. REMEDIATION OF SPENT ENGINE OIL POLLUTED SOIL USING MUSHROOM

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CERTIFICATION

This is to certify that this work was carried out by IKPONMWOSA ZABAYOR of the Department of Soil Science and Land Management, Faculty of Agriculture, University of Benin Benin-city.

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Date:	Date:

DEDICATION

My research work is heartily dedicated to God almighty and to my parents Mr and Mrs Zabayor for their love, understanding and support towards my academics, aspiration and goal.

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I want to give glory and honour to God almighty for being a source of strength, drive, motivation and inspiration unto me this very hour. I can not sincerely thank him enough. He is faithful indeed.

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ABSTRACT

The experiment was conducted at Faculty of Agriculture experimental farm, located at the back of the new faculty building of the University of Benin, Benin city, with the aim to determine the potentiality of mushroom in the removal of heavy metals from spent engine oil polluted soils.

The experiment was a 5m x 3m experiment in completely randomised design with 3 replications. The spent engine oil was applied and was left for 2 weeks before mushrooms were added to the soil. The Soil samples were then analyzed using Standard laboratory analytical methods. Some of the physical and chemical parameters of the soil tested were pH, total nitrogen, total organic carbon, available phosphorus, total hydrocarbon content, potassium, calcium, magnesium, and sodium.

The result showed that most of the parameters tested including pH, total nitrogen, total organic carbon, available phosphorus, total hydrocarbon content, potassium, calcium, magnesium, and sodium increased at all levels of spent engine oil treatments, while the exchangeable acidity decreased at all levels of spent engine oil treatments, thereby breaking down complex detrimental element from the SEO which is finally absorb by the plant used.

The result from the experiment was not significant due to the limited time used in carrying out the experiment.

CHAPTER ONE

1.0 INTRODUCTION

The disposal of spent engine oil (SEO) into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics. This oil, also called spent lubricant or waste engine oil, is usually obtained after servicing and subsequently draining from automobile and generator engines (Anoliefo and Vwioko, 2001) and much of this oil is poured into the soil. There are relatively large amounts of hydrocarbons in the used oil, including the highly toxic polycyclic aromatic hydrocarbons (Wang *et al.*, 2000). Nevertheless, this is dependent on the local environmental conditions and on the kind of soil constituents present in the soil-water system.

Spent engine oil is also referred to as based crankcase oil, is a brown to black liquid produced when new mineral based crankcase oil is subjected to high temperature and high mechanical strain. Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment. Contamination of soils with spent engine oil is one of the attendant environmental problems associated with industrialization and the dependence on petroleum and it's by products. Soil pollution occurs when contaminants that lead to change in the physical or chemical properties of the soil are introduced in the soil. The solid components of soil are usually clustered together in form of aggregates, thus creating a system of interconnected voids of various sizes filled with either air or water. These voids facilitate the accumulation of substances that settle on them easily thus resulting to pollution. Soils are dynamic bodies in equilibrium with environmental forces acting upon them. Alloway, B. J. (2000). The contamination or pollution of any environment with spent engine oil and related products poses a threat to the life of aquatic and terrestrial organisms including humans living in such polluted environments. This is due to the potential toxicity, mutagenicity, and carcinogenicity of crude oil products when present in high concentrations (Adebusoye *et al*., 2007). These contaminants affect the chemical composition and physical matrix of soils culminating to the loss of soil fertility, change in ecosystem and displacement of communities (Okpokwasili and Nnorom, 1990; Chikere and Azubuike, 2013). These dangers in combination with others can alter population dynamics and disrupt trophic interactions and structure of natural communities within an ecosystem (Bejarano and Michel, 2010). The potential dangers resulting from spent engine oil pollution have driven man into the search for environmentally friendly approaches to reclaim polluted environments, particularly crude oil polluted soil (Okpokwasili and Nnorom, 1990; Chikere and Azubuike, 2013).

Previous studies had revealed that the spent engine oil pollutants often resulted to insufficient aeration of the soil due to the displacement of air from the spaces between the soil particles, retard growth of plants, results in chlorosis of leaves and dehydration of plants. Soil contamination with hydrocarbons causes extensive damage of body system since accumulation of pollutants in animals and plant tissue may cause death or mutations (Sheetal, 2012).

Remediation is an innovative environmental friendly approach to remediate soil which can be used to remediate both organic and inorganic contaminants (Salt *et al*., 1998). The contaminated soils including areas of agricultural fields and their remediation is a serious issue (Khan, 2005). As trace elements cannot be degraded they are considered as non-biodegradable. They may be accumulated in the tissues of living organisms, therefore they only moved from one place to the other (Kumpiene *et al*., 2008). Conventional methods of bioremediation are based on the physical and chemical approaches. Combination of them can be used for better efficiency. However, these methods can be more expensive, time consuming and damaging for the environment, compared with the biological approach.

mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground, on soil, or on its food source. The standard for the name "mushroom" is the cultivated white button mushroom, Agaricus bisporus; hence the word "mushroom" is most often applied to those fungi (Basidiomycota, Agaricomycetes) that have a stem (stipe), a cap (pileus), and gills (lamellae, sing. lamella) on the underside of the cap. Mushroom" also describes a variety of other gilled fungi, with or without stems, therefore the term is used to describe the fleshy fruiting bodies of some Ascomycota. These gills produce microscopic spores that help the fungus spread across the ground or its occupant surface. Mushrooms and other fungi play a role in the development of new biological remediation techniques (e.g., using mycorrhizae to spur plant growth) and filtration technologies (e.g. using fungi to lower bacterial levels in contaminated water) (Kulshreshtha *et al*., 2014)

The main objective of this study therefore, is to use mushroom to remove some heavy metals from the spent engine oil contaminated soil.

