EFFECTS OF CHEMICAL AND ORGANIC FERTILIZER TREATMENTS ON BACTERIAL POPULATIONS AND DIVERSITY FROM SELECTED MAIZE FIELDS IN LUSAKA PROVINCE

by

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This dissertation is being submitted to the University of Zambia in partial fulfillment of the requirements for the Master of Science Degree in Applied Microbiology.

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DECLARATION

I, **Namasiku Lubasi**, declare that this master dissertation represents my own work. It has not previously been submitted for a postgraduate degree or any award at the University of Zambia or any other institution. All cited works and materials from other sources have duly been acknowledged and references thereby given.

Signature……

Date……

CERTIFICATE OF APPROVAL

This dissertation submitted by Namasiku Lubasi is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Applied Microbiology at the University of Zambia.

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ABSTRACT

Microbes are the primary agents responsible for the biogeochemical cycling of nutrients, promoting plant development, and suppressing disease. However, not much is known about how chemical fertilizers affect the populations, distribution and survival of soil microorganisms which are essential for enhancing soil structure in soils. To gain a better understanding of the effects of chemical fertilizers on bacterial community diversity, soil samples were collected from 20 representative locations in Lusaka Province of Zambia to investigate how soil bacterial diversity is shaped following the application of chemical (compound D) and organic (manure) fertilizers. The sampling was done between May to June after farming season. Soil physicochemical properties were analyzed. Genomic DNA was extracted from purified sub-cultured bacteria using Qiagen DNA extraction kit and PCR amplified using 16S rRNA primers and amplicons were sequenced. Analysis of variance (ANOVA) was carried out using Minitab (version 17.1.0.0) and a phylogenic tree was generated in MEGA 11. Little variation was recorded in soil temperature which ranged between 29 – 32˚C and the only exception was in soil temperatures of samples collected from the eastern farms (East 1 – East 5) for both organically and chemically fertilized maize fields. The pH of chemically fertilized soils ranged from 4.9 – 6.78 while organically fertilized soils had higher pH ranging from 6.77 – 7.05. The ANOVA test showed that variance in the soil moisture content and organic matter significantly affected and shaped the bacterial communities of the soils. The isolated soil bacteria were predominantly Gram-positive. The colonies mainly composed of bacteria with bacillus and cocci cell shape. Oligotrophic bacteria dominated in chemically fertilized soils. Different fertilization treatments had different impacts on the soil bacterial communities. Chemically fertilized soils had metabolic and stress-resistant bacteria including *Exiguobacterium auratiacum, Gottfriedia acidiceleris, Mycrobacterium paludicola, Lysinibacillus fusiformis* and *Peribacillus frigoritolerans* capable of metabolizing polysaccharides and proteins. Soils to which organic fertilizers were applied had copiotrophic bacteria including *Actinobacteria* and *Arthrobacter* capable of degrading unusual polymeric compounds. Therefore, it is important to understand the dynamics of chemical and organic fertilizers on soil bacteria to help devise agricultural practices that maintain soil health and fertility as well as maintain good crop yields.

Keywords: Bacterial diversity, chemical fertilizer, organic fertilizer, oligotrophic, copiotrophic.

DEDICATION

I dedicate this degree to my parents, Mr. Jacob Lubasi and Mrs. Rabecca Pumulo Silenga Lubasi for always supporting me throughout all my studies.

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LIST OF ABBREVIATIONS

BF	Bio-fertilizer
CF	Chemical fertilizer
CK	Control farm
DNA	Deoxyribonucleic acid
EA	Exchange acidity
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic Acid
HCl	Hydrochloric acid
N	Nitrogen
NK	Nitrogen + Potassium
NP	Nitrogen + Phosphorus
NPK	Nitrogen + Phosphorus + Potassium
NorgS	Non-organic soil
NPKOM	Nitrogen + Phosphorus + Potassium + Organic matter
PCR	Polymerase chain reaction
PK	Phosphorus + Potassium
OC	Organic carbon
OF	Organic fertilizer
OM	Organic matter
OrgS	Organic soil
OUT	Operational taxonomic unit
rRNA	Ribosomal Ribonucleic acid
SOM	Soil organic matter
TAE	Tris-acetate-EDTA

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND

Soil possesses physical, chemical and biological properties. Physical properties are determined by the soil matrix such as the texture, color of soil, porosity and structure. Chemical properties are related to the properties that directly affect plants and other organisms' nutrition such as pH, soil organic carbon, moisture content, soil organic matter. The biological properties of the soil are determined by its organic and inorganic composition (Idowu *et al*., 2019). Soil health is "the state of the soil being in sound physical, chemical, and biological condition hence having the capability to sustain the growth and development of plants and soil biota, retention and filtration of water and nutrient recycling (Idowu *et al*., 2019)." Regrettably, the overdependence on, and long-term use of chemical fertilizers has led to an imbalance in soil nutrients and a number of negative effects on the soil health.

Fertilization is an important measure of agricultural production as it improves soil nutrients and increases crop yield. Scientific investigations have revealed that low soil fertility is the primary constraint to crop yields (Ogbodo, 2013). Therefore, the intensification of agricultural processes has increased the demand on the use of chemical inputs such as fertilizers and mechanical processes, often at the expense of biologically-mediated processes (Liu *et al*., 2021). The term "fertilizer" refers to any synthetic or mineral compound substance created specifically to increase crop yield and three types of fertilizer are recognized as being chemical, organic or bio-fertilizers (Christians *et al*., 2016).

Chemical fertilizers are currently used due to their positive effects on crop plant growth and productivity. However, it is interesting to note that only 10 - 40 percent of fertilizers applied are directly absorbed and used by plants. The unabsorbed fraction is in the form of insoluble inorganic salts or leached into adjacent water bodies which is a threat to soil health and biodiversity (Bai *et al*., 2020).

Soil microorganisms and their enzyme activity are important indicators in the characterization of soil fertility and play a vital role in soil material transformation and energy flow (Liu *et al*., 2021). Soil bacteria through their physiological activities such as respiration are critical for maintenance

of soil fertility and ecosystem functions such as, nutrient transformation and recycling, and organic matter decomposition. Although the sensitivity of soil microbes to mineral fertilizer inputs is not an established fact (Chen *et al*., 2021), the role of different microorganisms in ecosystem function and agricultural productivity in response to different fertilizers, though complicated, has long been well established (Li *et al*., 2020).

The activity of soil microorganisms is an essential element of soil health (Marschner, 2003). Bacteria, fungi, actinomycetes, protozoa, algae and viruses make up soil microbial fauna and have many essential and basic functions in the soil such as improving soil fertility, nutrient recycling, increased productivity by increasing limited nutrient availability, and decomposition of inorganic and organic matter (Mele and Crowley, 2008).

Soil microbial diversity and populations are influenced by a number of factors such as soil fertility, agricultural practices, soil moisture, soil temperature and soil aeration, soil pH, organic matter content, nature of the soil and microbial associations (Olajire-Ajayi *et al*., 2015). Variations in any one or more of these factors can result in microbial shift which can affect soil fertility (Jeorgensen *et al*., 2010). Long-term use of chemical fertilizers has a profound effect on the biochemical properties of the soil, leading to changes in microbial populations. However, little has been reported on the long-term fertilization on soil microbial diversity (Jeorgensen *et al*., 2010).

A few studies have reported that the long-term application of chemical fertilizer reduces microbial biomass and dehydrogenase activity, while organic fertilizers have been reported to increase it (Jeorgensen *et al*., 2010). In a study by Ren *et al*., (2020) on the effects of the continuous use of nitrogen fertilization on diversity and composition of soil bacteria, a reduction in operational taxonomic units (OTU) was observed under different N application rates which might have been as a result of the changes in pH of the soil.

This study investigated the impacts of soil microbes in soil ecosystems with particular emphasis on the effects chemical fertilizers have on soil microbial diversity.

1.2 STATEMENT OF THE PROBLEM

Agriculture productivity world-wide is facing considerable problems such as low soil organic carbon (SOC) stock, low fertilizer use efficiency as well as the imbalance between nutrient removal and addition to the soil (Pahalvi *et al*., 2021). The whole scenario of agriculture is at a

confluence and there is need to rethink, improve agricultural packages and processes in order to meet the food needs of millions of people. Furthermore, improvement and maintenance of soil fertility and sustaining crop production are of global importance. Therefore, the management of soil health is necessary for securing sustainable agricultural production and sustenance of biodiversity (Dar & Bhar, 2020).

Understanding the effects of fertilizer treatments on soil bacterial diversity is essential for predicting their broader ecological implications, including soil nutrient cycling, plant-microbe interactions, and ecosystem stability (Lian-Jie *et al*., 2021). By elucidating these relationships, the research aims to provide insights into the sustainability and long-term productivity of agricultural systems in Lusaka Province.

The study seeks to identify key environmental factors associated with fertilizer treatments that influence soil bacterial diversity. Factors such as nutrient availability, pH levels, and organic matter content will be measured and correlated with bacterial community composition to elucidate the mechanisms driving observed changes in soil microbial communities (Lin *et al*., 2019).

This study aimed at investigating the impact of chemical and organic fertilizer treatments on soil bacterial diversity within selected maize fields in Lusaka Province of Zambia. By comparing bacterial community composition and abundance in soils treated with different fertilizers, this research seeks to assess the potential effects of these agricultural practices on soil health and bacterial diversity (Olajire-Ajayi *et al*., 2015).

Overall, this research addresses a significant knowledge gap regarding the effects of fertilizer treatments on soil bacterial diversity in maize fields, with implications for both agricultural productivity and environmental conservation in Lusaka Province.

1.3 AIM OF THE STUDY

The aim of the study was to assess the impact of chemical fertilizers on soil bacterial populations and diversity from selected maize fields in Lusaka.

1.4 SPECIFIC OBJECTIVES

The objectives of the study were to:

1. Determine the physicochemical properties of soil samples collected from selected maize fields with different fertilizer regimens in Lusaka Province;
2. Determine the effects of different fertilizer regimes on soil bacteria populations and communities from selected maize fields within Lusaka Province and
3. Identify the bacterial species from soil samples collected from selected maize fields with different fertilizer regimens in Lusaka Province.

1.5 RESEARCH QUESTIONS

1. What are the physicochemical properties of soil samples from selected maize fields with different fertilizer treatments in Lusaka Province?
2. How do different fertilizer treatments impact soil bacterial populations and communities in soils from selected parts of Lusaka Province?
3. Are there any differences in taxonomic composition of bacteria from soil samples treated with chemical as opposed to organic fertilizers?

1.6 SIGNIFICANCE OF THE STUDY

Sustainable agriculture relies on practices that minimize negative environmental impacts while ensuring long-term productivity (Qamar *et al*., 2018). This research provides insights into the sustainability of fertilizer management strategies by evaluating their effects on soil microbial diversity. Understanding the effects of chemical and organic fertilizer treatments on soil bacterial diversity is crucial for maintaining and enhancing soil health in maize fields (Qamar *et al*., 2018).

By identifying fertilization practices that support diverse and resilient soil microbial communities, the study can contribute to the development of sustainable agricultural practices that promote soil fertility, nutrient cycling, and overall soil ecosystem functioning (Johns, 2017).

Chemical fertilizers are associated with environmental risks such as nutrient runoff and soil degradation. By comparing the effects of chemical and organic fertilizers on soil bacterial diversity, the study can inform strategies for mitigating these risks. Organic fertilizers, for

example, may promote microbial diversity and contribute to soil conservation efforts by enhancing soil structure and water retention (Olajire-Ajayi *et al*., 2015).

Based on the findings, the study intends to offer evidence-based recommendations for optimizing fertilizer management practices to enhance soil health and microbial diversity in maize cultivation. The research can support policy initiatives aimed at incentivizing environmentally friendly farming practices and promoting soil health. These recommendations will be tailored to the local agricultural context in Lusaka Province, with the aim of promoting sustainable and environmentally friendly farming practices. By advancing knowledge in this field, the research can inform future studies on soil ecology, nutrient cycling, and agricultural sustainability. Additionally, the data generated by this study can serve as a valuable resource for researchers studying similar ecosystems and agricultural practices globally.

Soil fertility comprises three interrelated components, namely, physical, chemical and biological. Biological fertility varies greatly within soils due to the differences in soil conditions. It is highly complex and dynamic and dependent on the biota and how they interact with other components of the soil Fertile soils teem with soil microbes due to their ability to recycle nutrients and biodegrade organic matter which directly contribute to the biological fertility of soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 TYPES OF FERTILIZERS

Chemical fertilizers contain nutrients and elements such as nitrogen, urea and phosphates. Certain chemical fertilizers have a nitrogenous base, whereas others have a phosphate or potassium base. A combination of potassium, nitrophosphate, ammonium phosphate, and other nutrients are frequently found in complex chemical fertilizers (Christians *et al*., 2016).

Naturally created, organic fertilizers are made of organic materials and include carbon. They are often made of plant-based fertilizers like compost and biosolids and animal wastes like guano, manure, slurry, and meat processing wastes (Christians *et al*., 2016).

Biofertilizers are living microbes that enhance plant nutrition by either mobilizing or increasing nutrient availability in soil and mostly include symbiotic bacteria such as Rhizobium, Azotobacter, Azospirilium as well as symbiotic fungi such as Mycorrhiza, Algal Biofertilizers and Azolla (Mitter *et al*., 2021).

Nitrogenous Fertilizers

This type of fertilizer contains nitrogen in the form of ammoniacal nitrogen and amide nitrogen (Pahalvi *et al*., 2021).

Phosphorus Fertilizer

Phosphorus fertilizer contains available phosphate and this type of fertilizer is a vital terrestrial fertilizer required in less amounts compared to nitrogen fertilizer (Chandini *et al*., 2019). This includes rock phosphate and basic slag.

Potassium Fertilizer

Potassium fertilizer is composed of muriate (potassium in chloride form) and sulphate of potash (potassium in non-chloride form), (Pahalvi *et al*., 2021).

2.2 IMPACT OF CHEMICAL FERTILIZERS ON SOIL PROPERTIES

According to Ullah *et al*., (2008) the chemical properties of soil are influenced by different sources of soil nutrients (organic and chemical). Results from the study conducted by Ullah *et al*., (2008)

showed that soil pH varied significantly with the treatments, it significantly increased in maize fields with organic manure application and in combined applications but decreased with only chemical fertilizer application. The observations in this study by Ullah *et al*., (2008) were in agreement with those from a study by Yadvav *et al*., (2017), who observed that organic matter decreased in soils treated with chemical fertilizer and increased with all types of organic manure application. However, soil organic matter was recorded in higher quantities when organic fertilizers were applied in combination with inorganic fertilizers, an observation which was also supported by Yavitt *et al*., (2021).

Results from a study reported by Ogbodo (2013), showed that soils in the agricultural sites with records of continuous inorganic fertilizer application sampled were very acidic to strongly acidic while the exchange acidity (EA) was very high at all sites. Their results also indicated that Nitrogen fertilizers were strongly associated with acidification in the West African region with an average annual increase in aluminum (Al) saturation of 10 percent which rose to critical Al toxicity levels of 30 percent after only a few years of cropping. Organic carbon (OC) ranged from low to high (0.51 - 1.84 percent); The cation exchange capacity (CEC) was very low at all sites (1,654.5), while the base saturation ranged from very low to low (42.0650.10) (Ogbodo *et al*., 2013). Total nitrogen and phosphorus exchange were moderate (ranging from 0.090.19 to 5.7024.8); potassium, calcium, magnesium and sodium ranged from very low to low (0.050.32, 0.865.10, 0.302.0 and 0.090.25), respectively, between sites. However, the microbial population in the soil was stable and consistent with the normal microbial population for natural agricultural soils. Lime application and supplemental use of organic and mineral fertilizers have been recommended as a good strategy to manage the fertility of these soils. This study identified the negative impact of long-term input fertilizer applications (used without complementary liming and/or organic amendments) to be primarily acidification.

The impact of fertilizers on soil pH appears not to be clear. Results from a study conducted by Titilola (2006), appeared to have provided evidence that suggested fertilizer use had no substantial bearing on soil pH over two years of continuous use. In this study, different fertilizer treatments such as organic, inorganic plus organic fertilizer, no fertilizer, and inorganic fertilizer were used. It was observed that the level of organic carbon decreased by approximately 17, 44, 47, and 59 percent, respectively (Titilola, 2006). Furthermore, a reduction in total nitrogen was equally

observed across every plot and in the absence of any fertilizer, a decrease in the amount of available phosphorus from its original value of 4.72 mg/kg to 3.37 mg/kg was noticed. However, other treatments such as inorganic plus organic and inorganic fertilizers showed an increase of 8 percent and 9 percent respectively. Not only this, exchangeable potassium levels dropped in all plots despite the type of fertilizer used. These reductions varied from 25 percent for organic fertilizer to 53 percent for inorganic fertilizer. On the other hand, Effective Cation Exchange Capacity (ECEC) increased by 16 percent with the addition of organic fertilizer, while it decreased in the other plots.

