

**USE OF BIOCHAR TO ENHANCE BIOREMEDIATION OF AN OXISOL
CONTAMINATED WITH DIESEL OIL**

BY

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DECLARATION

I do hereby declare that this thesis has been written by me and that it is the record of my own research work. It has not been presented for another degree elsewhere. Works of other researchers have been duly cited by references to the authors. All assistance received has also been acknowledged.

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DEDICATION

This work is affectionately and humbly dedicated to my caring Mother and her sisters, my cousin Thomas Oyom and to all those who took an interest and encouraged me in my academic pursuit.



ACKNOWLEDGEMENT

Glory be to God for bringing me this far in my academic pursuit. I wish to express my sincere gratitude first and foremost to my family, for every form of support and sacrifice throughout all these years of my education.

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ABSTRACT

Oil pollution is a worldwide threat to the environment, especially in oil producing countries, and the remediation of oil-contaminated soils is a major challenge for environmental research. Bioremediation is a useful method for restoring oil contaminated soils because of its cost effectiveness and environmental friendliness. However, the process is very slow in soils with low pH. Soils of the Western Region of Ghana where most of the country's oil activities take place are classified as Oxisols. These soils are acidic in nature and have soil conditions unfavourable for effective biodegradation of petroleum and its products. Application of biochar to soils is currently gaining considerable interest globally due to its potential to serve as a liming agent and in raising soil pH in different soil types. It is against this background that the present study was carried out to investigate the effectiveness of biochar as a soil conditioner in enhancing microbial degradation of diesel oil in the Ankasa series (Plinthic acruox) sampled from the Western Region of Ghana and the subsequent growth of cowpea in the bioremediated soils. The acidic soil was contaminated with diesel oil at 100 mL/kg soil. Two biochar types, from rice straw (RB) and from saw dust (SB), were applied to the contaminated soils at 0, 65, 130, 195 and 260 Mg/ha. The treated soils were incubated and sampled for determination of the hydrocarbon utilizing bacteria (HUB) population, total aerobic heterotrophic bacteria (HET) population, change in soil pH and the amount of oil degraded at 10 days interval for 40 days. In another experiment the soil was contaminated with diesel oil at 100 mL/kg soil, amended with RB at 195 Mg/ha and fertilized with N and/or P in the form of ammonium nitrate and single super phosphate, respectively at 60 kg/ha. These treated soils were also incubated and sampled for analysis of hydrocarbon utilizing bacteria (HUB) population and the amount of oil degraded at 10 days interval. Cowpea was sown into the residual soils and harvested 6 weeks after planting for the determination of the number of nodule formed, shoot and root dry weights. In the first

experiment, results showed that all the biochar treatments significantly ($p < 0.05$) increased the amount of diesel oil degraded, HUB and HET populations when compared to the control. The RB treatments significantly ($p < 0.05$) enhanced diesel oil degradation more than the SB treatments. Results also showed that RB at 260 Mg/ha resulted in the highest amount of diesel oil degraded but was not significantly ($p > 0.05$) different from RB at 195 Mg/ha. Soil pH, soil organic carbon, total exchangeable bases, effective cation exchange capacity, and base saturation were also significantly ($p < 0.05$) increased by the biochar treatments. Soil available P increased significantly ($p < 0.05$) for the RB treatments. However, total N and exchangeable acidity significantly ($p < 0.05$) decreased when amended with biochar. X-ray diffraction analysis showed that these biochars contained large quantities of carbonates and oxides. Fertilizing RB (195 Mg/ha) with N and/or P significantly ($p < 0.05$) increased amount of diesel oil degraded and HUB population 40 days after incubation when compared to RB only treatment. Fertilization with N and/or P enhanced shoots and roots dry weights of cowpea. Addition of N was inhibitory to nodulation, however, P fertilization enhanced nodulation. In conclusion, the enhanced degradation was attributed to the presence of large quantities of carbonates and oxides in the biochars which might have served as liming agents, improved the soil microbiology and other chemical properties. Further studies should be conducted on the application of combination of rice straw biochar, nitrogen and phosphorus, in the bioremediation of oil under acidic condition should be conducted in the field to confirm its effectiveness for future recommendation.

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CHAPTER ONE

INTRODUCTION

The increase in urbanisation and mechanised agriculture has resulted in an increase in use of petroleum and its products (Ekpo and Nya, 2012). Accidental and deliberate crude oil spills have been and still continue to be a significant source of environmental pollution, and pose a serious environmental problem, due to the possibility of air, water and soil contamination (Trindade et al., 2005). For example, approximately 6×10^7 barrels of oil was spread over 2×10^7 m³ soil and 320 oil lakes were created across the desert during the first Gulf War in Kuwait (Al Saleh and Obuekwe, 2005). Other sources of oil contamination include leakage from storage containers, refuelling of vehicles, wrecks of oil tankers carrying oil and improper disposal by mechanics when cleaning tankers (Hill and Moxey, 1980).

Crude oil contamination of land negatively affects certain soil parameters such as the mineral and organic matter content, the cation exchange capacity, redox properties, available basic cations, available N and P and pH value (Wyszkowski and Ziolkowska, 2008). As crude oil creates anaerobic condition in the soil, coupled with water logging and acidic metabolites, the result is high accumulation of aluminum and manganese ions, which are toxic to plant and microbial growth (Odu, 1981). Pollution of the soil environment by crude oil can limit its protective function, upset metabolic activity, unfavourably affect soil chemical characteristics, reduce fertility and negatively influence plant production (Gong et al., 1996). Crude oil can bioaccumulate in food chains where they disrupt biochemical or physiological activities of many organisms thus, causing carcinogenesis of some organs, mutagenesis in the genetic material, and impairment in reproductive capacity and/or causing hemorrhage in the exposed population (Onwurah et al., 2007). Crude oil was found to reduce growth, photosynthetic rate, stem height,

density, and above ground biomass of *Spartina alterniflora* and *S. patens* and may cause their death (Krebs and Tamer, 1981). Severe crude oil spill in Cross-River state, Nigeria, has forced some farmers to migrate out of their traditional home, especially those that depend solely on agriculture (Onwurah et al., 2007). This is because petroleum hydrocarbons ‘sterilize’ the soil and prevent crop and microbial growth and yield for a long period of time (Onwurah 1999a). The negative impact of oil spillages remains the major cause of depletion of the Niger Delta of Nigeria’s vegetative cover and the mangrove ecosystem (Odu, 1981).

Many published articles have documented the potentials of native microorganisms to degrade oil both in the laboratory (eg. Lawson et al., 2012) and in field trials (eg. Bragg et al., 1994). However, these potentials are to a large extent curtailed in unfavourable soil conditions. There are 30 or more different genera of bacteria and fungi known to degrade hydrocarbons intrinsically and are found in almost any soil or aquatic environment, but they need help to degrade oil effectively (Bragg et al., 1994). Help is needed more especially in Oxisols, where biodegradation is usually slower. Oxisols are acidic in nature and are characterized by low nitrogen, phosphorus, organic carbon, basic cations (Ca, Mg, K, Na), and microbial activities (Beinroth et al., 1990). According to Walworth et al. (2005), Oxisols create such unfavourable conditions for microbial degradation that, there is about near zero degradation in the soil.

Ghana discovered crude oil in 2007 at Cape Three Points in the Western Region and commercial production started in late 2010. However, on December 26, 2009, the country experienced its first spillage of about 584 barrels of low-based mud drilling fluid into the sea and the second mud spill of seven barrels occurred on March 23, 2010 (Daily Graphic, 2010). Even though these spillages were not into soils, many people have concerns about future soil

contamination. Besides, soils of the Western Region of Ghana, where most of the oil activities take place are classified as Oxisols with low pH, because of the leaching of basic cations as a result of high rainfall. These acidic soils have high levels of Fe and other heavy metals, and are dominated by acidic cations like Al^{3+} and H^+ . These acidic soils also do not create favourable conditions for the cultivation of legumes because nodule initiation and formation, nitrogen fixation and growth are adversely affected due to low pH and unavailable phosphorus. It is well established that nitrogen fixing plants, either legumes or actinorhizal plants, require phosphate for adequate growth and root nodulation (Huss-Danell, 1997 and Marschner, 1995).

The processes leading to the eventual removal of hydrocarbon pollutants from the environment have been extensively documented and involve the trio of physical, chemical and biological alternatives. However, bioremediation which is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition, is an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to CO_2 and H_2O (da Cunha, 1996). It also provides highly efficient and environmentally safe clean-up tools (Margesin, 2000). This technology accelerates the naturally occurring biodegradation under optimized conditions such as oxygen supply, temperature, pH, the presence or addition of suitable microbial population (bioaugmentation), nutrients (biostimulation) and water content (Trindade et al., 2005). However, there is the need for further studies towards optimizing the process conditions for the application of bioremediation strategies in diverse climatic zones especially in extreme environments (such as acidic soils). This is so because, the effectiveness of bioremediation is often a function of the microbial population and how it can be enriched and maintained in an environment such as acidic oil polluted soil.

The need to modify unfavourable soil conditions to expedite microbial degradation has therefore become necessary. Biochar applications have been shown to increase soil pH, improve nutrient storage, ECEC, increase soil carbon content, increase water holding capacity, decrease aluminum toxicity, decrease tensile strength, change microbiology of the soil, decrease greenhouse gases (N_2O and CH_4) emissions from the soil, improve soil conditions for earthworm populations and improve fertilizer use efficiency (Downie et al., 2009; Sohi et al. 2009; Mbagwu and Piccolo 1997; Piccolo et al., 1996; Piccolo and Mbagwu, 1990). Most literature have concluded that, the greatest positive effects of biochar were seen on acidic, free-draining soils, with other soil types, specifically calcarosols showing no significant effect (either positive or negative). Literature have also shown that biochar can stimulate soil microbial activities (Jones et al., 2011a, Jones et al., 2011b and Lehmann et al., 2011). It has a higher sorption affinity for a range of organic and inorganic compounds, and higher nutrient retention ability compared to other forms of soil organic matter (Bucheli and Gustafsson 2000, 2003; Allen-King et al. 2002).

These multiple potential benefits of biochar, combined with the fact that it can potentially be a relatively low-cost and environmentally friendly tool for soil reclamation, provide incentive for more research. Hence, the present study seeks to rely on these numerous benefits of biochar in acidic soils, to create a favourable condition for the microorganisms involved in crude oil degradation to thrive and work effectively at a faster rate as well as provide favourable soil condition for crops like grain legumes that are sensitive to low pH. Although the use of biochar to enhance crude oil degradation is not a new development in Ghana especially, specific attention has not been focused on its use in acidic soils in the Western Region which has most of its soils dominated by Oxisols. The use of biochar has become necessary because, experts argue that the other physiochemical cleaning methods such as burying, evaporation, dispersion and washing of

contaminated soils cause geological damage which might even exceed the damage caused by the polluting oil (Bartha,1986), and they are also very expensive and not environmentally friendly. It would therefore be appropriate to research into the use of biochar in oil contaminated soils in Ghana that has just discovered oil in commercial quantities.

The objectives of the present study therefore are to investigate

1. the effects of biochar on microbial degradation of diesel oil in acidic soils.
2. the effects of supplementing biochar with nutrients on microbial degradation of diesel oil.
3. the growth and nodulation response of cowpea in oil-remediated soil.

Hypothesis

H₀: Amendment of acidic soil with biochar does not enhance microbial diesel oil degradation and subsequent crop growth.

H_A: Amendment of acidic soils with biochar enhances microbial diesel oil degradation and subsequent crop growth.

CHAPTER TWO

LITERATURE REVIEW

2.1 What is biochar?

Biochar is an extremely complex stable form of carbon produced by the controlled heating of plant and/or animal material (biomass feedstock) at high temperatures (350 – 600°C) in a low oxygen environment (Jenkins and Jenkinson, 2009). This definition includes chars and charcoal, and excludes fossil fuel products or geogenic carbon (Lehmann *et al.*, 2006). The technique of heating in a low oxygen environment is called pyrolysis.

Biochar's complex chemical structure is defined by the feedstock it is made from and the temperature conditions used in its manufacture. Biochar is a form of charcoal but is different in that, biochar is produced in controlled conditions so that most of the carbon is converted to usable products (Jenkins and Jenkinson, 2009). Charcoal usually has a total carbon content of over 75% whilst biochar often has much less total carbon (often 40-75%) but it has a higher mineral content, containing minerals such as Calcium (Ca), Potassium (K), Phosphorus (P) and Nitrogen (N) (Jenkins and Jenkinson, 2009).

The characteristic of any biochar is a function of the material from which it is made and the temperature conditions used to make it. The range of biochars available could be considerable, representing a wide range of feedstock, temperature, residence times and heating rates used in their creation (Jenkins and Jenkinson, 2009). Incorporation of biochar into soil is shown to affect the preexisting soil properties in ways attributed to the physical and chemical properties of biochar.

2.1.1 Physical properties of biochar

Unlike the structure of graphite which consists of aromatic rings arranged in perfectly stacked and aligned sheets, biochar is made of irregular arrangements of C containing O and H and, in some cases, minerals depending upon feedstock (Lehmann and Joseph, 2009). Charred biomass consists of recalcitrant aromatic rings as well as more easily degradable aliphatic and oxidized carbon structures (Lehmann, 2007). Key physical features of most biochars are their highly porous structure and large surface area which can provide refuge for beneficial soil microorganisms, such as mycorrhizae and bacteria, and influences the binding of important nutritive cations and anions (Atkinson et al., 2010).

Biochar is often macro porous in nature which reflects cellular structures in the feedstock from which it is produced, which is potentially important for water holding and adsorption of soil (Sohi et al., 2010). When added to soil, biochar appears to divide rapidly into particles of silt size or less due to abrasion, shrink-swell, and other physical weathering processes (Brodowski et al., 2007). Process temperature is the main factor governing surface area, increasing in one study from $120 \text{ m}^2 \text{ g}^{-1}$ at $400 \text{ }^\circ\text{C}$ to $460 \text{ m}^2 \text{ g}^{-1}$ at $900 \text{ }^\circ\text{C}$ (Day et al., 2005). Low temperature biochar is stronger than high temperature products with regards to adsorptive properties, but it is more brittle and prone to abrading into finer fractions once incorporated into soil (Sohi et al., 2010).

2.1.2 Chemical properties of biochar

Much research has produced unequivocal proof that biochar is not only more stable than any other amendment to soil and increases nutrient availability beyond a fertilizer effect, but its stability and nutrient retention properties make it more effective than any other organic material in soil (Lehmann and Joseph 2009). Chemical and physical properties such as high charge

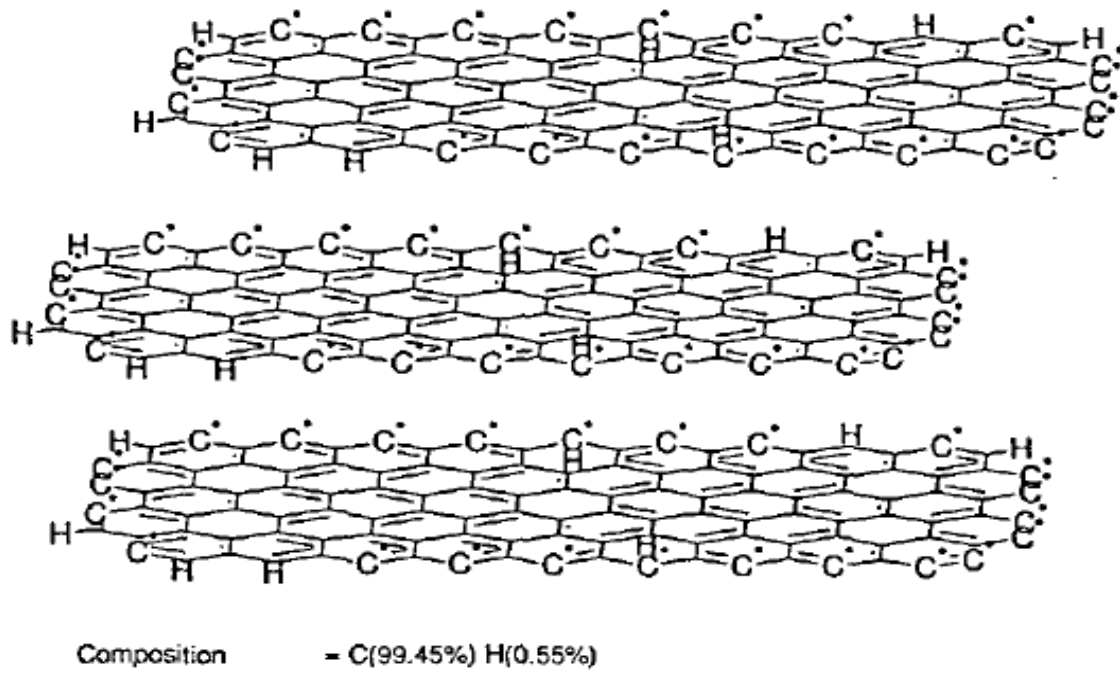
density and its particulate nature along with specific chemical structure, and high microbial and chemical stability, all contribute to greater nutrient retention and resistance to microbial decay than other organic matter (Atkinson et al., 2010).

Baldock and Smernik (2002) determined that thermal treatment of organic materials at temperatures $> 200^{\circ}\text{C}$ induces significant variations in their chemical composition. Changes in chemical composition, as measured by ^{13}C nuclear magnetic resonance (NMR) indicated that changes with increased pyrolysis temperature included a conversion of O-alkyl C to aryl and O-aryl furan-like structures, which are more chemically active oxygen-containing carbon ring (Baldock and Smernik, 2002). Research suggests that biochar created at low temperatures may be suitable for controlling the release of fertilizer nutrients while high temperatures would lead to a material similar to activated carbon (Sohi et al., 2010).

2.1.3 Structural composition of biochar

Thermal degradation of cellulose between 250 and 350°C results in considerable mass loss in the form of volatiles, leaving behind a rigid amorphous C matrix (Baldock and Smernik, 2002). As the pyrolysis temperature increases, so does the proportion of aromatic carbon in the biochar, due to the relative increase in the loss of volatile matter (initially water, followed by hydrocarbons, tarry vapours, H_2 , CO and CO_2), and the conversion of alkyl and O-alkyl C to aryl C (Baldock and Smernik, 2002; Demirbas 2004). Around 330°C , polyaromatic graphene sheets begin to grow laterally, at the expense of the amorphous C phase, and eventually coalesce (Demirbas 2004; Baldock and Smernik, 2002). Above 600°C , carbonization becomes the dominant process. Carbonization is marked by the removal of most of the remaining non-C atoms and consequent

relative increase of the C content, which can be up to 90% (by weight) in biochars from woody feedstocks.



(Antal and Gronli, 2003; Demirbas, 2004).

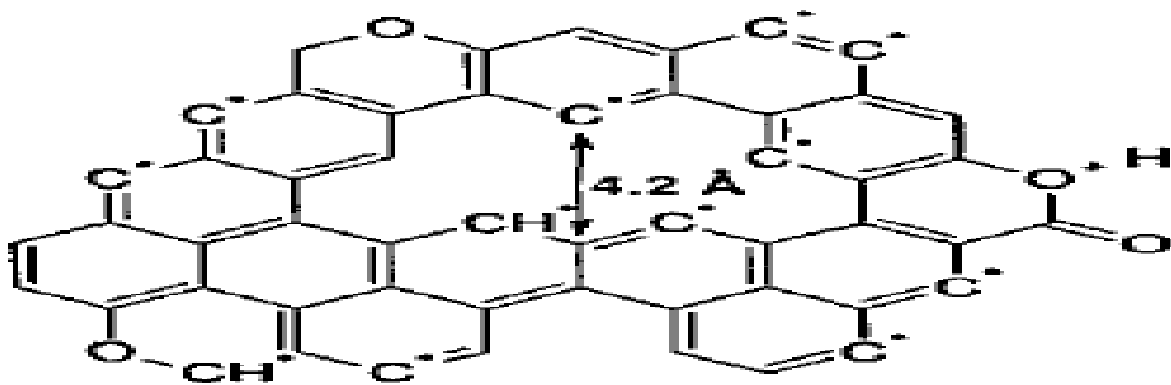


Fig. 2.1 Putative structure of charcoal (adopted from Bourke et al., 2007).

It is commonly accepted that each biochar particle comprises of two main structural fractions: stacked crystalline graphene sheets and randomly ordered amorphous aromatic structures (Fig. 2.1). Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorus (P) and Sulphur (S) are found predominantly incorporated within the aromatic rings as heteroatoms (Bourke et al., 2007). The presence of heteroatoms is thought to be a great contribution to the highly heterogeneous surface chemistry and reactivity of biochar.

2.1.4 Chemical composition and surface chemistry of biochar

Biochar is produced from biomass and is predominantly composed of recalcitrant organic C with contents of plant micro and macro-nutrients retained from the starting feedstock. It is known from research on wildfire occurrence and the development of Anthrosols (*e.g. Terra Preta* soils) in the Amazon that charcoal can remain in the soil for hundreds to thousands of years (Agee 1996; Lehmann and Rondon 2006). Consequently, biochar can rapidly increase the recalcitrant soil C fraction of soil. The C in biochar is held in aromatic form which is resistant to decomposition when added as a soil amendment (Amonette and Joseph 2009), making it a C sequestration tool. However, composition varies by feedstock type and conditions of pyrolysis (Downie 2009). Actual C contents can range between 172 g kg⁻¹ and 905 g kg⁻¹. Nitrogen content ranges from 1.8 g kg⁻¹ to 56.4g kg⁻¹, total P from 2.7g kg⁻¹ to 480g kg⁻¹ and total potassium (K) from 1.0g kg⁻¹ to 58g/ kg (Chan et al., 2007; Lehmann et al. 2002, Lima and Marshall 2005).

Biochar also contains varying concentrations of other elements such as Oxygen (O), Hydrogen (H), Nitrogen (N), Sulphur (S), Phosphorus (P), base cations, and heavy metals (Goldberg 1985; Preston and Schmidt, 2006). The outer surfaces contain various O and H functional groups and the graphene sheets may contain O groups and free radicals (Bourke et al., 2007). Additionally,

biochar has been produced with a range of pH values between 4 and 12, dependent upon the starting feedstock and operating conditions (Lehmann 2007). Generally, low pyrolysis temperatures (< 400° C) yield acidic biochar, while increasing pyrolysis temperatures produce alkaline biochar. Once incorporated into the soil, surface oxidation occurs due to reactions of water, Oxygen (O₂) and various soil agents (Cheng et al., 2006; Lehmann, 2007). The cation exchange capacity (CEC) of fresh biochar is typically very low, but increases with time as the biochar ages in the presence of O₂ and water (Cheng et al. 2008; Cheng et al., 2006; Liang et al., 2006).

Biochar composition is highly heterogeneous, containing both stable and labile components (Sohi et al., 2009). Carbon, volatile matter, mineral matter (ash) and moisture are generally regarded as its major constituents (Antal and Gronli, 2003) and Table 2.1 summarizes ranges their relative proportion ranges in biochar as commonly found for a variety of source materials and pyrolysis conditions (Antal and Gronli, 2003; Brown, 2009).

Table 2.1 Ranges of the relative proportion range of the four main components of biochar (weight percentage) as commonly found for a variety of source materials and pyrolysis conditions (adapted from Brown, 2009; Antal and Gronli, 2003)

Component	Proportion (w w⁻¹)
Fixed carbon	50-90
Volatile matter (e.g. tars)	0-40
Moisture	1-15

The relative proportion of biochar components determines the chemical and physical behaviour and function of biochar as a whole (Brown, 2009), which in turn determines its suitability for a site specific application, as well as transport and fate in the environment (Downie, 2009). For

example, coarser and more resistant biochars are generated by pyrolysis of wood-based feedstocks (Winsley, 2007). In contrast, biochars produced from crop residues (e.g. rye, maize), manures and seaweed are generally finer and less robust (lower mechanical strength). The latter are also nutrient-rich, and therefore, more readily degradable by microbial communities in the environment (Sohi et al., 2009). The ash content of biochar is dependent on the ash content of the biomass feedstock. Grass, grain husks, straw residues and manures generally produce biochar with high ash contents, in contrast to that from woody feedstocks (Demirbas 2004). For instance, manure (e.g. chicken litter) biochars can contain 45% (by weight) as ash (Amonette and Joseph, 2009). Moisture is another critical component of biochar (Antal and Gronli, 2003), as higher moisture contents increase the costs of biochar production and transportation for unit of biochar produced. Keeping the moisture content up to 10% (by weight) appears to be desirable (Collison et al., 2009). In order for this to be achieved, pre-drying the biomass feedstock may be a necessity, which can be a challenge in biochar production.

Breaking and rearrangement of the chemical bonds in the biomass during processing results in the formation of numerous functional groups (e.g. hydroxyl -OH, amino-NH₂, ketone -OR, ester -(C=O)OR, nitro - NO₂, aldehyde -(C=O)H, carboxyl -(C=O)OH) occurring predominantly on the outer surface of the graphene sheets (e.g. Harris, 1997; Harris and Tsang, 1997) and surfaces of pores (van Zwieten et al., 2009). Some of these groups act as electron donors, while others as electron acceptors, resulting on coexisting areas which properties can range from acidic to basic and from hydrophilic to hydrophobic (Amonette and Joseph 2009). Some functional groups also contain other elements, such as N and S, particularly in biochars from manures, sewage sludge and rendering wastes.

2.1.5 Pore size distribution and connectivity of biochar

Biomass feedstock and the processing conditions are the main factors determining pore size distribution in biochar, and therefore its total surface area (Downie et al., 2009). During thermal decomposition of biomass, mass loss occurs mostly in the form of organic volatiles, leaving behind voids, which form an extensive pore network. Biochar pores are classified in this review into three categories (Downie et al., 2009), according to their internal diameters (ID): macropores (ID >50 nm), mesopores (2 nm < ID < 50 nm) and micropores (ID < 2 nm). These categories are orders of magnitude different to the standard categories for pore sizes in soil science. The elementary porosity and structure of the biomass feedstock is retained in the biochar product formed (Downie et al., 2009). The vascular structure of the original plant material, for example, is likely to contribute for the occurrence of macropores in biochar, as demonstrated for activated carbon from coal and wood precursors (Wildman and Derbyshire, 1991). In contrast, micropores are mainly formed during processing of the parent material. While macropores have been identified as a ‘feeder’ to smaller pores (Martinez et al., 2006), micropores effectively account for the characteristically large surface area in charcoals (Brown, 2009).

The development of microporosity in biochar, which is linked to an increase in structural and organizational order, has been shown to be favoured by higher pyrolysis temperature and retention times, as previously demonstrated for activated carbon (e.g. Lua et al., 2004). For example, increasing pyrolysis temperature from 250 to 500°C enhanced the development of micropores in chars derived from pistachio-nut shells, due to increased evolution of volatiles. Similarly, heating rate and pressure during processing have also been found to influence the mass transfer of volatiles produced at any given temperature range, and are therefore regarded as key contributing parameters influencing pore size distribution (Antal and Grønli, 2003).

2.1.6 Feedstock and its influence on biochar characteristics

Feedstock is the term conventionally used for the type of biomass that is pyrolysed and turned into biochar. Feedstock is, along with pyrolysis conditions, the most important factor controlling the properties of the resulting biochar. Firstly, the chemical and structural composition of the biomass feedstock relates to the chemical and structural composition of the resulting biochar and, therefore, is reflected in its behaviour, function and fate in soils. Secondly, the extent of the physical and chemical alterations undergone by the biomass during pyrolysis (e.g. attrition, cracking, microstructural rearrangements) is dependent on the processing conditions (mainly temperature and residence times).

Pyrolysis of wood-based feedstocks generates coarser and more resistant biochars with carbon contents of up to 80%, as the rigid ligninolytic nature of the source material is retained in the biochar residue (Winsley, 2007). Biomass with high lignin contents (e.g. olive husks) have shown to produce some of the highest biochar yields, given the stability of lignin to thermal degradation, as demonstrated by Demirbas (2004).

Whereas woody feedstock generally contains low proportions (< 1% by weight) of ash, biomass with high mineral contents such as grass, grain husks and straw residues generally produce ash-rich biochar (Demirbas, 2004). These latter feedstocks may contain ash up to 24% or even 41% by weight, such as rice husk (Amonette and Joseph, 2009) and rice hulls (Antal and Grønly, 2003), respectively. The mineral content of the feedstock is largely retained in the resulting biochar, where it concentrates due to the gradual loss of carbon (C), hydrogen (H) and oxygen (O) during processing (Demirbas, 2004). The mineral ash content of the feedstock can vary widely and evidence seems to suggest a relationship between mineral ash and biochar yield

(Amonette and Joseph, 2009). Many different materials have been proposed as biomass feedstocks for biochar, including wood, grain husks, nut shells, manure and crop residues, while those with the highest carbon contents (e.g. wood, nut shells), abundance and lower associated costs are currently used for the production of activated carbon (e.g. Lua et al., 2004; Martinez et al., 2006; González et al., 2009).

Crystalline silica has also been found to occur in some biochars. Rice husk and rice straw contain unusually high levels of silica (220 and 170 g kg⁻¹) compared to that in other major crops (van Zwieten et al., 2007). High concentrations of calcium carbonate (CaCO₃) can be found in pulp and paper sludge (van Zwieten et al., 2007) and are retained in the ash fraction of some biochars. Regarding the characteristics of some plant feedstocks, Collison et al. (2009) go further, suggesting that even within a biomass feedstock type, different compositions may arise from distinct growing environmental conditions (e.g. soil type, temperature and moisture content) and those relating to the time of harvest.

In general, wood biochars had higher total C, lower ash content, lower contents of total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), sodium (Na), sulfur(S), and copper(Cu), and lower potential CEC and exchangeable cations than poultry litter biochars, whereas tree leaf biochars were generally intermediate (Singh et al., 2010). Much of the mineral content of the feedstock remains in the resulting biochar, where it is concentrated due to the loss of C, H and O during pyrolysis (Amonette and Joseph, 2009). There is considerable variation in the content of many elements especially N and P due to feedstock characteristics and range of production temperatures. Feedstocks typically high in N, P, K and S are sewage sludge, animal manures and biosolids.

2.1.7 Temperature and its influence on biochar characteristics

High temperature biochar pyrolyzed at 700 °C has recalcitrant characteristics and is advantageous when the chief objective is to remove atmospheric CO₂ and sequester C in soil for millennia (Keiluweit et al., 2010). However, synchrotron-based near edge X-ray absorption fine structure (NEXAFS) spectra have revealed that biochars produced at high temperatures are typically poorly crystalline (Keiluweit et al., 2010). This implies that some metals in the C lattice may possibly be volatilized, and that the mineral fraction will be less (Bridgwater and Boocock, 2006). Therefore, these biochars would consequently have lesser reactivity in soils than lower temperature biochars, which tend to have a better impact on soil fertility (Steinbeiss et al., 2009).

