EFFECT OF DIFFERENT CONCENTRATIONS OF SOME AGROCHEMICALS ON SOIL MICROBIAL DENSITY AND CROP GROWTH

BY

OPARAH, JENNIFER UZOAMAKA

REG NO: 20124764758

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN ENVIRONMENTAL MICROBIOLOGY

MAY, 2021.

CERTIFICATION

This is to certify that this research work titled **"Effect of Different Concentrations of Some Agrochemicals on Soil Microbial Density and Crop Growth"** was carried out by **Oparah Jennifer Uzoamaka** with registration number **20124764758** of the Department of Microbiology, Federal University of Technology Owerri, in Partial Fulfillment of the Award of a Master of Science (M.Sc.) Degree in Environmental Microbiology.

.....
Prof. Wesley Braide
(Supervisor)

19/05/2021
.....
Date

.....
Dr. (Mrs.). E.E. Mike-Anosike
(Co-Supervisor)

19/05/2021
.....
Date

.....
Prof. C. E. Nwanyawu
(HOD Microbiology)

19/05/2021
.....
Date

.....
Prof. F.O.U. Osuala
(Dean, School of Biological Science)

28/06/21
.....
Date

.....
Prof. C.C. Eze
(Dean, Post-Graduate School)

.....
Date

.....
Prof. D. Arutopin
(External Examiner)

19/05/2021
.....
Date

ii

DEDICATION

This research work is dedicated to Almighty God for his grace, provisions and guidance throughout this program. His name alone will be glorified in my life. I also dedicate this work to my parents Mr. Richard and Mrs. Patience Oparah for their wonderful encouragements and supports all the way. God bless you both.

ACKNOWLEDGEMENTS

My sincere and unreserved gratitude goes first to the Almighty God for his favour, love, grace, provision and divine mercy throughout the period of this progamme. I would like to express my sincere gratitude to my supervisors Dr. W. Braide and Dr. (Mrs.) E.E. Mike-Anosike for their immense support all through this program. Your patience, encouragement and immense help throughout this research work. Am very grateful to the both of you.

I would like to immensely thank my HOD Prof. C. E. Nwanyawu for his wonderful assistance, advice and motivation towards making this research work a success. Your patience and understanding is second to none, a million thanks to you sir. I also wish to thank in a very special way, all my lecturers in FUTO. My former Postgraduate Coordinator of Microbiology, Dr. I. E. Adieze is not left out for his tireless effort toward seeing that I complete this programme.

I am sincere grateful to my wonderful course mates E. Egbadon, S. Adeleye, Mrs. P. N. Ogu, and Mrs. C. C. Ukaefu, for being there for me in different ways, I really appreciate you all.

Finally, I want to use this opportunity to thank my parents, Mr. Richard I and Mrs. Patience C. Oparah, not forgetting my sister Mrs. Olivia Chukwu for their support and prayers. May God bless you all.

`

TABLE OF CONTENTS

CHAPTER THREE

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The effects of insecticide and herbicide pollution on soil nutrients, soil microbes as well as Pinto beans (*Phaseolus vulgaris*) and maize cultivated on the soil was studied. Insecticide herbicide were used to impact soil samples across concentration gradients. Pinto beans (*Phaseolus vulgaris*) and maize seeds were planted in separate experiments to monitor the effects of the agrochemicals on the cultivars. The microbial load, diversity and the physicochemical characteristics of the soil before and after pollution were determined using standard methods. Chlorophyll contents of the leaves were also determined after planting. Results shows that Bacterial isolates namely *Enterococcus* sp 54(44%), *Staphylococcus* sp 8(7%), *Bacillus* sp 46(37%) and *Micrococcus* sp 15(12%) and fungal isolates such namely *Penicillium* sp 41(35%), *Saccharomyces* sp 67(56%), *Yeast* sp 4(3%), *Geotrichum* sp 6 (5%) and *Aspergillus* sp 1(1%) were recovered from treated and untreated soil with the percentages representing after planting. The findings from the study showed that the herbicide treated soils indicated that the total heterotrophic bacterial count (THBC) increased after cultivation, while the total heterotrophic fungal count (THFC) decreased after cultivation. On the other hand, insecticides treated soils recorded a general decrease in THFC and THBC after cultivation. The total nitrogen (TN), available phosphorus (AP) and exchangeable potassium (EP) had significant differences before and after planting. The cultivars generally had poor growth characteristics recorded as well as chlorophyll content. This study demonstrates the negative effects of the use of insecticides and herbicides against non-target species and call for the need for strict regulations against their use especially against permissive threshold concentrations.

Key words: Heterotrophic fungal count (THFC), Total nitrogen (TN), Available phosphorus, Pinto beans (*Phaseolus vulgaris*), total Heterotrophic bacterial count (THBC).

CHAPTER ONE

1.0 INTRODUCTION

Microorganisms are the primary soil decomposers, driving key ecosystem processes such as organic matter decomposition, nutrient cycling and thereby improves plant productivity (Paul *et al.,* 2007). Therefore, agricultural practices affecting soil microorganisms are of particular interest. Modern agriculture worldwide uses a variety of pesticides which includes insecticides, nematicides, herbicides and fungicide to optimize crop production (Das and Mukherjee, 2008). However, continuous application of pesticides may result in soil pollution threatening processes driven by soil microorganisms and, thereby affecting soil fertility (Lopez *et al.,* 2002).

Pesticides are defined under the Federal Environmental Pesticide Control Act (FEPCA) as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (insects, rodents, nematodes, fungus, weeds and other forms of terrestrial or aquatic plants or animals like bacteria or other microorganisms) or any substance or mixture of substances intended for use as a plant regulators or desiccant (Mishra *et al*., 2001).

Pesticides are directly toxic to pest having indirect effects to soil invertebrates (Mishra *et al.*, 2001). They may be fungicides (toxic to fungus), insecticides (toxic to insects), nematicides (toxic to nematodes) and herbicides (toxic to herbs). According to Mishra *et al* (2001) it was observed that physicochemical properties of the soil, nature of substrates and environmental degradation determine the persistence of pesticides in nature. Excessive persistent and biological active residues endanger non-target organisms, prove hazardous and make the pest control operational uneconomical.

The consequences of agrochemical on soil biota may be direct and far reaching. Organisms which are of benefit to agriculture and which may be affected include, those responsible for: Organic matter decomposition and soil aggregation, breakdown of toxic compounds both metabolic by-products and agrochemicals inorganic transformation that make available nitrate, sulphate, and phosphates as well as essential elements like iron and manganese, and nitrogen fixation into forms usable by higher plants (Piao *et al*., 2001).

An ideal pesticide should be toxic only to the target organisms, biodegradable and should not leach into the groundwater (Pesticide, 2006). Unfortunately, this is rarely the case and the widespread use of pesticides in modern agriculture is of concern (Bishnu *et al*., 2009). Owing to their xenobiotics characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated biotransformation in the soil. Inactivation of nitrogen-fixing and phosphorus solubilizing microorganisms is observed in pesticide contaminated soil (Lopez *et al.,* 2012).

Recent studies show that some pesticides disturb molecular interactions between plants and N-fixing rhizobacteria and consequently inhibit the vital process of biological nitrogen fixation (Piao *et. al.,* 2001). Similarly, many studies show that pesticide reduces activities of soil enzymes that are key indicators of soil health. Application of pesticides may also influence many biochemical reactions such as mineralization of organic matter, nitrification, dentrification, methanogenesis and so on. (Piao *et. al.,* 2001). The most obvious effect is that of the direct toxicity of applied pesticide to the susceptible microbial species. Other microorganisms become resistant to the pesticide and can increase their biomass because of decreased competition (Rafatullah *et al.,* 2010).

Degradation of pesticides involves biotic and abiotic transformation processes. Biotic transformation is mediated by microorganisms, while abiotic transformation involves

processes such as chemical and photochemical reactions (Fenner *et al.*, 2013). The specific degradation processes for a given pesticide are determined by its structure and by the environmental conditions it is exposed (Cazaja *et al*. 2020). Photochemical transformations require sunlight, available only in the topmost part of lakes or rivers, plant surfaces or submilimeter soil layers (Fenner *et al.*, 2013).

Pesticides are rapidly degraded by microorganisms when used as a carbon source (William *et al.* 2002). In the work carried out by (William *et al.* 2002), it was observed that out of 10 microorganisms isolated and screened for degradative capabilities towards endosulfan degradation, the strains isolated were *Fusarium ventricosum* and *Pandoraea* sp. which degraded about 90% and 83% of 100ppm endosulfan respectively, in 15days using the pesticides as a carbon source.

Some pesticides breakdown faster and more easily than others, having shorter "half-lives" but some may remain longer in soil (Gill *et al.*, 2014). The more recalcitrant a pesticides is, the more damage it can cause to microorganisms, soil and the whole environment (U.S Environmental protection Agency, 1999). Pesticides like chlordane and paraqunt have high persistence half-life more than 100 days, while endosulfan, classified as organochlorine are persistent in the soil with an estimated half-life of 9months to 6yrs. It is one of the most commonly detected pesticide in U.S water (William *et al.*, 2002).

1.1 Statement of Problems

The soil microorganisms such as bacteria, fungi, algae and nematodes play important role in soil nutrition through their role in decay of plants and other organic matters in soil as nutrifiers. Anything that disrupts their activities could be expected to affect the nutritional quality of soils and would therefore, have serious consequences on crop yield (Lilane *et al.*, 2020). Also, microorganisms that live in soil can be killed not only by chemicals applied

directly to the soil, but also by those that reach the soil in drift from ariel sprays or washed off foliage which in turn affect the breakdown of some kinds of dead leaf material into its organic constituents and in the incorporation of these materials into the soil structure (Mishra *et al.*, 2001).

However, pesticides serve useful purposes, concern has been expressed regarding their possible effects on environment. Pesticides may affect living organisms in the soil in the following ways: They may be directly toxic to microorganisms in soil. They may affect the soil organisms genetically to produce population resistant to the pesticides. They may have sub-lethal effects that result in alterations in behaviour or changes in metabolic or reproductive activity. They may be taken into bodies of soil flora or fauna and passes on to the other organisms.

1.2 Aim of the Study

The aim of the study is to determine the effect of different concentrations of agrochemicals (pesticides) on soil microbial diversity, density and crop growth.

1.3 Objectives of the Study

The objectives of the study are:

a) To isolate and characterize the microorganisms in soil sample before and after treatments with pesticides.
b) To determine the physico-chemical quantity (N_1 P_1 K) of soil before and after inoculation.
c) To determine the effect of different concentration of some agrochemicals on plant growth (leaf length, stem height and girth).
d) To determine nitrogen and chlorophyll contents of harvested crop cultivars.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil

Soil is the mixture of minerals, organic matter, gases, liquids and countless organisms that together support plants life. Two general classes of soil are topsoil and subsoil (Voroney, 2006). Soil is a natural body that exists as part of the pedosphere (the outer-most layer of the earth that is composed of soil and subject to soil formation processes) and which performs four important functions: It is a medium for plant growth. It is a means of water, storage, supply and purification. It is a modifier of the atmosphere of earth and it is a habitat for organisms all of which modify the soil.

Soil is considered to be the "skin of the earth" with interfaces between the lithosphere, hydrosphere, atmosphere of earth and biosphere. Soil consists of a solid phase (minerals and organic matter) as well as a porous phase that holds gases and water.

Soil itself is very complex. It would be wrong to think of soils as just a collection of fine mineral particles. Soil also contains air, water, dead organic matters, and various types of living organisms. The formation of soil is influenced by organisms, climate, topography, parent material, and time.

2.1.1 Organic Activity

A mass of mineral particles alone do not constitute a true soil. True soils are influenced, modified, and supplemented by living organisms. Plants and animals aid in the development of a soil through the addition of organic matter (Cheesebrough, 2000). Fungi and bacteria decompose this organic matter into semi-soluble chemical substance known as humus. Layer

soil organisms like earthworms, beetles, and termites, vertically redistribute this humus within the mineral matter found beneath the surface of the soil (Cheng, 1999).

Organic activity is usually profuse in the near surface layers of a soil. For instance, one cubic centimeter of soil can be the home to more than 1,000,000 bacteria. A hectare of pasture land in a humid mid-latitude climate can contain more than a million earthworms and several millions insects. Earthworms and insects are extremely important because of their ability to mix and aerate soil. Higher porosity, because of mixing and aeration, increases the movement of air and water from the soil surface to deeper layers where roots reside. Increasing air and water availability to roots has significant positive effects on plant productivity. Earthworms and insects also produce most of the humus found in a soil through the incomplete digestion of organic matter (Cheng, 1999).

2.1.2 Translocation

When water moves downward into the soil, it causes both mechanical and chemical translocations of material. The complete chemical removal of substances from the soil profile is known as leaching. Leached substances often end up in the groundwater zone and then travel by groundwater flow into water bodies like rivers, lakes, and oceans. Eluviation refers to the movement of fine mineral particles (like clay) or dissolved substances out of an upper layer in a soil profile (Cheng, 1999). The deposition of fine mineral particles or dissolved substances in lower soil layer is called illuviation (Cheesebrough, 2000).

2.1.3 Soil Texture

The texture of a soil refers to the size distribution of the mineral particles found in a representative sample of soil. Also, the look and feel of a soil is referred as soil texture, it is

the arrangement of soil profiles into aggregates and is determined by size and type of particles that make up the soil.

The size of the ex-rock pieces (now the inorganic soil particles) varies substantially from large bits of gravel to much smaller clay pieces. The soil particles are actually referred based on their size: Gravel particles are greater than 2mm in diameter. Sand particles between 0.2mm and 0.02mm in diameter. Silt particles between 0.02m and 0.002mm in diameter. Clay particles less than 0.002mm in diameter.

Sand particles are large with small surface area. Therefore, sand drains easily, has poor ability to retain moisture, little chemical activity, and little nutrient bonding. Silt particles have limited surface area, little chemical activity, little nutrient bonding, may compact under heavy traffic, poor air and water movement. Clay particles are small but have large surface area. Therefore, water adheres very well to clay. It has the high ability to retain moisture (however, this water can be hard even impossible for the plants to use). Very chemically active and good nutrient bonding.

2.1.4 Soil pH

Soil supports a number of inorganic and organic chemical reactions. Many of these reactions are dependent on some particular soil chemical properties. One of the most important chemical properties influencing reactions in a soil is pH. Soil pH is primarily controlled by the concentration of free hydrogen ions in the soil matrix (Nester *et al.*, 1998). Soils with a relatively large concentration of hydrogen ions tend to be acidic. Alkaline soils have relatively low concentration of hydrogen ions (Sine, 1992). Hydrogen ions are made available to the soil matrix by the dissociation of water by the activity of plant roots, and by much chemical weathering reaction.

Soil fertility is directly influenced by pH through the solubility of many nutrients. At a pH lower than 5.5, many nutrients become very soluble and are readily leached from the soil profile. At high pH, nutrient become very soluble and plants cannot readily extract them. Maximum soil fertility occurs in the range 6.0 to 7.2 (Bradgett *et al*, 1999).

2.1.5 Soil Profiles

Most soil have a distinct profile or sequence of horizontal layers. Generally, these horizons result from the processes of chemical weathering, eluviation, illuviation, and organic decomposition. According to Pidwirny, layers found in a soil profile are up to five which can be represented in a typical soil: O, A, B, C, and R horizons

O horizon

This is the topmost layer of most soils. It is composed mainly of plant litter at various levels of decomposition and humus (Pidwirny, 2013).

A horizon

This is found below the O layer. This layer is composed primarily of mineral particles and has two characteristics: It is the layer in which humus and other organic materials are mixed with mineral particles, and it is a zone of translocation from which eluviation has removed finer particles and soluble substances, both of which may be deposited at a lower layer. Thus the A horizon is dark in color and usually light in texture and porous. The A horizon or organic accumulation, and a lower horizon shows loss of material by eluviation (Pidwirny, 2013).

Bhorizon

This horizon is a mineral soil layer which is strongly influenced by illuviation. Consequently, this layer receives material eluviated from the A horizon. The B horizon also has a higher bulk density than the A horizon due to its enrichment of clay particles. The B horizon may be colored by oxides of iron and aluminum or by calcium carbonate illuviated from the A horizon (Pidwirny, 2013).

C horizon

C horizon is composed of weathered parent material. The texture of this material can be quite variable with particles ranging in size from clay to boulders. The C horizon has also not been significantly influenced by the pedogenic processes, translocation, or organic modification (Pidwirny, 2013).

R horizon

This is the final layer in a typical soil profile. This soil layer simply consists of unweathered bedrock.

2.1.6 Soil Structure

Soil structure describes the arrangement of the solid parts of the soil and of the pore space located between them. It is determined by how individual soil granules clump or bind together and aggregate, and therefore the arrangement of soil pores between them. Soil structure has a major influence on water and air movement, biological activity, root growth and seedling emergence.

The arrangement of soil aggregate into different forms gives a soil its structure. The nature processes that aid in forming aggregates are: wetting and drying, freezing and thawing,

microbial activity that aids in the decay of organic matter. Activity of roots and soil animals, and adsorbed cations (Pidwirny, 2013).

