MINERAL LEVELS OF SOME PLANTS AND THEIR LEAF EXTRACTS AND THEIR EFFECTS ON SOIL pH AND GROWTH RATE OF SELECTED NON-LEUNIOUS PLANTS

BY

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DECLARATION

I hereby declare that this is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

This thesis is dedicated to my creator the Almighty God, dear parents Esther Wanjiku and Stephen Mwangi for financial support, wife Regina Wambui and children Daniel, Stephen and Washington for emotional and spiritual support.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
FYM	Farm Yard Manure
MDG	Millennium Development Goals
NPK	Nitrogen, Phosphorous and Potassium
SNK.	Student-Newman-Keuls
UV	Utra Violet

ABSTRACT

Essential plant nutrients are necessary for plant growth, However, some of these nutrients are added as inorganic fertilizers due to high demand of food arising from fast growth in population which has necessitated the need to expand agricultural activities. This has lead to overuse of commercial inorganic fertilizers leading to drop in soil pH. Low pH reduces the availability of essential nutrients to plants and also low rate of decomposition of organic matter that is required to increase buffering capacity of the soil. Soil acidity is reduced by liming, which is an expensive exercise and does not add fertility to the soil. Leaves and leaf extracts of different plants have been suggested as an alternative to inorganic fertilizer and liming materials. However their efficacy has not been tested. The study, therefore, aimed at investigating the effect of growth rate of non-leguminous plants, including wheat **(***Triticum* aestivum), kale (*Brassica oleraceae var acephala*) and coriander (*Coriandrum sativum* L)., grown on acidic soils treated with leaves and leaf extracts of plants known to have high buffering capacity and mineralisation. The leaves and leaf extracts for this study were applied to the soil with low pH of 4.75. Leaves and leaf extracts were mixed depending on levels of macro-nutrients and mineralization of selected plants found in Nyandarua County. The growth rates of wheat **(***Triticum* aestivum), kale (*Brassica oleraceae var acephala*) and corriander (*Coriandrum sativum* L) was monitored by comparing the dry mass of the uprooted seedlings from the experiment and control experiments for every fourteen days for a period 60 days. Determination of macronutrients in leaf extracts was done using flame photometry, atomic absorption spectrometry (AAS), UV/visible spectrometry and turbidmetry. Data analysis was done using analysis of variance (ANOVA) and student-newman-keuls (SNK) test. The mean pH value of leaf extracts ranged from 4.58 ± 0.27^{a} to 7.44 ± 0.04^{d} for 60 days. The highest mean levels in leaves (µg/g) K^{+}; 189.95 ± 0.17^{c}; Ca^{2+}, 367.33 ± 0.67^{e}; Mg^{2+}, $114.33^{e}\pm0.33$; PO_4^{3-}, 55.38 ± 0.23^{f}; NO_3^{-}, 322.25 ± 0.40^{f}; and SO_4^{2-}, 56.48 ± 0.23^{e}. There was significant difference in levels of macronutrient in soil treated with leaves and leaf extract (Le), commercial fertilizers (Cf) and untreated soil (Us). The mean growth rate for wheat (dry mass) was 1.27 ± 0.13^{b} g/wk (Le), 1.26 ± 0.12^{b} g/wk (Cf) and 0.32 ± 0.02^{a} g/wk(Us) while in (height) was 7.29 ± 1.43^{b}cm/wk (Le) 6.20 ± 1.95^{b}cm/wk (Cf) 3.98 ± 0.97^{a}cm/wk (Us). For kales 0.20 ± 0.01^{a} g/wk (Le), 0.30 ± 0.02^{a} g/wk and (Cf), and 0.03 ± 0.01^{b} g/k (Us) while in (height) 2.15 ± 0.85^{c} cm/wk (Le), 2.57 ± 0.88^{b} cm/wk (Cf) and 1.04 ± 0.02^{a} cm/wk (Us). The mean growth rate for corianders was 0.16 ± 0.01^{a} g/wk (Le), 0.17 ± 0.02^{a} g/wk (Cf), and 0.10 ± 0.01^{a} g/k (Us) while in (height) 1.85 ± 0.56^{b}cm/wk (Le), 1.86 ± 0.58^{b}cm/wk (Cf) and 0.79 ± 0.18^{a} cm/wk (Us). Soil treated with leaves and leaf extract (Le), soil treated with commercial fertilizers (Cf) and untreated soil (Us), respectively. Mean values with the same small letters are not significantly different (P >0.05 SNK test). It was established that there was no significant difference in the growth rate of both wheat and kales grown on soil with commercial fertilizers (Cf), and Le. The results of this study indicate that leaves and leaf extracts of plants studied can be used as an alternative to inorganic fertilizers and should be recommended for agro-forestry in unproductive areas with low pH.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Throughout the world today man is challenged by the task of producing enough food due to fast growth in population. There has been, therefore, the need to expand the agricultural activities to meet this challenge. Since the land owned by nuclear family is no longer sufficient for large scale farming due to subdivision, there is urgent need to increase the yield to feed the growing population. Food insecurity has, therefore, been associated with compromised crop production due to overuse of commercial inorganic fertilizer in small pieces of land which tend to be the ultimate solution in maintaining high yields of food crops (Buleav, 1984). Too much overuse of inorganic fertilizer has led to low soil pH which reduces the availability of essential nutrients to the plants (Nyle, 1990). This results in low rates of decomposition of organic matter. It also leads to depletion of micronutrients leading to production of food of low quality nutritionally (Islam *et al*., 2007). Unless the problem is addressed, the soil will eventually become unproductive. The organic matter is a necessity to increase buffering capacity of the soil and also releases nutrients to the plant.

The soil pH is increased by liming, which is an expensive exercise and does not add fertility to the soil. There is need for alternative method to the application of inorganic fertilizers and liming materials. Increased human population has not only increased overuse of the land but also has resulted in low yields leading to encroachment of forest cover, which, in turn, leads to reduced rainfall (Lepp, 1981; Tsadilas, 2000). Therefore, there is need to look for an alternative to inorganic fertilizers that can also increase the forest cover. Studies have shown that certain plants have litter that can decompose easily

releasing plant nutrient and increasing buffering capacity of soil (Murungi, 1990; Njagi, 2008). *H. annuus* (L) (sunflower) and *T. diversifolia (H.A. Gray*) have leaf extracts with high rate of humification, mineralisation and buffering capacity (Njagi, 2008). Therefore plants whose leaves decompose easily can be used as sources of organic manure. The study was aimed at practical determination of effect on growth rate of *Brassica oleraceae var acephala* (kale), *Coriandrum sativum* L (coriander) and *Triticum aestivum* (wheat) dry mass and height grown on acidic soil treated with leaves and leaf extracts of plant known to have higher buffering capacity and mineralisation.

1.1.1 Commonly used inorganic fertilizers and their effect on soil

The commonly used and available inorganic fertilizers are mainly for supplying the major macronutrient to plants. The percentage compositions of nitrogen, phosphurous and potassium are usually given in the order NPK major (macronutrients). These fertilizers include

i) Diammonium phosphate

ii) Calcium ammonium nitrate

iii) Urea (46:0:0, 20:20:0, 23:23:0)

iv) Trisuper phosphate, TSP (0:46:0)

v) Mono ammonium phosphate, MAP (9:46:0, 19:19:19, 17:17:17)

Inorganic fertilizer tends to change soil pH if applied for a long period since they are acidic in nature (Kennedy, 1992). The lowering of soil pH has negative effect on the growth of the plants and soil organisms and, therefore, reduces expected yields. This is because the acid deposition leads to leaching of essential nutrients such as calcium,

magnesium and potassium (Murungi, 1990). Soil with high buffering capacities may not be affected much by application of acidic fertilizers. Buffering capacity is the ability of an ecosystem to maintain a constant pH, despite addition of an acid or a base. Buffering is important in preventing rapid lowering of pH in soils (Nyle, 1990).

Soil with high limestone and silicate effectively neutralize the hydrogen ions released from fertilizers that come in contact with water and are able to maintain normal pH. Soils especially those found covering granite and igneous rocks lack buffering material. They are slightly acidic and are not able to neutralize acidic effect. Studies have shown that areas with high organic content are not affected by acidic deposition and it is believed that the decayed products of these areas buffer the effects of acid (Murungi, 1990).

1.1.2 Organic fertilizers and organic farming

Organic farm manure produced in the farm itself serves various fertilization purposes. They are important primarily because of their organic content. All soils require the supply of organic matter as a carrier of utilizable energy and nutrient for soil organisms as well as many other reasons (Cooke, 1982; Muchukuri *et al*., 2004). Organic manure used in farms is mainly made of decayed plants. The quality of manure depends on the types of plants used to make it. Therefore, there is need to find plants that would be suitable to provide high quality manure. However decomposition rate of leaves various plants and levels of their macronutrients of have been investigated (Murungi, 1990; Njagi, 2008). Most local indigenous trees have been replaced with exotic trees. It has been reported that some of these exotic plant produce litter that take too long to decompose, and decomposition products may be acidic (Murungi, 1990). It is believed that organic matter plays an important role in buffering capacity of the soil (Njoroge,

1999). Leaves with high buffering capacity have relatively high level of essential elements (Murungi, 1990).