A study carried out by Gaskin et al. (2008) showed that biochars produced at 500 °C concentrated their most essential plant nutrients; namely P, K, Ca, and Mg. This subsequently led to considerably higher quantities in the final biochar product. Consequently, biochar that is produced with the key role of being a soil fertility amendment needs to be specifically aimed at carbonizing the biomass material under moist conditions and at low temperatures (Novak et al., 2009). Investigations conducted on the effect of different pyrolytic temperatures on pine chars showed that there was a reduction in the organic content with increasing pyrolytic temperature in the range of 300 to 700 °C. These studies also showed that the weight loss of chars declined from 37 % to 24 % when the biomass was pyrolyzed at 500 °C during different time intervals comprising 10 to 300 minutes. Therefore, it was suggested that pyrolytic temperatures play a more important role than pyrolytic time to carbonize pine wood (Zhou et al., 2009). Other studies revealed that the pyrolysis temperature has an effect on the yield of biofuel and biochar. An increase in temperature resulted in a reduction in the recovery of biochar, while the concentration of carbon increased (Daud et al., 2001; Demirbas, 2004).

In a recent study, Cao and Harris (2010) investigated the effect that different heating temperatures have on the physical, chemical, and mineralogical properties of dairy-manure derived biochar. The untreated air dried biochar was dried at a room temperature of 25 °C and 500 °C respectively and used for comparative purposes. It was found that the following properties increased as a result of increased temperature during pyrolysis; specific surface area (SSA), ash content, pH and concentrations of P, Ca, and Mg. The SSA increased exponentially between 200 and 500 °C. The increase in ash content was due to the high presence of calcite and quartz minerals in the manure. At a temperature of 500 °C, the biochar produced more than 95 % ash, thus indicating the complete combustion of C. The pH increase was dependent on the heating temperature. Initially, the untreated manure at room temperature was alkaline at pH 7.5-8.0. At 200 °C, the pH declined to neutrality at about pH 7 (Cao and Harris 2010). At temperatures of 300 °C and above, the C began to ash, and subsequently increased the biochar pH to above 10.0, where it became constant (Cao and Harris 2010). In addition, the mean total P, Ca, and Mg concentrations increased from 0.91 %, 3.23 %, and 1.11 %, respectively at 100 °C to 2.66 %, 9.75 %, and 3.02 % at 500 °C. The total P, Ca, and Mg increases were attributed to increasing temperature.

2.2 Biochar's Impact on Soil Performance

- Improve nutrients availability, storage and CEC
- Increase soil carbon content
- Increase Water Holding Capacity
- Increase soil pH

- Decrease Aluminum toxicity
- Decrease tensile strength
- Change microbiology of the soil
- Decrease greenhouse gases emissions from the soil (N_2O and CH_4)
- Improve soil conditions for earthworm populations
- Improve fertilizer use efficiency.
- Increase soil water infiltration and permeability
- Sorption of organic and other chemical substances

2.2.1 Improvement of Nutrient Availability, Storage and CEC

2.2.1.1 Biochar effect on soil N

Lehmann et al. (2006) have suggested that biochar can adsorb both NH_4^+ and NH_3^- from the soil solution thus reducing solution inorganic N at least temporarily, but perhaps concentrating it for microbial use. Biochar is an N depleted material having a uniquely high C/N ratio (839). It is also possible that some amount of decomposition might have occurred when fresh biochar is added to soil (Schneour, 1966; Liang et al, 2006), which could induce net immobilization of inorganic N already present in the soil solution. Gundale and DeLuca (2006) reported that biochar addition to soil caused reduction in ammonification compared to the control due to adsorption and reduced the potential for NH_3 volatilization. The reduction could be due to high C/N ratio of biochar and greater potential for N immobilization (Lehmann et al., 2006).

It should be noted however that, immobilization potential associated with biochar additions to soil would be greatly limited by the recalcitrant nature of biochar (DeLuca and Aplet, 2007).

Biochar has the potential to catalyze the reduction of N_2O to N_2 ; potentially reducing the emission of this important greenhouse gas to the atmosphere, and thus biochar could directly or indirectly influence denitrification. The process of denitrification requires the presence of substrate (available C) and a terminal electron acceptor, such as NO_3^- (Stevenson and Cole, 1999).

2.2.1.2 Biochar effect on P availability

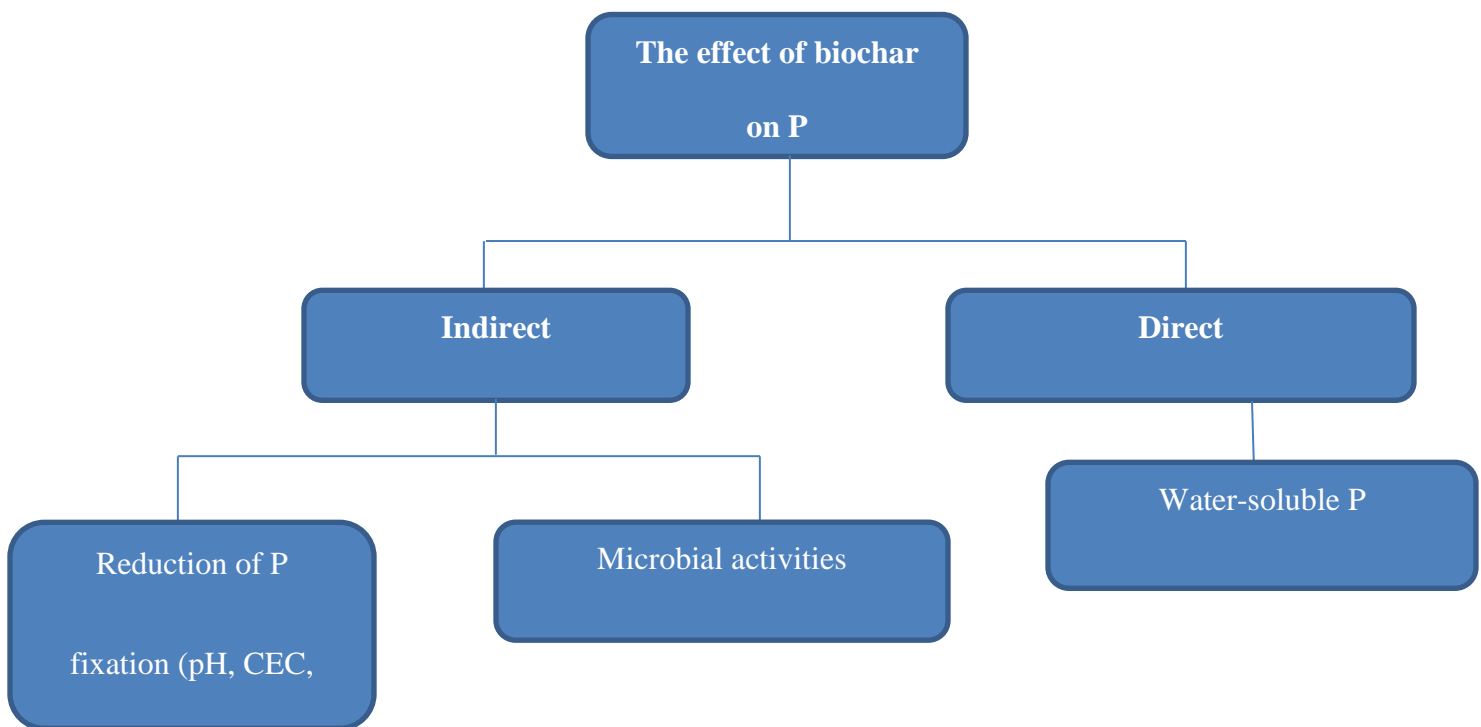


Fig. 2.2 Effect of biochar on soil (Cowie A. et al., 2006)

Soils found in tropical regions are particularly poor in plant available phosphorus resulting in P deficient environments. These soils contain sesquioxides that have the ability to strongly sorb phosphate (Turner et al. 2007), and thereby creating a sink on the availability of inorganic

phosphorus for plants (Oberson et al., 2006). Sandy textured soils give biochar the potential to ameliorate P leaching in soils, therefore, it is expected that P will increase with increasing levels of biochar additions (Novak et al., 2009). Addition of black charcoal to the previously mentioned Ferralsol and Anthrosol was correlated with increased phosphorus nutrition and plant uptake. Higher crop growth observed in this Anthrosol compared to the Ferralsol was largely an effect of elevated phosphorus and other nutrient availability along with comparatively low nutrient leaching (Lehmann et al., 2003).

The availability and, subsequently, the adsorption of phosphorus is highly pH dependent. Increases in soil pH are likely to influence P availability, with available forms most common between pH of 4 to 8.5 (Atkinson et al., 2010). The availability of some elements toxic to plant growth, particularly at low pH, such as Al, Cu and Mn, can be reduced by biochar incorporation while the availability of other elements can increase, with biochar induced increases in soil pH enhancing solubility of phosphorus as well as N, Ca, Mg and Mo (Atkinson et al., 2010). The increasingly investigated characteristics of biochar uphold a reputation for it to help ameliorate problems of poorly fertile soils. Additions were investigated to determine if biochar could contribute to improving fertility of a sandy, acidic soil (Novak et al., 2009). Phosphorus concentration in leachate was found to decrease with increasing biochar application (Novak et al., 2009). The decrease was attributed to a combination of reactions such as retention of $o\text{-PO}_4^{3-}$ through ligand exchange reactions involving oxygen-containing functional groups on the biochar surface, adsorption of $o\text{-PO}_4^{3-}$ by Fe and Al oxides and hydroxides, and by adsorption and precipitation by Ca, Mg-phosphates (Bohn et al., 1979).

Additions of biochar to soils result in alterations with effects that are beneficial to phosphate solubilizing bacteria (PSB), whose activities increase soil P (Lehmann et al., 2007).

2.2.1.3 Biochar effect on other nutrients

Incorporation of biochar into acid soils increased soil cation exchange capacity (CEC) and adsorption capacity of the soils for selected nutrients (Steiner et al., 2008; Novak et al., 2009; Sohi et al., 2010), especially in tropical and subtropical regions. Many studies have analyzed biochar's effect on nutrient availability and leaching, and have shown that it clearly has an influence on nutrient transformations. The extent of this influence depends highly on the ion of interest and the properties of biochar obtained from the feedstock and soil environment. The sources of organic matter used as biochar feedstocks are shown to alter the availability of key macronutrients such as N and P, and some metal ions such as Ca and Mg, when incorporated into the soil (Atkinson et al., 2010).

Both increasing and decreasing nutrient uptake and biomass productivity have been reported following biochar additions to soil and the effect of biochar additions on nutrient availability is not yet entirely clear (Lehmann et al., 2003). Large proportions of black carbon in an Anthrosol of the Amazon basin was found to have significantly higher availability of P, Ca, Mn, and Zn than a nearby Ferrasol, minimal nutrient leaching, and increased plant uptake of P, K, Ca, Zn, and Cu (Lehmann et al., 2003).

2.2.1.4 Biochar effect on CEC

Biochar was shown to increase the cation exchange capacity (Lehmann et al., 2003). Evidence suggests the cation exchange capacity (CEC) of biochar is consistently higher than that of the whole soil, clay minerals, or soil organic matter (Sohi et al., 2010). Soil CEC increases are due to carboxylate groups on the surfaces of the biochar itself and exposed carboxylate groups of organic acids sorbed by the biochar, both of which contribute negative surface charge to biochar

particles (Novak et al., 2009). Simultaneously, increases in charge density per unit surface of organic matter develop, which equate with a greater degree of oxidation, or increases in surface area for cation adsorption, or a combination of both (Atkinson et al., 2010).

2.2.2 Biochar effect on soil organic carbon

A change in microbial abundance and community structure may affect not only biochar mineralization itself, but also mineralization of other soil C (Busscher et al., 2010). The commonly observed greater microbial biomass has been presented as a reason for a greater decomposition of soil C (also called priming) in the presence of biochar (Wardle et al., 2008). The fact that this has generally not been observed beyond an initial greater mineralization after fresh biochar additions (Hamer et al., 2004; Wardle et al., 2008; Zimmerman et al., 2011) suggests different explanations for the C loss observed in these studies that may instead be related to physical export of C, changes in nutrient contents or pH (Lehmann and Sohi, 2008). Also, labile substances in biochars (such as condensable volatiles as found in smoke) may stimulate microbial activity shortly after biochar application to soil (Fischer and Bienkowski, 1999; Uvarov, 2000; Das et al., 2008; Steiner et al., 2008a), but these are mineralized within a relatively short period of time (Cheng et al., 2006). Longer incubations (beyond one year) and field trials have shown that biochars decrease mineralization of other soil C (Kuzyakov et al., 2009; Kimetu and Lehmann, 2010; Zimmerman et al., 2011). However, the conundrum of greater microbial biomass yet lower soil C respiration still warrants closer examination. Interestingly, similar observations of greater microbial biomass yet lower metabolism have been made in waste water treatment, where biofilms on sand showed greater removal and

mineralization rates of dissolved aromatic C than biofilms on activated carbons (Koch et al., 1991) that typically have large surface areas (Downie et al., 2009).

It is possible that CO₂ precipitates as carbonates on biochar surfaces that have high pH and abundant alkaline metals, which would explain reduced detection of CO₂ evolved, despite measured increases in microbial biomass. For example, lipases have been shown to sorb well to activated carbon matrices with long life and high activity (Quirós et al., 2011). So called “immobilization” of enzymes on materials such as biochar is by now used in many industrial processes that allow stable conditions for optimum enzyme activity (Novick and Rozzell, 2005).

A dominance of certain groups of microorganisms, such as coenocytic fungi degrading simple C compounds (e.g., Zygomycota) was observed when corn biochar was added to a temperate Alfisol, whereas, abundance of septate fungi (such as Basidiomycota, known lignin degraders) and Ascomycota) decreased (Jin, 2010). An increase in fungi that metabolize simpler sugars would be in accordance with greater microbial biomass and sorption of labile C compounds on biochar surfaces, rather than the inaccessibility of sorbed organic matter (Jin, 2010).

2.2.3 Biochars’ effect on water holding capacity of the soil

Biochar incorporation into a soil can have widespread impacts on the intrinsic properties of a soil. Water holding capacity is influenced by both the mineral and organic components of a soil (Glaser et al., 2002). Higher levels of organic matter are associated with higher water holding capacity and Glaser et al. (2002) found water retention to be 18% higher in terra preta than in adjacent soils, a difference believed to be attributed to the higher biochar content and higher levels of organic matter associated with charcoal in these soils. The high stability of biochar, due to the extensive structure of aromatic carbons, offers potential for providing long-term

modification to soil water holding capacity through its generally macro porous nature (Sohi et al., 2010). It is found that the long-term effect of biochar on available moisture will be positive in sandy soils dominated by larger pores, than in neutral or in medium-textured soils, and potentially detrimental in clay soils (Sohi et al., 2010). Gaskin et al. (2007) determined moisture release curves for a loamy sand field soil to which different amounts of biochar were added. The highest application rate was determined to have a significant effect on volumetric water content, double that of the control soil containing no biochar (Gaskin et al., 2007).

Tryon (1948) studied the effect of charcoal on the percentage of available moisture in soils of different textures and found different responses among soils. In sandy soil, the addition of charcoal increased available moisture by 18% after adding 45% biochar by volume, while no changes were observed in loamy soil, and soil available moisture decreased in the clayey soil. The high surface area of biochar can lead to increased water retention, although the effect seems to depend on the initial texture of the soil. Improved water holding capacity with biochar additions is most commonly observed in coarse-textured or sandy soils (Gaskin et al., 2007; Glaser et al., 2002). The impact of biochar additions on moisture content may be due to increased surface area relative to that found in coarse-textured soils (Glaser et al., 2002). Therefore, improvements in soil water retention by biochar additions may only be expected in coarse-textured soils or soils with large amounts of macro pores. Additionally, a large amount of biochar may need to be applied to the soil before it increases water retention.

2.2.4 Biochar effect on pH and aluminum toxicity

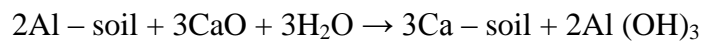
Hydrogen (H^+) and aluminum (Al^{3+}) ion dominance in the soil exchangeable complex causes acidity which limits crop yield and utilization of many essential nutrients by plants and microorganisms (Black, 1993; Chintala et al., 2012a). Liming to remediate acidic soils has a

longer history than the use of any other forms of soil amendments (McLean, 1971). Biochar contains some alkaline materials and has relatively high pH (Steiner et al., 2007; Gaskin et al., 2008) and, thus, can neutralize soil acidity and increase the pH of acid soils (Chan et al., 2008; Novak et al., 2009). Effects of biochar incorporation on pH and exchangeable acidity of acid soils have been reported (Chan et al., 2007; 2008; van Zwieten et al., 2010). Biochars can have pH values of below 4 or above 12, depending on feedstock type and pyrolysis temperature (Lehmann, 2007a; Chan and Xu, 2009). Similar to nutrient and C changes, the effects of pH changes induced by biochar will largely depend on the pre-existing soil pH, the direction and magnitude of change (Novak et al., 2009).

Higher pyrolytic temperature ($>400^{\circ}\text{C}$) was observed to produce biochars with alkaline pH (Novak et al., 2009). Before applying these biochars to acidic soils as amendment, it will be necessary to analyze their composition and liming potential. The physical and chemical characteristics of any amendment determine its effectiveness as liming agent (Barber, 1984). The liming effect of any amendment can be determined by studying soil indices such as soil pH and exchangeable acidity (Wong and Swift, 1995; Wang et al., 2009). Liming potential of a material can also be predicted by its properties such as calcium carbonate equivalence (Mokolobate and Haynes 2002) and ash alkalinity (Noble et al., 1996). The ameliorating effect of biochars on acidic soil was assumed to be consistent with their composition and properties which depend on biomass feedstock type and pyrolytic conditions (Noble et al., 1996).

Soil pH has the potential to undergo a change when either the biochar or a cation in the biochar reacts with the soluble monomeric Al species, or alternatively displaces it from the exchange sites of clay or soil organic matter (Sparks, 2003). Depending on the biochar biomass used, basic

cations such as Ca, K, Mg, and silicon (Si) can form alkaline oxides or carbonates during the pyrolysis process (Noble et al., 1996). Following the release of these oxides into the environment, they can react with the H⁺ and monomeric Al species, raise the soil pH, and decrease exchangeable acidity (Novak et al., 2009). Furthermore, research conducted by Novak et al. (2009) on pecan shell derived biochar revealed that there was a high concentration of calcium oxide (CaO) in the biochar, which neutralizes soil acidity as follows:



The reaction describes the reduction in exchangeable acidity whereby Ca replaces the monomeric Al species on the soil exchangeable sites and generates alkalinity. Subsequently, there is an increase in soil solution pH as a result of the reduction of the readily hydrolysable monomeric Al and the subsequent formation of the neutral [Al(OH)₃]⁰ species (Sparks, 2003).

2.2.5 Biochar effect on soil biology

The soil biota is vital to the functioning of soils and provides many essential ecosystem services. Understanding the interactions between biochar when it is used as a soil amendment, and the soil biota is therefore vital. It is largely through interactions with the soil biota, such as promoting arbuscular mycorrhizal fungi (AMF) as well as influencing on water holding capacity, which lead to the reported effects of biochar on yields (Steiner et al., 2008; Kolb et al., 2009). Soil is a highly complex and dynamic habitat for organisms, containing many different niches due to its incredibly high levels of heterogeneity at all scales. On the micro scale, soil is often an aquatic habitat, as micro pores in soil are full of water at all times, apart from during very extreme drought, due to the high water tension which exists there (Steiner et al., 2008; Kolb et al., 2009). This is vital for the survival of many microbial species which require the presence of water for mobility as well as to function (Steiner et al., 2008; Kolb et al., 2009). Indeed, many soil

organisms, specifically nematodes and microorganisms such as protozoa enter a state of cryptobiosis, whereby they enter a protective cyst form and all metabolism stops in the absence of water (Steiner et al., 2008; Kolb et al., 2009). When biochar application leads to an increased water retention of soils, it seems likely therefore that this will have a positive effect on soil organism activity, which may well lead to concurrent increases in soil functioning and the ecosystem services which it provides.

Organisms in the soil form complex communities and food webs and engage in many different techniques for survival and to avoid becoming prey, ranging from hiding in safe refuges, through to conducting forms of chemical ‘warfare’ (Zackrisson et al., 1996). Biochar, due to its highly porous nature, has been shown to provide increased levels of refugia where smaller organisms can live in small spaces which larger organisms cannot enter to prey on them. Microorganisms within these micro pores are likely to be restricted in growth rate due to relying on diffusion to bring necessary nutrients and gases, but as this occurs in micro pores within the soil, this demonstrates that microorganisms utilizing these refugia almost certainly would not be reliant of decomposition of the biochar for an energy source. This is likely to be one of the mechanisms for the demonstrated increases in microbial biomass (Steiner et al., 2008; Kolb et al., 2009), and combined with the increased water holding potentials of soil is a possible mechanisms for the increased observed basal microbial activity (Steiner et al., 2008; Kolb et al., 2009). However, due to the complexities of the soil system and its biota, it is probable that many more mechanisms are at work. For example Kolb et al. (2009) demonstrated that while charcoal additions affected microbial biomass and microbial activity, as well as nutrient availability, differences in the magnitude of the microbial response was dependent on the differences in base nutrient availability in the soils studied. However, they noted that the influences of biochar on the soil microbiota acted in a relatively similar way in the soils they studied, albeit at different

levels of magnitude, and so suggested that there is considerable predictability in the response of the soil biota to biochar application.

There is some evidence that the positive effects of biochar on plant production may be attributable to increased mycorrhizal associations (Nisho and Okano, 1991). The majority of studies concerning biochar effects on mycorrhiza show that there is a strong positive effect on mycorrhizal abundance associated with biochar in soil (Harvey et al., 1976; Ishii and Kadoya, 1994). The possible mechanisms were hypothesised by Warnock et al. (2007) to include (in decreasing order of currently available experimental evidence)

- a) Alteration of soil physico-chemical properties
- b) Indirect effects on mycorrhizae through effects on other soil microbes
- c) Plant–fungus signaling interference and detoxification of allelochemicals on biochar
- d) Provision of refugia from fungal grazers

2.2.6 Biochar effect on climate change

Incorporation of biochar into soils increases the locking of atmospheric carbon dioxide (CO₂) through a C-negative process (Glaser et al., 2009) and thus reduces emission of greenhouse gases such as CO₂, methane (CH₄), and nitrous oxide (N₂O) compared with its feedstock (Lehmann et al., 2006; Spokas and Reicosky, 2009).

Biochar is primarily composed of both single and condensed ring aromatic C, and subsequently has a mutual high surface area per unit mass and a high surface charge density (Lehmann 2007a). The biochars largely composed of single-ring aromatic and aliphatic C mineralize more rapidly in comparison to those composed of condensed aromatic C (Lehmann, 2007a; Novotny et al.,

2007). Spectra using NEXAFS reveal that aromatic and quinonic compounds are more common when aliphatic groups are lost at 400 °C (Keiluweit et al., 2010). Lehmann (2007a) reported that biochar may be an alternative to renewable energy because it is not carbon neutral, but rather carbon negative. This implies that because biochar is formed by a carbon negative process, it may serve as a long-term terrestrial sink of carbon. The carbon negative process means that the feedstock parent material used to manufacture biochar initially withdraws organic carbon from photosynthesis and decomposition carbon cycle pathway (Lehmann, 2007b). This process is then followed by storing this organic carbon in the soil, thus causing it to accumulate over time (Glaser, 2007). Relative to merely using fresh material to store C, because biochar decomposes over a long period of time, it is able to create the slow release of CO₂ into the atmosphere over an extended period, and thus reduces CO₂ emissions (Gaunt and Lehmann, 2008). Therefore, because biochar is able to gain CO₂ from the atmosphere, it would circumvent from the contribution of climate change, and hence aid in reducing global warming (Lehmann, 2007a).

It is generally accepted that reducing atmospheric concentrations of CO₂ by permanently sequestering C in the soil could reduce the impact of climate-related damage. Increasing soil organic carbon (SOC) storage by conventional soil management practices such as conservation tillage, no-till, and perennial cropping systems can take many years and there is uncertainty about the C sequestration potential of these systems (Baker et. al., 2007; Denman et al., 2007). By contrast, application of biochar to agricultural soils is an immediate and easily quantifiable means of sequestering C and is rapidly emerging as a new management option that may merit high value C credits (McHenry, 2008; Glaser et al., 2009; Tenenbaum, 2009; Steinbeiss et. al., 2009).

In many studies where biochar has been shown to reduce N₂O fluxes, a number of mechanisms have been proposed based mainly on prior knowledge of the requirements of nitrifiers and denitrifiers. These include: (i) enhanced soil aeration (reduced soil moisture) inhibiting denitrification due to more oxygen being present; (ii) labile C in the biochar promoting complete denitrification *i.e.*, dinitrogen (N₂) formation; (iii) the elevated pH of the biochar creating an environment where N₂O reductase activity is enhanced thus promoting N₂ formation and higher N₂/N₂O ratios; and (iv) a reduction in the inorganic-N pool available for the nitrifiers and/or denitrifiers that produce N₂O, as a result of NH₄⁺ and/or NO₃⁻ adsorption, greater plant growth, NH₃ volatilisation loss, or immobilisation of N. Increases in N₂O fluxes have been attributed to: (i) the release of biochar embodied-N or priming effects on SOM following biochar addition; (ii) biochar increasing soil water content and improving conditions for denitrification; and (iii) biochar providing inorganic-N and/or carbon substrate for microbes (Knoblauch et al., 2011; Scheer et al., 2011; Taghizadeh-Toosi et al., 2011; Clough et al., 2010; Singh et al., 2010; Van Zwieten et al., 2010b; Zhang et al., 2010; Spokas and Reicosky, 2009; Yanai et al., 2007).

2.2.7 Biochar effect on sorption of hydrophobic organic compounds (HOCS) and others

The sorption of anthropogenic hydrophobic organic compounds (HOC) (e.g. PAHs, polychlorinated biphenyl - PCBs, pesticides and herbicides) in soils and sediments, is generally described based on two coexisting simultaneous processes: absorption into natural (amorphous) organic matter (NOM) and adsorption onto occurring charcoal materials (Cornelissen et al., 2005; Koelmans et al., 2006). Comparatively to that of NOM, charcoals (including soot) generally hold up to 10 to 1000 times' higher sorption affinities towards such compounds (Chiou and Kile, 1998; Bucheli and Gustafsson, 2000, 2003). It has been estimated that black carbon (BC) can account for as much as 80 to 90% of total uptake of trace HOC in soils and sediments

(Cornelissen et al., 2005), and that it applies to a much broader range of chemical species than previously thought (Bucheli and Gustafsson, 2003; Cornelissen et al., 2004).

Biochar application is therefore expected to improve the overall sorption capacity of soils (Chiou 1998), and consequently, influence toxicity, transport and fate of trace contaminants, which may be already present or are to be added to soils. Enhanced sorption capacity of a silt loam for diuron (Yang and Sheng, 2006) and other anionic (Hiller et al., 2007) and cationic (Sheng et al., 2005) herbicides has previously been reported following the incorporation of biochar ash from crop (wheat and rice) residues. The relative importance of these latter studies is justified by the fact that charring of crop residues is a widespread agricultural practice (Hiller et al., 2007).

Previous studies have convincingly demonstrated that adsorption to charcoals is mainly influenced by the structural and chemical properties of the contaminant (i.e. molecular weight, hydrophobicity, planarity) (Cornelissen et al., 2004, 2005; Zhu and Pignatello, 2005; Zhu et al., 2005; Wang et al., 2006), as well as pore size distribution, surface area and functionality of the charcoal (e.g. Wang et al., 2006; Chen et al., 2007). For example, sorption of tri- and tetra-substituted-benzenes (such as trichlorobenzene, trinitrotoluene and tetramethylbenzene) to maple wood charcoal (400°C) was sterically restricted, when compared to that of the lower size benzene and toluene (Zhu and Pignatello, 2005).

In fact, experimental evidence has recently demonstrated that organic structures in the form of BC (including biochar) or NOM, which are equipped with strong aromatic π -donor and -acceptor components, are capable of strongly adsorbing to other aromatic moieties through specific sorptive forces other than hydrophobic interactions (Keiluweit and Kleber, 2009). Although a large body of evidence is available on the way the characteristics of HOC influence sorption to biochars, the contribution of the char's properties to that process has been far less evaluated. It is

generally accepted that mechanisms leading to an increase in surface area and/or hydrophobicity of the char, reflected in an enhanced sorption affinity and capacity towards trace contaminants, as demonstrated for other forms of BC (Jonker and Koelmans, 2002). The influence of pyrolysis temperatures mostly in the 340-400°C range (James et al., 2005; Zhu et al., 2005) and feedstock type (Pastor-Villegas et al., 2006) on such phenomena has been recently evaluated for various wood chars by a number of authors. Interestingly, sorption to high-temperature chars appears to be exclusively by surface adsorption, while that to low-temperature chars derive from both surface adsorption and (at a smaller scale) absorption to residual organic matter (Chun et al., 2004).

The influence of micro pore distribution on sorption to biochars has been clearly demonstrated by Wang et al. (2006). Diminished O functionality on the edges of biochar's graphene sheets due to heat treatment (e.g. further charring), resulted in enhanced hydrophobicity and affinity for both polar and non-polar compounds, by reducing competitive adsorption by water molecules (Zhu et al., 2005; Wang et al., 2013). The treated char also revealed a consistent increase in micropore volume and pore surface area, resulting in better accessibility of solute molecules and an increase in sorption sites (Wang et al., 2013).

The underlying sorption mechanism, including the way it is influenced by a wide range of factors inherent to the contaminant, to the char material and to the environment, remains far from being fully understood (Fernandes and Brooks, 2003). In this context, it is vital to comprehensively assess the environmental risk associated to these species in biochar-enriched soils, while re-evaluating both the use of generic OC-water distribution coefficients (Jonker et al., 2005) and of remediation endpoints (Cornelissen et al., 2005). For instance, remediation endpoints (undetectable, non-toxic or environmentally acceptable concentrations, as set by regulatory

agencies) for common environmental contaminants in biochar-enriched soils would need to be assessed based on dissolved (bioavailable) concentrations rather than on total concentrations (Cornelissen et al., 2005). In order to achieve that, prior careful experimental evaluation of the contaminant distribution, mobility and availability in the presence of biochar is paramount.

