ECOLOGICAL RISK ASSESSMENT AND PHYTOREMEDIATION OF SPENT ENGINE OIL CONTAMINATED SOILS OF SELECTED MECHANIC WORKSHOPS IN IMO STATE

BY

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CERTIFICATION

We hereby certify that this dissertation "**Ecological Risk Assessment and Phytoremediation Of Spent Engine Oil Contaminated Soils Of Selected Mechanic Workshops In Imo State**" was carried out by JOHNPAUL NNAWUIKE AZORJI with Reg. No: 20164024848, in partial fulfillment of the requirements for the award of Doctor of Philosophy (Ph.D) in Environmental Conservation and Management in The Department of Biology, Federal University of Technology, Owerri.

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DEDICATION

This dissertation is dedicated to my late parents Mr. Lawrence and Mrs. Beatrice Azorji for their immense contributions to my life.

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ABSTRACT

Assessment of the ecotoxicological risk of indiscriminate disposal of spent engine oil on terrestrial ecosystem was investigated using physicochemical, biochemical, and bioterance assays. Soil samples were collected from a depth of 0-20cm and analyzed using standard analytical protocols. Acute toxicity tests were conducted based on germination and seedling establishment of higher plants (*Zea mays*, *G. maxV. unguiculata*), Earthworms (mortality rate) and microorganisms (inhibitory effects) which covered a wide range of short-term lethal and sub-lethal endpoints used for risk characterization and analyzed using Probitanalysis. Results obtained showed that majority of the mechanics who are within 20-30 age bracket were found in Owerri (22.42%) while the least was found in Okigwe (12.98%); on weekly basis, 42 vehicles are serviced in Owerri; 27 inOrlu while 21 is been serviced in Okigwe respectively. Total annual volume of spent engine oil generated (in litres) was highest in Owerri zone (124,489.36L) while the least value was obtained in Orlu zone (18,434.52L). A significant volume 168 (49.56%) of the spent engine oil is been disposed into the immediate environment with others being sold off 41 (12.09%); given out for free 66 (19.47%); and taken by car owners 64 (18.88%). *Axonopuss compressus*, *Aspillia africana*, and *Chromolaena odorata* species were the dominant plant species identified in the order: *Axonopuss compressus>Aspillia africana> Chromolaena odorata.* Mean values of physical and chemical properties of soil indicated alteration of parameters analyzed relative to the control. The mean concentrations of heavy metals across the zones ranged from Pb(1.045 ± 0.1 to 1.534 ± 0.2), As (1.761 ± 0.3 to 1.805 ± 0.3), Cr (0.272 ± 0.01 to 0.273 ± 0.01), Cd (0.302 ± 0.02 to 0.606 ± 0.03), Ni (0.918 ± 0.03 to 0.932 ± 0.03), Co (1.278 ± 0.1 to 1.324 ± 0.2), Fe (63.927 ± 3.34 to 69.563 ± 3.53), Cu (12.446 ± 2.14 to 12.964 ± 2.42), Zn (156.121 ± 28.01 to 156.137 ± 28.12), and Al (1.353 ± 0.2 to 1.353 ± 0.12) mg/kg with a distribution pattern of Zn>Fe>Cu>As>Al>Co>Pb>Ni>Cd>Cr. Concentration of heavy metals were significantly ($p<0.05$) higher at the polluted sites than the control and above WHO permissible limits. Mean concentration of PAHs ranged between Owerri zone (0.03 ± 0.01 to 1.980 ± 0.36mg/kg) Orlu zone (0.004 ± 0.001 to 1.614 ± 0.10 mg/kg), and in Okigwe zone (0.01 ±0.001 to 1.418 ± 0.19mg/kg) with distribution pattern of Phe>Nap>Pyr>Mepl>Ind>Acpt> Flu>Bbf>B(ghi)p>B(g)p>Bkf>Da>An>Acph across the zones.Maximum Concentration of TPH was recorded in Owerriwhile the minimum value was gotten from the control site. Mean values of TPH were in the order: Owerri>Orlu>Okigwe>control. Result of acute toxicity assay showed that *Zea mays* exhibited more sensitivity than *V. unguiculata* and *G. max*. The microbial absorbance rate depended on the dose and type of organism in the order of: *Acinetobacter>Enterobacter> Bacillus species >Pseudomonas.* Mortality in earthworm was noted as concentration increased. Values of Risk Quotient for *Zea mays, V. unguiculata, G. max* and earthworm ranged from low risk to very high risk based on the estimated PNEC values. The microorganisms differed slightly in biotolerance to spent engine oil exposure based on estimated risk quotients. Exposure to spent engine oil posed minimal risk to *Pseudomonas sp., Bacillus sp.*, and *Acinetobacter sp.,* at the estimated PNECs with values less than unity (<1). Risk quotient values for *Enterobacter sp*. indicated a high risk with values above unity. Variable concentrations of heavy metals were accumulated by the plants from soil and were stored in the root, shoot and leaf except for *A. compressus* that accumulated values less than one (<1) for Pb and As. Metal accumulation pattern were in the order *C. odorat>A.africana>A.compressus*. *C. odorata* and *A. africana* exhibited characteristics typical of a phytoextractor while *A. compressus* could be applied as a phytostabiliser of spent engine oil polluted soils. The sensitivities exhibited by the organisms exposed to spent engine oil indicate the possibility of using them as bioindicators and for assessing the efficacy of phytoremediation process. Spent engine oil adversely affected the organisms in each scenario in a dose dependent manner. Overall, these findings proved that spent engine oil greatly altered soil properties and also repressed the growth of organisms in the ecosystem thereby strengthening the need to curb indiscriminate disposal of spent oil on the environment by mechanics in the business of auto repairs to forestall possible pollution problems of other components of the food chain.

Keywords: Spent Engine Oil, Indigenous Plants, Ecosystem, Risk Assessment, Phytoremediation, Bioassays, *Zea Mays, G. Max, V. Unguiculata*, Earthworm, Risk Quotient.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Ecological risk assessment entails the process of estimating the potential effects of anthropogenic activities on a natural resource (D'Souza, Vadakkeveedu, Kumar, Anish, Harikripa, & Keshava, 2022). Such anthropogenic activities include but not limited to various industrial, mining and Agricultural practices, pharmaceuticals and urban runoff (Gulnihal, Morghan, Gadde, Isah & Tahera, 2021). Indiscriminate disposal of wastes emanating from such activities have been a major devastating ecological problem in the world today (Odjegba & Sadiq, 2002; Oh, Cao, Li, & Cheng, 2014).

Unsustainable use and management of the ecosystems is leading to increased soil degradation and subsequent loss of key resource that is fundamental to life. Thus, management of ecosystems for continuity and sustainable ecosystem health is quite apt and deserves urgent attention considering the increasing anthropogenic pressure on the soil due largely to intensive material use by man (Farombi, Adebayo, & Oyekanmi, 2013). With recent advances and exploits in technology, developing and developed countries of the world are currently facing an upsurge in ecological and toxicological problems as a result of unregulated release of toxic contaminants to the environment (Ibe, Duru, Isiuku & Akalazu, 2021).

The environment comprises of various kinds of ecosystems among which are air, water (including marine and or freshwater), soil/sediments, plus various forms of biota. The influence of any contaminant on such a diverse ecosystems are further exacerbated by physical, chemical, biological, geological, climatic, factors including their combined interrelationships. This has prompted growing body of evidence on the extent to which pollution has caused ecosystem degradation (Okonokhua, Ikhajiagbe, Anoliefo, & Emede**,** 2007).

Environmental contamination can be classically defined as the alteration of the natural surrounding largely as a result anthropogenic activities of man's inactions and actions through direct or indirect effects of the changes in the energy pattern, radiation levels and chemical and physical constitution and abundance of organisms (USEPA, 2021; Prabhat & Rai, 2016). However, environmental contamination does not necessarily connote measurable damage to the biotic components of the ecosystems.

In recent decades, environmental pollution by petroleum and petrochemical products has been a trending issue of global concern (Abioye, Agamuthu, & Abdul, 2012; Mandri & Lin, 2007). In Nigeria, as in many developing countries, soil pollution occasioned by indiscriminate disposal of petroleum and its allied products is considered a serious ecological issue (Emoyan, Ejecha, Onochab & Tesic, 2020; Nkwoada, Alisa, & Amakom, 2018; Udebuani, Okoli, Nwaigwe & Ozoh, 2011). This ecological issue is rising as a result of the prevalent mode of disposal of these wastes in the environment. Hence, it is an issue of optimum importance to protect the ecosystem from industrial contamination which is continually threatening the terrestrial and aquatic ecosystem due to increasing anthropogenically released chemical agents that are capable of causing damage to the flora and fauna in the ecosystem (Nwachukwu, Azorji, Adjero, Green, Igwe, & Nnadozie, 2020).

Petroleum and their allied products are a critical part of modern life and a cornerstone of any modern industrial strategy (Abenchi*,* Okunola, Zubairu, Usman, & Apene, 2010; Bakare, 2016). Environmental problems posed by indiscriminate disposal of petroleum products have thus far contradicted their original beneficial use (Bamiro & Osibanjo, 2004a). In recent years, there has been growing concern over environmental pollution by petroleum products, mounting public apprehension over the ecological and human health effects and the consequence of global warning. However, since the commercial exploration and exploitation of petroleum products in Nigeria since 1958 (Okoh, 2003), petroleum and its allied products have become the lynchpin of the Nigerian economy with the petroleum exploration, exploitation and distribution activities leading to the pollution of land and waterways (Bamiro & Osibanjo, 2004b). A typical case in point is the Niger Delta region of the country where oil exploration and exploitation are carried out on regular basis (Njoku, Akinola, & Oboh, 2009).

The challenge of soil contamination is a major one because it reduces the value of industrial profitability as the sustainable development of environmentally safe practices is threatened through its negative impact on flora and fauna (Monthakarn, Apichaya, Thotsapol, Khemika, Mark & Sarun, 2022; Vidali, 2001). Contamination generated through industrial practices targeted at producing energy have also been implicated in the generally negative effects of ecological balance as noxious gases like carbon monoxide and sulphur dioxide are released as a result of this (Ajunwa, Odeniyi, Garuba, & Onilude, 2018).

Human activities in mining, industrialization, mechanization, urbanization, transportation and intensification of agriculture have thus far reached a rogue level thereby leaving a legacy of contaminated land (soil) around the world (Oluseyi, Adetunde, & Amadi, 2014). This has resulted to the generation of several wastes products (Onwuka, Chude, & Ogwuegbu, 2012) which are either deliberately applied on soil as fertiliser, pesticides etc (Lauhanen, Koukkanen, & Vahaoya, 2004) or through runoff/spill as solids, crude oil or spent engine oil. Some of these waste products can be converted into some important byproducts that can be re-used to meet with the challenges arising from increasing population of Nigeria (Nwite & Alu, 2015). In other words, they can be recycled into manures and fertilisers for increased productivity (Onwuka *et al.,* 2012). However, other toxic substances are more persistent and last for years or even centuries in the environment, in other words, such substances cannot be put into use or any beneficial secondary use and therefore pose a serious threat to the flora, fauna, and human being at large (Adelowo, Adeoye, & Sridhar, 2006).

Good examples of such chemosynthetic pollutants are spent engine oil which is usually obtained after servicing and subsequent draining off from automobile and generator engines (Sharif, Sadeghi, & Akbarpour, 2007). Spent engine oil is perceived as any lubricating oil that has served its purpose in a vehicle, been drained off from the area of application and considered not fit for its initial purpose because it is contaminated by physical or chemical impurities (Adeleye, Yerima, Nkereuwem, Sadiq, Shiaka, Onokebhagbe, & Amoo, 2021; Blodgette, 2001).

Spent engine oil has a dark to brown color and has been reported to be harmful to the soil ecosystem (Adedokun & Ataga, 2007). As the engine oil is running while in the vehicle, it picks up a number of additional compounds and dirt from engine wear (Uchendu & Ogwo, 2014). Because of the additives and subsequent contamination, spent engine oil has been considered to be more deleterious than crude oil (Abioye *et al*., 2012). Spent engine oil is a mixture of various chemicals such as low and high molecular weight (C_{15}-C_{20}), aliphatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricate additives and decomposition of products (Onwuka *et al*., 2012).

As reported by Jahir & Syed (2011), high percentage of PAHs, TPH and trace metals (Zn, Pb, Cr and Fe) are contained in spent engine oils than fresh oils. Some of the metals in the used engine oil could dissolve in water, sip through the soil easily and may eventually be found in surface water and groundwater. The highly toxic PAHs found in spent engine oil have been implicated in indirect secondary effects on the ecosystem like loss of soil organisms, loss of aquatic life, mutation during reproduction of organisms at various trophic levels of the ecosystem and cancer in humans (Abioye, *et al.,* 2012). Pollution of the environment by indiscriminate disposal of spent engine oil has been documented to be the most remarkable ecological contaminant considered nearly as extensive as crude oil pollution in most parts of Nigeria today (Odjegba & Sadiq, 2002; Emoyan *et al*., 2020).

In Nigeria, automobile workshops are sited in government allocated areas known as mechanic villages. Activities carried out in these designated areas include but not limited to panel beating, vulcanizing, charging of car batteries, spray painting, repairs and servicing of motor vehicles (Udebuani, Titoju, Onweremadu, Abara, & Alaekwe, 2016). Wastes emanating from these activities include spent engine oil, worn-out parts, packaging materials, used batteries, metal scraps and stripped oily sludge (Aloysius *et al.,* 2013). Unfortunately, these waste products are disposed indiscriminately by the artisans who know little about the resultant environmental harm. Spent engine oil is considered as an ordinary waste by majority of the workers who dispose it indiscriminately on soil surface (Uchendu & Ogwo, 2014). This practice of disposal into gutters, water drains, open plots and farms is a common phenomenon among the artisans (Odjegba & Sadiq, 2002).

More so, the engine oil thickness makes it possible to persist in the environment for an extensive period of time, resulting in the disruption of ecological integrity. Reports have illustrated the adverse impacts of spent engine oil that seep into water bodies through run-offs from rainfall as well as the persistence in soil layers (Phulpoto, Qazi, Mangi, Ahmed & Kanhar, 2015). There have also been indications of these compounds subsequently affecting aquatic and soil life forms (Sethy, Jha, Sahoo, Shukla, Tripathi, & Puranik, 2011). These challenges have sparked interests in remediation systems that enable management of indiscriminate disposal of spent engine oil as a chemical pollutant from the environment.

The contamination of the environment, particularly the terrestrial ecosystems by spent engine oil indicators such as heavy metals, TPH and polycyclic aromatic hydrocarbons is of considerable serious problem in the society because the environment is a direct receptacle for waste products generated in the space within the environment (Opuene & Okafor, 2007; Olubunmi *et al*., 2013). Moreover, these contaminants have been documented to be carcinogenic, mutagenic and teratogenic in nature. Therefore, there is need to ascertain potential ecological risks organisms are exposed to as a result of co-contamination in the ecosystem. Conversely, it is consequential to take the ecological study of these toxicants very seriously to avert their potential effect and costly consequence of their contamination effects if not checked.

Among the various methods of estimating ecosystem health and productivity is the process of ecological risk assessment. This process entails estimating the likelihood that a particular contaminant may pose a threat to the ecosystem. In other words, it is the probability of occurrence of an undesired ecological impact. Ecological risk is assessed using a process that involves problem identification, exposure analysis, effects assessment and finally risk characterization (Udebuani, Omoniyi, Akharame, Fatoki & Opeolu, 2021).

Chemical analysis of soil samples are not enough to describe the risk of co-contaminated soils such as spent engine as it does not evaluate the combined effects of stressors present at a contaminated site (Udebuani *et al.,* 2021). Different bioassay techniques have been designed for screening of soil toxicity (Juvonen, Martikainen, Schultz, Joutti, Ahtiainen, & Lehtokari, 2000). It has been established that species representing each trophic level can be included into a test battery to confirm the actual toxicity of contaminated sites. The use of bioassays with different organism can indicate the actual stress as a result of complex mixture of contaminants and provide the real risk to terrestrial and aquatic ecosystem (Udebuani *et al.*, 2021).

For instance, soil microorganisms (such as bacteria and fungi) plays an essential role in biomass decomposition, biogenic element circulation, which makes nutrients available to plants (primary producers), biodegradation of impurities, and maintenance of soil structure (Tunira & Krishan 2010); earthworms have been tagged “ecosystem engineers” because of their indispensable role in mixing of soil constituents, aeration, and maintenance of soil fertility and recycling (Weizhong, Wenjun, Xiao & Shuli, 2022; Langdon, Piearce, Meharg, & Semple, 2003). As earthworms burrow through the soil, they create pores through which oxygen and water can enter and carbon dioxide can leave the soil. The terrestrial plant on the other hand plays a prominent role in the ecosystem as primary producers in addition to supporting all life forms (Eapen & D’Souza, 2003). All these characteristics make microbes, earthworm and plants suitable for biomonitoring and assessment of soil quality representing the terrestrial compartments.

In search of a cleaner and safe environment as well as the adverse effects of contaminants in the ecosystem, scientists have designed methods of remediation of polluted sites. Some of these methods include physical, chemical, and thermal remediation technologies (Nedijim, 2021). Commonly used physical methods include booming and skimming, manual removal, mechanical removal, water flushing, sediment relocation and tilling and soil washing, vapor extraction, encapsulation etc (Verma, Sankhla, Jadhav, Parihar, & Awashti, 2021). Unfortunately, some of these technologies are environmentally destructive and expensive and do not completely decontaminate the site thereby adding to the existing problem. This has been attributed to the facts that not only are they harmful to the habitats but they also rid the soil of available nutrients and microorganisms.

Fortunately, a number of promising alternative have been developed using indigenous plants, fungi, and bacteria to clean-up contaminated sites. Plants and microorganisms have been separately utilized for the remediation of pollutant substances under the broad themes of phytoremediation and microbial remediation (Helder, Strik, Hamelers, Kuijken, & Buisman, 2012). This targets the breakdown of component parts of pollutants leading to their conversions into less toxic wastes like spent engine oil that remain after disposal on agricultural soils (Helder *et al*., 2012). Microbial techniques include the use of microorganisms to break down organic pollutants and immobilize toxic metals; phytoremediation (remediation or environmental cleanup using plants) which involves growing metal tolerant plants on contaminated soil to cover and stabilize otherwise unvegetated soils that can be a source of soil or water pollution.

Over time, it has been established that even with adoption of pollution prevention measures by regulatory bodies, contaminants still persisted and subsequently affects organisms in the ecosystem even at low level. This phenomenon has been attributed to their resistance to natural degradation processes, and has been into soil and eventually into water courses and other constituents of the ecosystem. This therefore, has created a hiatus for research on ecological risks and possible remediation techniques using eco-friendly and environmentally safe technology such as phytoremediation.

Previous studies on phytoremediation of spent engine oil contaminated soils have established that certain plants can bioaccumulate, translocate and volatilize petroleum hydrocarbons from contaminated soil matrixes (Nwaichi, Chukwuere, Abosi, & Onukwuru, 2021; Bolanle-Ojo & Sridhar, 2020). The choice of phytoremediation technology is premised on the fact that it is characterized by low cost, low demand for infrastructure and low carbon footprint; also phytoremediation is considered environmental friendly technology (Ukaoma, Okechukwu, Ezea,

& Nnoli, 2015). The phytoremediation technology also helps in recovering the basic processes that define ecosystems functionality and sustainability (Maria, Pilar, Alvarenga, Kate, Carmody, Pogrzeba, & Gerhard, 2020). Thus it is imperative to complement and integrate ecotoxicological studies with phytoremediation technology considering the potential effects of spent engine oil and their interactions on the ecosystem.

Therefore, this project thus seeks to bring to the fore potential ecological risks associated with spent engine oil contamination on terrestrial ecosystems and the unique diversity of indigenous spent oil tolerant plant species and apply them in target phytoremediation-based removal of spent oil polluted soils indiscriminately disposed in Imo State, and the Nigerian environment at large. Targeting contaminating substances like spent engine oil will enhance the industrial and environmental viability of this process and thereby validate its acceptability as a new technique for militating against the challenges of spent engine oil pollution currently bedeviling terrestrial ecosystems in Imo state and Nigeria at large.

1.2 Statement of Problem

Engine oil is a vital and adaptable resource that is utilized in a variety of ways for a wide range of purposes, but its negative environmental effects after disposal has thus far contradicted its benefits (Ibe *et al*., 2021; Yahaya, Abubakar, & Abdu, 2021; Nwachukwu *et al*., 2020). One of the most essential long-term consequences of spent engine oil is their destructive effects on the ecosystems' biodiversity. This could also be very disastrous for human health and socioeconomics. Effluents from spent engine oil have been implicated in degradation of soil quality; exert genotoxic and mutagenic effects (Luter, Akaahan, & Attah, 2011); influence physiological parameters in plants and soil microbial biomass (Nwachukwu *et al*., 2020); and cause health defects among mechanics (Yahaya *et al.,* 2021). This could portend a serious future threat to the ecosystem and services they render (Yousef & Tayel *et al.*, 2004). Again, while these waste substances are known to contain toxic chemicals such as heavy metals, TPH, and PAHs which can be deleterious to humans even at low concentrations, potential ecological risks and the levels of these contaminants are not well known (Ibe *et al.*, 2021). In 2005, PAHs, a very hazardous component of spent engine oil ranked 7th in the biennial ranking of chemicals deemed harmful which may pose the greatest possible risk to human health (Obinia, Okafor, & Afiukwa, 2013). With the prevalence of automobile mechanic workshops in rural and semi-urban areas, and the increasing number of vehicles being serviced/repaired on daily basis, there is the tendency of lack of proper disposal of spent engine oil (Kelechi, Njoku, Akinola, & Temitope, 2011). This thereby leads to increased spent oil residues on soil surfaces with the potential run-off into the ecosystem leading to contamination of soil and aquatic ecosystems and injuries to other life-forms.

1.3 Aim and objectives of study

The overall aim of this study was to assess the ecotoxicological risks of spent engine oil in terrestrial ecosystem and the possible remediation of these contaminants using indigenous plant species in Imo State.

Specifically, the study was carried out to:

1. determine the use and disposal method of spent engine oil generated in selected mechanic workshops in Imo State,
2. identify indigenous plant species growing in the vicinity of the selected auto mechanics,
3. determine the physical and chemical properties of spent engine oil polluted and unpolluted soils,

4. evaluate the acute toxicity of concentrations of spent engine oil on test biota (plants, microorganisms, earthworms),
5. characterize the ecological risk of spent engine oil against test biota (plants, microorganisms, earthworms).
6. screen the selected indigenous plant species for biotolerance to spent engine oil contamination,
7. evaluate the screened plant species for suitability in the phytoremediation of spent engine polluted soils.

1.4 Research Hypothesis

The following null hypotheses were tested in this study:

1. Spent engine oil impacted soil alters physicochemical properties, increases heavy metal load, TPH and PAHs in the ecosystem,
2. Spent engine oil inhabiting plant species are better phytoremediators of spent engine oil polluted soils,
3. Selected plant species identified at the auto mechanic workshops have the ability to tolerate and extract TPH from soil polluted with spent engine oil.
4. Spent engine contamination poses a potential ecological risk on terrestrial ecosystems,

1.5 Justification of the study

With the increasing unregulated disposal of spent engine oil on bare soil, it has become imperative to ascertain the ecological risks organism are exposed to in the ecosystem (Yousef *et al*., 2004; Alexander, 2000). Ecological assessment of polluted sites is usually based on physical and chemical analysis. However, physical and analysis does not allow for evaluation of potential risks organisms are exposed to. Bioindicators have become complementary tools to better predict the real risk posed by soil contamination to the ecosystem. The use of bioassays can detect negative effects of complex mixture of contaminants such as spent engine oil in the ecosystem. Plants are the primary producers in an ecosystem, Earthworms play prominent role in the maintenance of soil fertility and nutrient cycling while microorganisms are essential for decomposition. In ecotoxicological assessment of soil contaminants, the use of organisms representing each trophic level is very important as they allow for direct impact contaminant assessment. This makes plants, earthworms and microorganisms essential for monitoring ecosystem health. Alongside the toxic effects of spent engine oil on ecological, economic and human, is the high cost of current clean-up techniques necessitating the need for relatively less expensive and environmentally friendly options such as phytoremediation. Phytoremediation is considered as the most optimal technique for restoration of contaminated soils because it is ecofriendly, cheap nature, and can be applied in ecologically sensitive areas. As at the time of carrying out this research, studies on the ecological risk of spent engine oil on terrestrial ecosystem using a battery of ecotoxicological tests is still scanty. Thus, it has become imperative to integrate ecological risk assessment with phytoremediation using indigenous plant species.

1.6 Scope of the research work

This research work involved a holistic study on the ecotoxicological risk of spent engine oil on the terrestrial ecosystem and use of indigenous plant(s) in phytoremediation of such contaminants with focus in Imo State. The study was limited to *in vitro* and pot experiments using spent engine soil in the screen house. The test plants were restricted/grown in these pots and the soil and plants analyzed accordingly. Acute toxicity bioassays were used to assess the toxic effects of the contaminants to the test organisms; Plant growth parameters (germination, root and shoot length) were used as indicators of plant health while heavy level, Total Petroleum hydrocarbon degradation, was used as indicators of soil restoration through phytoremediation technology.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Generalities of soil contamination

The soil represents an indispensable part of the earth's crust comprising of the most diverse and complex ecosystems on earth (Adeleye *et al*., 2021; Okere, Azorji, Iheagwam, Emeka, & Nzenwa, 2021). Aside from providing the fundamental ecological support for the majority of plants, the soil is the habitat for a large diversity of animals (vertebrates and invertebrates) and microorganisms (Hongmei, Jingbo & Mengping 2021; Osama, Namat & Fares, 2021). Contamination of the soil by various anthropogenic activities such as industrial activities, agrochemicals, or improper disposal of wastes of various kinds is recognized as a global challenge and there is growing evidence that the ecosystems are being degraded at an alarming rate (Krishna & Dasaram, 2021; Akcin, 2021).

Soil contamination can be defined as the occurrence of contaminants in soils above threshold level causing deterioration or loss of one or more of soil functions and subsequent alteration of ecosystem integrity (Hongmei *et al*., 2021; Panagos, Liedekere, Yingini, & Montanarella, 2013; Mirsal, 2008). The most common soil contaminants of global concern are the organics (petroleum hydrocarbons, polynuclear aromatic hydrocarbons, solvents, pesticides) and the inorganics (heavy metals) (Lado, Hrngl & Reuter, 2008).

Petroleum and its allied products are the cornerstone of Nigerian economy and an essential element of the vast majority of its mechanized transportation system as well as the primary feedstock for many of the petrochemical industries (Abiodun, 2012). In a study to assess the volume of spent oil generated in an auto mechanic village in Imo state, Udebuani, *et al*, (2016) reported about 1.4 million liters of spent engine oil being generated annually in Nekede mechanic village. Their study, though restricted to Nekede mechanic village, further revealed that gross lack of knowledge of the environmental consequences of spent engine oil could be attributable to indiscriminate dumping of spent engine oil on bare soil. According to Opeyemi (2004), about 300 million liters of lubricating oil were being sold per year, and further estimated that about 150 million liters of used oil were being generated per year.

In a related study, Osubor & Anoliefo (2004) reported that Nigeria accounts for about 87 million liters of spent engine oil annually and adequate attention has not been given to it proper disposal. In other words, lack of adequate and sustainable disposal method still persists. Though this estimate of spent engine oil generation may appear very subjective, it gives an insight into the likely scale of the problem currently bedeviling the soil ecosystem (Akpakpavi, 2015). This leads to the understanding that there is a need for proactive measures towards safeguarding the soil ecosystem in other to eschew alteration of ecosystem integrity for the benefit of man (Ajunwa *et al.,* 2018).

As opined by Okere *et al*, (2019), lack of proper industrial waste management and disposal system is an unavoidable problem in Nigeria as a result of increasing population, rapid urbanization, industrialization, and lax environmental laws. This assertion is apt given the prevalent method of spent engine oil disposal system that generates hazardous pollutants of various kinds. The majority of these contains pollutants that subsequently pave their way into soil resulting to the release of toxic elements to the environment (Donkor, Bonzongo, Nartey, & Adotey, 2005; Obini & Afiukwaa, 2013). Consequently, the soil ecosystem is continuously being polluted; resulting to an adverse effect on the terrestrial ecosystem and the biodiversity they support (Okere, *et al.,* 2021).

Several decades ago many Western nations raised environmental standards to reduce the contamination of soil (UN-HABITAT, 2010). Nevertheless practices such as rampant dumping and unregulated disposal (Onwurah, Ogugua, Onyike, Ochonogor, & Otitoju, 2007) still typify main disposal strategy for most developing countries like in Nigeria. Though illegal, but the poor strategies persist possibly due to weak law enforcement and needed research. This is unsustainable since such practices impact soil, land and water bodies as well as the general wellbeing of host communities (Marshal & Farahbaksh, 2013).

Obviously, a top down approach is needed as a dire necessity to educate the public on the ecological implications of inappropriate discarding of spent engine oil into the environment by highlighting the damages due to such activities (Agamuthu, 2001). It is essential to point that the environment is the nature capital from which man should organize and sustainably explore for good living. Issues and challenges associated with spent engine oil contamination are well documented in literature (Yahaya *et al.*, 2021; Ibeh *et al*., 2021; Okonokhua *et al*., 2007). This is not surprising considering the enormous environmental, health, and ecological related implications; and the increasing number of vehicles being serviced daily. In industrialized nations, Marshal & Farabbakhsh (2013) highlighted public health, environment, resource scarcity, climate change, public awareness and participation as key drivers of spent engine waste management strategy.

Engine oil is an indispensable component of every modern society because of its multifaceted functions. It is a major product of petroleum product that helps the engine to function optimally (Boudewijns, Bakkers, Sturm, & Melchers, 2006). The lubricant oil protects automotive components by forming a wear-resistant film between moving surfaces, transports various protective chemical additives and inhibits corrosion (Yahaya *et al*., 2021). However, engine oil performs under harsh conditions inside the engine with its combination of heat and high pressure, combustion activities and generation of chemical residues. In this harsh operating system, the oil eventually gets dirty and additives and other chemicals break down and the oil requires regular changing (Leke, Akaahan, & Simon, 2011).

Spent engine oil is oil that have been used and as a result contaminated by chemical impurities which contributes to environmental degradation (Ekperusi & Aigbodion, 2014). Available literature shows that spent engine oil is a very dangerous soil pollutant regarded to be more toxic than crude oil (Onwuka, *et al.*, 2012). It is usually obtained after servicing and subsequent draining from automobile and generator engines (Sharifi, *et al*., 2007). As a result of its chemical composition, world-wide dispersion and effects on the environment, spent engine oil is considered a serious environmental challenge which contributes to mutagenicity and carcinogenicity with global ramifications.

Indiscriminate disposal of spent engine oil into gutters, water drains, open plots and farms still typify most disposal methods among artisans in the business of auto repairs in Nigeria (Osubor & Anoliefo, 2004; Odjegba & Sadiq, 2002). This has been attributed to the fact that most the artisans know little about the deleterious effects of spent engine oil on the ecosystem (Zitte, Awi-Waadu, & Okorodike, 2016). The concerns over ecosystem disturbance by indiscriminate disposal of spent engine oil have led to the need to detect variations in biotic and abiotic integrity of different trophic levels by assessing the potential risks on the ecosystem (Ameh, Mohammed-Dabo, Ibrahim, Ameh, Azienge, & Tanimu, 2011).

2.2 Disposal methods of spent engine oil

There are a number of reported cases of indiscriminate disposal of spent engine oil on the immediate environment (Plate 2.1). A survey of most cities in Nigeria showed that there are little or no organized disposal practices for waste engine oil (Nkwoada & Amakon, 2018; Udebuani *et al.*, 2016; Obinia & Afiukwaa, 2013; Nwachukwu, Feng, & Alinnor, 2011). Bamiro & Osibanjo (2004) had estimated the total national used oil generating capacity in Nigeria to be over 200 million litres per annum in 2004. In a study to appraise the volume of spent engine oil generated in Nekede mechanic village, Udebuani, Chidiogo, Ifeanyi, Okoli, Harriet, Nwigwe & Patrick (2011) reported a total of 1,469,678.08 litres. In a related study by Zitte *et al*, (2016), a total of 1628.50 litres of spent engine oil was recorded at Portharcourt area of Rivers State.

Plate 2.1 Spent engine oil disposed into the immediate environment by auto mechanics

Global demand for engine oil stood at 8,642 Kilo tons in 2020. In Nigeria, increasing demand for engine oil is expected to reach 339.52 Kilo tons by 2026. According to Nigerian lubricant market report (2021), increasing drain intervals from auto mobiles dropped from estimated 200 million litres per annum as a result of limitations ocaasioned by Covid-19 pandemic in 2019, it is expected to rise by 2026. This gives an insight into the volume of spent engine oil been disposed into the environment (Bamiro & Osibanjo, 2004a; Odjegba & Sadiq, 2002).

Unfortunately, the disposal of waste oil into gutters, water drains, open vacant plots, farms and so on is a common practice by motor mechanics (Noln, Harris, & Cavanaugh 2002; Omorowa & Agu, 2017). Conspicuously, large quantities of used oil generated in the garages in the country tend to be dumped around the cities in drains which ultimately contaminate and pollute the soil, water bodies including rivers, lagoons, streams, etc through runoff (Akpakpavi, 2015). Undoubtedly, this indiscriminate dumping of waste oils in the water bodies in the country could largely be attributed to the mechanics lack of knowledge regarding the physical, chemical, biological and environmental hazards of the used oils (Omorowa & Agu, 2017; Akpakpavi, 2015; John & David, 2009).

2.3 Composition of Spent engine oil

Spent engine oil is one of the components of crude oil comprising of up to 20-70 carbon atoms in its chain and more than 75% C-alkanes (Ayandele, 2012). Most of the C-alkanes in the base oil have long alkyl side chains (Bagherzadeh-Namazi, Shojaosadati, & Hashemi-Najafabadi, 2008). They are made from a heavier and thicker petroleum hydrocarbon base stock derived from crude oil with additives that help in maintaining a lubricating film between moving parts of a car or machine engines. Spent engine oil which is also known as used crankcase oil is a brown to black liquid produced when new Crankcase oil is subjected to high temperature and mechanical process (Achuba & Peretiemo-Clark, 2008; Onwuka & Igwe, 2010).

Spent engine oil is known to contain a number of several different mixtures of chemical contaminants from engine wears such as heavy metals (lead, zinc, chromium, barium, arsenic) etc, polychlorinated biphenyls, lubricative additives, chlorodibenzofurans, decomposition products, aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) that come from engine parts as they wear down (Ayandele, 2012; Adenikpekun *et al.*, 2008) and this contribute to chronic hazard, which include mutagenicity and carcinogenicity in the ecosystem (Bonchan, Britz, & Stanley, 2000). These contaminants get to the environment as a result of deliberate and accidental discharge/spill by motor and generator mechanics (Odjegba & Sadiq, 2002) and from the exhaust system during engine use and due to engine leaks (Osubor &Anoliefo, 2003).

2.3.1 Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are large group of organic compounds comprising of two or more fused aromatic rings ranging from two-ring naphthalene and naphthalene derivatives to complex ring structures containing up to 10 rings (Patel, Shaikh, Jain, Desai & Madamwar, 2020; Muze *et al*., 2020; Obinia *et al.,* 2013). They are released into the environment during incomplete combustion and pyrolysis of organic material such as coal, wood, fuel, tobacco, and meat (Franco, Adelaide, Nardocci, & Günther 2008; Sabate & Solanus, 2006). More than 100 different PAHs are one of the most significant classes of organic compounds that have recently caused a growing concern regarding harmful effects to humans and other living organisms (Nganje, Edet, & Ekwere , 2007a; Nganje, Edet, & Ekwere, 2007b).

PAHs are environmental pollutants derived from both anthropogenic or pyrogenic (like oil leakages, oil spills, and combustion of fossil fuels) and natural or petrogenic (like geogenic, forest fires, and volcanic eruption) origin with serious concern to ecosystem health and human through bioaccumulation (Nganje, Neji, Ibe, Adamu, & Edet, 2014). Although PAHs are found in coal and petroleum products, the main route of introducing PAHs into the environment is by the incomplete combustion of organic matter from sources such as motor vehicles, coal-fired plants, home heating furnaces and forest fires (Muze *et al.,* 2020; Yongyong *et al*., 2014).

PAHs are also released when coal, petroleum products, wood, urban solid wastes or old tires are burned (Patel, Singh, Patel, Jain, Amin, & Madamwar, 2019). Other sources of PAHs include oil refineries, coal gasification plants, steel mills, and aluminium plants. According to USEPA (2021); Nganje, *et al.,* (2014) PAHs are among the list of priority compounds considered hazardous to the environment. Because of their hydrophobic nature and low solubility, PAHs are resistant to biodegradation and can bioaccumulate in the environment through the food chain. Due to their inherent properties, PAHs are persistent pollutants having a wide range of biological toxicity; remediation of PAHs from the environment has been a global concern.

The levels of PAHs in soils around auto mechanic villages have been documented by previous researchers. Obinia *et al,* (2013) reported elevated levels of PAHs in an auto mechanic village in Abakiliki. Based on their findings, six out of sixteen (16) USEPA priority PAHs were detected at varying concentration. Their result further revealed that benzo[b]flouranthrene were the dominant group in the soil samples assayed. A study by Muze *et al*, (2020) to assess the effect of activities of auto mechanic at Ala Ojii Aba and Elekahia Port Harcourt River State revealed that recorded values for PAHs within the study areas were above the recommended levels of 1,000ug/kg, 1,500ug/kg and 5mg/kg recommended by clean-up guidelines by Denmark, Netherlands and Australia respectively. They attributed the high levels of PAHs to the activities of auto mechanic in the study areas.

In a study to ascertain the level of PAHs in Ghana, high concentrations of PAHs were reported by Gyasi (2014) in Kumasi Metropolitan assembly. Studies by Ibe *et al*, (2020) of PAHs in soils of the abandoned sections of Orji mechanic village in Owerri revealed a total of 14 PAHs detected in their soil samples with mean concentrations ranging from 1.22-0.02 mg/kg. Their result further revealed that Dbh had the highest percentage (53%) of occurrence in all the sampled soils. In a similar study to assess the concentration and source evaluation of 16 priority PAHs in soils of selected vehicle parks in southern Nigeria, Emoyan, Ejecha, & Godswill (2020), reported that the study area was contaminated with varying concentrations of PAHs.

2.3.1.1 Toxicity of Polycyclic Aromatic Hydrocarbon

Toxicity of PAHs varies in their effects on both human and ecosystem health (Rengarajan Rajendran, Nandakumar, Lokeshkumar, Rajendran, & Nishigaki, 2015; Abdel-Shafy & Mansour, 2016). Majority of the PAHs have been reported to be mutagenic, carcinogenic, teratogenic, and immunotoxic to living organisms, including microorganisms, animals, plants and humans (Rengarajan *et al*., 2015; Bolden, Rochester, Schultz, & Kwiatkowski, 2017). According to Abdel-shafy & Mansour, (2016), PAHs toxicity has been documented on aquatic life through runoff. The route of exposure, duration, and exposure dose are important parameters for the severity of PAHs' toxic effects (Rajpara, Dudhagara, Bhatt, Gosai, & Dave., 2017). As opined by Zheng, Xing, Hu, Zhang, Zhang, & Zhu, (2018) PAHs from different sources showed different risk levels, and they calculated incremental lifetime cancer risk (ILCR) in humans for soil-bound PAHs upon the three different exposure routes; the highest cancer risk was found for ingestion, i.e., 98.1–99.3%, followed by dermal contact, i.e., 0.66–1.83%, and inhalation, i.e., 0.03–0.04%. Toxic effects of PAHs may also vary according to factors such as pre-health status and age of the organism.

PAHs have been implicated in acute health effects such as eye irritation, vomiting, diarrhea, confusion, skin irritation, and inflammation (Abdel-Shafy & Mansour, 2016). Naphthalene, anthracene, and benzo(a)pyrene, chemical compounds found in PAHs are direct skin irritants and skin sensitizers for animals and humans (Rengarajan *et al.*, 2015). Chronic health effects including eye cataracts, kidney and liver damages, breathing problems, decreased immune function, lung malfunctions, and asthma-like symptoms have also been reported (Abdel-Shafy & Mansour, 2016). Naphthalene can cause the breakdown of red blood cells if inhaled or ingested in high amounts (Rengarajan *et al.*, 2015).

As opined by Patel *et al,* (2020), the carcinogenic potency of a PAH is associated with the structural features and the complexity of the molecule: a more complex compound is usually more potent. A number of epidemiological studies have demonstrated that exposure to environmental PAHs was associated with elevated lung cancer, breast cancer, skin cancer, and esophageal cancer in humans (Rybicki, Neslund-Dudas, Nock, Schultz, Eklund, Rosbolt, Bock, & Monaghan, 2006). Some studies have shown that people with occupational exposure to PAHs suffered from increased risk of prostate cancer (Ibeh *et al*., 2021; Muze *et al.*, 2020; Rybicki *et al*., 2006).

