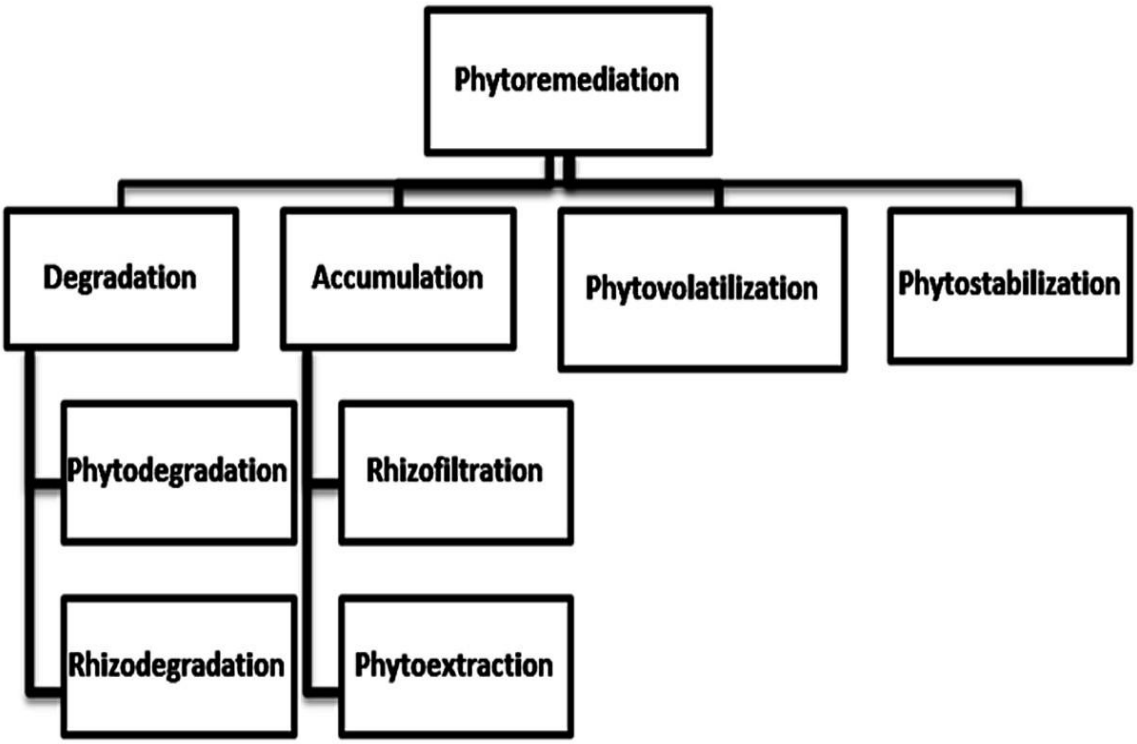

Figure 2.2 Mechanisms in phytoremediation

Source: Sarma and Lee (2018).

2.6.1.1 Phytoextraction

Phytoextraction is also called phytoaccumulation, and it refers to the uptake and translocation of contaminants in the soil by plant roots into the above ground portions of the plants. Phytoextraction is primarily used for the treatment of contaminated soils (USEPA, 2000). To remove contamination from the soil, this approach uses plants to absorb, concentrate, and precipitate hydrocarbons from contaminated soils into the above ground biomass (shoots, leaves, etc.) Discovery of hydrocarbon hyperaccumulator species demonstrates that plants have the potential to remove

hydrocarbons from contaminated soils (Isah, 2013). A hyperaccumulator is a plant species capable of accumulating 100 times more hydrocarbon than a common non-accumulating plant (Irshad *et al.*, 2015).

2.6.1.2Rhizofiltration

Rhizofiltration is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations. It is the adsorption or precipitation onto plant roots or absorption of contaminants in the solution surrounding the root zone. Rhizofiltration is typically exploited in groundwater (either *in situ* or extracted), surface water, or wastewater for removal of metals or other inorganic compounds (Subhashini and Swamy, 2015).

Rhizofiltration is similar to phytoextraction, but the plants are used primarily to address contaminated ground water rather than soil. The plants to be used for cleanup are raised in greenhouses with their roots in water rather than in soil. To acclimatize the plants, once a large root system has been developed, contaminated water is collected from a waste site and brought to the plants where it is substituted for their water source. The plants are then planted in the contaminated area where the roots take up the water and the contaminants along with it. As the roots become saturated with contaminants, they are harvested (Odiyi and Abiya, 2016). Sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their ability to remove lead from water, with sunflower having the greatest ability (Raskin and Ensley, 2000). In one study, after only one hour of treatment, sunflowers reduced lead concentrations significantly (Raskin and Ensley, 2000).

The advantages associated with rhizofiltration are the ability to use both terrestrial and aquatic plants for either *in situ* or *ex situ* applications. Another advantage is that

contaminants do not have to be translocated to the shoots. Thus, species other than hyperaccumulators may be used (Odiyi and Abiya, 2016). Terrestrial plants are preferred because they have a fibrous and much longer root system, increasing the amount of root area (Raskin and Ensley, 2000). Disadvantages and limitations include the constant need to adjust pH, plants may first need to be grown in a greenhouse or nursery; there is periodic harvesting and plant disposal; tank design must be well engineered; and a good understanding of the chemical speciation/interactions is needed (Odiyi and Abiya, 2016).

2.6.1.3 Phytovolatilization

Phytovolatization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (Odiyi and Abiya, 2016). Phytovolatilization also involves contaminants being taken up into the body of the plant, but then the contaminant, a volatile form thereof, or a volatile degradation product is transpired with water vapor from leaves (USEPA, 2000). Phytovolatilization may also entail the diffusion of contaminants from the stems or other plant parts that the contaminant travels through before reaching the leaves (Raskin and Ensley, 2000). Phytovolatilization can occur with contaminants present in soil, sediment, or water. The advantage of this method is that the contaminant may be transformed into a less toxic substance (Odiyi and Abiya, 2016).

2.6.1.4Phytostabilization

This is also referred to as in-place inactivation. It is primarily used for the remediation of soil, sediment, and sludge (USEPA, 2000). It is the use of certain plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of

plants (rhizosphere). This process reduces the mobility of the contaminant and prevents migration to the groundwater and it reduces bio-availability of hydrocarbon into the food chain (Isah, 2013).

This technique can also be used to reestablish vegetation cover at sites where natural vegetation fails to survive due to high hydrocabon concentrations in surface soils or physical disturbances to surface materials. Hydrocarbon-tolerant species is used to restore vegetation at contaminated sites, thereby decreasing the potential migration of pollutants through wind erosion and transport of exposed surface soils and leaching of soil contamination to groundwater. Phytostabilization takes advantage of the changes that the presence of the plant induces in soil chemistry and environment. Some of the advantages associated with this technology are that the disposal of hazardous material/biomass is not required and it is very effective when rapid immobilization is needed to preserve ground and surface waters (Zand*et al.*, 2016). The presence of plants also reduces soil erosion and decreases the amount of water available in the system (Notor*et al.*, 2017). However, this clean-up technology has several major disadvantages including: contaminant remaining in soil, application of extensive fertilization or soil amendments, mandatory monitoring is required, and the stabilization of the contaminants may be primarily due to the soil amendments (Odiyi and Abiya, 2016).

2.6.1.5 Phytodegradation

Phytodegradation is also referred to as phytotransformation. It involves the degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plant tissues (Zhang *et al.*, 2010). When the phytodegradation mechanism is at work, contaminants are broken down after they have been taken up by the plant. As with phytoextraction and phytovolatilization, plant uptake generally occurs

only when the contaminants' solubility and hydrophobicity fall into a certain acceptable range. Phytodegradation has been observed to remediate some organic contaminants, such as chlorinated solvents, herbicides, and munitions, and it can address contaminants in soil, sediment, or groundwater (Pospisil and Yamamoto, 2017).

2.6.1.6 Hydraulic control

This is the control of the water table and the soil field capacity by plant canopies. Phytoremediation projects employing hydraulic control generally use phreatophytic trees and plants that have the ability to transpire large volumes of water and thereby affect the existing water balance at the site. The increased transpiration reduces infiltration of precipitation (thereby reducing leaching of contaminants from the vadose zone) or increases transpiration of groundwater, thus reducing contaminant migration from the site in groundwater plumes. Hydraulic control can therefore be used to address a wide range of contaminants in soil, sediment, or groundwater (USEPA, 2000). It should be noted that hydraulic control is also a feasible phytoremediation mechanism for control of groundwater contamination in particular, because the characteristics of the contaminants are not as relevant to the success of the technique.

2.6.1.7Rhizodegradation

Rhizodegradation is also referred to as phytostimulation. Rhizodegradation refers to the breakdown of contaminants within the plant root zone, or rhizosphere. It is believed to be carried out by bacteria or other microorganisms whose numbers typically flourish in the rhizosphere. Studies have documented up to 100 times as many microorganisms in rhizosphere soil as in soil outside the rhizosphere (USEPA, 2000). Microorganisms may be so prevalent in the rhizosphere because the plant exudes sugars, amino acids, enzymes, and other compounds that can stimulate bacterial growth. The roots also provide additional surface area for microbes to grow on and a pathway for oxygen transfer from the environment (Shen *et al.*, 2017). The localized nature of rhizodegradation means that it is primarily useful in contaminated soil, and it has been investigated and found to have at least some successes in treating a wide variety of

mostly organic chemicals, including petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, pesticides, polychlorinated biphenyls (PCBs), benzene, toluene, ethylbenzene, and xylenes (USEPA, 2000). It can also be seen as plant-assisted bioremediation, the stimulation of microbial and fungal degradation by release of exudates/enzymes into the root zone (rhizosphere) (Zhang*et al.*, 2010).

2.6.1.8Advantages of phytoremediation

i. The cost of the phytoremediation is lower than that of traditional processes both *in situ* and *ex situ*
ii. The plants can be easily monitored
iii. The possibility of the recovery and re-use of the pollutant (by companies specializing in "phyto mining")
iv. It is potentially the least harmful method, because it uses naturally occurring organisms and preserves the environment in a more natural state.
v. It preserves the topsoil, maintaining the fertility of the soil (Ali *et al.*, 2013)
vi. Increase soil health, yield, and plant phytochemicals (Yahia and Daniel, 2018)
vii. The use of plants also reduces erosion and metal leaching in the soil (Ali *et al.*, 2013).

2.6.1.9 Limitations of phytoremediation

i. Phytoremediation is limited to the surface area and depth occupied by the roots (Yahia and Daniel, 2018).
ii. Slow growth and low biomass require a long-term commitment
iii. With plant-based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater (without the complete removal

of the contaminated ground, which in itself does not resolve the problem of contamination)

iv. The survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil.

v. Bio-accumulation of contaminants, especially metals, into the plants which then pass into the food chain, from primary level consumers upwards or requires the safe disposal of the affected plant material.

vi. When taking up heavy metals, sometimes the metal is bound to the soil organic matter, which makes it unavailable for the plant to extract (Sarma, 2011).

2.6.1.10 Types, uptake and transport of pollutantsby plant

There are two major classes of contaminants: organic and inorganic. Organic contaminants include different compounds such as petroleum hydrocarbons, chlorinated solvents, halogenated hydrocarbons such as trichloroethylene (TCE), and explosives such as trinitrotoluene (TNT). When compared to inorganics, the organic pollutants are relatively less toxic to plants because they are less reactive and do not accumulate readily (Zhang *et al.*, 2017). Inorganic compounds include heavy metals such as mercury, lead, and cadmium, and nonmetallic compounds such as arsenic and radionuclides like uranium (Irshad *et al.*, 2015).

The use of plants to remove organic pollutants was derived from the observation that organic pollutants disappear more quickly from vegetated soil than from barren soil (Pospisil and Yamamoto, 2017) and this was later confirmed in studies of plant-mediated degradation of petroleum contaminants (Subhashini and Swamy, 2015). As organic compounds are usually man-made and xenobiotic in plants, there are no transporters for their uptake and the usual mechanism of uptake is by simple diffusion

(passive uptake). When organic contaminants come into contact with roots, they may be sorbed to the root structure. The hydrophobic or hydrophilic nature of the organic compounds also determines their possible uptake. Hemicellulose in the cell wall and the lipid bilayer of plant membranes can bind hydrophobic organic pollutants effectively (Pospisil and Yamamoto, 2017).

2.6.1.11 Factors affecting phytoremediation

There are several options for the remediation of contaminated sites using plants. Different phytotechnologies have already been put into practice and each one uses different plants or plant properties. Faster growth rate, high biomass, hardiness, and tolerance to pollutants are some of the favorable plant properties being exploited for remediation. In addition, various biological processes such as plant-microbe interactions can affect the remediation efficiency (Kiran and Prasad, 2017). Among these, plant uptake of water and contaminants, plant-microbe interactions, enhanced microbial activity in the rhizopshere, fate and transport of contaminants in plant root zone, further translocation, and tolerance mechanisms are of paramount importance in developing improved phytoremediation technologies (Notor*et al.*, 2017).

2.7*Urena lobata*

Urena lobata, commonly known as Caesarweedor Congo jute,is a tender perennial, variable, erect, ascendant shrub or subshrub measuring up to 0.5 meters (1.6 ft) to 2.5 meters (8.2 ft) tall. The stems are covered with minute, starlike hairs and often tinged purple. Considered a weed, it is widely distributed in the tropics (Davidson, 2011).Each individual plant grows as a single stalk that freely sends out bushy stems. The leaf shape is palmately lobed (having lobes that spread out like fingers on a hand). Like the stem, the leaves also have tiny hairs (Plate 2.1). Flowers of the plant are pinkviolet and grow

one centimeter in width. The fruit is also hairy and may stick to clothing or fur (Centre for Aquatic and Invasive Plants CAIP, 2015).

PlateI:*Urena lobata*, Source; (Davidson, 2011)

2.7.1 Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malvales

Family: Malvaceae

Genus: *Urena*

Species: *U. lobata*

Binomial name:*Urena lobata L.*

2.7.2Invasiveness and uses

The plant can invade areas of ecological disturbance as well as eroded places, crop plantations, and pastures. It grows as an annual plant in most areas and the plant has been reported to grow rapidly from 0.5 meters (1.6 ft) to 2 meters (6.6 ft) by the end of its first year of growth(International Rice Research Institute, IRRI, 1999).

2.8 *Melissa officinalis* (Lemon balm)

Melissa officinalis (Lemon balm), also known as common balm, or balm mint, (Plate 2.2) is a perennial herbaceous plant in the mint family Lamiaceae and native to southcentral Europe, the Mediterranean Basin, Iran, and Central Asia, but now naturalized in the Americas and elsewhere(Zirkle, 2001).

PlateII:*Melissa officinalis*, Source:

2.8.1Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Lamiales

Family: Lamiaceae

Genus: *Melissa*

Species: *M. officinalis*

Binomial name:*Melissa officinalis L.*

The plantattract bees, hence the genus *Melissa* (Greek for "honey bee"). The leaves are used as a herb, in teas, and also as a flavouring. The plant is used to attract bees for honey production. It is grown as an ornamental plant and for its oil (to use in perfumery). The essential oil, and the extract are used in traditional and alternative medicine, including aromatherapy. Lemon balm is used in some toothpastes (Greer, 2017). The plant has been cultivated at least since the 16th century, but research is still being conducted to establish the safety and effects of lemon balm(Shakeri*et al.*, 2016).

2.9 Biodegradation of Spent Engine Oil

Biodegradation in other terms is a treatment process whereby contaminants or pollutants are metabolized into non toxic or less toxic compounds by microorganisms naturally existing in a given environment. Microorganisms can utilize many of the petroleum

hydrocarbon constituents as a source of carbon and energy producing carbon dioxide and water as by-products. Once all of the contaminants have been consumed by microorganisms, the microbial population becomes dormant or dies out. Biodegradation can take place under aerobic or anaerobic conditions in the presence of other suitable electron acceptors such as nitrate, sulfate, or carbonate. Extracellular surfactant secreted by microorganisms, enhances the process of biodegradation (Abdulsalam*et al.,* 2012).

Microbial degradation is the major mechanism for the elimination of used petroleum products from the environment (Jadhav *et al.,* 2017). Soils contain very large numbers of microorganisms which can include a number of hydrocarbons utilizing bacteria and fungi (Oyewole*et al.,* 2019). Microorganisms are capable of breaking down many complex molecules by adaptation of their degradative enzyme system (Langenbach, 2013).

Some microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions (Shahida *et al.*, 2015).

2.10 Plant-Microbe Synergism for Hydrocarbon Degradation

It has been known for long that plants, like animals, have microbiota which are present in their endosphere, rhizosphere and phyllosphere. This include plants' normal flora

which consists of a few dominant species called the core microbiome which are constantly associated with a given plant irrespective of environmental influence; the major microbiome which determines plant fitness and few other microbes in the endosphere whose roles are not clearly understood (Nataraja *et al.,* 2019). Plant-microbe interactions can be beneficial, harmful or neutral based on the effects to the host (Imam *et al.*, 2016). Different types of interactions are known to exist including mutualism, pathogenesis, and parasitism (Singh *et al.,* 2019). Earlier studies by Paungfoo-Lonhienne*et al.* (2010) have demonstrated a predatory relationship in which microbes enter root cells and are later digested to release nitrogen for growth. There are enough evidences that plant-microbe associations dramatically influence each other's lifestyles and health trajectories (Zhang *et al.,* 2014;O'Banion*et al.,* 2019).

The interaction between plants and microbes has been exploited to remove environmentalcontaminants from soil which offers a cheaper, safer, and eco-friendly alternative to available methods(Singh *et al.,* 2019). Although soil contamination with pollutants affects biological functions, synergisticplant-microbe interaction plays a crucial role in improving soil quality and plant performance (Velmourougane*et al.,* 2017). In this process, microorganismsdegrade organic contaminants or make inorganic pollutants bioavailable for uptake by plants (Chaudhry *et al.,* 2005). For the microbes to grow, multiply and subsequently degrade contaminants, they requireessential nutrients from plants, while plants benefit from the detoxification of pollutants by the microbes (Siciliano and Germida, 1998; Manoharachary and Mukerji, 2006).The key roles played by microorganisms in microbe-assisted phytoremediation of petroleum hydrocarbonsare plant growth promotion. This is made through minimizing phytotoxicity, promotingextensive root system, improving pumping capacity, providing mobilization due to surfactant production,enhancing stabilization due to secretion of chelators and

detoxification as a result of sequestration oncell walls (Thijs *et al.*, 2016). Studies by Montalbán*et al* (2017) have shownthat endophytic bacteria significantly decreased contaminant-induced stress and increased contaminantuptake into the plants. Conversely, plants offer the microbes a micro-environment, nutrients, electron acceptors, growth factors and water for growth (Ashraf *et al.*, 2013). There is increasing efforts to furtherelucidate plant-microbe interaction and how the networks operate in the environment (Sahu *et al.*,2020). Recently, focus has been shifted to using genetic and bioinformatics approaches to give clear understanding of the interconnectivity between plants and microbes in remediation processes (Agarwal *et al.*, 2020; Sharma *et al.*, 2020).

2.11 Interaction in the Rhizosphere

Rhizosphere is the term used to describe the portion of soil surrounding plant root system and under its influence (Shukla *et al.*, 2011; Correa-Garcia *et al.*, 2018). It is indefinite soil zone with a varying microbial abundance and diversity in which substantial microbial alteration in the soil is pronouncedadjacent to roots and subside as it far away. Rhizoplane is the external surface of plant root togetherwith any closely adhering particles of soil or debris (Manoharachary and Mukerji, 2006). To obtain rhizoplane soil, plant roots are gently removed from soil and transferred to a fresh sterile solution and shaken vigorously (Sharma *et al.*, 2020). The size of this zone is determined by the soil type, plant type and soil conditions (Manoharachary and Mukerji, 2006). Microorganisms are found in three distinct sites of the rhizosphere: (i) theendosphere; (ii) the rhizoplane usually as biofilm and (iii) the soil (ectorhizosphere) influenced by the plant roots (Rohrbacher and St-Arnaud, 2016). Although the rhizosphere covers some distance around the root in soils, its size and shape is difficult to assess despite the fact that recent understandings showed that it is quasi-stationary

(Kuzyakov and Razavi, 2019). The rhizosphere associated with peanut and soybean roots was estimated to reach about 0.2 mm thick using electron microbeam analysis and scanning electron microscope (Sharma *et al.*, 2020).

Rhizosphere microorganisms are either harmful or beneficial. The beneficial effects occur in either of the following ways (Bais *et al.*, 2006):

i). The first hypothesis suggests that there is aggressive colonization of roots by beneficial microbes which displaces the harmful ones and consequently leads to promotion of plant growth.

ii). The second hypothesis suggests that the beneficial microbes directly attack and kill the harmful ones. Beneficial microorganisms may produce hormones such as auxins and kinetins that bring about plant growth promotion. The stimulatory effect on microorganisms in the rhizosphere by plants is called the rhizosphere effect (Manoharachary and Mukerji, 2006; Nie*et al.*, 2010).

The predominant microbial species that inhabit the rhizosphere are fungi and bacteria (Bais *et al.*,2006). Rhizosphere effect may increase fungal and bacterial abundance by 2 - 20 times greater than inthe bulk soil (Phillips, 2008). Due the large number of microbes in the rhizosphere, the available nutrients become limited and as a result, there is high competition for nutrients. Therefore, different microbial species have evolved special adaptations for survival ranging from antagonism to synergism, both among themselves and with the plant. Due to the wide microbial diversity, several kinds of interactions within the microbial community and between the host plants are possible. The understanding of fundamentals of these interactions is essential for their use in plant growth promotion and remediation of contaminated soils (Hrynkiewicz*et al.*, 2009).

2.12 Metabolism of Hydrocarbons by Plants

Following exposure to petroleum hydrocarbons, plants withstand their effect by lowering, transforming, and degrading the harmful contaminants in specialized cells adapted for detoxification process (Sun *et al.*, 2015). Once in the plants' rhizosphere, they drift to the roots but some lipophilic compounds limit their uptake or cause their accumulation in the partly suberized cortex of the root. Hydrocarbon lipophilicity and its adsorption capacity to soil particles limit its uptake by plants. Hydrocarbons with lower log K_{ow} are water soluble and not firmly attached to roots and passively transported through plant membranes; whereas, those with higher log K_{ow} (> 3.0) can only adsorb to the surface of the roots with high proportion of lipids – uptake and translocation is restricted (Siciliano and Germinda, 1998; Farreland Germinda 2002).

However, there are divergent views on the ability of plants to uptake hydrocarbons where plants' inability to uptake hydrocarbon is an approved standard by the Canadian Council of Ministers of the Environment; CCME, 2008). A number of findings are in support of this standard (Lu *et al.,* 2010; Nwaaichi*et al.,* 2011). Some other studies by different researchers however, are of the opinion that plant uptake hydrocarbon at different capacity depending on their physiology (Basumatary*et al.,* (2012); Naidoo and Naidoo, 2016; Patowary*et al.,* 2017; Anyasi and Atagana, 2018). Despite these findings, Hunt *et al.* (2018) described them as numerically inconsequential and generally lack reliable data to back their conclusions; because majority of the investigations were not aimed at determining hydrocarbon uptake and/or its distribution but focused on determining the rates of phytoremediation. Furthermore, methodological inconsistencies, inadequate description of environmental conditions and analytical procedures and irreconcilable measurements marred the findings (Doucette *et al.*, 2018; Hunt *et al.,* 2018).

Where hydrocarbon uptake is believed to have taken place, the compounds are prevented from detoxification and metabolism but transferred into symplast to avoid the suberized casparian strips barrier in the root endodermis. They are later translocated by the transpiration stream along the xylem into other tissues of the plant (root and shoot) (Kathi and Khan, 2011). The metabolism is enzyme catalyzed and occurs in three phases. Phase-I is catalyzed by P-450 enzymes complex responsible for transformation reactions like hydroxylation, N and O-alkyl group removal and Sulphyl group oxidation. In phase-II of the metabolism, conjugation of the earlier transformed compounds with polar molecules of plants origin occurs (Kvesitadze*et al.*, 2009; Pandey and Bajpai, 2019). This stage is central in hydrocarbon detoxification by plants and it is facilitated by the activities of transferases (Aken*et al.*, 2010). If the formed conjugates are soluble, they can totally disintegrate into CO_2 and H_2O for the plant's benefit, but if they are insoluble, they are transferred by exocytosis to the apoplast and become part of the cell wall (Kathi and Khan, 2011; Schwitzgue, 2017). This describes the Phase-III or last stage of hydrocarbon metabolism in plants.

2.12.1 Phytoxicity of petroleum hydrocarbons

Petroleum hydrocarbons induce toxic effects on different plant species during germination and growth (Agbogidi, 2010) especially in heavily contaminated environment (Chaineau*et al.*, 1997). Plants seeds are seriously damaged due to the fact that some oil fractions have the capacity to wet and strongly penetrate into seed coat and embryo, which result to destruction and loss of seed viability (Kathi and Khan, 2011; Ismail *et al.*, 2019). There are reports that show that phytotoxic effects on seeds is correlated with hydrophobic properties of oils that prevent and/or reduce exchange of water and gases which disrupts the metabolism or cause acute toxicity that destroys the embryo (Amadi*et al.*, 1993). After emergence, hydrocarbons are known to reduce

growth and yield of crops even at low concentrations (Ali, 2019). Individual hydrocarbon fractions are ideal for testing hydrocarbon toxicity and as such, it is obscure to figure out the toxicity of petroleum mixture without knowing the parent constituents. The amount of TPH observed depends on the nature of solvent used in hydrocarbon extraction in which volatile compounds are lost during solvent concentration, which cause wrong estimate of plant hydrocarbon contents (Ali, 2019). However, Chaineau*et al.* (1997) have shown that light aromatics and naphtha to be more phytotoxic in seven different plant species. Studies by Somtrakoon and Chouychai (2013) have shown the toxicity of different PAHs on the germination and growth of sweet corn, waxy corn, and rice in which both single and mixed PAH treatment delayed germination and growth. Petroleum hydrocarbons impede plant growth by reducing the growth rate, soil fertility and plants resistance to pests and diseases (Wang *et al.*, 2017).

2.13Role of Root Exudates in Phytoremediation

Traditionally, plant root system is known to offer support and conduction of nutrients and water to the aerial parts, however, studies have shown that plants also release substantial amounts of organic molecules to soil through discharge from roots or exudation (Rohrbacher and St-Arnaud, 2016). Plant roots exudation can be active or passive (Hoang *et al.*, 2021), and may amount to 40% of a plant's total photosynthate (Gerhardt *et al.*, 2009). Different types of complex (organic acids, sugars, phenolic compounds, polysaccharides, and humic compounds) and simple (amino acids, monosaccharides etc.) organic molecules, are secreted through plant roots and they are collectively referred to as root exudates (Rohrbacher and St-Arnaud, 2016; Hoang *et al.*, 2021). These exudates provide nutrient source for the growing microbes at the rhizospheric regions and help in effective colonization (Singh *et al.*, 2019). In addition

to mucilage secreted from roots, worn out cells from root caps, decayed roots and starvation of the root cells also serves as source of nutrients for the microbes (Gupta *et al.,* 2020).There is a great diversity in the type and abundance of plant exudates which is a function of plant species, its age, health status and external biotic and abiotic influences (Liu *et al.*, 2019). The quantity of exudates in the rhizosphere varies and are more concentrated at the root tips and lateral branching (Shukla *et al.,* 2011).

Root exudates can be grouped into four based on the way they are produced. There are passive exudates, secondary plant metabolites, lysates and mucilage (Martin *et al.*, 2014; Gupta *et al.,* 2020). Different plant species secret specific exudates and the primary constituents in the exudates dictate the rhizosphere community structure (Zhang *et al.*, 2014; Mhlongo *et al.*, 2018). Root exudates stimulate microbial community shift in contaminated soils through two different ways: alteration of microbial catabolic genes expression and specific selection of microbial strains (Siciliano *et al.,* 2003; Gupta *et al.*, 2020). Rhizospheric microorganisms significantly relay on exudates as carbon and energy sources. Since most of the exudates are readily available sources of nutrients, microbial species become easily attracted through chemotaxis, leading to colonization and increased biomass (Hoang *et al.*, 2021). Plant roots serve as attachment sites for microbes and provide oxygen for metabolic activities including contaminant degradation (Martin *et al.,* 2014). As a result, beneficial rhizosphere microbiome may be selectively attracted towards roots thereby leading to increased metabolic activities (Correa-Garcia *et al.*, 2018). There is evidence that certain exudates specifically trigger enzymatic pathways for degradation of particular hydrocarbon compounds. They may also act as analogues to particular contaminants especially if they have related chemical structures (Correa-Garcia *et al.*, 2018).

Likewise, root exudates actively modulate the composition, diversity, and microbial activities in therhizosphere. The availability of organic contaminants for microbial metabolism is equally enhanced bythe exudates (Correa-Garcia *et al.,* 2018). Some of the root exudates (e.g. phenolics and flavonoids) act as inducers of genes for degradation pathways by rhizosphere microorganisms due to their resemblance with contaminants and as a result, catabolic genes for contaminants are boosted within the rhizosphere (Hoang *et al.*, 2021). Studies by Shukla *et al.* (2011) revealed that degradation of PAHs and their derivatives in *Sorghum* sp. rhizosphere might be linked to enzymatic activity of oxidoreductases released from the roots as exudates. However, root exudates offer special benefits to their host plant in addition to that of the microbes.Research findings have shown that the growth of competing plant species close to the host is inhibitedthrough root exudation (Schandry and Becker, 2019). They also use exudates to attract beneficial microbesand regulate rhizosphere microbial community composition (Vieira *et al.,* 2020). Flavonoidspresent in root exudates of legumes activate the *Rhizobium melilotig*enes coding for the nodulationprocess (Becard *et al.*, 1995). The root cells are protected by defense proteins like phytoalexins andother unknown chemicals from pathogenic bacteria (Flores *et al.*, 1999). In some cases, the plants andmicrobially produced compounds are further degraded to yield allelopathic or other toxic compounds,which are inhibitory to pathogenic microbes (Velmourougane*et al.*, 2017).

Table 2.3: Various compounds in root exudates of different plant species

Group	Compounds
Amino acids	a-Alanine, b-alanine, asparagines, aspartate, cystein, cystine, glutamate, glycine, isoleucine, leucine, lysine, methionine, serine, threonine, proline, valine, tryptophan, ornithine, histidine, arginine, homoserine, phenylalanine, c-Aminobutyric acid,a-Aminoadipic acid
Organic acids	Citric acid, oxalic acid, malic acid, fumaric acid, succinic acid, acetic acid, butyric acid, valeric acid, glycolic acid, piscidic acid, formic acid, aconitic acid, lactic acid, pyruvic acid, glutaric acid, malonic acid, tetronic acid, aldonic acid, erythronic acid
Sugars	Glucose, fructose, galactose, ribose, xylose, rhamnose, arabinose, desoxyribose, oligosaccharides, raffinose, maltose
Vitamins	Biotin, thiamin, pantothenate, riboflavin, niacin
	nine, cytidine, uridine
Enzymes	Acid/alkaline-phosphatase, invertase, amylase, protease
Inorganic ions and	HCO_3 , OH, H^+ CO_2ÆH$_2$ gaseous molecules

Source: Velmourouganeet *al*. (2017)

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Description of Study Sites

3.1.1 Niger State

Niger State whichwas created on the 3rdFebuary 1976 lies on latitude 80º to 11º30' North and Longitude 03º 30' to 07º 40' East (Baldrian*et al*., 2000). The State is bordered to the North by Zamfara State, West by Kebbi State, South by Kogi State, South West by Kwara State, North-East by Kaduna State and South East by the Federal Capital Territory (FCT). The State also has an International Boundary with the Republic of Benin along Agwara and BorguLocal Government Areas (LGAs) to the North West. Land mass is 76, 469.903 Square Kilometers which is about 10% of the total land area of Nigeria out of which about 85% is arable (Niger State Bureau of Statistics, 2012). The majority of the populace in the State (85%) are farmers while the remaining15% are involved in other vocations such as white collar jobs, business, craft and arts.

Niger State experiences distinct dry and wet seasons with annual rain fall varying from 1,100mm in the northern parts to 1,600mm in the southern parts. The maximum temperature (usually not more than 34ºC) is recorded between March and June, while the minimum is usually between December and January. The rainy seasons last for about 120 days in the northern parts and about 150 days in the southern parts of the State (Niger State Bureau of Statistics, 2012).Niger State has twenty five (25) Local Government Areas. Four different study sites were used for this study namely, Minna, Bida, Suleja and Tegina, all located in Niger State, Nigeria (Figure 3.1a and Figure 3.1b).

3.1.2 Minna

Minna is a city in Middle Belt Nigeria. It is the capital of Niger State, one of Nigeria's 36 federal States.Cotton, guinea corn (sorghum), and ginger are the main agricultural products of the city. Yam is also extensively cultivated throughout the city. The economy also supports cattle trading, brewing, shea nut processing and gold mining. The estimated population of Minna according to the 2006 population census result is a total of 304,113 (NPC, 2006).

3.1.3Bida

Bida is a Local Government Area in Niger State. The LGA has an area of 51 km² andBida is the second largest city in Niger State with an estimated population of 266,008 according to the population census of 2006 (NPC, 2006). It is located southwest of Minna, capital of Niger State, and is a dry, arid town. Districts found in Bida include Katcha, Enagi, Baddeggi, Agaie, Pategi, Lemu and Kutigi. The town is known for its production of traditional crafts, notably glass, bronze articrafts and brass wares. The major ethnic group is the Nupe (Nigerian Library Association, NLA, 2017).

3.1.4 Suleja

Suleja is a city in Niger State, Nigeria, with an estimated population of 260, 240 (NPC, 2006), Suleja,located north of Abuja, is the capital of the Suleja Emirate. Today Suleja is well known as an exporter of Gbari pottery. Cotton weaving and dyeing, with locally grown indigo, and mat making are traditional activities, but farming remains the chief occupation. Local trade is primarily in agricultural products (Rea, 2003).

3.1.5Tegina

Tegina is a town in Rafi LGA, Niger State, Nigeria. Various Kainji languages such as Kambari, Kamuku, Hausa and Fulani are spoken in and around Tegina. The estimated

population of Tegina is 24,037 (NPC, 2006). The predominant occupation of the dwellers is farming, cattle rearing and trading. The predominant languages spoken in Tegina are Kambari, Kamuku and Hausa.

