KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI, GHANA DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY COLLEGE OF SCIENCE

EFFECT OF SOME COMMONLY USED HERBICIDES ON SOIL MICROBIAL POPULATION

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MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

BY

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Science (MSc.) Degree, and that, to my best of knowledge it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Herbicide application has become an integral part of vibrant agricultural productivity in the whole world since its benefit has been overwhelming over the years. However, its toxic impact on the non-target soil microorganisms which play roles in degrading organic matter, nitrogen and nutrient recycling and decomposition needs to be considered. In the present study, the effect of four (4) most commonly used herbicides in Ghana; Atrazine, 2, 4-D amine, Glyphosate and Paraquat on soil microorganisms was assessed over a period of fifteen continuous days (exposure period). The herbicide treatments were the normal recommended field rate, (6.67 mg active ingredient per gram of soil for Atrazine, 6.17 mg for 2, 4-D amine, 5.56 mg for Glyphosate, and 2.46 mg for Paraquat), half the recommended field rate and double the rate. Bacterial and Fungal population were then determined at a five-day interval up to the 15^{th} day after treatment. The data gathered from bacterial enumeration was logarithmically transformed before graphs of mean bacterial were plotted against the exposure period for each selected herbicide. Bacterial population and percentage organic matter did not show any significant differences relative to the exposure period in this study ($p < 0.05$). However, the deleterious impact of the herbicides was seen as Paraquat treatment resulted in reduction in the bacterial population for five, ten and fifteen Days after treatment (DAT) in treatment halved the recommended field rate. Glyphosate follows with 69.3%, 12.7%, and 18.0%; 2,4-D amine had 44.8%, 33.5%, and 21.6%; and lastly Atrazine had 41.8%, 44.5% and 13.6% bacterial population 5DAT, 10DAT and 15DAT respectively. The inhibition effect on the fungal population was very specific as some fungi (*such as A. Niger, Trichoderma viride, Collectotrichum gloeosporioides, A. flavus, Mucor, Penicillium, Curvularia lunata*) which were present in the baseline (control) did not appear in the treatment and vice versa. Percentage organic matter for the treatment did not vary much with the baseline determination (control) but the impact was observed in the various levels of treatments for all the herbicides. A similar study should be conducted on a normal field condition where herbicide treatments would be carried out on a normal field condition since most of the previous studies had the herbicide treatment carried out under laboratory condition. It will also be very appropriate if further research work is carried out to identify the specific components of these herbicides which favour the growth and development of certain beneficial microorganisms such as fungi and bacterial.

DEDICATION

Special dedication to Mama Elizabeth Nketiah

I just want to say

Thank you for being a Mother

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CHAPTER ONE

INTRODUCTION

1.1 Background

The use of herbicides in agriculture has over the years contributed tremendously to both food and cash crop production all over the world of which Ghana is not an exception. But one of the challenges undermining the farming business (Ntow *et al.*, 2006), has been the invasion of many common weed species due to favorable environmental conditions such as abundance of rainfall, adequate sunlight, fertile soil etc. in Ghana. As a result, manufactures have adopted flooding the market with all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth (Sebiomo *et al.*, 2011). Perhaps, the efficacy of these herbicides in controlling the target weeds has resulted in the application of these chemicals by most farmers.

The soil serves as the repository for all agricultural contaminants, function as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influences the soil fertility (Rosli *et al.*, 2013), through organic material degradation, organic matter decomposition and nutrient cycling (De-Lorenzo *et al.*, 2001and Hutsch, 2001). Nonetheless, over application of these chemicals inhibit some of these natural processes, and decreases the performance of the non-target organisms (Subhani *et al.*, 2000). However, some soil organisms use these herbicides in the process of degradation as carbon energy source for their metabolic activities.

Numerous studies have shown that the level of contamination of soil with these chemicals depends on the persistency of the herbicides in the soils environment, the quantity, frequency of application and the toxicity of the chemical. However, most of these herbicides are designed to persist longer

enough to have the desired effect on the weeds (Greer *et al.*, 1990). The fate of herbicide applied onto the soil environment is governed by two major processes; transfer and degradation. The transfer process involves percolation, runoff, flora and fauna uptake, sorption and desorption, for which the applied chemicals remain physically intact in the soil environment. The degradation processes includes microbial decomposition, plant detoxification, chemical breakdown and photodecomposition which are chemically engineered. These two processes determine the persistency of herbicides, its efficacy for weeds, as well as its potential for soil and ground water contamination. (Subhani *et al.*,2000). Therefore there is the need to understand the factors affecting the degradation processes of herbicide in order to adopt effective strategies to reduce its persistent period within the soil environment.

A large number of the populace in Ghana can‟t read and understand herbicide label. This has resulted in the contamination of streams, rivers and ground water which is an important natural resource (Baran *et al.*, 2007). These contaminations do not pose danger to only the non-target organisms and the environment but exposes human beings to many health implications. Hence, the need to study the effects of some of these herbicides which are commonly used in Ghana in order to assess their inhibitory effects on some of the beneficial microorganisms in the soil.

1.2 Problem Statement

The role of herbicides in modern agriculture is very significant since they have contributed immensely to food production. However, the effectiveness of these herbicides to control the target

organisms remain the major priority of the farmers, with little or no consideration given to the non target microorganisms whose contribution to soil fertility is very vital. Farmers apply the herbicides to the target organisms without paying due attention to the herbicide producer‟s recommended rate of application as well as improper way of disposing the excess herbicides after its application. These pose a challenge to the normal functioning of the microorganisms in the soil.

1.3 Justification

Herbicide application has become the main strategy weed control for both agricultural and nonagricultural purposes in Ghana. The effect of these chemicals on the non-target soil microorganisms is very profound. These microorganisms play critical role in the decomposition of organic materials, nutrient recycling as well as organic matter degradation, which in turn affect soil fertility and plant growth. A decline in the population of these beneficial organisms has direct correlation on their performance and decreases the available organic material which provides their needed carbon energy source and subsequently leads to poor soil fertility.

1.4 The Aim and Objectives

1.4.1 **The main objective**

The main purpose of the study was to determine the effect of some commonly used herbicides on soil microbial population:

1.4.2 The specific objectives were

To determine:

a. The population of soil bacteria and fungi, and organic matter content of soil
b. The effect of herbicide contamination on soil bacterial population
c. The effect of herbicide contamination on soil fungal population
d. Organic matter content in the herbicide contaminated soil

CHAPTER TWO

LITERATURE REVIEW

2.1 Herbicides

Herbicides are chemicals which are applied for the purpose of controlling, regulating or inhibiting the growth of weeds. Weed control is a major agricultural activity which has attracted the attention of mankind in the history of agricultural business (Holm and Johnson, 2009). Perhaps this attention stems from the fact that weeds have the capacity to influence the development and the yield of crops by competing with crops for available soil nutrients, space, water, sunlight and air, as well as habouring other invasive pest (Wyss *et al.,* 2001). The past four decades have seen a large influx of herbicides being introduced into the market as pre and post-emergent herbicide in many parts of the world (Sebiomo *et al.,* 2011), and as the effectiveness of these herbicides is realized, farmers will increase its application proportionately to meet their production target without giving due cognizance to the side effect of the herbicide in the soil environment. Due to its numerous health implications many concerns are being raised on the excessive application of herbicides into our agricultural soils since it has the potential of contaminating ground water and other water bodies (Ayansina *et al.,* 2003).

On daily basis people are exposed to these harmful synthetic chemicals that have been released into the soil environment through both commercial and domestic activities (Clausen *et al.,* 2002, Droz *et al.,* 2005). Pesticides can enter watercourses through direct leaching from soils or in association with eroded soil or sediment (Stoate *et al.,* 2001). The herbicides can also enter through drains, storm sewers and other man-made routes (Gavrilescu, 2005). These can not only

deleteriously affect the quality of the water; they also cause an additional financial burden resulting from the need for additional purification. The leaching of herbicides into freshwater environment has been of particular concern due to the potential impacts that the compounds may have on aquatic plants (Peterson *et al.,* 1994). These herbicides are designed to be toxic to their target organisms, degrade into harmless metabolites and disappear before it moved into and adversely endanger the life of other environmental compartments (Greer *et al.,* 1990). However, pesticides and its allied chemicals are design to persist longer enough into the soil environment in order to achieve the desired aim of controlling the target organism, but their metabolites vary from their persistency and toxicity (Sebiomo *et al.,* 2011; Landa *et al.,* 1994).

2.2 Types of herbicides

Herbicides can be separated into two broad categories: those applied to the soil before weeds have emerged (pre-emergence herbicides) and those applied directly to visible weeds (postemergence herbicides). Herbicides can also be categorized as being either residual or non residual type. Residual herbicides have a lasting effect on the soil. How long weed growth is prevented by an application of residual herbicide depends on how quickly it is broken down on the soil by sunlight, microbial activity, or soil chemistry, and whether the herbicide is volatilized or leached below the upper inch of soil. Non-residual herbicides have little or no effect except on weeds that are present at the time of application (Holm and Johnson, 2009). Finally, some herbicides are effective only on grasses, some only on broadleaf herbs, and others show degrees of activity against both types of vegetation. The use of residual herbicides in particular should be limited to specific needs. The routine use of residual herbicides may increase the chance of creating a bare soil environment around trees (with an increased risk of soil erosion, tree rack, and cold temperature injury to tree

roots). And it may facilitate the development of weed populations that are difficult to control with currently available herbicide option.

2.3 Mode of Action of herbicides

The mode of action is the way in which the herbicide controls susceptible plants. It usually describes the biological process or enzyme in the plant that the herbicide interrupts, affecting normal plant growth and development (Holm and Johnson, 2009). In other cases, the mode of action may be a general description of the injury symptoms seen on susceptible plants. Some herbicide modes of action comprise several chemical families that vary slightly in their chemical composition, but control susceptible plants in the same way and cause similar injury symptoms.

Herbicides can also be classified by their "site of action," or the specific biochemical site that is affected by the herbicide (Miller *et al.,* 2008). The site of action is a more precise description of the herbicide‟s activity; however, the terms "site of action" and "mode of action" are often used interchangeably to describe different groups of herbicides. Knowing and understanding each herbicide‟s mode of action is an important step in selecting the proper herbicide for each crop, diagnosing herbicide injury, and designing a successful weed management program for your production system. Over-reliance on a single herbicide active ingredient or mode of action places heavy selection pressure on a weed population and may eventually select for resistant individuals (Holm and Johnson, 2009). Over time, the resistant individuals will multiply and become the dominant weeds in the field, resulting in herbicides that are no longer effective for weed control. Simply rotating herbicide active ingredients is not enough to prevent the development of herbicide-resistant weeds. Rotating herbicide modes of action, along with other weed control methods, is necessary to prevent or delay herbicide-resistant weeds. Many weeds have developed

"cross resistance" and are resistant to multiple herbicides within a single mode of action (Miller *et al.*, 2008). Therefore, it is important to not only rotate herbicide active ingredients but also to rotate modes of action to prevent herbicide-resistant weed populations from developing. One of the most effective ways to rotate herbicide modes of action is through crop rotation. Weeds that have developed "multiple resistances" are resistant to herbicides from two or more modes of action (Miller *et al.*, 2008).

2.4 The role of herbicide in modern Agriculture

Herbicides are an undeniable part of modern agriculture, used to control weeds from flower gardens to agricultural crops. Although often taken for granted, without these important products, food production would decline, many fruits and vegetables would be in short supply and prices would rise (Paloma, 2011). Herbicides can be used safely and effectively. But if proper care is not taken, herbicides can harm the environment by contaminating soil, surface and ground water, and ultimately kill wildlife. Also, the modern human is constantly exposed to a variety of toxic chemicals primarily due to changes in life style (Paloma, 2011). The food we eat, the water we drink, the air we breathe, and the environments we live in are contaminated with toxic xenobiotics.