Results from a study by Chakraborty *et al*., (2011) suggested that livestock manure helps enrich soil with organic matter and nutrients which feeds microbes in soil. This allows them to make nutrients available to plants in a natural and biological process and eventually had a positive impact on bacterial diversity after long-term chemical fertilization as well as improved the soil pH overtime. Another study by Wu *et al*., (2021) established that combinations of organic and chemical fertilizers showed an increase in soil microbial activity, improved soil's physical and chemical properties, enhanced nutrient availability in citrus tree organs, and promoted citrus growth and photosynthesis.

Nemera *et al*., (2018) in Ethiopia concluded that the pre- and post-treatment soil test indicated that the shift in topographical class was not a consequence of the treatments as it was with the control or without treatments. This study also established that there was an insignificant change in soil pH (Nemera *et al*., 2018). Furthermore, increased total nitrogen and organic carbon levels at chemical fertilizers were noted from a comparison of before and after soil sample analyses. Because urea fertilizer is a good source of nitrogen, chemical fertilizers tend to enhance total nitrogen and organic carbon.

2.3 IMPACTS OF CHEMICAL FERTILIZERS ON SOIL BACTERIAL DIVERSITY

The upsurge and overuse of chemical fertilizers around the world has been reported (Lin *et al*., 2019). Globally, chemical fertilizer usage is estimated to be around 190 billion tons, with China ranking first in chemical fertilizer usage and accounting for 25 percent of world usage (NBSPRC, 2020).

Long-term use of NPK has been found to deplete soil organic matter (SOM), especially in arid and semi-arid regions or where monoculture crops (e.g., maize) are grown (Zhang *et al*., 2010). Furthermore, an increase in SOM of mineral fertilizers was found only when applied in combination with organic supplements. Additionally, it was also noted that the quality of SOM greatly affects the composition of the microbial communities in the soil (Zhang *et al*., 2010).

Results from a study by Dinca *et.al*., (2022) have demonstrated that organic fertilizers can directly stimulate the growth of specific microbial populations by providing nutrients, hence resulting in an increase in the total number of microorganisms and enhancing microbial activity. Furthermore, it was also established that combinations of organic and inorganic fertilization increased microbial biomass which was not observed in inorganically fertilized soils (Dinca *et al*., 2022). The long-term nitrogen application has been observed to increase microbial biomass, however, this increase is only significant in soils with pH less than 5 while continuous use of N fertilizers has been reported to have a stabilizing or temporary effect on soil pH.

Results from an experimental study by Enebe and Babalola (2020) using high throughput next generation sequencing and a metagenomic approach, indicated that regardless of the fertilizer regimen, *Proteobacteria* and *Bacteroidetes* were distributed across all samples (inorganic, organic and control). Their results obtained by Enebe and Babalola (2020) appeared to suggest that lower amounts of nitrogen-based fertilizer, higher amounts of organic manure, and the untreated control, supported the enrichment and selection for *actinomyces* and *Proteobacteria.* Lower quantities of organic compost manure boosted the propagation of Bacteroidetes. Additionally, Firmicutes were found to be in abundance in low organic manure and also higher inorganic fertilized soil. Furthermore, fungi were found to be selected and enriched by both higher and lower amounts of compost manure, whereas archaea were found to be enriched by higher inorganic fertilized soil and lower compost manure soils. The study therefore concluded that different types of fertilizer impacted the maize rhizosphere microbial network differently while organic treatments resulted in the strong microbial network (Enebe & Babalola, 2020).

Treatment of crop stands with NPK fertilizers has been observed to negatively-affect microbial network, shape and abundance in an agricultural soil (Doran & Zeiss, 2000). In some cases, long-term chemical fertilizer applications result in a significant loss in SOM and causes a decrease in

porosity and nutrient availability and strongly affects the number of microorganisms and the qualitative selection of entire communities of soil microorganisms (Doran & Zeiss, 2000) while on the other hand, long-term fertilization with organic matter improves soil quality and enhanced yield, these findings are also supported by Song *et al*. (2015) in northeast China.

A study by Lin *et al*., (2019) in China showed that chemical fertilizer significantly increased Shannon`s diversity indices in rhizosphere soil in comparison to all treatments with the majority of the phyla identified assigned to *Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Gemmatimonadetes* and *Cyanobacteria.* On the other hand, soils treated with organic fertilizer showed an abundance of *Burkholderriales, Myxococcales, Streptomycetales, Nitrospirales, Ktedonobacterales, Acidobacterales, Germmatimonadales* and *Solibacterales* (Lin *et al*., 2019). Furthermore, a redundancy analysis was carried out to investigate the relationship between soil chemical properties and dominant genera. It was observed that OrgS samples were positively associated with higher abundance of *Catenulispora, Candidatus solibacter, Burkholderia paraburkholderia, Gemmatirosa, Nitrospira* and *Rhizomicrobium* (Lin *et al*., 2019). OrgS were negatively associated with the abundance of *Acidobacterium, Acidothermus and Acidicaldus* which appeared to be positively associated with NorgS. The abundance of *Acidobacter, Catenulispora, Burkholderia paraburkholderia, Gemmatirosa, Nitrospira, Candidatus solibacter, Rhizomicrobium* and *Sorangium* was found to be strongly associated with soil pH.

In contrast, Wang *et al*., (2020) showed that long-term application of chemical fertilizers NPK significantly affected the microbial community structure by significantly reducing the alpha diversity of soil microbial communities. Results from this study demonstrated that fertilization had an influence on the *r* and *k – strategists* (Wang *et al*., 2020). Generally, the *r – strategists* grow fast when the substrate is abundant, these include members of the Copiotrophic bacteria such as *Proteobacteria,* particularly *Alpha* and *Beta-Proteobacteria, Firmicutes, Actinobacteria and Candidatus Saccharibacteria.* On the other hand, *k-strategists* can grow when resources are limited, these include oligotrophic bacteria such as *Acidobacteria, Gamma-* and *Delta-Proteobacteria, Gemmatimonadete, Verrucomicrobia and Chloroflexi* (Francioli *et al*., 2016). Application of chemical fertilizers has been reported to enrich the *k*-strategist bacterial community. It was observed that the long-term PK treatment enhanced bacterial richness and diversity more than NK, NP, NPK of organic manure addition (Francioli *et al*., 2016). Phylogenetic analyses

performed in a long-term organic and chemical fertilization experiment in a sandy loam soil in northern China showed *Proteobacteria* as the dominant taxonomic group in the soil, this was followed by an abundance of *Acidobacteria* and *Gemmatimonadetes* (Ge *et al*., 2008).

A study by Cui *et al*., (2018) in China found that long term application of NPK chemical fertilizers enhanced the abundance of *Verrucomicrobia* and *Nitrospiraceae,* while the use of manure influenced the abundance of *Deltaproteobacteria* and *Myxococcales.* A combination of NPKs and manure perpetuated the proliferation of Actinobacteria and Planctomycetes. This study also found that exchangeable Magnesium, soil organic carbon (SOC) and alkali-hydrolysable nitrogen (AN) are the vital factors in shaping bacterial communities in the rhizosphere (Cui *et al*., 2018). This is supported by the experimental results from a study reported by Kamaa *et al*., (2011) which highlighted how continuous application of N fertilizers led to a net loss of soil organic matter and a drop in soil pH in their samples which resulted in the decline of bacterial communities. According to a study done in Kenya by Kamaa *et al*., (2011), it was established that the bacterial community structure and diversity were affected negatively by the use of nitrogen and phosphorus-based fertilizers (Kamaa *et al*., 2011).

2.4 ORGANIC FERTILIZERS AND THEIR INFLUENCE ON SOIL BACTERIAL COMMUNITIES

Gradual decomposition of organic matter in soil is facilitated by microorganisms (Davidson, Jansen, 2006) (Bardgett, van der Putten, 2004). These microorganisms make available the nutrients from crop residues back into the soil. It is because of this decomposition that microbial soil fauna food chains are maintained (Stewart *et al*., (2005). Olajire-Ajayi *et al*., (2015) added that organic fertilizers over a long period of time create a conducive and healthy environment, compared to their inorganic counterparts that work rapidly, but fail to avail a sustainable environment. Additionally, Olajire-Ajayi *et al*., (2015) postulated that the long-term use of inorganic fertilizers depletes the soils of the organic matter dependent organisms, resulting in the eventual disappearance of these organisms in that soil.

Furthermore, a study by Nakhro and Dkhar (2010), which compared the use of organic versus inorganic fertilizers, observed that organically treated soil had the highest number of

microorganisms (fungi and bacteria) and microbial biomass carbon. In contrast, one study on the long-term effect of chemical fertilizer in North-western China discovered that, chemical fertilizers had less influence than other soil treatments on microbial composition and diversity (He *et al*., 2008).

In an article on peanut monocropping by Chen *et al*., (2021), chemical fertilizer treatment was found to cause changes in bacterial community structure and reduced diversity as compared to organic fertilizers and also showed the prevalence of the bacterial pathogen *Ralstonia solanacearum* which is a causative agent of peanut wilt disease. Organic fertilizers on the other hand had more effectively increased the diversity of soil microorganisms and altered bacterial community structure.

Another study carried out at Fengqiu in northern China by Yuan *et al.,* (2008) showed that community structures of bacteria significantly differed between treatment soils. OM and PK treatments revealed a trend towards clear community structure, higher abundance and diversity compared to other treatments. Phylogenetic analysis indicated that *Proteobacteria* (30.5 percent) were dominant in soil, followed by *Acidobacteria* (15.3 percent), *Gemmatimonadetes* (12.7 percent) and so on. The bacterial community structures of the four N-containing treatments (NK, NP, NPK, and 1/2NPKOM), as well as CK appeared to be more similar, indicating that nitrogen fertilizers can be considered a key factor in counteracting the effects of other fertilizers on the microbial community.

Results from a study by Kamaa *et al*., (2011) conducted in Kenya showed that organically treated soils supported bacteria that clustered far from inorganically treated soils. Organically treated soils had a positive impact on bacterial and fungal variety with or without inorganic inputs. These results mirrored those from a study by Zhang *et al*., (2017) which confirmed how long-term fertilization of natural manure (with or without NPK application) caused C usage sample shifts and increased beneficial soil bacteria. Furthermore, Jeorgensen *et.al.,* (2010) confirmed that the long-term application of farmyard manure in aggregate with natural farming practices caused an elevated accumulation. This was in tandem with the findings of Maly *et al*., (2009) who further confirmed that long-time period mineral fertilization increased the percentage of r-strategists in soil.

Nonetheless, Kibunja *et al*., (2010) determined that the continuous application of chemical fertilizers on soil resulted in a drastic reduction of soil biota and a deterioration in soil pH, hence prompting the lessening of bacterial groups on this treatment. Therefore, bacterial and fungal communities are considerably affected by the fertilization input type.

2.5 MOLECULAR TAXONOMY IN EVALUATING SOIL MICROBIAL COMMUNITIES

The need to understand how the interactions between soil properties and microbial communities are affected by different fertilizers necessitates the application of molecular taxonomy methods. Several studies have employed techniques like 16S rRNA gene sequencing, metagenomics, and functional gene analysis to understand these relationships.

A study conducted by Chakraborty *et al.* (2011) utilized the 16S rRNA gene sequencing technique to demonstrate how livestock manure positively influenced bacterial diversity in soils subjected to long-term chemical fertilization. The addition of manure to soil increased soil biomass and cultivatable microorganisms thus enhancing enzyme activities and respiration (Chakraborty *et al.* 2011). The findings of this study emphasized the potential of organic amendments in restoring microbial diversity. Similarly, Yuan *et al.* (2008) investigated the impact of different nitrogen-containing treatments on soil bacterial communities using 16S rRNA gene analysis. In this study, they identified shifts in community structures and highlighted the role of nitrogen fertilizers in altering soil microbial compositions, specifically affecting Proteobacteria and Acidobacteria abundance.

On the other hand, Wu *et al.* (2021) employed metagenomic approaches and next-generation sequencing to establish the effects of combined organic and chemical fertilizers on soil microbial activity and nutrient availability in citrus orchards. Their findings emphasized the important relationship between the enhancement of microbial biomass and nutrient availability in response to combined fertilization. In the same vein Enebe and Babalola (2020) utilized a shotgun metagenomics approach to assess the microbial community in the maize rhizosphere under inorganic and organic treatments. Their study revealed differential impacts on microbial networks, validating how varying fertilizer types affected the abundance and selection of specific microbial groups in the rhizosphere.

The use of Functional gene analysis was evident when Cui *et al.* (2018) investigated the impact of long-term application of NKP chemical fertilizers on bacterial communities. Their work revealed specific associations between fertilizer inputs and the abundance of bacterial groups, and furthermore emphasizing the influence of magnesium, soil organic carbon, and alkali-hydrolysable nitrogen on shaping bacterial communities. Finally, in a very interesting study, Nemera *et al.* (2018) integrated molecular taxonomy data with soil property analyses, noting insignificant shifts in soil pH due to different treatments. This study highlighted the potential influence of chemical fertilizers in increasing total nitrogen and organic carbon levels, particularly from urea-based fertilizers.

Overall, the application of molecular taxonomy methods, including 16S rRNA gene sequencing, metagenomics, and functional gene analysis, has significantly advanced our understanding of soil microbial communities under diverse fertilization practices. These techniques provide insights into microbial diversity, functional potential, and how their interplay with soil properties is affected by various fertilizer applications

The reviewed literature showed variations in the conclusions drawn from the various studies. Some studies suggested that organic fertilizers promoted bacteria community diversification while others contradicted these findings. In Zambia and southern parts of Africa, there appears to be little in terms of literature to clarify the picture. This study was conducted to assess the impact of chemical fertilizers on soil bacterial diversity from selected maize fields in Lusaka.

CHAPTER THREE
MATERIALS AND METHODS

3.1 STUDY AREA

This study was carried out in Lusaka Province within a 50 km radius from the Lusaka Central Business District as depicted in Figure 3.1. Soil samples were collected from selected farms that either used chemical fertilizer or organic fertilizer in their maize fields.

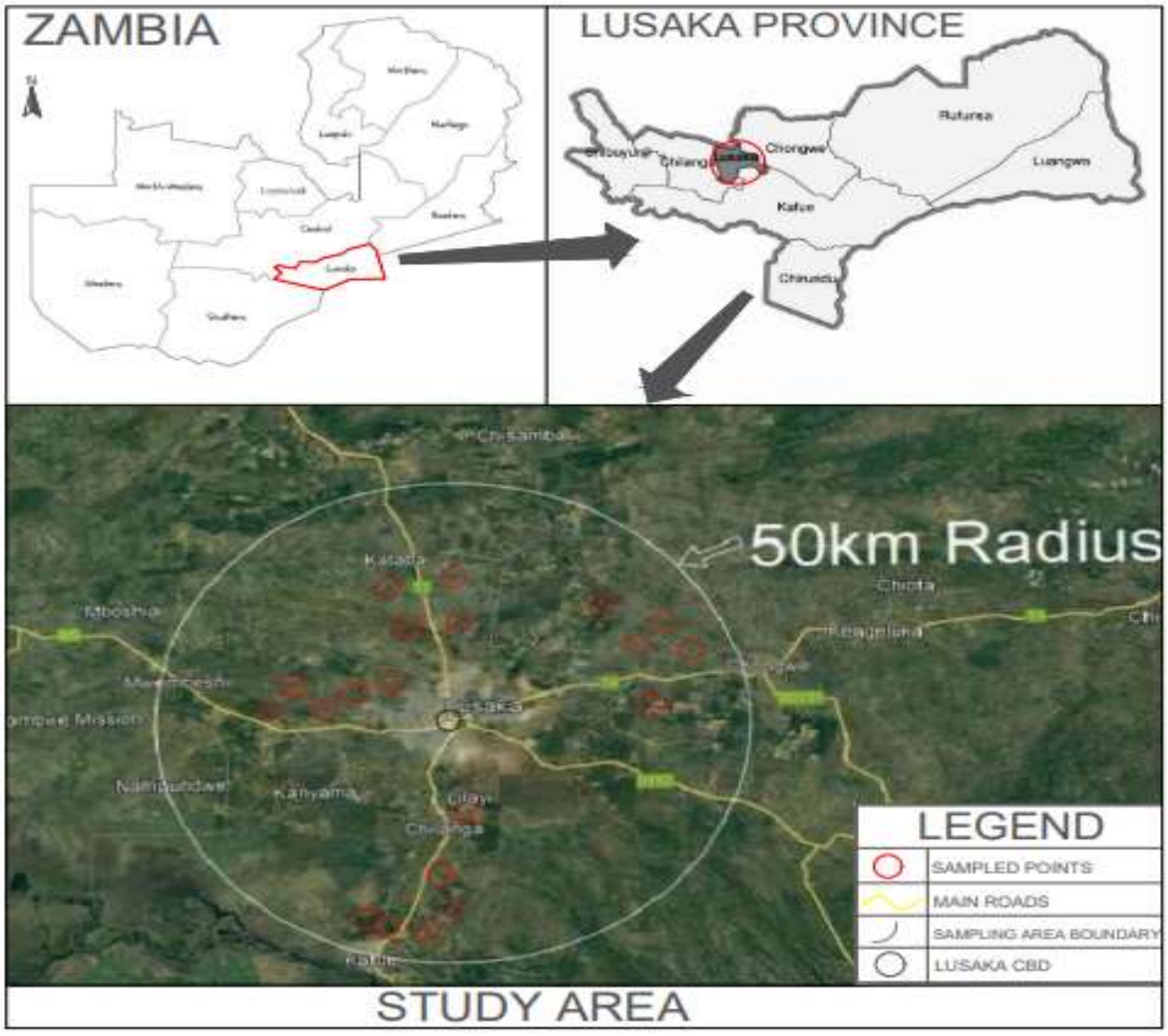


Figure 3.1: Location map of the study area showing soil sample collection points

Source: Google Maps (30th July, 2022.)