2.2.8 Impact of biochar on crop productivity

Positive yield effects from biochar addition were reported by Kimetu et al. (2008), who were able to establish that the impact was in part due to non-nutrient improvement to soil function. Improved fertilizer use efficiency was pin-pointed as an explanation for biochar maintaining crop yields after forest clearance in Amazonia, in essentially a recreation of terra preta (Steiner et al., 2008a). Biochar-amended plots receiving NPK sustained higher crop yield compared to control plots where yield declined rapidly. Results from semi-arid soils in Australia have shown positive response of rice to biochar in combination with fertilizer in pot trials (Chan et al., 2007), and in Indonesia maize and peanut yields were enhanced where bark charcoal was applied in combination with N fertilizer in the field (Yamato et al., 2006). The view that nutrient management and pre-existing soil nutrient status determine crop response to biochar was supported by a study on rice (Asai et al., 2009), where statistically higher first-season yield was observed only when biochar (at a low rate) was applied together with fertilizer N and in a low-yielding crop variety. Yield was lower than the control in an equivalent treatment using a high-yielding (and thus N-demanding) variety. However, some studies show no significant yield response, for example at low rates of application in an Australian study in wheat (Blackwell et al., 2007). A pot study of cowpea showed higher biological nitrogen fixation with biochar addition due to nutrient effects (Rondon et al., 2005); higher yield and N uptake reported in pot trials using radish (Chan et al., 2007, 2008). A key consideration highlighted in several studies is

the potential for biochar to immobilize previously plant available N. This could be from the mineralization of labile, high C-to-N fractions of biochar drawing N into microbial biomass, sorption of ammonium, or sequestration of soil solution into fine pores.

The increase in crop yield with biochar application has been reported elsewhere for crops such as cowpea (Yamato *et al.*, 2006), soybean (Tagoe *et al.*, 2008), maize (Yamato *et al.*, 2006; Rodríguez *et al.*, 2009), and upland rice (Asai *et al.*, 2009). Haefele (2007) and Haefele *et al.* (2008) discussed the possibility of biochar applications for rice-based cropping systems. Reichenauer *et al.* (2009) applied biochar in tsunami-affected paddy fields in Sri Lanka, and the experimental results showed that the application of 2 t rice-husk-charcoal ha⁻¹ increased the grain yield from less than 4 t ha⁻¹ for the control treatment to more than 5 t ha⁻¹ for the biochar treatment.

2.3 Petroleum and its' products

2.3.1 Origin and formation of petroleum oil.

Even though disagreement exists about the origin of petroleum oil, years of research by geologists has resulted in a reasonably clear understanding of how crude oil forms in the earth's crust, its composition, and how it occurs. Ideas about the origin of oil follow two different lines of thinking: organic theories and inorganic theories (Hedberg, 1969).

One of the earliest inorganic theories originated with Arab philosophers who, in about 850 A.D., suggested that water and air combined with fire to produce sulfur and mercury. The sulfur and mercury then combined with "earth" and, at great subterranean temperatures, yielded "naft" (naphtha) and "qir" (asphalt) (Hedberg, 1969). Two nineteenth-century scientists, Louis Joseph

Gay-Lussac (1850) and Alexander von Humboldt (1859) proposed that oil formed as a result of impregnation of marine sediments by subaqueous hot springs. Another nineteenth-century idea was that oil formed when hot alkalis combined with carbon dioxide deep in the earth's interior (Hunt, 1996). A Russian chemist, Dimitri Mendeleev (Mendeleev was also the "inventor" of the Periodic Table), believed that percolating water encountered iron carbide deep in the earth, generating hydrocarbons. Other scientists, noting that methane occurs in trace amounts in volcanic gases and in fluid inclusions in igneous rocks, assumed that it was "sweated" out of the earth's interior throughout geologic time, rose in the crust, changed into heavier hydrocarbons, and finally accumulated into the petroleum deposits we use today (Hedberg, 1969).

Hypotheses suggesting an organic origin for oil is also old. Oil and coal were linked by some naturalists as early as the sixteenth century. Abundant imprints of leaves, stems, and other evidence of vegetation left little doubt as to the origin of coal (Rogers, 1863). Chemists discovered that small amounts of oil could be distilled from coal in the laboratory and postulated that this occurred in nature as well. Geologists had problems with this idea though, because the primary oil-producing strata lacked associated coals, and naturally-occurring oils were chemically different from the oils derived from the distillation of coal. Other nineteenth-century workers believed that oil was derived from terrestrial vegetation, which was washed into the sea and deposited with the sediments containing the petroleum. Problems with this idea include the fact that some oil is produced from rocks containing only marine fossils, and also the high temperatures needed to convert wood into liquid organic matter are not geologically reasonable. By the late 1800's and early 1900's, the prevailing view was that crude oil represents an accumulation of hydrocarbons that were originally produced by living organisms, both plants and animals and coal came from the accumulations of dead plants (Tissot and Welte, 1984).

Other scientists tried to explain the origin of oil in other ways. The occurrence of hydrocarbons in meteorites has been well known to scientists since the mid-1800's (Tissot and Welte, 1984). In the early 1930's, astronomers learned that methane is a major component of the large outer planets – Jupiter, Saturn, Uranus, and Neptune. Because it was believed that all the planets in our solar system were closely related in origin, some researchers concluded that the raw materials for hydrocarbons must have been present in the substances from which the primordial earth accreted 4.6 billion years ago. By the 1950's, such reasoning led astronomer Fred Hoyle to argue that the deep earth must contain vast untapped reserves of oil just awaiting our technological ability to find and exploit them (Tissot and Welte, 1984). This idea is still favored by a small group of scientists.

It is now accepted today by most geologists that oil was formed millions of years ago from a combination of hydrocarbons synthesized by living organisms and hydrocarbons formed by thermal alteration of organic matter in sedimentary rocks (Carter, 2004). Ten to twenty percent of the oil in the earth's crust is thought to form from living organisms, whereas 80 to 90 percent is formed by thermal alteration (Edwards, 1997). Marine planktons are the major components in both methods of natural crude oil formation (Edwards, 1997). Several lines of evidence support this contemporary view of the origin of petroleum:

1. Oil is rarely found in rocks that formed before life developed on the earth;
2. Oil contains compounds derived from the pigments of living organisms;
3. The ratio of carbon isotopes in oil is similar to that in organic matter;
4. Hydrocarbon compounds found in oil affect polarized light in the same way that hydrocarbons and other compounds synthesized by living organisms affect polarized light;
5. The structures of many oil compounds are similar to those of fats and waxes found in living organisms and, therefore, could be formed from them.

When organisms die, bacteria attack their remains. These bacteria require oxygen, and if oxygen is plentiful, destruction of the organic remains is complete. Abundant remains of marine plankton, however, sometimes accumulate along with mud in stagnant underwater environments. The aerobic bacteria use up any dissolved oxygen quickly. Anaerobic bacteria, which obtain their oxygen from dissolved sulfur compounds and hydroxides in the pore waters of the mud, then take over. These bacteria consume most of the easily decomposable compounds in the organic matter, such as carbohydrates and proteins. As the muds are buried by an increasingly thicker cover of sediment, physical and low temperature chemical reactions continue to alter the chemical structure and composition of much of the organic matter. At even deeper burial depths, rising temperatures and pressures cause the organic debris to decompose further to form crude oil (Edwards, 1997). The muds compact and become shale. Petroleum migrates from the shale (it is “squeezed” out) and travels through more porous and permeable strata until it encounters a trap. Recent ideas about the origin of oil are based on a long history of scientific investigation. Some perceptive geologists intuitively reached the same conclusions over 100 years ago. For example, Henry Rogers, the first State Geologist of Pennsylvania (where oil was first discovered, in 1859), thought that Devonian black shales were the source of the oil found in the sandstones of Pennsylvania (Carter, 2004). He suggested that “...the greater portion of the oil and gas is derived from the marine [fossil organic matter] in the carbonaceous shales.”

2.3.2 Composition and Classification of Petroleum Hydrocarbons

Petroleum has been known for several years to occur in the surface seepage and was first obtained in pre-Christian times by the Chinese (Alloway and Ayres, 1993). The modern petroleum industry had its beginning in Romania and in a well-sunk in Pennsylvania by Colonel

E. A. Drake in 1859 (Alloway and Ayres, 1993). The principal early use of the product of the petroleum industry was for the replacement of expensive whale oil for lighting. Today, its consumption as a fuel and its dominance in the world market as a source of chemicals has diversified tremendously. Petroleum is defined as any mixture of natural gas, condensate, and crude oil. Crude oil which is a heterogeneous liquid consisting of hydrocarbons comprised almost entirely of the elements hydrogen and carbon in the ratio of about 2 hydrogen atoms to 1 carbon atom. It also contains elements such as nitrogen, sulphur and oxygen, all of which constitute less than 3% (v/v). There are also trace constituents, comprising less than 1% (v/v), including phosphorus and heavy metals such as vanadium and nickel. Crude oils could be classified according to their respective distillation residues as paraffins, naphthenes or aromatics and based on the relative proportions of the heavy molecular weight constituents as light, medium or heavy. Also, the composition of crudes may vary with the location and age of an oil field, and may even be depth dependent within an individual well. About 85% of the components of all types of crude oil can be classified as either asphalt base, paraffin base or mixed base. Asphalt base contain little paraffin wax and an asphaltic residue (Atlas, 1981). The sulphur, oxygen and nitrogen contents are often relatively higher in comparison with paraffin base crudes, which contain little or no asphaltic materials. Mixed crude oil contains considerable amount of oxides of nitrogen and asphalt.

On a structural basis, the hydrocarbons in crude oil are classified as alkanes (*normal* or *iso*), cycloalkanes, and aromatics. Alkenes, which are the unsaturated analogs of alkanes, are rare in crude oil but occur in many refined petroleum products as a consequence of the cracking process. Increasing carbon numbers of alkanes (homology), variations in carbon chain branching (*iso*-alkanes), ring condensations, and interclass combinations e.g., phenylalkanes, account for the

high numbers of hydrocarbons that occur in crude oil. In addition, smaller amounts of oxygen – (phenols, naphthenic acids), nitrogen- (pyridine, pyrrole, indole), and sulfur- (alkylthiol, thiophene) containing compounds, collectively designated as “resins” and partially oxygenated, highly condensed asphaltic fraction occur also in crude but not in refined petroleum (Atlas and Bartha, 1973).

2.3.3 Sources of petroleum in the environment

Petroleum hydrocarbon pollution of the environment may arise from oil-well drilling production operations, transportation and storage in the upstream industry, and refining, transportation, and marketing in the downstream industry (Oberdorster and Cheek, 2000). Petroleum hydrocarbon pollution could also be from anthropogenic sources (Oberdorster and Cheek, 2000). Some non-combusted hydrocarbons escape into the environment during the process of gas flaring. Until recently, the bulk of the associated gas produced during drilling in Nigeria, was flared (Okoh, 2002).

Sources of petroleum and its products in the environment also include accidental spills and from ruptured oil pipelines (Hill and Moxey, 1960). Today the international oil and gas-pipelines span several million kilometers and this is growing yearly. Just like any other technical appliance, pipelines are subject to ‘tear and wear’, thus can fail with time (Beller, *et al.*, 1996). Spilled petroleum hydrocarbons in the environment are usually drawn into the soil due to gravity until an impervious horizon is met, for example bedrock, watertight clay or an aquifer (Adedokun and Ataga, 2007). Poor miscibility of crude oil accounts for accumulation of free oil on the surface of ground water and this may migrate laterally over a wide distance to pollute other zones very far away from the point of pollution. Industrial and municipal discharges as well as urban run-offs,

atmospheric deposition and natural seeps also account for petroleum hydrocarbon pollution of the environment (Adedokun and Ataga, 2007). Table 2 (Baker, 1983) is a summary of some sources and estimated quantity of crude oil released into some named ecosystem. It is worthy of note that groundwater is one of the many media by which human beings, plants and animals come into contact with petroleum hydrocarbon pollution.

Table 2.2 Sources and estimates of crude oil or its products released into the environment(Baker, 1983)

Sources	Quantities (K. tones)
Transportation accidents	390
Tanker operations (washings)	710
Atmospheric/fuel combustion	300
Municipal, industrial and surface run-offs	1,400
Natural seeps / erosion	300

2.3.4 Distribution and methods of enumerating petroleum degrading microorganisms

Hydrocarbon degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats. Similarly, hydrocarbon degrading cyanobacteria have been reported (Challana *et al.*, 2004; Lliros *et al.*, 2003), although contrasting reports indicated that growth of mats built by cyanobacteria in the Saudi coast led to preservation of oil residues (Barth, 2003). Typical

bacterial groups already known for their capacity to degrade hydrocarbons include *Pseudomonas*, *Marinobacter*, *Alcanivorax*, *Microbulbifer*, *Sphingomonas*, *Micrococcus*, *Cellulomonas*, *Dietzia*, and *Gordonia* groups (Brito *et al.*, 2006). Molds belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Amorphoteca*, *Neosartorya*, *Paecilomyces*, *Talaromyces*, *Graphium* and the yeasts *Candida*, *Yarrowia* and *Pichia* have been implicated in hydrocarbon degradation (Chaillan *et al.*, 2004).

Although experimental and climatic conditions differed considerably in each study, some general trends have indicated that Gram negative *Proteobacteria* and *Cytophaga-Flavobacterium-Bacteroides* group dominate during bioremediation of petroleum contaminated soils and the diversity shifts with time to these groups (Macnaughton *et al.*, 1999; Kaplan and Kitts, 2004). These groups were usually associated with the fast degradation phase and their abundance was positively correlated to hydrocarbon attenuation (Macnaughton *et al.*, 1999; Kaplan and Kitts, 2004). Gram positive bacteria if detected are never diverse and dominant during bioremediation (Kaplan and Kitts, 2004). However, recent reports have shown that Gram positive bacteria mainly the *Actinobacteria* can actually dominate during bioremediation of petroleum hydrocarbon owing to their metabolic versatility and their widespread occurrences both in pristine and hydrocarbon polluted soil (Bell *et al.*, 1998; Bouchez-Naitali *et al.*, 1999; Bogan *et al.*, 2003; Margesin *et al.*, 2003; Larkin *et al.*, 2005; Hamamura *et al.*, 2006; Quatrini *et al.*, 2008).

Reports in literature on the actual numbers of hydrocarbon utilizers are at variance with one another because of the methodological differences used to enumerate petroleum-degrading microorganisms. The initial method involved the use of hydrocarbons incorporated into agar-based medium (Horowitz *et al.*, 1978). This approach has its problems. In some cases, a high correlation has been found between growth on agar and media containing hydrocarbons as the

sole carbon source, and the ability to rigorously demonstrate hydrocarbon utilization by isolates from these media in liquid culture. In other studies, only a low percentage of isolates from agar-based media could be demonstrated to be capable of hydrocarbon utilization. The use of silica gel-oil medium for the enumeration of petroleum degrading microorganisms has been recommended (Walker and Colwell, 1976), suggesting that counts of petroleum degraders be expressed as a percentage of the total population rather than as total numbers of petroleum degraders *per se*. Also, the ability to utilize hydrocarbons is widespread, even in environments not subjected to high levels of hydrocarbon pollution. Atlas (1978) reported that quantitative differences in the distribution of hydrocarbon utilizers were relatively unimportant over large geographic distances.

The Most Probable Number (MPN) procedure has also been tried as a substitute for the plate count technique for the estimation of hydrocarbon utilizing microorganisms, since it eliminates the need for a solidifying agent and permits direct assessment of the ability to actually utilize hydrocarbons. The use of liquid media for MPN also permits removal of trace organic contaminants and allows for the chemical definition of a medium with a hydrocarbon as a sole source of carbon. This technique thus incorporates the specificity for counting only hydrocarbon utilizers and eliminates the problem of counting organisms growing on other trace organic contaminants (Braddock and Catterall, 1999). The problems of culture techniques arise from the fact that most (90–99%) of the species making up competent degrading communities do not form colonies when current laboratory-based culture techniques are used (MacNaughton *et al.*, 1999). However, the application of molecular techniques for the analysis of the microbial communities that take part in *in situ* hydrocarbon biodegradation activities is helping to address these problems. The measurement of lipid biomarkers, specifically, phospholipid fatty acids (PLFA), together with nucleic acid-based molecular techniques for fingerprinting the 16S ribosomal DNA

(rDNA) component of microbial cells is a powerful combination of techniques for elucidating the microbial ecology of actively bioremediating communities (Stephen *et al.*, 1999). Lipid biomarker-based techniques measure the lipid profiles of microbes in the environment irrespective of culturability, thereby avoiding culture bias (White *et al.*, 1998). These methods provide insight into several important characteristics of microbial communities, especially the viable biomass, community structure, and nutritional status or physiological stress responses of the gram-negative bacteria (White *et al.*, 1998). Molecular genetic techniques allow researchers to examine microbial communities without cultivation using universal primers 16S rRNA gene primers (Borneman *et al.*, 1996). PCR has been particularly useful for detecting genes involved in the degradation of xenobiotic compounds (Joshi and Walia, 1996). Despite the shortcomings of cultivation-based techniques, standard culture methods are still adequate for site evaluation to determine whether indigenous bacteria are capable of degrading the contaminants.

2.3.5 Reasons for Persistence of Hydrocarbons in the Environment

Generally, petroleum hydrocarbons' biodegradation is a naturally slow process governed by their complex chemical structure, interaction with other molecules, toxicity, molecular recalcitrance and the insolubility in water which makes microbes in the soil unable to completely access the compounds thus increasing their persistence in the environments (van Eyk, 1997; Banerji et al, 1995, Bamforth and Singleton, 2005). Polycyclic Aromatic Hydrocarbons (PAH) refers to fused benzene ring aromatic hydrocarbons (in linear, angular or clustered arrangements) that are very recalcitrant due to their complex structures and stable molecular bonds with a high affinity for soil material and the longer they are left to accumulate in soils the more difficult they are to eliminate mainly because of their hydrophobic nature which gives them high persistence in the environment (Juhasz and Naidu, 2000).

Alexander (1994) also revealed that many chemical reactions that these hydrocarbons require for them to be degraded are foreign to micro-organisms present in the contaminated soil and thus bioremediation is not achieved while if microbes present in the soil metabolize these compounds by similar transformation pathways, adaptation to differentiate the metabolites generated may delay the biodegradation process. Bamforth and Singleton, (2005) also revealed that the binding of PAH's with other contaminants such as petroleum hydrocarbons and heavy metals can prolong their persistence in environments as this association depletes the oxygen level needed by microbes for the transformation while anaerobic transformation of PAH's is limited.

2.3.6 Effect of Petroleum on the Soil

2.3.6.1 Physical effects

The moisture content of a soil following oil application will either increase or decrease, depending on the site. McGill (1980) reported that soil will 'wet-up' in poorly drained low areas where oil will naturally pool whereas on slopes the movement of oil over the soil surface will result in a non-wettable dry soil. Usually well aerated contaminated soil will have a tendency to dry out and be more prone to soil erosion (McGill, 1978; Rowell, 1975; Loynachan, 1979).

Different authors have reported that oil polluted soils generally 'wet-up' more slowly in comparison to non-polluted soils (Toogood et al, 1977; Volk, 1980). Volk (1980) attributed this to the non-polar characteristics of oil. Watts et al (1982) reported that spills of certain types of oil, especially those with high viscosity, may result in poor or restricted water infiltration and a concomitant loss in wetting. The application of oil to soil also can create a soil crust, further restricting the infiltration of water and oxygen into the soil profile (McGill and Nyborg, 1975; Volk, 1980). Some workers have observed soil dispersion and disintegration of soil structure

after application of oil (Ellis and Adams, 1961; Rowell, 1975) whereas others have observed aggregation of oil-treated soil (Giddens, 1976; Raymond et al, 1976). Udo and Fayemi (1975) and others (Ellis and Adams, 1961; Giddens, 1976) have observed that after the oil is decomposed and converted to normal soil organic matter, the soil structure is improved.

2.3.6.2 Chemical Effects

One of the major effects of oil on soil is the inhibition of nitrification of ammonium-N to nitrate-N (Ellis and Adams; 1961; Schwendinger, 1968; Odu, 1972; Udo and Fayemi, 1975; McGill, 1977). Extractable phosphorus and potassium levels also may become depressed (Udo and Fayemi, 1975; Loynachan, 1979) or may increase (Ellis and Adams, 1961; Watts et al, 1982) in soil following oil contamination. It is generally accepted that available soil nutrients, notably nitrogen, are immobilized by soil microorganisms following oil application (Ellis and Adams, 1961; Schwendinger, 1968; Giddens, 1976; McGill, 1977).

According to Ellis and Adams (1961), the increase in phosphorus could be explained on the basis of a more favourable pH and the fact that some phosphorus could be brought into solution by reducing conditions that make iron phosphates more soluble. Volk (1980) reported that the increase in potassium found after oil addition to soil likely was related to the release of potassium initially present in the oil. There usually is a high demand for oxygen by soil microorganisms following oil addition to soil and oxygen levels in oil contaminated soil may become depleted, contributing to anaerobic or reducing conditions (Volk, 1980). Several authors have reported that anaerobic or reducing conditions also can result in increased solubility of manganese and iron in oil contaminated soil (Ellis and Adams, 1961; Schwendinger, 1968; McGill, 1977).

An increase in total nitrogen, organic carbon and organic matter also has been reported (Ellis and Adams, 1961; Watts et al, 1982) as has an increase in the C: N ratio of oil treated soil (Udo and Fayemi, 1975). Some authors also have reported that the soil pH tends to shift to neutral values after hydrocarbon addition to both acidic and alkaline soils (Vanlooocke et al, 1975). In addition, it has been reported that oil spills increased soil temperature from 1° C to 10° C (McGill, 1980) and this is believed to be related to solar warming associated with the darker colour of the oiled soil (Johnston, 1970; Raymond et al 1976) and to a lack of vegetative cover which would normally shade the soil.

2.3.6.3 Biological effects

Petroleum in whatever form is toxic to plants and soil microorganisms (Adenipekun and Kassim, 2006, Adenipekun et al., 2009). Previous study by Cook and Westlake (1974) had described that oil spill kills agricultural plants or inhibits the growth of the entire vegetation cover. Plants have been described as the first victims of oil spill on land ecosystem (Odijimi and Oghalu, 2006). De Jong (1980) reported that oil in soil creates unsatisfactory conditions for both plants and microbial growth probably due to insufficient aeration of the soil. Oil readily penetrates the pore spaces of the soil following any spill with heavier friction and this subsequently impedes photosynthesis and microbial growth (Bossert and Bertha, 1984).

2.4 Remediation of contaminated soils

2.4.1 In-situ Methods for Soil Remediation

In situ methods are used at the contamination site. Soil does not need to be excavated, and therefore exposure pathways are minimized. Some of these methods include;

2.4.1.1 Volatilization

In situ volatilization causes mechanical drawing or air venting through the soil. A draft fan is injected or induced, which causes an air flow through the soil, via a slotted or screened pipe, so that air can flow but entrainment of soil particles is restricted (Sparks, 1993). Some treatments, e.g., activated carbon, are used to recover the volatilized contaminant. This technique is limited to volatile organic carbon materials (Sparks, 1993).

2.4.1.2 Phytoremediation

The use of plants to decontaminate soils and water (phytoremediation) can be quite effective. It is an emerging technology that promises effective, inexpensive, and less intrusive clean up and restoration of oil-contaminated environments (Stomp, et al., 1993; Schnoor, et al., 1995). Phytoremediation involves plants that aid in the restoration of contaminated ecosystems (Cunningham and Berti, 1993). A green plant is a solar-driven, pumping, and effective filtering system endowed with measurable loading degradative and fouling capacities (Salt, et al., 1995). There are hundreds of plant species that can detoxify pollutants. For example, sunflowers can absorb uranium, certain ferns have high affinity for As, alpine herbs absorb Zn, mustards can absorb Pb, clovers take up oil, and poplar trees destroy dry-cleaning solvents (New York Times, 2001). Recently the brake fern (*Pteris vittata*) was found to be an As hyperaccumulator (Brooks, 1998) and very effective in remediation of a Central Florida soil contaminated with chromated copper arsenate (Ma et al., 2001). Brake ferns extracted 1,442 to 7,526 mg/kgAs from contaminated soils (Brooks, 1998).

Salt marsh plants are able to take up hydrocarbons from oil-contaminated sediment and increase the hydrocarbon or total lipid fraction of the aerial portions of plants (Lytle and Lytle, 1987).

There are three established mechanisms by which plants decontaminate oil polluted sites and these are direct uptake of petroleum hydrocarbons into their tissues; release of enzymes and exudates that stimulate the activity of hydrocarbonoclastic microbes and direct biochemical transformation (enzymes) of petroleum hydrocarbons; enhancement in the degradation of the contaminants in the rhizosphere due to mycorrhizal fungi and the activity of soil microbial consortia (Schnoor et al., 1995). Plants that are resistant to crude oil toxicity such as black poplar and willows, as well as miscanthus grass (elephant grass) have been found to be effective in the remediation of oil polluted soil (Shank and McEwan, 1998). In the marsh environment *Spartina patens*, *Sagittaria lancifolia*, *Spartina alterniflora* and *Juncus roemerianus* are considered ecologically and economically important in phytoremediation (Lytle and Lytle, 1987). *Dioscorea sp* can metabolise petroleum hydrocarbons such as n-hexadecane (Hardman and Brain, 1977). Cytochrome P450 and peroxidases found in the plant *Dioscorea composita* are involved in the biotransformation of this hydrocarbon (Vega-Jarquin et al., 2001). One major setback in phytoremediation is that the plants tend to be competing with the hydrocarbonoclastic microbial population for available nitrogen and phosphorus (Vega-Jarquin et al., 2001). However, phytoremediation can accelerate the reduction of oil concentration in both surface and deep soil, and thus restore crop sustaining potential and reducing marsh erosion after a spill.

2.4.1.3 Leaching

This method involves leaching the in-place soil with water and often with a surfactant (a surface-active substance that consists of hydrophobic and hydrophilic regions; surfactants lower the surface tension) to remove the contaminants. The leachate is then collected downstream of the site, using a collection system for treatment and/or disposal (Sparks, 1993). The use of this

method has been limited since large quantities of water are often needed to remove the pollutants and, consequently, the waste stream is large and disposal costs can be high. The effectiveness of a leaching technique also depends on the permeability, porosity, homogeneity, texture, and mineralogy of the soil, which all affect the desorbability (release) of the contaminant from the soil and the leaching rate of contaminants through the soil (Sparks, 1993).

2.4.1.4 Vitrification

In in situ vitrification the contaminants are solidified with an electric current, resulting in their immobilization. Vitrification may immobilize pollutants for as long as 10,000 years. Since a large amount of electricity is necessary, the technique is costly (Sparks, 1993).

2.4.1.5 Isolation or Containment

With this method, contaminants are held in place by installing subsurface physical barriers such as clay liners and slurry walls to minimize lateral migration (Xu et al., 1997). Scientists and engineers have also added surfactants to clay minerals (organo-clays) to enhance retention of organic pollutants (Xu et al., 1997) and used organo-clays in liners to minimize the mobility of pollutants and in wastewater treatment (Soundararajan et al., 1990).

2.4.1.6 Passive remediation

With this method, natural processes such as volatilization, aeration, biodegradation, and photolysis are allowed to occur; these processes may cause decontamination (Leavin and Gealt, 1993). Passive remediation is simple and inexpensive and requires only monitoring of the site.

Factors that affect this type of remediation include biodegradation, adsorption, volatilization, leaching, photolysis, soil permeability, groundwater depth, infiltration, and the nature of the contaminant (Leavin and Gealt, 1993).

2.4.2 Non In-situ Methods of Soil Remediation

Non-in-situ methods involve removal of the contaminated soil usually by excavation, and the soil is then treated on-site, or transported to another location and then treated. With these methods there are obviously concerns about exposure of the contaminants in the moving and hauling process. Some of these methods include;

2.4.2.1 Land treatment

With this technique, the contaminated soil is excavated and spread over land so that natural processes such as biodegradation or photo-degradation can occur to decontaminate the soil (Sparks, 1993). The land area is prepared by grading to remove rocks and other debris and the area is surrounded by berms to lessen runoff. The soil pH is adjusted to 7.0 to immobilize heavy metals and to enhance the activity and effectiveness of soil microbes (Sparks, 1993). Nutrients are also added for microbial stimulation. The contaminated soil is then spread on the site and mixed with a different soil to enhance the contact between the contaminant and microbes and to promote aerobic conditions (Sparks, 1993).

2.4.2.2 Thermal treatment

With thermal treatment, the excavated soil is exposed to high (about 500°C) heats using a thermal incinerator. The high temperature breaks down the pollutants, and the released volatiles are then collected and moved through an afterburner and combusted or recovered with solvents (Sparks, 1993).

2.4.2.3 Asphalt incorporation

With this method, contaminated soils are put into hot asphalt mixes. These mixtures are then used in paving. The asphalt and soil are heated while they are mixed. This causes volatilization or decomposition of some of the contaminants. The remaining pollutants are then immobilized in the asphalt (Sparks, 1993).

2.4.2.4 Solidification or Stabilization

This technique involves the addition of an additive to excavated, contaminated soil so that the contaminants are encapsulated. The mixture is then landfilled. Thus, the contaminants are not free to move alone; however, they are not destroyed. This method has been employed to minimize inorganic pollutant contamination (Sparks, 1993).

2.4.2.5 Chemical extraction

In this treatment the excavated soil is mixed with a solvent, surfactant, or solvent/surfactant mixture to remove the contaminants. The solvent/surfactant and released contaminants are then separated from the soil. The soil is then washed or aerated to remove the solvent/surfactant and

the latter is then filtered for fine particles and treated to remove the contaminants. This technique is expensive and is not often used (Sparks, 1993).

2.4.2.6 Excavation

With this method, the contaminated soil is removed and disposed elsewhere (e.g., a landfill). Landfills usually contain liners, such as clay, that diminish the mobility of the contaminants, or the landfills should be located on sites where the soil permeability is low (Sparks, 1993). Landfills require large land areas and often pose hazards for humans. Excavation and disposal costs are high, and there are also liability problems, safety concerns, odor production, potential runoff and groundwater contamination problems (Sparks, 1993).

