CHAPTER ONE

INTRODUCTION

1.1 Background

Lubricating oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Mandri and Lin, 2007). It is used to lubricate the parts of an automobile engine, in order to keep everything running smoothly (Hagwell et al., 1992). Large amounts of lubricating oils composed of long-chain saturated hydrocarbons (base oil) and additives that are used in car engines. The main components of the base oil are cyclic alkanes (c-alkanes). Long-chain hydrocarbons and c-alkanes are known as recalcitrant to microbial degradation (Bagherzadeh et al., 2008). The base oil contains C_{16}-C_{36} hydrocarbons, and more than 75% c-alkanes. The rings number of c-alkanes in the base oil is from 1 to 3 and any ring contains 5 or 6 members. Most of the c-alkanes in the base oil have long alkyl side chains (Koma *et al.*, 2003). Components of car engine base oil by Koma *et al.,* (2001) are shown in appendix 1.

The most important characteristic of the lubricating oil for automotive use is its viscosity. Fresh motor oil contains a higher percentage of volatile and water soluble hydrocarbons that would be a concern for acute toxicity to organisms (Boonchan et al., 2000). Used motor oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs) that could contribute to chronic hazards including mutagenicity and carcinogenicity (Hagwell et al., 1992; Boonchan et al., 2000). As the usage of petroleum hydrocarbon products increased, soil contamination with diesel and engine oils is becoming one of the major environmental problems (Mandri and Lin, 2007). Prolonged exposure to high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Mishra et al., 2001; Propst et al., 1999, Lloyd and Cackette, 2001).

In addition, PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment (Van Hamme et al., 2003). The illegal dumping of used motor oil is an environmental hazard with global ramifications (Blodgett, 2001). The release of oil into the environment causes environmental concern and attracts the public attention (Roling et al., 2002).

In Africa and some developing countries about 20 million gallons of waste engine oil are generated annually from mechanic workshops and discharged carelessly into the environment (Faboya, 1997; Adegoroye, 1997). Out of which only one liter is enough to contaminate one million gallons of freshwater (USEPA, 1996). Apart from this, used engine oil renders the environment unsightly and constitutes a potential threat to humans, animals and vegetation (ATSDR, 1997; Edewor et al., 2004; Adelowo et al., 2006). Several components of the oil, e.g. solvents and detergents added during the blending process, aliphatic hydrocarbon and PAHs distilled from crude oil, and metals from engine wear are either toxic in themselves or can combine with products of combustion to generate carcinogens and endocrine disrupters, (USEPA 1996, ATSDR 1997).

The waste-lubricating oil, otherwise called spent oil or used-lubricant, obtained after servicing and subsequent draining from automobile, generators and industrial machines is disposed off indiscriminately in many countries, for example, in United States about 500 million gallons of used oil are being disposed indiscriminately every year (Anoliefo and Vwioko 1995: Adesodun and Mbagwu, 2008). This waste-oil usually contains appreciable amount of toxic hydrocarbons and heavy metals such as Va, Pb, Al, Ni, Fe, Cr and Zn (Whisman et al., 1974).

Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Oil spillages into the environment have become one of the major problems. Spillages of used motor oils such as diesel or jet fuel contaminate our natural environment with hydrocarbon (Husaini et. al 2008). The hydrocarbons spread horizontally on the ground-water surface and partition into groundwater, soil pore, space air and to the surfaces of soil particles (Plohl et al., 2002). Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater) especially by PAHs has drawn public concerns because many PAHs are toxic, mutagenic, and carcinogenic (Bumpus 1989; Clemente et al. 2001; Cerniglia and Sutherland 2001). Before lubricating oil is used, they consist of a base lubricating oil (a complex mixture of hydrocarbons, 80 to 90% by volume) and performance enhancing additives (10 to 20% by volume). They are altered during use because of the breakdown of additives, contamination with the products of combustion, and the addition of metals from wear and tear of the engine. Therefore, the composition of waste oil is difficult to generalize in exact chemical terms. It is recognized that the major components consist of aliphatic and aromatic hydrocarbons (such as phenol, naphthalene, benz(a)anthracene, benzo(a)pyrene, and fluoranthene (Environment Canada, 1994). According to the US Coast Guard Emergency Response Notification System (ERNS), used motor oil is one of the most commonly spilled petroleum products in U.S (U.S. Coast Guard, 1993).