Organic farming relies on developing biological diversity in the field to disrupt habitat for organisms and the purposeful maintenance and replenishment of the soil fertility (Baker *et al.,* 2002).The farmers do not use synthetic pesticides and inorganic fertilizers. On the other hand, organic food is expensive but better to consume because it is far less likely to contain synthetic residue than conventional food (Lampkin, 1990). Organic farmers feed soil biota and build soil organic matter with cover crops, compost, and biologically based soil amendment (Baker *et al*., 2002), resulting in healthy plants that are able to resist diseases and insect predation (Lampkin, 1990).

There is need to investigate the effect of selected plants with high levels of macronutrients in leaves and high mineralisation (Njagi, 2008) on rate of growth of selected non-leguminous plants and pH of acidic soils. This research evaluated eight plants that showed potential resources of organic manure as alternatives to inorganic fertilizers.

1.2 Problem statement and justification

Food is a necessity for life because various foods provide energy, nutrients, healing and immune boost in our bodies (Paraphoria, 2004). This fact is today extensively exploited in search for cheap soil nutrients to supplement the already expensive commercial inorganic fertilizers and liming applications. It has therefore become imperative to monitor the natural pH and nutrients of soil and where necessary, to treat them with processed leves and leaf extracts in order to optimize the performance, especially in the

rural areas where the poor farmers cannot afford expensive inorganic fertilizers. The inorganic fertilizers end up polluting the rivers and lakes resulting in eutrophication. It has been noted that overuse of inorganic fertilizers increases soil acidity resulting in nutrient leaching and poor yield or production of low quality nutritionally (Njagi, 2008). Some leaves and leaf extracts have been found to reduce the acidity of the soil and release valuable nutrients to significant levels (Njagi, 2008). This study, therefore, aimed at applying the leaves and leaf extracts which raise the pH of the soil as well as adding nutrients to the soil and determining their effect on growth rate of selected non-leguminous plants. Since the study aimed at practical application of macronutrient from tree leaves and leaf extracts in a real controlled situation, it had the potential of uplifting the economic standards and provide cheap alternative production of food which would enhance fulfilment of the millennium development goals (MDGs) of poverty reduction. If the proposed intervention results in significant upgrading of the soil, the entire method of cheap production of wheat, kale and coriander canl be considered for a pilot project to various parts of the country to investigate general trend of soil improvement and improved quality crops at low cost.

1.3 Research questions

The study sought to answer the following questions.

i) What are the levels of macronutrients in the leaves and leaf extracts of the selected plant species?

ii) What is the maximum pH of leaves each selected plant soaked in distilled water?

iii) What is the effect of combined leaves and leaf extracts on pH of acidic soil?

iv) What is the effect of combined leaves and leaf extracts on growth rate (based on dry mass and height) of *T. aestivum* (wheat), *B. acephala* (kale) and *C. sativum* (coriander)?

1.4 Hypotheses

i) Macro-nutrients present in leaves of selected plants and in their leaf extracts have significant effect on the growth rate of non-leguminous plants.

ii) Selected plant leaves and leaf extracts can raise and optimise the pH of acidic soil.

1.5 Objectives

1.5.1 General objectives

To analyze for macronutrients in leaves and leaf extracts from selected plants and investigate the effect on growth rate on selected non-leguminous food crop/vagetables grown on acidic soil.

1.5.2 Specific objectives

The specific objectives of this study were to:

i) Determine the levels of K, N, Mg, P, Ca and S in leaves and leaf extracts of selected plants and pH of the latter.

ii) Determine the pH of acidic soils before and after treatment with combined leaves and leaf extracts from selected plants.

iii) Monitor the height and dry mass of *T. aestivum* (wheat), *B. acephala* (kale) and *C. sativum* (coriander) grown on soil treated with combined leaves and leaf extracts every two weeks for a period of 60 days.

1.6 Significance and anticipated output

The results of this study are important as they seek to identify plant species with high levels of macronutrients. The study also seek to promotes awareness of specific plant species as potential, cheap and easily available organic fertilizer. If exploited sustainably, these plant species could help to meet some of the millennium development goals (MDGs) in providing cheap alternative soil supplements in food production, and thus promoting a healthy nation. This will reduce poverty among local communities. The analytical results also provide important baseline data in the application of leaves and leaf extracts to improve growth rate of non-leguminous food crops as well as maintaining soil macronutrient levels. The growth of the plant species will increase forest cover and contain soil erosion. The results of this study will be availed to the ministry of agriculture and recommended for use by field officers to advise the farmers to plant trees whose leaves provide materials for making organic fertilizer.

1.7 Scope and limitation of this study

The study was done in Nyandarua County in Central province, Kenya. It involved leaves and leaf extracts of *Hellianthus annuus* (L) (Sunflower), *Cordia africana* Lam (Muringa), *Croton macrostachyus* Del (Mutundu), *Jacaranda mimosifolia* D. Don (Jacaranda), *Manihot esculenta* C. Crankz (Cassava), *Carica papaya* L (Pawpaw), *Vitex keniensis* Turril (Meru oak) and *Tithonia diversifolia* (H.A. Gray) (Maruru). The leaves and leaf extracts were used to treat acidic soils collected from the same area, after which *T. aestivum* (wheat), *B. acephala* (kale) and *C. sativum* (coriander) were grown in the area and changes in dry mass and height were monitored at interval of 14 days for a period of 60 days. Seasonal variations and age of the plant from which the leaves were collected were not considered.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Plants

Modern classification of plants is mainly based on structural similarities and differences. The class of plants whose embryo of the seed has two cotyledons is referred as dicotyledon. The leaves of these plants are broad and have network of veins. The cross-section of the stem reviews vascular bundles arranged in ring with a pitch and narrow cortex. A vascular cambium is usually present in stems and roots resulting in secondary growth. They have tap root system. A cross-section reveals a centrally placed star shaped xylem with phloem alternating with arms of the xylem. Their flowers have floral parts in fours or fives or their multiples. For this study, *T. aestivum* (wheat), *B. acephala* (kale) and *C. sativum* (coriander) were chosen because they are non-leguminous plants and for their fast growth rate as well as source of vegetables and grains.

2.1.1 Nutrient requirements by plants

Plants require about seventeen elements for growth and development. About 95% to 99.5% of fresh plant tissue is made up of carbon, hydrogen and oxygen. Only 0.5% remains as ash after plant tissue is dried and burned (Muchukuri *et al.,* 2004). This represents a very small proportion of the total plant and usually it is the absence of one or more elements found in the ash that limits plant growth and development. Plant growth is never retarded by lack of carbon, hydrogen and oxygen as long as carbon (IV) oxide in the air is plentiful, and thus emphasis is placed on the soil capacity to supply essential elements (Muchukuri *et al.,* 2004). There are thirteen nutrient elements, and of these, six are referred to as macro-nutrients because they are required by plant in relatively higher quantities than others. These are nitrogen, phosphorus, potassium, calcium, magnesium

and sulphur. They are applied as inorganic fertilizers for almost all crops. Seven of the essential elements are referred to as micro-nutrients or trace elements and are required by plants in very small amounts. These are iron, manganese, zinc, copper, molybdenum (known as heavy metals) boron and chlorine (non metals). There are others such as cobalt, silicon, sodium and aluminium which are referred to as beneficial elements (Muchukuri *et al.,* 2004). A summary of the nutrients required by plants is given in Table 2.1

Table 2.1 Classification of nutrients required by plants

Classification			**Elements**
Essential nutrients	Macronutrients	Major	N, P, K
		Secondary	S, Ca, Mg
	Micronutrients	Heavy metals	Fe, Mn, Zn, Cu, Mo
		Non metals	B, Cl
Beneficial elements			Na, Si, Co, Al

Source: (Muchukuri *et al*., 2004)

2.1.2 The role of macro-nutrients in plant nutrition

Nutrients are said to be deficient in soil if the plant is unable to meet its nutritional needs from the soil during its growth period. The symptoms of deficiency are normally manifested as discolouration or malformation leaves. Disorder symptoms depend on the following. The function of that element, whether it is readily translocated from older leaves to younger ones (Young, 1976).

The best indicator of nutrient deficiency is the plant itself but confirmation of deficiency may be confirmed in by laboratory analysis of leaf tissue. The visual method of detecting deficiency or toxicity has the following limitations: Symptoms can be due to microbiological, organic, moisture, aeration and physical factors, climate, insect and

plant diseases. Symptoms can only appear if deficiency or excess is extreme (Muchukuri *et al*., 2004). Individual macro-nutrients are discussed in the following subsections.

2.1.3 Nitrogen

Nitrogen is essential for plant growth and reproduction. It plays a very important role in protein formation and is a constituent of protein and protoplasm of all living cells. It forms part of chlorophyll molecule and makes plants succulent with deep green colour. Nitrogen encourages vegetative growth which is necessary in crops where leaves are harvested such as kales and corianders. Components of amino acids which are the building block of proteins are needed for growth and development of all living tissues. Nitrogen content of most protein varies from 14 - 18 % (Muchukuri *et al*., 2004). It is a very important constituent of chlorophyll which is associated with high photosynthetic activity, vigorous vegetative growth and dark green colour.

Lack of nitrogen is the most common cause of nutritional plant stress (Groot *et al.*, 1991). The conventional farmer relies on artificially fixed bagged nitrogen whereas the organic farmer relies on the Rhizobia bacteria in symbiotic association with legumes to make nitrogen available for plant nutrition (Glinsiki and Witold, 1985; Lampkin, 1990). Nitrogen is the only nutrient which can be lost at appreciable amount to the atmosphere (Witold, 1985). The gaseous nitrogen is lost as ammonia, while nitrogen oxide and molecular nitrogen may also be lost in solution form as nitrate ion. For most non-leguminous plants, the nitrate ion is the primary source of nitrogen (Marschner, 1986). Over the past century, anthropogenic activities have nearly halved the source availble of available nitrogen; invasive nitrophilous species have become more dominant in wetland ecosystems, altering the species composition and diversity (Vitousek *et al*. 1997).