3.2 STUDY DESIGN

A *Chemical fertilizer* is defined as any inorganic material of wholly or partially synthetic origin that is added to soil to sustain plant growth, while *Organic fertilizers* are substances that are derived from the remains or byproducts of natural organisms which contain the essential nutrients for plant growth (Enebe *et al*, 2020). This study was a cross sectional study of soil samples from maize fields that were organically and inorganically fertilized. The samples were collected between May – June of 2022 after maize cultivation. Soil samples were collected across a transect from each field and two treatments were set for this study. These were: OF – organically fertilized maize fields (control group) where farmers utilized cow dung as the main form of manure and CF – Chemically fertilized maize fields (experimental group) where chemical fertilizers with the N, P, K composition of 10, 20, 10 was used.

Soil samples were collected from twenty selected maize fields, of which 16 soil samples were collected from chemically-fertilized fields and 4 from fields treated with organic fertilizers as shown in Table 3.1. Four soil samples were collected in the northern direction from chemically fertilized maize fields only. These were labeled North 1 – North 4. Four samples were collected from chemically fertilized maize fields in the eastern direction labeled East 1 – East 4, and one sample was collected from an organically-fertilized maize field labeled East 5. Four samples were collected from chemically fertilized maize fields in the western direction labeled West 1 – West 4, and one sample was collected from an organically fertilized maize field labeled West 5. Four samples were collected from chemically fertilized maize fields in the southern direction labeled South 1 – South 4 and two samples were collected from organically fertilized maize fields labeled South 5 and South 6.

Table 3.1: Selected soil sampling sites

Area	Number of maize fields sampled		Total
	Chemically fertilized	Organically fertilized	
North	**North 1 Chemical – North 4 Chemical**	**0**	**4**
East	**East 1 Chemical – East 4 Chemical**	**East 5 Organic**	**5**
West	**West 1 Chemical – West 4 Chemical**	**West 5 Organic**	**5**
South	**South 1 Chemical – South 4 Chemical**	**South 5 Organic & South 6 Organic**	**6**
Total	16	4	20

3.3 SOIL SAMPLING

Before sampling, the designated area was cleared of top organic matter, grass and other vegetation. Soil samples were randomly collected at 4 points in the field and homogenized into one sample from the top 0-15cm depth Ogbodo (2013) (Figure 3.2).

Figure 3.2. Selected chemically fertilized maize field from south of Lusaka Province, A and collection of soil samples from a chemically fertilized maize field in the southern direction B.

The sampling spatula was sterilized by spraying with 70 percent ethanol at each sampling point. Soil samples were passed through a mesh, which was sterilized after each soil sample by rinsing with 70 percent ethanol, to remove debris and stones and packaged in sterile Ziploc bags. The soil samples collected in each field were transported in a cooler box with ice packs and separated at the laboratory. One part was stored at 4°C and used for microbiological and molecular analysis. The other part was air-dried for 24hours and sieved using a 2-mm sieve (Figure 3.3).

Figure 3.3: Air dried soil samples collected from organically and inorganically fertilized maize fields used for this study before analysis (Picture taken by the author).

3.4 SOIL PHYSICOCHEMICAL PARAMETERS ANALYSIS

The collected soil samples were analyzed for physical and chemical soil quality parameters including temperature, pH, electrical conductivity (EC), organic carbon (OC), total nitrogen (N), available phosphorus (P) and potassium (K) using methods and equipment shown in Table 3.2.

Table 3.2: Methods used for testing soil physicochemical parameters

No.	Parameter (Units)	Method	Reference
1.	Temperature (°C)	Mercury thermometer	
2.	pH	pH meter	
3.	Electrical conductivity (mS/cm)	Electrical conductivity meter	
4.	Moisture content (%)	Moisture Analyzer	
5.	Organic matter (%)	Walkley & Black Method	(Walkley & Black, 1934)
6.	Total Nitrogen (%)	Macro Kjeldahl Method	(Kjeldahl, 1883)
7.	Available phosphorus (mg/kg)	Bray I Method	(Bray & Kurtz, 1945)
8.	Potassium (cmol/kg)	Atomic Emission	

3.4.1 Soil Temperature

The soil temperature was determined at the time of sampling using four mercury thermometers which were placed at four points around the area where samples were collected. Temperature was taken before collection of the soil samples at each site to achieve accurate recording of the temperature before any disturbances were made to the soil. Average temperature readings were recorded for each site.

3.4.2 Soil pH

Soil pH was measured by mixing the air-dried soil sample with (0.01M) of calcium chloride (1gram soil to 2.5 ml 0.01M calcium chloride) on a shaker for one hour and measured using a pH meter.

3.4.3 Soil Electrical Conductivity

A portion of 10 g air-dried soil was weighed and transferred to a 100ml plastic bottle and 50ml of distilled water was also added. The bottle was closed with a stopper and the mixture was shaken on the shaker for 1hr. The solution was filtered using a filter paper and the filtrate was collected for the measurement of electrical conductivity.

To measure electrical conductivity, the conductivity meter was prewarmed for 15 minutes, and calibrated using a solution of 0.01M KCl which gave a conductivity of 1.412mS/cm at 25°C.

3.4.4 Moisture Content

The soil moisture content was measured using a moisture analyzer (Shangai Shangpu instrument and equipment co. Ltd). From the air-dried soil samples, 10 g of each soil sample was weighed and placed in the moisture analyzer, after 10 mins, the reading was taken note of. The figures recorded were the soil moisture content.

3.4.5 Soil Organic Matter

To determine the soil organic matter, 1g of soil was weighed and transferred to an Erlenmeyer flask, 10ml of 1N potassium dichromate solution and 20ml of Qualify-concentrated sulfuric acid was added to the flask. The mixture was mixed gently for one minute in rotations without leaving soil residues on the sides of the flask. The mixture was then let to sit for thirty minutes then diluted with 200ml using deionized water. Then 10 mls of phosphoric acid, 0.2g ammonium fluoride and 10 drops of diphenylamine indicator were added to the solution.

The solution was back titrated using 0.5N ferrous ammonium sulfate solution. The color turned dull green with chromos ions at the beginning and then shifted to turbid blue as the titration proceeded. At the end point, the turbid blue solution sharply shifted to brilliant green.

3.4.6 Soil Potassium

Potassium was measured by adding 50 ml of 1M ammonium acetate to 10g of soil, and the mixture was shaken for 30 minutes on a shaker. Afterwards, the mixture was filtered and the filtrate was read using a calibrated atomic absorption spectrometer (Perkin Elmer A Analyst 400).

3.4.7 Soil Total Nitrogen

The soil sample was weighed (1g) and placed in a 500cm³ Kjeldahl flask. In an empty flask 0.03g of starch was added to make a blank sample. Thereafter, 10cm³ of concentrated sulphuric acid was added and the mixture was swirled thoroughly.

A catalyst mixture of Sodium Sulphate/Copper Sulphate and Selenium weighing 3g was added and the flask was placed on the Kjeldahl digestion stand where it was supported at an angle of less than 450°C from the horizontal to reduce the danger of bumping and spattering. In soils containing high amounts of organic matter, 1g of liquid paraffin was added to avoid frothing. The flask was heated cautiously until water was removed and frothing had ceased. The heat was increased and regulated so that the H_2SO_4 condensed about one-third of the way up the neck of the flask. After the digest had cleared, gentle boiling continued for 1hr. During that time, the flask was rotated on a stand at intervals.

The digest was then carefully transferred into a clean 100ml plastic container up to the fill-up mark. From the 100mls retained from above, 10mls was transferred into the distillation apparatus. Thereafter, 10ml of 10M NaOH was added into the same distillation apparatus and distilled for 5 minutes collecting the distillate in 20ml boric acid (H_3BO_3) indicator.

The distillate was titrated with 0.01M standard HCI until color changed from green to pink. The result was corrected by subtracting the titration value (cm³) of an equally treated blank using 0.03g of pure starch.

3.4.8 Soil Available Phosphorus

To test for phosphorus, the soil samples were sieved through a 2 mm sieve. After which, 3g of the soil sample was placed into a 15cm^3 centrifuge tube and 21cm^3 of the extracting solution was added.

The samples were shaken for 1 min on a mechanical shaker, there after the suspension was centrifuged at 2000rpm for 15 mins. This was followed by pipetting 5cm^3 of the supernatant into a 25cm^3 volumetric flask. Thereafter, 10cm^3 of distilled water was added.

This was followed by the addition of 4 cm^3 of reagent B and make up to volume with distilled water. The color was allowed to develop for 15 mins and there after P-content was determined in the solution on a spectrophotometer at 882nm.

A set of P-content standard solutions containing 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg dm^3 P were prepared.

The percentage (%) absorbance for all standard P-solutions was read and a calibration curve was drawn. The soil sample was read and the reading was extrapolated on the calibration curve to obtain mg dm^3 of P in the solution.

3.5 ISOLATION AND IDENTIFICATION OF BACTERIA FROM THE SOIL

3.5.1 Serial dilutions

The soil samples were serially diluted using sterile distilled water (Figure 3.4) at a ratio of 1g of soil to 10 mls sterilized distilled water (soil 1g: water 10ml) for the stock solution. 1ml of the stock solution was taken with a sterile micropipette and transferred into serial blanks containing 9mls distilled water. This procedure was repeated by adding 1ml from tube 1 to 9mls distilled water in tube 2, 1ml from tube 2 was added to 9mls in tube 3 and repeated for 5 tubes. Thereafter, 0.1ml samples were pipetted from selected dilutions and spread-plated onto nutrient agar Petri dish using a sterile L-shaped glass rod. The Petri dishes were incubated at 28°C for 24hours. Colony forming units were calculated using the formula below;

$$\text{CFU/ml} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{Volume of culture plated in ml}}$$

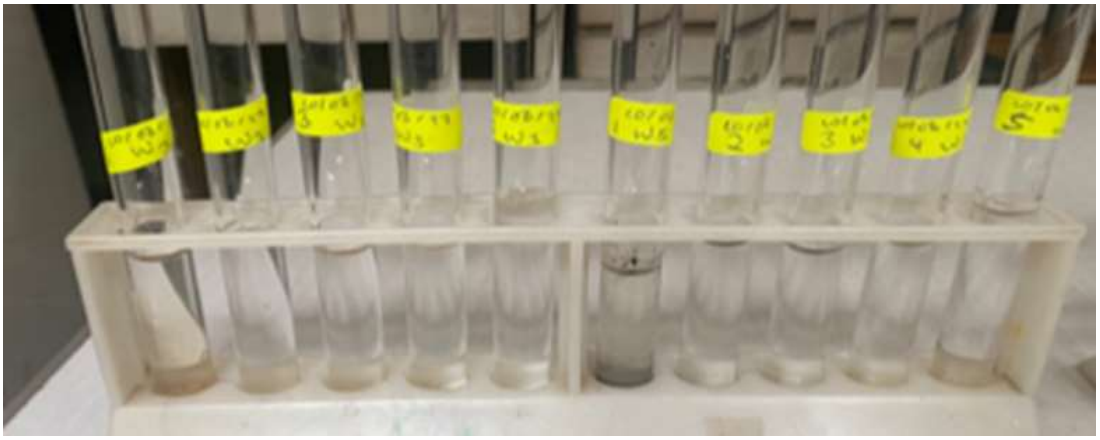


Figure 3.4: Serial dilutions for two of the samples collected from the western direction. Soil samples were diluted to produce dilutions ranging from 10^{-1} to 10^{-6}.

3.5.2 Sub-culturing and Gram staining

After 24 hours of incubation, the Petri dishes were observed for growth and unique colonies were sub-cultured for the purpose of making pure culture. The subcultures were Gram-stained.

3.5.3 Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from overnight cultures using a Qiagen DNA extraction kit (Inqaba Biotechnical Industries (Pty) Limited, (South Africa). A cell suspension was formed by suspending the bacterial colonies in nuclease free-water to facilitate the DNA extraction. Thereafter, 20µl of proteinase K, and 200 µl of the lysis buffer (AL) was added to each bacterial suspension and vortexed and spun down to remove the splashes from the lid to the bottom followed by incubation at 56°C for 10minutes. This was followed by the addition of 70 percent ethanol for precipitation. The contents were added to the spin column of a silica membrane to which the DNA binds and columns were spun at 3.914 x g for 1minute. This was followed by the addition of 500 µl of AW1 and AW2 to the columns to wash the DNA which was eluted using buffer AE into new eppendorf tubes. The DNA quality was measured using a NanoDrop Q5000 by using 1µl of the DNA. The NanoDrop Q5000 was blanked and calibrated using AE buffer after every 4 readings.

3.5.4 Polymerase Chain Reaction

PCR was performed using the one Taq quick load 2x master mix made by biolabs (Inqaba Biotechnical Industries (Pty) Limited, (South Africa). The PCR master mix comprised of 10 µl of one *Taq* quick load 2x master mix, 6 µl of nuclease free water, 1µl of 16S rRNA forward primer, 1µl of 16S rRNA reverse primer (Table 3.2) and 2 µl of the DNA template. The PCR thermal cycling conditions were as follows; initial denaturation was done at 95°C for 4minutes, the next 40 cycles were at 95°C for 40 seconds, annealing at 58°C for 1 minute and extension at 4°C for 1 minute repeated for all 40 cycles. The final extension was done at 72°C for 7 minutes and rest at 4°C to infinity. The primers used were as shown in Table 3.3 below;

Table 3.3: 16S rDNA primer sequences (Inqaba Biotechnical Industries (Pty) Limited, (South Africa).

Primer	**Sequence**
16s F	5’ – AGAGTTTGGATCCTGGCTCAG – 3’
16s R	3’ – CGCCGACCTAGTGGAGGA – 5’

3.5.5 Determination of DNA Quality Using Agarose Gel Electrophoresis

One percent agarose gel was prepared by transferring 1g of agarose powder into 100 ml of 1X TAE buffer (Biological Industries Israel Beit Ltd (Israel) which was heated to melt the agarose in a microwave oven. Ethidium bromide was added to a final concentration of 0.5 µg/ml to the melted agarose and the gel was transferred to a gel-casting tray where it was let to solidify. After which, 4µl of the PCR products was loaded onto the gel and the electrophoresis was run at 100 V for 30 minutes. The ladder marker used was a 1000 bp ladder (Inqaba Biotechnical Industries (Pty) Limited, (South Africa). The results were viewed and photographed using a UV transilluminator.