1.1 Aims and objectives

The specific objectives are to determine;

1. The effect of spent oil on some physical and chemical properties

2. To evaluate the potential use of mushroom in remediation of spent engine oil polluted soils.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Spent engine oil (SEO)

Spent engine oil (SEO) is typically referred to as used motor oil that have been collected from mechanical workshops, garages, and industry sources such as hydraulic oil, turbine oils, process oil and metal working fluids. Spent engine oil is a mixture of several different chemicals including low and high molecular weight (C15-C20) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, lubricant additives, chlorodibenzofurans, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they wear and tear down (Wang *et al*., 2000). Spent auto engine oil is obtained during routine maintenance of automobile and power generating engines; and often indiscriminately disposed into gutters, municipal drainage systems, open vacant plots and farms in Nigeria by auto technicians and allied artisans with workshops on the road sides and open places (Anoliefo and Vwioko, 2005). Spent engine oil contains complex mixtures of paraffinic, naphthalenic and aromatic petroleum hydrocarbons and various contaminants that may contain one or more of the following: carbon deposits, sludge, wear metals and metallic salt, aromatic and non aromatic solvents, water (as waterin-oil emulsion), glycols, silicon based antifoaming compounds, fuel, polycyclic aromatic hydrocarbons [PAHs] and miscellaneous lubricating oil additive materials (Ayoola and Akaeze, 2012). Engine oil becomes contaminated as a result of physical and chemical reactions. Metals from engine from time to time erode into the engine oil forming impurities.

2.2 General Properties of Engine Oil

The general properties of engine oil according to Wikipedia (2015) include the following:

1. Viscosity index: Oils behave differently at different temperatures. As temperatures drop, the hydrocarbon molecules in mineral oils start to line up and stick together. This causes the viscosity of the oil to increase, which makes it harder for it to lubricate an engine. At high temperatures, the opposite happens and the oil's viscosity decreases, making it less effective at protecting moving parts.

2. TBN (total base number): TBN is a measure of the oil's alkalinity. Alkalinity in oil is important because the combustion process produces acids which can attack metals and other materials in an engine, increasing wear. When oil is new, the TBN is highest. Over time, TBN decreases until finally the oil reaches a point where it cannot absorb any more acids and the acidity of the oil in the engine will start to rise. Most often, it is this depletion of TBN which signals that oil is 'worn out' and due to be changed.

3. Pour point: Pour Point is the lowest temperature at which the oil can still be poured out of a container.

4. Flash point: Flash point is the temperature at which the vapor of the oil will start to combust, but not continue to burn when mixed with air.

5. Noack volatility: Noack volatility is an oil property testing in which oil is heated to a temperature of 250˚C for one hour, after which the percentage of weight lost by the oil is measured. This indicates the extent to which the lighter-weight fractions of the oil are

volatilized and lost to the atmosphere. Any oil that volatilizes easily performs poorly because it quickly becomes thick.

6. Shear stability: Shear stability is an expression of how well the oil stands up to mechanical shear loads. In an internal combustion engine, oil is subjected to extreme shear loads as parts slide past each other. Oils with poor shear stability will 'shear out' and lose viscosity.

7. Oil weight: Most oils used in automotive and truck applications are multi-grade oils. This is indicated by the familiar nomenclature like 10W-30 or 10W-40. The first number is the winter weight of the oil. It indicates how the oil behaves when cold. The second number (30, 40, etc) is the nominal viscosity of the oil at 100˚C. Thus, a '10W' indicates how the oil behaves as "straight" 10 weight oil when cold. A 10W-30 behaves the same as "straight" 30 weight oil when it is hot.

2.3 Composition of Spent engine oil

Used mineral-based crankcase oil is a complex mixture of low and high (C15-C50) molecular weight aliphatic and aromatic hydrocarbons, lubrication additives, metals, and various organic and inorganic compounds. The chemical composition of used mineral-based crankcase oil varies widely and depends on the original crude oil, the processes used during refining, the efficiency and type of engine the oil is lubricating, the gasoline combustion products, the additives added to the fuel and to the original oil, and the length of time that the oil remains in the engine. The oil is typically 73-80% weight/weight aliphatic hydrocarbons (primarily alkanes and cycloalkanes with l-6 rings); 11-15% nonaromatic hydrocarbons; 2-5% diaromatic hydrocarbons; and 4-8%

polyromantic hydrocarbons (Vasquez-Duhalt 1989). The lubrication additives, which are approximately 20% of the oil, consist primarily of zinc diaryl, molybdenum disulfide, zinc dithiophosphate, metal soaps, and other organometallic compounds. Detergents and dispersants constitute 2-15% of the additives (Vasquez-Duhalt 1989).