Deficiency symptoms include stunted growth (in young corns) and yellow-green foliage because of reduction of chlorophyll in the leaf, and hence loss of green colour (chlorosis) (Watson *et al.*, 2004). In more mature plants, yellowing of lower leaves following the mid ribs in typical v-shaped fashion is observed but the leaf margin remains green and "firing" that is dying of the lower leaves is also observed (Bertand *et al.*, 2005; Ryoung *et al*., 2006).

Much of the nitrogen reserve is stored in the soil as organic matter and most of this organic fraction is found in the upper soil horizons. At surface mines, the upper soil horizons are usually removed and stockpiled prior to disturbance. The storage of topsoil allows for relatively rapid conversion of organic nitrogen to soluble nitrate (NO_3^-) and is subject to leaching or conversion to nitrogen gas (denitrification) which volatilizes out of solution into the atmosphere. Thus, when stored topsoil is spread on a disturbed landscape, nitrogen reserves may be depleted or altered by several chemical and biological phenomena and the healthy cycling of nitrogen through the ecosystem inhibited or prevented (Munshower, 1994). Commonly, nitrogen fertilizer is land applied to reclaimed mine sites where revegetation is desired. Tilling is generally not necessary to incorporate the nitrogen into the soil because of the leaching ability of nitrogen. Although, it is often tilled in since nitrogen fertilizer is often incorporated with other macronutrient fertilizer which do need tillage. Nitrogen in soils can be in different forms. Nitrogen is very dynamic and is constantly changing chemical species and concentrations. In most soils, nitrate is the common ionic form of plant-available nitrogen, but this element may also exist as ammonium (NH_4^+) or nitrite (NO_2^-) as well as other ions. Nitrogen is also incorporated in organic matter and microbes. When organic matter decomposes by microbial processes or when the microbes themselves die

and decompose, nitrogen is released in various forms into the soil solution (Brady and Weil, 1999).

2.1.4 Phosphorus

Phosphorous is an important element in establishment of crops. It encourages the formation, development and establishment of roots particularly secondary roots (Brady and Weil, 1999). Phosphorous is a constituent of many specific compounds making up the plant structure. Phosphorous is needed for cell division as it is a part of nucleoproteins which are involved in cell reproduction process. It forms part of a chemical essential to the synthesis and degradation of carbohydrates. Phosphorous is essential for seed germination, root development, flowering, fruit formation and crop maturation. It hastens the ripening of the fruits and strengthens the skeletal structure of the plants, thereby preventing lodging in cereals thus countering the effects of excess nitrogen. Phosphorous affects the quality of certain crops, especially forages and vegetable whose palatability improves when sufficient phosphorous is available (Young, 1976). It increases plant resistance to diseases. Its most essential function is in energy storage and transfer. It is necessary for all cell division, growth of root and shoots tissue and developing seed and kernel of grain.

Deficiency of phosphorus leads to stunted growth, slow emergence and slow growth of most annual crop. It leads to off colour green foliage with purple venation especially on the underside of leaves (Muchukuri *et al.,* 2004). It also makes petioles have a purple (accumulation of anthocyanin) cast, root development becomes poor, less fruits are produced and plant often look spindly stunted. Wheat and barley take up their phosphate in the early stage of growth (Ryoung *et al.,* 2006). Starvation during this period cannot be

rectified by a good supply later (Lampkin, 1990; Jungi *et al.,* 2005). Excess phosphate over the amount required by crops sometimes depresses the yields (Young, 1976; Ganeshamurthy and Sammi, 2000).

2.1.5 Potassium

Potassium is associated with protein activity, primarily in the maintenance of a positive ion balance to satisfy negative ion charges on the protein. It is required in enzyme activation where cell division is taking place rapidly and where primary tissues are formed (Rhodes *et al*., 1990). It provides osmotic regulation that makes plants to "pull" or draw water into the plant roots. It affects transportation by regulating stomata opening. It is required for production of high energy phosphate molecule which makes translocation of sugar produced by photosynthesis possible as well as other functions of energy. Uptake of nitrogen and protein of synthesis require potassium.

Deficiency of potassium symptoms includes development of whitish spot on non-leguminous plants leaves as well as marginal yellowing and browning. Shortening of internodes, dwarfing, small size of fruits and seeds. Loss of green colour, marginal discoloration from the base to the tip starting with older leaves leads to their premature death of plant. An excess of nitrogen relative to potassium renders leaves susceptible to fungal and bacterial diseases and reduces resistance to drought (Ryoung *et al*., 2006).

The original sources of potassium are the primary minerals, such as micas (biotite and muscovite) and potassium feldspar (orthoclase and microcline) (Brady and Weil, 1999). As these minerals weather, the potassium becomes more available as readily exchangeable and soluble potassium, which can be adsorbed by plants roots. At any one

time, most soil potassium is in primary minerals and nonexchangeable forms. In relatively fertile soils, the release of potassium from these forms to the exchangeable and soil solution forms that plants can use directly, may be sufficiently rapid to keep plants supplied with enough potassium for optimum growth. Conversely, in relatively nonfertile soils, the levels of exchangeable and soluble potassium may have to be supplemented by outside sources, such as in the form of chemical fertilizers, poultry manure, or wood ashes. Without these additions, the supply of available potassium will likely be depleted over a period of years and the productivity of the soil will likewise decline (Brady and Weil, 1999).

2.1.6 Magnesium

It is a component and the only metallic constituent of chlorophyll molecule. It is an activator and component of many plant enzymes. It is a structural component of ribosome, stabilizing them in the configuration necessary for protein synthesis (Muchukuri *et al.*, 2004). Magnesium is a primary constituent of chlorophyll, and without chlorophyll plants would fail to carry out photosynthesis, and leaf tissue will turn yellow, then brown, and finally become necrotic. Magnesium also serves as a structural component in ribosomes, stabilizing them in configuration necessary for protein synthesis.

Magnesium deficiency would cause the proportion of protein nitrogen to decrease and that of non-protein nitrogen to increase. This is especially important in forage crops where high protein levels are necessary to produce a high quality feed. The most common sources of magnesium fertilizer are dolomitic limestone (for agricultural fields) or epsom salts (for gardens). Deficiency symptoms include; regular yellow-white strips

on leaves followed by chlorosis and development of brilliant colours. Necrosis may develop interveinally or a long margin or tips of leaves and some plant the leaves curl (Ding *et al*., 2006).

2.1.7 Calcium

Calcium is a constituent part of cell walls and affects permeability of protoplasmic membrane that surrounds each living cell. It controls the uptake of water by the cell colloids. It enhances nitrate (NO_3^-) and is therefore, interrelated with nitrogen metabolism. It is essential for cell elongation and division. Excess calcium may however restrict the trace elements availability and should be avoided (Lampkin, 1990). Calcium deficiency leads to failure of terminal buds to shoot and epical tips of roots to develop which causes the non-leguminous plant growth to cease. It makes the tips of the leaves to be glued together, root rotting, spotted or necrotic areas on the leaves and back of the terminal buds, low fruit weight and low dry matter (Tuna *et al.*, 2005).

In corn, calcium deficiency prevents the emergence and unfolding of new leaves while in fruits vegetables calcium deficiency causes disorders such as blossom-end rot in tomato and bitter pit in apples. Special attention must be given to crops which are unable to obtain sufficient calcium from the soil, such as peanuts, tomatoes, and celery; as well as to crops that have high calcium requirements, such as alfalfa, cabbage, potatoes, and sugar beets. The primary source of calcium as a fertilizer is lime, which is also used to neutralize soil acidity.

2.1.8 Sulphur

Sulphur is required for synthesis of sulphur containing amino acids, cystine, cystein and methionine which are essential components of protein. It is used in the formation of disulphide bonds between polypeptide chains in the synthesis of other metabolites including coenzyme A, biotin, thiamine or vitamin B1 and glutathione. It is a component of S-containing substances like S-adenosylmethionine, formylthionine, lipoic acid and sulfolipid. It is also required in synthesis of chlorophyll though not a constituent (Young, 1976).

Sulphur deficiency leads to pronounced retardation effect on non-leguminous plants growth and is characterized by uniformly chlorotic plant that is stunted, thin and spindly. Lack of sulphur also leads to diseases susceptibility (Dubuis *et al.*, 2005).

2.2 Soil

The soil on which plants grow provides a store house for minerals, chemicals, water and air. It is the anchor for plants (Trudgill, 1988). Soil consists of about 45% mineral portion, 25% air, 25% water and 5% organic matter by volume as shown in the Figure 2.1.