3.5.6 Purification of PCR Product

The PCR products were purified using a Zymo DNA Clean and concentrator (Zymo Research). In a 1.5ml microcentrifuge tube, 500µl of DNA binding buffer was added to 100µl of the PCR product. The solution was mixed briefly by vortex. The mixture was transferred to the Zymo-spin column in a collection tube and centrifuged for 30seconds. The flow through was discarded and this was followed by the addition of 200µl of DNA buffer to the column. The solution was centrifuged for 30seconds and the wash step was repeated. Thereafter, 6ul of DNA elution buffer was added to the column matrix and the solution was incubated at room temperature for 1minute. The column was transferred to a 1.5ml microcentrifuge tube and centrifuged for 30seconds to elute ultra-pure DNA.

3.5.7 Brilliant Dye PCR

The master mix for this PCR was prepared for the forward and reverse reaction. The forward master mix consisted of 1µl brilliant dye terminator v 3.1, 3.5µl 5X sequencing buffer, 0.375 10µM of 16s forward primer, 12.18µl of nuclease free water and added 3µl of the template. The reverse master mix consisted of 1µl brilliant dye terminator v 3.1, 3.5µl 5X sequencing buffer, 0.375 10µM of 16s reverse primer, 12.18µl of nuclease free water and added 3µl of the template. PCR was run under the following conditions; the first denaturation was at 96°C for 45 seconds, the next 30 cycles were at 96°C for 10second, annealing at 50°C for 5seconds, extension at 60°C for 2minutes, which was repeated for 30 cycles and the final extension was at 4°C till infinity.

3.5.8 Sequencing Reactions

The precipitation reactions were done by adding 2µl of 125mM EDTA and 2µl of 3M Na Acetate to the big dye products. The solution was mixed by tapping. After tapping, 90µl of molecular grade

ethanol was added to the mixture and the mixture was incubated for 10minutes in a dark room at room temperature. This was followed by centrifuging the mixture for 20minutes at 4°C at the speed of 0.5873 x g.

Afterwards, the supernatant was removed and 20µl of 70 percent ethanol was added, without mixing, the mixture was centrifuged at 0.5873 x g for 10minutes at 4°C. The supernatant was removed and 70 percent ethanol was added to the mixture and centrifuged again at 0.5873 x g for 10minutes at 4°C. The samples were dried in the vacuum dryer for 10minutes while covered with aluminum foil. After the vacuum drying, 20µl of HIDI (highly deionized) formamide was added, then vortexed and denatured at 95°C for 2 minutes and extension at 4°C till infinity. The products of the sequencing reactions were added in the sequencing plate in the SeqStudio Genetic Analyzer (Thermo Fisher Scientific).

3.6 STATISTICAL ANALYSIS

Analysis of variance was carried out using Minitab (2017). RStudio was used to test the significance, standard deviation and standard error in means between parameters in organically fertilized soils against chemically fertilized soils.

CHAPTER FOUR

RESULTS

4.1 PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL SAMPLES FROM SELECTED MAIZE FIELDS

Data were collected to determine selected physical properties including temperature, pH, Electrical conductivity, moisture content and organic matter. The chemical properties included phosphorus, potassium and nitrogen. Excel was used to generate graphs, charts and results are discussed below.

4.1.1 Temperature

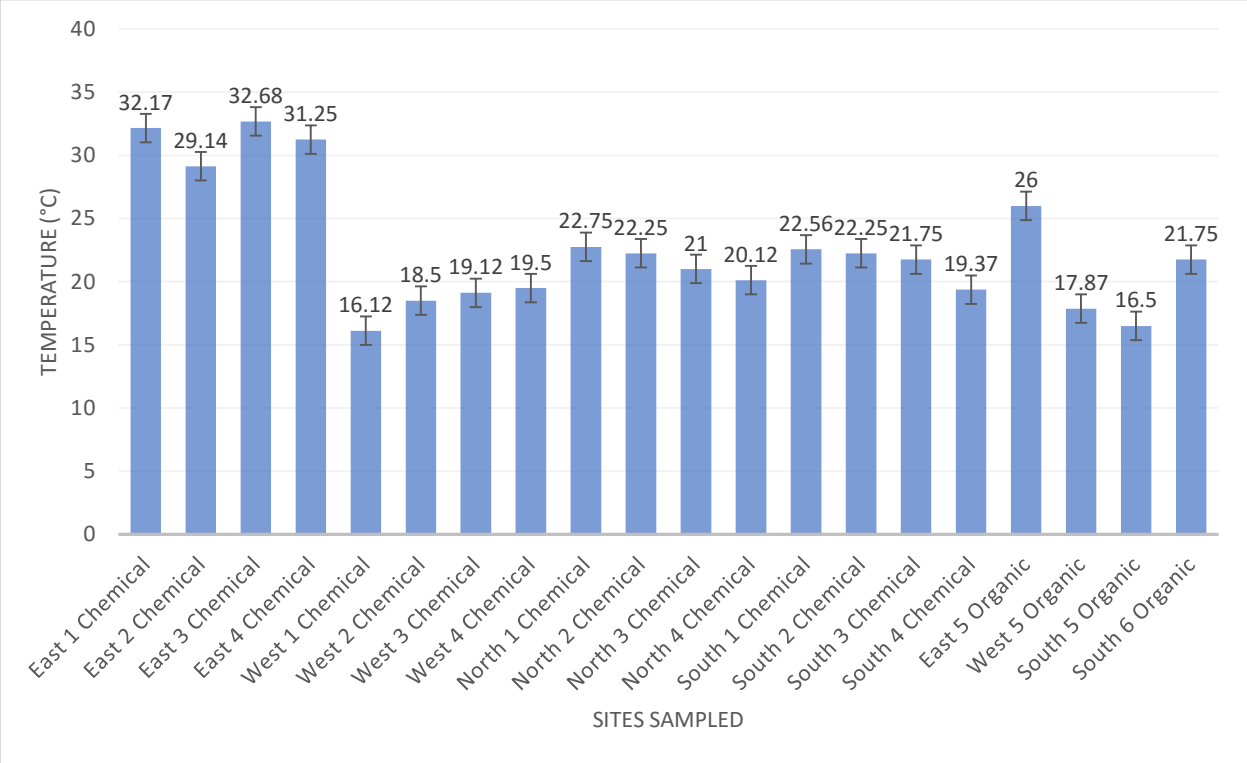


Figure 4.1: Soil temperatures recorded at each selected sampling site.

The average temperatures measured at each sampling point based on the type of fertilization used are shown in figure 4.1. Bars labeled “Chemical” represent temperature of soil samples collected from fields that were treated with chemical fertilizers while those labeled “Organic” represent temperature of soil samples collected from fields that were treated with organic fertilizers. Temperatures did not vary much in organically fertilized soil and chemically fertilized soils except

for the four chemically fertilized soil samples collected in the eastern part of Lusaka Province with the highest temperature from the fields sampled being 32.68°C. ANOVA using Minitab showed that there were no significant differences in the soil temperature in chemically fertilized soils and organically fertilized soils.

4.1.2 pH

Figure 4.2: Soil pH levels recorded in organically and chemically fertilized soil samples in selected maize fields

Results in Fig 4.2, showed that most chemically fertilized soils tended to have a pH lower than 6.8 which implied they were acidic soils while organically fertilized soils were all observed to have pH ranging from 6.7-7 which implied, they were neutral soils. From the analysis of variance of the pH calculated using Minitab, significant differences in pH were recorded in soils treated with the two different fertilizer types. Soils sampled from organically fertilized soils had a higher pH as compared to those from inorganically fertilized soils.

4.1.3 Electrical conductivity

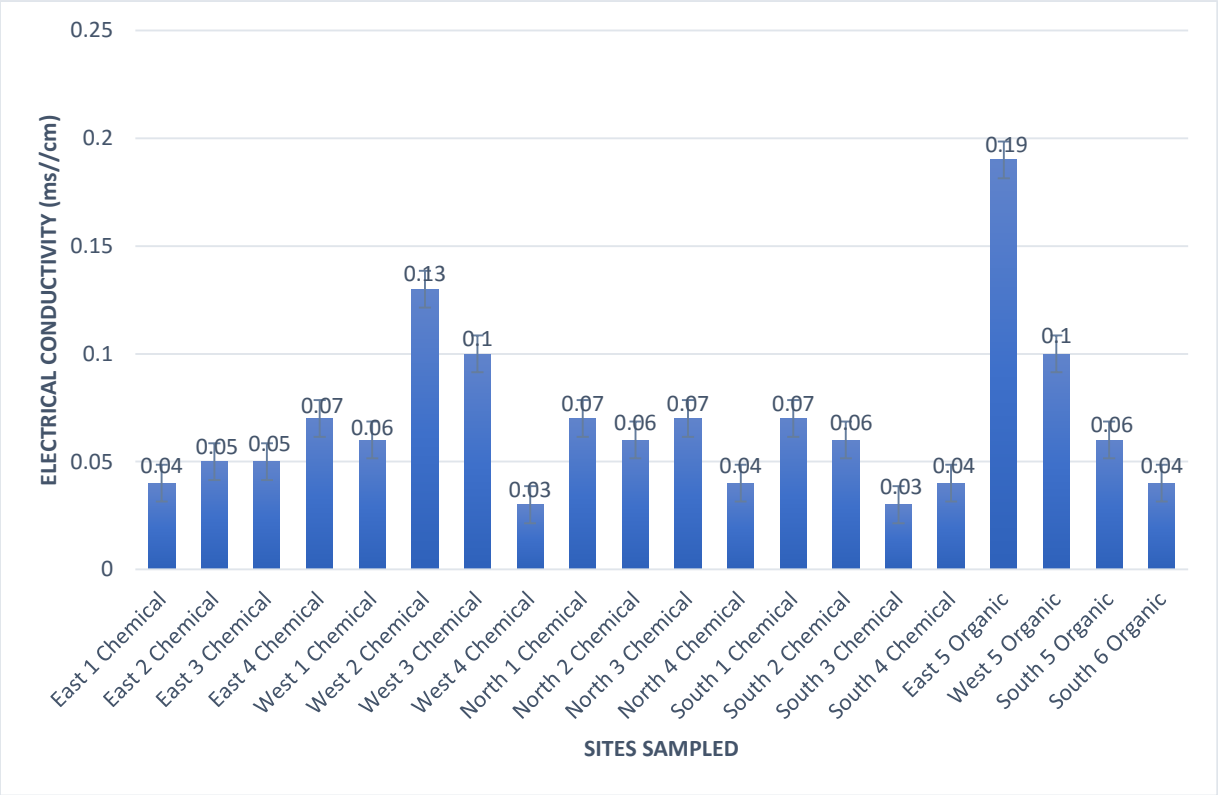


Figure 4.3: Electrical conductivity recorded in organically and chemically fertilized soil samples in selected maize fields

The soil electrical conductivity varied based on location of sampling site as shown in figure 4.3. It was observed through ANOVA (Minitab) that there are significant differences EC in soils of the two different fertilizer treatment types. The electrical conductivity in soil samples from organically fertilized fields had a higher mean than the electrical conductivity in chemically fertilized soils. As seen in Figure 4.3, soil samples from organically fertilized soils had a higher electrical conductivity compared to those from chemically fertilized soils.

4.1.4 Moisture content

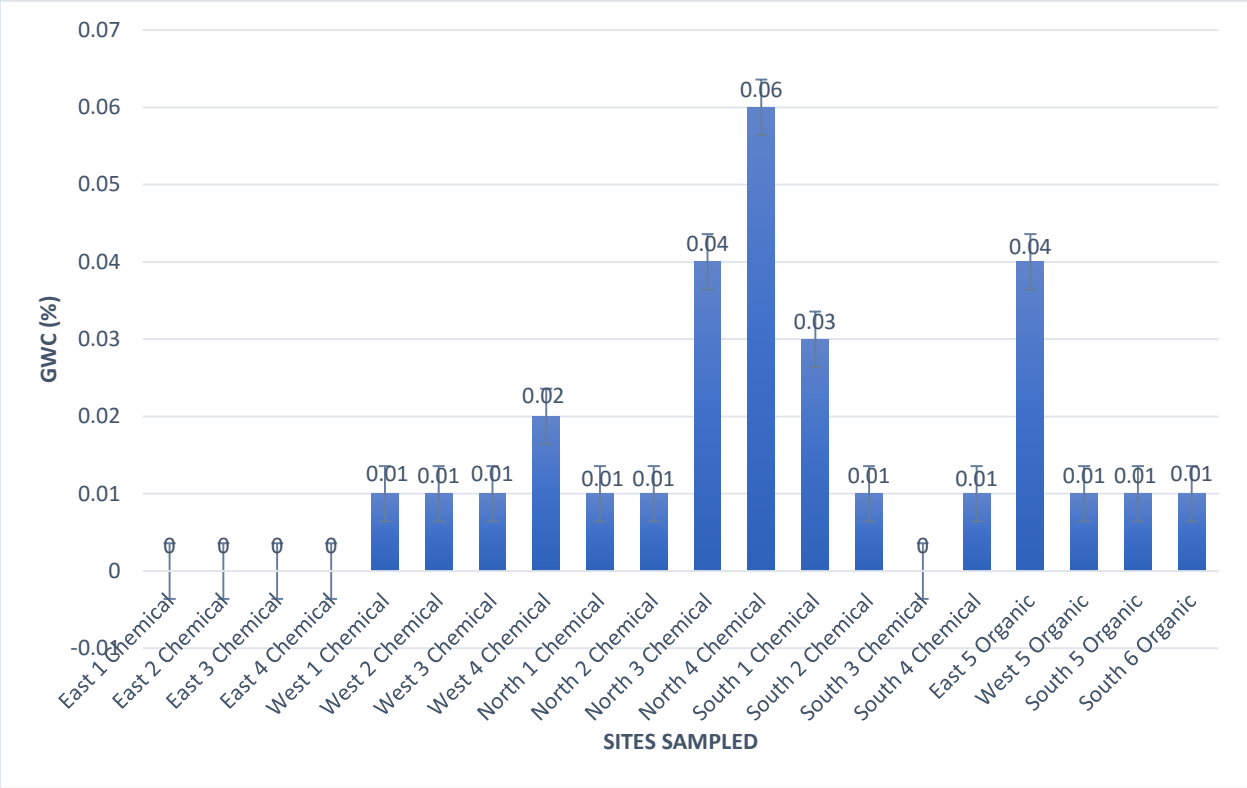


Figure 4.4: Moisture content recorded in organically and chemically fertilized soil samples in selected maize fields

According to Fig 4.4, after conducting ANOVA, the results above showed that there were significant differences in the soil moisture content in the two treatments.

4.1.5 Organic matter

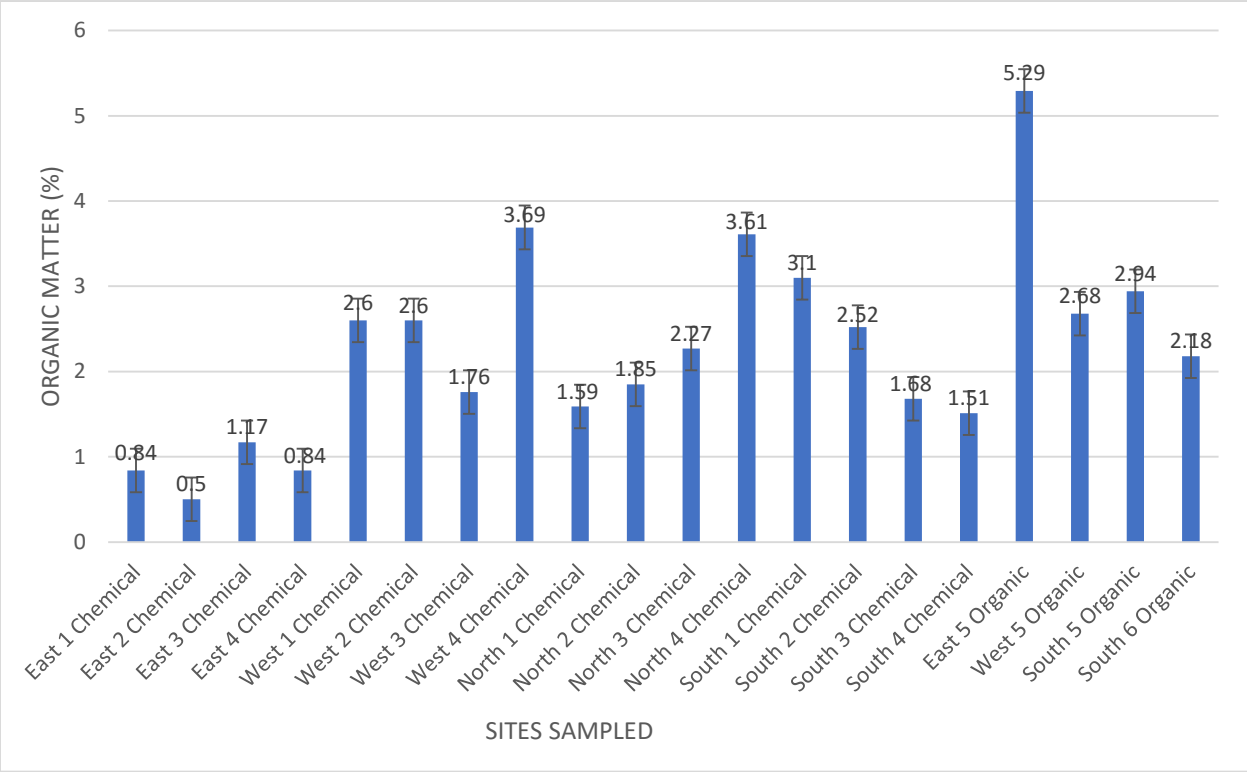


Figure 4.5: Soil organic matter content in recorded in organically and chemically fertilized soil samples in selected maize fields

From the information in Fig 4.5, ANOVA showed that there are significant differences in organic matter in the two treatments. The organic matter content had a higher mean value in organically fertilized soils than in chemically fertilized soils. This simply means that soil samples from fields that used organic fertilizer had a higher content of carbon in the soil unlike those from chemically fertilized fields.