2.5 The fate of herbicide in soil after its application

The fate of herbicides in the surface depends to a high degree on the ability of the microbial population to degrade the herbicides – ideally by complete mineralization of the parent compound into carbon dioxide (CO_2) and transfer of the chemical through certain physical processes. Degradation of herbicides is often considered to decrease with depth (Wood *et al.*, 2002, Albrechtsen *et al.*, 2001), and can be faster in subsoil than in the topsoil (Mills *et al.*, 2001). Bioavailability and biodegradation are intrinsically linked with abiotic factors such as compound

sorption to soil. Bioavailability is a measure of the potential of chemicals for entry into biological receptors. It is specific to the receptor, the route of entry, time of exposure, and the matrix containing the contaminant (Anderson *et al.*, 2000). Sorption may also decrease with depth because of decreasing organic matter content which on the other hand may increase the bioavailability (Bending and Rodriguez-Cruz, 2007). Herbicides biodegradation involves a wide variety of microorganisms including bacteria and fungi operating under dynamic anaerobic and aerobic conditions (Larsen *et al.*, 2000). It is suggested that biodegradation of herbicides in soil ecosystems can only take place through the synergistic interactions of a microbial consortium, the activity of which is affected by many soil physical and chemical properties, as well as the nature and extent of the herbicides contamination. Many herbicides have proven resistant to microbial biodegradation and therefore persist in the environments in which they are found. Enhanced biodegradation of herbicides in agricultural soils and the ability of microorganisms to adapt and rapid breakdown of some herbicides have resulted in economically significant pest control failures (Racke *et al.*, 1990). This recognition of microbial degradation as a primary means of degrading many herbicides in soil ecosystems prompted the development of biodegradable herbicides, insecticides, and fungicides in the mid 1970‟s (Racke *et al.*, 1990). Ideally, these herbicides would persist only long enough to complete their intended mission or benefit and then degrade to harmless products. Numerous interactions between the solid, liquid and gaseous phases of soil and between living and biotic components of soil significantly influence the environmental fate of herbicides in soil. Not much has been done on the degradation of herbicides in chalk or limestone despite the importance of this environment as water resource (Chilton *et al.*, 2005, Johnson *et al.*, 2003). However, the complexity and heterogeneity of this environment and the challenges in drilling and sampling these setting often being hard rock may be the reason for not having much work on investigating into the fate of herbicides (GEUS, 2009). Transfer is a physical process in which the

herbicides molecules remain intact; it includes sorption-desorption, runoff, percolation, volatilization and absorption by crop plants or animals (Subhani *et al.,* 2000). Factors that affect the volatility of herbicides (temperature, humidity, vapour pressure, soil organic and moisture can influence biodegradation rates, in that the extent to which they volatilize through air pockets of the soil, or escape from the surface, affect their concentrations in the solid phase of the soil and, as consequence, their bioavailability (Howell, 2011). The volatilization of herbicides can be influenced by soil moisture content and may be facilitated by a proposed wicking or capillary effect, through which more compounds that are soluble are brought to the soil surface more rapidly (Adawiah, 2008). Soil moisture content has been identified as a key factor influencing herbicides transport within the soil, and along with temperature, was incorporated into a model for volatilization from surface soil (Adawiah, 2008, Cohen *et al.,* 1986).

Photochemical **Plant uptake** **volatilization degradation**

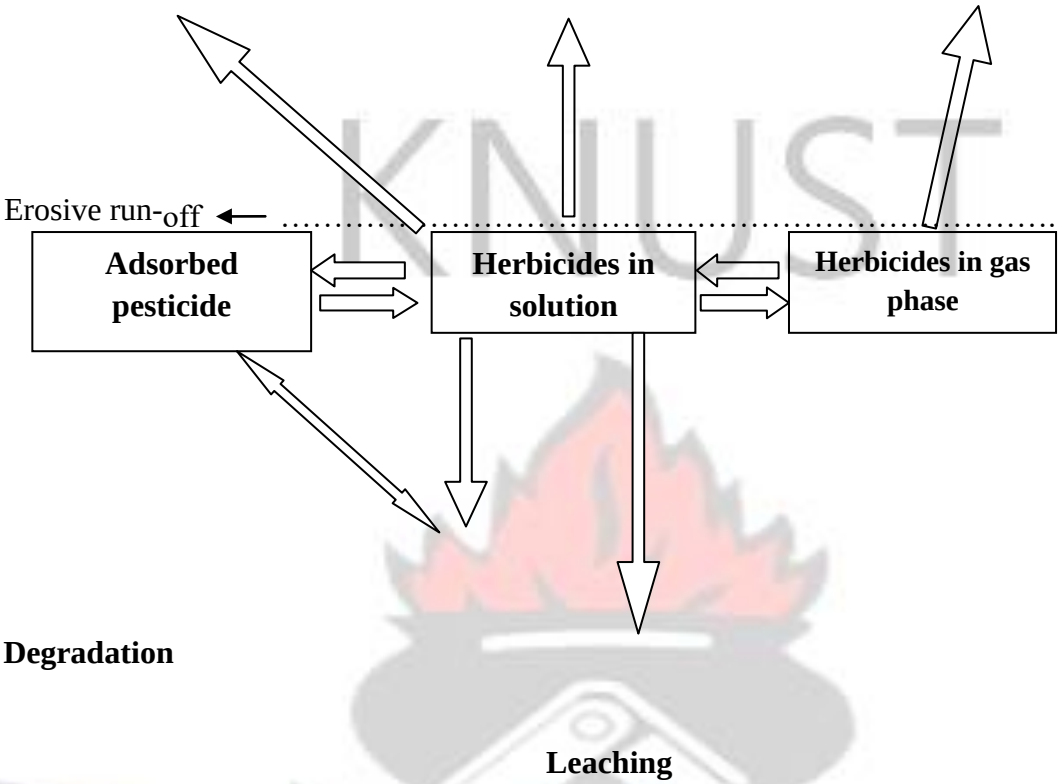

Figure 2.1: A conceptual diagram of the factors that determine the behavior and fate of herbicides in natural environment (Howell, 2011)

Runoff is the movement of water over a sloping surface. It can carry herbicide dissolved in water and pesticides sorbed to eroding soil. The pesticides are either mixed in the water or bound to eroding soil. Runoff can also occur when water is added to a field faster than it can be absorbed into the soil. Herbicides may move with runoff as compounds dissolved in the water or attached to soil particles. The amount of herbicides runoff depends on slope, texture of the soil, soil moisture content, amount and timing of a rain event and type of herbicides used (Tiryaki *et al.,* 2010; Reichenberger *et al.,* 2007, Kerle *et al.,* 2007). Soil erosion by water consists of two processes: i) the detachment of soil particles from the soil surface, and ii) their subsequent transport down slope. Detachment is caused by raindrop impact and also by the abrasive power of surface runoff,

especially when the runoff water flow has concentrated (Schnürer *et al.*,2006; Tiryaki *et al.,* 2010). Herbicides lost in runoff and erosion events leave the field either dissolved in runoff water or adsorbed to eroded soil particles. However, for most herbicides losses via runoff are considered far more important than losses via erosion, because the amount of eroded soil lost from a field is usually small compared with the runoff volume (Leonard, 1990; Tiryaki *et al.,* 2010,). Leaching is the downward movement of herbicides in the soil through cracks and pores. Soil normally filters water as it moves downward, removing contaminants such as herbicides. Soil and pesticide properties, geography and weather can influence the movement of pesticides (leaching). Herbicides that leach through soils may reach ground water (Toth and

Buhler, 2009). Soil properties (organic matter, soil texture and soil acidity), herbicides properties (solubility, adsorption and persistence), herbicides application (rate of application and application method), and weather conditions are the factors affecting leaching. Leaching potential is also affected by certain characteristics of the herbicide, including water solubility, electrostatic properties, vapor pressure, and photodecomposition. Because numerous complex interactions can occur between herbicides and the soil environment, it is impossible to accurately generalize leaching behavior for a wide range of possible soil situations. In addition, certain soil microorganisms and living weeds can sometimes metabolize absorbed herbicides, rapidly or gradually altering them to non-phytotoxic forms that may have different leaching characteristics.

Photodegradation is the breakdown of pesticides by sunlight (Tiryaki *et al.,* 2010). All herbicides are susceptible to photodegradation to some degree. The intensity of sunlight, length of exposure, and properties of the herbicides affect the rate of photodegradation. Pesticides that are applied to foliage or to the soil surface are more susceptible to photodegradation than herbicides that are incorporated into the soil. Herbicides may break down faster inside plastic-covered greenhouses

than inside glass greenhouses, since glass filters out much of the ultraviolet light that degrades herbicides (Kerle *et al.,* 2007, Tiryaki *et al.,* 2010). Chemical degradation occurs when herbicides reacts with water, oxygen, or other chemicals in the soil. As soil pH becomes extremely acidic or alkaline, microbial activity usually decreases. However, these conditions may favor rapid chemical degradation. Chemical breakdown is the breakdown of herbicides by chemical reactions in the soil. The rate and type of chemical reactions that occur are influenced by the binding of herbicides to the soil, soil temperature; pH levels (Kerle *et al.*, 2007*)***.**

2.6 Factors affecting herbicide after its application

Herbicide characteristics that determine their performance after its application are the site of uptake by the weeds, solubility, adsorption, persistence, leaching potential, photodecomposition, and volatility. An understanding of these factors will result in more effective herbicide use (Moomaw *et al.,* 1992).

2.6.1 The site of uptake by the weeds

After being taken up, herbicides kill the weed seedling by interfering with photosynthesis, protein synthesis, enzyme systems, cell division etc. Maximum herbicide performance results when the herbicide is placed by rainfall, irrigation, or mechanical incorporation in the soil zone of weeds (Moomaw *et al.,* 1992). For this reason the herbicide must always be kept between the first 0-3 inches of the soil. This is also true for herbicides that affect germinating weed seed. As the weed shoot passes through the herbicide zone, uptake occurs and the seedling is killed. If the herbicide is mechanically incorporated too deeply or excessive leaching occurs, poor weed control will

result. Dilution occurs if the herbicide is distributed in a larger volume of soil (Moomaw *et al.*, 1992; FAO, 2001). Atrazine, Bladex, and Sencor/Lexone are root-absorbed herbicides. As the root emerges from the germinating weed seed, herbicide is absorbed and the weed is killed. Control of some deeper germinating large-seeded broadleaf weeds, such as velvetleaf, cocklebur, sunflower, jimsonweed, and morning glory (Moomaw *et al.*, 1992), may be improved if a root-absorbed herbicide like Atrazine is mechanically soil incorporated 2 to 3 inches deep. Roots of the deeper germinating weed seed then encounter more herbicide (Moomaw *et al.*, 1992; Friedrich, 2004; Pimental, 1995).

