THE USE OF BIOCHAR TO REMEDIATE TWO COASTAL SAVANNAH SOILS CONTAMINATED WITH ATRAZINE AND PARAQUAT

BY

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has neither in whole nor in part been presented for another degree elsewhere. Works by other researchers have been duly cited by references to the respective authors and all assistance received acknowledged accordingly.

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Dr. E.K. Nartey (Co-Supervisor) Date

DEDICATION

This work is dedicated to the Almighty God for His gift of love, guidance and protection all these years. It is also dedicated to the memory of my late dad, Jacob Benjamin Sam and my mum Grace Ekua Sam who contributed immensely towards my success.

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ABSTRACT

The use of atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2, 4, 6-triazine) and paraquat (1, 1'-Dimethyl-4, 4'-bipyridinium dichloride) as alternatives to manual or mechanical weeding in the developing world is becoming inevitable. The frequent application of these two pesticides has led to contamination of soils with dire attendant environmental consequences.A study was therefore, conducted to evaluaate the ability of cocoa and rice husk-derived biochar types to enhance the remediation of atrazine and paraquat contaminated Acrisol (Adenta series) and Vertisol (Akuse series) and to ascertain the effectiveness of the two biochar types in actively supporting the growth of beneficial soil microorganisms to enhance the degradability of the pesticide in contaminated soils. The two soils were amended with each of the two biochar types at 10 t/ha and thereafter contaminated with the each of the two pesticides at three rates of zero, normal application and ten times normal application rates and replicated three times in a completely randomized design. The moisture contentwas kept at 80% field capacity and the samples incubated for 90 days.. Total heterotroph count, microbial biomass C and N and C and N mineralized were determined at 10 day intervals for the entire duration of the experiment. Results showed that biochar amendment stimulated growth of heterotroph counts in both the Akuse and Adenta series with the highest mean counts of 66 x 10^5cfu/g soil being obtained from the Akuse series amended with cocoa husk biochar and contaminated with atrazine at ten times the normal rate of application. Microbial biomass carbon was also high for the biochar amended soils especially for the cocoa husk biochar amended soil. The use of paraquat had a depressive effect on the total heterotroph counts. Application of paraquat did not significantly depress the microbial biomass carbon especially in the cocoa husk biochar amended soils contaminated with ten times the normal rate of atrazine. Microbial activity was also high in the biochar amended soils compared to the un-amended soils

and contaminated with pesticide. Degradation of atrazine at normal rate of application was faster in both soil series amended with rice husk biochar as compared to the soils amended with cocoa husk biochar and the un-amended. At ten times the normal application rate of atrazine, degradation was fast in the cocoa husk biohar amended Akuse and Adenta series. There was less degradation of paraquat in both soils; however, at normal application rate of paraquat, degradation was faster in the rice husk biohar amended soils i.e. for both Adenta and Akuse series. The high composition of the nutrients in the cocoa husk biohar biochar especially the available P materials make it a suitable material to be used as soil amendment in the highly acid soils in Ghana.

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CHAPTER ONE

1.0 Introduction

Soil is the most intricate and dynamic natural resource comprising of air, water, inorganic and organic solids and living organisms (microorganisms, animals and plants). It forms an interface between the atmosphere and the biosphere performing many crucial functions both from an ecological and non-ecological perspective (Guimarães, 2010). Among the ecological services the soil plays are primary food production, regulation of biogenic gases and the earth's climate, biogeochemical cycling, the maintenance of biodiversity, that is providing 'habitats' for various organisms: microorganisms, plants and animals (Blum, 2005; Hodson, 2010).

In spite of the many services the soil provides, persistent exploration and exploitation of the soil as a resource, incorrect agrochemical use, unrestrained combustion, as well as growths in population, urbanization and increased industrial activities have led to the introduction of synthetic materials into the soil environment (Mielke et al., 2004; Roy et al., 2005; Chen et al, 2007). The soil is polluted with these displaced organic and inorganic chemicals as a result of human activities (such as industrial, mineral extraction, poor waste disposal and agricultural activities).

In Ghana, agriculture and mining are the key contributors to soil pollution (Asante and Ntow, 2009). Agriculture remains a major factor in many sub-Saharan Africa countries including Ghana with about 40% share in the Gross Domestic Productivity (GDP) (Asante and Ntow, 2009),with subsistence farming commonly being practiced by most of the farmers (Duku et al., 2011). However, the need for the developing nations to increase agricultural production and to retain the organoleptic characteristics of fresh foods for extended period has led to an increase in the use of pesticide in Ghana.

Pesticides have become the fastest and most reliable means of reducing the high pre and post-harvest losses due to pest and disease with an estimated 87% usage among farmers (Dinham, 2003). Thus farming is fraught with abuse of pesticides particularly in the cocoa and cotton plantations, vegetable farms and rice fields as agriculture production surges (Asante and Ntow, 2009). However, accompanying the increased use of pesticides is environmental and health problems that are on the rise due to indiscriminate use and inappropriate handling of the chemicals. Most users of these pesticides are often ignorant, and lack the necessary training coupled with the ease of availability of highly toxic pesticides which are often not well labeled, poorly packaged and recklessly promoted (Ntow et al, 2005). Hazardous pesticides are often sprayed ten to twenty times more than the recommended rate leading to an accumulation in the soil environment (Focant, 2001).

In the soil environment, the fate of pesticides has become a huge problem with respect to efficient control of pests, non-target organism exposure and movement off-site (Hafez and Thiemann, 2003) possibly because of their harmful effects on microorganisms in the soil (Araújo et al., 2003) which may in turn disturb the fertility of the soil (Schuster and Schröder, 1990). An ideal pesticide must be biodegradable, not leach into ground water and should be toxic to target organism only. Regrettably, in modern agriculture this is hardly the case of pesticides use (Johnsen et al., 2001). The product has contaminated soils all around the world (Mench et al., 2010). According to the EEA,(2005),soil system is normally endowed with a large resilience; therefore damage often only becomes obvious when it is extreme.

1.1 Problem statement

It is projected that less than 10% of total pesticide applied generally gets to target pest and most of the pesticide remains unused and enter into the ecosystem (Fogarty and Tuovinen, 1991). This

problem arises from misapplication or over dependence on pesticides. Despite their persistence in the environment, the tendency to accumulate and become toxic to non-target organisms including humans, use of pesticide cannot be discontinued. Thus a suitable mitigation scheme is essential to make pollutants less movable and toxic to receptors. In Ghana, there has been no known attempt to remediate soils contaminated with pesticide for safe cultivation of agricultural crops.

Among the countless exisiting soil remediation technologies for detoxification and decontamination of soils contaminated with pesticide, bioremediation appears to be cost effective and environmentally (Haggblom, 1991). Generally in nature, microorganisms work to degrade organic products in the soil making use of nutrients in the soil. Modifying the soil environment can assist the organisms to accelerate the pesticide degradation (Singh, 2008). This can potentially be achieved by bioremediation methods such as the addition of geosorbents, for example biochar, zeolite, and activated carbon to the soils contaminated (Kah and Brown, 2007). Because of the extensive and frequent usage of pesticides in Ghana and the fact that most of the nation's farmers are poorly resourced, any technology that would seek to help remediate pesticide from the soil environment must be cheap and easy to adopt and apply.

Biochar is a highly porous material containing functional groups such as the carboxylic and phenolic groups which influence its surface chemistry such as CEC and surface acidity etc. It also has large surface area that greatly influences its sorption properties (Tang et al., 2013). In addition, it has high oxides and carbonate contents and high pH which vary with feedstock (Zheng et al., 2013). These characteristic of biochar imply that the material has sorption properties that may vary with feedstock and pyrolysis temperature.

The principal sorption process of organic compounds unto charred materials is adsorption, mainly attributed to the recently formed atomic surfaces and micropores, while absorption dominates that

of uncharred biomass(Wang & Xing ,2007).According to Loganathan et al. (2009), there was a decline in atrazine mineralisation, when the soil was amended with 1% (w/w) wheat char ascribing the decline in bioavailability of the pesticide to the rise in sorption of the herbicide on the char. With the high K and P contents of cocoa pod, it is likely charring the feedstock anaerobically will raise substantially the pH and P of the biochar produced. This high pH and P material when added to especially acid soil will encourage the proliferation of microbes to remediate pesticides that may have contaminated the soil. The high polyphenol content of cocoa husk when liberated on charring could also serve as sites for adsorption of pesticides in soils. Though several works exist in literature on the use of biochar in pesticide sorption, very little attention has been paid to the use of cocoa pod and rice husk biochar as soil amendements to create a congenial environment for microbial mediated remediation of pesticides.

1.2 Justification

Use of biochar is gradually being recognized as an environmental-friendly method, particularly as a climate change mitigation approach and remediation tool in recent years (Tang et al., 2013). Intrest in bochar as soil amendment is increasing because not only can it mitigate climate change by sequestrating C from atmosphere into soil (Lehmann, 2007;Marris, 2006), it also improves soil properties, enhance soil productivity by increasing moisture and nutrients retention (Lehmann et al., 2006) and microbial activity (Lehmann et al., 2011) hence increasing crop productivity. Several kinds of biomass such as crop residues, agricultural by-products, wood waste, forestry residues, organic portion of municipal solid waste, industrial waste and manures serve as raw material sources for biochar production, (Duku et al, 2011).

To boost the production of rice in Ghana, a national policy was put in place to reduce rice importation and has led to the generation of large volumes of rice husk in rice growing areas.

Rice husk waste accounts for 23% of the total paddy weight (Frimpong–Manso et al., 2011). It is highly unsuitable as a soil amendment because of its high C: N ratio which makes its breakdown difficult. Thus the material piles up breeding rodents such as mice, which in turn attract snakes to these sites.

Ghana has become the second largest producer of cocoa beans with about 1,000,000 metric tonnes of the beans being produced in 2012 (USDA, 2012). It is estimated that to produce one tonne of marketable cocoa beans between 25,000 and 30,000 pods would have to be cracked (STCP, 2007). The cracked cocoa pods are dumped near the farms becoming an important supply of disease inoculum to the crop growing in the field (Figueira, 1993). In the past, ashed cocoa pods were used in soap preparation, but with the invasion of imported soap, the locally manufactured soap is no longer attractive to Ghanaians leading to the piling up of the cocoa husk. Thus any technology that will transform the pods into a valuable material will go a long way in reducing the amount of cocoa waste produced.

Pyrolysis is likely to reduce the size of bio-solids, remove pathogens and organic matter is changed into bio-fuel, bio-oil and biochar (Lu et al., 2011; Domı´guez et al., 2006). Biochar pyrolysed from agricultural waste contain high elemental carbon, some nutrients including a large amount of exchangeable cations. Biochar has a large surface area and with its high nutrient composition especially P (Hossain et al., 2010, 2011; Lu et al., 2011), it can be exploited for use as soil amendment to boost microbial population and activities to degrade pesticides.

It is in the light of these that this study was carried out to elucidate the effectiveness or otherwise in using cocoa pod and rice husk biochar as an amendment in remediating pesticide contaminated soils in some soils of Ghana.

For wide applicability and to be able to transfer the results to most agricultural soils of the country,

it is also important for a trial to be carried out on both light, Adenta (Acrisol) and heavy, Akuse (Vertisol) textured soils.To be able to transfer the results to other agricultural soils of the country, it is also important for an agronomic trial to be carried out on both light, Adenta (Acrisol) and heavy, Akuse (Vertisol) textured soils..

The objectives of the study, therefore, were to

- Evaluate the effectiveness of two feedstock-derived biochars (cocoa and rice Husk) to enhance the remediation of atrazine and paraquat contaminated Acrisol (Adenta series) and Vertisol (Akuse series).
- Gain a better understanding of how biochar actively supports the growth of beneficial soil microorganisms and enhance their activities in pesticide contaminated soils.

1.3 Hypothesis:

HO: Addition of biochar to Ghanaian soils enhances degradation of pesticides.

HA: Addition of biochar to Ghanaian soils doesn’t enhance degradation of pesticide.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

Agriculture sustainability is crucial for human's survival given the rapid rate of the population growth. Sustaining or enhancing crop yields on remaining farm lands, either on a large or small scale, is very necessary since expanding agriculture into remaining natural vegetation is damaging. Pest control is among the controlling factors of agriculture particularly under tropical climatic conditions. The control of pests biologically is normally inadequate and mechanically is either strenuous by hand or demanding with machinery. Thus the use of pesticides have, therefore, become an inevitable tool in controlling the pests of various field crops worldwide including Ghana (Amoah, et al., 2006). Their persistent use leads to build up of toxic residues in the ecosystem especially the soil. Thus integrated pest management systems and also soil remediation programmes for already contaminated soils such as bioremediation is being adapted to help overt the difficulties associated with the usage of pesticides. In the ensuing paragraphs, literature is reviewed on biochar, bioremediation and pesticides in the soil environment.

2.1 Pesticide

According Food and Agricultural Organization (FAO) any substance intended for inhibiting, destroying or regulating pests is a pesticide. The pests comprises vectors of human or animal disease, undesirable plants or animals species causing damage or meddling with the production, handling, storage, transport or marketing of agricultural produce and inputs . Substances intended for use as a plant growth regulator, defoliant, desccant or agent for thinning fruit or stopping the

premature fall of fruit and substances applied to crops either before or after harvest to keep the commodities from declining during storage and transport are pesticides (WHO/FAO, 2005).

In agriculture, pests have been a threat to productivity because of their destructive activities and the economic damage they cause to crops before and after harvesting. Extensive use of pesticide is, therefore, due to the advantages they offer. They are effective and reliable in keeping crops healthy and prevent them from being destroyed by pests' infestation. Pesticides work fast in emergency situations especially when crops are under immediate threat of infestation. It is estimated that about one thousand four hundred (1400) pesticide ingredients are used in the agricultural sector, (Kolpin et al., 1998). Ghana currently consumes about 25% of these pesticides (Ntow et al, 2006). Aside the beneficial effects of pesticides, its negative effects on environmental quality and health have been well researched worldwide and create a major issue that contributes to worries at local, regional, national, and international scales (Ntow, 2001, Cerejeira et al., 2003).

Pesticide residues pollute soils and water, persist in the crops, move in to the food chain and are consumed by humans through food and water. Natural habitat deterioration and biodiversity losses as a result of pesticide use is well documented (Sattler et al., 2006). An occurrence of pest resurgence, resistance development to pesticides, pest outbreaks and non-target species destruction has been reported. Intensive pest management is generally required for vegetables production as it's attract a wide range of pests and diseases (Dinham, 2003).

Misapplication of highly toxic pesticides such as paraquat is among the unsuitable pests control practices in agriculture production in Ghana which results in pesticide contamination of the produce itself as well as the environment. While there is an increased concern by Ghana's elite

about the adverse effects of pesticide on the environment and the condition of the country's natural resources, little scientific research has been done to address the matter, (Ntow, 2001).

Safe pesticide management plays a vital part in the context of efforts to attain harmless, sound and viable production of vegetables. According to Bull (1982), management of pesticide includes all phases of the safe, effective and economic use in control of pesticides. Appropriate use of pesticides in Ghana means taking into consideration the wellbeing, social and economic realities of life. It entails using pesticides which can carefully be applied only when necessary in the right health, social and environmental perspective (Osafo and Frempong, 1998).

2.2 Uses of Pesticides

Pesticides are used generally to control organisms considered harmful to biodiversity and their products. In the tropical environments, the loss of crops due to pests, plants diseases and competition from weeds is enormous. According to the United State of America Data Programme (2003), to destroy vectors like mosquitoes that can transmit deadly diseases like West Nile virus, malaria and yellow fever pesticides are used. Pesticides can inhibit illness in humans that could be triggered by mouldy food. Animals can also be protected from parasitic infections such as fleas by pesticides. Pesticides are also used in clearing weeds that may cause environmental damage. Application of pesticides to ponds and lakes control algae and plant for instance wiregrasses that can impede activities like swimming and fishing causing the water to look or smell unpleasant. Wooden arrangements of houses such as ceilings, doors, and window frames can also be protected from pests such as termites may be controlled by pesticides. Pesticides have been used in grocery stores and food storage facilities to reduce post-harvest losses of produce such as grains by rodents and insects (Clarke et al., 1997). Various studies found that not using pesticides reduced yields by 10% (Adeyeye and Osibanjo, 1999) and aslo a ban on pesticides in the United States of

America may possibly cause increase in food prices, job loss and a surge in global hunger (Repto and Balige, 1996). Although the World Health Organization has given a solid backing to fighting malaria with pesticides, each use brings some related hazards. Proper use of pesticide nevertheless reduces these accompanying hazards to a level considered tolerable by Pesticide Regulatory Agencies such as United State Environmental Protection Agency. Widespread use of pesticides, therefore, is due to the advantages they offer, that is, keeping crops healthy and preventing them from being wasted by diseases and infestation.

2.3 Herbicides

Herbicides are generally pesticides used in the control of weeds. The use of herbicides to control weeds has a number of merits over other methods. The advantages include the control of weeds between rows as well as within rows and use of pre-emergence herbicides provide weed free environment for the crop from the beginning (Baker & Terry, 1991). Herbicides also improve crop growth by suppressing weeds at later stages and when the soil is not workable owing to incessant rains (Singh et al., 1996). Weeds which are difficult to control by physical or mechanical means are easily controlled by herbicides. In some cases, herbicide helps reduce the need for pre planting tillage and used as a zero tillage method. Uses of herbicides enhance the ability to cover a large area in a particular growth stage saving cost and time compared to manual weeding (Singh et al., 1996).

Like any other control methods, herbicides usage has its own drawbacks including leaching and run off into nearby fresh water (lakes, lagoons and rivers) and ground water, causing substantial harm to neighboring desirable organisms. Herbicides also contaminate the environment, especially the soil and kill non-targeted organisms. They are expensive and misuse and prolonged usage can cause resistance in some weeds. They have adverse effects on the human body

particularly those who apply it causing nausea, frequent headaches and chronic fatigue.

Several of the herbicides used to protect crops are grouped into chemical families: organochlorines, organophosphates, carbamates, phenoxy and benzoic acids (2, 4-D), triazines (atrazine), paraquat and glyphosate (Miller, 2002).

2.3.1 Atrazine

Atrazine, 2-chloro-4-(ethylamine)-6-(isopropyl amine)-s-triazine, a selective pre- and post-emergence herbicide is one of the most extensively used herbicides worldwide (Stevens & Sumner, 1991). Atrazine kills weeds by attaching to the quinone-binding protein in photosystem II, hence, hindering the photosynthetic electron transport. Atrazine is absorbed through roots of weed, depending on the time of application and weed species some foliage absorption can take place (Burken & Schnoor, 1997). Atrazine is the most common and extensively used herbicide on cereal farms and is mobile in the soils (Battaglin et al., 2003; Ribeiro et al., 2005). If atrazine is applied to the soil before a heavy downpour or in the event of irrigation can be washed out from the root zone into ground water resources.

Atrazine is frequently used in combination with other herbicides as compared to other chlorinated herbicides (Chan & Chu, 2005; Eldridge et al., 1999; USEPA, 2003). Due to the persistence of atrazine in soils, in addition to their runoff to surface and groundwater, severe environmental problems emerges even though it has a low toxicity on humans(Dos Santos et al, 2004).

As a result of atrazine's low biodegradability and high potential to contaminate e surface waters and groundwater is a pollutant of environmental concern (Chan & Chu, 2005). Though atrazine is a banned or a controlled substance in numerous countries, 100 mg/L to 1µg/L concentrations have

been reported in surface waters (Parra et al., 2004). The acute toxicity of 40 herbicides were compared utilizing nine diverse means of action on the green alga Raphidocelis subcapitata, Ma et al. (2006) establish that photosynthesis was the most delicate process, and atrazine was among the most toxic herbicides used.

Atrazine can be degraded chemically and biologically in soil. It is difficult to differentiate between the biological and chemical source of atrazine degradation. For example, hydroxyatrazine can result from either chemical hydrolysis (Armstrong and Konrad, 1974) or from metabolism by some soil fungi (Owen, 1989). Although N dealkylation can occur as a result of chemical degradation (Lerch and Li, 2001), it is also an essential process of biological degradation and might be brought about by fungi and bacteria present in the soil (Lin et al, 2005; Weber, 1977). It has been found that some microorganisms remove the ethyl side chain preferentially (Skipper and Volk, 1970; Schiavon, 1988), while others preferentially remove the isopropyl side chain (Lin et al, 2005). Even though atrazine has toxic effects on existence, its metabolites, including deethylatrazine and deisopropylatrazine, are currently presumed to be less toxic than their parent molecule (Kross et al., 1992; USEPA 2002). Nonetheless, particularly in agricultural soils, deethylatrazine and deisopropylatrazine, which keep the chlorine atom, are considered phytotoxic (Hounout et al., 1998).

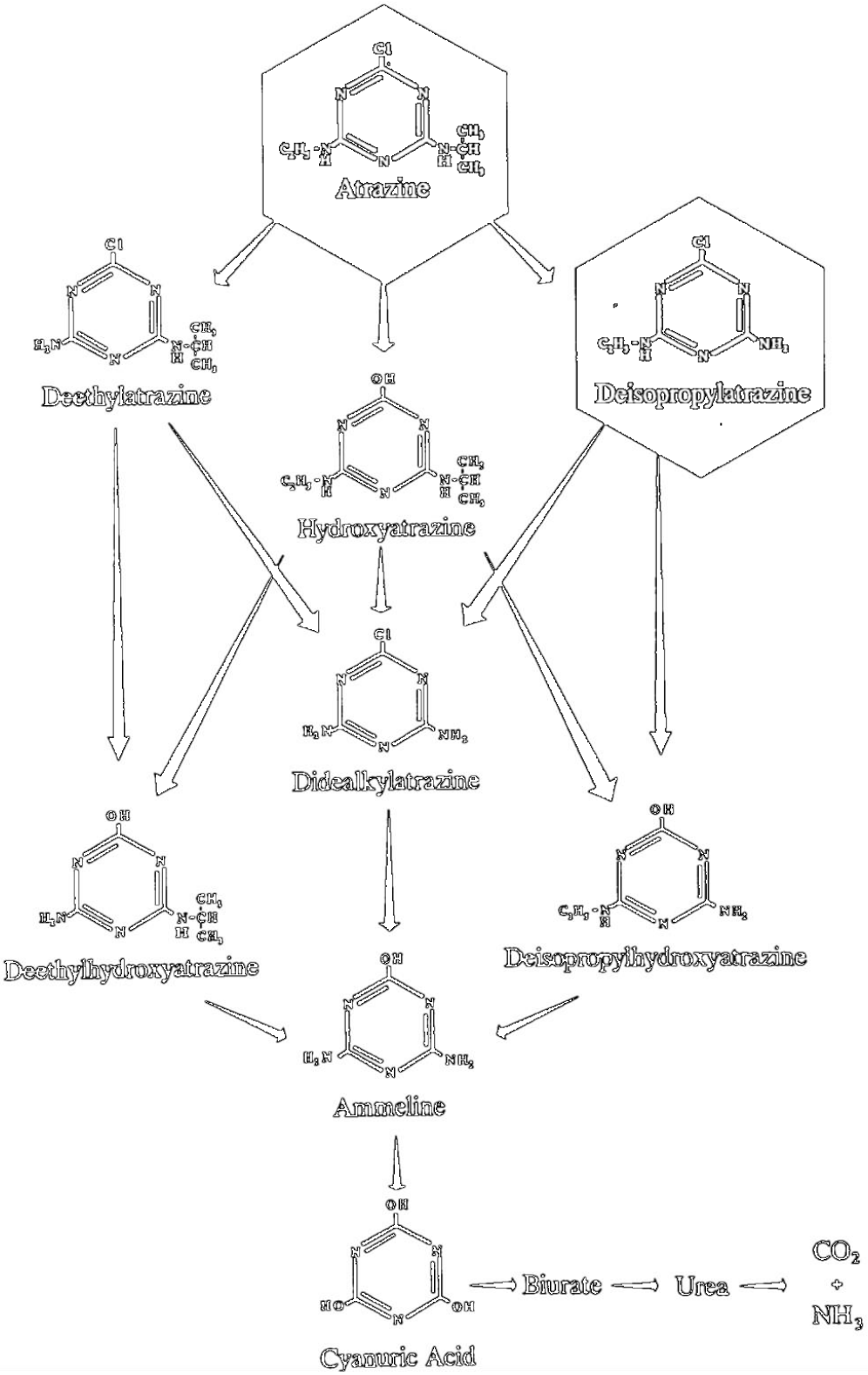


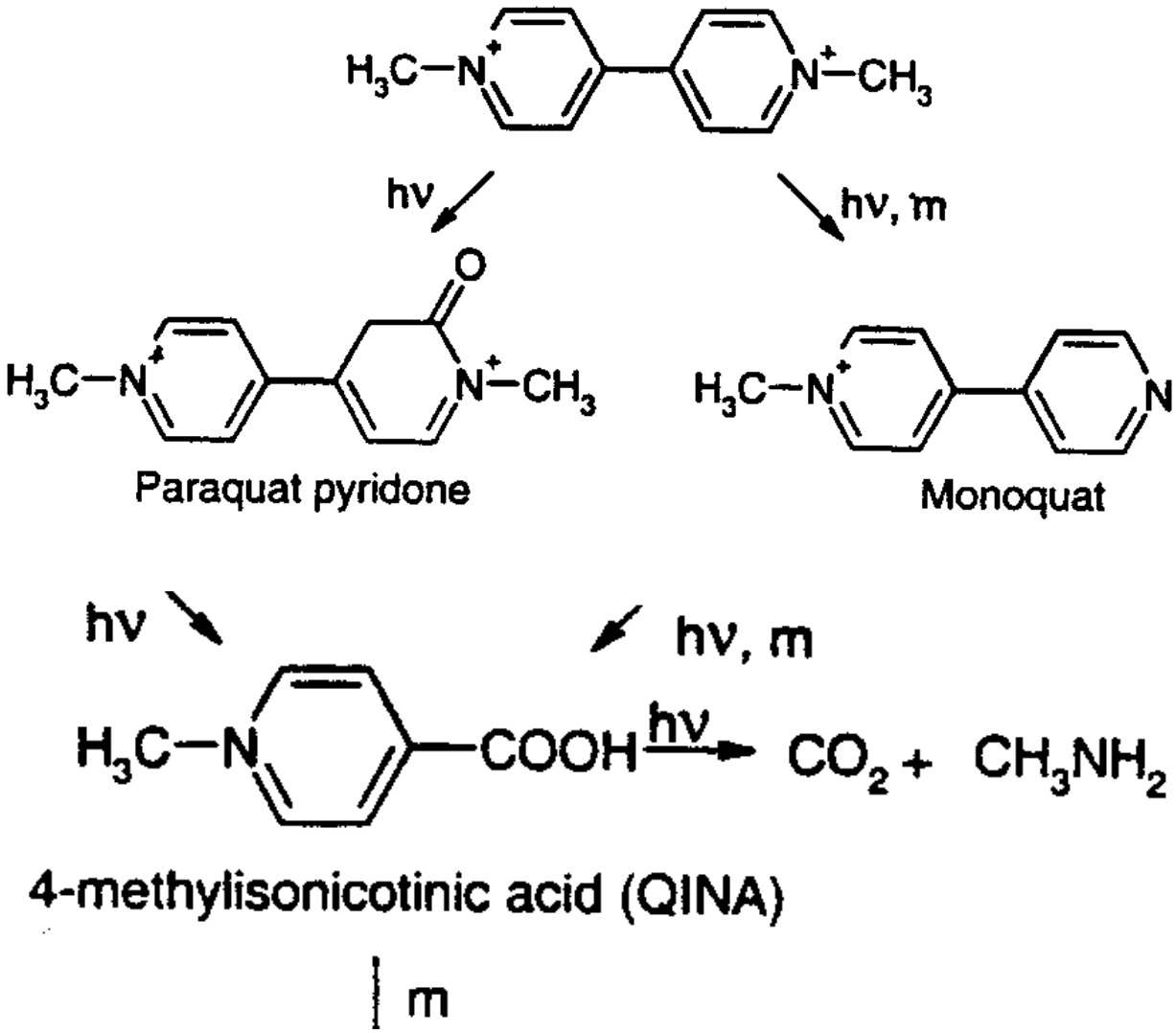
Fig 2.1 Degradation of Atrazine

2.3.2 Paraquat

Paraquat (1, 1'-dimethyl-4, 4'-bipyridylium dichloride) is a broad-spectrum contact herbicide widely used around the world for all spectra of weeds due to its excellent effect within plant cells in crop protection and horticultural use. It is a fast acting, non-selective herbicides, foliar-applied and can destroy tissues of weeds on contact and by translocation within the weeds (Suntres, 2002; Bromilow, 2003). The use of paraquat has brought substantial benefits to agriculture production globally.