1.2 Effects of Used lubricating Oil on Human Health

Aromatics hydrocarbon are considered to be the most acutely toxic component of petroleum products, and are also associated with chronic and carcinogenic effects

(Anderson et al., 1974). Aromatics are often distinguished by the number of rings they possess, which may range from one to five (Anderson et al., 1974). Lighter, mono-aromatics (one ring) compounds include benzene, toluene, ethylbenzene, and xylenes (NOAA, 1995). Aromatics with two or more rings are referred to as polyaromatic hydrocarbons (PAHs) (Anderson et al., 1974). Used lubricating oil contains several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo(a)pyrene (a PAH). Upshall et al., (1992) reported that motor oil had a density of 0.828 g/ml and contained 14% aromatics and 65.4% aliphatics (by weight). In their study, the sum of 26 individual PAHs represented 0.17% of the oil, or 1.2% of the aromatic fraction.

Chronic effects of naphthalene, a constituent in used motor oil, include changes in the liver and harmful effects on the kidneys, heart, lungs, and nervous system. Due to their relative persistence and potential for various chronic effects (like carcinogenicity), PAHs (and particularly the alkyl PAHs) can contribute to long term (chronic) hazards of jet fuels in contaminated soils, sediments, and groundwater. Like several individual PAHs, used lubricating oil has been shown to be mutagenic and teratogenic (Cao et al., 2009). The results are mixed, but some immunological, reproductive, fetotoxic, and genotoxic effects have been associated with a few of the compounds found in used motor oil. (Verdin et al., 2004).

1.3 Bioremediation of contaminated soil

Extensive petroleum hydrocarbon exploration activities often result in the pollution of the environment, which could lead to disastrous consequences for the biotic and abiotic components of the ecosystem if not restored. Remediation of petroleum contaminated system could be achieved by either physicochemical or biological methods. However, the attendant negative consequences of the physicochemical approach are currently directing greater attention to the exploitation of the biological alternatives (Okoh, 2006). Bioremediation of petroleum hydrocarbon contaminated soils has been recognized as an efficient, economic, versatile, and environmentally sound treatment (Margesin and Schinner, 2001). Harder (2004) estimated that bioremediation accounts for 5 to 10 percent of all pollution treatment and has been used successfully in cleaning up the illegal dumping of used engine oil. It is based on the capacity of microorganisms to degrade organic pollutant compounds, such as hydrocarbons. These compounds are important soil pollutants because of the high toxicity of the polycyclic aromatic hydrocarbon (PAH) fraction (Mancera-López et al., 2008). According to the Environmental Protection Agency (EPA), 16 PAHs have been reported as carcinogenic and mutagenic compounds (Verdin et al., 2004), so it is necessary to remove them from contaminated sites. Studies have reported several bacteria and filamentous fungi species with the capacity to mineralize or to degrade PAHs (Boonchan et al., 2000).

Microbial remediation of a hydrocarbon–contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra et al., 2001; Eriksson et al., 1999). A large number of *Pseudomonas* strains capable of degrading PAHs have been isolated from soil and aquifers (Johnson et al., 1996; Kiyohara et al., 1992). Other petroleum hydrocarbon-degraders include *Yokenella* spp., *Alcaligenes* spp., *Roseomonas* spp., *Stenotrophomonas* spp., *Acinetobacter* spp., *Flavobacter* spp., *Corynebacterium* spp., *Streptococcus* spp., *Providencia* spp., *Sphingobacterium* spp., *Capnocy-tophaga* spp., *Moraxella* spp., and *Bacillus* spp. (Antai, 1990; Bhattacharya et al., 2002). Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer periods of time to grow as compared to their bacterial counterparts (Prenafeta-Boldu et al., 2001). However, some hydrocarbon degradation studies have been carried out using white rot fungi such as *Phanerochaete chrysosporium, Pleurotus ostreatus and Trametes versicolor* (Yateem et al., 1998; Mollea et al., 2005). It would be interesting to develop the bioremediation process further using fungi, because of their capacity to incorporate rapidly into the soil matrix. Furthermore, they have the ability to grow in environments with low nutrient concentrations, low humidity and acidic pH (Potin et al., 2004; Mollea et al., 2005).