The wetting /drying and freezing /thawing action as well as root or animal activity push particles back and forth to form aggregates. Decaying plant residues and microbial byproducts coat soil particles and bind particles into aggregates. Absorbent cations help form aggregates whenever a cation is bonded to two or more particles.

Aggregates are described by their shape, size and stability. Aggregate types are used most frequently when discussing structure (Pidwirny, 2013).

Table 2.1: Structural Type and Description of Soil

Type	Description
Granular	Rounded surface
Crumb	Rounded surfaces but larger than granular
Subangular blocky	Cube-like with flattened surfaces and rounded corners
Blocky	Cube-like with flattened surfaces and sharp corners
Prismatic	Rectangular with a long vertical dimension and flattened trip.
Columnar	Rectangular with a long vertical dimension and rounded top
Single grain	No aggregate of course particles when dry
Structureless	No aggregation of fine particles when dry.

(Paul *et al.*, 2007)

Soil structure is one of the defining characteristics of a soil horizon. A soil exhibits only one structure per soil horizon, but different horizons within a soil may exhibit different structure. All of the soil forming factors, especially climate, influences the type of structure that develops at each depth. Granular and crumb structure are usually located at the soil surface in the A horizon. The subsoil, predominantly, the B horizon, has subangular blocky, blocky, columnar is prismatic structure. (Paul *et al.,* 2007).

2.2 Soil Fertility

In Southern Africa the most limiting factor to agricultural productivity is soil fertility (Ramaru *et al.*, 2000). Soil fertility is defined as the condition of soil that enables it to provide nutrients in adequate amounts and in proper balance for the growth of specified plants when other growth factors such as light, water, temperature, and physical condition of soil are favourable (Vander Watt and Van Rooyen, 1995).

Soil fertility can also be defined as the ability of the soil to supply essential plant nutrients and soil water in adequate amounts and proportions for plant growth and reproduction in the absence of toxic substances which may inhibit plant growth (Tan *et al.,* 2003).

Soil fertility depends on such factors affecting soil formation as climate, parent material and topography. The way in which soil is used is particularly important. The principal means of regulating supply especially in mobile forms available to plants is the addition of mineral and organic fertilizers (Tabatabai, 1994). The introduction of legumes into crop rotations and improvement of conditions promoting the activity of nitric bacteria and other organisms that take up nitrogen from the atmosphere are also important. Excessive acidity is corrected by liming, and excessive alkalinity by the application of gypsum (Stenberg *et al.,* 1998).

Soil fertility is markedly decreased by the presence of such toxic chemical compounds as ferrous oxide and mobile aluminum compounds, which usually accumulate in waterlogged soils. The moisture supply can be controlled by farming practices and hydraulic engineering measures (Snow retention, early spring harrowing, irrigation, drainage). Soil with good aeration and adequate moisture are the most fertile. Low fertility is often due to pathogenic organisms. Their removal by chemical agents (Sterilization, application of fungicides or nematocides) and by farming procedures (crop rotation, cultivation) increases soil fertility sharply. Correct use of soils not only prevents a decrease in their fertility but yields a steady increase (Tabatabai, 1994).

2.2.1 Factors Affecting Soil Fertility

a. **Parent material:** Soil parent material is the material that soil develops from, and may be rock that has decomposed in place, or material that has been deposited by wind, water, or ice. The character and chemical composition of the parent material plays an important role in determining soil properties, especially during the early stages of development (Braimoh *et al.*, 2005).

   Parent material composition has a direct impact on soil chemistry and fertility. Parent materials rich in soluble ions-calcium, magnesium, potassium, and sodium, are easily dissolved in water and available to plants. Limestone and basaltic lava both have a high content of soluble bases and produce fertile soil in humid climate (Bardgett *et al.*, 1999). If parent materials are low in soluble ions, water moving through the soil removes the bases and substitutes them with hydrogen ions making the soil acidic and unsuitable for agriculture. Soil developed over sandstone are low in soluble bases and coarse in texture which facilitates leaching (Bardgett *et al.*, 1999). Parent material influence on soil properties tends to decrease with time as it is altered and climate becomes more important.

b. **Climate:** Soils tend to show a strong geographical correlation with climate, especially at the global scale. Energy and precipitation strongly influence physical and chemical reactions on parent material. Climate also determines vegetation cover which in turn influences soil development (Debosz *et al.,* 1999). Precipitation also affects horizon development factors like the translocation of dissolved ions through the soil. As time passes, climate tends to be a prime influence on soil properties while the influence of parent material is less (Voroney, 2006).

   Climate affects both vegetative production and the activity of organisms. Hot, dry desert regions have sparse vegetation and hence limited organic materials available for the soil (Bardgett *et al.,* 1999). The lack of precipitation inhibits chemical weathering leading to coarse textured soil in arid regions. Bacterial activity is limited by the cold temperatures in the tundra causing organic matter to build up. In the warm and wet tropics, bacterial activity proceeds at a rapid rate, thoroughly decomposing leaf litter. Under the lush tropical forest vegetation, available nutrients are rapidly taken back up by the trees. The high annual precipitation also flushes some organic material from the soil. These factors combine to create soils lacking much organic matter in their upper horizons (Voroney, 2006).

c. **Topography:** This has a significant impact on soil formation as it determines runoff of water, and its orientation affects microclimate which in turn affects vegetation (Winding *et al.,* 2005). For soil to form, the parent material needs to be relatively undisturbed so soil horizon processes can proceed. Water moving across the surface strips parent material away, impeding soil development. Water erosion is more effective on steeper, unvegetated slopes (Alef and Nannipieri, 1995).

2.2.2 Ecological Significance of Soil Microbes

Microorganisms play an essential role in maintaining soil fertility; cycling nutrients, influencing their availability, improving soil structure, supporting healthy plant growth, degrading organic pollutants. Some soil bacteria and fungi cause plant diseases, others are antagonistic to plant pathogens and invertebrate pests (Winding *et al.,* 2005). The rhizosphere provides a region of increased microbial activity in which certain groups of bacteria and fungi are more likely to proliferate than in the bulk soil (Debosz *et al.*, 1999).

Microorganisms such as some rhizosphere originate from the seed but majority are derived from the soil in which a plant is growing and they will be returned to the soil, therefore bulk soil and rhizosphere reciprocate impact on microbial communities. This is especially important in the case of plant pathogenic microorganisms and microbial antagonists to pests and pathogens (Alef, 1995).

Any one group of microbes is unlikely to perform with maximum efficiency under all circumstances, so genetically diverse populations are needed to provide continuation of important soil processes.

Certain soil microorganisms such as mycorrhizal fungi can also increase the availability of mineral nutrients (e.g. phosphorus) to plants. Other soil microorganisms can increase the amount of nutrients present in the soil. For instance, nitrogen fixing bacteria can transform nitrogen gas present in the soil into soluble nitrogenous compounds that plant roots can utilize for growth (Piao *et al*., 2001). These microorganisms, which improve the fertility status of the soil and contribute to plant growth have been termed “biofertilizers” and are receiving increased attention for use as microbial inoculants in agriculture (Anderson 1998). Similarly, other soil microorganisms have been found to produce compounds (such as

vitamins and plant hormones) that can improve plant health and contribute to higher crop yield. These microorganisms are called phytostimulators.

Other soil microorganisms produce compounds that stimulate the natural defense mechanisms of the plant and improve its resistance to pathogens. Collectively, these soil microorganisms have been termed "biopesticides" and represent an emerging and important alternative (i.e. biological control) to the use of chemical pesticides for the protection of crops against certain pathogens and pests (Piao *et al.,* 2001).

2.2.3 Soil as a Habitat for Microorganisms

Soil is one of the more complex and highly variable habitats on earth. Any organisms that make their home in soil have had to diverse multiple mechanisms to cope with variability in moisture, temperature, and chemical change so, as to survive, function and replicate. Within a distance of less than one millimeter (<1mm), conditions can vary from acid to base, from wet to dry, from aerobic to anaerobic, from reduced to oxidized, and from nutrient-rich to nutrient poor (Atlas and Bartha, 1998). Along with spatial variability there is variability over time, so organisms living in soil must be able to adapt rapidly to different and changing conditions. Variations in the physical and chemical properties of the soil are therefore important determinants of the presence and persistence of soil biota.

The physical and chemical characteristics of a soil are different in different parts of the soil profile. Generally, the upper layers of a soil and root have more organic matter than the lower layers. The origin of the soil determines the way the soil is packed, creating spaces and surfaces that are either accessible or inaccessible to soil organisms (Wardle, 2004)

Roots can alter the soil environment for living organisms and play an important role in supplying nutrients for organisms that live around them. Roots are dynamic structures,

especially when young, releasing carbon compound that can be readily used as an energy and carbon source by many soil organisms. However, over time roots change and older roots have different surface properties and releases different compounds to younger roots. As roots age, the outer layers of the roots die, providing a source of organic material as well as a habitat for soil organism (Martius *et al.*, 2001).

Soil is considered as a continually changing and complex environment for organisms. Part of this complexity arises because the organisms themselves contribute to some of the changes that occur within the soil (Wardle, 2002).

The habitat available to a soil organism depends on its size: the soil environment of bacteria will be quite different to that of an earthworm for a bacterium, important aspects of the soil environment include:

Microhabitat (structure)

Surface of soil particles, pore space, roots, dead organic matter (plant, animals or microbial), water films.

Physical and chemical characteristics

The change of soil particles (positive or negative), degree of aeration, pH, salinity, temperature, nutrients.

Biological aspects

Other living organisms (including roots)

Bacteria are usually attached to the surfaces within soil pore or fragments of organic matter. They may attach themselves using flagella or fine hair-like fribrillae, although not all bacteria have these structures. Another method of attachment occurs through the production of

polysaccharide gums that are released through the cell wall of some bacteria. Certain fungi also produce gums that actually help bacteria attach to soil particles (Savonen, 1997).

Fungi experience a similar soil environment to that of bacteria, but the scale is greater. Fungi spread much further through the soil than bacteria do and will encounter a greater variety of soil environments protozoa and mites both feed on bacteria but only the protozoa are small enough to enter some soil pores (Williams *et al.,* 2002).

While the soil environment obviously has a great effect on soil organisms they in turn may affect their physical environment. The breakdown of organic matter by soil organisms changes the structure of the soil, which leads to changes in the habitats available for soil organisms (Wardle, 2004).

Soil habitat differ greatly depending on land use. For a similar soil type within the same climatic zone, a forest soil will generally have a greater diversity of habitats for soil organisms than a cultivated agricultural soil. These differences are primarily associated with a greater diversity of plant species and heterogeneity of the soil itself (Bonkowski, 2004).

The number of soil organisms varies greatly between the surface and very deep layers in the soil profile. The main reason for this is because the supply of plant organic matter that is essential for many soil organisms is almost absent lower in the soil profile (Piao *et al.,* 2001).

2.3 Pesticides

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others.

Pesticide is a chemical used to prevent, destroy, or repel pests (Gill *et al.,* 2014). It is also defined as a chemical that is used to kill animals or insects that damage plants or crops.

Pesticide can also be said to be a chemical or biological substance designed to kill or retard the growth of pests that damage or interfere with the growth of crops, shrubs, trees, timber and other vegetation, desired by humans. Practically all chemical pesticides, however are poisons and pose long time danger to the environment and humans through their persistence in nature and body tissue. Most of the pesticides are non-specific, and may kill life forms that are harmless or useful.

2.3.1 History of Pesticides

One of the first pesticides was sulfur, used by the Chinese in around 1000BC to control bacteria and mold (fungus). Sulfur is still widely used today. For example, it is used in fungides to control diseases on both agricultural and ornamental plants and in wine industry, sulfur is used to control unwanted bacterial growth in empty wine barrels and is commonly added to wine to kill unwanted yeast (Bezbaruah and Saikia, 1990). The Chinese also pioneered the use of arsenic containing compounds to control insects.

Arsenic has a long history of use both as an insecticide and herbicide, and also as a medicine. Arsenic trioxide was used as a weed killer (herbicide) in the late 1800s, and lead arsenate, containing both lead and arsenic was used as an insecticide, particularly in orchards prior to the development of synthetic pesticides following world war II (Kamrin, 1997)

Plant – based Pesticides

Plants have provided several other important nonsynthetic pesticides. In the late 1600s nicotine, an extract from tobacco leaves, was recognized as a patent insecticide and is now a limited use as a pesticide. Another group of nonsynthetic insecticides is pyrethrums, which are harvested and refined from chrysanthemums. The strychnine tree, Nux vonica, contain strychnine used to kill rodents. Plant extracts are useful for controlling pests, but they are

often difficult to purify and produce in large quantities. Consequently, the modern use of plant-based pesticides did not significantly increase until advances were made in synthetic chemistry and pest biology.

Synthetic – Pesticides

Synthetic chemistry advanced rapidly in the 1930s and by the early 40s, a range of new pesticides had been developed, including organochlorine insecticides like Dichlorodiphenyltrichloroethane (DDT). In 1937 the first organophosphate compounds were synthesized by a German Chemist Paul Muller. These very potent compounds were kept secret during World War II and were originally developed as potential chemical warfare agents. After the war, these organophosphate compounds were re-purposed as insecticides, and many of the organophosphate insecticides continue to be used today.

Herbicides were developed during World War II in order to increase food production and create possible warfare agents. In 1946, the first commercial available chlorine-based herbicides were marketed to kill broadleaf plants. This class of compounds includes 2, 4 – D (2, 4 – Dichlo-ophenoxyacetic acid) and 2, 4, 5 –T (2, 4, 6 – Trichlorophenoxyacetic acid).

2.3.2 Classification of Pesticides

There are two types of pesticides classifications: chemical pesticides and biopesticides.

Chemical pesticides

They are further divided into four types based on their origin.

Organophosphate pesticides: These are the chemical substances which are produced due to reaction between phosphoric acid and alcohols. Although a few organophosphate (OP) formulation remain available for vector, their use has dramatically decreased because of

resistance to Op_s, the potential for non-target effects and the development of alternative products. Members of this group contain phosphorus in their molecules. Products currently labeled for vector control include naled, malathion, and some formulations of dursban (CICAD, 1999).

Organophosphate are considered by most to pose a greater human risk for pesticide applicators than other families of pesticides. This affects the nervous system by inhibiting the action of enzyme acetyl cholinesterase (AChE). It causes irreversible blockage leading to accumulation of the enzyme which results in over stimulation of muscles. These mainly include insecticides, herbicides etc. (CICAD, 2004).

Organochlorines (chlorinated hydrocarbons): this represent one of the first groups of pesticides synthesized, and include the well-known insecticide DDT. Most of other organochlorines used for arthropod control includes chlordane, dieldrin, and lindane.

These are endocrine disrupting agents which affect the hormonal systems of the body, acts as duplicates of the normal hormones and thus causing adverse health problems. They remain in environment for a long time by breaking down slowly and accumulating in the fat tissues of animal example DDT (Dicloro diphenyl trichloroethane) (CICAD, 2004).

Carbamates: These are chemically similar in structure to organophosphates(Op_s), but whereas OP_s are derivatives of phosphoric acid, carbamates are derivatives of carbamic acid. The bond formed for inhibition in carbonates is less durable and therefore is reversible (JMPR, 2004).

Pesticides in this group used for vector control include carbaryl (sevin) for dusting rodent burrows to control fleas, propoxur (Baygon) for use against insect pests and certain brands of bee and wasp control sprays.

Carbamates also pose a relatively high risk for human poisoning. Some carbamates are herbicides (Morals *et al.,* 2012).

Pyrethroids are synthetically produced molecules that are chemically similar to pyrethrins, a natural insecticides. Pyrethroids are not persistent. They break down quickly in sunlight, and are rarely present after few days. Most pyrethroids are synergized with Piperonyl butoxide (PBO) which acts as a synergist. Synergists are materials that are not necessarily pesticidal by themselves, but have the effect of increasing the toxicity of insecticides with which they are mixed. Without PBO, insects treated with the dose of most pyrethroids would be knocked down, but would eventually survive. Several generations of pyrethroids have been produced with the latest formulations being effective at extremely small doses (JMPR, 1995a).

Pyrethrins and pyrethroids are now among the most common public health pesticides in California, especially for the control of adult mosquitoes. Their use now far outstrips that of conventional synthetic pesticides such as organochlorines and organophosphates because of their ability to easily pass through the exoskeleton of insects. Few examples are deltamathrin, cypermethrin etc. (CICAD, 1999).

Pyrethroid are derivatives of ketoalcoholic esters of chrysanthemic and pyrethrioc acids, and are potent nuero poisons, endocrine disruptors and causes paralysis (CICAD, 2004).