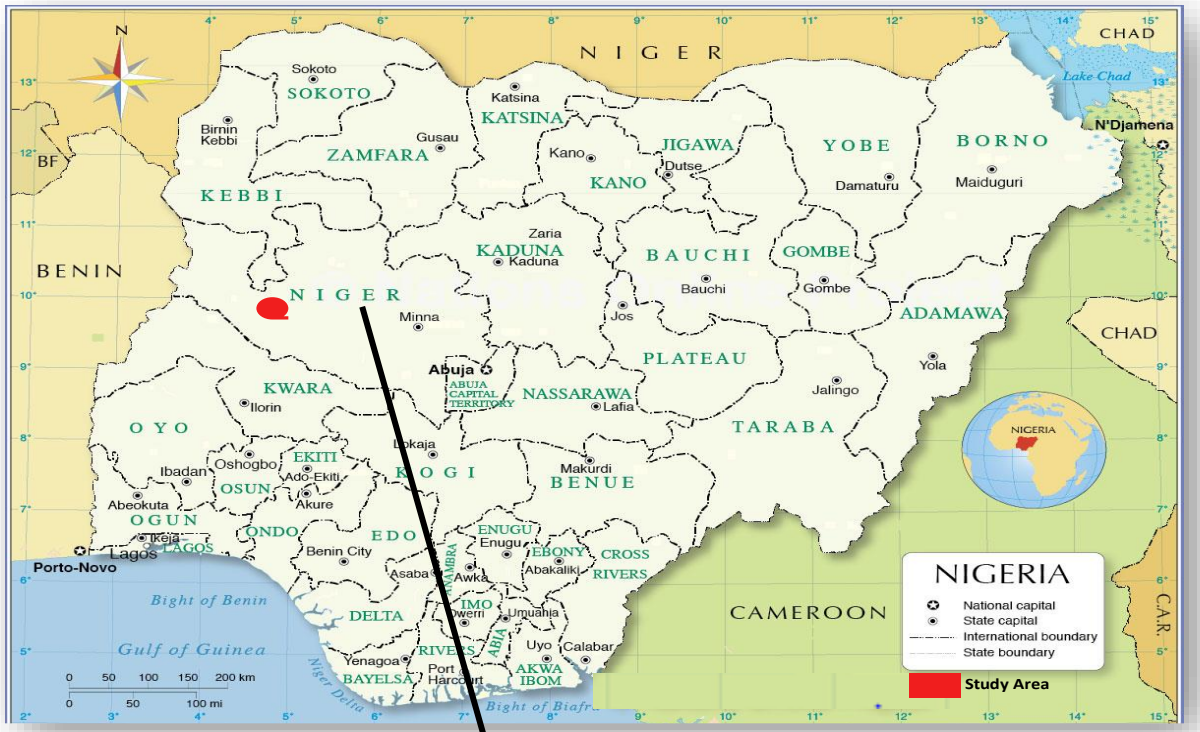

Figure 3.1a: Map of Nigeria Showing Niger State

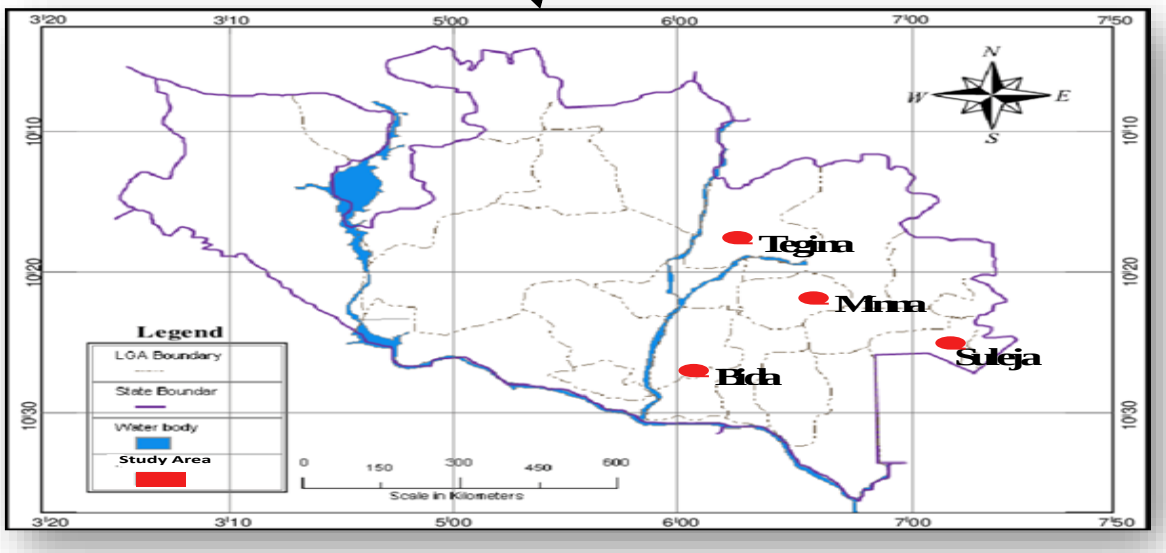

Figure 3.1b: Map of Niger State Showing Study Area

Source: Department of Geography, Federal University of Technology Minna.

3.2 Preliminary Survey of Automobile Workshops

Major automobile workshops were identified in each of the locations namely, Minna, Bida, Suleja and Tegina (Figure3.1b). These locations were chosen due the presence of heavy duty vehicles and clusters of automobile shops. The different types of plants, grasses and shrubs present were counted and five dominant plant samples from each location were collected in polythene bags for identification and preservation. This survey was done for one(1) month,after which two (2) plants that were found consistent in all of the four locations were chosen for the phytoremediation study. However, the plants fulfilled atleast 80% of the following conditions:

i. The plants were abundant in all the locations,
ii. Were found in all the locations selected
iii. Must be propagateable
iv. Rapid growth rate
v. Tolerance to pollutant
vi. Extensive root system
vii. High biomass

3.3Experimental Design

The experimental design for the phytoremediation study was a complete randomized design (CRD) as presented in Table 3.1

Table 3.1 Design of the Phytoremediation Studies

Treatments	Treatment Code	Details of the Treatment
1	PS1	Soil (5kg) alone
2	PS2	Soil (5kg)+ Plant 1
3	PS3	Soil (5kg)+Plant 2
4	PS4	Soil (5kg)+SEO (50cl)+ Plant 1
5	PS5	Soil (5kg)+ SEO (70cl) + Plant 1
6	PS6	Soil (5kg)+ SEO (50cl) + Plant 2
7	PS7	Soil (5Kg) + SEO (70cl) + Plant 2

Keys: PS= Phytoremediation study, SEO= Spent Engine Oil

3.3.1Experimental setup

The two (2) plants wereidentified by a Plant Biologist from the Federal University of Technology Minna, (plant 1: *Melissia officinalis* plant 2: *Urena lobata*) that were found consistent in all four locations were used for this study and two concentrations(50 and 70cl)of Spent lubricating oil was used to pollute the soil, and was studied for seven months. The plants were raised in nurseryfrom seeds for two weeks before introducing them to the SEO polluted soil.The phytoremediation study wasconducted according to the methoddescribed by Baldrian*et al.* (2000) and the set up wasas follows:

Fivekilogrammes (5kg) of moistened soil was poured in a 20 litre plastic container,in duplicates.Treatment one: only soil (for control), Treatment two: Soil + Plant 1, Treatment three: Soil + Plant 2, Treatment four: Soil + 50cl of Spent engine oil + Plant 1, Treatment five: Soil + 70cl of Spent engine oil + Plant 1, Treatment six: Soil + 50cl of Spent engine oil + Plant 2, Treatment seven: Soil + 70cl of Spent engine oil + Plant 2. This set up was studied for seven months and the experimental layout was conducted at the biological garden of the Federal University of Technology, Minna.

3.4 Collection of Samples

i. Soil samples were collected from 5 randomly selected automobile workshops. At each sampling point (25 metres on each side), samples were collected in triplicates in a clean labeled polythene bag; each from the depth 0 – 15 cm using a hand auger and transported to the laboratory for analysis.

ii. Soil samples were also collected from non-contaminated areas at about 50 metres from the contaminated sites, which served as control.

iii. Soil samples from rhizosphere of plants were collected after uprooting the plant and the soil attached to the root was gently shaken and soil sample collected in clean and sterile polythene bag and transported to the laboratory for analysis.

iv. Spent engine oil was collected from an automobile workshop in clean jerry-can from automobile workshop in Minna, Niger State and transported to the laboratory for the phytoremediation studies.

v. Plant samples were collected from the vicinity of the automobile workshops in polythene bags for identification and phytoremediation studies

vi. Soil samples were collected every month, from the various treatments as indicated in the experimental design, in clean polythene bags using hand trowel and transported to the laboratory for microbiological analysis, physical and chemical analyse and for phytoremediation studies.

3.5 Enumeration of Bacteria

Total aerobic heterotrophic bacteria (TAHB) were enumerated by spread plate technique, by inoculating 0.1mL of serially diluted sample onto Nutrient agar (NA) plates. Spent engine oil (SEO) degrading bacteria (SEODB) were enumerated on spent engine oil agar (SEOA, 1.2g KH_2PO_4, 1.8g K_2HPO_4 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$,

0.1g NaCl, 0.01g $FeSO_4.7H_2O$ and 20g agar per liter, pH 7.4; supplemented with 0.1% SEO). The Nutrient agar plates were incubated at 35 ± 2^0C for 48 hours while the SEOA plates were incubated at 35 ± 2^0C for 5 days, as described by Ijah*et al.* (2008). Colonies, whichappeared on the plates, were counted and expressed as colony forming units per gram of soil (CFU/g). Pure cultures were obtained by repeated sub-culturing and maintained on agar slants for further characterization and identification(Holt *et al.* 1994;Cheesebrough, 2006).

3.6 Enumeration of Fungi

A 0.1mL aliquot of appropriate dillutions of SEO polluted soil sample was inoculated into replicate Petri dishes containing Sabouraud dextrose agar and incubated for 5days at room temperature (28^0C). An oil agar medium (SEOA, 1.2g KH_2PO_4, 1.8g K_2HPO_4 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 0.01g $FeSO_4.7H_2O$ and 20g agar per liter, pH 7.4; supplemented with 0.1% SEO and 0.5g 0f Chloraphenicol)was used for the isolation of SEO utilizing mycoflora, and incubated at room temperature for 9days as reported by Chukwura (2016). Colonies formed were counted and expressed as cfu/g. Pure cultures were obtained by repeated sub-culturing and maintained on agar slants for further characterization and identification.

3.7Characterisation and Identification of Microbial Isolates

The bacterial isolates were identified based on the Taxonomic Schemes of Cowan and Steel (1974) and characterized using standard biochemical tests. Gram staining and the biochemical tests were carried using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*1994) and Cheesebrough (2006). Some of the tests carried out are presented in Appendix A.

The fungal isolates were characterized based on the color of aerial and substrate hyphae, shape and kind of asexual spores, presence of foot cell, sporangiophore, conidiophores, and characteristics of spore head. A small portion of mycelial growth was carefully picked and placed in a drop of lactophenol cotton blue on a slide and covered with cover slip. After microscopic examination, the fungal isolates were identified by comparing their characteristics with those of known taxa using the schemes of Nagamani*et al.* (2006).

3.8 Screening of Bacterial Isolates for Potential to Utilize Spent Engine Oil

Selected bacterial isolates were grown separately in nutrient broth at 28 ± 2^0Cfor 24hours. The utilization of spent engine oil as the sole carbon and energy source by the selected bacterial isolates was determined using the mineral salt medium (MSM) of Zajic and Supplisson (1972). Five millilitres (5ml) of mineral salts medium were dispensed in each bottle containing 0.05ml of spent engine oil. After sterilization at 121^oC for 15 minutes, the bottle was allowed to cool before being inoculated with 0.1ml of Nutrient broth grown culture bacterial isolates. The bottles were incubated at room temperature (28 ± 2^0C) for 10 days according to the methods of Ijah and Antai (2003a). The growth of the organism in the oil medium at the end of incubation was determined by the use of spectrophotomer to examine the degree of turbidity of the oil medium.

3.9 Screening of Fungal Isolates for Potential to Utilize Spent Engine Oil

Ability of fungal isolates to utilize spent engine oil (SEO) as sole carbon source was determined. Pure culture of each isolate was inoculated into mineral salt medium (Nagamani*et al*. 2006) broth incorporated with sterile SEO (1% v/v), redox indicator 2,6-dichlorophenol indophenols (2% w/v) and Tween 80 (0.1% v/v). The control flask had no organism. Incubation was done at room temperature ($28\pm2°C$) with constant

shaking at 180rev/min for 7days. The aliquots in the flasks were monitored daily for color change from deep blue to colorless. After 7days incubation, 5ml was collected from each flask and centrifuged (5000rpm) at room temperature for 5minutes and the supernatant wasread using a HACH 2010 portable data logging spectrophotometer at 600nm (Chukwura*et al.,* 2016).

3.10 Molecular Identification of Isolated Microorganism

(i) Extraction of DNA

For further characterization and identification of bacterial isolates, the chromosomal DNA of the organisms was extracted using the Qiagen Dneasy Blood and Tissue Kit (Cat. 69506). The growth from the broth was pelletized in a well labelled seven 1.5mL microcentrifuge tubes, two hundred microliter (200µl) Buffer AL was added to each of the tubes and mixed by vortexing. The tubes were incubated at 56^0C for 10 minutes. Two hundred microliter (200µl) of ethanol (96%) was added and mixed thoroughly by vortexing. The mixture was pipetted into a DNeasy Mini spin column in a 2 mL collection tube and centrifuged at 6000 x g (8000 rpm) for 1 minute. The flow-through and collection tubes were discarded. The spin columns were placed in new 2 mL collection tubes. Five hundred microliter (500µl) of Buffer AW1 was added to the spin column and centrifuged at 6000 x g for 1 minute. The flow-through and collection tube were discarded and the spin columns were placed in new 2 mL collection tubes, five hundred microliter (500µl) of Buffer AW2 was added to the tubes and centrifuged at 20,000 x g (14,000 rpm) for 3 minutes. The flow-through and collection tube were discarded and the spin column was carefully removed to avoid contact with the flow-through. The spin column was transferred into new 1.5mL microcentrifuge tubes, 200µl of Buffer AE was added to the centre of the spin column for elution of the genomic

DNA and then incubated for 1 minute at room temperature and centrifuged at 6000 x g for 1 minute. DNA quality and concentration was checked by running 2µl of the diluted DNA sample on 1% agarose gel. Accurate DNA quantification was carried out using a NANODROP®2000 spectrophotometer (Thermo Scientific Inc.) (Altschul*et al.*, 1990).

(ii)Polymerase Chain Reaction (PCR) Amplification of DNA

Polymerase chain reaction amplification of the extracted DNA was carried out with the 16S primer. Polymerase chain reaction was carried out in a total volume of 25µl containing 100ng of genomic DNA, 2.5µl of 10× PCR buffer, 1µl of 50mM $MgCl_2$, 2µl of 2.5mM dNTPs (Thermo Scientific), 0.1µl Taq polymerase (Thermo Scientific), 1µl of DMSO, 1µl each of forward and reverse primers and 11.3µl of H_2O. Touch-down PCR was used for amplification as follows: initial denaturation step of 5minutes at 94°C, followed by 9 cycles each consisting of a denaturation step of 20 seconds at 94°C, annealing step of 30 seconds at 65˚C, and an extension step of 72°C for 45 seconds. This was followed by another 30 cycles each consisting of a denaturation step of 20 seconds at 94°C, annealing step of 30sec at 55˚C, and an extension step of 72°C for 45sec. All amplification reactions were performed in a GeneAmp® PCR System 9700, Applied Biosystems. Polymerase chain reaction amplicons was loaded on 1.5% agarose gel and run at 100volts for 2 hours (Altschul*et al.*, 1990).

(iii)DNA Sequencing

For sequencing, the amplicons with single band wasselected from the amplified products and purified using manufacturer's protocol (QIAquick PCR Purification Kit, cat. No.28106). Sequencing wasperformed using a Big Dye terminator cycle sequencing kit (Applied BioSystems), Unincorporated dye terminators was then purified and precipitated using ethanol EDTA solution. The pellets were then re-dissolved in

HiDiformamide buffer (Applied Biosystems Cat No. 4311320). Sequencing was performed using 3130xl Genetic Analyser. The resulting patterns were then compared with the 16s rRNA nucleotide sequences present in BLAST tool of Genbank at NCBI (Altschul*et al.*,1990; Lodish*et al.,* 2004).

3.11 Determination of Total Petroleum Hydrocarbon (TPH)

This was determined gravimetrically by diethyl ether cold extraction method of Adesodun and Mbagwu (2008). Ten (10) grams of soil sample was weighed into 250ml capacity conical flask and 50 mL of diethyl ether was added, and shaken for 30minutes in an orbital shaker. The diethyl ether extract was filtered with Whatman No. 1 filter paper, the liquid phase of the extract was measured at 420nm wavelength using spectrophotometer. The TPH in soil was estimated with reference to the standard curve. total petroleum hydrocarbon data were fitted to first- order kinetics model of Yeung *et al.* (1997) and Abioye*et al.* (2011) as presented in equation 3.1

$$y = ae^{-kt} \qquad (3.1)$$

Where: y represents the residual hydrocarbon content in soil (g kg-^{1}), a stands for the initial hydrocarbon content in soil (g kg^{-1}), ‘k’ is the biodegradation rate (d-1) and ‘t’ is time (days). The model predict the biodegradation rate and half-life of hydrocarbons within the soil. Half-life was then calculated using the model of Yeung *et al.* (1997) as Half life = ln(2)/k. This model was using the assumption that the degradation rates of hydrocarbons positively correlate while using hydrocarbon pool size in the soil.

3.12Gas Chromatography Mass- Spectrophotometry Analysis of Residual SEO

After 1, 4 and 7 months, the residual oil was extracted from the soil by solvent extraction. The oil extracts from the soil samples wereanalyzed using GC-MS (QP2010

PLUS, Shimadzu, Japan) to determine the hydrocarbon degradation. GC-FID analyses were performed using a Chrompack CP 9000 gas chromatograph with an FID detector using splitless injection as described by Paula *et al.* (2012). A WCOT Fused Silica, stationary phase: CPSIL-8 CB (25 m × 0.25 mm i.d. with 0.4-µm film thickness) column was used. Nitrogen was used as carrier gas and hydrogen and oxygen were used as FID gases. Maestro software was used for data acquisition and processing. Volumes of 1 µL were injected using a 10^{-} µL microsyringe.

3.13 Physical and Chemical Analyses of the Samples (Polluted and Unpolluted Soil)

3.13.1 Determination of pH

The pH of the soil sample was determined by the potentiometric method. Ten grams of each sample was introduced into a 50 millilitre capacity beaker after which 20 millilitres of 0.01 M calcium chloride solution was added and stirred. The sample was allowed to stand for 30 minutes with occasional stirring so that the sediments could settle. The glass electrode of Testronic digital pH meter (Model 511) was immersed into the suspension to determine the pH reading of the sample. The test was repeated for each sample and the average of the duplicate samples were recorded (Ajao *et al.*, 2011).

3.13.2Determination of moisture

Moisture content of the soil samples was determined using the gravimetric methods described by Black (1965) and Agbenin (1995). The moisture-can was weighed using an electronic weighing balance. The can and the samples were weighed and transferred to a hot spot convectional oven (Genlab, MIN0150). The samples were dried in the oven at 105^{0}C for 5 hours. The samples were transferred to a desiccator and allowed to cool. The weights of the oven-dried samples were obtained using the electronic balance and the percentage moisture content was calculated thus using equation 3.2

% Moisture content = $\frac{B-C}{B-A} \times 100$ (3.2)

Where:

A= Weight of moisture can (grams)

B=Weight of can + Wet sample (grams)

C=Weight of can + Oven-dried sample (grams)

3.13.3 Determination of organic carbon

This was carried out using the method of Agbenin (1995) in which 1 g of 0.5 mm sieved soil samples was weighed in duplicates and transferred to a 250 mL capacity Erlenmeyer flask. Ten (10) millilitres of 1M $K_2Cr_2O_7$ solution was introduced into each flask and swirled gently. Twenty (20) milliliters of concentrated H_2SO_4wasadded rapidly using an automated pipette, directing the stream into the suspension. The flask was immediately swirled gently until the sample and reagents were mixed, and swirled more vigorously for one minute. The flask was rotated again and allowed to stand on a sheet of asbestos for 30 minutes after which 100 mL of distilled water was added. Three drops of the indicator (Barium-diphenylamine-Sulphonate) was added and then titrated with 0.5 M ferrous sulphate solution. As the end point was approached, the solution took on a greenish cast and then changed to dark green. At this point, the ferrous sulphate was added drop by drop until the colour changed sharply from blue to red (maroon colour) in reflected light against a white background. The blank was prepared in the same manner but without the sample to standardize the dichromate. The percentage carbon was calculated using equation 3.3:

% Organic carbon in soil

$$= \frac{(\text{me } K_2Cr_2O_7 - \text{me } FeSO_4) \times 0.003\ 100 \times (f)(\text{air-dry basis})}{1\text{g of air-dry soil}} \quad (3.3)$$

where:

Correction factor, f= 1.33

me = Molarity of solution x cm^3 of solution used

% Organic matter in soil = % Organic Carbon ×1.729 (IITA, 1979).

3.13.4 Determination of total nitrogen

In carrying out this experiment, the Macro-Kjeldahl method described by Black (1965) and Agbenin (1995) was used. Five (5) grams of each soil sample was weighed and moistened with a small amount of water into a Kjeldahl flask. Forty (40) milliliters of concentrated H_2SO_4 and three Kjeldahl tablets were added and the mixture was heated at 150^o C for 2 hours and at 390^o C for 4 hours. After the digestion, the mixture was cooled, filtered and made up to 100 cm^3 with distilled water. A 10 millilitre aliquot of the filtrate was introduced into the reaction flask and 10 milliliters of 10 M NaOH solution was added. The solution inlet of the apparatus was corked and steam distilled. The distillate was collected in a 50 milliliters capacity conical flask containing 5 milliliters of boric acid (4%) with two drops of mixed indicator (0.02g methyl red mixed with 0.1g bromocresol green and 43.8 milliliters of ethanol and 16.2 milliliters of distilled water). Moistened red litmus paper was used to determine the presence or absence of NH_3 coming directly from the condenser. The distillate was titrated with standardized 0.1M HCl. The total nitrogen was calculated using equation 3.4:

$$\%\text{Nitrogen} = \frac{(\text{Volume of HCl } cm^3 \text{sample} - \text{Volume of HCl } cm^3 \text{ blank}) \times 0.14 \text{ x df}}{\text{Volume of aliquot} \times \text{Weight of sample}} \quad (3.4)$$

where:

HCl= Volume of Hydrochloric acid in cm^3

df=Dilution factor.

3.13.5 Determination of particle size and textural class of soil

In carrying out this experiment, the hydrometer method described by IITA (1979) was used. Fifty (50) grams of oven dried soil sample, which was passed through a 2 mm sieve was introduced into a milkshake mix cup and 50 cm^3 of 5% sodium hexa-metaphosphate was added along with 100 cm^3 of distilled water. Stirring rod was accustomed to mix the sample, this was allowed for 30 minutes. The contents of the cylinder were thoroughly mixed by inversion several times until all soil was in suspension. The cylinder was positioned on a flat surface area and time observed. Immediately, the hydrometer was introduced into the soil suspension slowly till the hydrometer was floating. The first reading on the hydrometer was taken 40 seconds after the cylinder wasset down. The hydrometer was removed and temperature of the suspension recorded.

After the first hydrometer reading, the suspension was allowed to stand for 3 hours before a second reading was considered. In addition, the temperature from the suspension was considered. According to IITA (1979), the first reading measures the percentage of silt and clay in suspension. The second reading indicates the percentage of total clay in the suspension. Results were corrected to a temperature of 20^oC. In addition, 2.0 was subtracted from every hydrometer reading to compensate for added dispensing agent. The percentage weight of sand, silt and clay for the soils were calculated using equation 3.5 (IITA, 1979):

$$C = R - R_L + 0.36\ T \times \frac{100}{50}(3.5)$$

where:

C = Corrected hydrometer reading

R = Reading of the sample

R_L = Reading of the blank

T = Change in temperature (^oC)

After the values for silt and clay had been determined, the value for sand was obtained by subtracting the values of silt and clay from 100. The soil was classified using the textural triangle.

3.13.6Determination of available phosphorus

For this determination, the Bray No. 1 method of Bray and Kurtz (1945) was used. One gram of air dried soil sample that was passed through a 2 mm sieve was weighed into a 15 milliliter centrifuge tube and 7 cm^3 of 1 M NH_4F and 25 milliliters of 0.5 M HCl to 460 milliliters distilled water (extracting solution). The mixture was shaken for 1 minute on a mechanical shaker and the suspension centrifuged at 2000 rpm for 15 minutes. Two milliliters of the clear filtrate was introduced into a 20 cm^3 test tube, 5 cm^3 of distilled water and 2 cm^3 of ammonium molybdate solution was added. The content was mixed properly and 1 cm^3 of $SnCl_2$.$2H_20$ dilute solution was added and mixed again. After 5 minutes, the percentage transmittance was measured on the spectrophotometer at 660 nm wavelength. A standard curve within the range of 0-1µg P/ml (or ppm P) was prepared. The optical density (OD) of the standard solution was

plotted against the μg P/ml (or ppm P) and the content of extractable phosphorus in the soil was calculated using equation 3.6 Bray and Kurtz (1945):

$$\text{ppm P} = \frac{\text{Off curve reading} \times \text{Dilution factor} \times \text{Volume of extract}}{\text{Initial weight of soil}} \quad (3.6)$$

3.13.7 Exchangeable cations and cation exchange capacity in soil

a) Determination of Sodium and Potassium

Sodium and potassium were determined according to the methods of IITA (1979) and Agbenin (1995). To five grams of each sample, 30 cm^3 of 1 M NH_4OAc was added and shaken on a mechanical shaker for 2 hours. It was centrifuged at 9000 g for 10 minutes and the clear supernatant was carefully decanted into a 100 cm^3 volumetric flask. Another 30 cm^3 of NH_4OAc solution was added and shaken for 30 minutes. It was centrifuged at 9000 rpm for 10 minutes and the supernatant was transferred into the same volumetric flask. This was repeated and the supernatant was transferred into the same volumetric flask. It was made up to the 1 litre mark with the NH_4OAc solution. The potassium and sodium was determined on a flame photometer (Jenway PFP-7) after calibration with sodium and potassium standards.

(b) Determination of Mg^{++} and Ca^{++}

This was done according to the method of Agbenin (1995) using the disodium ethylenediamine tetra-acetic acid (EDTA) titration procedure. Calcium and magnesium was determined first and then calcium, after which the value of magnesium was obtained by subtracting the value of calcium from magnesium and calcium.

A reference end point was first determined by mixing 5 cm^3 of 1MNaOH with 5 drops of calcon, and diluted to 100 cm^3 with distilled water and then titrated with Na_2-EDTA solution. Five cubic centimetres aliquot of the sample extracts were introduced into a flask in which 100 cm^3 of water, 5 cm^3 of 1M NaOH and 5 drops of the indicator (Eriochrome Black-T) wasadded. It was titrated against Na_2-EDTA solution to obtain the end point, which was indicated by the matching of the colour of the solution to the reference end point. Blank titration was carried out as earlier done and subtracted from the sample reading.

Five cubic centimetres (5cm^3) of the sample solutions was introduced into each titration flask and diluted to 100 cm^3 with water. Fifteen (15) cubic centimetres of buffer solution (ammonium hydroxide), 10 drops of the indicator and 2cm^3 oftriethanolamic solution was added to each flask. They were titrated against Na_2-EDTA solution from red colour to a clear blue. Blank titration was carried out in the same manner and subtracted from the sample reading. The centimetre-equivalent of calcium and magnesium was determined using equation 3.7 of Agbenin (1995):

$$\text{C.eq. } Ca^{2+} + Mg^{2+}/100g \text{ soil} = M \times V \times df \times \frac{\mathbf{100}}{\mathbf{S}} \quad (3.7)$$

Where:

M = Molarity of the EDTA

V = Volume of EDTA used

df = Dilution factor

S = Original weight of soil

(c) Determination of Ca^{++}

A reference point was first obtained by mixing 5cm^3 of 1M NaOH with 5 drops of calcon and diluted to 100 cm^3 with water and then titrated with Na_2-EDTA solution. Five (5) cubic centimetres aliquot of the sample extract was introduced into a flask after which 100 cm^3 of water, 5cm^3 of 1 M NaOH and 5 drops of indicator was added. This mixture was titrated with Na_2-EDTA solution to obtain the end point which was indicated by matching of the colour of the solution to the reference end point. The blank titration wascarried out in the same manner and subtracted from the sample reading. The value of calcium was calculated using equation 3.8:

If X cm^3 of Na_2-EDTA solution was required for titration,

Ca (gkg^{-1} soil) – X(cm^3) × volume of solution (3.8)

10 × 5 cm^3 aliquot × sample wt (g)

Value obtained was subtracted from Mg^{++} + Ca^{++} to get Mg^{++}.

3.14 Analysis of Spent Engine Oil for Trace and Heavy Metals

The Spent engine oilsamples were digested with a mixture (3:1) of concentrated nitric acid and hydrofluoric acid in microwave assisted Kjeldahl digestion. Each microwave extraction vessel was added with 6ml of nitric acid and 2ml of hydrofluoric acid together with 0.8 g spent engine oil sample. The vessel was capped and heated in a microwave unit at 800 W to a temperature of 190^0C for 20 min with a pressure of 25 bars. The digested samples were diluted to 50 mL and subjected to analysis of the metals by atomic absorption spectrophotometer using flame atomization (Kai *et al.*, 2012).

3.15Remediation of Spent Engine Oil Polluted Soil

3.15.1 Microbial counts and identification

Changes in microbial population of the respective treatments were assessed by spread inoculating 0.1 ml of serially diluted sample onto Nutrient agar plates (NA) for the enumeration of total aerobic heterotrophic bacteria, and incubating at 30^0C for 48 hours. Spent Engine oil degrading bacteria (SEODB) were enumerated on spent engine oil agar (SEOA). The spent engine oil plates were incubated at 30^0C for 5 days. The colonies that developed after incubation were counted and expressed as colony forming units per gram (cfu/g) of soil sample.The bacterial isolates were identified as described in section 3.7of the present study.

Total heterotrophic fungi were enumerated by spread plating 0.1 ml of the serially diluted SEO polluted soil on Sabouraud Dextrose Agar (SDA) and incubating at room temperature (28 ± 2^0C) for 3-5days. Spent engine oil degrading fungi were enumerated on spent engine oil agar (SEOA) in which 50ug/ml of streptomycin and 30ug/ml of penicillin were added to inhibit the growth of bacteria. This was then incubated at room temperaturefor 7days. The fungal colonies that developed after incubation were counted and expressed as colony forming units per gram (cfu/g) of the soil sample(Cheesebrough, 2006).

The cultural characteristics of the pure isolates were noted and the microscopic features of both the filamentous fungal and yeast isolates were observed using the wet mount technique (Cheesebrough, 2006). Pure cultures were stored on SDA slants for further characterization. Lactophenol cotton blue stain was used as mordant. Biochemical tests such as urea hydrolysis, sugar fermentation tests and assimilation of carbon compounds were conducted to further characterize the yeast isolates.The taxonomic schemes of Nagamani*et al.* (2006) and Cheesbrough, (2006) was used to identify the fungi.

3.15.2Extraction, identification of the exudates from the rhizosphere of the plants used

The roots of *Melissia officinalis* and *Urena lobata* was collected, dried at room temperature, and ground using a blender. Five grams of each of the parts were weighed using an electronic weighing balance and cool extraction was carried out using Diethylether; Whatman filter paper was used to filter the extract. The extract was analysedusing for GCMS (described in section 3.12 of the present study) to determine the different compounds exuded from the roots of the plants used.

3.15.3 Analysis ofheavy metals in the soil

The acid digestion method was used to determine the concentration of heavy metals in the soil samples (Saparna*et al.*, 2011). An amount of 2 g of each soil sample was introduced in a screw capped Erlenmeyer flask and 15 mL of 4 N nitric acid was added to it. After that, the flask was placed in a hot water bathat 80^0C for 12 hours. Then, the samples were passed through a filter paper and the heavy metals in the filtratewere determined using the atomic absorption spectrophotometer. The dry ash extraction method was used to determine total concentration of the heavy metals in the plant samples (Saparna*et al.*, 2011). To do this, 2g of each plant sample was put in a porcelain crucible. The samples were placed in an oven at 55°C for 2 hours. Then, 5 mL of 2N hydrochloric acid was added to the samples. The samples were passed through the filter paper and the heavy metal was determined using the atomic absorption spectrophotometer.

3.15.4 Determination of heavy metals in the harvested plants

After harvesting, plant shoots and roots were separated from soil, carefully washed first with tap water, and then with distilled water for several times. All the samples were air-

dried for seven days. The samples were then oven-dried at 60^0C to a constant weight and dry biomass weights were recorded and ground to powder using a horizontal grinder (Kai *et al.,* 2012). The dried samples were digested with a mixture (3:1) of concentrated nitric acid and hydrofluoric acid in microwave assisted Kjeldahl digestion. Each microwave extraction vessel received 6 mL of nitric acid and 2 mL of hydrofluoric acid together with 0.8 g of plant sample. The vessels were capped and heated in a microwave unit at 800 W to a temperature of 190^0C for 20 min with a pressure of 25 bars. The digested samples were diluted to 50 mL and subjected to analysis of the metals by atomic absorption spectrophotometer using flame atomization. Results were expressed on dry weight basis of each component (Kai *et al.,* 2012).

3.15.5 Evaluation of phytoremediation factors

i. Bio-concentration factor (BCF): This was calculated using the metal concentration ratio in plant roots to that in soil (Yoon *et al.,* 2006; Nazir *et al.,* 2011) and is given as follows: BCF = metal concentration in root/metal concentration in soil.

ii. Translocation factor (TF): This is the ratio of metal concentration in plant shoot to that in plant root. TF = metal concentration in plant shoot/metal concentration in plant root (Yoon *et al.,* 2006; Ameh*et al.,* 2019).

iii. Biological accumulation coefficient (BAC): This is defined as the concentration of metals in plant shoots divided by metal concentration in soil (Nazir *et al.,* 2011) and is given as follows: BAC = concentration of metal in plant shoots/metals concentration in soil.

iv. Enrichment factor (EF): This is the ratio of metal concentration in plant leaves to metal concentration in soil. EF = concentration of metal in leaves/concentration of metal in soil (Lorestani*et al.,* 2011).

3.15.6 Determination of total petroleum hydrocarcabon (TPH)

Total petroleum hydrocarbon (TPH) was carried out as decribed in Section (3.11) of this present study.

3.15.7 Gas chromatography mass- spectrophotometry analysis of residual SEO

Gas chromatography mass- spectrophotometry analysis of residual spent engine oil was carried out as described in section (3.12) of this present study.

3.16Data Analysis

The data that were generated from the microbial counts wereanalysed in triplicates and data generated from SPSS (Version 20) was reported as Mean± Standard Error. One way analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) were used to determine significant differences, considering a level of significance of less than 5% ($P<0.05$).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Plants found within the vicinity of automobile workshops

The counts of the different plants found within the vicinity of the automobile workshops are represented in Appendix B, which showed that*Melissa officinalis*was more in Suleja (52 stands) and across all the workshops visited (with a total number of 171), closely followed by *Urena Lobata*with 50 stands and a total number of 173 in Bida.

