

EFFECT OF AQUEOUS NEEM (*Azadirachta indica* A juss) LEAF  
EXTRACT ON SELECTED SOIL CHEMICAL AND BIOLOGICAL  
PROPERTIES

BY

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A dissertation submitted to the University of Zambia, Directorate of  
Research and Graduate Studies, in partial fulfilment of the requirements  
for the degree in Master of Science in Integrated Soil Fertility  
Management

THE UNIVERSITY OF ZAMBIA

November 2015

## DECLARATION

I, Lubungo Aswell Chewe, do hereby declare that this dissertation is my own independent work, and to the best of my knowledge, it has never been submitted for the award of any degree to this or any other university.

Signature: -----

Date: -----

### **CERTIFICATION OF APPROVAL**

The University of Zambia has approved the dissertation of Mr. Lubungo Aswell Chewe as fulfilling part of the requirements for the award of the degree of Master of Science in Integrated Soil Fertility Management.

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## ABSTRACT

Zambia has soils with low plant available nutrients due to leaching, depletion of organic matter and acidification. One way of addressing this situation is by the application of organic materials such as aqueous neem leaf extract. No known documented research has been conducted in Zambia to evaluate the effects of neem products on soil biological and chemical properties when used as a soil amendment. Possible effects may include the addition of nutrients which may be used by the soil microorganisms or the chemicals that may have negative impacts on soil microorganisms resulting in reduced activity and population. This study was a greenhouse experiment designed to evaluate the effects of aqueous neem leaf extract on selected soil chemical and biological properties when applied as a soil amendment. Five treatments of neem leaf concentrations in replicates of four were arranged in a Completely Randomized Design. The aqueous neem extract concentrations included 2 %, 5 %, 10 %, 15 %, 20 % and 0 % as a control. The leaf extract was applied to 5 kg of soil in each plastic pot on a weekly basis for the first five consecutive weeks. In addition, each week, for ten consecutive weeks, 100 g of soil was removed from each pot. The soil which was removed from the pots was incubated for 7 days and analysed for soil microbial biomass and activity using the Chloroform Fumigation and Incubation (CFI) method and soil respiration method, respectively. Neem leaves used for making the extract had 2.58, 1.77, 1.5, 0.4 and 0.1 % of Ca, K, N, Mg and P respectively. These levels of nutrients were comparable to those in *Confrey* and *Tithonia* commonly used for making leaf extracts. The results also showed that 10 % and 20 % concentrations of aqueous neem leaf extract were significantly different from each other. The results at week 10 showed that there were non-significant differences among treatments 2 %, 5 % and 15 % but cumulative soil microbial activity at 10 % concentration of aqueous neem leaf extract had increased by 16 % from the control. This increase in the microbial activity can be attributed to increasing amounts of the nutrients available to the soil microorganisms. Microbial activity at 20 % concentration of aqueous neem leaf extract reduced by 3 % compared to the control attributable to the possible toxic effects of the secondary metabolites at this high concentration. The cumulative soil microbial biomass for 2 %, 5 % and 10 % concentrations of aqueous neem leaf extract had reduced by 19 %, 10 % and 3 % respectively. An increase in cumulative soil microbial biomass was recorded at 15 % and 20 % by 8 % and 4 % respectively.

## **DEDICATION**

This dissertation is affectionately dedicated to my late father Kingford Mumba Lubungo for the care, support and love he gave me before he died. I also dedicate this dissertation to my late mother Josephine Chipulu Maliko for the nine months she took care of me in her womb, advice, love and care she showed to me before she died. I should not forget to dedicate to my children Josephine Chipulu Lubungo, Aswell Chewe Lubungo (Jnr), Hellen Lubungo, Lameck Lubungo, Rabison Lubungo and Taonga Lubungo for being very understanding throughout the three years I was at the University of Zambia and for being the source of my inspiration for working hard towards this achievement. It is also important to remember my brothers Jolly Chewe, Alex Lubungo, Betram Lubungo, Petty Lubungo (Late), Malio Joseph (Late) and my sisters Gladys Lubungo, Lydia Lubungo and Patricia Lubungo for being very understanding during this period when I was pursuing my studies.

## **ACKNOWLEDGEMENTS**

I wish to express my sincere thanks to my Principal Supervisor, Dr Alice Mutiti Mweetwa, who was there throughout to encourage me to continue with my research even when I felt like abandoning it because of some challenges I encountered. I thank her for sparing her precious time. She was patient, tolerant and provided valuable guidance until the successful completion of this research work. I was not going to achieve this without her.

May I also thank my Co- supervisor, Dr Benson H Chishala for all his concerns, advice and the input into the laboratory and statistical analysis. I would also like to extend my special thanks to Mr Gideon Musukwa, the Chief Technician and other Laboratory technicians in the Department of Soil Science for being patient, tolerant and for guiding me during the Laboratory analysis. I wish to thank the other members of staff in the School of Agricultural Sciences and especially the staff from the Department of Soil Sciences for all the support when I was doing my course and research work.

May I also recognise the significant contributions from the Staff from Kasisi Agricultural Training Centre especially Mr Daniel Kalala who was my collaborator at the centre for providing me with the research materials. It is also important to recognise the members of staff from National Institute for Scientific and Industrial Research (NISIR), Plant Sciences Research Centre based in Kitwe for allowing me to carry out my experiment at this institution and for all the valuable help and guidance during the period I was there.

I extend my heartfelt gratitude to the former Provincial Agricultural Coordinator (PACO), Copperbelt Province, Mr Simon Manyerekete for facilitating my study leave. I also thank the entire staff of the Ministry of Agriculture and Livestock in Chililabombwe District and Ministry Headquarters for the moral, spiritual and financial support.

Lastly, I would like to thank my children and other family members for being with me during the study period.

## TABLE OF CONTENTS

DECLARATION .....	ii
CERTIFICATION OF APPROVAL .....	iii
ABSTRACT.....	iv
DEDICATION .....	v
ACKNOWLEDGEMENTS .....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES .....	xii
ACRONYMS AND ABBREVIATIONS .....	xiii
CHAPTER ONE .....	1
1.0 INTRODUCTION .....	1
1.1 Statement of the problem .....	1
1.2 Main objective .....	2
1.3 Specific objectives .....	2
1.4 Hypothesis.....	2
1.5 Justification/rationale .....	2
CHAPTER TWO .....	3
2.0 LITERATURE REVIEW .....	3
2.1 Uses of neem.....	3
2.2 Chemistry of neem.....	3
2.3 Neem as a soil amendment.....	4
2.4 Soil microorganisms .....	5
CHAPTER THREE .....	7
3.0 MATERIALS AND METHODS.....	7

3.1 Site description.....	7
3.2 Soil sampling and characterization .....	7
3.2.1 Soil reaction (pH).....	7
3.2.2 Total nitrogen.....	7
3.2.3 Available phosphorus.....	8
3.2.4 Soil organic matter.....	8
3.2.5 Exchangeable bases and Cation Exchange Capacity .....	9
3.2.6 Electrical conductivity .....	9
3.2.7 Texture .....	10
3.2.8 Bulk density .....	11
3.3 Source of neem leaves .....	12
3.4 Sampling and chemical characterization of neem leaves.....	12
3.4.1 Total nitrogen.....	12
3.4.2 Phosphorus, Magnesium, Potassium and Calcium .....	13
3.5 Preparation of aqueous neem leaf extract .....	13
3.6 Chemical characterization of aqueous neem leaf extract.....	14
3.7 Greenhouse experiment .....	14
3.8 Determination of soil microbial biomass and activity .....	14
3.8.1 Soil microbial biomass.....	14
3.8.2 Soil microbial activity.....	16
3.9 Statistical analysis of data.....	16
CHAPTER FOUR.....	17
4.0 RESULTS AND DISCUSSION .....	17
4.1 Nutrient composition of neem leaves.....	17
4.2 Chemical composition of aqueous neem leaf extract.....	18
4.2.1 Ammonium nitrogen (NH <sub>4</sub> -N) and Nitrate nitrogen (NO <sub>3</sub> -N) .....	18



4.2.2 Exchangeable bases (K, Ca and Mg) .....	19
4.2.3 Phosphorus (P) .....	21
4.2.4 Electrical conductivity (EC).....	21
4.2.5 Soil reaction (pH).....	22
4.3 Initial soil chemical and physical properties.....	22
4.3.1 Nitrogen (N) and Organic matter (OM).....	23
4.3.2 Exchangeable bases (K, Ca and Mg) and Cation Exchange Capacity (CEC).....	23
4.3.3 Phosphorus (P) .....	24
4.3.4 Electrical conductivity (EC).....	24
4.3.5 Soil reaction (pH).....	24
4.3.6 Bulk density and Texture .....	24
4.4 Effect of aqueous neem leaf extract on soil chemical properties .....	25
4.4.1 Nitrogen (N) and Organic matter (OM).....	25
4.4.2 Exchangeable bases (K, Ca and Mg) and Cation Exchange Capacity (CEC).....	26
4.4.3 Phosphorus (P) .....	28
4.4.4 Electrical conductivity (EC).....	29
4.4.5 Soil reaction (pH).....	30
4.5 Effect of aqueous neem leaf extract on cumulative soil microbial activity .....	31
4.6 Effect of aqueous neem leaf extract on cumulative soil microbial biomass .....	33
CHAPTER FIVE .....	36
5.0 CONCLUSION AND RECOMMENDATIONS.....	36
5.1 Conclusion .....	36
5.2 Recommendations.....	36
REFERENCES .....	37
APPENDICES .....	42
Appendix 1: Selected chemical properties of aqueous neem leaf extract.....	42

Appendix 2: Effect of aqueous neem leaf extract on soil chemical properties .....	43
Appendix 3: Effect of aqueous neem leaf extract on cumulative soil microbial activity .....	44
Appendix 4: Effect of aqueous neem leaf extract on cumulative soil microbial biomass .....	45

## LIST OF TABLES

Table 1. Uses of different parts of the neem tree and their uses .....	3
Table 2: Nutrient composition of neem leaves .....	17
Table 3: Initial selected soil chemical and physical properties.....	23

## LIST OF FIGURES

Figure 1: NH <sub>4</sub> -N and NO <sub>3</sub> -N contents of different concentrations of neem leaf extract.....	19
Figure 2: K, Ca and Mg contents of different concentrations of neem leaf extract.....	20
Figure 3: P content of different concentrations of neem leaf extract.....	21
Figure 4: Trends in Electrical conductivity for different concentrations of neem leaf extract.....	22
Figure 5: N and OM content of the soil treated with different concentrations of neem leaf extract at the end of experiment.....	26
Figure 6: Exchangeable cations and CEC of the soil treated with different concentrations of neem leaf extract at the end of experiment.....	27
Figure 7: P content for soil treated with different concentrations of neem leaf extract at the end of experiment.....	29
Figure 8: Trends in EC for soil treated with different concentrations of neem leaf extract at the end of experiment .....	30
Figure 9: Trends of cumulative soil microbial activity for soils treated with different concentration of aqueous neem leaf extract.....	32
Figure 10: Trends of cumulative soil microbial biomass for soil treated with different concentrations of aqueous neem leaf extract .....	34

## ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
Bt	<i>Bacillus thuringesis</i>
CEC	Cation Exchange Capacity
CFI	Chloroform Fumigation and Incubation method
CRD	Completely Randomized Design
EC	Electrical Conductivity
HPLC	High Performance Liquid Chromatography
ISFM	Integrated Soil Fertility Management
KATC	Kasisi Agricultural Training Centre
LSD	Least Significant Difference
NISIR	National Institute of Scientific and Industrial Research
NMRS	Nuclear Magnetic Resonance Spectroscopic Analysis
OPPAZ	Organic Producers and Processors Association of Zambia
PACO	Provincial Agricultural Coordinator
UNZA	University of Zambia

# CHAPTER ONE

## 1.0 INTRODUCTION

The Neem tree (*Azadirachta spp*) is a member of the Meliaceae family or the Mahogany family. In India, it is called Indian neem tree, Indian lilac or Margosa tree (Barceloux, 2008). There are two species of neem that have been reported, *Azadirachta indica* (A) Juss which is native to the Indian sub-continent and *Azadirachta excels* kack which is confined to Phillipines and Indonesia (Jattan *et al.*, 1995). *Azadirachta excels* kack grows as a wild tree in India, Bangladesh, Burma, Pakistan, Sri Lanka, Malaysia, Thailand, and Indonesia.

There are different products that come from the different parts of the tree such as seed, bark and leaf. Different neem products can be obtained; these include granules, oil, cake and leaf extracts (Ogbuevu, 2011). With these products, neem tree is mainly used as a source of human medicine, pesticide, fodder, insecticide, herbicide and organic manure.

Presently, neem tree can successfully grow in more than 72 countries worldwide across Asia, Africa, Australia, North, Central and South America (Almed *et al.*, 1989). The height of the tree ranges from 15 to 25 m (Cseke *et al.*, 2006). According to Ogbuewu (2011), neem tree can grow up to 30 m with limbs of 15 m in length. Neem tree can grow within an altitude range of 0 to 1500 m, and can tolerate temperatures up to 40°C and a mean annual rainfall of 400 to 1200 mm. Neem tree can grow in soils ranging from neutral to alkaline, but the optimum soil pH range is 6.2 to 7. It can also thrive on shallow, stony, sandy soils or where there is a hard calcareous or clay pan near the surface. In an ideal, habitat neem tree can live up to 150 to 200 years.

### 1.1 Statement of the problem

Most countries in sub-Saharan Africa and Zambia, in particular, have low soil productivity attributed to declining fertility levels (Sanchez *et al.*, 1997; Truter *et al.*, 2000; Crasswell *et al.*, 2001; Munsanje, 2007). Some organisations such as Kasisi Agricultural Training Centre (KATC) and Organic Producers and Processors Association of Zambia (OPPAZ) promote the use of organic materials such as neem cake and liquid organic manure as soil amendments to address this challenge and enhance crop yields among small scale farmers. Aqueous neem leaf extract

has been shown to positively affect soil chemical characteristics. However, its impact on soil microbiology has not yet been completely assessed.

## **1.2 Main objective**

To evaluate the effects of aqueous neem leaf extract applied as a soil amendment on selected soil chemical and biological properties.

## **1.3 Specific objectives**

In order to achieve the main objective, the following were the specific objectives:

- To characterize the elemental composition of soil, neem biomass and aqueous leaf extract
- To determine the effect of aqueous neem leaf extract application on soil microbial biomass
- To determine the effect of aqueous neem leaf extract application on soil microbial activity

## **1.4 Hypothesis**

Application of aqueous neem leaf extract (tea) does not negatively affect soil microbial biomass and activity when applied as a soil amendment.

Application of aqueous neem leaf extract (tea) does not negatively affect soil chemical properties when applied as a soil amendment.

## **1.5 Justification/rationale**

Several studies have been conducted to evaluate the effects of neem-based pesticides. However, there is no known documented research conducted in Zambia that has investigated the effects of neem products on soil biology when used as a soil amendment. Possible effects may include addition of substrates which may be used as a source of nutrients or inhibitory chemicals that may reduce the activities and populations of soil microorganisms. Hence, this study was conducted to provide information on the elemental composition of aqueous neem leaf extract as a soil amendment, and its effects on selected chemical and biological properties. This information is necessary for guiding farmers on the application of this amendment.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Uses of neem

Neem is mainly used for human medicine and as a pesticide, fodder, insecticide, herbicide and soil amendment (Table 1).

Table 1. Uses of different parts of the neem tree and their uses

Parts/Uses	Seeds	Leaves	Bark	Branches	Fruits
Medicine (Animal)		X			
Medicine (Human)		X	X		
Crop protection (Field)	X	X			
Crop protection (Storage)	X	X			
Timber				X	
Fuel				X	
Religious festivals		X			
Food					X
Soil amendment/Fertilizer	X	X			

SOURCE: Vitosh *et al.*, 1995

#### 2.2 Chemistry of neem

The neem tree is rich in terms of its chemical composition. It contains more than 300 plant secondary compounds (Koul *et al.*, 2004). The major chemical constituents of neem are terpenoids and liminoids. The major active component in the liminoids are the azadirachtin, 3 deacetyl-3-cinnamoylazadirachtin, 1-tigloyl-3-acetyl-11-methoxyazadirachtin, 22, 23 dihydro-



23B-methoxyazadirachtin, nimbanal, 3-tigloylazadirachtol, 3-acetyl-salannolide, nimbidiolide, margocin, margocinin and margocilin. The terpenoids, the major active components are the isoazadirolide, 6-nimbocinolide, nimbonone, nibonolone, methylgrevillate and margosinone (Ogbuewu, 2011). Azadirachtin is usually found in the highest concentration in the seeds of neem. The most active compounds are the terpenoids which are found in the fruits, seeds, twigs, stems, leaves and the bark. The most prominent and commercially produced limonoid that has been used is the Tetranortriterpene azadirachtin found in the seed kernels.

Based on its chemistry, various parts of the neem tree are used to cure more than 100 diseases (Ogbuewu, 2011; Joshi *et al.*, 2008; Vanka *et al.*, 2001; Mbah *et al.*, 2007) and to control different crop pests and insects (Koul *et al.*, 2004; Cseke *et al.*, 2006; Brahmachari, 2004). Neem has also been shown to control different pathogens (Hoque *et al.*, 2007; Harikrishnan *et al.*, 2008).

### **2.3 Neem as a soil amendment**

Neem is also widely used in agriculture as a soil amendment. Neem tree improves the fertility of the soil and water holding capacity (Murovat *et al.* 2008). It also has a unique property of calcium mining which influences soil pH, typically changes acid to neutral soils. Neem has a well-developed and deep rooted system that allows it to extract nutrients and water from lower soil levels and to control soil erosion by holding the soil particles firmly.

Different parts of the neem tree are used as soil amendments. For example, the neem cake, the residue left after extracting oil from the seeds, is used as organic manure (Kumar *et al.*, 2007). The neem cake has been shown to increase the organic matter content of the soil (Murugan *et al.*, 2011). The neem cake is also used to enhance the efficiency of nitrogen fertilizers by reducing the rate of nitrification. Neem cake coated or admixed with urea has been shown to increase nitrogen assimilation by plants through this slow release of N compared to untreated urea (Ketkar 1983). The reduced rate of nitrification has been attributed to the inhibitory effect on nitrifying bacteria that facilitate the oxidation of ammonium to nitrate (Ketkar 1983). The fact that neem inhibits nitrifying bacteria brings about the need to determine potential negative effects on other useful soil organisms that facilitate different nutrient cycling processes.

Neem leaves could be used directly for the preparation of vermin compost which can be used as fertilizer (Gajalakshmi, 2004). Neem leaves can also be used for the preparation of aqueous neem leaf extract; this leaf extract has traditionally been prepared by simply soaking chopped or ground leaves in water (Ketkar, 1983). This extract is applied as a source of nutrients for a number of crops by drenching the soil around the plant. The quality of the extract depends on various factors including the levels of nutrients as well as the release patterns into solution (Palm *et al.*,1997). While other plant species used for preparing different liquid organic manures or fertilizers have been characterized for nitrogen, phosphorus, potassium, calcium and magnesium (Hartemink, 2003; Gachengo *et al.*, 1999), a need exists to characterize the neem leaves used in the preparation of the extract and to determine the elemental composition of the extract itself. As a soil amendment, it is also important to determine the changes brought about in the chemical, physical and biological properties of the amended soil. This information is useful for the determination and subsequent recommendation of the appropriate application rates.

## **2.4 Soil microorganisms**

The five major groups of microorganisms found in the soil include: algae, actinomycetes, bacteria, fungi and protozoa (Alexander, 1997). These soil microorganisms are a very important element of a healthy soil as they perform functions that benefit higher plant life. Examples of beneficial organisms include: *Rhizobia* that lives in symbiotic association with legumes and fix atmospheric nitrogen; fungi and bacteria involved in soil organic matter decomposition and carbon cycling; nitrifiers that oxidize ammonium to nitrate; mycorrhizae-forming fungi that enhance nutrient and water uptake and other organisms involved in various transformations. Soil microorganisms require a source of nutrients and can sometimes compete with plants for nutrients when they are limiting. This is especially where nitrogen, phosphorus and potassium, that are required in large quantities, are concerned. Soil microorganisms utilize various substrates such as amino acids, vitamins, carbohydrates, lipids and fatty acids, lignin and other undefined metabolites found in the soil (Ketkar 1983). Their nutrients are primarily derived from the organic matter they subsist on but also utilize available inorganic sources of nutrients.

Soil microbial biomass is the part of organic matter in the soil which includes living microbial components such as bacteria, actinomycetes, fungi, algae, protozoa and microfauna (Dubey *et*

*al.*, (2002). Soil microbial biomass plays a role in structure formation, and stabilization of the soil Structure.

Typically, biomass carbon ranges from 1 to 5% of soil organic matter and is generally expressed in milligrams of carbon per kilogram soil or micrograms of carbon per gram dry weight of soil (Jenkinson, 1988). Estimations of the microbial biomass have usually involved treatment of the biomass as a single component, although it is known that a diversity of populations with different biochemical characteristics is present (Jenkinson, 1988). In a nutrient-poor ecosystem, the microbial biomass acts as sink and source of nutrients (Dubey *et al.*, 2002).