Biopesticides

They are group of pesticides that are considered relatively non-toxic to humans and are also environmentally safe. The Environmental protection Agency (EPA) defines biopesticides as types of pesticides derived from natural materials like animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal application and are considered biopesticides. Biopesticides fall into three (3) major classes:

Microbials pesticides: these consists of a microorganisms (e.g bacterium, fungus, virus, nematode, or protozoan) as active ingredient. Microbial pesticides contain many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that control certain weeds, and other fungi that kill specific insect: (JMPR 2009b). The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis* (Bt). Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bt's control much larvae found on plants, other Bt's are specific for larvae of flies and mosquitoes. The target insect species are determined by whether, the particular Bt produces a protein that can bind to larval gut receptor, thereby causing the insect larvae to starve (JMPR, 2009b).

Biochemical pesticides: They are naturally occurring substances that control pests by non-toxic mechanisms. Biochemical pesticides include substances such as insect sex pheromones that interfere with mating, as well as various scented plant extracts that attract insect pest to traps (Moral *et al.,* 2012).

The action of biochemical pesticides is based on the interruption of natural growth processes of arthropods. They are not particularly selective among arthropod species, but generally have extremely low toxicity for vertebrates, including people. Insect growth regulations (IGRs) chitin inhibitors, plant growth regulators, enzymes bio repellents or attractants and, hormones, are chromosterilants are also included in this group (JMPR, 2009a).

Plant incorporated protectants (PIPs): These substances are produced by plants naturally, but the gene necessary for production of pesticides is introduced into the plant through genetic engineering. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plants own genetic material. Then the plant, instead of the Bt

bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by Environmental Health Criteria.

Table 2.2: Classification Based on the Pests they Control.

PESTICIDE	TARGET
Insecticides	They act especially on insecticide
Algaecides	Control or kill growth of algae
Herbicides	Control or kill weeds
Bactericides	Acts against bacteria
Fungicides	Acts against fungi
Rodenticides	Kills or prevents rodents e.g. rats
Larvicides	Inhibits growth of larvae
Repellents	They tend to repel pest by its tastes or small
Desiccants	They act on plants by drying their tissues
Ovicides	They inhibit the growth of eggs of insects and mites
Virucides	Acts against viruses
Molluscidies	They inhibit or kill molluses i.e snails that are disturbing the growth of plants or crops
Acaricides	They kill arachinds like mites
Nematicides	They tend to kill nematodes that acts as parasite on plants
Avicides	These are used to kill birds
Moth balls	These are used to stop any damage to cloths by moth larvae or molds
Lampricides	These are designed to target larvae of lampreys which are jawless fish like vertebrates in the river
Piscicides	They are substances that act against fishes

JMPR (2009a)

Though pesticides are designed to kill or inhibit organisms that cause damage to the crops or animals, they have harmful effects on other organisms that must not be effected and tend to pollute the environment. If used in high quantities they can be lethal sometimes. Biopesticides are used instead of chemical pesticides as the negative effects are low compared to chemical pesticides (Armstrong and Stile, 1994).

2.4 Fate of Pesticides in the Soil

Pesticides are important to the success of agriculture as well as being important in helping maintain good published. During the past two decades, however this increase has caused great concern over the presence of pesticides in the environment and the threat they may pose to wildlife and humans.

Although pesticides are indispensable in modern agriculture, their use and misuses can lead to serious water quality problems. Fish kills, reproductive failure of birds, and acute illnesses in people have all been attributed to the ingestion of pesticides or exposure to pesticides. Usually this is the result of misapplication, careless storage or careless disposal of pesticides and containers (Alef, 1995).

Once a pesticide is applied, several things may happen. It may be taken up by plants or ingested by animals, insects, worms or microorganisms in the soil, it may move downward in the soil and adhere to soil particles, or it may dissolve, it may volatilize, it may be broken down into less toxic compounds, it may be leached or moved out of the plant's root zone by rain or irrigation water, or it may be carried away by runoff water or erosion (Bishnu*et al.,* 2009).

2.4.1 Factors Affecting Fate of Pesticides

There are four factors which affect the fate of pesticides. They are as follows: properties of the pesticide, properties of the soil, conditions of the site, management practices.

2.4.2 Pesticide Properties

Pesticide properties which affect movement to groundwater include solubility, adsorption, volatility, and degradation.

Solubility: Chemicals which dissolve reality in water are said to be highly soluble. As water seeps downward through the soil, it carries with it water-soluble chemicals. This process is called leaching. Highly soluble pesticides, therefore have a tendency to be leached from the soil to groundwater.

Adsorption: Many pesticide do not leach because they are adsorbed or tightly held by soil particles. Adsorption depends not only on the chemical, but also on the soil type and amount of soil organic matter present (Amalin, 2009).

Volatility: Highly volatile chemicals are easily lost to the atmosphere, similar to the evaporation of water. If a pesticide is highly volatile and not very water soluble, it is likely to be lost to the atmosphere and less will be available for leaching to groundwater. Highly volatile compounds may become groundwater contaminants, however if they are highly soluble in water.

Degradation: Another chemical property affecting leaching potential is the pesticide's rate degradation in the soil. Pesticides are degraded or broken down into the chemical forms by sunlight, microorganisms in the soil, and a variety of chemical and physical properties. The longer the compound lasts before it is broken down, the longer it is subject to the forces of

leaching. Many chlorinated hydrocarbons are highly persistent in soil, but they have not been found in groundwater because of their low solubility and strong adsorption to soil particles.

2.4.3 Soil Properties

Soil properties affecting the movement of pesticides include soil texture, soil permeability, and organic matter content (Cooper, 1996).

Soil texture: This is determined by the relative proportions of sand, silt and clay. Texture affects movement of water through soil and therefore affects the movement of dissolved chemicals, such as pesticides. The coarser the soil, the faster the movement of the percolating water, and the less opportunity for dissolved chemicals.

Soil permeability: Soil permeability is a measure of how fast water can move downward through a particles soil. Water moves quickly through soils with high permeability. They also lose dissolved chemicals with the percolating water. In highly permeable soils, the timing and methods of pesticide application need to be carefully designed to minimize leaching losses.

Organic matter content: Soil organic matter influences how much water a soil can hold and how well it will be able to absorb pesticides. Increasing the soils organic content, through practices such as application of manure or plowing under cover crops, increases the soil's ability to hold both water and dissolved pesticides in the roots zone where they will be available to plants and to eventual degradation (Das and Mukherjee, 2008).

2.4.4 Site Conditions

The conditions of the site where a pesticide is applied can also affect the movement of the pesticide. Such conditions include the depth to groundwater, geologic conditions, and climate.

Depth to groundwater: The shallower the depth to groundwater, the less soil there will be to act as a filter. Also there will be fewer opportunities for degradation or adsorption of pesticides. Therefore, extra precautions need to be taken to protect groundwater in area that is to closer to the ground surface. In humid regions, groundwater may be only a few feet below the surface of the soil. If rainfall is high and soils are permeable, water carrying dissolved pesticides may take only a few days to percolate downward to ground water. In arid region, groundwater may be several hundred feet below the soil surface, and leaching of pesticides to groundwater may be a much slower process.

Geologic condition: In addition to depth to groundwater, it is important to look at the permeability of the geologic layers between the soil and groundwater. Highly permeable materials, such as gravel deposits, allow water and dissolved pesticides to freely percolate downward to groundwater layers of clay, on the other hand, are much less permeable and, therefore inhibit the movement of water. Groundwater quality is most vulnerable in areas where permeability of geologic layers is rapid.

Climate: Areas with high rates of rainfall or irrigation may have layer amounts of water percolating through the soil and therefore, are highly susceptible to leaching of pesticides, especially if the soils are highly permeable (Downing *et al.*, 2004).

2.4.5 Management Practice

Management practices include the method used to apply the pesticide and the rates and timing of application.

Application Methods: Another factor determining leaching potential is the way in which a pesticide is applied. Injection or incorporation into the soil, as in the case of nematicides, mates the pesticides most readily available for leaching. Most of the pesticides which have

been detected in groundwater are ones which are incorporated into the soil rather than being sprayed into growing crops (Rafatullah *et al*., 2021).

Pesticide rates and timing: The rate and timing of a pesticides application also are critical in determining whether it will leach to groundwater. The larger the amount used and the closer the time of application to a time of heavily rainfall or irrigation, the more likely that some pesticide will leach to groundwater. Particular care should be taken when practicing chemigation (the application of pesticides and fertilizers to agricultural crops with irrigation water) because of the risks of back-siphoning and leaching (Rafatullah *et al*., 2021).

2.4.6 Good Management Practices

Good management practices can help prevent unwanted pesticide movement. There are several good management practices to consider when working with pesticides. They are as follows: following pesticide label directions, mix pesticide and calibrate equipment accurately, avoid spills and back-siphoning, dispose of pesticide wastes and containers properly, eliminate unnecessary pesticide applications and use other pest control methods if possible, consider such factors as the weather, soil type, location, timing and application methods before applying pesticides, irrigate properly by controlling the quantity and timing of irrigation, and always maintain records of pesticide use (Hildebrandt *et al.*, 2001)

2.5 Effect of Pesticides on the Physicochemical Properties of Soil and Microorganisms

Due to continuous use of pesticides in agriculture, appreciable quantities of pesticides and their degraded products may accumulate in the ecosystem leading to serious problem to man and the environment (Eddleston, 2000).

Pesticides which enter the soil environment are subject to a variety of degradative processes. The overall degradation of a pesticide from soil results from a combination of mechanisms such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and surface runoff (Barcelo and Hennion, 1997). The degree to which each mechanism will contribute to the overall degradation of the pesticide is in turn dependent on the physicochemical properties of the pesticide (for example, water solubility, sorptive affinity), characteristics of the soil (. pH, organic matter content, microbial biomass, redox status), environment condition (for example, temperature, moisture) and management practices (for example, application rate, formulation type). Within each of these variables there are complex interactions and interdependencies which are difficult to quantify in situ (Barcelo and Hennion, 1997).

Soil properties potential influence the behaviour of pesticides in soils. Microorganisms are vital for soil fertility and for the degradation of organic matter and pollutants.

Microbial biomass is an important indicator of microbial activities and provides direct assessment of the linkage between microbial activities and the nutrient transformation and other ecological processes (Locke *et al.,* 1995). Generally, a decrease in soil respiration reflects the reduction in microbial biomass or increase in respiration implies the enhanced growth of bacterial population (Mathur, 1999).

Some microbial groups are capable of using applied pesticide as a source of energy and nutrient to multiply, whereas the pesticide may be toxic to other organisms (Lioney *et al.,* 1988). Likewise sometimes, application of pesticides reduces microbial diversity but increase functional diversity of microbial communities even demonstrate the tendency of reversible stimulatory/inhibitory effects on soil microorganisms (Muir, 2004).

Pesticides application may also inhibit or kill certain group of microorganism and outnumber other groups by releasing them from the competition. For instance, Liong *et al.,* (2001) reported that fungicides applications killed or inhibited the activity of certain fungi which led to a rapid flush of bacterial activity. Sometimes, initially microbial population is affected by pesticide application but with time after a period of acclimation, the population merely returns to normal or even increases (Pier *et al.*, 1998). This is an indication if changes in microbial catabolic capabilities that may be either due to induced pesticide degradation capabilities or due to a change within the microbial community.

2.5.1 Benefits of Pesticides

Improving productivity: Tremendous benefits have been derived from the use of pesticides in forestry, public health and the domestic sphere and of causes, in agriculture. This result has been achieved by the use of high-yield varieties of seeds, advanced irrigation technologies and agricultural chemicals (Waren, 1998). Pesticides have been on integral part of the process by reducing losses from the weeds diseases and insect pests that can markedly reduce the amount of harvestable produce. Environews Forum, stated that considerable economic losses would be suffered in yield and economic margin that result from pesticides use. Moreover, in the environment most pesticides undergo photochemical transformation to produce metabolites which are relatively non-toxic to both human beings and the environment (Kole and Bagchi, 1995).

Quality of food: It has been observed that a diet containing fresh fruits and vegetables far outweigh potential risks from eating very low residue of pesticides in crops (Brown, 2004). Increasing evidence shows that eating fruit and vegetable regularly reduces the risk of many cancers, high blood, heart diseases, diabetes, stroke and other chronic diseases.

Lewis *et al.* (2011) discussed the nutritional properties of apples and blueberries in the US diet and concluded that their high concentrations of antioxidants act as protectants against cancer and heart disease. Lewis attributed doubling in wild blueberry production and subsequent increase in consumption chiefly to herbicide use that improved weed control.

Decrease the cost of food: Because the use of pesticide improves crop yields, crop protection technologies also impact the cost of food. Without crop protection chemicals, food production would decline, many fruits and vegetables would be in short supply and prices would rise. Helping to keep food prices in check for the consumer is another large benefit of pesticides.

Consumer benefits: Pesticides allow consumers to consume high quality produce that is free of insect blemishes and insect contamination. Pesticides that reduce or eliminate insect damage allow the consumer to purchase high-quality produce free of insect fragments (Ross, 2005).

Vector disease control: They are most effectively tackled by killing the vectors. Insecticides are often the only practical way to control the insects that spread deadly diseases like malaria. Disease control strategies are crucially important also for livestock (Bhatia, 2004).

2.5.2 Other Benefits of Pesticides

Household pest control: Pesticides products are used to control termites roaches, ants, rats and other pests.

Industry and Infrastructure: Herbicides are used to control vegetation that clogs navigable and other waterway or threatens to obstruct highway, utility and railroad rights of way.

Recreation areas: Pesticides are used to protect and enhance lawns, gardens, public parks, playing field, lakes and ponds for public enjoyment.

Human health: Many agricultural commodities are vulnerable to attack by aflatoxins and insect control is necessary to prevent its passage from insect to plant. Aflatoxin, a carcinogen can cause liver and other cancers in humans, lower the body's normal immune response, and can impair growth in children. Crop rotation chemicals are used to control insect damage that leads to aflatoxin contamination.

2.6 Hazards of Pesticides

Impact on humans: Pesticides are hazardous to a person's health. Pesticides are stored in the colon where they slowly but surely poison the body (Behera and Singh, 1999). This might not be realized, but anyone eating organic apple for instance, is eating over 30 different pesticides that have been sprayed on the apple. Even if a piece of fruit such as apple is washed, there are still many pesticides lingering on it and they could seeped into the fruit or vegetable (Warren, 1998).

In some studies, pesticides have been linked to cancer, Alzhaimer's disease, and even birth defects. Pesticides also have the potential to harm the nervous system, the reproductive system, the reproductive system and the endocrine system. Pesticides can even be very harmful to fetuses because the chemicals can pass from the mother during pregnancy or if a woman nurses her child (Liroff, 2000). Although one piece of fruit would not kill, but if they build up in one's body, they can be potentially detrimental to the health and should be avoided as much as possible.

The high risk group exposed to pesticides include production workers, formulators, sprays, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be higher because the processes included are not risk free. In industrial settings, workers are at increased risk since they handle various toxic chemical including pesticides raw materials, toxic solvent and inert carrier (Hurley *et al.*, 1998)

Chronic effects of pesticide exposure may include adverse effects on neurological function, cancer, reproductive harm, reduced growth and development, and birth defects. Much of the evidence of chronic effects is based on studies of adult workers who are exposed to a mixture of chemicals every day, making it difficult to pinpoint specific pesticides. The effects of individual pesticides during specific periods of fetal like, infancy, and early development have been studied in laboratory animals. Little research on the chronic effects of pesticides has been done directly on children, and even less on farm children (Murray and Lopez, 1996).

Neurological effects

In adults, exposure to pesticides and herbicides has been reported to confer an increased risk of early-onset.

Parkinson's disease is one of the long-term neurological problems which particularly shortened attention span and reduced coordination, have been reported in adults overexposed to organophophate pesticides (U.S. EPA, 1996). Studies have revealed that some pesticides appear to target the developing brain during the critical period of cell division, thereby leading to lasting behavioral aberrations. Not only do organophosphate pesticide interfere with a critical nerve impulse transmitter, but they also can permanently change the number of receptors in the brain for this neurotransmitter (U.S. EPA., 2001).

Subtle neurological effects may also occur in human children. A recent study compared preschool children in two farming communities in Mexico, one with heavy pesticide use and one with little or no pesticide use. The children living in the area with heavy pesticide use had strikingly impaired hand-eye coordination, decreased physical stamina, short-term memory impairment and difficulty drawing, compared with the less exposed children. Also it was noticed that exposed children had aggressive and anti-social behaviour compared to their less exposed counterparts (USGS, 1999).

Studies have shown that lead, a known neurotoxicant has lasting effects on attention span, intelligence, and behaviour. Infants and children are more susceptible to the toxic effects of lead than are adults, because their brains are still developing. Similarly, infants and children are also more susceptible to other neurotoxicants, including pesticides (Waskon, 1994).

Cancer

Examples of pesticides which are known carcinogens in animals and are still used around human today include pentachlorophenol, 1, 3-dichloropropene, and dichlorvos (WHO, 2001).

Studies of farm populations indicate that adults exposed to pesticides may be at increased risk for cancers of the lymphatics and blood, stomach, prostrate, tests, brain, and soft tissues (Liong *et al.,* 2001).

U.S. EPA (1996) observed in their study that children with leukemia were three to nine times more likely to have a parent who used pesticides in the home or garden during pregnancy or lactation.