Paraquat is immobilized on clay soil fractions shortly after application (Tucker et al, 1967). The adsorption of paraquat by clay colloids renders the herbicide unavailable for microbial degradative attack, and consequently, paraquat may remain unaltered, albeit biologically inert, in the soil for many years with negligible rates of loss from the environment (Riley et al, 1976;Smith et al, 1978). In the short period of time between its application and deactivation by soils, however, paraquat may be degraded by soil microorganisms (Burns, 1970). Studies on the microbial degradation of herbicides in soils have often proved difficult (Hills and Arnold,1978), and owing to the adsorption of paraquat, studies on paraquat degradation in situ have been particularly so. Thus, the majority of studies have been carried out in vitro. Several microorganisms have been reported as being capable of the in vitro degradation of paraquat (Funderburk and Bozarth, 1967). In the majority of the cases, however, paraquat degradation was shown to be extremely variable in both extent and rate, and evidence of only one degradation product other than CO_2 has been reported, the N-methyl betaine of isonicotinic acid (Funderburk and Bozarth, 1967). The latter product has, however, been shown to be a major intermediate in the photolytic degradation of paraquat (Slade, 1965). A strain of the soil yeast *Lipomyces starkeyi* is capable of rapid, efficient, and complete in vitro degradation of paraquat when the herbicide is used as a sole source of culture

nitrogen (Anderson and Drew. 1972; Baldwin et al, 1966). This aerobic degradation is effected over a wide range of pHs and temperatures (Anderson and Drew. 1972). The only product of paraquat degradation identified was CO_2, and no mechanism for the degradation of paraquat was proposed.



$$CH_3NH_2 + CO_2 + \text{formate} + \text{oxalate} + \text{succinate} \rightarrow NH_3 + CO_2 + H_2O$$

hv = Photolytic degradation

m = Microbial degradation

Fig 2.2 Degradation of paraquat.

2.4 Factors Influencing Degradation of Pesticide in Soil

Pesticides undergo a series of reactions in the soil environment before degradation takes place. The intensity of each of degradation mechanism depends on the pesticide physico-chemical properties, soil characteristics, environmental conditions as well as management practices. In this study the relevant factors that influence the pesticides degradation is presented.

2.4.1 Pesticide Structure

A pesticide's physico-chemical property and intrinsic biodegradability is governed by its structure. The susceptibility of a compound to biotransformation is brought about by minor changes in structure. Polar groups such as COOH, OH and NH_2 influence a site's vulnerability to attack by microbes to speed degradation.But other organic compounds such as halogens or alkyls makes the pesticide more resistant to degradation (Cork and Krueger, 1991). Slight alterations in the position of polar groups or substituents type in the same class of pesticides also affects degradation rate (Topp et al., 1997). Atrazine is an example of an asymmetrical mono-chloro-bisalkylamino-s-triazine, which contains in its chemical structure an aromatic hexameric and symmetrical ring constituted by three carbon and three nitrogen atoms in alternate positions (Fig.2.3).

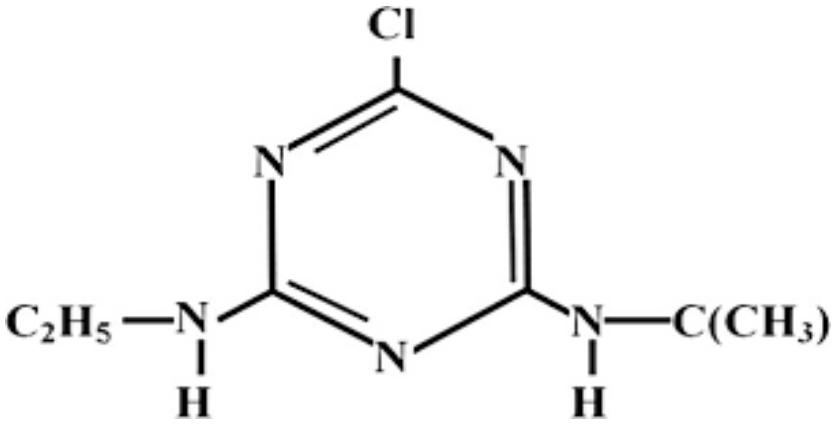


Fig 2.3 Chemical structure of Atrazine (Sene et al, 2010).

Paraquat (1, 1'-dimethyl-4, 4'-bipyridylium dichloride) is bipyridylium quaternary ammonium herbicide characterised by positive charges thus most absorb to negative surface charges. In the soil paraquat bioavailability is rapidly reduced by adsorption. Over a wide range of soils there is more than enough evidence to demonstrate that paraquat adsorption is capable of deactivating the mutiple applications. Furthermore this implies that paraquat is in effect immobile in soils and cannot leach into ground water.

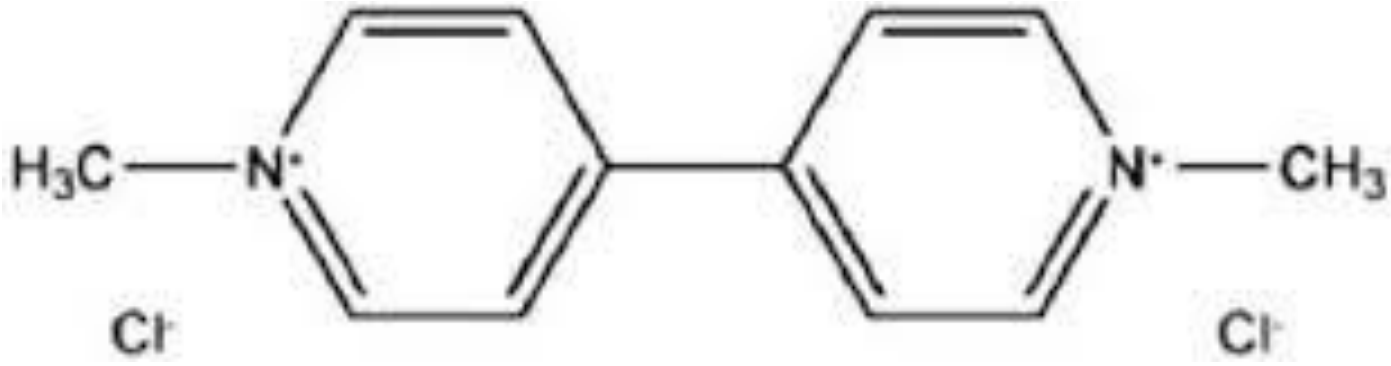


Fig 2.4 Chemical structure of paraquat (Dos Santos et al, 2011)

2.4.2 Pesticide Concentration

Concentration of pesticide application is an important parameter in determining the rate of biodegradation. The degradation kinetics of many pesticides approach first order; the rate of degradation decreases roughly in proportion with the residual pesticide concentration (Topp et al., 1997). Gupta and Gajbhiye (2002) reported that the half-life of flufenacet in three Indian soils, viz., Inceptisol, Vertisol and Ultisol, varied from 10.1 to 31.0 days at low rate (1.0 µg g-1 soil) compared to 13.0 to 29.2 days at high rate (10.0 mg kg^{-1} soil) of application. Butachlor at higher application rate in soil had reduced degradation rate which could be attributed to limitation in the number of reaction sites in soil and the toxic effect on microorganisms or enzyme inhibition (Prakash and Devi, 2000). Studies done by Yu et al (2003), showed that butachlor degradation was dependent on the rate of application and type of soil, hence reported half-lives of butachlor in

non-rhizosphere, wheat rhizosphere and inoculated rhizosphere soils ranged from 6.3 to 18.0 days at 1.0 mg kg-^{1}, 2.9 to 19.9) days at 10.0 mg kg-1 and (10.8 to 23.2) days at 100.0 mg kg-1.

2.4.3 Pesticide Solubility

Low solubility pesticides have a tendency to be more resistant to microbial degradation than high solubility compounds. Microorganisms can only benefit from the fraction of the pesticide dissolved in soil solution. The pesticide dissolution rate would, therefore, be regulated by biodegradation rate (Cork and Krueger, 1991).

McCall et al. (1980) suggested that the organic carbon water partition coefficient (Koc) value provides a reliable estimate of a chemical's mobility in soil. Atrazine has a Koc value of 170 which indicates that it is moderately mobile. Pesticide degradation products are usually more soluble than the parent molecule. Differences in molecular symmetry, and therefore polarity, can account for higher solubilities of structurally modified atrazine (Esser et al., 1975). With the use of soil thin-layer chromatography, Somasundaram et al. (1991) found that both the octanol water partition coefficient (Kow) and water solubility significantly correlated with the mobility of atrazine and hydroxyatrazine.

2.4.4 Soil Types

Degradation of pesticides in soil is inflenced by soil properties such as, clay content, organic matter and pH (Gupta and Gajbhiye, 2002). The soil plays a very vital role in pesticide degradation by providing a suitable environment for the degradative microorganisms. Sorption of pesticides by soil particles governs their bioavailability affecting their persistence. The type of clay and organic matter are important soil parameters that influence the activity of pesticide degrading microorganisms.

Even though paraquat is strongly adsorbed by almost all soil types, it interacts differently with components of soil such as organic matter and clay (Summers, 1980). Studies have proved that 300 mg of paraquat ion/kg of soil was adsorbed, 2500 - 3000 and 7500 -8500 mg/kg, by kaolinite and montmorillonite, respectively (Coats et al, 1964). The study established that adsorption by montmorillonite was greater than the adsorption in kaolinite and this was due to the large surface area of the former's expanding clay lattice structure as compared to nonexpanding clay lattice of the latter. However, at higher concentrations of paraquat, ammonium ions were found to be capable of partially substituting paraquat in both soil and kaolinite, but to a very limited magnitude in montmorillonite due to adsorption occurring via several mechanisms. By using X-ray diffraction further research works have provided an understanding into the processes of paraquat adsorption (Summers, 1980; Knight and Denny, 1970).

Studies have shown atrazine adsorption/desorption on the soil particles are very vital factors, which decides its environmental fate (Moreau-Kervevan and Mouvet, 1998). Other research works found that with atrazine adsorption on clay irrespective of its chemical nature, acidic H-montmorilonite provided enhanced function compared to near neutral Na-montmorillonite (Bailey et al., 1968). It has been observed that with an increase in the surface charge density of smectities, atrazine adsorption is reduced and the process is also reversible (Barriuso et al., 1994) with slight negative and positive attraction on some clay detected. Atrazine desorption from alluvial samples, tile and sand low in organic matter were hysteretic (Roy and Krapac, 1994). Downward movement and leaching of atrazine is considerably reduced since it is readily adsorbed on clayey soils or muck than on low organic matter and clay soils (El-Bestawy, 2013).

2.4.5 Soil Moisture

Microbes always need a film of water around them to function, and it also serves as solvent for pesticides movement and diffusion. In very dry soils, degradation is very slow as pesticide transformation rate mostly increases with increasingwater content. Anaerobic pesticide degradation is prevalent in very wet soils such as rice paddies than aerobic degradation, as atmospheric oxygen diffusion rate in the soil is very restricted. Pesticide degradation can also be accelerated or retarded by low oxygen transfer at high moisture content (Walter-Echols and Liechtenstein, 1978). Under aerobic condition, atrazine and trifluraline degrade more rapidly than underaerobic condition (Ponnamperuma, 1972).

2.4.6 Temperature

Temperature effect on the degradation of a pesticide is influenced by the molecular structure of the pesticide. Adsorption of pesticides in soil is influenced by temperature which in turn controls the hydrolysis and solubility of pesticides (Racke et al., 1997; Burns, 1975). While adsorption and desorption mechanisms are exothermic and endothermic respectively, it is estimated that as adsorption decrease with rise in temperature, pesticide solubility will decrease. Microbial activity is accelerated by temperature increase and thermophiles are likely to govern reactions at high temperatures.

An increase in temperature stimulates microbial activity and at certain temperature some ecological groups tend to dominate. A study of the effect of rimsulfuron on the growth and activity of microbial biomass under laboratory conditions at varying conditions of temperature in a silty clay loam soil Perucci et al. (1999). The onset and magnitude of the effects of rimsulfuron were temperature dependent and generally slight and transitory. Rimsulfuron hydrolyses rapidly in soil under conditions of high temperature (Vischetti et al., 2000). The maximum growth and activity of

microorganisms in soils occur at 25-35°C (Alexander, 1977) and pesticide degradation is optimal at mesophillic temperature range of around 25-40°C (Topp et al., 1997).

2.4.7 Soil pH

Pesticide adsorption is influenced by soil pH, as well as its degradation abiotically and biotically (Burns, 1975). Soil pH has an effect on the pesticide molecules sorptive behavior on organic and clay surfaces and hence, the speciation chemically, bioavailability and movement (Hicks et al., 1990). Pesticides susceptible to alkaline or acid catalyzed hydrolysis greatly influence effect of pH on degradation (Racke et al., 1997). In acidic or neutral solutions paraquat is chemically stable however in alkaline solutions especially at pH > 12 is hydrolyzed (Topp et al., 1997).

All microbial species are able to survive only within a specific pH range. Additionally, soil pH may influence availability of nutrients. Biodegradation of most pesticides is ideal at pH 7 (neutral); the standard range is pH 6 – 8 (US EPA, 2006).

Generally heterotrophic bacteria and fungi prefer a near neurtality pH, with fungi being more acid tolerant (Atlas, 1978). Therefore under extreme pH conditions such as salt marshes and hot springs, pH would have a negative impact on the capability of microbes to degrade pesticides (Patrick and DeLaune, 1977).

2.4.8 Soil Organic Matter

The presence of organic matter may alter the behaviour of pesticides in soils. Soil organic matter can either decrease the microbial mediated pesticide degradation by stimulating pesticide adsorption processes or enhance microbial activity (Perucci et al., 2000) by co-metabolism (Nair and Schnoor, 1994; Thom et al., 1997). The addition of organic materials to flooded soils enhanced the bacterial degradation of some organo-chlorine insecticides such as BHC, DDT, methoxychlor

and heptachlor (Yoshida, 1978). Microbial degradation of linuron (Hicks et al., 1990) and pencycuron (Pal et al., 2005) in non-sterile soils were stimulated by organic matter amendment. A certain minimum level of organic matter (greater than 1.0%) is essential to ensure the presence of an active autochthonous microbial population that can degrade pesticides (Burns, 1975). The species diversity arising from such situation may increase the presence of sufficient number of enzyme systems that are able to attack pesticide molecules (Farmer and Morrison, 1964; Butcher et al., 1969).

2.4.9 Soil Biotic Components

Pesticides degradation under non-sterile condition is faster when contrasted with degradation under sterile condition showing the function of microbes in the degradation of pesticide. Various works have indicated pesticide degradation in soil by microorganisms (Banerjee et al., 1999; Hafez and Thiemann, 2003; Sukul and Spiteller, 2001). The pesticides breakdown in soils is achieved by a range of biotic processes including the utilization of pesticides as carbon, energy and nitrogen sources. Microorganisms can likewise degrade pesticides co-metabolically (Blazes and Edwards, 1980). It is advantageous to study a new pesticide for degradation including the persistence of metabolites produced in diverse agroclimatic conditions. For the introduction of new pesticide, it is worthwhile to study its degradation including the persistence of metabolites formed in different agroclimatic situations.

2.5 Effects of Pesticides on Soil Microbiological Parameters

The population of soil microbes may change both quantitatively and qualitatively in numerous means by pesticides. The utmost noticeable impact is the immediate toxicity of applied pesticide to the susceptible species of microbes (Matsumara and Boush, 1971). As a result of reduced competition, different microbial species get to be resistant to the pesticide and can build their

biomass. Different parameters for example, soil microbial biomass, identification of various microbial populaces, estimation of functional or particular soil enzyme activity have been used to measure potential inhibitory impact of a pesticide (Topp et al., 1997). Studies of microbes depend more on soil microbial biomass than the numbers of soil microbes. Normally just a little portion of the microbial population is evaluated using microbial strategies; under research conditions the population number of these is non-culturable in artificial media (Trevors, 1998).

2.5.1 Soil Microbial Biomass

The living portion of organic matter in soil including microorganisms smaller than 5-10 µg is termed as soil microbial biomass. Generally microorganisms include bacteria, actinomycetes, algae, protozoa and micro fauna. Faunas greater than 5-10 cubic micrometer and roots of plants and earthworms, are excluded (Sparling, 1985).). As indicated by Duah-Yentumi and Johnson (1986), carbofuran and simazine have no noticeable hindering impact on soil microbial biomass; however continous utilizations of paraquat essentially diminished microbial biomass, generally the fungal biomass. Significantly variable impacts may exist on soil microbial biomass created by single or continous utilizations of various pesticides (Duah-Yentumi and Johnson, 1986).

A native fungal strain isolated from corn field soil readliy made use of atrazine as a nitrogen source (Singh et al., 2004). Another study also established that diverse groups of pesticide can be degraded or mieralised by several bacteria and fungi present in soils (Briceno et al., 2007). Also amending the soil with organic materials can stimulate pesticide adsorption, movement and biodegradation. In the same field frequent pesticide application for a number of years influenced the build up of a vigorous microbial population in soil with the ability to degrade known pesticides (Hicks et al., 1990).

Additionally the species of soil microoganisms were able to degrade alachlor, atrazine, monocrotophos and 4-chlorophenol (Yu et al, 2006; Bhadbhade et al 2002).A trazine degradation has been found to be dependent on alachlor degradation. The genera *Arthrobacter*, *Clavibacter*, *Nocardia*, *Rhodococcus*, *Nocardioides*, and *Streptomyces* give the impression as pesticide degrading *Actinomycetes* (De Schrijver and De Adage, 1999). Moststudies on the influence of different pesticides, particularly the herbicides were done mostly under laboratory conditions and barely reflects field. Pesticides, when connected at field rate, don't impede microbial biomass.

2.5.2 Microbial Activity

The metabolic state of microorganisms is not determined by evaluating microbial biomass and ergosterol. Alongside microbial biomass, microbial activities have to be similarly evaluated for right assessment for the functioning of the ecosystem and soil disturbing effects because of perturbations (Brookes et al., 1987).

2.5.2.1 Soil Respiration

Active living cells require constant energy supply, which the heterotroph acquires via the decomposition of organic matter with the evolution of CO_2 and H_2O. Thus by assessing evolution of CO_2 or uptake of oxygen, metabolic processs of microorganisms can be evaluated (Nannipieri et al., 1990). One of the oldest but still the most frequently employed parameter for assessing microbial activity in soil is soil respiration. It can be examined both in un-amended and amended soils. Basal respiration discloses total possible microbial activity (Dark, 1990). Substrate induced respiration is a gauge of the complete physiologically active portion of the soil microflora (Anderson and Domsch, 1978). The availablity of carbon to organisms is expressed by a combination of the basal and substrate induced respiration (Cheng et al., 1996). To depict the microbial status of soil both methods were usually used as bio indicators of health or quality of the

soil (Gregorich et al. 1994; Pankhurst et al., 1995). Comparable to other metabolic processes, soil respiration depends on the physiological state of the microbial cells and as affected by a few soil constituents. Soil respiration is often used in assessing the lateral effects of chemicals, for instance, pesticides and heavy metals (Alef, 1995). The level of inhibition impact relies on the influence of the intensity as well as on the exposure time of the microorganisms to stress. Atrazine has been found to essentially increase the soil respiration after 96 h of incubation (Tu, 1992).

2.5.2.2 Ecophysiological Quotients (qCO_2 and QR)

The ratio of soil basal respiration to microbial biomass (specific respiration of the biomass or microbial metabolic quotient , qCO_2) taking into account Odum's hypothesis of biological system progression (1969) was suggested as a preference rate of alterations in the physiological condition of the microbial group and its activity in light of unsettling influence (Anderson and Domsch, 1985, 1990). Jones and Ananyeva (2001) found a similar correlation between microbial metabolic quotient and pesticide degradation rate constant in diverse soil biological communities. Microbial metabolic quotients increments because of disturbances brought on after pesticide application as a result of microorganisms use of a huge portion of their energy budget for maintenance rather than cell synthesis (Anderson and Domsch, 1990). Organic matter amended soils, generally, showed minimal qCO_2 values than the un-amended soils, showing a higher usage of the substrate for growth of the cell than cell maintenance (Perucci et al., 2000).

The chemical characteristics of the pesticide applied and also the diversity of the indigenous microorganisms are likely to determine the potential stress (Jones and Ananyeva, 2001). The extent of the reaction to pesticide affected stress or upsetting influence should be contrasted with that of normally happening stressors, for example, dampness (Domsch, 1984). The respiration per

unit of biomass is a more delicate marker of toxic impacts than the respiration rate or the measure of biomass alone (Beelen and Doelman, 1997).

The proportion of basal soil repiration to substrate-induced respiration (QR) has beenutilized to evaluate the impacts of different perturbations in soil ecosystems (Anderson and Domsch, 1985; Insam and Domsch, 1988). Basal soil respiration is by and large ascribed generally to the metabolically dormant population and that of substrate-induced respiration to the metabolically activated population (Ohya et al., 1988). Pencycuron application at field rate had no inconvenient impact on the eco-physiological parameters however not at higher rates (Buddy et al., 2005).

2.5.3 Transformation rate of pesticide with Soil Microbiological Processes

Microbial processes had the most impact on the degradation of pesticides in soil (Alexander, 1994). Investigation of relationship between ecosystem properties, the size and makeup of microbial biomass and pesticide degradation limit might be helpful for evaluation of environment and landscape dynamics of pesticides. No connections were found between microbial biomass and degradtion of 2, 4-D and atrazine (Passage et al., 1994; Ghani et al., 1996). It was opined that this relationship may be helpful for creating approaches for assessing and foreseeing the fate of pesticides in different ecosystems (Voos and Groffman, 1997).

In microbial biomass C content gave parabolic curves ($p<0.05$ in all cases)inThe relationship between pesticide degradation and microbial biomass C content gave parabolic curves ($p<0.05$ in all cases) under all conditions tested for rimsulfuron, imazamox and benfluralin (Vischetti et al., 2002;Perucci et al., 1999; Vischetti et al., 2000). They proposed that quadratic equations may be useful in order to deduce the trend of soil microbial biomass in relation to pesticide concentration. From these equations it was possible to observe that the trend of the parabolic curves were similar

and independent of initial concentrations. These relationships helped in modeling behaviour of soil microbial biomass after pesticide treatment.

2.6 Microbial degradation and bioremediation

Microbial degradation is the procedure of breakdown of by and large intricate, organic pollutants (contaminants) to smaller, simpler products by the activities of microorganisms (Sharma, 2010). These natural substances serve as the microbial nourishment source or substrate. Degradation includes a sequence of biological degradation steps (pathways) that will finally result into the oxidation of the parent compound and frequently into generation of energy. Complete biodegradation or mineralisation includes oxidation of parent compound to form carbon dioxide and water, a procedure giving both carbon and energy for growth and propagation of microbial cells (Sharma, 2010).

Microbial degradation is the process of breakdown of generally complex, organic pollutants (contaminants) to smaller, simpler products by the activities of microorganisms (Sharma, 2010). These organic substances serve as the microbial food source or substrate. Degradation involves a series of biological degradation steps (pathways) that finally result into the oxidation of the parent compound and often into energy generation. Complete biodegradation or mineralisation involves oxidation of parent compound to form carbon dioxide and water, a process providing both carbon and energy for growth and reproduction of microbial cells (Sharma, 2010).

Bioremediation is ‘the use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere’ (Sharma, 2010). Bioremediation has been defined as “the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes” (Office of Technology Assessment, 1991). This technology is based on

the premise that a large percentage of the pollutants are readily biodegradable in nature (Atlas, 1984, Prince, 1993). It relies on biological processes to minimize an unwanted environmental impact of the pollutants. The microorganisms, in particular have the abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds (Sharma, 2010).

Bioremediation has emerged as one of the most promising treatment options for pesticide contamination. During bioremediation, microbes utilize chemical contaminants in the soil as carbon and energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. For example, atrazine and paraquat can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water (Nester et al., 2001). When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic respiration prevails. Generally, inorganic compounds such as nitrate, sulfate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation (State of Mississippi, Department of Environmental Quality, 1998).

Bioremediation strategies are based on the application of various methodologies to increase the rate or extent of the biodegradation process. The success of oil spill bioremediation depends on the ability to optimize various physical, chemical, and biological conditions in the contaminated environment. The success of pesticide bioremediation depends on our ability to establish and maintain conditions that favour enhanced pesticide biodegradation rates in the contaminated environment.

Pesticide bioremediation in soil can be promoted by stimulation of the indigenous microorganisms, by introducing nutrients and oxygen into the soil (biostimulation) (Seklemova et al., 2001) or through inoculation of an enriched microbial consortium into soil (bioaugmentation) (Richard and Vogel, 1999; Barathi and Vasudevan, 2001).

2.6.1 Bioaugmentation

Bioaugmentation involves the introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms (Biobasics, 2006). Although pesticide-degrading microorganisms are widespread in nature, bioaugmentation is been deliberated as a prospective strategy for pesticide-bioremediation since. The rationale for adding pesticide-degrading microorganisms is that indigenous microbial populations may not be capable of degrading the wide range of pesticides present in a particular soil (Leahy and Colwell, 1990). Other conditions under which bioaugmentation may be considered are when the population of indigenous degrading microbes is low, the speed of decontamination is the primary factor, and when seeding may reduce the lag period to start the bioremediation process (Forsyth et al., 1995).