2.6.2 Herbicide Persistency

"The length of time a herbicide remains active in the soil is refer to as the „persistence or its residual life" (Curran, 1998). How long a herbicide remains active in a soil system is expressed by a half-life value. Half-life is a period of time it takes for 50 percent of a herbicide in the soil to degrade. Half-life will vary with soil microbial populations, soil moisture, soil temperature, pH, and farming practices (Moomaw *et al.*, 1992; Vencill, 2002). There are so many factors that determine the length of time an herbicide will persist in the soil or within the environment. These factors falls into three categories: soil factors, climatic conditions, and herbicidal properties – which strongly interact with one another (Curran, 1998). Soil factors affecting herbicide persistence include soil composition, soil chemistry, and microbial activity. Soil composition is a physical factor determined by the relative amount of sand, silt, and clay in the soil (the soil texture), as well as by the organic matter content (Racke *et al.*, 1990; Curran, 1998). An important chemical property of the soil that can influence herbicide persistence is pH (Curran, 1998; Adawiah, 2008), and can influence the persistence of some herbicides especially the triazines and sulfonylureas. Chemical and microbial breakdown, two ways herbicides degrade in soil, often are slower in higher pH soils,

in particular, above 7.0. In addition, in higher pH soils lesser amount of these herbicides are bound to soil particles, making more available for plant uptake (Ahn *et al.,* 2002,; Curran, 1998). The microbial aspects of the soil environment include the types and abundance of soil microorganisms present in the soil. The degradation processes by soil microorganisms probably are the most important pathways responsible for the breakdown of herbicides. The type of microorganisms (fungi, bacteria, protozoans, etc.) and their relative numbers determines how quickly decomposition occur (Smith *et al.,* 1993; Curran 1998), and their activities are strongly affected by moisture, temperature, pH, oxygen and mineral nutrient supply. Soil composition affects herbicidal activity and persistence through soil-herbicide binding (adsorption), leaching, and vapor loss (volatilization) (Cohen *et al.,* 1989; Curran, 2008; Moomaw *et al.,* 1992). The climatic variables involved in herbicide breakdown are moisture, temperature, and sunlight (photodegradation). Herbicides degradation rates generally increases as temperature and soil moisture increase, because both chemical and microbial decomposition rates increase with higher temperatures and moisture (Curran, 1998; Boland *et al.,* 1999).

2.6.3 Herbicide Solubility

"Solubility refers to the amount of herbicide that will dissolve in water", (Moomaw *et al.,* 1992). Relatively insoluble herbicides require more rainfall for activation. Rainfall or irrigation is needed within five to seven days of herbicide application for best results. The amount, duration, intensity, and frequency of rainfall are important relative to herbicide solubility. Slow, gentle rains effectively move most herbicides into the soil (Clausen *et al.,* 2004). With high intensity rainfall and associated runoff, less soluble herbicides may not be activated. Too little rainfall may not move herbicides far enough into the soil for good performance. Too much rainfall can move certain herbicides deeper into the soil than desirable (Moomaw *et al.,* 1992; Sheng *et al.,*2001). Soils that

are near field capacity require less rainfall for herbicide activation than soils very low in soil water. When pre-emergence herbicides are applied to soil with ample moisture, but rainfall does not occur to activate the herbicide, poor weed control may result. This is because soil moisture conditions are ideal for weed seed germination before the herbicide is activated (Morillo *et al.,* 2000; Moomaw *et al.,* 1992). Highly water soluble herbicides move downward more readily with soil water. However, the percent organic matter, and the type and percentage of clay particles present in a given soil affect movement of herbicides dissolved in soil water (Moomaw *et al.,* 1992).

2.6.4 **Leaching Potential**

Protecting groundwater from contamination is a high priority. Leaching of herbicides and other herbicides can occur as rainfall or irrigation water moves down through soil. Leaching potential of various herbicides depends on factors such as solubility, amount and frequency of rainfall, soil adsorption, persistence, and soil texture and structure (Moomaw *et al.,* 1992). How these factors interact to affect leaching potential can be illustrated by Atrazine. Atrazine has low solubility and a medium sorption index, which indicates low leaching potential (Moomaw *et al.,*1992; GEUS, 2007). However, since Atrazine has a relatively high half-life, the leaching potential is high. As a result, the Atrazine label carries a groundwater advisory statement against using the product on well drained sand and loamy sand soils where groundwater is close to the soil surface (Subhani *et al.,* 2002; Sebiomo *et al.,* 2011).

Again, factors influencing whether herbicides will be leached into groundwater include characteristics of the soil and herbicide, and their interaction with water from a rain or irrigation (Tiryaki *et al.,* 2010). Leaching can be increased when: (I) the pesticide is water soluble, (II) the soil is sandy, (III) a rain-event occurs shortly after spraying, and (IV) the herbicides is not strongly

adsorbed to the soil (Anonymous, 2009). Leaching of water and dissolved herbicides to depth in soil occurs by matrix flow and preferential flow. Matrix flow is the slower transport process in which the simultaneous movement of herbicides with water is determined by the physical-chemical properties of the herbicides. Such movement is dependent on its water solubility, vapor pressure (Cessna, 2009; Tiryaki *et al.*, 2010).

2.6.5 Photodecomposition

Photodecomposition, which is the breakdown of a chemical by light, may occur when some herbicides are left on the soil surface for an extended period without rain. Herbicides that are subject to photodecomposition usually volatilize from the soil surface, thus requiring soil incorporation (Moomaw *et al.*, 1992). Pre-emergence herbicides remaining on the soil surface for long periods without rain may lose some effectiveness by photodecomposition and volatilization. Shallow incorporation with a rotary hoe or harrow is recommended to prevent photodecomposition and volatilization if rainfall does not occur within five to seven days of herbicide application.

2.6.6 Volatilization

Volatilization is the process of solids/liquids converting into a gas, which can move away from the initial application site. This movement is called vapour drift. Vapour drift from some herbicides can damage nearby crops. Volatilization from moist soil is determined by the moisture content of the soil, and by the herbicides‟ vapor pressure, sorption, and water solubility. Herbicides volatize most readily from sandy and wet soils and in Hot, dry or windy weather and small spray drops increase volatilization. Where recommended, incorporating the herbicide into the soil can help

reduce volatilization (Anonymous, 2009). The rate of volatilization of herbicides from soil depends upon properties of the chemical and of the soil. On the other hand post application volatilization represents further significant herbicide input into the troposphere for several days/weeks after application (Glotfelty *et al.*, 1984). The dominant factors that affect volatilization from soil and crops are vapor pressure, Henry‟s law constant (K*h*,is defined as the concentration of herbicides in air divided by the concentration in water) and water solubility of herbicides, as well as its persistence in the soil or plant surface, and environmental conditions. K*h* characterizes the tendency for a herbicide to move between the air and the "soil water." The higher the K*h*, the more likely that a herbicide will volatilize from moist soil. In general, pesticides with K*h* index values of less than 100 have a low potential to volatilize from moist soil. Pesticides with K*h* index values above 10,000 have a high potential to volatilize (Kerle *et al.*,2007*)*

2.7 The role of soil microorganism in the soil

Microbial communities can be considered as architects of soils (Rajendhran and Gunasekaran, 2008), and many ecosystem services that are linked to terrestrial ecosystems, including plant production, safeguarding of drinking water or C sequestration, are closely linked to microbial activities and their functional traits (Torsvik and Øvreås, 2002). The biotic components of the ecosystem do not function in isolation but there is a whole lot of complex interaction between the physical and the chemical component of the environment. This close interplay between abiotic conditions and the soil biosphere is one of the most fascinating issues as far as earth sciences are concerned, with huge implications on environmental as well as human health (Van Elsas *et al.*, 2006).

Generally, the majority of fungi and bacteria present in soils are considered to be beneficial to higher plants by: a) direct association with roots (mycorrhizae, nodule forming bacteria); b)

breakdown and release of minerals from organic matter present in the soil resulting in essential element availability increases to higher plants; c) parasitizing harmful or disease causing microorganisms or; d) suppressing growth, reproduction or activity of harmful disease causing microorganisms through other interactions (Schulz *et al.,* 2013) such as chemical inhibition. However, any change in environmental conditions such as food supply, temperature, moisture, oxygen supply, etc., can result in changes which cause one or many types of soil microbes to become temporarily dominant over the others. Saprophytic fungi – convert dead organic material into fungal biomass, carbon dioxide (CO_2), and small molecules, such as organic acids. These fungi generally use complex substrates, such as the cellulose and lignin, in wood, and are essential in decomposing the carbon ring structures in some pollutants (Tugel and Lewandowski, 2001; Butler *et al.,* 1998). A few fungi are called "sugar fungi" because they use the same simple substrates as do many bacteria. Like bacteria, fungi are important for immobilizing, or retaining, nutrients in the soil **(**Schulz *et al.,* 2013). In addition, many of the secondary metabolites of fungi are organic acids, so they help increase the accumulation of humic-acid rich organic matter that is resistant to degradation and may stay in the soil for hundreds of years (Tugel and Lewandowski, 2001). In exchange for carbon from the plant, mycorrhizal fungi help solubolize phosphorus and bring soil nutrients (phosphorus, nitrogen, micronutrients, and perhaps water) to the plant (Schulz *et al.,* 2013). One major group of mycorrhizae, the *ectomycorrhizae* grow on the surface layers of the roots and are commonly associated with trees. The second major group of mycorrhizae are the *endomycorrhizae* that grow within the root cells and are commonly associated with grasses, row crops, vegetables, and shrubs. Many fungi help control diseases. For example, nematode-trapping fungi that parasitize disease-causing nematodes, and fungi that feed on insects may be useful as biocontrol agents (Tugel and Lewandowski, 2001).

2.8 The effect of herbicide on the soil microbial biomass

Agrochemical manufacturers constantly pursue the development of agrochemicals that are: (i) effective against target organisms, (ii) not persistent in the environment, (iii) and have low toxicities to non-target organisms (Carlisle and Trevors, 1988). However, the excessive use of agrochemicals in conventional crop management has caused serious environmental and health problems, including loss of biodiversity and certain human disorders (Liu *et al*., 1999; Ghorbani *et al*., 2010). Regardless, herbicides are widely used in modern agriculture to control weedy plant species (Liu *et al.*, 1999). High crop productivity requires protection of crops against competition from weeds and attack by pathogens, and herbivorous insects (Oerke and Dehne, 2004). The heavy utilization of pesticides and, their persistence and transfer into trophic food webs can however cause major environmental contamination (Imfeld and Vuillemier, 2012). Similarly, concern regarding their effect on non-target organisms has grown considerably (Nyström, Björnsäter and Blanck, 1999; Sebiomo *et al.*, 2011). Serious questions are being raised about the potentially harmful effects of pesticides on consumers and the ecosystem. There is increasing concern that herbicides not only affect target organisms but also non-target organisms such as microbial communities present in the soil environment (Haney, *et al.*, 2002; Sebiomo *et al.*, 2011). These non-target effects may impact on many important soil functions such as organic matter degradation and the nitrogen cycle (Sebiomo *et al.*, 2011). Ignoring the potential nontarget detrimental side effects of any agricultural chemical, may therefore have dire consequences for food security, such as rendering soils infertile, crops non-productive, and plants less nutritious. The soil ecosystem can be altered by herbicides through direct and indirect effects on various components of the soil microflora, including saprophytes, plant pathogens, pathogen antagonists or mycorrhizae (Lévesque and Rahe, 1992; Ghorbani *et al*., 2008; Sanyal and Shrestha, 2008), which can result in

increased or decreased disease incidence. Phytotoxicity, and disease enhancement, are two of the most commonly reported problems of herbicide use on crops. It is generally accepted that herbicide-induced weakening of a plant can predispose the plant to infection by facultative pathogens (Lévesque and Rahe, 1992).