4.1.6 Available Phosphorus

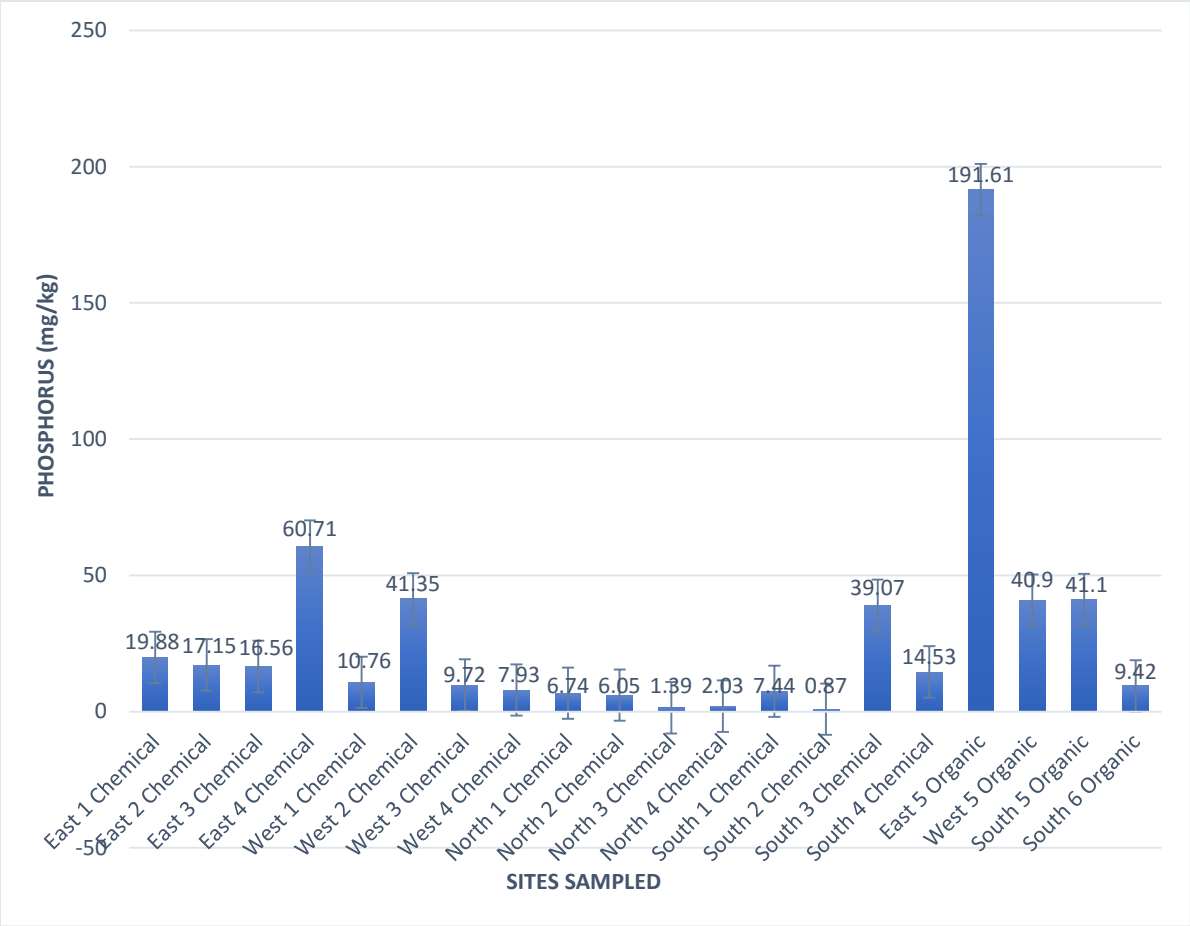


Figure 4.6: Available phosphorus values recorded in organically and chemically fertilized soil samples in selected maize fields

According to Fig 4.6 above, the available phosphorus was higher in organically fertilized soils with an exception of one chemically fertilized field in the eastern direction which had a significant difference in the level of available phosphorus as well as one organically fertilized field that had a significantly low phosphorus content level. The results above, an ANOVA showed that there were significant differences in the two treatments. Organically fertilized soils had a higher available phosphorus content in the soil as compared to samples from chemically fertilized fields.

4.1.7 Total Potassium

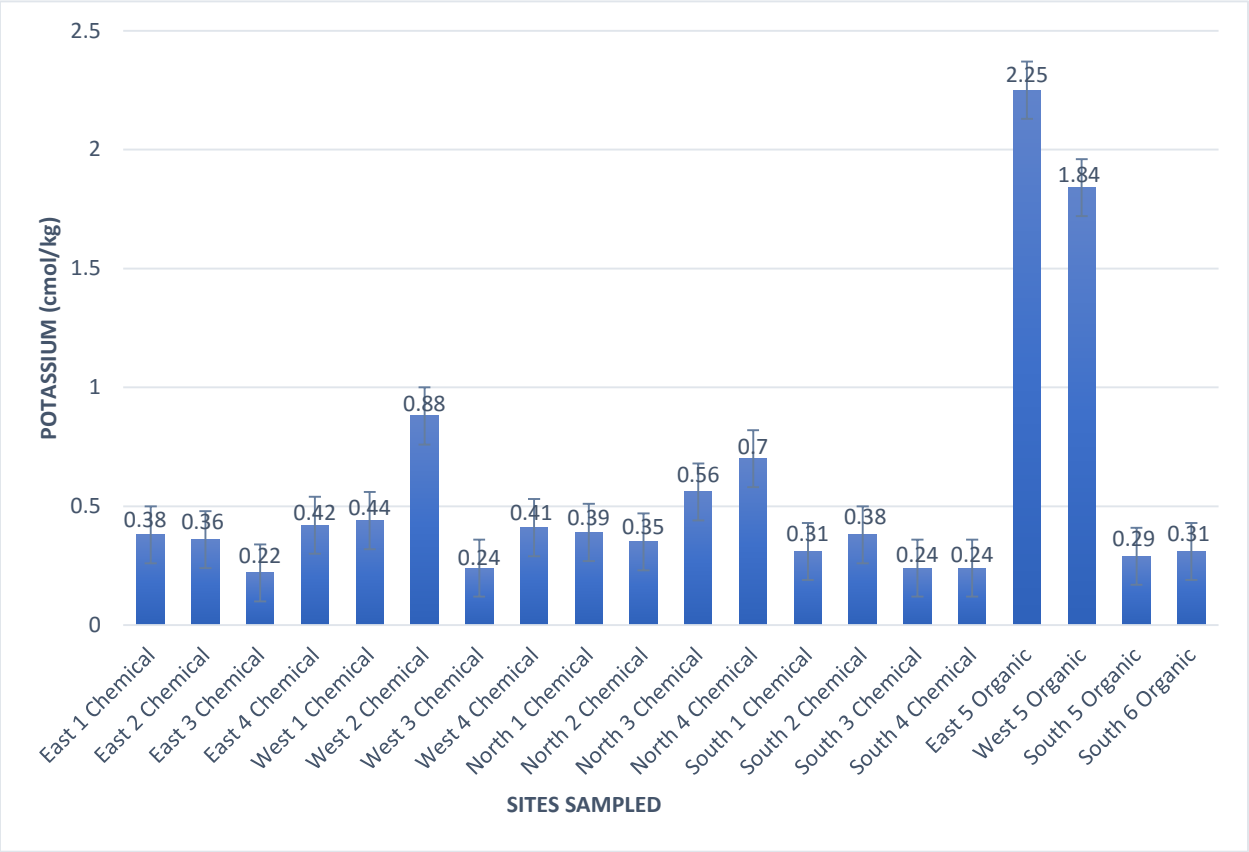


Figure 4.7: Variation of potassium values recorded in organically and chemically fertilized soil samples in selected maize fields

After statistically analyzing the results from Fig 4.7 above, it was equally observed that there were significant differences in the potassium content of the two treatments. Organically fertilized soils had a higher potassium content in the soil as compared to samples from chemically fertilized fields.

4.1.8 Total Nitrogen

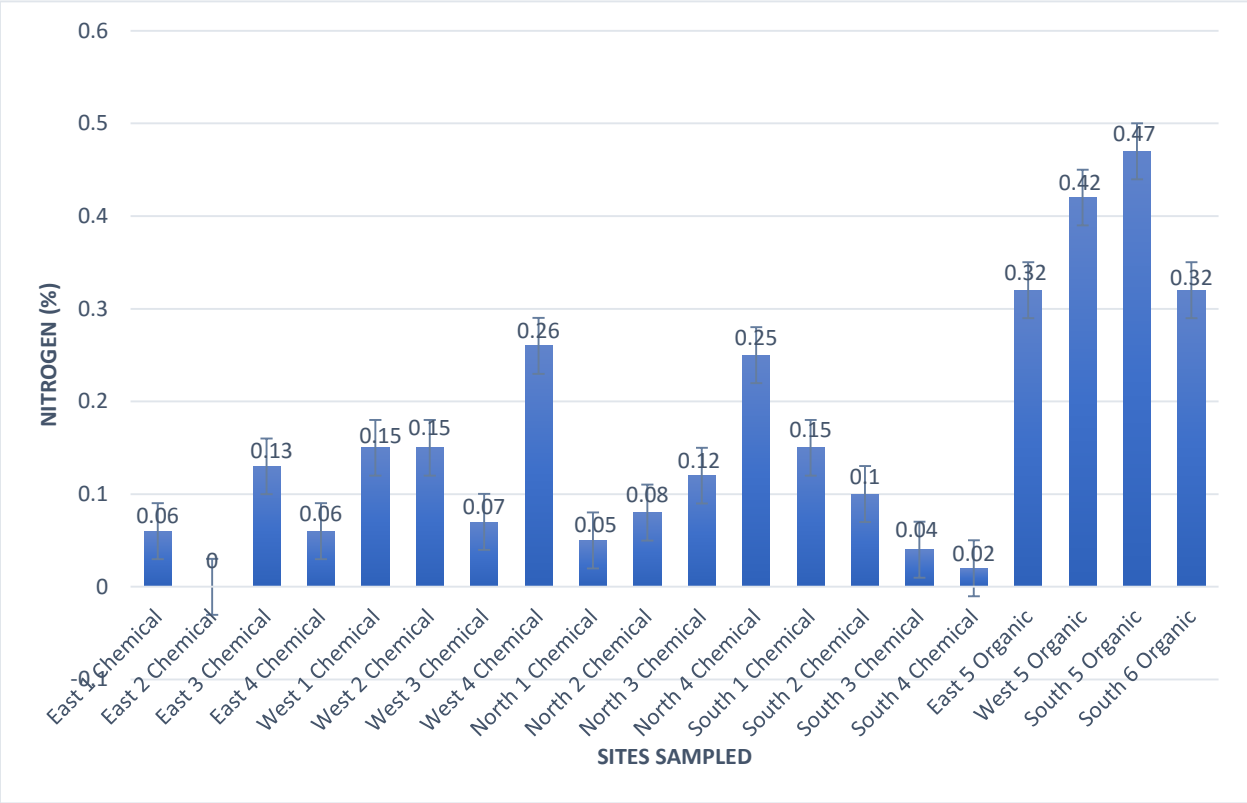


Figure 4.8: Variation of nitrogen values recorded in organically and chemically fertilized soil samples in selected maize fields

According to Fig 4.8, significant differences were equally observed in the nitrogen content of the soils from the two treatments. Organically fertilized soils had a higher total nitrogen content in the soil as compared to samples from chemically fertilized fields.

4.2 MICROBIOLOGICAL ANALYSIS

4.2.1 Bacterial Population

According to Figure 4.9, the bacterial populations in organically fertilized maize fields were observed to have colony forming units ranging from $8.5x10^7$ cfu/ml - $18.4x10^7$ cfu/ml while colony forming units in chemically fertilized maize fields were observed to range from $3.3\ x10^7$ cfu/ml - $18.6\ x10^7$ cfu/ml.

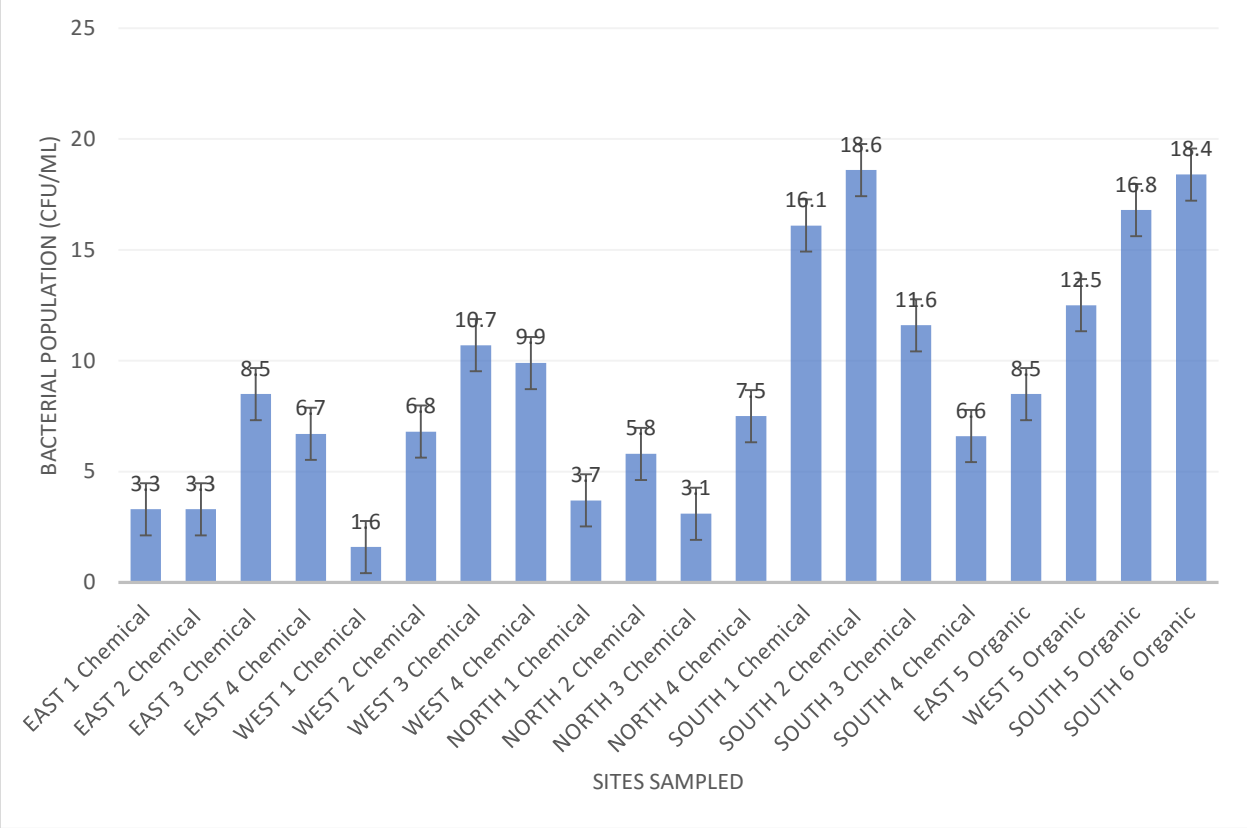


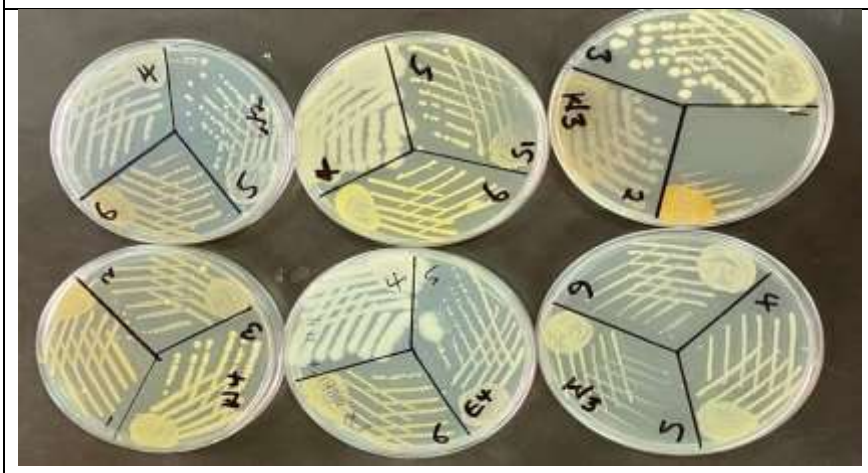
Figure 4.9: Colony forming units at $x10^7$ showing bacterial populations observed in organically and chemically fertilized soil samples from selected maize fields in the study.