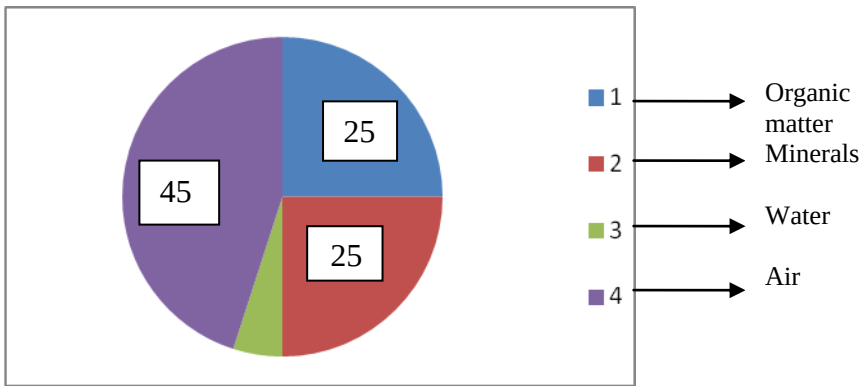


Figure 2.1 Components of soil

Source: (Muchukuri *et al.*, 2004).

The chemical that make the mineral portion of the soil are shown in the Table 2.2.

Table 2.2 Mineral composition of soil

Oxide	Approximate percentage
SiO_2	76
Al_2O_3	12
Fe_2O_3	5
CaO	1
MgO	1
K_2O	2
All others	3

Source: (Muchukuri *et al.*, 2004)

All others include titanium, sodium and very small amount of nitrogen, phosphorous, boron manganese, zinc, copper, molybdenum, chlorine and many other elements. Of three main elements, silicon, aluminium and iron, only iron is known to be essential for plant growth and only in minute amounts (Knuti *et al.,* 1984; Muchukuri *et al.,* 2004). The great mass of the soil mineral fraction only act as anchor to the plant root and as a skeleton on which to hang the colloidal clay and humus. The essential available nutrients required for plant nutrition are found in very small colloidal fraction of the soil (Knuti *et al.,* 1984; Muchukuri *et al.,* 2004) as discussed in Section 2.1.

2.2.1 Modern farming and related problems

Various fertilizers, pesticides and cultivation techniques used in modern farming have been associated with rapid decline of soil microbial activity. In particular, the use of fungicides has been shown to shift the balance of soil organisms from fungi to bacteria. Other pesticides and inorganic fertilizers have been shown to be directly toxic to soil fauna (Lampkin, 1990). The major problems associated with current agricultural practise that use inorganic fertilizers and pesticides include the following:

i) Damages soil structure: Soil structure is defined as the ‘spatial heterogeneity of the different components or properties of soil’ (Dexter, 1988). This identifies the importance of considering a hierarchy of structure, which finds resonance with the concepts of self- similarity over a range of dimensions as expressed in fractal geometry (Young *et al*., 2001). The range of particle sizes found for the mineral and organic components of soil is enormous, ranging from fine clay particles, around 10^{-7}m in diameter, through silt, sand, and gravel, to stones up to several centimeters in diameter. If all of these fractions existed as independent particles then most of our soil resource could not be used for crop growth and agriculture. It is only through the influence of soil structure that soils possess the qualities required for plant growth and agricultural production. These include a balanced supply of water and oxygen to the root system of the growing plant, the ability to form seedbeds, a mechanical anchorage for the root system, the capacity to buffer inputs of rainwater by infiltration, drainage and evaporation (Dexter, 1988). Many of these functions are critically dependent on soil porosity which also provides an environment in which soil micro-organisms live and make their contribution to the turnover of carbon and some of the major nutrients (Ranjard and Richaume, 2001).

ii) Damages the environment: The contamination of surface water takes place from pesticides, fertilizers in agricultural runoff leading water water borne diseases occurring as a result kill millions of people in developing countries.

iii) Creates potential health hazards in food: The addition of materials to soils by man comes from the use of agricultural chemicals such as herbicides, fungicide, and insecticides from dust fall and precipitation and use of

fertilizers and contaminated water. Chemical fertilizers are a source of trace metals either added deliberately or as an impurity, for example Cd, As and Pb, common trace metals in rock phosphates and also in superphosphates

iv) Is energy-intensive system: This may be defined as the unfavorable change of our surroundings, wholly or largely by a by-product of man's actions through direct or indirect effects of changes in energy patterns, radiation levels, chemical and physical constitution and abundances of organisms. These changes may affect man directly or through his supplies of water for agricultural purposes.

Therefore, this agricultural practice needs to be discouraged, alternative practices developed and those that already exist (such as organic farming) improved.

2.2.2 Effect of pH on availability of nutrient to plants

The pH of soil influences the rate of nutrient released by weathering and solubility of all materials in the soil. Generally, the more the acidic a soil is, the more mobile will be elements such as iron, zinc, manganese, copper and other minor elements (Hesse, 1972). At very low pH values, soil may contain toxic chemicals of aluminium and manganese, which are responsible for poor plant growth. Low pH values for soil can induce minor element deficiencies and destroy many micro-organisms and, in particular, nitrifyers are inhibited (Muchukuri *et al*., 2004). Some soils have variable charge decreasing at low pH and those with cation exchange capacity (CEC) from organic sources also lose this charge at low pH as carbohydrate group are protonated or bind aluminium ions. As a result, nutrients cations such as K^+, Ca^{2+} and Mg^{2+} may be leached from the soil which then becomes less fertile (Carter and Arocena, 2000). Initially, these cations are replaced

by protons, but at sufficient low pH (below 5.0), protons are replaced by positively charged aluminium species (Gathua, 1994; Kennedy, 1992). Most mineral compounds of nitrogen and sulphur except calcium sulphate are highly soluble at any pH and are subject to rapid leaching. The highest rate of mineralisation of these nutrients is between pH of 6.0 and 8.0. Beyond pH 8.0, available sulphur is from minerals other than organic matter (Muchukuri *et al*., 2004)

Phosphorous compounds have low solubility even when added as fertilizers, they are likely to react in the soil and form material of low solubility. A pH between 6.5 and 7.5 is usually the best for phosphorous availability. Iron and aluminium phosphates precipitate at high pH (Muchukuri *et al.,* 2004). At pH values above 7.0, complex insoluble calcium phosphate are formed. Thus the solubility of both native and applied phosphorous may be seriously impaired, which hinders phosphorous absorption and utilisation in plants (Brady, 1996).

Potassium is soluble at any pH but is removed from solution by sorption. Some is held at exchange site but some is held more tightly. Exchangeable calcium and magnesium are lost by leaching as the acidity of the soil gradually increases. The uptake of calcium ions by maize root is markedly reduced as pH is lowered from 5.0 to 3.0. Excess protons are found to decrease magnesium uptake (Kennedy, 1992).

Metallic cation such as iron, manganese, copper and zinc form compounds that decrease in solubility at high pH. Therefore, raising soil pH by liming acidic soil can induce these cations deficiency (Lawrence, 1990). On the other hand iron availability is generally reduced by low pH. Boron leaches at very low pH and its solubility is low at very high

pH. Molybdenum, which leguminous plants require in relatively high amounts, is less available in acidic soils. However, liming increases its solubility. Generally for most crops, soil pH values of 5.5 to 7.5 are suitable for availability of most nutrients (Muchukuri *et al.*, 2004).

2.3 Toxicity in acidic soils

Toxicity is attributed to factors associated with the presence of aluminium and manganese species (Alan, 1993). These become progressively more soluble as pH decreases. The aluminium and manganese toxicity are discussed in the following subsections.

2.3.1 Aluminium toxicity in soils

Toxicity from aluminium is probably the most serious growth limiting factor for plants in acidic soils (with pH under 5.5). Mild effects of aluminium toxicity are lack of vigour in root growth and branching. It may also displace calcium and magnesium ions from colloids and interfere with access by the plant to magnesium even when present. The other effects of elevated aluminium activity in soil are greater fixation of inorganic phosphate in form unavailable to plants (Stevenson, 1994). Plants don't need aluminum to grow and it is not an essential plant nutrient. Since many plant species are sensitive to micro-molar concentrations of aluminum, the potential for soils to be aluminum toxic is considerable (Charles, 2000). Its toxicity is the primary limitation to crop production on approximately 50% of the world's potentially arable lands, including many in developing countries, where food security is most tenuous (Emmanuel and Peter, 1995). The most striking effect of aluminum in acid soils is stunting of the root system. Toxic aluminum cations (Al^{3+}) solubilized by the acidity rapidly inhibits root growth and subsequent

uptake of water and nutrients (Bona *et al*., 1991; Baligar and Fageria, 2001). Restricted root systems can lead to nutrient deficiencies or drought symptoms of the crop even if moisture is available further down the soil profile (Charles, 2000). Fortunately, most of the Al^{3+} is bound by ligands or occurs in other non-phototoxic forms such as aluminosilicates and precipitates. However, solubilization of toxic aluminum cations is enhanced by low pH of acidic soil. When the soil pH is lower than 5.5, Al^{3+} is released to the soil and enters into root tip where it causes inhibition of cell elongation and cell division leading to root stunting accompanied by reduced water and nutrient uptake (Pandal *et al*., 2009).

The avoidance mechanism includes exclusion of Al from sensitive sites such as Al exclusion from root and organic compounds exudation for forming Al complex. The exudation of organic acids such as malate, citrate, succinate, and oxalate from roots has been suggested to play a role in aluminum exclusion (Nursyamsi *et al.*, 2002). Several recent studies have shown that Al resistance in wheat involves exclusion of Al from the root apex (Delhaize *et al.*, 1993). Malate has been reported as the predominant organic acid released in Al-tolerant genotypes into the plant, from the root tips of the wheat where it binds to Al, thereby, preventing its entry (Kochian *et al.*, 2004). It is also observed that as the root of *T. aestivum* (wheat) grows continuous organic acid exudation through the soil maintains the Al-chelating barrier around the root apex as it encounters new regions of acidic soil. The reduction of aluminium toxicity by farmyard manure (organic matter) is considered to occur through aluminium precipitation, chelation on organic colloids or by complexation of soluble aluminium by organic molecules, especially organic acids (Hue and Amien, 1989).