2.8.1. Negative herbicidal effects

The usage of herbicides may have indirect impacts on the whole ecosystem. These indirect impacts may be relatively severe since herbicide effects on target as well as non-target organisms may disrupt community structure and ecosystem function. Applied pesticides ultimately reach the soil in large amounts where they accumulate, leading to pesticide residues which can be ingested by invertebrates, absorbed by plants or broken down into other toxic products (Subhani *et al*., 2000). There is a significant response of soil microbial activity to herbicide treatment, either directly to the herbicide or to the breakdown products of the herbicide. Adaptation of microbial communities to increasingly higher herbicide concentrations and chemical residues can occur over weeks of continuous treatment (Sebiomo *et al.*, 2011).

2.8.2. Microbial biomass

Herbicides have been shown to affect microbial biomass in soil. For instance, the use of the uracil herbicide group, with the active ingredient bromacil, reduces microbial biomass significantly, an effect that can last up to 11 months after application (Sanders and Screstha, 1996). A significant reduction in microbial biomass can consequently delay the breakdown of this active ingredient. Furthermore, severe stress on soil microflora caused by bromacil may interfere with the ability of microbes to degrade the herbicide during repeated applications (Sanders and Screstha, 1996). Similarly, the application of imazethapyr to a silty loam and a loamy soil leads to a shift in the soil

community structure. Soil microbial biomass carbon (C) is reduced after imazethapyr application (Zhang and Luo, 2010a).

2.8.3. Fungi

Plant-herbicide-pathogen interactions can have negative repercussions that should not be ignored. For example, when the roots of plants that have been treated with herbicides die, they become colonized by facultative parasites such as *Pythium* spp., *Rhizoctonia solani* Kühn and *Fusarium* spp. as a result of the exudation of sugars and other carbon sources from the dead roots (Sullivan, 2004). *Rhizoctonia* root disease of wheat increased when a mixture of paraquat and diquat was applied close to the sowing date (Roger *et al.*, 1994). The problem was due to a lack of competing organisms, and was overcome by allowing a greater time between applications and sowing date (Roger *et al.*, 1994), in order to allow for competition by soil micro-organisms. It has been observed that the application of glyphosate or paraquat in bean fields also results in an increase of *Pythium* spp. in the soil (Descalzo *et al*., 1998).

2.9.4. Bacteria

Herbicides have been shown to have negative impacts on soil bacterial populations, either directly or indirectly. For example, no decrease in bacterial numbers in soil treated with Atrazine was observed, yet untreated soil showed an eightfold increase in bacterial numbers. Although repeated application of Atrazine did not affect the abundance of bacteria producing hydrolytic enzymes, a transient inhibition of bacterial growth was observed during the first week of application. The mere observation that bacterial numbers did not increase nor decrease with Atrazine application does not suggest that this herbicide has no effect on the bacterial populations. In fact, the increase in bacterial numbers in untreated soil suggests that the Atrazine does in fact negatively affect bacterial

populations. Soil bacterial populations have also been shown to be much lower, during the first week after herbicide application, in soils treated with

Atrazine, prim extra, Paraquat and Glyphosate respectively (Sebiomo *et al.*, 2011), while Paraquat has also been shown to greatly stress and inhibit bacterial populations temporarily (Kopytko *et al.*, 2002). Glyphosate has also been observed to cause a decrease in pseudomonad populations, which antagonize fungal pathogens in soil (Kremer & Means, 2009). It has also been observed that alachlor and paraquat are toxic to bacteria (Sahid *et al.*, 1992).

2.8.5. Other micro-organisms

Certain herbicides have been shown to be toxic to some soil fauna. For instance, Paraquat has been shown to be toxic to non-target organisms, such as *Collembola*. Similarly, Zaltauskaite and Brazaityte (2011) observed that the application of three herbicides with different active ingredients, namely amidosulfuron, iodosulfuron, and sodium salt, caused 50-100% mortality of the micro-invertebrate *Daphnia magnaI* due to runoff into drainage sites and rivers. Atrazine application to soil may also affect certain Collembola species, such as *Entomobrya musatica* Stach (Al-Assiuty and Khalil, 1996). Effects include direct toxicity and negative effects on reproduction and the fecundity of the animals which could adversely affect abundance and development of the organism (Al-Assiuty and Khalil, 1996). In contrast, Sabatini *et al.*, (1998) observed no direct effect of the herbicide triasulfuron at recommended field rate on the Collembola species, *Onychiurus pseudogranulosus* Gisin. Atrazine may however, be taken up through the body surface, even when applied at the recommended field rate, and lead to a direct lethal effect (Sabatini *et al.*, 1998). Atrazine and monuron have been shown to decrease the number of wireworms and springtails in grassland soils. In addition, atrazine has also been shown to reduce earthworm populations in grassland soils. Any impact herbicides may have on soil fauna may adversely affect plant health

due to a decrease in mineral and oxygen availability as a result of less channeling in soil. A further effect is less predation of potential plant pathogenic organisms by other soil fauna (Brown *et al*., 2001). Whatever effect herbicides have on soil fauna, it can result in a shift in the soil faunal community which will have a positive or negative impact on ecosystem functions.

2.8.6. Positive herbicidal effects

Most herbicides used at normal field rates are generally considered to have no major or longterm effect on gross soil microbial activities (Subhani *et al*., 2000; Zabaloy *et al.,* 2011). However, some reports indicate that herbicide application to soil may lead to the proliferation of general or specific organisms which can utilize a particular chemical in the herbicide for nutrition (Paulin *et al.*, 2011). This observation can be substantiated by the fact that certain herbicides, especially hormone-based types, can disappear from the soil due to microbial decomposition. The degradation process by soil micro-organisms is probably the most important pathway responsible for the breakdown of herbicides (Curran, 1998; Subhani *et al*., 2000). The synergistic interaction of the microbial community in the rhizosphere may also facilitate degradation of recalcitrant compounds (Costa *et al.,* 2000). For instance, Atrazine concentration decreases in the rhizosphere compared to non-vegetated areas (Costa *et al.*, 2003). The degradation of atrazine is higher in a rhizosphere dominated system, where the half-life is 7 days, compared to non-vegetated soil where the half-life is greater than 45 days (Costa *et al*., 2003). Similarly, mesotrione, a selective herbicide used for maize crops, applied at the recommended field rate is quickly dissipated from achernozem soil type and has no consistent impact on soil microbial communities Chernozem soil: Black, humus-rich grassland soil (Crouzet *et al*., 2010).

This suggests that the herbicide is degraded by soil microorganisms. However, Crouzet *et al.*, (2010) also stated that mesotrione, at doses far exceeding the recommended field rates, has an impact on non-target soil organisms.

2.8.6.1. Microbial biomass

The amount of herbicide available to soil micro-organisms depends on various factors, including available nutrients, pH, temperature, and moisture, although these factors differ in importance depending on the pesticide involved (Weber *et al*., 1993). For instance, the application of bentazon at the recommended field rate to soil does not significantly affect the microbial community, even in the absence of microbial degradation (Allievi *et al*., 1994). The addition of Atrazine to a semi-arid soil with low organic matter content, resulting in increased microbial activity, can be explained by adaptation of the resident microbial community to the xenobiotic

(Moreno *et al*., 2007).

2.8.6.2. Fungi

Fungi react differently to herbicides, even within the same genera. For instance, three different Basidiomycete species were reported to have different degradation rates on the herbicides chlortoluron, isoproturon and diuron. *Ceriporiopsis subvermispora* degraded chlortuloron 18%, isoproturon 60% and diuron 18%; *Coniophoraputeana* 13%, 69% and 38% respectively, and *Phlebia radiate* 33%, 25% and 82%, respectively (Khadrani *et al*., 1999). Claims have been made that repeated application of Atrazine does not affect the number of viable fungi in any way (Cole, 1976), suggesting that herbicides can elicit different reactions by different fungi. Certain fungal species are benefitted by herbicide addition, while others are inhibited. This could lead to the false perception of increased total microbial activity, while in actual fact only a specific population of organisms which are able to utilize the specific herbicide increased. For instance, herbicides may

reduce the severity of plant diseases by stimulating certain microbial antagonists which can suppress soil pathogens (Katan and Eshel, 1973).

2.8.6.3. Bacteria

The degradation of Atrazine in soils is a result of the activity of bacteria which are able to use the compound as a source of carbon (C) or nitrogen (N) (Mandelbaum *et al.*, 1993). An increase in soil microbial respiration observed after Atrazine addition could thus be due its utilization as a substrate for micro-organisms such as *Pseudomonas* spp. (Mandelbaum *et al*., 1993). The stimulation of bacterial populations in soil by Atrazine (Ros *et al*., 2006) as well as the stimulation of aerobic heterotrophic bacterial populations by glyhosate, 2,4-DDichlorophenoxyacetic acid (2,4-D), and metsulfuron (Zabaloy *et al.*, 2008) has also been documented. Kremer & Means (2009) reported that glyphosate increases the proportion of bacteria able to oxidize manganese (Mn).

2.9 The selected Herbicide

2.9.1 Paraquat

Paraquat, a herbicidal pesticide, is one of the most widely used herbicides in the world. Paraquat is quick-acting and non selective, killing green plant tissue on contact (Ogamba *et al.*, 2011). Paraquat is used to control broad-leaved weeds and grasses, being less effective on deep rooted plants such as dandelions. It does not harm mature bark, and is thus widely used for weed control in fruit orchards and plantation crops, including coffee, cocoa, coconut, oil palms, rubber, bananas, vines, olives and tea, ornamental trees and shrubs and in forestry. Other uses include weed control in alfalfa, onion, leeks, sugar beet, and asparagus. It is used for weed control on non-crop land and

can be used as a defoliant for cotton and hops before harvesting. Paraquat is used as a desiccant for pineapples, sugar cane, soya beans and sunflower (Tomlin, 1994). In pineapples, for example, Paraquat is applied after harvest to accelerate the drying out process and enabling plants to be burnt after 3-5 weeks, compared to 13 weeks after the alternative cutting and natural drying. Paraquat is increasingly used to destroy weeds in preparing land for planting in combination with no-till agricultural practices which minimize ploughing and help prevent soil erosion. Paraquat binds rapidly and tightly to clay materials in soils, and when adsorbed it is biologically inactive. It also binds to humus and other organic material: this results in no, or very low soil residues or leaching into water sources. Multiple spray trials showed Paraquat residues in soil from 22-58 mg/kg. Under field conditions, the residual Paraquat is slowly redistributed.

Long-term field studies have shown degradation rates of 5-10% per annum, which is thought to prevent saturation of the carrying capacity of the soil and to prevent adverse effects on micro flora and other soil organisms or on crop growth. In sandy soils with low organic content Paraquat may be more readily released into ground water.

2.10.2 Glyphosate

Glyphosate is one of the most commonly used herbicides worldwide. Glyphosate (N(phosphomethyl glycine), the active ingredient in the commercial product Roundup, is a broad spectrum, non-selective and post-emergent herbicide, developed in 1971 by Monsanto (Franz *et al.*, 1997). Since 1997, its agricultural use has increased considerably as a result of the introduction of genetically-engineered "Roundup Ready" glyphosate tolerant varieties of soybean, cotton and maize (Giesy *et al.*, 2000; Woodburn, 2000). It has become one of the most commonly-used herbicides for agricultural weed control worldwide (Giesy *et al.*, 2000; Kolpin *et al.*, 2006), and

also for domestic and industrial weed control in gardens or along rail tracks (Woodburn, 2000; Kolpin *et al.,* 2006)**.** Recent studies have shown that Glyphosate can stimulate microbial activity (Busse *et al.,* 2001; Haney *et al.,* 2000, 2002) and few studies have found any evidence that it has harmful effects on soil microorganisms (Busse *et al.,* 2001). Results of standardized tests with Glyphosate formulations for submission to regulatory agencies indicate no long-term effects on microorganisms in soil even at rates that exceed maximum use rate. In addition, independent researchers have reviewed numerous laboratory and field studies investigating the effects of Glyphosate on soil bacteria and fungi (Felsot, 2001; Giesy *et al.,* 2000). Although some laboratory test have shown effects on nitrogen-fixing bacteria (Moomaw *et al.,* 1992; Santos and Flores 1995) and soil fungi (Estok *et al.,* 1989; Busse *et al.,* 2001), effects are typically observed only under artificial laboratory conditions and at glyphosate concentrations well above normal field application rates.