2.4 Impact of Spent Engine Oil on Human Health

Health effects due to waste engine oil result from exposure to it. Owing to the physicochemical properties of used oil, the dermal route is the most susceptible route of exposure (Christopher *et al*., 2011). The contaminants in used oil can induce a variety of illnesses and diseases in humans and other mammals through inhalation, ingestion or skin contact. Observed effects include lipid pneumonia, lipid granuloma in the lung, eczematous and contact dermatitis, folliculitis, oil acne, and melanosis. Used oil can induce cancer, principally squamous cell cancer of the skin and scrotum, bladder and liver cancer (Bamiro and Osibanjo, 2004). These effects can be attributed largely to the presence of PAHs in used oil; benzene, toluene, and chlorinated solvents can also contribute to this toxicity. PAHs are formed by incomplete combustion of organic matter, such as oils. Seven PAHs have been classified as probable human carcinogens. PAHs are generally rapidly absorbed upon inhalation, ingestion or exposure through the skin. Heavy metals tend to concentrate in the environment (eg, in plants, animals and aquatic species), and humans may come into contact with them, causing a wide range of illnesses such as cancer, anemia, skin ulcerations and cardiovascular disease (Mckeagan 1992). The resultant health impacts of used mineral-based crankcase oil depends onwhether the chemicals are taken up, stored or excreted by the host body after exposure and on the individual properties of the chemicals in the oil. Studies on cattle that swallowed used mineral-based crankcase oil showed that lead and other metals in the oil were absorbed and distributed to various organs, such as the liver and kidney. Studies on mice showed that the PAHs that build up in used mineral-based crankcase oil were absorbed when the oil was applied to the skin (Granella and clonfero,1991). Polychlorinated biphenyls (PCBs) are principally found in used transformer oils, but it is believed that there are now very low levels of PCBs remaining in oils. Although the incidence of PCBs in used oil is low, the health effects from exposure are very serious. PCBs are highly persistent and can accumulate to high levels in human tissue. This can cause serious health effects, including liver damage, respiratory problems, cancer promotion, endocrine disruption and neurotoxicity (Mckeagan 1992).

2.5 Effect of Heavy Metals On Aquatic Environment

Heavy metals are highly persistent, toxic in trace amounts, and can potentially induce severe oxidative stress in aquatic organisms. Thus, these contaminants are highly significant in terms of ecotoxicology. Moreover, metals are not subject to bacterial degradation and hence remain permanently in the marine environment (Woo *et al*., 2009). Contamination of a river with heavy metals may cause devastating effects on the ecological balance of the aquatic environment, and the diversity of aquatic organisms becomes limited with the extent of contamination (Ayandiran *et al*., 2009). Heavy metals released into aquatic systems are generally bound to particulate matter, which eventually settle down and become incorporated into sediments. Surface sediment therefore is the most important reservoir or sink of metals and other pollutants in aquatic environments. Sediment-bound pollutants can be taken up by rooted aquatic macrophytes and other aquatic organisms (Peng *et al*., 2008). Because a major fraction of the trace metals introduced into the aquatic environment eventually become associated with the bottom sediments, environmental degradation by metals can occur in areas where water quality criteria are not exceeded, yet organisms in or near the sediments are adversely affected (Gurrieri J.T., 1998). Diatom community structure can be affected by high levels of micropollutants, and in particular by metals, which are often found in rivers. Once heavy metals are accumulated by an aquatic organism, they can be transferred through the upper classes of the food chain. Carnivores at the top of the food Chain including humans, obtain most of their heavy metal burden from the aquatic ecosystem by way of their food, especially where fish are present so there exist the potential for considerable biomagnifications (Ayandiran *et al*., 2009).

2.6 What is Soil?

Soil is the loose surface material that covers in most land. It consists of inorganic particles and organic matter. Soil provides the structural support to plants used in agriculture and is also their source of water and nutrients. Soils vary greatly in their chemical and physical properties. Processes such as leaching, weathering and microbial activity combine to make a whole range of different soil types. Each type has particular strengths and weaknesses for agricultural production.(Leeper *et al*., 1993)

2.7 Physical Properties of Soil

The physical characteristics of soils include all the aspects that you can see and touch such as:

- texture
- colour
- depth

- structure
- porosity (the space between the particles)
- stone content.

Soil structure contributes to soil and plant health allowing water and air movement into and through the soil profile. Soil stores water for plant growth and supports machine and animal traffic. While some soils are naturally better structured than others, some physical characteristics of soils can be changed with good management. It is important to monitor the physical characteristics of soil to understand soil condition. It is also important to ensure that management practices are not contributing to the decline of the soil. An example of this is excessive traffic causing compaction and reducing the amount of macropores, or spaces between the aggregates, therefore reducing the amount of air and water into and through the soil (Leeper *et al*.,1993)

2.8 Chemical Properties of Soil

Eight chemical elements comprise the majority of the mineral matter in soils. Of these eight elements, oxygen, a negatively-charged ion (anion) in crystal structures, is the most prevalent on both a weight and volume basis. The next most common elements, all positively-charged ions (cations), in decreasing order are silicon, aluminum, iron, magnesium, calcium, sodium, and potassium. Ions of these elements combine in various ratios to form different minerals. More than eighty other elements also occur in soils and the earth's crust, but in much smaller quantities. Soils are chemically different from the rocks and minerals from which they are formed in that soils contain less of the water soluble weathering products, calcium, magnesium, sodium, and potassium, and more of the relatively insoluble elements such as iron and aluminum. Old, highly weathered soils normally have high concentrations of aluminum and iron oxides (Leeper *et al*., 1993)

Chemical properties of soils depend on the following factors:

Inorganic matter present in the Soil: The mineral content of the soil is the major factor that differentiates various types of soil. It is so because of its abundance in the soil.