The microbial biomass is very sensitive and therefore, changes according to soil physiological properties, season, cropping pattern and tillage among other factors (Jenkinson, 1988). This parameter, therefore serves as an ecological marker. The soil environment and physical factors affecting microbial biomass and its activity are classified as chemical, physical and biological factors.

The application of neem leaf aqueous to the soil affects the soil microbial biomass. Soil microbial biomass would therefore be a good indicator of the effects on soil biology of the known anti-microbial activities of neem-based products. On the other hand, the changes in soil chemical and physical properties would be a good indicator of neem leaf aqueous as a soil amendment.

Soil contains a variety of microorganisms. These live microorganisms respire and produce carbon dioxide (CO<sub>2</sub>). The evolved carbon dioxide can be measured and assessed as an index of soil microbial activity and decomposition of organic matter. Therefore, the rate of carbon dioxide (CO<sub>2</sub>) production during soil respiration is an indication of the rate of degradation of the substrate and of the activity of the microbiota (Dubey *et al.*, 2002).

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Site description

Soils used in the study were collected from Chililabombwe District on the Copperbelt, Zambia. The geographical location of Chililabombwe district is approximately 12.3667°S and 27.8278°E. The area is in Agro-ecological Region III of Zambia receiving an average annual rainfall of 1 200 mm with mean annual temperature ranging from 16°C to 23°C and a cropping season of six months starting from November to March.

#### 3.2 Soil sampling and characterization

A total of 60 sub samples were randomly collected to a depth of 0 – 20 cm using the grid method. The sub samples were mixed thoroughly to make a homogenized composite sample. The composite sample was divided into two parts; one sample was subjected to chemical and physical characterization while the other sample was carried on ice in cooler boxes and kept refrigerated until potted for the greenhouse experiment.

The soil subjected to chemical and physical characterization was air dried and passed through a 2 mm sieve before it was analysed for the following: soil reaction (pH), total nitrogen, available phosphorus, soil organic matter, electrical conductivity, exchangeable bases and cation exchange capacity, bulk density and texture.

##### 3.2.1 Soil reaction (pH)

The soil reaction was determined using 0.01 M CaCl<sub>2</sub> (Mclean, 1973). The determination was done by weighing 10 g of 2 mm sieved air dried soil into a 50 mL beaker and adding 25 mL of 0.01 M CaCl<sub>2</sub>. Thereafter, the suspension was shaken for 10 minutes before it was allowed to settle for 30 minutes. The soil reaction was determined using glass-calomel electrodes connected to a pH meter.

##### 3.2.2 Total nitrogen

The total nitrogen was determined using the Kjeldahl method (Kjeldahl, 1983). The procedure was done in two phases digestion and distillation. A gram of air dried soil was weighed and

transferred into a digestion tube to which 4 g of mixed catalyst was added followed by 10 mL of concentrated sulphuric acid. The samples were then digested on the digestion blocks until the colour cleared. The digested samples were distilled. The amount of total nitrogen in the soil (%N) was determined as follows:

$$(S_T - B_T) \times 0.01 \times 1.401 / n \times DF$$

Where  $n$  – weight of oven dry soil used

$S_T$  – Titre, mL standard acid for sample

$B_T$  – Titre, mL standard acid for blank

DF – Dilution Factor

### 3.2.3 Available phosphorus

Available phosphorus was determined using the Bray 1 method (Bray *et al.*, 1945). Air dried soil (3 g) was weighed into 15 mL centrifuge tube before adding 21 mL of Bray 1 extraction solution. The suspension was then shaken for one minute on the mechanical shaker. Thereafter, the suspension was filtered and 5 mL of the filtrate was pipetted into a 25 mL volumetric flask. To which, 10 mL of distilled water was added. The mixture of ammonium molybdate, potassium antimony tartrate and ascorbic acid (Reagent B) was added to develop a blue colour for 15 minutes. Thereafter, P content in the solution was determined on a spectrophotometer at 882 nm. A similar treatment was given to the blank as well. The amount of P (mg/kg) was then calculated as follows:

$$\text{Reading (mg/L)} \times \text{Volume extract (mL)} / 1000 \text{ mL} \times (1000 \text{ g/kg}) / \text{weight sample} \times DF$$

Where: DF – Dilution Factor

### 3.2.4 Soil organic matter

The soil organic matter was determined using Walkely and Black method (Allibon, 1965). A gram of air dried soil was weighed into a 250 mL conical flask. The pipette was used to add 10 mL of 1N  $K_2Cr_2O_7$  before adding 20 mL of concentrated sulphuric acid. The suspension was swirled gently until soil and solution were thoroughly mixed and then swirled more vigorously for 1 minute. The mixture was then left to digest for 30 minutes. Thereafter, 150 mL of distilled

water was added followed by 10 mL of concentrated sulphuric acid and 10 drops of diphenylamine indicator. Then, it was titrated with ferrous sulphate solution. A blank titration was carried out throughout the procedure to standardize the dichromate solution. Soil organic matter content (%OM) was then determined as follows

$$(a - b) 0.8$$

Where a – Reading for the blank

b – Reading for the sample

### 3.2.5 Exchangeable bases and Cation Exchange Capacity

The exchangeable bases ( $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ ) were extracted by weighing 10 g of air dried soil into 250 mL conical flask. Then, 50 mL of ammonium acetate was added to the suspension and thereafter the suspension was shaken on the reciprocal shaker for 30 minutes. The suspension was filtered using number 42 Whitman filter paper before  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$  were measured in the filtrate (Doll *et al.*, 1975). The concentration (Cmol+/kg) of each cation was then determined as follows:

$$\text{Reading (mg/L)} \times \text{Volume of extractant (L)} \times \text{DF} / \text{Mass of soil} \times \text{Equivalent weight}$$

Where: DF – Dilution Factor

The cation exchange capacity in the soil was determined using ammonium acetate method (Doll *et al.*, 1975). Air dried soil (10 g) was weighed and put on the filter paper. A procedure was followed by pouring four times 25 mL of ammonium acetate, ethanol and 1 M KCl to come up with the filtrate. Thereafter, 10 mL of the filtrate was distilled before placing the sample in the digestion tube. Then, magnesium oxide (catalyst) and 10 mL of boric acid was added to the suspension. The distillation was done for 15 minutes followed by titration. The Cation Exchange Capacity was the amount of exchangeable cations per unit weight of soil.

### 3.2.6 Electrical conductivity

The electrical conductivity (EC) was determined using soil-water extraction method (Doll *et al.*, 1975); 10 g of air dried soil to which 50 mL of distilled water was added. The suspension was

then shaken for 30 minutes before it was filtered and electrical conductivity was then measured. The conductivity readings for temperature were corrected.

### 3.2.7 Texture

Soil texture was determined using the Hydrometer method (Paul, 1965). Air dried soil (50 g) was weighed and placed in a dispensing cup. Then, 50 mL of 5% calgon solution-sodium metaphosphate as a dispersing agent was added to the solution. The dispensing cup was filled up to half with distilled water and it was then stirred continuously for 5 minutes before it was transferred to the sedimentation cylinder. A stream of distilled water was used to complete the transfer and to bring the level of the liquid to the 1 litre mark. Immediately the temperature was measured and the plunger was inserted while holding the cylinder firmly and moving the plunger up and down to mix the contents thoroughly. After 20 minutes, the hydrometer was lowered carefully. The readings were taken at 40 seconds to determine silt and clay content. The suspension was not mixed again and the hydrometer reading was taken at 8 hours to determine clay content and the temperature reading was also taken at the same time. The texture of the soil was then determined by calculating particle size distribution as follows

#### % Sand

$$\% \text{ (Silt + Clay)} = (40 \text{ seconds reading} - C1 \pm C2 \times 100/50) \dots\dots\dots\%$$

$$\% \text{ Sand} = 100\% - \% \text{ (Silt + Clay)} = \dots\dots\dots\%$$

$$\% \text{ Clay} = (2 \text{ hours reading} - C1 \pm C3) \times 100/50 = \dots\dots\dots\%$$

$$\% \text{ Silt} = 100\% - \% \text{ Sand} - \% \text{ Clay} = \dots\dots\dots\%$$

Where C1 = Dispersing agent reading of the correction factor or the reading of the blank at 40 seconds or 2 hours

$$C2 = 40 \text{ Seconds correction}$$

$$C3 = 2 \text{ hours correction}$$

Then using the USDA Textural triangle, the texture class was determined (Paul, 1965).

### 3.2.8 Bulk density

The soil clods were collected at the time of soil sampling for determining bulk density. Bulk density was determined using the Paraffin Clod method (Blake, 1965). A soil clod was coated with paraffin, to prevent water penetration. The coated clod was then dipped in water to determine its volume. The bulk density was determined as follows:

$$A = B - C/C \times 100$$

Where A = Soil moisture content (%)

B = Mass of air dry clod

C = Mass of oven dry clod

The mass of oven dry clod (C) was determined as follows

$$C = 100/100 + A \times B$$

Where A = Soil moisture content (%)

B = Mass of air dry clod

C = Mass of oven dry clod

Then to calculate the bulk density, the following was done

$$D = E/F \quad \text{Where } D = \text{Bulk density for oven dry clod}$$

E = Weight of oven dry clod

F = Volume of clod

To obtain the volume of the soil clod, a cube approximately 3 x 3 x 3 cm was cut and tied onto a thread. The combined weight of the clod and the thread were determined using a top loading balance. The soil clod was then coated with melted paraffin. The coated soil clod was weighed in order to determine the volume of the paraffin based on the density of 0.9 kgdm<sup>3</sup>. A 500 cm<sup>3</sup> beaker was weighed on a top loading balance after it was filled with water up to three quarters



and the weight was recorded. Then, the coated clod was suspended in water and the weight was recorded. The gain in weight was equal to the weight of water displaced by the coated ped. The volume of the soil clod was determined as the difference between the volume of the coated soil clod and that of the paraffin.

### **3.3 Source of neem leaves**

Neem leaves were collected from Kasisi Agricultural Training Centre in Chongwe District in Agro- ecological Region II of Zambia, located 30 km East of Lusaka. The geographical location of Kasisi Agricultural Training Centre is approximately 15°10'25.30" South and 28° 31'55.12" East and 1214 m above sea level.

### **3.4 Sampling and chemical characterization of neem leaves**

The young fresh neem leaves were randomly collected from four sample trees which were approximately ten years old. The leaves were mixed thoroughly to make a homogenized composite sample before putting in paper bags.

The four replicate samples of neem leaves were oven dried at 60°C for 72 hours and analyzed for various elements.