Glyphosate inhibits protein synthesis via the shikimic acid pathway in bacteria and fungi; (Franz *et al.,* 1997), and one of its surfactants, polyoxyethylene tallow amine, is toxic to species of bacteria and protozoa (Tsui and Chu, 2003). Glyphosate has generally been found to be innocuous to soil High rate applications, in contrast, have been found to stimulate microbial respiration (Stratton and Stewart, 1992; Haney *et al.,* 2000; Busse *et al.,* 2001), and affect nutrient cycling processes. Glyphosate is a P-containing amino acid that functions both as a sole P source for in vitro microbial growth and as a readily available C and N source when degraded in soil (Busse *et al.,* 2001).

2.10.3 Atrazine

Atrazine has low solubility and a medium sorption index, which indicates low leaching potential. However, since Atrazine has a relatively high half-life, the leaching potential is high. As a result,

the Atrazine label carries a groundwater advisory statement against using the product on well drained sand and loamy sand soils where groundwater is close to the soil surface (Moomaw *et al.,* 1992). Globally, atrazine is used in the production of maize, sorghum, sugar cane, pineapples, chemical fallows, grassland, macadamia nuts, conifers, and for industrial weed control (Hicks, 1998), with its biggest market in maize production. In Europe, its use is concentrated on maize, orchards and vineyards (Tomlin, 2000), it is mainly used for maize, forestry, roses, and grassland (ACPEA, 1993). Atrazine is also applied in combination with many other herbicides, for example with simazine, another triazine chemical (Tomlin, 2000).

2.9.4. 2,4-D Amine

The effects of long-term applications of the herbicide 2,4-D on the soil microbial community, for example, have been analyzed by studying the microbial biomass, soil respiration, N mineralization, nitrification, urease and phosphatase activity. None of these experiments detected significant effects of the herbicide treatment (Biederbeck *et al*., 1987). 2,4-D amine are superior if leguminous cover crops or annual broad-leaf weeds are the dominant species present (USEPA, 2005). For post emergence weed control in the field with conventional or reduced tillage, 2,4-D amine is an extremely competitive products in terms of price per acre. As a selective herbicide, 2,4-D is used to control broadleaf weeds in a variety of settings from crops, rights-of-way, lawns, forests to aquatic settings (Burns and Swean, 2012). 2,4-D has a favorable environmental profile, and its exposures are expected to be minimal in both terrestrial and aquatic environments. It is rapidly broken down by microbial action in the soil and does not persist, accumulate or leach to groundwater under conditions of proper use. In field studies conducted across the U.S. under actual use conditions, 2,4-D had an average soil half-life of only five days with a range of less than two

to about 13 days. Although not strongly absorbed by soil, 95 percent or more of the residues were limited to only the top six inches of soil, and the maximum depth any residues were found was only 24 inches (USEPA, 2005).

2.10 Statistics on the use of herbicide in Ghana

Herbicide use has become very popular in with 84% of rice areas treated with herbicides as a result of its cheap price (Ragasa *et al.,* 2013)

Table 2.1 Cost difference between herbicide use and manual weeding

CASE	Without herbicides	With herbicides	Differences
Number of person-days for weeding (per ha)	211	86	125
Average daily wage (cedi/person-day)	7	7	0
Herbicide rate (liter/ha)	0	8	8
Price of herbicide (cedi/liter)	8	8	0
Total costs for weeding (cedi/ha)	1477	666	811

Source:

Assumptions are based on the average computed from CRI / SARI /IFPRI (NOVEMBER 2012 - FEBRUARY 2013) (Ragasa *et al.* , 2013)

Herbicides usage in Ghana continues to increase as agricultural production intensifies. However, associated with the increased use of herbicides are environmental and health problems which have risen due to indiscriminate use and inappropriate handling of the herbicides (Asante and Ntow, 2009). It is estimated that 87% of farmers in Ghana use pesticides to control pest and disease on

vegetable (Dinham, 2003). Out of this 87% pesticide used, 44% are herbicide, 33% are insecticides, and 23% are fungicide (Ntow *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

3.1.1. Geographic Area and Climate

Abadwum is in the Adansi North District near Akrokerri, Ashanti region, Ghana. Its geographical coordinates are latitude 6° 18' 0" North and longitude 1° 40' 0" West. The area therefore falls within a typical Tropical region of Africa, which characteristically experiences high temperatures and high rainfall with the mean annual temperature of 27 ^{0}C. Double maxima rainfall regime is experienced in the District. The annual total rainfall is between 1250 mm and 1750 mm. This puts the Adansi North District into a Semi-Equatorial climatic region (Adansi North district Assembly, 2006). Relative humidity is high about 80% in the rainy season and 20% in the dry season.

3.1.2. Topography and Soil

The District has an undulating terrain with more than half the total area rising to an average height of about 300 meters above sea level. In general the District is located in a hilly area. For this reason though there is land, most of it is not available for farming purposes since it is hilly. The major soil types in the District are Forest Ochrosols which develop well under moderate rainfall between 900 mm and 1650 mm and develop under forest vegetation with very rich humus content. The soils are well developed with well-defined profiles which supports meaningful agricultural production (Adansi North district Assembly, 2006).

3.2 Soil Sampling

The top soil (up to 5cm depth) sample was collected from oil palm plantation in Abadwum (Adansi-North district) with no prior herbicides treatment. The soil was collected from different points within the plantation and bulked together. It was shaken to mix it thoroughly and portion taken for onward laboratory analysis. The samples were sieved using a 2.0 mm mesh size to remove stones and plant debris for the laboratory analysis.

3.3 Herbicide Selection

The herbicides were obtained from a local agricultural input dealer in Akumadan in the Ashanti region. The selected herbicides were the most commonly used ones which contain the following active ingredients: Paraquat (Sun-Paraquat 200 SL), Glyphosate (Sunphosate 360 SL), 2, 4-D amine (720 SL) and Atrazine (Agrazine 500).

3.4 Soil Treatment

The soil treatment was carried out in three (3) different concentrations double the recommended field rate (RFR), half the (RFR) and normal the (RFR) over an interval of five days for fifteen days (15) exposure period in addition to the control sample. The rate of treatment was by the manufacturer of the herbicides recommended rate of 2.4 mg of the active ingredient per a gram of soil for Paraquat, 5.56 mg per gram of soil for Glyphosate, 6.17 mg per gram of soil for 2,4-D Amine and 6.67 mg per gram of soil for Atrazine. Each of the treatments was in three replicates.

Formula for calculating the treatments:

$$\text{Y (mg/g)} = \frac{\text{RFR (g a.i / ha)}}{\text{Am. AiF (g a.i / L) x 450 L/ha}} \times \frac{1000\ \text{mg}}{1\text{g}}$$

Where;

- ✓ Y - milligrams of chemical per gram of soil
- ✓ RFR- recommended field rate
- ✓ Am. AiF - amount of active ingredient in for

3.5 Enumeration of microbial population

3.5.1. Baseline determinations (Control)

This was the point where the bacteria and fungi population in the soil was determined without any chemical treatment to serve as the baseline to compare with the soils that were treated with the various herbicides. The soil organic matter was determined before the chemical treatment and after treatment.

3.5.2. Bacteria

The enumeration of the bacteria population was done using Pour Plate Counter. The plate count agar was prepared by suspending 20.5 g of dehydrated medium (powder) in one litre of distilled

water. The content was heated and boiled for one minute with constant agitation until the powder was completely dissolved. The agar was poured into a flask and sterilized in an autoclave at 121 ^{0}C. One gram of each treated soil sample was weighed and serially diluted. 1 ml aliquot was taken from an inch below the surface with sterilized 1ml pipette and placed in an empty sterile plate. 15 ml of the melted plate count agar which has been cooled to 45 ^{0}C was poured into the diluted sample. This was swirled to ensure that the mixture was thoroughly mixed and cooled to solidify on a flat laboratory bench before incubation was done under a lamina flow. These labelled specimens were inverted to prevent it from being soaked through condensation. Incubation was done at room temperature of 25 ^{0}C for 24 – 48 hours. Total viable colony on each plate was counted using the colony counter and the data recorded.

3.5.3. Fungi

The enumeration of the fungi was done by using Potato Dextrose Agar (PDA) supplemented with each of tetracycline and streptomycin to inhibit bacterial growth. The PDA was prepared by weighing 200 g of freshly peeled and washed potato in the laboratory. It was then boiled, mashed and the pulp squeezed through a fine sieve. 20 g agar was added and boiled to dissolve and again 20 g dextrose was added and boiled to dissolve and make up to one litre with water. The content was then sterilized at 15 psi for 20 minutes in an autoclave. 1 ml of the test samples was added to a sterile Petri dish and then a required amount of sterile, molten agar was added to the test sample. The content was cooled to 45 ^{0}C and swirled gently to mix well before it was allowed to solidify. Incubation of the fungi was done under a lamina flow at room temperature of 25 ^{0}C for

48 hours and identified with reference to Bergey‟s manual of systematic bacteriology. The total number of a particular organisms on each plate was identified and scored based on a maximum count of four (4) on a particular plate (Barnett and Hunter, 1972; Alexopoulos and Beneke, 1968).

3.6 Soil organic matter

The organic matter content was determined by the wet combustion (Walkley and Black, 1934). One gram of the sample soil was weighed out into a 500 ml Erlenmeyer flask and 10 ml of 1.0 N Potassium dichromate ($K_2Cr_2O_7$) solution added using a burette (Potassium dichromate oxidizes Carbon in the organic matter, itself being reduced in the process). This was followed by the addition of 20 ml conc. H_2SO_4 to generate heat to facilitate the reaction between carbon and Cr_2O. The mixture was swirled for one minute to ensure that the solution was in contact with all the particles of the soil. The flask and the content were allowed to cool on an asbestos sheet for 30 minutes. Two hundred milliliters of distilled water was added, followed by 10 ml orthophosphoric acid (to sharpen the colour change at the end point of titration). One milliliter of diphenylamine indicator was added and the solution titrated with 1.0 M normal ferrous sulphate solution until the colour changed to blue, and then finally to a green end-point.

The titre value was recorded and the blank solution corrected. Organic carbon was calculated using the formula below:

$$\% \text{ Organic C in Soil} = \frac{(\{\text{m. e. } K_2Cr_2O_7 - \text{m. e. } FeSO_4\} \times 0.003 \times f \times 100)}{\text{Weight of Soil}}$$

Where;

m.e. = milli equivalent = normality of solution × ml of solution used 0.003

= m.e. weight of C

f = correction factor = 1.33

% Organic matter was calculated using the formula:

Percentage (%) organic matter = Percentage organic carbon × 1.724

3.7 Statistical Analysis

Data generated from bacterial enumeration was subjected to logarithm transformation and subsequently expressed in graphs whilst data obtained from fungi enumeration was expressed in tables. Analysis of Variance (ANOVA) was run to compare the means of the different exposure periods of the herbicide. The data was again subjected to Duncan Multiple Range Test (DMRT) to compare the mean values between the baseline determinations and chemical treatments and to bring out the differences that exist between the treated soils.