2.4.3 In-situ microbial bioremediation

Because it treats contaminants in place instead of requiring their extraction, in-situ bioremediation takes care of these shortcomings in the other clean-up processes. Consequently, bioremediation is likely to yield faster results, take a few to several years compared to for example, a few to several decades for the pump-and-treat technology (Testa and Winegardner, 1991). The microbiological decontamination of oil-polluted soils has been assessed to be an efficient, economic and versatile alternative to physiochemical treatment (Bartha, 1986) even though the rate of hydrocarbon biodegradation in soils is affected by other physiochemical and biological parameters. While capital and annual operating cost may be higher for bioremediation, its shorter operating time should compensate in a reduction of total cost (Testa and Winegardner, 1991). Other factors that may contribute to cost reduction in bioremediation compared to pump-

and-treat method include reduced time required for site monitoring, reporting and management, as well as reduced need for maintenance, labour, and supplies (National Research Council, 1993). Furthermore, the surface treatment methods that are part of pump-and-treat systems typically use air stripping and/or carbon treatment to remove contaminants from the water or soil. The process is mainly that of transferring the contaminant to another medium (the air or the land) instead of destroying it (Testa and Winegardner, 1991).

Potential advantages of bioremediation compared to other in-situ methods include destruction rather than transfer of the contaminant to another medium; minimal exposure of the on-site workers to the contaminant; longtime protection of public health; and possible reduction in the duration of the remedial process (Bartha, 1986). The advantages of the bioremediation systems over the other technologies have been summarized (Leavin and Gealt, 1993) as follows: can be done on site i.e, in-situ application; keeps site destruction to a minimum; eliminates transportation costs and liabilities; eliminates long-term liability; biological systems are involved, hence often less expensive; and can be coupled with other treatment techniques to form a treatment train.

2.4.3.1 Basic understanding of bioremediation principles

Simply defined, bioremediation is the use of biological systems to destroy or reduce the concentrations of hazardous wastes from contaminated sites. Such systems have the potentially broad-spectrum site applications including ground water, soils, lagoons, sludge and process waste-streams, and it has been used in very large scale applications such as the shoreline cleanup efforts in Alaska, resulting from the oil tanker “Exxon Valdez” oil spill in 1989 (Caplan, 1993). Bioremediation strategy can be as simple as applying a garden fertilizer to an oil-contaminated

beach, or as complex as an engineered treatment “cell” where soils or other media are manipulated, aerated, heated, or treated with various chemical compounds to promote degradation (Hildebrandt and Wilson, 1991). The bioremediation strategy of choice ultimately will depend on the peculiarity of the contaminated site. Many published articles have documented the potentials of microorganisms to degrade oil both in the laboratory (Lawson et al., 2012) and in field trials. A number of the scientific papers including several review articles covered aspects of the biodegradation process as well as results from controlled field experiments designed to evaluate degradation rates in various environments (Gunkel and Gassmann, 1980; Atlas, 1981; Halmos, 1985). Furthermore, some studies carried out following major oil spills like the Amoco Cadiz have assessed oil degradation in the environment and confirmed the reliability of bioremediation process. Crude oil is a complex but biodegradable mixture of hydrocarbons, and the observation that hydrocarbon degraders can be enriched in many, if not most, types of environments (Atlas, 1981) have contributed to the development of oil bioremediation techniques (Margesin and Schinner, 1997). Although the optimum temperature for biodegradation of petroleum products has generally been found to be in the range of 20 - 30°C (Atlas and Bartha, 1992), local environmental conditions may select for a population with a varying optimum temperature.

Three primary ingredients for microbial bioremediation are: 1) presence of a contaminant, 2) an electron acceptor, and 3) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved (State of Mississippi, Department of Environmental Quality, 1998). Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of

attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic chemicals such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such contaminants into less toxic forms.

Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are nutrient availability, moisture content, pH, and temperature of the soil matrix. Inorganic nutrients including, but not limited to, nitrogen, and phosphorus are necessary for microbial activity and cell growth. It has been shown that “treating petroleum-contaminated soil with nitrogen can increase cell growth rate, decrease the microbial lag phase, help to maintain microbial populations at high activity levels, and increase the rate of hydrocarbon degradation” (Walworth et al., 2005). However, it has also been shown that excessive amounts of nitrogen in soil cause microbial inhibition. Walworth et al. (2005) suggest maintaining nitrogen levels below 1800 mg nitrogen/kg soil or water for optimal biodegradation of petroleum hydrocarbons. Addition of phosphorus has benefits similar to those of nitrogen, but also results in similar limitations when applied in excess (State of Mississippi, Department of Environmental Quality, 1998).

2.4.3.2 Bioremediation technologies for crude oil contaminated sites

Hydrocarbon compounds such as petroleum are essential elements of life. Since they do not naturally occur in the forms most useful to humans, they can be hazardous (Adriano et al., 2007). Fuel and lubricating oil spills have become a major environmental hazard to-date. The contamination of the environment with petroleum hydrocarbons provides serious problems for

many countries (Adriano et al., 2007). Scientists have conducted research on cost-effective clean-up techniques with minimal long-term damage to the environment. Biodegradation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as an efficient, economic, versatile and environmentally sound treatment (Margesin et al., 1997). The extent of hydrocarbon biodegradation in contaminated soils is critically dependent upon three factors: a) the creation of optimal environmental conditions to stimulate biodegradative activity, b) the predominant petroleum hydrocarbon types in the contaminated matrix and c) the bioavailability of the contaminants to microorganisms (Margesin et al., 1997). Additionally, petroleum hydrocarbon degradation is also affected by the molecular composition of the hydrocarbons (Marques-Rocha et al., 2000). The effectiveness of bioremediation often is a function of the microbial population and how they can be enriched and maintained in an environment (Ajisebutu and Alofe, 2004).

Microorganisms with the ability to degrade crude oil are ubiquitously distributed in soil and mine environments (Venkateswaran et al., 1995). However, when few or no indigenous degradative microorganisms exist in a contaminated area or when there is no time for the natural enrichment of a suitable population; inoculation (bioaugmentation) can be a realistic option (Marques-Rocha et al., 2000). Microbial enzyme activity assay is a simple and rapid method to indicate the cycling of nutrients in soil (Pepper et al., 1995). Enzymes may originate from biotic (viable cells) or abiotic (extracellular) components and their very specific reactions may only allow a small fraction of the total population being detected in the test. Therefore, soil enzyme activities may not always show a strong correlation with other soil biological parameters (Anderson, 1990). Dehydrogenase is involved in the electron transport system to remove the oxidative substrate, and has been found in correlation with the oxygen uptake and organic substrate removal rates in aerobic systems. In artificially contaminated soils, abiotic processes such as volatilization and

adsorption on soil colloids (clay minerals and humus particles) play an important role in the decontamination of petroleum hydrocarbons (Ajisebutu and Alofe, 2004). A part of the mineral oil hydrocarbons added to soils remains undetectable (Venosa et al., 1992). N-alkenes with the intermediate chain length (C10 - C24) are degraded most rapidly (Antai and Mgbomo, 1993). Short chain alkenes like kerosene are toxic for many microorganisms but they generally evaporate rapidly (Antai and Mgbomo, 1993). Very long chain alkenes like gas oil are increasingly resistant to biodegradation (Atlas, 1998). Kerosene consists of light hydrocarbons, so its abiotic reduction is greater than gas oil (Antai and Mgbomo, 1993). Soil dehydrogenase activity reflects a broad range of microbial oxidative activities. Dehydrogenase activity strongly relates to the number of microorganisms in all cases, so dehydrogenase activity can be considered as an indicator for aerobic biodegradation of TPH (Atlas, 1998). Microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Ijah and Antai, 1988; Ezeji et al., 2005; Antai and Mgbomo, 2006).

The simplest method of bioremediation of oil polluted soil is in situ land treatment. This technology utilizes standard farming procedures such as ploughing the oil-polluted soil with a tractor, periodical irrigation and aeration (Bento et al., 2005). This technology embraces the use of aerobic microorganisms to degrade the PHC and other derivatives to carbon dioxide and water, or other less toxic intermediates. Experience has shown that when land-farming technology is properly executed for PHC contaminated soil, non-volatile components of petroleum and other related products are rapidly immobilized, so may not be leached out (Bento et al., 2005). This technology may involve nutrient enrichment in the form of fertilizer application or further manipulation of site conditions such as inoculations with selected or adopted microbial population, mixing and aeration of the soil surface, pH adjustment and irrigation (Bento et al., 2005). Using this technology, an enhancement in the decontamination of

50 cm topsoil of an area previously polluted with crude oil was achieved (Compeau, et al., 1991). Possible enhanced soil fertility recovery for such oil polluted agriculture soil has been demonstrated in soil microorganism experiments where germination and growth of sorghum grains were improved after treatment with adapted *Azotobacter* inoculum (Onwurah, 1999a).

Composting technology is becoming important in the treatment of oil polluted coastal area. It involves the mechanized mixing of contaminated soil or sediment with compost-containing hydrocarbonoclastic bacteria, under aerobic and warm conditions (Chaineau et al., 2005). Through the addition of corn slash (post-harvest leaves and stems), microbial nitrogen fixation has been co-optimized with petroleum hydrocarbon degradation (Paerl, et al., 1996).

A bioreactor is essentially an engineered system in which biochemical transformation of materials is promoted by optimizing the activity of microorganisms, or by “in vitro” cellular components of the microbial cells (enzymes) (Mueller et al., 1991). Bioreactors for the remediation of oil-polluted soil utilize an aqueous slurry phase system. Slurry bioreactor is considered as one of the fastest bioremediation technologies because contaminants can be effectively transported to the microbial cells (Mueller et al., 1991).

An attractive alternative to the slurry bioreactors for treating oil-contaminated soils is the rotating drum bioreactors since they can handle soils with high concentrations of petroleum hydrocarbons (Gray et al., 1994; Banerjee et al., 1995). The fluid phase enhances transport of nutrients and “solublized” or dispersed PHC contaminants to the degrading bacteria. With a bioreactor, temperature, pH and other parameters are optimized for degradation. The rotating drum bioreactor incorporated with blade impellers inside was demonstrated to be effective in decontaminating hydrocarbon-polluted soil (Hupe et al., 1995). The contaminated soil must be excavated, mixed with water and introduced into the reactor. Generally the rate-limiting factors

in any bioreactor system used for crude oil degradation are, the degree of solubilisation through bio-surfactant production and the level or concentration of active biomass of hydrocarbonoclastic bacteria maintained in the system (Stroo, 1992). Degradation products in bioreactors are easily monitored and input regulated. Bioreactors are however intrinsically more expensive than in situ or land treatment technologies because they are specialized (Hupe et al., 1995).

Bio-innoculation, is one of the primary mechanisms of ultimate removal of petroleum hydrocarbons from polluted environments (Atlas, 1988; NRC, 1985). The acceleration of bioremediation is the objective of this process. Seeding a contaminated environment with strains of bacteria that are tolerant and capable of degrading a high percentage of the contaminating petroleum hydrocarbons, and thus supplementing the natural resident microbial population has proven to be useful in bioremediation (Mueller, 1992). The relative success of such adapted (oxotic) bacteria when added to crude oil polluted site will depend on a number of factors including competitive interactions with the native bacteria, their rate of growth in the system as well as their tolerance to the physico-chemical environment (Leahy and Colwell, 1990).

Table 2.3 shows some novel microbial systems that have been applied in PHC degradation. Some of these cultures have been developed as proprietary products through selection of genetically able (not engineered) microorganisms from mixed cultures that are found in a natural contaminated environment as opposed to the genetically engineered strains of microorganism.

Table 2. 3 Successfully used microbial system or strains in bioremediation of oil polluted environment

Microbial system or strain	Remediation mechanism	Reference
<i>Pseudomonas aeruginosa</i> (UG2)	Production of biosurfactant that emulsifies crude oil	Scheibenbogen <i>et al</i> 1994
<i>Pseudomonas sp/ Azotobacter vinlandii</i> consortium	Optimization of nitrogen fixation for crude oil metabolism and co-metabolism	Onwurah , 1999b Onwurah, and Nwuke, , 2004
DBCRS (IBS blend of hydrocarbon-degrading microbes)	Adapted microbial consortium that degrades many components of crude oil	Adams and Jackson 1996
* <i>Rhodococcus</i> * <i>Acinobacter</i> * <i>Mycobacteria</i>	Adapted / tolerant to petroleum hydrocarbon	*Balba, 1993

Assessment of the utility of inoculation or seeding oil spill sites with selected or adapted microorganisms has, thus far, been inconclusive (Pritchard and Costa, 1991). However, seeding with high density of the microbial cells increases the success of the operation (Onwurah and Nwuke 2004). The utility of adapted microbial consortium and nutrients in bioremediation of oil-polluted environment has been demonstrated (Adams and Jackson, 1996).

2.4.3.3 Principle of aerobic degradation of hydrocarbon

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Figure 2.4 shows the main principle of aerobic degradation of hydrocarbons (Nilanjana Das and Preethy Chandan, 2010). The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases (Bamforth and Singleton, 2005). Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate (Bamforth and Singleton, 2005).

Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis (Bamforth and Singleton, 2005). The degradation of petroleum hydrocarbons can be mediated by specific enzyme systems. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants (Wentzel et al., 2007). The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied (Wentzel et al., 2007).

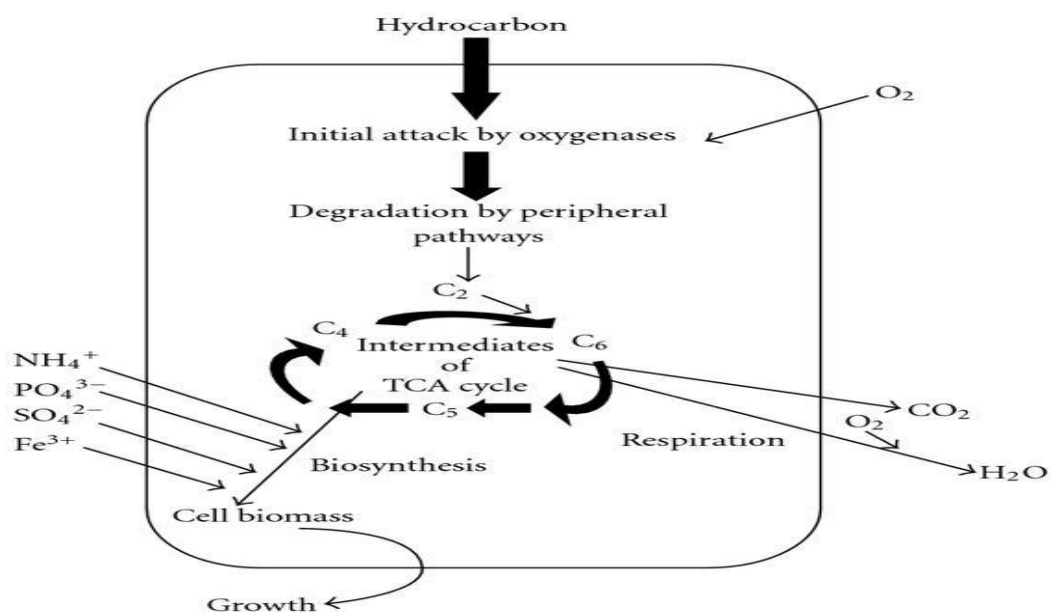


Fig. 2.3 Principle of aerobic degradation of hydrocarbon

(Nilanjana Das and Preethy Chandan, 2011)

The mechanism however differs based on the microbes involved as the transformation pathways for bacterial metabolism is different from that of fungi which is either lignolytic or non-lignolytic (Wentzel et al., 2007). For bacterial degradation, the benzene (aromatic) ring of the Polycyclic Aromatic Hydrocarbons (PAH) oxidised to cis-dihydrodiols by the action of dioxygenase enzymes, followed by the dehydrogenation of these dihydrodiols by dehydrogenase enzymes to form dihydroxylated intermediates and with further metabolism using catechols, carbon-dioxide and water are formed (Cerniglia, 1993, Cerniglia, 1997; Juhasz and Naidu, 2000; Bamforth and Singleton, 2005).

Cerniglia (1993) described fungal degradation of PAH's as quite different from that of bacteria as fungi do not use PAH's as their only source of carbon (energy) but metabolise the PAH's to detoxified chemical compounds. There are two types of metabolism of PAH's carried out by

non-lignolytic fungi i.e, fungi that do not grow on wood and the lignolytic fungi i.e, white rot fungi. The lignolytic fungi can produce lignin peroxidase enzymes while several of them produce both non-lignolytic and lignolytic enzymes. However, it is still unclear the contribution of each enzyme to the breakdown of the PAH's. (Bamforth and Singleton, 2005; Wentzel et al., 2007)

2.4.3.4 Mixed versus pure cultures in microbial degradation of crude

Biodegradation of complex hydrocarbon usually requires the cooperation of more than a single species. This is particularly true in pollutants that are made up of many different compounds such as crude oil or petroleum and complete mineralization to CO₂ and H₂O is desired (Van-Hamme and Odumeru, 2002). Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with overall broad enzymatic capacities are required to bring the rate and extent of petroleum biodegradation further (Sorkhoh et al., 1995; Van-Hamme and Odumeru, 2002). Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh et al., 1995; Rahman et al., 2002). This strongly suggests that each strain or genus has their roles in the hydrocarbon transformation processes (Rahman et al., 2002).

Further evidence for the cooperation of mixed cultures in biodegradation is apparent when Sorkhoh and co-workers (1995) observed a sequential change of the composition of the oil-degrading bacteria over a period of time in sand samples that were contaminated with oil. Venkateswaran and Harayama (1995) reported similar observations in sequential enrichments in medium containing residual crude oil. In an earlier study using pure cultures, it was reported that after exhaustive growth of one strain on crude oil, the residual oil supported the growth of a second and third strain of bacteria (Horowitz et al., 1975; Rahman et al., 2002).

Following the above findings, many studies of petroleum transformation have employed mixed bacterial or bacterial–fungal cultures in efforts to maximize biodegradation (Rahman et al., 2002). Rambeloarisoa et al. (1984) demonstrated a consortium of 8 strains made up of members of 6 genera to be able to effectively degrade crude oil. Interestingly, only 5 of these strains were able to grow in pure cultures using a variety of hydrocarbons. However, when the other 3 strains were removed from the consortium, the effectiveness of the mixed culture was remarkably reduced. This further supports the theory that each member in a microbial community has significant roles and may need to depend on the presence of other species or strains to be able to survive when the source of energy is limited and confined to complex carbons. The degradative capacity of any microbial consortium is the result of the capacities of the individual strains forming the association (Rambeloarisoa et al., 1984).

Komukai-Nakamura and co-workers (1996) reported the sequential degradation of Arabian light crude oil by two different genera. The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have been attributed to the effects of synergistic interactions among members of the association (Komukai-Nakamura et al., 1996).

2.4.3.5 Bioaugmentation in microbial degradation of crude oil

The effectiveness of bioremediation is often a function of the microbial population or consortium and how it can be enriched and maintained in an environment (Onwurah, 1999). Microorganisms with the ability to degrade crude oil are ubiquitously distributed in soil and marine environments, (Venkateswaran and Harayama, 1995). However, when few or no indigenous degradative microorganisms exist in a contaminated area or when there is no time for the natural enrichment of a suitable population, inoculation (bioaugmentation) can be a realistic option (arquez-rocha et

al.,2001). Inoculation of bacteria with hydrocarbon biodegradation capabilities shorten the time of the treatment (Marquez-rocha et al., 2001). The same ecological principles that influence biodegradation with the native microorganism population, in general, will also govern the effectiveness of the inoculation, regardless of whether they are natural isolates or genetically engineered microorganisms (Liu and Suflita, 1993). Some researchers have reported that inoculation had no positive, or only marginal effects on oil biodegradation rates. Microorganisms able to degrade organic pollutants in cultures may fail to function when inoculated into natural environments, because they may be susceptible to toxins or predators in the environment (IUPAC, 2001; Van Hamme et al, 2003). They may use other organic compounds in preference to the pollutant, or they may be unable to move through the soil to the contaminated site (IUPAC, 2001). The successful use of microbial inocula in soils requires that the microorganisms contact the contaminant (IUPAC, 2001). Physical adsorption to soil particles or filtration through small pores may limit the transport of organisms, (Margesin and Schinner, 1997).

Bacteria that can degrade oil constituents are ubiquitous hence, to date there is little convincing evidence that bioaugmentation (addition of more bacteria) significantly enhances either the rate or the extent of oil biodegradation in most environments (IUPAC, 2001; Van Hamme et al, 2003). Bioaugmentation was not shown to speed the mitigation of oil spills on marine shorelines, freshwater wetlands, salt marshes, or soil (IUPAC, 2001). Bioaugmentation with competent degrading strains of bacteria can stimulate the rate and extent of biodegradation of these compounds in appropriate environments (King et al., 1990). It is possible to engineer microbes that show enhanced oil degrading capabilities in the laboratory, but such microbes are unlikely to have a major impact in environmental settings (Van Hamme et al, 2003). First of all, from the moment the oil enters the environment, local microbes begin colonizing the oil droplets (Mueller

et al., 1992). A laboratory strain would have to be able to displace the indigenous microbes which are well-adapted to local conditions (Mueller et al., 1992). Designing microbes that are both more efficient at degrading oil and well adapted to the particular environmental conditions is a daunting challenge, which has not yet been achieved (Mueller et al., 1992). It is important to remember that microbial communities, not individual microbes, are involved in degrading all the various constituents of oil (Komukai-Nakamura et al., 1996). The level of metabolism in each individual microbe (which is what theoretically could be increased in an engineered microbe) is not what limits the rate of biodegradation (Komukai-Nakamura et al., 1996).

2.4.3.6 Effect of Additives on Biodegradation (biostimulation)

2.4.3.6.1 Dispersants

Dispersants do nothing to get rid of the oil they merely change its physical form (Blackburn, 1998). The dispersants may themselves have harmful effects; however, they are added in low concentrations relative to the oil and are less toxic than the oil itself (Blackburn, 1998). In principle, dispersants enhance biodegradation by increasing the surface area and availability of the oil to the microbes. Studies of the effect of dispersants specifically on biodegradation rates are difficult to design and execute because conditions vary so widely depending on the type of spill, the local environment, and weather conditions (Blackburn, 1998).

Based on their chemical structures, the dispersants that have been used on oil spills should themselves be fully biodegradable, but biodegradation rates have not been extensively studied (Margesin and Schinner, 2001). A number of bacterial species produce compounds that function

as dispersants and these compounds are now produced commercially for use in the food, cosmetic, crop treatment, and bioremediation industries (Marquez-Rocha et al, 2000).

2.4.3.6.2 Nutrients

Nutrients including nitrogen, phosphorus, and iron are essential to any biological process and crude oils are naturally deficient in these major nutrients (Margesin and Schinner, 2001; IUPAC, 2001). Many ecosystems are naturally nutrient-poor. Thus, when an oil spill results in a sudden increase in available food (oil hydrocarbons), there may not be enough nutrients in the ecosystem (soil or water) to support microbial growth (Bragg et al., 1994). Nutrient addition to relieve this limitation is one tool to enhance bioremediation and various strategies have been employed to provide nutrients in a suitable form (IUPAC, 2001; Choi et al., 2002). Like all living organisms, microbes have many nutritional requirements including nitrogen, phosphorus and other nutrients (Bragg et al., 1994). These substances are found in nature but may be present in limiting quantities. When food levels are high (for example, after an oil spill), the microbes can only degrade the hydrocarbons as fast as the availability of other nutrients allows (IUPAC, 2001). If nitrogen and phosphorus levels are very low, biodegradation of oil constituents will take place slowly (Bragg et al., 1994).

One of the best understood examples of a large scale addition of nutrients was in response to the Exxon Valdez oil spill, where nutrient addition indeed enhanced oil degradation in the ecosystems of Prince William Sound (Bragg et al., 1994). Theoretically, nutrient addition could have unintended consequences that upset the natural ecosystem balance, but no such consequences have been reported (Bragg et al., 1994).

2.4.3.7 Factors affecting biodegradation of oil in soil

Microbes are able to consume oil because they have the genetic instructions to produce oil-degrading enzymes. But just as crops grow faster with the right amount of light, water, and fertilizer, microbes can degrade oil much more quickly when environmental conditions are optimal. Some of the important factors are:

2.4.3.7.1 Bioavailability of the oil in soil

Contaminants can adsorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation (Chaillana et al., 2006). Thus in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type (Chaillana et al., 2006; State of Mississippi, Department of Environmental Quality, 1998). Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content (Chaillana et al., 2006). In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants (State of Mississippi, Department of Environmental Quality, 1998). Additional research findings of the existence of thermophilic bacteria in cool soil also suggest that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants (Perfumo et al., 2007; Chaillana et al., 2006). It is suggested that contaminants adsorbed to soil particles are mobilized and their solubility increased by high temperatures (Perfumo et al., 2007).

2.4.3.7.2 Physical nature of oil

If the oil is in a single large slick, there is less surface area for the microbes to gain access to the oil, so degradation is slower (Merkl et al., 2005). Furthermore, if the oil is heavy and viscous, the biodegradable components must first diffuse through that thick matrix to the oil-water interface so that the microbes can access those (Chaillana et al., 2006; Merkl et al., 2005). The lighter the oil, the faster its diffusion, making the biodegradable compounds more available to the microbes (Chaillana et al., 2006; Merkl et al., 2005).

2.4.3.7.3 Chemical nature of oil

Biodegradation rates vary depending on the particular hydrocarbons that make up the spilled oil (Chaillana et al., 2006; Aichberger et al., 2005). Oil is composed of thousands of different compounds, some may be preferred food sources and be consumed very quickly, others are degraded more slowly or not at all (Aichberger et al., 2005; Chaillana et al., 2006). In a marine environment like the Gulf of Mexico, hydrocarbons in which carbons are arranged in an unbranched chain can degrade quickly, in a matter of days or weeks (Vidali, 2001). Hydrocarbons that have a branched structure, or those in which the carbons are arranged in multiple rings (known as polycyclic aromatic compounds), can be far more difficult to biodegrade and therefore persist longer (Vidali, 2001). The most recalcitrant fractions of crude oil including resins and asphaltenes can last for millennia (Vidali, 2001).

2.4.3.7.4 Availability of nutrients

Like all living organisms, microbes have many nutritional requirements including nitrogen, phosphorus and other nutrients (Bragg et al., 1994). These substances are found in nature but may be present in limiting quantities. When food levels are high (for example, after an oil spill),

the microbes can only degrade the hydrocarbons as fast as the availability of other nutrients allows (Chaillana et al., 2006). If nitrogen and phosphorus levels are very low, biodegradation of oil constituents will take place slowly (IUPAC, 2001).

2.4.3.7.5 Availability of oxygen

The enzymatic process of breaking down oil is usually most rapid in the presence of oxygen (Chaillana et al., 2006; Nilanjana Das and Preethy Chandan, 2011). Theoretically, given enough oil and other nutrients, microbial populations could grow so quickly that they exhaust the oxygen from the soil or water in the vicinity of an oil spill (Bamforth and Singleton, 2005). In practice, oxygen has not proven to be as important a limiting factor as a nutrient in restricting oil degradation in soils and oceans, although degradation rates could be slow if a spill occurred in a location where oxygen levels are low (Chaillana et al., 2006; Bamforth and Singleton, 2005).

2.4.3.7.6 Availability of moisture

All soil microorganisms require moisture for cell growth and function (National Research Council, 1993). Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells (National Research Council, 1993). However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration (Chaillana et al., 2006). Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process (Trindade et al., 2005). Soil moisture contents “between 45 and 85 percent of the water-holding capacity (field capacity) of the soil or about 12 percent to 30 percent by

weight” are optimal for petroleum hydrocarbon degradation (Chaillana et al., 2006; US EPA, 2006).

2.4.3.7.7 Temperature

Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms (Nester et al., 2001). Generally, “speed of enzymatic reactions in the cell approximately doubles for each 10°C rise in temperature” (Nester et al., 2001). There is an upper limit to the temperature that microorganisms can withstand.

Most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbons, are mesophiles which have an optimum temperature ranging from 25°C to 45°C (Chaillana et al., 2006; Nester et al., 2001). Thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60 °C (Perfumo et al., 2007). This finding “suggested an intrinsic potential for natural attenuation in cool soils through thermally enhanced bioremediation techniques” (Perfumo et al., 2007).

2.4.3.7.8 Soil pH and type

Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the optimum range is pH 6 – 8 (State of Mississippi, Department of Environmental Quality, 1998; US EPA, 2006).

Soil type is an important consideration when determining the best suited bioremediation approach to a particular situation (Chaillana et al., 2006). In situ bioremediation refers to treatment of soil in place. Soil texture directly affect the soils porosity, in as much as permeability of soil to air and water is a function of soil texture. Fine-textured soils like clays have low permeability, which prevents biovented oxygen and nutrients from dispersing throughout the soil (Chaillana et al., 2006). It is also difficult to control moisture content in fine textured soils because their smaller pores and high surface area allow it to retain water (Chaillana et al., 2006). Fine textured soils are slow to drain from water-saturated soil conditions, thus preventing oxygen from reaching soil microbes throughout the contaminated area (US EPA, 2006).

2.4.3.7.9 Other microbes

Natural microbial communities are diverse, with many different types of microbes that both compete and cooperate (Chaillana et al., 2006). The complex interactions that characterize healthy, natural microbial communities are only beginning to be understood, but interdependence is the norm (Perfumo et al., 2007). This is one reason why adding microbes to oil spills in the hope of speeding degradation is challenging; outsiders (i.e., artificially introduced microbes) have a hard time breaking into the existing community structure and competing with the local species that have evolved together over the millennia in a particular habitat (US EPA, 2006).

2.4.3.8 How fast is the oil being consumed?

One question that scientists measuring oil degradation rates want to answer is "how fast is the oil being consumed?" Another is "when will the oil be gone?" The two questions are linked, of

course, but unlike gasoline consumption in a car's gas tank, microbial degradation does not proceed linearly over time (Atlas and Philp, 2005; Ezeji et al., 2005). So, if one-half of the oil is gone in one week, it does not mean that all of the oil will be gone in two weeks (Atlas and Philp, 2005). Generally, degradation rates slow as the oil concentrations decrease, making it difficult to calculate a certain end-point (Ezeji et al., 2005). It also slows as the more readily degradable components are used up, leaving behind the more recalcitrant components (Antai and Mgbomo, 1989).