2.3.2 Total Petroleum Hydrocarbons

TPHs are one of the most common soil contaminants in the environment. Total petroleum hydrocarbons (TPH) is the term used to describe a broad family of heterogeneous compounds that are found in crude oil and whose main chemical constituents are carbon and hydrogen atoms. The use of petroleum products for heating, industry and transportation is leading cause of TPH release into the environment through long-term leakage, indiscriminate spill and operational failures (Achuba & Peretiemo-Clark, 2008).

As they exist as a mixture of so many different compounds is more practical to quantify them in environmental samples as a group of congeners rather than separately (Achuba & Peretiemo-Clark, 2008). TPH can be divided into groups (fractions) of petroleum hydrocarbons that act alike in the soil or water. It can be distinguished two main fractions: aromatics and aliphatics, which in turn, can be subdivided in additional groups containing individual compounds with carbon chains of different length (Abioye *et al.*, 2012).

TPH entering the environment can affect all environmental compartments: water, air, and soil (Wang, Liu, Li, & Sun, 2014). When TPH is released to water, light TPH fractions will float forming thin surface films, while heavier TPH fractions will accumulate in the sediment at the bottom of the water (Okere *et al.*, 2021). In addition, some TPH compounds released to the soil may evaporate into the air while others may move downwards, dissolve into the groundwater and move away from the release area (Tripathi, Gaur, Dhiman, Gautam, & Manickam, 2009).

2.3.2.1 Toxicity of Total Petroleum Hydrocarbons

TPH contamination of soil is a major source of concern. Once released into soil, the volatility of can pose a fire or even explosion hazard, especially when vapors enter confine spaces (Ashutosh, Mukesh & Pavan, 2022). The resident contaminants can interfere with the nutrients and water transmission and thus lead to soil contamination and alteration of ecosystems (Ali, Hamid, Mohammad, Frank & Manuela, 2022). Most times, weathered petroleum residuals may stay bound to soil particles and be retained in soil for years. Although these contaminants may benefit the oil degraders as a carbon source, they are still toxic to the majority of soil biota. TPH pollutants have been reported to alter the ecology and the physiology of bacteria and fungi (Tripathi *et al.*, 2009). TPH contamination may destroy the aesthetics by inducing offensive odor, taste or appearance in environmental media. Contamination of soil by TPH is not only a concern for the soil itself, but is also a potential threat to other ecosystems.

The release of TPH to the environment does not always lead to exposure and toxicity to human beings. This only occurs if coming in contact with the substance of concern. According to Karam & Al-wazzan, (2021) toxicity effects on the individual depend on several aspects such as:

- pathway of exposure (i.e. by oral, dermal or inhalation exposure),
- time and number of exposures (acute, chronic),
- dose and physical form of the substance and
- individual factors (e.g. genetic background, sex, age, diet, lifestyle, overall health state).

2.3.3 Heavy Metals

According to Ali, & Khan, (2018), metals are chemically defined as elements that conduct electricity, have a metallic luster, are malleable and ductile, form cations and have basic oxides. Besides, in the scientific literature heavy metal has been generally referred to metals and semimetals (metalloids) associated with toxicity effects or chemical hazards rather than other intrinsic physicochemical properties (Kabata-Pendias, 2011). Tchounwou, Yedjou, Patlolla, & Sutton (2012) opined that heavy metals are groups of metal with a density greater than 5 g/cm^3 (i.e., specific gravity greater than 5). Examples of heavy metals are Pb, Cd, Cu, Hg, Sn, and Zn, etc. most of them when found above threshold levels in the environment leads to toxicity in various ecosystems.

Heavy metals entry into the environment can be through natural and anthropogenic (human induced) sources (Andrey, Melinda, Natalie, Kath, Dan, & Jennifer, 2022). Natural or geological sources of heavy metals in the environment include weathering of metal-bearing rocks and volcanic eruptions (Nagayoti, Lee, & Sreekanth, 2010). Anthropogenic or human induced sources of heavy metals include mining and extraction of different elements from their respective ores. Heavy metals released to the atmosphere during mining, smelting, and other industrial processes return to the land through dry and wet deposition (Ali & Khan, 2018).

Heavy metals are fundamentally categorized as essential and nonessential. Essential heavy metals are important for living organisms for normal functioning of the ecosystem and may be required in the body in quite low concentrations. Nonessential heavy metals have no known biological role in living organisms. Examples of essential heavy metals are Mn, Fe, Cu, and Zn, while the heavy metals Cd, Pb, and Hg are toxic and are regarded as biologically nonessential (Rahim, Ullah, Khan, & Haris, 2016).

2.3.3.1 Toxicity of heavy metals

Certain heavy metals have been reported to be carcinogenic, mutagenic, and/or teratogenic to different species depending on dose and duration of exposure (Ali, Khan, & Sajad, 2013). Some organisms appear to be more sensitive to heavy metals than others. The mechanisms by which heavy metals affect different organs, tissues, and systems in different organisms are very complex, and so far some of them are not fully explored (Ngo, Gerstmann, & Frank, 2011). In spite of the fact that most heavy metals, called essential heavy metals, play important roles in biological systems, they are generally toxic to living organisms depending on dose and duration of exposure (Ali & Khan, 2018).

In their study to ascertain heavy risk assessment of heavy metals in vegetables grown around quarry sites in Okigwe, Abara, Udebuani, Okeke, & Adjeroh, (2020) documented elevated levels of Pb and Cd which could pose a carcinogenic and mutagenic risk to the consumers in the study areas. According to Wang & Shi (2001), metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis. Several studies have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic (Yedjou & Tchounwou, 2006) cadmium (Tchounwou, Ishaque, & Schneider, 2001), chromium (Patlolla, Barnes, Field, Hackett, & Tchounwou, 2009), lead (Yedjou & Tchounwou, 2008), and mercury (Sutton, & Tchounwou, 2008). This is because of their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance (Syeda, Ghazala, Azizullah, Muhammad, & Zeshan, 2022; Sutton, & Tchounwou, 2008)

As opined by Okere *et al.*, (2020), humans are exposed to toxic heavy metals in the environment through different routes including ingestion, inhalation, and dermal absorption. People are more exposed to toxic metals in developing countries (Wittman, 2002). Generally, people have no awareness and knowledge about exposure to heavy metals and its consequences for human health, especially in the developing countries (Becker, Kaus, Krause, Lepom, Schulz, & Seiwert, 2002). People may be exposed to heavy metals in the work place and in the environment.

2.4 Environmental implications of spent engine oil contaminated soil

Previous authors have reported deleterious effects of spent engine oil soil contamination on the ecosystem. Studies by Echiegu, Amadi, Ugwuishiwu, & Nwoke, (2021), observed a significant decrease in soil properties subjected to various concentrations of spent engine oil. Agbogidi & Ilondu (2013) stated that soil contaminated with spent engine oil has significant effect on reducing the germination response and subsequent performances including the biomass production of *Moringa oleifera* seedlings. Moreover, Nwadinigwe & Oyiga (2009) reported a significant decrease in height, number of leaves, leaf area, and number of flowers, fruits and dry weight of *Solanum gilo* with increase in foliar spray of petroleum hydrocarbon.

Uchendu & Ogwo (2014), studied the effects of spent engine oil on soil properties in an auto mechanic village and observed that the soil Pb values were predominantly acidic. Their study further revealed that cation ion exchange capacity of the soil was relatively low implying low fertility of the soil with varying levels of Pb contamination which exceeded the permissible level limits. Their study suggested a well-coordinated waste oil collection programme by the Government and concerned private sectors to minimize indiscriminate disposal of spent engine oil.

Nkwoada, *et al*, (2018), reviewed an article on pollution in Nigerian auto mechanic villages, and the findings revealed that environmental contamination is one of the major problems that plague the Nigerian auto mechanic villages. Their findings showed that 95% of the studied auto mechanic villages are located in the cities or nearby urban settlement. They further suggested a concerted effort to remediate the sites, continuous assessment of pollutants around auto mechanic villages using biomonitoring and phyto-monitoring (phytoremediation) to avoid further environmental degradation.

In a study to ascertain the pollution status heavy metals in spent engine oil contaminated soil in Gwagwalada area of Abuja, Orji *et al*, (2018) observed a significant variation of heavy metals concentration with Pb having the highest concentration and Cd the least. The study further revealed that Pb had a moderate potential ecological risk factor and a very high contamination factor suggesting the need for adequate management and monitoring to deter further contamination of the land which could eventually affect farmland, ground and surface water thereby reducing the bioaccumulation of heavy metals across the food chain.

Farombi *et al*, (2013) conducted a study on the Physicochemical Properties of Soil Contaminated with Refined Petroleum Oil in Eluama Community, Abia State, Nigeria. Their results revealed that organic carbon, organic matter, calcium and magnesium increased with increase in pollution while nitrogen, potassium, sodium and phosphorus decreased with increase in pollution. The pH became more basic as pollution decreases. In addition, concentration of heavy metals increased as soil increases with pollution. Their result further revealed that the polluted soil when compared with the control (soil from unpolluted farmland) is unsuitable for agricultural activities as full remediation has not taken place except remediation can be hastened.

Donkor *et al,* (2004) opined that used mineral based crankcase oil known as spent engine oil is subjected to high temperature and high mechanical pressure. This spent engine oil is a common and toxic environmental contaminant which is not naturally found in the ecosystem. Large amounts of these spent engine oil is liberated into the environment during the process of oil change which ultimately dispersed into gutters, nearby water channels, open vacant plot and form lands.

Kayode, Olowoyo, & Oyedeji, (2009), evaluated the Effects of Pollution with Spent Lubricating Oil on the Physical and Chemical Properties of Soil. The results of their study revealed that pollution of vegetative soil with spent lubricating oil altered the physical and chemical properties of the soil. They further recommended that public awareness should be intensified, in the study area, on the detrimental effects of spent engine oil pollution and its indiscriminate disposal should be discouraged. Strict laws must be made to guide against its indiscriminate disposal in the study area while the existing laws on pollution should be reviewed with the aim of being made more effective and complementary with the present trend. They also suggested that there should be effective implementation of the laws on pollution in the study area.

Achuba & Clarke, (2008) conducted a research work on the effect of spent engine oil on soil pH as well as activity of selected enzymes (catalase and dehydrogenase) was studied. The result from their study revealed that spent engine oil caused a slight change in soil pH relative to the control. Their results further revealed that spent oil alters soil biochemistry.

Nwachukwu *et al*, (2011) worked on three automobile mechanic villages (Okigwe, Nekede and Orji) in which three samples from each mechanic villages were collected for the study of metals concentration and concluded that the degree of mechanic activity or population of mechanics and their workshops density affects the degree of metal enrichment in the mechanic village soil. Orji mechanic village, they opined, has the slope and drainage area with less workshop density, which supports dispersion of metal contaminants from the mechanic village thereby reducing metal concentration in the soil.

Agbogidi (2010) reported that a marked change in properties occurs in soil polluted with petroleum hydrocarbons, affecting the physical, chemical and microbiological properties of soil. Changes of soil properties due to contamination of petroleum derived substances can lead to water and oxygen deficit as well as shortage of available nitrogen and phosphorous. In a related study, Okonokhua *et al*, (2007) reported that plant height, root number and root length of maize grown in spent engine oil-contaminated soil were affected.

According to Adenipekun (2008), engine oil affects the moisture content in *Corchorus olitorius* Linn. Ogbuehi *et al*. (2011) reported a significant decrease in biochemical parameters including fiber and carbohydrate content in cowpea (*Vigna unguiculata*) growing in soil contaminated with spent engine oil. Agbogidi & Ilondu (2013) stated that soil contaminated with spent engine oil has significant effect on reducing the germination response and subsequent performances including the biomass production of *Moringa oleifera* seedlings. In *Zea mays,* leaf formation was disrupted, stunted growth and high mortality rate was observed when it was exposed to soils contaminated with used oil (Njoku *et al.*, 2021). This resulted to low yield and poor quality of grains observed during harvest for *Zea mays* and *Arachis hypogea* (Ogbuehi *et al*., 2011).

2.5 Application of bioindicators in contaminated soil characterization

The soil is a dynamic and complex ecosystem comprising of a variety of organism at different trophic levels (Akin-Obasola, 2019; Ogbuehi, Omotayo, & Obianime, 2015). In other to ascertain the extent contamination of a pollutant in the environment, contaminated soils are usually subjected to routine characterization encompassing physical and chemical (Udebuani *et al.*, 2021). Many authors have reported soil contamination of specific sites using threshold values (Okere *et al*., 2021; Nwachukwu *et al*., 2020; Orji *et al*., 2018; Ikpe, Ebunilo, & Okovido, 2018) to determine their associated risks in the ecosystem. However, several restrictions of chemical methodologies have been reported that limited their suitability for the assessment of soil contamination (Mari, García-Lorenzo, María, Martinez-Sánchez, Carmen & Pérez-Sirvent, 2020). First of all, chemical analyses require previous knowledge on the compounds of interest and their intermediary metabolites because they are unable to detect all soil contaminants (Leitgib, Kálmán, & Gruiz, 2007).

Once released into the environment, the risks associated to pollutants cannot be solely determined according to their toxicity. In fact, very toxic substances can pose a little threat if they are not easily available to organisms. The term bioavailability refers to the biologically active fraction of a contaminant, i.e. the ability of an environmental pollutant to reach an organism (or a target part of it) and cause some effect (Landis, Sofield, & Yu, 2011).

Njoku, Akinola, & Nkemdirim, (2014) in a study on Phytotoxicity Assay of Crude Oil Using Different Accessions of Sorghum bicolor revealed that Chromosomal aberrations ranging from vagrant, bridged anaphase, c-metaphase amongst others were noticed in the cells of the seedlings exposed to the different treatments. The results of their study shows that although the different assay parameters show that crude oil can affect different plants in the different ways, 07/125 is more sensitive to crude oil pollution. They suggested that further studies can be carried out on the more resistant accessions to determine their suitability for remediation of crude oil contaminated sites.

Chukwu & Odunzeh, (2006) conducted a study on relative toxicity of spent lubricant oil and detergent against benthic macro-invertebrates of a West African estuarine lagoon. Their results further suggested that the estuarine benthic macro invertebrates, which play key roles in the environment, may serve as useful *in-situ* sentinels for biomonitoring studies of petroleum pollutants in fragile aquatic ecosystems such as the Lagos lagoon.

2.6 Ecotoxicology

Ecotoxicology is the study of deleterious or toxic effects of pollutants on living organisms (biota) at both community and population levels of the ecosystem. According to the British Toxicology Society, ecotoxicology is the study of toxic effects of chemicals on the aquatic and terrestrial ecosystems. By extension, ecotoxicology entails the study of fate and effect of toxicants in the ecosystem (Bascietto, Hinckley, Plafkin, & Slimak, 2003). The essence of ecotoxicology is to predict and possibly prevent potential undesirable events in the natural ecosystems by conducting ecotoxicity assays or assessments on chemicals that may be disposed into the environment.

Ecotoxicology is interdisciplinary encompassing disciplines such as ecology, toxicology, chemistry, epidemiology and pharmacology (Panagos, Van, Yigini, & Montanarella, 2013). This implies that the knowledge of environmental chemistry of pollutants (chemicals); evaluation of its effects on the ecosystem, lethal and sublethal effects on organisms are essential components of ecotoxicology. The practice of ecotoxicology was based on two premises; firstly, as an applied field within the framework of regulatory environmental protection; and as a more scientific aspect that study the mechanisms underlying the fate and effects of contaminants on the organisms in the ecosystem.

As an aspect of ecotoxicology known as terrestrial ecotoxicology is the subfield that studies, evaluates and quantifies the effects of toxic substances on the diversity and function of soil-based plants and animals (García-Lorenz, Pérez-Sirvent, Martínez-Sánchez, & Molina-Ruiz, 2012). The ecotoxicological assessment of soils is strongly influenced by the complexity of the soil matrix and its interactions with polluting substances (Leitgib, Kálmán, & Gruiz, 2007).

After reaching the terrestrial compartment, pollutants are usually bound to the solid phase of the soil but their bioavailable fractions can be dissolved in the soil pore water (Posthuma, Suter, & Traas, 2002). Furthermore, soil-occurring phenomena like sorption, partitioning, and speciation strongly affect soil contaminants and must be taken into consideration since they will ultimately determine changes in toxicity and biodegradation rates of pollutants in the ecosystem (Tarazona Fernandez, & Veja, 2006).

2.6.1 Ecological Risk Assessment

Ecological risk assessment is defined as the probability that adverse ecological effects will occur as a result of exposure environmental stressors released due to human activities such as disposal of chemicals and natural catastrophes (Udebuani *et al.*, 2021). Stressors in this context are used to describe physical, chemical and biological agent in the ecosystem capable of causing adverse ecological effects at the community, population and even ecosystem level. The term “adverse ecological effects” is used to mean a wide range of disturbances such as mortality and ecosystem alterations (D'Souza, Vadakkeveedu, Kumar, Anish, Harikripa, Keshava, 2022; Hoffman, Rattner, Burton, & Cairns 2001)

Ecological risk analysis is a prediction coupled with evaluation to estimate the possibility/probability of harm (risk) for a particular toxicant (hazard). Manifestation of risks of a particular hazard follows three main routes: an activity (such as disposal of pollutants), a pathway for transport, a receptor (here referred to as the organism that could be harmed at the exposure point). In other words, risk analysis provides a well structure approach for appraising the nature of and strength of the relationship between cause and effect (Patra, Bhownfik, Bandopadhyay, & Shanna, 2004).

The risk assessment of contaminated soils can be performed through different bioassays which are categorized according to their duration, the number of species involved and their complexity (Abara, Udebuani, Okeke, Ifeyinwa, & Adjeroh, 2019). At the same time, the ecological relevance of each test is correlated with its complexity, costs and duration (Römbke &Notenboom, 2002). Because of these limiting parameters, the most complex tests are only rarely applied (OECD, 2006).

2.6.2 The process of risk assessment

2.6.2.1 Risk Valuation

Four techniques exist for the risk evaluation which includes: hazard identification, dose-response assessment, exposure assessment and risk. The techniques are resultants of consistent further concern of creation of significant potential exposure risk due to combination of diverse elements of the scheme (Iwuoha, Osuji, & Horsfall, 2012).

2.6.2.2 Hazard identification

It implies the study of an environmental situation to ascertain the probability of an organism (including humans) getting exposed to a potential harm causative environmental stressor (Kamunda, Mathuthu, & Madhuku, 2016; Bhatt, 2005). Hazard identification is benched on enquiry of agents/chemicals present at any location, their concentration, and spatial distribution (Khan, Cao, Zheng, Huang, & Zhu, 2008). This is the foremost phase of risk evaluation, which deals with data gathering and consequent detection or recognition of probable environmentally sustainable antidote. Oluseyi, Adetunde, & Amadi**,** (2014) term it as Tier 1 risk assessment where a desktop study is completed to ascertain chronological activities from the past, which could have enhanced contamination. Furthermore, the geographical dynamic details of the area are summarized from maps and models, which enable identification of probable sources, pathways, human and ecological receptors. Consequently, a semi-quantitative Conceptual Site Model (CSM) is considered and designed (USEPA, 2021), which investigates the potency of the characteristics of the contaminants to be harmful. Literature or established government or organizations databases are domiciled with data about the latent environmental harmful characteristics of the identified contaminant. Animal studies outcomes serve as benchmarks for testing for environmental hazards due to quality and ethical consideration associated with using humans. Further to hazard identification it is pertinent to get information on concentration levels of contaminant, dispersion, distribution and changes as time changes, moreover elements which influence fate, transport and exposure are fully considered (USEPA, 2000).

2.6.2.3 Dose-response assessment

This involves the categorisation of the affiliation between the dose receipt by receptor, which could be an organism or ecosystem and the consequent adverse impact occurrence on the involved receptor (Iwuoha, Osuji, & Horsfall, 2012). Hence it estimates the relationship among duration, level with rate of recurrence of exposure plus deleterious effect potential. The linear or nonlinear nature of the exposure and response is influenced by the receptor. A nonlinear scenario assumes subsistence of a threshold with no effects for doses lower than threshold, but with effect starting at when the threshold is surpassed (Iwuoha *et al.*, 2012; Oluseyi, Adetunde, & Amadi., 2014). The evaluated human populace daily oral exposure reference dose, without a significant lifetime threat of deleterious effect characterises nonlinear effect (USEPA, 2021; Oluseyi *et al.*, 2014). While a linear situation is the case in carcinogenic compounds with no threshold value, instead the dose shows a straight connection amid the response, with a linear increase as dose increases. To calculate, for instance, the lifetime surplus risk of cancerous effect according to USEPA (2000), is domed on the affiliation being characterized by a slope factor, which multiplies the dose. Moreover, the toxicant concentration level against the Maximum Contaminant Level (MCL), assessment could be calculated. Hence the site condition could be grouped as potential harm to the receptor if MCL is exceeded **(**USEPA, 2000; Oluseyi *et al.*, 2014).

2.6.2.4 Risk Characterization

Risk characterization estimates the occurrence of a dangerous impact based on exposure conditions explained in the exposure evaluation (USEPA, 2005). Moreover, it comprises the explanation of assessment, including the preceding steps uncertainties. It may perhaps be the last phase measure of risk evaluation. The data generated in preceding phases are summarized by the risk characterization. Important elements of the risk characterization phase are transparency, clarity, consistency and reasonability. According to Oluseyi *et al.*, (2014), transparency involves the clear description of the processes, assumptions and uncertainties with the outcomes of the result of each stage in the evaluation; clarity indicates comprehension of the outcome of the risk evaluation, while consistency implies doing the evaluation in alignment with relevant regulatory framework, along the line of study or operation. Finally, data from well researched scientific results that forms the foundation for decision is the main take of reasonability.

Risk evaluation has been subjected to uncertainty investigation in the recent past because of the aggregated final uncertainty from conglomeration of modeled parameters value uncertainty (Thomsen *et al.,* 2015). Probabilistic risk evaluation is employed to evaluate the risk. The technique of probabilistic risk assessment is utilized to estimate the risk. So, study of an aspect which requires determining the potential cancerous and non-cancerous impact on receptors will require integration of above explained characteristics and processes. USEPA, (2005) opines the essence of risk characterization is quantification of cancerous risk and hazard indices.

2.6.2.5 Exposure assessment

According to Bhatt (2005) exposure assessment is the procedure of evaluating or determining the amount, rate of occurrence, or interval of human or ecological exposure to agents that are prevailing in the environment, or have future existence potential. It aims to appraise the scale, rate and period of the pollutant effect. Exposure assessment might approximate the amount of mass discharge on receptors and/ or points of compliance. Direct measurements, calculations or even simulation models may be utilized to estimate the contaminant pathway to the point of compliance and or receptor from the origin (Kamunda, Madhuku, & Mathuthu, 2016).

Though direct measurement is least considered, the key receptor as documented in USEPA's guidelines on human health risk is human being. This is as opined in their definition of exposure as interaction or contact between an agent and an observable peripheral of a person such as skin and body opening (USEPA, 2005). In Denmark, according to Denmark Environmental Protection Agency guidelines for remediation of sites that are contaminated, key receptor is the groundwater (US EPA, 2021). Characteristically, the exposure routes are three- dermal, ingestion and inhalation. IPCS (2004) posits that these manner or contaminant entrance route in an organism on contact, is comparable to contaminants entrance into a person or inhabitants after contact (IPCS, 2004).

In ecotoxicological risk assessment, external exposure is more often considered, although it can be used to estimate an internal exposure based on toxicokinetic models. External environmental exposure is typically quantified in terms of the predicted environmental concentrations (PECs). Often different environmental compartments (water, air, soil, and sediment) are considered, and PECs are calculated for each of them. In order to derive a PEC, both an assessment of the emissions of the stressor and environmental modeling of its subsequent fate are required.

Ecotoxicological data are often applied to determine the highest dose or concentration at which there will be no adverse effects to a certain endpoint. This concentration is often referred to as the predicted no-effect concentration (PNEC) and is ideally derived from dose-response curves (from acute or chronic data). In practice, dose-response curves are not always available for the stressor and endpoint of interest.

It is pertinent to state that these concentrations must then be divided by an assessment factor that varies between 10 and 10, 000, depending on available data in order to obtain a PNEC. The assessment factor is defined as the uncertainty factor or the application factor. Assessment factors are not based on mechanistic models but rather on experience from effect assessment (Czerniawska-Kusza & Kusza, 2011).

By convention, if the PEC is higher than the PNEC, that is, if the quotient PEC/PNEC is > 1, it indicates potential risk of the contaminant under study. But if the PEC/PNEC < 1, no risk is envisaged at this point (Kasemets, *et al.*, 2003). The PEC and PNEC may also be expressed not as single numbers but as ranges or even probability distributions in order to conduct a more detailed risk characterization (Khan, Cao, Zheng, Huang, & Zhu, 2008).

2.6.3 Toxicity Evaluation of Pollutant in soil matrix

Toxicity is an inherent characteristic of most pollutants such as spent engine oil. Toxicity is a term used to describe the potentials of a toxicant to cause adverse effects on the ecosystem. In this context, toxicity is a function of dose (pollutant concentration) and the duration of exposure. Based on duration of exposures, the toxicity tests can be divided into acute toxicity test, and chronic toxicity test. Acute toxicity can be defined as deleterious effects experienced by the organisms during short-term exposure to toxicants (OECD, 2000). Acute toxicity tests are principally designed to determine the dose/concentration of a test material producing deleterious effects on a group of test organisms during short-term exposure under controlled laboratory conditions (Abara *et al*., 2019; Akin-Oshoba, 2019).

The easily detectable deleterious response is mortality of the exposed organisms and lack of movement, inhibition of germination (in plants) and lack of response to gentle prodding are generally used criteria for the death of organisms. Therefore, most common acute toxicity tests are acute lethality tests. Usually 50% (LC50) response is the most accepted and reproducible measure of toxicity and 96 hour is the standard exposure time, because it covers the period of acute lethal action (ISO, 2011).

2.6.4 Risk assessment with terrestrial plants

Terrestrial plants have been reported to one of the commonly used tool for biomonitoring ecosystem health (Fernandes, Soare, Braga, Robotim, Ferreira, Fidalgo, Pereira, & Cachada 2021; Azorji, Okechukwu, Udebuani, & Duru 2021a; Njoku, Akintola, & Oshodin 2011). The evaluation of the toxic effects of a pollutant is an essential component of the ecological risk assessment of that compound. Plants are primary producers in every ecosystem. For this reason, they form an essential trophic level of any ecosystem. Further, since all chemicals introduced into the environment ultimately find their way into terrestrial and aquatic ecosystems, terrestrial and plant toxicity evaluations are particularly critical. Terrestrial plant bioassays have been used for several decades for determining herbicidal effectiveness in the agricultural and horticultural industries.

Germination and early seedling establishment test is the most common plant test for heavy metal toxicity in soils (Signh & Jain, 2003). In addition, various life history parameters have been used, such as germination, seedling growth, plant height, leaf number and area, pod number and length (in legumes), biomass production, dry matter production, reproduction (Baran & Tarnawski, 2013).

Olfa *et al.*, (2012) conducted a study on toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays. In their study Total petroleum hydrocarbons (TPH)-contaminated soil samples were collected from an oilfield in Sfax, Tunisia. Two types of bioassays were performed. The average germination rate, calculated 8 days after sowing, varied between 64 and 74 % in low contaminated soils and less than 50 % in highly contaminated soils indicating toxicity of petroleum hydrocarbons.

Terrestrial plants are important for life because they are primary producers, and support all other life forms (Eapen & D'Souza 2005). The role of plants in soil development, stabilization, and nutrient cycling is essential. These characteristics make terrestrial plants representative organisms for monitoring and assessment of soil quality. These tests represent direct contact tests or solid-phase tests and their main advantage is that interaction occurs between soil and test organisms, so that the mobility and bioavailability of the contaminant is easily assessed.

2.6.5 Risk assessment with earthworms

Earthworms are relevant test-organisms in ecotoxicological tests because they are common in a wide range of soils, representing 60-80 % of the total soil animal/invertebrate biomass (Dada *et al.,* 2016). Earthworms have intimate contact with the soil and are the base of many food webs. They are also known to accumulate large concentrations of metals into their tissues when exposed to contaminated soils (Naseer, Pratiksha, Tabassum, Channgam, Shahid & Tasneem, 2022). Earthworms play an essential role in maintaining the structure and fertility of soils; recycling nutrients, increasing aeration and drainage, and can constitute an important component of the diet of birds, reptiles or small mammals (Naseer, *et al.,* 2022; Allen, 2002). Thus, earthworms are useful biological indicators of pollutants in soil (Mohamed, Servane & Jean-Bernard, 2022).

Toxicity in earthworms can be measured in two ways: mortality and sub-lethal effects. Toxicity may affect survival, growth, reproduction (cocoon production and viability), behaviour (soil selection and perhaps level of activity), metabolism, pigmentation and composition of the earthworm communities. Chukwu & Odunze (2006) recorded that *E. fetida* had a 56 day LC50 of 100 mg/kg for exposure to arsenate. Dada *et al*, (2016) demonstrated an LC50 of 100 mg/kg arsenate for an 8-day exposure of *L. terrestris*, and 400 mg/kg for 2-day exposure. Ekperusi & Aigbodion (2015) found no difference in survivorship of *E. fetida* between contaminated (mean 58 mg As/kg) and control soils (<11 mg As/kg).

2.6.6 Risk assessment with microorganism

Because of their important roles in the ecosystem as decomposers, microorganisms have been extensively used in ecotoxicological studies. Bacteria is ubiquitous, and have been documented to be the main biological agents responsible for the removal both organic and inorganic compounds in the environment (Mohamed, *et al*., 2022; Langdon, *et al.*, 2003). They also aid in recycling of mineral nutrients and their activities are essential for purification of aquatic ecosystem. In other words, toxic effects of chemicals could influence these biological activities of the microorganisms and subsequently affect ecosystem health (Kohler, Hofmann, Volsgen, Thurow, & Koch, 2001).

Microorganisms have been reported to be an essential biomonitoring tool in ecotoxicological studies (Landis *et al.*, 2011). The test directly measures chemical toxicity (inhibitory effect) to the bacterium. Toxicity is quantified by measuring the reduction in growth in response to chemical stressors in liquid or solid samples. Originally developed for evaluating effluents and elutriate samples, the procedure has been modified for directly testing solid matrices such as sediments and soils. This test is easy to run, and results can be obtained in a matter of hours. The test also has widespread acceptance and well-established protocols (OECD, 2011).

2.7 Remediation techniques

Remediation of contaminated soils falls into four major types viz: chemical, physical, thermal or biological techniques (phytoremediation and bioremediation). Most of the remediation techniques focus on exploiting or altering soil chemistry to either remove contaminants from the soil or to reduce their solubility and bioavailability (Jidere & Akamigbo, 2009). Chemical remediation is based on chemical oxidation that elimantes harmful compounds from the contaminant. This technique is relative fast but may negatively impact the surrounding ecosystem. Physical method (which includes excavation and washing) involves transportation of contaminated soil from source to an area for disposal, while in the second part (washing), contaminated soil is washed with organic solvents which eventually removes the contaminants (Anukwa, Onuoha, Nkang, & Nkereuwem, 2021). Again, the high cost of chemicals and threat to flora and fauna makes this method less applicable. Thermal technique entails desorption of and incineration which is very expensive with its attendant environmental pollution (Singh & Jain, 2003).

In view of this, plant based remediation technique known as phytoremediation is by far considered the most optimal remediation technique. It is a simple, vital, cost-effective, low-labor-intensive, widely acceptable, eco-friendly, sustainable, reliable, and promising technology which is applicable in large areas, particularly when native, ecologically and socioeconomically valuable plants are used for the remediation (Alford, *et al*., 2010).

2.8 Phytoremediation

Phytoremediation, also known as green-remediation is defined as the cleanup of contaminated sites using unique diversity of plants (Igwe, Nzegbule, Azorji & Okafor, 2016). This is a remediation technique that uses plants to detoxify contaminated soils. It presents an efficient, "green clean," environmental, low cost, and eco-friendly technology that uses plants to reduce or remove inorganic and organic pollutants from environment (Pilon-Smits, 2005; Lee, Baek, Kim, Kim, Kim, Kwon, & Bae, 2007). The term phytoremediation consists of the Greek prefix "phyto" which means "plant" and the Latin suffix *"remedium*" which means "renew, able to cure or restore" (Wikipaedia, 2021).

2.8.1 Characteristics of plants used in phytoremediation process

Plants ideal for phytoremediation of polluted sites should fulfill four main requirements which include:

- fast grow rate and high biomass production,
- deep roots system,
- easily harvestable aboveground portion,

- ability to accumulate large amounts of metals in shoot.

2.8.2 Advantages and disadvantages of phytoremediation

Phytoremediation presents many advantages compared to other remediation techniques of these advantages include:

- it can be performed with minimal environmental disturbance;
- it is applicable to a broad range of contaminants, including many metals with limited alternative options and radionuclides;
- possibly less secondary air and water wastes are generated than with traditional methods;
- soil can be left at the site after contaminants are removed, rather than having to be disposed or isolated;
- does not need to use heavy vehicles and devices which damage soil.
- it is cost-effective for large volumes of water having low concentrations of contaminants;
- topsoil is left in usable condition and may be reclaimed for agricultural use

However, several drawbacks and limitations are also associated with phytoremediation of polluted soil such as:

- a long time is often required for remediation;
- the treatment is generally limited to soils at a meter from the surface and groundwater within a few meters of the surface;
- climatic or hydrologic conditions may restrict the rate of growth of plants that can be utilized;
- the ground surface at the site may have to be modified to prevent flooding or erosion; contaminants may still enter the food chain through animals/insects that eat plant
- material containing contaminants;

- soil amendments may be required

2.8.3 Mechanisms of phytoremediation

Plants remove contaminants from the soil through various techniques. These diverse ways in which plants interact with contaminants for eventual removal or degradation is referred to as phytoremediation techniques (Wikipaedia, 2021). Phytoremediation of contaminated soil can take place by phytoextraction, phytofilteration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation or phytodesalination (Hussain, Siddique, Arshad, & Saleem, 2009; Pilon-Smits 2005; Hellström 2004; Ali, Khan, & Sajad, 2013).

2.8.3.1 Phytoextraction is also known as phytoaccumulation, phytoabsorption and phytosequestration. It results in the uptake of contaminants from soil or water by plant roots, translocation to and accumulation in aboveground biomass.

Characteristics of plants able to perform phytoextraction include:

- Ability to accumulate and tolerate high concentrations of metals in harvestable tissue;
- Rapid growth rate;
- High biomass production (This results in more metal removed per planting).

2.8.3.2 Phytofilteration is the removal of contaminants from water or wastewater by plants. This may be rhizofilteration (using plant roots), blastofilteration (using plant seedlings) or caulofilteration (using excised plant shoots). This is the dominant mechanism of remediation in wetlands.

2.8.3.3 Phytostabilization (also known as phyto- immobilization), as the name suggests, is the use of plants to stabilize contaminants in soil, thus reducing mobility and bioavailability in the environment. This prevents migration of contaminants to groundwater and the food chain. Phytostabilization can either prevent erosion, leaching, and runoff or convert contaminants to less bioavailable forms (Pilon-Smits, 2005). It is more a containment technique than a decontamination technique.

2.8.3.4 Phytovolatilization is the uptake of pollutants from soil by plants, followed by their conversion to a volatile form and subsequent release into the atmosphere. The limitation faced by this technique is that the contaminants are transferred from one environmental medium (soil) to another (air) (Wikipaedia, 2021).

2.8.3.5 Phytodegradation is the breakdown of contaminants by plants with the help of plant enzymes (e.g dehalogenase and oxygenase) and other molecules in root exudates. This technique is limited to organic contaminants as inorganic contaminants are not readily biodegradable.

2.8.3.6 Rhizodegradation (also called phytostimulation) is the breakdown of organic pollutants in soil by microorganisms in the rhizosphere of plants. In the rhizosphere, soil microbial activity is stimulated to about 10-100 times by secretion of plant root exudates containing carbohydrates, amino acids, and flavonoids (Ali *et al.,* 2013).These exudates provide additional carbon and nitrogen sources for soil microorganism, thus facilitating microbial growth.

2.8.3.7 Phytodesalination refers to the use of halophytic plants for removal of salts from salt-affected soils to enable them to support normal plant growth (Wikipaedia, 2021).

2.8.4 Phytoremediation of spent engine oil contaminated soil

Some studies have been carried out to ascertain the phytoremediation potentials of *Chromolaena odorata, Aspillia africana and Axonopus compressus*. Jampsari & Saeng-Ngam, (2021) reported that *C. odorata* accumulated more Cd in their root parts with a translocation factor of about <1 reducing their effects on the ecosystem. Their findings suggested that *C. odorata* is a better phytoremediator than *I. patula* and *G. pseudochina* in cadmium contaminated soils. Literatures abound on the application of local hyperaccumulator species for the remediation of co-

contaminated soils such as spent engine oil polluted soils. In a study by Nwadinigwe & Obi-Amadi (2014) to determine phytoremediation potentials of spent engine oil polluted soils, they observed that *Pennisetum glaucum* significantly reduced the percentage of hydrocarbons in spent engine oil polluted soil.

Jambhulkar & Juwarkar , (2009), experimented remediation possibilities of As, Cd, Pb, and Zn on medium contaminated spent engine oil polluted soil on seven plant species with a different trace element accumulation capacity and remediation potential they found good accumulation capabilities and remediation effectiveness of *Salix dasyclados* similar to studied hyper accumulators. Oseni, Dada, & Adelusi, (2015), reported appreciable amount of lead uptake in *C. odorata* grown in spent engine oil polluted soils.

In a study to determine the phytoremediation potentials of *C. odorata*, and *S. acuta* in Lead polluted soil, Osadolo & Animetu, (2018) reported that the two plants accumulated appreciable amounts of heavy metals in the root, shoot and leaf. Their study further revealed that the two plants exhibited the characteristics of a phytostabiliser because their transfer factors were less than one. Hussain, *et al*, (2009) reported 99.96 and 99.76% reduction in Ni content of soil in a study to evaluate potentials of *B. deflexia* and *P. scrobiculatum* grass in spent engine oil polluted soil.

2.8.5 Selection of plants for phytoremediation

Of all the remediation techniques currently in use, phytoremediation has proven to be more economical and ecofriendly process (Azadeh, Ebrahim, & Masoud, 2007). The ability of plants to remediate polluted sites is often attributed to the microorganisms resident in the rhizopheere under the influence of the roots (Jones, Hodge, & Kuzyakov, 2004). Prior to any phytoremediation study, an exhaustive plant selection from the local population must be made to screen plants for potential application. However, research reports on plants that have phytoremediation potentials are scanty and fairly undocumented in tropical areas; particularly in Nigeria (Ngumete *et al*., 2018; Chukwu, Anoliefo, & Ikhajiagbe, 2017; Anoliefo, 2006). Therefore, screening plant species to determine those with the capacity to grow on hydrocarbon polluted soils remains an essential step in phytoremediation technology (Messou, Coulibaly, Doumbia, & Gourene, 2013).

Selection of plants from the populations of the metal-polluted sites for their recovery is now the method (Ariyakanon & Winaipanich, 2006), as they are adapted to soil and climatic conditions to the zone, which should make phytoremediation a much easier task (Anoliefo, Vwioko & Mpamah, 2003). One of the strategies that can be adopted when working in phytoremediation is the use of native hyperaccumulator plants of high biomass, mainly those adapted to the climatic and soil conditions of the polluted site.

Interest in phytoremediation has grown significantly following the identification of metal hyperaccumulator plant species. Hyperaccumulators are conventionally defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common non-accumulator plants. Thus, a hyperaccumulator will concentrate more than 10 ppm Hg; 100 ppm Cd; 1,000 ppm Co, Cr, Cu, and Pb; 10,000 ppm Ni and Zn (Anyansi & Atagan, 2016; Idris, Abdullah, Titah, Latif, Abasa, Husin, Hanima, Ayub & Ashraf 2016). To date, approximately 400 plant species from at least 45 plant families have been reported to hyperaccumulate metals. Most hyperaccumulators bioconcentrate Ni, about 30 absorb Co, Cu, and/or Zn, even 11 fewer species accumulate Mn and Cd, and there are no known natural Pb hyperaccumulators (Andrew, Thomas, Chloe, Smith, & Thompson, 2007).