Reproductive and Developmental Toxicity

Numerous pesticides are known or suspected reproductive toxicants. Examples include the fungicides benomyl and vinclozolin, as well as the fumigants methyl bromide and metam sodium (Tanabe *et al*., 2001).

People who live in agricultural regions or undergo occupational exposure to pesticides are at increased risk of a variety of adverse reproductive outcomes. An investigation done in California reported that maternal occupational exposure to pesticides was associated with more than a doubling of the risk of still-birth. Numerous types of birth defects, particularly limb-reduction defects have been associated with pesticide exposures in human studies.

Endocrine Disruption

Many currently used pesticides are now known to interfere with normal hormonal function in animals. For example, vinclozolin and iprodione, popular fungicides both break down into metabolite that interferes with testosterone and other androgens (Martens and Bremner, 2002).

Several organochlororine pesticide including DDT, methoxychlor, endosulfan and dicofol acts as an anti-estrogen, and are also toxic to the nervous system. Atrazine a popular herbicide can disrupt ovarian function, cause mammary (breast) tumors in animals, interfere with the binding of steroid homones and the breakdown pathway of estrogen (CICAD, 1999). Because the endocrine system in animals is almost identical with the human, meaning that the effect observed may be relevant to human health. Disruption of hormone function can permanently alter normal development of the fetus and child.

2.6.1 Impact on Environment

Pesticides can contaminate soil, water, turf and other vegetation. Apart from killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risk to non-target organisms.

Surface water contamination

Pesticides can reach surface water through runoff from treated plants and soil. Contamination of water by pesticides is widespread. The US Geological Survey (USGS1998) found that concentrations of insecticides in urban streams commonly exceeded guidelines for protection of aquatic life. According to USGS 1999, more pesticides are detected in urban streams than in agricultural streams.

The herbicides 2, 4-D, diuron and prometion, and the insecticides chlorpyifos and diazinon, all commonly used by urban homeowners and school districts, were among the 21 pesticides detected most often in surface and ground water across U.S.A (U.S Geological Survey, 1998). Trifluralin and 2, 4-D were found in water samples collected in 19 out of 20river basins studied (Bevans *et al.*, 1998).

Groundwater contamination

Groundwater pollution due to pesticides is a worldwide problem. According to USGS at least 143 different pesticides have been found in groundwater. Before the mid-1970s, it was through that soil acted as a protective filter that stopped pesticides from reaching groundwater. Studies have now shown that this is not the case (Waskon. 1994).

During one survey in India, 58% of dividing water sample drawn from various hand pumps and wells around Bhopal were contaminated with organochlorine pesticides above the EPA standards (Kole and Bagchi, 1995). Once groundwater is polluted with toxic chemicals, it may take many years for the contamination to dissipate or be cleaned up. Cleanup may also be very costly and complex, if not impossible (O' Neil and Raucher, 1998).

Soil contamination

Pesticides like carbonates, fungicides and some organophosphous insecticides can be moved from soil by runoff and leaching, thereby constituting a problem for the supply of drinking water to the population. The pesticides are retained by soils to different degrees depending on the interactions between soil and pesticides properties. The most influential soil characteristic is the organic matter content. The larger the organic matter content, the greater the adsorption of pesticides (Muir, 2004).

Heavy treatment of soil with pesticides can cause population of beneficial soil microorganisms to decline. Overuse of chemical fertilizers and pesticides have effects on the soil organisms that are similar to human overuse of antibiotics. Indiscriminate use of chemicals might work for a few years but after a while, there aren't enough beneficial soil organisms to hold onto the nutrients (Savonen, 1997). For instance, plant depends on a variety of soil micro-organisms to transform atmosphere nitrogen into nitrates which plants can use.

Herbicides like triclopyr inhibits soil bacteria that transform ammonia into nitrite (Pell *et al*., 1998), glyphosphate reduces the growth and activity of free-living nitrogen-fixing bacteria in soil (Santos and Flores, 1995). Mycorrhizal fungi grow with the roots of many plants and aids in nutrient uptake. These fungi can also be damaged by herbicides in the soil.

Contamination of air and non-target vegetation

Pesticide sprays can directly hit non-target vegetation, or can drift or volatilize from the treated area and contaminate air, and non-target plants. Some pesticides drift occurs during every application, even from ground equipment (Glotfelty and Schomburg, 1989). As much as 80% of an applied pesticide can be volatilized within a few days of application (Majewski and Capel, 1995).

According to the USGS, pesticides have been detected in the atmosphere in all sampled areas of USA (Savonen, 1997). Nearly every pesticide investigated have been detected in the rain, air, fog, or snow across USA at different time of the year (U.S. Geological Survey, 1999).

Herbicides are designed to kill plants, so it is not surprising that they can injure or kill desirable species if they are applied directly to such plants, or if they drift or volatilize onto them. In addition to killing non-target plants outright, pesticide exposure can cause sublethal

effects on plants. Phenoxy herbicides and 2, 4-D, can injure nearby trees and shrubs if they drift or volatilize onto leaves (Dreistadt *et al.,* 1994). Exposure to herbicide glyphosate can severely reduce seed quality (Locke *et al.*, 1995). It can also increase the susceptibility of certain plants to disease (Brammall and Higgins, 1998).

Non-target organisms

Pesticides are found as common contaminants in soil, air, water and on non-target organisms in the urban landscapes. Once in the environment, they can harm plants and animals ranging from beneficial soil microorganisms, insects, non-target plants, fish, birds, and other wildlife. Chloropyrifos a common contaminant or urban streams, is highly toxic to fish.

In addition to fish, other marine or freshwater animals are endangered by pesticide contamination. Exposure to great concentrations of persistent and toxic contaminants such as DDT (1, 1, 1-trichloro – 2, 2 – bisethane) and PCBs has adverse effects on reproductive and immunological functions in captive or wild aquatic mammals (Colborn and Smolen, 1996).

Herbicides on water have devastating effects on aquatic plants. Algae is a staple organism in the food chain of aquatic ecosystem. Impacts of the herbicides atrazine and alachlor on algae and diatoms in streams showed that even at fairly law levels, the chemical damaged cells, blocked photosynthesis, and stunted growth in varying ways.

The herbicide oxadiazon is also toxic to bees, which are pollinators. Non-target birds may also be killed if they ingest poisoned grains set out as bait for pigeons and rodents (U.S EPA, 1998). Exposure of eggs to 2, 4-D reduced successful hatching of chicken eggs and caused sterility in pheasant chicks (Colborn and Smolen, 1996).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Location and Collection

The soil used in this study was collected from a farmland at Nekede, Owerri West, Imo State Nigeria with no history of herbicide application to avoid enhanced degradation of the agrochemicals. The soil sample was collected from about 15-20cm below the soil surface using soil auger into a sterile polyethene bag and taken to the laboratory for analysis. Debris and other plant materials were removed manually before sieving the soil through a 2mm stainless steel sieve (Fulekar, 2014).

3.1.1 Agrochemicals Used: Agrochemicals used were bought from commercial suppliers in Owerri Main Market (Ekeonuwa) in Owerri town Imo State, Nigeria. Two different types of agrochemicals were used, which included herbicide (Atrazine and S- metolachor) and pesticide (Deltamethrin).

3.1.2 Sample Description of Agrochemicals

a) **Primextra**: This is a pre-emergence, broad spectrum herbicide for annual weed control maize and sorghum. It contains 290g/litre S-metolachor and 370g/litre Atrazine. The herbicide is a white to beige fluid paste (liquid), with purity of 94% and manufactured by Syngenta Australia PTY LTD.

Mode of action

Metachlor is mainly taken up through the roots of germinating seeds and seedlings. Weeds are therefore killed before emergence, at emergence or shortly after emergence. Root uptake is less pronounced than seed uptake. Atrazine can be taken up by roots and leaves of plants.

b) **Deltamethrin**: This is an insecticide belonging to the pyrethroid family. Pyrethroids are the man-made versions of pyrethrins, natural insecticide from chrysanthemum flowers, amber colour, manufactured by Shreeji Pharma International Vadodara India, and with the purity of 98%.

Mode of action

Deltamethrin can kill insects by direct contact or if they eat it. It disrupts their normal nervous system function giving a quick knockdown effect.

3.2 Experimental Method

3.2.1 Preparation of Soil Sample: Forty kilograms (40kg) of soil sample was weighed into a nursery bag. The quantity was repeated into thirty six (36) bags extra and labelled appropriately.

3.2.2 Preparation of Agrochemicals: Nine hundred milliliters (900ml) of water was used to dilute 100ml of herbicide giving a total volume of 1000ml (10%v/v) herbicide. The same thing was done for insecticide.

3.2.3 Spiking of Soil with Agro-Chemicals: The nursery bags containing the soil were labeled according to the agrochemicals impacted in each of them and the seeds to be planted in each.

Six (6) bags for each chemical and a particular type of seed as shown in the experimental design Table 3.1

Table 3.1: Experimental Design of Agrochemicals

Chemical + Seed	Code	Number of bags
Herbicide + Beans	HB	6
Herbicide + Maize	HC	6
Pesticide + Beans	PB	6
Pesticide + Maize	PC	6
Pesticide + Herbicide +Beans	PHB	6
Pesticide + Herbicide +Maize	PHC	6
Soil + Seed (Control)	SO	1

The spiking was done using the same concentration of agro-chemicals but different volumes in this order: 10ml, 20ml, 30ml, 40ml, and 50ml.

3.3 Analysis of Soil

3.3.1 Physiochemical Analysis

Determination of total nitrogen: Ten grams (10 g) of soil sample was accurately weighed (air-dried and ground to pass 0.5 mm sieve) into a dry 500 ml Macro-kjeldahl flask and 200 ml of distilled water was added. The flask was swirled for 5 minutes and allowed to stand for 30minutes.

One tablet of mercury catalyst and 10 g of K_2S0_4 were added. Thirty milliliters of concentrated H_2S0_4 was also added through an automatic pipette. The flask was cautiously heated at low temperature on the digestion stand. When the water has been removed, the heat was increased until the digest cleared. The mixture was boiled for 5hours. The heating was regulated during the boiling so the H_2S0_4 condenses about middle of the way up neck of the flask. The flask was allowed to cool and about 100 ml of water was slowly added to the flask.

The digest was carefully transferred into a clean macro kjeldahl flask (750ml capacity). All the sand particles were retained in the original digest because sand can cause severe bumping during kjeldahl distillation. The sand residues were washed with 50 ml of distilled water four times and an aliquot was transferred into the same flask. Fifty milliliters of H_3B0_3 indicator solution was put into a 500 ml Erlenmeyer's flask which was then placed under condenser of the distillation apparatus. Seven hundred milliliters Kjeldahl flask was attached to the distillation flask by opening the funnel stopcock. Distillation commenced immediately. The condenser was kept cool (below 30 ^{0}C) by allowing sufficient cold water to flow through and by regulating heat to minimize frothing and prevent suck back. About 150 ml distillate was collected and distillation was then stopped (Michalowski *et al.,* 2013).

Determination of exchangeable Potassium (K)

Fifty three point five grams (53.5 g) 0f ammonium chloride was dissolved in distilled water and the mixture was made up to 1 litre which gave us 1 M NH_4CL. Twelve point five grams (12.5 g) of soil was accurately weighed into a 250 ml beaker. Hundred milliliters (100 ml) of 1 M NH_4CL solution was added to it. The mixture was left overnight. The soil was filtered and leached to a total volume of 250 ml with the 1 M NH_4CL. Potassium in the extract was determined using a flame photometer (Wang and Scott 2001).

Determination of phosphorous

Thirty seven grams (37 g) of NH_4F was dissolved in distilled water and diluted to 1liter to get ammonium fluoride (NH_4F) and stored in polyethylene bottle. Also 20-22 ml of concentrated HCL was diluted to 500 ml with distilled water under fume hood. To get the extract of the solution, 15 ml of 1.0 ml NH4F and 25 ml of 0.5M HCL were added to 460 ml of distilled water. Three grams (3.0 g) of air dried soil (passed through 2mm sieve) was weighed into a 50 ml centrifuge tube and 20 ml of extraction solution was added. The mixture was shaken for 1minute on a mechanical shaker and the suspension was centrifuged at 2000 rpm for 15 minutes. It was then filtered through Whatman N0.42 filter paper and organic particles were washed into an acid-washed container. Phosphorous in the extract was determined.

All these soil analysis were carried out on the soil sample before planting and after planting (Wang and Scott 2001).

3.3.2 Microbiological Analysis:

3.3.2.1 Media Preparation

The media used were prepared according to the manufacturer's specification. The media used includes nutrient agar and sabourauds dextrose agar (SDA). The nutrient agar was used to

determine the total heterotrophic bacterial count, while sabourauds dextrose agar was used to determine total heterotrophic fungal count.

Preparation of Nutrient Agar

Twenty eight grams (28 g) of the nutrient agar powder was weighed and dispensed in 1000 ml of distilled water. The mixture was allowed to soak for ten minutes (10mins) and swirled thereafter. The mixture was sterilized at 121^{o}C for 15minutes. It was cooled to 47 ^{o}C, thoroughly swirled and then poured into plates.

Preparation of Sabourauds Dextrose Agar (SDA)

Sixty two grams (62 g) of the sabourauds dextrose agar powder was weighed and dispensed into 1000 ml of distilled water and was allowed to soak for 10minutes. The mixture was swirled and sterilized with an autoclaving at 121^{o}C for 15minutes and allowed to cool at 47^{o}C. The cooled media was thoroughly swirled and poured into plates.

3.4 Isolation of Organisms

One grams (1 g) of soil sample was weighed out and suspended in nine milliliters (9 ml) of water. The suspension was stirred and allowed to settle. Thus form the microbial community and ten-fold serial dilution was carried out on it by transferring 1ml of the suspension into9ml of sterile water in Bijou bottle. The bottle was properly shaken and from this, subsequent dilutions were performed unto 10^{-5}.

An aliquot portion (0.1m) of 10^{-5} dilution were inoculated onto freshly prepared surface-dried nutrient agar for bacterial count. Same aliquot portion of 10^{-5} were also inoculated into freshly prepared surface-dried sabourauds dextrose agar (SDA) for fungal count.

The inocula were spread plated with a sterile glass rod (dipped into alcohol and allowed to burn-off on contact with blue Bunsen burner flame) and incubated at 40^{o}C for 24hrs for heterotrophic bacterial count and 28^{o}C (room temperature) for 78hours for total heterotrophic fungal count (Cheesebrough, 2000). This analysis was carried out on the soil before and after planting.

Colonies formed on the plates after incubation and total heterotrophic bacterial and fungal counts were counted by dividing the Petri-dish into four quadrants at the reverse surface. Total colony forming unit per-gram was expressed as (Cfu/g) and the percentage occurrences of bacterial and fungal isolates were calculated before and after planting.

$$\text{Percentage occurrence (\%)} = \frac{\text{The number of a specie}}{\text{The total number of organisms isolated}} \times \frac{100}{1}$$

To identify the fungi, a drop of lactophenol cotton blue was dropped on a slide, a few strands of fungal hyphae were then taken with a sterile needle from the fungi growth and placed on the slide. It was gently covered with a cover slip, and viewed under the microscope with a high power objective (x100).

3.5 Preparation of Seeds (Sterilization) and Seeds Plant

Thirty milliliters (30 ml) of hypo bleach was diluted in 70 ml of water, the solution was used to sterilize the seeds (*Phaseolus vulgaris* and maize grains) by soaking them in the solution for 30mins. The soil samples were watered, and then the seeds were sieved out and planted.

3.6 Monitoring of Parameters

After one (1) week, the cultivars that grew were monitored by measuring the leaf length, stem length and stem girth with a measuring tape every week for six (6) weeks.

3.7 Harvesting of Plants (Determination of Wet and Dry Weight)

After six (6) weeks, the plants were uprooted. Plants from each soil sample were tied together and the sands on the roots were removed. The plants were then weighed using the weighing balance to determine wet weight. The plants were later spread on a board and air dried for one (1) week. The dried plants were also weighed to determine the dry weight.

3.8 Determination of Chlorophyl Content

Fifty milliliters (50 ml) ethanol was pipetted into beaker and fresh leaves of the plants were cut and soaked into the ethanol and left overnight. The supernatant was withdrawn and absorbance was measured at six hundred and sixty four nanometer (664 nm) and six hundred and forty eight nanometer (648 nm) in spectrophotometer.

CHAPTER FOUR

4.0 RESULTS

The findings in table 4.1 and 4.2 had it that *Bacillus* and *Enterococcus* species dominated the bacterial isolates while *Saccharomyces* and *Penicillium* species were the dominant in fungal isolates. *Fusarium, Mucor* and *Streptomyces* species were not detected upon analysis after planting.