It was observed that chemically fertilized soil samples had high bacterial populations of the same type of bacteria while those from organically fertilized fields had a wider variety of bacteria noticed through morphological appearance such as the color of the colony, texture of the colony, how it tinted the agar on the petri dish and the gram stain and shape observed under a microscope.

4.2.2 Colony Characterization

Bacterial diversity was determined by observing the different types of colonies on nutrient agar plates from different sampling sites. Colonies were characterized based on texture, color, morphology and tint of colonies on agar plates.

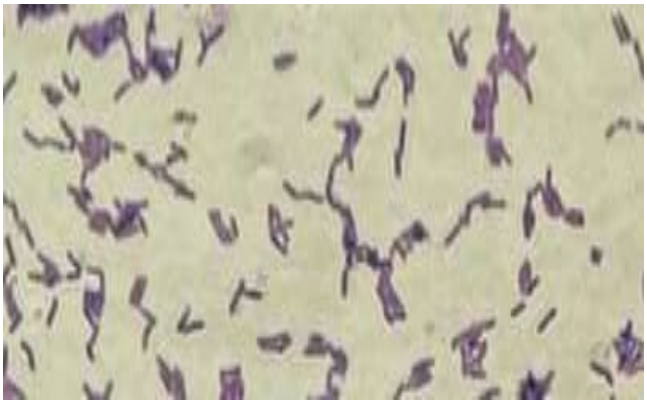
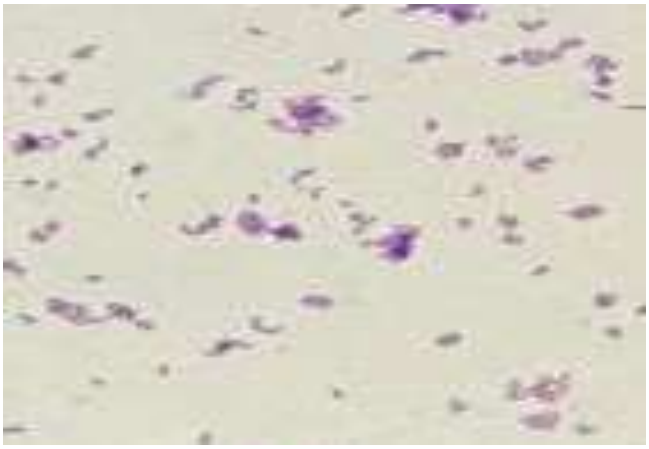
Table 4.1: Colony characterization of bacteria isolated from organic and chemically fertilized maize fields.

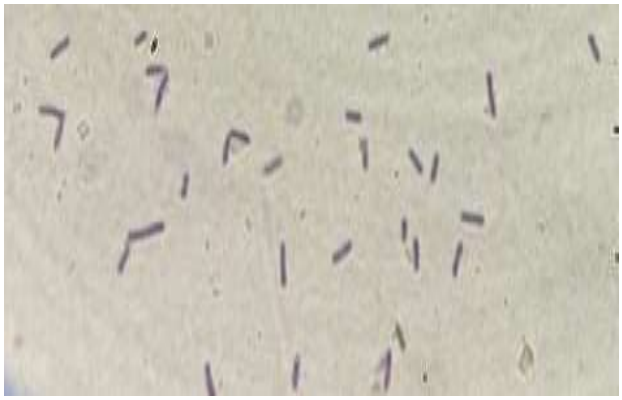
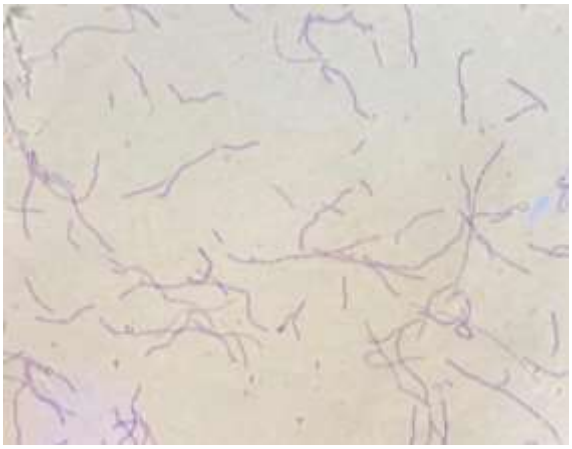
RESULTS	COMMENTS
	Nutrient Agar plates after incubation at 21°C for 24 hours
	Pure colonies after re-streaking selected bacterial colonies. Approximately 30 colonies were re-streaked.

4.2.2.1 Gram staining

The isolated cultures were gram stained as a first step for bacterial grouping. From the images below in Table 4.5, it was observed that the bacteria cultured and Gram stained from chemically fertilized soil samples and organically fertilized soil samples were predominantly gram-positive bacteria.

Table 4.2: Gram-staining results of selected bacteria isolated from organic and chemically fertilized maize fields.

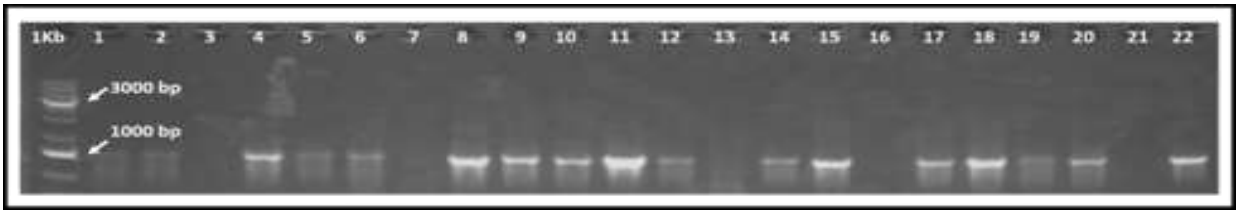
RESULTS	COMMENTS
	Image of *Bacillus amyloquefaciens* isolated from chemically fertilized maize fields. Gram positive Bacillus (rod-shaped) Magnification = x1000
	Image of *Lysinibacillus fusiformis* isolated from chemically fertilized maize fields Gram positive Bacillus (rod-shaped) Magnification = x1000

	Image of *Priesia aryabhatari* isolated from both organically and chemically fertilized maize fields. Gram positive Bacillus (rod-shaped) Magnification = x1000
	Image of *Actinobacteria* viewed at 100X isolated from organically fertilized maize fields Gram positive Bacillus (rod-shaped) Magnification = x1000

4.3 BACTERIAL MOLECULAR ANALYSIS

4.3.1 Gel Electrophoresis Results

Genomic DNA was analyzed on 1 percent agarose and its concentration assessed by NanoDrop spectrophotometer. The DNA was then PCR amplified using the 16S rRNA gene-targeting primers and the amplicons were analyzed on 1 percent agarose and viewed under a gel documentation system and the results presented in Figure 4.10. All of the samples produced an amplicon of slightly lower than 1000 bp except for samples 3, 7, 13, 16 and 21. The PCR was repeated and similar results with better amplification were obtained for samples 1 to 3 and successful amplification for the sample 7 and 21.



A

B

Figure 4.10. Agarose gel electrophoresis analysis of first PCR amplification of genomic DNA A, and repeat PCR, B for samples extracted from bacteria isolated from organically-fertilized (lanes 2, 7 and 11), chemically fertilized (lanes 1, 3, 4, 9, 15, 16, 17, 20 and 22) and from both organically and chemically fertilized (lanes 5, 6, 8, 14, 19, 21) selected maize fields in Lusaka Province.

4.3.2 Identification of Isolated Bacteria

A total of 22 samples were sequenced, out of which, only 17 were successfully assembled into contigs of acceptable sizes. When these were analyzed by the basic local alignment search tool (BLAST) at the NCBI database (www.ncbi.nlm.nih.gov) they enabled the identification of bacteria to species level at 98 percent identity. From those 17, 9 were from chemically fertilized soil samples, namely *Exiguobacterium auratiacum*, *Bacillus anthracis*, *Gottfriedia acidiceleris*, *Microbacterium paludicola*, *Bacillus amyloliquefaciens*, *Lysinibacillus fusiformis*, *Solibacillus silvestris*, *Bacillus subtilis* and *Peribacillus frigoritolerans*, while 3 were from organically

fertilized soil samples, namely *Arthrobacter*, *Actinobacteria* and *Planococcus rifietoensis* whereas 5 were from both organic and chemically fertilized soil samples, namely *Neobacillus drentensis*, *Staphylococcus succinus*, *Kocuria posea*, *Priestia aryabhatari* and *Bacillus proteolyticus*.

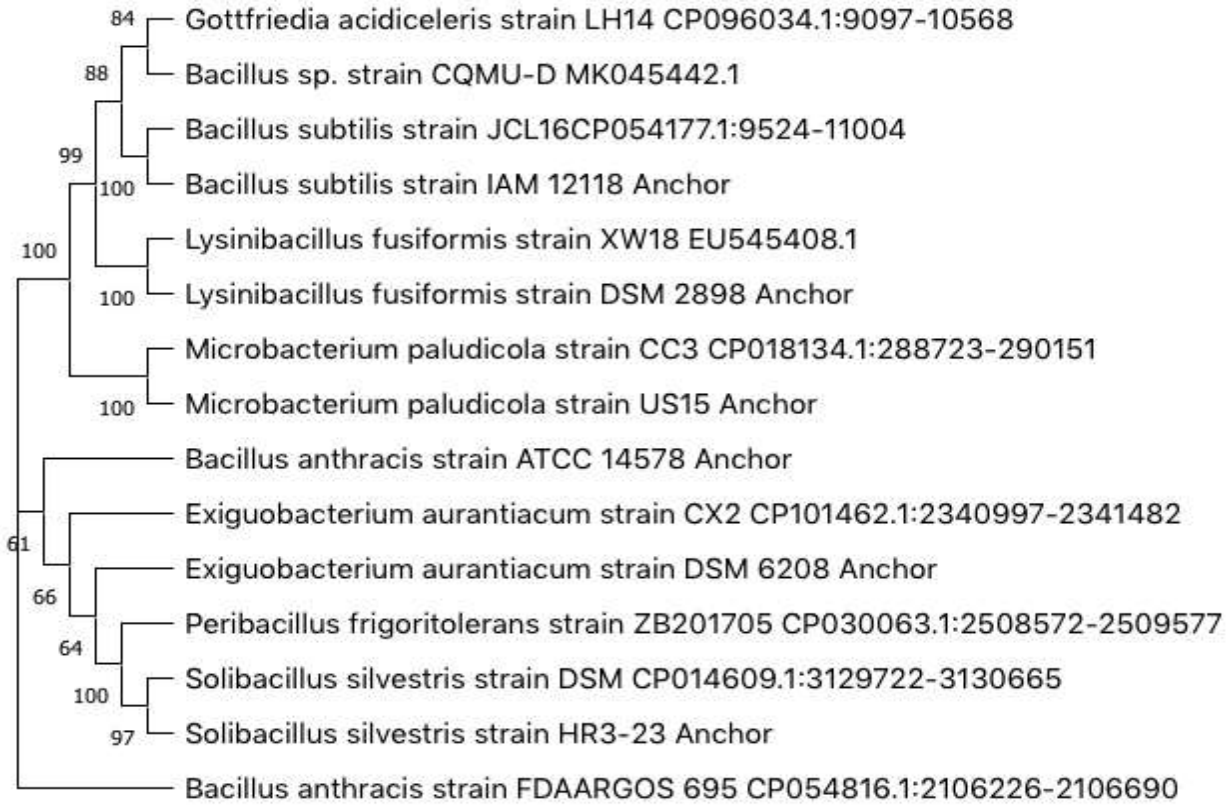


Figure 4.11: Neighbor-joining phylogenetic tree with a bootstrap value of 1000 iterations based on sequences identified from chemically fertilized maize fields during the study and anchoring sequences from the worldwide web.

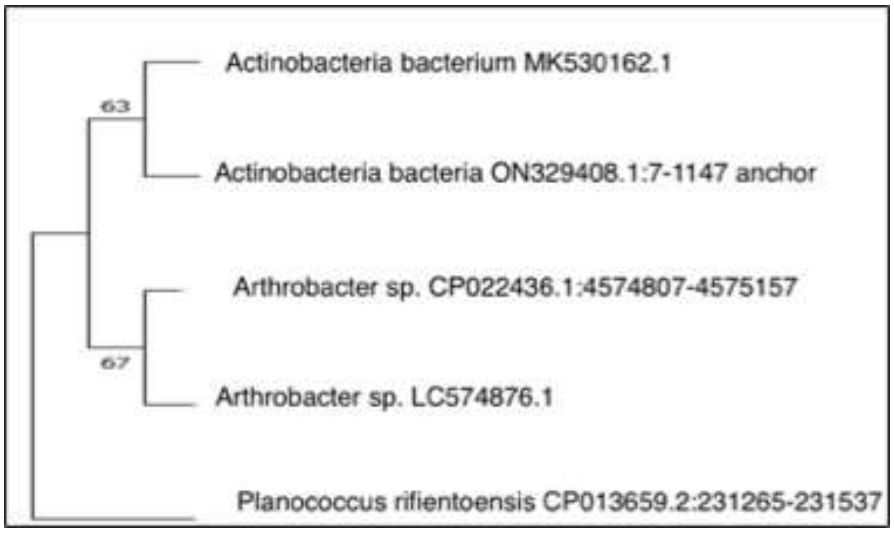


Figure 4.12: Neighbor-joining phylogenetic tree with a bootstrap value of 1000 iterations based on sequences identified from organically fertilized maize fields during the study and anchoring sequences from the worldwide web.

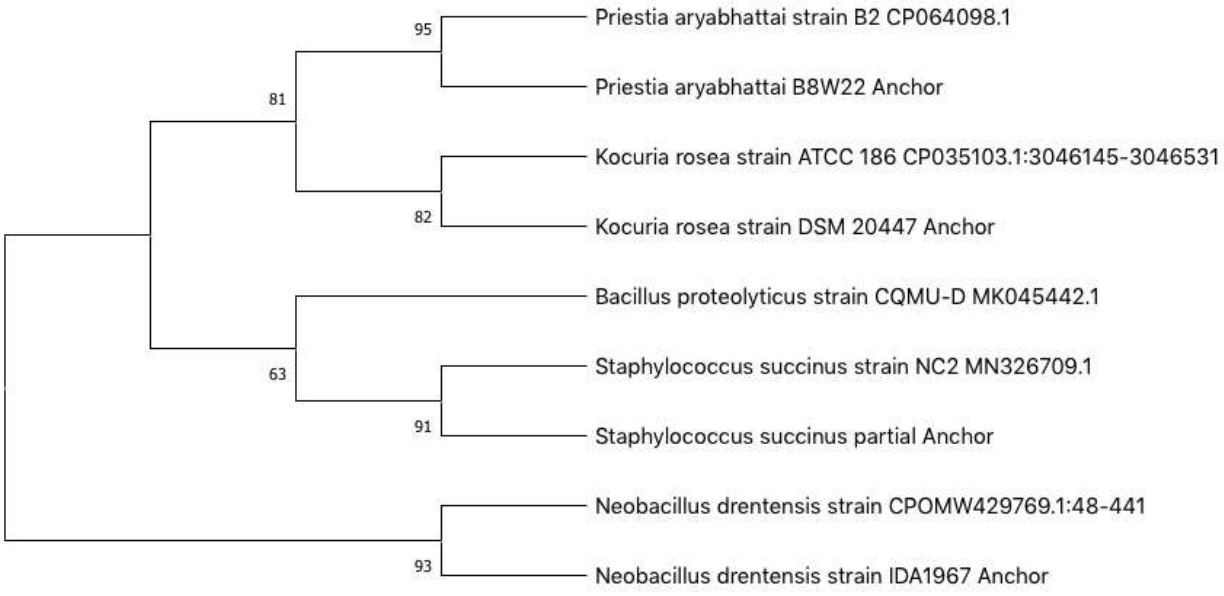


Figure 4.13: Neighbor-joining phylogenetic tree with a bootstrap value of 1000 iterations based on sequences identified from chemically and organically fertilized maize fields during the study and anchoring sequences from the worldwide web.