Selection of plant species depends on several factors such as their ability to treat the concerned pollutants, to achieve their remedial properties and for their adaptability to other site-specific factors (Azorji *et al.*, 2021). The most preferred vegetation characteristics include adaptation to local climates, depth of the plant's root structure, ability of the species to flourish in the type of soil present, ability to extract or degrade the concerned contaminants to less toxic form, fast growth rate, ease of planting and maintenance and the uptake of large volumes of water by evapotranspiration (Idris *et al.*, 2016). It has been reported that care should be taken into consideration during the selection process to prevent the introduction of non-native species into the areas where those species are absent (Doty, 2008). Plant species that are benign under most circumstances may become a problem when introduced into a new area (Alvarez, Berla, Sheffield, Cahoon, & Jez, 2009).

In a preliminary survey carried out to identify spent engine oil tolerant plant species at selected auto mechanic workshops in Imo State Nigeria, Azorji *et al*., (2021) recorded a total of 137 plant species belonging to fifteen families. In their findings, the *Asteraceae* dominated with 22%, followed by *Poaceae* (17%), *Gramineae* (12%), *Leguminoseae* (9%), *Sterculiaceae* (8%), and *Araceae* (7%). The prevalence of *Asteraceae* and *Poaceae* plant species across the sampled areas suggested that they are spent engine oil tolerant and could be applied in phytoremediation technology.

Raymond & Harrison (2018) profiled plants species growing at petroleum contaminated site for possible inclusion for phytoremediation of polluted sites and recorded a total of 28 native plant species from different families growing in and around hydrocarbon-impacted soil in the vicinity of vandalized pipelines carrying petroleum products were collected and studied for their ability to grow in a hydrocarbon-impacted soil and remove the PHC from the impacted soil. According this study, some of the plants demonstrated the ability to grow in soil with high levels of the total petroleum hydrocarbons (TPH), which shows that they may be tolerant to hydrocarbons in soil and could potentially phytoremediate a hydrocarbon-contaminated soil. *Chromolaena odorata, Aspilia africana, Chloris barbata, Pasparlum vaginatum, Bryophyllum pinnatum, Paspalum scrobiculatum, Cosmos bipinnatus, Eragrostis atrovirens, Cyperus rotundus*, and *Uvaria chamae* showed tendencies to phytoremediate contaminated soil.

Nguemte, *et al*, (2018) in their study on Floristic surveys of hydrocarbon-polluted sites in some Cameroonian cities (Central Africa) documented a total of 106 species belonging to 76 genera and 30 families were identified on hydrocarbon polluted sites. Their study a plant species inventoried at the site would be a guide to develop inexpensive, green and sustainable technologies for cleanup.

Idris *et al*, (2016), screened and identified Plants growing at a Petroleum Contaminated Site in Malaysia for Phytoremediation. Their study was based on bioaccumulation coefficient (BAC) values for arsenic (As) and leads (Pb). The selected plants, *Melochia corchorifolia L., Ludwigia octovalvis (Jacq.) P. H. Raven, P. vaginatum, Cyperus sphacelatus Rottb*., are potential as phytoremediators while *L. octovalvis* and *Melastoma malabathricum* L. are potential Pb phytoremediators.

A study carried out by Chukwu *et al*, (2017) to select metal tolerant species and distribution in and around metal based industries in Benin City showed that *Eragrostis tenella* occurred most frequently in all the sites studied, followed by *Amaranthus spinosus, Eleusine indica*, while *Cucurbita pepo* occurred least. Their study further revealed that family *Poaceae*, was identified in all the sites visited. The study documented a total of 720 metal tolerant plant species. Azorji, *et al*, (2021), conducted a similar study in selected auto mechanic villages in Imo State and reported about 128 plant species in the study sites. Their study showed that the *Asteraceae* and *Poaceae* were the dominant plant families.

Messou e*t al*, (2013) conducted a study on Plants diversity and phytoaccumulators identification on the Akouedo landfill in Abidjan, Côte d'Ivoire. They inventoried a total of 130 taxa belonging to 39 families. The most frequent families (36.9% of the total taxa) were Poaceae, Euphorbiaceae and *Cyperaceae*. The dominant taxa on the old waste dumpsite having an average density superior to 5 plants/m^2 and occurring less frequently on the control site were *Alternanthera sessilis, Amaranthus spinosus, Cyperus rotundus, Cyperus iria, Eleusine indica, Euphorbia glomerifera, Ipomoea triloba, Portulaca oleracea* and *Trianthema portulacastrum*. These plant species may be indigenous phytoaccumulators on the Akouedo landfill.

In a similar study, Ashutosh & Misra (2012) assessed the floral distributions at municipal waste dumpsites in relation to their soil properties. Among 34 plant species investigated on these sites, 13 species were identified as highly adaptive to these waste dumpsites. *Nepeta hindostana* (IVI-112.57); *Croton bonplandianum* (IVI-76.4); *Cassia tora* (IVI-51.99); *Ricinus communis* (IVI-34.37); *Achyranthes aspera* (IVI-34.32); *Calotropis procera* (IVI-39.08) and *Amaranthus spinosus* (IVI-23.19) are some of the pioneer adaptive plants of these dumpsites. The study may prove significant in selecting appropriate plant species for further phytoremediation studies under control conditions and will be helpful in understanding the role of soil properties in vegetation establishment and development on such sites.

Anoliefo, Ikhajiagbe, Okonofhua, & Diafe (2006) surveyed plant species and their families in auto mechanic workshop in Asaba and Benin City. Their study showed that *Peperomia pellucida* occurred most in all the sites visited with 55% frequency. They opined that the high rate of occurrence of a particular plant species in the frequency table suggests that such plants are tolerant and may be introduced as a possible phytoremediation agent.

Eshalomi-Mario & Tanee (2015) carried out a phytodiversity assessment in abandoned solid Waste Dumpsites in Port Harcourt, Solid waste and discovered that dumpsites altered and favored the growth of diverse species. This study revealed the ability of some plants species to thrive in any given situation. According to their findings, uncontrolled deposition of solid waste in the environment is posing serious threat to species diversity as it gives rise to the growth of alien species that can eliminate the native species of the area. The dumpsite had more of herbaceous species and higher species diversity than the control site which indicates that the solid waste alters the requirements in the soil for plants growth and development, hence affecting phytodiversity.

Consequently, the future prospects in phytomanagement of spent engine oil polluted soils require a better knowledge of ecological risks organisms are exposed to as a result of indiscriminate disposal of spent engine oil; and application of native species that are the best adapted to local region which is important for maintaining ecosystem services and quality of human life of the people (Bitala, 2008; Ariyakanon, & Winaipanich, 2006). Taken together, knowledge on the potential ecological risks of chemical compounds in the environment can offer great prospects for improving ecosystem health, phytoremediation technologies and cleanup of polluted environments (Asante, Agusa, Subramanian, Ansa-Asare, Biney & Tanabe, 2007). All these observations can be useful for the selection of suitable plant species capable to endure the environmental stress associated with spent engine oil contaminated areas and for the development of successful phytomanagement of degraded sites on a global scale.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Imo State southeast Nigeria. Imo State has a total of twenty seven (27) Local Government Areas (L.G.A). The people of Imo State are basically of Igbo extraction having similar cultural activities like other Igbo communication and they speak Ibo language. The state is influenced by urban sprawl whereby smaller communities merge together. There are several activities that go on in the state, notable among them is automobile workshops scattered all over the nooks and crannies of the state from which spent engine oil and other effluents containing pollutants are deliberately or accidentally spilled on any available space by the mechanics in the business of auto repairs.

Till date, there is no regulation on sitting of the auto workshops; as a result, they are located at random or directly in front of residential or commercial areas. This exposes the soil ecosystem and residents to various kinds of pollutants.

Imo State is divided into three political zones; the three zones are: Owerri zone, Okigwe zone and Orlu zone. Based on the foregoing, study sampling was done to reflect the zones.

3.1.1 Geography

Geographically, Imo State is bordered by Abia State on the East, River Niger and Delta State to the West, Anambra State on the North and Rivers State to the South. The State lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E with an area of about 5,100 sq km^3. However, its population is about 4,927, 5634 (NPC, 2006), which has increased geometrically as at the time of this study. The topography is mainly plain to slightly gentle rolling lands (Onweremadu, 2007a; Onweremadu, Akamigbo, & Igwe, 2007b).

3.1.2 Climate

The Climate is humid tropical with a minimum and maximum temperature of 20 ^{0}C and 32 ^{0}C (Onweremadu, *et al*, 2007). The mean annual temperature ranges from 27-28 ^{0}C with a relative humidity ranging from 70-80 %. The State is characterized by two major climatic seasons, rainy and dry seasons. The rainy seasons that prevails from April with its peak in July and September and dry season that lasts from November to March. Mean annual rainfall of about 2500mm (Onweremadu *et al*, 2007).

3.1.3 Vegetation

The general vegetation of the state is rainforest. The climate in the state favors the cultivation of crops like oil palm (*Elaeis guineensis*), Cassava (*Manihot esculentus*), Plantain (*Musa paradisiaca*), Mango (*Mangifera indica*), Coconut (*Cococ nucifera*),Yam (*Dioscorea Species*), Maize (Zea mays). Other plants found in the area include grasses such guinea grass (*Panicum maximum*), elephant grass (*Pennisetum purpureum*). The original vegetation has been extensively modified in many places by industrialization and urbanization.

3.1.4 Activities at the study sites

At the various auto mobile workshops, activities carried out by the artisans include but not limited to disassembly and assembly of machine spare parts, changing of oil in vehicles, car spraying and general maintainance/repair of vehicle parts. Through these activities, spent engine oils and other solvents containing petroleum hydrocarbons are indiscriminately dumped or spilled on every available space (Udebuani *et al.*, 2011). Materials that are considered to be waste are either sold as scraps or burnt which exacerbates the emission of heavy metals, TPH, PAHs and other pollutants.

3.2 Selection of sampling site

The study was carried out to cover the three zones of Imo State. Each zone was further subdivided into three sub-zones making a total of nine (9) sub-zones for sample collection. Prior to the commencement of the study, a reconnaissance visit was made within the three zones (Owerri, Orlu, and Okigwe) of the state in other to select suitable sites for the study. From each sub-zones, three (3) sampling sites were randomly selected making a total of twenty seven (27) sampling sites (9 samples per zone). The study spanned from 2019 to 2021.

3.3 Estimation of disposal method, benefits and perception of environmental risks of used engine oil generated in the study areas

For data collection, a multistage approach was employed that involved personal observation, face to face interaction. A modified version of Omorowa & Agu, (2017); Akpkpavi, (2015) questionnaire was used to elicit information from the respondents based on willingness to participate in the study. The questionnaire survey elicited information on the age distribution, disposal method, benefits, volume of oil generated and perception of environmental risks by the mechanics. A total of 350 mechanics made up the sample size. A copy of the questionnaire can be found in Appendix 4.

3.4 Collection and characterization of plants species at the auto mechanics

Identification and documentation of plant species growing on mechanic workshops was done with slight modifications from earlier reported procedures (Anoliefo *et al.*, 2006; Ashutosh, Tripathi & Misra, 2012). At each mechanic site, plant species was identified around 5 metre radius and subsequently segregated based on family and species. The specimens which could not be identified *in situ* were taken to the taxonomy unit of the Department of Biology, Federal University of Technology, Owerri for characterization and proper identification by a renowed

Plant Taxonomist, Dr. C.M. Duru. Frequency of occurrence was estimated as the rate (%) at which a plant species appeared in the subset of the sampling sites (Anoliefo *et al*., 2006). Relative frequency (RF) gives information about the rate of occurrence of species in a community (Gillet, 2000). In this study, relative frequency of the plant species was estimated using the formular:

$$\mathrm{RF} = \frac{\mathrm{Fa}}{\mathrm{nr}}\ X\ 100$$

Where RF = relative frequency of the taxon; Fa = absolute frequency of the taxon; nr = total number of quadrats.

3.5 Collection of soil samples

Each auto mechanic workshop was subdivided into three quadrants (5m space between sampling points) from where soil samples were collected and subsequently bulked/homogenized to form composite. Following the European Union soil studies, 500g from composite is recommended for laboratory analysis (Tóth & Montanarela, 2013). The samples were collected with an improvised stainless steel soil augur of approximately 7.5cm at a depth of 0 – 20 cm per site after the removal of the plant debris and coded accordingly; Owerri zone (Owerri soil sample 1 – 9 coded OWSS1 – OWSS9), Orlu zone (Orlu soil samples 1 - 9 coded ORSS1 – ORSS9) and Okigwe zone (Okigwe soil sample coded OKSS1 – OKSS9 respectively) under stringent controls from about 5km away from the study sites and coded as Owerri, Orlu and Okigwe soil sample control respectively (OWSSC, ORSSC and OKSSC). This depth was chosen because the surface soil appears to be the first locus of input of soil pollutants where they tend to accumulate on a relatively long term basis (Abenchi *et al*., 2010). This implies that measurable concentration of pollutants could be present at this depth if assessed (Aloysius *et al*., 2013). Soil samples for PAH and TPH were stored in a dry glass bottle free of grease (to avoid cross contamination) while samples for heavy analysis were stored in polythene bags (Muze *et al*., 2020). Sampling design for this study was predicated on two premises: first, the need to spread sampling sites objectively across the auto mechanic workshops in Imo State and to ensure that the sites' pollutant level is adequately depicted. In all, a total of 27 soil samples (9 from each zone) were collected from the entire study areas (Plate 1). The sites were named using a landmark around the sampling site and the locations were geo-referenced using POP 4 Android Phone Installed Global Positioning System (GPS) and subsequently presented on a map (Figure 3.1). Table 3.1 depicts the location code, name and geographic coordinates of sampling points respectively.

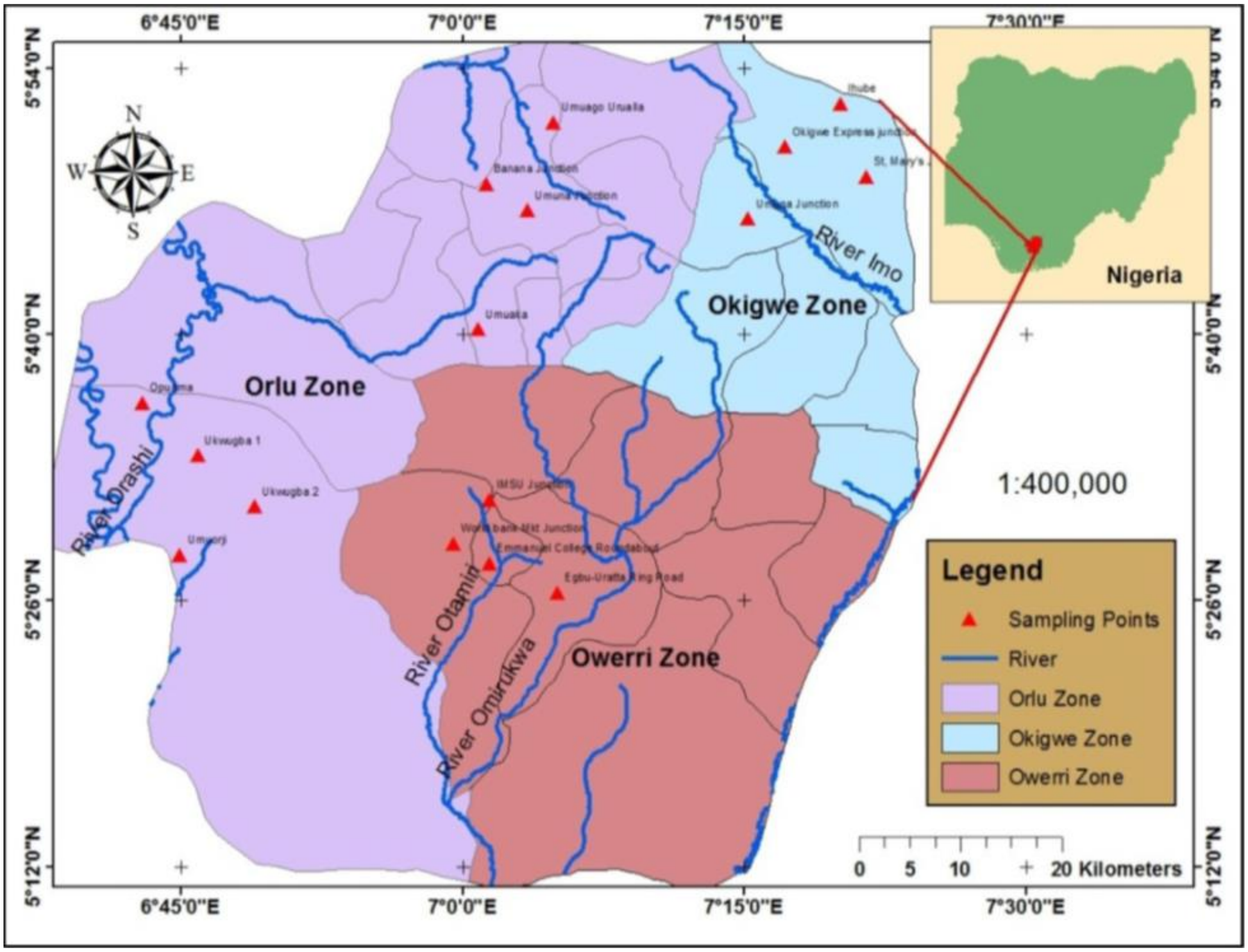

Fig 3.1: Locations of sampling areas/points

Plate 3.1: Soil Samples Collected from the Study Areas

Table 3.1: Location Code, Name and Geographic Coordinates of Sampling Points

S/N	LOCATION CODE	LOCATION NAME	LATITUDE	LONGITUDE
1	OWSS1	Umuekwune	05^{0}20’11.6’’N	007^{0}04’58.8’’E
2	OWSS2	Imerienwe	05º21’01.4”N	007º04’60.8’’E
3	OWSS3	Obiangwu	05º17’39.3”N	007º05’03.0’’E
4	OWSS4	Uvuru, Mbaise	05º21’01.4”N	007º04’60.8’’E
5	OWSS5	Oboama Ezinihitte	05º17’39.3”N	007º05’03.0’’E
6	OWSS6	Egbu road	005°28I19’’N,	007°03I14’’ E
7	OWSS7	Along police College Nekede	005°24I56’’ N,	007°00I42’’ E
8	OWSS8	Along Owerri timber market	05°28I 16’’ N,	007°02I21’’ E
9	OWSS9	Orji mechanic village	005° 31′ 39"N	007° 3′ 50" E
10	OKSS1	Umuka	05̊ 48′ 21.23″N	007̊ 57′ 50.85″E
11	OKSS2	Aro-Ubaha	005̊50′10.18″N	007̊ 59′ 14.76″E
12	OKSS3	Agbuala	005̊ 47′ 58.45″N	007̊ 58′ 53.02″E
13	OKSS4	Ihube	005^o 52’ 0 N	007^o 22’ 0 E
14	OKSS5	Ehime Mbano	5°43'310"N	7°14'800"E
15	OKSS6	Umuokpara	5˚40'1198’’N	6˚50'
16	OKSS7	Alaike-Ogbaku	005^o 52’ 0 N	007^o 22’ 0 E
17	OKSS8	Ama-eze ogii	5°43'310"N	7°14'800"E
18	OKSS9	Aro-okigwe	5˚40'109’’N	6˚50'1132’’E
19	ORSS1	Dikenafai	005·75784’1’’N	007.7111’33’’ E
20	ORSS2	Amaraku	005.6531’33’N	007.13116’99’’E
21	ORSS3	Attah	005.6156’33’’N	007.131178’99’’E
22	ORSS4	Njaba	005.75784’1’’N	007.7111’33’’ E
23	ORSS5	Umuna	005.6531’33’N	007.13116’99’’E
24	ORSS6	Ama-ifeke	005.6156’33’’N	007.131178’99’’E
25	ORSS7	Okporo	005.75784’1’’N	007.7111’33’’ E
26	ORSS8	Eziachi	005.6531’33’N	007.13116’99’’E
27	ORSS9	Owerre-nkwoji	005.6156’33’’N	007.131178’99’’E
28	OWSSC	Umudioka	005.75784’1’’N	007.7111’33’’ E
29	OKSSC	Amike	005.6531’33’N	007.13116’99’’E
30	ORSSC	Umutanze	005.6156’33’’N	007.131178’99’’E

Legend: OWSS=Owerri soil sample, OKSS= Okigwe soil sample, ORSS= Orlu soil sample, OWSSC= Owerri soil sample control, OKSSC= Okigwe soil sample control, ORSSC= Orlu soil sample control.

3.5.1 Sample pretreatment

Composite soil samples were air-dried in electric oven at a temperature of 40°C approximately for 30 minutes and subsequently disaggregated using a wood pestle, sieved with 2 mm mesh to give fine earth fraction following the method of de Zorzi, *et al,* (2005). The resulting fine earth fraction (< 2 mm) was subjected to the various physical and chemical determinations in the laboratory.

3.5.2 Physical and chemical determination of soil samples

Soil sample analysis was carried out at the soil laboratory unit of the International Institute of Tropical Agriculture (IITA), Ibadan. The following Physical and chemical properties of the soil samples were determined:

3.5.2.1 The particle size determination

Particle size distribution for texture determination was carried out using the Bouyoucous hydrometer method. 40g of 2mm sieved soil was weighed into an extraction bottle before 100ml of 5% calgon (sodium hexametaphosphate) solution was added to the bottle. The suspension was mechanically shaken for 30 minutes and thereafter transferred into 1L graduated sedimentation cylinder. Distilled water was added to make up the 1 liter mark after which a plunger was inserted into the cylinder to stir the suspension vigorously bymoving the plunger up and down several times. Finally, hydrometer readings were taking at 45minuites in the first instance, then after 5 minutes (for silt) and later at 5hours (for clay). The sand component was obtained by decanting the suspension from the sedimentation cylinder through 0.2mm sieve, washed with pipe-borne water and the left over fraction was oven dried at 1050C over a 24 hour period. The oven dried samples were retrieved and kept in desiccators for cooling and weight measurement subsequently. The sand, silt and clay proportions of the soils were then calculated for as follows:

$$Silt\ (\%) + Clay\ (\%) = \frac{corrected\ hydrometer\ reading\ at\ 5\ mins}{\text{Sample weight (g)}}\ x\ 100 \qquad (1)$$

$$\% \ clay = \frac{collected\ hydrometer\ reading\ at\ 5hrs}{\text{sample weight (g)}} \quad x\ 100 \qquad (2)$$

$$\% \ silt = equation\ 1 - equation\ 2 \qquad (3)$$

$$\% \ sand = 100\text{-}equation\ 1. \qquad (4)$$

The textural classes were read off on the textural triangle based on USDA textural classification.

3.5.2.2 Bulk density

The bulk density was determined on clod soil samples taken by driving a metal corer into the soil horizon of investigation. The sand samples were then taken to the laboratory where they were oven dried and weighed. Data on bulk density was obtained by derivation using the formula:

$BD = (Ms/Vt)\ g/cm^3$

Where BD = Bulk density

Ms = Mass of oven dried soil and

Vt = total volume of core sampler

3.5.2.3 Soil Moisture Content

Soil moisture content was determined by the hanging water column technique as described by Obi (2000). Hanging water column procedure involved collection of undisturbed core soil samples. The metal cores used had dimensions of 4.8 cm (internal diameter, ID) and 5.6 cm height. After saturation for 24 hours, the cores were used to measure soil water retention at matric potential of -6 kPa and then oven-dried at 105^{o}C for 24 hours. Soil moisture content was subsequently measured gravimetrically and recorded.

3.5.2.4 pH

The soil pH was determined by preparing a 1:2 soil/water suspension. 10g of sieved air dried soil was weighted into a beaker and added 20ml of distilled water. The mixture was mechanically stirred (end-over-end) for about 20-30 minutes at laboratory room temperature and allowed to stand for another ten (10) minutes to enable solution to settle. The pH meter was standardized using a buffer solution at pH 4.0 and 7.0 before the pH electrode was inserted into the suspension and readings recorded as pH for water. The same procedure was carried out on 0.01 M $CaCl_2$ at a 1:1 ratio of soil solution. Thorough washing of the electrodes was done between measurements of buffer solutions and between buffer solutions and soil solutions (Rayment & Higginson, 2011). The pH reading was obtained using the Metrohn (Bie & Beinstsen) 691 meter at 25.8^0C.

3.5.2.6 Electrical conductivity

Electric conductivity was determined by preparing a 1:2 soil/water suspension. 10g of sieved air dried soil was weighted into a beaker and added 20ml of distilled water. The mixture was mechanically stirred (end-over-end) for about 20-30 minutes at laboratory room temperature and allowed to stand for another ten (10) minutes to enable solution to settle. The Bie & Berntsen A-S E587 Conduct meter was calibrated to 1000 μscm-1at 25.8^0C. The conductivity cell was dipped into the supernatant, moving it up and down slightly without disturbing the slurry and corresponding readings taken when the system had stabilized (Rayment & Higginson, 2011).

3.5.2.6 Total Nitrogen content

Total Nitrogen was determined by weighing 1g of sieved air dried soil sample into 250ml digestion tube. 2.0ml of deionized water was added and swirled for a few minutes, allowed to stand for 30minutes. 2.2g of catalyst mixture (Selenium) and 8ml of conc. H_2SO_4 were added and heated in a fume digestion chamber. Inside the digestion chamber, samples were heated until they became bleached. They were removed and allowed to cool under laboratory temperature. The Markham distillation apparatus was prepared by adding 5mlof 40% of H_3BO_3 to each 100ml of borosilicate beaker/flask. Approximately 25ml 60% solution of (400 g) of NaOH was carefully add in each flask and attached to the distillation unit; The resultant mixture was distilled for about 5 minutes after which 2% Boric acid indicator was added to 5ml of collected distillate (ie.2g of Boric acid in 100ml. of distilled water). The distillate was titrated with standardized 0.01m Hydrochloric acid (HCl). The end point was indicated by a colour change from pale green to faint pink. These were in turn used to evaluate for percentage soil nitrogen (% N) as follows:

% N = (a – b) x 0.01 x 14 x v x 100 / 1000 x w x al

Where,

v = final vol. of digestion

w = weight of the sample taken in grams.

al = aliquot of the solution taken for analysis- 5ml.

0.01 = Molarity of acid (0.014)

14 = molar weight of nitrogen

3.5.2.7 Total organic carbon and organic matter

Total organic carbon was determined by weighing 0.5g of air-dried sieved soil into a 250ml flask wetted with 10ml of dichromate solution. 20ml of concentrated H_2SO_4 was added to the flask and swirled to achieve a homogenous mixture before allowing stand for about 30minutes. Subsequently, 200ml of distilled water, 10ml of orthophosphoric acid and 2ml of barium diphenylamine sulphate indicator were added in that sequence and titrated with the ferrous ammonium sulphate solution from its blue colour to a green end point. Titer values were subsequently used to calculate for percentage carbon as follows:

% Carbon = [10 – (X N) x 0.3] / W

Where 10 = dichromate solution (ml)

X = ml. of ferrous ammonium sulphate solution required for the titration

N = normality of ferrous ammonium sulphate solution

W = weight of soil sample in gram.

3.5.2.8 Organic Matter content (%) of soil was calculated for, by multiplying % carbon with 1.724. where 1.724 is the conversion factor (Blacky *et al.*, as cited in Spectrum Analytic, 2005).

3.5.2.9 Determination Exchangeable Potassium, Calcium, Magnesium and Sodium

These parameters were determined by extracting 10g of sieved air-dried soil sample with 20ml of each cation and sieved through Whatman filter paper. The extract was mixed with 77.08g of Ammonium acetate dissolved in a 100ml beaker and stirred mechanically for about an hour. Thereafter, samples were taken for flame photometer reading (meq/100g). The resultant reading for individual parameter was further subjected to calculation to ascertain actual values for nutrients, as follows: % = (AAS reading/1000) x (vol. of extract/1000) x (100/wt of soil).

3.5.2.10 Effective Cation Exchange Capacity

The Effective Cation Exchange Capacity was determined by calculation using the values obtained (in meq/100g) in an equation formulated by "Spectrum Analytic Incorporation" as follows: **(**AAS = Atomic absorption spectrophotometer and Meq= mil-equivalent).

METHOD 1*: Use if a buffer pH (BpH) is available. C.E.C. = (lb K ÷ 780) + (lb Mg ÷ 240) + (lb Ca ÷ 400) + [12 × (7- BpH)],*

If buffer pH is 7.0 or greater, use a 0 value as the remainder...Example: (7.0 - 7.1) = 0 ***METHOD 2:*** *Use if Buffer pH is not available. C.E.C. = [(lb K ÷ 780) + (lb Mg ÷ 240) + (lb Ca ÷ 400)] × Factor.*

3.5.2.12 Available phosphorus

Available phosphorus was by the Bray-2 method. This method involved weighing 2 g of soil sample into a test tube. Then, 20 ml of 0.03 NH_4F in 0.1NHCl was added to the sample of soil in the test tube. The test tube was closed and shaken for a minute. It was allowed to settle and filtered with 608 filter paper. 1ml of the filterate was pipetted into a 50 ml of volumetric flask. Then, 7ml of distilled water, 1ml of NH_4 molybdate and 1ml of ascorbic acid were added to the sample. The flask was made up to the mark with distilled water and allowed to stand for 15 minutes before taking the reading. The available phosphorus was read off from the standard curve obtained from optical density using a colorimeter.

3.5.2.12 Total Exchangeable Acidity (EA)

The titrimetric method using 1 NKCl extract of McLean (1982) was used in the determination of total exchangeable acidity (Al^{3+} and H^+).

ECEC = TEB + TEA

where;

ECEC = Effective cation exchange capacity (cmolkg^{-1} soil)

TEB = Total exchangeable bases (cmolkg^{-1} soil)

TEA = Total exchangeable acidity (cmolkg^{-1} soil).

3.5.3 Heavy Metal Determination

In the laboratory, soil samples were stored in cardboard boxes and then dried with a fan at 36–40°C until constant weight was reached (Tóth, & Montanarela, 2016). Then they were disaggregated using a wood pestle, sieved at 2 mm, the volume was reduced by quartering and rifling to obtain the test sample from the laboratory samples (de Zorzi, *et al*., 2005). About 1.0g weight of the air dried soil sample was weighed and digested using Aqua Regia. This method which is consistent with USEPA 3050 and ISO standard 11466 as reported by Guadino *et al.* (2007), involves treating a soil sample with a 3:1 mixture ratio of hydrochloric (HCl) and nitric (HNO_3) acids (Gaudino, Galas, Belli, Barbizzi, de Zorzi, Jaćimović, & Jeran, 2007). The nitric acid destroys organic matters and oxidizes sulphide material. It reacts with concentrated hydrochloric acid to generate aqua regia: $3HCl+HNO_3 \rightarrow 2H_2O+NOCl+Cl_2$. Quantitatively, the digested samples were transferred into a 125ml plastic container, filtrated with 50ml de-ionized water and metal analysis performed with Buck scientific Atomic Absorption Spectrophotometer (Model GFA-EX7i, Shimadzu Corporation, Japan) using air acetylene flame with a digitalized read out system. This involves direct light source emitting a narrow spectral line of characteristic energy. A hallow cathode lamp is used to excite the free atoms of trace metal of concern in the flame. The detection limits of Cd, Pb, Cu, Cr, Co, Mn, Fe, As, Ni and Zn were set at 0.001. Concentration of representative metal standard solution was run along the wavelength and its absorbance reading recorded. All results are expressed in mg/kg.

3.5.4 Determination of PAHs in soil samples

With slight modifications from previous reports (Emoyan *et al*, 2020; Obinnia *et al*, 2013), about 500 mg of the soil samples were dissolved using a mixture of 25 mL n-hexane and acetone (7:3 v/v). A microwave extraction arrangement was used with its pressure carefully controlled for 45 minutes. After cooling, the extract was filtered with a Whatman glass fiber filter in a glass bottle, followed by a concentration of the extract to 1.5 mL using a rotatory evaporator. Concentration of the PAHs was determined by the GC Guadrupole Mass Spectrometer (GC-MSD) (Agilent 5975 MSD). The sample was separated into its components by chromatographic separation using a capillary column of an internal diameter of 30 m×0.25 mm and film thickness of 0.25 μm, HP- 5MS and a helium carrier gas of high purity (99.5%), having a flow rate of 1 mL/min from a steel cylindrical pipe. The chromatographic separation conditions include an injector temperature of 250˚C and an initial 70˚C temperature of the chromatographic column which was held for 1 minute. The temperature increase by 30˚C/min to 200˚C, by 35˚C/min to

250˚C, and by 10˚C/min to 300˚C and this was maintained for 25 minutes. The PAH content of the soil samples was subsequently quantified from the resulting chromatogram and expressed in mg/kg

3.5.5 Determination of TPH concentration of soil samples

The TPH content of the soil samples was extracted according to the methods described by previous rsearchers (Ibe *et al.,* 2021; Yahaya *et al*., 2020; Ogoko, 2014). About 10 g of the soil sample was carefully mixed with 150 mL dichloromethane which was used as the extraction solvent and extracted for 4 hours 30 minutes. This was done in the presence of 2.5 g of dried sodium sulfate and 300 µg/mL of 1-chloro-octadecane as a surrogate standard. 0.3 g of silica was introduced into the extraction mixture after the extraction to facilitate the adsorption of polar materials like animal fats and oil from vegetable materials. The extracts were later passed through a Whatman glass fiber filter for filtration. The separation and determination of TPH contained in the soil samples were carried out with Gas Chromatography equipped with Flame Ionization Detector (GC-FID) (Agilent 6890N). A concentrated 3 µL of the sample was introduced into the GC column with a micro-syringe previously rinsed with dichloromethane (blank) and the sample. The TPH was determined at a specific chromatogram in mg/kg. Full sampling operations from identification of sampling points to producing sample portion for chemical evaluation is illustrated in Figure 3.2.

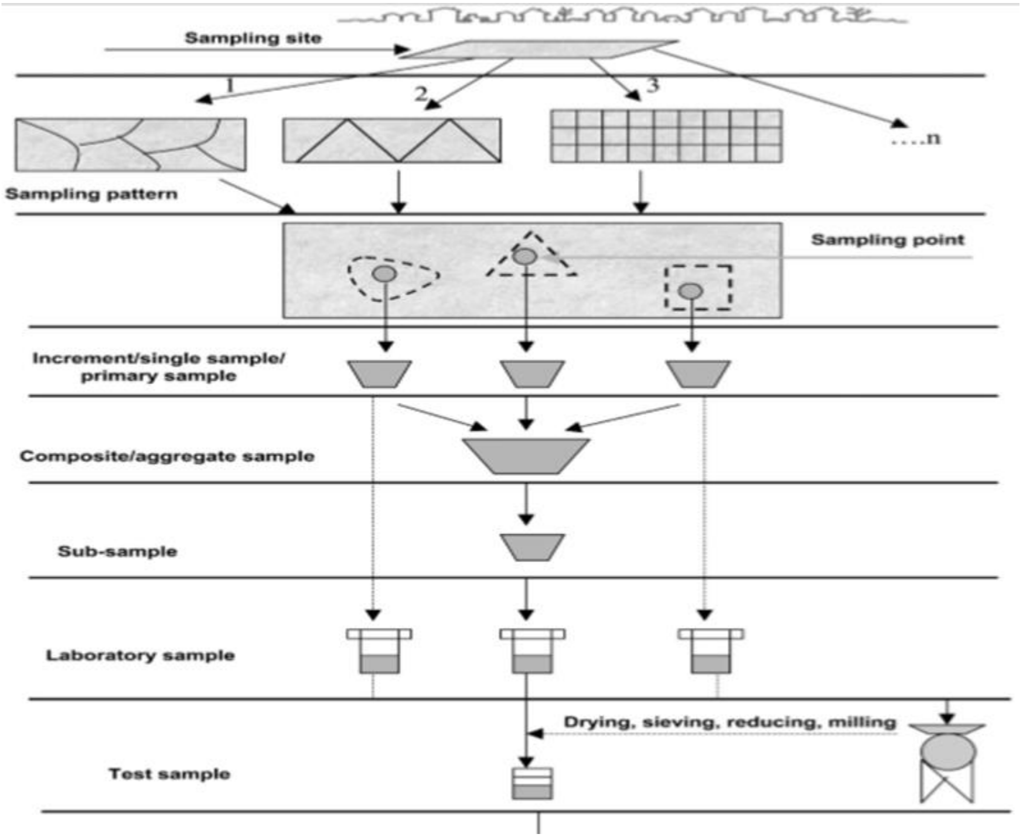

Figure 3.2: Pictorial representation of soil Analytical operations carried out in this study.

Source: de Zorzi, *et al*, (2005).

3.6 Terrestrial Acute Tests on test organisms (Plants, earthworm and Microorganisms)

OECD (2006) suggests a set of ecotoxicological bioassays in other to characterize a soil matrix. In this study, the choice about which ecotoxicological bioassay to perform were benchmarked on different factors such as sensitive and response time, cost-effectiveness, reproducibility, ease of measurement, and diagnostic ability. Based on the above considerations, three different species representing each trophic level of the ecosystem were chosen for the study (OECD, 2006). The selected test organisms are soil microorganism, earthworm and plants. All acute tests on test species (Microorganisms, earthworm and plants) followed International Standard Guidelines (OECD, 2006). Terrestrial ecotoxicity tests were carried out using soil polluted with various concentrations of spent engine oil as recommended by the ISO and OECD guidelines (ISO, 2008; OECD, 2004). To evaluate the impact on the critical events that occurs in the life of organisms; germination tests (inculcating root and shoot inhibition) was performed to assess phytotoxicity; survival and growth inhibition tests were performed for soil invertebrates (Microorganisms and earthworms). The effective/lethal concentration (EC_{50} or LC_{50}) of the contaminant that inhibited 50% growth in each of the test organisms were estimated and used for risk characterization in each scenario.

3.6.1 Collection of spent engine oil

Spent engine oil used for the acute toxicity study was sourced in 5L container from various auto mechanic workshops in Imo State, bulked and used for the study. Information on the location, car type, and age type of engine and duration of oil used are depicted in Table 3.2 below:

Table 3.2 Details of spent engine oil used for the study

Details	Owerri zone	Okigwe zone	Orlu zone
Car type	Lexus 350 XL	Toyota	Sienna
Age	5	7	3
Brand of oil	AZ	AZ	AZ
Engine type	Petrol	Petrol	Petrol
Duration of usage	4	6	3

3.6.2 Plant seedling emergence and growth inhibition assay

The essence of this test is to assess the effect on seedling emergence and early growth of terrestrial plants on exposure to polluted soil in the ecosystem. In accordance with OECD 208 guideline, the test gives an idea of the sensitivity of plants during the early stage of development/establishment and provides data as to whether the pollutants inhibits or enhances the growth of the terrestrial plants under study. In line with the aim of this study, the test was performed using three test plants (OEDC, 2006).

3.6.3 Collection and preparation of seeds

Seeds used in this study were collected from the gene bank of International Institute of Tropical Agriculture, Ibadan. The accessions are: TVu-13606 (*Vigna unguiculata* TGm-504 (*Glycin max*) and TZm-30181 (*Zea mays*). This species were chosen due to their unique properties such as uniformity, (as they are readily available from the sources which produce them consistently), uniformity in the seedling growth and amenability to testing in the laboratory, giving reliable reproducible results. For these reasons, the plant species are included in the list of OECD guideline for the testing of chemicals in terrestrial plants (OECD, 2006). Seed viability test was carried by water floatation method following the method of Njoku *et al*, (2011). The concentration was prepared by mixing appropriate volumes of distilled water to spent engine oil to obtain 0, 4%, 8%, 12% and 16% (v/v). Each concentration served as a treatment. Ten viable seeds of each accession were placed in 26mm Petri dishes lined with cotton wool and moistened with the measured concentration of spent engine oil. The growth chamber was set at 22^0C with relative humidity of approximately 58.9%. The seeds in each Petri dish were moistened with each level of contaminant and monitored for germination seedling growth for 14 days (OEDC, 2006). The number of germinated were recorded every morning at 9am. The experiment was laid out in Completely Randomized Design with three replicates each. Seeds were considered as germinated when the root reached 2 mm long. Seeds that germinated from treatment were added cumulatively to obtain percentage germination. The reaction of the test plants to exposed contaminant at various concentrations was assessed in reference to control using phytotoxicity test parameters: seed germination percentage and rate of germination, seedling root and shoot lengths after germination and recorded as follows:

Percentage germination = $\frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown}$ X 100 (Agbogidi, 2010)

Rate of germination: The germination rate was determined as the coefficient of velocity of germination, as:

CVG = $\frac{CV}{100}$ = $\frac{1}{t}$

Where t = mean germination time, and CV coefficient of velocity.

In time, since the mean rate increases and decreases with 1/t not with t (Onwusiri, Aguoru, & Akomolafe, 2017).

Seedling root and shoot elongation was measured with the aid a graduated meter rule (cm).

Seedling dry and fresh weights were determined using the method of Onwusiri *et al*, (2017).