CHAPTER FOUR

RESULTS

4.1 Effect of the exposure period of herbicide in relation to the baseline determination

The bacterial population after the herbicide (Atrazine) treatment was higher in the baseline (Control) in all the exposure period followed by the treatment doubled the recommended field rate (RFR). Even though, there was a steady declining rate of the bacterial population in relation to the exposure period, but somehow the Atrazine application above the recommended field rate supported the growth of soil bacteria.

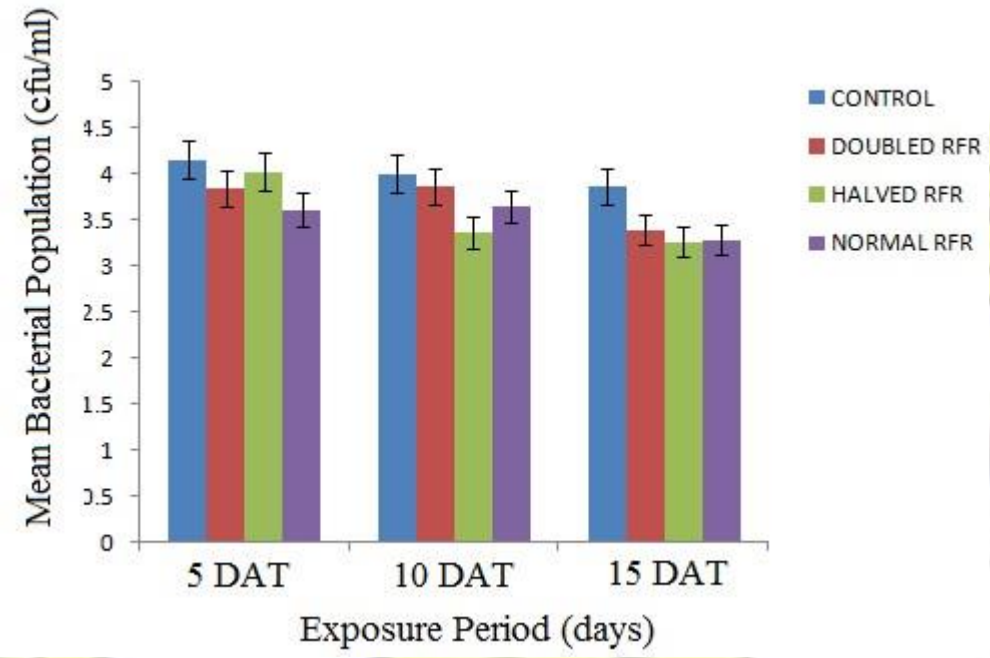

Figure 4.1 Mean Bacterial Population with Atrazine treatment in relation to the exposure period

DAT, is the days after treatment, RFR, is the recommended field rate.

This is the graph showing the mean bacterial population of soil treated with Atrazine herbicide against fifteen days exposure period. According to figure 4.1 the baseline determination recorded

the highest bacterial population followed by herbicide treatment doubled the normal recommended field rate with the exception of the first five days after the herbicide treatment. In the first five days the herbicide treatment halved the company‟s recommended field rate recorded second highest bacterial population, but recorded the least bacterial population in both the 10 and 15 DAT exposure periods.

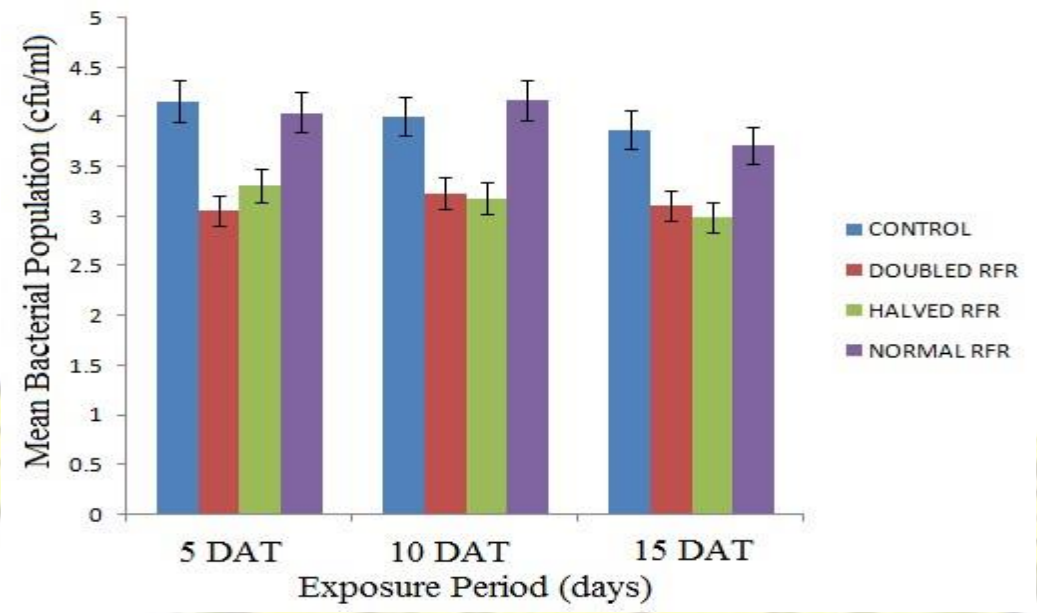

Figure 4.2: Mean values of Bacterial Population after 2, 4-D amine treatment in relation to the Exposure periods

DAT, is the day after treatment, RFR, is the recommended field rate.

This is the graph showing the mean bacterial population of sample soil treated with 2, 4-D amine herbicide against fifteen days exposure period. According to figure 3, the bacterial population in the sample treatment based on the normal recommended field application rate did not differ much through out the exposure period. However, bacterial population decreased in the sample treatment doubled the normal recommended field rate in relation to the controlled sample in all the exposure period. But a close look at the halved sample treatment reveals gradual decline in the bacterial population from day five (5) up to day fifteen (15) after treatment.

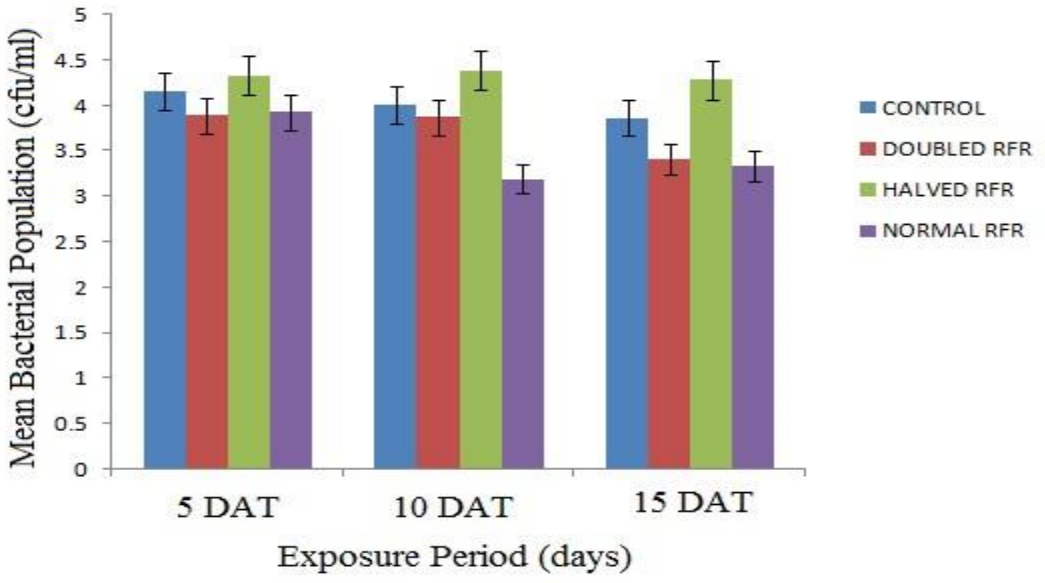

Figure 4.3: Mean Bacterial Population after Glyphosate treatment over the exposure periods.
DAT, is the day after treatment, RFR, is the recommended field rate.

This is the graph showing the mean bacterial population of soil treated with Glyphosate herbicide against fifteen days exposure periods. Bacterial population in the treatment halved the recommended field rate increased constantly from the first five day after treatment to the fifteen days after treatment suggesting an improvement in the bacterial growth and development in relation to the controlled experiment. Again, decrease in bacterial population was recorded in the fifteen days after treatment with Glyphosate in both treatments doubled and normal the company"s recommended rate of application. But bacterial population declined steadily throughout the exposure period might be due to a shortage of carbon source which was provided in the treated samples.

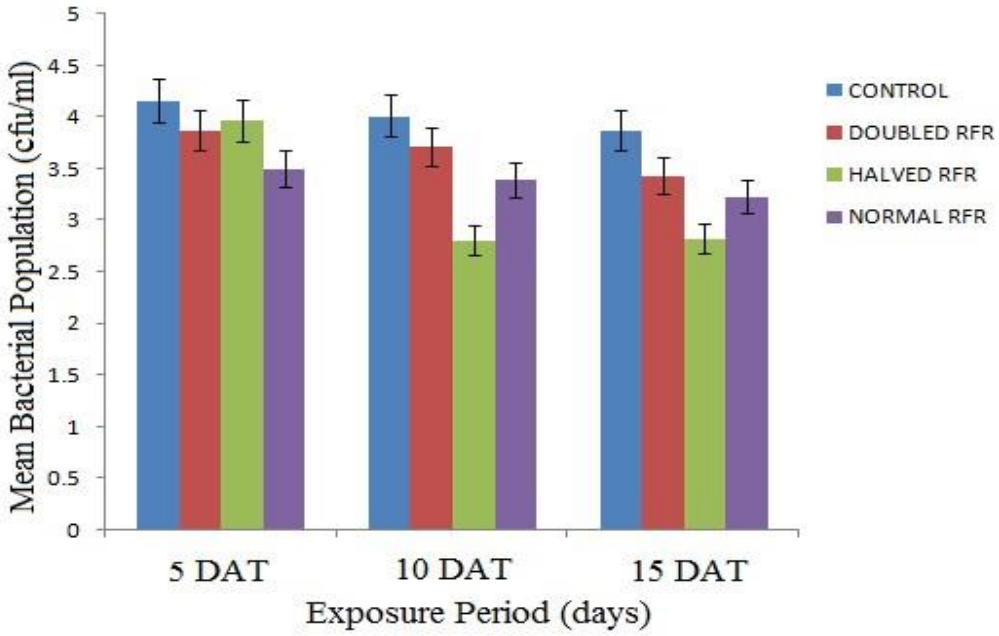

Figure 4.4: Mean bacterial population after Paraquat treatment against exposure periods

DAT, is the day after treatment, RFR, is the recommended field rate

This is the graph showing the mean bacterial population against fifteen exposure periods for sample soil treated with Paraquat. According to the figure, the baseline determination recorded the highest bacterial population and subsequently declined gently from the first five days (5 DAT) to day fifteen (15 DAT). The first five days after Paraquat application revealed an increased bacterial population beyond the entire treated sample halved the recommended field rate, but it sharply declined after day five to day fifteen. This decreasing trend in all the treatment towards the fifteen days suggests a decline in carbon source to support the initial population of bacteria‟s.

4.2 **Effect of different exposure period and concentration of the selected herbicides on fungal population**

Enumeration of fungi was scored on the basis of four (4) counts or scored on a plate under the different concentration and three different exposure periods for Atrazine, 2, 4-D amine, Glyphosate

and Paraquat was recorded. The cumulative fungal population of the four herbicides under different concentration in relation to the recommended field rate (RFR) was calculated and seven specific fungi were recorded from the plates. The seven fungi recorded were represented with letters (a-g) as indicated in a table below for three different exposure periods. From the table 4.1 a cumulative score of 13 fungi was recorded as against a zero cumulative record for the baseline.