2.3.2 Manganese toxicity

Manganese toxicity is required in trace amounts but has narrow optimum range in the soil solution and can be toxic if this is exceeded. Its toxicity results from the destruction of organic complexation which it is fixed. It has the general effects of heavy metals on proteins when present at high concentrations, but can also inhibit some enzymes at high concentrations around 10^{-6} Molar, for example, glutamine synthetase interfering with nitrogen assimilation (Kennedy, 1972). Toxicity due to manganese can be corrected by enriching the soil with organic matter which increases the organic fixation of manganese and limits the lowering of the pH by action (Marcello, 2005).

Symptoms of manganese toxicity generally fall into two types. The most diagnostic feature is the darkening of leaf veins, usually on older foliage. The blackening associated with the veins is due to the accumulation of dark crystals of insoluble manganese dioxide in tissues within or alongside the veins and is usually visible to some degree on both sides of symptomatic leaves. In some plants, the dark crystals will accumulate outside the leaf tissues at the base of trichomes. A second less diagnostic symptom of manganese toxicity is interveinal chlorosis with leaf cupping or necrotic blotching of foliage. Many factors could cause such symptoms on a wide array of plants, but this symptom resulting from manganese toxicity is displayed most notably in the cabbage family (*Brassica spp*) and in lettuce (*Lactuca sativa L*) (Kandasamy, 1989).

Causes of manganese toxicity in plants are simply supply of too much manganese in fertilizers. Many "complete" fertilizers contain sufficient micronutrients for normal plant growth. The use of micronutrient supplements in the belief that deficiencies exist or that extravagant amounts of micronutrients might improve plant growth or appearance has

resulted in manganese toxicity in a number of cases with foliage ornamentals.Moreover plants are often grown in a soil with a low pH. Lowering of soil pH to 5.0 or below can solubilise manganese and other metallic micronutrients that subsequently may be taken up in excessive amounts by plants.

Knowing what situations can lead to manganese toxicity problems, the following treatments are suggested. The most obvious treatment for manganese toxicity is to discontinue fertilizer applications that contain manganese. Fertilizer labels need to be read carefully to determine if micronutrients (manganese, iron, copper, zinc, molybdenum, and so on) are included in the formulation. Dolomite or lime can be affected to raise soil pH. This is especially effective if soil pH has been mistakenly lowered or if soil has gradually acidified as a result of continuous cultivation without regard to soil pH. Raising the pH of soil may not provide sufficient protection from manganese toxicity in all cases.

2.4 Effect of pH on decomposition of organic matter in soils

Bacteria, protozoa, and fungi decompose all materials produced by plants. Studies have shown that microbial composition is changed by acid. At pH of 4.0, fungi predominate while at pH of 7.0 bacteria are predominant species. Studies have shown that the breakdown of leaf litter depend not only on pH but also the type of the plant (Greenland and Hayes, 1981; Murungi, 1990). Soils with adequate organic matter usually have pH values of about 7.0 (Muchukuri *et al*., 2004).

2.5 Effect of pH on soil organisms

The full soil life ranges from soil fauna (which includes the insects such as springtails,

termites, beetles, flies ants, bees and wasps, mites, various invertebrates such as flat worms, nematodes, snails, slugs and earthworms. centipedes and millipedes) to the soil micro-organisms (covering a wide range of different types of bacteria, fungi and actinomycetes). These are important for turnover of nutrients, turnover of energy contained in organic matter and for many chemical weathering processes which take place in the soil (Lynch, 2000). Maintaining the correct soil pH is important for both the faunal population and for chemical nutrient availability. In low pH soil biological activity is reduced, slowing the turnover of organic matter and reducing the release of nutrient (Lampkin, 1990). Earthworms cannot tolerate acidic soils with a pH much below 5.0, so that under these conditions, mineral and organic matter are not blended together. Research by soil scientist has clearly shown the benefits of encouraging earthworms in terms of soil structure formation (Lampkin, 1990). Many micro-organisms provide polymers, particularly polysaccharides, which provide cementing action between soil particles. There is also some evidence that fungi, such as vascular and arbuscular mycorrhizae also have a role in promoting soil aggregate stability (Lampkin, 1990)

Bacteria function better in mineral soils at intermediate and higher pH values, the activity being markedly curtailed when the pH drops below 5.5. Fungi are particularly facultative, flourishing more or less satisfactorily at the soil pH. In normal soils therefore, fungi predominate at the lower pH values but at intermediate and higher ranges they meet strong competition from the bacteria and actinomycetes and hence, must yield the field to some degree. Nitrification and nitrogen fixation takes place vigorously in mineral soils only at pH values above 5.5 (Hansen and Henriksen, 1989). In general, soils in intermediate pH range (6-7) perhaps present the most satisfactory biological regime (Stefan and Horvath, 2004).

2.6 Mineralization and humification

2.6.1 Mineralisation

When an organic material is added to a soil, it undergoes decomposition. Most organic matter decomposition relates to enzymic oxidation by organism living in soil or at its surface (Nyle, 1990). The breakdown of complex organic structure also leads to the formation of a variety of more simple, inorganic product, a process known as mineralisation (Coleman, 1989). It is an important process and sources of nutrients like nitrogen, sulphur and phosphorus (Cobo *et al.*, 2002). Cations such as calcium, magnesium and potassium are also released (Ellis and Mellor, 1995). The soil must provide these nutrients for plants in a suitable form and rate at which they can be used. Plants take up nutrients primarily in the form of electrically charged ions (NO_3^-, $H_2PO_4^-$, K^+, Mg^{2+}, and Ca^{2+}) (Dahlin *et al*., 2005).

The soil must be the bank for these ions and hold them safely, yet make them readily available on demand. The positive ions are held in the soil by negatively charged site, these absorptive sites are referred to as colloids. The ability of the soil to hold cations is measured by the cation exchange capacity (CEC) (Olszewska, *et al.,* 1998). Two important contributors to the soil CEC are clay particles and humus (Wilding *et al.*, 1983). Clay mineral are impure silicates structures, the impurities in clay carry smaller positive charge than the silica they replace, resulting in the clay carrying the net negative charge (Kabata and Pendias, 1985).

Soil organic matter contain carbonyl groups which dissociate to provide negatively charged sites. These typically have twice the charge of clay minerals, so even low level of organic matter can be a significant contribution to the CEC (Dubuis *et al*., 2005). The

process of chelation by which organic matter can hold and buffer the trace element is also important. Organic matter can act as scrubbers' to avoid toxic level of trace elements and in some cases can be used specifically for this purpose (Lampkin 1990; Greenland and Szaboles, 1994).

2.6.2 Humification

Humus is the presence functional groups such as amino, hydroxyl and carbonyl in humid compounds which give rise to high CEC and the ability to complex with clays, thus contributing to soil, structure although the highest molecular weight compounds are the most stable and, therefore, play a better role (Yeloff and Manquoy, 2006). Soil organic matter can be divided into three broad categories, Fresh and incompletely decomposed plants and animal residues (active organic matter). Product of advanced decomposition of organic residues and products resynthesized by micro organisms (protein like substances, organic acids, carbohydrates, gums, waxes, fats tannins and lignins). High molecular weight humic substances, fulvic acids, humic acids and humin which are generally resistant to further biological decomposition (Morill *et al.,* 1982)

Humification (or decomposition of organic matter) is an extremely complex process. At first the simple compound such as starches and sugars are decomposed followed by proteins, cellulose, hemicellulose and, finally, the most resistant compounds such as tannins. Gradually, the material is decomposed and the dark coloured substance known as humus is formed (Ponge, 2003). Humification involves a complex set of reactions of various decomposition products to produce large, complex molecular chain or polymers (Stevenson, 1994; Frimmel and Christman, 1988). Generally, humic substance refers to product resulting from decomposition of plant and animal residues (Chil, 2005). They are

composed of complex heterogenous mixture of organic compounds and are characterised as being yellow to brown in colour of high refractive molecular masses and refractory. They are defined as complex mixtures of organic acids (Townshend, 1995; MacCarthy *et al*., 1990). Because they act as exchangers, these humic substances, will also regulate plant nutrition to certain extent (Alloway, 1995). Some of these compounds may be taken up directly by the plants, possibly as result of mycorrhizal associations, and activate physiological and biological processes in the plant. The effect of this may be to stimulate or regulate growth or to assist the plant resistance to pathogenic attacks.

2.7 Organic Farming

Organic farming is production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators and live stock feed additive (Lampkin, 1990). Maximum feasible organic farming system rely on crop rotation, crop residues, animal manures, off-farm wastes and aspects of biological pest control to maintain soil productivity and tilth to supply plant nutrients and to control insects, weeds and other pests (Nancy and Mary, 1990). Preservation of soil structure, earthworms, micro-organism and larger insect is essential to organic system. Therefore, the protection of the environment is fundamental ‘must’ for organic farmer and not something that can be considered at the end if profit allows (Mekkaoni, 2000)

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2.7.1 The biological life in the soil

Soil contains enormous of different organisms, varying widely in size and function. All of them, however, play an important role in the mobilization of nutrients in the soil. Each of these organisms has as specific role or function within soil. Producers, those organisms which are able to use the suns energy through photosynthesis to form complex

carbon compounds, are less obvious in the soil than other ecosystems. Only the algae are photosynthetic, although they can also grow in the absence of light if simple organic solutes are also available. The blue green algae, which occur in great abundance under certain conditions, are also able to fix nitrogen from the atmosphere, while receiving energy directly from the soil (Lampkin, 1990). The vast majority of the soil organisms function as consumers and decomposers. Other organisms specialize in breaking down organic matter of both plant and animal origin into simple inorganic forms (Nancy and Mary, 1990). Studies have shown that organically managed soils have higher levels of microbial activities, as indicated by the number and species of fungi, rates of cellulose (straw) break down activity of enzymes such as dehydrogenase, urea and phosphate all crucial for the release of nutrients to the growing crops (Lampkin, 1990).