#### **3.4.1 Total nitrogen**

Total nitrogen (N) was determined using the modified Kjeldah method (Kalra *et al.*, 1991). A gram of oven dried ground plant material was put into a digestion tube then 10 mL of a mixture of sulphuric acid and salicylic acid was added. After 30 minutes, 1.0 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O was added and the mixture was shaken. Thereafter, 10 mL of sulphuric acid and 1.0 g of catalyst mixture were added. The samples were digested at 410°C until the colour cleared. The digestion tubes were removed from the digestion block and allowed to cool for 15 minutes. Then distillation was done; thereafter the distillate was titrated with 0.1M HCl. Total tissue nitrogen was determined as follows

$$\text{mgN/Kg} = (S_T - B_T) \times 0.1 \times 1.4 \times 10 / \text{Weight of oven dry matter}$$

and

$$\% \text{ N} = \text{mgN/Kg} / 10\ 000$$

Where  $B_T$  = Blank Titre

$S_T$  = Sample Titre

### 3.4.2 Phosphorus, Magnesium, Potassium and Calcium

Determination of P, Mg, K and Ca was done using the Dry Ashing method (Cotteinie *et al.*, 1982). Crucibles were cleaned by heating on a hot plate with a 10%  $HNO_3$ . The crucibles were then dried in an oven at  $80^\circ C$  for atleast 30 minutes and allowed to cool. Then, 1.0 g oven dried ground plant materials were weighed into the clean crucibles and placed into a cold muffle furnace. The sample was then ashed at  $450^\circ C$  and then cooled in a desiccator and transferred quantitatively into a 100 mL beaker by means of 20 mL 1.0M  $HNO_3$ . The sample was then digested for 30 minutes before being filtered into a 100 mL volumetric flask. The filter paper was washed several times with distilled water and then filled to the mark. Then, calcium, potassium, magnesium and phosphorus in the plant tissues were determined by Atomic Absorption Spectrometer (AAS). The following calculations were used to determine the actual amounts of the elements

$$\% \text{ P in Plant Tissue} = \text{Reading (mg/L)} \times 10^{-3} \times 25 \times \text{DF}$$

$$\% \text{ Ca, Mg or K} = \text{Reading (mg/L)} \times 25 \times 10^{-3} \times \text{DF}$$

Where DF = any further dilutions of the samples

### 3.5 Preparation of aqueous neem leaf extract

There are several methods of preparing the aqueous neem leaf extract based on the use, whether as a pesticide or as a soil amendment. For this study, the extract was prepared as a soil amendment according to the procedure developed by KATC (2004).

The young fresh neem leaves collected from the four sample trees were mixed thoroughly to make a homogenous composite sample and apportioned into: 1, 2, 3 and 4 kg sub- samples. To arrive at these different concentrations, each sub-sample was placed in a securely tied sack and immersed in a drum containing 20 litres of water. The concentration of the extract based on the weight of leaves and volume of the water used to make the tea were 2%, 5%, 10%, 15% and

20%, respectively. For example, 1 kg of sub-sample in 20 liters of water was equivalent to the 10% concentration.

The drums were covered with polythene bags to prevent any foreign material from falling into the mixture. The mixture was vigorously stirred on a daily basis to allow proper leaching of the nutrients from the leaves into water. The sacks with the leaves were carefully removed after 14 days; to remove any debris, the extract was passed through a 2 mm sieve.

### **3.6 Chemical characterization of aqueous neem leaf extract**

In order to characterize the aqueous neem leaf extracts, standard laboratory analyses methods for water and liquid organic manure (Chapman *et al.*, 1961) were used to analyze for N, P, K, Ca, Mg, pH, and EC.

### **3.7 Greenhouse experiment**

To determine the effects of the different concentrations of aqueous neem leaf extract on soil microbial biomass and activity, the extract was applied to 5 kg of soil in a pot at a rate of **3 600 l/ha** every week for 5 consecutive weeks. This was done following the practice of small scale farmers in Zambia.

The six concentrations of aqueous neem leaf extract were arranged as a greenhouse experiment in a Completely Randomized Design (CRD) with four replications. Every week for 10 weeks, 100 g of soil from each pot was removed and analyzed for soil microbial biomass and activity using the Chloroform Fumigation and Incubation method and Soil Respiration method, respectively. After the 10 weeks, the soil remaining in the pots was collected and analysed for some selected chemical properties according to the methods described above.

### **3.8 Determination of soil microbial biomass and activity**

#### **3.8.1 Soil microbial biomass**

The soil microbial biomass was determined using Chloroform Fumigation and Incubation (CFI) method (Jenkinson *et al.*, 1981). Soil (100 g) was collected from each pot and further divided into two 50 g sub samples. The soil sub samples were put into small metal containers and moistened with distilled water to 70 % field capacity. The soil samples were placed into two separate desiccators. In one desiccator, the soil was fumigated with 40 mL chloroform placed in

a beaker. The purpose was to kill the microorganisms in the soil. The soil in the other desiccator was not fumigated. Thereafter, the two desiccators were incubated at room temperature for 72 hours. At the end of the fumigation, the beaker containing the chloroform was removed from the desiccator in an open place outside.

The soil microbial biomass was then determined by calculating the amount of evolved carbon dioxide carbon for the fumigated and non-fumigated samples using soil respiration method also described in 3.8.2

Once CO<sub>2</sub>-C evolved from fumigated and non-fumigated samples were calculated, then, soil microbial biomass (CO<sub>2</sub>-C mg/kg wet soil) was determined as follows:

$$C = F_c / 0.45$$

Where C = soil microbial biomass carbon (CO<sub>2</sub>-C mg/kg wet soil)

$F_c = (\text{CO}_2\text{-C evolved from fumigated soil in 10 days incubation period}) - (\text{CO}_2\text{-C evolved from non-fumigated soil in 10 days incubation period})$

0.45 = fraction of killed biomass mineralized to CO<sub>2</sub> over 10 days incubation period

To use this method, a number of basic assumptions were summarized by Jenkinson *et al.*, 1981 as follows:

- Carbon in dead organisms was more rapidly mineralized than that in living organisms.
- Soil fumigation with chloroform lead to a complete kill of the microorganisms in the soil.
- The number of the microorganisms killed in the unfumigated soil is negligible compared to that in the fumigated soil.
- The only effect of soil fumigation is to kill the microbial biomass
- The fraction of dead biomass C mineralized over a given time period does not differ in different soils.

### 3.8.2 Soil microbial activity

The soil microbial activity was determined using the soil respiration method (Dubey *et al.*, 2002). The soil microbial activity was determined by weighing 50 g of soil which was not fumigated. The soil sub samples were put into small metal containers and moistened with distilled water to 70 % field capacity.

Soil in small metal containers was placed in the plastic containers and small bottles containing 5 mL of potassium hydroxide were put in the same containers. A bit of water was put into the containers before incubating for ten days in the cupboard at room temperature. After 10 days of incubation, potassium hydroxide in small bottles which had trapped carbon dioxide was titrated. Two 2 drops of phenolphthalein were added to the KOH and back titrated with hydrochloric acid (HCl) until the red colour of the dye disappeared. The hydrochloric acid reading of the burette was noted. Subsequently, 2 drops of methyl orange were added and hydrochloric acid added until the yellow colour of the second dye turned pink. The amount of HCl consumed between the colours shifts corresponded to the amount of CO<sub>2</sub> which was fixed. The amount of soil microbial activity (soil respiration) was determined using the following formula

$$r = (a-b) \times 10t \times 1.2 \times 20/n$$

Where a – cm<sup>3</sup> HCl consumed between the two colour shifts

b – cm<sup>3</sup> HCl consumed between the two colour shifts (control)

10t – titre of HCl x 10 (correction factor for t being different from 0.1M HCl)

n – Days of incubation

1.2 – equivalent amount of C for 1 cm<sup>3</sup> 0.1M HCl

20 – Conversion factor for 50 g of wet soil

### 3.9 Statistical analysis of data

To determine the effects of aqueous neem leaf extract on soil chemical and biological characteristics, all data were subjected to Analysis of Variance (ANOVA) at 95% level of confidence using the SAS Package version 9.1. The means were separated and compared using Least Square Difference test at the same confidence level.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 Nutrient composition of neem leaves

The nutrient content of neem leaves used to prepare aqueous neem leaf extract is showed in Table 2 below.

Table 2: Nutrient composition of neem leaves

Nutrients	Leaf content (%)
Nitrogen	1.50±0.05
Phosphorus	0.09±0.01
Potassium	1.77±0.10
Calcium	2.58±0.29
Magnesium	0.35±0.14

Mean values of four replicates ± Standard Error of means

Neem leaves contained 2.58% of calcium which was highest, followed by potassium which had 1.77% and nitrogen at 1.5%. The nutrient level of phosphorus was 0.09% while the least nutrient content of neem leaves was magnesium at 0.35%. The composition of neem leaves is comparable to other sources of aqueous leaf extract such as confrey (*Symphytum officinale*) and tithonia (*Tithonia diversifolia*). Confrey leaves were reported to have 5.3 % potassium, 1.8 % nitrogen and 0.5 % phosphorus. Tithonia leaves from Northern part of Zambia recorded 4.2 % potassium, 2.9 % nitrogen and 0.18 % phosphorus (Malama, 2001). Neem leaves are therefore a good source of nutrients for the preparation of aqueous neem leaf extract.

## **4.2 Chemical composition of aqueous neem leaf extract**

In order to determine the amounts of nutrients being added to the soil in the different concentrations of the aqueous neem leaf extracts, the extracts were analysed and results were presented in appendix 1 and Figures 1 – 4 below.

The concentrations of the nutrients depended on the aqueous neem leaf extract concentrations. The lowest concentrations were in the control (0%) and the highest in the 20% extract for all selected chemical properties.

The chemical composition of aqueous neem leaf extract is attributable to various factors, these include: the nature of structural compounds in the neem leaves, the duration of extraction, the age of the tree, the part of the trees from which the leaves were collected, and also the method of preparation (Blunden, 1991). The extract in the current study was prepared according to the method of KATC (2004); fresh leaves were put in sacks and immersed in water for fourteen days. This is as opposed to the alternative method where the neem leaves are washed with tap water and then they are sun dried before they are ground to powder form. This method of preparing the leaf aqueous extract has been shown to result in higher levels of nutrients than when applied dry. This phenomenon has also been observed in the case of manure; soaking of manure makes the nutrients more readily available than when applied as dry (Mafongoya *et al.*, 1997).