Organic matter present in Soil: Though these matters present in very small quantities but they play important role in deciding the fertility of the soil.

Colloidal properties of Soil: Colloids are mainly of two types:

Clay Colloids: they are important for the adsorption of a large quantity of water.

Organic Colloids: these help in increasing the moisture and nutrient retention capacity of the soil.

the pH of Soil: The measure of the chemical reaction which a soil shows is expressed by its pH value. The pH value of soil determines its acidic or basic nature. (Lotusarise 2021). Low pH values indicate acidic soil, and a high pH indicates alkaline conditions. Most complex plants grow only in the soils with levels between pH 4 and pH 10 but optimum pH varies with the plant species. In arid and semi-arid regions, soils tend to be alkaline and soils in humid regions tend to be acidic. To correct soil alkalinity and to make the soil more productive, the soil can be flushed with irrigation water. Strongly acidic soils are also detrimental to plant growth, but soil acidity can generally be corrected by adding lime to the soil. The most important effect of pH in the soil is on ion solubility, which in turn affects microbial and plant growth. A pH range of 6.0 to 6.8 is ideal for most crops because it coincides with optimum solubility of the most important plant nutrients. Some minor elements (e.g., iron) and most heavy metals are more soluble at lower pH. This makes pH management important in controlling the movement of heavy metals (and potential groundwater contamination) in soil. Lime requirement, or the amount of liming material needed to raise the soil pH to a certain level, increases with CEC(cation exchange capacity). To decrease the soil pH, sulfur can be added, which produces sulfuric acid. (Lotusarise 2021).

2.9 Biological Properties of Soil

Organic matter in the soil improves soil structure and increasing the nutrient and water holding capacity of the soil. Organic matter also provides a food supply for soil biology. Soils with low organic matter can have a 'poor' structure, hold little water, and erode or leach nutrients easily. The exception is cracking clay soils where clay minerals have the main effect on the structure. Soils with high organic matter levels have a 'good' structure, good water-holding capacity, and reduced erosion and nutrient leaching.

Biological properties include:

organic matter

soil organisms

the presence of disease-causing organisms

The total role of biologic processes in soil formation includes the presence and activities of living plants and animals as well as their non-living organic products. Living plants contribute to soil formation in two basic ways.

(i) Biomass i.e. the production of organic matter the biomass both above the soil as stems and leaves and within the soil as roots. It provides the raw material of organic matter in the O horizon and in lower horizons. The decomposer organisms process this raw material, reducing it to humus and ultimately to its initial components carbon dioxide and water.

(ii) Nutrient Recycling: It involves the cycling of nutrients from the soil in dead plant tissues. Nutrient recycling is a mechanism by which nutrients are prevented from escaping through the leaching action of surplus soil water moving downward through the soil.

Animals living in the soil play an important role in biologic processes of soil. e.g. earthworms rework the soil not only by burrowing but also bypassing the soil through their intestinal tracts.

Some of the important factors which decide the biological behavior of soil are:

Respiration rate: CO2 evolution under standard laboratory conditions or at the field.

Potential N/C mineralization: Increase in mineral Nitrogen or Carbon content under standard laboratory conditions.

Earthworms: Density of earthworms.

Bacterial biomass: Total bacterial biomass for a given soil mass.

Bacterial diversity: It can be determined by functional groups, or describing genetic diversity.

Presence of pathogens: By different pathology techniques, from cultures to DNA profiling. (Lotusarise 2021)

2.10 Effect of Heavy Metals on Soils

Heavy metal pollution not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community (Yao *et al*., 2003). Therefore, heavy metals are considered as one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd, Zn, Cr, and Pb (Hinojosa *et al*., 2004). Heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes (Shun-hong *et al*., 2009). Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms. Conversely, long-term heavy metal effects can increase bacterial community tolerance as well as the tolerance of fungi such as arbuscular mycorrhizal (AM) fungi, which can play an important role in the restoration of contaminated ecosystems (Mora *et al*., 2005). Chen *et al*, (2010) suggested that heavy metals caused a decrease in bacterial species richness and a relative increase in soil actinomycetes or even decreases in both the biomass and diversity of the bacterial communities in contaminated soils. Karaca *et al*, (2010) reported that the enzyme activities are influenced in different ways by different metals due to the different chemical affinities of the enzymes in the soil system. Cd is more toxic to enzymes than Pb because of its greater mobility and lower affinity for soil colloids. In general, an increase of metal concentration adversely affects soil microbial properties e.g. respiration rate, enzyme activity, which appears to be very useful indicators of soil pollutions. In case of soil contaminated with lead (Pb) slight change was observed in the soil microbial profile.