2.7.2 Fertilizers in organic farming

The fertility of the soil is not only has implication in terms of soil degradation, it also has an important impact on the health of the crops, animals and human beings which depend on it. The manure helps to modify the physical conditions of the soils, by improving water holding capacity, aeration, drainage and friability. Darker colour of organic matter means that the soil warms up faster (Oswal, 1983). Organic fertilizers provide energy needed for increasing microbiological activity (Gichuru *et al.*, 2003). It also helps to protect crop from temporary gross, excess mineral salt and toxic substances and from rapid fluctuations in soil reaction by means of their high absorption capacity exerting a ‘buffering’ action. In this way, they can counter the effect of excess liming as far as the availability of micronutrients is concerned. In addition, nutrients are released more slowly from manures than from the inorganic fertilizers; therefore, the desirable effect of the compost manure can last for more than one season (Njoroge, 1999).

Farmers who have domestic animals may take farm yard manure (FYM) which consist of the animal excrete and bedding materials usually straw in varying quantities and varying stages of decomposition (Lampkin, 1990). These manures have advantage of supplying essential nutrients either directly or indirectly by eliminating aluminium toxicity or by producing organic acids, thereby increasing nutrients availability (Nziguheba *et al.*, 1998). Organic manure is made by composting plants residues, animal dung, ash and green vegetation in Kenya (Njoroge, 1999). Some of the plants reported for making compost are young grass hay, cow pea hay, soya bean hay, maize stalk, leaves and wheat straw (Omare *et al.*, 2003; Njoroge, 1999). "Plant teas" for top dressing are made with broad leaved young shoots that can easily decompose in water such as those of stinging nettle, comfrey, amaranthus and *Tithonia diversifolia (H.A. Gray*) (Nancy and Mary, 1990).

2.7.3 Quality of organically produced foods

The quality of food stuffs is usually assessed by appearance, technological suitability and nutritional value and content of harmful substances.

2.7.3.1 Appearance

Organically produced vegetables, tomatoes and potatoes under controlled experimental conditions have been found to taste better and have fewer "off flavours" than conventionally produced ones. However potatoes produced organically tended to disintegrate more on cooking (Lampkin, 1990).

2.7.3.2 Technological suitability

Technological suitability here refers to post harvest processes associated with food

product before reaching the consumer. Organically grown crops with slower rate and greater physiological maturity at harvest have longer storage life (Lampkin, 1990). Respiration rates and enzyme activity have been shown to be lower in organically produced vegetables leading to reduced storage losses (Lampkin, 1990). Better storage life of spinach grown in organic fertilizers may be associated with lower free amino acid content, a nutrient attractive to bacteria and related closely to levels of nitrogen in the fertilizer used. The organically manured spinach had the lowest level of biochemical changes and number of total bacteria (Lampkin, 1990).

In more than 75% of the cases, the post-harvest behaviour of crops which had received only organic manure were superior and that increasing levels of nitrogen use (inorganic fertilizers) were particularly detrimental (Lampkin, 1990). The greatest difference occurred with respect to perishability, shrivelling and colonisation by epiphytic micro-organisms; peroxidise activity, nitrite formation and vitamin C breakdown (Yona *et al*., 2005; Lampkin, 1990). Higher dry matter levels in organically produced vegetables can be advantageous as far as some specific processing such as pickling are concerned (Lampkin, 1990).

2.7.3.3 Nutritional quality and harmful substances

The risk of food grown organically being contaminated with pesticide residues is much smaller than with conventionally produced crops (Danny *et al.,* 2002). Also there is no evidence that occurrence of natural toxins is more prevalent in organic systems than the convectional systems (Lampkin, 1990; Williams, 2002; Danny *et al.*, 2002). The use of significant quantities of mineral fertilizers affects the nutritional composition of crops. Hypomagnesaemia (milk fever) in daily cow is linked to luxurious uptake of nitrogen

fertilizers at the expense of magnesium (Lampkin, 1990). Studies have shown that increased use of nitrogen fertilizer result in not only higher nitrate levels, but also higher levels of amino acids, oxalates and other undesirable compounds as well as lower levels of vitamin C, calcium, phosphorous, magnesium, sodium and trace elements (Yona *et al*., 2005; Lampkin, 1990; Rajendra, 2005).

The use of organic fertilizers and appropriate soil management practices in organic agriculture means that a much wider and more balanced range of nutrients is available to crops than is the case when readily soluble NPK fertilizer is applied and taken up directly by the plants (Rajendra, 2005). The problem associated with increased use of inorganic fertilizers requires encouragement of use of organic fertilizers from plants especially for poor rural farmers. So, there is need for identifying plants whose litter (leaves) have high levels of nutrients which are released fast to crops and at the same time raise soil pH and hence the need for this study.

2.8 Methods used for analysis

Several techniques for determination of metallic elements are currently in use. These include; the atomic absorption spectroscopy (AAS) (Taylor *et al*., 2006), inductively coupled plasma mass spectroscopy (ICP-MS) (Conor, 2006), the inductively coupled plasma atomic emission spectroscopy (ICP-AES) and energy dispersive X-ray fluorescence (EDXRF) spectroscopy (Cherop**,** 2009). In this study atomic absorption spectrometer (AAS) is used for analysis magnesium and calcium (Taylor *et al*., 2006). The UV/Visible spectrometer (Okalebo *et al*., 2002) and volumetric (Nkana, 2001) are used to determine nitrogen, sulphur and phosphorous. In this study, atomic absorption spectrometry, flame photometry and U/V visible spectrophotometer were used because

of their high sensitivity and availability.

2.8.1 Atomic absorption spectrometry (AAS)

The AAS has been used in determination of many metals. This method is largely free from spectral or radiation interferences because each metal has its own characteristic absorption wavelength and the source of lamp are composed of the element being determined. In a view of the high accuracy and precision, selectivity, sensitivity and speed at which analysis is done the method has been adopted in this study.

Principles of AAS

The AAS is a method of elemental analysis that works on the principle of absorption of radiation energy by free atoms. The concentration of an element is measured by the absorption of radiation with characteristic frequency by free atoms of an element. Light of certain wavelength produced by monochromatic or hollow cathode lamp emits spectral lines corresponding to energy required for excitation of an element of interest. The analytical signal is obtained from the difference between the intensity of the source in the absence of the element of interest and the decreased intensity obtained when the element of interest is present in the optical path. Absorption of light is associated with transition process from one steady state to another, for instance the case of a steady state O and J where Eo < Ej, the O-J transition results in the absorption of light with the frequency given in Equation (2.1).

$$Voj = \frac{Ej - Eo}{h} \quad \text{.....Eq.2.1}$$

Where
h = Plank's constant
V = Frequency
E_o = Energy at ground state
E_j = Energy at the excited state
$O - J$ – the transition stimulated by absorption of external radiation.

The number of atoms in the excited state relative to the number in the ground state is given by Maxwell –Boltzmann law (Skoog *et al*., 1998), given by Equation 2.2

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} \exp\left(\frac{E_0 - E_1}{KT}\right) \quad \text{..... Eq.2.2}$$

Where
N_1= Number of atoms in the excited state
N_0 = Number of atoms in the ground state
g_1 and g_o – Statistical weight of excited and ground state respectively
K = Boltzmann′s constant E_O= Energy at ground state
T=Absolute Temperature
E_j = energy at the excited state

The relative fraction of atoms in excited state is dependent on temperature whereas intensity is independent of temperature. Sample solution is aspirated through nebulizer into the air/acetylene or nitrous oxide/acetylene flame (Taylor *et al.,* 2006). An electrically heated graphite furnace is used when very high sensitivity is required. The sample solution gets dispersed into mist of droplets and then evaporated into dry salt. The dry salt goes into vapour dissociate into atoms that absorb resonance radiation from external source. The unabsorbed radiation is allowed to pass through the monochromator which isolates spectral lines. The isolated analyte line falls on the detector and the output of which is amplified and recorded. The parameter measured is absorbance (A) and related to concentration by the Equation 2.3

$$A = \log I_0 / I = \varepsilon c l \quad \text{.....Eq 2.3}$$

Where
A = Absorbance
I_0 = Incidence radiation
I = Attenuated radiation
ε = Molar absorptivity ($Lmol^{-1}cm^{-1}$)
c = Concentration ($moldm^{-3}$)
l = Path length (cm)

Since the relationship between absorbance (A) and concentration (C) is linear over a wide range of concentration (Beer′s law), standards are used to obtain calibration curve

from which concentration of analyte is established through interpolation method (Vanloon, 1980). The most important components of atomic absorption spectroscopy (AAS) are:

(a) Radiation source

There are two types of radiation sources namely

i) Continuous source

The continuous source gives a wide range of radiation band includes deuterium lamp and mercury vapour lamp. It is less sensitive because only a small band of radiation passed by monochromator is absorbed while a large portion falls on the detector.

ii) Hollow cathode source

It is commonly used in atomic absorption spectroscopy (AAS) instrument and made up of metallic or alloy of element of interest. Hollow cathode lamp consists of a tungsten anode and cylindrical cathode sealed in a glass tube that is filled with neon or argon gas at a pressure of 1-5 torr.