Plate 3.2: Phytotoxicity bioassay set-up

3.6.4 Microbial growth inhibition bioassay

Due to its short duration, reproducibility and simplicity, microbial growth inhibition assay has been widely incorporated in routine ecotoxicological assessment of pollutants in water, sediment and soil (USEPA, 2021). This test was performed in accordance with OECD (2006) protocols.

3.6.4.1 Collection and Processing of soil samples

Soil samples were collected with sterile containers from polluted and unpolluted sites based on the technique adopted by Phulpoto, Qazi, Mangi, Ahmed, & Kanhar, (2015) with slight modifications. Precisely 5g of each sample collected from each site was dissolved in 50 mL of sterile distilled water and placed in flasks (250 mL capacity). The sample containing flasks was incubated at 37 ^{o}C for 2 hours. After incubation, approximately 5 mL of each sample was used as an inoculum for enrichment procedure. Microbial load (THB) were carried out using a surface spreading technique after which serial dilutions ranging from 10^{-1} to 10^{-10} of both soil samples were carried out on general purpose medium (Nutrient Agar). Triplicate of each dilution were prepared and incubated at 28 ^{0}C for 36 hrs before enumeration. Results were presented in colony forming units (CFU/g) calculated on 1 g of dry matter of soil.

3.6.4.2 Pre-isolation sample enrichment with test pollutant

An enrichment technique involving the use of a mineral salt media (MSM) containing 0.5 g/L $MgSO_4$, 0.2 g/L $CaCl_2$, 13.6 g/L KH_2PO_4, 5 g/L $(NH_4)_2.SO_4$, 0.05 g/L $FeSO_{4.}7H_2O$, 15 g/L Na_2HPO_4 (Yonetanmi, 2004) was prepared by mixing components and autoclaving at 121 ^{o}C for 15 minutes at 15 *psi*. The soil suspension (5 mL) was aseptically added into flasks containing 100 mL of prepared MSM broth enriched with spent engine oil at 0%, 4%, 8%, 12% and 16% (0 mL, 0.4mL, 0.8mL, 1.2mL and 1.6mL) as carbon source in the concentration of 100 ppm. The experimental set-ups were incubated at 37 ^{o}C for 10 days under agitation (150 rpm). The total microbial growth absorbance was measured in every 24 hrs at 600 nm and recorded.

3.6.4.3 Isolation and identification bacteria isolate

Experimental set-ups with the highest absorbance values connoting higher microbial presence were used for microbial isolation. Primary isolation of bacteria was carried out using nutrient agar by plating out 0.1 mL of samples on appropriately prepared culture. Evaluations inculcated presumptive, confirmatory as well as completed experiments (Bagherzadeh-Namazi, Shojaosadati, & Hashemi-Najafabadi, 2008). The bacteria isolates were identified according to colonial morphology, surface, shape, size, margin and pigmentation on nutrient agar medium. The microscopic examination including Gram-staining, and biochemical tests including citrate utilization, starch hydrolysis, methyl red, Voges-Proskauer test (MR-VP), triple sugar iron test (TSI) for lactose, dextrose, sucrose, glucose and mannitol fermentation, carbohydrate fermentation, H_2S production, indole production test, urease test, catalase test, citrate test, indole test and sugar fermentation test were carried out according to standard procedures and identified using Bergey's Manual of Systematic Bacteriology.

3.6.4.4 Toxicity assay

Biotolerance of bacteria isolates to different levels of spent engine oil was carried out using UV spectrophotometer at 600 nm. 1mL of the test bacteria were inoculated into 20mL spent engine oil broth (prepared using enrichment method described above) having different concentration of spent oil as carbon source. The optical density was monitored and reading taken 24 hourly for 120 hours starting with the 0 hr reading.

3.6.5 Earthworm bioassay test

The soil used in this study was collected from the Botanical garden of the Federal University of Technology, Owerri Imo State at a depth of 0-20cm bulked together and taken to the laboratory for routine physicochemical analysis (OECD, 2000; 2006). While in the laboratory soil samples were air dried and passed through <2mm mesh. Approximately 10g of sieved soil sample was used for physicochemical analysis. Spent engine oil used in this study was pooled from three different auto mechanic workshops within Obinze and homogenized in 5L container to form composite and kept in the laboratory before use.

3.6.5.1 Collection and acclimatization of earthworms

The earthworms were collected immediately after rainfall while crawling around plant debris to seek shelter. Criterion used for collection of the earthworm was maturity (presence of clitellum) and liveliness (active response when anterior segment is prodded). Subsequently, the earthworms were aseptically put into transparent bottles and taken to the laboratory for acclimatization to laboratory condition and were regularly checked on a daily basis to ascertain their health condition (Ekperusi & Aigbodion, 2015). The earthworms were fed with 10 % finely ground *sphagnum* peat pellets and soil from source of collection in other to simulate natural condition under which the earthworms thrive in accordance with OECD protocols (2006). The species used in this study was authenticated by earthworm taxonomist according to the method of Yan *et al.*, (2000). All earthworms were allowed to acclimatize in the laboratory for five (5) days before use. Clitellate adult earthworms (OECD, 2000, ISO, 2011; ISO, 2007) with average live weight of 0.8 g were selected for the study.

3.6.5.2 Test procedure

The test was carried out under stringent bioassay procedures with modifications from previous authors (Abara *et al*., 2020; Akin-Obasola, 2019; Ekperusi & Aigbodion, 2015). Prior to the commencement of the toxicity assay, a range finding test was carried out to ascertain the concentration that would kill 100% and 0% of the worm (Abara *et al.*, 2020). The setup was arranged in complete randomized design with three replicates per treatment and monitored for 72 hr. During this time, failure to respond to external stimuli when prodded was used as criteria for death (OECD, 2006).

Plate 3.3: Spiking of of different concentrations spent engine oil in experimental soil

3.6.5.3 Definitive test (acute toxicity)

The selected worms were exposed to five concentrations (0, 4%, 8%, 12%, and 16%) of spent engine oil. Ten (10) worms were used in each concentration and replicated thrice for the earthworm acute toxicity assay. Nine rectangular plastic containers measuring 9 cm in breadth, 15 cm in length and 8 cm in height with cover lid and clips on both sides of the edges were purchased from the local market. 5 kg (ISO, 2011) of the sun-dried soil was then weighed into each of the containers. With the aid of a glass beaker, the different concentrations of the spent engine oil was thoroughly spiked into each of the containers having 5 kg of soil and moistened with distilled water to water holding capacity (about 70%) of the soil and allowed to stabilize for 24 hr (OECD, 2006). A netting material cut into sizes was placed on top of each of the containers and the cover lid frame placed on top of it to hold it firmly with the help of the clips on both sides of the containers (Plate 3.4). This was done to avoid escape of the earthworms and allow free flow of oxygen into the treatments. The setup was placed inside the laboratory and checked morning and evening on a daily basis and monitored for 7 and 14 days. Mortality rate was recorded using inability of the worms to respond when prodded at the anterior and posterior end (Akin-Obasola, 2019).

Plate 3.4: Experimental setup for earthworm toxicity test

Table 3.3 Distribution of the earthworms during acute toxicity test

TREAMENTS (%)	T_1	T_2	T_3	T_4	T_5	**CONTROL**	**TOTAL**
REP I	10	10	10	10	10	10	60
REP II	10	10	10	10	10	10	60
REP III	10	10	10	10	10	10	60
TOTAL NO OF WORMS	30	30	30	30	30	30	180

3.6.5.4 Assessment of burrowing index

The burrowing and survival responses of the exposed earthworms at various concentrations to soil contamination was evaluated using the protocol of the Organization for Economic Co-operation and Development, OECD, (2008) as reported by Dada, *et al*, (2016) with minor modifications. The time (T) taken for all worms or the last worm to burrow in each concentration level was recorded. Each worm was regarded to have completely burrowed if no part of its body is seen on the soil surface. Readings for each container was taken only where all the worms completely burrowed. Inability of one or more worms to completely burrow in a container was taken as an evidence of avoidance for that particular toxicant concentration; hence, burrowing time was recorded in such cases.

3.6.5.5 Assessment of survival responses

Mortality was assessed at 7th and 14th days of exposure (Dada, *et al*., 2016). After 14 days in the laboratory, the soil, including the worms, in each container was poured into a plastic tray and spread out into a thin film in order to count live worms (OECD, 2006). Worms were regarded as dead if they did not respond to mechanical stimulus of touch at the front end (Dada *et al*., 2016).

The pH of the soil medium used was 6.8 ± 0.1 while moisture content was maintained at 35 ± 2% (OECD, 2008).

3.6.7 Ecological Risk assessment of Spent Engine Oil against test organisms

To estimate the actual potential risks probable from toxic compounds in the ecosystem, risk assessment via the aspects including exposure assessment and risk characterization are paramount (Kamunda *et al.,* 2016). According to USEPA (2021), to estimate the potential ecological risk in soil, dust, air and water compartments, the algorithm described below could be incorporated.

In this study, ecotoxicological risk assessment of spent engine oil against different species that represent the trophic levels (microbes, plants and earthworm) was determined based on the ratio between the Measured Environmental Concentration (MEC) and the Predicted No Effect Concentration (PNEC). This ratio is denoted as Risk Quotient (RQ) (Sanderson, Johnson, Wilson, Brain, & Solomon, 2003).

Thus,

$$RQ = \frac{MEC}{\text{PNEC}} \qquad \textit{eqn. (1)}$$

Where MEC = Measured Environmental Concentrations,

RQ = Risk Quotient,

PNEC = Predicted No Environmental Concentration.

The values for PNECs were obtained from acute toxicity tests using microorganisms, plants and earthworms based on average estimation of EC_{50} (effective concentration that killed 50% of the organisms), and LC_{50} (lethal concentration that killed 50% of the organisms) respectively. The MEC was derived from average TPH concentration in soil (Gonzalez-Naranjo & Boltes, 2014).

The corresponding PNECs values were derived by dividing the toxicological dose descriptors (LC50 and EC50) obtained in this study by appropriate Assessment Factor (AF) of 1000 (for plants and earthworm) and 10 (for microorganisms) as shown in equation 2 (OECD, 2006; Sanderson *et al*, 2003).

$$\text{PNEC} = \frac{LC50/EC50}{Assessment\ Factor} \qquad eqn\ (2)$$

The use of an Assessment Factor (dimensionless) highlights the uncertainty of the defined extrapolation method in accordance with OECD (2006); USEPA (2021). In other words, it takes into account the inherent uncertainties emanating from laboratory and natural environment.

Different risk levels are traditionally determined by the resulting Risk Quotients (RQ) values according Yamamoto *et al*,, (2011) (minimal risk: less than 0.1, median risk: $0.1 \leq RQ < 1$, and high risk ≥ 1). However, in this study, the method reported by Sanderson *et al*, (2003) was adopted to characterize ecological risk level: lower than 0.1 is implies “acceptable risk”; between 0.1 and 1 “needs further survey” and equal to or higher than 1, “needs detailed evaluation.”

The approach used in this study was based on an estimate of the incidence of adverse effects that occur in the spent engine oil exposure at the measured concentrations. Values were measured in mg/L or mg/kg. Summary of ecotoxicological risk assessments carried out in this study is depicted in Table 3.4 below.

Table 3.4 Summary of acute toxicity tests performed in this study

Test type	Source of organisms	Test chamber	Effect criteria	Growth condition	Exposure time (h)	Endpoint	Method
Species							
Seed Germination /inhibition	IITA, Ibadan	Controlled Environment	EC_{50}	Tempt.: 22^{0}C±10^{0}C; potoperiod: 16-hr light; Humidity 70^{0} ±25%	14 days	Germination and early seedling growth inhibition	OECD, (2008;2006)
	IITA, Ibadan	Controlled Environment	EC_{50}	Tempt.: 22^{0}C±10^{0}C; potoperiod: 16-hr light; Humidity 70^{0} ±25%	14 days	Germination and early seedling growth inhibition	OECD, (2008)
	IITA, Ibadan	Controlled Environment	EC_{50}	Tempt.: 22^{0}C±10^{0}C; potoperiod: 16-hr light; Humidity 70^{0} ±25%	14 days	Germination and early seedling growth inhibition	OECD, (2008)
Earthworm							
	Soil	Controlled Environment	LC_{50}	Tempt.: 22^{0}C±10^{0}C; potoperiod: 12-hr light; Humidity 70^{0} ±25%	14 days	Mortality, Tendency to burrowing	OECD 222 (2004)
Microorganisms	Laboratory cultured	Controlled Environment	EC_{50}	Tempt.: 22^{0}C±2^{0}C	120 hrs	Growth Inhibition	OECD 222 (2006)

Legend: OECD=Organization for Economic Co-operation and Development; LC_{50}= Lethal Concentration; EC_{50}= Effective Concentration.

3.7 Phytoremediation of spent engine oil using three indigenous plants

3.7.1 Pot experiment

Phytoremediation potentials of the selected hyperaccumulator species from the various auto mechanic workshops were evaluated *in vivo* by conducting pot experiments in the screen house. Three plant species (*Chromolaena odorata, Axpnopus compressus and Aspillia africana*) were selected based on phase one of this study as well as their dominance at mechanic workshops.

3.7.2 Collection and processing of soil samples

Soil samples with no history of oil pollution were collected from a depth of 0-20 cm within the Federal University of Technology Owerri using auger of approximately 7.5cm diameter and taken to the laboratory for pre-planting soil analysis. The soil samples were air-dried and pre-sieved with <2mm sieve and used for physicochemical determination. Vegetative parts of the plant species (stem cuttings of *Chromolaena odorata, Axonopus compressus* and *Aspillia africana*) were collected from an area with no trace of spent oil pollution and stabilized in the nursery for two (2) weeks by which time an average of four (4) fully expanded leaves per plant had been developed before been used for the study.

3.7.3 Experimental design and treatment application

Equal volume of pre-sieved soil (20kg) was filled in Polythene bags and arranged at 0.5m spacing between polybags and 1m between replications and perforated at the base to avoid water logging. Concentrations of spent engine oil spiked included 0, 4%, 8%, 12% and 16% (v/w) and which was allowed to stabilize for two (2) week to simulate condition of natural spill (Plate 5). For the treatment, the control soil was not spiked with spent engine oil. Each treatment was replicated thrice and arranged in a complete randomized design. Two weeks after spiking with different concentrations of spent engine oil, about 100g of soil samples were collected at the surface from each replicate, homogenized and sent to the laboratory for soil analysis prior to planting (Tóth, & Montanarela, 2013). This was done to track the initial level of pollutants of concern in spent engine oil before transplanting (Njoku *et al*., 2016).

Plate 3.5: Experimental set up for phytoremediation studies

3.7.4 Transplanting of seedlings into plant pots

Two weeks after nursery establishment of the seedlings, plants with three to four fully expanded leaves were selected for transplanting into the polythene bags containing the respective treatments/control; one plant per pot. Each of the polythene of was watered with 200mls of water a day before transplanting. Overall, three plant species (*Chromolaena odorata*, *Axonopus compressus* and *Aspillia africana*) were tested in five treatment soils each having three replicates.

3.7.4.1 Data collection

The observations on the growth of the plant species were recorded at two (2) weeks interval with the following parameters determined as follows:

- **Plant height (cm):** the perpendicular distance from the ground level to the tip of the longest branch was measured with the aid of meter rule on three randomly selected plants and the average data was recorded.
- **Number of leaves per plant** (NL): the total number of leaves found in the plants was counted from three randomly selected plants and the average data was recorded.

3.7.4.2 Plant Harvesting

Plants were carefully harvested after 12 weeks of treatment and washed with distilled water to rid them of soil particles. The plants were sorted according to treatment and separated into roots, shoots and leaves and placed in paper envelops before oven drying. The three replicates from each treatment were pooled together to give composite sample from which data from the following parameters were taken:

- **Fresh weight** (FW) (g): the weight of the above ground fresh biomass of the three randomly selected shoots was recorded.
- **Root number** (RL) (cm): the number of roots was counted from the three randomly selected plants and the average data was recorded.

- **Fresh weight** (FW) (g): the three randomly selected roots were washed and allowed to air dry and the fresh weight was recorded.

3.7.4.3 Laboratory studies

Post analysis of soil and plants tissues (root, shoot and leaf) were carried out to track the phytoremediation potentials of *Chromolaena odorata* and *Aspillia africana* at the various treatment levels.

3.7.4.4 Determination of heavy metals in plant parts (Root, shoot and leaf)

Each subsample was oven-dried at 70°C for 24 hours. Acid digestion method of Yusuf *et al.* (2003) was used for the digestion of grounded plant samples. 1 g each of this was weighed into 50mL capacity beaker, followed by addition of 10mL mixture of analytical grade acids: HNO3; H_2SO_4; $HClO_4$ in the ratio 1 : 1 : 1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at temperature of 70°C until about 4mL was left in the beaker. Then, a further 10mL of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4mL. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50mL with distilled water. Quantitatively, the digested samples were transferred into a 125ml plastic container, filtrated with 50ml de-ionized water and metal analysis performed with Buck scientific Atomic Absorption Spectrophotometer (Model GFA-EX7i, Shimadzu Corporation, Japan) using air acetylene flame with a digitalized read out system (Sabate, *et al.*, 2006). Results were expressed in mg/kg.

3.7.4.5 Determination of TPH in Plant Tissues (Root, Shoot and leaf)

The plants parts from the different treatments were separately extracted in Carbon tetrachloride. The extracts were then analyzed using IR spectroscopy following the method of Raymond & Harrison, (2018).

3.7.4.6 Determination of Phytoremediation Potentials of the selected Plants

Each plant per treatment was examined to determine the percentage removal of contaminants as well as the total accumulation of the contaminants in the root, shoot and leaves. The Biological Accumulation Coefficient (BAC), and Transfer Factor was estimated and the results was used to assess the phytoextraction, phytostabilization and hyperaccumulation potentials of the test plants.

The biological accumulation coefficient (BAC) is the concentration of contaminant in the plant shoots divided by the metal concentration in the soil (Raymond & Harrison, (2018):

(a) **Bioaccumulation Coefficient (BAC)** $= \frac{\textbf{metal content in shoot}}{\textbf{metal content in soil}}$

BAC factor > 1 indicates that the plant species has the ability to store contaminants from the soil into the shoots.

Biological Transfer Factor is the ratio of the contaminant concentration in the plant shoot divided by that of the plant root. BTC values > 1 indicate that the species has potential to accumulate contaminant from root to the aerial part of the plants.

(b) **Biological transfer coefficient =** $\frac{\textbf{metal content in shoot}}{\textbf{metal content in root}}$

Statistical analysis

Data collected was presented in charts and tables. Mean separation was done using Duncan Multiple Range Test. Probit analysis was carried to determine the EC50 and LC50 of each organism used in acute toxicity test. All statistical analysis was run using SAS package, 20 version.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Age distribution of automobile mechanics in the study area

The age distribution of automobile mechanics in the study areas are presented on Table 4.1. The results obtained showed that the mechanics in the age group between 20 to 30 years are highest in number of mechanics participating in the job. The result further shows that mechanics within the age of 20 to 30 years were higher in number in all the sample areas compared to other age groups. This is followed by the age group between 31 to 40 years. The least values were obtained from the age group of 60 years and above. Results also showed that automobile mechanics in Owerri had the highest number of mechanics in the age group of < 20 years, 20 to 30, 31 to 40 years respectively. However, the lowest number of mechanics within the age groups <, 20, 20 to 30 and 31 to 40 years were obtained from Okigwe. The total number of mechanics and percentage participation were highest in age group between 20 to 30 with a value of 173 (51.03%) while Owerri showed the highest number of mechanics and percentage age group participation with a value of 142 (41.29%) while the lowest percentage participation was obtained at Okigwe area with a value of 88 (25.96%). However, the number of mechanics and percentage age groups obtained in Owerri was significantly different from that of other study areas with a distribution of Owerri> Orlu> Okigwe respectively.

Table 4.1: Age distribution of automobile mechanics in the study area

	Participant locations			
Age groups (years)	**Owerri**	**Orlu**	**Okigwe**	**Total**
≤20	12(3.54)	10(.2.95)	4(1.18)	26(7.67)
20-30	76(22.42)	53(15.63)	44(12.98)	173(51.03)
31-40	43(12.68)	32(9.44)	28(8.26)	103(30.39)
41-50	9(2.65)	11(3.25)	10(2.95)	30(8.85)
≥60	2(0.59)	3(0.88)	2(0.59)	7(2.06)
Total	142(41.29)	109(32.15)	88(25.96)	339(100)

4.1.2 Number of vehicles serviced per day and annual volume of spent engine oil generated in the study areas

The frequency and percentage distribution of mechanics according to the number of vehicles serviced per week and annual volume of spent engine oil generated in the study areas is shown in Table 4.2 and Appendix VI respectively. Results obtained showed that the number of vehicles serviced in Owerri, Orlu and Okigwe mechanics are 42(46.7%), 27(30.0%) and 21(23.5%) respectively. The highest volume of spent engine generated was obtained from Owerri (124,489.36), while the lowest was gotten fron Okigwe area (18,434.52) respectively. The total annual volume of spent engine oil generated (in litres) was 171, 354.88 from the entire study area.

Table 4.2: Number of vehicles serviced per day and annual volume of spent engine oil generated in the study areas

Treatments	Locations		
	Owerri	**Orlu**	**Okigwe**
Volume of SEO removed (L)	4	4	4
Mean NVS/mechanic/week	7.7	4.5	3.9
Mean volume of SEO generated/vehicle	3.95	3.95	3.95
NVS/day	53.90	20.25	15.15
NVS/week	323.4	121.5	90.9
NVS/annum	16816.80	6318.0	4726.80
SEO generated/day	212.91	79.99	59.84
Volume of SEO generated/week	1277.43	479.93	359.06
Volume of SEO generated /annum	124,489.36	28,431.0	18,434.52

Legend: L = Litre, V = Volume, SEO = Spent engine oil, NVS = Number of vehicles serviced

4.1.3 Frequency and percentage disposal methods of spent engine oil by auto mobile mechanics

The frequency and percentage disposal methods of spent engine oil by auto mobile mechanics in the mechanics villages are presented in Table 4.3. The results obtained showed that a good number of mechanics dispose the spent engine oil in their immediate environment 168 (49.56%). Both the frequency and percentage disposal methods were highest with those that dispose in immediate environment compared to others. This is followed by mechanics that give it out freely to end users 66 (19.47%). The results also shows that mechanics also sale spent engine oil to other people to the frequency and percentage disposal rate of 41 (12.09%). However, whichever way spent engine oil is disposed, wether sold, given out freely or taken by the owner, all may eventually land back to the environment.

Table 4.3: Result showing Frequency and percentage disposal methods of spent engine oil by auto mobile mechanics

Methods of disposal	F	Percentage (%)
Sold	41	12.09
Poured into immediate environment	168	49.56
Given out for free	66	19.47
Taken by car owners	64	18.88

Legend: F = Frequency

4.1.4 Frequency and percentage uses of spent engine oil by auto mobile mechanics

The various uses of spent engine oil by mechanics and people in mechanic villages are presented in Table 4.4. The result obtained showed eight (8) different uses of spent engine among the mechanics. The result showed that Lorry/Truck reuse of spent engine oil had the highest frequency and percentage use 74(21.83%) compared to other uses. This was followed by Timber cutting 51(15.04%) in which spent engine oil is bought and robbed on the Saw for easy cutting of the wood. The mechanics use spent engine specifically in control of mosquitoes in the environment 48 (14.16%). The least frequency and percentage use of spent engine oil was obtained from those that pour it in a pit laterine 18(5.31%). Spent engine oil was also used in pest control 38 (11.21%), marking play ground 25 (7.37%), and treating roofing timber 43 (12.69); the frequency and percentage use is also significant. However, in all its use, spent engine oil eventually finds its way back into the environment.

Table 4.4: Result showing Frequency and percentage uses of spent engine oil by auto mobile mechanics

	Frequency	**%**
Lorries/trucks Reuse	74	21.83
Poured in pit latrine	18	5.31
Mosquito	48	14.16
Timber cutting	51	15.04
Pest control	38	11.21
Marking play ground	25	7.37
Treating roofing timber	43	12.69
Treating fencing post	42	12.39

4.1.5 Knowledge of health, environmental risk and possible training on disposal methods of spent engine oil

The knowledge of health, environmental risk of spent engine oil and possible training on disposal methods by the mechanics are shown in Table 4.5. The result obtained showed that the three hundred and eighty seven 387 (84.66%) of the mechanics do not have any knowledge of the health risk associated with exposure to spent engne oil compared to fifty two 52 (15.34%) mechanics who said they are aware of the health risk associated with exposure to spent engine oil. Also, two hundred and forty three 243 (71.68%) mechanics have no knowledge of the environmental risk of exposure to spent engine oil compare to ninety six 96 (28.32%) mechanics that said they do not have the knowledgeof it. Fourteen mechanics 14 (4.12%) have been trained on the disposal methods of spent engine oil. However, three hundred and twenty five 325 (95.87%) have not been not trained on how to handle spent engine oil. The no knowledge of health, environmental risk, and training of disposal methods of spent engine oil oil were significantly higher ($P<0.05$) than the yes response of the study.

Table 4.5: Knowledge of health, environmental risk and possible training on disposal methods of spent engine oil

Parameters	Responses	Frequency	Percentage	Chi-Square	P-Value
Health risk	Yes	52	15.34	11.37	.121
	No	387	84.66		
Environmental risk	Yes	96	28.32	23.76	.273
	No	243	71.68		
Training on hazards	Yes	14	4.12	.235	.104
	No	325	95.87		

4.1.6 Plant families found on spent engine oil polluted soils of auto mechanic in Owerri, Okigwe and Orlu zones.

Table 4.6 shows plant species found growing on spent engine oil polluted soils of auto mobile mechanic villages. The result obtained showed that nine (9) plant families were found in spent engine oil contaminated soil of Owerri and Orlu mechanic villages, however, eight (8) plant families were found in Okigwe mechanic villages. However, these three zones recorded the same plant families. *Poaceae* species had the highest number of occurrence in all the three zones, with Okigwe zone having the highest number of *Poaceae* (23), and total number of individual plants to be 217. This was followed by *Euphorbiaceae* which was more abundant in spent engine oil polluted soils of Okigwe mechanic villages. *Asteraceae* family was higher in all the sampling areas. The percentage frequency of occurrence results showed the Poaceae to be significantly higher in spent engine oil polluted soil of Orlu mechanic villages compared to others. However, *Asteraceae* and *Poaceae* were significantly high in spent engine oil polluted soil of Owerri and Okigwe respectively. A total number of individual plants that was counted for spent engine oil polluted soils in Owerri, Okigwe and Orlu mechanic villages were 812, 607 and 426 respectively.

Table 4.6: Plant families found on spent engine oil polluted soils of auto mechanic in Owerri, Okigwe and Orlu zones.

Zones	Families	No Of Occurrence(n)	Total No of Ind. Plants (N)	% Frequency of Occurrence
Owerri	*Asteraceae*	9	188	**23.15***
	Poaceae	19	213	**26.23****
	Amaranthaceae	7	119	14.65
	Euphorbiaceae	*5*	*122*	*15.02*
	Convolvulacea	*1*	*36*	*4.43*
	Malvaceae	*3*	*39*	*4.80*
	Gramineae	*1*	*16*	*1.97*
	Lamiaceae	*2*	*63*	*7.75*
	Portulacaceae	*5*	*16*	*1.97*
			812	***100.00***
Okigwe	*Asteraceae*	*10*	*179*	*29.48**
	Poaceae	*23*	*217*	*35.74***
	Amaranthaceae	*8*	*121*	*19.93*
	Euphorbiaceae	*12*	*33*	*5.43*
	Lamiaceae	*4*	*25*	*4.15*
	Cyperaceae	*8*	*12*	*0.32*
	Piperaceae	*4*	*1*	*0.16*
	Curcubitaceae	*1*	*12*	*0.32*
			607	***100.00***
Orlu	*Poaceae*	*17*	*213*	*50.00*
	Amaranthaceae	*4*	*53*	*12.44*
	Euphorbiaceae	*2*	*17*	*3.99*
	Convolvulacea	*3*	*16*	*3.75*
	Malvaceae	*1*	*5*	*1.17*
	Commelinacea	*2*	*10*	*2.34*
	Malvaceae	*1*	*9*	*2.11*
	Asteraceae	*11*	*97*	*22.76*
	Portulacaceae	*3*	*6*	*1.40*
			426	***100.00***

4.1.7 Relative frequency percentage occurrence of indigenous plant species growing on spent engine oil polluted soils of Owerri, Okigwe and Orlu mechanic villages

Results of the relative frequency of occurrence by species identified in the vicinity of auto mechanic workshops and control sites in displayed in Table 4.7. The result indicated that in all the sites studied, *Axonopuss compressus* (belonging to Poaceae family) recorded the highest relative frequency of occurrence (78.0%), followed by *Aspillia africana* (72.6%) and *Chromolaena odorata* (68.0%) in that order. There was a variation in the relative frequency of plant species across the zones. However, *Axonopuss compressus* remained the most common plant species in all the three zones studied with slight variation; Owerri (68%), Okigwe (77%) and Okigwe (57%). The dominance of the plant species were in the order: *Axonopuss compressus>Aspillia africana>Chromolaena odorata*.

Potential phytoremediators of spent engine polluted soils where ranked based on taxa with relative frequency >10% in each site under study as shown in Table 4.7.1. The plant species were ranked thus: *Axonopuss compressus* 78.0 (39.3)> *Aspilla* africana 72.6 (30) > *C. odorata*, 68.0 (21.3) > *Eleusine indica* 42.3 (21.6) > *Talinum triangulare* > 40.0 (17.0) > *Ixora coccinea*, 24.6 (11.) >*Mimosa pudica*, 24.3 (4.0) > *Steculia africana*, 23.0 (16.0) >*Andropogon gayanus*, 18.6 (19.0)>*Platostoma africana*, 18.6(2.6) >*Chloris babata*,14.6 (2.6)>*Gomphrena celosioidas*,16.33 (7.0)>*Panicum maximum* 16.6 (13.0)>*Andropogon tectorum*, 12.6 (9.3) >*Amaranthus spinosus*, 12.66 (6.3)>*Paspalum conjugatum*, 12.0 (4.6)> *Pepperomia pellucida* 12.0 (5.0) >*Sterculia urceolata* 11.6 (3.6)>*Sida acuta*, 10.0 (13.6)> *Portulaca oleracea*, 10.3 (8.3).

4.7 Relative frequency of occurrence by species identified in the vicinity of auto mechanic workshops and control sites

		Relative frequency (%)			
Families	**Plant Species**	**Owerri**	**Okigwe**	**Orlu**	**Means**
Amaranthaceae	*Gomphrena celosioidas,*	18(9)	21(11)	10(1)	16.33(7.0)
	Amaranthus spinosus	11(5)	20(7)	7(0)	12.66(6.3)
Asteraceae	*Aspilla africana*	73(33)	66(18)	79(39)	72.6(30)
	C. odorata,	88(19)	72(38)	44(7)	68.0(21.3)
	Mimosa pudica	18(3)	0(0)	19(3)	24.3(4.0)
Commelinacea	*Commelina Benghalensis*	12(1)	0(0)	0(0)	4.0(0.3)
Convolvulacea	*Ipomoea vagans*	8(2)	0(0)	3(1)	3.6(1.0)
Cyperaceae	*Cyperus rotundus*	1(1)	3(0)	1(0)	1.6(0.3)
	Mariscus flabelliformis	5(0)	0(0)	8(2)	4.3(0.6)
	Cyperus esculentus,	0(0)	0(7)	0(0)	2.3(0.0)
	Carex stricta,	1(0)	7(2)	0(0)	2.6(0.6)
	Scirpus cespitosus	0(0)	0(5)	0(2)	0.0(2.3)
Curcubitaceae	*Cucurbita pepo*	8(1)	0(0)	0(0)	2.6(0.3)
Euphorbiaceae	*Sabastiana chamaelea,*	1(0)	0(0)	0(0)	0.3(0.0)
	Dysopsis baill	0(0)	0(5)	0(0)	0.0(1.6)
	Phyllanthus amarus	9(3)	0(9)	0(0)	3.0(4.0)
	Croton hirtus	0(0)	0(11)	0(0)	0.0(3.6)
	Ixora coccinea	19(0)	17(0)	0(33)	24.6(11.0)
Gramineae	*Orinus longiglumis,*	10(6)	1(0)	0(0)	3.6(2.3)
	Paspalum vaginatum	11(9)	0(0)	1(0)	4.0(0.0)
	Chloris babata,	14(4)	11(3)	19(1)	14.6(2.6)
	Eragrostis atrovirens	0(0)	10(2)	0(0)	3.3(0.6)
Lamiaceae	*Hyptis laceolata*	0(0)	1(8)	11(2)	4.0(3.3)
	Platostoma africana	7(0)	6(1)	17(7)	18.6(2.6)
Malvaceae	*Steculia africana,*	28(5)	19(21)	22(16)	23.0(16.0)
	Sterculia urceolata	12(3)	22(8)	1(0)	11.6(3.6)
	Sida acuta	15(9)	3(13)	12(19)	10.0(13.6)
	Sida corymbosa	0(5)	0(0)	3(1)	1.0(2.0)
Poaceae	*Axonopuss compressus*	68(42)	77(19)	89(57)	78.0(39.3)
	Andropogon gayanus	17(33)	0(7)	39(17)	18.6(19.0)
	Eleusine indica	49(28)	33(19)	45(18)	42.3(21.6)
	Andropogon tectorum	15(19)	10(0)	13(9)	12.6(9.30
	Panicum maximum	20(16)	19(12)	11(8)	16.6(13.0)
	Paspalum conjugatum	10(9)	9(0)	17(5)	12.0(4.6)
	Chloris pilosa	0(1)	0(0)	18(5)	6.0(2.0)
	Cynodon dactylon	7(3)	0(0)	12(8)	6.3(4.0)
Portulacaceae	*Talinum triangulare*	39(18)	43(16)	38(17)	40.0(17.0)
	Portulaca oleracea	12(10)	19(7)	0(8)	10.3(8.3)
Piperaceae	*Pepperomia pellucida*	0(9)	17(3)	19(3)	12.0(5.0)

Table 4.7.1 Potential phytoremediators of TPH based on relative frequency and abundance

Plant species	**R.F (>10%)**	**Mean ranking**
Gomphrena celosioidas,	16.33(7.0))	10
Amaranthus spinosus	12.66(6.3)	16
Aspilla africana	72.6(30)	2*
C. odorata,	68.0(21.3)	3*
Mimosa pudica	24.3(4.0)	7
Ixora coccinea	24.6(11.)	6
Chloris babata,	14.6(2.6)	11
Platostoma africana	18.6(2.6)	9
Steculia africana,	23.0(16.0)	8
Sterculia urceolata	11.6(3.6)	17
Sida acuta	10.0(13.6)	18
Axonopuss compressus	78.0(39.3)	1*
Andropogon gayanus	18.6(19.0)	9
Eleusine indica	42.3(21.6)	4
Andropogon tectorum	12.6(9.3)	13
Panicum maximum	16.6(13.0)	12
Paspalum conjugatum	12.0(4.6)	15
Talinum triangulare	40.0(17.0)	5
Portulaca oleracea	10.3(8.3)	20
Pepperomia pellucida	12.0(5.0)	14

Legend*:* R.F. = Relative Frequency

4.1.8 Physical and chemical properties of spent engine oil polluted and unpolluted soils

4.1.8.1 Physical properties

The physical and chemical parameters of soil samples collected from spent engine oil polluted and unpolluted soils from the three study areas are displayed in Table 4.10. The particle size distribution of soil shows that there was no particular trend in the distribution of sand content across the zones. All the zones revealed a high sand fraction in Owerri (76 ± 6.14), Orlu (78 ± 6.21) and Okigwe (79 ± 6.99) with the control site recording slightly higher value (80 ± 7.09). The highest sand fraction was recorded in Okigwe (79 ± 6.99) while the least was observed in Owerri zone (76 ± 6.14). Percentage silt content in Owerri, Orlu and Okigwe zones were: 63 ± 6.13, 74 ± 7.68, 77 ± 7.73 and 77 ± 7.73 with the highest value of 83 ± 7.23 recorded at the control site.

Percentage clay content varied in the sampling zones with the highest values recorded at Orlu (10.33 ± 2.31) and Okigwe zones (10.19 ± 2.23) and least values of 6.4 ± 1.96 recorded at Owerri zone while the control site recorded a mean value of 10.19 ± 2.23. Mean moisture content of soil ranged from 26 ± 3.17 in Owerri to 39.34 ± 3.69 in Orlu with least value obtained at Okigwe zone (39.06 ± 3 .52) while the control site recorded 40.06 ± 3.46. The mean bulk density was 3.8 ± 4.19 in Owerri, 4.0.48 ± 4.43 in Orlu and 4.0.69 ± 4.47 in Okigwe zone with 4.0.34 ± 3.19 recorded at the control site.

4.1.8.2 Chemical properties

With respect to the chemical properties of soil, the mean pH ranged between 4.2 ± 1.26 9 to 4.5 ± 1.38 with a slightly higher value of 6.0 ± 1.41 at the control site. The Organic carbon content of the soil followed a decreasing pattern with Owerri zone having highest mean value of 13.68 ±

3.79 followed by Orlu zone (11.34 ± 3.54). The control had the least value of Organic carbon content with a mean value of 8.23 ± 3.05. Organic matter content of the soil followed a visibly regular pattern in all the zones. The organic matter content in Owerri, Orlu and Okigwe zones were: 0.34 ± 0.18 0.37± 0.16 0.38 ± 0.19 respectively. Total nitrogen content was higher at the control site compared with the polluted sites. The mean values of 0.93 ± 0.39 was obtained in Owerri, 0.96 ± 0.39 in Orlu and 0.99 ± 0.40 in Okigwe zone with respect total nitrogen content. The available phosphorus was observed to be marginal at the polluted sites relative to the control sites with mean values of 1.01 ± 0.67, 1.01 ± 0.67 and 1.03 ± 0.68 in Owerri, Orlu and Okigwe zones respectively.

The spent engine oil polluted soil indicated a somewhat similar pattern for Ca, Mg, K, and Na. Calcium content ranged from 1.21 ± 0.88 to 1.16 ± 0.86 with the control site having mean value of 3.33 ± 0.97 Cmol/kg^{-1} . Magnesium content of the soil in all the sites were 4.50 ± 0.56, 4.46 ± 2.13 and 4.49 ± 2.13 with highest value 6.12 ± 2.13 recorded at the control site. Mean values for potassium was highest at the control site in comparison with the polluted sites. Orlu zone had 4.37±1.54 while Owerri zone recorded 4.42 ± 1.71. Least value of 3.9 ± 1.11 was recorded in Owerri zone. Soil sodium ranged from 1.0 ± 0.09 to 1.22 ± 0.16 with the highest mean value of 2.39 ± 0.32 recorded at the control site. Total exchangeable acidity significantly varied in Owerri, Orlu and Okigwe with mean values of 11.33 ± 3.14, 15.34 ± 3.38, 16.31 ± 4.07, and 16.31 ± 4.07 at the control site. However, Okigwe zone recorded the higher mean value of Total Exchangeable Acidity (TEA) (16.31 ± 4.07) while the lowest value of TEA was gotten from Owerri zone (11.33 ± 3.14) Total exchangeable base ranged from 42.87 ± 3.74 to 44.62 ± 4.38 with a mean value of 61.00 ± 5.39 at the control site. Electrical conductivity showed mean values of 97.80 ± 16.29 in Owerri, 99.66 ± 17.61 in Orlu, 99.71 ± 19.17 in Okigwe, and 104.5 ±

1.03 at the control. However, the minimum and maximum values of EC was obtained from Orlu (97.80 ± 16.29) and Owerri (99.66 ± 17.61) zones respectively. Effective cation exchange capacity values were 74.62 ± 8.51 in Owerri, 85.44 ±9.33 in Orlu and 97.50 ± 10.13 in Okigwe and 90.5 ± 11.07 at the non-active site (control).