Table 4.1: Distribution of bacterial and fungal isolates before planting

Sample code	Bacteria distribution	Fungal distribution
PC_{20}	*Bacillus* species	*Fusarium* sp, *Steptomyces* sp, *Penicillium* sp, *Saccharomyces* sp
PC_{10}	*Bacillus* species	*Aspergillus* sp, *Penicillius* sp, *Saccharomyces sp, Mucor sp*
PB_{50}	*Enterococcus* sp, *Micrococcus* species	*Penicillium* sp, *Streptomyces* sp, *Saccharomyces* sp
PB_{40}	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Aspergillus* sp, *Penicillium* sp, *Saccharomyces* sp
HC_{30}	*Micrococcus* sp, *Enterococcus* sp, *Bacillus* sp	*Aspergillus sp, Fusarium sp, Saccharomyces sp, Mucor sp*
PHB_{10}	*Staphylococcus* sp, *Bacillus* sp,	*Penicillum sp, Mucor sp, Saccharomyces sp*
PHC_{20}	*Bacillus* sp	*Penicillum* sp, *Fusarium* sp, *Mucor* sp, *Saccharomyces* sp
PHB_{20}	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Penicillum* sp., *Saccharomyces* sp, *Aspergillus* sp, *Fusarium* sp, *Mucor* sp,
HC_{40}	*Bacillus* sp, *Staphylococcus* sp	*Saccharomyces* sp, *Penicillum* sp, *Mucor* sp
PHC_{30}	*Micrococcus* sp, *Bacillus* sp	*Aspergillus* sp, *Saccharomyces* sp, *Fusarium* sp, *Mucor* sp
PC_{50}	*Bacillus* sp, *Staphylococcus* sp *Saccharomyces* sp	*Penicillum* sp, *Mucor* sp, *Saccharomyces* sp, *Streptomyces* sp, *Aspergillus* sp, *Fusarium* sp
PHB_{30}	*Staphylococcus* sp, *Bacillus* sp *Enterococcus* sp	*Fusarium* sp, *Penicillum* sp, *Saccharomyces* sp, *Mucor* sp
PB_{30}	*Bacillus* sp, *Micrococcus* sp *Enterococcus* sp	*Aspergillus* sp, *Penicillium* sp, *Saccharomyces* sp, *Mucor* sp
HC_{20}	*Micrococcus* sp, *Enterococcus* sp, *Bacillus* sp	*Penicillum* sp, *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp

SO_C	*Micrococcus* sp, *Staphylococcus* sp, *Bacillus* sp	*Aspergillus sp, Penicillium* sp, *Mucor, Saccharomyces* sp
PHC_{10}	*Enterococcus* sp, *Bacillus* sp	*Saccharomyces* sp, *Penicillum* sp, *Geotrichum candidium*
HB_{50}	*Staphylococcus* sp, *Bacillus* sp	*Fusarium* sp, *Penicillum* sp, *Geotrichum candidium*
HC_{50}	*Micrococcus* species	*Aspergillus* sp, *Penicillum* sp, *Saccharomyces* sp
HC_{10}	*Staphylocuccus* sp, *Bacillus* sp, *Micrococcus* sp, *Enterococcus* sp	*Geotrichum candidium, Saccharomyces* sp
PC_{40}	*Bacillus* species	*Geotrichum* sp, *Saccharomyces* sp, *Fusarium* sp
PHB_{40}	*Enterococcus* sp, *Bacillus* sp	*Penicillum* sp, *Saccharomyces* sp, *Mucor* sp
HB_{10}	*Micrococcus* sp	*Penicillium sp, Saccharomyces sp, Geotrichum candidium, Mucor sp*
HB_{40}	*Bacillus sp, Staphylococcus sp, Micrococcus sp*	*Penicillum sp, Saccharomyces sp*
PHB_{50}	*Micrococcus sp, Bacillus sp, Staphylococcus sp, Enterococcus sp*	*Saccharomyces* sp, *Penicillum* sp
PC_{30}	*Bacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Enterococcus* sp,	*Aspergillus* sp, *Fusarium* sp, *Mucor* sp
PH_{20}	*Bacillus* sp, *Micrococcus* sp	*Saccharomyces* sp, *Mucor* sp, *Fusarium* sp
PHC_{50}	*Micrococuccus* sp, *Enterococcus* sp	*Penicillum* sp, *Saccharomyces* sp
HB_{20}	*Bacillus* sp	*Saccharomyces* sp
HB_{30}	*Staphylococcus* species	*Geotrichum candidium, Saccharomyces* sp

Table 4.2: Distribution of bacterial and fungal isolates after planting

Sample code	Bacterial distribution	Fungal distribution
PHC_{40}	*Staphylococcus* sp, *Enterococcus* sp	*Penicllium* species
H_{20}	*Staphylococcus* sp	*Penicillum* sp, *Saccharomyces* sp, *Yeast*
PC_{50}	*Enterococcus* sp, *Bacillus* species	*Penicillum* sp, *Saccharomyces* sp
PHC_{50}	*Enterococcus* sp, *Bacillus* sp	*Penicillum* species
HC_{20}	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Saccharomyces* sp, *Penicillum* sp
PB_{10}	*Enterococcus* sp, *Staphylococcus*sp	*Penicillum* sp, *Saccharomyces* sp
PHB_{40}	*Enterococcus* sp, *Staphylococcus*sp, *Bacillus* sp	*Penicillium* species
HB_{50}	*Enterococcus species*	*Saccharomyces* sp, *Penicillium* sp.
HC_{50}	*Micrococcus* sp, *Staphylococcus* sp	*Saccharomyces sp, Penicillum* sp.
PC_{40}	*Micrococcus sp, Enterococcus* sp	*Saccharomyces* sp *Aspergillus sp, Penicillium* sp, *Saccharomyces* sp
HP_{30}	*Staphylococcus* sp, *Micrococcus* sp,	*Saccharomyces* sp
HC_{30}	*Bacillus* sp, *Enterococus* sp	*Saccharomyces* species, *Penicillium* sp
PHB_{30}	*Enterococcus* sp, *Bacillus* sp	*Saccharomyces* sp, *Geotrichum* sp
PC_{20}	*Enterococcus* species	*Penicillum* sp, *Saccharomyces* sp, *Geotrichum* sp
HB_{10}	*Micrococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Penicillium* species
PC_{30}	*Bacillus* species	*Saccharomyces* sp, *Penicillium* sp.
PHB_{50}	*Enterococcus* species	*Penicillium* sp, *Geotrichum* sp
PHB_{20}	*Bacillus* sp, *Enterococcus* sp	*Saccharomyces* sp,
PC_{10}	*Staphylococcus* sp, *Bacillus* sp,	*Saccharomyces* species

	Enterococcus sp	
HC_{40}	*Bacillus* sp, *Enterococcus* sp	*Saccharomyces* species
HC_{10}	*Bacillus* sp, *Enterococcus* sp	*Saccharomyces* sp, *Yeast*,
HB_{40}	*Enterococcus* sp, *Staphylococcus* sp	*Saccharomyces* species
PHC_{30}	*Bacillus* sp, *Enterococcus* sp	*Saccharomyces* sp, *Yeast*
SO_C	*Enterococcus* sp, *Bacillus* sp	*Saccharomyces* species
PB_{30}	*Bacillus* sp, *Enterococcus* sp,	*Saccharomyces* species
PHC_{20}	*Bacillus* sp, *Enterococcus* sp,	*Saccharomyces* species
PB_{40}	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Yeast*,*Saccharomyces* sp
PB_{50}	*Micrococcus* sp, *Bacillus* sp, *Enterococcus* sp	*Penicillium* species
PHC_{40}	*Staphylococcus* species	*Penicillium* species
PHB_{30}	*Micrococcus* species	*Saccharomyces* species
PC_{30}	*Bacillus* species	*Penicillium* species

4.1. Microbial community assessment before and after planting

Table 4.3 shows the percentage(%) occurrence of bacterial and fungal isolates before and after planting the cultivars. The bacterial population present in the soil include *Staphylococcus* sp 13(14 %), *Micrococcus* sp 16(19%), *Enterococcus* sp 16(19 %), and Bacillus sp 36(44%). On the other hand, fugal isolates obtained include, *Steptomyces* sp 3(2%), *Geotrichum* sp. 7(6 %), *Fusarium* sp 11(10 %), *Aspergillus* sp. 11(10%), *Mucor* sp 18(16 %), *Penicillium* sp 26(24%),*and Saccharomyces* sp 31(23%). After planting, the microbial community assessed revealed *Staphylococcus* sp 8(13%), *Micrococcus* sp 15(11 %),*Bacillus* sp 46(34%) and *Enterococcus* sp 54(40 %), while the fungal isolates include *Yeast*4(3%), *Geotrichum* sp 6(5%), *Aspergillus* sp 1(0.8%), *Penicillium* sp 41(34 %) and *Saccharomyces* sp 67(56 %).

Therefore specie of bacteria belonging to *Bacillus*, *Staphylococcus*, *Micrococcus* and *Enterococcus* were isolated ,whilespecies of fungi belonging to the genus *Fusarium, Saccharomyces, Aspergillus*, *Penicillium, Mucor*, *Streptomyces* and *Geotrichum* were isolated from both the treated and untreated soils before and after planting.

Table 4.3: Percentage (%) occurrence of bacterial and fungal isolates before and after planting Isolates

Organism	Before	After
Bacteria	*Staphylococcus* sp. 13(14)	*Micrococcus* sp. 15(11)
	Micrococcus sp. 16(19)	*Staphylococcus* sp. 18(13)
	Enterococcus sp. 16(19)	*Bacillus* sp. 46(34)
	Bacillus sp. 36(44)	*Enterococcus* sp. 54(40)
Fungi	*Streptomyces* sp. 3(2)	*Aspergillus* sp. 1(0.8)
	Geotrichum sp. 7(6)	Yeast 4(3)
	Aspergillus sp. 11(10)	*Geotrichum* sp. 6(5)
	Fusarium sp. 11(10)	*Penicillum* sp. 41(34)
	Mucor sp. 18(16)	*Saccharomyces* sp. 67(56)
	Penicillum sp. 26(24)	
	Saccharomyces sp. 31(23)	

Key: Numbers in parenthesis are percentage (%) of occurrence

Others are the total number each isolate occurred.

4.2 Effect of combination of insecticide and herbicide application on the total nitrogen (TN), available phosphorus (AP), and exchangeable potassium (EP) of soils cultivated with Pinto beans (*Phaseolus vulgaris*)and maize cultivar.

Table 4.4 shows the total nitrogen (TN), available phosphorus (AP), and exchangeable potassium (EP) before and after planting. The pesticide impacted soil samples with *Phaseolus vulgaris* decreased in the TN content after planting at 10-20% (v/v) concentration of the pesticide. However, an increase was recorded at higher concentration. Similarly, soil

cultivated with maize cultivar had increase in TN at 30-40%(v/v), while a decrease was recorded at 10 and 20% (v/v).

The *Phaseolus vulgaris* cultivated herbicide impacted soil recorded decrease in TN after cultivation at 10-30% concentration, while an increase was recorded at 40-50% (v/v) concentration. In the study with maize cultivated soil sample, it was observed that there was a general increase in TN after cultivation except at a concentration of 30% of the herbicide.

Mixtures of the pesticide and herbicide impacted in *Phaseolus vulgaris* cultivated soil recorded a general increase in TN for all the concentrations after cultivation. Similar findings were obtained for the maize cultivated soil except at concentration of 10-20%(v/v).

The AP of the impacted soil samples cultivated with *Phaseolus vulgaris* recorded a decrease in AP for the pesticides except at 30%; herbicides increased before planting except 10% and 20% (v/v), herbicides after except at 50% and mixtures of pesticide and herbicide except at 10%. On the other hand, the soil cultivated with maize recorded a general increase with the mixture of herbicide and pesticide impacted soil; herbicide impacted soil except at 20%; and the pesticides at a concentration of 40%.

For the application of pesticide in soil sample cultivated with *Phaseolus vulgaris*, there was a relative increase in the exchangeable potassium (EP) at all concentrations after planting except at a concentration of 30%. On the other hand, similar polluted soil cultivated with maize experienced a relative decrease in EP at most of the concentrations except at 10% and 30%. On herbicide impacted soil, there was a general decrease in the EP of the soil samples. Similar soil cultivated with maize had it that the EP generally increased at all concentrations except at 50%. The combination of the toxicants on *Phaseolus vulgaris* impacted soil, there was an increase in EP as concentration decrease till a concentration of 30% after which the concentration increased with increase in concentration after cultivation. Similar soil

cultivated with maize recorded an increase at concentration 10% and 40% and decrease in EP at other concentrations.

Table 4.4: Physicochemical properties of soil samples before and after planting

	Concentration (%)									
	Before					After				
Plant/parameter	10	20	30	40	50	10	20	30	40	50
Pesticide + Beans (PB)										
Total nitrogen (g/kg)	0.10	0.12	0.06	0.09	0.11	0.10	0.02	0.12	0.14	0.12
Exchange potassium(mg/kg)	0.23	0.26	0.17	0.25	0.14	0.21	0.22	0.27	0.10	0.07
Available phosphorous (mg/kg)	0.50	0.48	4.09	12.06	0.32	16.12	15.95	19.64	7.71	0.4
Pesticide + Maize (PC)										
Total nitrogen (g/kg)	0.10	0.13	0.07	0.13	0.09	0.09	0.10	0.09	0.14	0.07
Exchange potassium(mg/kg)	0.06	0.10	0.14	0.21	0.10	0.12	0.07	0.24	0.14	0.06
Available phosphorous (mg/kg)	2.31	6.36	2.56	9.24	1.67	7.12	6.13	10.15	6.92	7.80
Herbicide + Bean (HB)										
Total nitrogen (g/kg)	0.12	0.14	0.08	0.10	0.07	0.07	0.08	0.03	0.12	0.11
Exchange potassium(mg/kg)	0.27	0.23	0.20	0.23	0.08	0.09	0.06	0.04	0.17	0.06
Available phosphorous (mg/kg)	0.33	0.93	3.36	4.12	10.13	5.10	7.80	16.82	17.56	1.10
Herbicide + Maize (HC)										
Total nitrogen (g/kg)	0.06	0.06	0.10	0.11	0.10	0.11	0.07	0.07	0.25	0.11
Exchange potassium(mg/kg)	0.15	0.17	0.13	0.12	0.24	0.29	0.20	0.19	0.14	0.15
Available phosphorous (mg/kg)	5.16	12.82	0.69	1.84	2.24	18.11	6.00	15.70	16.35	14.93
Pesticide + Herbicide + Beans (PHB)										
Total nitrogen (g/kg)	0.10	0.12	0.12	0.13	0.07	0.09	0.13	0.13	0.16	0.11
Exchange potassium(mg/kg)	0.29	0.24	0.25	0.20	0.19	0.18	0.12	0.26	0.25	0.23
Available phosphorous (mg/kg)	16.24	0.21	9.61	12.11	15.23	7.65	2.51	15.75	16.70	15.40
Pesticide + Herbicide + Maize (PHC)										

Total nitrogen (g/kg)	0.09	0.09	0.10	0.12	0.10	0.08	0.09	0.13	0.17	0.15
Exchange potassium(mg/kg)	0.09	0.18	0.25	0.22	0.21	0.24	0.13	0.12	0.26	0.09
Available phosphorous (mg/kg)	1.76	6.76	9.63	7.37	2.55	17.79	8.67	18.24	14.36	14.06
Control (SO)										
Total nitrogen (g/kg)	0.10					0.09				
Exchange potassium(mg/kg)	0.12					0.16				
Available phosphorous (mg/kg)	0.16					19.10				

Key: PB – Pesticide + Beans (*Phaseolus vulgaris*)

PC – Pesticide + Maize

HB - Herbicide + Beans

HC - Herbicide + Maize

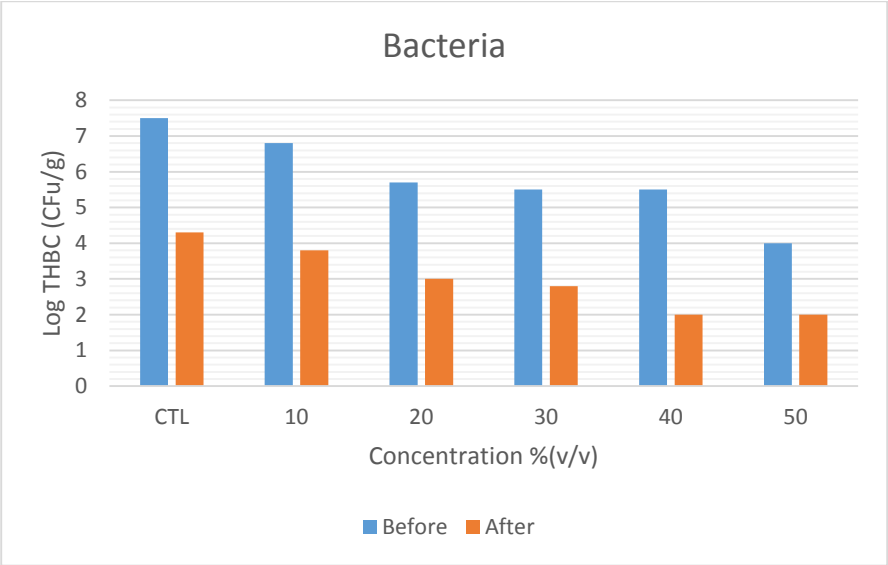
PHB – Pesticide + Herbicide + Beans

PHC - Pesticide + Herbicide + Maize

SO - Control

4.3 Effect of combination of insecticide and herbicide application on the microbial population of soils cultivated with *Phaseolus vulgaris* (beans)

The effect of combination of insecticide and herbicide application on the microbial population of soils cultivated with beans was also determined before and after planting. Apart from the control sample, there was a marked decrease in population of bacteria after cultivation. The THFC also followed similar trend except that there was an increase in fungal load at concentration 40-50% of the agrochemicals combined.