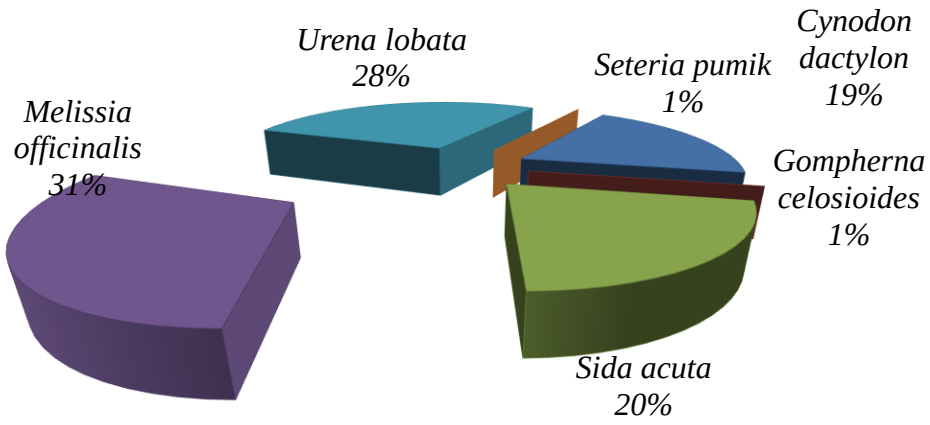

Figure 4.1: Percentage Counts of Plants found within the vicinity of Automobile workshops

A

B

C

D

Plate III: Automobile Workshop at: A= Minna, B= Suleja, C=Tegina, D= Bida

Table 4.1: Major plants found within the vicinity of automobile workshops

Plant	Minna	Bida	Suleja	Tegina
Cynodondactylon	√	√	×	×
Gompherynacelosioides	×	√	√	×
Sida acuta	√	×	√	×
Melissia officinalis	√	√	√	√
Urena lobata	√	√	√	√
Seteria Pumik	×	×	√	√

*Where √= Present,× = Absent

4.1.2 Total aerobic heterotrophic bacterial (TAHB) counts in soil from automobile workshops

The results (Figure 4.2) revealed that the automobile workshops in Minna, hadplants in its vicinity in which the rhizosphere harboured high aerobic bacterial counts ($1.42\times10^7\pm2.41\times10^7$cfu/g) followed by Suleja ($1.11\times10^7\pm1.89\times10^7$ cfu/g) while Tegina and Bida had low counts of ($6.75\times10^6\pm1.15\times10^7$cfu/g) Statistical analysis revealed that there were significant differences($p<0.05$) across the locations.

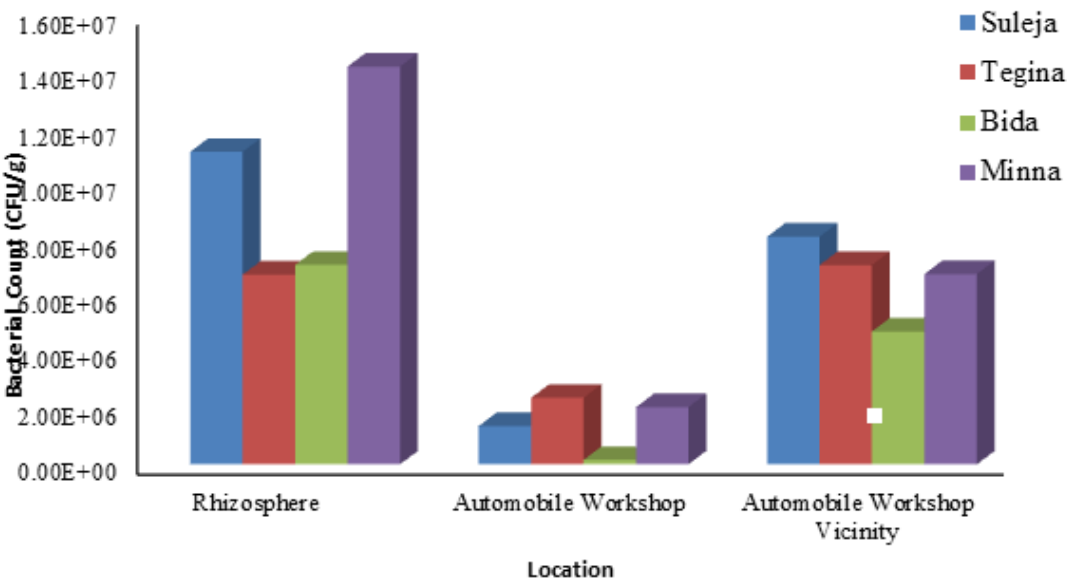

Figure 4.2: Total aerobic heterotrophic bacterial (TABH) counts in soil from the rhizosphere and automobile workshops

4.1.3 Total fungal counts in soil from automobile workshops

The resultsof the total fungal counts in the rhizosphere showed that rhizosphere of plants found in automobile workshops in Bida had the highest counts($7.10\times10^{6}\pm1.20\times10^{7}$cfu/g), while Tegina had the least counts($1.70\times10^{6}\pm2.86\times10^{6}$cfu/g) when compared to Minna and Suleja (Figure 4.3). Statistical analysis revealed that there were significant differences ($p<0.05$) among the locations. Automobile workshop vicinity had a relatively lower fungal counts when compared to the rhizosphere soil.Bida had the highest fungal counts($4.73\times10^{6}\pm8.03\times10^{6}$cfu/g) while Minna had the least counts($1.02\times10^{6}\pm1.72\times10^{6}$cfu/g) in relative terms to Suleja, Tegina (Figure 4.3). Statistical analysis revealed that there was no significant difference ($p>0.05$) among the locations.The total fungal counts in soil from the automobile workshop had the lowest fungal counts when compared to the rhizosphere and the automobile workshop vicinity, with Bida having the highest counts ($1.36\times10^{6}\pm2.26\times10^{6}$cfu/g) while Tegina and

Minna had the least count ($3.41\times10^5\pm5.71\times10^5$cfu/g) Suleja had mean counts of ($1.01\times10^6\pm1.73\times10^6$cfu/g). Statistical analysis showed that there was no significant difference ($p>0.05$) among the stations.

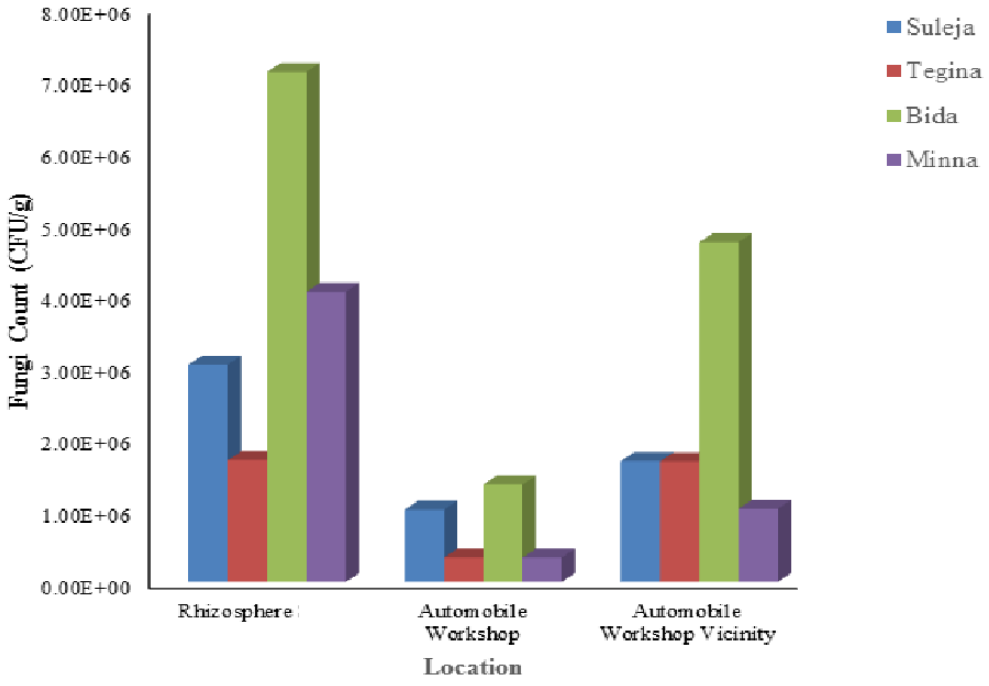

Figure 4.3: Total fungal counts of soil from various automobile workshops

4.1.4 Total aerobic heterotropic bacterial counts (TAHBC) in soil remediated with *Melissia officinalis* and *Urena lobata*

Figure 4.3 shows the total aerobic heterotrophic bacterial counts (TAHBC) obtained from soil alone (SA), soil and plant one (SP1), soil and plant two (SP2), soil and plant one polluted with 50cl of SEO (SP1 50cl), soil and plant two polluted with 50cl (SP2 50cl), soil and plant one polluted with 70cl of SEO (SP2 70cl), soil and plant two polluted with 70cl SEO (SP2 70cl). The results (Figure 4.4) revealed that the TAHBC were higher in SA, SP1 and SP2 than SP1 (50cl), SP2 (50cl), SP1 (70cl) and SP2 (70cl)

from June to December. It was observed that higher counts were obtained between August and October. Lower counts were observed between June and October and increased in November and December (Figure 4.4).The bacterial counts were lower in the month of June and July and the lowest counts were observed in soil with plant two polluted with 70cl of spent engine oil (SP2 70cl) and soil with plant one polluted with 50cl of spent engine oil (SP1 50cl), (Appendix C). The highest bacterial counts were observed in October in soil with plant one (SP1), $2.83\times10^{7}\pm1.89\times10^{7}$cfu/g (Appendix C). Statistical analysis revealed that there were significant differences ($p<0.05$) among the treatments.

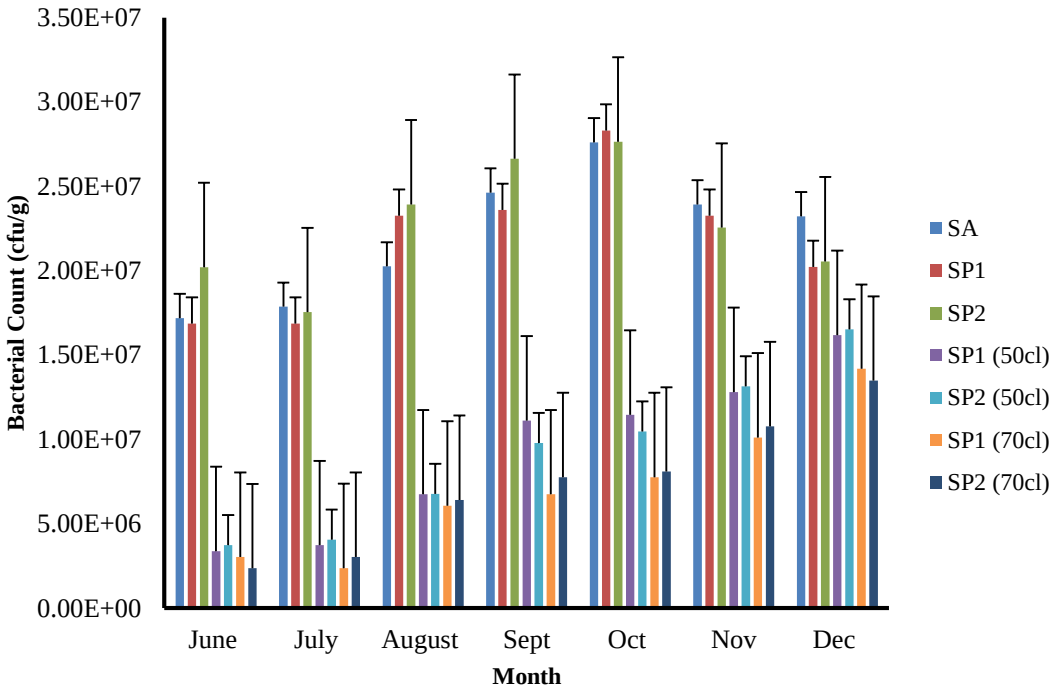

Figure 4.4: Total aerobic heterotrophic bacterial (TAHBC) counts in remediated soil

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

4.1.5 Total fungal counts in Soil remediated with *Melissia officinalis* and *Urena lobata*

Figure 4.5 shows the total fungal counts ranging from $9.01\times10^6\pm5.17\times10^6$cfu/g(in September SP1) to $1.02\times10^6\pm5.17\times10^6$cfu/g (in July SP2 70cl). Generally, the fungal counts (Apendix D) were low in June and July. Soil polluted with 70cl of SEO and plant two (SP2 70 cl)had the lowest fungal countswhileSP1 (September) had the highest count (Figure 4.5).

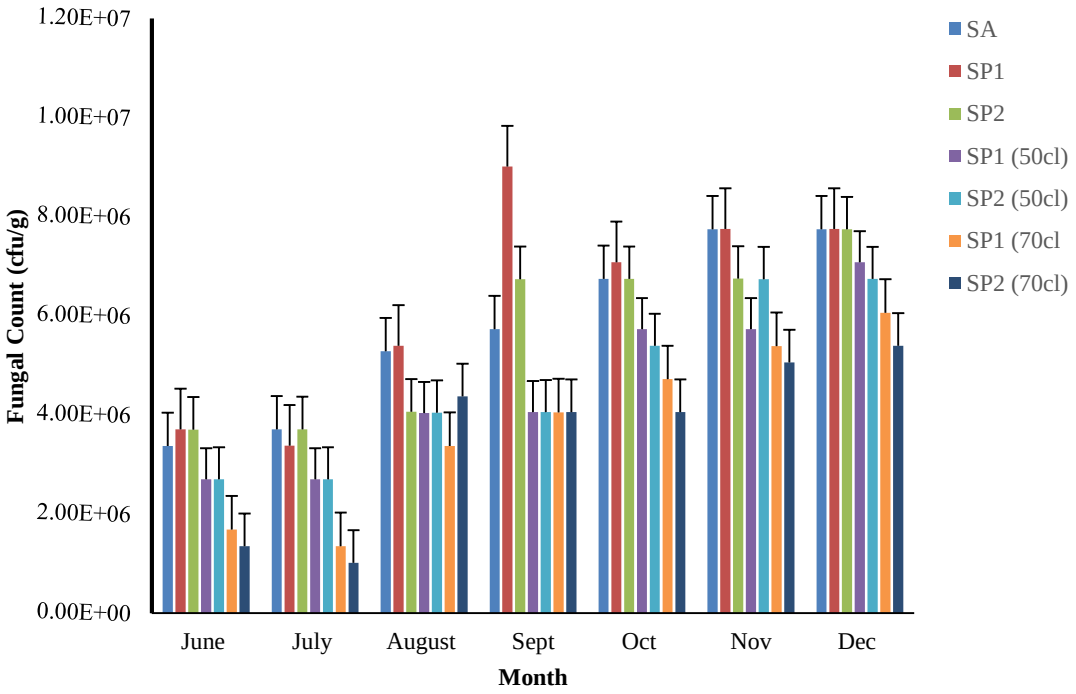

Figure 4.5: Total fungal countsof SEO remediatd soils

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

4.1.6 Oil utilizing bacterial counts in soil remediated *Melissia officinalis* and *Urena lobata*

Figure 4.6 shows the oil utilizing bacterial counts in the remediated soil. The oil utilizing bacterial counts were low and ranged from $2.32\times10^3\pm5.17\times10^3$cfu/g (SA in December) to $1.44\times10^3\pm5.17\times10^3$cfu/g (SP1 June), (Appendix E). Statistical analysis revealed that there were significant differences at ($p<0.05$) among the treatments from June to December.

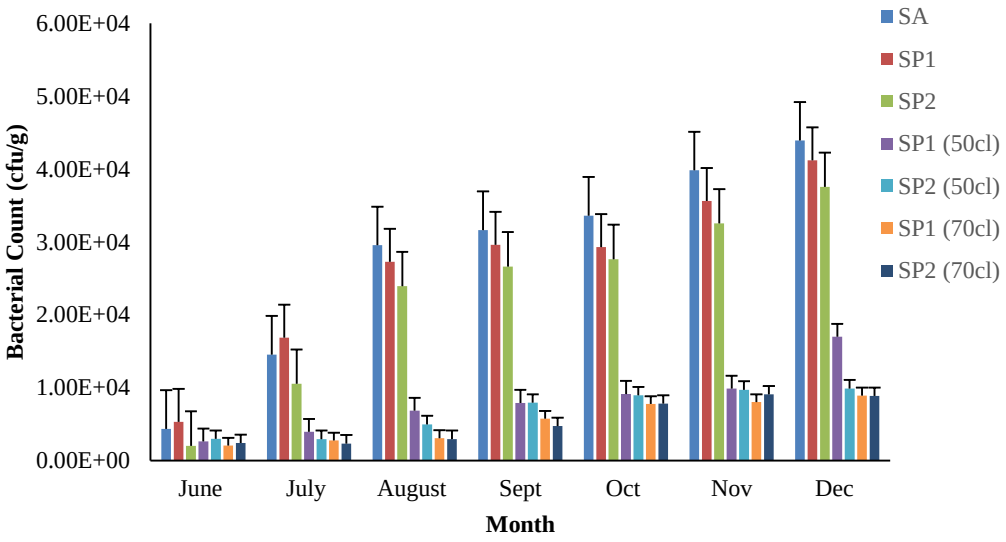

Figure 4.6: Oil utilizing bacterial counts in remediated soil

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

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4.1.7 Oil utilizing fungal counts in remediated soils

Figure 4.7 shows the oil utilizing fungal countsof the remediated soil. The counts were low when compared to the total fungal counts. June and July had the lowest counts, while September had the highest counts (Figure 4.7).

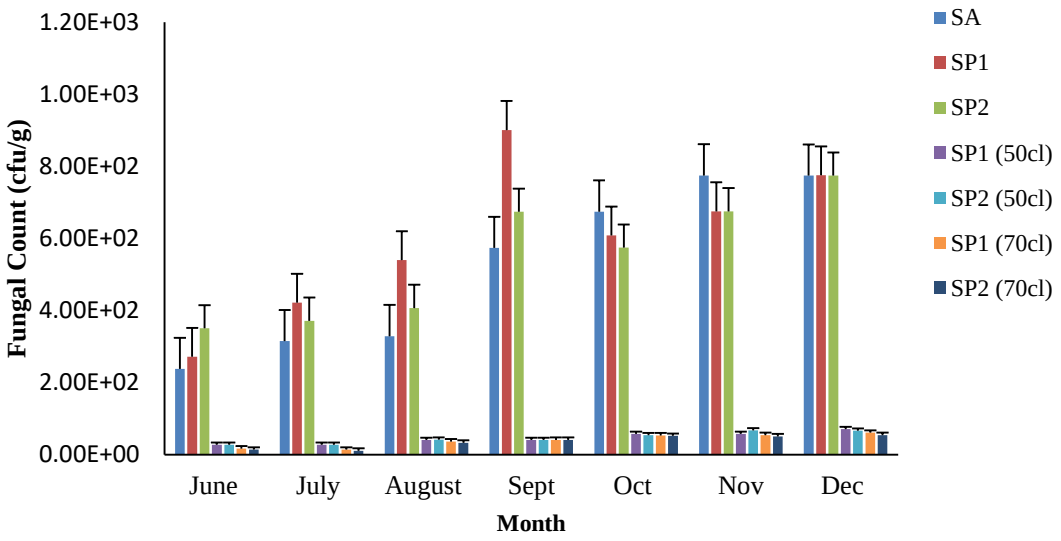


Figure 4.7: Oil utilizing fungal counts inremediated soil

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

4.1.8 Identification and Frequency of Occurrence of Microorganisms in the Remediated Soils

4.1.8.1 Bacteria

Bacteria isolated from the remediated soil were *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeroginosa,* and *Staphylococcus epidermidis* (Table 4.2).In soil alone (SA),*Bacillus subtilis* had the highest frequency of occurrence (9.16%) followed by *Bacillus megaterium* with5.34% (Table 4.3) while *Staphylococcus epidermidis* had the lowest occurrence (2.29%), in soil remediated with *M. officinalis* alone (SP1)*Staphylococcus epidermidis* had the lowest frequency of occurrence (0.76%). Similar patterns of results were observed in soil remediated with *U.lobata* alone (SP2)*Bacillus subtilis* had the highest frequency of occurrence (8.39%) while *Staphylococcus epidermidis* had the lowest frequency of occurrence (0.76%).

Table 4.2: Morphological and Biochemical Characteristics of Bacterial Isolates

Code	Gram	Shape	Catalase	Coagulase	Starch Hydrolysis	Oxidase	MSA	Urease	Citrate	MR	VP	Bacterial Isolate (Identified)
MA_i	+	Rod	+	-	+	-	-	-	+	-	-	*Bacillus subtilis*
MA_{ii}	+	Cocci	+	+	-	-	+	-	+	-	-	*Staphylococcus aureus*
BA_{ii}	-	Rod	+	-	-	+	-	-	+	-	-	*Pseudomona aeruginosa*
TC_{ii}	+	Rod	+	-	+	-	-	-	+	-	-	*Bacillus megaterium*
SC_{Ii}	+	Cocci	+	-	-	-	+	-	+	+	-	*Staphylococcus epidermidis*

KEY; +: Positive, -: Negative, MSA= Mannitol Salt Agar, VP= Voges Proskauer, M.R= Methyl red

Table 4.3: Frequency of occurrence of bacterial isolates in the experimental setup

Bacteria	SA(%)	SP1(%)	SP2(%)	SP1 (50) (%)	SP2(50) (%)	SP1(70) (%)	SP2(70) (%)
Bacillus subtilis	12(9.16)	7(5.34)	11(8.39)	8(6.10)	5(3.81)	4(3.05)	4(3.05)
Staphylococcus aureus	4(3.05)	2(1.52)	2(1.52)	1(0.76)	2(1.52)	2(1.52)	2(1.52)
Bacillus megaterium	7(5.34)	4(3.05)	4(3.05)	4(3.05)	5(3.81)	3(2.29)	2(1.52)
Pseudomonas aeruginosa	6(4.58)	3(2.29)	3(2.29)	3(2.29)	3(2.29)	1(0.76)	1(0.76)
Staphylococcus epidermidis	3(2.29)	1(0.76)	1(0.76)	2(1.52)	2(1.52)	1(0.76)	1(0.76)
Total	32 (24.42)	21(16.03)	22 (16.79)	18 (13.74)	17 (12.97)	11 (8.39)	10 (7.63)

SP1 50: Soil polluted with 50cl of spent engine oil and treated with *Melissia officinalis*, SP2 50: Soil polluted with 50cl of spent engine oil and treated with *Urena lobata*, SP1 70: Soil polluted with 70cl of spent engine oil and treated with *Melissia officinalis*, SP2 70: Soil polluted with 70cl of spent engine oil and treated with *Urena lobata*.

4.1.8.2 Fungi

Table 4.4 shows the fungal isolates in the remediated soilidentified as*Aspergillus flavus, Aspergillus fumigatus, Penicillium notatum, Aspergillus niger*and *Fusarium oxysporium*. In soil alone (SA) *A. niger* had the highest frequency of occurrence (8.19%) closely followed by *A. flavus* (4.09%) while the fungi with the least occurrence was *P. notatum* (0.81%)*F.oxysporium* had frequency of occurrence of 1.63%(Table 4.5).

Table 4.4: Cultural and Morphological Characteristics of Fungal Isolate

Isolates code	Cultural Characteristics	Microscopic Characteristics	Inferences
F1	Green colony with granular surfaceanda brown reverse coloration on SDA	Septate hyphae, hyaline and coarsely rough conidiophores.	***Aspergillus flavus***
F2	Gray colony with granular surface, white edges and a black reverse coloration on SDA	Septate hyphae, and short smooth-walled conidiophores.	***Aspergillus fumigatus***
F3	Black colony with granular surface and black reverse	Septate hyphae. Dark brown large globose conidial heads.	***Aspergillus niger***
F4	Pink centered white colony with cottony surface anda brown reverse coloration on SDA	Septate hyphae, canoe shaped macroconidia.	***Fusarium oxysporium***
F5	Bluish –green colony with cottony surface, white border and a brown reverse coloration on SDA.	Septate hyphae with unbranched conidiophores and secondary branches (metulae).	***Penicillium notatun***

Table 4.5: Frequency of occurrence of fungal isolates in the experimental setup

Fungi	SA(%)	SP1(%)	SP2(%)	SP1(50) (%)	SP2(50) (%)	SP1(70) (%)	SP2(70) (%)
Aspergillus flavus	5(4.09)	7(5.73)	8(6.55)	3(2.45)	4(3.27)	1(0.81)	1(0.81)
Aspergillus fumigatus	3(2.45)	2(1.63)	3(2.45)	2(1.63)	2(1.63)	2(1.63)	2(1.63)
Penicillium notatum	1(0.81)	1(0.81)	1(0.81)	2(1.63)	2(1.63)	1(0.81)	1(0.81)
Aspergillus niger	10(8.19)	12(9.83)	11(9.01)	7(5.73)	8(6.55)	4(3.27)	5(4.09)
Fusarium oxysporium	2(1.63)	1(0.81)	3(2.45)	3(2.45)	2(1.63)	1(0.81)	1(0.81)
Total	21(17.21)	23(18.85)	24(19.67)	17(13.93)	18(14.75)	9(7.37)	10(8.19)

SP1 50: Soil polluted with 50cl of spent engine oil and treated with *Melissia officinalis*, SP2 50: Soil polluted with 50cl of spent engine oil and treated with *Urena lobata*, SP1 70: Soil polluted with 70cl of spent engine oil and treated with *Melissia officinalis*, SP2 70: Soil polluted with 70cl of spent engine oil and treated with *Urena lobata*.

4.1.9 Screening of isolates for SEO utilization

Table 4.6 shows the growth of bacteria in mineral salt broth enriched with spent engine oil (SEO) as the sole carbon source for 28 days.

Table 4.6: Utilization of spent engine oil by bacterial isolates

Coded BacterialIsolates	**Utilizationof spent engine oil after(Days)**	**Extentof growthofSEO after 28 days**	**Optical density (OD) 490nM**
Bacillus subtilis B1	2	+++	0.410
*Staphylococcus aureus*B2	5	+	0.930
*Bacillus megaterium*B3	3	++	0.630
*Pseudomonasaeruginosa*B4	4	++	0.610
Staphylococcus epidermidis B5	6	+	0.942
Bacillus subtilis B6	2	+++	0.422
Staphylococcus aureus B7	5	++	0.820
Bacillus megaterium B8	3	+++	0.632
Pseudomonas aeruginosa B9	3	+++	0.702
Staphylococcus epidermidis B10	7	++	0.832
Staphylococcus aureus B11	7	+	0.911
*Bacillus megaterium*B12	3	+++	0.465
Bacillus subtilis B13	2	+++	0.472
*Staphylococcus aureus*B14	4	++	0.820
Pseudomonas aeruginosa B15	4	++	0.700
*Bacillus subtilis*B16	3	+++	0.470
*Bacillus megaterium*BI7	5	++	0.752
Bacillus megaterium B18	5	++	0.670

+: minimal growth, ++:moderate growth, +++: maximum growth, SEO: spent engine oil

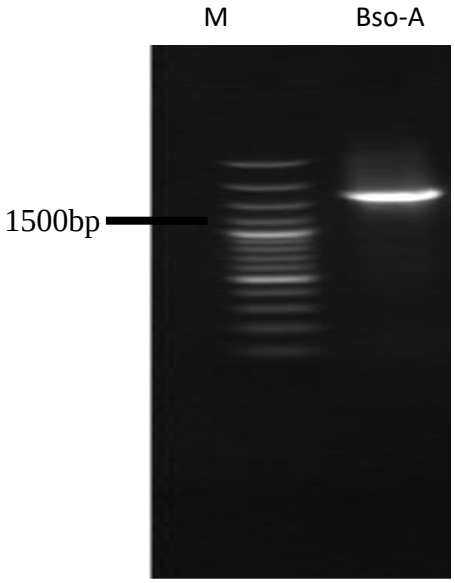
Table 4.7: Utilization of spent engine oil by fungal isolates

Coded Fungal Isolates	Utilization of spent engine oil after(Days)	Extent of growth of SEO after 28 days	Optical density (OD) 490 nM
Aspergillus flavus F1	7	+++	0.540
Aspergillus fumigatus F2	6	++	0.670
Penicillium notatum F3	8	+	0.800
Aspergillus niger F4	5	+++	0.400
Fusarium oxysporium F5	8	+	0.780
Aspergillus niger F6	6	++	0.503
Aspergillus fumigatus F7	7	++	0.760
Aspergillus niger F8	7	+++	0.570
*Fusarium oxysporium*F9	8	++	0.840
Aspergillus fumigatus F10	7	++	0.650
Aspergillus niger F11	7	+++	0.530
Aspergillus niger F12	7	++	0.522
Aspergillus flavus F13	8	+++	0.610
*Aspergillus niger*F14	6	++	0.510
*Aspergillus flavus*FI5	6	++	0.720
*Aspergillus niger*F16	6	+++	0.430
*Aspergillus flavus*F17	7	++	0.780
Aspergillus niger F18	6	++	0.660
Aspergillus flavus F19	6	+++	0.690
Aspergillus niger F20	6	+++	0.540
Aspergillus niger F21	6	++	0.583

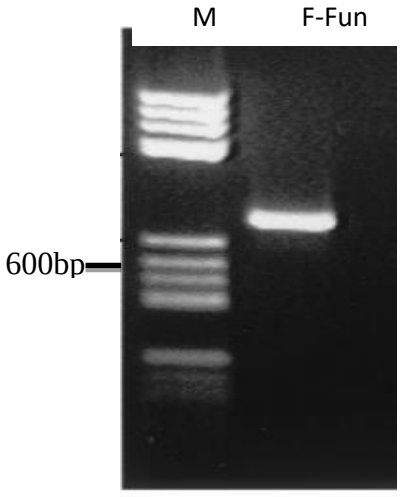
+: minimal growth, ++:moderate growth, +++: maximum growth, SEO: spent engine oil

4.1.10 Molecular identification of selected spent engine oil utilizers

The agarose gel of the amplified bacteria and fungi are shown in (Plate IV and V), revealing the 16S rRNA of 1500 base pairs and ITS of 600 base pair (bp) sequences respectively.While the identified organisms with the assigned accession numbers from the GenBank is presented in Table 4.8



PlateIV: Agarose gel of amplified bacterial 16S rRNA sequences of 1500 Bp. M= 1Kb; 1= Bso- A



PlateV: Agarose gel of Amplified Fungi ITS sequences of 600 Bp. M= 1Kb; 1= Rso- A

Table 4.8: Identified organisms with the assigned accession numbers from the GenBank

Sequence – ID	**Organism**	**Accession Numbers**	**Locus (bp)**	**HC-Gas**
Bso-A	*Bacillus subtilis*	EE681738	1500	E
F-Fun	*Aspergillus niger*	HQ659967	600	M

Key: HC= hydrocarbon, E= ethane, M= methane

Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequence, showing the phylogenetic relationships between identified bacteria and the most closely related strains from the GenBank.

Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequence, showing the phylogenetic relationships between identified bacteria and the most closely related strains from the GenBank is shown in Figure 4.8.

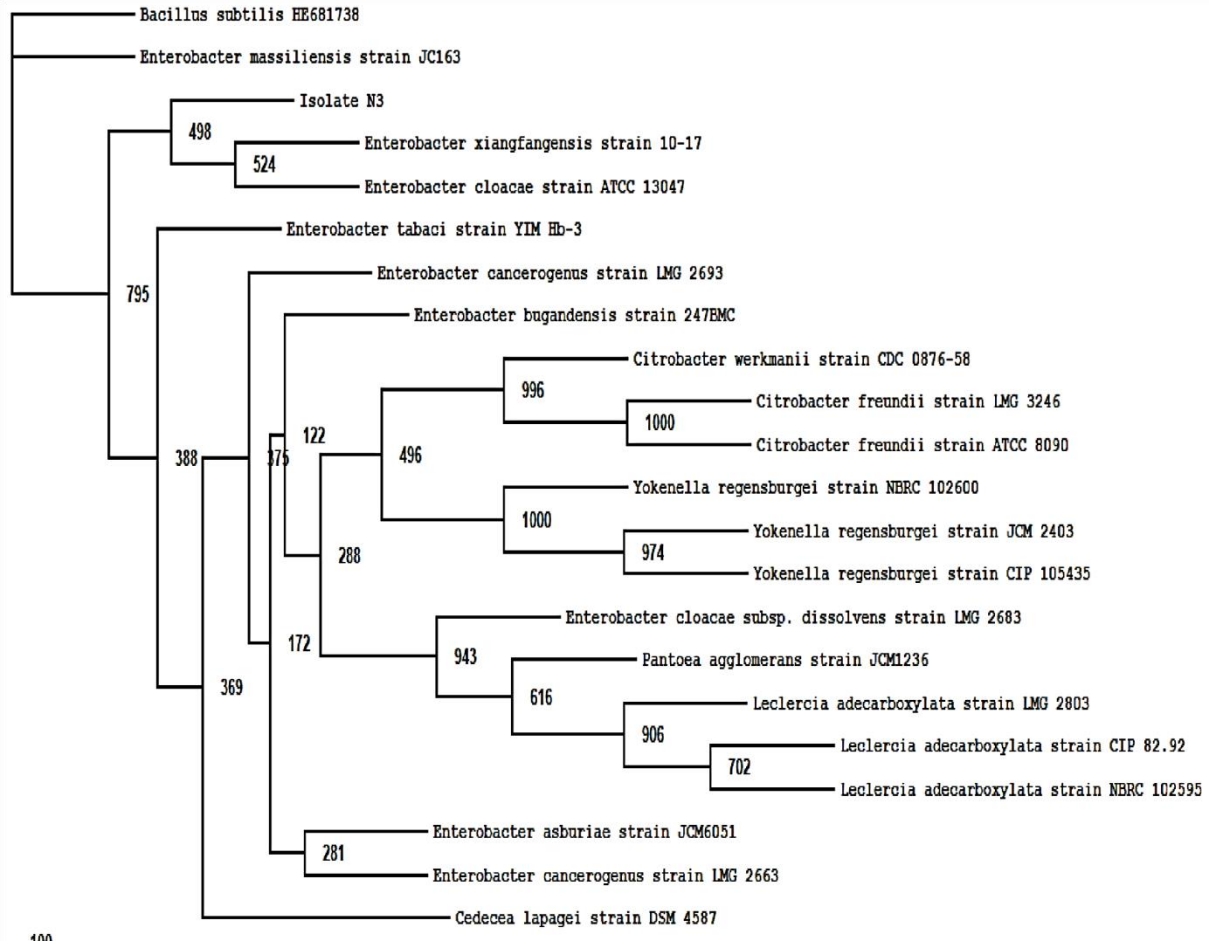


Figure 4.8: Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequence, showing the phylogenetic relationships between identified bacteria and the most closely related strains from the GenBank.

4.1.11 Residual total petroleum hydrocarbon (TPH) in spent engine oil polluted soil remediated with *Melissia officinalis* and *Urena lobata*

At one month of the phytoremediation of spent engine oil polluted soil with *M. officinalis* and *U. lobata*, it was observed that soil polluted with 50cl of SEO and remediated with *M. officinalis* (SP1 50)had the highest percentage biodegradation of 16.31% while the lowest percentage biodegradation was 7.11% in soil polluted with 70cl of SEO and remediated with *U. lobata* (SP2 70cl). After four months of phytoremediation, soil polluted with 50cl of SEO and remediated with *M. officinalis* (SP1 50)had the highest percentage biodegradation of 33.61% followed by SP250 (20.10%), SP1 70 (19.31%) and SP2 70 (17.10%) in that order (Table 4.9).

Table 4.9:Biodegradation of spent engine oil in soil remediated with *Melissia officinalis* and *Urena lobata*

Treatment	Biodegradation (%) after (months):		
	1	4	7
SP1 (50)	16.31 ± 0.01^{a}	33.61 ± 0.01^{b}	56.61 ± 0.01^{c}
SP2 (50)	14.13 ± 0.01^{a}	20.10 ± 0.01^{b}	50.55 ± 0.01^{c}
SP1 (70)	9.32 ± 0.01^{a}	19.31 ± 0.01^{b}	37.9 ± 0.01^{c}
SP2 (70)	7.11 ± 0.01^{a}	17.10 ± 0.01^{b}	32.1 ± 0.01^{c}

SP1 (50): Soil polluted with 50cl of spent engine oil and treated with *Melissia officinalis*, SP2 (50): Soil polluted with 50cl of spent engine oil and treated with *Urena lobata*, SP1 (70): Soil polluted with 70cl of spent engine oil and treated with *Melissia officinalis*, SP2 (70): Soil polluted with 70cl of spent engine oil and treated with *Urena lobata*.