The phylogenetic tree generated using bacterial isolates from chemically fertilized soils in figure 4.12 showed the formation of 3 paraphyletic clades which divide further to form 9 monophyletic clades. The majority of the bacterial isolates from chemically fertilized maize fields appeared to be Gram positive bacteria belonging to the phylum Bacillota, class Bacilli, order Bacillales dividing into different family, genus and species such as *Bacillus anthracis*, *Bacillus amyloliquefaciens*, *Lysinibacillus fusiformis*, *Solibacillus silvestris*, *Bacillus subtilis* and *Peribacillus frigoritolerans*.

The phylogenetic tree generated using bacterial isolates from organically fertilized maize fields in figure 4.13 showed the formation of 1 monophyletic clade and 1 paraphyletic clade with an abundance of Bacillus bacteria with some that belong to Phylum Actinomycetota such as *Actinobacteria* and *Arthrobacter*.

The phylogenetic tree generated using bacterial isolates that appeared in both chemically and organically fertilized maize fields in figure 4.14 showed the formation of 1 monophyletic clade and 1 paraphyletic clade with an abundance of gram-positive bacteria belonging to the phylum Bacillota and a few that belong to Phylum Actinomycetota such as *Kocuria rosea.* According to the phylogenetic tree generated, it was observed that the bacterial species isolated were of the same ancestral lineage as those retrieved from the worldwide web. Most of the bootstrap values were observed to be above 70 percent with a few ranging between 60–70 and showed high bootstrap confidence levels.

4.4 STATISTICAL ANALYSIS

Statistical analysis was carried out using Minitab for the analysis of variance and RStudio was used to calculate the significance at $p \leq 0.05$ as well as the standard deviation and standard error of means in organically and chemically fertilized maize fields.

Table 4.4.1: Fertilizer treatment means of selected Biological, Chemical and Physical characteristics of soil under organic and inorganic fertilizer treatments.

Parameter	**Fertilizer Treatment Means**		**T-test at p≤0.05**
	Chemical	**Organic**	
Available Phosphorus (mg/kg)	16.39	70.82	A B
CFU (cfu/ml)	86.42	140.57	A A
E. C (ms/cm)	2.01	3.27	A B
Moisture content (%)	0.01	0.02	A B
Organic matter (%)	0.11	0.38	A B
pH	5.58	6.72	A B
Temperature (° C)	23.16	20.53	A A
Total Nitrogen (%)	0.11	0.38	A B
Total Potassium (cmol/kg)	0.41	1.17	A B

Mean ± SE in columns followed by the same letter do not differ significantly according to Tukey's test at $p \leq 0.05$.

From the table 4.3 above, it can be concluded that temperature and colony forming units had no significant differences in their means while the other parameters analysed had significant differences in their means. This implies that colony forming units and temperature do not influence affect bacterial communities.

Table 4.4.2: Standard deviation and Standard error of means of selected Biological, Chemical and Physical characteristics of soil under organic and inorganic fertilizer treatments.

Parameter	**Mean**	**Standard Deviation**	**Standard Error**
Available Phosphorus	0.3860	0.32844770	0.073443139
CFU	97200000	51802865	11583473
Electrical conductivity	0.0680	0.03819617	0.008540923
Moisture Content	0.0085	0.004893605	0.001094243
pH	5.8155	0.74235383	0.165995363
Organic Matter	5.5050	1.36013738	0.304135963
Temperature	22.6325	5.043419	1.127743
Total Nitrogen	1.8550	0.95226876	0.212933768
Total Potassium	29.2875	13.25907271	2.964818791

The average pH level is 5.82, with a standard deviation of 0.74, indicating moderate variability around the mean. The standard error of 0.17 suggests that the mean pH is estimated with moderate precision.

The average electrical conductivity is 0.068, with a low standard deviation of 0.038, indicating low variability, and a standard error of 0.0085, indicating high precision in the estimated mean.

The average organic matter is 5.51, with a standard deviation of 1.36, suggesting moderate variability, and a standard error of 0.304, indicating moderate precision.

The average total nitrogen content is 1.86, with a standard deviation of 0.95, indicating moderate variability, and a standard error of 0.213, suggesting moderate precision.

The average available phosphorus content is 0.39, with a standard deviation of 0.328, indicating moderate variability, and a standard error of 0.073, indicating moderate precision.

The average potassium content is 29.29, with a high standard deviation of 13.26, indicating high variability, and a standard error of 2.965, suggesting moderate precision.

The mean CFU at concentration 5 is 97,200,000, indicating the central tendency of CFU measurements, with a high standard deviation (SD) of 51,802,865 suggesting significant variability, and a standard error (SE) of 11,583,473 reflecting a relatively precise mean estimate due to the large sample size. The average temperature recorded is 22.63°C, with a moderate SD of 5.043 indicating some fluctuations, and SE of 1.128 showing that the mean temperature is estimated with reasonable precision. The mean moisture content is 0.0085, reflecting a low overall moisture content, with a low SD of 0.0049 indicating consistent measurements, and SE of 0.0011 showing the mean is estimated very precisely, suggesting individual measurements are close to the mean

Overall, the data shows varying levels of variability and precision across different chemical properties. Variables like Electrical conductivity show low variability and high precision, while variables like potassium show high variability with moderate precision.

This suggests that while some properties are consistently measured across samples, others show a wider range of values, which might require further investigation to understand the underlying causes.

CHAPTER FIVE

DISCUSSION

5.1 EFFECTS OF TREATMENTS ON SOIL PHYSICOCHEMICAL PROPERTIES

The physical and chemical characteristics of the different fertilizer treatments in the selected sampled maize fields are shown in Fig 4.1 - 4.8. The composition of bacterial communities was greatly influenced by the environment including soil properties and nutrition.

According to Figure 4.1 which shows the variations in soil temperatures, high temperatures were observed to be recorded in the selected maize fields in the eastern direction of the study area in both organically and chemically fertilized maize fields ranging between 29°C - 32°C. This could be as a result of evaporation, solar radiation as well as precipitation. However, soil temperatures do not affect soil health and fertility but just affect bacterial activities.

A comparison of pH values obtained showed most chemically fertilized soils had a low pH of < 5.70 with an exception of West 2, North 3, North 4, South 1 and South 2 chemically fertilized fields (Fig 4.2). The lowest pH value observed was in field East 3 which had a pH value of 4.71 while on the other hand, the highest pH value was observed in field East 5 Organic which had a pH value of 7.05. This entails that there is a possibility that the soil pH in field East 3 could have declined over time due to fertilization practices. These findings were consistent with those of Wang *et al*., (2020) who reported a decline in soil pH following the application of fertilizer that resulted in soil acidification. Chemical fertilizers soil pH overtime especially if they are applied annually (Wang *et al*., 2020). The nitrification process, which converts ammonium-nitrogen to nitrate-nitrogen, releases hydrogen ions (H+) that can decrease soil pH and cause acidification. The pH value of organically fertilized soils was significantly higher than that of chemically fertilized fields ($p < 0.05$). Organic matter added as manure can act to help buffer the soil against a decrease in pH by improving the buffer capacity of the soil, increases the soils ability to resist a change in pH and increases soil organic carbon (Wang *et al.*, 2019).

Electrical conductivity showed variances in organically and chemically fertilized maize fields (Fig 4.3). The highest electrical conductivity was observed in East 5 Organic with East 1 chemical recording one of the lowest values. It is generally accepted that the higher the soil porosity (higher moisture content), the greater the ability of soil to conduct electrical currents. Electrical

conductivity does not directly affect plan growth but indirectly indicates nutrient availability and salinity levels in growth mediums. Low electrical conductivity levels typically indicate low nutrient concentrations which causes nutrient deficiencies and slowed growth rate, this is supported by a study conducted by Titilola (2006).

The selected sample sites recorded significantly different moisture content values from both organically and chemically fertilized maize fields (Fig 4.4). The difference in distribution of values could be as a result of the particular soil types' water holding capacities. Soil water holding capacity is affected by the soil texture and organic matter content. In a study by Ouattara *et al*., (2006), it was observed that plants that received chemical fertilizers reduced the soil water more than non-fertilized plants. It was equally proposed that organic matter input significantly improved soil water content. The sampled organically fertilized soils sampled in this research had high organic matter content which justifies why field East 5 organic had a higher moisture content as compared to East 1 – East 4.

Organic matter, available phosphorus, potassium and nitrogen were observed to have the highest values in organically fertilized fields. Chemically fertilized fields East 1- East 4 in the eastern direction recorded the lowest organic matter content between 0.5 - 1.17 percent while the organically fertilized field East 5 recorded the highest organic matter content of 5.29 percent overall (Fig 4.5). After carrying out ANOVA, it was observed that there were significant differences in the soil organic matter content of soils from organically and chemically fertilized maize fields. This entails that organically fertilized soils had higher carbon content in them unlike in chemically fertilized soils. This is supported by a study conducted by Roelcke *et al.,* (2004) in which long term excessive use of chemical fertilizers contributed to reduced soil organic matter (SOM) content, with a consequent decline in agricultural soil quality and an increase in soil acidification and environment pollution. It is equally important to note that carbon is a substrate for most organisms hence there is a higher chance of survival for different microorganisms in an environment that has readily available source of nourishment.

Phosphorus is one of the key macronutrients required for plant growth and metabolism. Chemical fertilizers that contain phosphate temporarily increase phosphorus levels in soil that plants can access. However, overtime reactions with iron and aluminum in acidic soils or calcium and

magnesium in alkaline soils can reduce the availability of phosphorus fertilizers (Roelcke *et al.*, 2004). One advantage of using organic fertilizers is that the nutrients (nitrogen, phosphorus and potassium) are supplied more gradually comparing to chemical fertilizers. This, because organic fertilizers only release nutrients when bacteria break down the organic matter (Roelcke *et al.*, 2004). In this study, it was observed that the available phosphorus was higher in organically fertilized fields than in chemically fertilized fields. This is seen in a comparison between chemically fertilized fields East 1 – East 3 which had phosphorus levels ranging between 16.56 – 19.88mg/kg while East 4 had a value of 60.71 which is considerably higher than other chemically fertilized soils (Fig 4.6). Organically fertilized field East 5 recorded the highest value of 191.61mg/kg. The conversion of soluble phosphorus into insoluble phosphates involves microorganisms. Phosphorus is necessary for maintaining a balance between other plant nutrients and ensuring normal crop growth. It is important to note that phosphorus is an important nutrient responsible for promoting root and shoot growth as well as vigorous seedling growth.

The availability of potassium in the soil can increase with various amendments. In a study conducted by (Vinoth *et al.,* 2023). it was observed that the application of organic manure to the soils increased potassium release and decrease potassium fixation in soils. Variations in the values of potassium showed a significant difference in the values of potassium especially organically fertilized fields East 5 and West 5 recording 2.2cmol/kg and 1.84cmol/kg, respectively (Fig 4.7). Chemically fertilized fields showed lower recordings with the lowest recorded in East 3 with a value of 0.22cmol/kg, West 3 with a value of 0.24cmol/kg, and South 3 and South 4 with a value of 0.24cmol/kg. It is important to note that potassium is responsible for increasing disease resistance and water stress tolerance hence crops grown in soils with less potassium have a high risk of contracting diseases.

Nitrogen available in soils is in a form that cannot be used by plants. Soil bacteria are responsible for the conversion is the available nitrogen into a form usable by the plants. Nitrogen based fertilizers are alternatively used to fertilize plants, however, overuse of these fertilizers causes problems (Vinoth *et al.,* 2023). Firstly, the nitrogen fertilizers sink into the soils creating conditions that favor the growth of weeds rather than native lants. Excess nitrogen is washed away in waterways causing eutrophication (Vinoth *et al.,* 2023). Organic fertilizers on the other hand increased the Total Nitrogen (STN) content of the soil and had a greater effect on plant uptake of

native soil nitrogen than mineralization (Vinoth *et al.,* 2023). Available nitrogen values observed were quite high in organically fertilized fields such as West 5 and South 5 which recorded values above 0.40 than chemically fertilized fields such as East 2 which had a very negligible amount of nitrogen and South 4 which had the least amount of 0.02 (Fig 4.8). This simply depicts that low nitrogen levels in soil can in turn affect the yield of the crops in those soils.

This clearly indicates that the continuous use of chemical fertilizers has an effect on the soil physicochemical parameters which in turn affect the bacterial diversity in those particular soils. In conclusion, it was observed that organically fertilized soils had more conducive physicochemical properties that could support bacterial growth as compared to chemically fertilized soils which in turn helped to maintain the soil health (Vinoth *et al.,* 2023).

5.2 EFFECTS OF DIFFERENT TYPES OF FERTILIZER TREATMENTS ON BACTERIAL POPULATION

Analysis of variance showed no significant differences in bacterial colony forming units under different fertilization treatments, and these were consistent with previous results like those of Hartmann (2014). The main reason for this would be that organic materials contain a variety of nutrient elements which can increase exogenous carbon, promote the formation of aggregate structures and provide good conditions for the growth and reproduction of bacteria (Liu *et al*., 2021). However, despite the similarities in bacterial populations, most of the bacteria identified under chemically fertilized selected maize fields showed an abundance of non-beneficial bacteria hence making them irrelevant to the promotion of soil health but rather hazardous. Chemical fertilizers can have negative impacts on soil bacteria, including killing them and reducing the diversity. Higher doses of fertilizers like urea increase ammonium toxicity in bacteria (Damodaran *et al*., 2016) whereas phosphorus and potassium fertilizers reduce substrate induced respiration in bacteria (Bolan *et al*., 1996). This negatively affects the soil food web as other members of the food web disappear once the bacteria, fungi, nematodes and protozoa are gone.

According to Fig 4.9, it is observed that chemically fertilized fields South 1 and South 2 had high colony forming units' values of 1.6 and 1.87 x 10^8 cfu/ml as compared to the other chemically fertilized fields. Organically fertilized fields South 5 and South 6 had the highest colony forming unit values of 1.68 and 1.85 x 10^5 CFU/ml with slight variations compared to the chemically fertilized maize fields South 1 and South 2. This simply implies that there were no significant

differences in the abundance of bacterial populations found in organically and chemically fertilized maize fields. However, chemically fertilized maize fields had a high population of the same type of bacterial colonies which was observed when cultured while organically fertilized soils showed a wider variety of colonies when cultured. This was indirectly affirmed by Liu *et al.,* (2011) that increased nitrogen fertilizer inputs suppressed soil community richness.

Nitrogen fertilizers have both positive and negative effects on the richness of soil communities. Combining nitrogen fertilizers with bio-organic fertilizers can increase the abundance of copiotrophic bacteria and decrease the abundance of oligotrophic bacteria (Liu *et al.,* 2011). Biochar combines with nitrogen fertilizers lead to an increase in the capabilities of soil microbes involved in nitrogen fixation and nitrification (Liu *et al.*, 2011).

However, the excessive use of nitrogen fertilizers can lead to a decrease in pH and increase the available nitrogen in soils which can result in a lower bacterial species richness. This in turn reduces soil fertility, enzyme activity as well as microbial community diversity (Liu *et al.*, 2011). The application of organic fertilizers caused a significant increase in bacterial community structure and richness. Organic fertilizers provide carbon compounds that soil microbes use to increase their growth and biomass (Liu *et al.*, 2011).