2.4.3.9 Fate of microbes after degradation

The microbes that degrade oil are part of the local food web; they are consumed by other microbes that are, in turn, consumed by larger predators (Rittman, 1990). When the oil is gone, the oil-degrading microbes, with less food available, stop dividing so rapidly and eventually return to pre-spill abundance (Rittman, 1990). However, determining whether the species composition is exactly as it was pre-spill is complicated at the microbial level, because microbial communities are dynamic systems (Paerl. et al., 1996). It is difficult to determine exactly what the community looked like before the oil spill and we know little about the dynamics of natural populations, even in the absence of obvious disturbances (Paerl, et al., 1996). Further complicating matters, several kinds of bacteria may carry out similar functions.

The relative numbers of these 'redundant' groups may change after an oil spill, but the overall functionality of the community largely remains the same (Paerl, et al., 1996). The functional stability of microbial communities makes it difficult and perhaps unnecessary to determine whether a community is exactly the same as it was before the spill (Olson, and Tsai, 1992). However, no evidence has been found that a bloom of oil-degrading microbes crowds out other

microbial species and drives them to extinction (Leahy, and Colwell, 1990). Even at the height of oil-degrading activity, oil-degrading microbes make up only a small percentage of the total microbial community (Leahy, and Colwell, 1990).

2.5 Oxisols and low pH(soil acidity)

In the Soil Taxonomy classification a soil order of tropical soils that are old, deeply developed, and lacking in horizons wherever well-drained; heavily weathered, low in cation exchange capacity, and low in fertility are called Oxisols (Beinroth et al., 1996). Oxisols are typically found in tropical climates. They are characterized by being deep, red, very strongly weathered soils (Beinroth et al., 1996). Very strongly weathered means that, most of the original minerals, those inherited from the parent material, have been changed to new, secondary minerals (Beinroth et al., 1996). Oxisols, by definition have less than 10% of the original weatherable minerals remaining (Beinroth et al., 1996). Oxisols are low in pH hence, acidic in nature.

Soils that are acidic have *pH* values less than 7 on the *pH* scale (SSSA, 1997). Theoretically, soil acidity is largely associated with the presence of hydrogen and aluminum ions in exchangeable forms (Brady, 2001; Fageria and Baligar, 2003). Thus, the higher the concentrations of these ions in soil solution, the higher the acidity. Most acid soils have been found to be low in fertility, have poor physical, chemical, and biological properties (He et al., 2003). Crop production on such soils is seriously constrained, particularly in areas where proper management measures have not been put in place (He et al., 2003). Generally, soils have natural buffering capacity by which they are able to resist changes in soil *pH* upon marginal increases in their acidity or alkalinity (Black, 1968). This natural buffering ability of soils, however, varies from region to region as the parent

materials and the unconsolidated soil strata differ greatly in their physico-chemical properties (Black, 1968).

2.5.1 Major Constraints of Soil Acidification

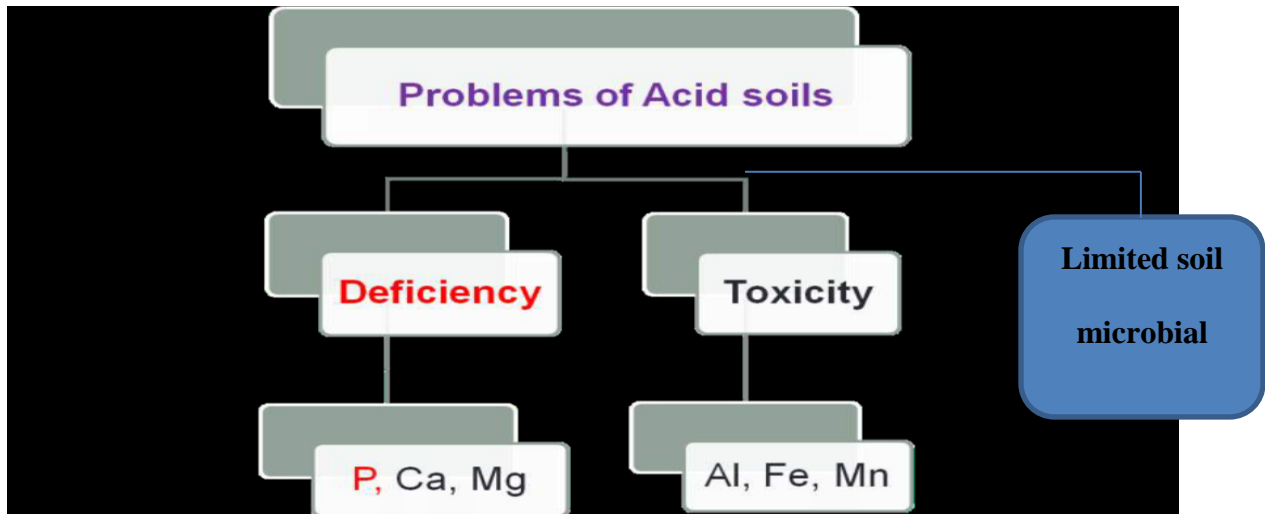


Fig. 2.4 Major Constraints of Soil Acidification (Cowie A. et al., 2001)

2.5.1.1 Aluminum and hydrogen toxicity

A high concentration of aluminum is not desirable for growth of most plants and soil microorganisms except for some few plants species like rice (Howeler and Cadavid, 1976) and tropical legumes (Andrew et al., 1973). Concentration of soil solution Al^{3+} above 1ppm often causes direct toxicity to most important soil factors (Edwards et al., 1981). The problem is particularly severe below pH 5.0 but it may occur as high as pH 5.5 in kaolintic soils (Carvalho et al., 1980). Thus, little or no exchangeable aluminum is present in soils at higher pH values (Reynolds, 1973). The acid nature of soils having exchangeable aluminum is a result of the

hydrolysis of aluminum in the soil solution with the resultant production of hydrogen ions (Sanchez, 1976) ;

- $\text{Al}^{3+} + \text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})^{2+} + \text{H}^+$
- $\text{Al}(\text{OH})^{2+} + \text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})_2^+ + \text{H}^+$
- $\text{Al}(\text{OH})_2^+ + \text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})_3 + \text{H}^+$

The levels of aluminum in the soil solution also depend on the soil organic matter and salt content (Horst et al., 1983). In organic soils, Al is complexed very strongly by the organic fraction, therefore very little Al^{3+} present (Horst et al., 1983). Evidence as reported by Alexander (1980), suggests that aluminum toxicity limits the microbial breakdown of organic matter in some strongly acid soils.

2.5.1.2 Manganese toxicity

Manganese is very soluble at pH values lower than 5.5 and at that pH and lower; manganese is in toxic levels (Reynolds, 1973). Manganese toxicity is probably the second most important growth limiting factor after aluminum toxicity in acid soils (Foy, 1973). Manganese toxicity can also occur at higher soil pH values in poorly drained or compacted soil where reducing conditions favour the production of divalent manganese (Foy, 1984). Highly weathered soils that have large sesquioxide clay content often contain large amounts of Mn (Foy, 1984). According to Kamura

and Nishitani, (1977) and Bromfield, (1979), the availability of manganese in soils is closely related to the activities of microorganisms that can oxidize the soluble and toxic divalent manganese to the tetravalent non-toxic form. Toxic levels of manganese in the soil diversely reduces growth and development of soil organisms especially microorganisms and plants (Brammer, 1962).

2.5.1.3 Calcium deficiency

Many acidic soils have very low amounts of exchangeable Ca and a low Ca saturation of the CEC (Tisdale and Nelson, 1975). The higher rainfall common to acid soils often leaches essential mineral elements such as Ca from the soil and makes them deficient in such soils (Tisdale and Nelson, 1975). Calcium deficiency in acidic soils causes a whole lot of disorders in plants grown on them and more importantly, limits the growth and development of soil microorganisms (Tisdale and Nelson, 1975). A calcium saturation of approximately 25 to 30% appears to be adequate for supplying the Ca requirements of most plants and microbes (Foy, 1984).

2.5.1.4 Magnesium deficiency

The effect of the soil pH value on Mg availability is probably due to an antagonism of both Al and H on Mg uptake, particularly when the percent Al saturation is high (Marshall, 1970).

Neutralization of Al and H is necessary for optimum Mg availability. Since Mg is an essential mineral, its deficiency in the soil limits the growth and development of plants and microorganisms in it (Marshall, 1970). This is basically because these organisms need such minerals to synthesize their cytoplasmic or protoplasmic constituents (Marshall, 1970).

2.5.1.5 Phosphorus deficiency

Phosphorus like nitrogen, potassium, calcium, magnesium and sulphur is classed as a macro or major nutrient. Acid soils are notorious for having low levels of phosphorus in both the surface and sub soil (Kamprath, 1973; Pearson, 1975). Of the many essential functions that P has in the soil, its role in energy storage and transfer is singly the most important (Tisdale and Nelson, 1975; Roberts, 1976). Phosphate compounds act as 'energy currency' within organisms (Tisdale and Nelson, 1975; Roberts, 1976). Energy obtained from photosynthesis and metabolism of carbohydrates is stored in phosphate compounds for subsequent use in growth and reproductive processes (Kamprath, 1973; Pearson, 1975).

In acid soils, the retention of phosphates results largely from the reaction with iron (Fe) and aluminum (Al) and their oxides and hydroxides (Sanchez, 1976; Tisdale and Nelson, 1990). It has been confirmed by Birch (1951) that, soil phosphorus availability is depressed at high

acidity. Hence, acidic soils have low levels of microbial and plants activities (Tisdale and Nelson,1990).

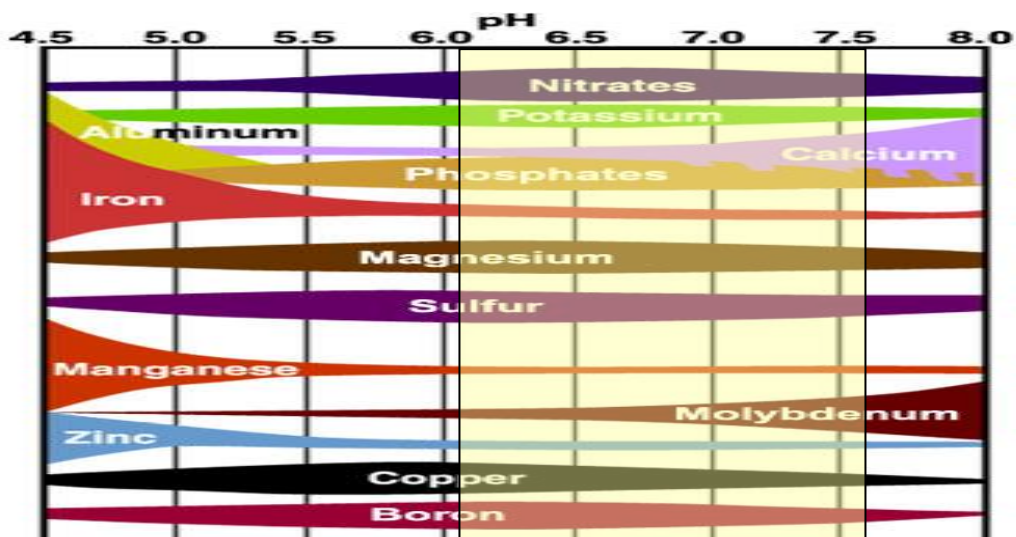


Fig. 2.5 Most Nutrients are Highest and Most Toxins are Lower at pH 5.5-7.0
(Source;<http://www.traylorchemical.com/images/faqs/phchart.jpg>)

2.5.2 Types (pools) of soil acidity

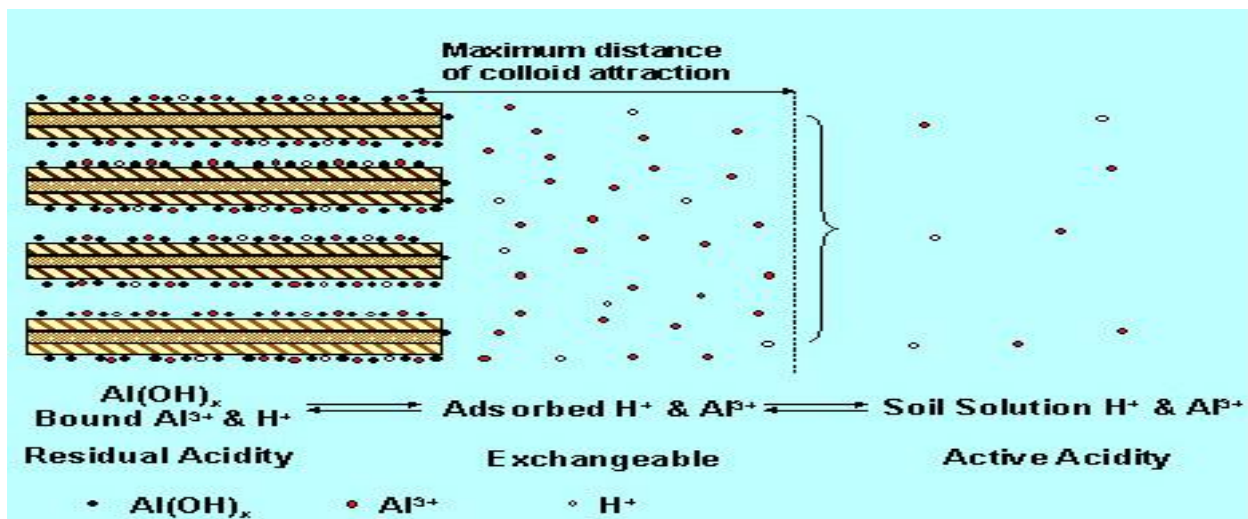


Fig. 2.6 Pools of soil acidity (Brady and Weil, 2004).

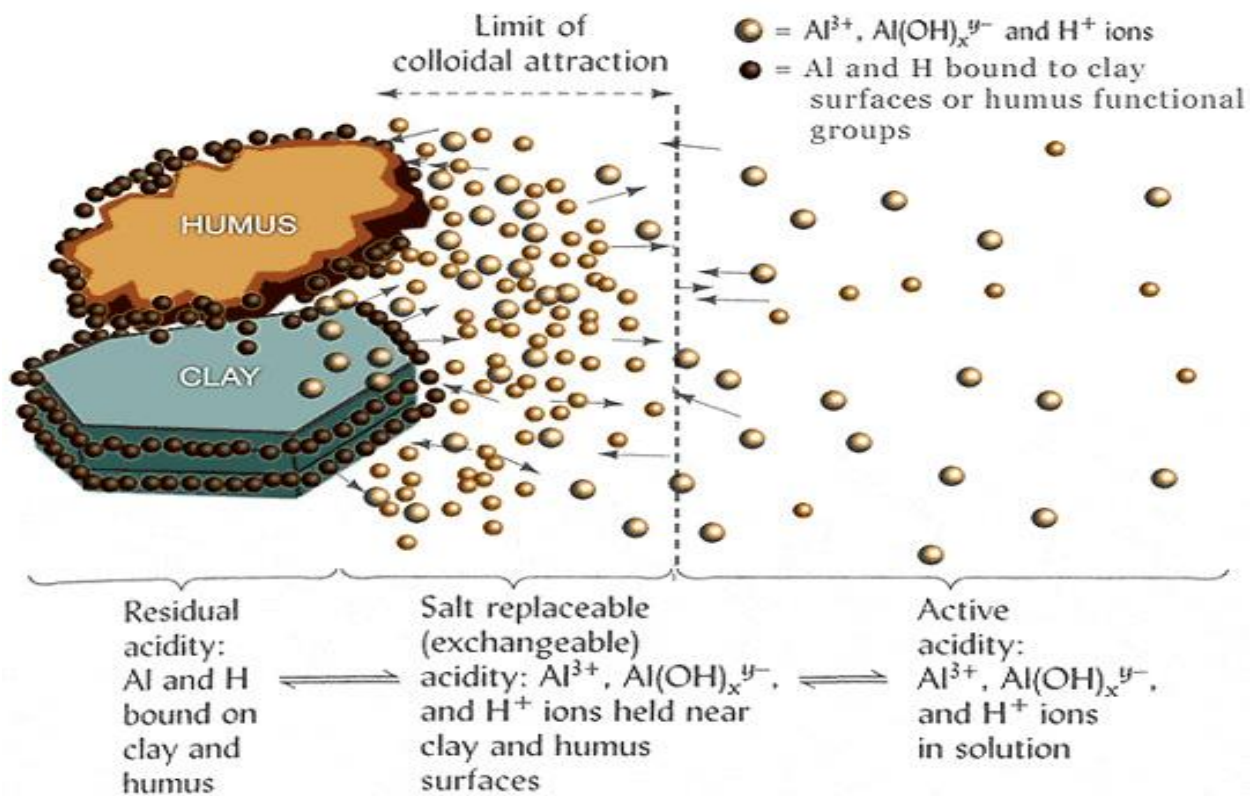


Fig. 2.7 Types of soil acidity (Brady and Weil, 2004).

2.5.2.1 Active acidity

This is the H^+ concentration in soil solution and it determines the pH of the soil. This is the type of soil acidity upon which plant growth reacts (Brady and Weil, 1996). Together with exchangeable and residual activity, they make up the total acidity of the soil. Active acidity is relatively very small compared to the exchangeable or reserve acidity. Most soil chemists indicate that the exchangeable and residual acidity together are 1000 times greater than the active acidity in acid sandy soils and it may be even 50,000 to 100,000 times larger in acid clayey soils (Brady and Weil, 1996). However, active acidity is very important to the plant and soil microbes since it determines the solubility of many elements and nutrients in the soil solution.

2.5.2.2 Exchangeable or salt replaceable acidity

This is due to the exchangeable acidic cations Al^{3+} and H^+ on the colloidal surface (Brady and Weil, 1996). On addition of a salt like KCl to the soil, the cations Al^{3+} and H^+ at the exchange site are released into soil solution by exchanging with K^+ . The Al released undergoes hydrolysis to produce more H^+ into soil solution, which lowers the soil pH further (Brady and Weil, 1996).

2.5.2.3 Residual acidity

This is the soil acidity that remains after active and exchangeable acidity have been neutralized (Brady and Weil, 1996; Moore et al., 1998). Residual acidity is associated with aluminum hydroxy ions and with Al^{3+} and H^+ ions that are bound in non-exchangeable forms by organic matter and silicate clays (Brady and Weil, 1996). This form of acidity is greater than either active or exchangeable acidity (Moore et al., 1998).

2.5.3 Causes of Soil Acidity

2.5.3.1 Weathering and leaching

Usually, the parent rocks or materials from which soils are formed are the main provenance for both essential and non-essential plant nutrient elements (Bolland et al., 2004). If the parent material is acidic, there will be acidifying effect and alkaline effect on the other hand will manifest, if the parent material is basic (Moore et al., 1998). During weathering both acidic and basic cations are released into the soil (Moore et al., 1998). The influx of these nutrient elements to the soil is, however, annulled through leaching, where most of the basic cations (Ca^{2+} , Mg^{2+} , Na^+ and K^+) that would counteract the acidity effects of the acidic cations (mainly H^+ and Al^{3+}) are removed from the soil, resulting in the preponderance of these acidic cations which give rise

to soil acidity (Moore et al., 1998). This process is very effective in areas where there is high temperature and where precipitation exceeds evaporation and plant transpiration to induce weathering and leaching (Bolland et al., 2004). By reason of Ghana's location on the globe, most of the soils particularly those in the south-western parts are naturally highly weathered and leached rendering them acidic and less fertile, hence the predominance of Oxisols and Ultisols (Dwomo and Dedzoe, 2010).

2.5.3.2 Organic matter decomposition

In the course of their lives, plants and animals take up nutrients in various forms. When these plants and animals expire, the process of decomposition supervenes, whereby these organic tissues are broken down into humus with a concomitant release of many sundry chemicals (Moore et al., 1998). As a corollary of this eternal natural cycle, acids are either formed or consumed (Moore et al., 1998). Generally, organic matter contains reactive substances such as carboxylic and phenolic groups which behave as weak acids. The dissociation of these groups results in the release of H^+ ions which are responsible for acidity (Seatz and Peterson, 1964). Decaying organic matter also produces CO_2 which reacts with H_2O to form weak carbonic acids (Moore et al., 1998). The conversion of organic N to mineral N through nitrification can also increase soil acidity (Moore et al., 1998). Brady (2001), however, indicated that the contribution to acid soil development by decaying organic matter is generally very small and it is only the accumulated effects that might ever be measured.

2.5.3.3 Acid rain

Rainfall is characteristically acidic and it is made so by oxides of sulphur and nitrogen fed into the atmosphere from the internal combustion engines, the burning of coal and agricultural activities (Moore et al., 1998). The acidity of the rain which can have a pH value between 4 and 4.5 (Brady, 2001) is imparted to the soil through precipitation (Coy et al., 1990). The quantity of H_2SO_4 and HNO_3 that is brought to the earth in acid precipitation globally is enormous but the amount falling on a given hectare in a year which can induce any significant changes in pH is somewhat low, particularly in the less industrialized countries like Ghana. With time however, the cumulative effects of acid precipitation can influence both soils and plants that grow in them (Brady, 2001).

2.5.3.4 Crop production and removal

Obviously the main aim of any agricultural production system is to produce saleable products. However, this often saddled with some problems such as soil acidification. The respiration of plants and micro-organism in the soil is necessary for their survival. However, this result in large quantities of carbonic acids with acidulating effects being produced (Moore et al., 1998). According to Black (1968) however, this effect is relatively small because most of the carbonic acids decompose and are lost to the atmosphere as CO_2 . Also, the uptake of nutrients by plants results in partitioning of acidity into the soil and alkalinity into the plant (Tang and Rengel, 2003). Crops absorb basic cations such as Ca^{2+} , Mg^{2+} and K^+ and also NH_4^+ from the soil solution for their nutrition (Bolland et al., 2004). Consequently, there is a release of H^+ from the plants in order for electrical balance to be established, particularly when plants absorb nutrient in the form of NH_4^+ (Moore et al., 1998). The release of these H^+ ions has an acidulating effect in the soil (Tisdale and Nelson, 1975). When these crops are subsequently harvested from the field,

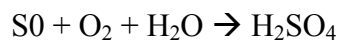
or burnt and washed away via surface run-off, these basic cations which are responsible for counteracting the acidity developed by other processes are lost and the net effect is increased acidity (Chen and Barber, 1990). The amount of these nutrients removed by cropping, however, depends on the type of crop grown, the part of crop harvested, and stage of growth at harvest (Moore et al., 1998). The leaves and stem portions of the plant contain larger amounts of these basic nutrients than the grains. Therefore, high-yielding forages such as Bermuda grass, hay and alfalfa have a larger effect on soil acidity than grains when harvested (Bolland et al., 2004).

2.5.3.5 Application of acid forming fertilizers

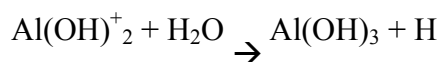
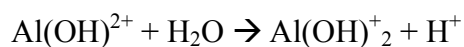
Generally, as a result of high temperatures and other natural phenomena such as precipitation and incessant leaching of nutrients, there is deterioration of the soils' inherent capacity to support crop production (FAO, 2004). The continuous use of agricultural lands without appropriate management measures to ensure their rejuvenation has also given rise to many infertility problems (FAO, 2004). The levels of organic matter and most of the essential plant nutrients, particularly nitrogen and available phosphorus are low in most Ghanaian soils (Dwomo and Dedzoe, 2010). By reason of this intrinsic poverty of most of the soils, most farmers depend on external sources of fertilization to replenish the soil of its fertility (FAO, 2004). The reliance on chemical fertilizers far surpasses that of organic amendments for soil fertility replenishment in Ghana (Dwomo and Dedzoe, 2010). Chemical fertilizers mostly used in Ghana include ammonium sulphate (AS), muriate of potash (MOP), urea, single and trisuperphosphate (SSP and TSP), etc. (FAO, 2004). There is no denying the fact that significant successes in terms of crop yields have been recorded in so many places via the use of such chemical fertilizers. Albeit these fertilizers are vital for high yields, their use is saddled with many ramifications such as soil acidification (Moore et al., 1998).

2.5.3.6 Oxidation of elemental sulphur

Oxidation of elemental sulfur produces sulfuric acid which dissociates easily



2.5.3.7 Hydrolysis of Al^{3+}



2.5.4 Effects of soil acidity on crop production

Soil acidity has been shown to have detrimental effects on plant growth by affecting plant nutrient availability and plant development (Boland et al., 2004). Two fundamental factors are associated with acid soil infertility; nutrient deficiencies such as P, Ca and Mg, and the presence of phytotoxic substances such as soluble Al and Mn (FAO, 2004). In the soil, plants absorb nutrients mainly in soluble forms. Under acidic conditions some of the vital nutrients such as P, Ca and Mg are made unavailable in the soil solution for plant uptake due to the abundance of elements such as Al and Mn. As mentioned in the preceding chapters, soil acidity is usually associated with H^+ and Al^{3+} (Marshall, 1970). Surprisingly, however, not so much deleterious

effects on plant growth as a result of increased activity of H^+ ions under acidic conditions have been documented compared to that of Al^{3+} (Black, 1968; Rao et al, 1993).

Soluble aluminum in the soil causes most of the problems associated with acidic soils (Black, 1968; Rao et al, 1993). The principal effects on plant growth from soluble aluminum in the soil solution is increased acidity via Al hydrolysis and reduced root proliferation and function, which is generally observed in the roots on the field as stunted, club shaped (Carvalho et al., 1980). This reduces the ability of plants to extract water and other nutrients from the soil. Several studies have also shown that when Al is in abundance, P is fixed as aluminum phosphate which is insoluble, hence making it unavailable for plant uptake (Fageria and Baligar, 2003). This is one of the main problems farmers in Ghana are faced with concerning acid soils. With the exception of molybdenum, the availability of micro-nutrients generally increases as soil acidity increases (Carvalho et al., 1980). Since micro-nutrients are needed by the plants in only minute quantities, plant toxicity and other detrimental effects occur with excess amounts (Fageria and Baligar, 2003).

2.5.5 Management of soil acidity

The preceding sections have shown that soil acidity is an ongoing natural process which can be augmented via anthropogenic activities (Brady and Weil, 1996). However, with appropriate management practices, soil acidity and its deleterious effects can be mitigated or prevented. Studies have shown that a wide array of possibilities exist for mitigating the effects of soil acidity (Brady and Weil, 1996). Some of the methods commonly used include liming, the use of acid tolerant crops (Sanchez, 1977), organic material addition and agroforestry (Ofori, 1971; Lathwell, 1979; Dennis and Issaka, 1986; Anane-Sekye, 1997).

2.5.5.1 Liming

Several liming materials such as crushed limestone (CaCO_3), dolomitic lime (CaMgCO_3), slaked lime (Ca(OH)_2), quick lime (CaO) etc., can be used to reduce soil acidity (Brady and Weil, 1996). They can be used either singly or in combined form. Studies have shown that apart from reducing the acidity of the soil by counteracting the effects of excess H^+ and Al^{3+} ions (Fageria and Baligar, 2005), liming also has several other benefits including, its ability to reduce the toxicity effects of some micro elements by lowering their concentrations while increasing the availability of plant nutrients such as Ca, P, Mo, and Mg in the soil (Naidu et al., 1990) and reducing the solubility and leaching of heavy metals (Lindsay, 1979; Sauvé et al., 2000). Application of lime is, however, affected by factors such as quality of the liming material, soil texture, soil fertility, crop rotation, conservation tillage, crop species and the use of organic manure (Fageria and Baligar, 2008). Lathwell (1979) conducted an experiment on improving the fertility of some acidic soils and the study revealed a concomitant increase in pH and Ca+Mg contents and a decrease in Al concentration of the soil. This altogether resulted in a mammoth increase in crop yield. According to Buri et al., (2005), a similar experiment was conducted and 1.0 t/ha and 2 t/ha lime were applied, 72% and 48% increases in yield respectively were recorded over no lime treatments.

2.5.5.2 Application of organic materials

For simplicity, the use of organic materials herein represents all forms of organic materials from both plants and animal origins. It has long been established that apart from improving the fertility, structure and some biological properties of the soil, organic materials have the capacity to reduce soil acidity and Al saturation (Brady and Weil, 1996). Though still enigmatic, a

number of possible mechanisms have been suggested by several workers in their quest to elucidate how this occurs.

Plant materials generally contain excess cations, and the balance between cations and anions is maintained by synthesis of organic acid anions such as oxalate, citrate and malate (DeWit et al., 1963). During microbial decomposition of plant materials, these organic acid anions are decarboxylated (Yan et al., 1996; Tang et al., 1999). Noble et al. (1996) therefore posited that the decarboxylation of organic acid anions in the process of plant residue decomposition requires a proton for this reaction to be complete. By consuming a proton, the hydroxyl ions increase and this results in an increase in alkalinity. Thus the greater the content of cations in plant material, the greater will be this effect. Plant materials such as legume residues (soybean, red clover, and acacia) were observed to have a substantially higher Ca, Mg, and total basic cation contents than the non-legumes (maize and sorghum), and consequently, ash alkalinity values were also higher (Bessho and Bell, 1992; Pocknee and Sumner, 1997; Wong et al., 1998).

Another mechanism was adduced by Wong et al. (1998) who indicated that functional groups associated with organic materials can also consume protons and in the process, increase the alkalinity of the soil. To this extent, all the mechanisms presented are related to organic acids. A study was conducted by Safo et al (1997) to assess the effects of Palm bunch ash (PBA) on growth of cowpea and the characteristics of a Ghanaian soil. According to the authors, the palm bunch ash is very hygroscopic, extremely basic (pH 10-12) and contains 25-34% K, 3.6-5.5% Ca, 1.6-3.6% Mg, 0.5-1.7% P, and approximately 0.1% N. Their study revealed an incredible increase in pH of about 2.5 units (4.7 to 7.2) and concluded that PBA applied at 4.0 mg/kg of soil may be as effective as limestone in ameliorating acidity in poorly buffered soils.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Soil sample collection

The soil sampling site is located in the Ankasa Wildlife Protected Area ((05°12.922'N, 002°39.031'W, Alt-209 ft) in Western Region, which is believed to an ancient rainforest with the highest biodiversity in Ghana (Castaldi et al., 2012). The forest has an area of about 500 km² and in 1976 became a wildlife protected area to preserve the forests from cutting, slashing and burning, and conversion to agro-ecosystems (Castaldi et al., 2012). The mean annual temperature is about 25°C and the mean annual precipitation is between 1500 to 2000mm, depending on the year, mainly concentrated from March to mid-July and from September to November, with a relative humidity ranging from 75% to 90 % (Castaldi et al., 2012). The soils are deeply weathered and highly acidic and are classified as Oxisols (Plinthic Acrudox) (Brammer, 1962). The soils developed on coherent biotite-rich granites of the Cape Coast complex, form the Ankasa association, consisting of “Abenia” series, the most widespread series on top of the hills, which alternates to the “Ankasa” series, along the slopes (Castaldi et al., 2012). The Ankasa series was sampled for the present study.