4.1.12 Physical and chemical properties of soil from automobile workshops in parts of Niger State

Table 4.10 – 4.13 shows the physical and chemical properties of the soils collected from different locations within the automobile workshops and the soil in the vicinity of the workshops located atBida,Tegina, Minna and Suleja. The pH of soil of Bida workshop and its vicinity (BABV) ranged from 4.9 to 6.13, Tegina (TATV) 5.16-6.25, Minna (MAMV) 4.77-6.53 while Suleja (SASV) had 5.03 – 6.07.

Table 4.10: Physicochemical properties of soil from different automobile workshops visited

Parameter	BV	BA	TV	TA	MV	MA	SV	SA
pH	6.13	4.9	6.25	5.61	6.53	4.77	6.07	5.03
Nitrogen (%)	0.46	0.29	0.52	0.33	0.71	0.26	0.39	0.31
Organic carbon (%)	9.19	5.6	7.63	3.39	8.11	3.75	7.35	4.52
Organic matter (%)	8.35	5.26	7.54	4.53	6.43	5.17	7.28	4.89
Phosphorus mg/kg	25.28	15.66	25.44	14.75	26.8	14.63	27.01	16.56
Na (Cmol/kg)	0.638	0.438	0.821	0.364	0.759	0.34	0.75	0.417
K (Cmol/kg)	0.65	0.33	0.71	0.31	0.76	0.27	0.62	0.25
Ca (Cmol/kg)	9.38	5.38	7.32	4.65	8.86	4.07	7.94	5.11
Mg (Cmol/kg)	6.6	4.84	7.18	3.66	7.36	5.25	7.05	3.87
Exchangeable acidity (Cmolkg)	0.96	0.7	1.88	0.59	2.02	0.54	1.27	0.62
Electrical conductivity	72	53	75	50	66	39	68	41
Sand	63.25	50.65	59.62	48.52	60.74	41.66	60.83	45.07
Clay (%)	20.75	31.45	27.41	30.14	25.37	29.54	23.36	28.69
Silt (%)	14.96	16.44	12.53	20.37	13.22	27.85	15.09	25.79
Soil moisture (%)	27.2	16.5	25.720	17.74	27.21	18.38	28.4	
Soil texture	Fine Sand	Granular	Fine Sand	Granular	Fine Sand	Granular	Fine Sand	
Soil structure	Sand	Sand	Sand	Sand	Sand	Sand	Sand	
Soil type	Garden soil	Field soil	Garden soil	Field soil	Garden soil	Field soil	Garden soil	
Soil color	Whitish Black	Light Black	Whitish Black	Light Black	Whitish Black	Light Black	Whitish Black	

BV: Bida (Automobile Workshop Vicinity), BA: Bida automobile workshop, TV: Tegina (Automobile Workshop vicinity), TA: Tegina automobile workshop, MV: Minna (Automobile Workshop Vicinity), MA: Minna automobile workshop SV: Suleja (Automobile Workshop Vicinity) SA: Suleja automobile workshop

4.1.12.1 Physical and chemical properties of Spent Engine Oil Polluted SoilRemediated with M. officinalis and U. lobata

The physical and chemical properties of the soil one month after phytoremediation are presented in Table 4.11. The pH was generally acidic and ranged from 4.18 to 5.03 in unpolluted soil habouring plants. In the polluted soil (50-70cl) remediated with plants (SP1, SP2) pH ranged from 4.37 to 5.2 while in SP1 (70cl) and SP2 (70cl), the pH ranged from 4.04 to 5.66 (Table 4.11).

Table 4.11: Physicochemical properties of SEO polluted soil after one month of phytoremediation

Parameters	SA	SP1	SP2	SP1(50)	SP2(50)	SP1(70)	SP2(70)
pH	4.31	5.03	4.18	5.2	4.37	4.04	5.66
Nitrogen (%)	2.17	1.24	1.41	0.98	1.65	2.15	3.63
Oxygen (%)	9.56	8.84	7.3	6.9	12.2	6.17	10.31
Organic matter(%)	15.68	16.14	14.64	14.83	16.32	14.77	15.48
Phosphorus (mg/kg)	37.11	35.62	26.57	34.15	34.81	34.63	36.05
Na (Cmol/kg)	0.95	0.721	0.67	0.544	0.821	0.506	0.859
K (Cmol/kg)	0.72	0.68	0.5	0.65	0.78	0.53	0.76
Ca (Cmol/kg)	4.65	7.02	4.19	4.56	5.81	5.14	4.8
Mg (Cmolkg)	7.85	7.68	5.72	5.63	9.52	6.25	8.77
Exchangeable acidity (Cmol/kg)	0.79	1.81	0.91	0.88	1.65	1.24	2.47
Electrical conductivity	88	70	61	76	78	73	85
Sand (%)	53.38	62.65	55.01	49.58	56.55	51.6	60.43
Clay (%)	23.61	25.01	27.11	30.44	21.25	25.46	25.17
Silt (%)	12.19	10.33	20.09	19.37	12.63	22.55	11.2
Soil moisture	21.6	19.5	19.2	21.3	20.4	18.62	22.2
Soil texture	fine sand	fine sand	fine sand	granular	fine sand	Granular	fine sand
Soil structure	Sand	Sand	Sand	Sand	Sand	Sand	Sand
Soil type	non-sticky	granular soil	non-sticky	field soil	granular soil	field soil	granular soil
Soil color	whitish black	whitish black	light black	light black	whitish black	light black	whitish black

A: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engin

Table 4.12: Physicochemical properties of SEO polluted soil after four months of phytoremediation

Parameters	SA	SP1	SP2	SP1(50)	SP2(50)	SP1(70)	SP2(70)
pH	5.39	5.33	4.88	4.7	5.52	5.65	4.73
Nitrogen	0.91	0.91	0.7	0.86	1.51	0.83	0.84
Oxygen	10.21	7.56	8.57	5.59	9.83	8.25	12.04
Organic matter	16.73	17.19	15.89	15.97	17.44	17.03	16.15
Phosphorus (mg/kg)	28.82	36.5	29.01	24.69	25.53	34.17	30.22
Na (Cmol/kg)	0.708	0.617	0.766	0.544	0.853	0.64	0.835
K (Cmol/kg)	0.866	0.581	0.664	0.743	0.719	0.531	0.752
Ca (Cmol/kg)	13.65	12.01	11.47	14.07	11.23	14.5	14.82
Mg (Cmol/kg)	7.51	4.88	7.25	5.37	7.58	4.96	9.03
Exchangeable acidity (Cmol/kg)	2.04	0.81	1.17	0.86	1.25	0.79	1.06
Electrical conductivity	69	61	81	79	72	70	85
Sand (%)	64.14	48.57	63.03	58.6	55.22	56.62	60.21
Clay (%)	22.77	26.09	20.16	28.59	25.44	31.34	21.54
Silt (%)	13.42	25.09	14.49	22.83	12.73	21.72	15.92
Soil moisture	24.1	19.3	22.3	28.3	27.7	20.5	25.80
Soil texture	fine sand	Granular	fine sand	Granular	fine sticky	Granular	fine sand
Soil structure	Sand	Sand	Sand	Sand	Sand	Sand	Sand
Soil type	non-sticky	field soil	non-sticky	field soil	granular soil	field soil	granular soil
Soil color	whitish black	light black	whitish black	light black	whitish black	light black	whitish black

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

Table 4.13: Physicochemical properties of SEO polluted soil after seven months of phytoremediation

Parameters	SA	SP1	SP2	SP1(50)	SP2(50)	SP1(70)	SP2(70)
pH	5.15	5.23	6.11	6.07	6.03	5.66	5.25
Nitrogen (%)	0.82	0.75	0.96	0.89	0.78	0.93	0.81
Oxygen (%)	4.33	4.17	5.85	5.32	6.2	6.3	0.462
Organic matter (%)	18.55	16.63	19.74	19.62	18.15	18.53	18.89
Phosphorus (mg/kg)	25.84	28.27	24.9	29.34	26.81	24.57	25.56
Na (Cmolkg)	0.701	0.69	0.43	0.562	0.688	0.446	0.677
K (Cmolkg)	0.614	0.708	0.573	0.686	0.591	0.659	0.552
Ca (Cmol/kg)	7.83	6.66	5.17	8.41	7.8	7.05	5.73
Mg (Cmol/kg)	9.38	7.66	5.95	8.15	8.64	7.61	6.78
Exchangeable acidity (Cmolkg)	1.43	2.22	2.84	1.71	1.06	0.89	0.91
Electrical conductivity	95	86	79	101	87	90	69
Sand (%)	57.26	59.4	51.06	63.03	61.22	58.72	48.54
Clay (%)	25.11	23.73	23.25	20.64	19.5	24.04	26.29
Silt (%)	11.03	12.62	24.15	15.19	12.26	20.77	22.91
Soil moisture	22.6	27.1	20.6	26.3	24.8	27.4	28.2
Soil texture	fine sand	fine sand	granular	fine sand	fine sticty	Granular	Granular
Soil structure	Sand	non plastic	Sand	Sand	non plastic	Sand	Sand
Soil type	non-sticty	granular soil	non-sticty	granular soil	granular soil	field soil	field soil
Soil color	whitish black	whitish black	light black	whitish black	whitish black	light black	whitish black

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

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4.1.13 Accumulation and translocation of heavy metals in *M. officinalis* and *U. lobata* used for phytoremediation of SEO

Table 4.14reveals the results of heavy metal accumulated and translocated by *M.officinalis* and *U. lobata* during remediation process. The bioconcentration factor (BCF)of iron (Fe) and cadnium (Cd)was greater than one (>1) and their translocation factor was less than one (<1) in soil polluted with 50cl and 70cl of spent engine oil and remediated with *M. officinalis* (SP1 50cl and SP1 70cl).The BCF for Lead (Pb) was less than one (<1) and translocation factor was greater than one in SP1 (50cl)while for Lead in SP1 (70cl), BCF and TF were both greater than one (>1). Also soil remediated with *U. lobata* (SP2 50cl) the BCF of Iron (Fe), Zinc (Zn),Cadium (Cd)greater than one (BCF >1) while the translocation factor less than one (TF<1). In SP1 (70cl) the translocation factor for Pb was more than one while the bioconcentration factor was less than one (TF>1 and BCF<1).

Table 4.14: Accumulation and Translocation of Heavy metals in *M. officinalis* and *U. lobata* used for the remediation study

Treatment	Heavy Metals	BCF	TF	BAC	EF
SP1 (50)	Fe	1.67	0.19	0.31	1.46
	Zn	0.06	0.68	0.04	0.06
	Cd	0.90	0.86	0.77	1.35
	Cr	0.02	0.00	0.00	0.01
	Pb	0.23	1.24	15.3	1.41
	Ni	0.23	0.69	0.16	0.18
SP1 (70)	Fe	3.80	0.19	0.52	2.45
	Zn	0.08	0.67	0.06	0.09
	Cd	1.71	0.86	1.47	2.57
	Cr	0.03	0.00	0.00	0.02
	Pb	1.46	1.23	17.5	16.2
	Ni	0.90	0.68	0.68	0.78
SP2 (50)	Fe	3.80	0.18	0.70	3.32
	Zn	1.20	0.67	8.17	1.22
	Cd	1.02	0.86	0.88	1.54
	Cr	.0.08	0.00	0.00	0.05
	Pb	19.8	1.24	24.5	22.7
	Ni	0.26	0.69	0.18	0.20
SP2 (70)	Fe	1.77	0.19	0.33	1.57
	Zn	0.13	0.68	0.09	0.13
	Cd	0.48	0.86	0.41	0.72
	Cr	0.01	0.00	0.00	11.7
	Pb	0.01	1.23	22.31	20.6
	Ni	0.14	0.69	0.09	11.9

Key: BCF- Bio-concentration factor = metal concentration ratio of plant roots to soil, TF (Translocation factor)= metal concentration ratio of plant root, BAC (Biology Accumulation coefficient) = metal concentration ratio of plant shoot to soil, EF (Enrichment Factor) = concentration ratio of plant leaf to soil, SP1= soil and *Melissia officinalis* SP2=soil and *Urena lobata*

The Plate VI and VII presented the *Melissia officinalis* grown on spent engine oil polluted soil and *Urena lobata* grown on spent engint oil polluted soil.

Plate VI: *Melissia officinalis* grown on spent engine oil polluted soil

Plate VII: *Urena lobata* grown on spent engint oil polluted soil

4.1.14 Gas chromatography-mass spectrophotometry (GC-MS) analysis of spent engine oil (SEO) used for the remediation study

Gas chromatography- mass spectrophotometry (GC-MS) analysis of the spent engine oil (Figure 4.9) used for this study revealed various hydrocarbonsincluding normal alkanes-Octanes (C_8H_{18}), Nonane (C_9H_{20}), Decane ($C_{10}H_{22}$), Undecane ($C_{11}H_{22}$), Dodecane ($C_{12}H_{26}$)and the aromatic compounds benzene, propylbenzene, p- xylene, o- xylene, ethylmethylbenzene (Table 4.15).

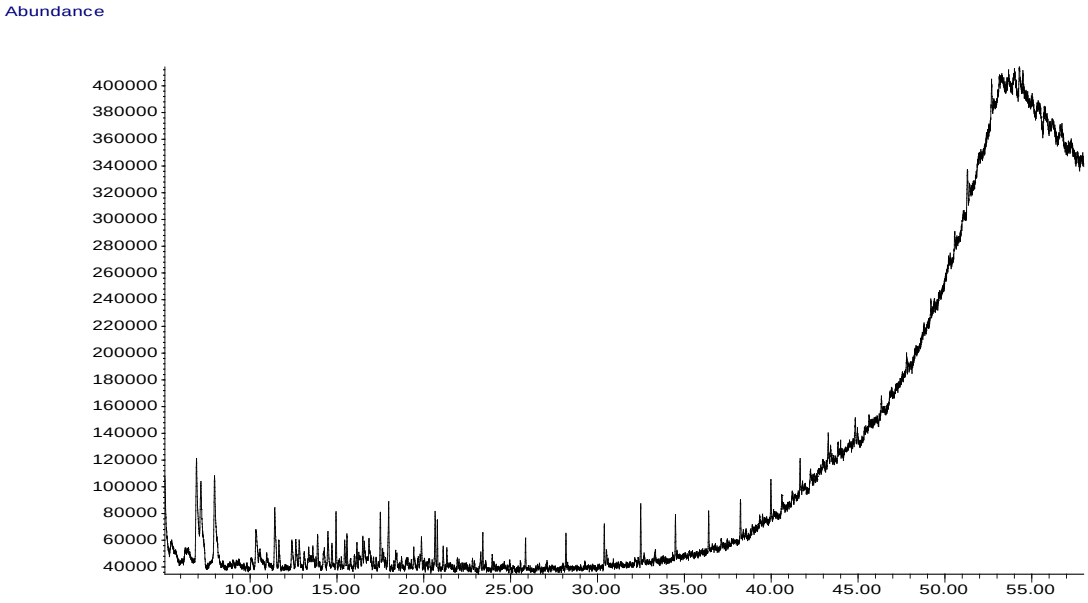

Figure 4.9: Gas chromatograms of spent engine oil (SEO) used for the remediation study

Table 4.15: Hydrocarbons in SEO used in the study

Retention Time (Minutes)	Area (%)	Compounds
6.266	0.55	Benzene, 3-Phenoxy, Boroxin,
6.912	9.79	Ethylbenzene
7.954	7.21	p-Xylene, Benzene
9.006	0.25	4,5-Dihydro-4-4-trifluoromethyl, Phenyl, 3H-Isoxazolo, Acridine
9.819	0.25	Propionic acid, 3,3,3-Trifluoro-2, hydroxymethyl, 1H-pyridin, Ethyl ester
10.042	0.30	Hydroxymethyl, 1H-pyridin, Phenyl
10.952	0.68	Trifluoromethyl
12.405	2.06	2-p-Methoxyphenyl, 8-Methyl-4-quinolyl, 2, Pyridyl ketone.
12.823	1.35	2-Butenedioic acid
13.115	1.16	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene
13.613	1.17	1H-Indole, 2-Carboxamide, 3, N-(2'-acetylphenyl)
13.790	0.25	6-Methoxy-4-methyl, 8-Nitro-5,ethoxyquinoline
13.882	1.54	Acridin-9-yl, 4-Trifluoromethoxyphenylamine, 3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene
13.996	0.31	1-Phenoxy-naphthalen-2-yl, Diazene
14.225	0.42	Phosphoric acid, Phenyl ester
15.249	0.39	3H-isoxazolo[3,4,5-k1]
15.444	1.09	1,3-Cyclopentadiene
16.010	0.85	1-Phosphacyclopentene
16.491	1.17	3H-isoxazolo[3,4,5-k1]
16.571	0.76	Diazene
16.846	0.77	Inolin-2-yl), 4-Quinolinecarboxylate
16.966	0.49	Imidazolo, Benzimidazol
17.109	0.41	2-Butenedioic acid
17.681	0.24	3,10-Dinitrodiftalone
17.790	0.41	Boroxin
17.979	3.39	Dodecane, Tridecane
18.276	0.34	1-Phosphacyclopent-2-ene
18.385	0.68	2-p-Methoxyphenyl-8-methyl-4-quinolyl-2-pyridyl, Ketone
18.625	0.31	2-Butenedioic acid
19.295	0.23	6-Methoxy-4-methyl-8-nitro-phenoxyquinoline
20.296	0.43	Coumarin, Trifluoroacetate
22.917	0.29	1H-Indole-2-carboxamide, 3-Phenyl-, N-(2'-Acetylphenyl

23.397	1.16	Tetradecane
26.653	0.44	Phenantroimidazole
27.094	0.34	2-Phosphabicyclo[3.1.0], Hex-3-ene
30.390	1.60	Pentacosane
30.516	0.76	4-Trifluoromethyl, 3H-isoxazolo
30.607	0.22	1,3-Difluoro-5-pentafluorophenyl, dimethylsilyloxybenzene
30.910	0.36	1H-Indole-2-carboxamide
32.490	2.08	Octadecane,Pentacosane
34.487	1.32	Nonadecane
38.229	1.20	Heneicosane
41.668	0.87	Tridecane

4.1.15GCMS of Spent Engine Oil (SEO) extracted from seo polluted soil one month after remediation with *Melissia officinalis* and *Urena lobata*

4.1.15.1 Spent engine oil (50cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometry (GC-MS) analysis of the spent engine oil extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month one (1) are presented in Figures 4.10 and 4.11 respectively. The result is presented in Table 4.16.

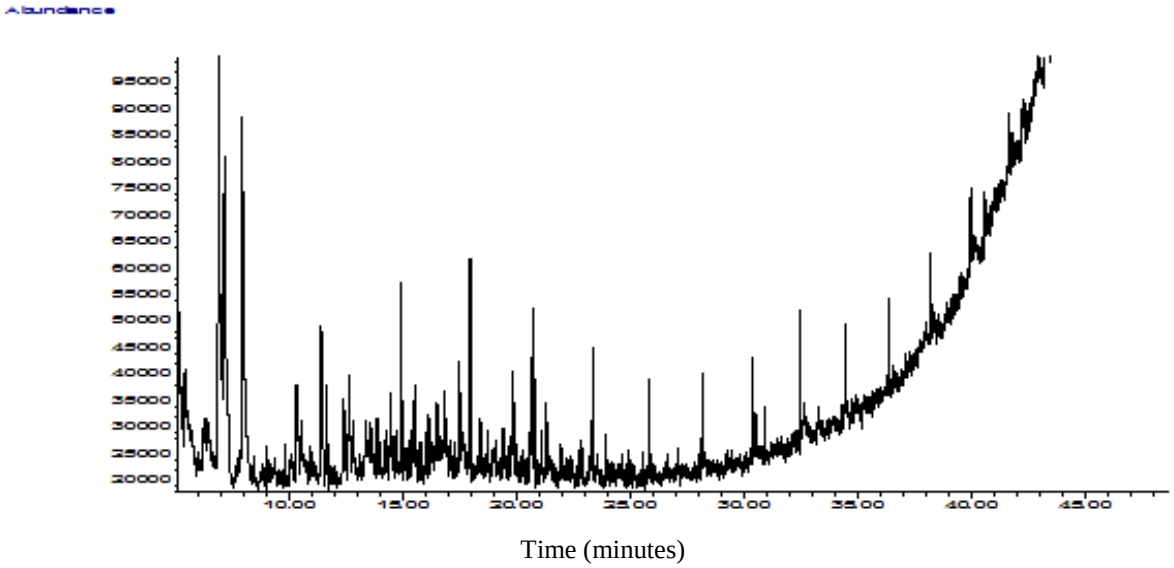

Figure 4.10: Gas chromatograms of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* at Month one (1)

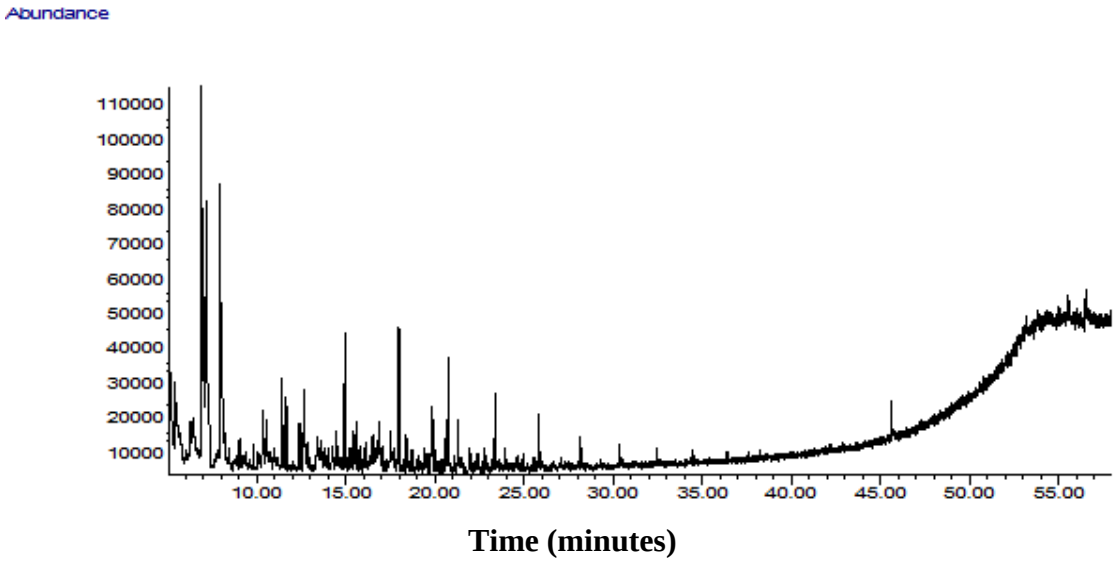

Figure 4.11: Gas chromatograms of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Urena lobata* at Month one (1)

Table 4.16 Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis*and *Urena lobata*at month one (1)

		Melissia officinalis			***Urena lobata***
Retention Time (Minutes)	Area (%)	Compound	Retention Time (Minutes)	Area (%)	Compound
5.430	1.44	Cyclohexanol, Nonane, 5-Methylene, Methylnonene	5.530	1.53	Nonane, 2-Methyl, 1-Nonene
6.254	0.71	Benzene, 1,1'-Phenoxy, Acetic acid, 4-Chlorophenoxy, Dodecyl ester, 2(1H)-Pyrimidinone, 4-4-Methylphenyl, 5-Phenoxy-6-phenyl	6.314	0.83	Acetic acid, Benzene
6.901	11.58	Ethylbenzene	6.931	7.81	Dodecyl ester, o-Xylene, Ethylbenzene
7.164	10.06	Benzene, 1,3-dimethyl, o-Xylene	7.265	9.06	Benzene
7.874	0.19	2(1H)-Pyrimidinone, 4-methylphenyl	7.844	0.21	2(1H)-Pyrimidinone, Phenoxy-6-phenyl
9.808	0.53	p-Chlorobenzylidine, Dioxybenzyl, Pyrimidine	9.808	0.42	Pyrimidine
10.329	1.57	Benzene, 1-Ethyl-3-methyl-	10.329	1.57	Ethyl-3-methyl, Undecyl ester
10.517	0.30	Acetic acid, 4-Chloro-2-methylphenoxy, Undecyl ester	10.607	0.55	4-Chloro-2-methylphenoxy
10.952	0.30	Butane-1,4-dione, 1-(4-Chlorophenyl), 4-Phenyl-3-2-thienyl	10.855	0.40	Butane-1,4-dione, 1-(4-chlorophenyl), -4-phenyl-3-(2-thienyl)-
11.416	3.00	Mesitylene	11.519	2.10	Acetic acid, Mesitylene
11.656	1.52	Decane	11.736	1.32	Nonane, Dodecane, Decane
12.017	0.25	2,4-Diamino-5-Chlorobenzylidine, Dioxybenzyl, pyrimidine	12.017	0.25	Pyrimidine, Hexadecane
12.623	2.21	1-Hexanol, 2-Ethyl	12.623	2.21	Methanoindene, Hexanol,
12.823	0.89	7-Methylenecycloocta-1,3,5-triene	12.823	0.89	7-Methylenecycloocta-1,3,5-triene
13.104	0.21	Quinoline, 2-Butenedioic acid, Dimethyl ester, Phosphoric acid	13.127	0.22	Quinoline, Butenedioic acid, 2,3-Dimethyl ester, Phosphoric acid, Phenyl ester
13.378	0.51	Pyrimidine	13.378	0.51	Chlorobenzylidine, Dioxybenzyl, Pyrimidine
13.590	0.42	Benzene, 1,2,4,5-Tetramethyl	13.467	0.42	1,2,4,5-Tetramethyl, Benzene

13.785	0.31	2-Phosphabicylohex-3-ene	13.677	0.31	Hex-3-ene
13.882	0.87	7-Methanoindene	13.732	0.88	3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene
14.002	0.35	Methyl-4-quinolyl, 2-Pyridyl ketone	14.122	0.50	Methyl-4-quinolyl
14.208	0.26	Benzene, 1-Methyl-1-silabenzocyclobutene	14.228	0.45	1-Methyl-1-silabenzocyclobutene
14.471	1.36	p-Cymene	14.375	0.61	p-Cymene
14.700	0.73	2(1H)-Pyrimidinone,	14.503	1.33	2(1H)-Pyrimidinone, 4-(4-methylphenyl)-5-phenoxy-6-phenyl-
14.940	2.43	Undecane, Hexadecane	14.710	1.63	Undecane, Hexadecane
15.084	0.32	5-Phenoxy-6-phenyl	15.185	0.42	Phenoxy-6-phenyl
15.255	0.46	2-Butylphenyl, 5,4-Biphenylyl, 1,3,4-Oxadiazole	15.345	0.51	5, 4-Biphenylyl, Oxadiazole
15.438	0.98	1-Methyl-1-silabenzocyclobutene, Benzene	15.528	1.08	Dimethylstyrene, 1,2,3,5-Tetramethyl
15.564	1.35	1,3,8-p-Menthatriene	15.536	0.30	Quinolin, 1,3,8-p-Menthatriene
15.764	0.27	Phosphoric acid	15.664	0.27	Phosphoric acid, Phenyl ester
16.005	0.70	2-Butenedioic acid, 2,3- Dimethyl ester	16.305	0.76	Dimethyl ester
16.142	1.07	2,4-Dimethylstyrene, Phenyl-1-butene	16.447	2.00	Phenyl-1-butene, Acetic acid
16.834	0.78	5-Phenoxy-6-phenyl	16.862	0.89	5-Phenoxy-6-phenyl, 4-Quinolinecarboxylate
16.897	0.32	Acetic acid, Dodecyl ester	16.881	0.22	4-Chlorophenoxy, Dodecyl ester
16.949	0.26	Methyl, 4-Quinolinecarboxylate	16.979	3.06	Triazolo(1,5-a)quinolin-2-yl)
17.246	0.43	Octane	17.348	1.12	1,8,8-Trimethyl-3-octane
17.493	1.43	Azulene	17.595	1.30	Coumarine, Acridin
17.779	0.23	2-Butenedioic acid	17.672	0.26	Quinolinecarboxylate, Dimethyl ester
17.967	3.35	Decane, Octane	17.981	2.51	Butenedioic acid, Dodecane, Decane
18.276	0.22	1-Phosphacyclopent-2-ene	18.336	0.33	1,2,3-Triphenyl-5-dimethylmethylene, 1-Phosphacyclopent-2-ene
18.379	0.58	Acetic acid, 4-Chlorophenoxy	18.472	0.69	4-Chlorophenoxy, dodecyl ester
18.442	0.46	2,4-Imidazolidinedione	18.531	0.46	Acetic acid, Ttrimethylsily, , 2,4-Imidazolidinedione
18.603	0.21	2-p-Methoxyphenyl, 4-Quinolyl-2-pyridyl ketone	18.623	0.22	Pyridyl ketone
18.706	0.27	1,3-Difluoro-5-pentafluorophenyl, Dimethylsilyloxybenzene, 2-Pyrazolin-5-one	18.744	1.20	2-Pyrazolin-5-one

18.952	0.21	2-Butenedioic acid	18.971	0.30	3-Methylphenyl, Phosphoric acid
19.078	0.28	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene	19.188	0.32	Cyclopent-2-ene
19.278	0.24	Phosphoric acid, Phenyl ester	19.318	0.24	Phenyl ester, 1-Ethylidene
19.661	0.56	2-Butenedioic acid	19.758	0.72	Dinitrodiftalone
19.781	0.64	3,10-Dinitrodiftalone	19.880	1.01	4-Phenoxy-naphthalene
19.856	1.49	Naphthalene, 1,2,3,4-tetrahydro-5-methyl	19.954	0.39	4-Chloro-2-methylphenoxy
19.964	0.28	Acetic acid, 4-Chloro-2-methylphenoxy-, Undecyl ester	20. 251	0.34	1-Phosphacyclopent-2-ene, 1,2,3-triphenyl-5-dimethylmethylene
20. 268	0.23	1-Phosphacyclopent-2-ene	20.775	1.17	Phosphoric acid, 1H-Indene
20.462	0.33	2-p-Methoxyphenyl	20.868	1.82	Tridecane, Dimethylsilyloxybenzene
20.645	2.15	1H-Indene, 1-ethylidene	20.896	0.45	Boroxin, tris(4-methylphenyl)-
20.771	1.91	Tridecane	21.942	0.61	N-(2,4,6-Trinitrophenyl)-2-naphthylamine
20.891	0.50	Boroxin	22.385	0.20	Silane
21.927	0.53	N-(2,4,6-Trinitrophenyl)	22.643	0.21	1,3-Difluoro-5-pentafluorophenyl
22.396	0.26	Silane	22.949	0.38	Phosphacyclopent-2-ene
22.631	0.25	1,3-Difluoro-5 pentafluorophenyl, Dimethylsilyloxybenzene	23.452	0.42	dodecyl ester
22.917	0.41	1-Phosphacyclopent-2-ene	24.161	0.29	5-p-Tolylamino-methylene, Thiazolidin-4-one
23.558	0.34	dodecyl ester	24.754	0.43	2-Pyrazolin-5-one
23.936	0.64	3,3,3-Trifluoro-2-hydroxy-2-pyridin, 2-Ethyl ester	24.922	0.40	Trifluoroacetate, Coumarin
24.170	0.29	3-Phenethyl, Thiazolidin-4-one	28.275	1.31	4-Phenoxy-Tridecane
24.834	0.34	2-Pyrazolin-5-one	29.257	0.22	Nonahexacontanoic acid, Ketone
24.931	0.41	7-Hydroxy-4-methyl, Coumarin, Trifluoroacetate	30.285	1.41	Pentacosane , Heptadecane
28.176	1.41	4-Phenoxytridecane	32.570	1.00	Octadecane
29.257	0.23	Pyridyl ketone	38.417	0.81	Carbonic acid, Octadecyl Vinyl ester
30.384	1.23	Pentacosane, Heptadecane			
30.510	0.61	1H-pyridin			
32.479	1.67	Octadecane			
38.218	0.93	Benzene, Carbonic acid, Nonahexacontanoic acid			

4.1.15.2 Spent engine oil (70cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometry (GCMS) analysis of the spent engine oilextracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month one (1) are presented in Figures 4.12 and 4.13 respectively.