2.11 Effect Of Spent Engine Oil On Some Soil Physical Properties

The significant increase in bulk density of spent engine oil treated soil could be attributed to compaction resulting from oil contamination we well as reduced porosity. The presence of spent engine oil in the soil increase the bulk density and decrease water holding capacity of the treated soil. Similarly, the soil porosity decreases with an increase in the concentration of spent engine oil in the soil. Spent engine oil at high concentration alters the physical properties of the soil, it destroys the structural classes as well as leads to increased bulk density of the soil, which can reduce root penetration of crops and subsequently impedes nutrients uptake from the soil (Kayode *et al*., 2009). An increase in the bulk density of soil especially above 1.4g/cm^3 may lead to reduction in crop yield (Janssen and Vander-Weert 1997). Spent engine oil affects the soil aeration in the polluted soil. Previous assertion by Bossert and Bartha (1984) revealed that crude oil readily penetrates the pore spaces of terrestrial vegetation on land following any spill with heavier friction, which may block the spores of the soil particles. Spent engine oil is likely to have similar effects on land (Kayode *et al* 2009). Rasaiah *et al* (1990) observed decreased soil water retention capacity in soils contaminated with spent engine oil.

2.12 Effect Of spent Engine Oil On Some Chemical Properties

The pollution of the soil with spent engine oil can also alter the chemical properties of the soil. It was observed that spent engine oil made the soil acidic and increased organic matter and carbon content were observed. This was due to the addition of carbon already present in the spent engine oil to carbon already present in the soil (Kayode *et al*., 2009). Spent engine oil in soil also reduces phosphorus, Nitrogen, Calcium and Magnesium content of the soil. Kayode *et al* (2009) observed that there was reduced nitrogen in the soil treated with spent engine oil. Spent engine oil in soil can inhibit microbial transformation of organic matter thus leading to low mineralisation of phosphorus. Ogboghodo *et al* (2004) noted that available phosphorus was low in soil treated with spent engine oil. The application of spent engine oil depresses the availability of exchangeable cations.

2.13 Effect Of Heavy Metals Polluted Soil On Plant Growth

Some of these heavy metals i.e. As, Cd, Hg, Pb or Se are not essential for plants growth, since they do not perform any known physiological function in plants. Others i.e. Co, Cu, Fe, Mn, Mo, Ni and Zn are essential elements required for normal growth and metabolism of plants, but these elements can easily lead to poisoning when their concentration greater than optimal values (Garrido *et al*., 2002). Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health (Sprynskyy *et al*., 2007). Absorption and accumulation of heavy metals in plant tissue depend upon many factors which include temperature, moisture, organic matter, pH and nutrient availability. Heavy metal accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil to plant transfer factors of the metals (Khan *et al*., 2008). Heavy metals are potentially toxic and phytotoxicity for plants resulting in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and reduced ability to fixate molecular nitrogen in leguminous plants (Guala *et al*., 2010).

2.14 What Is Remediation?

Remediation is an innovative environmental friendly approach to remediate soil which can be used to remediate both organic and inorganic contaminants (Salt *et al*., 1998). The contaminated soils including areas of agricultural fields and their remediation is a serious issue (Khan, 2005). As trace elements cannot be degraded they are considered as non-biodegradable. They may be accumulated in the tissues of living organisms, therefore they only moved from one place to the other (Kumpiene *et al*., 2008). Conventional methods of bioremediation are based on the physical and chemical approaches. Combination of them can be used for better efficiency. However, these methods can be more expensive, time consuming and damaging for the environment, compared with the biological approach. Currently, incineration is the most effective and common remediation practice, but this is extremely costly, in terms of money and energy used.

2.15 Mushroom and Bioremediation

Mushrooms have been shown to degrade diverse type of wastes; organic and inorganic pollutants due to extracellular enzymes they possess thereby converting the waste and pollutants into food of high quality, flavor and nutritive value. Therefore, mushroom may be a promising fungi for environmental bioremediation. (Wikipedia, 2019). Mushrooms have considerable potential in biotechnology and in bioremediation of waste materials. Gast *et al*., (1988) reported that mushroom have the potential to convert agricultural wastes and accumulated heavy metals namely Cd, Cu, Pb, Cr and Mn to valuable products. The uptake rate varied from species to species (Akjn *et al*., 2010; Demirbas 2002). (Isikhuemhen et al., 2003) reported that white-rot fungi are increasingly being investigated and used in bioremediation because of their ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants.

Lang *et al*. (1995) reported that lignin decomposing white-rot fungi show extraordinary abilities to transform recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAH‑s). They added that this unique capability may be used for decontamination of oil-polluted soils although lignocellulosic substrates must be supplied for the survival of fungal species in the soil. White-rot fungi have been used in bioremediation of polluted soils and accumulation of heavy metals. They have also been found to be involved in mineralization, bio-deterioration, biodegradation, transformation and co-metabolism (Bennet et al., 2002).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Site description

The trial was conducted at the experimental screen house department of soil science located near 305 farms at the back of the faculty building of the University of Benin. Benin city which ties between latitude 60 27' 16"N and longitude 50 41' 50"E. , with mean annual mean rainfall range of 1825mm ➔2025mm and 320 meter above sea level. This area lies within tropical rain forest zone, with a bimodal rainfall distribution pattern with peak at July/August and September and a lower precipitation period in August known as August-Break. It has a temperature range of 26.1^0C ➔26.6^0C (Aneric and Abagbonhi, 2015).