The Ankasa series (Oxisol- according to the USDA classification system) was sampled within the plough depth (0- 20 cm), conveyed to the laboratory and air-dried. Un-decomposed plant materials such as roots, sticks and other debris were removed and the remaining soil gently crushed and passed through a 2 mm sieve. The sieved soil sample was thoroughly mixed and stored in sacks for laboratory analyses and experiments.

3.2 Laboratory Analyses

3.2.1 Particle size distribution

Soil particle size distribution was determined by the modified Bouyoucos Hydrometer method described by Day (1965). Forty (40) g of soil was taken into a beaker followed by the addition of 60 mL of 6% H₂O₂ in order to destroy the organic matter in the soil. One hundred (100) mL of 5% calgon (sodium hexametaphosphate) solution was added. The suspension was allowed to stand for approximately 10 minutes and stirred with a mechanical stirrer for 30 minutes.

The suspension was then transferred into a graduated sedimentation cylinder using distilled water from a wash bottle and made up to the 1 liter mark with distilled water. The temperature of the suspension was recorded after equilibration. The content of the cylinder was then mixed thoroughly with the help of a plunger and hydrometer readings taken 5 minutes and 5 hours thereafter. The suspension was then poured into a 47- μ m sieve and the particles retained on the sieve washed with water and dried in an oven at 105 °C for 24 hours. The dried samples were then weighed to represent the sand fraction. Blank hydrometer readings of sodium hexametaphosphate solution at 5 minutes and 5 hours were taken. The particle size distribution was then determined using the formulae below;

$$(Clay + Silt)\% = \frac{5 \text{ minute hydrometer reading}}{\text{Sample mass}} \times 100 \quad (1)$$

$$(Clay)\% = \frac{5 \text{ hour hydrometer reading}}{\text{Sample mass}} \times 100 \quad (2)$$

$$Silt (\%) = (1) - (2) \quad (3)$$

$$(Sand)\% = \frac{\text{Oven dry mass (g) of particle retained on 47 } \mu\text{m sieve}}{\text{Sample mass (g)}} \quad (4)$$

3.2.2 Bulk density

Bulk density was determined using the core sample method. Core samples were taken from sampling location to represent the entire area. The soil surface was cleared and a core sampler was gently inserted into the soil with the help of a mallet. The soil surrounding the core sampler was then gently removed so that the sampler could be removed from the soil without disturbance. The ends of the sampler were leveled with a knife edge and covered. The sampled soils were then taken to the laboratory for bulk density determination.

In the laboratory, the content of the sampler was emptied into a clean moisture can with known weight (W_1). The moisture can together with its contents were oven dried for 72 hours at 105°C and thereafter, the weight was taken (W_2). Bulk density was calculated using the formula by Blake and Hartge, (1986).

$$\rho_b(\text{kg/m}^3) = \frac{M}{(\pi d^2/4)h} \quad [5]$$

Where

ρ_b = Bulk density of soil

M = mass of soil = $W_2 - W_1$

W_2 = Weight in grams taken after oven drying the moisture can and its contents.

W_1 = Weight in grams of empty moisture can.

$\Pi d^2/4 =$ area of core

d= diameter of core

h= height of core

$\Pi =$ constant=3.142

$(\Pi d^2/4) h =$ volume of core= volume of soil

3.2.3 Soil pH

Soil pH was determined at a soil to water ratio of 1:1 using an MV 88 Pracitronic pH glass electrometer. Ten (10) g of soil sample was weighed into a 50 mL beaker and 10 mL of distilled water was added (for water determination) and 10mL of 1N KCl to another 10g soil sample (for KCl determination). The solid-liquid suspensions were then stirred for 30 minutes. The suspensions were then allowed to equilibrate to room temperature. Using buffer solutions of pH 4.0 and 7.0, the pH electrometer was standardized. The standardized electrode was then inserted into the supernatant of the suspension to measure the pH of the soil sample.

3.2.4 Organic carbon

The wet combustion method of Walkley and Black (1934) as modified by Allison (1965) was used to determine the organic carbon content of the soil. Ten (10) ml of 0.167 M potassium dichromate ($K_2Cr_2O_7$) solution and 20 mL of concentrated sulphuric acid (H_2SO_4) were added to a 0.5 g soil which had been passed through a 0.5 mm sieve in an Erlenmeyer flask. The flask was then swirled to ensure full contact of the soil with the solution after which it was allowed to stand

for 30 minutes. The unreduced $K_2Cr_2O_7$ remaining in solution after the oxidation of the oxidizable organic material in the soil sample was titrated with 0.2 M ferrous ammonium sulphate solution after adding 10 mLs of orthophosphoric acid and 2 mLs of barium diphenylamine sulphonate indicator from a dirty brown color to a bright green end point. Standardization of the $K_2Cr_2O_7$ with the ferrous ammonium sulphate was done and the amount of organic carbon calculated by subtracting the number of moles of unreduced $K_2Cr_2O_7$ from the number of moles of $K_2Cr_2O_7$ present in the standardized titration.

The percent organic carbon was calculated as:

$$\% C = \frac{0.3 \times (10 - XN) \times 1.33}{W} \times 100 \quad [6]$$

Where % C = Percent organic carbon

X = Titre value (mL)

N = Normality of $Fe(NH_4)_2(SO_4)_2$

W = Weight of soil sample

3.2.5 Total nitrogen

The Kjeldahl method (Hesse, 1971) was used to determine total nitrogen. Two (2) g of air dried soil were weighed into 250 mL Kjeldahl flasks and selenium catalyst and 5 mL of concentrated sulphuric acid was added. The mixture was then heated on a digestion block until the digest became clear. The digest was then allowed to cool and transferred with distilled water into a 50 mL volumetric flask and made up to volume. A 5 mL aliquot of the digest was taken into a Markham distillation apparatus and 5 mL of 40% NaOH solution added. The liberated ammonia was collected into 5 mL of 2% boric acid to which three drops of methyl red and methylene blue

indicator mixture had been added. The distillate was back titrated against 0.01M HCl to a purplish end point. The amount of total N was then calculated from the number of moles of HCl consumed in the titration reaction.

The percent N was calculated as follows:

$$\% \text{ N} = \frac{0.01 \times \text{titre volume} \times 0.014 \times \text{volume of extract} \times 100}{\text{Sample weight (g)} \times \text{volume of aliquot (mL)}} [7]$$

Where 0.01 = Normality of HCl

0.014 = Milliequivalence of Nitrogen

3.2.6 Available phosphorus

Available phosphorus was determined by Bray and Kurt method (1945). A 4 g soil sample was weighed into an extraction bottle and 40 mL of extractant (0.03 M NH_4F in 0.025 M HCl) was added and shaken for about two minutes on a mechanical shaker. The soil-extractant mixture was filtered through a Whatman No.42 filter paper and a 10 ml aliquot was used to develop the colour using the Murphy and Riley method (1962). Phosphorus in the filtrate was determined using the molybdate-ascorbic acid method. A 10 mL aliquot of the filtrate was put into a 50 mL volumetric flask. The pH was adjusted by adding drops of p-nitro phenol indicator and few drops of 4 M NH_4OH until the solution turned yellow. The 2 mL of reagent B (1.056 g of ascorbic acid in 200 mL of reagent A) was added. Reagent A was made by dissolving 12 g of ammonium molybdate in 250 mL of distilled water plus 0.2998 g of antimony potassium tartarate to 1000 mL of 5 M H_2SO_4 , mixed thoroughly and made up to 2 liter with distilled water. A blank was prepared using the aforementioned procedure but without the soil. The spectrophotometer was calibrated using standard phosphorus solution by pipetting 5 mL of the standard phosphorus

solution into a 50 mL volumetric flask, followed by colour development as outlined above. The intensity of colour at a wavelength of 712 nm was measured on the spectrophotometer and then recorded. The P was calculated using the relationship below:

$$P(\text{mg/kg}) = \frac{(\text{spectrophotometer reading} - \text{blank}) \times (\text{volume of extract})}{\text{volume of aliquot} \times \text{weight of soil}} \quad (8)$$

3.2.7 Exchangeable cations

A 5 g soil was weighed into an extraction bottle and 50 mL solution of 1 M ammonium acetate (NH₄OAc, pH 7) was added. The bottle was shaken in a mechanical shaker for one hour and the content filtered through a Whatman No. 42 filter paper into clean empty bottles. Exchangeable calcium and magnesium in the extract were determined using the Atomic Absorption Spectrometer (AAS). Exchangeable Na and K were determined by flame photometer.

3.2.7.1 Potassium (K) Determination

The flame photometer was standardized such that 10 mg/kg of K gave 100 full scale deflections. The flame photometer after standardization was used to determine the concentration of potassium in 10 ml aliquot. The result was used in the calculation of the amount of potassium present in the soil as shown in the formula below.

$$\text{Exchangeable K (cmol/kg soil)} = \frac{R \times V \times 100}{\text{Weight of soil} \times 39.1} \quad \text{---[9]}$$

Where R= Flame Photometer reading for K (ppm)

39.1=Molecular weight of Potassium

V= Volume of extract (100 ml)

3.2.7.2 Sodium (Na) Determination

The flame photometer was standardized in a way that 10 mg/kg of Na gave 100 full scale deflections. After the standardization of the photometer, the concentration of sodium in 10mL aliquot was determined. The result was then used in the calculation of the amount of sodium (Na) present in the soil as shown by the formula below.

$$\text{Exchangeable Na (cmol/kg soil)} = \frac{R \times V \times 100}{\text{Weight of soil} \times 23} \text{ --- [10]}$$

Where R= Flame photometer reading for Sodium (ppm)

V= Volume of extract (100 ml)

23=Molecular weight of Sodium

3.2.7.3 Calcium (Ca) Determination

To a 10 ml aliquot of the sample solution, 10 ml of 10% KOH and 1ml triethanolamine (TEA) were added. Three drops of 1M KCN solution and a few crystals of cal-red indicator were then added after which the mixture was titrated with 0.02M EDTA solution from red to blue end point. The titre value was used in the calculation of calcium as shown below.

$$\text{Ca (cmol/kg)} = \frac{\text{Titre value} \times \text{N} \times \text{Vol.of extract} \times 100 \text{ (meq/100 g)}}{\text{Vol. of aliquot} \times \text{Weight of soil}} \dots\dots\dots(11)$$

Where N = Molarity of EDTA

3.2.7.4 Magnesium (Mg) Determination

To a 10 ml aliquot of the sample solution, 5 ml of ammonium chloride – ammonium hydroxide buffer solution was added followed by 1ml of triethanolamine. Three drops of 1M KCN solution and a few drops of Eriochrome black T solutions were added after which the mixture was titrated with 0.02M EDTA solution from red to blue end point. The end point titre value determines the amount of calcium and magnesium in the solution. The titre value of magnesium was then determined by subtracting the value obtained for calcium above from the new titre value obtained. The titre value of magnesium was then used for the calculation of the concentration of magnesium (Mg) as shown below.

$$\text{Mg (cmol/kg)} = \frac{\text{Titre value} \times \text{N} \times \text{Vol.of extract} \times 100 \text{ (meq/100 g)}}{\text{Vol. of aliquot} \times \text{Weight of soil}} \dots\dots\dots(12)$$

Where N = Molarity of EDTA

3.2.8 Exchangeable acidity (H⁺ and Al³⁺).

Ten (10) g of soil was weighed into a 100 mL extraction bottle and 50 mL of 1MKCl solution was added. The bottle and its content were placed on a mechanical shaker and shaken for 30 minutes. The soil suspension was then filtered through No. 31 Whatman filter paper into an empty clean bottle. Twenty five (25) mL aliquot was pipetted into a 100 mL conical flask and 2-

3 drops of phenolphthalein indicator was added for titration to a permanent pink end point against 0.01M NaOH. The titre value was recorded as titre for both H^+ and Al^{3+} . Ten (10) mL of NaF was added to the solution at the endpoint and back titrated against 0.01M HCl until a colourless end point was reached. The titre was recorded as the titre for Al^{3+} .

3.2.9 Effective Cation Exchange Capacity (ECEC)

The Effective Cation Exchange Capacity is equal to the sum of the exchangeable Ca^{2+} , Mg^{2+} , Na^+ , K^+ , H^+ , and Al^{3+} . (i.e. $Ca^{2+} + Mg^{2+} + Na^+ + K^+ + H^+ + Al^{3+}$).

3.3 Biochar production

Rice straw and saw dust were collected from the Soil and Irrigation Research Centre of the University of Ghana at Kpong and His Majesty Carpentry Shop at Kumasi, respectively. The biochar was produced in a kiln at Soil Research Institute (SRI, Kumasi) at 500°C pyrolysis temperature based on the recommendation of Lehmann et al. (2003). After the pyrolysis process, the biochar was grounded to small granules and passed through 2mm sieve in order to have the same particle size as that of the soil.

3.3.1 Biochar pH

A biochar to water ratio of 1:5 was prepared (appropriate for organic materials because of their ability to absorb more water) and pH determined as described above using the MV 88 Pracitronic pH glass electrometer.

3.3.2 Ca, Mg, P, Na and K of biochar

A 0.5g of the biochar sample was put into a crucible and then placed into a furnace for at least 1 hour at a temperature of 600°C. The ash was later digested with 10mL of nitric acid, accompanied by heating to speed up the rate of the reaction. It was then transferred into a 100mL flask and topped up with distilled water to the 100mL mark. 50mL of the solution was taken to determine Ca and Mg content on the atomic absorption spectrometer.

Ten (10) mL of ammonium metavanadate plus 10mL of ammonium molybdate were added to another 50mL of the solution and topped up with distilled water to the 100mL mark. Samples were taken from this and then read for P on the spectrophotometer at wavelength 470nm and K and Na were determined on the flame photometer.

Table 3.1 Some chemical properties of the biochar used

TREATMENT	RICE STRAW BIOCHAR	SAW DUST BIOCHAR
pH (1:5)	10.5	7.50
% Ash content	4.00	2.00
% Carbon	49.0	48.0
% Nitrogen	0.16	0.20
C:N Ratio	306.3	240.0
% P	0.26	0.03
% K	0.97	0.21
% Ca	0.74	0.67
% Mg	0.44	0.15
% Na	0.18	0.14

3.3.3 Mineral composition of biochar

The mineral composition of the two biochars was determined by the X-Ray diffraction method. The diffractogram of the two biochars are shown in Appendix 1 and 2. The analysis showed that Rice straw biochar contained large quantities of silica (SiO_2), calcite (CaCO_3), calcium diffeerate oxide (CaFe_2O_4), potassium chloride (KCl), siloxene (H_2OSi_2) and periclase (MgO). Saw dust biochar contained large quantities of silica (SiO_2), calcite (CaCO_3), Iron oxide (FeO) and graphite, as well as minute quantities of magnesium oxide (MgO), magnesium silicate (Mg_2SiO_4) and iron magnesium oxide (FeMgO_4).

3.4 Experiment 1 (Degradation of oil in biochar amended soils)

Two (2) kg of the sieved soil was weighed into an experimental pot and contaminated with diesel oil at 100 mL oil/kg of soil. The contaminated soils were then amended with rice straw (RB) and saw dust (SB) biochar at 0 (control), 50, 100, 150 and 200 g/pot (equivalent to 0, 65, 130, 195 and 260 Mg/ha as described by Chintala et al.(2013), replicated three times and completely randomised. The amended soils were maintained under same moisture content (15%; wt:wt basis). The set up was left to stand under a shade at ambient temperature (mean temperature of 29°C) and samples were taken at an interval of 10 days for the determination of hydrocarbon utilizing bacterial population, heterotrophic bacterial count and quantity of diesel oil degraded.

3.4.1 Amount of diesel oil degraded

Residual diesel oil in the contaminated soils was extracted using a modified method of Abu and Oji (1996). Soil samples were air-dried to constant weight and 5 g were placed conical flasks and

10 mL chloroform was added for extraction. The residual diesel oil was extracted by gently shaking the flasks for 5 min. Each extract was filtered through cotton wool in a funnel and collected in clean glass containers, closed immediately and analyzed for diesel oil content. Quantitative determination of diesel oil extracts was employed as described by Udeme and Antai (1998). A standard curve of absorbance (520 nm) against varying concentrations of diesel oil in chloroform was drawn after taking readings from a spectrophotometer. The oil concentrations were calculated from the standard curves.

3.4.2 Hydrocarbon-utilizing bacterial count

The hydrocarbon utilizing bacteria count was estimated using the modified mineral salts agar medium of Mills *et al.* (1978) and modified vapour phase transfer technique of Okpokwasili and Amancuhkwu (1988). One milliliter of each serial dilutions prepared as above was plated onto the modified mineral salts medium containing 10 g NaCl, 0.42 g MgSO₄.7H₂O, 0.29 g KCl, 0.53 g KH₂PO₄, 0.42 g NH₄NO₃, and 15 g agar in 1L distilled water (adjusted pH= 6.8). The modified vapour transfer technique involves spreading 0.5 mL of diesel (serving as carbon source) onto the mineral salts agar medium after setting and allowing the diesel oil to diffuse into the agar medium for about 1 hour before incubating at room temperature for 5 to 7 days. The number of colonies formed was used to estimate the hydrocarbon utilizing bacterial population.

3.4.3 Heterotrophic bacterial count

The total aerobic heterotrophic (TAH) bacterial population was estimated using the plate count method with nutrient agar as the growth medium. Ten (10) g of soil was sampled, tenfold serial dilution prepared; 1 mL of appropriate dilution was plated onto nutrient agar and incubated at

room temperature for 3 days after which the number of colonies formed was used to estimate the TAH bacterial population.

3.5 Experiment 2 (Biostimulation with mineral fertilizer)

Two (2) kg of the sieved soil was contaminated with diesel oil at 100 mL oil/kg of soil. The contaminated soils were amended with only N, only P, N + P, only biochar (195 Mg/ha), biochar + N, biochar + P, biochar + N + P. Ammonium nitrate was used as source of nitrogen at 60 kg/ha (NH_4NO_3) and single superphosphate as source of phosphorous at 60 kg/ha (P_2O_5). The used biochar was rice straw, ammonium nitrate and single superphosphate were used as the N and P sources, respectively. The treated soils were maintained under same moisture content (15%; wt:wt basis). The set up was left to stand under a shade at ambient temperature (mean temperature of 29°C) and samples were taken at an interval of 10 days for the determination of hydrocarbon utilizing bacterial population and quantity of diesel oil degraded as described above.

3.6 Experiment 3 (Plant culture in remediated soils)

Seeds of cowpea (*Vigna unguiculata*) were obtained from Crop Science Department of University of Ghana. The seeds were 100% viable after a petri dish viability test was performed. The seeds were sterilized in 70% ethanol and sown into the residual treated soils in Experiment 2. This was carried out two days after the end of experiment 2. The seeds were sown at five seeds per pot, thinned to two seeds per pot and percent germination determined 5 days after sowing. The plant culture was carried out in the screen house of the Ecological Laboratory of University of Ghana. Normal growth conditions were ensured and pots were irrigated whenever

needed to keep the soil moisture to field capacity. The seedlings were harvested 6 weeks after sowing, and nodule count, shoot and root dry weights were determined. The shoots and roots were oven dried at 70⁰C for 24 hours.

$$\% \text{ Germination} = \frac{\text{number of seeds germinated} \times 100}{\text{number of seeds sown}} \dots \dots \dots [13]$$

3.7 Statistical analysis

Analysis of variance (ANOVA) was performed for all the variables and parameters, considering all data collected and using the Genstat statistical software package (9th edition). The means were separated by the least significant study (LSD) test, considering a significant level of $p < 0.05$ throughout the study.

CHAPTER FOUR

RESULTS

4.1 Soil characteristics

Some of the physical and chemical properties of the top soil (0 – 20 cm) of Ankasa series used in the present study are shown in Table 4.1. The soil had sand content of 72.92%, 21.08% silt and 6.0% clay, and is therefore sandy loam in texture according to the USDA (2003) system of classification. The soil had a bulk density of 1300 Mg/m³ and a pH of 4.70. The soil had an electrical conductivity of 0.42(dS/m) and an organic carbon content of 1.09 % (1.88 % of organic matter). The soil also had available phosphorus, total nitrogen, total exchangeable bases and ECEC values of 2.0 mg/kg, 0.12 %, 1.28 cmol/kg and 2.83 cmol/kg respectively, and an exchangeable acidity of 1.55 cmol/kg.

4.2 Some Soil Properties After Biochar Amendment

4.2.1 Soil pH

The soil pH after biochar amendment of the contaminated soils is represented in Table 4.2.1. There was significant ($p < 0.05$) increase in soil pH of all the biochar treatments, 20 days after amendment (DAA). At 20 DAA, increasing rate of biochar application resulted in increase in pH however, there was no significant ($p > 0.05$) difference between 150 and 200 g/pot. The highest pH was observed in the soil amended with Rice Straw Biochar at 200g/kg soil (RB₂₀₀). The Rice Straw Biochar (RB) treatments generally raised pH of the soil more than the Saw Dust Biochar (SB) treatments. At 40 DAA there was general decrease in pH for all treatments but the decrement was not significantly ($p > 0.05$) different from that of 20 DAA. Statistically there was no change in soil pH of the control treatment during the experimental period.

Table 4.1 Some physio-chemical properties of the soil used

Soil properties	Values
Clay (%)	6.00
Silt (%)	21.08
Sand (%)	72.92
Soil Texture	Sandy loam
Bulk density (Mg/m ³)	1.3
pH (H ₂ O)	4.7
Electrical conductivity (dS/m)	0.42
OC (%)	1.09
Na (cmol/kg)	0.06
K (cmol/kg)	0.09
Mg (cmol/kg)	0.43
Ca (cmol/kg)	0.7
TEB (cmol/kg)	1.28
Base Saturation (%)	45.23
Exchangeable Acidity (cmol/kg)	1.55
ECEC (cmol/kg)	2.83
Total N (%)	0.12
Available P (mg/kg)	2.0

TEB = Total exchangeable bases, ECEC = Effective cation exchange capacity

Table 4.2 Soil pH after biochar amendment

TREATMENT	DAY 0	20 DAA	40 DAA
CONTROL	4.70a	4.78a	4.80a
RB ₅₀	4.70a	6.18b	6.09b
RB ₁₀₀	4.70a	6.54c	6.49c
RB ₁₅₀	4.70a	6.92d	6.88d
RB ₂₀₀	4.70a	7.28d	7.19d
SB ₅₀	4.70a	5.15e	5.12e
SB ₁₀₀	4.70a	5.61f	5.55f
SB ₁₅₀	4.70a	5.98b	5.90b
SB ₂₀₀	4.70a	6.24b	6.19b

* Means having subscript in common within each column do not have any significant difference at 5% probability.

*RB-Rice straw Biochar and SB-Saw dust Biochar, at either 50,100,150 or 200 g/kg soil.

4.2.2 Organic Carbon (OC), Total Exchangeable Bases (TEB), Base Saturation (BS), Effective Cation Exchange Capacity (ECEC), Total Nitrogen (TN), Available P and Exchangeable acidity

At the end of the experiment the addition of biochar significantly ($p < 0.05$) increased organic carbon content (OC), total exchangeable bases (TEB), base saturation (BS) and effective cation exchangeable capacity (ECEC) when compared to control values (Table 4.3). Each successive increase in the rate of application of biochar resulted in significant ($p < 0.05$) increase in the four properties mentioned above. RB₂₀₀ treatment gave the highest values.

Table 4.3 Some chemical properties of the treated soils at the end of experiment

Treatment/ Property	OC %	TEB cmol/kg	BS %	ECEC cmol/kg	TN %	Av.P mg/kg	Exc.Acidity cmol/kg
CONTROL	1.56	1.11	42.21	2.63	0.11	1.99	1.52
RB ₅₀	3.12	3.13	98.12	3.19	0.09	2.11	0.07
RB ₁₀₀	3.89	4.05	98.54	4.11	0.08	2.12	0.06
RB ₁₅₀	4.36	5.17	99.04	5.22	0.08	2.15	0.05
RB ₂₀₀	4.50	5.67	99.13	5.72	0.07	2.16	0.05
SB ₅₀	3.04	2.78	97.54	2.85	0.10	2.03	0.09
SB ₁₀₀	3.26	3.29	98.21	3.35	0.10	2.03	0.08
SB ₁₅₀	3.68	4.20	98.59	4.26	0.09	2.05	0.08
SB ₂₀₀	3.87	4.64	98.72	4.70	0.08	2.05	0.07
Lsd (5%)	0.160	0.200	0.095	0.017	0.020	0.060	0.027

Results also showed that addition of biochar significantly ($p < 0.05$) decreased the total nitrogen (TN) when compared to the control value, even though there were no significant ($p > 0.05$) differences among the biochar treatments. Even though addition of biochar increased available P at the end of the experiment when compared to the control treatment, significant ($p < 0.05$) differences were only observed for the RB treatments. Besides, there was no significant ($p > 0.05$) difference among the various levels of same biochar. The addition of biochar significantly ($p < 0.05$) decreased exchangeable acidity when compared to the control. The RB₁₅₀ and RB₂₀₀ were significantly ($p < 0.05$) lower than the other biochar treatments.

4.3 Amount of oil degraded

In experiment 1, the results obtained on the amount of diesel oil degraded in the oil contaminated soils amended with biochar at different rates of application are shown in Fig 4.1. There was a significant ($p < 0.05$) increase in cumulative diesel oil degraded as a result of addition of biochar when compared to the control. From 0 to 10 DAA, there was a gradual increase in the amount of oil degraded for all the biochar treatments and between 10 and 30 DAA the increment was sharp. Between 30 and 40 DAA the amount of oil degraded for RB₁₅₀ and RB₂₀₀ plateaued but the other biochar treatments continued increasing. At the end of the experiment, biochar application significantly ($p < 0.05$) increased the amount of oil degraded when compared to the control.

The highest amount of oil degraded was recorded in RB₂₀₀ (0.708g oil/kg soil), followed by RB₁₅₀ (0.698g oil/kg soil) but there was no significant ($p > 0.05$) difference between them. SB₅₀ (0.313g oil/kg soil) recorded the lowest amount of oil degraded among all the biochar treatments but was significantly ($p < 0.05$) higher than that of the control (0.099g oil/kg soil).

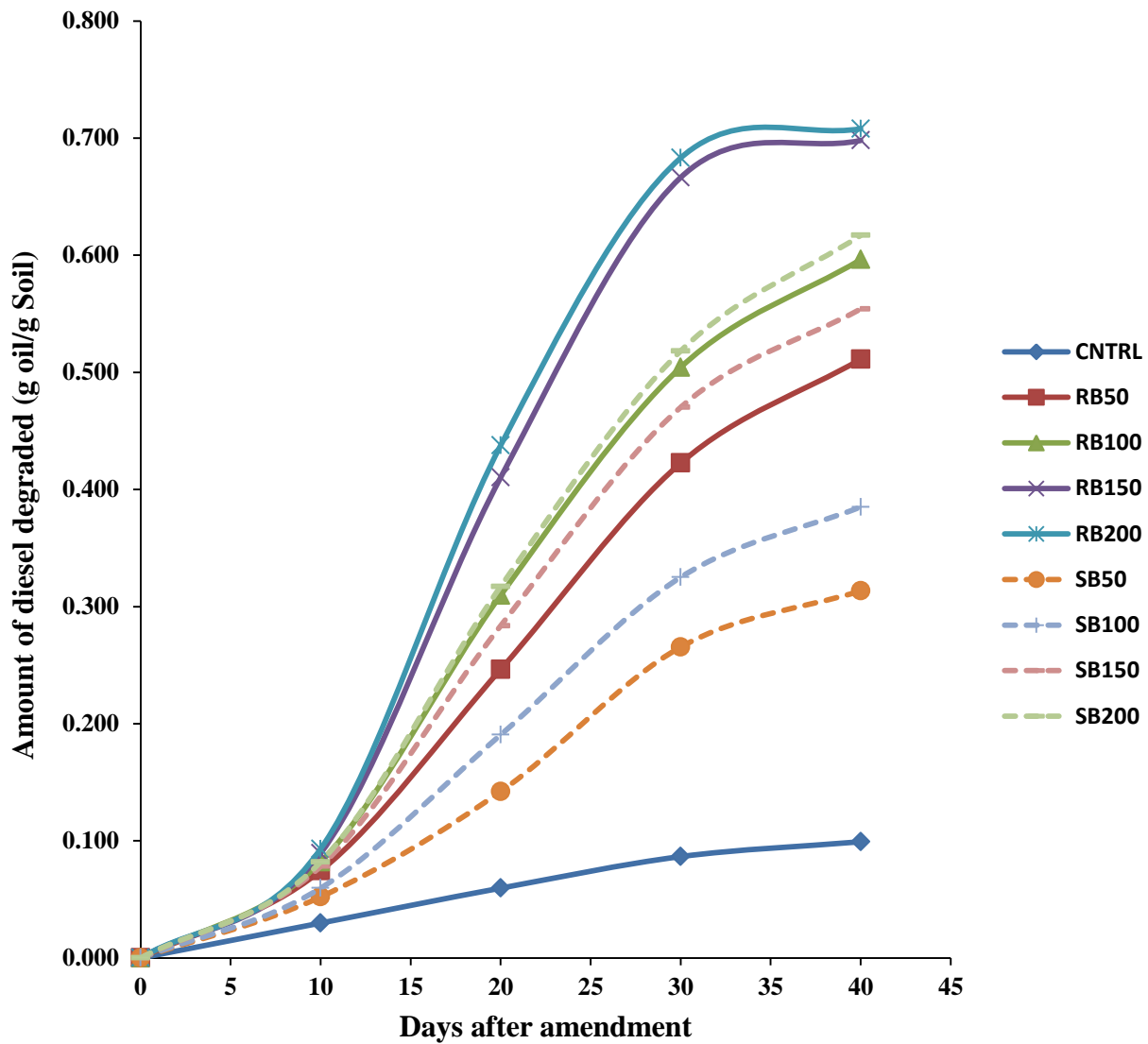


Fig.4.1 A graph of cumulative amount of diesel oil degraded in experiment 1

In experiment 2, the results obtained on the amount of diesel oil degraded in the oil contaminated soils amended with biochar and fertilized with N and/or P is shown in Fig 4.2. There was a significant ($p < 0.05$) increase in cumulative diesel oil degraded as a result of fertilizing biochar treatments with N and/or P when compared to the control and biochar only. There was

no significant ($p > 0.05$) increase in oil degraded when N or P only applied to non-biochar amended soils. However, application of combined N and P significantly ($p < 0.05$) increased the amount of oil degraded when compared to the control. From 0 to 10 DAA, there was a gradual increase in the amount of oil degraded for all the treatments.