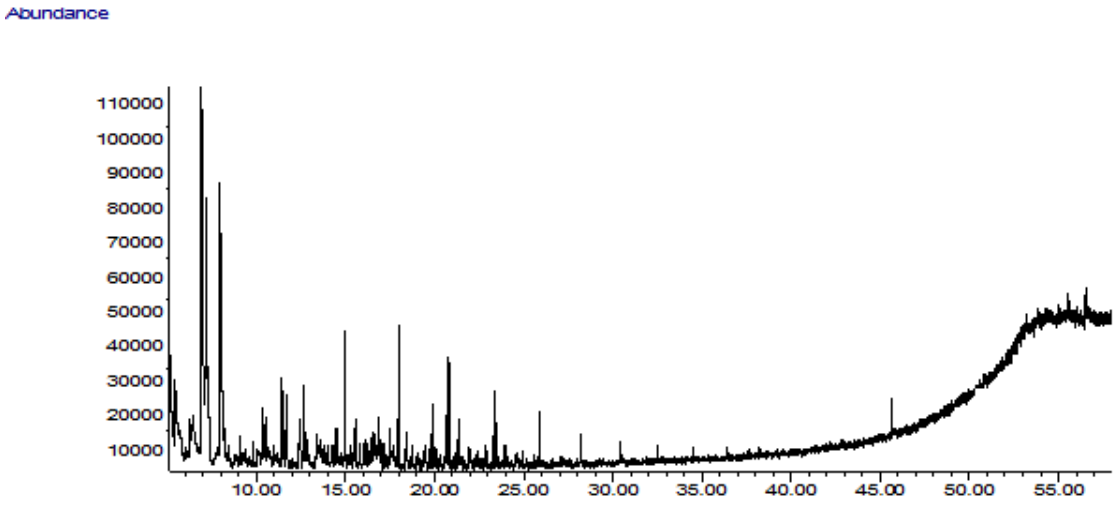

Figure 4.12: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* at Month one (1)

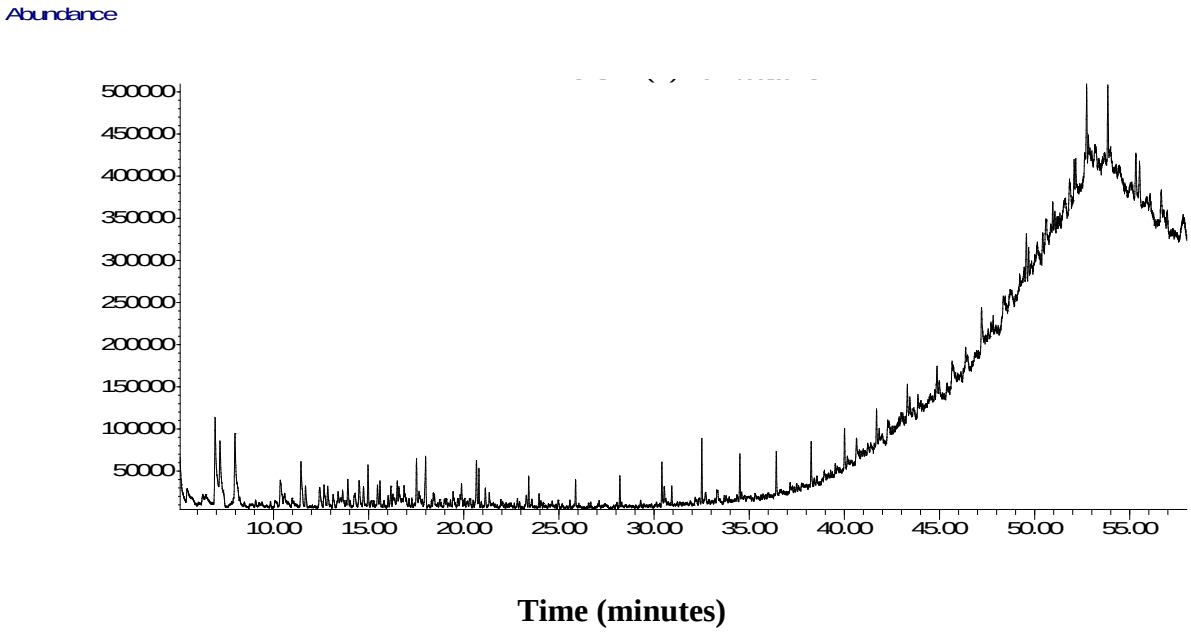

Figure 4.13: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Urena lobata* at Month one (1)

Table 4.17: Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis and Urena lobata* at month one (1)

Melissia officinalis			*Urena lobata*		
Retention Time (Minutes)	Area (%)	Compounds	Retention Time (Minutes)	Area (%)	Compounds
5.476	1.15	Acetic acid, Butyl ester	5.372	1.15	Hexyl ester
6.289	0.98	Benzene,4-phenoxy-Silane, 2-Fluorophenyloxy, 4-Diamino-5-pyrimidine	6.328	0.88	Silane, Chlorobenzylidine
6.930	8.41	Ethylbenzene	6.930	8.41	Tricosanoicacid, Ethylbenzene
7.187	7.29	p-Xylene	7.482	5.39	1,3-dimethyl, Nonane
7.977	6.46	o-Xylene, , Hexyl ester	7.977	2.04	2-Fluorophenyloxy, o-Xylene
8.223	0.30	Nonane, 4-Chlorophenoxy, Dodecyl ester	8.323	0.60	Dodecyl ester, , p-Xylene,
8.469	0.23	Tricosanoic acid, Benzene, 2(1H)-Pyrimidinone	8.516	0.37	Benzene, Pyrimidinone
8.761	0.27	p-Chlorobenzylidine	8.901	0.47	Pyrimidine, 3-Phenoxy, Butyl ester
9.053	0.33	2(1H)-Pyrimidinone, 2,3,5,6,7,8-Hexahydro-1-phenylthiazepine	9.155	0.42	2(1H)-Pyrimidinone, 4-(4-methylphenyl), Cyclopentane
9.842	0.27	Hydroperoxide	9.842	0.27	4-Methylphenyl, 1-Ethylbutyl
10.077	0.47	Trimethylsily1-benzyl, 1H-Indazol-3-acetate, Cycloheptatriene	10.077	0.47	1-Benzyl-1H-indazoloxy, Acetate, 1,3,5-Cycloheptatriene
10.357	2.40	1-Ethyl-3-methyl	10.260	1.70	1,2,3-trimethyl
10.580	0.70	Mesitylene	11.524	3.40	Mesitylene
10.987	0.49	1-Ethyl-4-methyl	11.685	0.22	Decane
11.685	1.22	Decane	12.652	0.43	1-Pentanol
12.652	1.51	1-Hexanol, 1-Pentanol	12.846	1.42	Hexahydro-4,7-Methanoindene, Cyclopropyl

12.846	1.20	Indane, Cyclopropyl	13.138	0.99	Tricosanoic acid, Indene
13.138	0.99	Indene	13.396	1.16	1-Hexanol, 1-Methyl-3-propyl
13.396	1.11	1-Methyl-3-propyl	14.331	0.30	3-methyl-, Oxalic acid, isobutyl nonyl ester, Nonane, 5-butyl-
13.813	0.24	Tricosanoic acid	14.510	1.67	Indane, o-Cymene, Cycloheptadiene
13.905	1.48	7-Methanoindene	14.983	1.86	Undecane, 2,4-Dimethylstyrene
14.031	0.30	Oxalic acid, Isobutyl, Nonane	15.376	0.40	4,4'-Dimethoxy-2'-hydroxychalcone, 2-methylpropionate
14.500	1.80	o-Cymene	16.028	0.42	7-Decadiene, Nonane
14.729	1.06	Decane	16.115	0.50	2,3-Dihydro-5-methyl
14.963	1.86	Undecane	16.274	0.72	Naphthalene, Borazine
15.267	0.30	2-Methylpropionate	16.503	1.07	1H-Indene, Bourbonene
16.028	0.52	Cyclohexane, 3,7-Decadiene	16.863	2.30	5-Chlorovaleric acid, Dimethylsilane
16.165	1.10	2,4-Dimethylstyrene, 1H-Indene	17.121	0.43	2-Phenylpropane, Propyl-Nonadecane
16.280	0.49	Borazine, Bourbonene, 1,3-Cycloheptadiene	17.713	1.16	Benzeneacetaldehyde, Azulene
16.773	0.60	Naphthalene	18.299	0.57	2-tert-Butyltoluene, 1,3-diethyl-5-methyl-, 2-tert-Butyltoluene
16.900	0.40	1-Chloro-2-methyl, 2-Phenylpropane, 5-Chlorovaleric acid	18.452	0.61	Tridecane, Heptadecane, 4-Quinolinecarboxylate
17.221	0.52	9-Methylheptadecane, 1-Hexanol, Nonadecane	18.746	0.49	4-Methyl-2H-benzopyrane, Iron
17.615	1.06	4-Methyl-2H-Benzopyrane, Azulene	19.026	0.38	3-Buten-1-ol, 4-Phenyl
17.923	0.52	Benzeneacetaldehyde	19.884	0.25	Pyrimidine, Tricosanoic acid
18.280	0.37	2-tert-Butyltoluene	20.056	0.29	Sulfurous acid, 2-Ethylhexyl, Octane, Heptanone
18.512	0.61	Undecane, Tridecane, Heptadecane	20.296	0.23	1-Hepten-3-ol, 1-Phenyl,

					Ethanone,
18.846	0.31	4-Quinolinecarboxylate, 4-Methyl-2H-Benzopyrane	20.491	0.32	Octane-2,4-dione
19.126	0.73	3-Buten-1-ol, 4-Quinolinecarboxylate	22.230	0.23	Tricosanoic acid
19.687	1.41	Diazacyclooctadeca-2,11-diene, Iron, Phenylenamine	22.820	0.40	4-Quinolinecarboxylate, Carbonic acid
20.176	0.49	Sulfurous acid, 2-Ethylhexylnonyl, Octane, 4-Heptanone	22.929	0.26	Biphenyl
20.397	0.33	1-Hepten-3-ol, Ethanone,	24.960	0.49	Silane
20.601	0.52	Octane-2,4-dione	25.578	0.46	1,1'-Biphenyl, 3-methyl-
20.970	1.25	1H-Pyrrolo[2,3-b]quinoxalin	28.055	0.42	3,3'-Dimethylbiphenyl
22.246	0.40	Tricosanoic acid	28.199	1.21	Eicosane
22.740	1.70	Carbonic acid	30.413	1.89	2,6,10-trimethylundecane, Heptadecane
22.937	0.86	Biphenyl, Inolin-2-yl	30.928	0.66	thyl-3-phenyl-
24.998	0.99	Silane	32.164	0.35	9H-Fluorene, Benz[a]azulene, Diphenylacetylene
28.869	1.01	Eicosane	32.708	0.74	Tritriacontane
30.843	0.79	Heptadecane	33.783	0.35	Nonahexacontanoic acid
32.164	0.95	9H-Fluorene, Benz[a]azulene, Diphenylacetylene	33.967	0.27	Hentriacontane, Octane-2,4-dione
40.009	2.69	Octatetracontane	40.209	1.66	Octatetracontane, 1-iodo-
41.691	1.85	Nonahexacontanoic acid, Eicosyl isobutyl ether	41.891	1.78	Carbonic acid, Eicosyl isobutyl ether

4.1.16GCMS of Spent Engine Oil (SEO) Extracted from SEO Polluted Soil Four Months After Remediation with *Melissia officinalis* and *Urena lobata*

4.1.16.1 Spent engine oil (50cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometry (GC-MS) analysis of the spent engine extracted from soil polluted with 50cl SEO are presented in figure 4.14 and 4.15. These compounds are similar to those identified at month one (1) while at month four (4), most of the compounds were further degraded to alcohols, acids and new isomers were formed (Table 4.18).

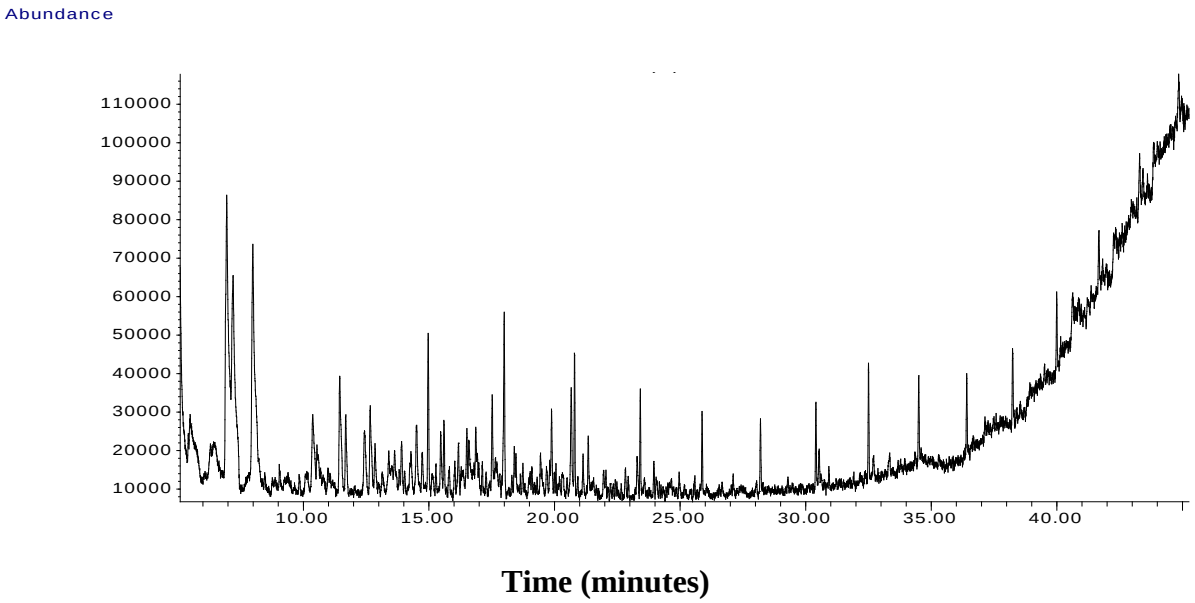

Figure 4.14: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* at Month four (4)

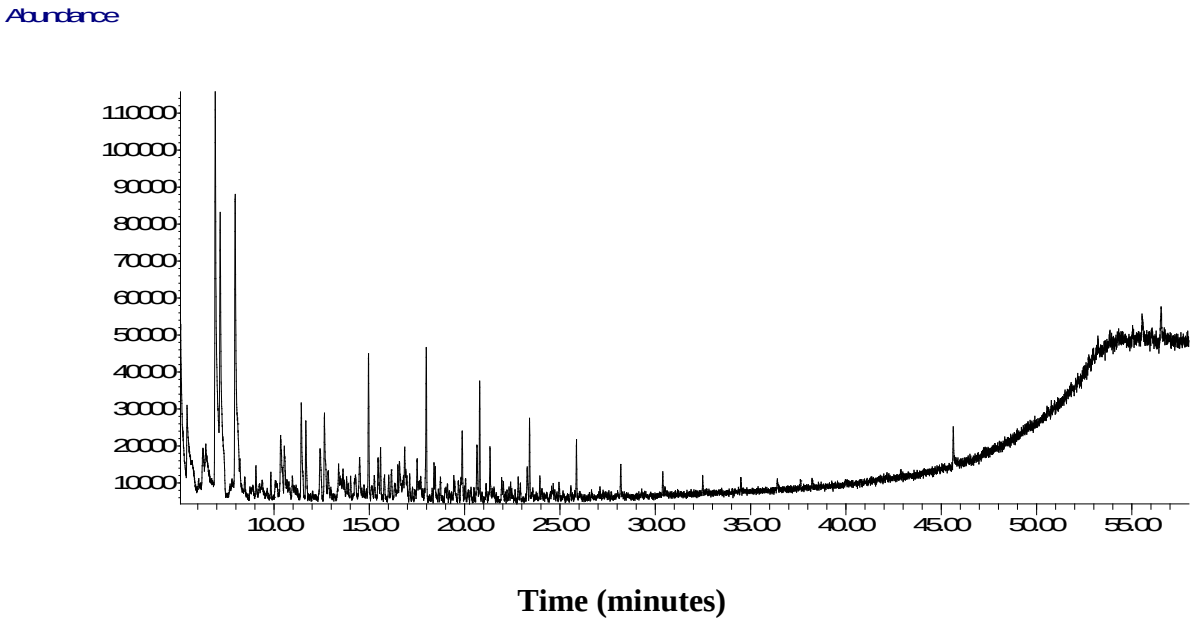

Figure 4.15: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Urena lobata* at Month four (4)

Table 4.18: Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis*and *Urena lobata* at month four (4)

Melissia officinalis			*Urena lobata*		
Retention Time (Minutes)	Area (%)	Compounds	Retention Time (Minutes)	Area (%)	Compounds
6.947	14.09	Ethylbenzene, 5-Phenoxy-6-phenyl	6.597	11.09	4-Chlorophenoxy
7.988	9.92	o-Xylene, Benzene	7.617	7.62	Benzene, 1,3-Dimethyl
8.463	0.31	Acetic acid, Dodecyl ester	8.463	0.31	Ethylbenzene, Acetic acid
8.766	0.30	Silane	8.766	0.30	Silane, dimethyl, 2-Fluorophenyloxy
9.041	0.33	Acetic acid, 4-Chloro-2-methylphenoxy, Pyrimidinone, 4-4-Methylphenyl	9.741	1.63	4-(4-Methylphenyl), 5-Phenoxy-6-phenyl
10.077	0.20	4-Chlorophenoxy	10.374	2.80	1,2,3-Trimethyl
10.374	2.80	Quinolone, Mesitylene	10.981	0.33	Diazacyclooctadeca-2,11-diene
10.981	0.33	Diazacyclooctadeca-2,11-diene	11.450	4.09	1-Ethyl-3-methyl, Mesitylene
11.690	2.15	Decane, Bicycloocta-1,3,5-triene,	11.690	2.15	Decane
12.663	2.81	1-Hexanol	12.663	2.81	1-Hexanol
12.852	1.29	Indane, 7 -Methyl-cyclopropyl	12.852	1.29	Indane, Bicycloocta-1,3,5-triene
13.144	0.54	8-Nitro-5-ethoxy, 6-Methoxy-4-methyl,	13.144	0.54	Quinolone
13.813	0.26	Acetic acid, 4-Chloro-2-methylphenoxy, Undecyl ester	13.813	0.26	Acetic acid, 4-Chloro-2-methylphenoxy, Undecyl ester
13.911	1.25	Pyrimidinone,	13.911	1.25	Pyrimidinone, 4-Methylphenyl
14.025	0.44	Tricosanoic acid	14.025	0.44	Tricosanoic acid
14.511	1.52	1-Ethyl-2,4-dimethyl, o-Cymene	14.511	1.52	o-Cymene
14.740	0.91	4,7-Methano-1H-indene	14.740	0.91	4,7-Methano-1H-indene
14.969	2.90	Undecane	14.969	2.90	Undecane
15.118	0.29	2-Phosphabicyclohex-3-ene	15.118	0.29	2-Phosphabicyclohex-3-ene
15.284	0.57	Coumarin, Trifluoroacetate	15.284	0.57	7-Hydroxy-4-methyl, Coumarin,

					Trifluoroacetate
15.461	1.38	1,3-Cyclopentadiene	15.461	1.38	1,3-Cyclopentadiene
16.028	0.79	Octane-2,4-dione	16.028	0.79	Octane-2,4-dione
16.171	1.25	2-Methyl-2-propenyl	16.171	1.25	2-Methyl-2-propenyl, 2-Butenyl
16.285	0.38	2-Butenedioic acid, Propionate	16.285	0.38	2-Butenedioic acid
16.508	0.76	1-Methyl-2-phenylcyclopropane	16.508	0.76	1-Methyl-2-phenylcyclopropane
16.863	0.45	Naphthalene	16.863	0.45	Naphthalene
17.126	0.44	4-Chloro-2-methylphenoxy	17.521	0.18	Azulene
17.521	0.18	Azulene	18.397	0.59	5-Ethyldecane
17.904	0.33	3-Fluorophenoxy, Dodecyloxy-	18.465	0.20	2(1H)-Naphthalenone, 3,4-Dihydronaphthalene
18.299	0.72	Trifluoromethylphenoxy	18.637	0.51	Aminocaproic acid, Hexadecyl ester
18.397	0.59	5-Ethyldecane, 3,4-DihydroNaphthalene	18.740	0.22	Quinolinecarboxylate
18.465	0.20	2(1H)-Naphthalenone,	18.980	0.43	2-Butenedioic acid, Dimethyl ester
18.637	0.51	6-Aminocaproic acid, 3,4-DihydroNaphthalene	19.444	0.69	1H-Indene
18.740	0.22	Quinolinecarboxylate	19.810	0.52	4-Chloro-2-methylphenoxy
18.980	0.43	2-Butenedioic acid	20.663	2.22	1-methyl-Benzocy cloheptatriene
19.444	0.69	1H-Indene	20.920	0.31	3,10-Dinitrodiftalone
19.810	0.52	4-Chloro-2-methylphenoxy	21.137	0.90	Benzocycloheptatriene
20.291	0.21	Coumarin, Trifluoroacetate	21.343	1.04	1(2H)-Naphthalenone
20.663	2.22	1-Methyl-Benzocycloheptatriene	22.825	0.60	Nonane
20.920	0.31	3,10-Dinitrodiftalone	23.415	1.62	Tetradecane, Eicosane
21.137	0.90	Benzocycloheptatriene	24.439	0.15	1-phenoxy-naphthalen-2-yl
21.343	1.04	1(2H)-Naphthalenone	24.513	0.57	Undecyl ester
22.825	0.60	Nonane	24.960	0.22	4-Chlorophenoxy

23.415	1.62	Tetradecane	25.171	0.28	Trimethylsilyl ether
24.439	0.15	1-Phenoxy-naphthalen-2-yl, Diazene	26.671	0.60	Oxadiazole
24.513	0.57	Undecyl ester	30.922	0.31	2-Pyrazolin-5-one
24.960	0.22	4-Chlorophenoxy	34.504	1.23	Tetracosane
25.171	0.28	2-t-Butylphenyl, 1,3,4-Oxadiazolethylcoumarin			
26.671	0.60	2-t-Butylphenyl, 1,3,4-Oxadiazole			
30.922	0.31	2-Pyrazolin-5-one			
34.504	1.23	Tetracosane			

4.1.16.2 Spent engine oil (70cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometry (GC-MS) analysis of the spent engine extracted from soil polluted with 70cl SEO as shown in Figure 4.16 and Figure 4.17. the equivalent results was presented in Table 4.19

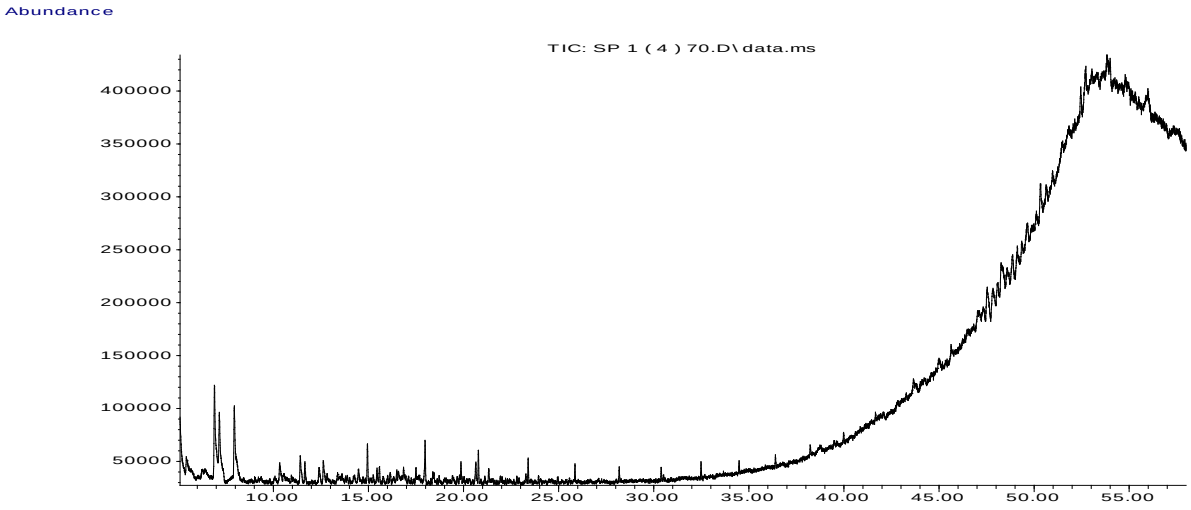

Figure 4.16: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* at Month four (4)

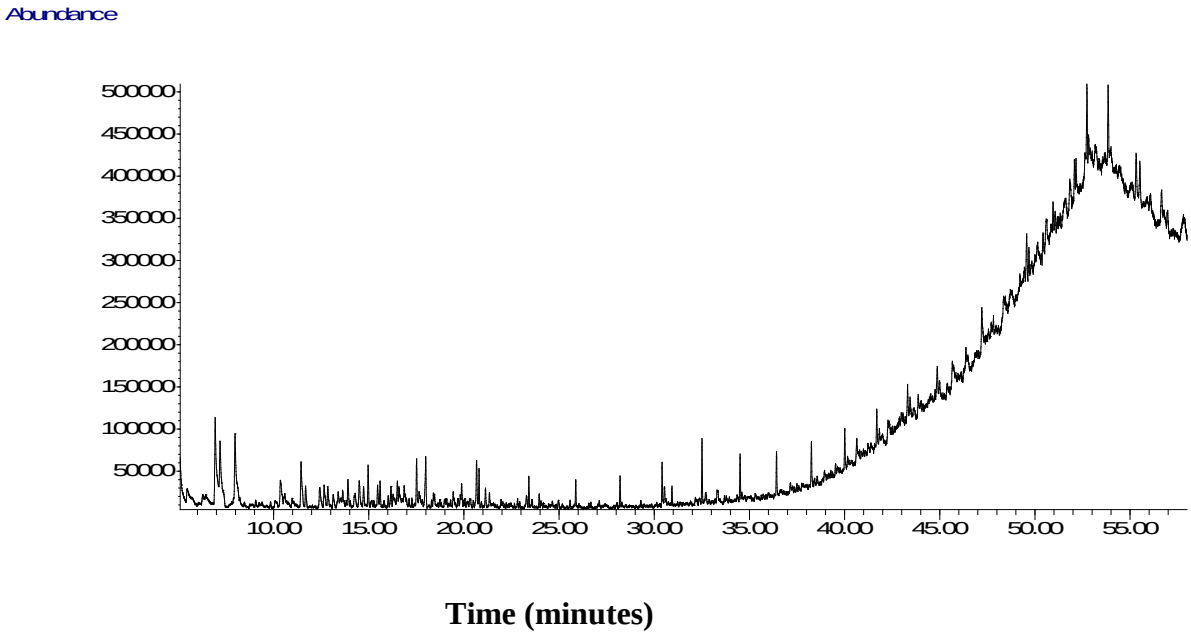

Figure 4.17: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Urena lobata* at Month four (4)

Table 4.19: Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis*and*Urena lobata*at month four (4)

Melissia officinalis			*Urena lobata*		
Retention Time	Area (%)	Compounds	Retention Time (Minutes)	Area (%)	Compounds
5.425	1.50	Benzene	6.832	10.29	4-Chlorophenoxy
6.232	0.40	Boroxin, 4-Methylphenyl	7.911	9.85	2(1H)-Pyrimidinone
6.901	13.67	Ethylbenzene, p-Xylene	8.560	1.55	Ethylbenzene
7.159	7.29	1,3-dimethyl-	8.766	0.30	1-Hexanol, Silane
7.948	8.25	o-Xylene	9.861	0.53	o-Xylene, Benzene Acetic acid
8.429	0.20	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene	10.187	0.42	4-Chlorophenoxy
9.814	0.41	Propionic acid, 3,3,3-Trifluoro-2-hydroxy-2-methyl-1H-pyridin, Ethyl ester	10.374	2.80	Acetic acid
10.043	0.32	Phosphoric acid, Phenyl ester	11.450	2.19	Mesitylene
10.512	0.25	2-Butenedioic acid, Dimethyl ester	11.670	0.50	Diazacyclooctadeca-2,11-diene
12.412	1.51	Trifluoromethyl, Acridin	12.663	2.81	Decane
12.623	2.96	2-Phenylpiperazine	12.852	0.30	Octa-1,3,5-triene
12.824	0.93	Phosphoric acid, 4-Methylphenyl	13.144	1.44	6-Methoxy-4-methyl
13.607	0.52	1,3-Difluoro-5-pentafluorophenyl, Dimethylsilyloxybenzene	13.856	0.30	Undecyl ester
13.888	0.77	4-Methoxy-phenyl, 1-Phenoxy-naph thalen-2-yl, Quinolyl-2-pyridyl ketone	13.941	0.50	7-Methanoindene, 2(1H)-Pyrimidinone
14.483	1.01	Phosphoric acid, 4-MethylPhenyl ester	14.025	2.34	1,3-Cyclopentadiene, 1H-indene
14.946	2.98	Tridecane, Undecane	14.511	1.52	Tricosanoicacid, o-Cymene

16.320	0.19	2-Phosphabicyclohex-3-ene	14.740	1.36	Octahydro-,4,7-Methano-1H-indene
16.491	0.99	Naphthylamine	14.889	2.00	Hex-3-ene, Undecane
16.566	0.37	Phosphoric acid, 4-Methylphenyl, Phenyl ester	15.118	1.29	6,6-Dimethyl, 2-Butenedioic acid
16.846	0.82	2-4-4-Trifluoromethyl, Propionate	15.284	0.57	Trifluoroacetate, 3,4-p-Chlorobenzylidine
17.109	0.58	2-Butenedioic acid, 2,3-Dimethyl ester	15.461	1.38	Methyl-5-methylene
18.443	0.51	Ethyl ester	16.028	0.79	Pyrimidine, Octane-2,4-dione
19.175	0.23	1,3-Difluoro-5 pentafluorophenyl, Dimethylsilyloxybenzene	16.355	1.00	Trifluoromethyl, Azulene
19.295	0.21	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene	16.612	0.56	Phenylcyclopropane
19.421	1.54	3,10-Dinitrodiftalone	16.800	1.55	Naphthalene
20.646	2.08	Butyrate	17.376	0.66	Trifluoroacetate
21.939	0.74	2-(t-Butylphenyl), 4-Biphenylyl, 1,3,4-Oxadiazole	17.543	3.11	Coumarin
23.398	1.54	Tetradecane	17.955	0.38	3-Fluorophenoxy, Propionate
30.110	0.18	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene	18.427	1.29	Naphthalenone, 5-Ethyldecane
			18.652	1.40	Naphthalenone, 1,2,3,4 -
			18.891	0.30	Quinolinecarboxylate
			19.346	0.73	Propionate, Trifluoroacetate
			19.444	0.69	1H-Indene, 2,3-dihydro-1,2-dimethy
			19.720	1.53	4-Chloro-2-methylphenoxy)-,
			19.879	1.55	1,2,3,4-tetrahydro-5-methyl-

	20.291	1.31	Coumarin, 6,6-Dimethyl-2,3,4-triphenyl
	20.764	2.30	Benzocycloheptatriene
	20.963	0.41	3,10-Dinitrodiftalone, Naphthalenone
	21.307	0.83	Benzocycloheptatriene
	22.247	0.59	7-Hydroxy-4-methyl-3-(2-thiophenyl
	22.955	1.60	Nonane
	23.500	3.62	Tetradecane, Eicosane
	24.301	0.45	4-Methoxy-phenyl
	24.513	1.78	3-Ethyl-5-propyl, Diazene
	24.880	1.22	4-Chlorophenoxy
	25.472	0.68	Oxadiazolethylcoumarin
	27.831	1.50	1,3,4-Oxadiazole
	32.852	0.41	2-Pyrazolin-5-one, 4-p-Methylaminophenyl
	34.744	1.23	Tetracosane

4.1.17 GCMS of Spent Engine Oil (SEO) Extracted from SEO Polluted Soil Seven Months After Remediation with *Melissia officinalis* and *Urena lobata*

4.1.17.1 Spent engine oil (50cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometric (GC-MS) analysis results of the spent engine oil extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month sevenare presented in figure 4.18 and 4.19 respectively. The results Table 4.20

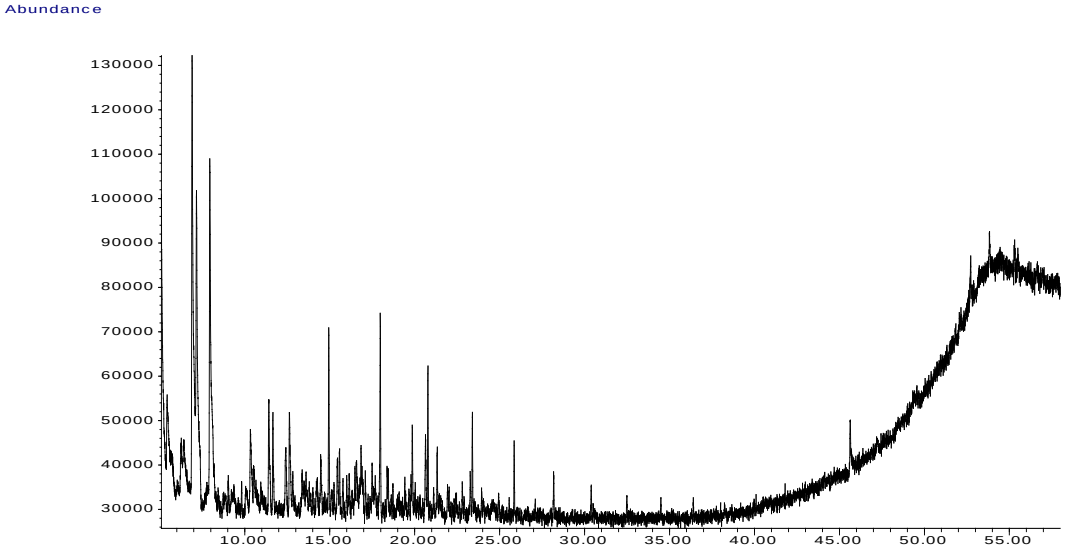

Figure 4.18: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* at Month seven (7)

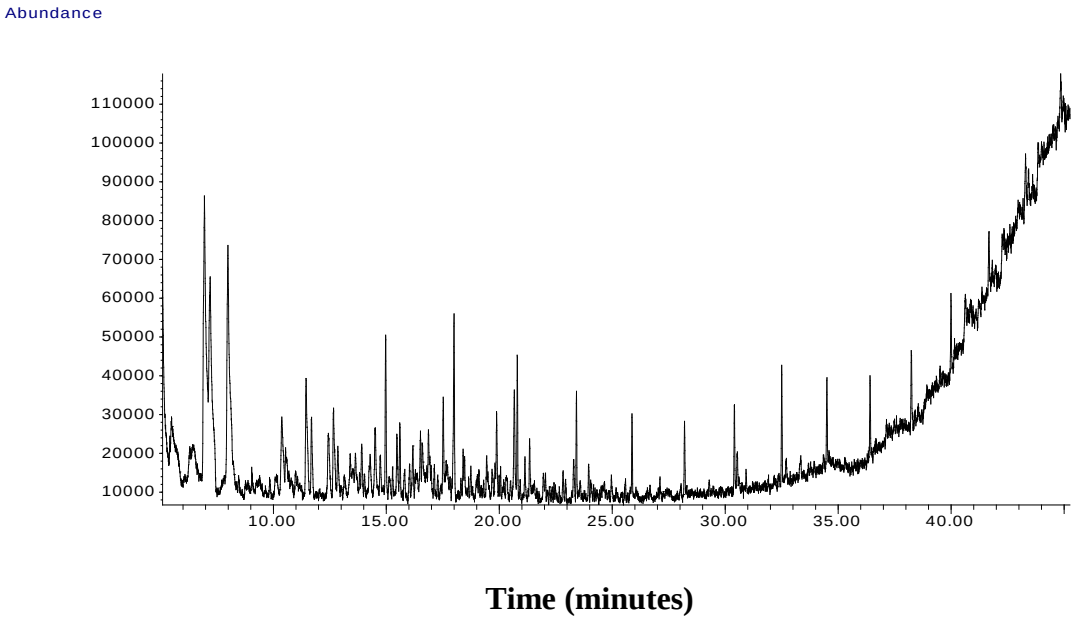

Figure 4.19: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Urena lobata* at Month seven (7)

Table 4.20: Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 50cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month seven (7)

Melissia officinalis			***Urena lobata***		
Retention Time (Minutes)	Area (%)	Compounds	Retention Time (Minutes)	Area (%)	Compounds
6.901	17.34	Ethylbenzene	6.941	8.24	4-Methoxy-phenyl
7.159	7.23	o-Xylene, p-Xylene	7.519	2.30	Benzene, 1,3-dimethyl-, o-Xylene, p-Xylene
8.726	0.21	Benzimidazoldione	8.861	1.20	Benzimidazol-7-dione, Ethylbenzene
9.030	0.69	4-Methoxy-phenyl, 1-Phenoxynaphthalen-2-yl, Diazene	9.068	0.50	1-Phenoxynaphthalen-2-yl, Diazene
9.619	0.26	Boroxin	9.687	0.50	4-Methylphenyl
10.329	1.71	Oxamide	10.348	3.18	N-(3-methylbenzyl), Boroxin
10.512	0.56	Pentafluorophenyldimethylsilyloxybenzene	10.967	0.21	Quinolone
10.941	0.33	6-Methoxy-4-methyl	11.315	1.36	Oxamide
11.656	2.37	Propionic acid	11.602	1.70	3,3,3-Trifluoro-2-hydroxyphenyl, Ethyl ester
12.406	0.63	3,3,3-Trifluoro-2- hydroxy-2-methy, Ethyl ester	12.411	1.60	Propionic acid
12.623	3.01	2-Phosphabicyclohex-3-ene,	12.323	2.11	6,6-Dimethyl, 2,3,4-Triphenyl
13.379	0.75	13-Dioxa-4,10- diazacyclooctadeca-2,11-diene, 2-p-Methoxyphenyl	14.324	1.50	Diazacyclooctadeca-2,11-diene
14.941	3.84	Tridecane, Undecane	14.842	6.04	Nonane
15.250	1.00	2-Butenedioic acid	15.310	1.30	2-Butenedioic acid, Dimethyl ester
15.461	1.69	1-Methyl-1H-pyridin	15.503	0.31	Dodecane
15.782	0.98	1-Phenoxy-naphthalen-2-yl	15.754	0.74	1-Phenoxy-naphthalene