### **4.2.1 Ammonium nitrogen (NH<sub>4</sub>-N) and Nitrate nitrogen (NO<sub>3</sub>-N)**

The levels of NH<sub>4</sub>-N in the different concentrations of aqueous neem leaf extract are presented in Figure 1 below, NH<sub>4</sub>-N ranged from 21.7 to 103.6 mg/l, with the control having 15.75 mg/l. The levels of NH<sub>4</sub>-N for the control, 2% and 5% concentrations were non-significantly different from each other.

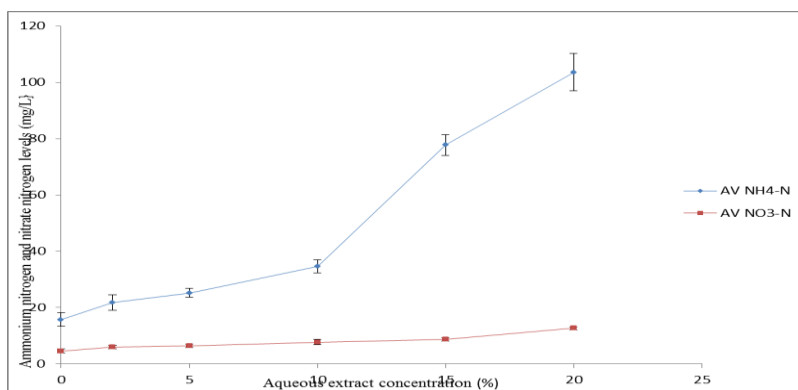


Figure 1: NH<sub>4</sub>-N and NO<sub>3</sub>-N contents of different concentrations of neem leaf extract

The levels of NO<sub>3</sub>-N for the different concentrations increased with the concentration of aqueous neem leaf extract and ranged from 5.98 to 12.60 mg/l. Significant differences were observed at 10% and above. Generally, it was observed that there was more ammonium nitrogen in aqueous neem leaf extract than nitrate nitrogen. This finding is in agreement with earlier findings by Moyin-jesu (2011) while most plants take up nitrogen in the nitrate form. It has to be reduced to ammonium prior to assimilation into organic compounds. In this regard, nitrate does not accumulate in plant tissues and will be found only in lower concentration than ammonium/ammonia.

Comparative evaluation of modified neem leaf, neem leaf and wood ash extracts showed highest levels for NH<sub>4</sub>-N and NO<sub>3</sub>-N in neem leaf extract (5.65 and 0.89 mg/l) followed by poultry manure extract (4.53 and 0.45 mg/l) then modified neem leaf extract (3.69 and 0.15 mg/l). The least level for NH<sub>4</sub>-N and NO<sub>3</sub>-N was recorded in wood ash (0.15 and 0.16 mg/l). The levels of both NH<sub>4</sub>-N and NO<sub>3</sub>-N in the different concentrations of aqueous neem leaf extract were far much higher than the levels recorded by Moyi jesu (2011). Minimal or no differences were observed in Figure 1 below in the levels for Nitrate nitrogen for the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract from the control.

#### 4.2.2 Exchangeable bases (K, Ca and Mg)

The levels of K in the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract were high 35.37, 54.87, 59.94, 67.88 and 115.95 mg/L respectively compared to the control (3.49 mg/L).



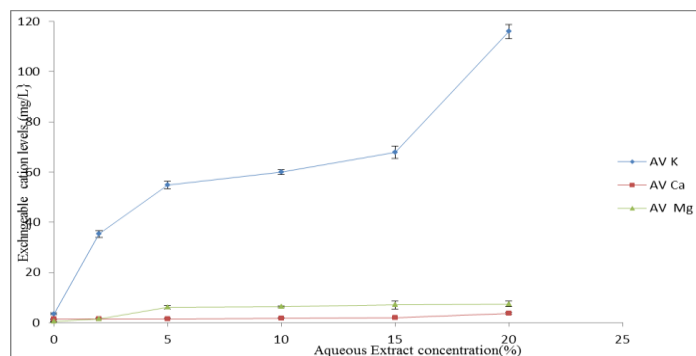


Figure 2: K, Ca and Mg contents of different concentrations of neem leaf extract

The levels of Ca in the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract were high 2.58, 2.98, 3.31, 3.77 and 4.50 mg/L respectively compared to the control (1.36 mg/L).

It was also observed that the more the extract was concentrated, the higher the levels for Mg. The levels of Mg according to the different concentrations (2, 5, 10, 15 and 20%) were: 1.49, 6.06, 6.28, 7.01 and 7.42 mg/L respectively compared to the control 0.34 mg/L.

These results indicate that the neem leaf extracts present a good source of nutrients for soil amendments and could be used in the place of wood ash extracts and poultry manure.

Calcium content were constant for the different concentrations of aqueous neem leaf extract and the levels were very minimal as compared to potassium.

The trends in magnesium for the different concentrations of aqueous neem leaf extract in Figure 2 below showed that it was constant from 5% to 20%. A minimal increase was recorded from the control to 5% concentration.

An earlier report has shown different trends in exchangeable cations. Moyin jesu., 2011 recorded higher levels of  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  in modified neem leaf, wood ash, neem leaf extract and the least was recorded in poultry manure extract as follows: K (3.2, 2.60, 1.67 and 0.97 mg/l respectively),  $Ca^{2+}$  (15.66, 15.00, 0.77 and 0.32 mg/l respectively) and  $Mg^{2+}$  (1.53, 1.00, 0.75 and 0.41 mg/l respectively).

Therefore, it was concluded that the levels of potassium ( $K^+$ ) were significantly higher with the concentration of aqueous neem leaf extract although it was constant for 5%,10% and 20%

concentrations. It was observed that the trends of calcium and magnesium ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) were also constant with the concentration.

### 4.2.3 Phosphorus (P)

The levels for P according to the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract were 0.24, 0.32, 0.33, 0.34 and 0.34 mg/L respectively compared to the control 0.16 mg/L. It was observed that the more the extract was concentrated, the higher the levels of Phosphorus. It was also observed that 2, 5, 10, 15 and 20% concentrations of aqueous neem leaf extract were higher than the control by 0.08, 0.16, 0.17, 0.18 and 0.18 mg/L respectively.

Moyin-jesu, 2011 reported the highest level of P in poultry manure extract (3.20 mg/l) and the second highest level of P was observed in modified neem leaf. The third level of P was recorded in neem leaf extract while the least content was recorded in wood ash. The levels for P observed by Moyin-jesu, 2011 were slightly higher than the levels observed in the different concentrations of aqueous neem leaf extract.

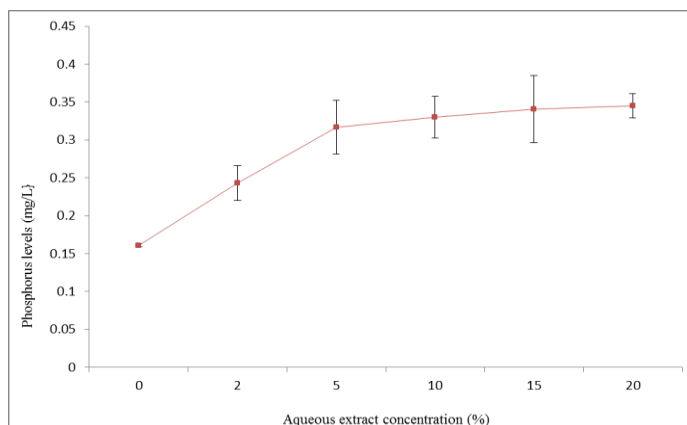


Figure 3: P content of different concentrations of neem leaf extract

### 4.2.4 Electrical conductivity (EC)

The levels for EC were also observed to increase with the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract as follows: 0.78, 1.39, 2.27, 2.95 and 4.00 mS/cm @ 25°C from the control (0.72 mS/cm @ 25°C).

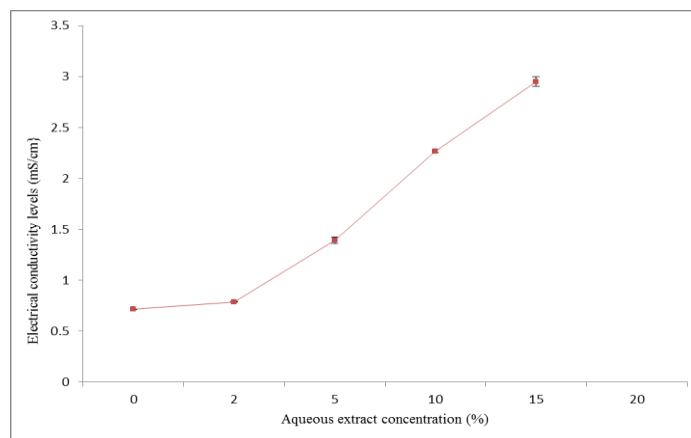


Figure 4: Trends in Electrical conductivity for different concentrations of neem leaf extract

#### 4.2.5 Soil reaction (pH)

The pH for the different concentrations (2, 5, 10, 15 and 20%) of aqueous neem leaf extract was weakly alkaline to alkaline as observed in the levels of pH as follows: 7.93, 7.94, 7.94, 7.89 and 8.10 respectively compared to the control was 7.12.

#### 4.3 Initial soil chemical and physical properties

Table 4 below gives the initial soil chemical and physical properties before the soil was treated with the aqueous neem leaf extract. The selected soil chemical properties included pH, calcium (Ca), potassium (K), nitrogen (N), cation exchangeable capacity (CEC), phosphorus (P), electrical conductivity (E.C), magnesium (Mg) and organic Matter (OM) while the selected physical properties included bulk density and texture.

Table 3: Initial selected soil chemical and physical properties

Soil Parameters	Values	Critical value for maize production
Soil pH (CaCl <sub>2</sub> )	6.40±0.05	4.5
Organic matter (%)	1.64±0.43	2.5
Total Nitrogen (%)	0.20±0.01	0.1
Phosphorus (mg/kg)	0.10±0.01	10.0
Cation Exchange Capacity (cmol +/kg)	8.00±1.29	14.0
Electrical Conductivity (mS/cm@250C)	0.10±0.02	-
Calcium (cmol+/kg)	8.10±0.72	0.1
Magnesium (cmol+/kg)	0.10±0.02	0.1
Potassium (cmol+/kg)	0.40±0.01	0.22
Bulk density (g/cm <sup>3</sup> )	1.50±0.15	-
Texture	Sandy Clay	-

Mean values of four replicates ± Standard Error of Means

#### 4.3.1 Nitrogen (N) and Organic matter (OM)

The Nitrogen content of the soil was 0.20% which was less than its critical value of 0.25% for maize production. Nitrogen content was moderately low probably due to low organic carbon in the soil since organic carbon is an index of soil fertility. Soils in Zambia are generally N-deficient. The organic matter content of the soil was 1.64 % which was also less than the critical value of 2.5%. The organic matter was low probably due to the fact that organic carbon in the soil was low.