A sharp increase in the amount of oil degraded was observed between 10 and 30 DAA for all biochar amended treatments but a steady increase for treatments without biochar. At the end of the experiment, fertilizing biochar treatments with N and/or P significantly ($p < 0.05$) increased the amount of oil degraded when compared to the control. The highest amount of oil degraded was observed in biochar + N + P (0.989g oil/kg soil) followed by biochar + N (0.951g oil/kg soil) but the difference between these treatments was not significant ($p > 0.05$). The lowest amount of oil degraded was observed in the P only treatment (0.123g oil/kg soil) and was not significantly ($p > 0.05$) different from the control.

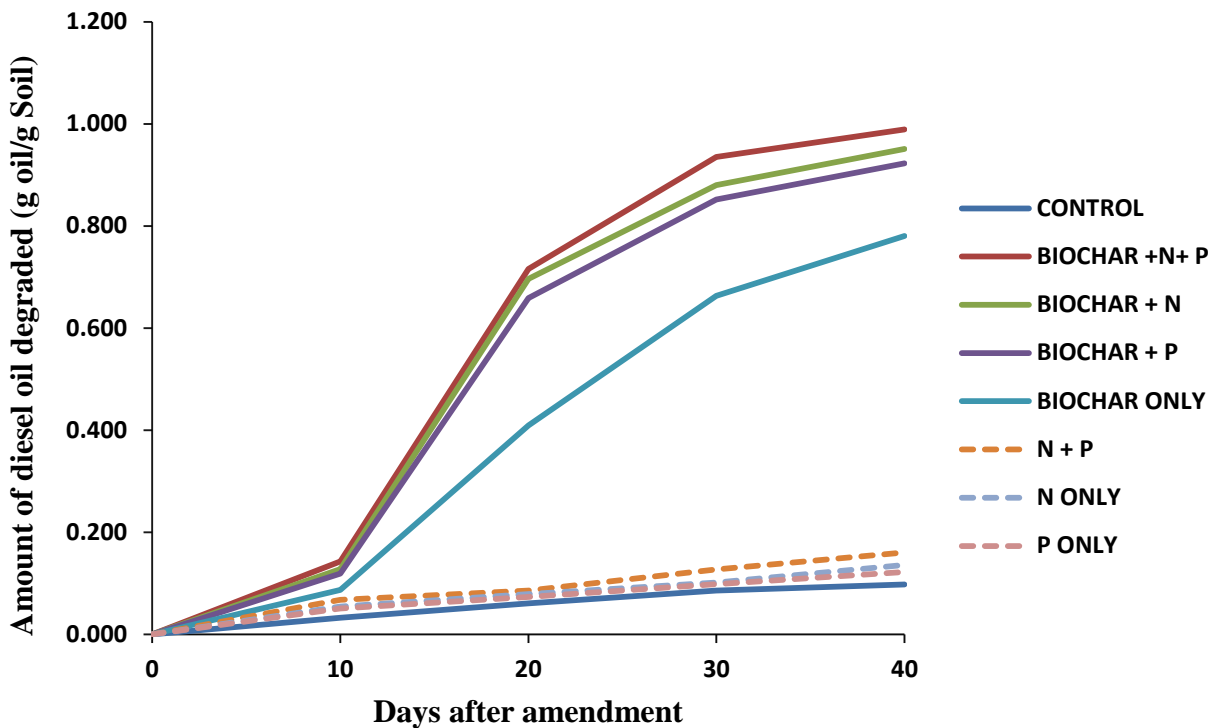


Fig.4.2 A graph of cumulative amount of diesel oil degraded in experiment 2

4.4 Hydrocarbon utilizing bacterial population

The growth of hydrocarbon utilizing bacteria (HUB) in the contaminated soils amended with biochar (Experiment 1) is presented in Fig. 4.3. The initial HUB population in the soil was 1.6×10^5 cfu/g but increased rapidly 10 DAA in all treatments. The peak HUB populations were observed at 20 DAA for all biochar treatments. RB₂₀₀ had the highest peak value (1.42×10^7 cfu/g) but not significantly ($p > 0.05$) different from RB₁₅₀ (1.38×10^7 cfu/g). These two peak values were significantly ($p < 0.05$) higher than the corresponding peak values of SB applications (SB₂₀₀, 8.5×10^6 cfu/g and SB₁₅₀, 8.0×10^6 cfu/g, respectively).

The HUB population of the control treatment however continued increasing. There was a decline in HUB populations after 20 DAA for the biochar treatments. At the end of the experiment, the

HUB populations of the biochar treated soils were significantly ($p < 0.05$) higher than the control value.

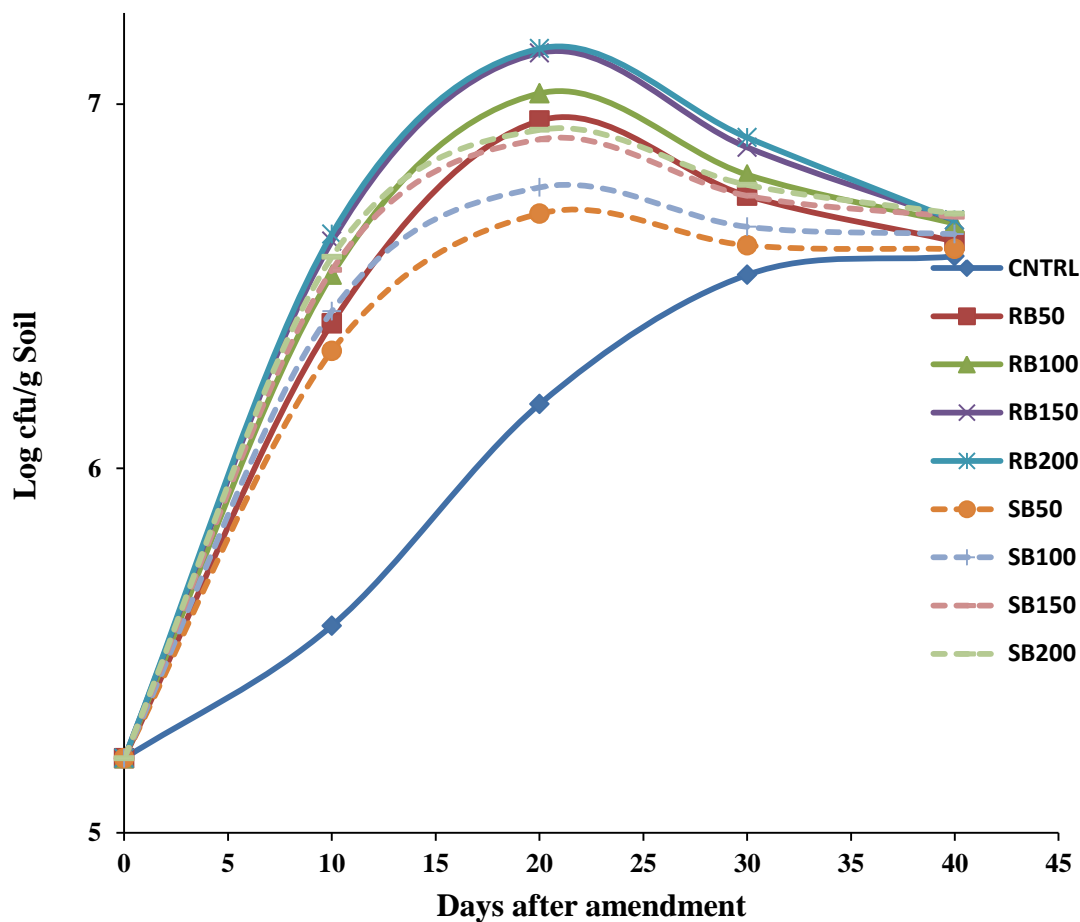


Fig.4.3 A graph of HUB populations of the contaminated soils amended with biochar (Experiment 1)

In experiment 2, the growth of hydrocarbon utilizing bacteria (HUB) in the contaminated soils is presented in Fig. 4.4. Between 0 and 20 DAA there was sharp increase in HUB population for all treatments. Biochar +N+P, Biochar+N, Biochar+P, N only and P only had their peak values at 30 DAA. Addition of biochar only had its peak value at 20 DAA. Addition of combined N+P had

steady increase in HUB population after 20 DAA. However, the control treatment continued increasing in population.

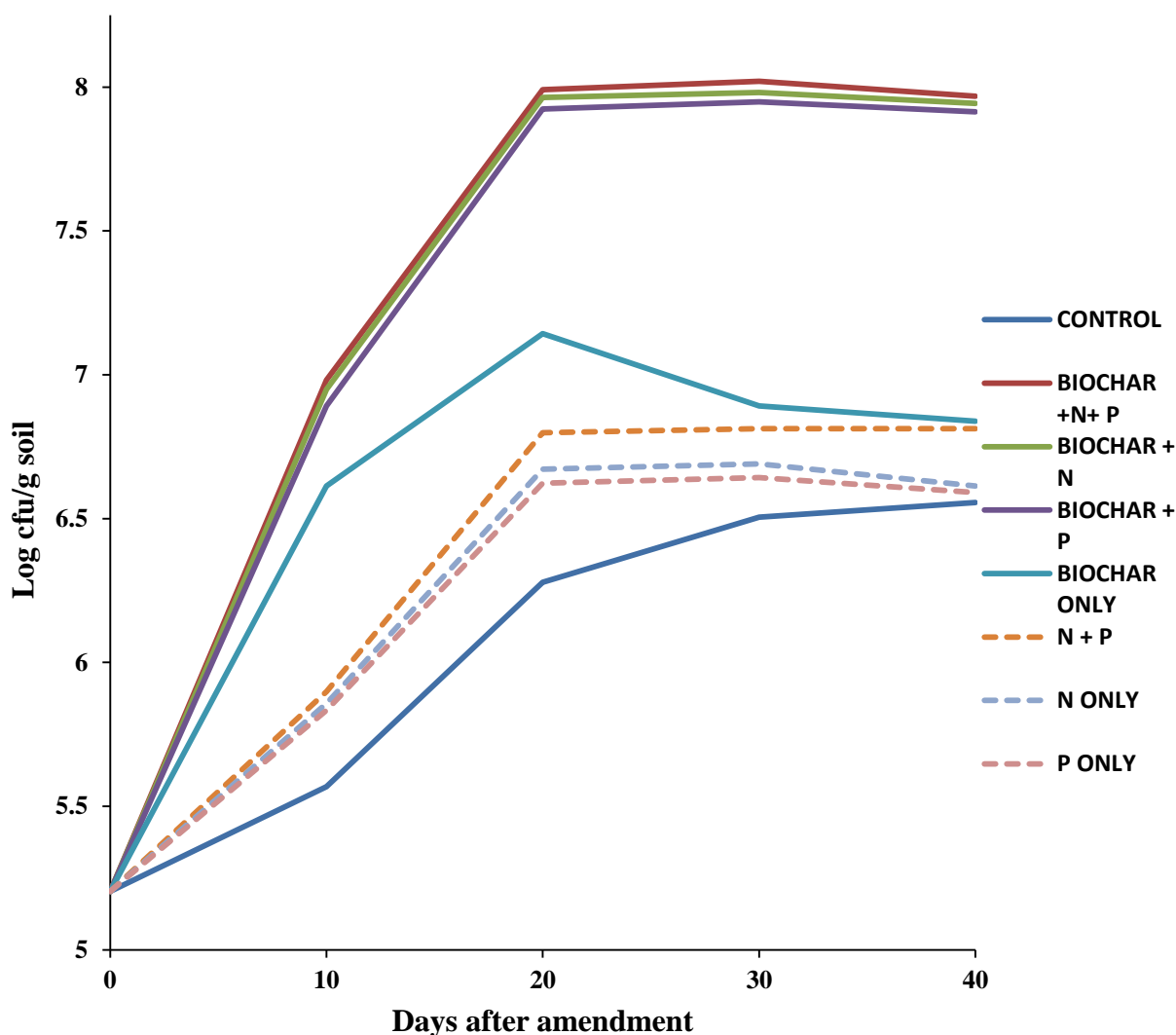


Fig.4.4 A graph of HUB populations of contaminated soils treated with biochar, N and P (Experiment 2)

At the end of the experiment, biochar+N+P, biochar+N and biochar+P treatments contained HUB populations that were significantly ($p < 0.05$) higher than the other treatments. The highest HUB population was observed in biochar+N+P treatment. The amendment of the contaminated soil

with biochar only had significant ($p < 0.05$) higher HUB population than N only, P only and N+P treatments.

4.5 Heterotrophic Bacterial Count

The population of the total heterotrophic bacteria (HET) in the contaminated soils amended with biochar is presented in Fig. 4.5. There was a sharp increase in the HET population for all biochar treatments until they peaked at 20 DAA. After 20 DAA, the heterotrophic bacterial population for all the biochar treatments declined.

The heterotrophic bacterial population in the control treatment however declined sharply between 0 and 10 DAA but increased afterwards. At the end of the experiment, the total heterotrophic bacterial populations of the biochar treated soils were significantly ($p < 0.05$) higher than the control treatment.

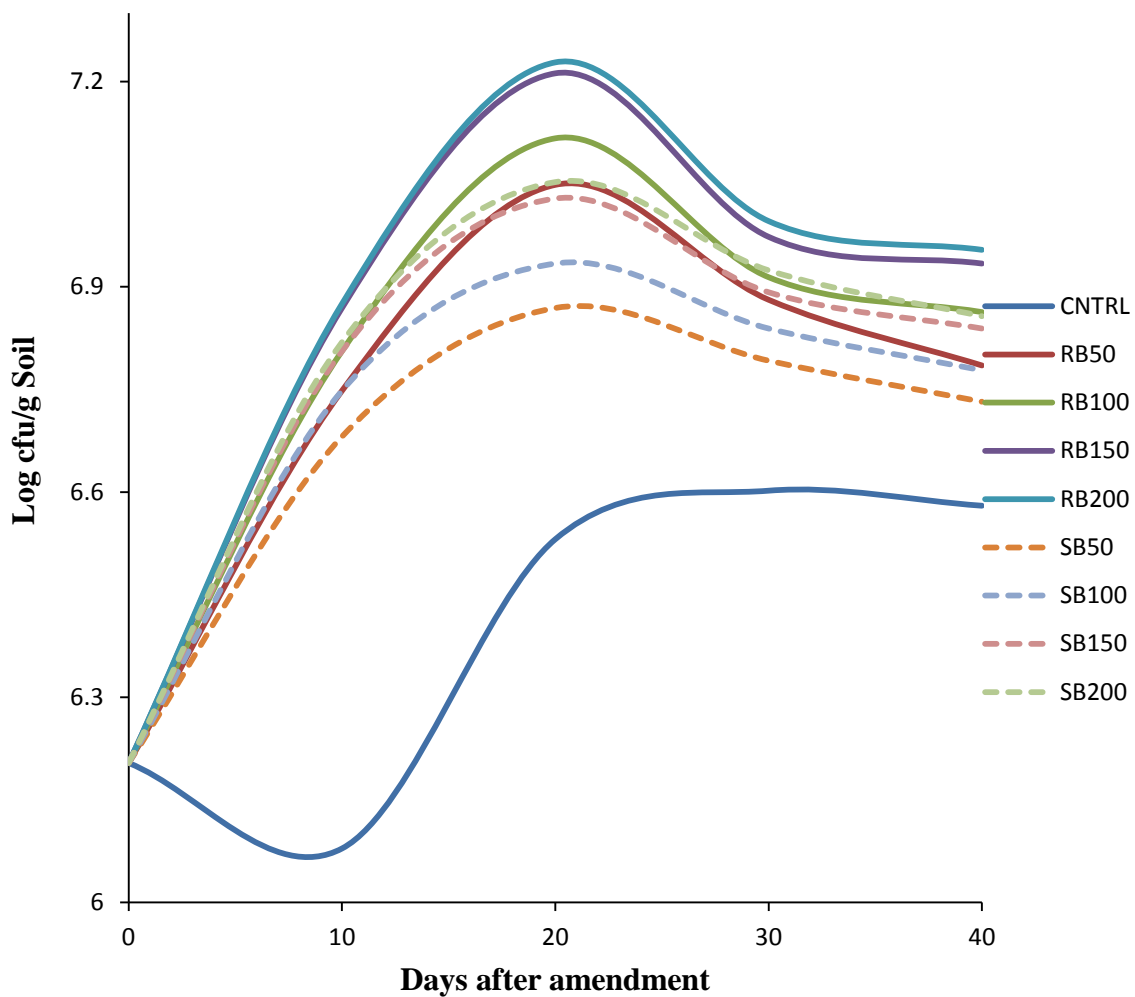


Fig.4.5 A graph of the total heterotrophic bacterial population in the contaminated soil amended with biochar.

4.6 Percentage of HUB present in total heterotrophic bacterial population (HET)

The percentage HUB in the contaminated soils is shown in figure 4.6. At the beginning of the experiment, only 10% of the entire heterotrophic bacterial population was HUB. This increased rapidly at 10 DAA and peaked at 20 DAA for all biochar treatments. At the peak day (20 DAA), the highest value was recorded for RB₂₀₀ (84.7%), followed by RB₁₅₀ (84.0%) and the lowest value was recorded for SB₅₀ (67.6%). Generally, the percentage values were higher for the RB

treatments when compared to the SB treatments. In the control however, the percentage continued increasing. Apart from SB₅₀ and SB₁₀₀, the other biochar treatments declined after 20 DAA. At the end of the experiment, the control treatment (89.5%) was significantly ($p < 0.05$) higher than the biochar treatments.

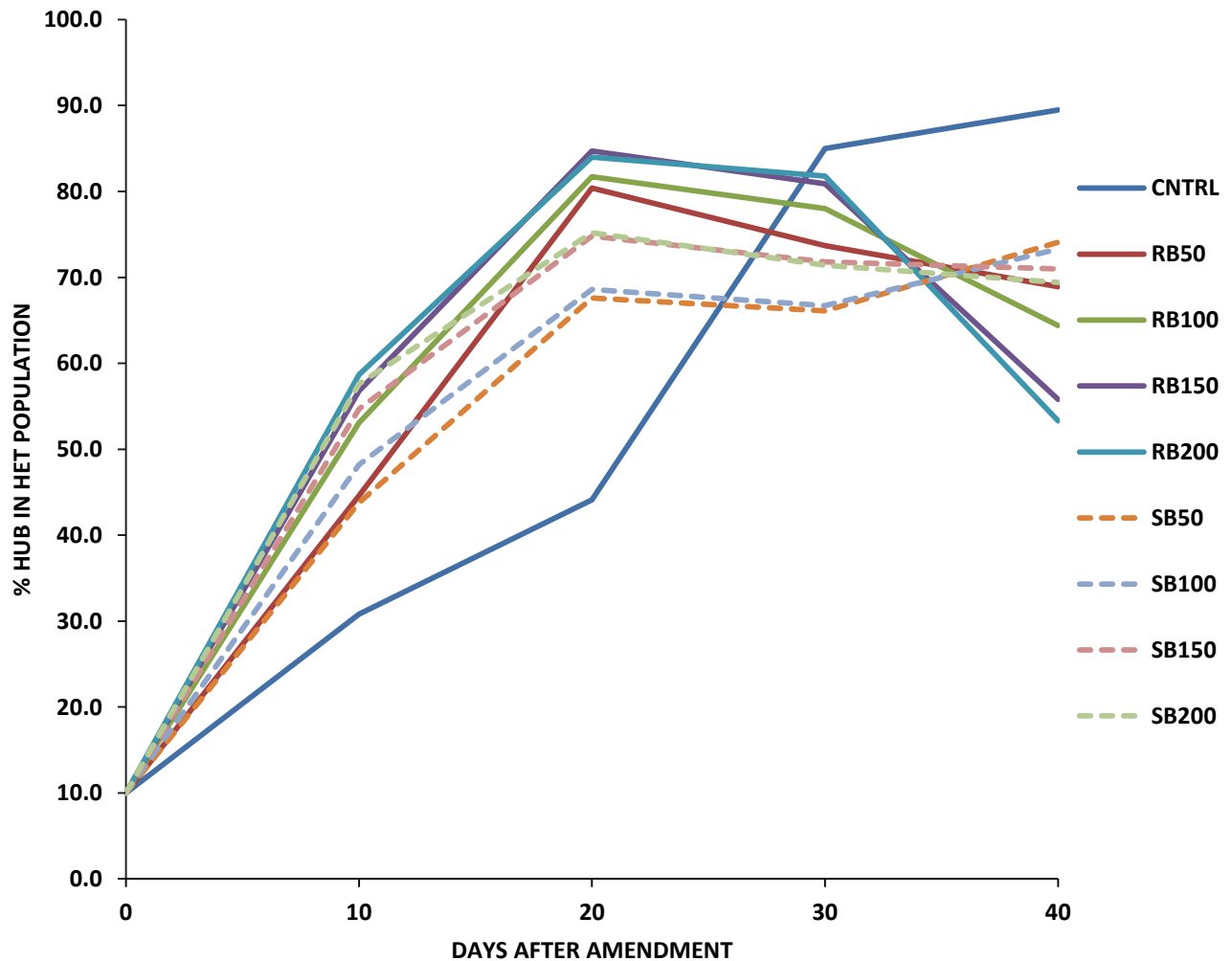


Fig.4.6 A graph of % HUB in HET population in experiment 1

4.7 Amount of oil remaining (%) in the soil after the treatment period (Exp. 2)

At the end of experiment 2, the amount of oil remaining in the treated soils before the cultivation of the cowpea is shown in Fig.4.7 below. The amount of oil remaining in the non-biochar treatments were in the range of 89 to 93%. This indicates high amounts of oil undegraded in these treatments. However, the biochar only, biochar + P, biochar + N and biochar + N + P treatments had low amounts of 36.8, 25.1, 22.2 and 20.2% respectively remaining in them.

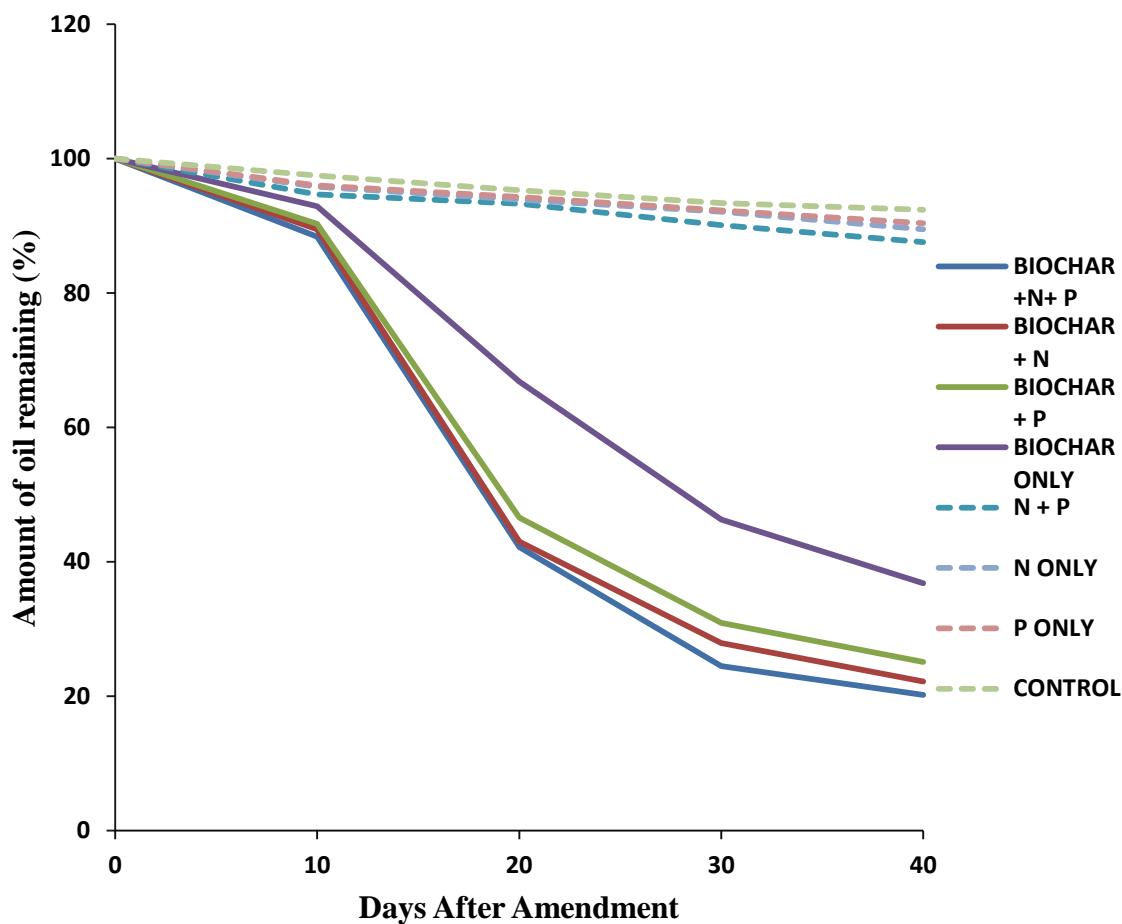


Fig.4.7 A graph of the amount of oil remaining in the treated soils at the end of Expt. 2

4.8 Percent germination of cowpea

The germination response of cowpea to biochar with or without N and P supplementation are shown in Fig.4.8. Compared to the control, all treatments containing biochar significantly ($p < 0.05$) enhanced germination of cowpea, while the enhancement by the others without biochar was not significant ($p > 0.05$). Amending soils with N or P only resulted in increased percent germination from 40 to 46.7%, with another slight increase when N was combined with P. Biochar only treatment resulted in 86.7% germination of cowpea, lower than the 100% for the biochar + nutrient treatments.

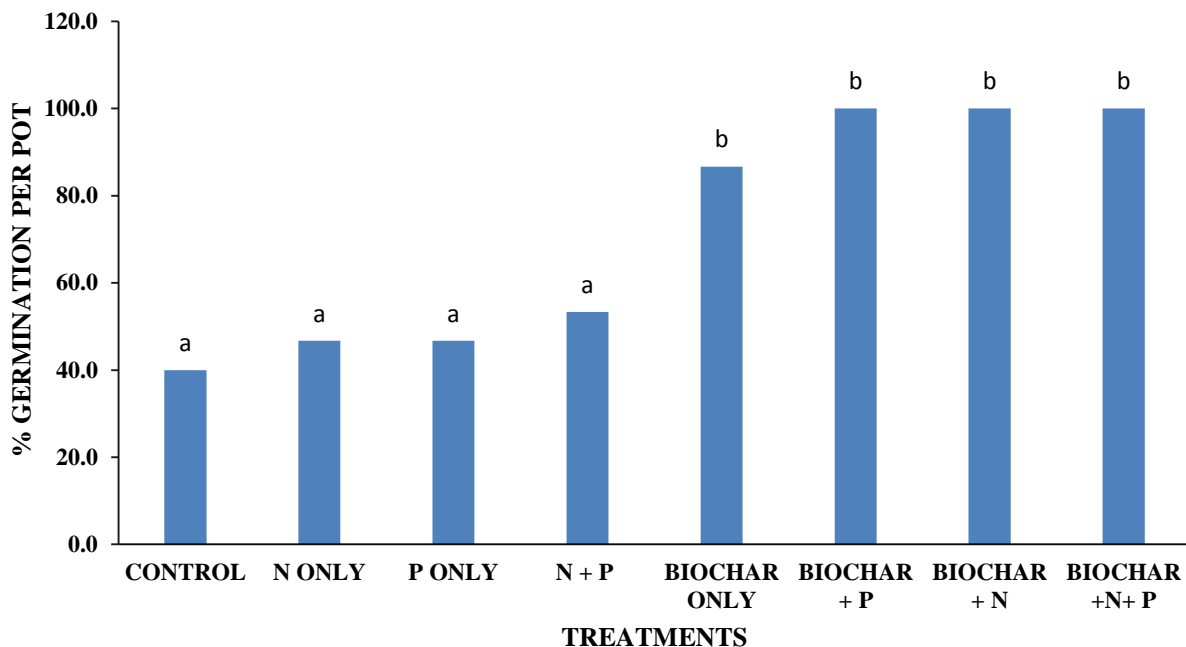


Fig.4.8 A graph of percent germination of cowpea per pot

* Means having the same letters on each bar do not have any significant difference at 5% probability.

4.9.1 Nodule count

Results on nodule formation in cowpea are shown in figure 4.9. Results showed that no nodule was formed in the unamended soils as well as in the soil amended with N only. Although the addition of P and its combination with N resulted in nodule formation on cowpea, the difference against the control was not significant ($p < 0.05$). However, the application of biochar with or without N and/or P significantly ($p < 0.05$) increased nodule number, with the highest number of nodules formed on the biochar + P treatment.