2.6.2 Biostimulation

Biostimulation also uses indigenous microbial populations to remediate contaminated soils. Biostimulation includes the growth of rate-constraining nutrients to quicken the biodegradation process. In many ecosystems that have been heavily contaminated with pesticide, nutrients deficiency is main the restricting variables in biodegradation. Biostimulation comprises of adding nutrients and different substances to soil to catalyze common weakening procedures. Most laboratoryexperiements have demonstrated that amending the soil with deficient nutrients has improved the rate of biodegradation. Application of nutrients sorts and focuses change broadly

relying upon the pesticide properties and the ecological conditions. Biostimulation is a more powerful approach in light of the fact that the option of corrupting microorganisms won't upgrade degradation more than straightforward supplement option (Lee et al, 1997; Venosa et al., 1996).

2.7 Advantages and Disadvantages of Bioremediation

Bioremediation has numerous advantages over the traditional methods of decontamination used. It is comparatively economical. Bioremediation is additionally a more environmentally friendly as it includes the subsequent degradation of pesticide to mineral products, such as carbon dioxide and water, while physical and chemical techniques typically remove the contaminant from one ecological environment into the other. Since it depends on natural process, it is less disturbing to the polluted site.

Bioremediation also has its disadvantages. It involves a various heterogeneous and complex procedures. The success of bioremediation relies on having the appropriate microorganisms under suitable natural conditions. Its operational use can be constrained by the structure of the pesticide. Bioremediation is additionally a normally a slow process, taking weeks to months to produce results, which may not be plausible when quick cleanup is required. Concerns likewise emerge about potential antagonistic impacts connected with the use of bioremediation agents. These incorporate the toxicity of bioremediation microbes themselves and metabolites with their attendant eutrophic impacts (Swannell et al., 1996).

2.8 Biochar

2.8.1 Origin and production

Biochar is a fine-grained geosorbent, prepared from the pyrolysis of organic materials different from charcoals in its use as a soil amendment. Biochar was at first connected to the survey and

archeological investigation of early human settlement and soils. These early investigations of soils which involved the planned blending of smoldered biomass in soils around human settlements started later enthusiasm for biochar (Lehmann et al., 2003). The Pre – Columbian Amazonians are believed to have utilized biochar to upgrade soil profitability. It is created by burning anaerobically different sorts of biomass, for example, agricultural crop residues, forestry residues, wood waste, organic portion of municipal solid waste (MSW) and animal manures (Solomon et al., 2007) in pits or trenches (Lehmann, 2007).

Biochar is created through an energy transformation process called pyrolysis, which is burning without oxygen to avoid complete burning of the natural biomass as happens in open flames (Sohi et al., 2009). Pyrolysis of biomass produces char, oils and gasses. Amid pyrolysis, the polymeric building blocks of biomass, in particular cellulose, hemicelluloses and lignin experience different procedures, for example, cross-connecting, depolymerisation and fracture at different temperatures. The yields of pyrolysis items rely on the type and nature of the feedstock, especially the lignin and procedure conditions, for example, temperature, weight, vapor evacuation arrangement time, warming rates and molecule size (McLaughlin, 2010). The quality and attributes of biochar, for example, thickness, molecule size dissemination, cinder content, dampness substance and pH rely on upon the sort, nature and starting point of the feedstock, together with pyrolysis response conditions (Zhang et al., 2008).

Pyrolysis is generally in three fundamental types; slow, moderate, and quick (Sohi et al., 2009; Chestnut, 2009; McLaughlin, 2010). As can be seen from Table 2.1, moderate pyrolysis and transitional pyrolysis both result in higher biochar yields, while quick pyrolysis gives higher fluid (bio-oil) yields. Biochar is rich in a recalcitrant type of carbon which is not oxidized by soil microorganisms. Biochar has been credited with numerous advantages, including the capacity to

improve the productivity of soils by improving nutrient and water holding capacities of soils (Block, 2010).

Notwithstanding a wide range of materials having been proposed as biomass feedstock for biochar, the suitability of every feedstock for such an application is subject to various physical, natural, and in addition monetary and logistical components (Verheijen et al., 2010, Kloss et al., 2012).

Recent research recommends that the kind of feedstock utilized for pyrolysis is more essential where biochar is to be used as a soil conditioner. Feedstock utilized on commercially include wood chip and wood pellets, tree covering, crop biomass including straw, nutshells, cocoa waste distiller's grain, bagasse from the sugarcane and bovine manure.

The natural proportions of carbon, oxygen and hydrogen are key feedstock parameters in commercial use and the nature of fuel items (Friedl et al., 2005). The extents of hemicellulose, cellulose and lignin content decide the proportions of unpredictable carbon (in bio-oil and gas) and balanced out carbon (biochar) in pyrolysis items. Feedstock with high lignin content create the most astounding biochar yields when pyrolysed at moderate temperatures of about 500 °C (Fushimi et al., 2003; Demirbas, 2006).

Table 2.1 The mean post-pyrolysis feedstock residues resulting from different temperatures and residence times.

Process	Conditions	Liquid (bio-oil)	Solid (biochar)	Gas (syngas)
Fast pyrolysis	Moderate temperature (~500 °C), short hot vapour residence time (<2s)	75%	12%	13%
Intermediate pyrolysis	Moderate temp~500℃, moderate hot residence time of 10 – 20 seconds.	50%	20%	30%
Slow pyrolysis, very long solids (Carbonization)	Low temp~ 400℃, very long solids residence time.	30 %	35 %	35 %
Gasification	High temp~ 400℃, long vapour residence time.	5 %	10 %	85 %

Source: (Verheijen, 2010).

2.8.2 Stability of biochar in the soil

Biochar has long been used to date archaeological deposits by quantifying its carbon-14 decay (Arnold and Libby, 1951). Biochar persists in the environment longer than any other form of organic carbon. Finely divided biochar has remained in soils in humid tropical climates, such as the Amazon, for thousands of years (Sombroek et al., 2003), resisting the rapid rates of mineralization common to organic matter in these environments and producing a distinct black colour.

2.8.3 Structural composition of biochar

Thermal breakdown of cellulose in organic biomass utilized as a part of the generation of biochar between 250ºC and 350 ºC results in huge mass loss of volatile compounds, leaving recalcitrant

amorphous C network. As pyrolysis temperature rises, so does the extent of aromatic carbon in the biochar, because of the relative increase in the loss of volatile matter. Water is lost first, followed by hydrocarbons, tarry vapors, H_2, CO and CO_2. Thereafter, is the transformation of alkyl and O-alkyl C to aryl C (Baldock and Smernik, 2002; Demirbas, 2004). At about 330 ºC, poly-fragrant graphene sheets start to grow laterally, at the expense of the amorphous C phase, which eventually coalesce. Above 600 ºC, non-C molecules are evacuated resulting in the relative increase in the C contents, a process known as carbonization. Carbonisation can be up to 90% by weight in biochars from woody feedstocks (Antal and Grönli, 2003; Demirbas, 2004).

2.8.4 Chemical composition and surface chemistry of biochar

Composition of biochar is highly heterogeneous, containing both labile and steady parts (Sohi et al., 2009). Carbon, volatile matter, mineral matter (ash) and moisture are for the most part viewed as the real constituents of biochar (Antal and Gronli, 2003). The relative proportion of biochar constituents influences the chemical and physical behavior of the material (Chestnut, 2009) and hence its suitability for a particular site application, and additionally fate and mobility in the enviroment (Downie, 2009 check is it Downie et al. 2009).

Higher moisture content increases costs of production and transportation for unit of biochar formed (Antal and Gronli, 2003). Keeping the moisture content up to 10% (by weight) is necessary (Collison et al., 2009). Pre-drying of biomass feedstock is therefore an important assignment in biochar production. Carbon content in biochar has been found to run between 172 to 905 g/kg, albeit organic carbon regularly represents under 500 g/kg, as reported by Chan and Xu (2009) for an assortment of source materials.

Total N differs somewhere around 1.8 and 56.4 g/kg depending on the feedstock (Chan and Xu, 2009). In spite of the apparently high total N content of biochar it may not automatically be available to crops, since N is for the most part present in an unavailable form with mineral N substance less than 2 mg/kg (Chan and Xu, 2009). The C: N proportion in biochar has been found to differ generally between 7 and 500 (Chan and Xu, 2009). Total P and total K in biochar extensively vary between 2.7 to 480 and 1.0 to 58.0 g kg-1, respectively (Chan and Xu, 2009).

The heterogeneous chemical composition of biochars is controls its surface chemistry, which in turn determines the interaction of the material with the organic and inorganic components of soil. Re-arrangement of the bonds in the biomass results in the development of various useful functional groups including hydroxyl, amino-NH_2, ketone - OR, ester - (C=O)OR, nitro - NO_2, aldehyde - (C =O)H, carboxylic - (C=OOH) on the external surface of the graphene sheets (Harris, 1997; Harris and Tsang, 1997) and surfaces of pores (van Zwieten et al., 2009). Some of these functional groups act as electron donors, while others as electron acceptors. These culminate in acidic, hydrophilic to hydrophobic properties of the material (Amonette and Joseph, 2009). The composition, distribution, relative proportion and reactivity of functional groups within biochar are subject to diverse elements, including the source material and the pyrolysis procedure utilized (Antal and Gronli, 2003).

As the pyrolysis temperature rises, so does the extent of sweet-smelling carbon in the biochar, with N contents peaking around 300 ^{o}C (Baldock and Smernik, 2002). Conversely, low pyrolysis temperatures (under 500 ^{o}C) favour the availability K, Cl (Yu et al., 2005), Si, Mg, P and S (Schnitzer et al., 2007). Temperatures below 500 ^{o}C promote the retention of nutrients in biochar (Chan and Xu, 2009). Biochar prepared from rice straw and husk of the same plant had higher C

and N in the starw with higher exchangeable bases such as Ca, K and Mg in the husk (Agusalim, 2010).

2.8.5 Cation Exchange Capacity and pH of biochar

The CEC of soils changes following incorporation of the biochar (Lehmann, 2007; Agusalim et al., 2010). This is as a result of leaching of hydrophobic compounds from the biochar (Briggs et al., 2005) or by increasing carboxylation of C through abiotic oxidation (Cheng et al. 2006; Liang et al., 2006). Ageing of biochar also increases its CEC (Glaser et al., 2001).

Considering the very large heterogeneity of biochar properties, biochar pH values are relatively homogeneous, that is they are largely neutral to basic. Chan and Xu (2009) reviewed biochar pH values from a wide variety of feedstocks and found a mean of pH 8.1 in a total range of pH 6.2 – 9.6. The lower end of this range seems to be from green waste and tree bark feedstocks, with the higher end from poultry litter feedstocks. When biochar is applied to soils, it helps to retain nutrients and makes them more available to plants mainly by adsorption to minerals and organic matter.

Biochar has an even greater ability than other soil organic matter to adsorb cations per unit carbon (Sombroek et al., 2003), due to its greater surface area, greater negative surface charge, and greater charge density (Liang et al., 2006). In contrast to other organic matter in soil, biochar also appears to be able to strongly adsorb phosphate, even though it is an anion.

2.8.6 Modes of biochar application

The mode of biochar application in soils can substantially affect soil processes and functioning, including the fate and behaviour of biochar particles in soil (Verheijen et al., 2010). Three primary methods of application are:

(a) Topsoil application

(b) Depth application

(c) Top-dressing

Biochar can be incorporated as a sole amendment or in combination with composts or manures. In conventional tillage systems, the biochar and compost or manure is generally mixed homogeneously throughout the topsoil at depths between 0-15/30 cm.

Depth application of biochar has been described mostly as 'deep-banded' application (Blackwell et al., 2007). The placement of the biochar directly into the rhizosphere is thought to be more beneficial for crop growth and less susceptible to erosion. The application can be either by pneumatic systems, which can operate at high rates, or by applying the biochar in furrows or trenches and subsequently levelling the soil surface.

The spreading of mostly the dust fraction of biochar to the soil surface and relying on natural processes for the incorporation of the biochar into the topsoil is top dressing. This form of application is considered mainly for situations where mechanical incorporation is not possible and in no-till systems, forests. It is also done in forests and pastures.

2.9 Agronomic benefits of Biochar

The ideal application rate for biochar depends on the particular soil type and crop management. It has been demonstrated that biochar has various uses. At the point when added to soil it can essentially enhance soil fertility and is also as a sink for carbon (Lehmann, 2007). Carbon along these lines is removed from the atmosphere in via sequestration (Zwietenoe, 2006; Davies, 2007).

Biochar can function as a soil conditioner by enhancing the physical and biological properties of soils, for example, water holding capacity and soil nutrients retention culminating in better crop growth (Sohi et al, 2009; De Gryze, 2010). Biochar raises: (i) raise soil pH, (ii) reduce aluminum toxicity, (iii) decrease soil elasticity, (iv) enhance soil conditions for earthworm population, and (v) enhance fertilizer use efficiency (McLaughlin, 2010; Schmidt and Noack, 2000; Cunha et al., 2009).

The combined addition of biochar and inorganic fertilizer can possibly increase crop yield, in this manner and reducing the amount of inorganic fertilizer use and importation (De Gryze, 2010; Quayle, 2010). Biochar application to hard setting soils in Australia, for example, decreased tensile strength and further enhanced plant growth (Amonette and Joseph, 2009; Gaskin et al. 2008). Additionally, the application rate of 5 tons/ha of biochar reduced fertilizer needs by 7% (Steiner et al., 2008). Sohi et al.,(2009) reported that crops grew three times faster in biochar amneded soils than in un-amended soils. Comparative similar results have been exhibited in Benin, Liberia, and South Africa (Sohi et al., 2009; Cunha et al., 2009).

In a study on the impacts of charcoal production on physical and hydrological properties of soil in Ghana, the saturated hydraulic conductivity of soils under charcoal furnaces increased considerably and there was significant rise in soil pH, electrical conductivity and exchangeable Ca, Mg, K, Na and P in the soil at a charcoal production site contrasted with neighboring soils under no charcoal production (Oguntunde et al., 2004, and Oguntunde et al., 2008). When mixed with organic matter, biochar enhanced the retention (De Gryze et al., 2010; Oguntunde et al., 2008; Major et al., 2009).

2.10 Environmental benefits of biochar

2.10.1 Carbon sequestration

Carbon sequestration is the removal and storage of carbon to keep it from being discharged to the atmosphere. A lot of carbon in biochar might have been sequestered in the soil for long periods evaluated to be hundreds to thousands of years (Lehmann et al., 2006; Bracmort, 2010; Ogawa et al., 2006, Woolf, 2008). While biochar in the long run mineralizes in soils, a small amount of it stays in an extremely stable structure with a carbon-14 age higher than that of the most seasoned soil organic matter (SOM) portions (Schmidt and Noack, 2000). This property of biochar gives it the possibility to be an important carbon sink.

Compared with other terrestrial sequestration procedures, for example, afforestation or re-forestation, carbon sequestration in biochar expands its storage time (Sohi et al., 2009; Ogawa et al., 2006). Decreases in N_2O and CH_4 emissions as a consequence of biochar application are added advantages of biochar (Steiner, 2010; Duku et al., 2011).

2.10.2 Effect of biochar on pesticide application

Enviromental fate and functioning of pesticides may be influenced by the additions of biochar. Wheat char has been found to be very effective as a sorbent for pesticides, and its presence (1% by weight) in soil contributed 70% to pesticide sorption (Sheng et al., 2005). Biochar application, offers a vital approach for reducing leaching of pesticides.

Herbicide persistence in soil may be influenced by herbicide sored by biochar (Spokas et al., 2009; Yu et al., 2009). Despite greater persistence of the pesticide residues in biochar amended soils, the plant uptake of pesticides decrease markedly with increasing biochar content of the soil (Yu et al., 2009). Studies have shown that 1% by weight biochar additions to soil and potting

medium induced a systemic resistance against two foliar fungal pathogens *(B. cinerea* and *L. taurica*) in both pepper and tomato plants, and to a pest (*P. latus*) in pepper plants (Elad et al., 2010).

2.10.3 Biochar and soil nutrient dynamics

Current global interest in biochar has been built largely on research conducted using highly weathered and infertile soils (Steiner et al., 2007; Kimetu et al., 2008; Asai et al., 2009; Major et al., 2010). Challenges in these highly weathered systems include prevention of nutrient loss via leaching and retention of nutrients in the root zone.

Schnell et al. (2012) found that topdressing up to 3 Mg/ha of sorghum-derived biochar on an Alfisol caused significant surface runoff P losses compared with the control soils and incorporating the biochar into soil reduced runoff P losses by 78%.

Brewer et al. (2012) amended a sandy mollisol with biochar made under various pyrolysis conditions and observed an increase in soil extractable P, K, Mn, and Fe compared with un-amended soil but little change in soil NO_3–N concentrations with biochar amendment compared with control soil. In calcareous system, there was no observed change in soil pH, cation, or P availability (Lentz and Ippolito, 2012).

Switch grass biochar pyrolysed at two different temperatures (250 ºC and 500°C) was added to two Aridisols and there was a two- to threefold decrease in leachate P concentrations with the lower versus higher temperature biochar (Ippolito et al., 2012 and Hass et al., 2012). This observation was probably due to retention of orthophosphate by surface functional groups, Fe and Al hydroxide sorption, and Ca and Mg phosphate precipitation (Novak et al., 2009).

Biochars produced at low pyrolysis temperatures showed less Ca, Mg, and NO_3–N leaching than

biochars produced at higher temperature. This was because biochars produced at 250°C contained substantial amounts of bioavailable C similar to the hydrochar produced by Gajić and Koch (2012) and these could reduce immobilisation.

Calcareous soils amended with biochar produced from bagasse at 400 to 800°C, NO_3–N was weakly sorbed to biochar but sorption increased with higher temperatures due to the formation of base functional groups (Kameyama et al., 2012) . Increased retention of nutrients and water in biochar micropores decreased NO_3- leaching and provided a greater opportunity for crops to utilize available NO_3–N.

2.10.4 Some negative impact of biochar

Most cases of decreased plant growth due to biochar application has been attributed to temporary high levels of pH, volatile or mobile matter (MM), and or nutrient imbalances associated with fresh biochar (McClellan et al., 2007). Biochar often can have an initially high (alkaline) pH, which is desirable when used with acidic, degraded soils. However, if soil pH becomes too alkaline, plants may suffer nutrient deficiencies. “Mobile matter” refers to tars, resins, and other short-lived substances that remain on the biochar surface immediately after production and can inhibit plant growth (McClellan et al., 2007; McLaughlin et al., 2009)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Soils, sampling and soil preparation

The study was conducted at a Screen House located behind the Ecological Laboratory, University of Ghana using two major coastal savannah soil series, locally classified as Adenta series (Brammer, 1960) and Akuse series (Brammer, 1967). The Adenta series has been classified as a Ferric Acrisol (Dowuona et al., 2011) with the Akuse series as a Typic Calciustert (Ason et al, 2014). The Adenta series is kaolinitic and acidic whereas the Akuse series is smectitic alkaline to neutral in soil reaction. The plough layer (0-20cm) of Adenta series was sampled from the University of Ghana Experimental Farms, Legon at a site located on latitude 05°39'03" N and longitude 00°11'13"W. Samples from the Akuse series were collected from a pristine site between Akuse and Akuse Junction in the Eastern Region of Ghana at latitude 6° 09 N, longitude 00° 04' E and at an altitude of 22 m above mean sea level. Undisturbed clod samples were collected for bulk density determination.

All foreign materials were removed from the soils (e.g. dry leaves, roots and twigs) and the soils were air dried. The soils sampled were then passed through a 2 mm sieve to obtain the fine earth fraction. After sample preparation, the soils were sent to the laboratory for routine physical and chemical analyses.

3.2 Physico-Chemical analyses

3.2.1 Bulk density

Bulk density was determined by the clod method for the Akuse series. Clods from the soil samples

were selected and oven-dried overnight at 105 ^{0}C and upon removal, cooled in a dessicator and the oven dried weight determined on a weighing balance. A 100 mL measuring cylinder was filled with very fine river sand to the 30 mL mark amidst gentle tapping and shaking. The clod whose bulk density was to be determined was carefully placed on the river sand in the measuring cylinder. A known volume (b mL) of the river sand in a second measuring cylinder was carefully poured onto the clod in the first cylinder amidst gentle tapping and shaking until the clod was completely covered with the river sand.

The final volume i.e. 30mL + b mL + volume of clod was noted.

The 30 mL + b mL volume was subtracted from the final volume to give the volume of the clod.

The bulk density was then determined using the equation below:

$$BD(Mg/m^3) = \frac{oven\ dry\ mass\ of\ clod}{volume\ of\ clod} - - - [1]$$

Where, BD is the bulk density

The bulk density for the Adenta series was determined as follows;

The bulk density was determined using the core method by Blake (1965). Soil samples were taken at each site using cylindrical metal core samplers (5 cm diameter x 5 cm height) after clearing the vegetation. A second core sampler of similar size was placed on the first and hammered into the soil far enough to fill the volume of the first core. The filled core sampler was carefully removed and both ends trimmed. The core samples were carefully transferred into moisture can with known mass (W_1) and dried at 105^0 C for two days (48 hours). The weight of dried soil plus moisture can was determined and recorded as W_2. The bulk density of soil was determined as the ratio of mass of dried soil to the volume of soil. It is assumed that the volume of the soil sample is equal to the

internal volume of the core sampler.

Thus the bulk density of the soil is calculated as:

$$Bulk\ Density(Mgm^{-3}) = \frac{W_2 - W_1}{V} - -[2]$$

3.2.2 Particle Size Analysis

The Bouyoucos Hydrometer method modified by Day (1965) was used to determine the particle size analysis of the soil. For each of the two soils, 40 g of the fine earth fractions were weighed into a beaker followed by the addition of 60 ml of 6% H_2O_2 in order to destroy the organic matter. Hundred (100) mL of 5% Calgon (sodium hexametaphosphate) solution was added. The suspension was shaken on a mechanical shaker for 2 hours. The suspension was transferred into a graduated sedimentation cylinder and was brought to the 1000 mL mark with distilled water. A plunger was inserted into the cylinder and moved up and down several times to mix the suspension thoroughly. A hydrometer was gently lowered into the content and the density of the suspension for silt and clay noted 5 minutes after the plunger was removed. A second hydrometer reading was taken after 5 hours to represent that of clay. The suspension was poured from the sedimentation cylinder into a 47-micron sieve and effluent discarded. Tap water was run through the sediment in the sieve to wash off the fine material. The sand particles left in the sieve were transferred into a moisture can, oven dried for 24 hours and the dry weight determined. The temperatures of the suspensions, T_1 and T_2 were respectively recorded during the 5 minute and 5 hour hydrometer readings. Blank hydrometer readings of sodium hexametaphosphate solution at 5 minutes and 5 hours were taken. The percentage of Sand, Silt and Clay were calculated based on the oven dry weight of the soil sample taken as follows

Clay (%) +Silt (%) =Hydrometer reading at 5mins sample weight (g) ×100---------- [3]

Clay (%) =Hydrometer reading at 5 hours sample weight (g) ×100---------- [4]

Silt (%) = [3] – [4] …… [5]

Sand (%) = 100 – [3] ……..... [6]

Temperature effects on density of the soil particles was accounted for using the relation provided by Day (1965). For every 1°C increase in temperature, above 19.5 °C, there is an increase of 0.3 in the density of the particles in suspension.

Correction for temperature = blank hydrometer reading – increase in weight of particles

3.2.3 Soil moisture determination

A 500 g soil sample was saturated with water and placed in a filter paper-lined-perforated plastic container of 15 cm diameter and 20 cm height. The top was covered with a plastic and allowed to drain for 3 days. Sub samples were then taken and oven dried at 105 ^{o}C for 24 hours. The gravimetric water content was determined as the difference in mass between moist soil and oven-dried soil per oven-dried soil. This was considered to be the moisture content of the soil at field capacity. The soil moisture content at 80% field capacity was then estimated.

3.2.4 Soil pH Determination

The pH of the soil in distilled water at soil to water ratio of 1:1was determined using an electrometric method (Bates, 1954). Twenty (20) grams of soil was weighed in duplicates into 50 mL beakers. Twenty (20) millilitres of distilled water was added and the soil-liquid suspension was stirred for 30 minutes. The suspension was allowed to stand for an hour for equilibration. The pH metre was standardised using buffer solutions of pH 4.0, 7.0 and 9.0. The electrode was inserted into the suspension to measure the pH of the samples. The pH of the soils was also determined in 0.01 M $CaCl_2$ as described in that of water except that in the salt, a soil to solution

ratio of 1:2 was used.

3.2.5 Determination of Soil Organic Carbon

The wet combustion method of Walkley and Black (1934) was used in determing the organic matter. Air dried and sieved soil (0.5 mm sieve) of 0.5 g was weighed into a conical flask. Dilute HCl was then added to the Akuse series to destroy the carbonates. Ten (10) ml of 0.167 M potassium dichromate ($K_2Cr_2O_7$) and 20 mL of concentrated sulphuric acid (H_2SO_4) were added. To ensure that all the soil particles were in contact with the solution the flask was swirled around and digested. The content of the flask was allowed to settle for 30 minutes. The unreduced potassium dichromate ($K_2Cr_2O_7$) remaining in solution after the oxidation of the oxidizable organic material in the soil sample was titrated with 0.2 M ferrous ammonium sulphate after adding 10 mL orthophosphoric acid and 2 ml of barium diphenylamine sulphonate (an indicator) until colour change from a dirty brown colour to a bright green end point. A standardization titration 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.

3.2.6 Total Nitrogen

The Kjeldahl digestion procedure as outlined by Anderson and Ingram (1993) was used in the determination of total nitrogen. Soil of 2 g was weighed into a 300 mL Kjedahl flask and selenium catalyst was added. Concentrated sulphuric acid of 5 mL was then added. The mixture was digested until the digest became clear. The flask was then cooled and the content transferred into a 100 mL volumetric flask. The content was made to the 100 mL mark with distilled water. Into a Markham distillation apparatus, a 5 mL aliquot of the digest and 10 mL of 40% NaOH were added and the mixture distilled. The liberated ammonia was collected in boric acid (H_3BO_3) to which

three drops of a mixed indicator containing methylene blue and methyl red had been added. The ammonium borate formed was then back titrated with 0.01M HCl from green to reddish end point. The percent N was calculated as follows:

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

Where

0.01 = Molarity of HCl

0.014 mg =molar mass of Nitrogen

3.2.7 Available Phosphorus Determination

Available P of the Akuse series was determined using the method of Olsen (1965) for the extraction of phosphate. Ten grams of the fine earth fraction was weighed into extraction bottles. A 100 mL sodium bicarbonate (pH 8.5) was added to the samples in the extraction bottles; it was then capped and shaken for 30 min on a mechanical shaker. The extracts were filtered using Whatman's No. 42 filter paper to obtain clear solution. A 10 mL aliquot was taken into a test tube and then in drop-wise, 1 mL of 1.5 M H_2SO_4 was added to decolourise the solution by settling the organic matter in it. It was then left in a refrigerator to cool for few minutes. The extracts in the test tubes were centrifuged, and gently decanted for colour development and phosphorus analysis. The concentration of P in the extracts was then determined using the Murphy and Riley method (1962). An aliquot of 1mL of the sample solution was pipetted into a 50 mL volumetric flask and a drop each of P-nitrophenol and ammonium hydroxide were added. Then, 8 mL of a solution containing concentrated sulphuric acid, ammonium molybdate, potassium antimony tartrate, and ascorbic acid were added. The content was topped up to the 50 mL mark with distilled water. The concentration of phosphate was then determined on a Philips' UV spectrophotometer at a

wavelength of 712 nm. Available phosphorus content of the soil was calculated as;

$$P = \frac{(spectrometer\ reading - blank\ reading) \times volume\ of\ extract}{Volume\ of\ aliquot \times sample\ weight} - - - -[8]$$

Available P of the Adenta series was determined using Bray 1 method (Bray and Kurtz, 1945). Five (5) grams of soil was weighed into a centrifuge tube in duplicates. Fifty (50) millilitres of Bray solution (0.03 M NH_4F +0.025 M HCl) was added (Bray and Kurtz, 1945). The tubes were shaken end-over-end on a mechanical shaker for 5 min and were then centrifuged at 2500 rpm for 5 min. The suspensions were each filtered through a No. 42 Whatman filter paper into a 50 mL Erlemeyer flask. Phosphorus in the filtrate was determined using the Murphy Riley method (1962) as described above.