Table 4.8: Physical and chemical properties of SEO polluted and unpolluted soil samples from the three study locations

Soil properties	**Locations (Value range)**			
	OWSS	**ORSS**	**OKSS**	**CONTROL**
Physical properties				
Sand (%)	76 ± 6.14	78 ± 6.21	79 ± 6.99	80 ± 7.09
Silt (%)	63 ± 6.13	74 ± 7.68	77 ± 7.73	83 ± 7.23
Clay (%)	6.4 ± 1.96	10.33 ± 2.31	10.19 ± 2.23	10.19 ± 2.23
MC	26 ± 3.17	39.34 ± 3.69	39.06 ± 3 .52	40.06 ± 3.46
BD	38 ± 4.19	40.48 ± 4.43	40.69 ± 4.47	40.34 ± 3.19
Chemical properties				
pH	4.2 ± 1.26	4.5 ± 1.38	4.5 ± 1.38	6.0 ± 1.41
OC (%)	13.68 ± 3.79	11.34 ± 3.54	10.28 ± 3.46	8.23 ± 3.05
OM (%)	0.34 ± 0.18	0.37± 0.16	0.38 ± 0.19	0.49 ± 0.12
TN (%)	0.93 ± 0.39	0.96 ± 0.39	0.99 ± 0.40	1.0 ± 0.21
Av. P (%)	1.01 ± 0.67	1.01 ± 0.67	1.03 ± 0.68	1.18 ± 0.82
Ca (Cmol/kg^{-1})	1.13 ± 0.77	1.16 ± 0.86	1.21 ± 0.88	1.33 ± 0.97
Mg (Cmol/kg^{-1})	4.50 ± 0.56	4.46 ± 2.13	4.49 ± 2.13	6.12 ± 2.13
K (Cmol/kg^{-1})	3..9 ± 1.11	4.37±1.54	4.42 ± 1.71	6.27 ± 1.98
Na (Cmol/kg^{-1})	1.0 ± 0.09	1.22 ± 0.16	1.20 ± 0.44	1.39 ± 0.32
TEA (Cmol/kg^{-1})	11.33 ± 3.14	15.34 ± 3.38	16.31 ± 4.07	16.31 ± 4.07
TEB (Cmol/kg^{-1})	44.62 ± 4.38	42.87 ± 3.74	43.00 ± 4.48	61.00 ± 5.39
EC (Cmol/kg^{-1})	97.80 ± 16.29	99.66 ± 17.61	99.71 ± 19.17	104.5 ± 1.03
ECEC (Cmol/kg^{-1})	74.62 ± 8.51	85.44 ±9.33	97.50 ± 10.13	90.5 ± 11.07

***Legend**: SEO=spent engine oil, OWSS = Owerri soil samples, ORSS=Orlu soil samples, OKSS=Okigwe soil samples; MC= moisture content, BD= bulk density, OC=organic carbon, OM=organic matter, TN= total nitrogen, Av. P=available phosphorus, TEA & TEB=total exhachangeable acidity and total exchangeable base, EC=electrical conductivity, ECEC= effective cation exchange capacity,*

4.1.9 Heavy metal concentration in polluted and unpolluted soils

Results of the mean heavy metal concentration in soil samples from polluted and unpolluted sites with spent engine oil from the investigated sites is shown in Table 4.11. Mean values of Lead fluctuated as follows: Owerri (1.534 ± 0.2), Orlu (1.045 ± 0.1) and Okigwe (1.475 ± 0.2). However, Owerri zone had the highest mean value of Pb (1.534 ± 0.2), while Orlu zone recorded the least value (1.045 ± 0.1). No value of Pb was detected in the reference (control) sites. The concentration of Arsenic ranged from 1.761 ± 0.3 to 1.805 ± 0.3 with no value detected at the control site. There was no significant difference at $p < 0.05$ between mean values of Arsenic at Owerri, Orlu and Okigwe zones. However, the hihest values of Arsenic was recorded in Owerri zone (1.805 ± 0.3) while the least mean value for Arsenic was observed at Orlu zone (1.761 ± 0.3). There was no significant difference at $P < 0.05$ among the mean values of Chromium is the study sites. Mean concentration of Chromium in Owerri, Orlu and Okigwe zone were: 0.273 ± 0.01, 0.272 ± 0.01, and 0.273 ± 0.01 with no value detected at the reference (control) site. Cadmium content of the soil varied as follows: Owerri (0.606 ± 0.03), Orlu (0.302 ± 0.02) and 0.596 ± 0.03 in Okigwe zone. There was no significant difference at $p<0.05$ among the concentration of Nickel across the zones. The highest value was obtained at Owerri zone (0.932 ± 0.03), while the least mean values was gotten from Orlu zone (0.918 ± 0.03), with a mean value of (0.235 ± 0.01) gotten from the control site. Similarly, no significant difference ($p < 0.05$) was observed between the Cobalt content of the polluted soils at Owerri and Orlu zones. Least mean value of Cobalt was observed at Okigwe zone with a value of 1.278 ± 0.1. There was a significant difference ($p>0.05$) in the mean Iron content of the soil samples. The highest mean value of Iron was obtained at the control site (76.784 ± 4.25), while Owerri, Okigwe and Orlu zones recorded mean values of 63.927 ± 3.34, 69.563 ± 3.53, and 65.487 ± 3.42 respectively.

Values for Copper content of the soil in Owerri, Okigwe and Orlu zones were: 2.453 ± 0.65, 12.964±2.42, 12.446 ± 2.14, and 12.653 ± 2.31. The lowest value Copper (2.453 ± 0.65) was observed at the control site. No significant difference was found among the Zinc content of the soil in all the study areas. Owerri zone recorded 156.137 ± 28.12, while Okigwe and Orlu zones had 156.125 ± 28.04, and 156.121±28.01 compared to mean values 146.642 ± 2.67 obtained at the control site. In the same vain, mean values of Aluminium indicated no statistically significant difference ($p<0.05$) in all the polluted sites. The mean values for each sampling zone were 1.353 ± 0.2 in Owerri, 1.352 ± 0.2 in Okigwe and 1.353 ± 0.2 in Orlu zones respectively. The control site recorded a mean value of 0.426 ± 0.02 for Aluminium.

Table 4.9: Heavy Metal concentration in spent engine oil polluted and unpolluted soils from Owerri, Okigwe and Orlu mechanic villages

Heavy Metals	WHO LIMIT(2006) (mg/kg)	CONTROL	OWERRI ZONE	OKIGWE ZONE	ORLU ZONE
Pb (mg/kg)	0.01	ND	1.534 ± 0.2^{a}	1.045 ± 0.1^{b}	1.475 ± 0.2^{a}
As (mg/kg)	0.09	ND	1.805 ± 0.3^{a}	1.761 ± 0.3^{a}	1.792 ± 0.3^{a}
Cr (mg/kg)	0.02	ND	0.273 ± 0.01^{a}	0.272 ± 0.01^{a}	0.273 ± 0.01^{a}
Cd (mg/kg)	0.05	ND	0.606 ± 0.03^{a}	0.302 ± 0.02^{b}	0.596 ± 0.03^{a}
Ni (mg/kg)	0.2	0.235 ± 0.01^{b}	0.932 ± 0.03^{a}	0.918 ± 0.03^{a}	0.931 ± 0.03^{a}
Co (mg/kg)	0.05	ND	1.324 ± 0.2^{a}	1.278 ± 0.1^{b}	1.312 ± 0.2^{a}
Fe (mg/kg)	30	76.784 ± 4.25^{a}	63.927 ± 3.34^{d}	69.563 ± 3.53^{b}	65.487 ± 3.42^{c}
Cu (mg/kg)	2.0	2.453 ± 0.65^{d}	12.964 ± 2.42^{a}	12.446 ± 2.14^{c}	12.653 ± 2.31^{b}
Zn (mg/kg)	0.02	146.642 ± 2.67^{b}	156.137 ± 28.12^{a}	156.125 ± 28.04^{a}	156.121 ± 28.01^{a}
Al (mg/kg)	-	0.426 ± 0.02^{b}	1.353 ± 0.2^{a}	1.352 ± 0.2^{a}	1.353 ± 0.2^{a}

Values are mean±SD triplicate samples; means having different superscript of letters along the row differ significantly at $P < 0.05$ using least significant difference(LSD). ND = Not detectedWHO = World Health Organisation .

4.1.10 Poly aromatic hydrocarbon concentration in polluted and unpolluted soils

The results of concentrations of Polyaromatic hydrocarbons from soil collected from the various auto mechanic workshops is presented in Table 4.10 while result of Total Petroleum Hydrocarbon content of the soil sample is presented in Figure 4.1. The result obtained from the various zones (Owerri, Orlu and Okigwe) revealed increased values of Acenapthylene (1.382 ± 0.2; 0.330 ± 0.04; 0.216 ± 0.03), Fluorene (1.091 ± 0.68; 0.917 ± 0.39; 0.391 ± 0.28), Indono (1,2,3-d) pyrene (1.300 ± 0.91; 0.339 ± 0.31; 0.091 ± 0.05), Methyinaphthaiene (1.330 ± 0.47; 1.130 ± 0.36; 1.016 ± 0.27), Naphalene (1.700 ± 0.11; 1.614 ± 0.10; 1.124 ± 0.09), Phenanthrene (1.980 ± 0.36; 1.176 ± 0.28; 1.418 ± 0.19), pyrene (1.402 ± 0.22; 1.311 ± 0.17; 1.176 ± 0.12) at varied concentrations compared to the unpolluted sites which PAHs were not detected. Other PAHs found in the soil samples at marginal concentrations fluctuated among Owerri, Orlu and Okigwe zones as follows: Anthracene (0.010 ± 0.001, 0.004 ± 0.001, 0.003 ± 0.001); Benzo (a) anthracene (0.011 ± 0.001, 0.005 ± 0.002, 0.005 ± 0 .002); Benzo (b) fluoranthene (0.018 ± 0.004, 0.017 ± 0.004, 0.014 ± 0..003); Benzo (c) pyrene (0.020 ± 0.005, 0.013 ± 0.003, 0.010 ± 0.001); Benzo (g,h,i) perylene (0.040 ± 0.07, 0.007 ± 0.002, 0.007 ± 0.002); Benzo (k) fluoranthene (0.884a ± 0.37, 0.070b ± 0.25, 0.070b ± 0.25, 0.019 ± 0.016); Chrysene (0.035 ± 0.19, 0.019 ± 0.14, 0.012 ± 0.011); Dibenzo (a,h) anthracene (0.044 ± 0.21, 1.010 ± 0.67, 1.030 ± 0.68); and Fluoranthene (0.170 ± 0.68, 0.091 ± 0.44, 0.007 ± 0.40) that measured values less than unity respectively. The control site indicated the presence of few PAH with respect to Anthracene (0.002±0.001), Benzo (c) pyrene (0.002±0.001), and Chrysene (0.002±0.001) respectively. In terms of abundance, PAH concentrations were higher in Owerri but lower at Okigwe zone with a distribution pattern of Owerri>Orlu>Okigwe>control.

4.1.10.1 Total Petroleum hydrocarbon concentration in polluted and unpolluted soils

TPH values recorded in the soil samples at the various auto mechanic workshops ranged from 1,932 mg/kg to 3,6520 mg/kg as displayed in Figure 4.1. The highest value of TPH was obtained in Owerri zone compared to Orlu and Okigwe zones with mean values of 2,582mg/kg and 1,932mg/kg respectively. TPH concentration in the soil samples was in the order: Owerri> Orlu> Okigwe> Control. However, the control site recorded the least value of TPH (681 mg/kg).

Table 4.10: Poly Aromatic Hydrocarbon concentrations of spent engine oil polluted and unpolluted soils of Owerri, Orlu and Okigwe mechanic villages

Parameters (mg/kg)	**Zones (Value range)**			
	Owerri	**Orlu**	**Okigwe**	**Control**
Acenaphthene	0.03 ± 0.01^{ab}	0.02 ± 0.01^{b}	0.01 ± 0.001^{c}	ND
Acenapthylene	1.382 ± 0.2^{a}	0.330 ± 0.04^{b}	0.216^{c} ± 0.03^{c}	ND
Anthracene	0.010 ± 0.001^{a}	0.004 ± 0.001^{b}	0.003^{c} ± 0.001^{c}	0.002±0.001^{d}
Benzo (a) anthracene	0.011 ± 0.001^{a}	0.005 ± 0.002^{b}	0.005 ± 0 .002^{b}	ND
Benzo (b) fluoranthene	0.018 ± 0.004^{a}	0.017 ± 0.004^{a}	0.014 ± 0.003^{b}	ND
Benzo (c) pyrene	0.020 ± 0.005^{a}	0.013 ± 0.003^{b}	0.010 ± 0.001^{c}	0.002±0.001^{d}
Benzo (g,h,i) perylene	0.040 ± 0.07^{a}	0.007 ± 0.002^{b}	0.007 ± 0.002^{b}	ND
Benzo (k) fluoranthene	0.884 ± 0.37^{a}	0.070 ± 0.25^{b}	0.019 ± 0.016^{c}	ND
Chrysene	0.035 ± 0.19^{a}	0.019 ± 0.14^{b}	0.012 ± 0.011^{c}	0.002±0.001^{d}
Dibenzo (a,h) anthracene	0.044 ± 0.21^{c}	1.010± 0.67^{a}	1.030 ± 0.68^{a}	ND
Fluoranthene	0.170 ± 0.68^{a}	0.091 ± 0.44^{a}	0.007 ± 0.40^{c}	ND
Fluorene	1.091 ± 0.68^{a}	0.917 ± 0.39^{b}	0.391 ± 0.28^{c}	ND
Indono (1,2,3-d) pyrene	1.300 ± 0.91^{a}	0.339 ± 0.31^{b}	0.091 ± 0.05^{c}	ND
Methyinaphthaiene	1.330 ± 0.47^{a}	1.130 ± 0.36^{b}	1.016 ± 0.27^{c}	ND
Naphalene	1.700 ± 0.11^{a}	1.614 ± 0.10^{a}	1.124 ± 0.09^{c}	ND
Phenanthrene	1.980 ± 0.36^{a}	1.176± 0.28^{b}	1.418 ± 0.19^{c}	ND
Pyrene	1.402 ± 0.22^{a}	1.311 ± 0.17^{b}	1.176 ± 0.12^{c}	ND

Values are mean±SD triplicate samples. Mean alone the row having different superscript of letters differ significantly at P≤ 0.05 level. ND = Not detected.

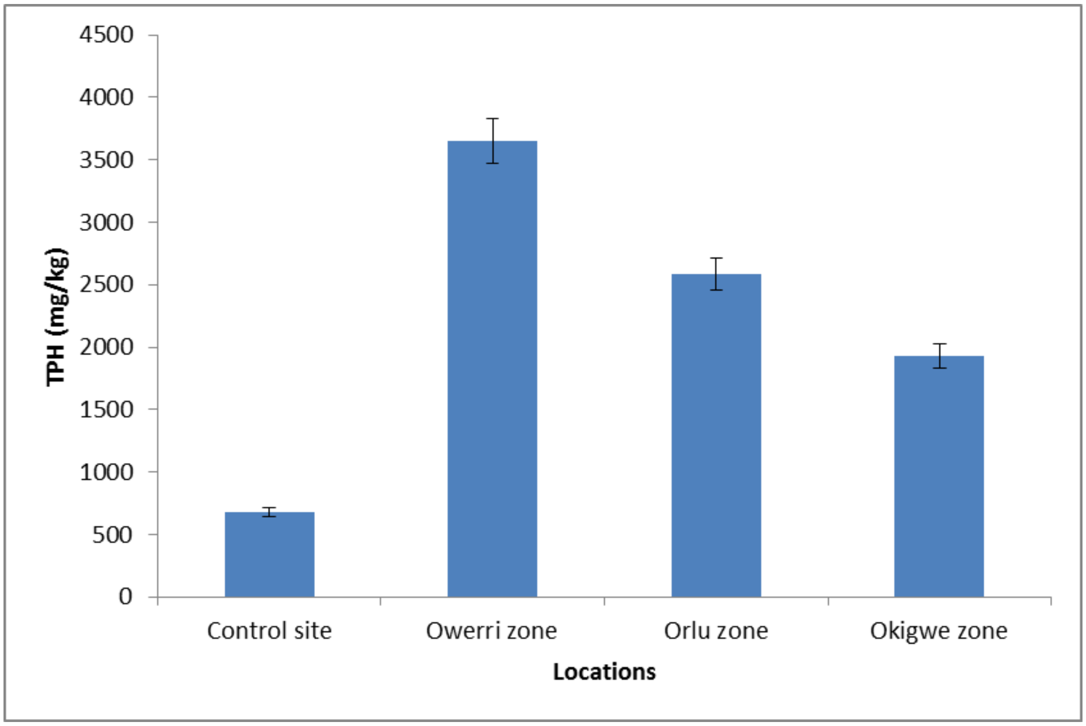

Figure 4.1: The Concentrations of Total Petroleum Hydrocarbons in spent engine oil polluted and unpolluted soils of Owerri, Orlu and Okigwe mechanic villages. ***Values are mean+ SD of triplicate samples.***

4.1.11 Effects of spent engine oil on early seedling estabilishment of test plants

4.1.11.1 Effects on germination

Percentage seed germination and average inhibitory effects on test plants (*Zea mays, G. max* and *V. unguiculata*) exposed to different concentrations of spent engine oil are presented in Figures 4.2 and 4.3. Generally, more seeds germinated from 0% concentration than in the other treatments. It was observed that the percentage germination fluctuated among the accessions as follows: *Zea mays* (78%), *G. max* (71.6%) and *V. unguiculata* (89.4%) respectively. In 4% concentration, seeds of *V. unguiculata*, *Zea mays*, and *G. max* more seeds emerged within 5 days of exposure in comparison with other treatments. More than 50% of the seeds of *V. unguiculata* emerged at 4% treatment level. Drastic reduction in germination percentage was observed at 8%, 12%, and 16% treatment when compared with seeds grown in control. There was a significant difference ($p<0.05$) in germination percentage among the test plants in the different treatments. The percentage germination of *G. max* at 8% treatment level was significantly lower relative to the control at 99.9% level of significance while the percentage germination of the seeds of *Zea mays* and *V. Unguiculata* were significantly lower at 99% significance level. At 12% treatment level, seeds of *G. max* performed better with values of 39.5% compared with *V. unguiculata* (31.7%) and *Zea mays* (23.6%) respectively. The percentage germination of the seeds of *Zea mays* was significantly lower in 16% treatment followed by *G.max* and *V. unguiculata* in that order. *V. unguiculata* showed strong tolerance to spent engine oil contamination with about 18.7% germination percentage at the highest concentration (16%) followed by *G. max* (17.8%) and *Zea mays* (15.2%).

As shown in figure 4.3, the germination inhibition of maize at 0% was quite minimal with a value of 2.09%. However, when the concentration reached 8%, germination inhibition progressed to 57.7%. The 4% concentration did not inhibit seed germination rate but slightly stimulated germination rate. However, a rapid increase in inhibition was found between 12 and 16 % concentration. The inhibition rate at 12% concentration was 88.5% which exceeded 50% indicating a significant effect on maize seedling establishment. The inhibition rate reached 89.8% at 16% concentration. No germination was observed inhibition was observed in 0% concentration in *V. unguiculata*. However, the rate of inhibition increased with elevated levels of spent engine oil concentration. Average inhibition rate was 48.9% at 8% concentration, not exceeding 50%, implying a strong tolerance of *V. unguiculata* to spent engine oil contamination. Generally, all concentrations had inhibitory effect on the germination rate with increasing concentration. Statistically significant inhibition rate was observed at 16% concentration, with a value of about 88.7% exceeding 50% at this level. 0% concentration resulted to no observable inhibition of germination in *Glycin max.* However, germination inhibition was observed with increase in concentration. The rate of inhibition at 4% concentration was 37.9% suggesting mild tolerance of *Glycin max* to spent engine oil contamination. The highest inhibition rate was recorded in 16% concentration with a value of 87.9% which exceeded 50% at this level.

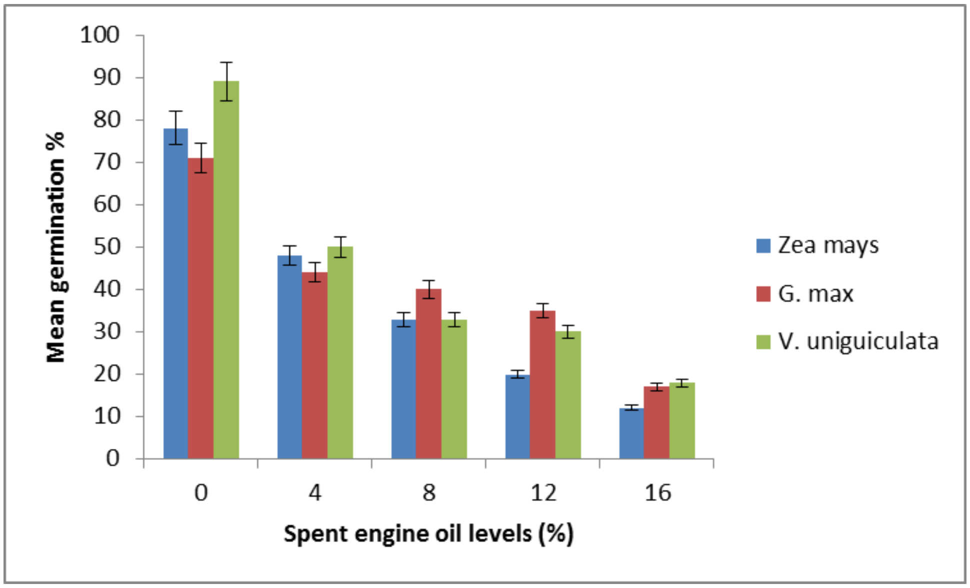

Figure 4.2: Percentage seed germination *Zea mays*, *G. max* and *V. unguiculata* exposed to spent engine oil polluted and unpolluted soils

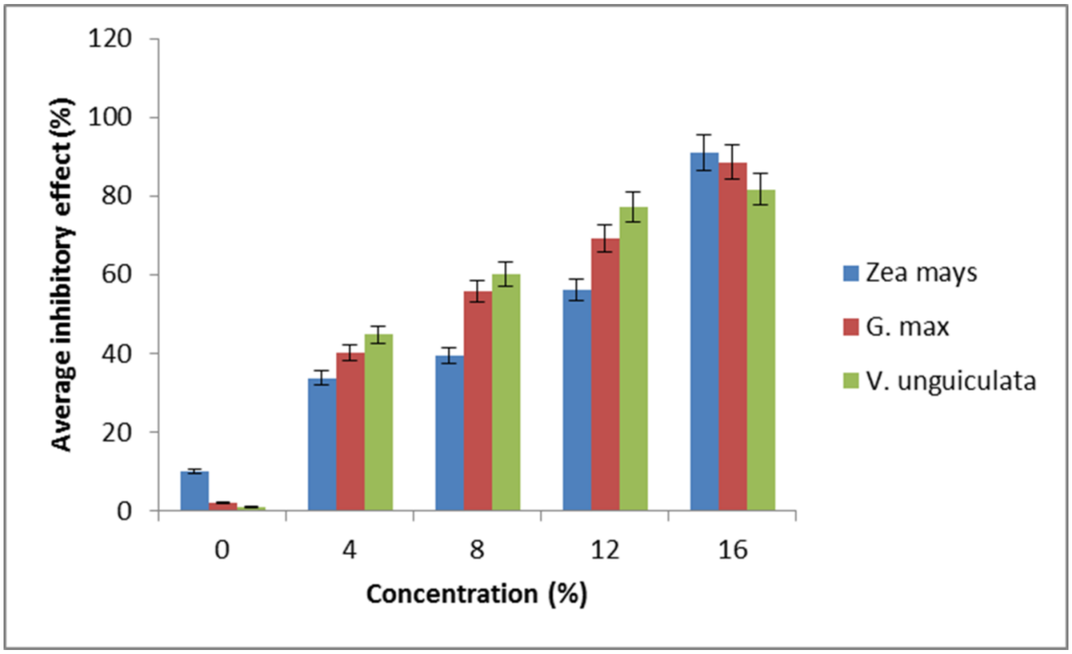

Figure 4.3: Average inhibitory effects of plant species exposed to different concentrations of spent engine oil.

4.1.11.2 Effects on early seedling establishment

The effect of spent engine oil treatments on early seedling establishment is displayed in presented in Table 4.11. Application of spent engine oil had a dose-dependent inhibitory effects on all the parameters assessed. Generally, the speed (rate) of germination from 0% was more vigorous than other treatments. The germination speed (rate) of *Zea mays* was generally more affected by all treatments than other species used in the bioassay. There was a significant difference ($p<0.05$) in the rate of germination in all the seedlings. There was a monotonic dose-dependent relationship in shoot length of among the plants. However, 0% treatment level recorded the highest shoot length with mean values of 11.6±1.16 while the lowest mean value of 5.7±1.01 was observed at 16% treatment level for *Zea mays*. There was no significant difference ($p>0.05$) in shoot lengths between 0% and 4% treatments in for *Zea mays* and *G. max*. The overall mean shoot lengths in *Zea mays, G. max* and *V. unguiculata* were significantly different from each at $p<0.05$ respectively. The shoot lengths of the different plant species were significantly shorter in 4% treatment level than the control ($p<0.05$). Generally, there was marked decrease in shoot length with corresponding increase in treatments.

The mean root lengths of the different plant species were generally higher in 0% than other treatment levels. No significant difference ($p>0.05$) exist between mean root lengths of *Zea mays* and *G. max* at 0 and 4% treatment levels. However, the root length of seedlings treated with 8% spent engine oil was longer than the roots of the seedlings of 12 and 16% contaminant levels respectively. The root lengths of *V. unguiculata* were significantly higher at16% treatment level than *Z. mays* and *G. max* treated at similar level. The seedling root lengths at 4%, 8%, 12% and 16% treatments were significantly shorter than the control treatment ($p<0.05$).

Seedling mean fresh and dry weights were affected by spent engine oil exposure in a concentration-dependent manner. The control recorded the highest mean seedling fresh and dry weights in all the test plants. No significant difference ($p>0.05$) was observed between 0% and 4% treatment among the test plants with respect to seedling fresh and dry weights. At 8% and 12% treatment levels, there was no significant difference ($p<0.05$) in mean seedling fresh weights in all the test plants. However, at 16% treatment level, a marked significant difference ($p>0.05$) was observed. Seedling dry weights followed a similar trend as the fresh weights. There was no significant difference ($p<0.05$) between mean seedling dry weights in *Zea mays* and *V. Unguiculata.* Also, there is no significant different ($p<0.05$) between seedlings dry weights for *G. max* at 8% and 12% treatment levels.

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Table 4.11: Germination rate, shoot and root length, seedling dry and fresh weights of test plants exposed to different concentrations of spent engine oil

Conc.(%)	Varieties	Germination rate	Shoot length (%)	Root Length (cm)	Seedling fresh Weight (kg)	Seedling dry Weight (kg)
0		15 ± 1.8^{a}	11.6 ± 1.16^{a}	9.4 ± 1.14^{a}	7.7 ± 1.09^{a}	6.3 ± 1.06^{a}
4	***Zea mays***	14 ± 1.24^{a}	11.0 ± 1.15^{a}	8.1 ± 1.11^{a}	6.9 ± 1.03^{a}	5.8 ± 0.84^{a}
8		9 ± 1.13^{b}	8.5 ± 1.13^{b}	7.6 ± 1.10^{ab}	5.3 ± 0.82^{b}	5.6 ± 0.71^{a}
12		4 ± 0.67^{c}	7.4 ± 1.07^{b}	6.3 ± 1.06^{b}	4.9 ± 0.72^{b}	4.3 ± 0.70^{b}
16		3 ± 0.41^{c}	5.7 ± 1.01^{c}	3.5 ± 0.43^{c}	3.3 ± 0.60^{c}	2.9 ± 0.44^{c}
4	***G. max***	14 ± 1.24^{a}	13.5 ± 1.12^{a}	7.8 ± 1.11^{ab}	6.6 ± 1.08^{b}	5.1 ± 0.69^{a}
8		13 ± 1.19^{a}	11.8 ± 1.17^{a}	6.2 ± 1.05^{b}	5.8 ± 1.02^{b}	4.3 ± 0.70^{b}
12		10 ± 1.14^{b}	9.0 ± 1.15^{b}	5.4 ± 0.84^{b}	5.1 ± 0.79^{b}	4.0 ± 0.67^{b}
16		5 ± 0.73^{c}	4.2 ± 0.69^{c}	4.1 ± 0.68^{c}	3.2 ± 0.40^{c}	$2\ 3\pm0.02^{c}$
4	***V.unguiculata***	14 ± 1.24^{a}	12.7 ± 0.03^{d}	9.1 ± 1.13^{a}	6.6 ± 1.9^{b}	5.1 ± 0.81^{a}
8		12 ± 1.17^{b}	11.5 ± 1.17^{a}	8.3 ± 1.11^{a}	5.6 ± 1.00^{b}	4.3 ± 0.70^{b}
12		8 ± 1.11^{b}	9.3 ± 1.16^{b}	7.7 ± 1.10^{ab}	5.4 ± 0.84^{b}	4.0 ± 0.67^{b}
16		3 ± 0.63^{c}	4.1 ± 0.68^{c}	5.2 ± 0.81^{b}	3.2 ± 0.59^{c}	2.9 ± 0.44^{c}

Values are mean+ SD of triplicate samples; Mean along the column having different superscript of letters differ significantly at $P\leq 0.05$ level according to Duncan Multiple Range Test (DMRT).

4.1.12 Burrowing responses of earthworm exposed to various concentrations of spent engine oil

The response of earthworm exposed to various concentration of spent engine in soil is displayed in Table 4.12 while the lethal concentration (LC_{50}) values at 7 and 14 days acute toxicity assay is depicted in Table 4.13. Results obtained showed that burrowing was concentration-dependent in all the treatments. Mean burrowing ranged from 51 to 21.3 minutes within the first 7 days of exposure. It was further observed that burrowing ranged from 46.3 to 20.3 after 14 days of exposure. There was a progressive decrease in burrowing vigour with increase in treatment. In treatment 0 to 8%, the earthworms burrowed within a relatively shorter time but burrowing was slowed when concentration reached 12% and 16% respectively.

The 7 and 14 day median lethal concentration (LC_{50}) of earworms exposure to various concentrations of spent engine oil is displayed in Table 4.13. There was a strong positive correlation ($r = 0.89$) between 7 and 14-day LC50 values. The 7th day LC_{50} values for 4, 8, 12, 16% treatment was 605, 742, 813 and 942 mg/kg respectively. The LC_{50} value for 16% treatment decreased from 942 to 912 mg/kg at the 14th day of exposure. The corresponding LC50 for the 14 day exposure were 598, 728, 794 and 912 mg/Kg. The result further showed that the LC_{50} value for 7 day exposure was the most toxic with a value of 942 mg/kg. Generally, the Probit line equation revealed a strong positive correlation between concentration and mortality rate in a dose-dependent manner. By implication, mortality rate increased with increase in concentration.

Table 4.12 : The time taken for earthworm to burrow in spent engine oil polluted and unpolluted soil

Days	Conc. (%)	Rep1	Rep 2	Rep 3	Mean
7	0	22	20	22	21.3
	4	38	36	37	37
	8	52	50	51	51
	12	NB	61	63	41.3
	16	NB	NB	62	20.7
14	0	22	20	19	20.3
	4	30	32	34	32
	8	46	45	48	46.3
	12	NB	49	NB	16.3
	16	42	52	50	48

Table 4.13: The Lethal concentration (LC_{50}) of SEO that affected earthworm exposed at 7 and 14 days of exposure.

Days	Conc. (%)	LC_{50} (mg/kg)	95% confidence limit		Slope ± S.E	D.f.	Probit line equation
			Lower	Upper			
7	0	0.00	0.00	0.00	0.00 ± 0.00	2	-
	4	605	116.56	245.44	3.32 ± 0.68	2	Y=3.32+5.47X
	8	742	125.72	250.11	5.65 ± 0.79	2	Y=5.65+6.94X
	12	813	211.78	271.97	6.63 ± 0.93	2	Y=6.63+7.22X
	16	942	316.56	338.22	8.21 ± 1.16	2	Y=8.21+9.46X
14	0	0.00	0.00	0.00	0.00 ± 0.00	2	-
	4	598	126.20	251.82	3.46 ± 0.71	2	Y=3.46+5.73X
	8	728	141.04	263.03	5.71 ± 0.84	2	Y=5.71+7.01X
	12	794	236.23	282.28	6.79 ± 0.99	2	Y=6.79+7.48X
	16	912	343.33	367.07	8.37 ± 1.23	2	Y=8.37+9.73X

LEGEND: D.F. = degree of freedom; S.E= standard error; LC50=lethal concentration. Conc. = concentration.

4.1.13 Total microbial load from spent engine oil polluted and unpolluted soils

The total heterotrophic bacteria (THB) load from spent engine oil polluted and unpolluted soil samples are displayed in Table 4.14. Average of 4.35 X 10^6 CFU/g were obtained from spent engine oil polluted site with corresponding value of 3.04 X 10^5 CFU/g for the reference site (control). There were noteworthy variations in the values for the two sampling sites with no significant difference ($p<0.05$) between polluted and unpolluted soil samples. A total of 7 bacteria species were isolated from the spent engine polluted soil while 5 bacteria species were isolated from unpolluted site. They were determined to be of bacteria genera *Pseudomonas, Bacillus, Acinetobacter, Enterobacter, Neisseria sp., E. coli, Streptococcus sp.,* and *Klebsiella sp.* respectively. The cellular shape and biochemical characteristics of the isolates were all congruent with these identifications. The probable identity of bacteria isolates and their corresponding domiciled microorganisms in polluted and unpolluted soil samples are displayed in Table 4.15.

Table 4.14: Total heterotrophic bacteria count from spent engine polluted and unpolluted soils

Sample ID	Sample type	GPS location	THC (CFU/g)
PS	Soil	05º17’39.3”N 007º05’03.0’’E	4.37 X10^6
US	Soil	005^0.6531’33’N 007^0.13116’99’’E	3.04X 10^5

Legend: PS= Polluted soil; UP= Unpolluted soil

Table 4.15: Bacteria isolates from spent engine oil polluted and unpolluted soils

Isolates	Polluted soil	Unpolluted soil
Neisseria sp.	+	+
E. coli	+	+
Streptococcus sp.	+	-
Pseudomonas sp.	+	+
Bacillus sp.	+	+
Acinetobacter sp.	-	+
Enterobacter sp.	+	-
Klebsiella sp.	+	-
TOTAL	**7**	**5**

Note: + and – Present and Not present.

4.1.14 Biotolerance of bacteria isolates exposed to different concentrations of spent engine oil

Biotolerance of bacteria species exposed to different concentrations of spent engine oil is displayed in Figures 4.4, 4.5, 4.6 and 4.7 respectively. As can be seen from the figures, optimum growth of the microbes as indicated by average optical density values were recorded at 4% treatment (lowest contaminant level) indicating that the microbes might have used up the spent engine as sole carbon source in the medium. There was significant difference ($p < 0.05$) in absorbance rate among all the concentrations relative to the control. The absorbance values indicated that *Pseudomonas species* (Figure 4.4) had a logarithmic increase in cell numbers within the first 72 hrs of incubation but decreased sharply with increase in concentration. The optical density ranged from 0.9 to 2.1 for *Pseudomonas species*. The absorbance of *Bacillus* species exposed to varying levels of spent engine oil as indicated in Figure 4.5 showed a stepwise decreased with increase in treatment level. The optical density ranged between 2.0 to 2.06. *Acinetobacter* and *Enterobacter* species followed a similar pattern in absorbance under spent engine exposure as shown in Figures 4.6 and 4.7. The organisms showed a logarithmic increase in absorbance rate which was concentration dependent. Generally, the organisms showed higher absorbance rate with decrease in contaminant level.

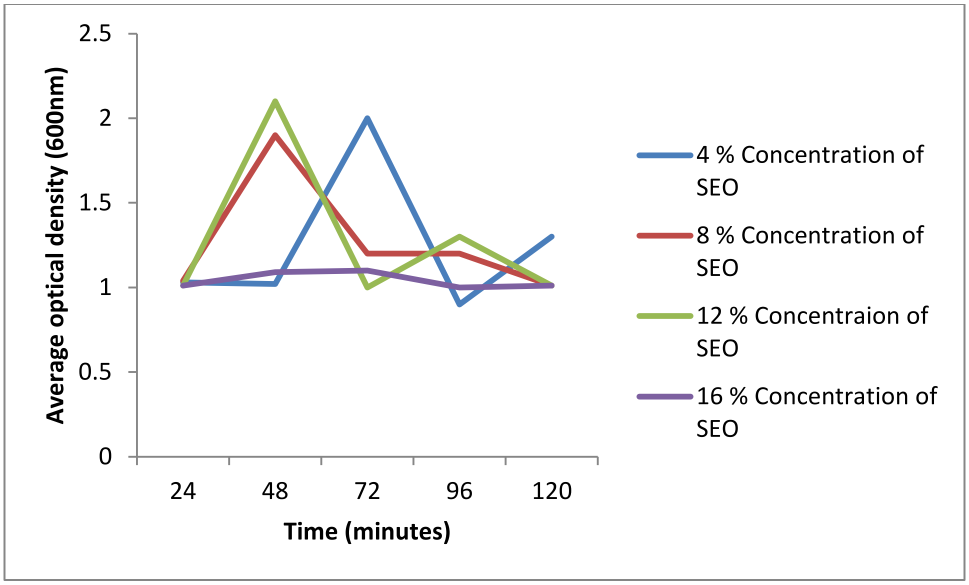

Figure 4.4: Biotolerance of *Pseudomonas* species exposed to different concentrations of spent engine oil polluted soil

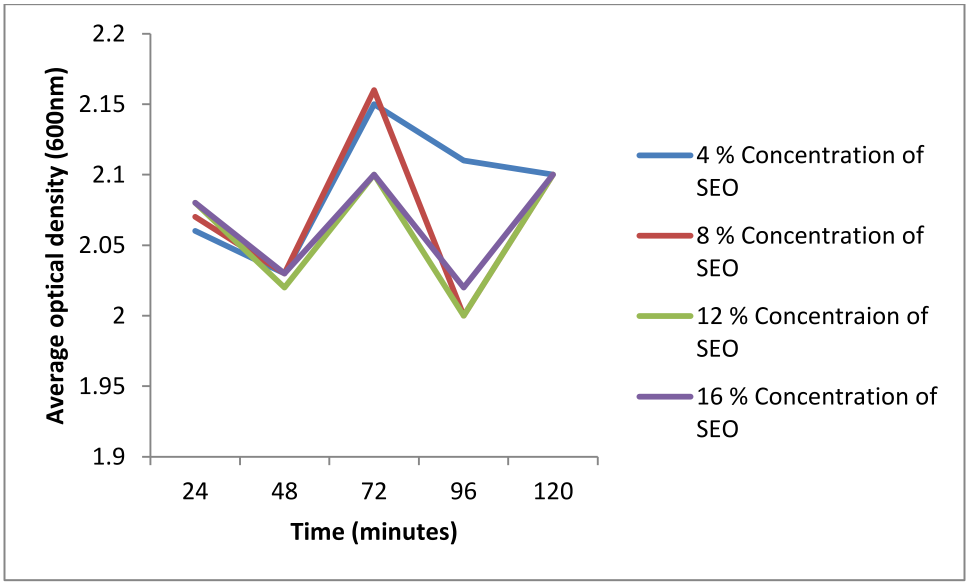

Figure 4.5: Biotolerance of *Bacillus species* exposed to different concentrations of spent engine oil polluted soil

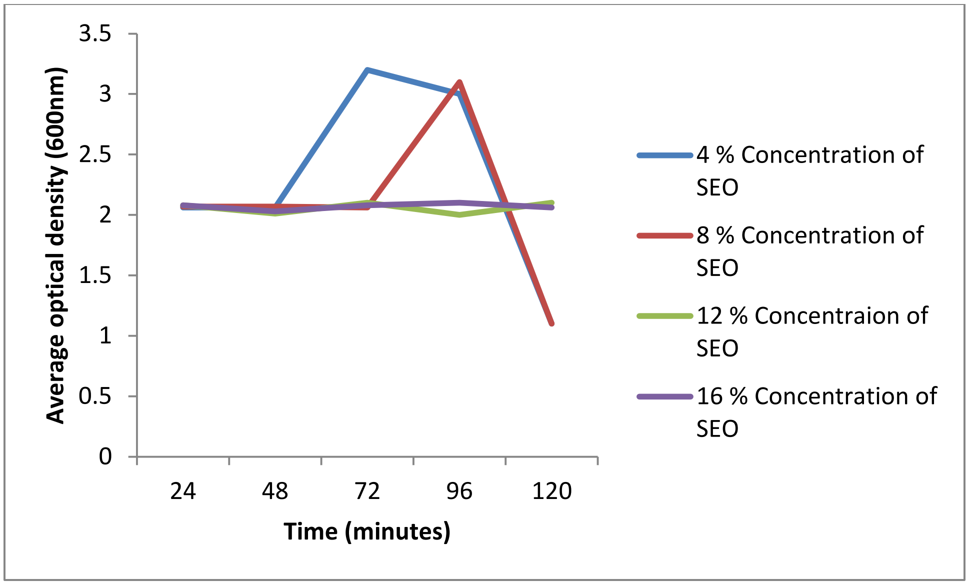

Figure 4.6: Biotolerance of *Acinetobacter species* exposed to different concentrations of spent engine oil polluted soil

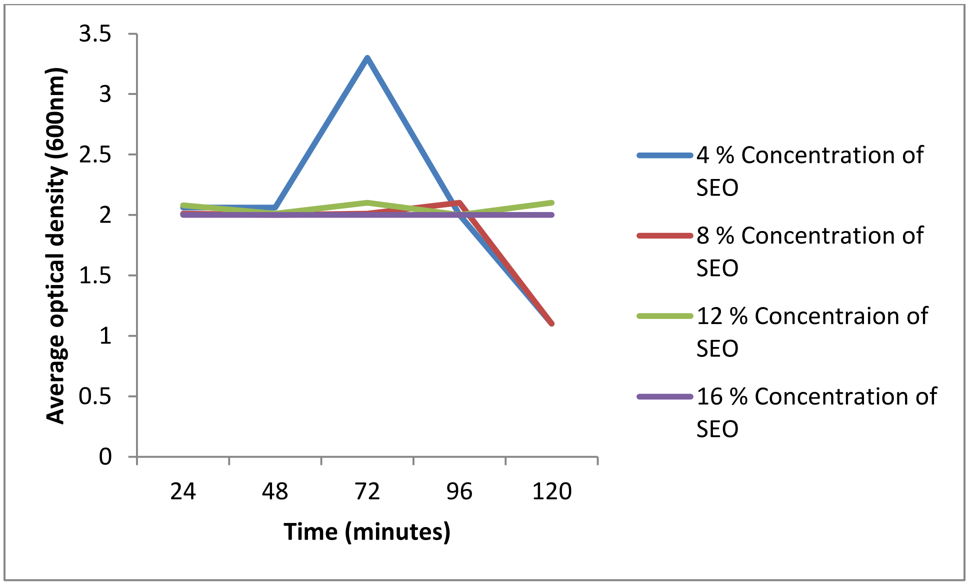

Figure 4.7: Biotolerance of *Enterobacter species* exposed to different concentrations of spent engine oil polluted soil

4.1.15 Toxicity and ecological risk characterization of spent engine oil to plants, earthworm and microorganisms

The Ecological risk Characterization based on acute toxicity tests, Measured Environmental Concentration with their corresponding risk quotients for plants, earthworm and microorganisms is presented in Table 4.16. In other to characterise the actual risk the test organisms are exposed to in the ecosystem, an estimate of the incidence of adverse effects that occurred as a result of spent engine oil exposure was determined at the Measured Environmental Concentration. The environmental effects were characterised by the calculation of Predicted No Effect Concentration (PNEC) based on average EC_{50} (Effective Concentration that inhibited 50% growth of the organisms) and LC_{50} (Lethal Concentration that inhibited 50% growth in the organisms) values obtained during ecotoxicity tests. In this study, the ecotoxicological risk was estimated using the ratio between Measured Environmental Concentration (MEC) and Predicted No Effect Environmental Concentration (PNEC) to obtain the corresponding Risk Quotients (RQ). By convention, $RQ<0.1$ indicates that no adverse effect is expected for the organism evaluated in its environment; if $0.1>RQ>1$, a potentially adverse effect should be considered, but at $1>HQ>10$, a moderate hazard must be expected. Additionally, $HQ>10$ indicates that a high risk is probable (EMEA, 2006).