CTL – Control

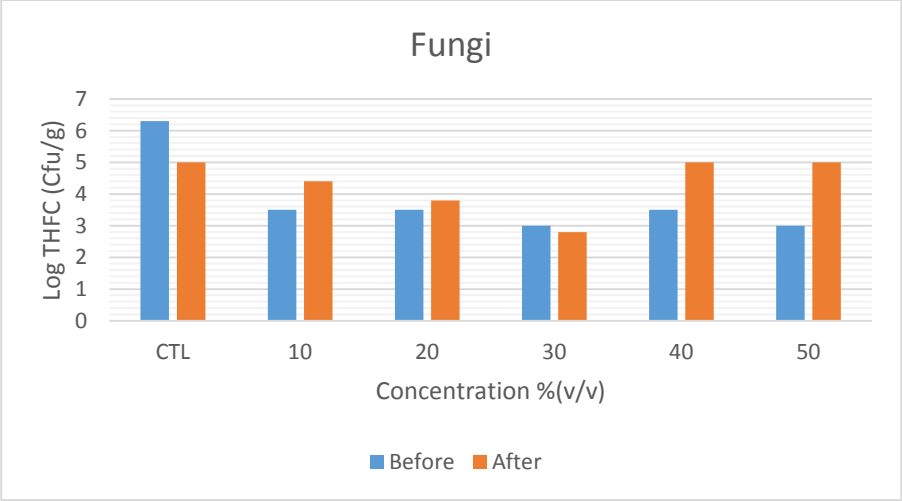
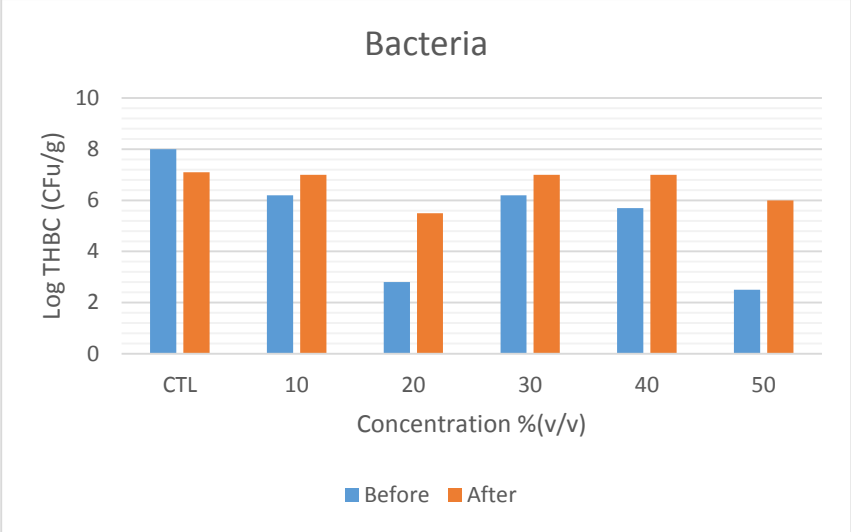


Fig 4.1: Effect of combination of Pesticide and herbicide on bacterial and fungal population of soil cultivated with *Phaseolus vulgaris* (beans).

4.4 Effect of combination of insecticide and herbicide application on the microbial population of soils cultivated with maize cultivar.

Figure 4.8 shows the effect of combination of insecticide and herbicide application on the microbial population of soils cultivated with maize. Findings indicated that the mixture of insecticide and herbicide resulted to a relative increase in THBC except at 20% and 50%. On the other hand, a relative decrease was observed in THFC.



CTL – Control

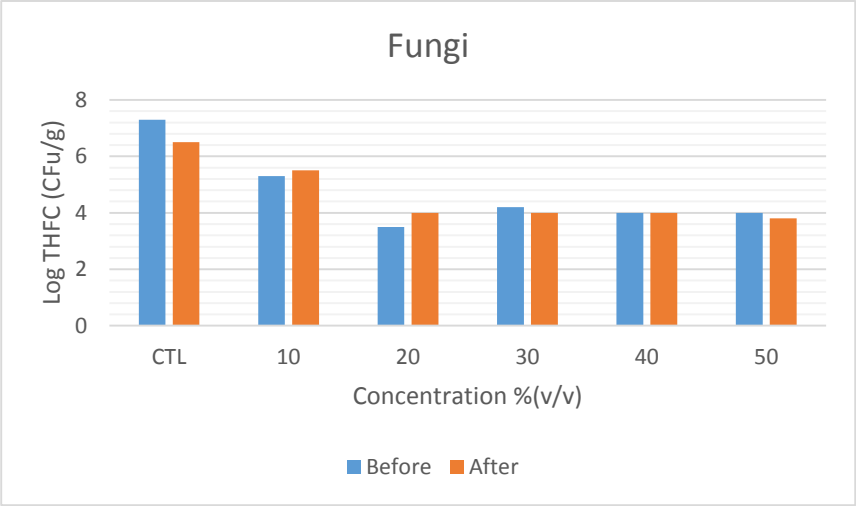


Fig 4.2 Effect of combination of pesticide and herbicide application on the microbial population of soils cultivated with maize cultivar.

4.5 Effect of the pesticide application on the plant growth parameters

Table 4.7 shows the effects of pesticide application on the growth parameters of maize cultivar over 6 weeks. It was observed that at week 1, there was an increased in seed dormancy caused by higher concentration of the pesticide at 40-50%. Despite the fact that the dormancy was overcome, the plants had an etiolated growth that was characterized by an increase in leave length with slender stems. The control plant had a normal growth with a great stem size and broad and short leaves. The growth pattern was seriously affected by increased concentration of the pesticide.

Table 4.8 shows the wet and dry weights of whole maize cultivars after harvest. The findings had it that the pesticide treated soils had negative effects on the weights and water contents of the whole plants as observed in the differences that occurred between the control and test experiments.

The effect of the pesticide on the chlorophyll content of the plant is shown in table 4.9. The chlorophyll content of the plant was also used as a yardstick to access the health of the plant. It was observed that higher chlorophyll content was observed in the control plant than all test plants at different concentrations. In addition, the chlorophyll content decreased with increase in concentration of the pesticide.

Table 4.5: Effect of pesticide on the growth of maize cultivar

		Pesticide [PC (%)]				
Weeks	**Parameter (cm)**	**0**	**10**	**30**	**40**	**50**
1	Leaf length	-	-	-	-	-
	Stem length	4	6	6	-	-
	Stem girth	0.4	0.6	0.4	-	-
2	Leaf length	6	11	13	-	-
	Stem length	5	7	7	4	4
	Stem girth	0.4	0.6	0.3	0.5	0.6
3	Leaf length	9	15	13	4	-
	Stem length	8	8	8	6	6
	Stem girth	1.0	1.0	1.8	0.7	0.5
4	Leaf length	10	20	20	13	4
	Stem length	8	5	10	8	8
	Stem girth	1.1	1.2	1.3	0.8	0.7
5	Leaf length	13	21	20	15	14
	Stem length	10	9	13	13	9
	Stem girth	1.6	1.6	1.6	1.6	1.1
6	Leaf length	19	26	28	28	17
	Stem length	10	10	14	13	19
	Stem girth	1.6	1.3	1.2	1.1	1.3

Key: 0 – Control (SO)

Table 4.6: Wet and dry weight of whole plants in pesticide impacted soil of maize (PC) sample

PC	Dry weight (gm)	Wet weight (gm)	Water content (gm)
0	11.46	20.54	9.08
10	5.35	13.79	7.94
30	1.19	9.63	7.94
40	3.96	10.30	6.42
50	4.44	10.77	6.33

KEYS: Control – (0) , PC – Pesticide maize

Table 4.7: Chlorophyl content of the leafs of maize plants in pesticide impacted soil sample (PC)

Sample	Absorbance(nm)	
PC	**664**	**648**
0	2.032	2.187
10	1.834	2.038
30	1.064	1.025
40	0.717	1.026
50	0.477	0.371

Key: 0 -Control

PC – Pesticide in maize cultivar

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others (Aktar *et al*., 2009). The results obtained from this work involves the use of insecticides and herbicides.

In agriculture and in control of diseases, man used a greater number of different chemicals. Among others, insecticides and herbicides are commonly encountered. Agrochemicals are the major soil, water and air pollutant and as a result, there is growing concern throughout the world at the way man is damaging his environment by injudicious use of pesticide to overcome problem of controlling insects, diseases, weeds and so on (Zhao *et al*., 2013). The microbes play an important role in the soil ecosystem, and their functions are very crucial in nutrient cycling and decomposition (Lorenzo *et al*., 2001).The increased use of pesticides in agricultural soils causes the contamination of the soil with toxic chemicals (Muñoz-Leoz *et al*., 2013). On the other hand, herbicides have the tendency to affect non-target plants at particular thresholds.

Despite this, some organisms have the capacity to resist concentrations of pesticides to particular thresholds. Moulds, yeasts and certain bacteria are dominant with this trait (Liliane and Charles, 2020). In this work, after planting, the microbial community assessed revealed *Enterococcus* sp 54(40%), *Staphylococcus* sp 8(13%), *Bacillus* sp 46(34%) and *Micrococcus* sp 15(11%) while the fungal isolates include *Penicillium* sp 41(34%), *Saccharomyces* sp 67(56%), Yeast 4(3%), *Geotrichum* sp 6(5%) and *Aspergillus* sp 1(0.8%). *Fusarium, Mucor*

and *Streptomyces* species were not detected upon analysis after planting which indicated their inability to resist concentrations of the insecticides and herbicides used.

The ability to resist concentration of agrochemicals is usually an adaptive characteristics and gives these group of microorganisms the ability to degrade the pesticides (Arisoy and Bull, 1998).

This research demonstrated that agrochemicals has significant effects on the microbial population, soil nutrients, as well as the survival of plants in the polluted soil. The soil quality was also affected as indicated in the physicochemical parameters. Ubuoh *et al.*(2012) reported that the addition of volumes of pesticides to soil samples reduced the microbial diversity drastically and proposes that this would invariably reduce the action of bacteria in the soil that might lead to decline in soil fertility in the farmland. In addition, previous researchers reported that the persistence of agrochemicals in soil loads to chemical degradation of the soil (Kamrin, 1997; Gupta, 2001).

In addition, agricultural runoff often contains developed levels of heavy metals from fertilizers and other agricultural chemicals applied to the fields (Gill and Garg). These chemicals are carried with rainfall runoff into rivers and streams, reservoirs, polluting water bodies and modifying aquatic habitats.

The direct effects of pesticides can be short; obvious in the first season after application of the pesticides or long term; if repeated additions have taken place. Indirect effects are usually long-term; takes more than one season to develop, and are due to changes in pH or changes in productivity, residue inputs and soil organic matter levels (Bune-mann and McNeill, 2004). These effects become important in agriculture when nutrient availability to plants and crop productivity are changed due to the effect.

A considerable amount of the applied pesticides frequently ends up in the soil, where it can undergo biological and physicochemical transformations. Once in the soil, microbial degradation is the main route of pesticide removal (Bending *et al*., 2006).Depending on several factors (for example, pesticide composition, soil type, soil physicochemical and biological properties), most pesticides frequently have slow rates of degradation in the soil environment (Fenner *et al*, 2013). Consequently, repeated application of pesticides can ultimately lead to their accumulation at concentrations detrimental to soil microorganisms (Munier-Lamy and Borde, 2000; Rice *et al*., 2002).

The fate of pesticides in the soil and the transport processes depend on the cumulative effects of the pesticide's characteristics (for example, absorptivity, solubility, volatility and degradation rate), the soil's characteristics (for example, texture and organic matter), the application methods used namely aerial or ground and the site conditions (e.g., topography, weather and irrigation) (Bhandari, 2014).

5.2 CONCLUSION AND RECOMMENDATION

It is then concluded that in spite of the importance of pesticide, used in agriculture, excessive use of pesticide can lead to soil degradation. Although in tropical soil, continuity of pesticide in soil and their effect on density and non-target soil organisms is minimum at a normal agricultural dose, their effect is obvious at population metabolism, with change in physiological and biochemical responses (Behera and Mishra, 2001). This implies that at doses higher than prescribed dose, the balance of the soil sub-system is altered. In other words, mineralization and humification processes would be very low leading to low nutritional values, hence poor crop yield that may lead to hunger and starvation.

Based on the conclusion, to minimize damage due to pesticide application to soil ecosystems, the following precautionary measures farmers should be properly guided on the use of agrochemicals in agriculture.

It is also recommended that a socio-ecological studies should be carried out in communities where there has been regular use of these chemicals. In addition, a molecular study should be carried out to discover how some microorganisms resist thresholds of pesticides that are toxic to other isolates. These will help to strengthen knowledge about the spectrum of activities of pesticide application in soils.

REFERENCES

Aktar, M.W., Sengupta, D. and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology.* **2**(1):1-12.

Alef, K. (1995). *Methods in Applied Soil Microbiology and Biochemistry.* Brace and Company Publishers. Academic Press London P 349

Alef, K. And Nanniperi, P. (1995). *Methods in Applied Soil Microbiology and Biochemistry (eds)*. Academic Press London, P 576.

Anderson, S. J. (1998). Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. *Science of The Total Environment Vol.***5**: 458-460.

Arisoy, M. and Bull, J. (1998). Biodegradation of chlorinated organic compounds by white-fungi. *Environmental Contamination of Tellurol.***60**: 875-876.

Armstrong, D. and Stile, M.E. (1994). A review of pesticide effect on microorganisms and microbial processes related to soil fertility. *Journal of Production Agriculture* **2**(1): 14-23

Barcelo, D. and Hennion, M.C. (1997). *Trace Determination of Pesticides and their Degradation Products in Water*. Elsevier, Amsterdam: 3

Bardgett, R. D., Roger, D. L., Phil, J. H., and Steve, C. J. (1999). Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Original Research Article,***31** (7): 1021-1030.

Behera, B. and Singh, S. G. (1999). Studies on Weed Management in Monsoon Season Crop of Tomato. *Indian Journal of Weed Science.***31** (1-2):67.

Behera, N. and Mishra, P. C. (2001). *Insecticide Pollution and Soil Fertility. Soil Pollution and Soil Organisms (ed.)*. Ashish Publishing House, Punjabi Bagh, New Delhi, pp. 63 – 83.

Bending, G. D., Lincoln, S. D., Edmondson, and R. N. (2006). Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. *Environ Pollut.***139**:279–87.

Bevans, H. E., Lico M. S. and Lawrence, S. J. (1998). *Water quality in California.* U.S. Geological Survey Circular, 1170.

Bhandari, G. (2014). An Overview of Agrochemicals and Their Effects on Environment in Nepal. Applied Ecology and Environmental Sciences, 2(2), 66-73.

Bhatia, R. (2004) *Agrochemicals as environmental pollutant.* In control of pesticide applications and residues in food. A guide and Directory.

Bishnu, A. K. Chakrabarti, A. Chakraborty and T. Saha, (2009). Pesticide residue level in tea ecosystems of Hill and Dooars regions of West Bengal, *India Environ. Monit. Assess,***149** (1-4): 414-457.

Bonkwoski, M. (2004). *Protozoa and plant growth.* The microbial loop in soil revisited.

Braimoh, A. K., Stein, A. and L. G. Velk Paul, (2005) *Identification and Mapping of Association among Soil Science* **170** (2): 137-148.

Bramnall R. A. and Higgins, V. J. (1998) *Microbial biomass in soil measurement and turnover.* In Soil Biochemistry, pp: 415-471.

Cheng, B. (1999). Identifying the characteristics of organic soil amendments that suppress soil borne plant diseases. *Soil Biochem*, **42** (2): 136-144.

Chessebrough, M. (2000). *"Medical Laboratory Manual for Tropical Countries".* Volume 3: Microbiology Tropical Health Technology, 14 Bevills Close, Doddington Cambridgeshire, England.

Colban, S. and Smolen, K. (1996). Effects of biofertilizer containing N-fixer, P and K Solubilizers and fungi on maize growth: a greenhouse trial. *Geoderma*; **125** (1): 155-166.

Concise International Chemical Assessment Document (CICAD), (1999). *Biphenyl*. Geneva, International Programme on Chemical Safety, 37pp.

Concise International Chemical Assessment Document (CICAD), (2004). Hydrogen cyanide and cyanides: *Human health aspects.* Geneva, International Programme on Chemical Safety 67pp.