The concentration of phosphorus was then determined with a Philips' UV spectrophotometer at a wavelength of 712 nm. Available phosphorus content of the soil was calculated with equation 8 above.

3.2.8 Cation Exchange Capacity (CEC)

Soil of 10 g was weighed into an extraction bottle and 100 mL of 1M NH_4Ac solution was added and shaken on a mechanical shaker for 30 minutes. Using a No. 42 Whatman filter paper, the suspension was filtered and the samples leached four times with 25 mL of alcohol (ethanol) to wash off excess ammonium. The residue was again leached four times with 25 mL acidified

KCl. Ten millilitres of the decanted leachate (filtrate) was pipetted into a Kjeldahl flask and 10 mL of 40% NaOH and 100 mL of distilled water were added. The solution was distilled and the distillate collected in boric acid to which three drops of methyl red and methylene blue mixture had been added. The distillate was back-titrated against 0.01M HCl until the end point. The titre value

was used to determine the CEC of the soil.

$$CEC(cmol/kg) = \frac{volume\ of\ extract \times molarityHCl \times titre\ value \times 10}{volume\ of\ aliquot\ * sample\ weight} - -[9]$$

3.3 Preparation of Biochar

The feedstocks used for biochar production in this study focused on two agricultural residue, cocoa husk (pod) and rice husk. The cocoa husks were collected from a farmer's field at Tafo and the rice husk from the University of Ghana Soil and Irrigation Research Centre (SIREC), Kpong. The collected samples were air dried oven-dried overnight at 80^{o}C and subsequently cut into small pieces.

A kiln at the Soil Research Institute of the Council for Scientific and Industrial Research, Kwadaso, Kumasi was used for biochar production at a temperature of 350^{o}C. After pyrolysis, biochar in the kiln was allowed to cool overnight to room temperature, washed to remove the ash, dried, crushed, sieved through <2 mm sieve and sealed in sack for use.

3.4 Characterization of Cocoa husk and rice husk biochar

3.4.1 Determination of biochar bulk density

An amount of the air-dried biochar sample was carefully packed amidst intermittent tapping on the laboratory bench (to ensure good packing) into a measuring cylinder to a pre-determined volume (Vt). The quantity of sample packed was then transferred into a moisture can and put in an oven at 105 ^{o}C to dry for 24 hours after which its mass (Ms) was recorded. The dry bulk density (ρb) of the biochar was then calculated from the relation:

$$\rho = \frac{Ms}{Vt} - - - [10]$$

3.4.2 pH of biochar

One gram of biochar (from each feedstock) rice husk and cocoa husk was weighed into a beaker and 10 mL of distilled water added, to give biochar water ratio of (1:10). This ratio was used to ensure enough volume of supernatant for immersion of electrode. The mixture was then stirred several times for about 30 minutes and left to stand for about an hour to allow most of the suspension to settle and also for the suspension temperature to equilibrate with the temperature in the instrument room. The pH values were then determined using a glass electrode pH meter-CG818, Schott Great. The electrode was then rinsed with distilled water and then immersed into the partly settled suspension and the reading on the pH meter recorded. The determination of pH of the samples was repeated using 1 M KCl solution according to the protocol outlined.

3.4.3 Organic carbon of biochar

Organic carbon was determined by the wet combustion method of Walkley and Black (1934). Thirty millilitres of 0.167 M potassium dichromate ($K_2Cr_2O_7$) solution and 20 mL of concentrated sulphuric acid (H_2SO_4) were added to 0.1 g of each of a less than 0.5 mm cocoa and rice husk in different conical flasks. To ensure full contact of the materials with the liquids, each flask was swirled and after which the mixture was allowed to stand for 30 minutes to ensure complete digestion.

After the oxidation of the oxidizable organic material in the biochar, the unreacted $K_2Cr_2O_7$ in solution was back titrated against 0.2 M ferrous ammonium sulphate solution after adding 10 ml of orthophosphoric acid and 2 ml of barium diphenylamine sulphonate indicator from dirty brown colour to bright green end point. A standardization titration of the $K_2Cr_2O_7$ with the ferrous ammonium sulphate was done. The concentration of oxidizable carbon in each of the samples was calculated indirectly from the number of moles of unreduced dichromate consumed by the ferrous

ammoinium sulphate.

3.4.4 Available phosphorus determination

Available phosphorus in the biochar samples was determined by the method of Olsen (1965). Into an extraction bottle, one gram of each biochar sample was weighed and 50 mL of sodium bicarbonate solution was added and shaken for 30 minutes on a mechanical shaker. The biochar-extractant mixture was filtered through a Whatman No.42 filter paper. A 10 mL aliquot was taken and 1 mL 1.5 M sulphuric acid (H_2SO4) added and centrifuged at 3000 rpm for 15 minutes. The concentration of P in each sample was then determined after colour development using the Murphy and Riley method as described in section 3.2.7. The intensity of the colour at a wavelength of 712 nm was measured with the spectrophotometer and recorded. The P was calculated using the formula in Section 3.2.7.

3.4.5 Determination of Total Phosphorus.

Total phosphorous was determined by digesting 0.2 g of biochar with 25 mL of a mixture of concentrated HNO_3 and 60% $HClO_4$ in the ratio of 2:3. Distilled water was added to the digest, filtered and made up to volume in a 100 mL volumetric flask with distilled water. The P colour in the digest was then developed as described by Murphy and Riley method (1962) and the P concentration read on a UV spectrophotometer at 712 nm wavelength. The P concentration in the biochar was then calculated as in equation 8.

3.4.6 Determination of Total Nitrogen

Total nitrogen was determined by a modified Kjeldahl digestion method (Bremner, 1965). The nitrogen in the sample was converted to ammonium by digestion with concentrated sulphuric acid using selenium as catalyst and addition of K_2SO_4 to raise the boiling point of the mixture. The

ammonium formed was determined by distilling the digest with a strong alkali (40% NaOH) and titrating with a standard acid.

Air dried biochar of 0.2 g was weighed in triplicates into Kjeldahl digestion flasks. The catalyst……. and K_2SO_4 were added. Concentrated H_2SO_4 of 5 mL was also added. The mixture was digested for about 30 minutes and est after cooling, the mixture was transferred into a 100 mL volumetric flask and made up to volume with deionized water. An aliquot of 5 mL was then pipetted into a Markham distillation apparatus and 5mL of 40% NaOH added and rinsed with deionized water to about 100 mL. A 5 mL boric acid solution to which few drops of mixed indicator (0.13 g of methyl red + 0.666 of methylene blue dissolved in 100 mL of 95% ethanol) had been added were put into a conical flask to trap the liberated ammonia. The distillate was then back titrated with 0.01 M HCl solution. Similar procedure was adopted for a blank which had no biochar sample to account for traces of N if any, in the reagents and water used. The concentration of N in the biochar was estimated from the number of moles of HCl consumed in the reaction with ammonium borate formed when the ammonia was trapped in boric acid.

3.4.7 Cation exchange capacity of the cocoa and rice husk biochar

Ten grams of each biochar were weighed into an extraction bottle and 100 mL of 1 M ammonium acetate solution added. The CEC of the material was then determined using the method outlined for soil CEC in section 3.2.8.

3.4.9 X-ray Diffraction

The two biochar types were ground into fine powder in a mortar ensuring that there was no cross contamination in order to identify the minerals that may be present in the biochar. An Empyrean X-ray diffractometer was used for the X-ray analysis. A Cu K-Alpha and Beta radiations were

produced using 40mA and 45kV power source. The ground samples were mounted on a divergence slit and diffraction patterns of the biochar samples were obtained by scanning the samples at a starting position of 1^{o} per minute between 3^{o} to 60^{o}.

The X-ray diffraction (XRD) patterns were acquired with a computer controlled Panalytical Empyrean X-ray powder diffractometer to determine the type of minerals present.

3.5 Incubation Study

The study was conducted in a screen house located behind the Ecological Laboratory, University of Ghana, Legon. Daytime temperatures in the screen house ranged from 32 to 35^{o}C.The relative humidity of the screen house was between 63and 84%. Exactly 8.0 kg of the Akuse series and 9.7 kg of the Adenta series were weighed into plastic buckets with perforated bottoms to a predetermined height to attain the respective field bulk density values of the two soils. The packed soils were left undisturbed for three weeks prior to the application of treatments to allow for stabilization of microbial activity.

The soil moisture content was kept constant at 80% of the field water capacity. Biochar as a factor was three i.e. control (zero biochar, cocoa husk biochar and rice husk biochar); two types of pesticide viz., atrazine and paraquat were applied, at three levels (zero pesticide application, normal application rate and ten times the normal application rate of each of the pesticide). The normal application rate used for atrazine was 3.5 kg active ingredient/ha whilst that for paraquat was 36.0 g. active ingredient/ha. Each treatment was replicated three times. Thus with two soils, three biochar types two pesticides, three pesticide rates and three replicates there was a 2 x 3×2×3×3 factorial experiment with the pots being arranged in a randomized complete design. There were thus a total of 108 pots. The whole experiment lasted for 90 days. Sampling of soil was

done on days 0, 10, 20, 30 and so forth to day 90. The two biochar types were applied at 10 Mg/ha.

3.6 Enumeration of microorganisms

3.6.1 Preparation of media

The microbial population during incubation studies was determined using the plate count method. The Nutrient agar medium was used for culturing the total bacteria.

The composition of the medium is shown below:

Ingredient	mass (g)
Tryptone	10
Meat extract	5
Sodium Chloride	5
Bacteriological agar	15

The medium was dissolved in 1 L distilled water and then sterilized in an autoclave.

3.6.2 Preparation of Ringer's solution

The Ringer's solution was used in preparation of serial dilutions to improve the viability of microbial cells instead of using ordinary distilled water. The composition of the Ringer's solution is:

Ingredient	mass (g)
NaCl	1.0
KCl	0.105
$CaCL_2.2H_2O$	0.1
$NaHCO_3$	0.05

These were dissolved in 1 L distilled water.

3.6.3 Total heterotroph counts

Enumeration of heterotrophs was done using the dilution plate count. Fresh soil of 1g was weighed and added to 9 mL sterilized Ringer's solution in a 10 mL dilution bottle. The resulting solution was the shaken on a rotary shaker to mix the solution uniformly. This established a 10^{-1} dilution; four other dilution bottles were each filled with 9 mL of the Ringer's solution. The bottles and their respective contents were sterilized by autoclaving at 121° C for 30 minutes. One milliliter (1mL) of the freshly shaken suspension (10^{-1}dilution) was immediately drawn and transferred into one 9 mL dilution bottle followed by shaking. This gave a 10^{-2} dilution, 1mL was drawn and transferred into bottle containing 9 mL Ringer's solution to establish a 10^{-3} solution. The transfer process was again repeated twice to establish 10^{-4} and 10^{-5} dilution, respectively.

3.6.4 Preparation of pour plates

At the end of the preparation of the dilution series, 1 mL portion of the 10^{-5} dilution was aseptically transferred with a micro pipette into sterile petri dishes that contained the set nutrient agar medium. The dish was rotated in both clockwise and anticlockwise directions to achieve complete mixing. The medium was previously sterilized by autoclaving at 121°C for 30 minutes at 103 kPa.

3.6.5 Incubation, Counting of Colonies and Calculation of Results

The inoculated plates were incubated at 30°C and heterotroph counts were made after 72 hours. The concentration of cells in the soil sample (C) was computed by dividing average number of colonies per plate by the dilution factor.

The concentration of cells was divided by the weight of oven dry soil (determined separately on a sub sample at the time of initial plating) to convert the results to dry weight basis. Differences among treatments were examined using analysis of variance.

3.7 Microbial biomass

3.7.1 Microbial Biomass Carbon

Soil microbial carbon was monitored under the different soil amendments. Chloroform fumigation and extraction (FE) method described by Ladd and Amato (1989) was used to determine the microbial biomass carbon. Ten grams of field - moist soil sample, after passing through a < 2mm sieve, was weighed into a fifty (50) mL volumetric flask marked with pencils to prevent ink from running in chloroform and placed in a vacuum desiccator lined with moist paper towels to prevent the desiccation of soil samples. A 50 mL volumetric flask containing 80 mL of alcohol –free chloroform with boiling chips was placed by the soil sample. The desiccator was then evacuated until the chloroform boiled for about five minutes and vented for fifteen minutes. These steps were repeated three more times, not venting the last time. A 50 mL volumetric flask containing soil sample (10 g) was placed in a separate desiccator without chloroform representing the unfumigated control soil sample.

The desiccators were covered with black polythene bag (darkness prevents the chloroform from breaking down) and allowed to stand at room temperature for 5 days (Anderson and Ingram,

1993). Five days after fumigation, 50 mL of 0.5 M K_2SO_4 solution was added to the soil samples to extract microbial carbon from the lysed microorganisms. The amount of microbial carbon in the extract was determined using the colorimetric method. An aliquot (5 mL) of the extract was pipetted into 250 mL Erlenmeyer flask. To this, were added 5 mL of potassium dichromate (0.1667 M) and 10 mL concentrated sulphuric acid. The resulting solution was allowed to cool for 30 minutes after which 10 mL of distilled water was added.

First 5 mL of potassium dichromate (0.1667 M) and 10 mL concentrated sulphuric acid were added to the anhydrous dexterous serving as the standard carbon source then allow to stand. Then a standard series of concentration was developed from this ranging from 0, 2.5, 5.0, 7.5, 10.0 mg/mL C. These concentrations were obtained when volumes of 0, 5, 10, 15 and 20 mL of a 50 mg/mL C stock were pipetted into labelled 100 mL volumetric flasks and made up to the mark with distilled water. The absorbance of the standard and sample solutions was read on a quant-Lambda 850 spectrophotometer at a wavelength of 600 nm. A standard curve was obtained by plotting absorbance values of the standard solutions against their corresponding concentrations. Extracted carbon concentration of the samples was determined from the standard curve. For biomass C, a k –factor of 0.35 (Sparling et al., 1990) was used. The following equation according to Sparling and West (1998) was used to estimate the microbial C from the extracted C:

$$Microbial\ C\ (mg) = Ec/k$$

$$But\ Ec\ =\ Fc - UFc$$

Where

Ec = the chloroform-labile C pool (EC), and is proportional to microbial biomass C

Fc = C from the fumigated sample

UFc = C from the unfumigated sample

k = the fraction of the killed microbial biomass extracted as carbon under standardized conditions

3.8 Measurement of soil microbial respiration

The gas entrapment method by Hutchinson and Mosier (1981) and Sullivan et al. (2010) was used in this study. A 10 mL solution of 1M NaOH was dispensed into a vial and placed under the plastic chamber to trap CO_2 evolved from the soil. Additional vials containing 10 mL of 1M NaOH placed in the transparent container with their lids on to exclude CO_2 evolved from the soil served as controls to account for the CO_2 trapped from the atmosphere. The trapping solutions were changed every 72 hours. After exposure of the alkali, the vials were removed, covered with lids (air tight seal) immediately and taken to the laboratory for analysis. The measurements were taken from day three (3) to day ninety.

3.8.1 Laboratory analysis of soil CO_2 efflux

At the end of each 72 hour incubation period, a 10mL solution of 1M $BaCl_2$ was added to the solutions from the experimental pots to precipitate the carbonates to facilitate determination of CO_2 evolved from the soil. The evolved CO_2 was then determined by titration. Excess NaOH in solution was titrated against 1.0 M HCl using phenolphthalein indicator after precipitating the carbonate formed with 1.0 M $BaCl_2$.

3.8.2 Chemical reactions and computation of evolved soil CO_2

At the end of each reaction, the 1.0 M $BaCl_2$ added precipitated the carbonate as insoluble $BaCO_3$. Each mole of CO_2 dissolved in the NaOH leads to the production of 2 moles of H^+ after addition of the $BaCl_2$. Thus each mole of dissolved CO_2 results in the neutralization of 2 moles of OH^-. The reactions that occurred were:

$$CO_2 + NaOH \rightarrow HCO_3^- + OH^-$$

$Ba^{2+} + HCO_3^- \rightarrow BaCO_3 + 2H^+$

$HCl + OH^- \rightarrow NaCl + H_2O$

The HCl neutralizes the remaining OH^-, so that the amount of CO_2 trapped in the NaOH can be calculated as:

mmol CO_2 trapped in the NaOH = 0.5 × (volume $_{HCl}$ blank – Volume $_{HCl}$ sample) × M $_{HCl}$ [3.3]

Where, M $_{HCl}$ is the molarity of the HCl used in the titration.

The amount of CO_2 trapped (mmol) in equation is converted to mg by multiplying it by the molar mass of CO_2 (44 $gmol^{-1}$). This amount of CO_2 (mg CO_2) measured by the opened circular area of the chamber in contact with the soil is expressed per m^2 per 72 hours of the sampling time (mg m^{-2}/72hrs).

3.9 Nitrogen Mineralisation

Five grams of the soil sample was weighed into a 100-mL centrifuge bottle and 50 mL of 2 M KCl solution added. The contents were shaken for 30 minutes on a mechanical shaker after which the sample was filtered through a Whatman No. 42 paper. Five millilitres of the filtrate was pipetted into a 100 mL micro Kjeldahl flask and 0.2 g of MgO was added. The flask was connected to a distillation apparatus and about 30 mL of the distillate was collected in 5 mL of 2% boric acid to which three drops of methyl red – methylene blue indicator mixture had been added. The distillate was then back titrated against 0.01 M HCl to a purplish end point for ammonium-N determination.

One millilitre of sulphamic acid and 0.2 g Devarda's alloy were then added to the contents of the flask and the distillate collected in a new conical flask containing 5 mL of 2 % boric acid and three drops of the mixed methyl red and methylene blue indicator. The distillate was then back titrated against 0.01 M HCl from a green to a purplish end point to account for the level of nitrate in the

soil. The respective concentrations of NH_4^+ and NO_3^- in the soil were then determined from the number of moles of HCl consumed in the two back titration reactions.

3.10 Quantitative measurement of Residual Pesticide

3.10.1 Paraquat

Fifty milliliter of methanol and 5mL of sodium dithionite were added to 5g soil sample and vigorously shaken at 270 rpm for 2 hours. The solution was then filtered using a Whatman No 42 filter paper. The filtrate was then measured by UV Vis spectrophotometer.

Standard solutions of 0, 0.5, 1, 2, 3, 4, and 5 µg/mL were prepared by adding 0, 1.25, 2.5, 5, 7.5, 10, and 12.5 mL of stock solution of paraquat 5 mL of sodium dithionite 1% (in 0.1 M NaOH) and then diluted with methanol up to 50 mL (Khoiroh, 2008). The solution was then measured based on UV-Vis spectrophotometer absorption at λ=345 nm.

3.10.2 Atrazine

Thirty milliliter of methanol was added to 10 g soil sample and vigorously shaken at 270 rpm for 30 minutes. The solution was then filtered using a Whatman No 42 filter paper. The filtrate was then measured by UV-Vis spectrophotometer.

Standard solutions of 0, 0.5, 1, 2, 3, 4, and 5 ug/mL were prepared by adding 0, 1.25, 2.5, 5, 7.5, 10, and 12.5 mL of stock solution of atrazine 20 µg/mL to methanol. The solution was then measured based on UV-Vis spectrophotometer absorption at λ=275 nm.

3.10 Statistical Analysis

The data of different experiments conducted were subjected to Analysis of variance and means were obtained using the software GENSTAT version 9 at a 5% level of significance (LSD) by

comparing the LSD. Where it was necessary Microsoft Excel software was used to summarize the values of mean into tables and graphs, including running correlation analysis where needed.

CHAPTER FOUR

4.0 RESULTS

4.1 Characterization of soil

The data on some selected chemical and physical properties of the Adenta and Akuse series used for the incubation study are presented in Table 4.1. The Adenta series has a higher sand content (70.3%) than the Akuse series (47.1%). The silt contents of the two soils were similar with values of 7.2% and 5.4% for Adenta series and Akuse series, respectively. The clay content was higher in the Akuse series than the Adenta series. The two soils also have medium dry bulk density of 1.31 and 1.41 Mg/m^3 respectively, for Adenta series and Akuse series.

In water (1:1) pH was slightly acidic for Adenta series and slightly alkaline for the Akuse series respectively (Table 4.1). When pH values were determined in 0.01 M $CaCl_2$, the values were lower than those determined in water for all the soils, making the net change in pH negative. This indicates that the soils have a net negative charge that may serve as potential sites for adsorption of positively charged compounds and also the soils were also deeply weathered due to the negative charges (Abekoe & Sahrawat, 2001).

Generally the organic carbon (OC) contents of the soils were low 6.0 and 9.1 g/kg for Adenta and Akuse series (Table 4.1), respectively. These are characteristic of soils in semi-arid ecosystems where the high rate of mineralization due to high temperatures reduces the accumulation of carbon (Dowuona et al., 2012).

Total nitrogen content of the soils is generally low following trends in variations similar to OC contents. Broad ratings of nitrogen measurements in soils were described by Landon (1984) as

very high (> 10 g/kg), high (5 - 10 g/kg), medium (2 - 5 g/kg), low (1 - 2 g/kg) and very low (<1 g/kg). The Adenta series had a total nitrogen content of 1.09 g/kg, similar to the Akuse series

Table 4.1 Some physico-chemical properties of the soils used.

Soil properties	Adenta series	Akuse series
Sand%	70.3	47.1
Silt%	7.2	5.4
Clay%	22.5	47.5
Textural class	Sandy Clay Loam	Sandy Clay
Bulk density Mg/m^3	1.31	1.4
pH (H_2O)	5.4	7.6
pH (0.01M $CaCl_2$)	4.9	6.2
Organic Carbon g/kg	6.0	9.1
Total Nitrogen g/kg	1.09	0.91
C:N ratio	5.50	10
Available Phosphorus mg/kg	4.8	8.93
CEC cmol/kg	8.12	37.6

1.33 g/kg reported by Asuming-Brempong et al. (2013) and 0.91g/kg for Akuse series. The Adenta series had a low available P content of 4.8 mg/kg which is consistent with values obtained for similar soils in the landscape (Darko, 2007) and Akuse series had 8.93 mg/kg. The CEC of the Akuse series is very high (37.6 cmol/kg) and about four times higher than the Adenta series (8.12 cmol/kg).

4.2 Characteristics of cocoa husk biochar and Rice husk biochar

Some physico-chemical properties of the cocoa husk biochar (CHB) and rice husk biochar (RHB) used in the study are summarized in Table 4.2. The CHB had a bulk density of 0.36 Mg/m^3, 0.14 Mg/m^3 higher than that of the RHB. Biochar pH values are relatively homogenous (Verheijen et al., 2010), largely neutral to basic. In this study, pH in water of cocoa husk biochar was 10.7 and 7.4 for rice husk biochar which could be described as very strongly alkaline and mildly alkaline. The pH values determined in 0.01M $CaCl_2$ were higher than those determined in water for all biochar samples making the net charge in pH positive. This suggests that the biochar samples have a net positive charge that may serve as potential sites for adsorption of negatively charged compounds.

The CHB and RHB had organic carbon (OC) content of 256.0 g/kg and 291.0 g/kg respectively, total nitrogen content was 3.5g/kg and 1.4g/kg respectively giving a carbon to nitrogen ratio (C:N) of 73.14 for cocoa husk and 212.14 for rice husk.

The cocoa husk biochar had a very high total and available P values of 4700 and 1100 mg/kg, respectively. The total P in the RHB was 1100 mg/kg and its available P approximately seven times lower than that in the CHB (Table 4.2).

The x-ray diffraction patterns showed that silica (SiO_2) was the main mineral common to the two samples (Fig 4.1). In addition to the silica, CHB had other minerals such as potassium hydrogen carbonate ($KHCO_3$), aluminum iron (iii) oxide ($AlFeO_3$) and iron oxide hydroxide (FeOOH) in appreciable quantities. The minor minerals in CHB include periclase (MgO) and magnesium oxide (MgO), magnesium carbonate ($MgCO_3$), and potassium magnesium carbonate ($K_2MgCO_3)_2$. The RHB had only Magnesium hydroxide ($Mg\ [OH]_2$) in addition to the silica.

Table 4.2 Chemical and physical properties of the soil amendments.

Properties	Cocoa Husk Biochar	Rice Husk Biochar
Bulk Density Mg/m^3	0.36	0.22
pH_w (H_2O)	10.4	7.4
pH_s (0.01 $CaCl_2$)	10.8	7.6
Total P mg/kg	4700	1100
Available P mg/kg	3897.7	531
CEC cmol/kg	81.67	38.8
Organic Carbon g/kg	256.0	291.0
Total Nitrogen g/kg	3.5	1.4
C:N ratio	73.14	212.14

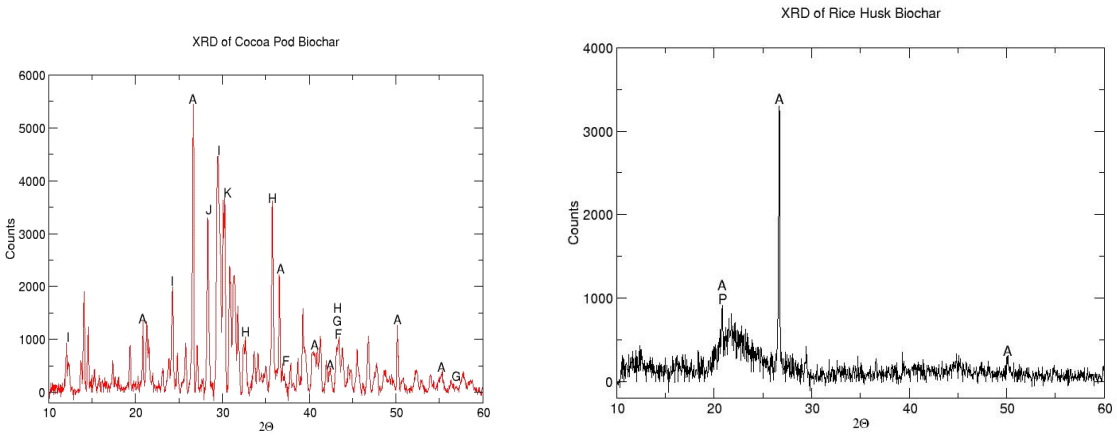


Figure 4.1 X-Ray Diffraction Spectra of Biochars.