As shown in Table 4.16, values of Risk Quotient for *Zea mays*, *V. unguiculata, G. max* and earthworm were all above unity (>1) indicating a potential adverse effects attributable to spent engine contamination to these plants investigated. This suggested that it is most likely that adverse ecological effects will occur when concentration of spent engine in soil for *Zea mays, V. unguiculata*, *G. max* and *earthworms* is higher than 15.33%, 13.04%, 17.20% and 48.03% (for earthworm) based on the estimated PNEC values. The microorganisms differed slightly in biotolerance to spent engine oil exposure based on estimated risk quotients. Exposure to spent engine oil posed minimal risk to *Pseudomonas sp., Bacillus sp.,* and *Acinetobacter sp.,* at the estimated PNECs with values less than unity (<1). Risk quotient values for *Enterobacter sp.* on the other hand indicated a high risk with values above 1. For the tested organism for which risk quotients of spent engine oil exposure were lower than 1, the implication is that ecological risk expected would be minimal or nil. On the other hand, when the risk quotient is greater than 1, it is considered that the organisms under study would be exposed to some level of risks in the ecosystem.

Table 4.16: **Ecological risk Characterization based on acute toxicity testing and Measured Environmental Concentration for plants, earthworm and microorganisms**.

Species Exposed	A.F.	PNEC(%)	$MEC_{(mg/kg)}$	MEC/PNEC	RQ
Zea mays	1000	15.33	50	**3.62****	<0.1RQ>0.1
V. unguiculata	1000	13.04	50	**3.83****	<0.1RQ>0.1
Glycin max.	1000	17.20	50	**2.09***	<0.1RQ>0.1
Earthworm	1000	48.03	50	**1.04***	<1RQ>1
Pseudomonas sp.	10	97.02	50	0.15	< 1
Bacillus sp.	10	83.01	50	0.60	<1
Acinetobacter sp.	10	73.10	50	0.68	<1
Enterobacter sp.	10	30.42	50	**1.64***	>1

Legend: *AF = assessment factor, MEC = measured environmental concentration, RQ = risk quotient.*

4.1.16 Effects of spent engine oil on growth of *C. odorata, A. africana* and *A. compressus*

4.1.16.1 Effects on plant height

The results of the plant heights for *C. odorata*, *A. africana* and *A. compressus* exposed to different concentrations of spent engine oil are presented in Tables 4.17. There was an observable significant decrease in mean plant height with a corresponding increase in spent engine oil levels across the weeks. The mean height of the control (0%) was significantly higher than that of plants exposed with 4, 8, 12 and 16% concentration of spent engine oil at $p<0.05\%$. Although mean plant height increased as the week progressed from 4WAP to 12WAP as recorded in treatment levels. A marked decrease in plant height was observed with increase in treatment in all the test plants. However, significant reduction was observed from the 10th to 12th WAP with 16% treatment level having the highest negative effect on the plant height followed by 12% treatment whereas the highest plant height was recorded in the control followed by treatment 4% level. There was no significant difference ($p<0.05$) between 4% and 8% treatment levels for *C. odorata* and *A. africana*, while *A. compressus* showed no significant difference ($p<0.05$) between 8% and 12% treatment levels respectively.

Table 4.17: Mean height of *C. odorata, A. africana* and *A. compressus* exposed to different concentrations of spent engine oil polluted and unpolluted soil after 12 weeks

Treats(%)	Varieties	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
0		9.0±1.60^{a}	10.1±1.64^{b}	12.8±1.77^{a}	14.8±1.82^{c}	16.8±1.87^{c}	20.3^{c}±2.01^{c}
4	*C. odorata*	6.8±1.23^{b}	7.8±1.15^{c}	9.5±1.20^{c}	12.3±1.23^{d}	14.6±1.59^{d}	18.3± 1.87^{d}
8		6.7±1.22^{b}	7.1±1.29^{c}	8.6±1.13^{c}	11.9±1.16^{d}	13.0±1.46^{d}	18.0±1.82^{d}
12		5.9± 1.17^{b}	6.9±1.05^{c}	8.0±1.07^{c}	10.8±1.10^{e}	12.8±1.34^{d}	16.8±1.77^{e}
16		4.8± 1.11^{c}	5.3±1.01^{d}	7.2± 1.05^{c}	10.1± 1.08^{c}	12.9±1.25^{c}	14.2±1.73^{e}
4	*A. africana*	5.3± 1.24^{c}	8.3±1.09^{c}	11.2± 1.11^{d}	13.2±1.58^{c}	17.2±1.74^{c}	20.8±2.06^{c}
8		5.0± 1.15^{c}	7.2±1.06^{c}	10.9±1.09^{b}	12.9±1.35^{d}	16.3±1.68^{c}	19.3±1.90^{c}
12		4.2± 1.00^{c}	7.0±1.04^{c}	9.9±1.24^{c}	12.0±1.29^{b}	16.2±1.65^{c}	17.3±1.79^{c}
16		3.0± 0.93^{d}	5.2±1.26^{d}	9.0±1.27^{c}	11.2±1.37^{c}	13.2±1.53^{d}	15 0±1.76^{e}
4	*A. compressus*	11.1± 1.76^{a}	13.2±1.79^{a}	15.2± 1.83^{a}	19.3±1.98^{a}	24.4±2.27^{a}	28.8±2.34^{a}
8		10.3± 1.67^{a}	12.8±1.79^{a}	13.2± 1.81^{b}	17.3±1.94^{b}	21.9±2.19^{b}	25.3±2.23^{b}
12		9.9±1.71^{a}	11.3±1.73^{b}	11.9±1.77^{b}	16.9±1.84^{b}	20.4±2.17^{b}	24.1±2.21^{b}
16		7.7± 1.31^{b}	10.3± 1.43^{b}	10.8±1.45^{b}	14.8±1.61^{b}	18.1±1.86^{c}	20.3±2.01^{c}

Values are means±S.E. Mean along the column having different superscript of letters differ significantly at P≤ 0.05 levels according to Duncan Multiple Range Test (DMRT).
WAP = weeks after planting

4.1.16.2 Effects on number of leaf

With respect to mean number of leaf, the highest number of leave was recorded in control (unpolluted soil) and this was statistically ($p<0.05$) different in comparison with other treatments as shown in Tables 4.18. Significant marked reduction was observed in soils treated 16% concentration compared to other treatments. There was a significant difference ($p>0.05$) between the various treatments and the control. Trend in marked leaf reduction was as follows: *A. compressus> C. odorata> A. africana.* Also observed in the leaf of test plants grown in higher concentration of spent engine oil are defoliation, yellowing of leaf, chlorosis and necrosis.

Table 4.18: Number of leaf of *C. odorata*, *A. africana* and *A. compressus* exposed to different concentrations of spent engine oil polluted and unpolluted soil after 12 weeks

Treats(%)	Varieties	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
0		11 ± 1.32^{a}	12 ± 1.35^{b}	13 ± 1.36^{d}	15.8 ± 1.47^{c}	19 ± 1.54^{ab}	23 ± 2.22^{d}
4	*C. odorata*	5 ± 0.22^{b}	5 ± 0.22^{c}	6 ± 1.24^{e}	10 ± 1.29^{de}	14 ± 1.50^{c}	16 ± 1.58^{ef}
8		6 ± 1.24^{bc}	7.1 ± 1.26^{bc}	8.6 ± 1.30^{d}	11.9 ± 1.34^{d}	13 ± 1.41^{c}	18 ± 1.52^{e}
12		4 ± 0.21^{c}	4 ± 0.21^{c}	4 ± 0.21^{f}	9 ± 1.32^{e}	13 ± 1.36^{c}	14 ± 1.50^{f}
16		8 ± 1.27^{b}	1 ± 0.01^{d}	3 ± 0.04^{f}	7 ± 1.25^{e}	11 ± 1.34^{d}	11 ± 1.34^{g}
4	*A. africana*	11 ± 1.3^{a}	13 ± 1.36^{a}	15 ± 1.55^{a}	19 ± 1.58^{a}	20 ± 1.74^{a}	33 ± 2.06^{a}
8		10 ± 1.29^{a}	10 ± 1.29^{b}	14 ± 1.50^{ab}	17 ± 1.35^{b}	18 ± 1.68^{b}	30 ± 1.90^{b}
12		8 ± 1.27^{b}	9 ± 1.32^{b}	13 ± 1.36^{b}	14 ± 1.29^{c}	15 ± 1.65^{b}	28 ± 1.79^{c}
16		7 ± 1.26^{b}	8 ± 1.27^{b}	11 ± 1.27^{c}	12 ± 1.35^{d}	13 ± 1.36^{c}	24 ± 1.76^{d}
2	*A. compressus*	3 ± 0.20^{d}	5 ± 0.22^{c}	6 ± 1.24^{e}	8 ± 1.27^{e}	9 ± 1.54^{d}	11 ± 1.32^{g}
8		1 ± 0.01^{d}	2 ± 0.18^{d}	3 ± 0.20^{f}	4 ± 0.21^{f}	4 ± 0.21^{e}	5 ± 0.22^{h}
12		2 ± 1.18^{d}	2 ± 0.18^{d}	3 ± 0.20^{f}	3 ± 0.20^{f}	4 ± 0.21^{e}	4 ± 0.21^{h}
16		1 ± 1.01^{d}	2 ± 0.18^{d}	3 ± 0.20^{f}	3 ± 0.20^{f}	4 ± 0.21^{e}	4 ± 0.21^{h}

Values are means±S.E. Mean alone the column having different superscript of letters differ significantly at P≤ 0.05 level according to Duncan Multiple Range Test (DMRT).WAP= Weeks After Planting.

4.1.16.3 Effects on root number fresh and dry weight of *C. odorata, A. africana* and *A. compessus* exposed to different concentration of spent engine oil

There was a progressive decrease in root number with increase in level of contaminant as shown in Table 4.19. The highest root number, fresh and dry weights were observed in control in comparison with other treatments. There was gross reduction in root number in *A. compressus, C. odorata* and *A. africana* compared with the control. *A. africana* produced the highest root number followed by *A. compressus* and then *O. odorata*. There was a significant difference ($p<0.05$) among the root number in all the test plants. The responses of the plants with regard to fresh and dry weights varied consistently with a corresponding increase in treatment than control.

Table 4.19: Mean Number of root, fresh and dry weight of *C. odorata, A. africana* and *A.* compessus exposed to different concentration of spent engine oil

Conc. (%)	Varieties	No of Roots	Fresh Weight (g)	Dry Weight (g)
0	***C. odorata***	22±1.43^{ab}	20.8±1.26^{b}	17.7±1.19^{b}
4		20±1.33^{c}	19.8±1.2^{bc}	16.9±1.18^{bc}
8		19±1.22^{c}	16.6±1.15^{d}	16.3±1.14^{bc}
12		17± 1.17^{d}	13.8±1.11^{e}	14.8±1.12^{c}
16		15± 1.11^{e}	27.3±1.31^{a}	20.3± 1.24^{a}
4	***A. africana***	23± 1.24^{a}	19.9±1.20^{bc}	16.8± 1.14^{bc}
8		21± 1.15^{bc}	19.0±1.19^{bc}	15.9±1.13^{c}
12		19± 1.00^{c}	17.9±1.17^{d}	14.5±1.12^{c}
16		15± 0.93^{e}	12.2±1.08^{e}	11.3±1.05^{d}
4	***A. compressus***	16± 1.76^{e}	21.8±1.28^{b}	18.6± 1.23^{b}
8		20± 1.67^{c}	21.0±1.27^{b}	16.3± 1.13^{bc}
12		18±1.71^{bc}	18.2±1.18^{bc}	13.3±1.10^{c}
16		21± 1.31^{b}	15.3± 1.13^{d}	11.2±1.04^{d}

Values are means±S.E. Mean alone the column having different superscript of letters differ significantly at P≤ 0.05 level according to Duncan Multiple Range Test (DMRT).

4.1.17 Metal accumulation in *C. odorata, A. africana* and *A. compressus* after 12 weeks of exposure to spent engine oil

Results of the analysis of selected heavy metals in below (soil) and aboveground (root, leaf and shoot) of *C. odorata, A. compressus* and *A. africana* after 12 weeks exposure to different concentrations of spent engine oil is presented in Figures 4.8 to 4.17 while Figure 4.18 shows the TPH accumulation in test plant tissues. As demonstrated in the figures, there was marked variation in heavy metal accumulation pattern in the different plant tissues (root, leaf and shoot). Amount of Iron extracted by the plant tissues varied. Iron accumulation in the root was highest in *C. odorata* with mean value of 27.39mg/kg, followed by *A. africana* (27.21mg/kg) while the lowest level accumulation of Fe was recorded in *A. compressus* with a mean value of 24.31mg/kg. Some level of Fe was also observed in the leaf. *C. odorata* accumulated 236.91mg/kg, while *A. africana* and *A. compressus* recorded 22.41mg/kg and 236.9mg/kg respectively. The highest phytoextraction rate was observed in *A. africana* and *C. odoara* while the least was recoreded in *A. compressus*. At the shoot tissue of the plants, Iron content fluctuated as follows: *C. odorata* (26.63mg/kg), *A. africana* (24.92mg/kg) and *A. compressus* (22.34mg/kg) respectively (Figure 4.8).

By the end of 12 weeks of the experiment, no amount of Cobalt was detected in the soil samples. The root system accumulation Cobalt by *C. odorata, A. africana* and *A. compressus* were 0.053mg/kg, 0.0143mg/kg and 0.0141mg/kg. 0.082mg/kg was recorded in the leaf of *C. odorata* with no values recorded for *A. africana* and *A. compressus* in the leaf (Figure 4.9). However, it was observed that the highest level of Cobalt (17.43 mg/kg) was found in the shoot of *C. odorata* followed by *A. compressus* with mean value of 0.142mg/kg and *A. africana* (0.053mg/kg) respectively.

Cadmium accumulation was recorded mostly in the root of *C. odorata* and *A. africana* (0.091mg/kg) while the least accumulation in root was recorded in *A. compressus* (0.068mg/kg). Equal level of Cadmium was observed in the shoot for each plant (0.078 mg/kg) as shown in Figure 4.10. The highest accumulation of Arsenic was observed in the shoot by *C. odorata* (12.44 mg/kg) while the least level (0.127 mg/kg) was recorded in the leaf. A similar value (0.127mg/kg) was also recorded for *A. africana* in the leaf (Figure 4.11).

Uptake of Manganese by *C. odorata* was more pronounced in the shoot when compared with other test plant parts. Equal mean values of 0.543mg/kg were recorded in the roots of the three plants under study. However, the leaf recorded equal mean values of 0.668mg/kg for *C. odorata* and *A. africana* (Figure 4.12).

The mean Lead concentration values in root were: *C. odorata* (0.063mg/kg); *A. compressus* (0.064mg/kg) and *A. africana* (0.048mg/kg). Increased level of accumulation in the aerial part of the plants was also observed with respect to Lead (Figure 4.13). The leaf of *C. odorata*, *A. compressus* and *A. africana* had 0.348mg/kg, 0.094mg/kg and 0.348mg/kg respectively. With respect to the shoot accumulation of Lead, *C. odorata* recorded the highest accumulation with mean value of 0.348mg/kg followed by *A. compressus* (0.064mg/kg) and *A. africana* (0.053mg/kg).

Ability of the plant species to phytoextract Zinc from soil also varied. Mean Zinc content in roots for *C. odorata*, *A. compressus* and *A. africana* were 1.038mg/kg, 4.69mg/kg and 4.266mg/kg; the leaf content were 2.203mg/kg, 4.495mg/kg and 3.761mg/kg. The shoot accumulations were 4.49mg/kg, 12.66mg/kg and 11.481mg/kg (Figure 4.14). Concentration of Nickel in root, leaf and shoot tissues varied as follows: *C. odorata* (3.55mg/kg), *A. compressus* (27.34mg/kg) and

2.761mg/kg; 28.23mg/kg, 27.34mg/kg and 22.17mg/kg; while 28.23mg/kg was observed in the shoot for *C. odorata* with nil recorded in *A. compressus* and *A. africana* (Figure 4.15)

The highest level of Copper in soil treated with *C. odorata* was high when compared with other test plants. In the root, concentration of Copper in *C. odorata, A. compressus* and *A. africana* are 5.72mg/kg, 0.068mg/kg, and 0.04mg/kg (Figure 4.16). Mean concentration of Copper also varied in the leaf and shoot as follows: 22.63mg/kg, 0.068mg/kg and 22.63mg/kg, 0.04mg/kg and 0.599mg/kg. The least accumulation of copper was recorded in *A. compressus* (0.04 mg/kg).

The uptake of chromium by the test plants indicated that *C. odorata* accumulated the highest level of chromium in root, leaf and shoot while the least accumulation was observed in *A. compressus*. In the root, the mean values of chromium recorded were *C. odorata* (8.21mg/kg), *A. compressus* (0.599mg/kg) and *A. Africana* (0.599mg/kg). In the leaf tissue, mean concentration were *C. odorata* (22.3mg/kg), and 0.14mg/kg for *A. compressus* and *A. africana* respectively. Mean accumulation by *C. odorata, A. compressus* and *A. africana* in the shoot were 22.30mg/kg, 0.588mg/kg and 0.589mg/kg (Figure 4.17).

4.1.18 TPH accumulation in test plant tissues

TPH concentration in the shoot revealed that the mean accumulation in *C. odorata, A. compressus* and *A. africana* were 13.77mg/kg, 10.34mg/kg and 0.068mg/kg. The result further showed that TPH content in the leaf of *C. odorata* accumulated the highest level of TPH (178.43 mg/kg) in the leaf followed by *A.compressus* (46.58 mg/kg) and *A. africana* (26.26 mg/kg). The presence of high levels of TPH in plant tissues indicates that the active uptake of hydrocarbons from the soil was taking place (Figure 4.18).

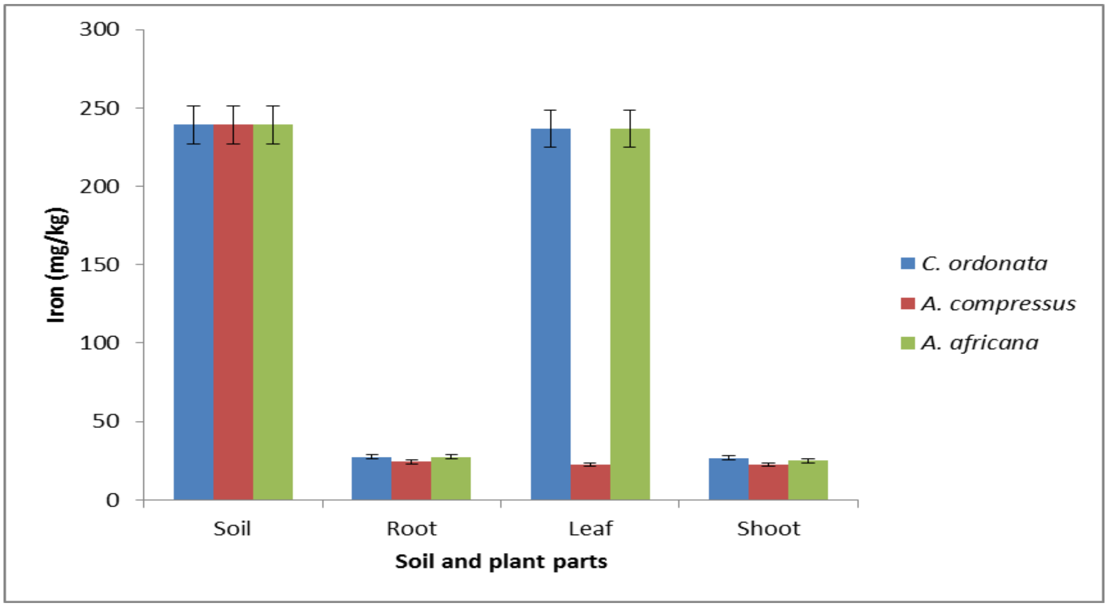

Figure 4.8: Concentration of iron in soil, root, leaf and shoot *of C. odorata, A. compressus* and *A. africana* after 12 weeks of exposure to spent engine oil.

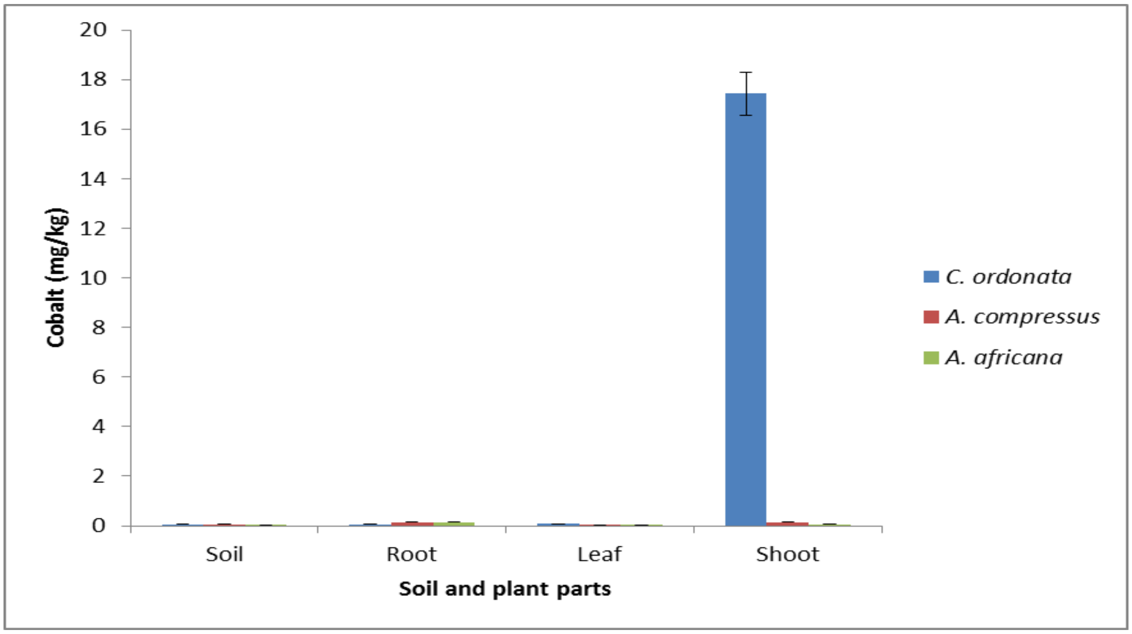

Figure 4.9: Concentration of cobalt in soil, root, leaf and shoot of *C. odorata, A. compressus* and *A. africana*

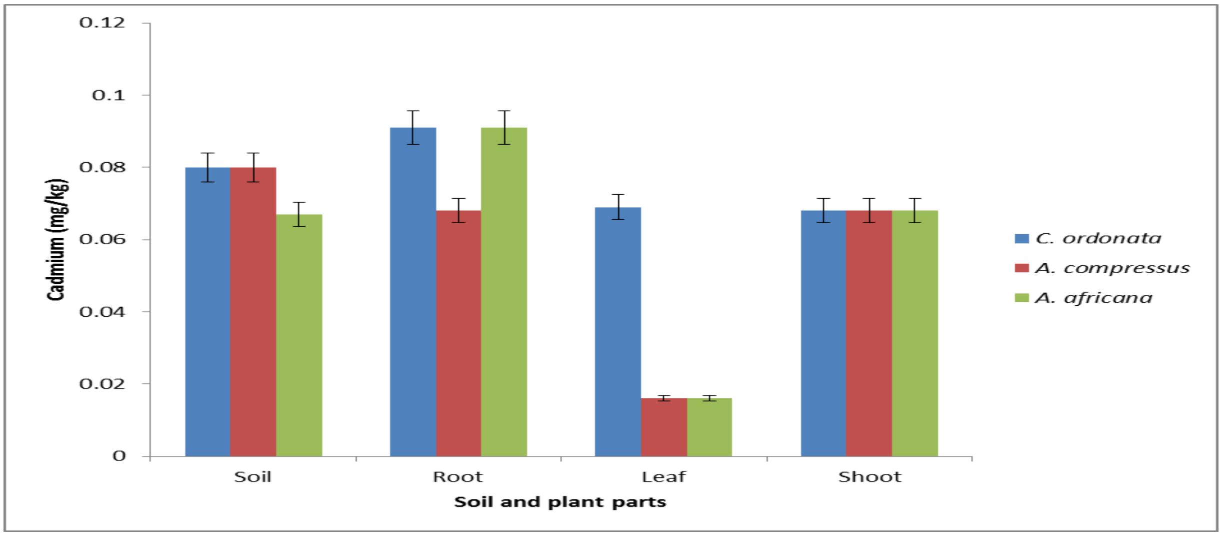

Figure 4.10: Concentration of cadmium in soil, root, leaf and shoot of *C. ordonata, A. compressus* and *A. africana*

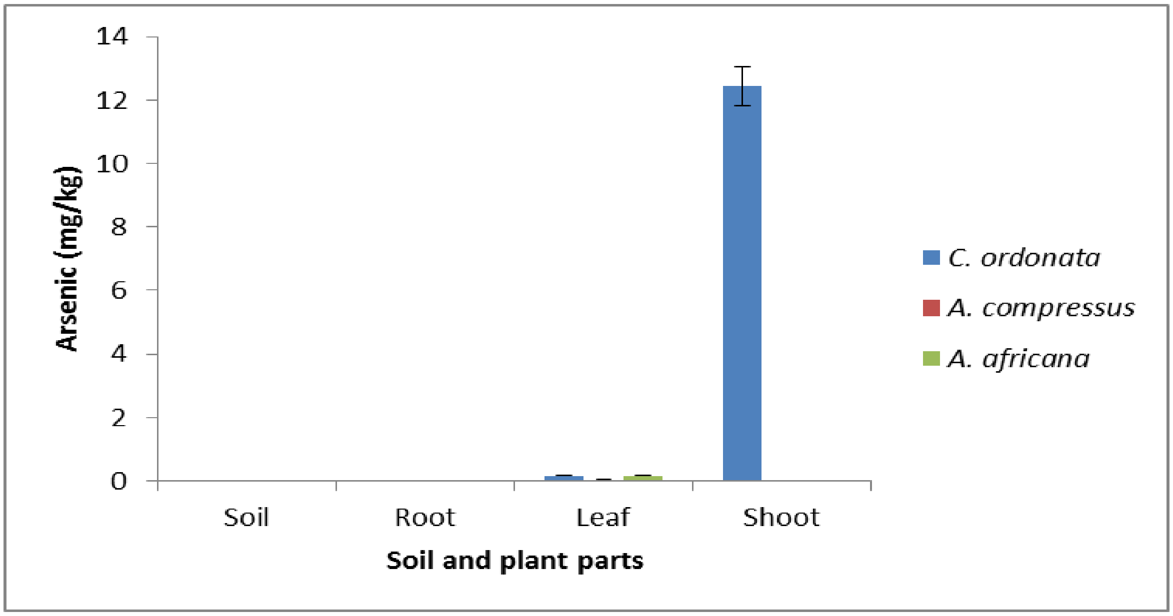

Figure 4.11: Concentration of arsenic in soil, root, leaf and shoot of *C. odorata, A. compressus* and *A. africana*

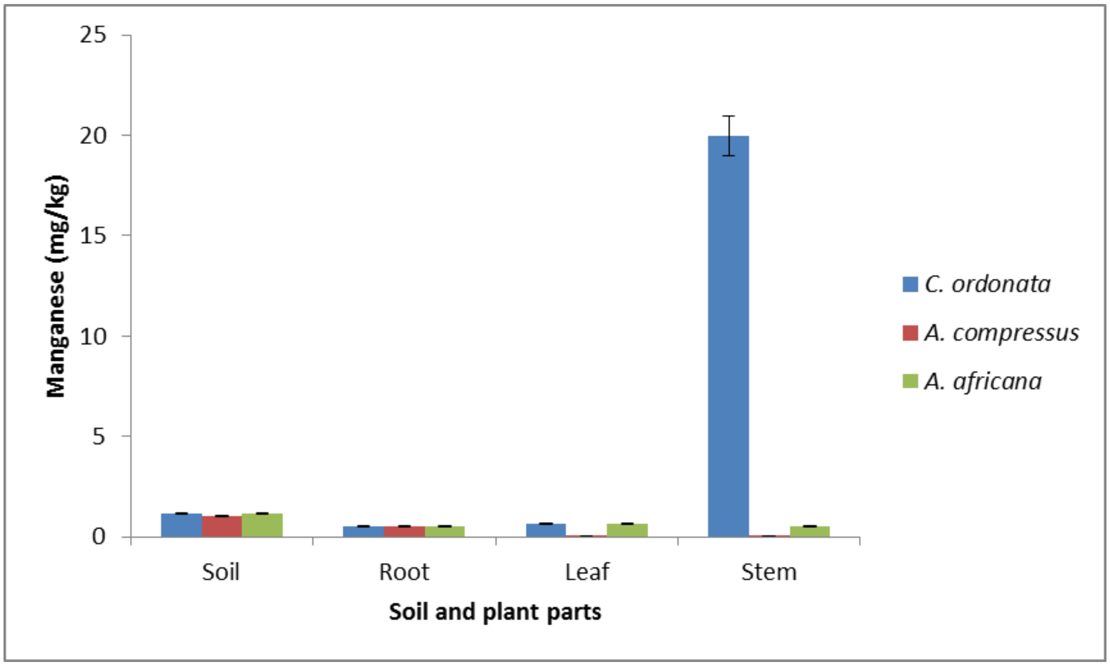

Figure 4.12: Concentration of manganese in soil, root, leaf and shoot of *C. ordonata, A. compressus* and *A. africana*

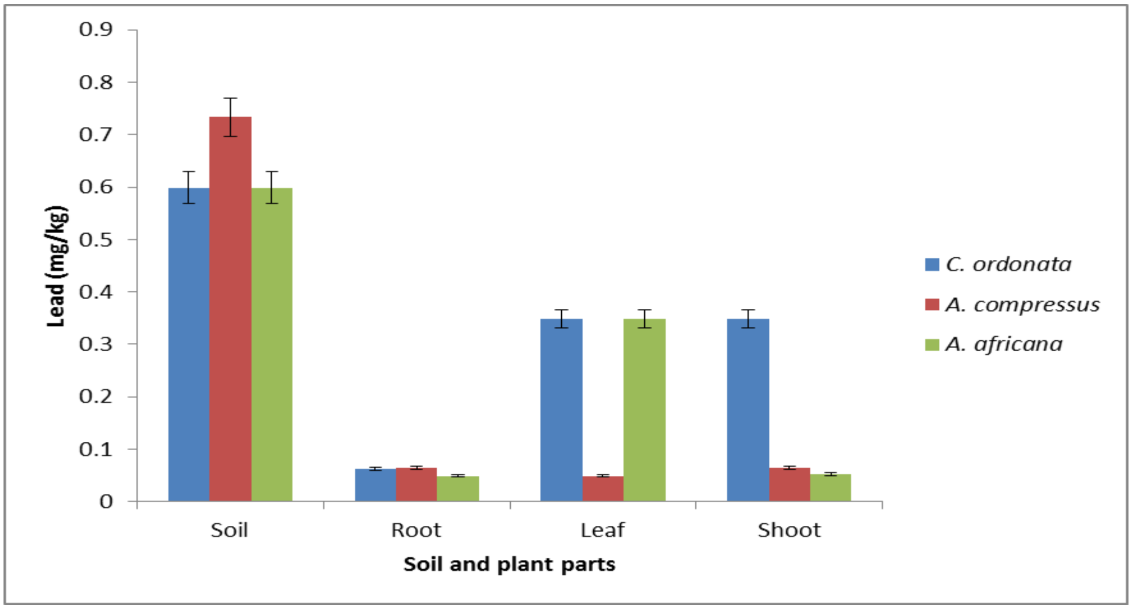

Figure 4.13: Concentration of lead in soil, root, leaf and shoot of *C. odorata, A. compressus* and *A. africana*

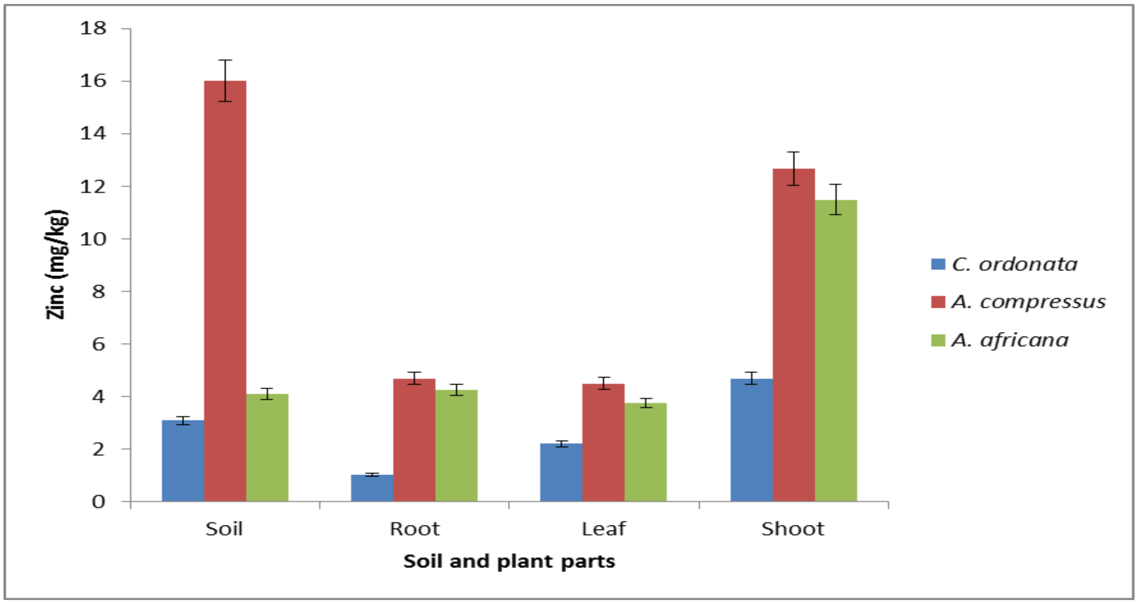

Figure 4.14: Concentration of zinc in soil, root, leaf and shoot of *C. odorata*, *A. compressus* and *A. africana*

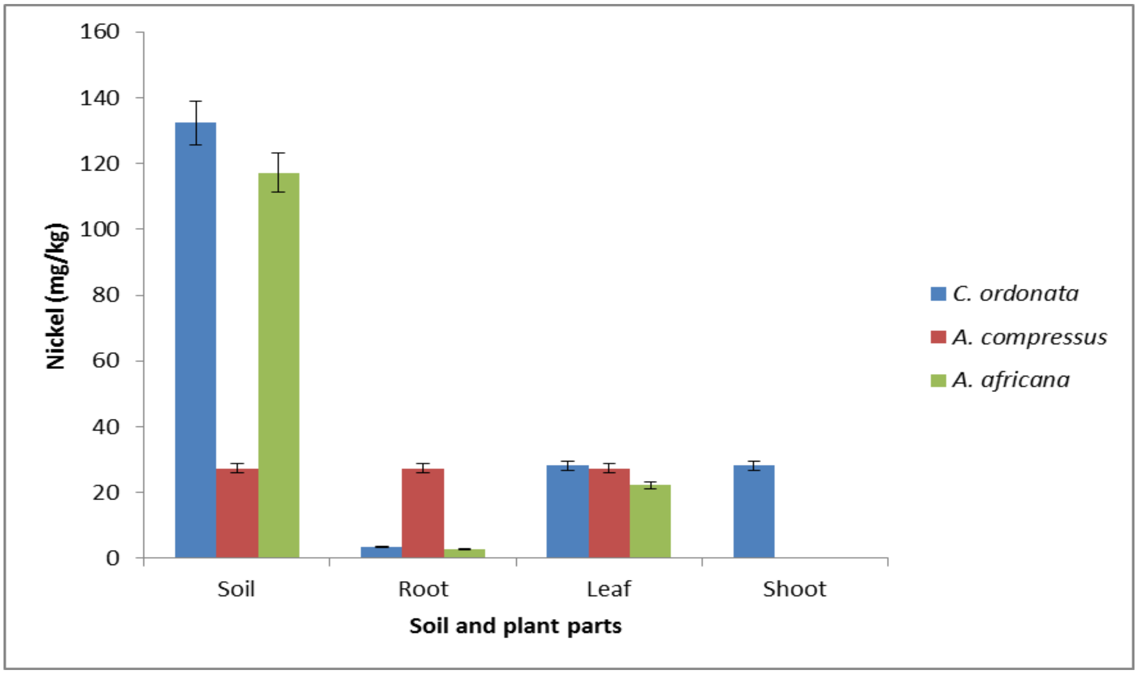

Figure 4.15: Concentration nickel in soil, root, leaf and shoot of *C. odorata*, *A. compressus* and *A. africana*

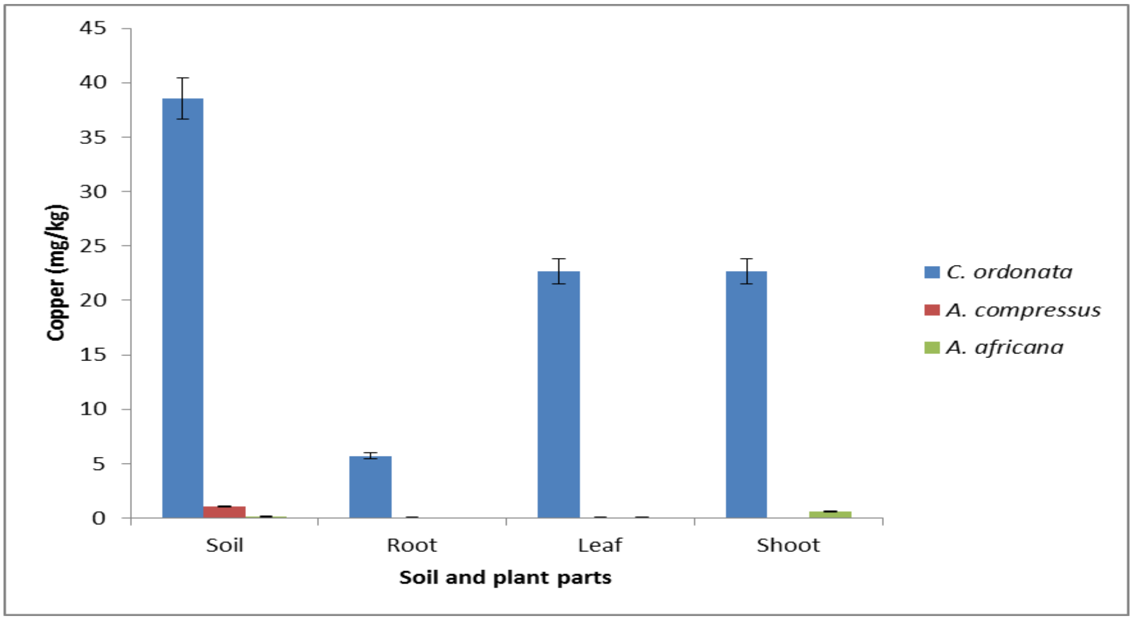

Figure 4.16: Concentration copper in soil, root, leaf and shoot of *C. odorata*, *A. compressus* and *A. africana*