(b) Atomizer The two types of atomizers are flame and electro thermal atomizers. In flame atomizer, the temperature is determined by flow rate and ratio of oxidant and fuel. In flame atomizer solvent is evaporated to produce solid molecular aerosol during dissolving process. Dissociation leads to atomic gas whereas some of the atoms ionize to give cations and electrons. In electro-thermal atomizer, few molecules of the sample are first evaporated at low temperature and ashed at higher temperatures in electrically heated graphite. After ashing, the temperature is increased from 2000-3000 ^{0}C to cause atomization of the sample.

(c) Monochromators

They are analyzers that present monochromatic radiation to the detector. They are filters, prisms or gratings that disperse or separate radiation so that selected wavelength corresponding to particular energy of the samples is transmitted. Diffraction grating is preferred to prisms as they offer accuracy over a wide range of wavelengths.

(d) Detectors Detectors convert radiation energy into electrical signal and include phototube, photomultiplier tube and photodiode array detector

(e) Read out system

These are digital and interfaced with microprocessors that allow the programming of various aspects, bringing simplicity in operation. However AAS is a single elemental method in which one element is determined in a series of samples and instrumental parameters optimized for the next element. Schematic diagram of atomic absorption instrument shown on Figure 2.2 shows the basic component of AAS.

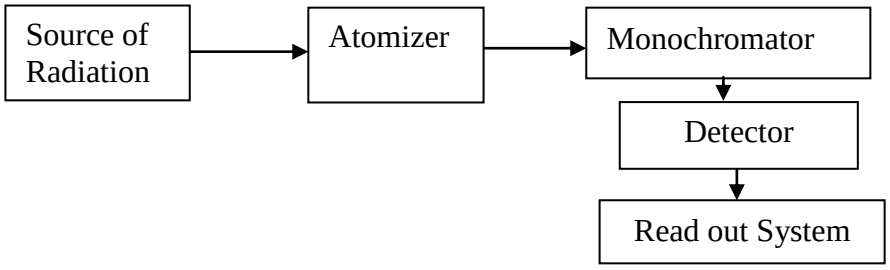


Figure 2.2: Schematic diagram of atomic absorption spectrophotometer

2.8.2 Flame Photometry

In flame photometry, the radiation that is emitted is measured since the flame is used for both atomisation and excitation. The sample solution is nebulised by a flow of gaseous oxidant, mixed with gaseous fuel and carried into flame where atomisation and excitation occurs.This process may be summarised in Figure 2.3 while flame photometer Plate 2.1

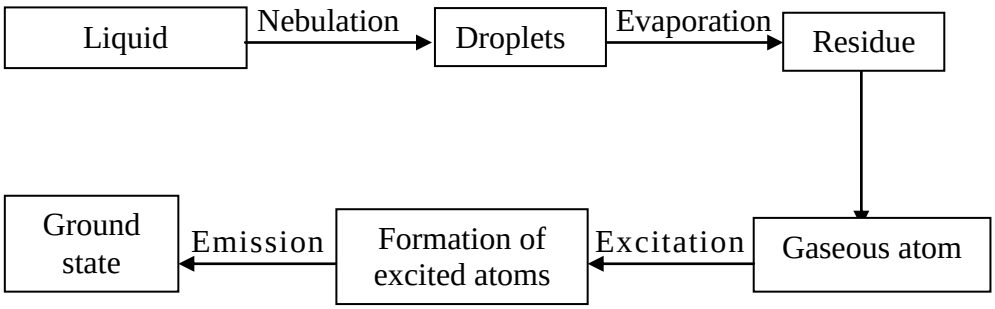


Figure 2.3: Schematic diagrams of processes taking place in the flame



Plate 2.1: Flame photometer used in this study

The relationship between emission intensity and concentration of the atom in the sample provides basis for quantitative analysis. The schematic diagram of flame photometry instrument is shown in the Figure 2.4

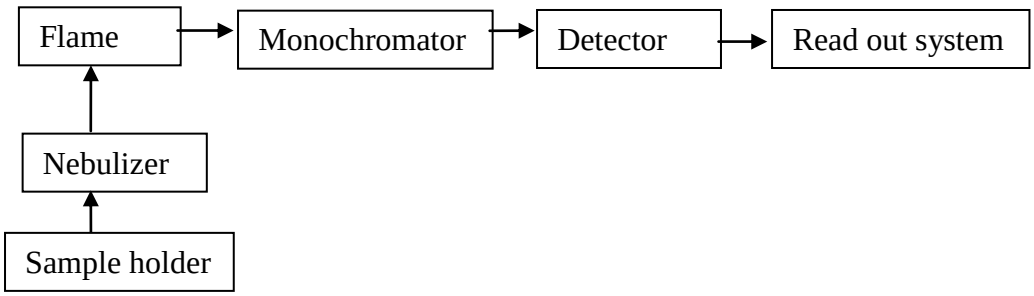


Figure 2.4: Schematic diagram of flame photometer

This is one of most important used analytical technique in determination of molecular species. It involves measurement of the amount of radiation absorbed by the molecules or

molecular ions. It is known for its rapidity, high selectivity, a reasonably high detection limit and affordable instrumentation.

2.8.3.1 Principle of UV/ Visible spectrophotometer

The total energy in a molecule is the sum of contribution from electronic, rotational, translational and vibrational energies, is given in Equation 2.5

$$Etot = Eel + Erot + Evib \quad \text{.....Eq 2.5}$$

The electronic energy is generally larger than the other three and electronic transitions ordinarily involve energies corresponding to UV/visible region of the electromagnetic spectrum. The molecular absorption in the UV/ visible region depends on the electronic structure of the molecule. There are three distinct types of electrons involved in organic molecules. These are designated as Sigma (δ), Pi (π) and nonbonding (n) electrons depending on molecular orbital they occupy. Energy absorbed in the UV/ visible region by molecules causes transition of valence electrons from ground state to excited state in the molecule. The transitions are $\delta \longrightarrow \delta^*$, $n \longrightarrow \delta^*$, $n \longrightarrow \pi^*$ and $\pi \longrightarrow \pi^*$ the star (*) refers to excited state .The transitions $n \longrightarrow \pi^*$ and $\delta \longrightarrow \delta^*$ are in the vacuum UV, where atmospheric gas absorb and are not used in the UV/visible spectroscopy. Modern spectrometers operate on range of 200-800 nm. Thus transition $\pi \longrightarrow \pi^*$ (ultraviolet) and $n \longrightarrow \pi^*$ (near UV and visible) are the ones normally involved in UV/visible spectroscopy measurements (Solomons, 1990).

A number of inorganic species also absorb in the UV/visible region. Many ions of transitional metal are coloured in solution and can be determined by spectrophotometric measurements. Other species like nitrites, nitrates, and chromate ions, oxides of nitrogen, elemental halogens on ozone show characteristic absorption peaks. Many non absorbing

analytes can be determined by causing them to react with chromophoric reagent to produce products that absorb strongly in ultraviolet and visible regions. The successful application of these colour forming reagents usually require that their reaction with analytes be forced to near completion (Skoog *et al.*, 1996).

The absorption of radiation by molecules obeys Beer-Lamberts law, which is in the simplified mathematical form states as given in Equation 2.6.

$A = \varepsilon c l$ .....Eq 2.6

Where
A = absorbance,
c = concentration in mole per litre;
ε = molar absorptivity
l= the path length in centimetres.

Since absorbance depend on concentration of the absorbing species, it is possible to quantitatively determine the amount of a given species present (skoog *et al*., 1998).

2.8.3 UV/ Visible spectrophotometer

The basic of UV/ Visible spectrophotometer instrument (Figure 2.5) is similar to that of AAS but no atomizer is required. Most important components are Radiation source, Monochromators, and Detectors

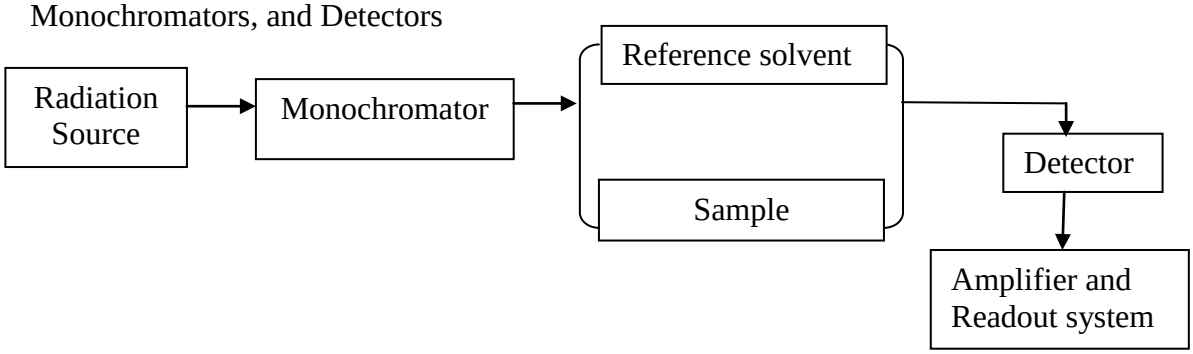


Figure 2.5: Summary of the important parts UV/ Visible spectrophotometer

2.8.4 Determination of pH

The pH of the solution is determined by the use pH meter which is an electrometric

method (Brady and Holum, 1993; Busaidi *et al*., 2005). The other method is calorimetric measurement but it not recommended for laboratory procedure because it is not very accurate (Hesse, 1972). A pH meter uses a glass electrode in measurement of pH. The electrode is made from a hollow glass tube filled with a special thin walled glass membrane at the bottom. This is filled partway with dilute solution of hydrochloric acid and a silver wire coated with a layer of silver chloride dipped into this solution. The potential of the electrode is controlled by the difference between hydrogen ion concentration inside and outside the thin glass membrane at the bottom. Since the hydrogen ion concentration inside the electrode is constant, the electrode potential varies with only concentration of the H^+ in the outside. This potential is proportional to the logarithm of the H^+ concentration and therefore to the pH of the outside solution. A glass electrode is always used with another reference electrode whose potential is constant and this gives a galvanic cell whose potential depend on pH of the solution into which the electrode is immersed. The pH meter (Plate 2.2) translates the potential hydrogen ion into the pH of the solution (Robert *et al.,* 1990; Brady and Holum, 1993).