Key: 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), H= Magnesium carbonate ($MgCO_3$), I= Potassium hydrogen carbonate ($KHCO_3$), J= Aluminum Iron (iii) Oxide ($AlFeO_3$), K= Iron oxide hydroxide (FeOOH), L= Iron oxide (FeO), M= Graphite, nitrated, N= Magnesium silicate (Mg_2SiO_4) O= Iron magnesium oxide ($FeMgO_4$), P= Magnesium hydroxide ($Mg[OH]_2$). Q= Potassium magnesium carbonate $(K_2MgCO_3)_2$, R= Calcium magnesium.

Mineralogical composition of biochar

Biochar type	Minerals present
Cocoa husk	A, f, g, h, I, J, K, q, r
Rice husk	A, p

Lower case = minor component

4.3 Pesticide influence on microbial counts in soils amended with biochar

The microbial counts in biochar amended soils before and upon pesticide application are herewith presented in the sections below.

4.3.1 Total Heterotroph Count when no pesticide was added to soils

The total heterotroph count (THC) patterns were similar in the two soils. The count was low initially and grew to peak values with time and then declined within the incubation period. The total heterotroph count in the control soils (or soils un-amended with biochar) ranged from 3 x 10^5 to 9 x 10^5 cfu/g soil for the Akuse series (AkC) and the Adenta series (AdC) from day 0 to day 90 (Fig. 4.2a). The counts were all significantly different with time ($p<0.05$) for both soils.

On amendment with biochar, total heterotroph count (THC) values were generally lower in the Adenta series than the Akuse series. Adenta series amended with RHB had higher THC than when amended with CHB. Significant differences among the RHB, CHB and the un-amended soils were recorded in days 60, 80 and 90 (Fig. 4.2a) for the Adenta series.

When the soils were amended with biochar, the total heterotroph count was higher for Akuse series amended with CHB as compared to the same soil amended with RHB (Fig. 4.2a). The highest count of 25-28 x10^5 cfu/g soil occurred in the Akuse series amended with CHB between 30 to 70 days of incubation, after which the total heterotroph count declined.

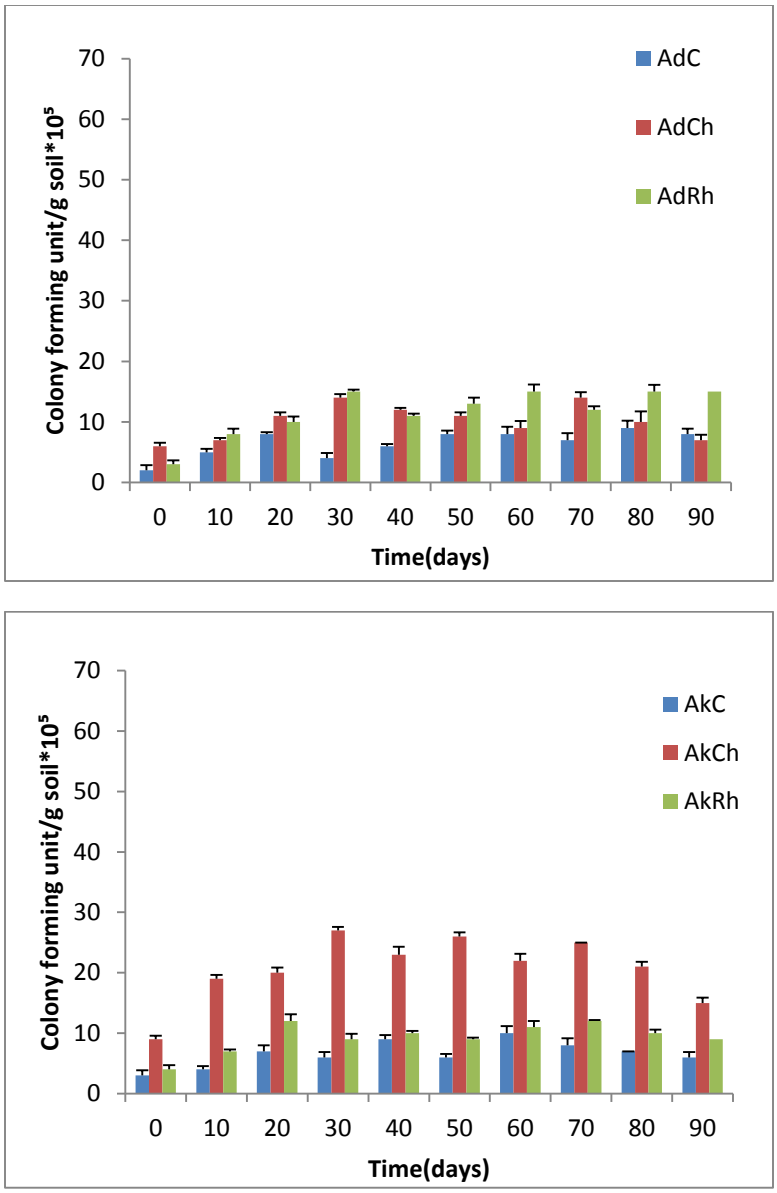


Figure 4.2a Total heterotroph count of bacteria in the un-amended and amended Adenta series and Akuse series when no herbicide had been applied.

(AdC-Adenta series un-amended with biochar, AdCh- Adenta series amended with CHB, AdRh-Adenta series amended with RHB, AkC-Akuse series un-amended with biochar, AkCh-Akuse series amended with CHB, AkRh-Akuse series amended with RHB)

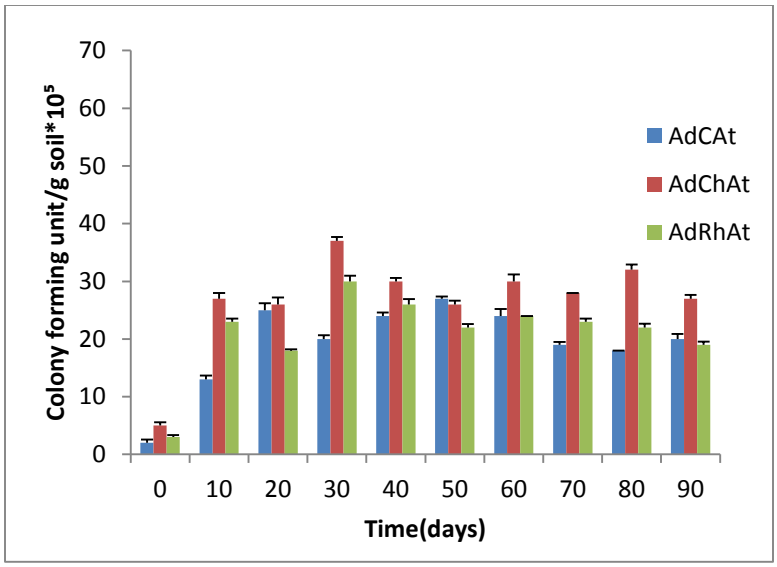
4.3.2 Total Heterotroph Count when normal rate of atrazine and paraquat were applied to soils

On application of the normal rate (3.5 kg a.i/ha) of atrazine to the Adenta series, higher THC were recorded for the CHB and RHB amended soil as compared to the control or the un-amended soil (Fig. 4.2 b). The highest total heterotroph counts of 37 x 10^5 and 32 x 10^5 cfu/g soil were respectively, obtained on days 30 and 80 for the CHB. Lower THC were observed for the Adenta series amended with RHB and these significantly varied with sampling time at $p < 0.001$ (Fig. 4.2b).

On applying paraquat at the normal recommended rate (36 g a.i./ha) to the biochar amended Adenta series, THC were generally lower than when the atrazine was applied ($p<0.01$) at the normal recommended rate (Fig 4.2 b). The THC for Adenta series amended with RHB was highest in days 20, 60 and 80, and significant differences existed with time.

Akuse series amended with CHB and contaminated with atrazine at the normal rate had high THC of 36 x10^5cfu/g soil on day 30 which declined thereafter with time (Fig. 4.2c) to the same soil. The THC in Akuse series amended with CHB was significantly higher than the same soil amended with RHB in days 10, 20, 30, 40 and 50.

Lower total heterotroph counts were recorded when paraquat was applied at the normal application rate to the Akuse series as compared to when atrazine was applied (Fig. 4.2c) to the same soil. The highest THC was obtained after 30 days of application (24 x10^5 cfu/ g soil). The THC counts upon application of the two pesticides on Akuse series were significant different during the 90 day incubation period, $p < 0.05$.



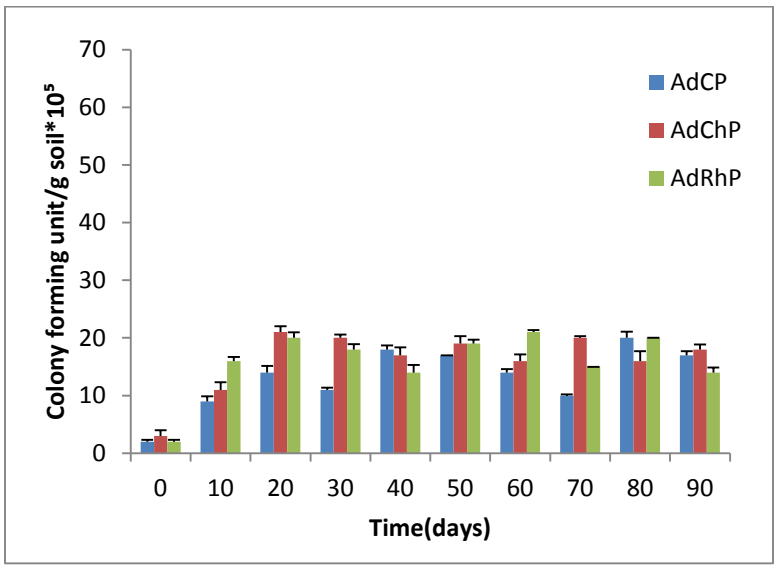
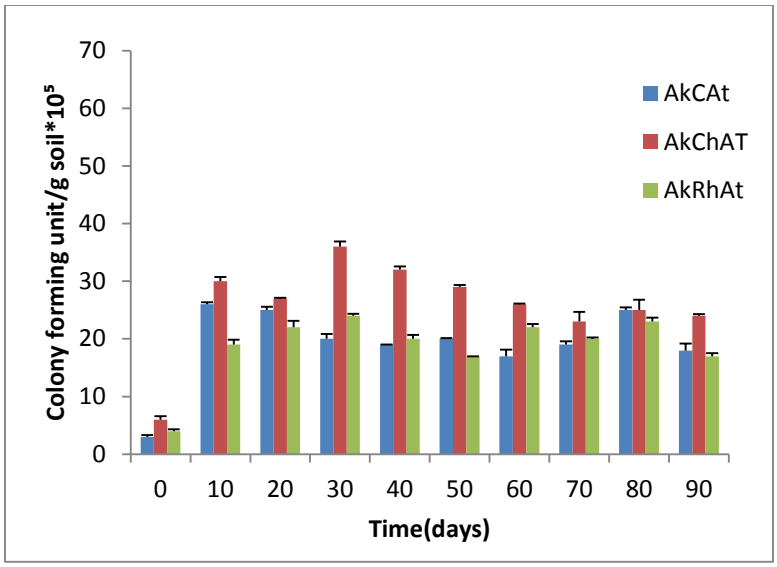


Figure 4.2b Total heterotroph count of bacteria when normal rate of herbicide application to the Adenta series.

(AdCAt-Adenta series un-amended with atrazine applied, AdChAt-Adenta series amended with CHB with atrazine applied, AdRhAt- Adenta series amended with RHB with atrazine applied, AdCP-Adenta series un-amended with paraquat applied, AdChP-Adenta series amended with CHB with paraquat applied, AdRhP- Adenta series amended with RHB with paraquat applied)



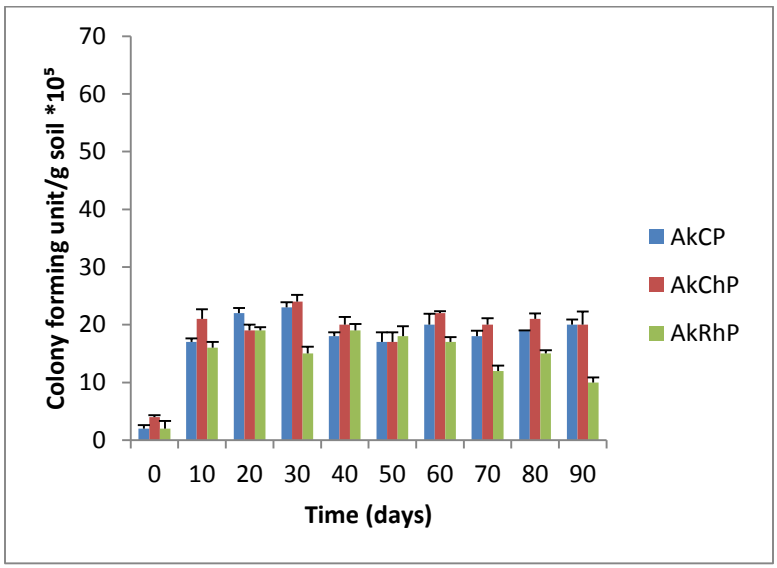


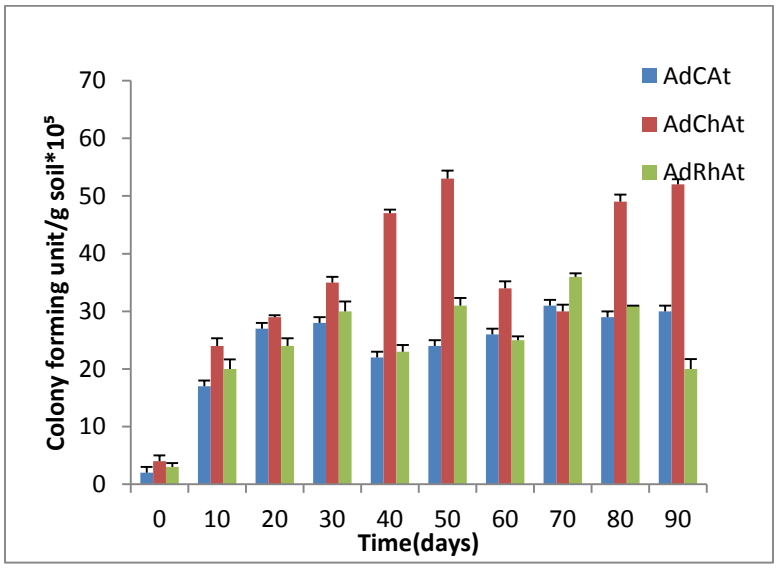
Figure 4.2c Total heterotroph count of bacteria in Akuse series that had normal rate atrazine and paraquat application.

(AkCAt-Akuse series un-amended with biochar with atrazine applied, AkChAt-Akuse series amended with CHB with atrazine applied, AkRhAt- Akuse series amended with RHB with atrazine applied, AkCP-Akuse series un-amended with paraquat applied, AkChP-Akuse series amended with CHB with paraquat applied, AkRhP- Akuse series amended with RHB with paraquat applied).

4.3.3 Total Heterotroph Count when ten times the normal rate of atrazine and paraquat were applied to soils.

High THC counts that of between 46 and 52 x 10^5 cfu/g soil were observed on days 40, 50, 80 and 90 for the Adenta series amended with CHB with no significant differences in THC among the sampling days (Fig. 4.2d).

The use of 10X the normal application rate of paraquat depressed THC in all soils amended with biochar such that the control soils had higher heterotroph counts than the CHB and the RHB amended soils (Fig. 4.2d).



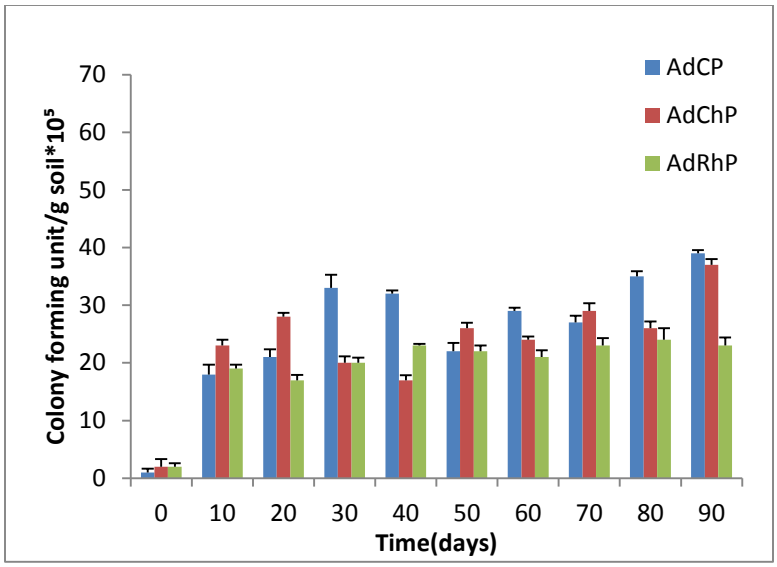
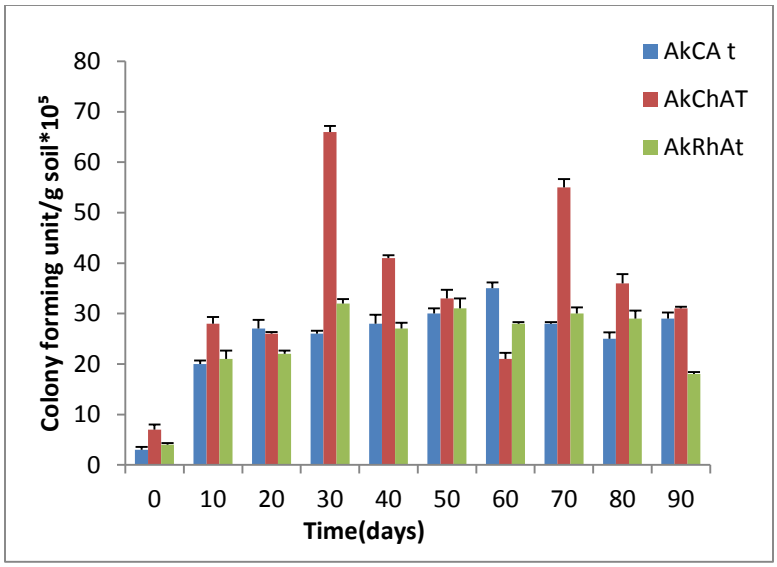


Figure 4.2d Total heterotroph count when the Adenta series was treated with atrazine and paraquat at 10X the normal application rate.

(AdCAt-Adenta series un-amended contaminated with atrazine, AdChAt-Adenta series amended with CHB contaminated with atrazine, AdRhAt- Adenta series amended with RHB contaminated with atrazine, AdCP-Adenta series un-amended contaminated with paraquat, AdChP-Adenta series amended with CHB contaminated with paraquat, AdRhP- Adenta series amended with RHB containated with paraquat).

In the Akuse series, when atrazine 10X the normal concentration was applied to the CHB amended soil, high heterotroph counts of 66 x 10^5 and 58 x 10^5 cfu/g soil respectively, occurred on days 30 and 70. Rice husk biochar amended Akuse series however, recorded lower THC and in most cases, there was no significant difference between the control treatments and the rice husk biochar amended soil (Fig. 4.2e).

When paraquat was applied, the un-amended Akuse series recorded higher THC than the biochar amended soils just as was observed in the Adenta series (Fig. 4.2e). On days 50 and 90, the un-amended Akuse series had the high THC of 30 and 32 x10^5 cfu/ g soil, respectively. Treatment differences between Akuse series treated with atrazine and Akuse series treated with paraquat were significant ($p<0.05$).



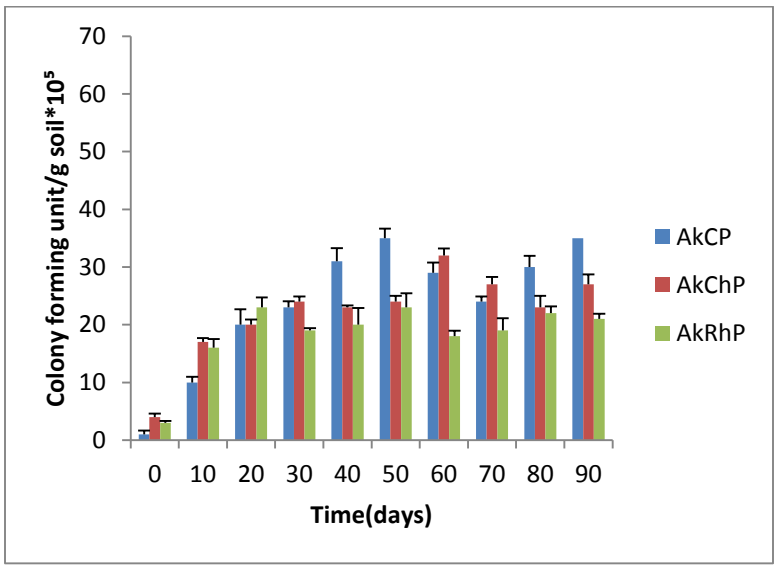


Figure 4.2e Total heterotroph count the when Akuse series was applied with 10X the normal recommended rate of atrazine and paraquat.

(AkCAt-Akuse series un-amended contaminated with atrazine, AkChAt-Akuse series amended with CHB contaminated with atrazine, AkRhAt- Akuse series amended with RHB contaminated with atrazine, AkCP-Akuse series un-amended contaminated with paraquat, AkChP-Akuse series amended with CHB contaminated with paraquat, AkRhP-Akuse series amended with RHB contaminated with paraquat).

4.4 Soil microbial biomass carbon

Soil microbial biomass carbon for the un-amended and amended soils upon application of the pesticides is presented herein.

4.4.1 Microbial biomass carbon when no atrazine and paraquat were applied to soils

Microbial biomass was low in all the treatments at the onset of incubation in both soils but with time, increase in microbial biomass was observed (Fig. 4.3a). In the Adenta series, slightly higher biomass was observed in the CHB than in the RHB amended soils (Fig. 4.3a).

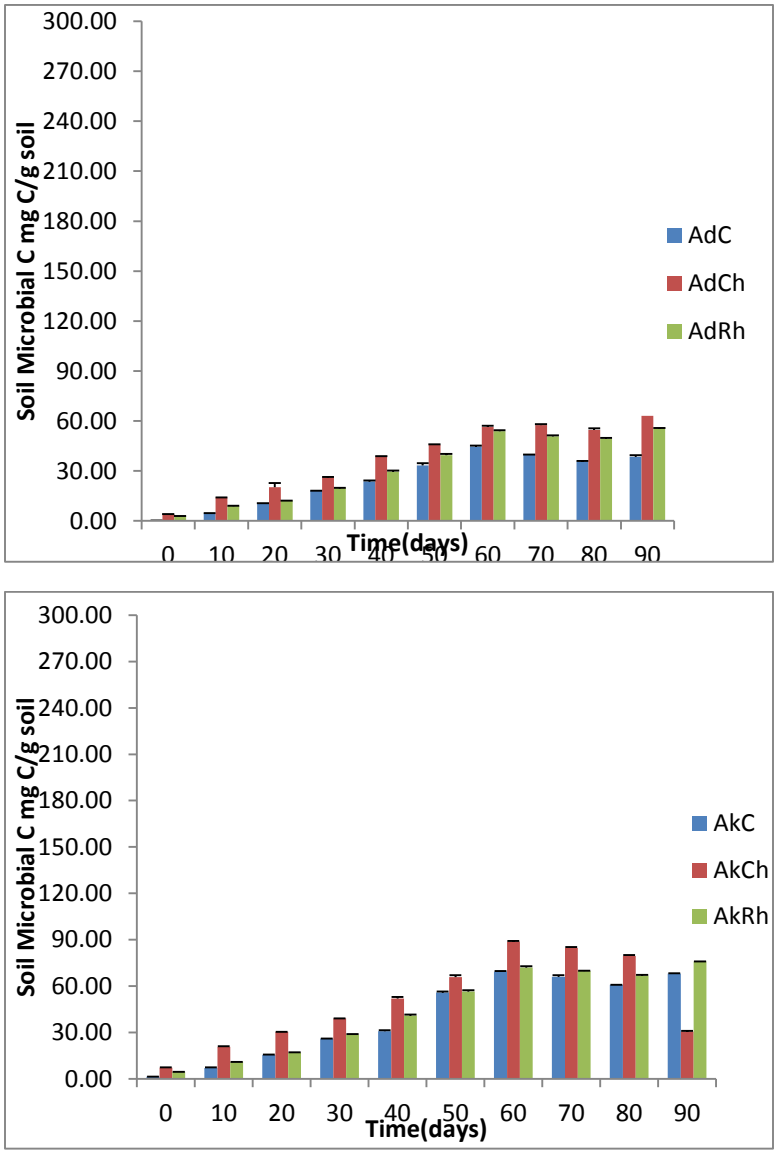


Figure 4.3a Microbial biomass C in the Adenta series and Akuse series when no atrazine and paraquat had been applied.
(AdC-Adenta series un-amended with biochar, AdCh- Adenta series amended with CHB, AdRh-Adenta series amended with RHB, AkC-Akuse series un-amended with biochar, AkCh-Akuse series amended with CHB, AkRh-Akuse series amended with RHB).
The un-amended soil had the least microbial biomass within the 90 day incubation period and was

not significantly different from the biochar amended Adenta series ($p > 0.05$) in the Adenta series.

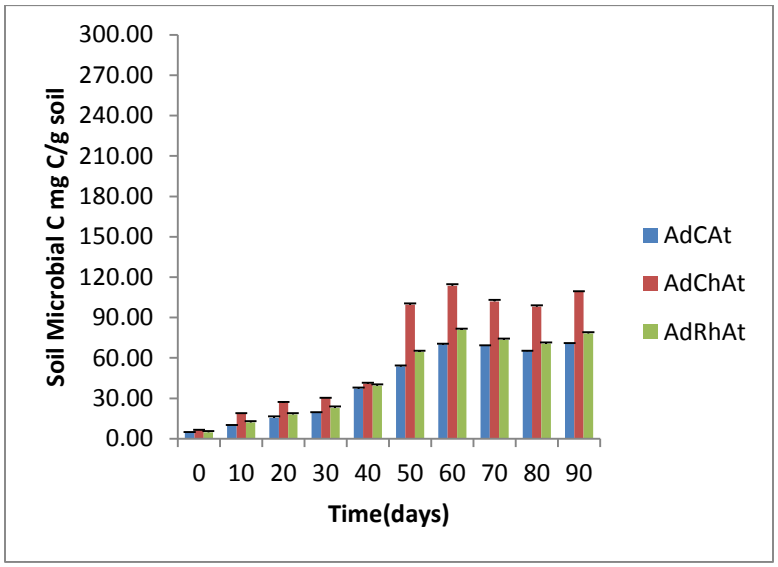
In the Akuse series, the CHB amended treatments recorded higher biomass than RHB amended soils and the control soil. High microbial biomass was recorded from days 60 to 90 for the CHB amended Akuse series (87.3 to 90.5 mg C/g soil). In the Adenta series however amending the soil with the CHB biochar types gave lower values of 56.44 to 63.07 mg C/g soil (Fig. 4.3a). Generally, higher microbial biomass was obtained for the Akuse series for all treatments as compared to the Adenta series. Differences were significant between treatments of the Akuse series and the Adenta series at all times ($p<0.01$).