3.2 Sources of material used

The spent engine oil (SEO) was obtained from a mechanic workshop along Uwelu road, Benin City. The soil was collected from 305 farms at the back of the faculty building of the University of Benin. Freshly grown mushroom was collected from 305 farms at university of Benin, Benin City.

3.3 Experimental layout

The experiment layout was a 5*3 experiment in completely randomized design with 3 replications and each replication had 5 sub levels.

3.4 Experimental procedure

90kg soil was collected and air dried for 4days after which the soil was sieved with a 2mm size filter to remove stones and other debris in order to obtain a fine soil. A general pollution was done. The polluted soil was left for 2 weeks so that the soil and spent engine oil can properly equilibrate before the mushroom was introduced. 2kg of the polluted soil was filled into 36 nursery pots, each replication had different levels of treatment. Watering was done twice a week, weeding was weekly by hand picking.

3.5. Laboratory Analysis

3.5.1. Soil Analysis

Samples taken from different replications were analysed for some physical and chemical properties using standard laboratory procedures. Parameters includes;

1. Soil pH÷ The pH of soils were determined using a glass electrode pH meter. 10g 0f soils were weighed into a plastic shaking bottle and 10ml distilled water added and stirred intermittently for 30 minutes with a glass rod. The pH was read in a table bench micro-processor pH meter which was standardized using two buffer solutions of pH 4.0 and pH 7.0 respectively.

2. Organic carbon was determined using Walkley-Black wet oxidation method (Walkley and Black 1934).

1g of soil samples were weighed into 250ml beakers, 10ml 0f Potassium dichromate added, 25ml Conc. H2SO4 added carefully and flaks swirl gently, while the mixtures, were allowed to cool and then 20-30mls of distilled water added and swirl gently to mix properly. 5 drops of ferroin indicator added and titrated with $FeSO_4 \cdot 7H_2O$ from dark green to reddish brown colour end-point. Organic Carbon percentage was calculated as follows:-

(Bml ➔Tml) * N * 0.003F * 100

Weight of sample used

3. Organic matter÷ The organic matter was computed by multiplying the value of the organic carbon by a standard value of 2.0 by Douglas W. Pribyl (2010)

4. Available Phosphorus reflects the available fraction of phosphorus to plants at the time. It can be determined in the laboratory using several methods but the one commonly used and used for this work is Bray No. 1 method by Bray and Kurtz (1945), as reported by Stevenson (1986).

5g of air-dried soils were weighed into plastic shaking bottles and 50mls of Bray 1 extracting solution added (0.025HCl + 0.03N NH_4F) and mixture shaken for few minutes. The suspension, filtered using whatman filter paper. The available phosphorus of the samples were lateer determined by using colorimetric Molybdem blue colour procedure (Riley and Murphy, 1972) and a standard curve prepared alongside the samples after reading off in a spectrophotometer at 882mu.

5. Exchangeable Acidity (H^+ + Al^{3+}) determined using titre procedures

6. Particle size:. The particle size Analysis by the hydrometer method as modified by Day (1965) used to know the textural class of soils. Soils and separated into percentage sand, silt and clay.

50g of prepared soil sample was weighed into a 250ml shaking bottle. 100ml of Calgon was added and was allowed to soak for 30 minutes, and add 10ml H_2O_2 (20%). 100ml of distilled water was then added and the suspension was transferred into a one litre measuring cylinder. The mouth of the cylinder covered with palm and was inverted several times until all soil is in suspension. The cylinder was placed on a flat surface and soil hydrometer placed inside the soil suspension after 40 seconds for the first reading and second reading was taken after 120 minutes, and temperature reading taken thereafter.

Calculations:

$$\% \text{ Sand} = 100 \rightarrow \frac{(\text{corrected 40sec. Hydrometer reading}) * 100}{\text{Weight of sample used}}$$

$$\% \text{ Clay} = \frac{(\text{corrected 2hrs hydrometer reading}) * 100}{\text{Weight of sample used}}$$

$$\% \text{ Silt} = 100 \rightarrow (\%\text{sand} + \%\text{clay})$$

7. Exchangeable base; Exchangeable cations, Ca, Mg, K and Na were extracted with 1N ammonium acetate solution (1N NH4OAc) buffered at pH 7.0 the Ca and Mg were determined from the extract using 0.01m EDTA (ethylenediaminetra-acetic acid) titration method as described by Black (1965), while K and Na were determine using flame photometer (Jackson, 1962).

8. Total Nitrogen *(C.A. Black, 1965)*

1g of soil were weighed into 500ml Kjeldahl flasks, 25mls of conc. H_2SO_4 added and digested using catalysts mixtures of K_2SO_4 + $CUSO_4$ + SeO_2 for 3hrs till the solution became clear. The flasks were allowed to cool own and the mixture turned into 100ml volumetric flasks and were made up to mark. The digests were distilled using 40% NaOH into a 4% Boric acid mixed indicators of methyl red and Bromocreesol green and the collected distillates titrated with 0.01N HCl.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Some Physical and Chemical Properties of the Soil Before Pollution

Table 4.1 shows some chemical and physical properties of the Soil before pollution and these properties include; pH, Total nitrogen, Total hydrocarbon content, Potassium, Calcium, Magnesium, Sodium, Hydrogen, Aluminium, Iron, Zinc, Chromium, Available phosphorus and Particle size distribution.