#### 4.3.2 Exchangeable bases (K, Ca and Mg) and Cation Exchange Capacity (CEC)

Potassium content was 0.4 cmol+/kg observed to be more than the critical value of 0.1 cmol+/kg. Levels of calcium (8.1 cmol+/kg) were more than the critical value of 0.1 cmol+/kg while the

value for magnesium was 0.1cmol+/kg which equals to the critical value of 0.10 cmol+/kg. There was need to replenish the soil in order to have more magnesium (Mg) in the soil.

The cation exchange capacity was 8.0 cmol+/kg recorded to be lower than the critical value of 14 cmol+/kg. The cation exchange capacity depends on the amount of soil organic matter and clay. The higher the organic matter in the highly weathered soils, the higher the cation exchange capacity (Sanchez *et al.*, 1997). It was observed that the cation exchange capacity was low and this was attributed to the low soil organic matter content as compared to the critical value for maize production.

#### **4.3.3 Phosphorus (P)**

Plant available phosphorus was 0.10 mg/kg which was below the critical value of 10.0 mg/kg for maize production. The low phosphorus content of the soil could be attributed to fixation even at pH 6.4. The other reason could be that the soil has inherently low levels of phosphorus.

#### **4.3.4 Electrical conductivity (EC)**

The electrical conductivity of the soil was 0.1 mS/cm @ 25°C which was falling in the salinity class of 0 – 2 mS/cm @ 25°C. Therefore, the soil is classified as not being hazardous for the soil microorganisms.

#### **4.3.5 Soil reaction (pH)**

According to the results in Table 4, the soil was slightly acid at pH 6.4. It implied that the soil was conducive for microbial activity as slightly acidic to neutral conditions of the soil tend to favour soil microbial activities (Alexander, 1997). The critical value of 4.5 is strongly acidic and it affects soil microbial activities even if some microorganisms do well in strongly acidic soils (Alexander, 1997).

#### **4.3.6 Bulk density and Texture**

The bulk density was 1.5 g/cm<sup>3</sup> which implied that the soil was loosened as opposed to compacted soils which usually have the bulk density more than 1.8 g/cm<sup>3</sup>. The soil was conducive for root penetration, movement of water and free movement for the microorganisms (Sanchez *et al.*, 1997).

The soil texture was Sandy Clay and this soil type is typical of Chililabombwe which is found in Region III and experiences high rainfall which is highly leached.

#### **4.4 Effect of aqueous neem leaf extract on soil chemical properties**

The results of chemical analysis for soils collected from the pots after the greenhouse incubation experiment are shown in appendix 2.

Worth noting is that aqueous neem leaf extract has been reported to be a good source of nutrients, particularly for nitrogen, phosphorus and potassium when it is applied as a soil amendment (Manu *et al.*, 1991).

##### **4.4.1 Nitrogen (N) and Organic matter (OM)**

It was observed that there were non-significant differences in the nitrogen levels among the different concentrations of aqueous neem leaf extract and the control after the experiment was concluded. It was further observed that there was no change between the initial nitrogen levels of the soil before and after the soil was treated with the different concentrations of aqueous neem leaf extract. This was despite the addition of nitrogen through repeated applications of aqueous neem leaf extract. The reason could be that the applied nitrogen could have leached down as supported by observations made by Martin and Duddles, (1984) that application of excess nitrogen could lead to its loss through leaching.

The results showed that there was non-significant difference in the levels of soil organic matter in the soil treated with the different concentrations of aqueous neem leaf extract and the control. It was further observed that there was no change in the nitrogen contents of the soil treated with 15% concentration of aqueous neem leaf extract as compared to the initial soil organic matter and that of the control. A reduction by 50% in the soil organic matter levels was observed in the other concentrations (2%, 5%, 10% and 20%) of aqueous neem leaf extract. The reason could be that the microorganisms were feeding on the soil organic matter and there was no replenishment. The diversity and activity of the soil microorganisms depends on organic matter (Sakala *et al.*, 2004).

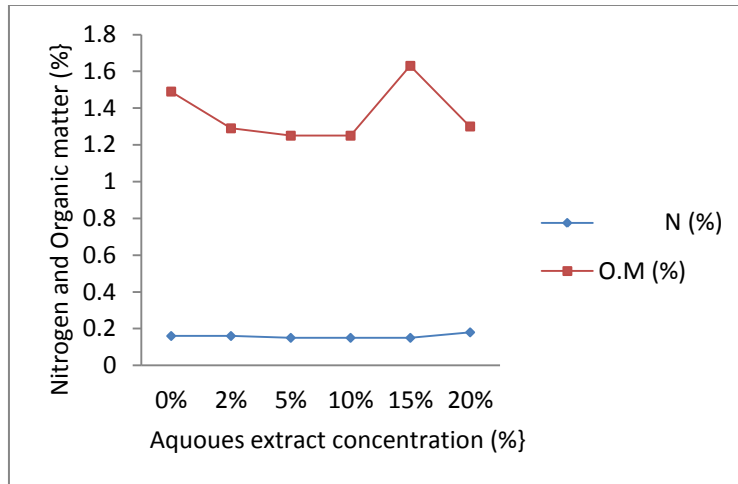


Figure 5: N and OM content of the soil treated with different concentrations of neem leaf extract at the end of experiment

The trends in figure 5 in organic matter content showed a reduction and maintained as compared to the control from 2% to 10% of aqueous extract concentration. A sharp increase was observed at 15% aqueous extract concentration. It was further observed that the organic matter declined at 20% aqueous extract concentration. The trends in nitrogen content of the soil at the end of the experiment showed that it was almost the same in the different concentrations of aqueous neem leaf extract. A slight increase was observed at 20% aqueous neem leaf extract concentration.

#### 4.4.2 Exchangeable bases (K, Ca and Mg) and Cation Exchange Capacity (CEC)

There were non-significant differences in the levels of the exchangeable bases (K, Ca and Mg) between the soils treated with different concentrations of aqueous neem leaf extract and the control. This was probably due to the fact the soil was from the same source and the sample size was the same. The other reason may be could be that the soil reaction was within the same range. Significant differences were only recorded in the levels of potassium (K) in the soils treated with 10% and 20% concentrations of aqueous neem leaf extract. The reason could be due to soil variabilities attributed to the nature of the season when soil was collected. It was further observed that there were non-significant changes in the levels of the exchangeable bases (K, Ca and Mg) in the soil from the start up to the end of the experiment. This is contrary to the observation made by Mudenda and Yerokun, (2008) that manure extracts are faster nutrient release source. The reason could be that the nutrients (K, Ca and Mg) were in excess such that they were leached down when the soils in the pots were moistened at 70% for the environment to

be conducive for the soil microorganisms. Therefore, it was concluded that aqueous neem leaf extract had non-significant effect on the soil exchangeable bases (K, Ca and Mg) of the soil due to leaching down and slow release of the nutrients.

There were non- significant differences in the cation exchangeable capacity (CEC) for the soils treated with the different concentrations of aqueous neem leaf extract and the control. It was further observed that there were no changes in the soil even after it was treated with aqueous neem leaf extract. This is attributed to the fact that there were no changes in the exchangeable bases. The reason could be that the cation exchangeable capacity depends on the texture, Ph and Organic Matter content. The soil had some clay in it and the organic matter was low despite being uniform. Therefore, it was concluded that aqueous neem leaf extract had non-significant effect on the Cation Exchange Capacity.

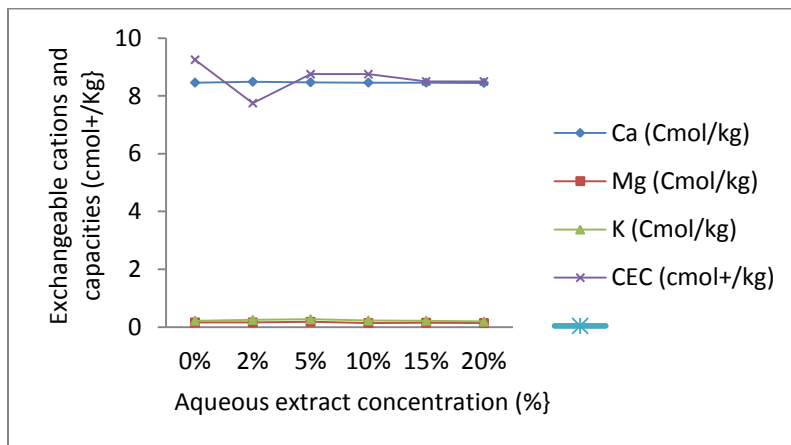


Figure 6: Exchangeable cations and CEC of the soil treated with different concentrations of neem leaf extract at the end of experiment

The trends in figure 6 for exchangeable cations showed that calcium was high as compared to magnesium and potassium. It was further observed that the levels of calcium were the same for all the concentrations of aqueous neem leaf extract compared to the control. It was also observed that the levels of magnesium and potassium were the same for the concentrations of aqueous neem leaf extract compared to the control. The levels of magnesium for the different concentrations of aqueous neem leaf extract were the same compared to the control. It was also observed that the levels of potassium were the same for the concentrations of aqueous neem leaf extract compared to the control.



The trends in figure 6 for the cation exchange capacity showed generally a reduction for the concentrations of aqueous neem leaf extract compared to the control. It was also observed that there was a further reduction in the cation exchangeable capacity at 2% concentration of aqueous neem leaf extract as compared to the other concentrations of aqueous neem leaf extract.

#### **4.4.3 Phosphorus (P)**

It is worth noting that there were non-significant differences in the phosphorus (P) levels in soils treated with the different concentrations of aqueous neem leaf extract and the control. It was further recorded that there were non-significant changes in the P levels in the soils treated with the different concentrations of aqueous neem leaf extract. This was due to the fact that the availability of phosphorus is largely determined by soil pH. The soil reaction was conducive for the availability of phosphorus and soil was collected from the same source. Therefore, it is concluded that the application of aqueous neem leaf extract had non-significant effect on P levels in the soil.

The trends in figure 7 for phosphorus content for the soil treated with different concentrations of aqueous neem leaf extract at the end of the experiment showed a reduction at 2% concentration of aqueous neem leaf extract compared to the control. This was also due to the fact that the soil had low levels of organic matter which is a major source of phosphorus although it is dependant on soil biological activities. A sharp increase in phosphorus content was observed at 5% as compared to the control. The phosphorus content was almost maintained at 10%, 15% and 20% concentrations of aqueous neem leaf extract as compared to the control. This could be attributed to high levels of soil biological activity as a result more phosphorus was released despite the low levels of organic matter in the soil.