4.4.2 Microbial biomass carbon when normal rate of atrazine and paraquat were applied to soils

When the Adenta series was contaminated with atrazine at the normal recommended rate, high microbial biomass carbon values were obtained from day 50 to day 90 on amendment with the CHB. However, there was no significant difference in microbial biomass carbon within that period of time (Fig. 4.3b). Microbial biomass carbon for both the un-amended soil and the RHB amended Adenta series was not significantly different from each other throughout the incubation period.

When paraquat was applied to the Adenta series at the normal field rate, depressive effect was observed on the microbial biomass carbon especially for the biochar amended soils. Thus at day 90, Adenta series amended with CHB and contaminated with atrazine had a microbial biomass carbon of 109.15 mg/g soil whilst the same treatment when contaminated with paraquat recorded a microbial biomass carbon of 87.90 mg/g soil (Fig. 4.3b). Significant differences in treatments were observed between Adenta series contaminated with atrazine and the Adenta series contaminated

with paraquat ($p<0.05$).



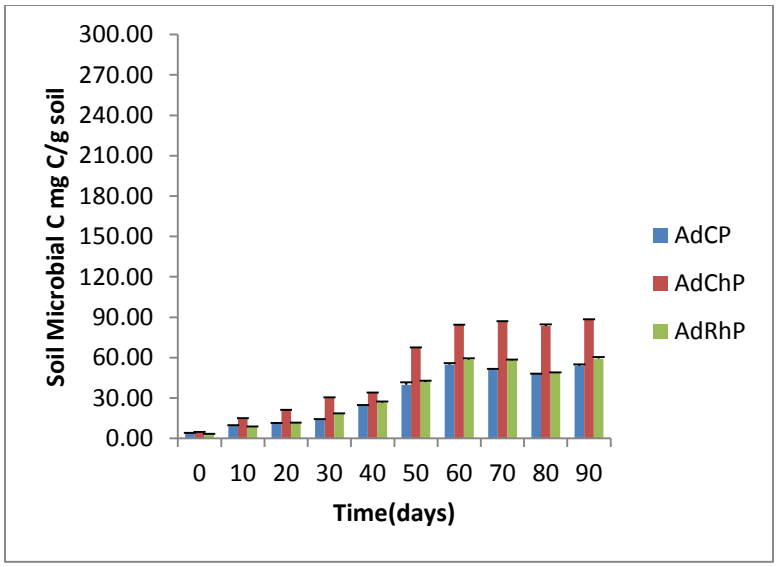


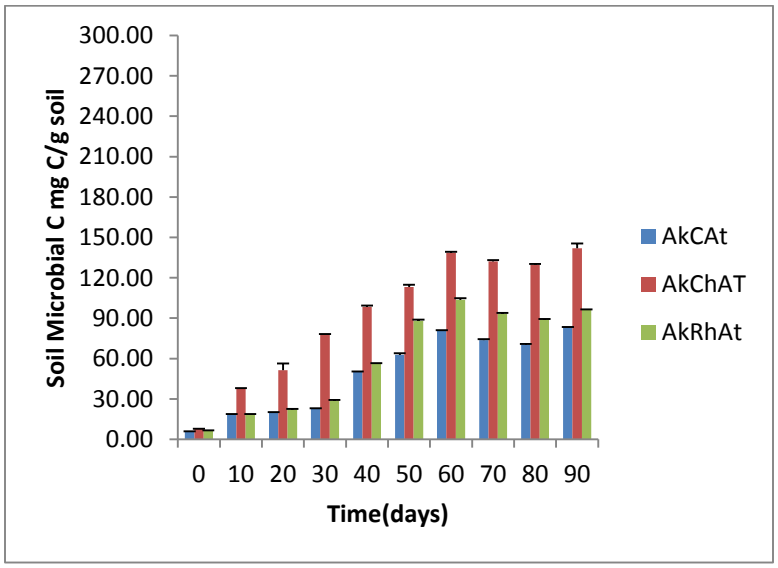
Figure 4.3b Microbial biomass C in Adenta series when normal rate of atrazine and paraquat had been applied.

(AdCAt-Adenta series un-amended with biochar with atrazine applied, AdChAt-Adenta series amended with CHB contaminated with atrazine, AdRhAt- Adenta series amended with RHB contaminated with atrazine, AdCP-Adenta series un-amended contaminated with paraquat, AdChP-Adenta series amended with CHB contaminated with paraquat, AdRhP- Adenta series amended with RHB contaminated with paraquat).

High microbial biomass carbon of between 113 and 148 mg/g soil was observed in the Akuse

series from day 50 to 90 for the CHB amended soil contaminated with atrazine (Fig 4.3c). Lower microbial biomass carbon values were observed for the RHB amended soil and the un-amended soil. There was no significant difference between un-amended and RHB amended soils when they were contaminated with atrazine during the period of incubation (Fig. 4c). Significant differences were observed between the un-amended and amended Akuse series when the normal rate of atrazine was applied ($p < 0.05$).

When paraquat was applied to both the un-amended and biochar amended Akuse series, high microbial biomass was also observed from days 50 to 90 with the CHB amended Akuse series even though there were no significant differences among microbial biomass carbon (Fig 4.3c). There were significant differences ($p<0.05$) between atrazine and paraquat contaminated Akuse series, irrespective of whether the vertisol was amended with biochar or not.



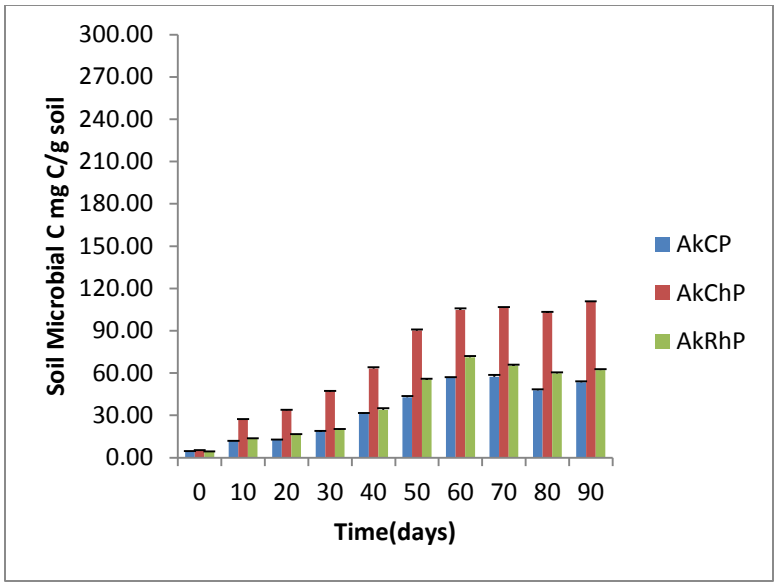
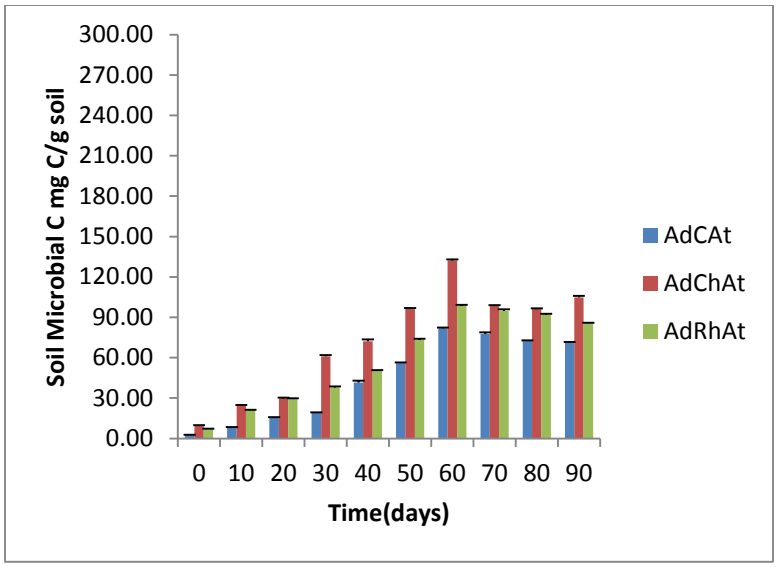


Figure 4.3c Microbial biomass C in the Akuse series normal rate of atrazine and paraquat had been applied.

(AkCAt-Akuse series un-amended with biochar and atrazine applied, AkChAt-Akuse series amended with CHB with atrazine applied, AkRhAt- Akuse series amended with RHB with atrazine applied, AkCP-Akuse series un-amended with paraquat applied, AkChP-Akuse series amended with CHB with paraquat applied, AkRhP- Akuse series amended with RHB with paraquat applied).

4.4.3 Microbial biomass carbon when ten times the normal rate of atrazine and paraquat were applied to soil

Microbial biomass carbon was highest on day 60 (132.40 mg/g soil) decreasing with time there after in the Adenta series amended with CHB. Higher microbial biomass carbon was obtained for the Adenta series amended with CHB as compared to the same soil amended with RHB and the un-amended (control) soil (Fig 4.3C)



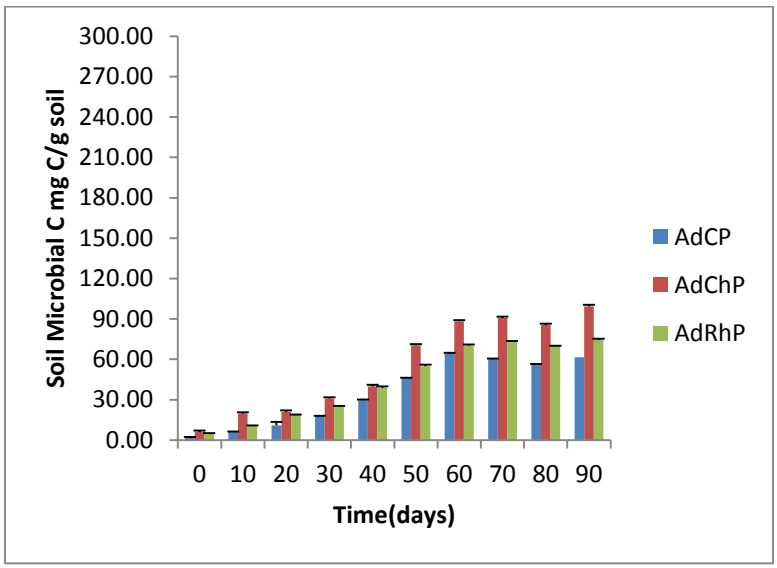


Figure 4.3d Microbial biomass C in Adenta series when ten times the normal rate of atrazine and paraquat were applied.

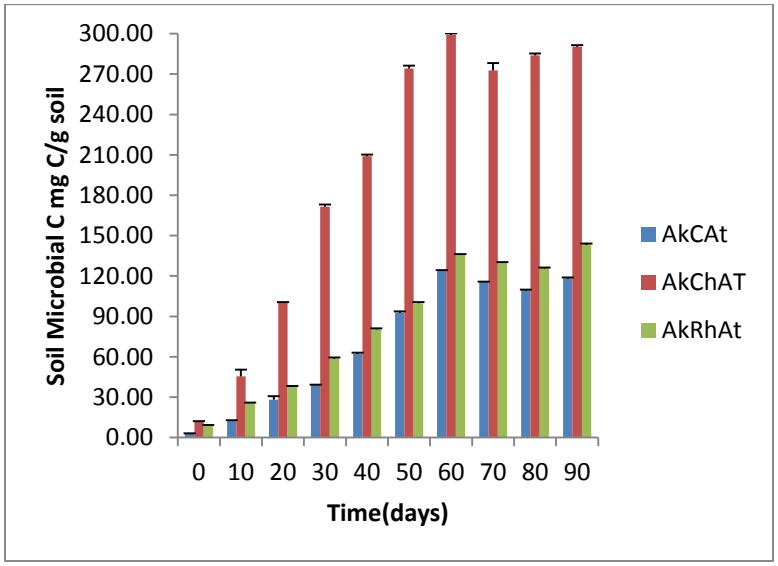
(AdCAt-Adenta series un-amended contaminated with atrazine, AdChAt-Adenta series amended with CHB contaminated with atrazine, AdRhAt- Adenta series amended with RHB contaminated with paraquat, AdChP-Adenta series amended with CHB contaminated with paraquat, AdRhP- Adenta series amended with RHB containated with paraquat).

When the Adenta series was contaminated with paraquat at ten times the normal application rate, the CHB amended soil had high microbial biomass carbon from days 60 to 90 (ranging from 88.18 to 99.44 mg/g soil). Depressive action of paraquat as compared to atrazine in the Adenta series was not significant ($p>0.05$), Fig 4.3d.

Akuse series amended with CHB and contaminated with atrazine ten times the normal application rate recorded very high microbial biomass values (Fig. 4.3e). Microbial biomass carbon was significantly higher for the CHB treatment than the soils amended with RHB and the unamended soils. From days 30 to 90, the highest microbial biomass carbon was recorded and the values ranged from 171.42 to 290.30 mg C/ g soil.

When paraquat was applied to the Akuse series, the highest microbial biomass carbon was observed from day 40 to day 80 with the microbial biomass carbon ranging from 110.71 to 104.83 mgC/g soil (Fig. 4.3e). With the paraquat contaminated Akuse series, significant differences existed between soils amended with CHB and the un-amended soils treatments ($p<0.05$).

Significant differences existed between Akuse series contaminated with atrazine and Akuse series contaminated with paraquat at ten times the normal application rate ($p< 0.05$) showing the depressive action of paraquat on microbial biomass in the Akuse series.



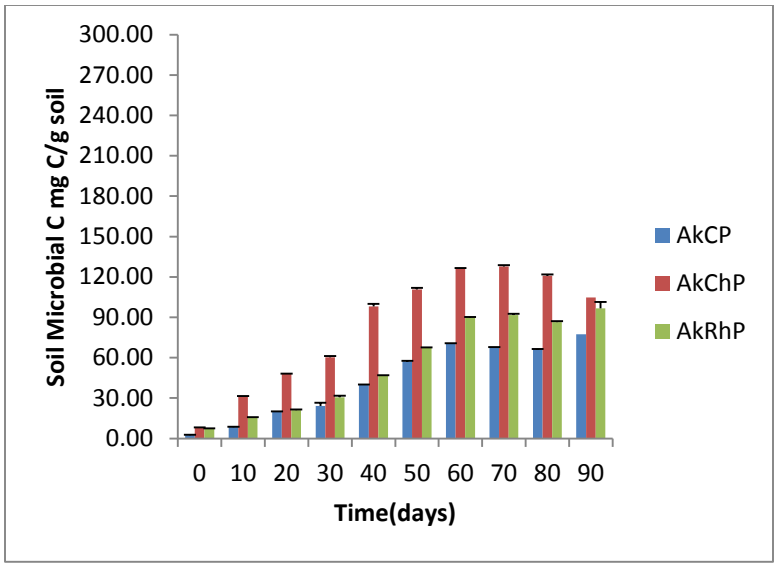
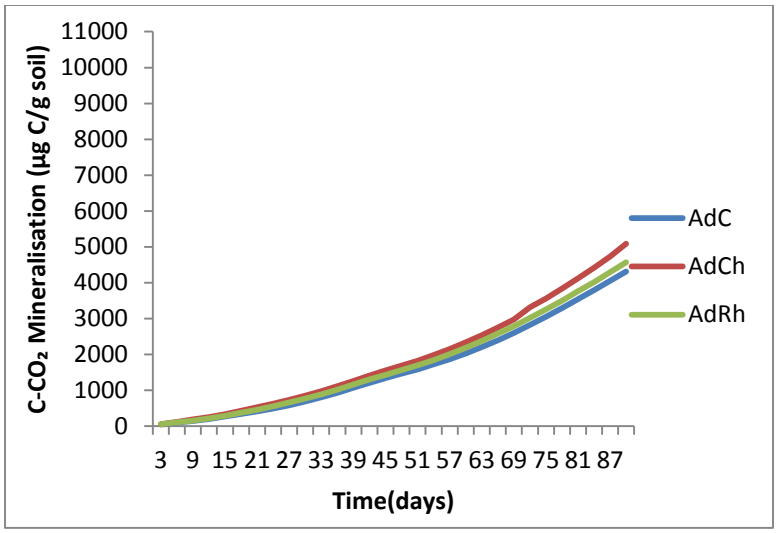


Figure 4.3e Microbial biomass carbon when ten times the normal rate of atrazine and paraquat were applied to Akuse series.

(AkCAt-Akuse series un-amended contaminated with atrazine, AkChAt-Akuse series amended with CHB contaminated with atrazine, AkRhAt- Akuse series amended with RHB contaminated with atrazine, AkCP-Akuse series un-amended contaminated with paraquat, AkChP-Akuse series amended with CHB contaminated with paraquat, AkRhP-Akuse series amended with RHB containated with paraquat).

4.5 Carbon mineralization in soils contaminated with pesticides and amended with biochar

Carbon mineralization increased with time in all treatments and in the two soils used. The highest carbon mineralization was observed in soils amended with CHB followed by soils amended with RHB. The un-amended soils (control) had the least carbon mineralization value at any point in time. Differences in C mineralized were not significant ($p > 0.05$) in soils not contaminated with pesticide (Fig 4.4a).



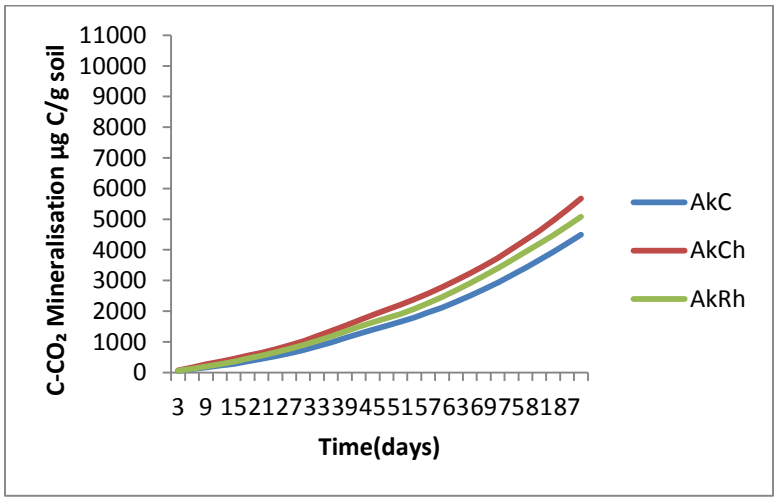


Fig 4.4a Carbon mineralization in the Adenta and Akuse series not contaminated with atrazine and paraquat.

When normal rate of atrazine was applied to the Adenta and Akuse series, the CHB amended treatments had the highest carbon mineralization values in both soil series (Figs. 4.4b). It is worthy of note that there was unusually high carbon mineralization of Akuse series amended with CHB and contaminated with atrazine at the normal application rate, making that treatments significantly different from the other atrazine amended soils especially from days 50 to 90 ($p<0.05$) (Fig. 4.4b). A similar observation was made when the Akuse series amended with cocoa husk biochar was contaminated with 10X atrazine (Fig. 4.4c). Generally, paraquat treated soils be it normal rate or ten times the normal rate of application, had lower carbon mineralization values

than soils treated with atrazine

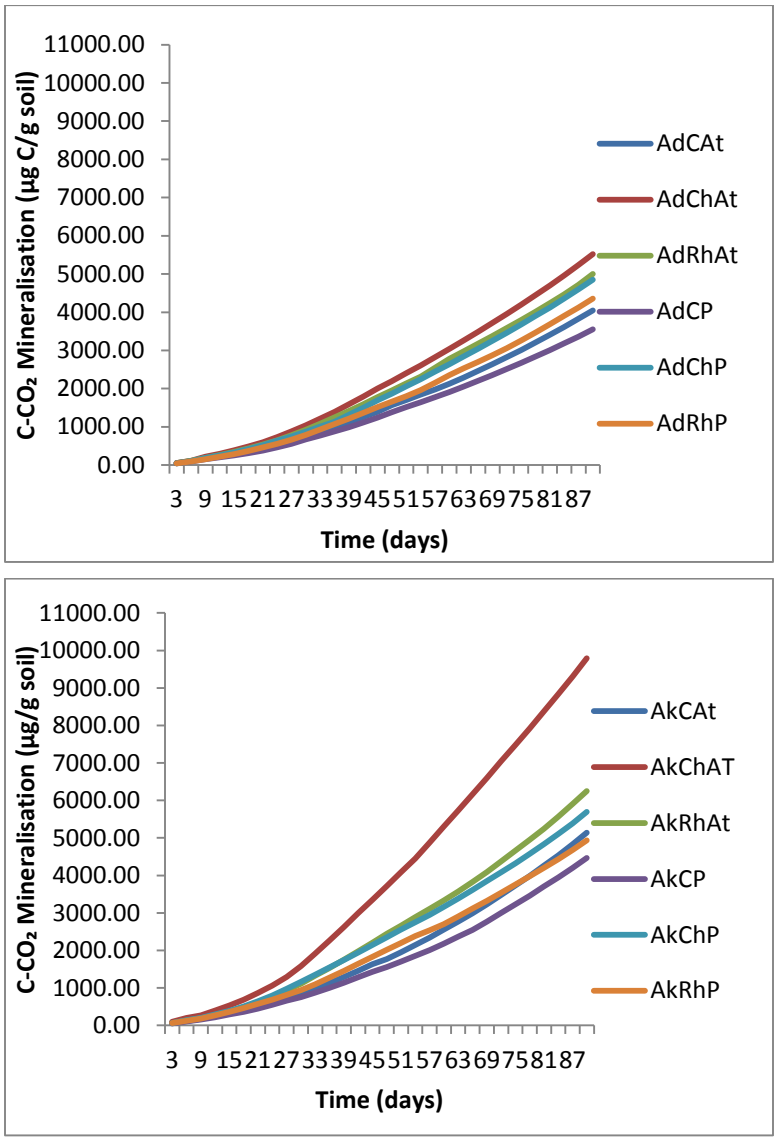
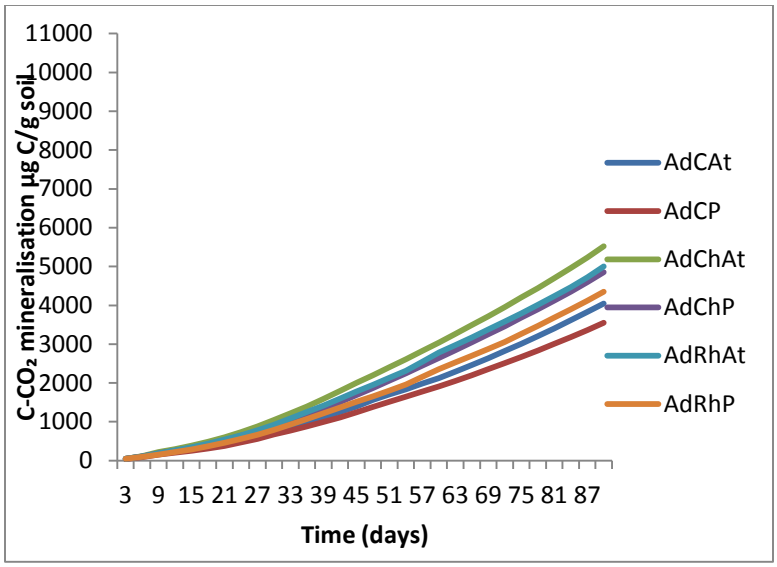


Figure 4.4b Carbon mineralization in the Adenta and the Akuse series when normal rates of atrazine and paraquat were applied.



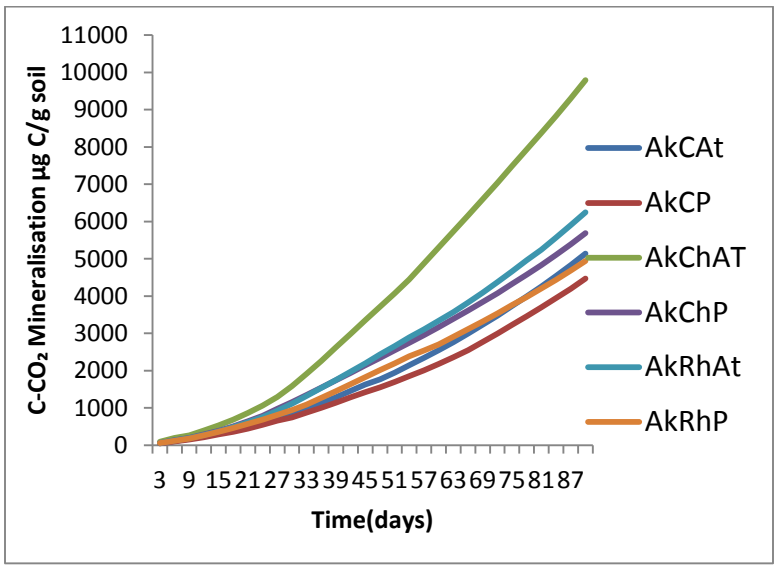


Figure 4.4c Carbon mineralization in the Adenta and the Akuse series when ten times the normal rates of atrazine and paraquat were applied.

4.5.1 Influence of pesticide on carbon mineralization rate of biochar amended and un-amended soils

Carbon mineralization rate of the un-amended Adenta series with no pesticide applied was lower (47.3 µgC/g soil) than the carbon mineralization rate of un-amended Akuse series (49.47 µgC/g soil) with no pesticide applied. Amendment of the Akuse series with CHB (AkChAt and AkChP treatments respectively) stimulated carbon mineralization rates to 62.46 µgC/g soil (Table 4.3).

The Akuse series amended with RHB (AkRhP and AkRhAt) was less stimulated and the carbon mineralization rate was 55.76 µgC/g soil when no pesticide had been added (Table 4.3). In the Adenta series, carbon mineralization rate was 55.91 µgC/g soil when amended with CHB (AdChAt and AdChP) whilst in the same soil amended with RHB (AdRhAt and AdRhP), carbon mineralization rate was 50.21 µgC/g soil ($p>0.05$) and no pesticide had been applied.

When the normal rate of application of atrazine and paraquat were added to the Akuse and Adenta series, carbon mineralization rates were intermediate between the soils that had not been treated with pesticide and the soils that 10X the pesticide treatment ($p<0.05$) (Table 4.3). Carbon mineralization rates were stimulated when the normal application rate of atrazine and paraquat were added to the Adenta and Akuse series compared to the same treatments that no pesticide had previously been applied. It was only AdCP where slight depressive effect was observed compared to the same treatment when no paraquat had been added. The highest carbon mineralization was observed for AkChAt (81.78 µg/g soil).

Table 4.3 Carbon mineralisation rate of Akuse and Adenta series between 30 to 50 days of incubation amended and un-amended with biochar and as influenced by pesticides at different rates.