Table 4.1: Physical and Chemical Properties of the Soil before Pollution

Properties	Values
pH	5.30
T.N (g/kg)	0.73
T.O.C (g/kg)	12.6
AV.P (mg/kg)	8.11
T.H.C (mg/kg)	N/D
K (cmol/kg)	0.25
Ca (cmol/kg)	0.70
Mg (cmol/kg)	0.23
Na (cmol/kg)	0.12
H+ (cmol/kg)	0.21
Al+ (cmol/kg)	0.12
Sand (g/kg)	889
Silt (g/kg)	58
Clay (g/kg)	55

Fe (mg/kg)	58.8
Mn (mg/kg)	9.54
Cr (mg/kg)	N/D

4.2 Physical and Chemical Properties of the soil two weeks after pollution

Table 4.2 shows the soil physical and chemical properties two weeks after contaminating the soil with spent engine oil before the application of mushroom. From the results, it was observed that the soil pH, Total nitrogen, Total organic carbon, AV.p, the macro and micro nutrients increases with increase in spent engine contamination.

Table 4.2: Physical and chemical properties of the soil two weeks after Pollution

Properties	Values
pH	5.64
T.N (g/kg)	0.83
T.O.C (g/kg)	45.3
AV.P (mg/kg)	10.5
T.H.C (mg/kg)	80.2
K (cmol/kg)	0.30
Ca (cmol/kg)	0.88
Mg (cmol/kg)	0.26
Na (cmol/kg)	0.16
H+ (cmol/kg)	0.13
Al+ (cmol/kg)	0.08
Sand (g/kg)	880

Silt (g/kg)	68
Clay (g/kg)	53
Fe (mg/kg)	105.3
Mn (mg/kg)	13.2
Cr (mg/kg)	6.1

4.3 Chemical Properties of the spent engine oil before application to the soil

Table 4.3 shows some chemical properties of the spent engine oil before application to the soil. The properties include; Total nitrogen, Total organic matter, Available Phosphorus, total hydrocarbon content, specific gravity, Cadmium, Chromium, Zinc, Lead, and Arsenic.

Table 4.3: Chemical Properties of the spent engine oil before application to the soil

Properties	**Values**
T.N (g/kg)	3.00
T.O.C (g/kg)	160.4
AV.p (mg/kg)	0.10
T.H.C (mg/kg)	4.250
SG	0.81
Cd (mg/kg)	3.26
As (mg/kg)	N/D

4.4 Chemical Properties of Mushroom

Table 4.4 shows the Chemical properties of mushroom used for the experiment. The properties includes; pH, Sodium, Calcium, Potassium, Magnesium, Copper, Zinc, Ion, Lead, Aluminium and Phosphorus.

Table 4.4: Chemical Properties of Mushroom

Properties	**Values**
pH	6.35
Na (cmol/kg)	1.289
K (cmol/kg)	41.803
Mg (cmol/kg)	26.061
Cu (mg/kg)	0.250
Zn (mg/kg)	9.582
Fe (mg/kg)	N/D
Pb (mg/kg)	N/D
Al (mg/kg)	N/D
P (mg/kg)	4.66.3

4.5 Particle size distribution after treatment

Table 4.4 shows the particles size distribution which are sand, silt and clay after the treatment of the soil. It was observed that the sand increased slightly after the application of spent engine oil from 880gkg- 884gkg in rep one, from 880gkg - 881gkg in rep two and 880gkg- 881gkg in rep three, with an average of 882gkg. The silt ranges from 59gkg in rep one, it then increased to 66gkg in rep two and reduced to 64gkg in rep three with an average of 63gkg when compared to the soil (68gkg) two weeks after pollution. The clay ranges from 55gkg in rep one, it reduces slightly to 51gkg in rep two and 43gkg in rep three with an average of 49gkg when compared to the soil (53gkg) two weeks after pollution. The textural class is sand.

Table 4.5 Particle size distribution

Replication	Sand (glkg)	Silt (g/kg)	Clay (g/kg)	Textural class
Rep 1	884	59	55	Sand
Rep 2	881	66	51	Sand
Rep 3	882	64	43	Sand
Mean	882	63	49	

4.6 chemical properties of Soil after treatment

Table 4.6 shows the Chemical properties of the experiment soil after treatment, and they include; pH, Total nitrogen, Total organic carbon, Available phosphorus, Total hydrocarbon, exchangeable bases (K, Ca, Mg, Na) and exchangeable acidity (H+, Al+).

4.6.1 Soil pH

Soil pH is a critical factor influencing the availability of elements of elements in the soil for absorption by plants. From the study, the pH of the soil ranges from 5.30- 5.58. This implies that the soil is slightly acidic. The pH is given as 5.50 in rep one, it increased slightly in rep 2 to 5.58 and decreased slightly to 5.55 with an average of 5.54 when compared to the soil two weeks after pollution (5.64). The results from this findings agrees with that of jia *et al*., (2009) who reported that an increase in the hydrocarbon increase soil pH.