Table 4.1. Cumulative fungal Population score of herbicide treatment under three different concentrations of Atrazine

	Specific Fungal Population and their cumulative score						
Herbicide Exposure period	a	b	c	d	e	f	g

5 DAT	3	2	2	3	1	0	0
10 DAT	2	6	0	2	2	0	0
15 DAT	2	5	4	0	1	0	0
Cumulative Score	7	13	6	5	4	0	0
BASELINE (Cumulative)	**4**	**0**	**5**	**0**	**0**	**0**	**3**

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label. See appendix A for above fungi

The above table shows the cumulative fungal population score of herbicide treatment under tree different concentration. From the table it is clearly seen that some of the fungi identified in the herbicide treated samples were not in the baseline determination. According to table fungi identified as *b, d, e, f,* and *g* were not identified as indicated. Fungi labelled (b) recorded the highest cumulative score of 13 with the highest enumeration occurring in the first ten days after treatment followed by fungi labelled (a) with the least cumulative fungi recorded for fungi (e) as compared to fungi labelled (g) in the baseline determination though it is absent in the herbicide treatments.

Table 4.2. Cumulative fungal Population score of herbicide treatment under three different concentration of 2, 4-D amine

	Specific Fungal Population and their cumulative score						
Herbicide Exposure period	a	b	c	d	e	f	g

5 DAT	4	0	4	0	1	2	0
10 DAT	7	0	0	3	2	0	0
15 DAT	7	0	4	0	1	0	0
Cumulative Score	18	0	8	3	4	2	0
BASELINE (Cumulative)	**4**	**0**	**5**	**0**	**0**	**0**	**3**

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label. See appendix A for the above fungi

The table above depicts the cumulative fungal population score of 2, 4-D amine herbicide treatment under three different concentrations for fifteen days continuous exposure period. Fungi labelled (a) had an impressive cumulative score of 18 with 7 fungi enumerated in each of 10 DAT and 15 DAT compared to cumulative score of 4 recorded in the baseline determination. According to the table some fungi were enumerated in the herbicide treatment (*b*, *d*, *e*, and *f*) but were absent in the baseline determination which might be as a result of a particular component of the herbicide favoring their growth and multiplication. The fungi labelled (f) recorded the least cumulative score of 2 though it was absent in the baseline determination.

Table 4.3. Cumulative fungal Population score of herbicide treatment under three different concentration of Glyphosate

	Specific Fungal Population and their cumulative score						
Herbicide Exposure period	a	b	c	d	e	f	g
5 DAT	2	6	2	3	1	0	3

10 DAT	1	11	0	0	0	0	4
15 DAT	0	1	1	0	0	0	3
Cumulative Score	3	18	3	3	1	0	10
BASELINE (Cumulative)	**4**	**0**	**5**	**0**	**0**	**0**	**3**

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The above table also shows the cumulative fungal population scores of Glyphosate herbicide treatment under three different concentrations at fifteen days continuous exposure period. According to the table 4.3 some fungi like (*b, d, e and f*) were absent in the baseline determination but were present in the Glyphosate treated sample. Fungi labelled (b) recorded the highest cumulative score of 18 with the highest enumeration occurring at the 10 DAT. It is followed by fungi labelled (g) which recorded cumulative score of 10 compared to a score of 3 in the baseline determination.

Table 4.4. Cumulative fungal Population score of herbicide treatment under three different concentration of Paraquat

PARAQUAT

	Specific Fungal Population and their cumulative score						
Herbicide Exposure period	a	b	c	d	e	f	g
5 DAT	4	2	4	2	0	0	0

10 DAT	3	6	1	0	2	0	0
15 DAT	2	3	1	2	1	0	3
Cumulative Score	**9**	**11**	**6**	**4**	**3**	**0**	**3**
BASELINE (Cumulative)	**4**	**0**	**5**	**0**	**0**	**0**	**3**

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The table reveals the cumulative fungal population scores of herbicide treatment under three different concentrations of Paraquat for 15 days continuous exposure period. It clearly seen from the table that some fungi such as (*b, d, e, and f*) were absent in the baseline determination but were present in the Paraquat treatment samples. Fungi labelled (b) which was absent in the baseline determination recorded the highest cumulative score of 11 with the highest population enumerated at 10 DAT. Least cumulative score of 3 each was recorded from fungi labelled (e) and (g).

4.5 Mean percentage organic matter under different concentration of four herbicides

The mean values of the % organic matter calculated for each of the four herbicides treatment under three different concentrations did not differ significantly with the baseline determination as can be seen in the table 11. The 2, 4-D treatment above the normal field application rate recorded a higher mean value of 3.88 ± 0.01 for the percentage organic matter as compared to 3.2 ± 0.01 in the baseline determination.

Table 4.5 Mean ± SE of % Organic matter of soil treated with the selected herbicides under three different concentrations

HERBICIDE TREATMENT	MEAN ± SE		
CONCENTRATION (mg a.i / g of soil)	NORMAL RFR (X)	DOUBLED RFR (2X)	HALVED RFR (0.5X)
ATRAZINE	3.07 ± 0.07	3.13 ± 0.08	3.03 ± 0.06
2, 4-D AMINE	3.59 ± 0.02	3.88 ± 0.01	3.06 ± 0.02
GLYPHOSATE	3.11 ± 0.02	3.15 ± 0.01	3.07 ± 0.03
PARAQUAT	2.95 ± 0.01	2.94 ± 0.01	3.13 ± 0.03
BASELINE DETERMINATION		**3.20 ± 0.01**	

Means and SE is the standard Error; RFR, is the recommended field rate of application.

The above table shows the mean plus the standard error of percentage organic matter of soil treated with the four selected herbicides under three different concentrations these chemicals. All the mean obtain followed similar trend which is not different from the mean value recorded for the baseline determination

CHAPTER FIVE

DISCUSSION

5.1 The effect of some selected herbicides on bacterial population

Generally, the effect of the four selected herbicides was not significant to the bacterial population at DMRT ($p<0.05$), but there was an appreciable change in microbial population in both bacteria and fungi. The three levels of herbicide concentration either impacted a detrimental effect on the microbial population or supported population growth structure of some of the organisms. In figure 4.1, the bacterial population gradually increased from ($6.945x10^3$ cfu/ml) of the first 5DAT to ($7.400x10^3$ cfu/ml) of the 10DAT exposure period with Atrazine treatment when the normal field application rate was doubled relative to the baseline which decreased from ($14.462x10^3$ cfu/ml) of the 5DAT to ($10.245x10^3$ cfu/ml) (See appendix B). This increase suggests the capacity of the organisms to degrade some aspect of the herbicide and utilize it as a carbon source to support their growth and multiplication (Wardle and Rahman, 1992).

The increase in bacterial population after the herbicide treatment is in support of the research conducted by Sebiomo *et al*., (2011), who also recorded an increased bacterial population in the first and second week of the same herbicide treatment. However, there was a sharp decrease in bacterial population which might be due to the fact that the rise in bacterial population became lethal with the subsequent increase in exposure period as reported by (Anderson *et al.*, 2000). Similarly, there was a steady decrease in bacterial population from 5DAT, 10DAT and 15DAT respectively, compared to the baseline determination in the current study. A study conducted by Rosli *et al.*, (2013) also recorded such free-fall decrease in microbial population under similar soil treatment in Malaysia.

Contrary, to the above trends where there is an increased microbial population from 5DAT to 10DAT and a subsequent decrease to 15DAT, bacterial population of 69.3% under normal field application rate declined sharply to 12.7% and rose again to 18.0% afer Glyphosate treatment (See fig. 4.3 and appendix C) . Glyphosate is a P-containing amino acid that functions both as a sole P source for in vitro microbial growth and as a readily available C and N source when degraded in soil (Busse *et al.,* 2001), hence, the sharp declination observed in table 4 under normal field rate might be due high mortality rate since high population would lead to fast depletion of the carbon source. Some studies report increased populations of actinomycetes and fungi after treatment with Glyphosate increased soil microbial biomass (Hanley *et al.,* 2002). In figure 4.4, Paraquat treatment recorded an impressive 87.2% bacterial population for the first 5DAT but the effect of its toxicity was felt from 10DAT (6.5%) to the 15DAT (6.4%) (See appendix C for details). Similarly, Rosli *et al.,* (2013) reported a drastic inhibition of both bacterial and actinomycetes populations by Paraquat to about 70 to 82% at recommended rate. The inhibitory capacity of Paraquat stems from the fact that it is known to be bounded strongly and coherently to soil component, including clay minerals and organic matter, therefore limits the access of microorganisms to Paraquat in soil water. The result of this study for Paraquat is consistent for all the treatment levels (Doubled, Halved and Normal recommended field rate of application).

5.2 The effect of some herbicides on soil fungal biomass and % organic matter

The fungal population was scored on the basis of four (4) maximum counts on a plate. With this as many as seven (7) different fungi were observed and are represented with the letters (a - g) (see appendix A for details). For this reason cumulative score for each herbicide treatment were tabulated taking into consideration the treatment levels as indicated in table (4.1 - 4.4). In this

study, a cumulative score of 7 was recorded for a particular fungus by Atrazine treatment compared to 4 score for the baseline determination. Again, cumulative score of 13 was scored as compared to zero (0) for the baseline (control). A laboratory study conducted by Estok *et al.,* (1989) and Busse *et al.,* (2001) confirm this inhibitory effect to some fungi. It can be seen from table 4.1 that at some exposure periods some fungi scored 0 relative to the baseline and even under normal field rate of the herbicide application. Most of these fungi were present in the baseline determination but were absent in the treatment samples. This means that some of the herbicides are toxic whilst others may be moderately toxic to some fungi. Claims have been made that repeated application of Atrazine does not affect the number of viable fungi in any way (Cole, 1976), suggesting that herbicides can elicit different reactions by different fungi. Certain fungal species are benefitted by herbicide addition, while others are inhibited. This trend is consists in all the herbicide treatment with some cumulative score as far as 18cfu/ml.

In this study the percentage organic matter of the control and the various herbicide treatments did not differ (P=0.05) much with the Mean ± SE of 3.2 ± 0.01 for the baseline determination. Only 2, 4-D amine recorded 3.88 ± 0.01 when the concentration was doubled compared to the normal field rate of application. According to table 4.5 it could be seen that almost all the % organic matter revolved around the baseline determination with no prominent significant differences at ($p<0.05$). This unaffected pattern of the percentage organic matter might be due to the short term nature of the exposure period for the herbicides treatment, since Sebiomo *et al.,* (2011) recorded from a study conducted in Nigeria that soil organic matter increased after continuous application from the second to the six week of treatment.

5.3 Implication of the study for conservation

The soil serves as the repository for all agricultural contaminants, but function as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influences the soil fertility Rosli *et al.,(* 2013), through organic material degradation, organic matter decomposition and nutrient cycling De-Lorenzo *et al.,*(2001)and Hutsch, (2001). These soil micro-organisms are greatly influenced by factors including the application of herbicides Pampulha *et al.*, (2007), which are applied in modern agricultural practices to attain optimum crop yield. Generally, every level of herbicide application either above or below the normal recommended field rate has deleterious effect be on the soil environment be it short or in the long-run. Hence, much caution should be exercised in our herbicide application.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of the study indicates that the presence of Atrazine, 2, 4-D amine, Glyphosate and Paraquat in the soil exert considerable change in the growth and development of soil microorganism. The toxic effect of some of the herbicide was felt shortly after its application whilst herbicide treatment like Paraquat had lasting effect on most microorganisms. For instance, the population of bacteria sharply increased to about 87.2% but steeply declined to 6.4% from the 10DAT to 15DAT. The pattern of change may vary as a result of differences in exposure period, the concentration of the active ingredient in the formulation, time of exposure, and so many environmental factors. This is supported by the view that microbial response to herbicides manifests itself in a variety of ways depending on factors including the herbicide itself, inherent micro-organism populations, herbicide concentration, exposure time, and chemical and physical characteristics of the soil.