Cooper, J. (1996). "Influence of pesticides on soil microbes and soil properties". In pesticides and their effect on soils and water, *ASA special publication***8**: 95-108.

Czaja K, Strucinski P., Korcz W., Minorczyk M., and Hernik A. (2020). Alternative toxicological methods for establishing residue definitions applied for dietary risk assessment of pesticides in the European Union food and chemical toxicology 137, 111120.

Das, V. and Mukherjee, D. (2008). Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. *Boil Fert Soils,***28**: 371-376.

Debosz, K. Ramussen P. H. and Pederson A. E. (1999). Temporal variations in microbial biomass carbon and cellulolytic enzyme activity in arable soils: effects of organic matter. *Appl. soil ecology*, **13** (3): 209-218.

Downing, J., Brar, B. and Dillon, N. (2004). Soil organic matter dynamics as affected by long-term use of organic and inorganic fertilizers under maize-wheat cropping system. *Nutr. Cycl Agroecosys* **81**: 59-69.

Dreistadt, S. H., Clark, J. K. and Flint, M. L. (1994). *Pest of landscape trees and shrubs.* An integrated pest management guide. University of California Division of Agriculture and Natural Resources. Publication 3359.

Dwivedi, S. K., Swarup, D., Sahadeb Dey and Patra, R. C. (2001). Lead poisoning in cattle near primary lead-zinc smelter in India. *Veterinary and human toxicology* **43**(2); 93-4

Eddleston, M. (2000). Patterns and problems of deliberate self-poisoning in the developing world. *QJ Med* **93**:715-31.

Fenner K., Canonica S., Wacket L. P and Elsner M. (2013). Evaluating pesticide degradation in the environment: blind spots and emerging opportunities **341**(6147) 732 – 758.

Fulekar, M. H. (2014). Rhizosphere Bioremediation of Pesticide by microbial consortium and potential microorganism. *Int. J. Cur. Microbiol. App. Sci.* **3**(7) 235-248.

Gill H. K and Garg H. (2014). Pesticide: Environmental impact and management strategies **8**:10,57399 - 5772

Glotfelty D. E. and Schomburg C. J. (1989). *Volatilization of pesticides form soil in reactions and movements of organic chemicals in soil*. Soil science society of America special Pub.

Gupta, P. K. and Mishra, P. C. (2001). *Pesticide Production in India.* An overview. Soil Pollution and Soil Organisms. Ashish Publishing House, Punjabi Bagh, New Delhi, pp.1-16.

Hildebrandt, J. A, Mcconnell, L. L; Rice, C. P. and Baker, J. E. (2001). Wet deposition and air-water gas exchange of currently used pesticides to a subestuary of the Chesapeake Bay. *Environ Sci. Tech***34**: 1462-1468.

Hurley, P. M., Hill, R. N. and Whiting, R. J. (1998). Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumours in rodents. *Environ Health Perspect* **106**:437.

Jang J. J. and Scott A. D. (2001). Determination of exchangeable potassium in soil using ion selective electrode in soil suspensions. European journal of soil science **52**: 143 – 150.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (2005a). Pesticide residues in food. Report 2-3. *Update on the revision of principles and methods for risk assessment of chemicals in food,* **223**: 265-269.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (2009b). Mesotrione. *Toxicological evaluation monograph*, WHO. pp. 242-305.

Joergenson, R. G. (1995). Microbial biomass. *Methods in applied soil microbiology and biochemistry academic press*, London, pp: 382-386.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR), (2004). Pesticide residues in food. Report 4.27 spices. *FAO plant production and protection paper Rome* 2004. **178**: 224

Kamrin, M.N. (1997). Effects of Longterm Herbicide Application on Soil Processes. *Environmental Quality*.**16**:257.

Karnrin A. M. (1997). Pesticide profiles: *Toxicity, environmental impact, and fate*. 704.

Khan, S., Cao, Q., Hesham, A.B., Xia, Y. and He, J. (2007). Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. *J.environ. Sci.,* **19**:834-840.

Khan, S., Hesham, A. L., Qiao, M., Rehman, S. and He, J. (2010). Effects of Cd and Pb on soil microbial community structure and activities. *Environ. Sci. Pollut.Res.,***17**: 288-296.

Kole, R. K. and Bagchi, M. M. (1995). Pesticide residues in the aquatic environment and their possible ecological hazards. *J Inland Fish Soc. Indian* **27**(2): 79-87

Lewis, M. A., Wang, J., Dai, J., and Chen, R. (2011). Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long term application of organic amendment and mineral fertilizer. *J. Soil sediment***11**:271-280.

Liliane T.N. and Charles T. N. (2020). Factors affecting yield of crops

Lioney, J., Beaudette, L., Hart, L. A. and Moutoglis, M. (1988) Methods of studying soil microbial diversity. *J. Microbial Methods***58**:169-88.

Liong, P. C., Hamzah, W. P. and Murugan, V. (2001). Toxicity of some pesticides towards freshwater fishes. *Malaysian Agric. J.***54**(3): 147-156.

Liroff, R. A. (2000). Balancing risks of DDT in the global POPs treaty. *Pestic. Safety News***4**:3.

Locke, D. Landivar, J. A. and Moseley, D. (1995). *The effects of rate and timing of glyphosate applications of defoliation efficiency, regrowth inhibition.* National Cotton Council of America: 1088-1090.

Lopez, M., Meinken, K., and Backhaus, H. (2012). Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic finger printing in addition to conventional testing procedures. *Appl. environs. Microbial,***64**: 284-2821.

Lorenzo, D. E., Scott, G.I. and Ross, P. E. (2001). Toxicity of pesticides to aquatic microorganisms: *Environ. Toxicol. Chem.,***20**: 84-98.

Majewski, M. and Capel, P. (1995). *Pesticides in the atmosphere: distribution, trends, and governing factors*. Volume one, Pesticides in the Hydrologic System. Ann Arbor Press Inc: 118.

Martens, D. A. and Bremner, J. M. (2002). Influence of herbicides on transformations of urea nitrogen in soil. *J. Environ Sci Health B***28**:377-395.

Martius, P., Gilman, G. C. and Metcalf, R. (2001). *Pesticide Production in India:* An Overview. Soil Pollution and Soil Organisms. pp. 1-16.

Mathur, S.C. (1999). Future of Indian pesticides industry in next millennium. *Pesticide information***24** (4): 9-23.

Mishra, S. D., Dwivedi, B. K. N. and Prasad, D. (2001): *Effect of Environmental Pollutant on Metabolism of Soil Nematodes.* Soil Pollution and Soil Organisms. pp. 31-44.

Morals S., Dias E and Pereira (2012). Carbamates: human exposure and health effects. Pp 21-38

Muir, D. C. G. (2004). Empirical and modeling evidence of regional atmospheric transport of current use pesticides. *Environ Toxico Chem.***23**: 2421-2432.

Munier-Lamy, C., and Borde, O. (2000). Effect of triazole fungicide on the cellulose decomposition by the soil microflora. *Chemosphere***41**: 1029–35.

Muñoz-Leoz, B., Garbisu, C., Charcosset, J., Sánchez-Pérez, J. M., Antigüedad, I. and Ruiz-Romera, E.(2013). Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil. *Sci. Total Environ*. **449**:345-354.

Murray, C. J. and Lopez, A. (1996). The effect on soil fertility of repeated applications of pesticides over 20years. *Pestic. Sci.***48**: 63-72.

Nester, E. W., Deng, W. L., Lizang, X., Gardon, M. P. and Comal, I. (1998). Agrobacterium vir D2 protein interacts with plant host Cyclophilins. *PNAS USA.* **95**: 7040-45.

O'Neil, W. and Raucher, R. (1998). *Groundwater public policy leaflet series.* The costs of groundwater contamination. http://www,dnr,state.wi.us/org

Paul, D., Michael, S., Richard, H. and William T. (2007) Microbial aspects of the interaction between soil depth and biodegradation of the Isoproturon. *Chemosphere*, **66**: 664-671.

Pell, M., Stengberg, B. and Torstensson, L. (1998). Potential dentrification and nitrification tests for evaluation of pesticide effects in soil. *Ambio***27**: 24-28.

Pesticide residue in food (2006). Joint FAO/WHO Meeting on Pesticide Residues. *FAO Plant Production and Protection Paper 187.* Italy, 3-12.

Piao, S. C., LIV, G. S., Wu, Y. and Xu, W. B. (2001). Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in montainous soil of Southwest China. *Biol. Fertil. Soils*, **33**(4): 347-350.

Pidwirng M. (2013) *Soil Microbiology, ecology and biochemistry*: Academic press.

Pier, A. B., Bernucci, I., Barmbilla, G., Consonni, D. and Pesatori, C. A. (1998). "The seveso studies on Early and Long-Term Effects of Dioxin Exposure: a Review*" Environ Health Pers Suppl.***106** (52): 5-20.

Rafatullah M., Sulaiman O., Hashim R. and Ahmed A. (2010). Removal of pesticide from water and waterwaste. *Journal of hazardous materials*, **170**(2-3), 969-977

Ramaru, J. Z., Mamabolo, Z. and J. Lekoro (2000). *Improving soil fertility management in South Africa:* London through participatory extension approaches. Managing Africa's Soils No. 19.

Rice, P. J., Anderson, T. A. and Coats, J. R. (2002). Degradation and persistence of metolachlor in soil: effects of concentration, soil moisture, soil depth and sterilization. *Environ. ToxicolChem.***21**:2640–8.

Ross, G. (2005). Risks and benefits of DDT. *The Lancet***366** (9499): 1771.

Santos, A. and Flores, M. (1995). Effect of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. *Appl. Microbiol.* **20**:349-352

Savonen, C. (1997). *Soil microorganisms object of new Osu service.* Good Fruit Grower: http://www.goodfruit.com/archive/1995/bother.html.

Sine, B. (1992) *Soil Ecology Cambridge University Press*, Cambridge United Kingdom.

Tabatbai, M. A. (1994). Soil enzymes. *Methods of soil analysis. Part 2.* Microbial and biochemical properties. Soil science society of America Madison, U.S.A, PP: 775-783.

Tan, Z. X., Lal, R., Seek, N. E., Calhonun, F. G., Gehring, R. M., and Parkinson, B. (2003). Identifying association among soil and site variables using canonical correlation analysis, *Soil Science***168** (5): 376-382.

Tanabe, S., Senthilkumar, K., and Subramanian, A. N. (2001). Accumulated features of polychlorinated biphenyls and organochlorine pesticides in resident from South India. *Arch. Environ Contam Toxicol.***34**: 387-397

Ubuoh, E. A., Akhionbare, S. M.O. and Akhionbare, W. N. (2012). Effects of Pesticide Application on Soil Microbial Spectrum: Case Study- Fecolart Demonstration Farm, Owerri-West, Imo State, Nigeria. *International Journal of Multidisciplinary Sciences and Engineering*, **3**(2): 34-39

United State Environmental Protection Agency (U.S. EPA) (1996). Pesticides, and toxic substances. Re-registration eligibility decision (RED): *Trifluralin.* Washington, D.C.

United State Environmental Protection Agency (U.S. EPA) (1998). Pesticides and toxic substances. Guidance for the re-registration of pesticide products containing glyphosate as the active ingredient Washington, D.C.

United State Environmental Protection Agency (U.S. EPA) (1999). *Spray drift of pesticides*. Washington D.C: office of pesticide programs. http://www.epa.gov./pesticides/citizens/spraydrift.htm.

United State Environmental Protection Agency (U.S. EPA) (2001). Managing small-scale application of pesticides to prevent contamination of drinking water. *Water protection practices bulletin*, Washington, D.C. EPA **816**-f-01-031.

United State Geological Survey (U.S.G.S.) (1995). *Pesticides in the Hydrologic System.* https://pubs.usgs.gov/fs/1995/0244/pesticides.html

United State Geological Survey (U.S.G.S.) (1998). *National Water Quality Assessment.* Pesticide national Synthesis Project. Pesticides in surface and ground water of the United States; Summary of results of the National Water Quality Assessment Program http:water.wr.usgs.gov/pnsp/allsum/fig 02.

United State Geological Survey (U.S.G.S.) (1999). *The quality of our nation's waters-nutrients and pesticides.* Circular 1225.http://water.usgs.gov/pubs/circ/circ1225

Voroney, R.P. (2006). *The Soil Habitat in Soil Microbiology, Ecology, and Biochem.* Academic press. p. 552.

Wardle, D.A. (2002). *Communities and ecosystems*: linking the above ground and belowground components. Princeton University Press.

Wardle, D.A. (2004). Trends in ecology and evolution. *Spatial soil ecology*.**17** (4): 177-183.

Warren, A.D. (1998). Soil microbiological parameters as indicators of soil quality under improved fallow management systems. *Plant and soil.* **202** (1): 97-107.

Waskon, R. (1994). *Best management practices for private well protection.* Colorado State Univ. Cooperative Extension, http://hermes.ecn.purdue.edu:8001/cgi/conventwq,7488.

William, R. M., Dmontet, S., Bufo, S. A. and Mazzatura, A. (2002). Effects of organic amendment and herbicide treatment on soil microbial biomass. *Biol. Fer. Soils***32**: 17-23.

Winding, A., Kerstin, H. R. and Rutgers, M. (2005). The use of microorganisms in ecological soil classification and assessment concepts. *Ecological and Environmental Safety*,**62** (2): 230-248.

World Health Organization report (WHO) (2001). Mental health: New understanding, new hope. *Geneva*: 88.

Xiaolan, Y., Fei, D and Xiangyang, L (2018). *Microbial Resources and Ecology-Microbial Degradation of Pesticides.* Natural Resources Conservation and Research. 22-29

Zhao, C., Xie, H., Zhang, J., Xu, J. and Liang, S. (2013). Spatial distribution of organochlorine pesticides (OCPs) and effect of soil characters: A case study of a pesticide producing factory. *Chemosphere*,**90**: 2381-2387.

APPENDICES

APPENDIX I

Table 1: Nutrient Agar

Composition	Mass(g)
Beef extract	3.0
Sodium chloride	6.0
Peptone	5.0
Agar	12.0
Distilled water	1000ml
pH	7.3

Table 2: Sabourauds Dextrose Agar

Composition	Mass(g)
Dextrose	40.0
Balanced peptone	10.0
Agar	12.0
Distilled water	1000ml
pH	5.6

APPENDIX II

Table 3: Biochemical Characteristics of Bacteria Isolated from Soil Sample before Planting

Sample code	Colony code	CAT	OXI	COAG	IN	MR	VP	CIT	URE	NO_3	GLU	SUC	LAT	MAL	MAM	XYL	IDENTITY OF ISOLATES
PC_{20}	$PC_{20\,(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{20\,(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{20\,(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{20\,(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{10\,(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PC_{10}	$PC_{10\,(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{10\,(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{10\,(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PB_{50}	$PB_{50\,(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PB_{50\,(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
PB_{40}	$PB_{40\,(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp

	$PB_{40\ (2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PB_{40\ (3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
$1HC_{30}$	$HC_{30\ (1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HC_{30\ (2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HC_{30\ (3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHB_{10}	$PHB_{10(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PHB_{10(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{10(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHC_{20}	$PHC_{20(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{20(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{20(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{20(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHB_{20}	$PHB_{20(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PHB_{20(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{20(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{20(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

	$PHB_{20(5)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{40(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{40(3)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PHC_{30(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{30(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$PB_{30(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$PB_{30(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PB_{30(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
HC_{20}	$HC_{20(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HC_{20(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HC_{20(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{20(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

SO_c	$SO_{c\,(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$SO_{c\,(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$SO_{c\,(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$SO_{6\,(4)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
PHC_{10}	$PHC_{10(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHC_{10(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{10(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
HB_{50}	$HB_{50(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{50(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
HC_{50}	$HC_{50(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{50(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HC_{50(3)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp

HC_{10}	$HC_{10(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HC_{10(2)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
PC_{40}	$PC_{40(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{40(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{40(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{40(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
HB_{10}	$HB_{10(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HB_{10(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HB_{10(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HB_{10(4)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
HB_{40}	$HB_{40(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

PHB_{50}	$PHB_{50(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{50(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{50(3)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
PC_{30}	$PC_{30(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PC_{30(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{30(3)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
PB_{20}	$PB_{20(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PB_{20(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$PB_{20(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHC_{30}	$PHC_{30(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{30(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp

HB_{30}	$HB_{30(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

Keys: CAT - Catalase M - Maltose

COAG - Coaglase S - Sucrose

OXI - Oxidase L - Lactose

IN - Indole G - Glucose

MR - Methyl red MN - Mannitol

VP - Vogues Proskauer

CIT - Citrate

Table 4: Biochemical Characteristics of Bacteria Isolated from Soil Sample after Planting

Sample code	Colony code	CAT	OXI	COAG	IN	MR	VP	CIT	URE	NO_3	GLU	SUC	LAT	MAL	MAM	XYL	IDENTITY OF ISOLATES
	$PHC_{40(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus*sp
HB_{20}	$HB_{20\ (1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{20\ (2)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{20(3)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{20(4)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{20(5)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
PC_{50}	$PC_{50(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{50(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