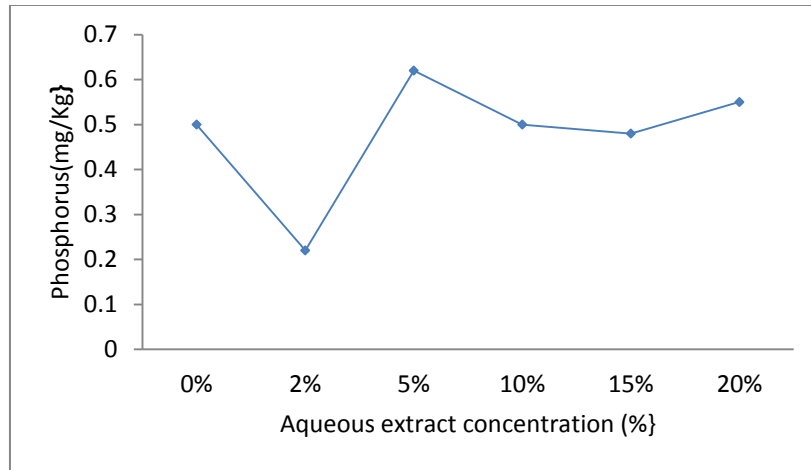


Figure 7: P content for soil treated with different concentrations of neem leaf extract at the end of experiment

#### 4.4.4 Electrical conductivity (EC)

It was observed that there were no significant differences between all the treatments (different concentrations of aqueous neem leaf extract) and the control. Despite the high levels of salinity (high EC) observed in the different concentrations of aqueous neem leaf extract in Table 3, non-significant changes were recorded in the EC of the soil over the period of the experiment. Hence, the salinity hazard levels of the soils were classified as non-hazardous as they all had the values not more than  $2.0 \text{ mScm}^{-1}$  @  $25^{\circ}\text{C}$ .

The trends in figure 8 for the electrical conductivity of the soil treated with different concentrations of aqueous neem leaf extract showed a reduction at 2%, 5%, 15% and 20% concentrations of aqueous neem leaf extract compared to the control. The electrical conductivity was maintained at 10% concentration of aqueous neem leaf extract.

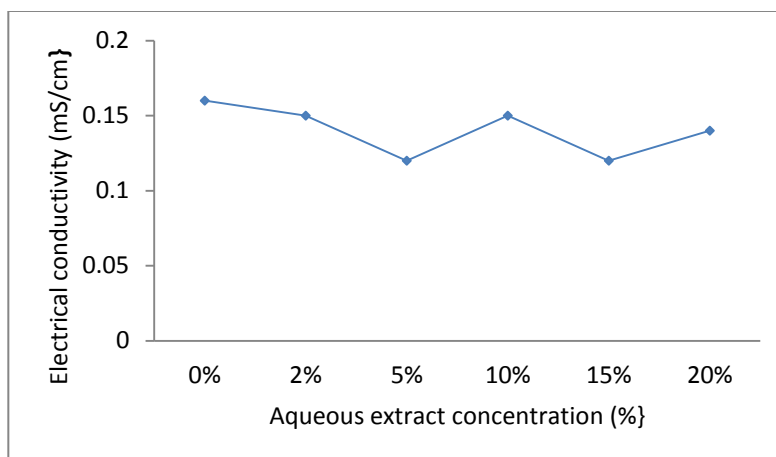


Figure 8: Trends in EC for soil treated with different concentrations of neem leaf extract at the end of experiment

#### 4.4.5 Soil reaction (pH)

It was observed that pH for the soils treated with the different concentrations of aqueous neem leaf extract were non significantly different from the control. It was further observed that there were non-significant changes in the pH of the soil contrary to the observation by Mudenda and Yerokun, (2008) that the aqueous neem leaf extract had no effect on soil pH. Mudenda and Yerokun, (2008) also recorded an average rise of pH to 6.21 and 6.76 from an initial of 5.9 in soils treated with manure extracts. This was attributed to protonation of organic ligands from dessication of water (Sakala, *et al.*, 2004). Previously, manure extracts have been reported to raise pH levels of soils (Wong *et al.*, 2000). The soil pH was still conducive for soil microorganisms which thrive in slightly acidic to alkaline soils (Sakala, *et al.*, 2004). In strongly acid or highly alkaline soils, the growing conditions for the microorganisms are poor, resulting in low levels of biological oxidation of organic matter. Therefore, it was concluded that there was non-significant differences in soil reactions despite the application of different concentrations of aqueous neem leaf extract.

#### **4.5 Effect of aqueous neem leaf extract on cumulative soil microbial activity**

There were non- significant differences in treatment effects of 2%, 5%, 15% and 20% and the control on soil microbial activity at 95% confidence limit at weeks 5 and 10 (refer to appendix 3). Significant differences were only observed at week 10 among treatment effects of 10% concentration and the other concentrations (0%, 2%, 15%, and 20%). There was non- significant difference between treatment effects of 5% and 10% concentrations of aqueous neem leaf extract. Significant differences were also recorded at week 5 among treatment effects between 10% and 20% concentrations. There were non- significant differences at week 5 among treatment effects (0%, 2%, 5%, 15%). There was a double increase in microbial activity from week 5 to week 10 in all the treatments including the control.

The cumulative microbial activity at week 10 for the control was 60.36 CO<sub>2</sub>-C mg/kg moist soils. Significant and positive effects were observed in 10% concentration of aqueous neem leaf extract which recorded 71.73 CO<sub>2</sub>-C mg/kg moist soils. The significant change in the soil treated with 10% concentration of aqueous neem leaf extract was recorded at week 10 which was 11.37 CO<sub>2</sub>-C mg/kg moist soil or 16%.

The cumulative microbial activity at week 5 for the control was 31.45 CO<sub>2</sub>-C mg/kg moist soils. Non- significant effect and no trend were observed among the treatments. Except that there was a doubling at week 5 and 10 and 10% treatment was the highest.

Conventry and Allan, (2001) reported that neem extracts had antimicrobial activity effect, inhibiting microorganism activity in soil and their activities. Kiran and Patra, (2003) attributed the antimicrobial activity of neem extract to meliacins (nimbin and nimbidin) which were active ingredients that were found in neem responsible for the inhibition of nitrification process. Conventry and Allan (2001) also reported that neem extract inhibited rhizobia in beans.

The aqueous neem leaf extract showed no negative effects on soil microbial activity because neem extracts broke down quickly in sunlight and in the soil (Robson, 2002). In this respect neem extracts are environmentally friendly compared to chemical fertilizers and pesticides which leach down the soil profile and kill the microorganisms (Kiran and Patra, 2003).

The other reason why aqueous neem leaf extract had non- significant effect on soil microbial activity was the method which was used to prepare the extract was different from the method that

was usually used to prepare the extract meant for use as a pesticide. When the extract was prepared for use as a pesticide, the neem leaves were washed with tap water and thereafter it was sun dried and ground to powder form before it was mixed with water in different concentrations. When preparing the extract for use as a soil amendment, the neem leaves were simply soaked in water for fourteen days in order to have enough nutrients released. When the extract was prepared by sun drying and ground to powder form, chemical compounds in it became more concentrated such that the effect on the microbial activity was negative. It may not have a negative effect if it is prepared by soaking in water which dilutes the solution.

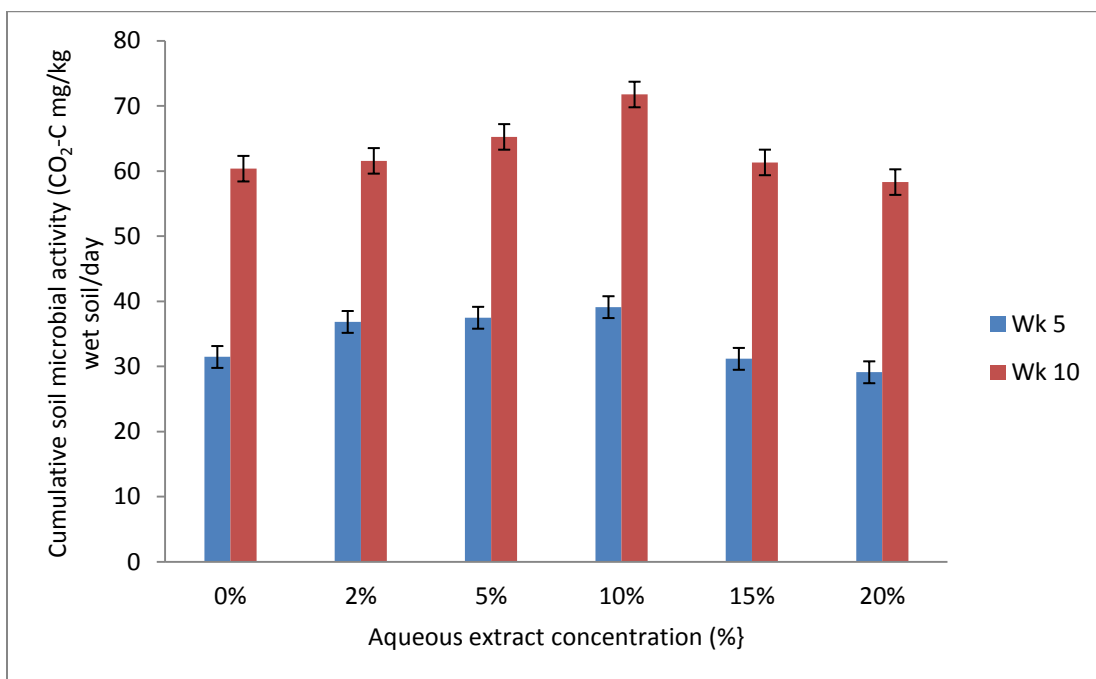


Figure 9: Trends of cumulative soil microbial activity for soils treated with different concentration of aqueous neem leaf extract

The trends of cumulative soil microbial activity for the soil treated with different concentrations of aqueous neem leaf extract at week 5 showed in figure 9 above that the soil microbial activities at 2%, 5% and 10% concentrations of aqueous neem leaf extract increased as compared to the control. The reason could be that the aqueous extracts were less concentrated to eliminate soil microorganisms hence increased microbial activity. The other reason could be that the soils treated with 2%, 5% and 10% concentrations of aqueous neem leaf extract had more substrate because the microorganisms depended so much on the extract. The soil microbial activities at

15% concentration of aqueous neem leaf extract was at the same level compared to the control. The soil microbial activity for soil treated with 20% concentration of aqueous neem leaf extract reduced as compared to the control. It was observed that 20% concentration of aqueous neem leaf extract was the possible threshold for anti-microbial activity. It also showed an inhibitory effect due to high concentration of chemical substances and reduced substrate.