16.486	0.50	3,10-Dinitrodiftalone	16.568	0.40	2,4-Imidazolidinedione
16.583	0.78	2-p-Methoxyphenyl, 2-Pyridyl ketone	16.602	0.48	2-p-Methoxyphenyl
16.846	0.50	2,4-Imidazolidinedione	16.746	1.42	3,10-Dinitrodiftalone
17.493	0.70	N-(2,4,6-Trinitrophenyl), 2-Naphthylamine	17.435	0.60	N-(2,4,6-Trinitrophenyl)-2-naphthylamine
17.973	4.97	Dodecane	17.981	2.07	Tridecane, Undecane
18.265	0.19	1-Phosphacyclopent-2-ene	18.261	0.29	1-Phosphacyclopent-2-ene, 1,2,3-triphenyl-5-dimethylmethylene
19.467	0.22	6-Methoxy-4-methyl	20.403	0.60	4-Chlorophenoxy, Dodecyl ester
20.033	0.40	Acetic acid, 4-Chlorophenoxy	20.744	3.11	Acetic acid, 1,2,3-Triphenyl-5-dimethylmethylene
20.634	2.51	1,2,3-Triphenyl-5-dimethylmethylene	21.219	1.21	Benzimidazol
21.332	1.13	Dimethyl ester	21.322	1.40	2-Butenedioic acid, 2,3- Dimethyl ester
21.452	0.22	1H-Indole-2-carboxamide, N-(2'-acetylphenyl)	21.532	0.19	N-2'-acetylphenyl
23.398	1.70	Tetradecane	23.875	0.75	Tetradecane
			33.453	0.30	1H-Indole-2-carboxamide, Dimethylsilyloxybenzene

4.1.17.2 Spent engine oil (70cl) polluted soil remediated with plants

Gas chromatography- mass spectrophotometric (GC-MS) analysis results of the spent engine oil extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month seven are presented in figure 4.20 and 4.21 respectively. The equivalent results is shown in Table 4.21

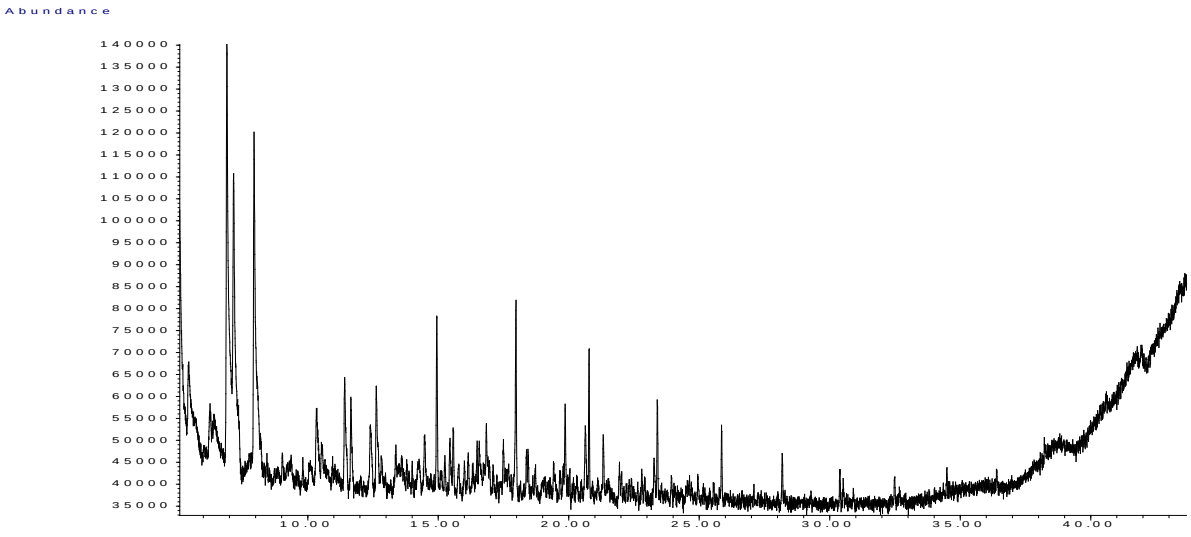

Figure 4.20: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* at Month seven (7)

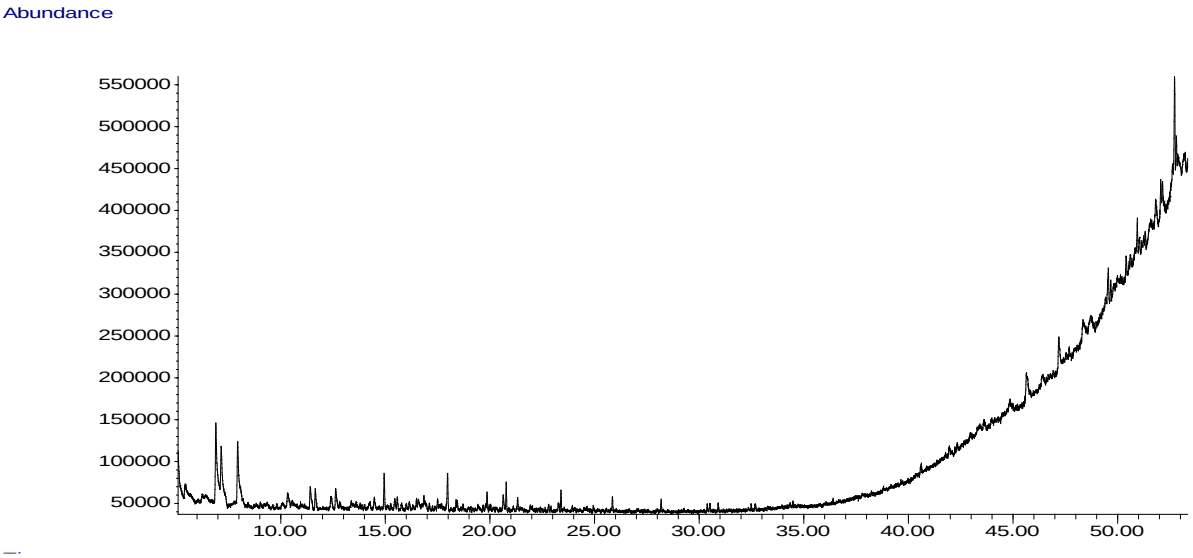

Figure 4.21: Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Urena lobata* at Month seven (7)

Table 4.21: Compounds from Gas chromatogram of spent engine oil (SEO) extracted from soil polluted with 70cl of SEO and remediated with *Melissia officinalis* and *Urena lobata* at month seven (7)

Melissia officinalis			*Urena lobata*		
Retention Time	Concentration Area (%)	Compounds	Retention Time	Concentration Area (%)	Compounds
6.901	19.62	Ethylbenzene	5.431	1.08	Benzene, 1-Phosphacyclopent-2-ene, 1,2,3 Triphenyl-5-dimethylmethylene
7.159	9.22	p-Xylene, o-Xylene	6.901	14.77	Ethylbenzene, p-Xylene
7.943	12.21	1,3-dimethyl	7.153	7.30	1,3-dimethyl
11.416	3.50	Benzene, Propionate	7.943	9.32	o-Xylene
11.650	3.34	Boroxin, 4-Methylphenyl	9.030	0.38	Propionic acid,
12.400	2.47	1-Methyl-1H-pyridin, Ethyl ester	9.213	0.16	2-p-Methoxyphenyl-8-methyl
12.629	2.95	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene	10.329	0.97	Phosphoric acid, 4-methylphenyl, Phenyl ester
14.941	3.87	Undecane	11.416	3.54	1,2,4-Trimethyl
15.570	1.62	4-Methoxy-phenyl, 1-Phenoxy-naphthalen-2-yl, Diazene	11.650	2.33	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene
16.148	1.03	2-Butenedioic acid, 3-Methylphenyl, Dimethyl ester	12.400	1.77	2-Phosphabicyclohex-3-ene, 6,6-dimethyl-2,3,4-triphenyl
16.571	1.47	Propionic acid, Methyl-1H-pyridin-, 2-Phenyl, Ethyl ester	12.623	2.76	2-Butenedioic acid, 3-Methylphenyl, Dimethyl ester
16.846	0.73	2,4-Imidazolidinedione, 3-Methylphenyl	13.316	0.23	2-Pyridyl ketone
17.098	0.57	1-Phosphacyclopent-2-ene	13.676	0.67	Quinoline
17.498	1.17	1-Phenoxy-naphthalen-2-yl, Diazene	13.796	0.41	4-Methoxy-phenyl, 1-Phenoxy-naphthalen-2-yl, Diazene
17.979	5.09	Dodecane	14.941	3.07	Tricosanoic acid
18.380	0.63	Trifluoromethyl, Acridin	15.444	1.37	Phosphoric acid, Phenyl ester

19.415	0.47	3,10-Dinitrodiftalone	16.846	0.66	Boroxin, 4-Methylphenyl
19.656	0.38	2-p-Methoxyphenyl-8-methyl, 2-Pyridyl ketone	17.258	0.31	1H-Indole-2-carboxamide, 3-Phenyl, N-(2'-Acetylphenyl)
19.799	0.30	4-Benzotriazol-2-yl, Xanthen-9-one	17.630	0.20	3,10-Dinitrodiftalone
19.862	2.09	2-Butenedioic acid, 2,3-Dimethyl ester	17.973	3.64	Dodecane
20.045	0.85	1H-Indole-2-carboxamide, N-2'-Acetylphenyl	19.421	0.34	3,10-Dinitrodiftalone
20.783	2.82	Tridecane	20.033	0.45	1,3-Difluoro-5-pentafluorophenyl, Dimethylsilyloxybenzene
21.109	0.40	1-Phosphacyclopent-2-ene	20.274	0.21	1-Phosphacyclopent-2-ene, 1,2,3-Triphenyl-5-dimethylmethylene
23.209	0.32	2-Phenylpiperazine,	20.640	1.87	Benzimidazol
23.392	2.03	Tetradecane	20.777	2.27	Tridecane
27.094	0.39	Boroxin, tris(4-methylphenyl)-	22.808	0.71	1-Phosphacyclopent-2-ene
34.493	0.61	Pyridyl ketone	24.628	0.21	4-Methylphenyl
			24.845	0.26	1-Phenoxy-naphthalen-2-yl
			30.390	0.82	2-Pyrazolin-5-one, 1,3-Diphenyl

4.1.18 Exudate extracted from *Melissia officinalis* and *Urena lobata* in SEO polluted soil grown

Exudates extracted from *Melissia officinalis* and *Urena lobata*were subjected to GCMS analysis and the compound found in (Table 4.22) and Figure 4.22 and 4.23 shows Gas chromatogram of exudates extracted from *Melissia officinalis* and *Urena Lobata*Tricosanoic acid, Benzene,Ethylbenzene, Benzene, 1, 3-dimethyl, o-Xylene, p-Xylene, 2(1H)-Pyrimidinone, 4-(4-Methylphenyl), Phenoxy-6-phenyl,Coumarin, Mesitylene Trifluoroacetate, 2(1H)-PyrimidinoneAcetic acid, (4-chlorophenoxy), Dodecyl ester, Acetic acid, 4-Chloro-2-methylphenoxy, Undecyl ester, Acetamide, N-(2-Cyano-4,5-diethoxyphenyl)-2-o-tolyloxy,Cyclopentanol, Diazacyclooctadeca-2,11-diene,Decane, Benzene,3-Phenoxy, 2,4-Diaminochlorobenzylidine, Dioxybenzyl,Pyrimidine, 4-Chlorophenoxy, Dodecyl ester, 1-Hexanol, 2-ethyl, Indane, Benzene, 2-propenyl, 4-Chloro-2-methylphenoxy, Tricosanoic acid, 2,3,5,6,7,8-Hexahydro-1-phenyl, 5-(p-Chlorophenylimino), Thiazepine, Oxamide, 1H-Indene, 2-(t-Butylphenyl), 1,3,4-Oxadiazole, Coumarin, Trifluoroacetate, Acetic acid, Iron, Phenylenamine-N'-amino, Naphthalene, Pyrimidine,Octane-2,4-dione, Vincamine, Azulene, 2-Phenylpiperazine, Boroxin, Sulfurous acid, ester, 2-Phenylpiperazine, 4-Quinolinecarboxylate, Propionate, Pyridine, 1,3,4-Oxadiazole, Acetic acid, Butyl ester, Acetic acid, butyl ester, Acetic acid, 4-Chlorophenoxy, Dodecyl ester, 4-chloro-methylphenoxy, Undecyl ester, Naphthalene, 1,2,3,4-tetrahydro-1, 5-dimethyl, Benzene, 3-Phenoxy, Tetradecane,Pentadecane,Tridecane, Naphthalene, Methanol, Heptafluoro-1- butyrate, Tricosanoic acid, 4-Trifluoromethyl, 2,4-Diaminochlorobenzidine, Pyrimidine, Chloro-2-methylphenoxy, Undecyl ester, Triazolo(1,5-a)quinolin-2-yl), Quinolinecarboxylate, 1,3,2-Dioxaphosphorinane, Silane, 2-Fluorophenyloxy, 2,4 Diamino-3,4-chlorobenzylidine, Pyrimidine, Coumarin, Trifluoroacetate, Tricosanoic acid, Acetic acid, 4-Chloro-2-methylphenoxy, Carbonic acid, Pentadecyl ester.

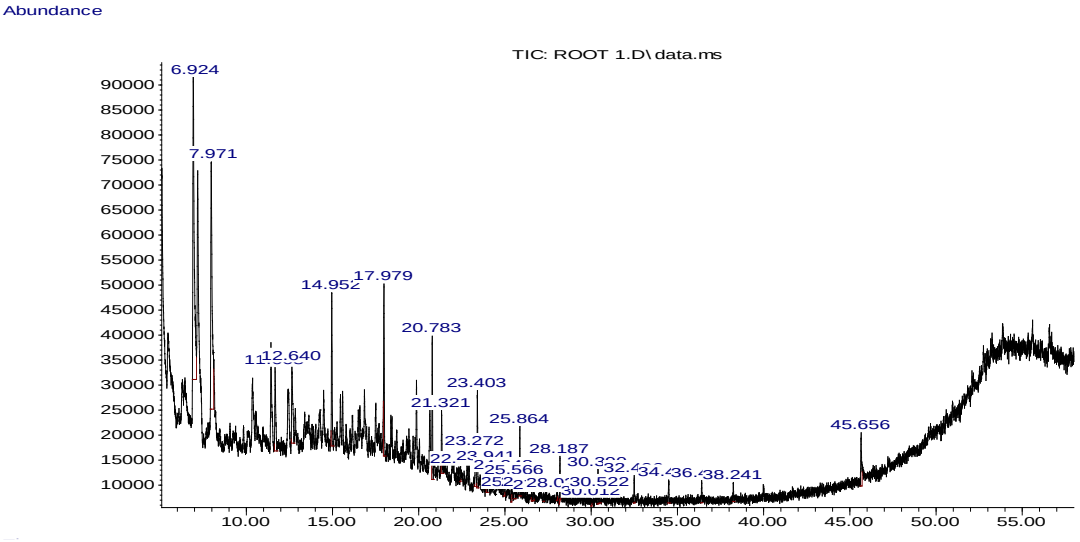

Figure 4.22: Gas chromatograms of exudates from *Melissia officinalis*

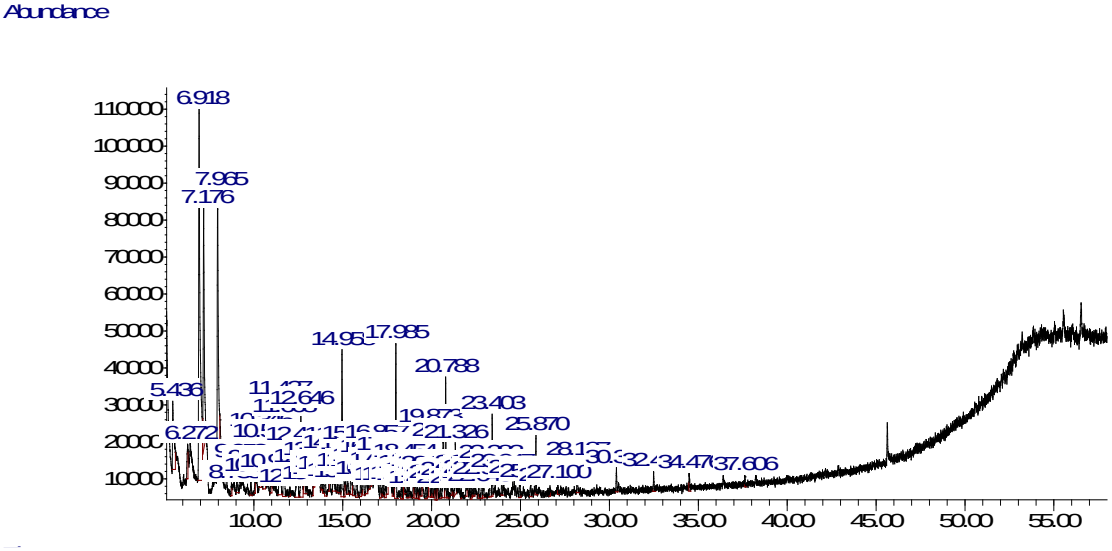

Figure 4.23: Gas chromatograms of exudates from *Urena lobata*`

Table 4.22: Exudates from the roots of *Melissia officinalis* and *Urena lobata*

Melissia officinalis			*Urena lobata*		
Retention Time	Area (%)	Compound	Retention Time	Area (%)	Compound
6.924	25.6	Ethylbenzene	5.436	2.87	Tricosanoic acid, Benzene
7.971	20.5	p-Xylene, Benzene, 1,3-dimethyl-	6.272	0.87	Ethylbenzene
11.668	4.56	1-Octanol, 2-butyl-, Decane Dodecane	6.918	16.47	Benzene, 1,3-dimethyl, o-Xylene, p-Xylene.
12.640	5.33	3-Tetradecene,	7.176	7.55	2(1H)-Pyrimidinone, 4-(4-Methylphenyl), Phenoxy-6-phenyl
14.952	6.45	Undecane	8.738	0.25	Coumarin, Trifluoroacetate, 2(1H)-Pyrimidinone
17.979	6.74	Dodecane,Pentadecane,Tridecane	9.053	0.72	Acetic acid, (4-chlorophenoxy), Dodecyl ester, Acetic acid, 4-Chloro-2-methylphenoxy, Undecyl ester
20.783	5.07	Tridecane, Octadecane	9.836	0.55	Acetamide, N-(2-Cyano-4,5-diethoxyphenyl)-2-o-tolyloxy.
21.321	2.48	Naphthalene, 1,2,3,4-Tetrahydro-1, 4-Dimethyl	10.065	0.30	Cyclopentanol, Diazacyclooctadeca-2,11-diene
22.425	0.49	Acetic acid, 4-Chlorophenoxy, Dodecyl ester, 4-chloro-methylphenoxy, Undecyl ester	10.958	0.52	Mesitylene
23.272	1.90	Naphthalene, 1,2,3,4-tetrahydro-1, 5-dimethyl, Benzene, 3-Phenoxy	11.427	3.28	Decane
23.403	3.40	Tetradecane ,Pentadecane, Tridecane	11.668	2.15	Benzene,3-Phenoxy, 2,4-Diaminochlorobenzylidine, dioxybenzyl], Pyrimidine, 4-Chlorophenoxy, Dodecyl ester
23.941	0.94	Naphthalene, Methanol, Heptafluoro- 1- butyrate	12.017	0.22	1-Hexanol, 2-ethyl
24.948	0.89	Tricosanoic acid, 4-Trifluoromethyl	12.646	3.11	Indane, Benzene, 2-propenyl
25.378	0.50	2,4-Diamino-5-[3,4-chlorobenzidine, Pyrimidine, Chloro-2-methylphenoxy, Undecyl ester	12.846	0.80	4-Chloro-2-methylphenoxy, Tricosanoic acid
25.566	0.82	Triazolo(1,5-a)quinolin-2-yl), Quinolinecarboxylate, 1,3,2-Dioxaphosphorinane	13.327	0.17	2,3,5,6,7,8-Hexahydro-1-phenyl, 5-(p-Chlorophenylimino), Thiazepine.

26.539	0.32	Silane, 2-Fluorophenyloxy	13.888	0.25	Oxamide, 1H-Indene
26.665	0.39	2,4 Diamino-3,4-chlorobenzylidine, pyrimidine	14.723	0.26	2-(t-Butylphenyl), 1,3,4-Oxadiazole.
27.243	0.34	10- Diazacyclooctadeca-2,11-diene	15.267	0.21	Coumarin, Trifluoroacetate, Acetic acid,
28.187	0.31	Nonadecane, Tetracosane, Eicosane	16.016	0.32	Iron, Phenylenamine-N'-amino
30.012	1.78	Coumarin, Trifluoroacetate	16.331	0.54	Naphthalene, Pyrimidine,Octane-2,4-dione,Vincamine
32.496	0.29	Tricosanoic acid, Acetic acid, 4-chloro-2-methylphenoxy	16.857	0.57	Azulene, 2-Phenylpiperazine, Boroxin, Sulfurous acid, ester
34.493	0.95	Carbonic acid, Pentadecyl ester	17.510	0.50	2-Phenylpiperazine, 4-Quinolinecarboxylat
			18.969	0.56	Propionate, Pyridine, 1,3,4-Oxadiazole
			19.673	0.82	Acetic acid, Butyl ester

Table 4.23: Summary of Various Compounds in Root Exudates from the Roots of *Melissia officinalis* and *Urena lobata*

Group	Compounds
Amino acids	Pyrimidine, Phenyleamine,

Organic acids	Acetic acid, Carbonic acid, Sulfuric acids, Tricosonic acid
Polysaccarides	Triflouroacetate, Coumarin
Vitamins	Thiazepine, Acetamide, Oxadiazole
Purines/ Nucleotides	Vicamine, Mesitylene
Inorganic ions and gaseous molecules	H,OH
Alcohols	Octanol, Cyclopentanol, Methanol
Ester	Buyl ester, Pentadecyle ester, Undeccyl ester

4.2 Discussion

4.2.1 Plants found within the vicinity of automobile workshops

The plant with the lowest count was *Cynodondactylon* with a total number of 43 stands while *Gomphrena celosioides*had a total number of 48 stands. Figure 4.1 shows the percentage representation of the counts of plants found within the vicinity of the automobile workshops with *M. officinalis* having 31% while *U. lobata* had 28%. Thus both plants have met some of the requirements for plants meant for the phytoremediation study. Other plants found in the vicinity of the automobile workshops in the four study locations (Plate 4.1) were *Cynodondactylon, Gomphrena celosioides, Sida acuta and Seteria pumik* (Table 4.1).

Melissia officinalis and *Urena lobata* were found in all study locations, this might be as a result of the nature of their roots (well established tap roots and high in number too), the nature of their leaves (they were broad and plenty too plants makes use of chloropyll found within their stomata to carryout photosynthesis which is a metabolic process employed by the plant to derive their nourishment needed for survival). Besides, it

might be that the plants were able to synthesize certain compounds (exudates) that were able to degrade the spent engine oil alone or in collaboration with the rhizospheric microorganisms, hence they were able to proliferate. Wu *et al*. (2019); Aransiola*et al.* (2021) used *Melissia officinalis* to remediate soil polluted with heavy metals.

Other plants identified have been reported to have phytoremediation potential. *Sida acuta* had better phytoremediating potential for Pb^{2+}, Cd^{2+} and Co^{2+} than*Durantaerecta* (Anarado*et al.*, 2018) *Gomphrena celosioides* and *Cynodondactylon* have been reported to be used in the remediation of kerosene polluted soil (Musa, 2016; Musa *et al*., 2019).

4.2.2 Total aerobic heterotrophic bacterial (TAHB) counts in soil from automobile workshops

The results of bacterial counts found within the various workshops showed that the counts were generally low when compared to the counts from both rhizosphere and the automobile workshop vicinity. The mean bacterial counts of the automobile workshop were higher in Tegina ($2.37\times10^6\pm4.01\times10^6$cfu/g), than either Suleja or Minna. Bida had the least bacterial counts of $1.56\times10^5\pm2.13\times10^5$cfu/g (Figure 4.2). Statistical analysis revealed that there was no significant difference ($p>0.05$) among the various automobile workshops.

The results of the bacterial counts found in the automobile workshops vicinity revealed that Suleja had the highest bacterial counts ($8.10\times10^6\pm1.38\times10^7$cfu/g) while Bida had the lowest bacterial counts ($4.73\times10^6\pm8.03\times10^6$cfu/g) as compared to Tegina and Minna (Figure 4.2). Statistical analysis revealed that there were significant differences ($p<0.05$) among the locations.

The high number of bacteria found in the rhizosphere and within the vicinity of the automobile workshops particularly in Minna and Suleja may be as a result of favorable

physical and chemical conditions of the soil that supported their growth and development. It is also possible availability of nutrients enabled both obligate and facultative organisms to survive and degrade the spent engine oil within the root region of the plants. This may also be a major pointer that the pollutant (SEO) might have not seeped too deeply into the soil, coupled with the fact that the plant exudates supported microbial growth for effective biodegradation. Soils of automobile workshops had the lowest bacterial counts due to the heavy presence of SEO found in the soil, probably only hydrocarbon degraders were able to grow and proliferate in SEO soaked environment. These results agree with the findings of Musa *et al.* (2019) and Abioye*et al.* (2021) which reported that soil polluted with hydrocarbons or its bye- products create hydrophobic condition that prevents the growth of some microorganisms, and allows some to proliferate due to their ability to sysnthesize enzymes that assist the organisms to withstand harsh environmental conditions and to degrade hydrocarbons.

4.2.3 Total fungal counts in soil from automobile workshops

The general decrease in fungal counts across the soil samples collected from the rhizosphere, automobile workshop vicinity and the automobile workshop might be as a result of the effect of the SEO that might have inhibited the growth of strict aerobic organisms hence only hydrocarbon degrading fungi were able to survive and grow in the soil. Limited nutrients in the soil could also be responsible. The implication is that there is competition for the limited supply hence only the fungi with adaptive and protective mechanisms will grow. This finding is in line with Oyewole*et al.*, (2019). The high counts in Bida might be due to the soil type (i.e physical and chemical properties), the plants and the rhizospheric organism might have degraded the SEO via natural attenuation.

4.2.4 Total aerobic heterotropic bacterial counts (TAHBC) in soil remediated with *Melissia officinalis* and *Urena lobate*

A general increase in the counts of bacteria was observed from June to December. Soils that were treated with 70cl of spent engine oil had the lowest counts closely followed by soil treated with 50cl of spent engine oil. Soil with plant one (SP1) and plant two (SP2) alone had very high bacterial counts (Figure 4.4). The low bacterial counts observed in June and July might be as a result of low amount of nutrients for the growth of the bacterial hence competition for the limited nutrients. It may also be as a result of unfavorable environmental conditions. However, the high bacterial counts observed from August to December may be as result of rainfall as it has been reported that rainfall increases the growth and proliferation of bacteria. Besides, the plants used for the remediation might have released their exudates that enhanced the proliferation of the bacteria in the rhizosphere (Zand*et al.*, 2016; Sharma *et al.*, 2018). Root nodules of some plants serve as habitats for microorganisms (Wu *et al.*, 2020).

4.2.5 Total fungal counts in Soil remediated with *Melissia officinalis* and *Urena lobate*

A significant increase was observed from August to December in the fungal counts with September having the highest counts (SP1). Statistical analysis showed that there were significant differences ($p<0.05$) among the treatments. The low fungal counts observed in June and July might have been due to the harsh environmental conditions caused by the pollutant (SEO). The high fungal counts observed from August to December might be as a result of rainfall, adaptation to the pollutant (SEO) and increase in the number of hydrocarbon degrading fungi which might have degraded the SEO to less harmful compounds which other non oil degraders can use as substrates for their metabolism.

The plants used for the treatment might also be a reason the increased numbers of fungi. These findings are in agreement with the findings of Ismaila*et al.* (2014).

4.2.6 Oil utilizing bacterial and fungal counts in soil remediated *Melissia officinalis* and *Urena lobata*

The oil utilizing bacterial counts of soil remediated with plants were higher than the unremediated soils. It was also observed that there was a gradual increase in oil utilizing bacterial counts as the months progressed from June to December (Figure 4.6). The increase in counts might be as a result of increase in the exudates associated with the root nodules of the plants, compounds which detoxified the hydrocarbons thereby allowing the proliferation of non oil degraders in the soil (Ravanbakhsh*et al.*, 2008).

The oil utilizing fungal count ranged from $9.01\times10^{2}\pm5.17\times10^{2}$cfu/g (in September SP1) to $1.02\times10^{1}\pm5.17\times10^{1}$cfu/g (in July SP2 70cl), (Appendix F). Statistical analysis showed significant differences ($p<0.05$) among the treatments. Differences in counts however, might be as a result of the ability of different fungi to adapt and utilize SEO, the environmental stress which in this case is the pollution of soil with SEO, affects their proliferation as it might have altered their metabolism rate (Zhang *et al*., 2009).

4.2.7 Identification and Frequency of Occurrence of Microorganisms in the Remediated Soils

Soil polluted with 50cl of spent engine oil and remediated with *M.officinalis* (SP1 50) *Bacillus subtilis* had the highest frequency of occurrence (6.10%) while *Staphylococcus aureus* had the lowest frequency of occurrence (0.76%). In soil polluted with 50cl of spent engine oil and remediated with *U.lobata*(SP2 50) *Bacillus subtilis* had the highest frequence of occurrence (3.81%) while *Staphylococcus epidermidis* and *Staphylococcus aureus* had the lowest frequency of occurrence (1.52%). It was observed that in soil polluted with 70cl of spent engine oil and remediated with *M.officinalis* (SP1 70) *Bacillus subtilis* had the highest frequency of occurrence (3.05%) while *Staphylococcus epidermidis* had the lowest frequency of occurrence (0.76%). In soil oil polluted with 70cl of spent engine oil and remediated with *U.lobata* (SP2 70) *Bacillus subtilis* had the highest frequency of occurrence (3.05%) and *Staphylococcus epidermidis* had the lowest frequency of occurrence, 0.76% (Table 4.3). These microorganisms have been reported by many researchers to play a major role in hydrocarbon degradation. (Ismaila*et al.*, 2014; Abioye*et al.*, 2021; Oyewole*et al.*, 2021). The proliferation of species of *Bacillus* and *Staphylococcus* in this study might be due to their active and efficient enzymatic system which aids their ability to consume and break down carbon compounds efficiently (Oladoja*et al*., 2019; Wu *et al*., 2019).

In soil remediated with *M.officinalis* alone (SP1), *A. niger* had the highest frequency of occurrence (9.83%) while *P. notatum* had the lowest frequency of occurrence (0.81%). In soil remediated with *U. lobata* alone (SP2), *A. niger* had the highest frequency of occurrence (9.01%) while *P. notatum* had the lowest frequency of occurrence (0.81%). Soil polluted with 50cl of spent engine oil and remediated with *M.officinalis* (SP1 50) *A. niger* had the highest frequency of occurrence (5.73%) while *P. notatum* and *A.*

*fumigatus*had the lowest frequency of occurrence (1.63%), while in soil polluted with 50cl of spent engine oil and remediated with *U. lobata* (SP2 50) *A. niger* had the highest frequency of occurrence (6.55%) and *P. notatum*, *A. fumigatus* and *F. oxysporium* had the lowest frequency of occurrence (1.63%), (Table 4.5). In soil polluted with 50cl of spent engine oil and remediated with *M.officinalis* (SP1 70) *A. niger* had the highest frequency of occurrence (3.27%) while *P. notatum* and *A. flavus and F. oxysporium* had the lowest frequency of occurrence (0.81%), soil polluted with 70cl of spent engine oil and remediated with *M.officinalis* (SP2 70) *A. niger* had the highest frequency of occurrence (4.09%) while *P. notatum* and *A. flavus and F. oxysporium* had the lowest frequency of occurrence (0.81%). Most of these fungi have been reported by other researchers to posses the ability to degrade hydrocarbon (Chikere*et al.*, 2009; Aransiola*et al.*, 2021). The proliferation of species of *Aspergillus* and *Penicillium* might be due to their enzymatic system which aid them to thrive efficiently also it might be due to their ability to form spores in unfavorable condition (Oyewole*et al.*, 2021).

4.2.8 Screening of isolates for SEO utilization

High growth rate was observed in *Bacillus subtilis* B1 with an optical density of 0.410 followed by *Bacillus megaterium*B3with an optical density of 0.630 while *Staphylococcus aureus* B2 had an optical density of 930 with the lowest growth rate hence could not utilize SEO as effectively as *Bacillus subtilis* (Table 4.6). This is an indication that *Bacillus subtilis* is capable of degrading and utilizing the SEO better than the other bacteria isolated. This result agrees with the findings of Oyewole*et al*, (2021), who identified species of *Bacillus* and *Pseudomonas* as efficient hydrocarbon utilizers and attributed their capability to withstand the toxic component of the hydrocarbons (Makut*et al*, 2022).

The growth of fungi in mineral salt broth enriched with SEO as the sole carbon source is shown in Table 4.7. The results revealed that *Aspergillus* niger F4 with an optical density of 0.400 had the highest growth rate hence it was able to utilize SEO more than all other fungi closely followed by *Aspergillus flavus* F1 with an optical density of 0.540, while *Penicillium notatum* F3 with an optical density of 0.800 had the lowest growth. Those fungi that could utilize SEO maximally were 8(38.10%) while those fungi that could utilize SEO moderately were 11(52.38%) and fungi that could utilize SEO minimally were 2(9.52%) (Table 4.7). *Aspergillus niger* and *Aspergillus flavus* utilized the SEO at maximum rate, thus they proliferated rapidly, probably due to the fact that the fungi had competent enzyme system (Oyewole*et al.* 2021).

4.2.9 Molecular Identification of Selected Spent Engine Oil Utilizers

The amplicon of the identified bacteria from the Rso-A sample falls within the expected amplicon size (1500 bp) for 16S rRNA gene conserve regions for all bacteria (Plate VIII). Also, all the amplicons of the identified fungi are within the expected range for the ITS region for all fungi (Plate IX). The names and accession numbers of the identified isolates are revealed in Table 4.8.

The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Figure 4.10). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using

the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

4.2.10 Residual Total Petroleum Hydrocarbon (TPH) in Spent Engine Oil Polluted Soil Remediated with *Melissia officinalis* and *Urena lobate*

After seven (7) months of the phytoremediation of the spent engine oil polluted soil, 55.61% oil biodegradation was obtained followed by SP2 50 with 50.55% oil biodegradation while SP1 70 and SP2 70 had 37.9% and 32.10 % oil biodegradation respectively (Table 4.9). The rates and total extent of SEO biodegradation were significantly different at ($p< 0.05$). The two plants showed great ability to remediate SEO polluted soil at 50cl SEO concentration which is intended to represent moderate pollution (Table 4.9). The high rates of biodegradation in the soil could be due to the long interaction of the pollutant with the plant and its associated rhizospheric microorganisms. Also the nature of the *M. officinalis* root might have enhanced the microbial growth which in turn led to the utilization of the SEO as their major carbon source. The implication of these results is that concentration of SEO influences rate and total extent of biodegradation and also the longer the time, the more biodegradation (Ijah and Antai, 2003; Musa, 2016). The finding agrees with the report of Zand*et al.* (2016) that the effectiveness of phytoremediation relies on the establishment of plants with sufficient biomass growth and active root proliferation, which can support a flourishing microbial consortium assisting contaminant dissipation in the rhizosphere.

4.2.11 Physical and Chemical Properties of Soil from Automobile Workshops in Parts of Niger State

Generally, the pH of these soils in all locations were acidic. The organic carbon was higher in soils in the vicinity of the automobile workshops (3.39 – 5.9%) than in soils within the automobile workshops in all four locations (7.35 - 9.19%). Similar observation was made with the organic nitrogen and phosphorus contents of the soil in all locations (Table 4.10). The soil within the automobile workshops had less moisture (16.50- 17.74%) than soil in the vicinity of the workshops (25.7 - 28.4%).