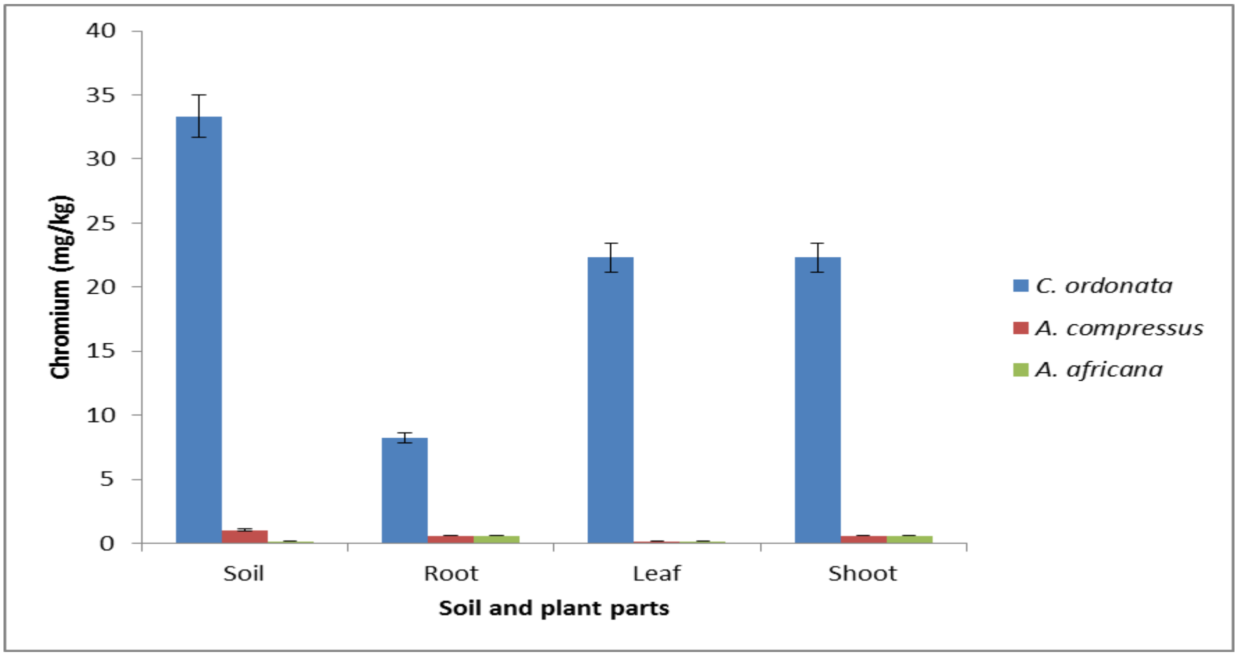

Figure 4.17: Concentration chromium in soil, root, leaf and shoot of *C. odorata*, *A. compressus* and *A. africana*

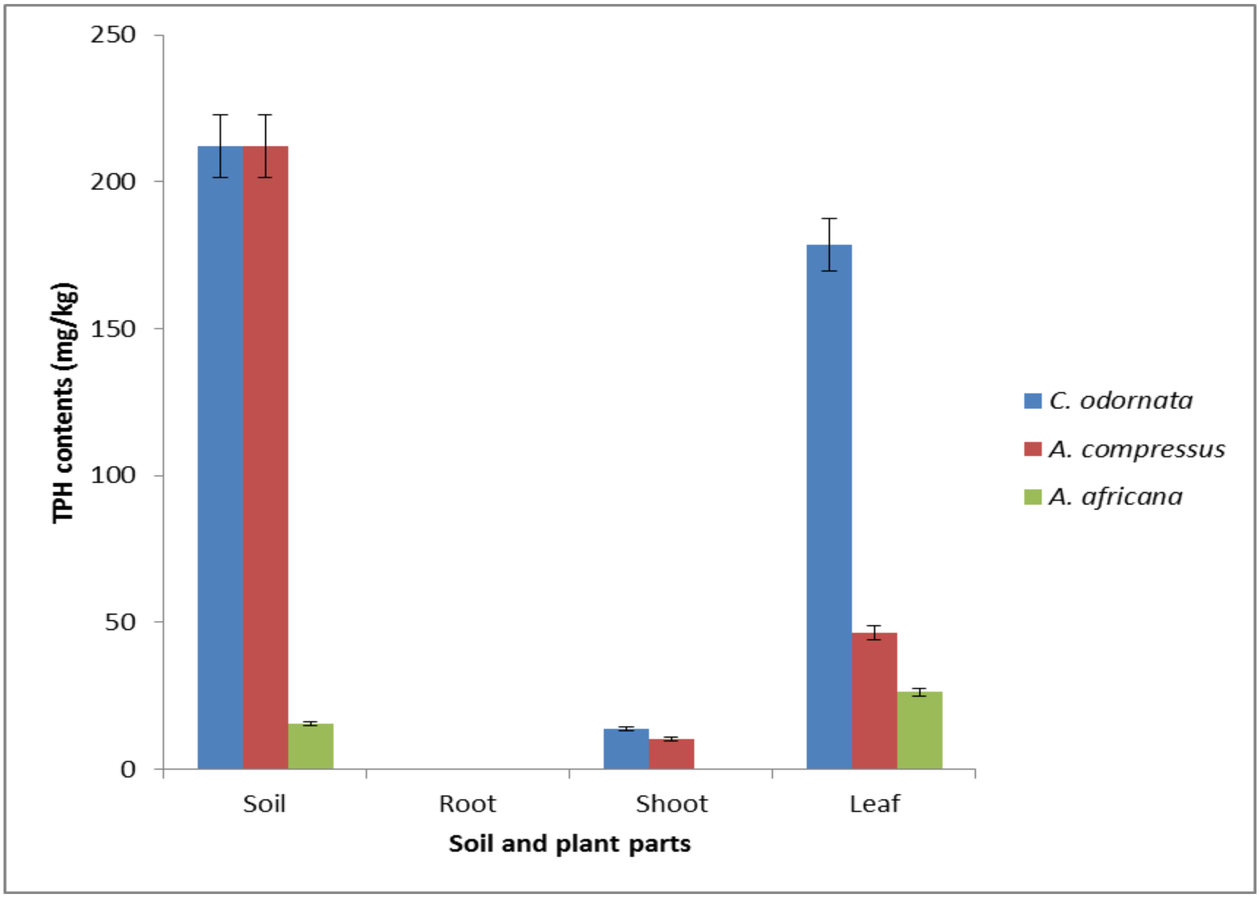

Figure 4.18: TPH contents in soil, root, leaf and shoot of *C. ordonata*, *A. compressus* and *A. africana*

4.1.18.1 Correlation between TPH concentration in soil and plant tissues

In other to understand the relationship between the concentration of TPH in soil and plant tissues, correlation analysis was performed as shown in Figure 4.19. Results obtained revealed that there was a significant and positive relationship ($r = 0.74$, $p = 0.004$) at 0.05 probability level between spent TPH content in the soil and root. This might be as result of increase in concentration of TPH in the soil also increases the accumulation of TPH by the plant species thus: $Y = 378.26 + 1.8914X$, where Y is the concentration of TPH in plants roots, and X is the concentration of TPH in soil. This means that TPH level in soil positively correlates with TPH in plant roots. In other words, increase in TPH level in soil increases TPH content in roots and vice versa.

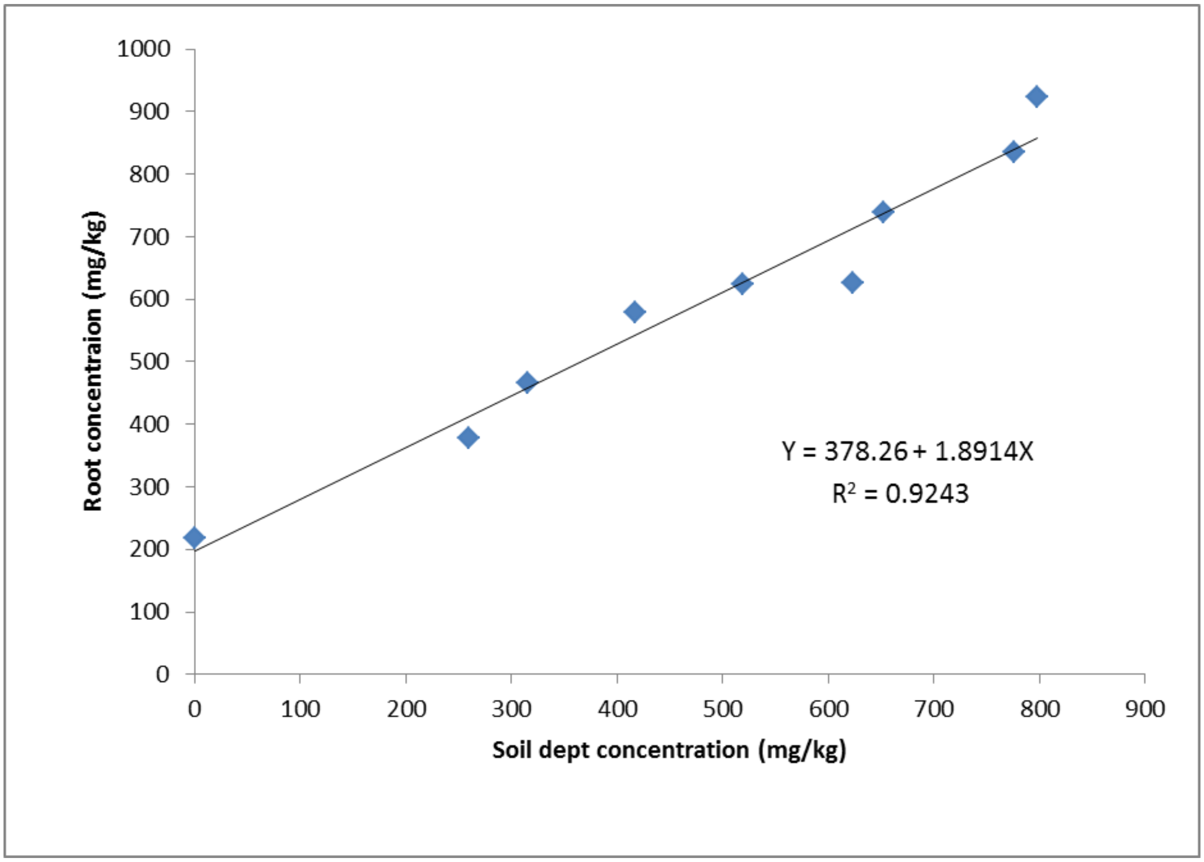

Figure 4.19. Linear regression between TPH concentration in contaminated soil and root

4.1.19 Percentage reduction of heavy metals by *C. odorata, A. africana* and *A. compressus*

Percentage biodegradation of spent engine oil in the soil by *C. odorata A. africana* and *A. compressus* is presented in Figures 4.20, 4.21, and 4.22. There was a significant percentage reduction in heavy metal content by *C. odorata* compared with the 0% concentration (left under natural attenuation). At 4%, 8%, 12% and 16% treatment level, *C. odorata* showed a concentration dependent percentage phytodegradation (between 77.0 and 96.8%) for Fe, Cd, Co, As, Mn, Pb, Cr, Ni, Cu and Zn after 12 weeks. It was also observed that the highest percentage reduction value was recorded in Fe at 16% (96.06%) treatment levels compared with with other treatments. *A. compressus* followed a similar pattern in percentage heavy metal biodegradation with the highest and least percentage reduction values of 85% for Zn and 3.01% for Pb. *A. africana* was observed to have biodegraded between 68.6% and 90.71% at the highest level of treatment with increase in concentration level (16%). Generally, the plant species (*C. odorata, A. africana* and *A. compressus*) significantly reduced concentrations of heavy metals after twelve weeks of growth in spent engine oil polluted soil.

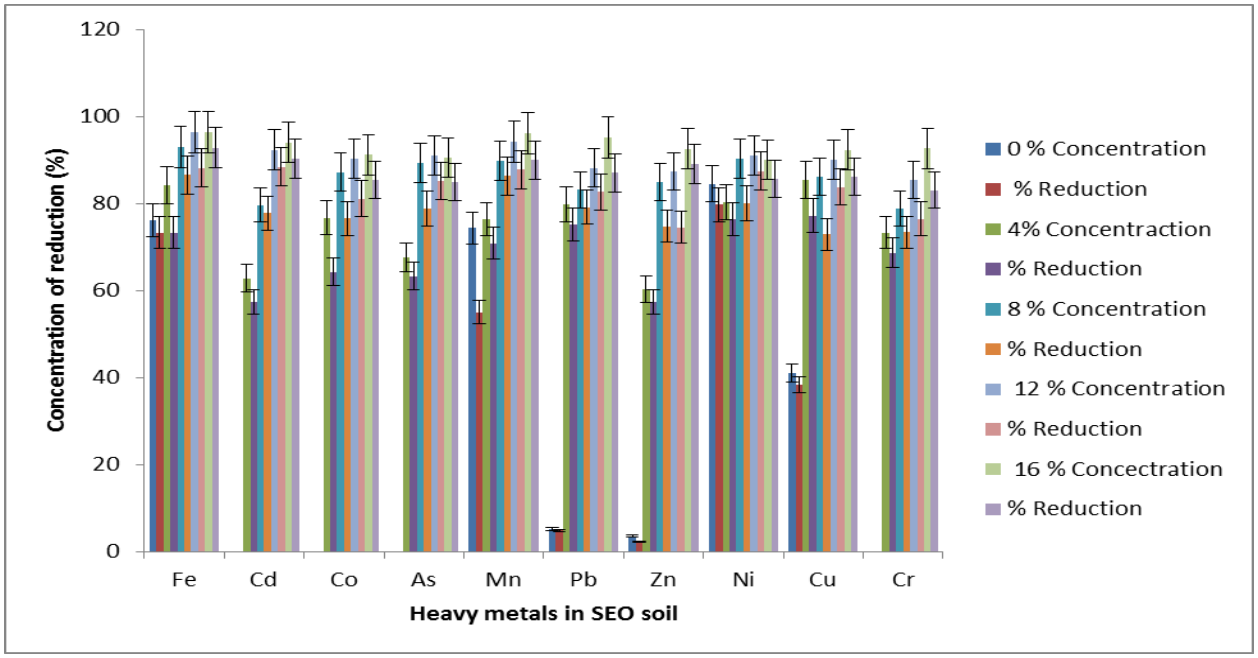

Figure 4.20: Percentage reduction of heavy metals in C. odorata exposed to SEO polluted and unpolluted soil

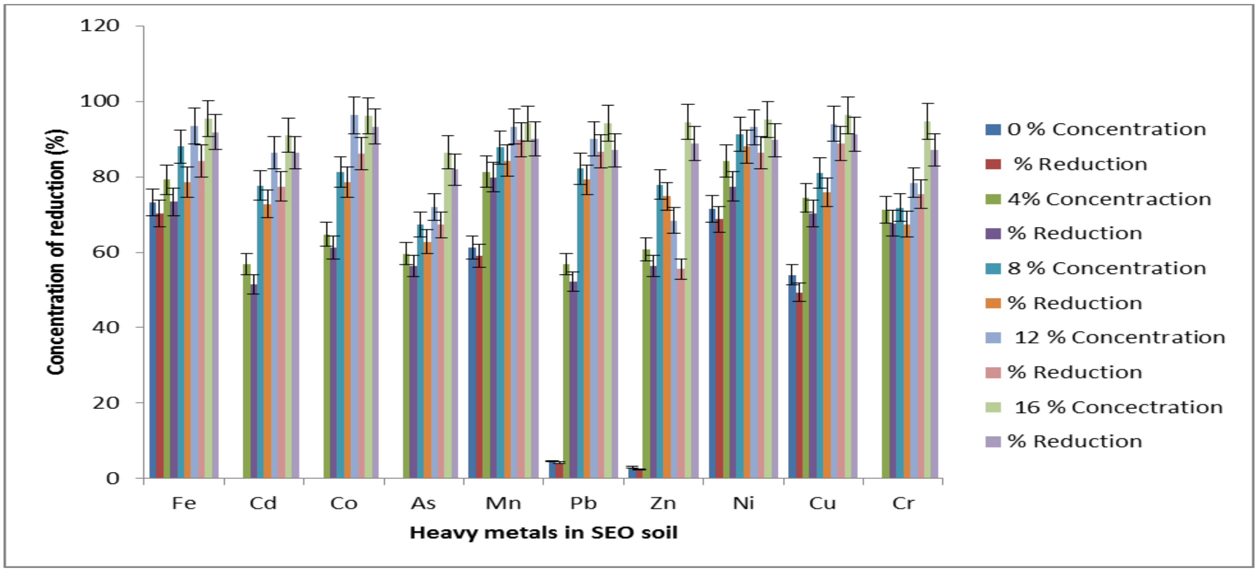

Figure 4.21: Percentage reduction of heavy metals in *A. compressus* exposed to SEO polluted and unpolluted soil.

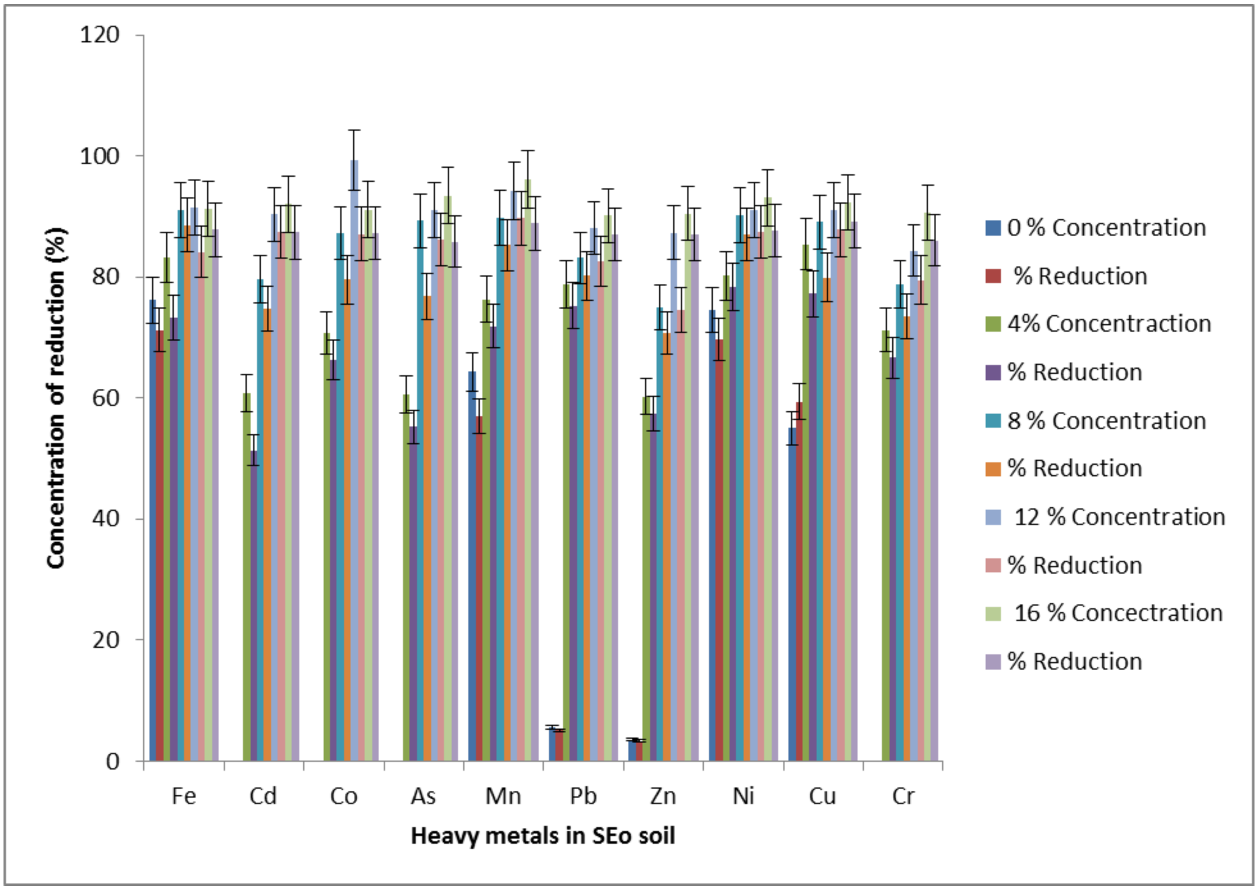

Figure 4.22: Percentage reduction of heavy metals in *A. africana* exposed to SEO polluted and unpolluted soil

4.1.20 Bioaccumulation Factors and Transfer Factors in Plants exposed to spent engine oil polluted soil.

Bioaccumulation and Transfer Factors in Plants *C. odorata A. africana* and *A. compressus*) exposed to to different concentrations of spent engine oil is presented Table 4.20 while the heavy metal accumulation pattern is presented in Table 4.20.1. Results obtained showed that within the belowgrooud (soil) and above ground (shoot) part of the plants, bioaccumulation factor ranged from 1.17 to 0.01 for Arsenic, 1.90 to 0.01 for Cadmium, 1.98 to 0.08 for Chromium, 2.71 to 0.03 for Cobalt, 1.60 to 0.10 for Copper, 8.50 to 0.03 for Iron, 2.91 to 0.50 for Manganese, 1.69 to 0.01 for Nickel, 0.19 to 0.01 for Lead and 5.21 to 0.01 for Zinc respectively. The maximum bioaccumulation factor was noted in *C. odorata* (6.40 mg/kg) for Copper while the minimum (0.01 mg/kg) was observed in *A. africana* for Lead. It was also observed that heavy metal bioaccumulation was higher in the spent engine oil oil treated plants than the control.

The ability of the plant species to transfer heavy metals from root to shoot (transfer factor) ranged from 1.12 to 0.01 for Arsenic, 2.65 to 0.01 for Cadmium, 1.08 to 0.01 for Chromium, 3.11 to 0.11 for Cobalt, 1.69 to 0.60 for Copper, 5.47 to 1.40 for Iron, 2.59 to 0.11 for Manganese, 1.90 to 0.01 for Nickel, 0.13 to 0.01 for Lead, and 6.81 to 3.61 for Zinc. The highest transfer factor was observed in *C. odorata* for Zinc (6.81 mg/kg) while minimum transfer factor was recorded in *A. compressus* for Cadmium (0.01 mg/kg). Bioaccumulation Factor for *A. africana A. compressus* and *C. odorata* were in the order: Zn> Cu> Fe> Mn> Ni> Co> Cr> Cd> As >Pb; Fe> Co> Mn> Ni> Zn> Cu> Cr> Cd> As> Pb and Cu> Fe> Zn> Co> Mn> Ni> As> Cd> Cr> Pb while the Transfer Factor followed: Fe> Zn> Mn> Cd> Cu> Ni> Co> Cr> As>Pb; Fe> Zn> Mn> Ni> Co> Cd> As> Mn> Cr>Pb and Zn> Fe> Cd> Ni> Cu> Mn> As> Cd >Cr>Pb respectively.

Table 4. 20: BCF and TF of heavy metals in above and belowground parts of *A. africana, A. compressus* and *C. odorata* exposed to different concentrations of spent engine oil contaminated soil

		Bioconcentration Factor (soil to shoot Ratio)									
Conc.(%)	**Species**	**As**	**Cd**	**Cr**	**Co**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
0		ND	ND	ND	ND	0.10	0.03	ND	ND	ND	0.01
4	***A. africana***	0.17	0.61	0.99	0.09	4.82	2.01	2.91	5.11	0.02	2.19
8		0.02	1.80	1.90	1.70	0.60	2.40	0.51	1.61	0.09	2.33
12		0.20	0.80	0.91	1.71	1.10	4.41	1.51	0.01	0.11	4.01
16		ND	0.01	1.98	2.71	1.60	4.50	1.81	1.69	0.19	4.11
4	***A. compressus***	0.11	ND	0.88	0.03	ND	3.40	0.50	1.06	0.07	1.70
8		0.12	0.80	1.22	1.71	1.50	2.40	2.51	1.61	0.07	1.61
12		0.42	1.80	1.32	2.21	1.57	3.41	2.54	1.61	0.09	1.71
16		0.45	1.90	1.96	2.51	1.61	5.10	2.51	1.21	0.09	1.71
4	***C. odorata***	0.03	0.81	0.08	0.71	3.00	3.41	1.11	1.01	0.01	1.01
8		1.33	1.82	0.38	1.18	4.30	3.48	1.21	1.21	ND	2.21
12		1.31	0.84	0.18	1.01	4.20	3.12	1.41	1.31	0.03	3.41
16		1.35	1.85	0.58	1.11	6.40	3.20	1.61	1.61	0.09	5.21
		Transfer Factor (Root to shoot Ratio)									
4		ND	0.01	ND	0.11	1.09	2.40	0.11	1.21	ND	3.61
8	***A. africana***	0.12	1.00	1.08	0.70	0.75	4.40	1.05	1.66	0.01	5.71
12		0.02	0.21	0.08	0.77	0.60	4.42	1.51	1.81	0.09	5.31
16		1.12	1.80	0.91	1.53	1.65	5.47	2.04	1.61	0.10	7.01
4	***A. compressus***	0.21	0.01	ND	0.71	1.69	2.40	0.11	1.71	ND	3.71
8		0.04	1.80	0.98	1.71	1.10	2.30	0.51	1.01	0.01	3.77
12		0.05	1.00	0.91	2.01	1.67	3.40	0.61	1.61	ND	4.71
16		0.19	0.30	0.93	2.71	1.69	3.44	0.53	1.61	0.01	4.79
4	***C. odorata***	0.04	1.90	ND	3.11	0.90	1.40	1.61	1.90	ND	5.71
8		0.10	0.01	0.01	2.80	1.50	1.46	2.50	0.01	0.10	5.10
12		0.01	2.65	ND	2.71	1.20	2.44	0.54	1.32	0.13	6.71
16		0.22	2.22	0.08	2.99	1.10	2.49	2.59	1.11	ND	6.81

LEGEND: CONC. = concentration; *BCF and TF =Bioconcentration Factor* and *Transfer Factor. ND= Not detected.*

Table 4.20.1. Heavy Metal Accumulation Pattern for *A. africana, A. compressus* and *C. odorata* exposed to different concentrations of spent engine oil contaminated soil

Species	**Heavy metal bioaccumulation pattern**
	BAF
A. africana	Zn> Cu> Fe> Mn> Ni> Co> Cr> Cd> As >Pb
A. compressus	Fe> Co> Mn> Ni> Zn> Cu> Cr> Cd> As> Pb
C. odorata	Cu> Fe> Zn> Co> Mn> Ni> As> Cd> Cr> Pb
	TF
A. africana	Fe> Zn> Mn> Cd> Cu> Ni> Co> Cr> As>Pb
A. compressus	Fe> Zn> Mn> Ni> Co> Cd> As> Mn> Cr>Pb
C. odorata	Zn> Fe> Cd> Ni> Cu> Mn> As> Cd >Cr> Pb

Legend: BAF= Bioaccumulation Factor, TR= Transfer factor.

4.2 Discussion

The auto-mechanics carryout numerous activities which expose the environment to pollution through indiscriminate disposal of wastes. The study showed that the majority of the automobile mechanics are males within the ages of 20-30 years (Table 4.1). This is not surprising as the job is physically challenging and strenuous which limits female participation. According to Omorowa & Agu (2017, males tend to do to more strenuous jobs while females are assigned to less physically demanding jobs in Africa. This result is similar to the finding of John & David (2009) that reported same age group among mechanics in Kampala District of Uganda. A study by Omorowa & Agu (2017) documented 21-26 age groups among auto mechanics in Benin City which is in line with the observation made in this study. This observation could be ascribed to poverty as an automobile service is one of the jobs used to alleviate poverty in Nigeria. Meachanics are recruited at a very tender age, especially those who are not willing to go to school/dropouts so that they will eventually grow into it. The finding that less number of people are involved in automechanic work at the age of 60 years corroborates with similar studies among automobile mechanics (Abu, Boadi-Kusi, Opuni, Kyei, Owusu-Ansah, & Darko-Takyi, 2016; Oche, Okafoagu, Oladigbolu, Ismail, Ango, Hashimu, & Ijapa, 2020; Amfo-Otu, & Agyemang, 2017). This can be linked to the fact that mechanic work requires active people who can carry heavy loads, and work under the sun for a long period of time which people above 60 years may not find comfortable to do at that age (Monney, Bismark, Isaac, & Kuffour, 2014). Again, the study observed that more number of young mechanics was found in Owerri compared to other zones**.** In a rapidly urbanizing society, it is assumed that cities open more opportunities than the rural areas. This observation may be attributed to the choice of working in a “happening city” and possible class of clients that would pay them well (Johnson & Bassey, 2016).

It was established that on the average, 3 to 9 number of vehicles are been serviced by the mechanics on weekly basis as seen in Table 4.2. This number is slightly higher than 3 to 7 reported by Udebuani *et al,* (2011) in a study at Nekede auto mechanic village but aligns with the findings of John & David (2009). As opined by Zitte *et al,* (2016), the more vehicles serviced and spent engine oil generated, the higher the volume that accumulates and has to be disposed into the environment. These activities further pollute the environment thereby causing harm on the ecosystem.

The study has also established the annual volume of spent engine oil generated in the study areas. Though the volume of spent engine oil generated in Owerri was greater than other study areas, this could be as a result of more anthropogenic activities taking place in this zone than others. Owerri zone is more metropolitan than Okigwe and Orlu zones. This result compares well with the findings of Zitte *et al,* (2016) who reported 1628.50 litres generated at Porthacourt area of Rivers State Nigeria but lower than the value of 1,469,678.08 litres reported by Udebuani *et al,* (2011) in Nekede mechanic village of Imo State. A study by John & David (2019) established that about 60 litres of spent engine are been generated annually at Kampala district in Uganda, which is lower than the values recorded in this study (Table 4.2). Similarly, Echiegu, Amadi, Ugwuishiwu & Nwoke (2021) recorded 279.25 Litres as volume of spent engine oil generated at auto mechanic village in Enugu State which is lower than the volume obtained in this study. The low volume of spent engine oil generated in Okigwe and Orlu areas could be attributed to less number of cars in operation there; as such areas are semi-developed urban areas.

The alarming volume of spent engine oil generated in the study areas indicates that there could be serious spent engine oil pollution in the soils of the study areas which could eventually alter ecosystem integrity. Spent engine oil contains toxic substances such as heavy metals and poly aromatics which when released into the environment, have the potentials to affect flora and fauna, contaminate water bodies through runoff (Ibe *et al.*, 2021; Obinia & Afiukwaa, 2013). When spent engine gets to the soil, it physically coates the body of an organism, causes behavioral changes which in turn causes hypothermia.

The study showed that large number of mechanics disposed spent engine oil within their immediate vicinity (Table 4.3). This is in agreement with the report of previous authors (Udebuani *et al* 2011; Zitte *et al*, 2016). The mechanics also acknowledged that they sell the oil off to other end users which may eventually be disposed into the environment. This is a major source of pollution of the environment. Toxic chemicals through these disposal methods enter the environment and alter the physical and chemical properties of the environmental components (Obinia & Afiukwaa, 2013).

However, the study established other beneficial applications of spent engine oil in the study areas**.** The most common benefits of spent engine oil according to responses from auto mechanics were: as pest repellant, given out to lorry drivers(for reuse), poured in pit latrine, road construction (where it is mixed with bitumen), timber cutting, pest control, marking playground, treating roofing timber, and treating fencing post respectively (Table 4.4). Spent engine oil is robbed on building poles to prevent wood rot and termite infestation. Lumbermen also use spent engine oil during felling of trees and in saws used for cutting trees. The oil is also used to marking lines on the timers and lubricates chain saws used for cutting wood into different sizes (John & David, 2019).

Most people prefer to use spent engine oil in marking playground because it is cheaper than lime which is costly and not readily available. According to the responses from the mechanics, spent engine is being poured into the laterine to kill maggots and flies, and also reduce bad odor from pits. In many urban poor areas, the management of of pit laterines is very poor resulting in quick filling up of the pits. These causes offensive odor and emergence of maggot from pit onto the floor of the laterine especially during the rainy seasons. This pit also acts as a breeding habitat for mosquitoes and other pests. Application of spent engine oil reduces the pests and delays the faecal degradation process in the pit (John & David, 2019). All of these many uses may result in the release of spent engine oil back into the environment with its attendant effects on the ecosystem. Upon release on the environment, the oil can seep into aquatic bodies causing harm to the living organisms that make up the food chain (Aiyesanmi, Oladele, Adelodun & idowu, 2020).

The study further established the health, environmental risk and training on disposal methods of spent engine oil among mechanics. The result showed that most of the mechanics (84.66%) know nothing about the health risk and environmental hazards (71.68%) associated with indiscriminate disposal of spent engine oil (Table 4.5). This is similar to the finding of Bamiro & Osibanjo, (2004); Inam, Edet, & Offiong, (2015). As knowledge of health, environmental risk and disposal methods are lacking; the mechanics are ignorantly exposed to toxic subtances which may have been inflicting some diseases and pain on them without their knowing it. As it was observed that no personal protective equipments (PPEs) are used by the mechanics to cover their body against the negative effects of these substances. Abdominal pains, coughing, irritating eyes, soar throat and other musculoskeletal disorders had been reported among auto mechanics (Monney, *et al.*, 2014).

Harmful chemicals like heavy metals, aliphatic and aromatic hydrocarbons present in spent engine can pose a serious health effect when accidentally ingested by the mechanics. These pollutants have been documented to cause haematological derangement, kidney damage and skin cancer (Omorowa & Agu, 2017). Health effects attributable to spent engine oil results from exposure to it (Omorowa, Agu, Okolie, Oghagbon, Sule, & Egbuta, (2015). A study by Ekeocha, Ogukwe, & Nikoro, (2017) opined that dermal contact is the most susceptible route of exposure among auto mechanics in Nigeria. Spent engine oil had been implicated to cause immunological, neurological, reproductive, genotoxic and carcinogenic effects in humans.

One of the major setbacks to successful application of phytoremediation technology is dearth of knowledge of plant species capable of phytoextracting metals from polluted soils. The results from this study revealed a number of plant species found growing on spent engine oil polluted sites. This is in agreement with the finding of Anoliefo *et al,* (2006). In all the sites visited, the *Poaceae* family recorded the highest frequency of occurrence. This was followed by the *Asteraceae*. Plants of the family *Portulacaceae, Cyperaceae, Piperaceae, Curcubitaceae* and *Malvaceae* were among the least prevalent plant families identified (Table 4.6). Similar trend had been reported by Chukwu *et al*, (2017) within metal scrap dumpsites in Benin City. *Cyperaceae* and *Piperaceae* were among the least prevalent plant families identified by Anoliefo *et al*, (2006) in auto mechanic workshops in Asaba and Benin City which aligns with the findings in this study.

Its was established that *Axonopuss compressus* (which belongs to the Poacea family) remained the most prevalent plant species in all the sites visited followed by *C. odorata* and *A. africana* as shown in (Table 4.7). The order of occurrence were *A. compressus>C. odorat >A. africana* in all the study areas. Raymond & Harrison (2018) observed a similar trend in hydrocarbon contaminated sites in Umuahia. Low frequency of occurrence by some of the plant species may not necessarily imply their inability to tolerate spent engine oil laden soils; it could be as a result of constant clearing/weeding at the sites due to the shrubby nature of the plants. A study by Ngumete *et al*, (2018) reported that plant species found growing in metal contaminated sites could be used as potential phytoremediators.

Most of the plant species identified at the spent engine oil polluted sites belong to grasses and legumes, which according previous reports makes them highly efficient in phytoremediation studies (Ukaoma *et al*, 2015; Njoku *et al*, 2013). According to Idris *et al*, (2016), plant species with potential tolerance to high TPH content are known to poses fibrous root system. Incidentally, the most dominant plant species (*C. odorata, A africana* and *A. compressus*) identified in this study poses fibrous root system. This corroborates the report of previous works (Ngumete *et al*, 2018; Chukwu, Anoliefo & Ikhajiagbe, 2017; Ogbo, Zibigha, & Odogu, 2009 Amadi, Agomuo, Akpobasaha, & Njoku, 2017).

Based on plant species rating with relative frequency >10% as opined by Ngumete *et al,* (2018), the dominant plant families identified in this study belong to Poaceae (*A. compressus*) and Asteraceae (*A. africana* and *C. odorata*). Species belonging to these plant families have been associated with decontamination of hydrocarbon polluted sites studies (Table 4.7.1). This is in tandem with the findings of previous researchers (Ramirez *et al,* 2021; Ariyo, 2020; Anyasi, *et al.*, 2018; Jidere *et al,* 2018). According to Njoku, Akinola, Nkemdilim, Ibrahim, & Olatunbosun (2014), these plant species can be used in remediation of hydrocarbon polluted soils.

Physical properties of soil such as texture and bulk density are known to influence movement of nutrients through the soil pores and water holding capacity of the soil (Vidali, 2001). The particle size distribution of soil highlights a general sandy loam textural classification irrespective of the location. This indicates that the soils are coarse textured with high content of sand, silt and then clay. According to Okoro, Chukwuma, Chukwuma, & Ugwu (2015), these attributes are typical of the soils of the study area. This could eventually lead to a low sorption capacity for metal ions present in the soil.

As opined by Brady & Weil, (2012); and Iwegbue, (2007), soils with higher percentage of sand are usually fragile in structure, well drained, well aerated and very erodible. The concentration of sand, silt, clay, moisture content, and bulk density followed the trend sand> silt>bulk density>moisture content>clay across the zones with their non-active sites (control) (Table 4.8). A study by Okoro *et al*, (2015) at auto mechanic villages in Imo state differed slightly in trend as follows: sand> clay> silt>moisture content>bulk density but aligned with sand>silt>bulk density>moisture content >clay sequence obtained by Otobong & Victoria (2017) at mechanic villages in Calabar; and Pam, Rufus, & Offem, (2013) at auto mechanic workshops in Benue State.

Soil moisture content describes the ratio of the mass of water held in the soil to the dry soil. There was no significant difference ($P<0.05$) among the moisture content of the soils in both contaminated and control sites. Nwite & Alu (2015) obtained a moisture content value of 47.98% which is in the range with value obtained in this study. Udebuani *et al*, (2013) obtained a slightly lower mean value of 14.88±0.5 at auto mechanic village in Owerri. The value of bulk density was lower than 1.17 g/mL reported by Umunnakwe, Aharanwa, & Njoku, (2020) at Orji auto mechanic village but aligns with 51.39g/mL documented by Udebuani, Onweremmadu & Allison (2013) in spent engine oil contaminated soils of Owerri Agricultural soils. This could be an indication of spent engine oil aided contamination of the soil (Emoyan *et al*., 2020). The high bulk density recorded in this study can be ascribed to higher compaction of the soils as result of the residents walking on the soils and to closure of air spaces by wastes such as used engine oils that are improperly disposed on mechanic village soils (Njoku, Mbah, Elom & Agwu, 2021). Soil compaction (high bulk density) restricts plant root growth by increasing resistance to root penetration (impedance) resulting in reduced nutrient uptake, nutrient deficiencies, and crop yield.