Treatment	*Carbon mineralisation rate (no pesticide applied) µg/g soil/day	Carbon mineralization rate (Normal application rate of pesticide) µg/g soil/day	Carbon mineralization rate (10 times normal application rate) µg/g soil/day
AdCAt	47.3	47.48	73.2
AdChAt	55.91	67.86	79.1
AdRhAt	50.21	55.02	76.0
AdCP	47.3	42.10	52.1
AdChP	55.91	59.22	62.1
AdRhP	50.21	50.70	49.1
AkCAt	49.47	55.42	60.1
AKChAt	62.46	81.78	91.2
AkRhAt	55.76	77.24	79.1
AkCP	49.47	47.43	62.3
AkChP	62.46	69.58	70.34
AkRhP	55.76	62.68	68.1
p	<0.05	<0.05	<0.05

*No pesticide (0 kg a.i/ha) had been added to soil therefore treatments AdCAt, AdChAt, AdRhAt, AdCP, AdChP, AdRhP, AkCAt, AkChAt, AkRhAt, AkCP,AkChP, AkRhP could be considered as $AdCAt_0$, $AdChAt_0$, $AdRhAt_0$, $AdCP_0$ and so forth for column 2 above.

When atrazine was added to the Adenta series at 10X the normal application rate, the mineralization rates of the un-amended soil was 73.2 µgC/g soil and 79.1µgC/g soil for CHB amended soil (Table 4.3). The same soil treated with paraquat, depression of carbon mineralization rate were observed especially with the RHB ($p<0.01$).

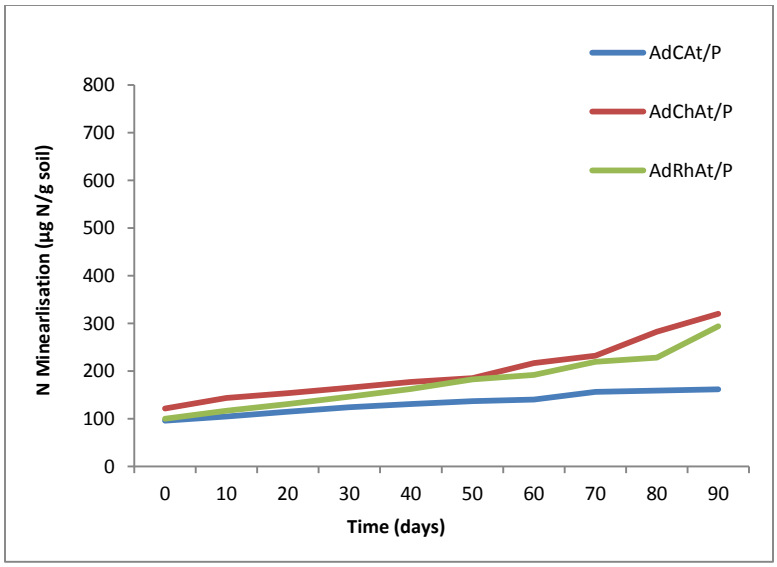
When the Akuse series was amended with CHB and was contaminated with 10X normal rate of atrazine, the highest carbon mineralization rate occurred with treatment AkChAt followed by AkRhAt treatment (91.2 and 79.1µgC/g soil). Carbon mineralization rates were lower in the Akuse series that 10X the normal application rate of paraquat have been added (Table 4.3) as compared to the same soil that atrazine 10X the normal application rate added.

4.6 Nitrogen mineralization

The N mineralization of un-amended and amended soils with and without pesticide application is presented in the ensuing sections.

4.6.1 Nitrogen mineralization when no atrazine and paraquat were applied to soils

Nitrogen mineralized increased with time in both the Adenta series and the Akuse series. By the 90th day, about 300 µgN/g soil had been mineralized in the Adenta series amended with cocoa husk biochar (Fig. 4.5a). Mineralization was generally higher in Adenta series amended with cocoa husk biochar as compared to the same soil amended with rice husk biochar. A similar observation was made with the Akuse series (Fig. 4.5a).



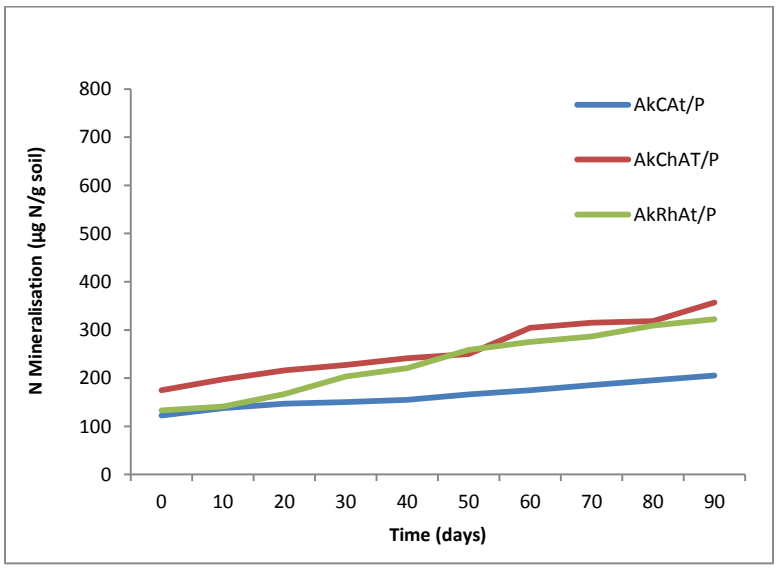


Figure 4.5a Gross N mineralization in the un-contaminated Adenta and Akuse series.

4.6.2 Nitrogen mineralization at normal rate of atrazine and paraquat applications

On application of the herbicides to the Adenta series, no significant differences ($p > 0.05$) in treatments were observed in nitrogen mineralization during the incubation period, (Fig. 4.5b).

In the Akuse series, nitrogen mineralization was in the order of cocoa husk biochar amended soils contaminated with atrazine (AkChAt) > followed by the same soil amended with rice husk and also contaminated with atrazine (AkRhAt) > the rest. Thus by day 90, N mineralized was 500 µg/g

soil in the AkChAt treatment and 410 µg/g soil in the AkChP treatment. The same treatments (AdChAt and AdChP) in the Adenta series resulted in 350 µg/g soil and 300 µg N /g soil of nitrogen mineralized (Fig. 4.5b).

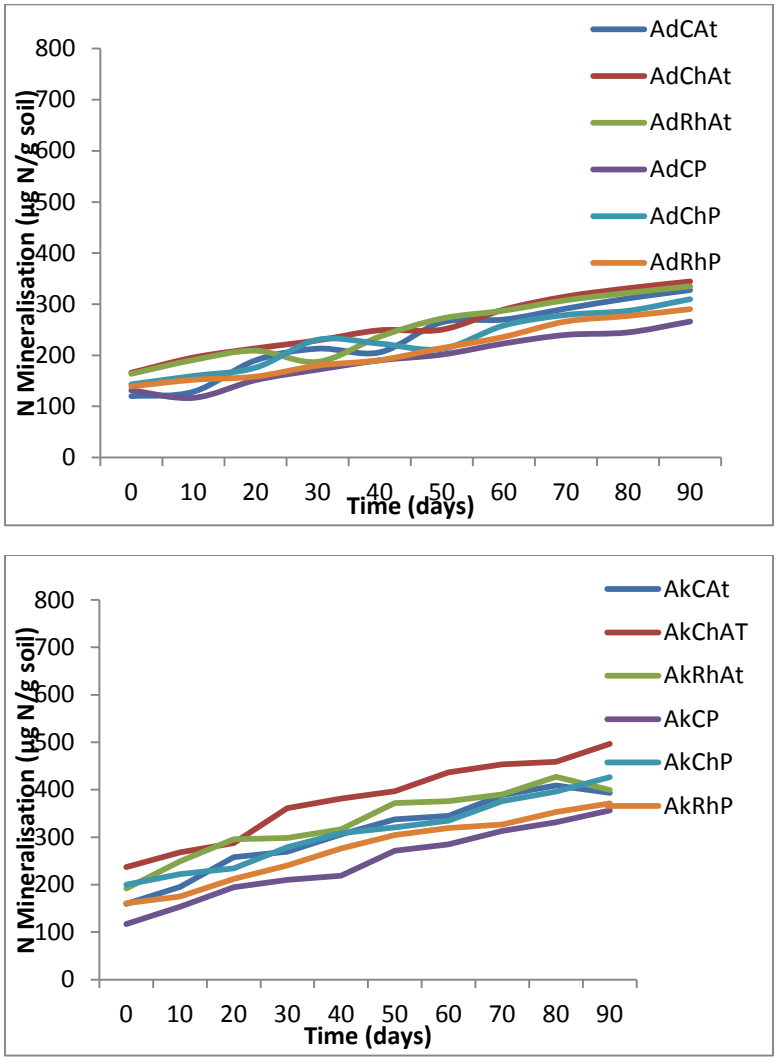


Fig 4.5b Gross nitrogen mineralization in Adenta and Akuse series at 1X normal atrazine and paraquat application rate.

4.6.3 Nitrogen mineralization at 10X normal rate of atrazine and paraquat applications

Significant differences in nitrogen mineralized was not observed with the different treatments in the Adenta series ($p>0.05$) when contaminated with 10X the normal rate of atrazine and paraquat. However, in the Akuse series, significant differences ($p <0.01$) were observed in treatments

especially by day 50, (Fig. 4.5c). When Akuse series was contaminated with atrazine upon amendment with CHB (AkChAt), gave the highest nitrogen mineralization throughout the incubation period followed by the atrazine contaminated RHB amended soil (AkRhAt). Akuse series treated with paraquat gave lower nitrogen mineralization during the period of investigation.

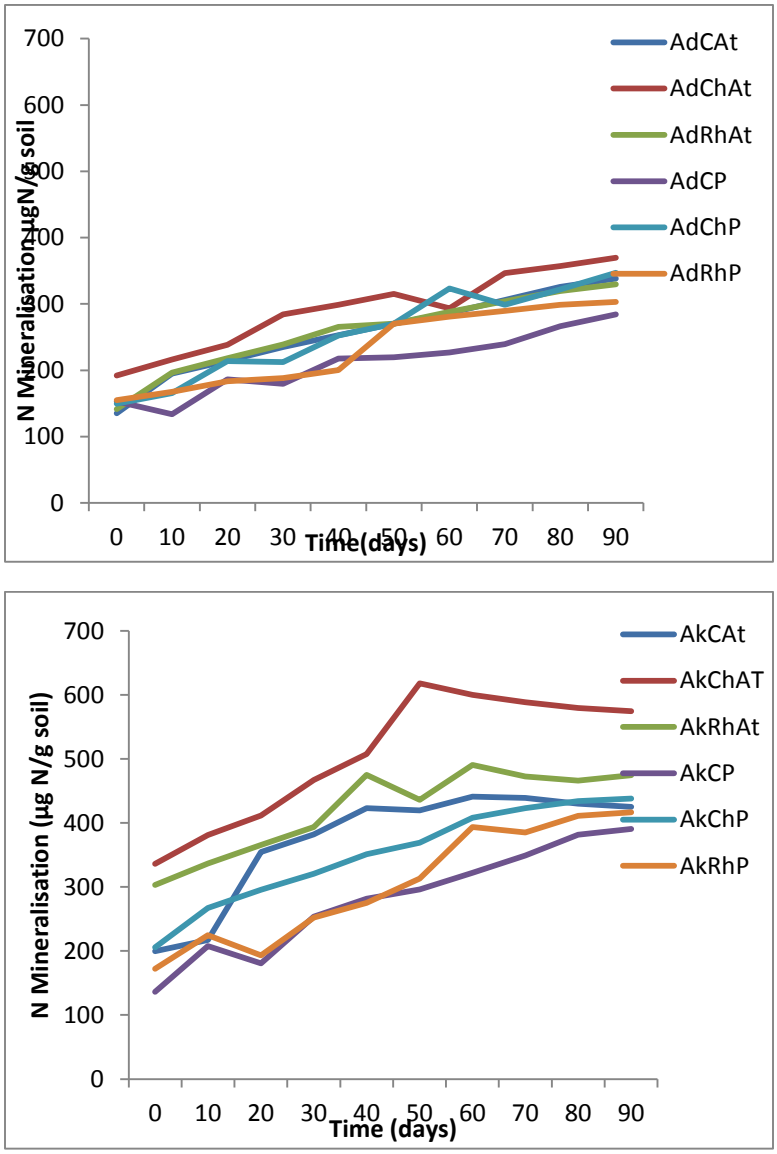


Figure 4.5c: Gross nitrogen mineralization in Adenta and Akuse series at 10X normal atrazine and paraquat application.

4.6.4 Nitrogen mineralization rates as influenced by concentrations of herbicide

Nitrogen mineralization rate of the un-amended Akuse series (AkCAt and AkCP) was higher than that of the un-amended Adenta series (AdCAt and AdCP) when no pesticide had been applied to both treatments (Table 4.4). When amended with biochar (CHB and RHB) and no pesticide had been applied, the nitrogen mineralization rate was almost the same for both soil series with rates of about 2 µg/g soil. For instance, RHB amended Adenta series not contaminated with atrazine (AdRhAt) and RHB amended Akuse series not contaminated with atrazine (AkRhAt) had the same nitrogen mineralization rates of 2.1µg/g soil and 2.15 µg/g soil, respectively (Table 4.4).

The application of atrazine and paraquat generally stimulated nitrogen mineralization rates in all the biochar amended Akuse series compared to the uncontaminated Akuse series i.e. no addition of atrazine or paraquat (Table 4.4). For the Adenta series depression of nitrogen mineralization was observed as compared to the same soil that no pesticide had been applied. The highest nitrogen mineralization rate was AkChAt (2.89 µg/g soil/day) followed by AkChP (2.66 µg/g soil/day) when normal rates of atrazine and paraquat had been, respectively, applied to the Akuse series amended with CHB. Generally, nitrogen mineralization rates were high in the Akuse series as compared to the Adenta series.

When ten times the normal rate of atrazine was applied, nitrogen mineralization rates were high for Akuse series as compared to the Adenta series. The highest nitrogen mineralization rate occurred when the un-amended Akuse soil was contaminated with paraquat (AkCP) followed by the same soil amended with RHB and contaminated with paraquat (AkRhP) (Table 4.4).

Table 4.4 Nitrogen mineralisation rate of biochar amended and unamended Akuse and Adenta series contaminated with the herbicides.

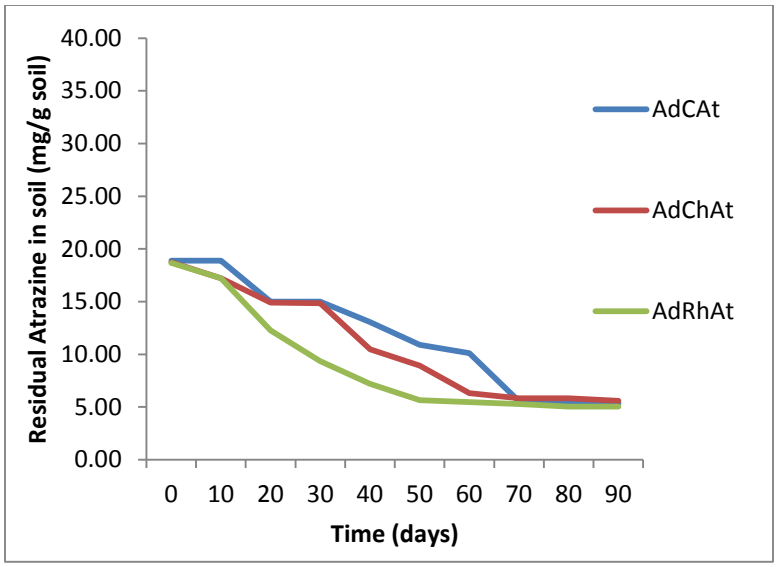
Treatment	*Nitrogen mineraliation rate 0-90 days where no pesticide was applied	Nitrogen mineralization rate 0-90 days where the normal rate (1X) of pesticide was applied	Nitrogen mineralization rate 0-90 days µg/g soil/day where 10X normal rate was applied
AdCAt	0.74	2.03	2.26
AdChAt	2.204	1.983	1.975
AdRhAt	2.15	1.902	2.08
AdCP	0.74	1.496	1.23
AdChP	2.204	1.85	2.20
AdRhP	2.15	1.679	2.1
AkCAt	0.92	2.6	2.50
AKChAt	2.02	2.89	2.65
AkRhAt	2.1	2.3	1.92
AkCP	0.92	2.66	2.82
AkChP	1.75	2.51	2.57
AkRhP	2.1	2.34	2.72
p	> 0.05	<0.05	>0.05

*No pesticide (0 kg a.i/ha) had been added to soil therefore treatments AdCAt, AdChAt, AdRhAt, AdCP, AdChP, AdRhP, AkCAt, AkChAt, AkRhAt, AkCP,AkChP, AkRhP could be considered as $AdCAt_0$, $AdChAt_0$, $AdRhAt_0$, $AdCP_0$ and so forth… for column 2 above.

4.7 Pesticide Degradation

The pesticide degradation of un-amended and amended soil is presented in the ensuing sections.

When the Adenta series was contaminated with atrazine at the normal rate of application, degradation was faster in the soil amended with RHB (AdRhAt) than when the same soil was amended with CHB and the un-amended Adenta series. Significant differences in treatments between AdRhAt and the others were observed on day 40 (Fig. 4.6a).



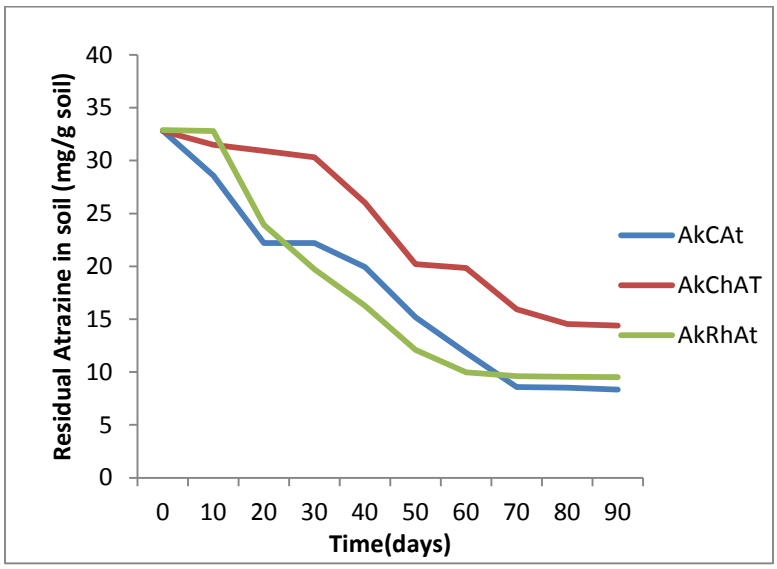


Figure 4.6a: Degradation of Atrazine at normal application rate in Adenta and Akuse series.

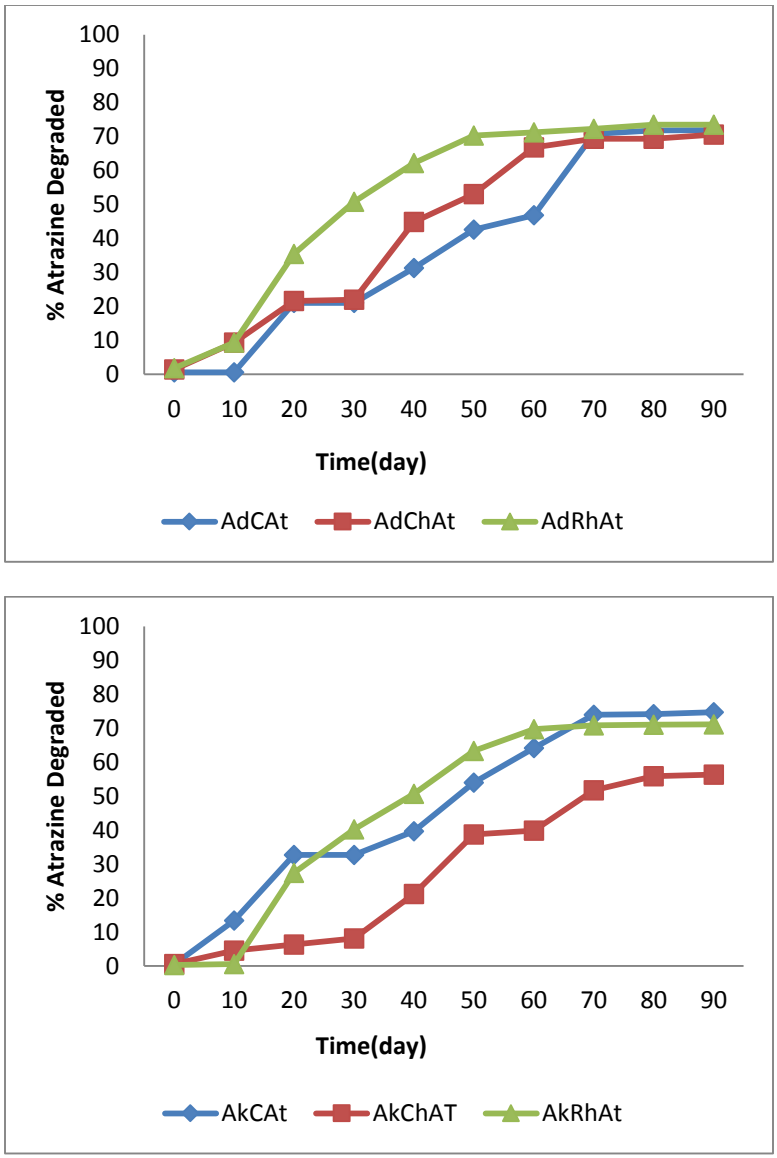


Figure 4.6b Percentage degradation of atrazine applied at the normal rate in the Adenta and Akuse series.

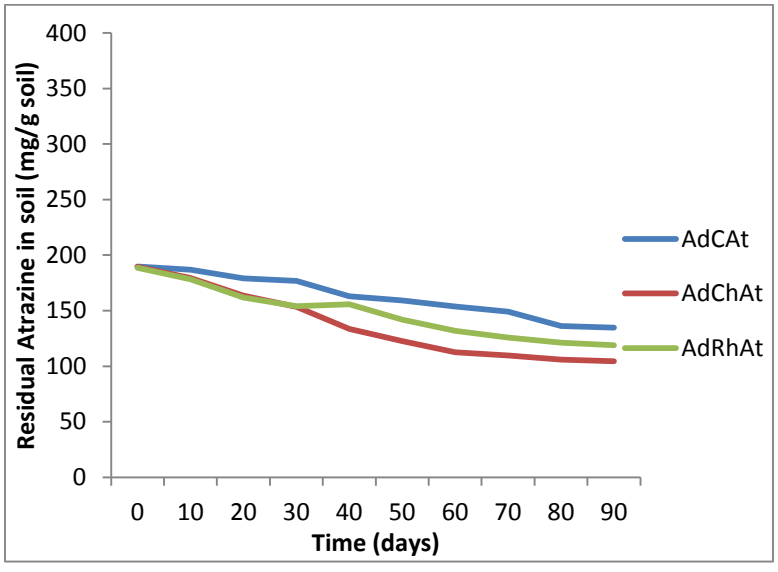
In the Akuse series, when the normal rate of atrazine was applied, degradation was faster in the RHB amended soil than the un-amended soil and the CHB amended soil (AkChAt) (Fig. 4.6a). There were significant differences in treatment between the AkChAt and the other treatments.

The percentage degradation was higher in the RHB and the un-amended soils than in soils amended with CHB when atrazine was applied at normal rate to both the Adenta and the Akuse series (Fig. 4.6b). At such an application rate, the atrazine was a carbon and nitrogen source for

microbes in the soil such as heterotrophs, hence the microbes made use of some of the pesticide for metabolic activities and the rest was degraded. Results also show that at the normal rate of pesticide application, indigenous soil microorganisms could effectively degrade the herbicides.

When 10X the normal application rate of atrazine was added to the Adenta series, degradation was faster in the Adenta series amended with biochars, initially with AdRhAt and later with AdChAt treatments. Treatment differences were significant in treatment from day 50 to 70 (Fig. 4.6c).

When 10X the normal application of atrazine was added to the Akuse series, degradation was fastest in the Akuse series amended with CHB compared to the same soil amended with RHB and the un-amended soil. Significant differences in treatments were observed between the AkChAt and the other treatments from days 50 to 70 (Fig. 4.6c). The percentage degradation of atrazine showed that when both soils are contaminated with atrazine at 10X the normal rate of application, CHB amended soils degraded atrazine fastest (Fig. 4.6d).



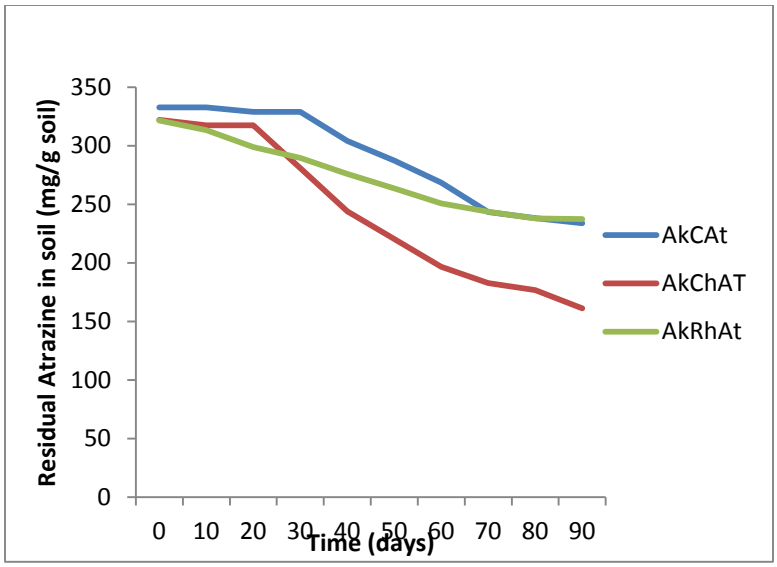
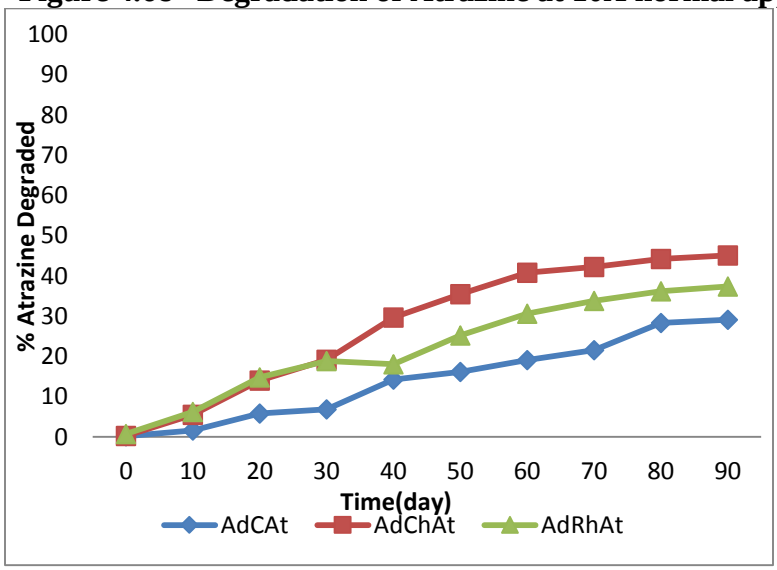


Figure 4.6c Degradation of Atrazine at 10X normal application rate.