The soil texture in all locations was granular or fine sand while the soil structure was sand (Table 4.10). These results agree with the findings of Ijah and Abioye (2003b) who observed a decreased pH in Kerosene polluted soil. Also the low moisture content observed is in agreement with the findings of Kayode *et al.* (2009) who concluded that the low permeability of the soil was due to blocked spores as a result of the spent lubricating oil, hence low infiltration of water.

4.2.11.1Physical and chemical properties of Spent Engine Oil Polluted Soil Remediated with M. officinalis and U. lobata

The pH was generally acidic and ranged from 4.18 to 5.03 in unpolluted soil habouring plants. It was observed that the soil organic carbon, Nitrogen and Phosphorus increased while moisture content decreased slightly one month after the phytoremediation process. The increase in organic carbon, Nitrogen and Phosphorus might be due to the plant used for the remediation of the spent engine oil polluted soil. This result agrees with the findings of Aransiola*et al*., 2021 that attributed the increase in Nitrogen and Phosphorus to the grass used, and stated that grasses have the largest root surface area, penetrate deep into soil, genetically diverse and easily grow under unfavorable soil conditions, fix nitrogen; thus, limiting microbial competition for nitrogen which

becomes limited in oil-contaminated sites. The physical and chemical properties of the soil remediated soil after four months of phytoremediation are presented in Table 4.12. The pH was generally acidic and ranged from 4.88 to 5.33 in unpolluted soil habouring plants. In the polluted soil (50-70cl) remediated with plants (SP1, SP2) pH ranged from 4.7 to 5.52 while in SP1 (70cl) and SP2 (70cl), the pH ranged from 4.73 to 5.65 (Table 4.12). Organic carbon, Nitrogen and Phosphorus increased greatly when compared to one month after phytoremediation. This might be due to the poliferation of rhizospheric microorganism degradation of the pollutant (SEO) into less toxic compounds that can be used up by the microorganisms for their metabolism and the plants used. These results agree with the findings of Siciliano and Germida (1998) and Manoharachary and Mukerji, (2006) reported that for the microbes to grow, multiply and subsequently degrade contaminants, they require essential nutrients from plants, while plants benefit from the detoxification of pollutants by the microbes.

After seven months of phytoremediation the physical and chemical properties of the remediated soil varied (Table 4.13). The pH was generally acidic and ranged from 5.23 to 6.11 in unpolluted soil habouring plants. In the polluted soil remediated with plants SP1 (50cl), SP2 (50cl) pH ranged from 6.03 to 6.07 while in SP1 (70cl) and SP2 (70cl), the pH ranged from 5.25 to 5.66 (Table 4.13). The pH slightly increase from acidic to moderately acidic range, Organic acid and Phosphorus increased while Nitrogen decreased slightly when compared to month four. The slight increase in pH might be due to the degradation of the spent engine oil in the soil by the activities of the plants and its associated microorganisms which might have helped to reduce the hydrophobicity of the experimental soil thus making the soil less acidic. The increase in organic carbon and Phosphorus might be as a result of the exudates secreted by the plants which aids in the proliferation of the microoraganisms thus helping in nutrient

cycling for improved soil conditions. These finding agrees with the report of Hoang *et al.* (2021) that different types of complex (organic acids, sugars, phenolic compounds, polysaccharides, and humic compounds) and simple (amino acids, monosaccharides etc.) organic molecules, are secreted through plant roots (exudates). These exudates provide nutrient source for the growing microbes at the rhizospheric regions and help in effective colonization and degradation of pollutants (Singh *et al.*, 2019; Abioye*et al.*, 2021a).

4.2.12 Accumulation and Translocation of Heavy metals in *M. officinalis* and *U. lobata* used for Phytoremediation of SEO

These results suggest that *Melissia officinalis* and *Urena lobata* employ phytostabilization and phytoextraction as their mechanisms for carrying out phytoremediation. This agrees with earlier postulations that when bioconcentration factor (BCF) and (TF) is greater than one that suggest that the plants mechanism of phytoremediation is phytoextraction and when BCF>1 and TF<1 then phytostabilization is the mechanism employed for remediation. These results agree with the findings of Lorestani*et al.* (2011) and Ali and Khadijeh, (2018). The implications of these findings suggest that both plants employed both phytostabilization and phytoextraction as their mechanisms for carrying-out phytoremediation.

It was observed that *M.officinalis* and *U. lobata* leaves turned yellow and were dropping off three months after planting (3MAP) (Plate 4.1 and Plate 4.2). Yellowing of the leaves of the plants could be caused by a number of conditions such as moisture stress; overwatering or underwatering are the most common culprits when a plant's leaves turn yellow if plants do not receive enough water, they drop leaves to prevent transpiration (essentially, a plant's way of sweating) to conserve water (Hu and Chen,

2020). The spent engine oil used to pollute the soil is hydrophobic hence preventing water from reaching the roots of the plants (which might be the major reason why the the leaves turned yellow). Besides, when the soil does not drain well, an overdose of water makes the soil waterlogged, excluding oxygen. Without oxygen, roots start to die (Brestic*et al.*, 2016). Normal aging: is another reason why plant leaves turn yellow and eventully drop. As plants age, the lower leaves turn yellow and drop off (Hu and Chen, 2020). This is simply a normal part of their growth, but in the case of the plants used for the study present they were not over aged, which means that the yellowing of leaves might be caused by water and oxygen tension created by the hydrophobic nature of the spent engine oil.

Plant leaves may also turn yellow if a plant is not receiving all of the nutrients it requires (Brestic*et al.*, 2016). This can be caused by chlorophyll and nitrogen deficiency. The presence of the SEO might be responsible for altering the normal nutrient cycle which the plants use to source for their required nutrients.

4.2.13 Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis of Spent Engine Oil (SEO) Used for the Remediation Study

The chromatograms showed that spent engine oil had more aromatic cycloalkanes than straight chain alkanes. It also contains the aliphatic unsaturated hydrocarbons such as diazene, butene, ethene and alkynes group (diemethylene). Besides aliphatic saturated hydrocarbons such as propane, hexadecane, decane, nonane, tetradecane, methylpropane and pentacosane were detected. The aromatic hydrocarbons present were the mono aromatics such as benzene, toluene, ethylbenzene and xylene (BTEX) and polycyclic aromatic hydrocarbon (PAHs), (Table 4.15). Non- hydrocarbons compounds detected were propionic acids, inulin, phosphoric acid and coumarins (Table 4.15).

Xylene compounds are abundant in SEO. It exists in three isomeric forms. The isomers can be distinguished by the designations *ortho*- (*o*-), *meta*- (*m*-) and *para*- (*p*-), which specify to which carbon atoms (of the benzene ring) the two methyl groups are attached. By counting the carbon atoms around the ring starting from one of the ring carbons bonded to a methyl group, and counting towards the second methyl group, the o-isomer has the IUPAC name of 1,2-dimethylbenzene, the *m*-isomer is 1,3-dimethylbenzene and the *p*-isomer is 1,4-dimethylbenzene. Of the three isomers, the *p*-isomer is the most industrially sought after since it can be oxidized to terephthalic acid (Fabri and Simo, 2000). Xylene is used as a solvent. In this application, with a mixture of isomers, it is often referred to as xylenes or xylol. Solvent xylene often contains a small percentage of ethylbenzene. Like the individual isomers, the mixture is colorless, sweet-smelling, and highly flammable. Areas of application include the printing, rubber, and leather industries. It is a common component of ink, rubber, adhesives, in thinning paints and varnishes, it can be substituted for toluene where slower drying is desired, and thus is used by conservators of art objects in solubility testing (Carson and Hladik, 2009). The main effect of inhaling xylene vapor is depression of the central nervous system (CNS), with symptoms such as headache, dizziness, nausea and vomiting. At an exposure of 100 ppm, one may experience nausea or a headache. At an exposure between 200 and 500 ppm, symptoms can include feeling "high", dizziness, weakness, vomiting, and slowed reaction time (Kandyala*et al.*, 2010), The side effects of exposure to low concentrations of xylene (< 200 ppm) are reversible and do not cause permanent damage. Long-term exposure may lead to headaches, irritability, depression, insomnia, agitation, extreme tiredness, tremors, hearing loss, impaired concentration and short-term memory loss. A condition called chronic solvent-induced encephalopathy,

commonly known as "organic solvent syndrome" has been associated with xylene exposure (Fuente *et al.*, 2012).

The presence of these compounds in SEO may be due to prolonged usage of the oil leading to its contamination by chemical impurities (Dominguez- Rosada and Pichtel, 2004). This observation is in line with the report of Diab (2008) that larger amount of aromatic hydrocarbons are contained in SEO than normal alkanes, this might be as result of the changes that took place during its usage (Dominguez- Rosada and Pichtel, 2004).

4.2.14GCMS of Spent Engine Oil (SEO) Extracted from SEO Polluted Soil After Remediation with *Melissia officinalis* and *Urena lobata*

The results revealed the numerous and higher peaks of compounds in *M.officinalis* remediated soil (Figure 4.8) than *U. lobata* remediated soil (Figure 4.9). This means that biodegradation of the hydrocarbons was faster and greater in *U. lobata* remediated soil. Hydrocarbons present in spent engine oil as presented in (Table 4.16) can be grouped into two major groups which are aliphatic (unsaturated and saturated phenyl-1-butene, 5-phenoxy-6-phenyl, methyl) and aromatic (mono and polycyclic aromatic hydrocarbons such as naphthalene, benzene, toluene ethylbenzeneand xylene). Other non-hydrocarbons present were acetic acid, pyrimidionne, hexanol and pyrimidione.

Many isomers also emerged when compared to compunds found in spent engine oil used for the study. The plants used for the remediation *M.officinalis* and *U. lobata* at different concentrations (50cl and 70cl) of the SEO and its associated microorganisms might have played a major role in the degradation of the compounds which led to the formation of the isomers such as conversion of: Benzene to Ethylbenzene, Butane to Butane-1,- dione and Methylene to Methylnone (Table 4.16). These results agree with

the findings of Musa (2016), who also found many of compouds in kerosene pollued soil too. The formation of this new isomer implies that the bonds between the parent compounds have been altered and this alteration points to the fact that microorganisms and the plants used for the phytoremediation were able to degrade them.

These results which are similar to what were observed in soil remediated with 50cl revealed the numerous and higher peaks of compounds in *M.officinalis* remediated soil (Figure 4.10) than *U. lobata* remediated soil (Figure 4.11). The results suggest that biodegradation of the hydrocarbons was faster and greater in *U. lobata* remediated soil. Hydrocarbons identified in the SEO extract are presented Table 4.17. The groups of hydrocarbon found were aliphatic, Aromatic, alkenes, alkylnes, mono and polycylic aromatic hydrocarbons. Other non hydrocarbon such as oxalic acid, borazine, bournbonene, boraxine and tricosonic acid were detected. These findings agree the findings of Musa (2016) who identified similar compounds when *Gomphrena cellosiode* and *Cyandondatylon* were used to remediate kerosene polluted soil.

At month four Some compounds were found to be consistent from month one to month four, the compounds include Ethylbenzene, Mesitylene, Indane, Decane, Dodecane, Pyrimidinone, Benzene, Fluorophenyloxy, Dihydronaphthalene, Naphthalene, Ethyldecane, Azulene and Undecane, Benzocycloheptatriene, 1(2H)-Naphthalenone, Acetic acid, 7- MethanoindeneBoroxin, Trifluoroacetate, Ethyldecane and 2-Pyrazolin-5-one (Table 4.18). Also many acids were identified such as Acetic acid, Phosphoric acid, Butenedioic acid and Propionic acid (Table 4.18). These have further confirmed that the plants and its associated microorganisms played a major role in the oxidation of the compounds thus leading to their breakdown to acids and alkanones. Abioye*et al.* (2021) and Aransiola*et al.* (2021) reported that some microorganisms have the ability to

synthesize enzymes that help them to adapt to unfavourable environmental conditions and also using other organic compounds as carbon source for their metabolism.

These compounds are similar to those identified in spent engine oil polluted soil (50cl), few compounds were consistent from month one to month four including Dihydronaphthalene, Naphthalene, Ethyldecane, Azulene and Undecane, Xylene, Ethylbenzene, Mesitylene, Indane, Decane, Dodecane, Pyrimidinone, Benzene, Fluorophenyloxy, Benzocycloheptatriene, 1(2H)-Naphthalenone, Acetic acid, 7-MethanoindeneBoroxin and Trifluoroacetate (Table 4.19). Also acids were identified such as Acetic acid, Phosphoric acid, Butenedioic acid and Propionic acid (Table 4.19)Microorganisms have varying mechanisms for adapting to and catabolizing petroleum hydrocarbons (i.e., the enzyme-catalyzed breakdown of inorganic and organic pollutants). Other species may aid in this process probably through symbiotic relationships (i.e., release of glucose to aid in proliferation of hydrocarbon-degrading species or secretion of surfactants to render the oil more bioavailable). Thus, to the plants and its associated microorganisms may have played a major role in the breakdown of the compounds to acids and alkanones. Abioye*et al.* (2021) and Aransiola*et al.* (2021) reported that some microorganisms have the ability to synthesize enzymes that help them to adapt to unfavourable environmental conditions and breakdown organic compounds to simpler compounds.

After seven month of the phytoremediation study the GC/MS results revealed that SEO polluted soil remediated with *U. lobata* had more pronounced hydrocarbons peaks than *M. officinalis* remediated soils. Compounds identified were Ethylbenzene, o-Xylene, p-Xylene, Benzimidazoldione, 4-Methoxy phenyl, 1-Phenoxynaphthalen-2-yl, Diazene, Boroxin, Oxamide, Pentafluorophenyldimethylsilyloxybenzene, 6-Methoxy-4-methyl, Propionic acid, 3, 3, 3-Trifluoro-2- hydroxy-2-methy, Ethyl ester. 2-

Phosphabicyclohex-3-ene, 13-Dioxa-4, 10-diazacyclooctadeca-2,11-diene, 2-p-Methoxyphenyl, Tridecane, Undecane 2-Butenedioic acid, 4-Methoxy-phenyl. The number of peaks observed in soil polluted with 50cl and remediated with *M. officinalis* was few when compared to soil polluted with *U. lobata*, the reduction in the number of peaks might be as a result of degradation by plants used for the remediation and its associated microogranisms found within the rhizosphere region of the plant while the increased observered might be due to the formation of isomers formed as a result of the oxidation and reduction reactions of their parent compounds. Besides formation of new isomers, also formation of these new isomers might be attributed to the reaction of the parent compounds with the plants exudate as well as the interactions of the compounds with enzymes synthesized by the rhizoshperic microorganism. This agrees with the findings of Otobong and Victoria (2017); Oyewole*et al.* (2019) and Abioye*et al.* (2021).

In soil polluted with 70cl spent engine oil and remediated with *Melissia officinalis* and *Urena lobata* at seven month the GC/MS results revealed that SEO polluted soil remediated with *U. lobata* had more pronounced hydrocarbons peaks than *M. officinalis* remediated soils, this is in contrast to what was observered in soil polluted with 50cl spent engine oil where few peaks where observed in soil remediated with *M. officinalis* when compared to that remediated with *U. lobata* figure 4.16 and 4.17. Compounds identified were 2-p-Methoxyphenyl, Tridecane, Undecane, 2-Butenedioic acid, 4-Methoxy-phenyl, Ethylbenzene, o-Xylene, p-Xylene, Benzimidazoldione, 4-Methoxy phenyl, 1-Phenoxy naphthalen-2-yl, Diazene, Boroxin, Oxamide, Pentafluoro phenyl dimethylsilyl oxybenzene, 6-Methoxy-4-methyl, Propionic acid, 3, 3, 3-Trifluoro-2-hydroxy-2-methy, Ethyl ester. 2-Phosphabicyclohex-3-ene, 13-Dioxa-4, 10-diazacyclooctadeca-2,11-diene.

Other compounds were found to be recalcitrant that is the compounds were present from month one (1) to month seven (7). These compounds include Ethylbenzene, Mesitylene, Indane, Decane, Dodecane, Pyrimidinone, Benzene, Fluorophenyloxy, Dihydronaphthalene, Naphthalene, Ethyldecane, Azulene and Undecane, Benzocycloheptatriene, 1(2H)-Naphthalenone, Acetic acid, 7- MethanoindeneBoroxin, Trifluoroacetate, Ethyldecane and 2-Pyrazolin-5-one, Benzimidazolidione, Tridecane, Diazene, o-Xylene, p- Xylene, 4- Methoxy-phenyl, Tetradecane, 2, 4-Imidazolidinedione, 3,10- DinitrodiftaloneBoroxin and Propionic acid (Table 4.20).

At month seven (7) it was observed that many compounds were completely degraded, hence they were not present among the compounds identified at month seven. Some the these compunds are 9H-Fluorene, Diphenylacetylene, Tritriacontane, Nonahexacontane, Hentriacontantane, Octatetracontane, IH-Pyrrolo[2,3-b] quinoxalin, Ethanone, Eicosylisobutyl ether, Inolin-2-yl and Carbonic acid. It should be noted that these compounds were present at month one and month four but were not detected at month seven. Several researchers reported that some plants have phytoremediative potentials due to their ability to degrade heavy metals, hydrocarbons and other environmental pollutants (Ugoh and Moneke 2011;Ismaila*et al.*, 2014; Zand*et al.*, 2016).

4.2.15 Exudate extracted from *Melissia officinalis* and *Urena lobata* in SEO polluted soil grown

The rhizosphere is the area of soil roots where most of the reactions are affected by plant roots. Root exudates are the chemical compounds that are secreted by roots and act as a source of food for soil microbes and play an important role in soil microbe and plant interaction. The soil microbes include bacteria, fungi and actinomycetes which are important for plant growth development and health of plants. Plant roots release a huge

variety of chemical compounds to attract and select microorganisms in the rhizosphere which induce different mechanisms by which plant associated microorganisms influence plant growth and development. Plant microbe interactions play important roles in a number of vital ecosystem processes, such as carbon sequestration and nutrient cycling initiates and intimates physical and biological communication between the soil microbes and plant roots, (Plate 4.6) shows the nature of root of *Melissia officinalis* and *Urena lobata* during phytoremediation process**.**

Besides providing the mechanical support, the plant roots assist in water and mineral nutrient uptake, which includes the functions like synthesis, secretion and accumulation of diverse group of chemical compounds which plays a vital role as source of chemicals in soil root ecosystem, (Table 4.22) contains some compound which are called Auxins like Naphthalene, 4-Chlorophenoxy. Auxins are any group of plant hormones that regulates growth, particularly by stimulating cell elongation in stem and also plays a vital role in cell division and differentiation, in fruiting development and root formation. Amino acids are another group of compound found in (Table 4.23) and an example is Phenyleamine and Pyrimidine and they are the building blocks of DNA and RNA that functions as a store for genetic information. Organic acids were Acetic acid which are fungicidal, herbicidal, microbiocidal and are pH adjusters, Tricosanoic acid which is a major constituent of plant which functions to provide structural intergrity to the plant, aids in their energy metabolic processes, serves as signal transduction mediator (invitro and extra cellular signals) and Carbonic acid.

Another compound which is of importance is Coumarin. Coumarins are a family of plant-derived secondary metabolites that are produced and secreted roots by the plants roots is an iron-mobilizing compound that aid in iron uptake from iron-deprived soils (Grillet and Schmidt, 2017). Members of the coumarin family are found in many plant

species. Besides their role in iron uptake, coumarins have been extensively studied for their potential to fight infections in both plants and animals. Coumarin activities range from antimicrobial and antiviral to anticoagulant and anticancer according to Gnonlonfin*et al.* (2012). Pyridine is used to dissolve other substances. It is also used to make many different products such as medicines, vitamins, food flavorings, paints, dyes, rubber products, adhesives, insecticides, and herbicides. Pyridine can also be formed from the breakdown of many natural materials in the environment.

Plant roots exude a broad range of compounds into the rhizospheric soil. Generally it has been found that microorganisms in rhizosphere live under conditions of nutrient starvation and are thus constantly looking for nutrients. Root exudates mostly include sugars, amino acids, peptides, vitamins, nucleotides,organic acids, enzymes, fungal stimulants, and also some other compounds which help in plant water uptake, plant defense, and stimulation (Pate *et al.*, 2001 ; Pate and Verboom 2009; Taylor *et al.*, 2009). Sugars, organic acids, lipids, flavonoids, enzymes, amino acids, proteins, aliphatics, and aromatics are examples of primary substance found within the root exudates (Shukla *et al.*, 2011). Among these, the organic acids have been found of great importance because of its role in providing substrate for microorganisms and acting as intermediate in both biological and chemical reactions in the soil (Wutzler and Reichstein, 2013). Root exudates can be divided into two categories: low molecular weight compounds which include amino and organic acids, sugars, phenolic compounds and other secondary metabolites and high molecular weight compounds e.g. polysaccharides and proteins (Badri and Vivanco, 2009).Various environmental factors such as soil type, pH, temperature, nutrient availability and the presence of microorganisms determine the quality as well as quantity of root exudates (Shukla *et al.*, 2011; Xue *et al.*, 2013). Mostly at the root tips concentration of exudates is found to be

greater and at the sites of lateral branching, decreasing with increasing distance from the root surface (Marschner*et al.*, 2011).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The roles of automobile workshops have received little or no serious attention due to the portion of land polluted by individual automobile workshop operators, but the danger is when the total landmass occupied by automobile operators in every State is calculated and sumed up together then it will be more glaring that they cover a vast expance of land which poses a serious concern to the environment. Plants thriving in the vicinity of automobile workshops in Minna, Bida, Suleja and Tegina in Niger State were identified as*Cyanodondactylon, Gomphrena celosioides, Sida acuta, Melissia officinalis, Urena lobata* and*Seteraipumik*. However, the two plants that were found in all locations and used for the phytoremediation study were *Mellissia officinalis* and *Urena lobabta*.The mechanisms of phytoremediation revealed in this study were phytoextraction and phytostabilization and these mechanisms were both employed at the same time by both plants.

A gradual increase in microbial counts was observed on the soil polluted with 50cl and 70cl of spent engine oil as the study progressed which indicated that the plants and its associated microorganisms were able to degrade the spent engine oil. The microorganisms identified were *Bacillus subtilis, Staphylococcus aureus, Bacillus*

megaterium, Staphylococcus epidermidis, Aspergillus niger, Aspergillus flavus, Fusarium oxysporium, Penicillium notatum and *Aspergillus fumigatus .Bacillus subtilis* strain EE681738and *Aspergillus niger* strain HQ659967were able to utilize the spent engine oil effectively.

The physicochemical properties of the remediated soil revealed that the pH gradually improved from slightly acidic to neutral pH which was suitable for growth of the plants and proliferation of microorganisms. Nitrogen and organic matter contents of the polluted soil were higher than the SEO free soil, while higher phosphorous, Sodium and Potassium contents were observed in SEO free soil than the oil polluted soil.

After seven (7) months of phytoremediation of spent engine oil polluted soil with *M. officinalis* and *U. lobata,*soil polluted with 50cl of SEO and remediated with *M. officinalis* had higher biodegradation (56.61%) than soil polluted with 70cl of SEO and remediated with *U. lobata* (50.55%). However, the two plants showed great ability to remediate SEO polluted soil at 50cl which represents moderate pollution.

GCMS analysis of the residual SEO revealed that many compounds were completely degraded, such as 9H-Fluorene, Diphenylacetylene, Tritriacontane, Nonahexacontane, Hentriacontantane, Octatetracontane, IH-Pyrrolo[2,3-b]quinoxalin, Ethanone, Eicosylisobutyl ether, Inolin-2-yl and Carbonic acid after seven months.

In the exudates generated during the phytoremediation process, many compounds such as plant secondary metabolites, amino acids, organic acids, phenolic compounds, and aromatic compounds were detected. These compounds might have played crucial roles in phytoremediation of the spent engine oil in the soil.

5.2 Recommendations

Based on the results obtained, the following recommendations are made:

1. *Melissia officinalis* and *Urena lobata* can be employed in remediation of hydrocarbon and heavy metal polluted soils.
2. Hydrocarbon degrading potentials of the microbial isolates, particularly *Bacillus subtilis* strain EE681738 and *Aspergillus niger* strainHQ659967 should be exploited for bioaugumentation ofoil spills in the environment.
3. Products such as propionic acids and mesitylene resulting from the microbial breakdown of the oil pollutants should be harnessed for industrial purposes. Propionic acids can be used for the production of herbicides, while mesitylene can be used for the production of polyesters resin curing agents, stabilizers and plasticizers.
4. Compounds in the plant exudates released during oil biodegradation should be harnessed for industrial purposes. Such compounds areacetic acids (for making textiles and photographic films), coumarins (treatment of postrate cancer), and oxamide (as nitrogen fertilizers for biostimulation of polluted environment)
5. Laws should be strictly enforced that automobile workshops be located in selected areas of the cities to avoid indiscriminate dumping of spent lubricating oil in the environment.

5.3 Contribution of Research to Knowledge

The study revealed and addressed gaps that existed in phytoremediation studies and established that tropical plants found in the Automobile Workshops in parts Niger State, in association with theirautochthonous microorganisms have the ability to remediate spent engine oil polluted soil.

The following research contribution were identified:

I. Plants found growing within the vicinity of Spent Engine Oil (SEO) polluted soil could be a possible solution in the remediation of SEO polluted soil.

II. The plants used for this study which were *Melissia officinalis* and *Urena lobata* has been established to have abilities to remediate spent engine oil.

III. The rhizospheric exudates of the plants *Melissia officinalis* and *Urena lobata* played a major role in the remediation of spent engine oil.

IV. Identified microorganisms such as *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger*and *Aspergillus flavus* (when used in an assisted capacity) could be used as a plant growth promoting microorganisms in the remediation of spent engine oil polluted soil.

V. It was also established from the GC/MS results of this study that the autochthonous microorganisms in association withthese plants,*M. officinalis* and *U. lobata* were able to completely degrade 9H-Fluorene, Diphenylacetylene, Tritriacontane, Nonahexacontane, Hentriacontantane, Octatetracontane.

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APPENDICES

Appendix A: Biochemical test for identification of bacterial isolates

(i) Gram staining

A thin smear of each of the pure 24 hour old culture was prepared on clean grease-free slides, fixed by passing over gentle flame. Each heat-fixed smear was stained by addition of 2 drops of crystal violet solution for 60 seconds and rinsed with water. The smear was again be flooded with Lugol's iodine for 60 seconds and rinsed with water, decolourized with 70% alcohol for 15 seconds and rinsed with distilled water. It was then counter stained with 2 drops of Safranin for 30 seconds and finally rinsed with water, then allowed to air dry. The smears was mounted on a microscope and observed under oil immersion objective lens (Cheesbrough, 2006).

(ii) Motility test

A sterile needle was used to pick a loop of the organism and stabbed onto nutrient agar in glass vials. The vials were incubated at 37^{o}C for 48h. Non-motile bacteria showed growth confined to the stab line with definite margins without spreading to surrounding area while motile bacteria showed diffused growth extending from the surface (Cheesbrough, 2006).

(iii) Catalase test

A small quantity of culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Production of gas bubbles indicated the presence of catalase enzyme (Cheesbrough, 2006).

(iv) Methyl red test

Five millimetres of glucose phosphate broth (1g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100ml distilled water) was dispensed in clean test tubes and sterilized by autoclaving at 121^oC for 15 minutes. The tubes were then inoculated with the test organisms and incubated at 37^oC for 48 hours. At the end of incubation, few drops of methyl red solution was added to each test tube and colour change was observed, for positive test was indicated by bright red colour while negative test were indicted by yellow colour (Olutiola*et al.,* 2000).

(v) Voges-proskaeur test

Five millimetre of glucose phosphate broth (1g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100ml distilled water) was dispensed in clean test tubes and sterilized by autoclaving at 121^oC for 15 minutes. The tubes were then inoculated with the test organisms and incubated at 37^oC for 48 hours. After incubation, 6% " alpha-napthtol and 6% Sodium hydroxide was added to about 1ml of the broth culture. A strong red colouration forming within 30 minutes indicated positive reaction (Olutiola*et al.,* 2000).

(vi) Indole test

Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates were inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37^oC for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 minutes to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicated a positive result while yellow colouration indicated a negative result (Cheesbrough, 2006).

(vii) Citrate utilization test

The test organisms were lightly inoculated on the Simmons citrate agar slant and incubated at 35^0C for 18 hours. (Some organisms required up to 7 days of incubation due to their limited rate of growth on citrate medium). The development of blue colour, denoting alkalization was observed. For positive result, growth was visible on the slant surface and the medium was an intense Prussian blue. Trace or no growth was visible and no colour change occured for negative result (Cheesbrough, 2006).

(viii) Starch hydrolysis

Nutrient agar was prepared with 1% soluble starch and sterilized by autoclaving at 121^0C for 15 minutes. The medium was poured into sterile plates and allowed to solidify at room temperature the plate was inoculated by streaking the organisms once across the mediun. The plates were incubated at 37^{o}C for 24hours after which they were flooded with Gram's iodine. Hydrolysed starch appears as a clear zone due to alpha amylase activity while reddish brown zones around the colony indicates partial hydrolysis of starch to dextrans (Cheesbrough, 2006).

(ix) Oxidase test

This test was used to differentiate *Pseudomonas* sp from other gram negative enteric bacteria based on the presence of a few drops of the enzyme indo-phenol Oxidase were added onto the colonies of the test organisms, Oxidase positive colonies quickly became dark – purple within 10 seconds (Cheesbrough, 2006).

(x) Coagulase test

The tube coagulase test was carried out zero point five millilitre 0.5ml of sterile nutrient broth was dispensed into test tubes and the test organisms inoculated into the broth. The

cultures were incubated at 37^{o}C for 30 minutes. Then 0.5ml of blood plasma was added and incubated at 37^{o}C for 4 hours and observed for sign of coagulation at 1 and 4 hours interval. For a positive result, the blood plasma was converted into a shift gel or soft gel, best observed by tilting the tube to the horizontal position, while negative result showed no coagulation (Cheesbrough, 2006).

(xi) Urease test

This was done to determine the ability of the isolated organisms to produce the enzyme, urease for the decomposition of urea. The colonies were inoculated heavily over the entire surface of the urea medium and incubated at 37^{0}C. Observation was made at interval of 24 hours, 48 hours and after overnight incubation. In urease positive cultures, the colour of the medium was changed from dark brown to red or purple, while white colour was observed in urease negative cultures (Cheesbrough, 2006).

Appendix B: Total aerobic heterotrophic bacterial (TBH) counts of Soil from various Automobile Workshops

Location	Rhizosphere Soil		Automobile Workshop		Automobile Workshop Vicinity	
	Bacteria (cfu/g)	Fungi (cfu/g)	Bacteria (cfu/g)	Fungi (cfu/g)	Bacteria (cfu/g)	Fungi (cfu/g)
Suleja	$1.11 \times 10^7 \pm 1.89 \times 10^{7a}$	$3.03 \times 10^6 \pm 5.17 \times 10^{6a}$	$1.36 \times 10^6 \pm 2.28 \times 10^{6}{}_{b}$	$1.01 \times 10^6 \pm 1.73 \times 10^{6}{}_{b}$	$8.10 \times 10^6 \pm 1.38 \times 10^{7a}$	$1.68 \times 10^6 \pm 2.87 \times 10^{6}{}_{b}$
Tegina	$6.75 \times 10^6 \pm 1.15 \times 10^{7a}$	$1.70 \times 10^6 \pm 2.86 \times 10^{6a}$	$2.37 \times 10^6 \pm 4.01 \times 10^{6}{}_{b}$	$3.41 \times 10^5 \pm 5.71 \times 10^{5}{}_{b}$	$7.09 \times 10^6 \pm 1.20 \times 10^{7a}$	$1.68 \times 10^6 \pm 2.88 \times 10^{6}{}_{b}$
Bida	$7.10 \times 10^6 \pm 1.20 \times 10^{7a}$	$7.10 \times 10^6 \pm 1.20 \times 10^{7a}$	$1.56 \times 10^5 \pm 2.13 \times 10^{5}{}_{b}$	$1.36 \times 10^6 \pm 2.26 \times 10^{6}{}_{b}$	$4.73 \times 10^6 \pm 8.03 \times 10^{6a}$	$4.73 \times 10^6 \pm 8.03 \times 10^{6}{}_{b}$
Minna	$1.42 \times 10^7 \pm 2.41 \times 10^{7a}$	$4.04 \times 10^6 \pm 6.89 \times 10^{6a}$	$2.03 \times 10^6 \pm 3.44 \times 10^{6}{}_{b}$	$3.41 \times 10^5 \pm 5.71 \times 10^{5}{}_{b}$	$6.77 \times 10^6 \pm 1.15 \times 10^{7a}$	$1.02 \times 10^6 \pm 1.72 \times 10^{6}{}_{b}$

*a Superscript – Significant difference across the column *b Subscript – No Significant difference ac

Appendix C: Total aerobic heterotrophic bacterial count of the soil from the experimental soil

	June	July	August	Sept	Oct	Nov	Dec
SA	$1.72\times10^{7}\pm 1.89\times10^{7a}$	$1.79\times10^{7}\pm 1.89\times10^{7a}$	$2.03\times10^{7}\pm 1.89\times10^{7a}$	$2.46\times10^{7}\pm 1.89\times10^{7a}$	$2.76\times10^{7}\pm 1.89\times10^{7a}$	$2.39\times10^{7}\pm 1.89\times10^{7a}$	$2.32\times10^{7}\pm .89\times10^{7a}$
SP1	$1.69\times10^{7}\pm 1.89\times10^{7a}$	$1.69\times10^{7}\pm 1.89\times10^{7a}$	$2.33\times10^{7}\pm 1.89\times10^{7a}$	$2.36\times10^{7}\pm 1.89\times10^{7a}$	$2.83\times10^{7}\pm 1.89\times10^{7a}$	$2.33\times10^{7}\pm 1.89\times10^{7a}$	$2.02\times10^{7}\pm 1.89\times10^{7a}$
SP2	$2.02\times10^{7}\pm 1.89\times10^{7a}$	$1.75\times10^{7}\pm 1.89\times10^{7a}$	$2.39\times10^{7}\pm 1.89\times10^{7a}$	$2.66\times10^{7}\pm 1.89\times10^{7a}$	$2.76\times10^{7}\pm 1.89\times10^{7a}$	$2.25\times10^{7}\pm 1.89\times10^{7a}$	$2.06\times10^{7}\pm 1.89\times10^{7a}$
SP1 (50cl)	$3.38\times10^{6}\pm 1.89\times10^{6a}$	$3.73\times10^{6}\pm 1.89\times10^{6a}$	$6.75\times10^{6}\pm 1.89\times10^{6a}$	$1.11\times10^{7}\pm 1.89\times10^{7a}$	$1.15\times10^{7}\pm 1.89\times10^{7a}$	$1.28\times10^{7}\pm 1.89\times10^{7a}$	$1.62\times10^{7}\pm 1.89\times10^{7a}$
SP2 (50cl)	$3.73\times10^{6}\pm 1.89\times10^{6a}$	$4.05\times10^{6}\pm 1.89\times10^{6a}$	$6.77\times10^{6}\pm 1.89\times10^{6a}$	$9.78\times10^{6}\pm 1.89\times10^{6a}$	$1.05\times10^{7}\pm 1.89\times10^{7a}$	$1.32\times10^{7}\pm 1.89\times10^{7a}$	$1.65\times10^{7}\pm 1.89\times10^{7a}$
SP1 (70cl)	$3.04\times10^{6}\pm 1.89\times10^{6a}$	$2.37\times10^{6}\pm 1.89\times10^{6a}$	$6.07\times10^{6}\pm 1.89\times10^{6a}$	$6.75\times10^{6}\pm 1.89\times10^{6a}$	$7.76\times10^{6}\pm 1.89\times10^{6a}$	$1.01\times10^{7}\pm 1.89\times10^{7a}$	$1.42\times10^{7}\pm 1.89\times10^{7a}$
SP2 (70cl)	$2.37\times10^{6}\pm 1.89\times10^{6a}$	$3.04\times10^{6}\pm 1.89\times10^{6a}$	$6.41\times10^{6}\pm 1.89\times10^{6a}$	$7.76\times10^{6}\pm 1.89\times10^{6a}$	$8.10\times10^{6}\pm 1.89\times10^{6a}$	$1.08\times10^{7}\pm 1.89\times10^{7a}$	$1.35\times10^{7}\pm 1.89\times10^{7a}$