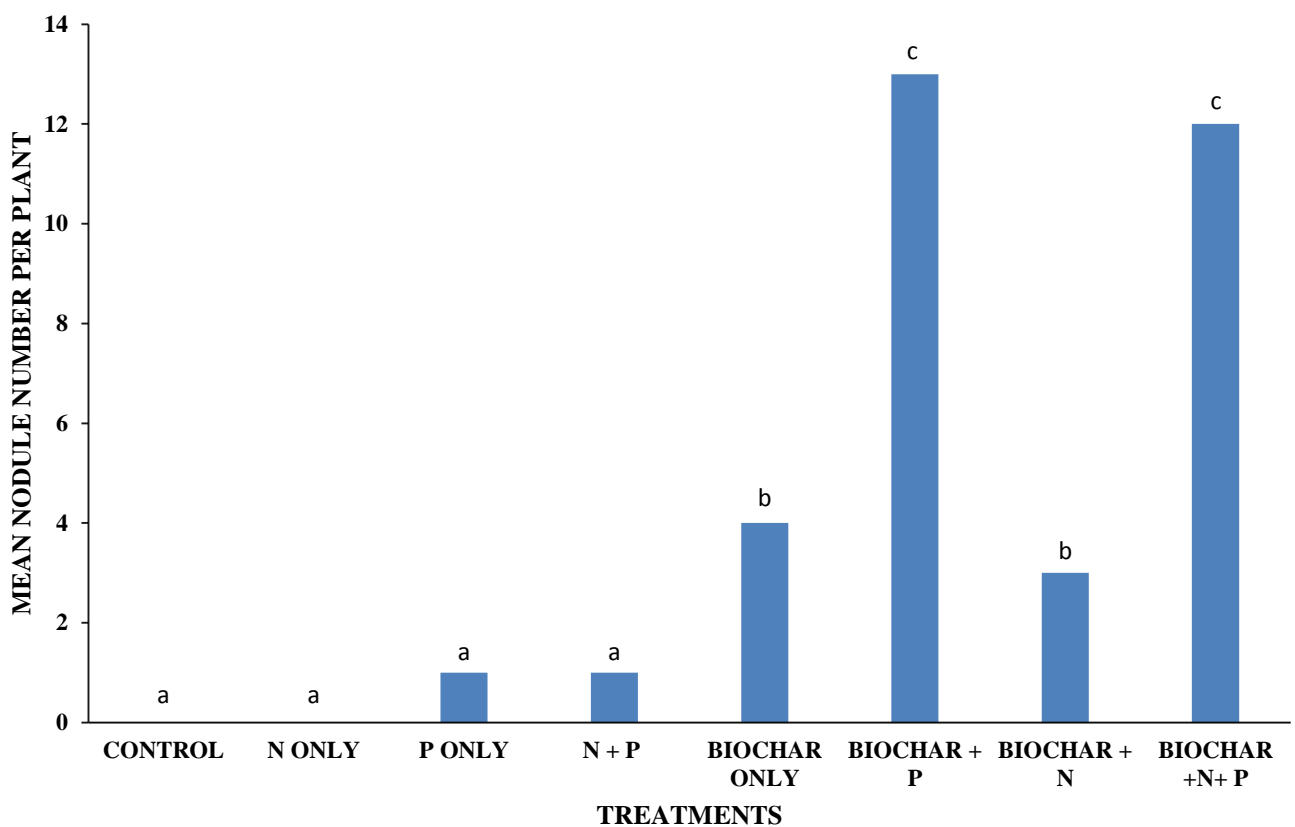


Fig.4.9 A graph of nodule count per plant

* Means having the same letters on each bar do not have any significant difference at 5% probability.

4.9.2 Shoot dry weight and root dry weight per plant

The shoot dry weights (SDW) and root dry weight (RDW) of the cowpea grown in the various amended soils are shown in Figure 4.9.1 and 4.9.2, respectively. The SDW and RDW of cowpea grown in soils amended with N or P only were not significantly ($p > 0.05$) different from the control treatment, but a combination of both resulted in significantly ($p < 0.05$) higher values. Further significant ($p < 0.05$) increases in SDW and RDW were obtained when soils were amended with biochar with or without N and/or P, with the highest increases occurring in the biochar + N + P amended soils.

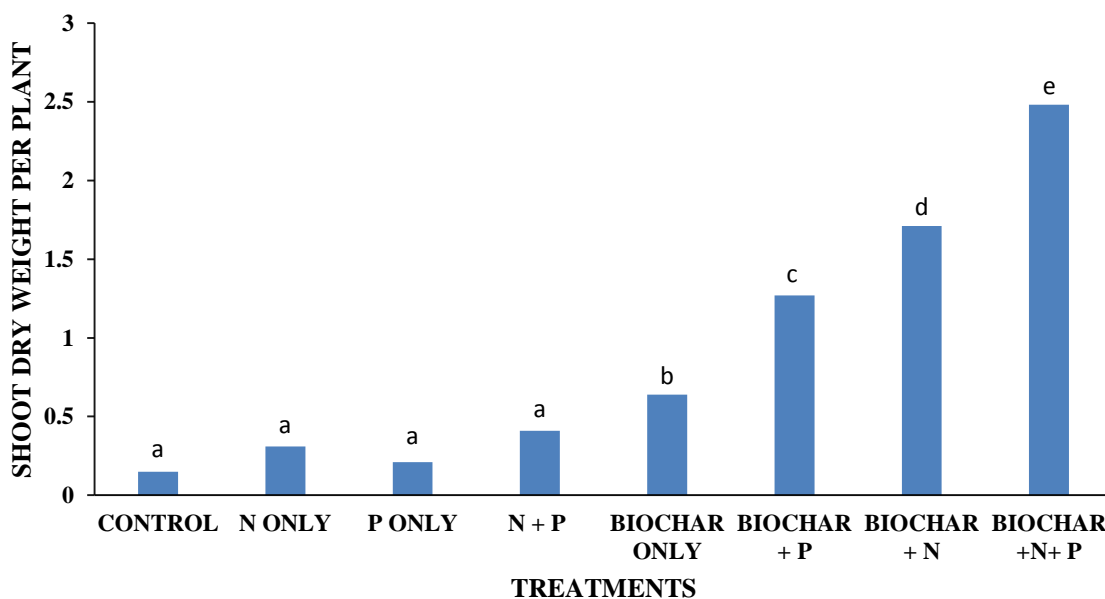


Fig.4.9.1 A graph of shoot dry weight per plant (g)

*** Means having letters in common for each bar do not have any significant difference at 5% probability.**

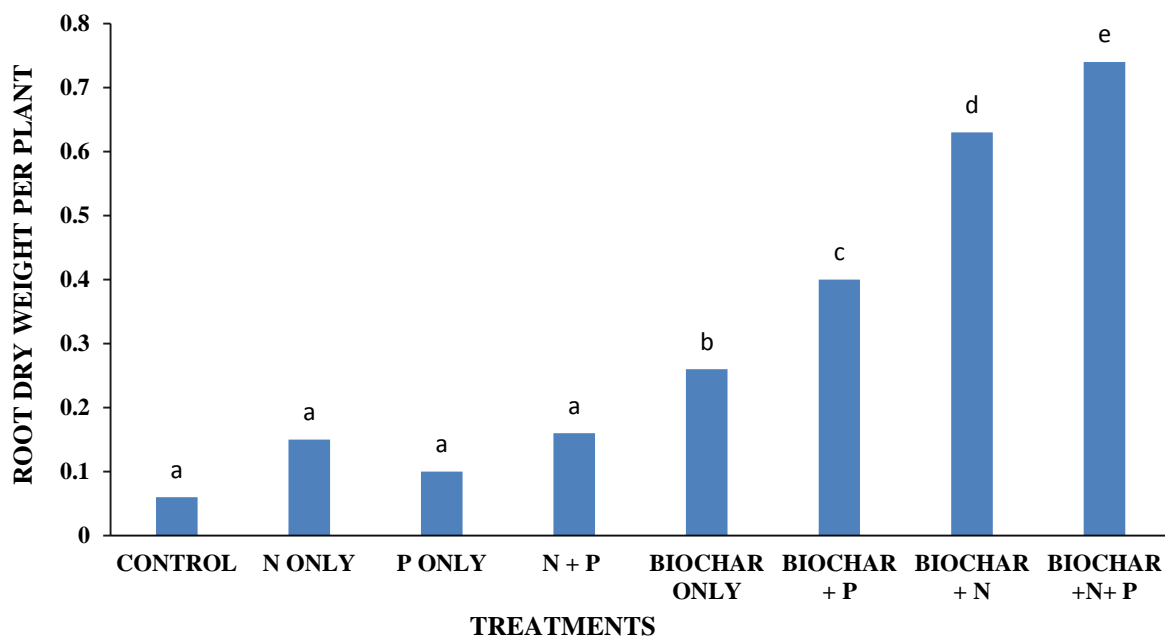


Fig.4.9. 2A graph of root dry weight per plant (g)

*** Means having letters in common for each bar do not have any significant difference at 5% probability.**

CHAPTER FIVE

DISCUSSION

5.1 Changes in Some Soil Properties after Biochar Amendment

5.1.1 Changes in soil pH after biochar amendment

The general increase in soil pH from day 0 to 20 DAA could be attributed to the liming effect of the two biochars because they contained large quantities of carbonates and oxides (explained in Chapter Three and Appendix 1 and 2). This is in conformity with work done by Glaser et al. (2002) and Lehmann and Rondon (2006), which suggested that biochar can serve as a liming agent, resulting in increased pH for a number of different soil types. Depending on the biochar biomass used, basic cations such as Ca, K, Mg, and silicon (Si) can form alkaline oxides or carbonates during the pyrolysis process (Noble et al. 1996). According to Novak et al. (2009), with the release of these oxides and carbonates into the environment, they can react with the H^+ and monomeric Al species, raise the soil pH, and decrease exchangeable acidity. The inherently low buffering capacity of the soil used (sandy loam), may also explain the rapid increase in the soil pH.

The pHs of RB treatments were 0.8 to 1.0 units higher than those of SB. This could be attributed to the high carbonates and oxides in the RB as compared to the SB treatments as also suggested by Amonette and Joseph (2009) and Wang et al. (2009). This is because biochar cations largely determine the resulting carbonate concentrations, making some biochars better liming agents than others (Amonette and Joseph, 2009; and Wang et al., 2009).

At 40 DAA, there was a decrease in the soil pH for all the biochar treatments even though not significant. This decrease could be attributed to the production of organic acids and phenolic substances which reduce the pH as a result of increased decomposition of cellulose and hemicellulose as documented by Abe et al.(1998). Cheng et al. (2006) also attributed the decrease in soil pH to the oxidation of C to form acidic carboxyl groups, which tend to lower the soil pH.

5.1.2 Changes in OC, TEB, BS and ECEC after biochar amendment

There were significant increases in soil OC, TEB, BS and ECEC at the end of the experiment for biochar treatments. The increase in the soil OC could be attributed to the introduction of the diesel oil, since diesel is mainly made up of hydrocarbons. According to Nigussie et al.(2012) and Lehmann (2007), during the conversion of biomass to biochar, about 50% of the original C is retained in the biochar, which offers considerable opportunity for creating a C sink. Hence the increased OC could also come from the biochar. The higher % OC in the RB as compared to the SB (Table 3.1) could explain the differences among the two biochar treatments. The increased TEB and BS could be attributed to increased cations by the two biochars at high pH. Cowie et al. (2001) reported that, most cations (Ca, Mg, K) are available at soil pH between 5.5 and 7.0, and deficient at soil pH less than 5.5.

The high amounts of basic cations in the RB relative to the SB, and the associated effect of increased pH could be the reason for the higher TEB and BS of the rice straw biochar amended soils as compared with those amended with saw dust biochar. The significant increase in ECEC can be attributed to the explanation documented by Liang et al. (2006) who stated that biochar has great ability to adsorb and retain cations in an exchangeable form due to its greater surface

area, and negative surface charge. The increase in ECEC could also be due to the increased negative charges arising from the increased exposed carboxyl groups of the biochar with time (Bot and Benites, 2005; Novak et al., 2009).

5.1.3 Changes in total N after biochar amendment

There was decrease in total N for all the biochar amended soils at the end of the experiment (40 DAA). The reduction could be due to the high C/N ratio of biochar (Table 3.1) and hence, N immobilization during mineralization of labile fraction of the biochar as documented by Deenik et al. (2010) and Lehmann et al. (2003). It could also be due to the high microbial populations associated with biochar addition and their high dependency on soil N for oil degradation as indicated by Lehmann et al. (2003). Camps (2009) and Yanai et al. (2007) suggested that, it is possible the N that exists within the biochar is not bioavailable when introduced to the soil, as it is bound up in heterocyclic form. This therefore implies that N in the biochar would not be readily available for microbial utilization, hence, the microbes would depend mainly on the N in the soil and would result in N immobilization. The soils amended with RB decreased in total N more than those amended with SB. This difference could be attributed to the high C/N ratio of RB, hence, greater potential for N immobilization as documented by Lehmann et al. (2006).

5.1.4 Changes in soil available P after biochar amendment

There were increases in available P for all biochar treatments and this could be due to the increased soil pH as a result of the biochar amendments. Birch (1951) confirmed that, soil phosphorus availability is depressed at high acidity, as the phosphate is largely fixed by Al and Fe. The increase was however larger in the soils amended with RB than those amended with SB,

and this could be attributed to difference in oxides and carbonates in RB. Kothamasi et al. (2006) also demonstrated an increase in other bacteria population such as the phosphate solubilizing bacteria (PSB) in the soil after biochar amendment hence, could also be the reason for the increase in the soil available P.

5.1.5 Changes in soil exchangeable acidity after biochar amendment

There was decrease in exchangeable acidity after amending the soil with biochar. This decrease could be attributed to the alkaline nature of the biochar used. Novak et al. (2009) documented that when Ca, Mg, and K oxides and carbonates in biochar are released into the soil environment, they react with the H^+ and monomeric Al species, raise the soil pH, and thereby decreasing exchangeable acidity. RB decreased soil exchangeable acidity more than the SB and this could probably be due to the higher amounts of carbonates and oxides.

5.2 Hydrocarbon utilizing bacteria population

There was initial increase in hydrocarbon utilizing bacterial population. The increase in the HUB population conforms to work done by Udeme and Antai (1998) and Lawson et al. (2012) who documented that, introduction of petroleum hydrocarbons into the soil encourages high microbial biomass. Joshi and Pandey (2011) also suggested that the percentage of petroleum utilizing bacteria in a particular environment appears to be an index of the presence of hydrocarbons in that environment. The HUB population peaked at 20 DAA for the biochar amended soils and declined afterwards but the population in the control treatment continued increasing. Lawson et al. (2012 and Atibila (2013) also observed HUB population peaking at 20 and 30 days after oil

contamination in some Ghanaian soils. This reduction could be explained by Rittman (1992) who suggested that, the microbes that degrade oil are part of the local food web; they are consumed by other microbes that are in turn, consumed by larger predators. Rittman (1992) further indicated that when oil is gone or is about finishing, the oil-degrading microbes, with less substrate available, stop dividing so rapidly and eventually return to pre-spill abundance. The continued increase in the control treatment could be attributed to availability of substantial amount of oil serving as carbon source for cell synthesis.

The addition of N and/or P to biochar resulted into higher HUB population. Atibila (2013) also observed higher HUB population when oil contaminated soils were amended with N and/or P. Bacteria require phosphorous for sugar phosphorylation, nucleic acid synthesis and other cellular processes and N for microbial cellular build up (Andrew and Jackson, 1996).

5.3 Amount of oil degraded

There was high cumulative increase in the amount of oil degraded for all the biochar treatments. This increment could be attributed to the corresponding increase in HUB population as a result of improved soil environment. According to Ajisebutu and Alofe (2004), the effectiveness of bioremediation often is a function of the microbial population and how they can be enriched and maintained in an environment. Okoh et al. (2001) also suggested that, the important factor in the degradation of petroleum oil is adequate bacterial biomass. The lack of a lag phase in the HUB and HET populations as shown in Fig. 4.3, 4.4, 4.5 and 4.6, but a contrary in the amount of oil degraded as shown in Fig. 4.1 and 4.2, could be attributed to the ineffectiveness of the HUB population during that period. The ineffectiveness could be due to the harsh conditions imposed by the low pH and its associated effects. There was further increment in the amount of oil

degraded when N and/or P was added to the biochar treatments. Similar result was observed by Atibila (2013) when oil contaminated soils were amended with N and/or P. Abu and Ogiji (1996) stated that, lipophilic fertilizers can be used to enhance microbial utilization of petroleum oil. According to Okoh and Trejo-Hernandez (2006), for effective bioremediation one of the requirements necessary is the availability of nutrients (nitrogen and phosphorus are essential) to support microbial growth and enzyme production.

5.4 Growth and nodulation of cowpea

The germination results showed that biochar + N and/or P treatments gave 100% germination of cowpea, biochar only treatment gave 86% while control, N and/or P treatments gave low values ranging between 40 and 47%. The low percent germination in these treatments might be due to the presence of high amount of oil in those soils before planting the cowpea (Fig. 4.7). This result supports the findings of Horne (1978) who stated that heavy crude oil pollution leads to poor germination due to poor soil aeration. Bossert and Bartha (1985) also observed a low germination of corn seeds in soil contaminated by crude oil. Similar results were reported by Gallegos-Martinez *et al.* (2000) who found a reduction in germination between 30-90% in the tropical native Mexican species subjected to soil contamination with crude oil. According to Adam and Duncan (2002), diesel oil has volatile components that contain light hydrocarbons capable of entering easily through the plant cell walls. These small hydrocarbon molecules that penetrate the plants can be phytotoxic, explaining the decrease in seed germination (Ogbo, 2009). Fresh crude oil has a coagulatory effect on the soil, binding the soil particles into water impregnable soil that seriously impairs water drainage and oxygen diffusion (Atuanya, 1987).

There were no nodules recorded for cowpea grown in the control and N amended soils. This could be attributed to the high amount of diesel oil in these soils. This finding agrees with a study by John et al. (2011) which revealed that symbiotic nitrogen fixing bacteria associated with legume is very sensitive to crude oil pollution. Jones et al. (2011) stated that diesel oil affected the survival or multiplication of rhizobia by altering the soil properties and hence decreasing their chances of invasion into the roots and subsequent nodulation process. The poor root development in the control and N amended soils could be attributed to very poor nodulation. The low pH for these treatments could also be responsible for the poor nodulation. Andrew et al. (1973) also reported of poor growth of a legume on acidic soil and concluded that, it could be due to the toxic effect of large amounts of Al in those soils, which impedes the uptake of other essential nutrients by the crop. According to Holding and King (1963), the more ineffective strains of rhizobia are found in soils at low pH. The control and the N-only treatments had very low pH (4.8) at the time of the planting of the cowpea, and could have been the reason for the lack of nodulation in those treatments, because they were ineffective at that pH and high oil concentration.

Addition of biochar and P enhanced nodulation, while, N was inhibitory. This result confirms the findings of Luse et al. (1975), Agboola and Obigbesan (1977), who reported increases in the number of nodules in cowpea due to phosphorus application. Phosphorus addition affects the growth and survival of rhizobia (O'Hara et al., 1988), nodule formation (Drevon and Hartwig, 1997), nodule functioning (Tang et al., 2001), host plant growth (Tsvetkova and Georgiev, 2003), root development and N₂ fixation (Danso, 1992).

The increase in nodulation as a result of biochar addition is in agreement with work done by Ogawa (1994) and Rondon et al. (2007), who reported that biochar addition increased nodulation

and N₂ fixation and subsequently, led to an increase of between 30 and 40% in the yield of beans (*Phaseolus vulgaris* L.). According to Lehmann et al. (2003), biochar addition to the soil stimulates root growth and abundance, resulting in increased chances of invasion of rhizobia to crop roots. Chen et al. (2009) attributed enhanced nodulation as a result of biochar addition to the sorption of compounds by biochar, which would have otherwise inhibited microbial growth. Kasozi et al. (2010) reported that, compounds such as catechol that are toxic to microorganisms were found to be strongly sorbed to comparatively high temperature biochars produced from ash rich corn stover.

It is well known that high soil N particularly mineral nitrogen, during initial growth retards nodule formation (Ezedinma 1964; Tewari, 1965). The inhibitory effect of high inorganic nitrogen on nodulation and nitrogen fixation has been reported by many researchers (Eaglesham et al., 1983; Becker et al., 1986; Waterer, 1992). According to Becker et al. (1986), nodulating legumes prefer easily absorbable forms of N to nodulation and fixation of atmospheric N because of the “cost of energy” involved in the later.

The cowpea plants grown in the soils amended with biochar + N and/or P had higher SDW and RDW than other treatments. This finding is similar to that of Baker (1970), Akinola et al. (2004), Merckl et al. (2004) and Agbogidi et al. (2006 and 2007) who found increase in dry matter yield of crops at lower concentrations of oil. This shows that the high content of oil in these treatments might have inhibited the growth of cowpea. The toxicity of petroleum hydrocarbons at higher concentrations has been linked to displacement of nutrients and nutrient uptake (Amadi et al., 1993); reduction in available phosphorus and total nitrogen (Baker-Coker and Ekundayo, 1995) and interference with soil chemotaxis by crude oil (Rosenburg *et al.*, 1992) culminating in growth retardation (Travern, 1992).

However, the higher dry matter yield observed in N-fertilised plants is indicative that N₂ fixation was not supplying the plants with all the nitrogen it needed for maximum growth as documented by Sanginga et al. (2000). Nitrogen is an integral component of many compounds, including chlorophyll and enzymes essential for plant growth processes. According to Walker *et al.* (2001), availability of N in the soil directly affects the relative growth rate of plants.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

Using biochar to enhance bioremediation of diesel oil in an acidic soil showed satisfactory results. Rice straw biochar enhanced degradation of diesel oil more than the saw dust biochar. The enhanced degradation of these biochar types was attributed to the presence of large quantities of carbonates and oxides in the biochars which served as liming agents, improved microbiology and other chemical properties of the soil. Addition of nutrients, nitrogen and phosphorus, to the biochar further enhanced oil degradation and supported growth of cowpea. Nodulation was improved by application of combined biochar and phosphorus.

It is therefore recommended that, the application of combination of rice straw biochar, nitrogen and phosphorus, in the bioremediation of oil under acidic condition should be conducted in the field to confirm its effectiveness. It is also recommended that, further and detailed investigation should be done to concretely explain the absence of a lag phase in the HUB population but a contrary in the amount of oil degraded.

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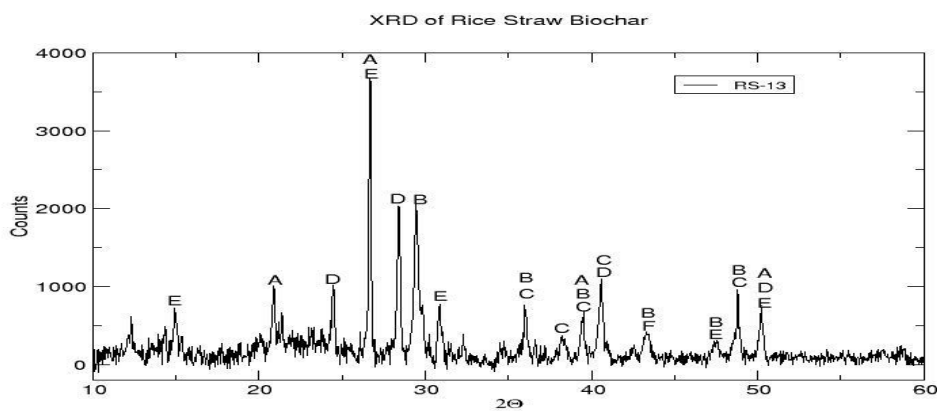
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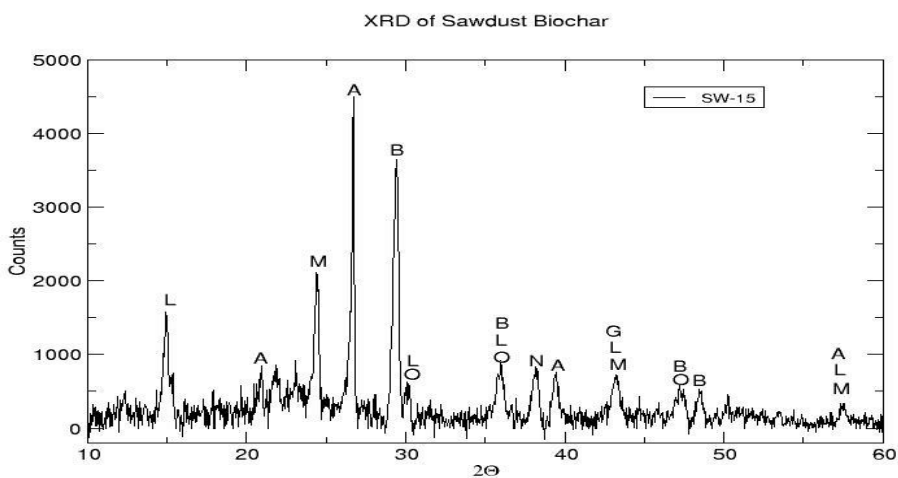
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APPENDIX



Appendix 1: X-Ray diffractogram of rice straw biochar



Appendix 2: X-Ray diffractogram of saw dust biochar

A= Silica (SiO_2), B= Calcite (CaCO_3), C= Calcium differate oxide (CaFe_2O_4), D= Potassium chloride (KCl), E= Siloxene (H_2OSi_2), F= Periclase (MgO), G= Magnesium oxide (MgO),), L= Iron oxide (FeO), M= Graphite, N= Magnesium silicate (Mg_2SiO_4) O= Iron magnesium oxide (FeMgO_4).

Appendix 3: Statistical Analysis of Data

Variate: SOIL_pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	17	29.12251.7131	17130.87	<.001	
Residual	36	0.0036	0.0001		
Total	53	29.1261			

Significant at 5%: LSD.= 0.38

Variate: ORG CARBON

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	8	18.7269	2.3409	625.16	<.001
Residual	18	0.0674	0.0037		
Total	26	18.7943			

Significant at 5%: LSD.= 0.16

Variate: TOTAL EXCH. BASES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	8	4.585E+01	5.732E+00	64481.72	<.001
Residual	18	1.600E-03	8.889E-05		
Total	26	4.586E+01			

Significant at 5%: LSD.= 0.20

Variate: TOTAL N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Trt	8	0.0039	0.0005	6.21	<.001
Residual	18	0.0014	0.0001		
Total	26	0.0053			

Significant at 5%: LSD.= 0.020

Variate: BASE SATURATION

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	8	8.451E+03	1.056E+03	1.056E+07	<.001
Residual	18	1.800E-03	1.000E-04		
Total	26	8.451E+03			

Significant at 5%: LSD.= 0.095

Variate: ECEC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	8	27.8832	3.4854	34854.00	<.001
Residual	18	0.0018	0.0001		
Total	26	27.8850			

Significant at 5%: LSD.= 0.017

Variate: AVAILABLE P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Trt	8	0.0816	0.0102	53.98	<.001
Residual	18	0.0034	0.0002		
Total	26	0.0850			

Significant at 5%: LSD.= 0.06

Variate: EXCH. ACIDITY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	8	5.6949	0.7119	8008.41	<.001
Residual	18	0.0016	0.0001		
Total	26	5.6965			

Significant at 5%:LSD = 0.0268

Variate: ROOT_DRY_WGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	7	1.3596	0.1943	398.43	<.001
Residual	16	0.0078	0.0005		
Total	23	1.3675			

Significant at 5%:LSD = 0.03822

Variate: SHOOT_DRY_WGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	7	14.9652	2.1379	221.07	<.001

Residual	16	0.1547	0.0097
Total	23	15.1199	

Significant at 5%:LSD = 0.1702

Variate: NODULATION

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	7	620.958	88.708	37.35	<.001
Residual	16	38.000	2.375		
Total	23	658.958			

Significant at 5%:LSD = 2.667

Variate: HUB POPULATION (STUDY 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAY	3	2.448E+00	8.159E-01	86456.17	<.001
RATE	3	4.692E-01	1.564E-01	16572.33	<.001
TRTMNT	1	3.264E-01	3.264E-01	34588.97	<.001
DAY.RATE	9	7.232E-02	8.036E-03	851.47	<.001
DAY.TRMTNT	3	1.771E-01	5.903E-02	6254.53	<.001
RATE.TRMTNT	3	3.232E-03	1.077E-03	114.16	<.001
DAY.RATE.TRMTNT	9	9.041E-04	1.005E-04	10.64	<.001
Residual	64	6.040E-04	9.438E-06		

Total 95 3.498E+00

Variate: HUB_POPULATION_STUDY_2

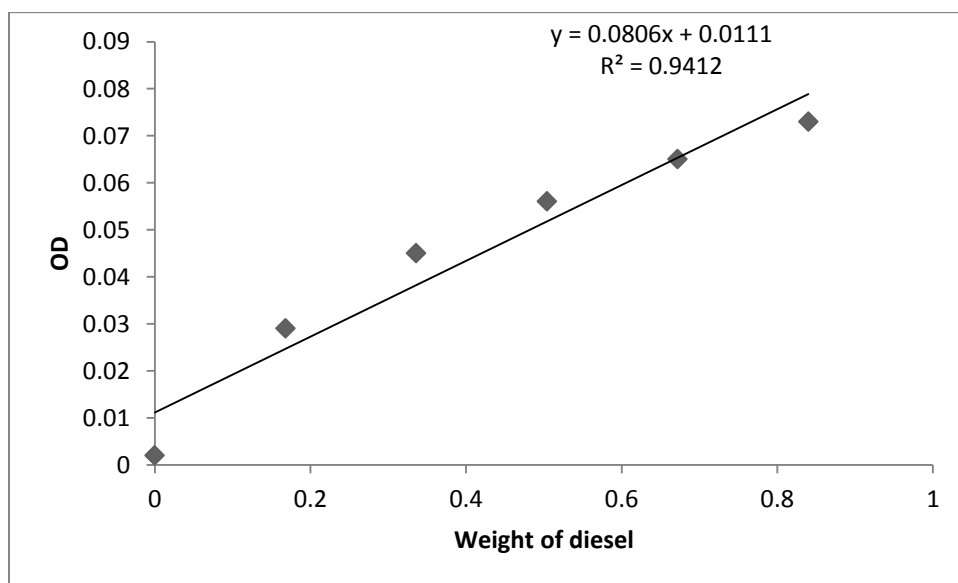
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAY	3	1.125E+01	3.751E+00	3.751E+06	<.001
TRTMNT	6	2.749E+01	4.582E+00	4.582E+06	<.001
DAY.TRTMNT	18	9.320E-01	5.178E-02	51778.60	<.001
Residual	56	5.600E-05	1.000E-06		
Total	83	3.967E+01			

Variate: AMOUNT_OF_OIL_STUDY_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAY	3	3.2E+00	1.0E+00	1.0E+06	<.001
RATE	3	5.0E-01	1.6E-01	1.6E+05	<.001
TRTMNT	1	3.3E-01	3.3E-01	3.3E+05	<.001
DAY.RATE	9	1.4E-01	1.5E-02	15717.92	<.001
DAY.TRTMNT	3	9.1E-02	3.0E-02	30602.37	<.001
RATE.TRTMNT	3	3.7E-03	1.2E-03	1249.62	<.001
DAY.RATE.TRTMNT	9	1.2E-02	1.3E-03	1357.62	<.001
Residual	64	6.4E-05	1.0E-06		
Total	95	4.3E+00			

Variate: AMOUNT_OF_OIL_STUDY_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAY	3	2.9E+00	9.9E-01	9.9E+05	<.001
TRTMNT	6	6.1E+00	1.0E+00	1.0E+06	<.001
DAY.TRTMNT	18	1.7E+00	9.5E-02	95589.63	<.001
Residual	56	5.6E-05	1.0E-06		
Total	83	1.0E+01			



Standard curve for determining the amount of diesel oil degraded.