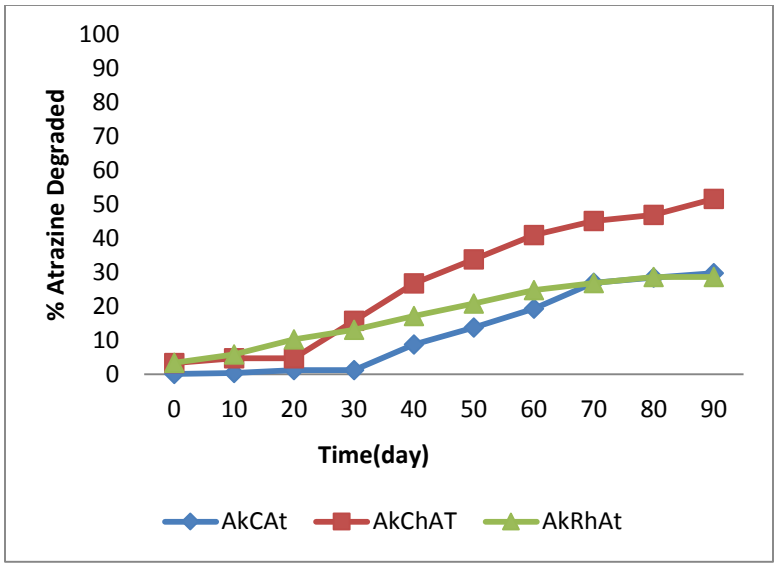
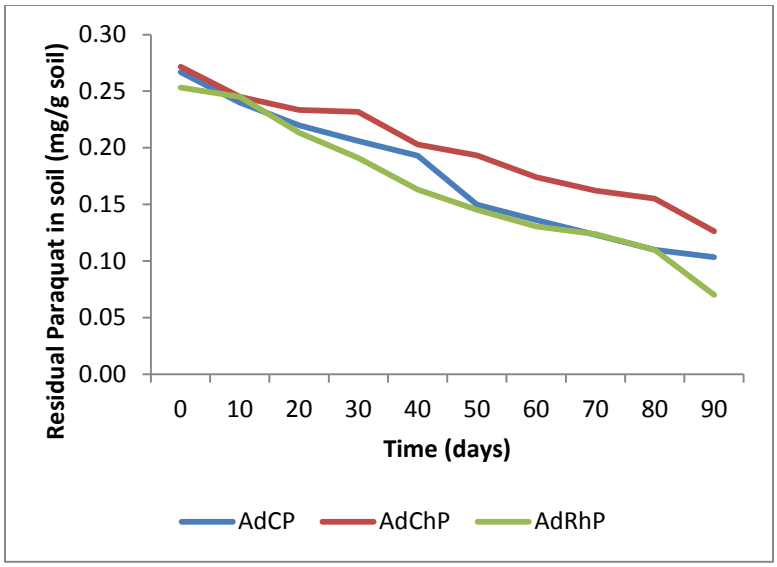


Figure 4.6d Percentage degradation of atrazine in the Adenta and the Akuse series at 10Xnormal application rate.

4.8 Degradation of paraquat in the Adenta and Akuse series

The results also showed that there was little degradation of this pesticide in both soils albeit more rapid degradation in Adenta than the Akuse series. Paraquat degradation was higher in the Adenta series amended with RHB than the same soil amended with CHB and the un-amended soil. Significant differences in treatments were observed in days 50-80 for the RHB amended Adenta series and other treatments (Fig. 4.6e).

In the Akuse series, a similar observation to that in the Adenta series was made with high degradation obtained in the RHB amended soil than the other treatments and in days 30 to 90 significant differences in treatments between the Akuse series amended with RHB and other treatments were observed (Fig. 4.6e).



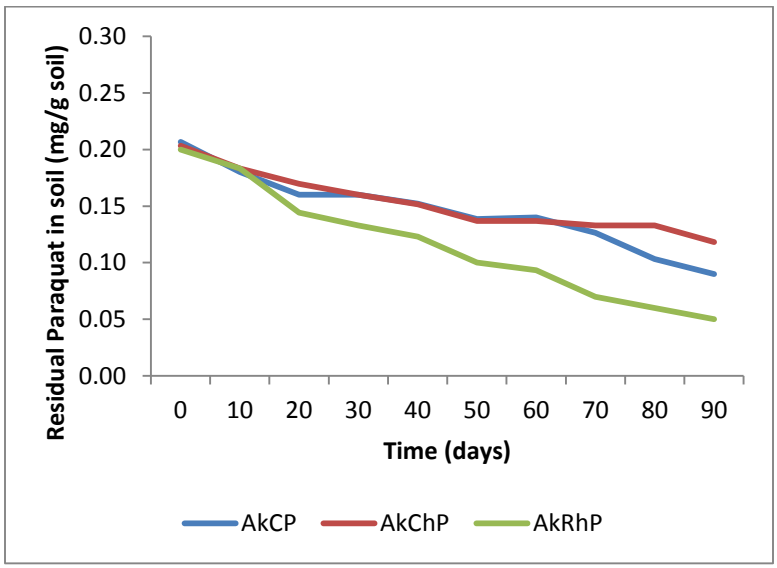
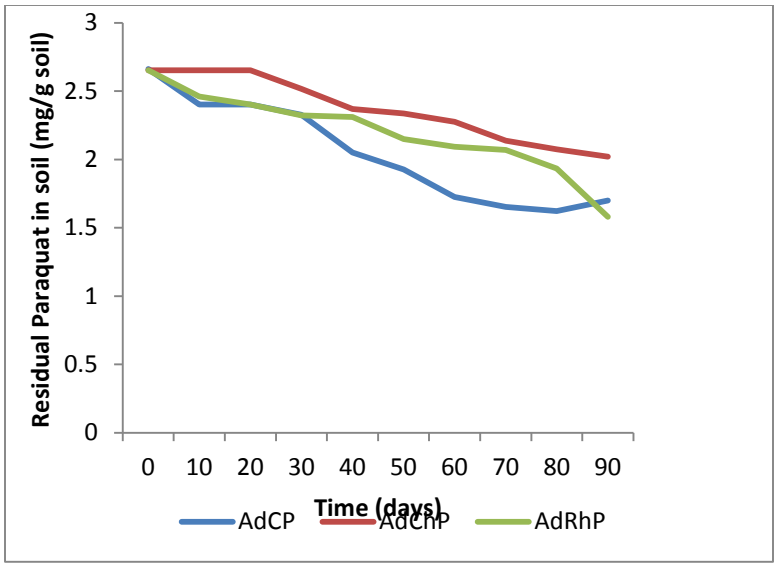


Figure 4.6e Paraquat degradation in Adenta and Akuse series at normal application rate.

Paraquat degradation at 10X normal application to Adenta series, degradation was faster in the control soil than in soils amended with biochars (Fig. 4.6f). Significant differences in treatment were observed between the un-amended soil and other treatments from days 40 to 80.

Degradation of paraquat in the Akuse series followed the same trend as observed when ten times the normal rate of application of paraquat was applied to Adenta series (Fig. 4.6f).



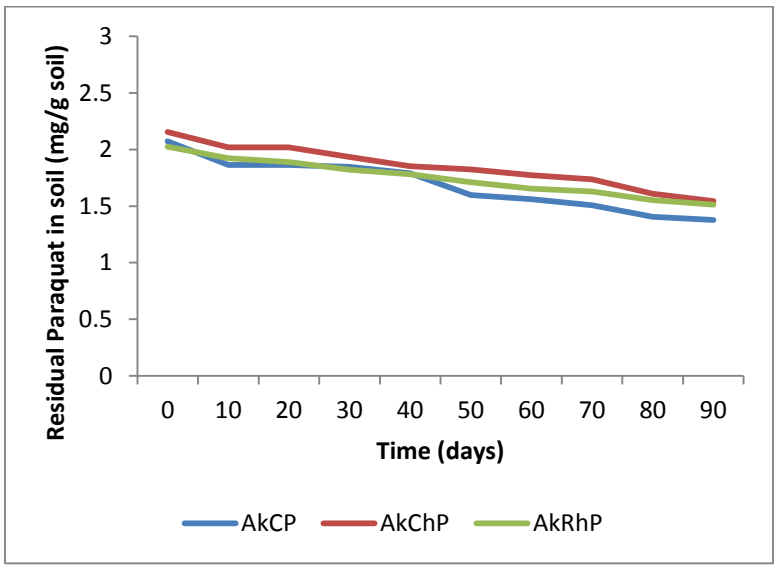


Figure 4.6f Paraquat degradation in Adenta and Akuse series 10X normal application rate.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Soil Characterization

The Adenta series is sandy clay loam in texture whilst the Akuse series is a sandy clay soil according to the USDA system of classification (Soil Survey Staff, 2003). The higher sand content of 70.3% in the Adenta series than the Akuse (47.1%) soil is due to the fact that the former has been formed from tertiary sand with the latter from garnetic ferrous hornblende gneiss (Brammer, 1962). The medium bulk density of 1.3 $Mg/m^3$of the Adenta series reflects the sandy clay loam characteristic of the soil in the landscape. The occurrence of montimorillonite claysin Akuse series gave it higher bulk density than the Adenta series. Also the existence of, garnetic ferrous hornblende make the Akuse series harden on drying to raise the bulk density. The higher bulk density of the Akuse series could also be due to its higher Ca contents (Dowuona, 1985) which would promote aggregation.

The Akuse series has a higher pH than the Adenta series probably because of the presence of $CaCO_3$ nodules found in the soil (Ahenkora, 1997). In the Adenta series the low content of clay high sand content together with a daily average temperature of 28^oC enhanced the rapid decomposition and low retention of organic matter added. Consequently organic carbon contents of the Adenta series are low with a concomitant low total nitrogen content of approximately 1.09 g/kg and available P of 4.8 mg/kg. It has been established that clay, holds more organic carbon, total N and P than sand and silt (Jones et al., 2006). For that reason, it is not unexpected that with its high clay content, the Akuse series has quite high organic carbon and available P. The higher

available P content in the Akuse series than the Adenta series is also attributed to the pH of 7.6 of the Akuse series as compared to the pH (H_2O) value of 5.4 of the Adenta series. This 7.6 value is closer to the second pK value of orthophosphoric acid (7.15) (Evangelou, 1998) than 5.4. Thus, more of the orthophosphate anion HPO_4^{2-} will be in soil solution.

The Adenta soil with a lower clay content of 22.5% is dominated by low activity clays mainly kaolinite (Eze, 2008). Additionally, its organic carbon content is very low and explains the soil's very low CEC value of 8.12 cmol(c)/kg. The fact that the Akuse series is 47.5% higher in clay content coupled with its higher organic carbon contents in part, accounted for its higher CEC. The four times higher CEC in the Akuse series than the Adenta series is also attributed to the presence in large quantities of high activity clays, particularly montmorillonite (Dowuona, 1985).

5.2 Characterization of biochar

The pH of the two biochar was more than 7.0 and that could be due to the type of pyrolysis (Streubel, 2011). Pyrolysis between 300º C to 600ºC, organic acids and phenolic substances are released during the cracking of hemicellulose and cellulose. These acids then combine with basic cations in the feedstock to form alkali salts with a concomitant increase in pH of the biochar (Shinogi and Kanri, 2003). It is evident that type of feedstock has accounted for differences in pH in the biochar types. The strongly alkaline pH (10.4) in the CHB is explained by the presence of appreciable quantities of $KHCO_3$, and $MgCO_3$ and small quantities of MgO which are all liming materials.

In all, cocoa husk biochar had the highest nutrient composition with respect to total N, P and available phosphorus. High composition of these nutrients in the CHB especially available P which is 3987.7 mg/kg coupled with its high pH and the abundance of liming materials makes it a

suitable material to be used as soil amendment in highly acid soils like the soil of the Western region of Ghana.

Generally as during pyrolysis as temperature as temperatures increases from 350 to 600^{o}C most of the N is lost (Streubel, 2011). This is because as pyrolysis temperature increases, the N forms pyridine-like complexes that reduce its availability (Bagreev et al., 2001). The concentration of N reduced during heating and this can be attributed to volatilization and also some N-containing structures in the biochar (e.g., amino acids, amines, amino sugars) are compressed into recalcitrant types and therefore may be unavailable for plant use (Cao and Harris, 2010). It is therefore a matter of consequence that all the biochar samples have low total N (<4 g/kg) contents. The relatively higher total N contents in the CHB which is about 1.6 times that in the RHB could be due to the higher fertilization regimes of cocoa in Ghana.

The sum of the total exchangeable cations that a biochar can hold or adsorb in is termed as cation exchange capacity (CEC) (Essay, 2013) and is also a reflection of the number of negative charges created on the surface of the material. The biochars had high CEC due to the high amounts of oxygen-containing functional groups (e.g. -CO [O] and -OH) (Yuan and Xu, 2012). Another study credited the high CEC of biochar to carboxylic groups' formation by oxidation on the edges of the aromatic backbone of biochar (Glaser et al., 2002). This may imply that cocoa pod biochar with the higher cation exchange capacity of 81.67cmol/kg contain these oxygen-containing functional groups than the RHB (38.8cmol/kg) biochar with the lower CEC. To measure a soil's nutrient retention capacity and the ability to protect groundwater from cation contamination CEC is used (Essay, 2013).

The available phosphorus of CHB was about seven times higher than the rice husk that makes it a

very rich source of phosphorus for microorganisms. The bulk density of biochar was low 0.22 Mg/m^3 for CHB and 0.33 Mg/ m^3 RHB. The bulk density of the 0-0.20m of the Akuse series was 1.40 Mg/ m^3 and 1.31 Mg/m^3 for Adenta series. It is therefore expected that the overall bulk density of the soil will reduce when biochar is added (Verheijen et al., 2010).

5.3 Effect of biochar amendments on microbial population of pesticide polluted soils

Microorganisms are capable of degrading pollutants such as pesticide in many soils. The populations of microorganisms vary in different soils, and the addition of organic material may stimulate the growth of heterotrophs. The organic material acts as an energy source for the heterotrophs. It is therefore, not surprising that addition of biochar in this study enhanced the growth of those organisms capable of using atrazine and paraquat for growth. Pesticide degrading organisms besides energy source require nutrients for their growth. These nutrients are obtained from the soil but often the concentrations of these nutrients are limiting.

To synthesize their cell membranes, microorganisms require phosphorus as phospholipids as it is an intrgral part of nucleic acids and for sugar phosphorylation (Andrew and Jackson, 1996). Adenosine triphosphate is very important in microbial proliferation as it is needed for energy transfer. The main active ingredient in ATP formation is P. Thus any medium in which P is readily available would facilitate the proliferation of microorganisms. Amelioration of soil with the two biochar types resulted in increased populations of bacteria due to the concentration of the nutrient in the amendments. Since degradation of contaminants in the soil is often linked to numbers of the degrading bacteria, increasing the number of bacteria acting on a particular substrate under any given set of conditions will result in faster degradation. This was exactly what was observed; addition of biochar increased both the population of the heterotrophs, with the concomitant decrease in the levels of pesticide remaining in the soil. This finding agrees with

studies by Chorom et al. (2010), noted that application of nutrients to polluted soils produced a significant effect on groth of soil bacteria growth; and also average bacterial growth in the treatment samples was significant different from the control. Studies by several researchers have indicated that, nutrients are necessary for biodegradation activities (Ijah and Antai, 2003; Adesodun and Mbagwu, 2008; Joo et al., 2007).

The total heterotroph growth increased in the first few weeks and decreased thereafter with time. Sang-Hwan et al. (2007) a comparable observation was made and established that degrading bacteria population increased fast during the first 30 days of 105 days experiment. However, with time, due to soil resistant components and with less nutrients remaining, the growth of bacteria and pesticide degradation decreased (Schaefer and Juliane, 2007). The non-significant difference in counts as time elapsed was probably due to the depletion of these inorganic nutrients. The higher fertility status of the Akuse series did not reflect in the total heterotroph count in the un-amended (control) soils. This could be due to the fact that the Akuse series used had had no known history of farming. Akuse series on amendment with CHB recorded higher bacteria population growth that peaked at 30 days after incubation than when amended with RHB due to the higher nutrient status of the CHB than the RHB. The heterotroph count in the Akuse upon amendment with CHB being higher than when its Adenta counterpart was amended with the same material shows the contribution of the native fertility of the soil medium. The vertisol has more organic carbon, total N and available P than the Alfisol. It therefore stands to reason that upon amendment with the same material, the Vertisol showed superior THC.

In some biochar-amended soils the rate of microbial growth increased (Pietikäinen et al., 2000; Steiner, 2004), and in waste water (Koch et al., 1991). The pores in biochar are thought to protect both bacteria and fungi against grazers or competitors (Ezawa et al., 2002; Thies and Rillig, 2009).

Thus when the soils were amended, THC was higher in the two soils than their respective un-amended counterparts.

Even though there was an increase in counts for both pesticides, paraquat had depressive effects on the total heterotroph count especially at high concentration of 10X the normal application rate. However, the decline in total heterotroph count in paraquat treated soil may be due to the fact that microbial populations were intolerant of paraquat and were susceptible to the products of soil-herbicide interactions, which could have possibly been bactericidal or fungicidal (Taiwo and Oso, 1997).

Some microorganisms have the ability to degrade herbicide, while others are adversely affected depending upon the application rate/dose and type of herbicide used (Ayansina and Oso, 2006; Sebiomo et al., 2011). Thus, the effects of herbicides on soil microbial population may be either stimulating or depressive depending on the agrochemicals (type/formulation and concentration), mode of application, groups of microorganisms and environmental conditions (Subhani et al., 2000; Zain et al., 2013).

The fact that irrespective of soil type, CHB boosted the population of the heterotrophs better than RHB when either of the two amended soils was contaminated with atrazine shows the contribution of the superior nutritive value of CHB in bacteria growth. The lower heterotroph count in the paraquat than the atrazine contaminated and non-significant difference in heterotroph count when paraquat was applied to both CHB and RHB amended soils confirms depressive nature of paraquat and suggests that higher P levels may have no effect on heterotroph count in the presence of paraquat. This is further evident in the higher heterotroph count in the un-amended soils contaminated with paraquat than the amended soils contaminated with the same herbicide especially between 20 and 40 days when the population of the bacteria population should have

peaked.

5.4 Microbial biomass carbon

Soil microbial biomass, the living portion of soil organic matter, is a mediator in alteration of added and native organic matter and functions as a labile pool for plant - available N, P and S (Jenkinson and Ladd, 1981). Microbial biomass activity of the soil is normally utilized in the characterization of the microbiological status of soil (Nannipieri et al., 1990) and to establish the influence of cultivation (Anderson and Domsch, 1993: Beyer et al., 1991) and field management on soil microbes.

The level of variation in microbial is based on the value of organic matter inputs biomass Schnurer et al. (1985). Though the microbial biomass content of soil is generally linked to C inputs, other factors such as nutrient availability, moisture and temperature can adjust the development and activity of the indigenous micro flora. The higher microbial carbon in the Akuse series than the Adenta series irrespective of whether the soils were amended or not corroborates the higher fertility and in particular, the higher organic carbon status of the Akuse series. With a higher organic carbon content and a higher clay content and a higher THC, it stands to reason that the soil has a higher microbial C. Higher level of microbial biomass C was recorded in the Akuse series amended with CHB and contaminated with ten times the normal application rate of atrazine because the microbes made use of the nutrients in the CHB and at the same time had an abundance of carbon from the atrazine and these stimulated the microbial growth and degraded the atrazine.

Paraquat had N just as atrazine. However it had a detrimental effect on microbial biomass carbon probably due to the higher chloride content than atrazine that has sterilizing effect on some microorganisms. Similar work showed that due to the binding effect of paraquat on soil organic

matter, the paraquat available for the microbes to degrade reduces in soil (Bollag and Lui, 1990). Paraquat is also toxic to some microorganisms. Being cationic in nature, it can directly bind with bacteria in the soil since most bacteria are negatively charged. This adsorption may have deleterious effect on the microbe thus inhibiting their growth and preventing them from effectively degrading the pesticide.

Unlike in the THC where amending the two soils with CHB did not have any effect when paraquat was applied at both normal and 10X normal application rate, microbial biomass carbon increased when amended with CHB and on contamination with paraquat. This could be due to the fact that other microorganisms which are non-heterotroph were stimulated by the higher nutritional content of the CHB accounting for the higher microbial biomass C. The fact that at the normal and 10X application rates of paraquat on the amended soils, THC was not significantly different from the un-amended soil but microbial biomass especially in the CHB amended soil differed and was even higher at 10X the normal application rate gives further credence to the assertion that other microorganisms different from the heterotrophs may have proliferated and or involved in the degradation of paraquat.

5.5 Pesticide Degradation

Biostimulation of soil microorganisms by the addition of CHB and RHB resulted in the degradation of the pesticide especially atrazine in the contaminated soils. This is evident in the highest C mineralized as CO_2 in the two soils amended with CHB and contaminated with atrazine. It is further corroborated by the highest mineralisation rate of 81.78 and 91.2 µg C/g soil/day in the Akuse series amended with CHB and contaminated with atrazine at normal and 10X normal rate, respectively. The fact that C mineralization rate was in the order of CHB > RHB > un-amended soil, irrespective of type of herbicide used shows the positive influence of biochar in

mineralization of C from the herbicide and also the superior quality of CHB compared to RHB in degradation of atrazine and paraquat in a Calcicustert and a Ferric Acrisol.

The two herbicides have carbon and if same levels of CHB are applied and CO_2 is a product of degradation then the treatment with the highest CO_2 should be the highest in degradability. This is further corroborated by the fact that the un-amended vertisol and Alfisol contaminated with paraquat had the least mineralizable C. The reason for increased biodegradation of pesticide in the amended soils as compared to the un-amended soil could be attributed to the increase in total heterotroph counts, subsequently leading to an increase in the biodegradation of the contaminant (atrazine and paraquat). Nutrients such as P in this study would be a very important ingredient for successful biodegradation of pollutants (Cooney, 1984). Hence the addition of nutrients is necessary to enhance the biodegradation of pollutants (Choi et al., 2002; Kim et al., 2004).

The atrazine content in all the treatments decreased with time; however the treatments containing CHB had the lowest content at the end of the 90 days, thus indicating that biochar played a vital role in the utilisation of pesticide by the microorganisms. Degradation of atrazine was higher in the Adenta series than in Akuse series as is reflected in the lower residual atrazine concentration in the Adenta soil than the Akuse soil despite the latter's higher fertility. The higher residual content in the Akuse series is attributed to its higher clay content which may have sorbed some of the herbicide protecting it from biodegradation. Adenta with a higher sand content would be more aerated and less adsorptive exposing the herbicide more for microbial attack.

The fact that at normal application rate of atrazine, residual atrazine levels was lowest when the two soils were amended with RHB whereas at 10X application rate residual atrazine was lowest when the soils were amended with CHB implies that at normal rate RHB should be the preferred biochar type for remediation whereas at very high contaminations of atrazine the CHB could be

employed. Conversely, excessive nutrient concentrations could hinder biodegradation activity (Chaillan et al., 2006); and numerous works have highlighted the adverse effects of high N, P, and K levels on the biodegradation of pesticides (Oudot et al., 1998; Chaineau et al., 2005) and more particularly on the aromatics (Carmichael and Pfaender, 1997). Perhaps, the over seven times higher available P in the CHB (3897 mg/kg) than the RHB (531 mg/kg) may have suppressed degradation of atrazine at the normal application rate.

The lower residual paraquat content in the RHB soils implies that at normal application rate RHB is a better material for bioremediation and further enforces the fact that the RHB is more effective in biostimulating the soils at lower concentrations of the two herbicides. The fact that at 10X higher concentration, the two biochar types did not have any effect on paraquat degradation as residual levels in the amended soils were higher than the un-amended soil shows the depressive nature of the herbicide. Thus the use of paraquat at very high concentrations should be discouraged as it may persist in the soil for a long time.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

In alleviating climate change, enhancing crop productivity, remediating polluted environment and agricultural wastes recyclying, biochar has observed to be valuable. The benefits of biochar are numerous, tangled and takes account of direct and indirect influences. For example, biochar has remedial effects which aids in removal of contaminants and create a cleaner and healthier soil which ensures the normal growth of different crops. The numerous benefits of biochar are interconnected to each other and they would form a beneficial group when one phase is triggered. Hence, biochar can actually be an attractive alternative in modern society to change the state of pesticide contaminated soils which is as a result of the need to increase agriculture produce.

Biostimulation of indigenous microorganisms to enhance the degradation of atrazine and paraquat in a Calcicustert and a Ferric Acrisol with cocoa husk and rice husk biochar charred at 350 ^{o}C showed promising results. When the two soils were amended with cocoa husk biochar atrazine degradation was enhanced. The biochar addition significantly stimulated the degradation of atrazine in all the two soils used in the present study. The study revealed that at normal application rate rice husk biochar is a better material for degradation of atrazine whereas at very high concentrations of atrazine, cocoa husk biochar should be the preferred choice. At normal application rate of paraquat rice husk biochar aids in bioremediation but at high concentration of paraquat rice husk and cocoa husk biochar at 350 ^{o}C have no effect on bioremediation on Adenta and Akuse series. The study also showed that paraquat had an adverse effect on microbial population and activity in both Adenta and Akuse series.

Further research should be conducted:

- With soils from other ecological zone to know the effect of biochar on these soils
- On the field to ascertain whether truly the biochar would enhance bioremediation of pesticide contaminated soils. Since conditions in the greenhouse maybe different from what pertains on the field
- On field soil contaminated with cocktail of pesticides
- Different feedstocks in each agroecological zone should be used, because of the economical cost.

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APPENDIX

Variate: total heterotroph count

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Day.Soil.Biochar.Pesticide	20	1995.73	99.79	9.86	<.001
Residual	216	2186.67	10.12		
Total	236	4182.4			

Significance at 5% LSD=5.120

Variate: C-CO_2 Mineralisation

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Day.Soil.Biochar.Pesticide	20	50527.5	2526.4	2.87	<.001
Residual	216	190228.5	880.7		
Total	323	3550838.9			

Significance at 5% LSD=47.759

Variate: Microbial Biomass Carbon

Source of variation	**d.f.**	s.s.	m.s.	v.r.	F pr.
Day.Soil.Biochar.Pesticide	20	41103.82	2055.19	23.63	<.001
Residual	216	18785.52	86.97		
Total	236	59889.34			

Significance at 5% LSD=15.008

Variate: N Mimeralisation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Soil.Biochar.Pesticide	20	76147.	3807.	3.77	<.001

Residual	216	218390.	1011.		
Total	236	294537.			

Significance at 5% LSD=51.172

Variate: Pesticide residue

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Day.Soil.Biochar.Pesticide	20	1480.01	74.00	3.38	<.001
Residual	216	4724.98	21.87		
Total	236	6205.08			

Significance at 5% LSD=7.527