The value of pH in this study depicts a general acidic soil. pH value obtained in this study is comparable with the range of 5.96-6.05 reported by Njoku, *et al.*, (2021) and the range of 4.71-7.00 obtained by Echiegu, *et al*, (2021). The result further revealed that pH from control sites were slightly higher than those obtained from the study sites. This is in conformity with previous reports on auto mechanic villages in Nigeria (Verla *et al*, 2015; Ahukaemere *et al*, 2016; Enyoh *et al*, 2017). According to Ahukaemere, (2012), soils in the Southeast regions are naturally acidic irrespective of anthropogenic activity. Chris-Emenyonu & Onweremadu (2011) also reported that soils of the area are highly erodible and this must have contributed to the low pH values obtained in this study.

pH among variables such as soil structure, the organic matter quantity and the cation exchange potential of the soil could control the circulation and enrichment of heavy metals in soil (Lin *et al.*, 2002). This could favour the precipitation and bioaccumulation of heavy metals in soil (Ujevic *et al.*, 2000), such as Mn, Zn and Ni . The high rainfall in the area could also be responsible for the acidic soil, which may cause soil erosion and consequent leaching of common cations such as Ca^{2+} and K^{+}, Mg^{2+} and Na+ (Ahukaemere, 2012). These macro nutrients are more soluble in acidic medium and hence neutralize the acidic property of the soil. Berardi (2003) opines that some anions such as Phosphate (PO_4^{3-}) and Sulphate (SO_4^{2-}) are acidic and could alter the acid-base load of the body when taken in high concentration and this could be obvious without a corresponding availability of basic cations (K, Ca, and Mg) that has potential of balancing it.

The organic carbon content ranged from 13.68 ± 3.79 to 10.28 ± 3.46 across the sampled zones with the control having the lowest value of 8.23 ± 3.05. This contradicts the work of Ndukwu, Onweremadu, Ahukaemere, Ihem, Nkwopara & Osujieke (2015) who documented higher organic carbon content in unpolluted site than in automobile polluted site but agrees with the findings of Chukwu & Udoh (2014) who reported higher OC values in crude oil polluted soils than the non-contaminated site. This could be as a result of hydrocarbon content of spent engine oil (Udebuani *et al*, 2013). Soil organic carbon is as a result of living or decomposing biological matter in soil. It is relatively available carbon as a fresh plant/animal remains in soil. Increases in organic carbon content in comparison with the control indicate beneficial effects of spent engine on soil chemical properties. This is in tandem with the report of Ndukwu *et al,* (2015) which opined that organic carbon and organic matter from spent engine oil can influence the ability of microbes to degrade pollutants in soil.

The Organic matters, Total Nitrogen Available Phosphorus obtained in this study were lower at the polluted sites compared to the control. This agrees with the findings of Nwachukwu *et al*, (2020). Organic matter is a labile pool of organic matter, present in the dissolved form in soil solution. It is comprised of leachates from plant residues and exudates from soil organisms and plant roots. It serves as energy substrate for soil microbial activity. Total nitrogen content content obtained compares well with the report of Nwachukwu *et al*, (2020). The available phosphorus was generally low when compared to the established critical values of 17 mg/kg for Agricultural production in Nigeria (Umeri, Onyemekonwu, & Moseri, 2017). This result agrees with the findings of Ndukwu, *et al*, (2015) who reported higher P values in control site than in automobile polluted site but disagrees with the findings of Chukwu & Udoh (2014) who reported higher P values in spent oil polluted soils than the non-contaminated site. Total Nitrogen for all the zones were below 0.15%, the critical value for tropical soils (Enwezor, *et al.*, 2020), and indicate high N deficiencies in the polluted sites. This might be an indication of spent engine oil contamination of the soil which had been previously reported to impact nutrient content of the soil (Ibe *et al*., 2021; Njoku *et al.*, 2021; Nwachukwu *et al.*, 2020; Verla *et al,* 2017; Udebuani *et al.*, 2016; Udebuani *et al.*, 2013).

The mean values of Calcium, Magnesium, Potassium and Sodium were generally low based on the critical values of 5.0 cmol/kg^{-1} (Amalu, 2009) as well as the control the sites. This may be attributed soil erosion and consequent leaching (Ahukaemere, 2012). In comparison with the control, there was no observable significant difference ($P< 0.05$) across the zones. A study by Otobong & Victoria, (2017) reported a similar trend as observed in this study. This according to Nwachukwu *et al,* (2020) can be attributed to the coarse textured nature of the soil which is commonly lower in clay and humus content. Availability of most macronutrients (nitrogen, potassium, calcium, magnesium, and sulfur) is optimal within a pH range of 6 to 7 and decreases outside this range. Typically, low soil pH (acidity) can lead to significant yield reductions due to nutrient deficiencies in crops. This could be the reason for low macronutrient content of soils observed in this study (Otobong & Victoria, 2017).

The TEA at the control sites was observed to be higher than the contaminated sites. The TEA and TEB were observed to be low in contaminated sites than the control. The value of TEB obtained in this was higher than that reported by Otobong & Ediene (2017). The TEB of most soil increases with corresponding increase in pH. At very low pH, TEB is generally low. The value of TEB obtained in this study implies a generally low fertility of the soil as a result of contamination with spent engine oil.

The electrical conductivity (EC) is a measurement of the dissolved material in an aqueous solution, which relates to the ability of the material to conduct electrical current through it (Nwachukwu *et al*, 2020). However, EC values at the control were higher than the contaminated soil. The observed higher EC values in this study could have been due to the textural class of the soil. This result is in line with the findings of Nwachukwu *et al*, (2012) at auto mechanic village in Imo State. EC usually correlates strongly to soil texture and Cation exchange capacity (CEC) (Verla *et al*, 2015). The ECEC followed a similar trend as EC. Highest value of ECEC was obtained at the control site when compare with the contaminated sites. This aligns with the report of Nganje *et al*, (2007). The ECEC of a soil affects soil fertility and plant growth. Macronutrients such as Calcium, magnesium, potassium, ammonium, and zinc are some important nutrient cations vital for plant growth. Soils with a higher ECEC values retain nutrient cations and maintain them in soil solution. Conversely, the nutrient cations are susceptible to leaching in soils with a low ECEC, and as a result, plants are most likely to develop nutrient deficiencies in these soils.

Heavy metal concentration in the soil samples also varied with increased values obtained at the polluted sites than the control as seen in Table 4.9. A similar pattern of elevated levels of heavy metals in auto mechanic workshops had been reported (Okoro *et al*, 2015; Nwachukwu, 2014). These high levels of heavy metals recorded at the polluted sites may be linked to the large amount of chemical pollutants domiciled in spent engine oil polluted soil. The concentration of Pb recorded in the soil samples was higher at the polluted sites relative to the control and higher than 0.01mg/kg by the WHO permissible limit. Mean value of Pb recorded in this study is lower than the value obtained by Uchendu & Ogwo, (2014) at Nekede mechanic village, but aligns with the values obtained by Orji *et al*, (2018) at spent engine oil contaminated soil of Gwagwalada.

Occupational and accidental exposures to high levels of Pb have been ascribed to various clinical effects including kidney damage, and effects on the nervous and reproductive systems. Other symptoms in occupational setting include anaemia, headache, insomnia, constipation and hypertension and low vitamin D production. In Pb contaminated soils, microbial enzymatic activity and organic matter breakdown can be impaired, together with reduced reproduction in nematodes. Controlled studies in laboratory setting have revealed effects of Pb on germination, seedling growth and metabolisms, photosynthesis and transpiration when exposed to concentrations higher than 100-1000 mg/kg^{-1}. Animals inhabiting Pb contaminated soils have been associated with kidney disorders and reduced red blood cell counts.

Arsenic was detected in all the polluted sites except the control but higher than the 0.009 mg/kg permissible limit by the WHO. The value of Arsenic obtained in this study was higher than 0.220±.00337mg/kg obtained by Ayodeji, Ogbole, Adewale & Abdulbasit (2019) in an auto mechanic village at Kogi state, Nigeria. Nwachukwu *et al.,* (2020) and Ololade (2014) in a related study, reported mean Arsenic values of 1.30±0.02mg/kg and 2.45±0.12mg/kg which is in line with the values obtained in this study. Implying that the soil samples were polluted with respect to Arsenic. The presence of Arsenic contaminated soils raises concern about direct exposure and the potential for localised pollution of soils and rivers.

The concentration of Chromium was higher at the polluted sites with no trace of Chromium detected at the control site. However, the mean values of chromium obtained in all the zones were within the permissible limit of 0.02mg/kg proposed by the WHO. Indicating that the soils from the auto mechanic workshops are unpolluted with respect to chromium. The range of chromium reported by Orji *et al*, (2018) was higher than that recorded from this study. In the same vain, other authors (Tóth, *et al*, 2016; Verla *et al*, 2015) had reported mean values similar to that obtained in this study. Chromosomal abnormalities have been recorded in workers exposed to elevated level of chromium in soil as having allergic reactions and the appearance of small pits in in the skin. In microorganisms, chromium exposure can have population effects and at the individual levels, impact on cell metabolism; in algal cells, growth and photosynthesis can be affected. In plants, chromium causes oxidative stress, affects photosynthesis and impairs enzymatic processes including nitrate reduction. Certain invertebrate species such as snails, larvae and crustaceans are sensitive to chromium pollution. Reduced reproductive capacity has been reported.

Cadmium levels in polluted sites recorded higher concentration in comparison with the control in all the sites but higher than 0.02 mg/kg WHO permissible limit in soil. This in in consonant with previous authors' reported higher mean values (0.6-3.5 mg/kg) of cadmium in soils of auto mechanic workshops (Luter, Akaahan, & Attah, 2011), but disagrees with mean value of 0.32mg/kg obtained by Ayodeji *et al*, (2019) at mechanic villages in Anyiba area of Kogi state. The health effects of cadmium are well documented from occupational and environmental exposure. Chronic inhalation of particulate cadmium from the workplace has been suspected of causing lung diseases. In plants, cadmium is easily taken up and is extremely toxic, disrupting enzyme activities as a result of its affinity for thiol group. An elevated level of cadmium has been linked to affect respiration, transpiration and photosynthesis, with visible symptoms such as leaf chlorosis and retarded growth. The presence of cadmium in soil can alter microbial activities. According to Ebong, Akpan, & Mkpene, (2008) the presence of Cadmium could be due to the dumping of PVC plastics, nickel-cadmium batteries, motor oil and disposal sludge in the auto-mechanic villages**.**

Concentration of Nickel in soil was detected in both polluted and unpolluted soil from the mechanic workshops with higher mean values recorded at the polluted site. However, the concentrations of Ni from the polluted sites were higher than 0.2 mg/kg established by the WHO. It is possible that there might be runoff from the polluted sites, hence the presence of Ni in the control site. Similar values of Ni had been reported by Johnbosco *et al.*, (2020). Ni concentration obtained by Orji *et al*, (2018) compares well with values obtained in this study, but higher than 0.069- 0.108 mg/kg obtained by Johnbosco *et al*, (2020). The main environmental impact of Ni in soil is phytotoxicity. In Ni polluted soils, plant uptake may be significant and lead to Ni concentrations in plants and crops that are an order of magnitude higher than background levels. Ni interacts closely with Fe and appears to inhibit Fe translocation from root to shoot. Outcomes of Ni toxicity include chlorosis, and impairments to photosynthesis, transpiration and N fixation. Nickel is known to accumulate in plants and with intake of too large quantities of Ni from plants grown on nickel rich soils (such as tea, beans, vegetables), there are higher chances of developing cancer of the lung, nose, larynx and prostrate as well as respiratory failures, birth defects and heart disorders.

Cobalt concentration of soil samples revealed a higher value at the polluted site compared with the control with no trace of cobalt. According to WHO permissible value of 0.05mg/kg, the auto mechanic soil samples were polluted with cobalt. This aligns with the findings of Warmate, Ideriah, Tamunobereton, & Udonam (2011). Iron, Zinc and Aluminium concentration were higher at the polluted sites than the control sites and exceeded the permissible limits set by the

WHO. Iron is an important micronutrient required for normal physiological processes in plants, but at elevated levels may pose threat to the flora and fauna in the ecosystem.

The high value of iron obtained in this study agrees with that reported by Shinggu, Ogungbuaja, Barminas, & Toma (2002).The increase in iron content of the soil might be as a result of waste generated in automobile workshops in the study area which includes solvent, hydraulic fluid, spent lubricants, metal construction works, welding of metals and iron bending. This result disagrees with the findings of Adelowo, Alagbe, & Ayandele (2006) where they noted that iron concentration in auto mechanic workshops fall within the permissible limits set by the WHO.

However, the mean concentration of Copper recorded in this study falls above the permissible limit of 2.0mg/kg which is the standard set by WHO. At this level in the soil, Copper has a massive concentration in the soil, which means the soil is contaminated with Copper. This result is congruent with the report by Otobong, & Victoria, (2017) who opined that areas with heavy vehicular traffic and higher tempo of anthropogenic activities of urban settlements have high soils contaminants than those with low vehicular traffic. Copper is bioaccumulative in some organisms. It bioaccumulates in some planktons, oyster and squid species, but rarely biomagnify in the food chain. Impacts in microorganisms are manifested by decrease in mineralisation of organic matter, available phosphorus and nitrogen. Impacts of copper have been observed in macro and microalgae.

Concentration of Zinc recorded at all sampling sites was above the permissible limit of 0.02mg/kg by the WHO. The study location has no industry it is thus believed that the increase of Zn levels in the study area was from the auto mechanic shops, since this element is found as part of many additives to lubricating oils. The concentration may be due to factors such as age of the mechanic workshops, volume of work done on each site, types of automobile service or repairs, type of lubricant commonly used, mode of wastes disposal and type of soil. The concentration of Zn in this study is low compared with many other studies (Nwachukwu *et al.*, 2011). Also, Omorowa & Agu, (2017) reported that high concentration of Zinc in heavy traffic zones indicate that fragmentation of car tyres are likely source of the metal.

Generally, man and other animals could directly take in heavy metals via inhalation of dusty soil (Ibe, *et al.*, 2021). Heavy metals such as Lead, Zinc, Copper, Cadmium and Nickel etc play disruptive role when they enter the body system in higher concentration than required amount (AC-Chukwuocha *et al.*, 2015). This often leads to Anemia, brain damage, anorexia, convulsion, vomiting and death (Bullut and Baysal, 2006; AC-Chukwuocha *et al.*, 2015).

Meanwhile plants in terrestrial ecosystem have been reported to bioaccumulate trace metals pollutants from soil in dumpsites, which consequently degrade the rate of phytodiversities (Amalu, 2009). Ali & Khan, (2018) opined that degradation of soil quality and decline in vegetation abundance are some of the issues associated with indiscriminate disposal of spent engine oil.

The overall trend for mean concentration of PAHs across the zones was as follows: Owerri> Orlu> Okigwe> control (Table 4.10). This indicates progressive high levels of pollution as a result of indiscriminate disposal of spent engine oil. This aligns with the findings of Ibe *et al*, (2021). However, it was observed that PAHs were recorded in the control sites. According to Ibe *et al*, (2021), detection of PAHs in the control sites could be linked to a plethora of factors such as topography. Mixtures of PAHs are among the pollutants that are considered carcinogenic in the environment (Tobiszewski & Namiesnik, 2012). They could be emitted from processes that occur naturally such as volcanic eruptions, biomass combustion, and diagenetic processes (Wang *et al.* 2011). Atmospheric deposition could also be ascribed to high PAH concentrations in areas considered unpolluted by virtue of the economic and other anthropogenic activities in such areas (Njoku, Ibe, Alinnor, & Opara, 2016; Ibe *et al*. 2020).

Also, the elevated levels of PAHs in the control may be linked to flooding in the area due to the high intensity of rain events which causes water overflow and heavy flooding resulting in substantial leaching and eroding of topsoil particles containing deposited or accumulated PAHs (Chukwuocha *et al*. 2017; Enyoh and Isiuku , 2020). This could result to eventual distribution of the PAHs to the floodplains and other areas having low elevation. These factors may have influenced the PAH levels observed at the control sites (Muze, *et al.*, 2020).

The values of PAHs recorded at the study sites were higher than the control sites in all the study areas. A study by Santos, Sausa, Soares, Frena, & Alexandre, (2018) recorded elevated levels of PAHs in sediments. The observed increase in PAHs across the study may be attributed to indiscriminate disposal of spent engine oil in all the studied sites. This is in agreement with the findings of Obinni *et al*, (2013). A study by Ogoko, (2014) at NNPC depot Aba recorded value in the range of 6.30-7.40 mg/kg which was higher than the values recorded in this study but aligns with the reported values recorded by Ibe *et al,* (2021). This according to Nwachukwu *et al.*, (2020) can be linked to indiscriminate disposal/management of spent engine oil on soil.

According to the United States Environmental Protection Agency (USEPA, 2006), PAHs pose a major threat in terms of their carcinogenic, mutagenic and teragenic effects on humans. Among these are Anthracene, Benzo (c) pyrene, Naphalene, Chrysene, Benzo (a) anthracene, Benzo (k) fluoranthene and Benzo (b) fluoranthene which were all observed at increased levels in this study. This finding agrees with the report of Adetunji, Opeolu, Olatunji, Fatoki, Jackson & Snyman, (2020). Benzo (c) pyrene, Naphalene, Chrysene, Benzo (a) anthracene, Benzo (k) fluoranthene and Benzo (b) fluoranthene are among the strongly carcinogenic PAHs in the environment (USEPA, 2006). The ability of PAHs especially Phe to induce neurotoxicity, cytotoxicity, DNA damage and reproductive disruption have been reported (Machdo, de, Hoff, Klein, Cordero, Lencina & Bianchinni, 2014). Some studies have also shown that PAHs can induce dioxin-like activity and weakened estrogenic responses (Ogoko, 2014; Villeneuve, Khim, Kannan. & Giesy, 2002).

TPH values recorded in the soil samples at the various auto mechanic workshops ranged from 3,6520 mg/kg to 1,932 mg/Kg as displayed in Figure 4.1. TPH concentration in the soil samples was in the order: Owerri> Orlu> Okigwe> Control. However, the control site recorded the least value of TPH (681 mg/Kg). The observed TPH at the control sites can be attributed to runoff/leaching from the source of dumping of spent engine oil (Ibe *et al*, 2021). The TPH concentrations were above 0.08 mg/kg and 1.7 mg/kg for the sample points and higher than background values by the Department of Petroleum Resources recommended maximum permissible limit of 1,000 mg/kg except for the control which was 681mg/Kg. The result further revealed elevated level of TPH at Owerri zone compared to other zones. This may be as a result of higher anthropogenic activities taking place at Owerri zone compared to other zones. Previous authors have reported higher levels of TPH in auto mechanic workshops compared to the values recorded in this study (Ololade, 2014; Farombi, Adebayo, & Oyekanmi, 2013).

Concentration of TPH obtained in this study is comparable with 1,923mg/kg reported by Akomah & Osayande (2018) at auto mechanic village in PortHarcourt. Alinnor & Nwachukwu, (2013) reported that soil samples in Rivers State, Nigeria were contaminated with TPH concentrations of 1534.7, 1438.0 and 1651.0 mg/kg at depths of 0.0 to 0.5 m, 0.5 to 1.0 m and 1.0 to 2.0 m respectively, which are in the same range of values obtained in this study. As reported by Iturbe *et al*, (2004), the soil of coastal Mexican refinery was heavily contaminated with hydrocarbons with appreciable level of TPH concentration of 130000 mg/kg which is higher than the values recorded in this study.

Toxicity of TPH in soil has been studied using a battery of ecotoxicological assays from various trophic levels of the ecosystem. Species such as plants, earthworm, and microorganisms and a range of acute toxicity to sublethal effects such as germination inhibition, root and shoot elongation, mortality in earthworm had been documented (Akin-Obasola, 2019). According to Environmental Protection Agency (USEPA) (2021) TPH is a huge threat to public health and safety via contamination of drinking water, causing fire and explosion hazards, diminishing air and water quality, compromising agriculture, destroying recreational areas, destroying habitats and food, and wasting non-renewable resources.

Sylvia, (2019) opined that once the soil is polluted by petroleum hydrocarbons (PHCs) the recovery may take several years. Petroleum residuals may stay bound to soil particles and remains in the soil for years. Although contaminants may benefit from carbon source as degrader they are still toxic to the ecosystem. PHCs may destroy the aesthetic by inducing offensive odour, taste or appearance in environmental media. The high contamination of the soil samples is an indication of indiscriminate spill of spent engine oil. Ogoko, (2014) had earlier stated that TPH compounds are generally carcinogenic, genotoxic, teratogenic and immunotoxic in nature. The presence of TPH at elevated levels far above permissible limit is a source of ecological concern.

Previous authors have recorded phytotoxic effect on germination and early seedling establishment of crops grown in spent engine oil contaminated soil (Nwachukwu *et al.*, 2020;

Onwusiri *et al*., 2017; Njoku *et al*., 2011). The cessation of seed germination by spent engine oil observed in this study is in line with previous research reports by Debojit, Jitu, & Sarada (2011); who opined that growth parameters in *Amaranthus hybridus* decreased as the concentration of crude oil contamination increased (Figures 4.2 and 4.3).

The toxic effect of spent engine oil used in this study was does-dependent. This finding is in agreement with the findings of Agbogidi & Ilondu (2013) in which the inhibitory effect of spent engine oil on germination and seedling growth of *M. oleifera* was found to be dose dependent. Also, they are in congruent with the data presented for *Glycine max, Vigna unguiculata* and *Zea mays* (Kayode *et al.,* 2009). It is possible that the embryo of the seeds could have been injured or killed if it comes in contact with the oil (Amadi *et al*., 2017).

According to Amalu, (2009), spent engine oil has the ability to prevent uptake of nutrient, water and oxygen required for seed germination. This could be as a result of volatile fractions of oil which have high wetting capacity and penetrating power; it enters the seed coat and kills the embryo. The presence of spent oil in the soil-plant micro-environment affects normal soil chemistry wherein nutrient release and uptake as well as amount of water get reduced (Nwoko Okeke, Agwu, & Akpan, 2007; Odejgba & Sadiq, 2002). The result also showed tolerance of plant species in this order: *V. unguiculata>G. max>Zea mays*. Agbogidi & Nweke (2005), Ogbo, (2009) and Agbogidi (2013) had opined that oil effect on plants are species and variety dependent (Table 4.11).

The use of plants as bioindicators of pollution had been documented by previous authors (Njoku *et al*., 2009). A marked dose-dependent reduction in germination was observed in all the accessions used. The EC_{50} expressed as the median effective concentration that resulted to 50%

inhibition of an organism. As observed in this study, the different plant species exhibited different abilities to withstand spent engine oil pollution. According to Njoku *et al,* (2021), this could be ascribed to differences in the genetic makeup of the plant species. Generally, the lower the EC_{50}, the higher the sensitivity of an organisms to chemical stressors in the environment. The value of EC50 values in this study shows that *Zea mays* was more sensitive to spent engine oil contamination than other plant species use.

Delayed burrowing could be attributed to the toxic nature of spent engine oil. This result is in agreement with the report of Dada *et al,* (2016) as shown in (Table 4.12). The LC50 values obtained in this study is lower than those reported by Dada *et al*, (2019) but aligns with values documented by Spurgeon & Hopkin, (2000). The relatively lower LC50 values obtained in this study shows higher toxicity of spent engine to earthworm (Table 4.13).

The values of total bacteria load from polluted and unpolluted soils are in the same range with 4.5 x 10^3CFU/mL obtained by Okere *et al*. (2021) but lower than 8.6 X 10^2 obtained by Udebuani *et al* (2013) from spent engine oil contaminated soils Owerri. Total bacterial count was higher in the polluted soil than the unpolluted soil (Table 4.14). It is possible that the microorganisms from spent engine oil polluted sites had more carbon source than those from unpolluted sites. Eight bacteria isolates were obtained and was screened down to four promising isolates connoting the highest absorbance rate based on preliminary studies (Table 4.15). The four pure bacteria species include: *Pseudomonas, Acinetobacter, Bacillus,* and *Enterobacter* species. It has been noted also that *Acinetobacter sp*. are wide spread in nature and can remove or degrade a wide range of organic such as phenol, toluene and heavy metals (Mandri & Lin, 2007). This is indication that the microbes were able to adapt and subsequently utilise spent engine as sole carbon source. Proliferation of such organism in polluted sites could be harnessed for bioremediation of spent engine oil polluted soils provided their homeostatic status is not altered by excess spent engine oil disposal. Biotolerance of the bacteria isolates as measured by optical density indicated a dose dependent absorbance rate by the microbes as shown in Figures 4.4 to 4.7. This is similar to the report of Ajunwa *et al*, (2018).

In this study, the ecological risk of spent engine oil was evaluated since these pollutants are most times disposed directly on soil with the possibility of seeping into nearby aquatic environment. The ecological risk characterisation through risk quotient analysis (MEC/PNECs) indicates that spent engine oil could have both human and biological impacts on each component of the food chain used in this study (microorganisms, plants and earthworm). The potential risk was high for plants and earthworm since the values were far more than unity (>1). On the other hand, the risk quotients for microorganisms were medium risk since the values were approaching unity 1 except for Enterobacter species with RQ .>1. Suggesting that potential ecological impact was more likely at the different PNECs measured. This observation in line with the report of Essien, Ebong, Asuquo & Olajire, (2012). Only a few studies have reported ecotoxicological risk due to spent engine oil in Nigeria. In a study by Valca´rcel *et al*, (2011), risk quotients were above 1. Essien, Ebong, Asuquo & Olajire, (2012) documented a risk quotient value greater than 1 for hydrocarbon contamination in the Niger Delta Region of Nigeria which is congruent with the findings in this study (Table 4.16).

In a related study, Yamamoto, Pereira, Cortez, Pusceddu, Santos, & Guimara˜es (2011) applied two cumulative risk evaluation approaches to estimate the risk due to the occurrence of seven *parabens* in water, but their hazard evaluation was based on toxicity data obtained for each compound individually, and did not include any synergistic or antagonist effects. Although their study employed a different approach, the idea was the same, to estimate the risk quotient in a real situation where an organism is exposed to a mixture of pollutants.

Growth parameters assessed in this study were plant height, number of leaf, number of root, fresh and dry weight respectively (ssee Tables 4.17, 4.18 and 4.19). Spent engine oil affected plant height at 4%, 8%, 12% and 16% treatment levels. Although there was a marked increase in plant height as the weeks passed with marked reduction as the concentration increased. The highest plant height performance was observed in control soil (0%) compared with other treatments. The spent engine oil in soil may have interfered with aeration, mineral availability, plant water relation and suitable warmth that are required for plant growth and development. This finding is in line with Adenipekun, (2008) who reported that treatment of soil with spent engine oil consistently inhibited plant growth.

Growth retardation could have been attributed to both soil-plant-water interrelations as well as a disruption in the xylem and phloem vessels due to the marginal soil structure (Agbogidi & Eruotor, 2012). As opined by previous authors (Sharifi *et al.*, 2007), plant growth stimulatory effect at 1 % spent engine oil contamination was observed when compared to the control for various plant species. Generally, the behaviour of the 4% spent engine oil in this study may be related to the findings of Nwoke *et al* (2007) who reported that contamination of soil with 1-5 % petroleum hydrocarbon normally act as a boost to soil organic matter. However, maximum growth inhibition was observed at the highest treatment level (16%) in all the plants. Increased contamination 12% and 16% had observable effects of the mean plant height at 12 weeks when compared to other treatments studied. The order of effects on plant height was as follows: *A. compressus*> *A. africana*> *C. odorata*. The result showed that spent engine oil retarded the height of the plants under study as evidenced by the reduction in growth of the plants. This corroborates the work of Nwachukwu *et al*, (2020).

The exposure of the test plants resulted to a decrease in leaf number with increase in concentration and the yellowing of the leaves. It is possible that photosynthesis might have been affected. According to a study by Olajuyigbe & Aruwajoye, (2014), leaf number was reduced by the hydrocarbon content of the spent engine oil in *Khaya senegalensis* and *Terminalia superba* similarly, a study by Nwoko, Okeke, Agwu, & Akpan (2007), documented that spent engine oil contamination hindered the growth and development of *Phaseolus vulgaris* seedlings which is consistent with the findings in this study. The changing colours and paleness of the leaves, leaf wilting and abscission, as well as darkening of lower portion of the stem region have also been previously attributed to diesel and spent engine oil contamination of soils (Nwoko *et al.,* 2007; Olajuyigbe & Aruwajoye, 2014; Fayinminnu & Abimbola, 2016). The severity of the effects of increasing treatment levels (from 8% to 16%) was initially high until the 8th week of the experiment, after which the leaf number stabilized.

Reduction in mean number of leaf recorded in this study compares well with the report of Kayode *et al.* (2009) on *Glycine max*, *Vigna uniguiculata* and *Z. mays L.*, and the findings of Adenipekun (2006) that used engine oil affect number of leaves in *Celosia argentea*. This implies that spent engine oil affected the leaf and this could be attributed to the large amount of hydrocarbons contained in the oil which includes the highly toxic PAHs (Table 4.18). This is in line with the report of Onwusiri *et al*., (2017). Ogbuehi, (2011) reported reduction in fresh and dry weight of soybean planted in crude oil polluted soil. The higher performance of control over other treatments (4%, 8%, 12%, and 16%) is an indication that the control were in higher supply of nutrients over the time with resultant decrease in fresh and dry yield. This is congruent with the findings of Onwusiri *et al*, (2017). According to Anoliefo (2001) reduction in number of roots and low biomass production could also be interpreted as being due to gross effects of the spent engine oil in the soil (Table 4.19). Multiple comparison of the growth properties measured indicated that the 16% concentration had the highest inhibitory effect on the plants under study. This is similar to the reported by Nwachukwu *et al*, (2020).

The accumulation of heavy metals in roots shoots and leaf may be an indication that the test plants (*C. odorata, A. africana* and *A. compressus*) posses the potential to remediate sites with low to medium contamination (Olajuyigbe, Fayinminnu, & Ayoade, 2019). This was evidenced by the survival and continued, though concentration-dependent reduction in growth of the test plants, in spent engine oil contaminated soils (Nwoko *et al.,* 2007). The ability of *C. odorata, A. africana* and *A. compressus* to grow in soils contaminated with spent engine oil suggests that the species may have the ability to phytodegrade the toxicants resident in spent engine oil (Figures 4.8 to 4.18). This result is similar to the findings of Lone *et al,* (2008). Similar growth responses have been reported for seedlings of *Terminalia ivorensis, Terminalia superba* and *Khaya senegalensis* (Olajuyigbe & Aruwajoye, 2014). A significant and positive correlation was recorded between TPH concentration in soil and plant tissue. This result is in agreement with the report of Raymond & Harrison (2018). The reason might be that increase in TPH content in soil resulted to increased level of TPH in plants.

Bioconcentration and transfer factors are two major parameters used in evaluating the rate of heavy metal uptake in in plants tissues and potentials for phytoremediation (Sharmar & Reddy, 2020; Mirsal, 2008). The ratio of metal concentration in plant root to soil (contaminant source) give the Bioconcentration factor (BCF) and the further ratio of root to shoot transfer of contaminant is referred to as Transfer factor (TF) (Yadav, Juwarkar, Kumar, Thawale, Singh, &

Chakrabarti, 2009). By convention, when both BCF and TF are >1 it means that the plants have potentials for phytoremediation of spent engine oil polluted soils (Chandra *et al.,* 2017). The BCF of all plant species exposed to different concentrations of spent engine oil was found more to be more than one (>1) for the majority of the heavy metals assayed, this is an indication that the plants were accumulating the metals from soil to root. Although bioaccumulation ratios of the plant species for some of the heavy metals indicated values less than unity (<1) to be classified as hyperaccumulators, the plants were identified as potential species that can succefully carry out phytoextraction of spent engine oil polluted sites. Bioconcentration pattern in the plant species was in the order: *C. odorata>A. africana>A. compressus* (Table 4.20). The result in this study showed that the plant species have the potentials to be used as phytoaccumulators. Results of this study agree with the report of previous researchers (Sharma, & Reddy, 2020; Pooja, Tripathi, Chandra, 2020). The variation in heavy metal accumulation pattern might be attributed to differences in the specific physiological characteristics of the test plants based on their genetic make-up (Sharma, & Reddy, 2020).

The ability of all plants to transfer metals from root to shoot which is an important parameter of phytoremediation potential also indicated that these tested plants showed TF > 1 for most of the heavy metals assayed (Table 4.20). The TF pattern was in the order: *C. odorata>A. compressus>A. africana*. A study by Oseni, Dada, & Adelusi, (2015) reported decontamination of Lead (Pb) contaminated soil with *C. odorata* which in tandem with findings in this study. Efe & Okpali, (2012) reported that *A. compressus* (Poaceae) degraded 47% and 48% in petroleum impacted soils. According to Ma, Komar, Zhang, Cai, & Kennelley, (2001), plant species with TF>1 are categorized as hyperaccumulators for metal accumulation from roots to shoot. According to Suleiman & Hamzar, (2018), the root appears to accumulate higher levels of heavy metals compared to other parts of the plants. This is because the root acts as a barrier for metal translocation and possibly protech the stem from metal contamination. This might have lead to higher ratios of transfer factor obtained in this study. This is in line with the report of Suleiman & Hamzar, (2018). In a related study by Wei & Chen, (2006), plant species with TF >1 actively mopped up metals to the aerial parts which makes them good phytoremeditors. TF>1 obtained in this study suggested a high metal accumulation property of these plant species for the translocation and metal movement to the aerial parts of plants. This result corroborates the findings of previous authors (Udoro & Essien, 2015; Njoku *et al.*, 2009 Ogbo, Zibigha, & Odogu, 2009).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Within the context of this study, it is evident that the quantity of spent of spent engine oil being disposed into the terrestrial and aquatic environments is quite alarming. The inferences drawn from the physical and chemical analysis of spent engine oil polluted and unpolluted soil showed that there was alteration of physicochemical properties and a gradual build-up of heavy metals, Polyaromatic hydrocarbons and, total petroleum Hydrocarbons in the soils of auto mechanic workshops in Imo State. Heavy metal concentration of the polluted soils where observed to be higher than the permissible limits of the World Health Organisation (WHO) for the majority of metals assayed. Of note are the higher levels of PAHs and TPH detected in the spent engine oil polluted soils which are the main constituents of spent engine oil. These pollutants are known to exert harmful effects on the quality of life with possible alteration of ecosystem integrity. Majority of the Polyaromatic hydrocarbons detected in this study are known to be mutagenic, tumorigenic and carcinogenic. This further reaffirms the need for holistic environmental assessment to estimate the health of the ecosystem. The negative influence of spent engine oil on the organisms was obvious and could portend a serious danger in the ecosystem if the rate of spent engine oil disposal persists. Bioassays with plants, earthworms and microorganism represent a direct contact test that measures the effect of a pollutant on test organisms. Plants are known as primary producers in the ecosystem supporting other forms of life. Earthworms are one of the key species with multiple economic importance in agriculture. Soil microorganisms are known to perform a number of vital processes in the maintenance of soil quality. Antimicrobial activities of spent engine oil on soil may distort the activities of these microbes in soil and differentially inhibit their growth with resultant alterations in ecological functionality of the soil. The negative effects of spent engine oil disposal may distort the proper functioning of the soil which could result to biodiversity loss with man at the receiving end of the food chain. The organisms showed varied sensitivity to contaminant levels, however for the most part, the spent engine oil had a negative impact on the organisms in each scenario. Nevertheless, considering inhibition in seed germination and early seedling growth, mortality in earthworms and variation in inhibition of microbial growth, it could be concluded that the pollutants present in spent engine were the main cause of ecotoxic effects observed on the organisms. By applying the risk quotient approach, a potential adverse ecological risk was obvious for *Zea mays, V. unguiculata*, *G. max* and Earthworm with a risk quotient greater than 1. The differential response and sensitivity of the test organisms used in this study suggested that they could be useful indicators for the assessment of soil quality and ecosystem health. The microbes (*Pseudomonas sp.*, *Bacillus sp.*, *Acinetobacter sp.*, and *Enterobacter sp.*) though were inhibited minimally, but were able to utilise spent engine as carbon source, thus making them potential candidates for bioremediation of spent engine oil contaminated sites provided their current homeostatic status is not altered by excessive spent engine oil contamination in the ecosystsem. For plants to thrive in heavy metal laden sites, they either phytostabilise the contaminants or accumulate them through their cellular structure. The ability *of O. odorata, A. africana* and *A. compressus* to bioaccumulate and transfer heavy metals in their aboveground tissues (root, shoot and leaf) suggested that these plants could be used to remediate spent engine oil contaminated sites. Thus, the three plant species have demonstrated the ability to tolerate heavy metal stress, grow and accumulate biomass in spent engine oil polluted soil. This underscore the need to further explore the adaptability of these indigenous plant species to heavy metals for their selective exploitation in phytoremediation of spent engine oil polluted sites. This study has successfully integrated ecotoxicological risks posed to terrestrial organisms by indiscriminate disposal of spent engine using risk quotient method and phytoremediation using indigenous plant species. High to medium ecotoxicological risk was observed in each test organisms due to the presence of pollutants contained in the spent engine oil. This underscores the need to enact laws probihiting indiscriminate disposal of spent engine oil on the environment. These findings validate the need for recycling and reuse of spent engine oil to forestall further pollution of the environment.

5.1.1 Contribution to knowledge

Within the context of this study, results obtained and discussions that followed, the following contributions to knowledge were made:

- Documented plant species capable of phytoremediating spent engine oil polluted sites in Imo State.
- The study has successfully elucidated the toxic effects of spent engine oil on terrestrial ecosystem by applying a battery of ecotoxicological tests at different trophic levels of the ecosystem in Imo State.
- This study has established risk quotient as a complementary, easy to measure, and cheap tool for assessing real risk organisms as a result of indiscriminate disposal of spent engine oil in the ecosystem.
- The study has established that *C. odorata,* and *A. africana* are potential phytextractors while *A. compressus* are proven potential phytostabiliser of spent engine oil polluted sites.

5.2 Recommendations

Based on the findings in this study and the subsequent discussions, the following recommendations are made:

- Training and awareness should be created to inform the auto mechanics on the toxic nature of spent engine oil and possible environmental hazards.
- Adequate plans should be made to conserve indigenous plant species identified at the study sites for restoration of hydrocarbon polluted sites.
- Given the level of sensitivities observed among the accessions used, *Zea mays*, *G. max* and *V. unguiculata* are recommended for biomonitoring of the ecosystem health.
- Since *O. odorata, A. africana* and *A.compressus* exhibited tenacity to withstand deleterious effects of spent engine oil, it is recommended that the plants should be subjected to field trial in phytoremediation studies within the tropics.
- More studies should be carried out to ascertain the effect of spent engine oil pollution on oxidative stress of the plants used in this study.
- More studies should be carried out to ascertain occupational health risk of spent engine oil among auto mechanics.
- Further study should be carried out to probe the impact of spent engine oil on DNA of the test organisms used in this study.
- More work should be carried out to investigate the molecular mechanisms of bioaccumulation and translocation of heavy metals in plant tissues.

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APPENDICES

APPENDIX I: IMAGES SHOWING MECHANICS AT THE VARIOUS SAMPLING SITES

APPENDIX II: AAS MACHINE USED FOR HEAVY METAL ANALYSIS

APPENDIX III: A COPY OF THE QUESTIONNAIRE USED FOR THE STUDY

RESEARCH QUESTIONAIRE

Dear Sir/Madam,
This questionnaire is for the collection of data in respect of ongoing research work. This study is mainly for academic purpose and all information supplied here will be treated with strict confidence

Please do fill the questionaire form to the best of your knowledge
(Please tick as appropriate).

S/N	QUESTIONS	RESPONSE(S)
1	What is your level of education?	Primary/Secondary/College/Tertiary/None
2	How long have you been a mechanic?	1-10, 10-15, over 20yrs
3	How many automobile vehicles do you service in a day?	1-5 5-10 10-15 20 and above
4	What quantity of used oil do you remove from a vehicle serviced	1-5L 5-10L 15L and above
5	a. How is the spent engine oil you removed from vehicle stored? b. What do you do with large quantity of spent engine oil collected	Plastic keg/no storage/drums
6	How do you dispose the spent engine oil you removed from a vehicle?	Sold/poured into the environment/given to people/given to clients/sales/reuse
7	Are you aware of the occupational health risk ofexposure to spent engine oil used oil? If yes, mention any	YES/NO
8	Are you aware of the environmental risksassociated with exposure to spent engine oil?	YES/NO
9	What other purposes do spent engine oil serve in the society Or Are you aware of any local way spent engine oil can be recyclingand used?	YES/NO
10	Have you had any talk or training on-hazards associated with exposure to spent engine oil?	YES/NO

Appendix V: Number of vehicles serviced per week in the three study locations

Locations	Weeks	F	Percentage (%)
OWERRI	1	0	0
	2	6	6.7
	3	7	7.8
	4	8	8.9
	5	8	8.9
	6	9	10
	7	4	4.4
Total		**42**	**46.7**
Mean		**7.7**	
ORLU	1	0	0
	2	3	3.3
	3	4	4.4
	4	7	7.8
	5	6	6.7
	6	3	3.3
	7	4	4.4
Total		**27**	**30.0**
Mean		**4.50**	
OKIGWE	1	0	0
	2	3	3.3
	3	2	2.2
	4	3	3.3
	5	6	6.7
	6	5	5.6
	7	2	2.2
Total		**21**	**23.3**
Mean		**3.90**	

Appendix VI: measuring of earthworm weight in the laboratory