5.3 EFFECTS OF DIFFERENT TYPES OF FERTILIZER TREATMENTS ON BACTERIAL DIVERSITY

Actinobacteria were the most abundant bacteria consistent with previous results reported in different systems whose abundance was relatively higher in treatments with organic fertilizer and are considered to be copiotrophic microorganisms (Liu *et al.*, 2021). Copiotrophic bacteria are organisms found in environments rich in nutrients, particularly carbon. Arthrobacter is commonly found in soils, aerial surfaces of plants and wastewater sediments. Some of the genera have the ability to use organic matter to grow and reproduce. Arthrobacter can degrade unusual polymeric compounds and plays an important role in biodegrading agrochemicals and pollutants (Liu *et al*., 2021). In this study, the abundance of these genera seemed abundant in treatments with organic fertilizers than those with chemical fertilizers, which was consistent with the findings of (Liu *et al*., 2021).

It was observed that both chemical and organic fertilizers can stimulate the growth of specific microbial populations by supplying nutrients leading to an increase in bacterial populations,

improving bacterial activity as well as determining a switch in bacterial diversity (Yuan *et al.*, 2008). The productivity and stability of agroecosystems is dependent on microbial diversity present in the soil. However, it was equally observed through many studies such as those done by Liu *et al*., (2021) and Lian-Jie, *et al*., (2021), that chemical fertilization reduced microbial diversity affecting plant beneficial microbial taxa. According to a study by Wang *et al*., (2020), it was observed that nitrogen fertilizer treatments lead to 15.1 percent increase in soil microbial biomass compared to unfertilized control plots and increased soil pH, however, the use of chemical fertilizer alone did not lead to remarkable increase in soil microbial abundance. It has generally been observed that chemical and organic fertilizers can directly stimulate the growth of specific microbial populations by supplying nutrients (Liu *et al*., 2021). However, intensive fertilization with mineral fertilizers can negatively affect soil biota by reducing the diversity of microorganisms and creating niches for pathogenic organisms Wang *et al*., (2020).

Chemically fertilized soils had an abundance of *Bacillus* species, most of which were oligotrophic microorganisms. Oligotrophic microorganisms take longer to grow and can grow in an environment with less nutrients. Availability of soil nutrients is usually linked with the transformation from oligotrophic microorganisms to copiotrophic microorganisms.

It was also observed that the bacterial community structures underwent changes under different fertilization treatments, consistent with the results of Lian-Jie, *et al*., (2021), who documented a clear separation of bacteria under different fertilization modes. Fertilization directly affects soil bacteria community structure by changing nutrients available in the soil and affecting the biological activities of bacteria in the soil.

Phylogenetic analysis indicated that *Exiguobacterium auratiacum, Bacillus anthracis, Gottfriedia acidiceleris, Mycrobacterium paludicola, Bacillus amyloliquefaciens, Lysinibacillus fusiformis, Solibacillus silvestris, Bacillus subtilis* and *Peribacillus frigoritolerans* were observed in chemically fertilized fields only. *Exiguobacterium* which was isolated from inorganically fertilized fields in the northern, western and southern direction is a genus of *bacilli* and a member of the low GC phyla of *Bacillota*. They are gram positive facultative anaerobes found in various habitats such a plant rhizosphere, freshwater, soil and even extreme environments such as marine waters (Enebe and Babalola 2020). They can grow in a wide range of temperature, salinities and pH values. These bacteria have a wide range of metabolic and stress resistant genes and can metabolize and utilize

a wide range of polysaccharides and proteins in their environment. These characteristics of *Exiguobacterium* explain why it is possible for it to be found in an acidic environment with a low supply of nutrients.

Despite the different types of bacteria found in both organically and fertilized soils being beneficial to the soil and plants, harmful bacteria were also detected in the samples, one of which was *Bacillus anthracis*, a gram-positive rod-shaped bacterium that causes anthrax in livestock and can be transmitted to humans (Bolan *et al*., 1996). This bacterium is found in soils with high calcium levels, moisture content and pH ranging from 5.0 – 7.0. In this study, this bacteria species was observed in soils sampled from the north (north 4), west (west 1) and east (east 1,3 and 4) which had similarities in soil physicochemical properties such as pH, electrical conductivity and nutrients such as phosphorus, potassium and nitrogen.

5.4 LIMITATIONS OF THE STUDY

1. The study may be limited by the number of maize fields and soil samples included in the analysis. While efforts were made to select representative sites across Lusaka Province, the findings may not fully capture the variability present within the region. Increasing the sample size and geographic coverage could enhance the robustness and generalizability of the results.
2. The study duration may be insufficient to capture long-term effects of fertilizer treatments on soil bacterial diversity. Soil microbial communities exhibit temporal dynamics and may undergo gradual shifts in response to changing environmental conditions and management practices. Longitudinal studies spanning multiple growing seasons would provide a more comprehensive understanding of these dynamics.
3. The study focused exclusively on maize fields, which limits the generalizability of the findings to other crop systems. Different crops may have distinct effects on soil microbial communities, and fertilizer responses may vary depending on crop species and rotation practices. Future research could explore the effects of fertilizer treatments on soil bacterial diversity in a broader range of cropping systems.
4. Soil bacterial diversity can be influenced by various environmental factors beyond fertilizer treatments, such as soil texture, climate, land use history, and microbial interactions. While efforts were made to control for confounding variables, the study design may not fully account for all potential sources of variability. Additional factors should be considered in future studies to provide a more comprehensive understanding of soil microbial ecology.
5. The methodologies used to assess soil bacterial diversity, such as sequencing of 16S rRNA genes, have inherent limitations and biases. PCR amplification, sequencing errors, and sample contaminations may introduce artifacts and affect the accuracy of microbial community analyses. Careful interpretation of results and validation using complementary techniques are essential to mitigate potential methodological biases.
6. The study focused primarily on taxonomic characterization of soil bacterial communities and did not assess functional attributes of microbial populations. Understanding the functional roles and metabolic activities of soil bacteria is crucial for elucidating their contributions to soil processes, nutrient cycling, and ecosystem functioning. Future

research could integrate functional analysis to provide a more holistic understanding of soil microbial ecology.

7. The findings of this study are specific to the environmental and agricultural context of Lusaka Province and may not be directly applicable to other regions with different soil types, climate conditions, and agricultural practices. Consideration of local context is important when interpreting and extrapolating study results to other geographic areas. Comparative studies across diverse regions could provide insights into the broader applicability of the findings.

Addressing these limitations and incorporating them into future research efforts will contribute to advancing our understanding of the effects of chemical and organic fertilizer treatments on soil bacterial diversity and inform sustainable soil management practices in agricultural systems.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This study provides valuable insights into the effects of chemical and organic fertilizer treatments on soil bacterial diversity within maize fields in Lusaka Province. Through comprehensive analysis and comparison of soil samples from different fertilizer treatment groups, several key findings have emerged:

1. Environmental factors such as soil pH, nutrient content, and organic matter levels play a significant role in shaping soil bacterial communities in response to fertilizer treatments. pH, in particular, emerged as a critical driver of bacterial community composition, highlighting the importance of soil pH management in agricultural systems. The long-term use of chemical fertilizers showed low levels of pH which were less than 5.7 in chemically fertilized soils indicating they were acidic soil and low amounts of nitrogen and organic carbon in some maize fields. Phosphorus and potassium were equally observed to be low in most chemically fertilized fields but significantly higher in organically fertilized fields.

8. The results demonstrated that fertilizer treatments significantly influence soil bacterial diversity in maize fields. Chemical fertilizers tend to reduce overall bacterial diversity compared to organic fertilizers. This reduction in diversity may be attributed to the selective pressure exerted by chemical fertilizers on specific microbial taxa.

9. Distinct patterns in bacterial community composition were observed between fields treated with chemical and organic fertilizers. Organic fertilizers promoted greater microbial diversity

and the enrichment of beneficial microbial taxa associated with nutrient cycling and soil health. In contrast, chemical fertilizers lead to shifts in microbial community composition, favoring taxa adapted to high nutrient availability but potentially compromising soil resilience.

10. After sequencing and blasting of the bacteria isolates, the resultant strains of bacteria observed in organically fertilized soils were dominantly copiotrophic bacteria such as *Arthrobacter, Actinobacteria* and some *Bacillus sp.* due to the abundance of nutrients in these soils while chemically fertilized soils promoted the growth of oligotrophic bacteria such as *Exiguobacterium aurantiacum* and *Microbacterium paludicola* that require fewer nutrient concentrations to grow and are stress resistant. These types of bacteria are equally highly resistant to changes in the environment and take longer to grow.

Overall, this study underscores the importance of considering soil microbial diversity in agricultural management decisions and highlights the potential of organic fertilizers as a sustainable alternative to chemical fertilizers in maize cultivation. By integrating these findings into agricultural practices and policy-making processes, the findings can work towards building resilient and environmentally friendly agricultural systems in Lusaka Province and beyond. Longitudinal studies tracking microbial community dynamics over multiple growing seasons could provide a more comprehensive understanding of the resilience and stability of soil ecosystems under different fertilization regimes. Integrating organic fertilizer amendments into farming practices can enhance soil fertility, improve crop yields, and contribute to long-term agricultural sustainability.

6.2 RECOMMENDATIONS

1. Application of fertilizers is essential for today's agricultural crop production system since it improves crop development and output while replenishing soil nutrients. But in order to prevent the various risks brought on by excessive fertilizer use, fertilizers should be used wisely and sustainably. To do this, the soil should first undergo thorough testing and analysis before fertilizer is applied. Therefore, it is important to adopt the integrated use of various nutrient supplements, such as chemical fertilizer, organic manures, biofertilizers, and other slow-release or controlled-release fertilizers, to ensure both improved and sustainable agricultural production and to protect the environment. Improved fertilizer nutrient usage efficiency, especially nitrogen fertilizers, should be used to eliminate the pollution risks caused by chemical fertilizers.

2. It is recommended that further studies be done on this subject matter in order to understand the exact changes that occur in the soil physicochemical properties and how those changes affect the bacterial populations in those soils and how this eventually affects the soil health and fertility.

3. It is important to assess the effects of using a combination of organic and inorganic fertilizers for maize growing in order to observe the bacterial growth and diversity in soils subjected to this treatment and how that affects soil fertility.

4. Furthermore, it is imperative to conduct further studies on the production of cost effective biofertilizers to be used for crop production can also be a great addition to the existing body of knowledge.

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APPENDICES

APPENDIX A: ETHICAL CLEARANCE CERTIFICATE

THE UNIVERSITY OF ZAMBIA

DIRECTORATE OF RESEARCH AND GRADUATE STUDIES

Great East Road Campus | P.O. Box 32379 | Lusaka10101 | Tel: +260-211-290 258/291 777

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APPROVAL OF STUDY

IORG No. 0005376

HSSREC IRB No. 00006465

2nd November, 2022

REF NO. NASREC-2022-OCT.-003

Ms. Namasiku Lubasi,
The University of Zambia,
School of Natural Sciences,
P.O. Box 32379,
LUSAKA

Dear Ms. Lubasi,

RE: "ASSESSMENT OF THE EFFECTS OF CHEMICAL FERTILIZER ON BACTERIAL DIVERSITY FROM SELECTED FARMS IN LUSAKA"

Reference is made to your protocol dated as captioned above. NASREC resolved to approve this study and your participation as Principal Investigator for a period of one year.

REVIEW TYPE	**ORDINARY REVIEW**	**APPROVAL NO. NASREC-2022-OCT.003**
Approval and Expiry Date	Approval Date: 2nd November, 2022	Expiry Date: 1st November, 2023
Protocol Version and Date	Version - Nil.	1st November, 2023
Information Sheet, Consent Forms and Dates	• English.	To be provided
Consent form ID and Date	Version - Nil	To be provided
Recruitment Materials	Nil	Nil
Other Study Documents	Questionnaire.	

APPENDIX B: PARTICIPANT INFORMATION SHEET

Instructions: Please read carefully through all the information before making your decision to participate.

Dear participant, my name is **Namasiku Lubasi**, a postgraduate student at the University of Zambia, pursuing a Master's Degree in Applied Microbiology. As a partial fulfillment of the master's program at the University, students are required to carry out research in their area of interest that will be of benefit to the general public and the body of knowledge.

With these reasons and many more below, I am carrying out a research on **"Effects of Chemical Fertilizers on Bacterial Diversity from Selected Maize Fields Here in Lusaka Province"**. So, the objective of this study is to determine the effects of chemical fertilizers on bacterial diversity.

My research requires me to analyze soil samples from maize fields in that use chemical fertilizers as well as those that use organic fertilizers in order to find out the types of bacteria found in these soils.

If you agree to take part in this research, I will ask you to grant me access to your maize field to sample soil which will be analyzed at the University of Zambia Microbiology Laboratory. It should take approximately 30 minutes to sample from 4 different points in your field.

Your participation in this research is completely voluntary. If at any point you wish to no longer take part in the research you have the right to withdraw at any time.

If you are not sure about anything mentioned above, please do not hesitate to ask me.

If you agree to take part you will be asked to sign a consent form. The consent form will not be used to identify you.

Thank you very much for your time and help.

APPENDIX C: INFORMED CONSENT FORM

Name of Researcher: Namasiku Lubasi

Programme: MSc. Applied Microbiology

School: The University of Zambia

Research Project: Effects of Chemical Fertilizers on Bacterial Diversity from Selected Maize Fields in Lusaka Province.

Instructions: Please tick ☑ either "Yes" or "No" to respectively agree or disagree to the following sentences;

a) I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐ Yes ☐ No

b) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

☐ Yes ☐ No

c) I understand that any information given by me may be used in future reports, articles or presentations by the research team.

☐ Yes ☐ No

d) I understand that my name will not appear in any reports, articles or presentations.

☐ Yes ☐ No

e) I agree to grant the researcher access to my maize field as required by the above study.

☐ Yes ☐ No

Name of Participant _____ Date _____ Signature/Thumb _____

Name of Researcher _____ Date _____ Signature _____

"A copy will be given to the participant and the original will be kept by the Researcher"

APPENDIX D: LOCATION DETAILS OF SAMPLED FARMS

EASTERN SITE

1. AREA: CHINKULI, NDULIKA.
   COORDINATES: 15° 17’ 01” S, 28° 34’ 33” E
2. AREA: CHINCHILI.
   COORDINATES: 15° 17’ 29” S, 28° 33’ 48” E
3. AREA: CHINCHILI.
   COORDINATES: 15° 17’ 27” S, 28° 33’ 51” E
4. AREA: MWASHINANGA.
   COORDINATES: 15° 17’ 50” S, 28° 33’ 07” E
5. AREA: CHINKULI, NDULIKA.
   COORDINATES: 15° 16’ 58” S, 28° 34’ 32” E.

WESTERN SITE

6. AREA: NDUNDU, KASUPE.
   COORDINATES: 15° 21’ 06” S, 28° 13’ 04” E
7. AREA: FURNGROOVE, LUSAKA WEST.
   COORDINATES: 15° 21`’ 14” S, 28° 10’ 35” E
8. AREA: SEKELELA, LUSAKA WEST.
   COORDINATES: 15° 21’ 35” S, 28° 10’ 03” E
9. AREA: NAKACHENJE, LUSAKA WEST.
   COORDINATES: 15° 21’ 52” S, 28° 10’ 51” E
10. AREA: NDUNDU, KASUPE.
    COORDINATES: 15° 20’ 57” S, 28° 13’ 09” E

NORTHERN SITE

11. AREA: MUTAKWA, MUNGULE.
    COORDINATES: 15° 13’ 36” S, 28° 10’ 33” E

12. AREA: MUTAKWA, MUNGULE.
    COORDINATES: 15˚ 13’ 29” S, 28˚ 10’ 45” E
13. AREA: ROCKFIELD, TEN MILES.
    COORDINATES: 15˚ 14’ 59” S, 28˚ 12’ 51” E
14. AREA: TEN MILES.
    COORDINATES: 15˚ 15’ 01” S, 28˚ 12’ 48” E

SOUTHERN SITE

15. AREA: MWALEKA, KAFUE.
    COORDINATES: 15˚ 44’ 09” S, 28˚ 11’ 43” E
16. AREA: SHIMABALA, KAFUE.
    COORDINATES: 15˚ 40’ 27” S, 28˚ 14’ 09” E
17. AREA: SHIMABALA, KAFUE.
    COORDINATES: 15˚ 17’ 29” S, 28˚ 33’ 48” E
18. AREA: SHIMABALA, KAFUE.
    COORDINATES: 15˚ 39’ 38” S, 28˚ 14’ 25” E
19. AREA: KASUSA, KAFUE WEST.
    COORDINATES: 15˚ 43’ 07” S, 28˚ 08’ 14” E
20. AREA: KASUSA, KAFUE WEST.
    COORDINATES: 15˚ 44’ 14” S, 28˚ 08’ 56” E