Almost all the herbicide inhibited the growth of some specific fungi; the reason was that some fungi which were found in the baseline determination were not seen after the herbicide treatment and vice versa.

In this experiment the % organic matter seems not to be influenced by the herbicide exposure and its concentration but studies have already shown that continuous application of herbicide leads to an increased organic matter. Again, any change in the microbial structure will have a proportional change in the % organic matter since dehydrogenase activities level in the soil is an indication of how fertile a particular soil is.

However, the significance of these herbicides in modern agriculture should not be relegated to the background when issues of productivity and food security are at stake.

6.2 RECOMMENDATIONS

On the basis of the findings of this study it is recommended that:

- A similar study should be conducted on a normal field condition where herbicide treatments would be carried out on a normal field condition since most of the previous studies had the herbicide treatment carried out under laboratory condition.

- Other studies should be conducted using some of the up and coming herbicides with different active ingredients to see their impact on the soil and its inhabitants.

- Further research work should be carried out to identify the specific components of these herbicides which favour the growth and development of certain beneficial microorganisms.

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CODING OF FUNGI IN THE TABLES

a---------- A. *flavus* **b**----------

Colletotrichumgloeosporioides

c---------- *A. niger d*----------

Tichodermaviride

e---------- Mucor

f---------- penicillium g----------

Curvularialunata

Fungi Enumeration
5 DAT

SN	PLATE	ORGANISM AND (SCORE)
01	AAB	*A. flavus (2); Colletotrichum gloeosporioides (2)*
02	AB	*A. niger (1); penicillium (1); A. flavus (1)*
03	AN	*A. niger (1); Tichoderma viride (3)*
04	PAB	*A. niger (2); A. flavus (2)*
05	PB	*A. niger (2); A. flavus (2)*
06	PN	*Trichoderma viride (2); Colletotrichum gloeosporioides (2)*
07	GAB	*Colletotrichum gloeosporioides (2); A. niger (2)*
08	GB	*Colletotrichum gloeosporioides (2); A. flavus (2)*
09	GN	*Colletotrichum (2); penicillium (1);* Trichoderma *viride (1)*
10	DAB	*Curvularia lunata (2); Penicillium sp. (1); A. flavus (1)*
11	DB	*A. niger (2); A. flavus (1); Penicillium (1)*
12	DN	*A. niger (2); A. flavus (2)*
13	CTL.	*A. niger (4)*

10 DAT

SN	PLATE	ORGANISM AND (SCORE)
01	AAB	*Colletotrichum gloeosporioides (2); Penicillium (1); A. flavus (1)*
02	AB	*Colletotrichum gloeosporioides (4)*
03	AN	*Trichoderma viride (2); A. flavus (1); Penicillium (1)*
04	DAB	*Trichoderma viride (3); Penicillium sp.(1*)
05	DB	*A. flavus (3); Penicillium sp.(1)*
06	DN	*A. flavus (4)*
07	PAB	*Penicillium (1); A. flavus (2); Colletotrichum gloeosporioides (1)*
08	PB	*A. niger (1); A. flavus(1); Penicillium sp.(1); Colletotrichum gloeosporioides (1)*

09 PN *Colletotrichum gloeosporioides (4)*

10 GAB *Colletotrichum gloeosporioides (4*)

11 GB Colletotrichum gloeosporioides (4)

12 GN *Colletotrichum gloeosporioides (3); A. flavus (1)* 13 CTL. *Mucor (3); A. niger (1)*

15DAT

SN	PLATE	ORGANISM AND (SCORE)
01	AAB	*A. flavus (2); Colletotrichum gloeosporioides (2)*
02	AB	*Colletotrichum gloeosporioides (2); A.niger (2)*
03	AN	*Penicillium sp. (1); Colletotrichum gloeosporioides (1); A. niger (2)*
04	DAB	*A. flavus (4)*
05	DB	*A. niger (2); Penicillium (1); A. flavus (1)*
06	DN	*A. niger (2); A. flavus (2)*
07	PAB	*Colletotrichum gloeosporioides (2); A. flavus (1); A. niger (1)*
08	PB	*Penicillium (1); A. flavus (1); Trichoderma viride (2)*
09	PN	*Mucor (3); Colletotrichum gloeosporioides (1)*
10	GAB	*Mucor (3); Colletotrichum gloeosporioides (1)*
11	GB	*Mucor (4)*
12	GN	*Mucor (3); A. niger (1)*
13	CTL.	*A. flavus (4)*

APPENDICE B

Bacterial counts in soil after the administration of different concentrations of a the selected herbicides

Chemical Treatment	Concentration (mg a.i / g)		Bacteria Enumeration (cfu / ml)		
			DAT 5	DAT 10	DAT 15
Control			1.4462×10^4	1.0245×10^4	7.406×10^3

A_{AB}		2X	$6.945x10^3$	$7.400x10^3$	$2.503x10^3$
A_B		0.5X	$1.0720x10^4$	$2.303x10^3$	$1.810x10^3$
SA_N	6.67	1X	$4.162x10^3$	4.455103	$1.903x10^3$
D_{AB}		**2X**	**$1.150x10^3$**	**$1.710x10^3$**	**$1.293x10^3$**
D_B		**0.5X**	**$2.060x10^3$**	**$1.544x10^3$**	**$9.93x10^2$**
D_N	**6.17**	**1X**	**$1.1145x10^4$**	**$1.4780x10^4$**	**$5.293x10^3$**
G_{AB}		2X	$7.792x10^3$	$7.455x10^3$	$2.570x10^3$
G_B		0.5X	$2.1227x10^4$	$2.4290x10^4$	$1.9180x10^4$
G_N	**5.56**	1X	$8.500x10^3$	$1.560x10^3$	$2.206x10^3$
P_{AB}		**2X**	**$7.507x10^3$**	**$5.205x10^3$**	**$2.730x10^3$**
P_B		**0.5X**	**$9.180x10^3$**	**$6.46x10^2$**	**$6.70x10^2$**
P_N	**2.46**	**1X**	**$3.173x10^3$**	**$2.460x10^3$**	**$1.716x10^3$**

ANOVA for treatment of the selected chemicals

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	139.729	4	34.932	1.292	0.35
	Within Groups	216.304	8	27.038		
Day5	Total	356.033	12			
	Between Groups	129.496	4	32.374	0.621	0.661
	Within Groups	417.253	8	52.157		
Day10	Total	546.749	12			
	Between Groups	92.483	4	23.121	0.916	0.499
	Within Groups	201.979	8	25.247		
Day15	Total	294.462	12			

ANOVA for dosage of the selected chemicals

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	55.607	2	27.804	0.965	0.417
	Within Groups	259.418	9	28.824		
Day5	Total	315.025	11			
	Between Groups	6.829	2	3.414	0.059	0.943
	Within Groups	524.446	9	58.272		
Day10	Total	531.275	11			
	Between Groups	26.745	2	13.372	0.474	0.637
	Within Groups	254.15	9	28.239		
Day15	Total	280.895	11			

ANOVA OF % ORGANIC MATTER

		Sum of Squares	df	Mean Square	F	Sig.
Day5	Between Groups	0.205242308	3	0.068414103	0.299499926	0.82505
	Within Groups	2.05585	9	0.228427778		
	Total	2.261092308	12			
Day10	Between Groups	0.279680769	3	0.093226923	0.317680672	0.81248
	Within Groups	2.64115	9	0.293461111		
	Total	2.920830769	12			
Day15	Between Groups	0.272826923	3	0.090942308	0.366661785	0.77892
	Within Groups	2.23225	9	0.248027778		
	Total	2.505076923	12			

APPENDICE C

Table A: Mean and % bacterial population of soil treated with Atrazine in three different concentration

Herbicide Treatment (mg a.i./g)	Mean and % Bacterial Population (cfu / ml)		
	5 DAT x10^3	10 DAT x 10^3	15 DAT x10^3
ATRAZINE			

Double RFR	6.945[a] (41.8%)	7.400[a] (44.5%)	2.503[a] (13.6%)
Half RFR	1.072[a] (19.9%)	2.303[a] (44.9%)	1.810[a] (35.1%)
NORMAL RFR	4.162[a] (39.5%)	4.445[a] (42.9%)	1.903[a] (18.1%)
BASELINE (CONTROL)	**14.462[a] (45.0%)**	**10.245[a] (31.8%)**	**7.406[a] (23.1%)**

Within the same column number followed by similar letter are not significant DMRT at ($p<0.05$). % Population in parenthesis while the means are in standard form for all the concentration of the herbicides. RFR, recommended field rate on the product label to be applied.

Table B: Mean and % bacterial population of soil treated with 2,4-D amine in three different concentration

Herbicide Treatment (mg a.i./g)	Mean and % Bacterial Population (cfu / ml)		
	5 DAT x 10^3	10 DAT x 10^3	15 DAT x10^3
2, 4 - D amine			
Double RFR	1.150[a] (27.7%)	1.71[a] (41.2%)	1.293[a] (21.6%)
Half RFR	2.06[a] (44.8%)	1.544[a] (33.5%)	0.993[a] (21.6%)
NORMAL RFR	11.145[a] (35.6%)	14.78[a] (47.4)	5.293[a] (16.9%)
BASELINE (CONTROL)	**14.462[a] (45.0%)**	**10.245[a] (31.8%)**	**7.406[a] (23.1%)**

Table C: Mean and % bacterial population of soil treated with Glyphosate in three different concentration

Herbicide Treatment (mg a.i./g)	Mean and % Bacterial Population (cfu / ml) 5 DAT10^3	10 DAT x 10^3	15 DAT x10^3
GLYPHOSATE			
Double RFR	7.792^a (43.7%)	7.455^a (41.8%)	2.57^a (17.1%)
Half RFR	21.227^b (32.8%)	24.29^b (37.5%)	19.18^a (29.6%)
NORMAL RFR	8.500^a (69.3%)	1.560^a (12.7%)	2.206^a (18.0%)
BASELINE (CONTROL)	**14.462^a (45.0%)**	**10.245^a (31.8%)**	**7.406^a (23.1%)**

Table D: Mean and % bacterial population of soil treated with Atrazine in three different concentration

Herbicide Treatment (mg a.i./g)	Mean and % Bacterial Population (cfu / ml) 5 DAT x 10_3	10 DAT x 10_3	15 DATx10_3
PARAQUAT			
Double RFR	7.510^a (48.6%)	5.200^a (33.7%)	2.730^a (17.1%)
Half RFR	9.180^a (87.2%)	0.680^a (6.5%)	0.670^a (6.4%)
NORMAL RFR	3.17 0^a (43.1%)	2.460^a (33.5%)	1.720^a (23.4%)
BASELINE (CONTROL)	**14.462^a (45.0%)**	**10.245^a (31.8%)**	**7.406^a (23.1%)**

Within the same column number followed by similar letters are not significant by DMRT at ($p<0.05$). Percentage Population in parenthesis while the means are in standard form for all the concentration of the herbicides, RFR, recommended field rate of the product label to be applied.