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

Appendix D: Total Fungal count of soil from the Setup (Experiment)

	June	July	August	Sept	Oct	Nov	Dec
SA	$3.37\times10^{6}\pm 5.17\times10^{6a}$	$3.71\times10^{6}\pm 5.17\times10^{6a}$	$5.29\times10^{6}\pm 5.17\times10^{6a}$	$5.73\times10^{6}\pm 5.17\times10^{6a}$	$6.74\times10^{6}\pm 5.17\times10^{6a}$	$7.75\times10^{6}\pm 5.17\times10^{6a}$	$7.74\times10^{6}\pm 5.17\times10^{6a}$
SP1	$3.71\times10^{6}\pm 5.17\times10^{6a}$	$3.38\times10^{6}\pm 5.17\times10^{6a}$	$5.40\times10^{6}\pm 5.17\times10^{6a}$	$9.01\times10^{6}\pm 5.17\times10^{6a}$	$7.08\times10^{6}\pm 5.17\times10^{6a}$	$7.75\times10^{6}\pm 5.17\times10^{6a}$	$7.75\times10^{6}\pm 5.17\times10^{6a}$
SP2	$3.70\times10^{6}\pm 5.17\times10^{6a}$	$3.71\times10^{6}\pm 5.17\times10^{6a}$	$4.07\times10^{6}\pm 5.17\times10^{6a}$	$6.74\times10^{6}\pm 5.17\times10^{6a}$	$6.74\times10^{6}\pm 5.17\times10^{6a}$	$6.75\times10^{6}\pm 5.17\times10^{6a}$	$7.74\times10^{6}\pm 5.17\times10^{6a}$
SP1 (50cl)	$2.70\times10^{6}\pm 5.17\times10^{6a}$	$2.70\times10^{6}\pm 5.17\times10^{6a}$	$4.04\times10^{6}\pm 5.17\times10^{6a}$	$4.06\times10^{6}\pm 5.17\times10^{6a}$	$5.73\times10^{6}\pm 5.17\times10^{6a}$	$5.73\times10^{6}\pm 5.17\times10^{6a}$	$7.08\times10^{6}\pm 5.17\times10^{6a}$
SP2 (50cl)	$2.70\times10^{6}\pm 5.17\times10^{6a}$	$2.70\times10^{6}\pm 5.17\times10^{6a}$	$4.05\times10^{6}\pm 5.17\times10^{6a}$	$4.06\times10^{6}\pm 5.17\times10^{6a}$	$5.40\times10^{6}\pm 5.17\times10^{6a}$	$6.74\times10^{6}\pm 5.17\times10^{6a}$	$6.74\times10^{6}\pm 5.17\times10^{6a}$
SP1 (70cl)	$1.69\times10^{6}\pm 5.17\times10^{6a}$	$1.35\times10^{6}\pm 5.17\times10^{6a}$	$3.37\times10^{6}\pm 5.17\times10^{6a}$	$4.05\times10^{6}\pm 5.17\times10^{6a}$	$4.72\times10^{6}\pm 5.17\times10^{6a}$	$5.39\times10^{6}\pm 5.17\times10^{6a}$	$6.06\times10^{6}\pm 5.17\times10^{6a}$
SP2 (70cl)	$1.35\times10^{6}\pm 5.17\times10^{6a}$	$1.02\times10^{6}\pm 5.17\times10^{6a}$	$4.38\times10^{6}\pm 5.17\times10^{6a}$	$4.06\times10^{6}\pm 5.17\times10^{6a}$	$4.06\times10^{6}\pm 5.17\times10^{6a}$	$5.06\times10^{6}\pm 5.17\times10^{6a}$	$5.40\times10^{6}\pm 5.17\times10^{6a}$

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

Appendix E: Oil utilizing bacterial counts of soil from the setup (experiment)

Samples	June	July	August	Sept	Oct	Nov	Dec
SA	$2.51\times10^{3}\pm$ 5.17×10^{3a}	$2.79\times10^{4}\pm$ 5.17×10^{4a}	$5.03\times10^{3}\pm$ 5.17×10^{3a}	$2.46\times10^{3}\pm$ 5.17×10^{3a}	$2.76\times10^{3}\pm$ 5.17×10^{3a}	$2.39\times10^{3}\pm$ 5.17×10^{3a}	$2.32\times10^{3}\pm$ 5.17×10^{3a}
SP1	$2.59\times10^{3}\pm$ 5.17×10^{3a}	$2.69\times10^{4}\pm$ 5.17×10^{4a}	$5.93\times10^{3}\pm$ 5.17×10^{3a}	$2.36\times10^{4}\pm$ 5.17×10^{4a}	$2.83\times10^{3}\pm$ 5.17×10^{3a}	$2.33\times10^{3}\pm$ 5.17×10^{3a}	$2.02\times10^{3}\pm$ 5.17×10^{3a}
SP2	$2.02\times10^{3}\pm$ 5.17×10^{3a}	$2.25\times10^{2}\pm$ 5.17×10^{2a}	$6.39\times10^{3}\pm$ 5.17×10^{3a}	$2.66\times10^{3}\pm$ 5.17×10^{3a}	$2.76\times10^{3}\pm$ 5.17×10^{3a}	$2.25\times10^{3}\pm$ 5.17×10^{3a}	$2.06\times10^{3}\pm$ 5.17×10^{3a}
SP1 (50cl)	$2.02\times10^{3}\pm$ 5.17×10^{3a}	$2.00\times10^{2}\pm$ 5.17×10^{2a}	$3.75\times10^{3}\pm$ 5.17×10^{3a}	$1.11\times10^{2}\pm$ 5.17×10^{2a}	$1.15\times10^{2}\pm$ 5.17×10^{2a}	$1.28\times10^{2}\pm$ 5.17×10^{2a}	$2.62\times10^{2}\pm$ 5.17×10^{2a}
SP2 (50cl)	$1.96\times10^{3}\pm$ 5.17×10^{3a}	$2.05\times10^{2}\pm$ 5.17×10^{2a}	$3.77\times10^{3}\pm$ 5.17×10^{3a}	$9.78\times10^{2}\pm$ 5.17×10^{2a}	$1.05\times10^{2}\pm$ 5.17×10^{2a}	$1.32\times10^{2}\pm$ 5.17×10^{2a}	$1.65\times10^{2}\pm$ 5.17×10^{2a}
SP1 (70cl)	$1.44\times10^{3}\pm$ 5.17×10^{3a}	$1.74\times10^{2}\pm$ 5.17×10^{2a}	$2.07\times10^{3}\pm$ 5.17×10^{3a}	$6.75\times10^{2}\pm$ 5.17×10^{2a}	$7.76\times10^{2}\pm$ 5.17×10^{2a}	$1.01\times10^{2}\pm$ 5.17×10^{2a}	$1.42\times10^{2}\pm$ 5.17×10^{2a}
SP2 (70cl)	$1.41\times10^{3}\pm$ 5.17×10^{3a}	$1.04\times10^{2}\pm$ 5.17×10^{2a}	$2.41\times10^{3}\pm$ 5.17×10^{3a}	$7.76\times10^{2}\pm$ 5.17×10^{2a}	$8.10\times10^{2}\pm$ 5.17×10^{2a}	$1.08\times10^{2}\pm$ 5.17×10^{2a}	$1.35\times10^{2}\pm$ 5.17×10^{2a}

Appendix F: Oil utilizing fungal counts of soil from the setup (experiment)

	June	July	August	Sept	Oct	Nov	Dec
	$2.37\times10^{2}\pm$	$3.15\times10^{2}\pm$	$3.29\times10^{2}\pm$	$5.73\times10^{2}\pm$	$6.74\times10^{2}\pm$	$7.75\times10^{2}\pm$	$7.74\times10^{2}\pm$
SA	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}
	$2.71\times10^{2}\pm$	$4.22\times10^{2}\pm$	$5.40\times10^{2}\pm$	$9.01\times10^{2}\pm$	$6.08\times10^{2}\pm$	$6.75\times10^{2}\pm$	$7.75\times10^{2}\pm$
SP1	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}
	$3.50\times10^{2}\pm$	$3.71\times10^{2}\pm$	$4.07\times10^{2}\pm$	$6.74\times10^{2}\pm$	$5.74\times10^{2}\pm$	$6.75\times10^{2}\pm$	$7.74\times10^{2}\pm$
SP2	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}	5.17×10^{2a}
	$2.70\times10^{1}\pm$	$2.70\times10^{1}\pm$	$4.04\times10^{1}\pm$	$4.06\times10^{1}\pm$	$5.73\times10^{1}\pm$	$5.7310^{1}\pm$	$7.0810^{1}\pm$
SP1 (50cl)	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}
	$2.70\times10^{1}\pm$	$2.70\times10^{1}\pm$	$4.1510^{1}\pm$	$4.0610^{1}\pm$	$5.4010^{1}\pm$	$6.7410^{1}\pm$	$6.6410^{1}\pm$
SP2 (50cl)	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}
	$1.69\times10^{1}\pm$	$1.3510^{1}\pm$	$3.6310^{1}\pm$	$4.0510^{1}\pm$	$5.2710^{1}\pm$	$5.3910^{1}\pm$	$6.0610^{1}\pm$
SP1 (70cl)	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}
	$1.35\times10^{1}\pm$	$1.0210^{1}\pm$	$3.2810^{1}\pm$	$4.0610^{1}\pm$	$5.1610^{1}\pm$	$5.0610^{1}\pm$	$5.4010^{1}\pm$
SP2 (70cl)	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}	5.17×10^{1a}

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

Apendix G: Experimental setup at Month one, four and seven respectively

Apendix H: Nature of root of a: *Melissia officinalis* b: *Urena lobata* during phytoremediation process

ɔendix I: DNA sequence data

Bacillus subtilis strain EE681738

GGTTAAGTTAGAAAGGGCGCACGGTGGATGCCTTGGCACTAGGAGCCGATG
AAGGACGGGCGAACACCGATATGCTTCGGGGAGCTGTAAGCAAGCTTTGAT
CCGGAGATTTCCGAATGGGGAAACCCACCACTCGTAATGGAGTGGTATCCA
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CGAAACGGGATTAGCCCAAACCAAGAGGCTTGCCTCTTGGGGTTGTAGGAC
ACTCTGTACGGAGTTACAAAAGAACGAGGTAGATGAAGAGGTCTGGAAAG
GCCCGCCATAGGAGGTAACAGCCCTGTAGTCAAAACTTCGTTCTCTCCTGAG
TGGATCCTGAGTACGGCGGAACACGTGAAATTCCGTCGGAATCCGGGAGGA
CCATCTCCCAAGGCTAAATACTCCCTAGTGACCGATAGTGAACCAGTACCGT
GAGGGAAAGGTGAAAAGCACCCCGGAAGGGGAGTGAAAGAGATCCTGAAA
CCGTGTGCCTACAAGTAGTCAGAGCCCGTTAACGGGTGATGGCGTGCCTTTT
GTAGAT

Aspergillus niger strain HQ659967

TGCGGGGCGGACGGGTGAGTTATAGCCTAGGAATCTGCCTGGTAGTGGGGG
ATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGT
GGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTA
GTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAG
GATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
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GTAAACGATGTCGACTAGCCGTTGGATCCTTTGAGATCTTAGTGCGCAGCTA
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TTTCGTAAGCAAACGCGAAGTACCCTTACCCTGGCCTTGAACATGGCTTGAG
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ATGGCTGTTCGTCAGCTCGTGTCGTGAGATTGGTGGGTTTAGTCCGGTTACC
GAAGCGCAACCTGTACATTAGGTTACCAGCACTCGGGGTGGCACTCTAGAG
ACTTGCCCGGTGTACATAACCCG

Appendix J: Growth of spent engine oil utilizing bacteria in oil medium

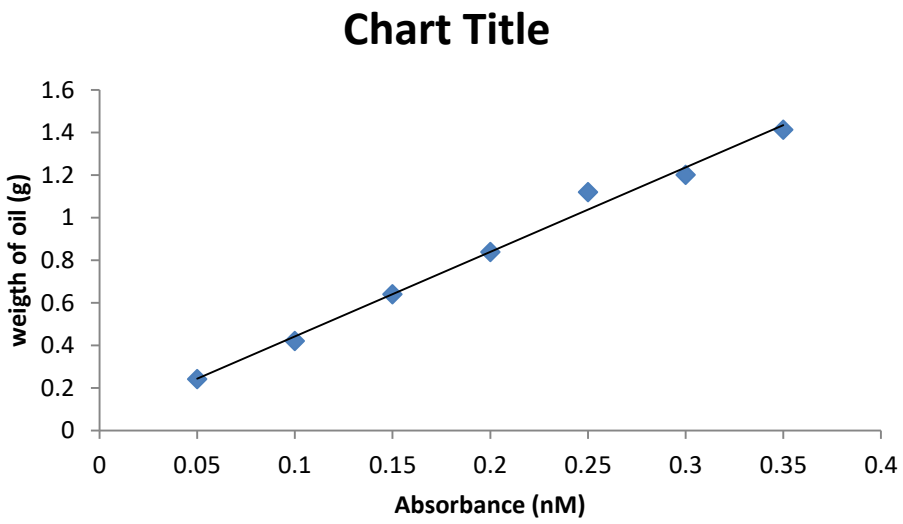
Isolate code	Day 7	Day 14	Day 21	Day 28
BS	1.1	1.4	1.2	1.23
BM	0.8	1.101	1.201	1.113
SA	0.4	0.801	0.6	0.301
SE	0.3	0.7	0.5	0.2
PA	0.53	0.6	0.4	0.32

Appendix K: Growth of spent engine oil utilizing fungi in oil medium

Isolate code	Day 7	Day 14	Day 21	Day 28
AN	1	1.2	0.9	0.85
AF	0.6	0.92	0.4	0.3
PN	0.5	0.8	0.401	0.35
AFM	0.5	0.82	0.3	0.2
FO	0.4	0.6	0.405	0.304

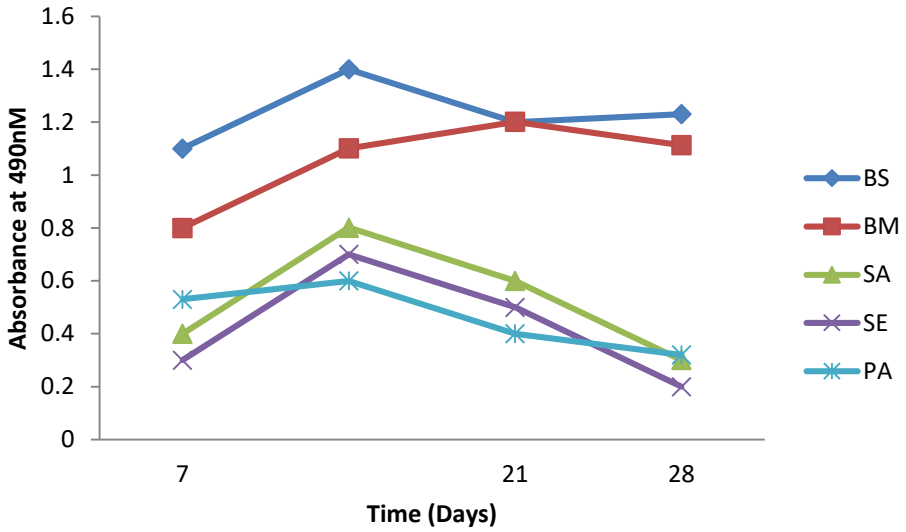
Appendix L: Standard curve for TPH determination

Weight of Spent engine oil (g)	Absorbance (Nm)
0.05	0.241
0.1	0.42
0.15	0.641
0.2	0.839
0.25	1.12
0.3	1.201
0.35	1.413



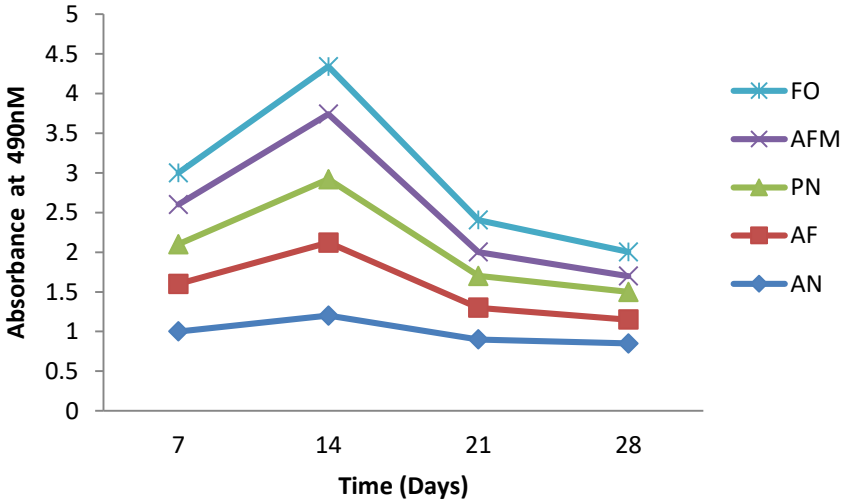
Standard Curve

Appendix M: Growth of spent engine oil utilizing bacteria in oil medium



Growth of spent engine oil utilizing bacteria in oil medium

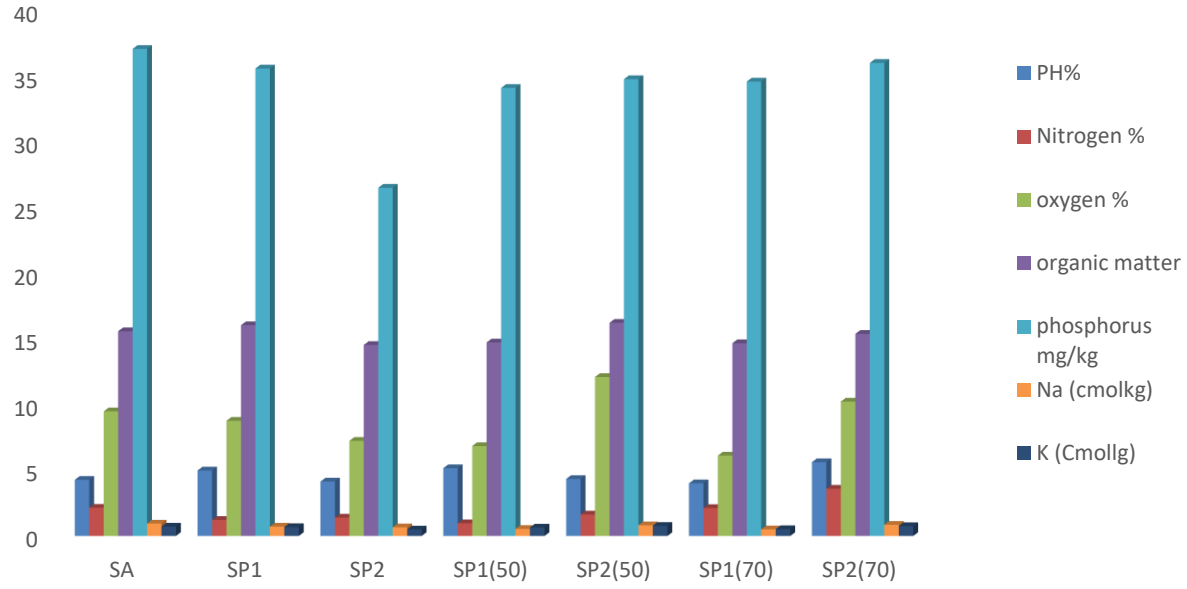
BS: *Bacillus subtilis*, BM: *Bacillus megaterium*, SA: *Staphylococcus aureus*, SE: *Staphylococcus epidermidis*, PA: *Pseudomonas aeroginosa*

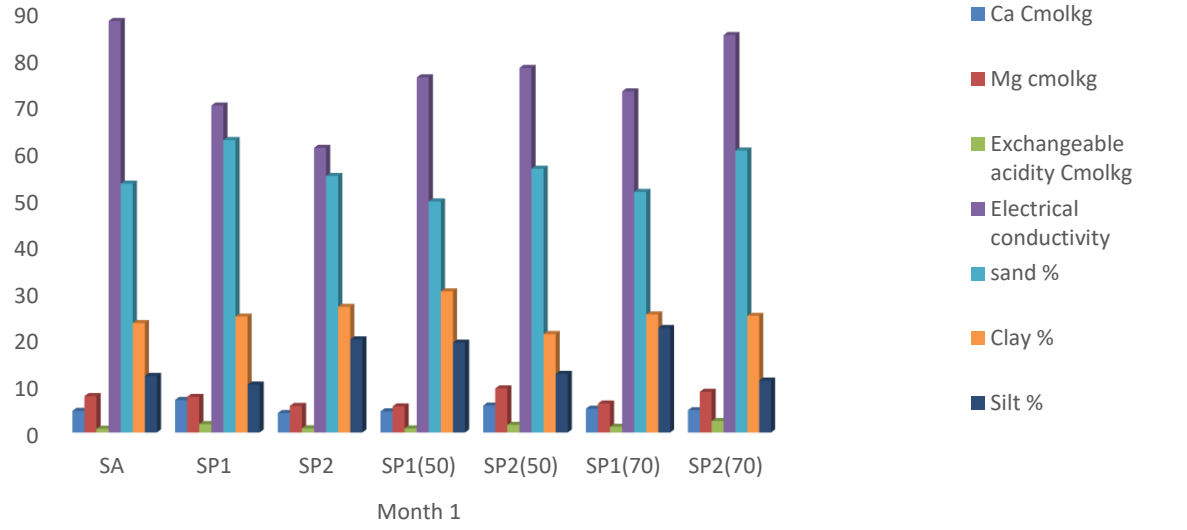


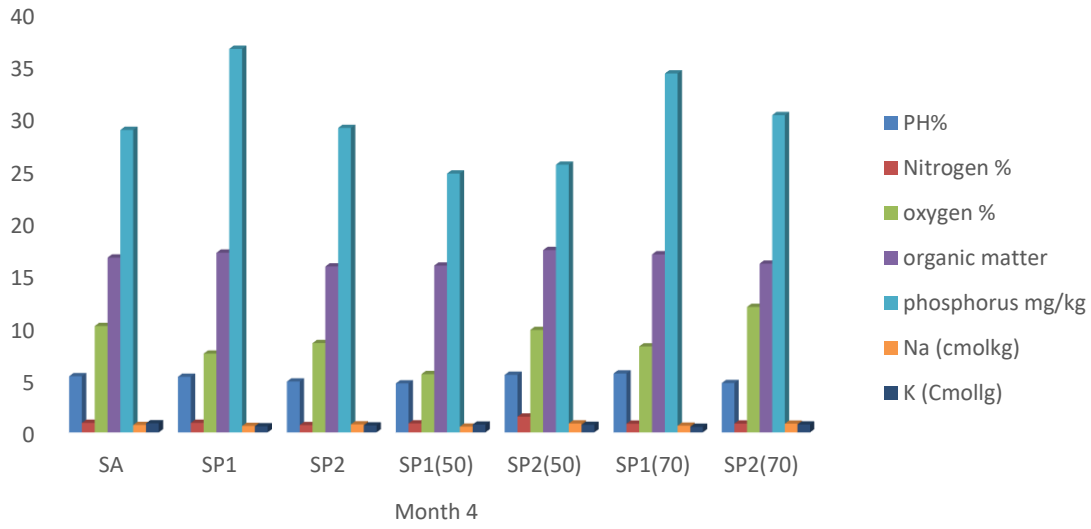
Growth of spent lubricating oil utilizing fungi in oil medium

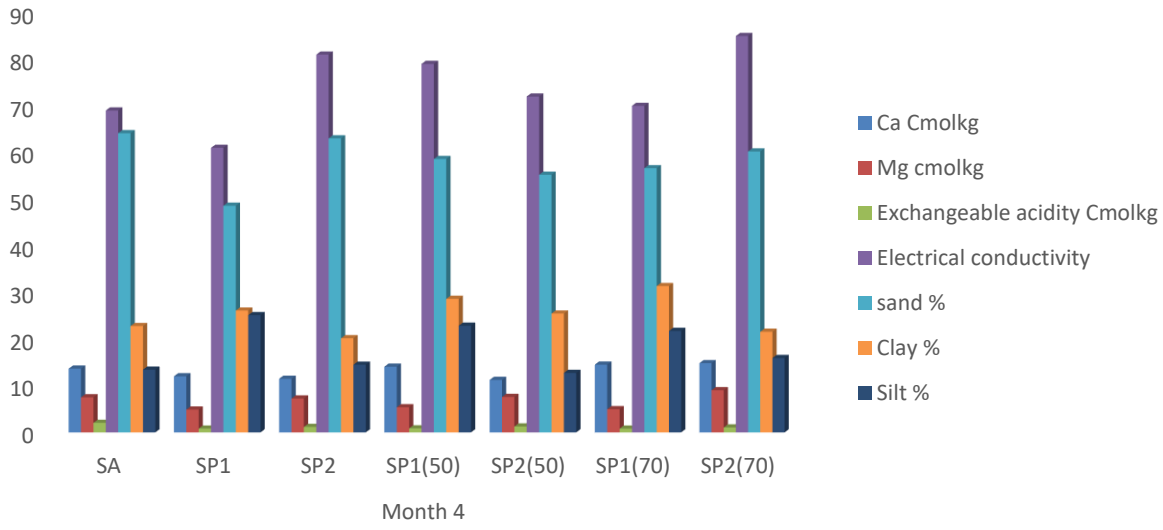
FO: *Fusarium oxysporium*, AFM: *Aspergillus fumigates*, PN: *Penicillium notatum*, AF: *Aspergillus flavus*, AN: *Aspergillus niger*.

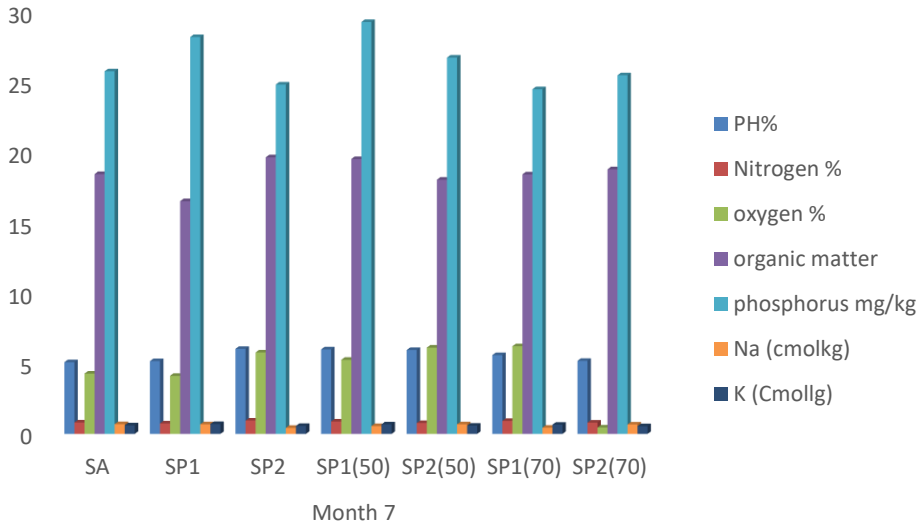
Appendix N: Physicochemical parameter






40
35
30
25
20
15
10
5
0
SA
SP1
SP2
SP1(50)
SP2(50)
SP1(70)
SP2(70)
Month 4
PH%
Nitrogen %
oxygen %
organic matter
phosphorus mg/kg
Na (cmolkg)
K (Cmollg)


90
80
70
60
50
40
30
20
10
0
SA
SP1
SP2
SP1(50)
SP2(50)
SP1(70)
SP2(70)
Month 4
Ca Cmolkg
Mg cmolkg
Exchangeable acidity Cmolkg
Electrical conductivity
sand %
Clay %
Silt %


30
25
20
15
10
5
0
SA
SP1
SP2
SP1(50)
SP2(50)
SP1(70)
SP2(70)
Month 7
PH%
Nitrogen %
oxygen %
organic matter
phosphorus mg/kg
Na (cmolkg)
K (Cmollg)

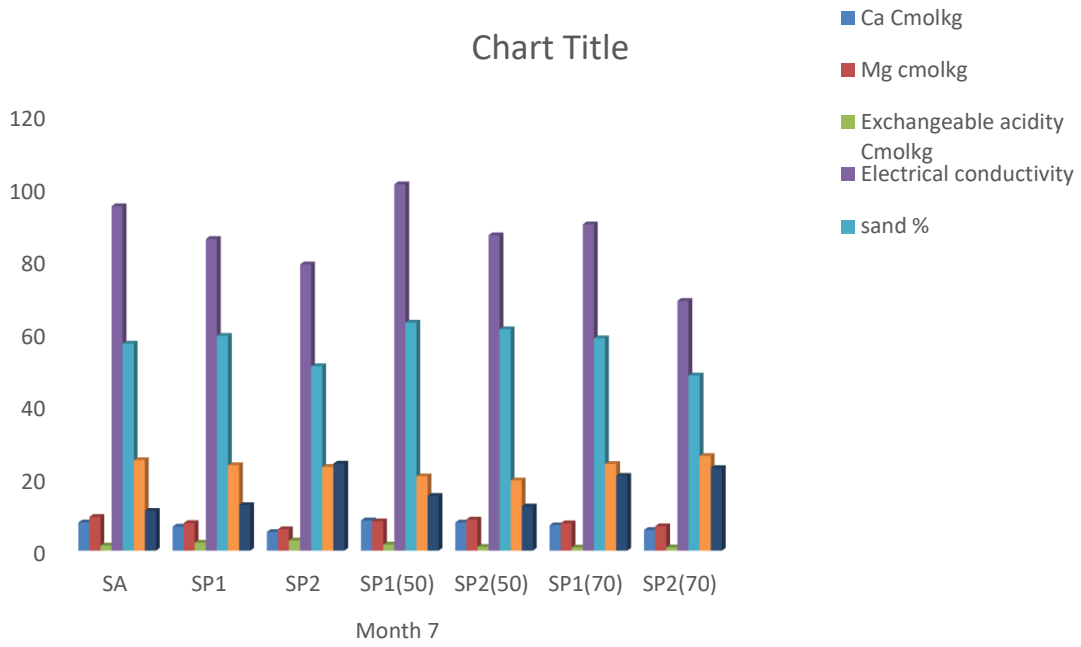


Chart Title
120
100
80
60
40
20
0
SA
SP1
SP2
SP1(50)
SP2(50)
SP1(70)
SP2(70)
Month 7
Ca Cmolkg
Mg cmolkg
Exchangeable acidity Cmolkg
Electrical conductivity
sand %

Appendix O:Count of Plants found within the vicinity of Automobile workshops

Plant	Minna	Bida	Suleja	Tegina
Cynodondactylon	26	17	0	0
Gompherynacelosioides	0	30	18	0
Sida acuta	29	0	25	0
Melissia officinalis	41	34	52	44
Urena lobata	38	50	49	36
Seteria Pumik	0	0	24	20

APPENDIX P: Heavy Metal Analysis of *Urena lobatal* and *Melissia officinalis* Plants

Coded samples	Fe	Zn	Cd	Cr	Pb	Ni
U. L (Leave)	3264.50	62.50	45.75	37.50	164.00	81.50
U.L (Stem)	1727.50	67.00	39.75	52.00	73.50	81.25
U.L (Root)	5624.00	39.75	47.50	17.25	197.50	102.50
M.O (Leave)	5271.00	59.00	54.00	11.50	227.00	96.25
M.O (Stem)	1120.50	39.25	31.75	0.00	245.50	83.75
M.O (Root)	6027.25	58.00	36.00	18.75	198.25	121.50

*U.L = *Urena lobatal*, M.O =*Melissia officinalis*, * Fe = Iron. Zn= Zinc, Cd = Cadmium Pb = Lead, Ni = Nickel.

APPENDIX Q: Heavy **S**oil heavy metal analysis result

(i) One month after phytoremediation

Sample	Cadmium Cd Mg/kg	Iron Fe Mg/kg	Zinc Zn Mg/kg	Chromium Cr Mg/kg	Nickel Ni Mg/kg	Mercury Hg Mg/kg	Lead Pb mg/kg
SP2(1) (50)	0.035	1.586	0.048	0.225	0.463	0.052	0.010
SA (1)	0.018	1.432	0.264	0.997	0.771	0.040	0.008
SP1(1) (A)	0.069	2.007	0.329	0.363	0.493	0.049	0.006
SP2(1) (70)	0.074	3.354	0.433	0.979	0.860	0.022	0.011
SP1(1) (70)	0.021	2.145	0.645	0.538	0.123	0.033	0.014
SP2(1) (A)	0.015	1.947	0.532	0.723	0.832	0.051	0.005
SP1(1) (50)	0.040	3.592	0.864	0.834	0.521	0.027	0.016

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.

(ii) Four month after phytoremediation

Sample	Cadmium Cd Mg/kg	Iron Fe Mg/kg	Zinc Zn Mg/kg	Chromium Cr Mg/kg	Nickel Ni Mg/kg	Mercury Hg Mg/kg	Lead Pb Mg/kg
SP2(4) (70)	0.016	0.964	0.704	0.597	0.678	0.003	0.035
SP2(4) (A)	0.025	1.327	1.253	0.753	0.037	0.037	0.041
SP2(4) (50)	0.006	1.671	0.719	0.899	0.150	0.015	0.055
SA (4)	0.007	2.265	0.584	1.452	0.013	0.052	0.038
SP1(4) (50)	0.018	1.005	0.183	0.827	07146	0.037	0.025
SP1(4) (Alone)	0.031	1.028	0.846	0.971	0.281	0.015	0.043
SP1(4) (70)	0.014	2.931	0.281	1.265	0.193	0.055	0.027

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata***.**

(iii) Seven month after phytoremediation

Sample	Cadmium Cd Mg/kg	Iron Fe Mg/kg	Zinc Zn Mg/kg	Chromium Cr Mg/kg	Nickel Ni Mg/kg	Mercury Hg Mg/kg	Lead Pb Mg/kg
SP2(7) (50)	0.002	1.976	0.901	0.621	0.845	0.008	0.004
SP1(7) (50)	0.005	2.498	0.628	0.780	0.967	0.004	0.011
SA (7)	0.060	4.753	0.757	1.018	0.731	0.007	0.009
SP1(7) (Alone)	0.037	3.853	0.622	1.041	0.675	0.027	0.015
SP2(7) (Alone)	0.071	2.005	0.832	0.808	0.392	0.015	0.008
SP2(7) (70)	0.019	2.060	0.745	0.907	0.595	0.006	0.014
SP1(7) (70)	0.052	1.437	0.417	1.117	0.645	0.015	0.022

SA: Soil alone, SP1: Soil and plant 1, SP2: Soil and plant 2, SP1 (50cl): Soil, Plant 1 and 50cl of Spent engine oil, SP2 (50cl): Soil, Plant 2 and 50cl of Spent engine oil, SP1 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, SP2 (70cl): Soil, Plant 1 and 70cl of Spent engine oil, Plant 1: *Melissia officinalis*, Plant 2: *Urena lobata*.