The trends of cumulative soil microbial activity for the soil treated with different concentrations of aqueous neem leaf extract at week 10 were similar to the trends observed at week 5. The trends showed in figure 9 above that the soil microbial activities for soils treated with 2%, 5% and 10% concentrations of aqueous neem leaf extract increased as compared to the control due to low concentrations of chemical substances and the soil condition was conducive. The increase in soil microbial activity was also attributed to increased substrate. The soil microbial activity for soil treated with 15% concentration of aqueous neem leaf extract had maintained compared to the control while it reduced for soil treated with 20% concentration of aqueous neem leaf extract. This is due to reduced substrate and high concentrations of chemical substances.

#### **4.6 Effect of aqueous neem leaf extract on cumulative soil microbial biomass**

There were significant differences in cumulative soil microbial biomass among the different treatments of various concentrations of aqueous neem leaf extract at weeks 10 (refer to appendix 4). Significant differences were observed only among 2%, 5% and 15% and the rest had no significant differences at week 10. There were non-significant differences in all the treatments at week 5. Treatment 15% showed the highest microbial biomass at weeks 5 and 10.

The cumulative microbial biomass at week ten for the control, 2%, 5%, 10%, 15% and 20% treatments were 68, 55, 61, 66, 74 and 71 CO<sub>2</sub>-C mg/kg wet soil respectively. The cumulative soil microbial biomass for 2%, 5% and 10% concentrations were lower than the control by 19%, 10% and 3% respectively. The cumulative soil microbial biomass for 15% and 20% were higher than the control by 8% and 4% respectively.

Soil microbial biomass, a living part of soil organic matter, is an agent of transformation for added and native organic matter and acts as a labile reservoir for plant available nitrogen,

phosphorus and sulphur (Jenkison and Ladd, 1981). The activity of microbial biomass is mainly used to characterize the microbiological status of a soil (Nannipieri *et al.*, 1990). It is also worth noting that the soil microbial properties are influenced by several factors such as variations in the soil moisture and temperature as well as nutrient supply (Campbell *et al.*, 1999).

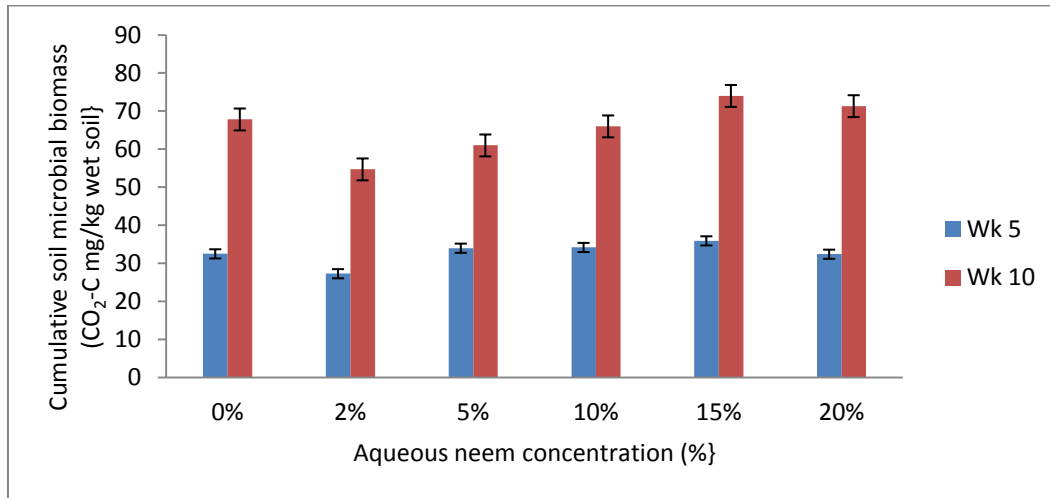


Figure 10: Trends of cumulative soil microbial biomass for soil treated with different concentrations of aqueous neem leaf extract

The trends of cumulative soil microbial biomass for soil treated with different concentrations of aqueous neem leaf extract at week 5 showed in figure 10 above that it was lower in soil treated with 2% concentrations of aqueous neem leaf extract compared to the control. This is due to the fact that the soil had low substrate and organic matter for the microbes to survive. The soil microbial biomass was almost maintained for soils treated with 5%, 10%, 15% and 20% concentrations of aqueous neem leaf extract compared to the control. This was a result of insufficient substrate in the soil.

The trends of cumulative soil microbial biomass for soil treated with different concentrations of aqueous neem leaf extract at week 10 showed in figure 10 above that it was lower in soils treated with 2% and 5% concentrations of aqueous neem leaf extract compared to the control there by showing negative effects. This is due to the fact that the soil had insufficient substrate. It was also clear that the soil microbial biomass for soil treated with 10% concentration of aqueous neem leaf extract was maintained compared to the control. The positive effects were observed in

soils treated with 15% and 20% owing to the fact that there was no residue effect over time since neem leaf extract was non synthetic.



## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

Neem biomass had the highest level of calcium (Ca) followed by potassium (K), nitrogen (N), magnesium (Mg) and the least was phosphorus (P). The chemical levels in aqueous neem leaf extract increased according to the concentration. The more concentrated the aqueous neem leaf extract, the higher the levels of the nutrients. Soil reaction (pH) for the soils treated with different concentrations of aqueous neem leaf extract were non-significantly different from the control. There were non-significant changes in the soil reaction (pH). Despite the high levels of electrical conductivity (EC) in the different concentrations of aqueous neem leaf extract, non-significant changes were recorded in the EC of the soil over the period of the experiment. The application of aqueous neem leaf extract had non-significant effect on P levels in the soil. Aqueous neem leaf extract had non-significant effect on the cation exchange capacity (CEC). Aqueous neem leaf extract had non-significant effect on the soil exchangeable bases (K, Ca and Mg) of the soil due to leaching down and slow release of the nutrients. The application of aqueous neem leaf extract had non-significant effect on the levels of nitrogen (N) and organic matter (OM) in the soil. Also the application of aqueous neem leaf extract had no significant effect on soil microbial biomass and activity. Application of aqueous neem leaf extract improved soil microbial biomass and activity due to the fact that the nutrients from the extract were quickly and readily released into the soil. Therefore all the different concentrations (2%, 5%, 15% and 20%) of aqueous neem leaf extract apart from 10% concentration had non-significant effect on soil microbial biomass and activity.

#### 5.2 Recommendations

There is need to carry out the similar research in the field where the small scale farmers should be involved in the management of a test crop using 10% concentration of aqueous neem leaf extract based on the findings from this research. In this research, the rate of application of aqueous neem leaf extract was 3 600 ha/L. Therefore, a research should be conducted to determine the effect of different rates of aqueous neem leaf extract on soil biology.

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## APPENDICES

**Appendix 1: Selected chemical properties of aqueous neem leaf extract**

<b>Treatments</b>	<b>NH<sub>4</sub>-N</b>	<b>NO<sub>3</sub>-N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>pH</b>	<b>EC</b>
			<b>mg/L</b>				<b>Cacl<sub>2</sub></b>	<b>mS/cm</b>
0%	15.75d	4.55d	0.16c	3.49e	1.36d	0.34b	7.12b	0.72e
2%	21.70d	5.95cd	0.24bc	35.37d	1.50cd	1.49b	7.93a	0.78e
5%	25.20dc	6.35cd	0.32ab	54.87c	1.54cd	6.06a	7.92a	1.39d
10%	34.65c	7.70bc	0.33a	59.94c	1.81bc	6.28a	7.94a	2.27c
15%	77.70b	8.75b	0.34a	67.88b	2.02b	7.01a	7.85a	2.95b
20%	103.60a	12.60a	0.34a	115.95a	3.75a	7.42a	8.06a	4.00a
LSD(p≤0.05)	10.73	2.04	0.08	5.28	0.40	2.42	0.32	0.07
CV (%)	15.56	17.90	20.03	16.32	13.59	30.20	12.83	22.38

Means followed by the same letter within a column are not significantly different

**Appendix 2: Effect of aqueous neem leaf extract on soil chemical properties**

<b>Aqueous extract concentration</b>	<b>0%</b>	<b>2%</b>	<b>5%</b>	<b>10%</b>	<b>15%</b>	<b>20%</b>	<b>LSD (p≤0.05)</b>	<b>CV (%)</b>
K(cmol+/kg)	0.22ab	0.25ab	0.27a	0.23ab	0.22ab	0.19b	0.07	19.20
Mg(cmol+/kg)	0.16ab	0.16ab	0.18a	0.14b	0.15ab	0.14b	0.04	16.92
Ca(cmol+/kg)	8.46a	8.49a	8.47a	8.46a	8.46a	8.45a	0.09	10.69
N (%)	0.16a	0.16a	0.15a	0.15a	0.15a	0.18a	0.03	13.32
OM (%)	1.49a	1.29a	1.25a	1.25a	1.63a	1.30a	1.03	15.46
CEC(cmol+/kg)	9.25a	7.75a	8.75a	8.75a	8.50a	8.50a	2.69	21.09
EC(mS/cm@25 <sup>0</sup> C)	0.16a	0.15a	0.12a	0.15a	0.12a	0.14a	0.05	22.31
pH(CaCl <sub>2</sub> )	6.55a	6.42a	6.53a	6.48a	6.58a	6.50a	0.20	12.02
P(mg/kg)	0.50a	0.22a	0.62a	0.50a	0.48a	0.55a	0.52	23.54

Means followed by the same letter are not significantly



**Appendix 3: Effect of aqueous neem leaf extract on cumulative soil microbial activity**

<b>Aqueous extract concentration</b>	<b>Cumulative microbial activity at week 5 (CO<sub>2</sub>-C mg/Kg moist soil/day)</b>	<b>Cumulative microbial activity at week 10 (CO<sub>2</sub>-C mg/Kg moist soil/day)</b>
0%	32ab	60b
2%	37ab	62b
5%	38ab	65ab
10%	39a	72a
15%	31ab	61b
20%	29b	58b
LSD (p≤0.05)	9	10
CV (%)	18	11

Means within a column with the same letter are not significantly different

**Appendix 4: Effect of aqueous neem leaf extract on cumulative soil microbial biomass**

<b>Aqueous extract concentration</b>	<b>Cumulative soil microbial biomass at week 5 (CO<sub>2</sub>-C mg/Kg moist soil)</b>	<b>Cumulative soil microbial biomass at week 10 (CO<sub>2</sub>-C mg/Kg moist soil)</b>
0%	33ab	68ab
2%	27a	55bc
5%	34ab	61bc
10%	34ab	66abc
15%	36a	74a
20%	32ab	71ab
LSD (p≤0.05)	8	12
CV (%)	16	12

Means within a column with the same letter are not significantly different