	$PC_{50(3)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PC_{50(4)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PC_{50(5)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHC_{50}	$PHC_{50(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHC_{50(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus*sp
HC_{20}	$HC_{20(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HC_{20(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{20(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PB_{30}	$PB_{30(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PB_{30(2)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PB_{30(3)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PB_{30(4)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp

PB_{10}	$PB_{10(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PB_{10(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PHB_{40}	$PHB_{40(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{40(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus*sp
HB_{50}	$HB_{50(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HB_{50(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HB_{50(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
HC_{50}	$HC_{50(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HC_{50(2)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HC_{50(3)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$PC_{40(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PC_{40(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp

	$PC_{40(4)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
HB_{30}	$HB_{30(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$HB_{30(2)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
HC_{30}	$HC_{30(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{30(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PHB_{10}	$PHB_{10(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{10(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{10(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{10(4)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{10(5)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PC_{20}	$PC_{20(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PC_{20(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp

HB_{10}	$HB_{10(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
	$HB_{10(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{30(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PC_{30}	$PC_{30(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{30(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PHB_{50}	$PHB_{50(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHB_{50(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PHB_{20}	$PHB_{20(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHB_{20(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
PC_{10}	$PC_{10(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PC_{10(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PC_{10(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp

HC_{10}	$HC_{10(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$HC_{10(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HC_{10(3)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$HC_{10(4)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
HB_{40}	$HB_{40(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
PHC_{30}	$PHC_{30(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHC_{30(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PHC_{30(3)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
$S0_c$	$S0_{c(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$S0_{C(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PB_{20}	$PB_{20(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
	$PB_{20(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp

PHC_{20}	$PHC_{20(1)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus* sp
	$PHC_{20(2)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp
PB_{40}	$PB_{40(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
	$PB_{40(2)}$	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	*Enterococcus*sp
PB_{50}	$PB_{50(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
PHC_{40}	$PHC_{40(1)}$	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
PHB_{30}	$PHB_{30(1)}$	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus* sp
PC_{30}	$PC_{30(1)}$	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	*Bacillus* sp

Keys: CAT - Catalase ; M - Maltose; COAG - Coaglase ; S - Sucrose; OXI - Oxidase; L - Lactose; IN - Indole
G - Glucose ; MR - Methyl red; MN – Mannitol; VP - Vogues Proskauer; CIT - Citrate

Table 5: Colonial and Microscopic Characteristics of Fungal Isolated Before Planting

SAMPLE CODE	COLONY CODE	COLONIAL CHARACTERISTICS	MICROSCOPIC CHARACTERISTIC	IDENTITY OF ISOLATES
PC_{20}	$PC_{20(1)}$	White hyphae on red background	Septate conidia with sickle cell shape	*Fusarium* sp
	$PC_{20(2)}$	Brown in colour with clear zone of inhibition	Fluffy thread like hyphae	*Streptomyces* sp
	$PC_{20(3)}$	Lemon green on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{20(4)}$	Moist and shiny with irregular shape and cream in colour	Fluffy thread like hyphae	*Streptomyces* sp
PC_{10}	$PC_{10(1)}$	Hyphae is septate conidia attached to sterigma	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PC_{10(2)}$	Lemon green on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{10(3)}$	Dull and dry with irregular shape, and clear zone of inhibition	Fluffy thread like hyphae	*Streptomyces* sp
	$PC_{10(4)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PB_{50}	$PB_{50(1)}$	Lemon green on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PB_{50(2)}$	White in colour with clear zone of inhibition	Fluffy thread like hyphae	*Streptomyces* sp
	$PB_{50(3)}$	Dull and dry cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PB_{40}	$PB_{40(1)}$	Hyphae in septate	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PB_{40(2)}$	Greenish spores on white shout	Septate hyphae, conidia	*Penicillium* sp

		hyphae	mop like	
	$PB_{40(3)}$	Dull and dry cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HC_{30}	$HC_{30(1)}$	Black space on short white hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$HC_{30(2)}$	Red background with white hyphae	Septate conidia with sickle cell shape	*Fusarium* sp
	$HC_{30(3)}$	Cream moist and shiny irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{30(4)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PHB_{10}	$PHB_{10(1)}$	White shout hyphae on light yellow background	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{10(2)}$	Fluffy white hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
	$PHB_{10(3)}$	Moist and shiny yellow with irregular shape and clear zone of inhibition	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PHC_{20}	$PHC_{20(1)}$	Light yellow background with white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHC_{20(2)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
	$PHC_{20(3)}$	Moist and shiny with yellow colour	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{20(4)}$	White hyphae on red background	Septate conidia with sickle cell shape	*Fusarium* sp
PHB_{20}	$PHB_{20(1)}$	White short hyphae on light	Septate hyphae, conidia	

		yellow background	mop like	*Penicillium* sp
	$PHB_{20(2)}$	Septate hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PHB_{20(3)}$	Moist shiny yellow coloured colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHB_{20(4)}$	Red background with white hyphae	Septate conidia with sickle cell shape	*Fusarium* sp
	$PHB_{20(5)}$	Mold with irregular non-septate hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
HC_{40}	$HC_{40(1)}$	Yellow coloured colonies that are moist and shiny	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{40(2)}$	Light yellow background with white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HC_{40(3)}$	Fluffy white hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PHC_{30}	$PHC_{30(1)}$	Septate hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PHC_{30(2)}$	Moist shiny yellow coloured colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{30(3)}$	White hyphae with red background	Septate conidia with sickle cell shape	*Fusarium* sp
	$PHC_{30(4)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PC_{50}	$PC_{50(1)}$	Light yellow background with white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{50(2)}$	Mold with irregular non-septate hyphae	Non septate hyphae, spores enclosed in a	*Mucor* sp

			sporangium	
	$PC_{50(3)}$	Clear zone of inhibition, moist and shiny yellow colonies with irregular shape	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{50(4)}$	Brown colour with regular shape and high shape concave with zone of inhibition	Fluffy thread like hyphae	*Streptomyces* sp
	$PC_{50(5)}$	Septate hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PC_{50(6)}$	Shout white hyphae on red background	Septate conidia with sickle cell shape	*Fusarium* sp
PHB_{30}	$PHB_{30(1)}$	Red background with short white hyphae	Septate conidia with sickle cell shape	*Fusarium* sp
	$PHB_{30(2)}$	White short hyphae on light yellow background	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{30(3)}$	Moist shiny yellow coloured colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHB_{30(4)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PH_{30}	$PH_{30(1)}$	Black spore on short hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PH_{30(2)}$	Green spore on short white hyphae	Septate conidia with sickle cell shape	*Fusarium* sp
	$PH_{30(3)}$	Dull and dry with irregular shape colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PH_{30(4)}$	Fluffy white hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
HC_{20}	$HC_{20(1)}$	Green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp

	$HC_{20(2)}$	Black spores on short hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$HC_{20(3)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
	$HC_{20(4)}$	Moist shiny yellow coloured colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
SO_C	$SO_{C(1)}$	Septate hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$SO_{C(2)}$	White short hyphae on light yellow background	Septate hyphae, conidia mop like	*Penicillium* sp
	$SO_{C(3)}$	Fluffy white hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
	$SO_{C(4)}$	Moist and shiny with irregular shape colonies that are cream on colour	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PHC_{10}	$PHC_{10(1)}$	Moist shiny yellow coloured colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{10(2)}$	Green spore on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHC_{10(3)}$	White hyphae on red background	Conidia septate and rectangular fork shape	*Geotrichum candidium*
HB_{50}	$HB_{50(1)}$	White hyphae on red background	Septate conidia with sickle cell shape	*Fusarium* sp
	$HB_{50(2)}$	Green spore on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{50(3)}$	White cotton wool-like hyphae with pinkish background	Conidia septate and rectangular fork shape	*Geotrichum candidium*
HC_{50}	$HC_{50(1)}$	Black spores on short white hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp

	$HC_{50(2)}$	Green spores on white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HC_{50(3)}$	Dull and dry irregular shape colonies cream colour	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HC_{10}	$HC_{10(1)}$	White cotton wool-like hyphae	Conidia septate and rectangular fork shape	*Geotrichum candidium*
	$HC_{10(2)}$	Moist and shiny circular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PC_{40}	$PC_{40(1)}$	White cotton wool-like hyphae	Conidia septate and rectangular fork shape	*Geotrichum candidium*
	$PC_{40(2)}$	Yellow spores on white cotton wool-like hyphae	Conidia septate and rectangular fork shape	*Geotrichum* sp
	$PC_{40(3)}$	Moist and shiny circular cream colonies with irregular shape	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{40(4)}$	Green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
PHB_{40}	$PHB_{40(1)}$	Green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{40(2)}$	Dull and dry cream irregular colony	Septate hyphae, conidia mop like	*Penicillium* sp
PB_{10}	$PB_{10(1)}$	Green spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PB_{10(2)}$	White short hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
HB_{10}	$HB_{10(1)}$	Lemon green spores on white shout hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{10(2)}$	Moist and shiny cream colony	Large gram positive	*Saccharomyces* sp

			spherical and oval budding cells	
	$HB_{10(3)}$	White cotton wool like hyphae	Conidia septate and rectangular fork shape	*Geotrichum candidium*
	$HB_{10(4)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
HB_{40}	$HB_{40(1)}$	Green spore on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{40(2)}$	Moist and shiny circular cream colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
			Large gram positive spherical and oval budding cells	
PHB_{50}	$PHB_{50(1)}$	Dull and dry cream colonies		*Saccharomyces* sp
	$PHB_{50(2)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{50(3)}$	Moist and shiny circular cream colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PC_{30}	$PC_{30(1)}$	Black spore on short hyphae	Hyphae septate, conidia globosed	*Aspergillus* sp
	$PC_{30(2)}$	Green on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{30(3)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
PB_{20}	$PB_{20(1)}$	Dry with irregular shape and clear zone of inhibition with low convex	Large gram positive spherical and oval budding cells	*Saccharomyces* sp

	$PB_{20(2)}$	White fluffy hyphae	Non septate hyphae, spores enclosed in a sporangium	*Mucor* sp
	$PB_{20(3)}$	Green spore on short white hyphae with light yellow background	Septate conidia with sickle cell shape	*Fusarium* sp
PHC_{50}	$PHC_{50(1)}$	Lemon green spore on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHC_{50(2)}$	Moist and shiny cream regular colonies with low convex	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HB_{20}	$HB_{20(1)}$	Moist, shiny, circular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HB_{30}	$HB_{30(1)}$	White cotton wool like hyphae	Conidia septate and rectangular fork shape	*Geotrichum candidium*
	$HB_{30(2)}$	Moist and shiny cream colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp

Table 6: Colonial and Microscopic Characteristics of Fungal Isolated After Planting

SAMPLE CODE	COLONY CODE	COLONIAL CHARACTERISTICS	MICROSCOPIC CHARACTERISTICS	IDENTITY OF ISOLATES
PHC_{40}	$PHC_{40(1)}$	Greenish spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHC_{40(2)}$	Lemon cream spores on white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
HB_{20}	$HB_{20(1)}$	Lemon green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{20(2)}$	Greenish spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{20(3)}$	Dull and dry cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HB_{20(4)}$	Moist and shiny cream regular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HB_{20(5)}$	Moist and shiny cream finger like colony	Filamentous hypae with a pointed end	*Yeast*
PC_{50}	$PC_{50(1)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium notatum*
	$PC_{50(2)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{50(3)}$	Dull and dry irregular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{50(4)}$	Lemon green spores on white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{50(5)}$	Moist and shiny pink	Large gram positive	*Saccharomyces* sp

		irregular colony	spherical and oval budding cells	
PHC_{50}	$PHC_{50(1)}$	Lemon green spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHC_{50(2)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Pencillium* sp
HC_{20}	$HC_{20(1)}$	Moist and shiny irregular golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{20(2)}$	Greenish spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium notation*
	$HC_{20(2)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PB_{30}	$PB_{30(1)}$	Lemon green spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PB_{30(2)}$	Dull and dry irregular cream colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PB_{30(3)}$	Moist and shiny cream regular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PB_{30(4)}$	Greenish spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
PB_{10}	$PB_{10(1)}$	Moist and shiny golden yellow irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PB_{10(2)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp

PHB_{40}	$PHB_{40(1)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium notatum*
	$PHB_{40(2)}$	Lemon green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
HB_{50}	$HB_{50(1)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HB_{50(2)}$	Moist and shiny circular yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HB_{50(3)}$	Greenish spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{50(4)}$	Moist and shiny irregular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HC_{50}	$HC_{50(1)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{50(2)}$	Moist and shiny golden yellow colonies	Septate hyphae, conidia mop like	*Penicillium* sp
	$HC_{50(3)}$	Lemon green spores on short white hyphae	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PC_{40}	$PC_{40(1)}$	Moist and shiny circular golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{40(2)}$	Moist and shiny cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{40(3)}$	Black spores on white short hyphae	Hyphae, conidia globosed	*Aspergillus* sp

	$PC_{40(4)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium notatum*
HB_{30}	$HB_{30(1)}$	Lemon green spores on white short hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$HB_{30(2)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HC_{30}	$HC_{30(1)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{30(2)}$	Moist and shiny golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PHB_{10}	$PHB_{10(1)}$	Greenish spores on short white hyphae with lemon background	Septate hyphae, conidia mop like	*Penicillium notatum*
	$PHB_{10(2)}$	Lemon green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{10(3)}$	White short hyphae with orange background	Conidia septate and rectangular fork shape	*Geotrichum candidum*
	$PHB_{10(4)}$	Moist and shiny cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHB_{10(5)}$	Moist and shiny small circular golden yellow colour	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PC_{20}	$PC_{20(1)}$	Lemon green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{20(2)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium notatum*

	$PC_{20(3)}$	Moist and shiny cream circular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{20(4)}$	Moist and shiny golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{20(5)}$	Moist and shiny colour pink colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{20(6)}$	White short hyphae with orange background	Conidia septate and rectangular fork shape	*Goetrichum candidum*
HB_{10}	$HB_{10(1)}$	Greenish spore on short white hyphae	Septate hyphae, conidia mop like	*Penicillium notatum*
	$HB_{10(2)}$	Lemon green spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
PC_{30}	$PC_{30(1)}$	Moist and shiny irregular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{30(2)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
	$PC_{30(3)}$	Dull and dry irregular cream colony	Septate hyphae, conidia mop like	*Penicillium* sp
PHB_{50}	$PHB_{50(1)}$	Dull and dry irregular cream colonies	Septate hyphae, conidia mop like	*Penicillium* sp
	$PHB_{50(2)}$	Yellow spores on white cotton wool like hyphae	Conidia septate and rectangular fork shape	*Geotrichum candidium*
PHB_{20}	$PHB_{20(1)}$	Moist and shiny on irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHB_{20(2)}$	Dull and dry green irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp

PC_{10}	$PC_{10(1)}$	Moist and shiny irregular circular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{10(2)}$	Moist and shiny circular golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PC_{10(3)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HC_{40}	$HC_{40(1)}$	Moist and dull cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{40(2)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$HC_{40(3)}$	Dull and dry cream irregular colonies with rough pink center	Filamentous hypae with a pointed end	*Yeast*
	$HC_{40(4)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
HB_{40}	$HB_{40(1)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PHC_{30}	$PHC_{30(1)}$	Moist and shiny small circular golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{30(2)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{30(3)}$	Pink rough colonies with	Filamentous hypae with	Yeast

		cream background	a pointed end	
SO_C	$SO_{C(1)}$	Moist and shiny small irregular golden yellow colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$SO_{C(2)}$	Moist and shiny small circular cream colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PB_{20}	$PB_{20(1)}$	Moist and shiny cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PB_{20(2)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PHC_{20}	$PHC_{20(1)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
	$PHC_{20(2)}$	Moist and shiny cream irregular colony	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PB_{40}	$PB_{40(1)}$	Dull and dry cream irregular colonies with rough centers	Filamentous hypae with a pointed end	Yeast
	$PB_{40(1)}$	Dull and moist cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PH_{50}	$PB_{50(1)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp
PHC_{40}	$PHC_{40(1)}$	Greenish spores on short	Septate hyphae, conidia	*Penicillium* sp

		white hyphae	mop like	
PHB_{30}	$PHB_{30(1)}$	Dull and dry cream irregular colonies	Large gram positive spherical and oval budding cells	*Saccharomyces* sp
PC_{30}	$PC_{30(1)}$	Greenish spores on short white hyphae	Septate hyphae, conidia mop like	*Penicillium* sp

APPENDIX III

Table 7: Parameter for Soil Sample Physico-Chemical Properties

	Low	**Medium**	**High**
T/N (g/kg)	< 0.1	0.1-0.2	> 0.2
Available (mg/kg)	< 10	10-20	>20
Exchangeable K (cmol/kg)	0.15	0.15-0.3	>0.3

FAO (2004)