Several different bioremediation techniques have been developed, but biostimulation is the one used most often (Head, 1998). This consists of the activation of native soil microorganisms through the addition of nutrients. Providenti et al., (1993) reported that efficient removal of contaminants require 1 x 10^3 $CFUg^{-1}$ of soil, although other factors to be considered are the molecular structure and bioavailability of the contaminants (Juhasz and Naidu, 2000). Biostimulation is relatively trouble-free and inexpensive, relative to other methods; it is the method of bioremediation most frequently used to mitigate soil contamination (Cunningham and Philip, 2000). Biostimulation causes a rapid depletion of the available pools of major hydrocarbons, is easy to maintain and is a cost effective treatment over large areas (Margesin and Schinner, 2001; Bento et al., 2005). In biostimulation, the activity of naturally-occurring microbes is stimulated by circulating water-based solutions through contaminated soils. Nutrients (nitrogen, phosphorus, carbon and others), oxygen, or other amendments, may be used to enhance bioremediation and contaminant desorption from subsurface materials (Van Deuren et al., 1997). It is possible that even if the native microorganism population is large enough, it does not have the ability to degrade components of high molecular weight or to emulsify insoluble compounds. Bioaugmentation could be used for the latter case. This technique is defined as the addition of pre-grown microbial cultures to perform a specific remediation task in a given environment (D'Annibale et al., 2006). The microbial cultures must have the ability to withstand different soil environmental conditions and to survive in the presence of other microorganisms (Riser-Roberts, 1998). Most bioaugmentation studies have been carried out using filamentous fungi inoculated into model soil systems and using contaminants of low molecular weight PAHs with up to four rings (D'Annibale et al., 2006). The interest of these microorganisms is their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin decay that can degrade high molecular weight, complex and more recalcitrant toxic compounds, including aromatic structures (Colombo et al., 1996). For the breakdown of complex aromatic structures, fungi–bacteria consortia are preferred due to the successful results reported. For example, the consortium comprising *S. maltophilia–P. janthinellum* degraded 44–80% of a chrysene, benzo[a]anthracene, benz[a]- pyrene and dibenz[a,h]anthracene mixture, in 100 days (Boonchan et al., 2000). This success has rekindled an interest in treating solid wastes generated by the petroleum industry (Alexander, 1994). According to the literature, bioaugmentation technology has mostly been used for the degradation of pure compounds (Gray et al., 2000). The mineralization of high concentrations of phenanthrene has been reported when successive inoculations were tested (Schwartz and Scow, 2001). According to the success of these results, it has been reported that the knowledge of new strains could be of interest to accelerate the remediation of zones polluted with high concentrations of hydrocarbons. During the last two decades, many investigations have been performed to determine the persistence of hydrocarbons in different natural environments and the possible role of the indigenous microflora on the degradation rate of these contaminants (Kastner et al., 1994; Balba et al., 1998).

Various factors may limit the rate of petroleum hydrocarbon degradation including a lack of essential nutrients such as nitrogen. Therefore, the addition of inorganic or organic nitrogen rich nutrients (biostimulation) is seen as an effective approach to enhance the bioremediation process (Hollender et al., 2003; Semple et al., 2006; Walworth et al., 2007) with positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation being widely demonstrated (Jørgensen et al., 2000; Margesin et al., 2000; Margesin and Schinner, 2001; Riffaldi et al., 2006; Margesin et al., 2007). Alternatively, amendment with nitrogen (particularly inorganic fertilizers) when applied at high concentrations may have deleterious effects (Bento et al., 2005; Walworth et al., 2007). In terms of deleterious effects, inorganic nitrogen fertilizers composed of nitrate and ammonium salts increase the salt concentration of soil pore water, lowering the soil osmotic potential and inhibiting microbial activity (Walworth et al., 2007). Therefore this study employed organic wastes [banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)] as a substitute to inorganic fertilizer which are expensive and presently insufficient for agriculture let alone the use for bioremediation to enhance the biodegradation of used lubricating oil in soil. Phytoremediation using two different plants was also considered in this study for enhancing biodegradation of the oil in soil.

1.4 Objectives

The objectives of this study are as follow:

i. To evaluate the effectiveness of the different organic wastes (banana skin, grain spent mushroom compost) in enhancing biodegradation of used lubricating oil in contaminated soil at different oil concentrations under laboratory and natural conditions.

ii. To determine the rate of biodegradation of hydrocarbon in contaminated soil and half-life of the used lubricating oil.

iii. To compare the performance of *Jatropha curcas* and *Hibiscus cannabinus* plant species in remediation of used oil contaminated soil.

iv. To determine the uptake rate of heavy metal in lubricating oil by *Jatropha curcas* and *Hibiscus cannabinus* plants.

v. To compare the effectiveness of biostimulation and phytoremediation in reclamation of used lubricating oil-contaminated soil.

vi. To determine the soil toxicity after biostimulation of oil-polluted soil using seed germination test.

vii. To isolate and screen potential microorganisms for used lubricating oil degradation from contaminated and uncontaminated soil.