4.6.2 Total Nitrogen

Results from this study shows that Total nitrogen increases with an increase in the amount of spent engine oil application. The Total nitrogen ranges from 0.73g/kg - 0.83g/kg. The Total nitrogen in rep one is given as 0.75g/kg, it redcued slightly to 0.73g/kg in rep two and increased slightly to 0.75g/kg with an average of 0.74 when compared to soil two weeks after pollution (0.83g/kg). The value for TN obtained from this study fall a little between the permissible limit of (1.1gkg-1 → 1.5gkg-1) recommended by Landon (2000) for tropical soil. Nitrogen is acknowledged to be a key limiting nutrient in most agricultural soil.

4.6.3 Total Organic Carbon

The Total organic carbon ranges from 12.6g/kg- 61.6g/kg. The Total organic carbon content of the soil increased after contamination. This was caused by the introduction of large amount of hydrocarbons into the soil which buttresses the position of Jason *et al*., (2004) that oil spills results in significant increase in present carbon because engine oil is essentially a mixture of carbon and hydrogen.

4.6.4 Available Phosphorus

The Available phosphorus ranges from 8.11g/kg - 20.1g/kg. The Available phosphorus was highest at rep one (20.1g/kg). This implies that Available phosphorus increased with increased spent engine oil. The Available phosphorus of the soil was within th critical level of 10-16mg/kg reported by Adebayo and Agboola (2001).

4.6.5 Total Hydrocarbon

Total hydrocarbon ranges from 47.5mg/kg - 142.5mg/kg. Total hydrocarbon was highest at rep two (142.5mg/kg) and it then reduced significantly to 47.5mg/kg in rep three with an average of 50.9mg/kg when compared to soil two weeks after pollution (80.2mg/kg).This finding corresponds with the findings of Wang *et al*., (2010).

4.6.6 Exchangeable Bases (K, Ca, Mg and Na)

The exchangeable bases increased with an increase in spent engine oil treatment. The results corresponds with the findings by Benka-coker and Ekundayo (1995). The Potassium values ranges from 0.24cmol/kg - 0.30cmol/kg. After treatment, it reduced slightly to 0.25cmol/kg with an average of 0.25cmol/kg when compared to soil two weeks after pollution (0.30cmol/kg).

The Calcium values ranges from 0.70cmol/kg -0.88cmol/kg. Although, application of spent engine oil increased the calcium concentration, this was below the critical level of 2.50cmol/kg reported by Akinride *et al*., (2005).

The Magnesium values ranges from 0.23cmol/kg - 0.26cmol/kg. After the introduction of spent engine oil, the value increased from 0.23cmol/kg - 0.26cmol/kg and reduced to 0.22cmol/kg after treatment with an average of 0.22cmol/kg.

The Sodium values ranges from 0.12cmol/kg - 0.16cmol/kg. It increased after the application of spent engine oil to 0.16cmol/kg and then reduced after the treatment to 0.12cmol/kg.

4.6.7 Exchangeable Acidity (H+ and Al^{3+})

H+ values ranges from 0.12cmol/kg - 0.21cmol/kg. From the result, it was observed that H+ decreased in the spent engine oil. H+ was highest before the introduction of spent engine oil (0.21cmol/kg). This result agrees with the findings of Osuji *et al*., (200) who noted that with the application of spent engine oil, H+ tends to decrease. Similar trends was also observed for Al^{3+}.

Table 4.6; Chemical Properties of Soil after Treatment

Reps	pH	T.N (g/kg)	T.O.C (g/kg)	AV.p (g/kg)	T.H.C (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	H+ (cmol/kg)	Al^{3+} (cmol/kg)
Rep 1	5.50	0.75	48.8	20.1	50.9	0.25	0.77	0.22	0.13	0.15	0.08
Rep 2	5.58	0.73	45.7	12.3	142.5	0.25	0.81	0.22	0.13	0.12	0.05
Rep 3	5.55	0.75	61.6	13.0	47.5	0.25	0.73	0.22	0.13	0.13	0.08
Mean	5.54	0.74	51.3	15.1	80.3	0.25	0.78	0.22	0.13	0.13	0.07

CHAPTER FIVE

5.0 Conclusion and Recommendations

The need to remediate soils in areas polluted with high concentrations of HEAVY METALS generates interest about environmental friendly remediation technologies. Remediation uses plants to decrease soil pollution to an acceptable level. It is necessary to find plant species with a good accumulation capacity and remediation capability. This study has shown the potential of Mushroom in the remediation of spent engine contaminated soil. The result obtained showed that the pH, total nitrogen, total organic carbon, available phosphorus, total hydrocarbon content, potassium, calcium, magnesium, and sodium increased at all levels of spent engine oil treatments, while the exchangeable acidity decreased at all levels of spent engine oil treatments combined with mushroom.

Therefore, sustainable rremediation with mushroom will explore the potentials of local and indigenous weed species with proven adaptability to the local environmental and ecosystem challenges. Such environmental friendly approach or alternative for the treatment of spent engine oil contamination will help in ecosystem restoration.

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