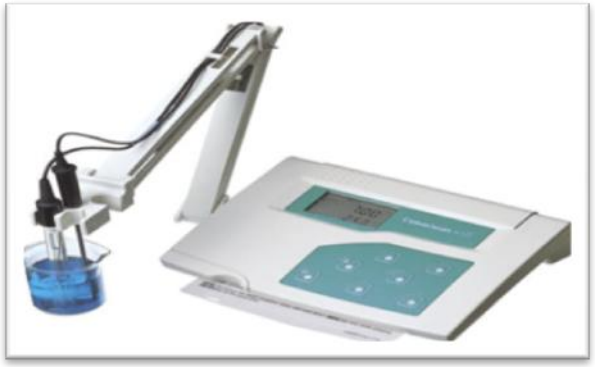


Plate 2.2: pH meter used in this study

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted at Rurii and Kasuku in Nyandarua County, Kenya. The area has an altitude raging between 1800 and 2200 meters above the sea level, the average annual rainfall ranges from 400-2200 mm and soil type is loam. Nyandarua County borders Laikipia County to the north, Nakuru County to the west, Nyeri County to east and Kiambu County to the south. The study area was selected because of the relatively small pieces of land subdivided into sections for plant fodder crops, non-leguminous plants for food and potatoes twice a year during the long and short rains. To produce high yields, many farmers apply inorganic fertilizers at least twice a season (for base and top dressing). However there are also poor farmers who cannot afford inorganic fertilizers and rely on organic fertilizer.

3.2 Research design

The experimental design on collection of leaves from plant was randomised. It involved use of leaves of *Jacaranda mimosifolia* D.Don (E1), *Cordia africana* Lam (L1), *Croton macrostachyus* Del (L2), *Vitex keniensis* Turril (L3), *Tithonia diversifolia* H.A. Gray (L4), *Manihot esculenta* C. Crankz (F1), *Carica papaya* L (F2) and *Helianthus annuus* L (F3) which were found to contain high levels of macronutrients and high buffering capacity. These leaves were further tested for the rate of mineralisation, their effect on pH of acidic soil and the effect of combined leaves and leaf extracts on growth rate of *T. aestivum* (wheat), *B. acephala* (kale) and *C. sativum* (coriander) in comparison with the same acidic soil treated with inorganic fertilizer NPK under a control experiment of untreated soil. Figure 3.1 shows the research design used in this study.

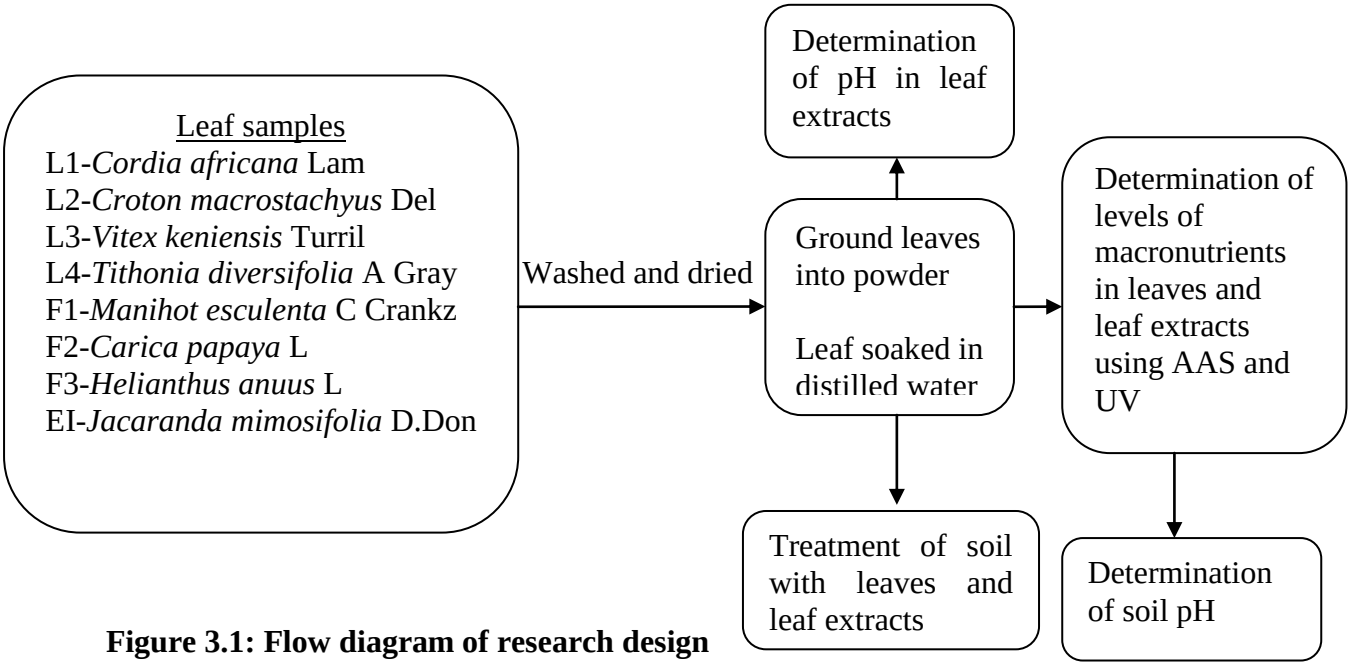


Figure 3.1: Flow diagram of research design

Figure 3.2 shows flow chart for monitoring of growth rate of each non-leguminous plant used in this study. Untreated soil Cs, Soil treated with NPK fertiliser Cf and soil treated with leaves and leaf extracts Le. In each container, there were 30 seeds planted of which 5 seedlings were uprooted after every 14 days for a period of 60 days for determination of height (cm) and dry mass (g).

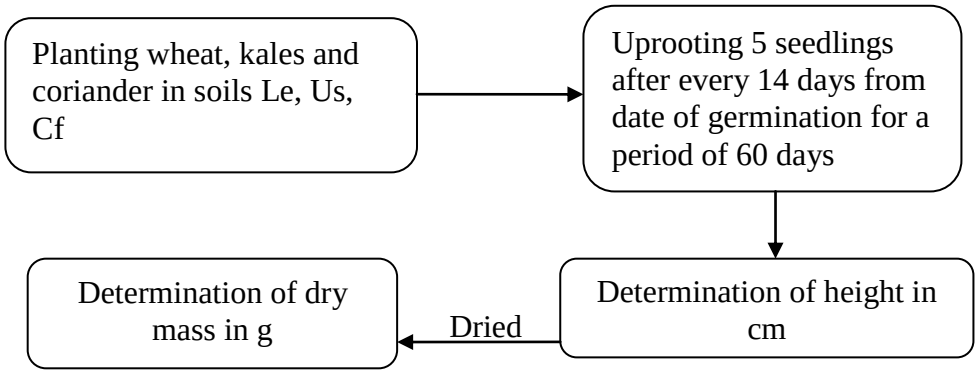


Figure 3.2: Monitoring of growth rates

3.3 Cleaning of apparatus

All apparatus were washed with liquid detergent, rinsed and then soaked in 1:1 analytical grade nitric acid for 48 hours. They were then be rinsed in aqua regia, followed by tap

water and then rinsed several times with distilled water. The glass wares were then dried in hot oven at 120 ℃. The cleaning procedure was repeated before each test.

3.4 Reagents and solvents

Analytical grade (AR) reagents sourced from Thomas Baker Chemicals Ltd Mumbai India, deionised and distilled water were used throughout the study. Weighing of the chemicals was done using a research analytical electronic balance (SHIMADZU, model ATY224, Shimadzu Philippines Manufacturing Inc (SPM)).

3.5 Sample collection and sample pre-treatment

Leave sampling was done in the month of April and May 2010 in Nyandarua County. In this season it is rainy and the trees are vegetative. During this season trees hinder sunlight from reaching the young food crops hence the need to prune them. The leaves and leaf extract obtained are used in this study. The sample size was calculated using Equation 3.1 as described by Gupta and Kapoor (1977):

$$n = \frac{s^2 t_x^2}{d^2 + s t_x^2 / N} \quad \text{.....Eq 3.1}$$

Where
n - Sample size
t_x - Critical value of t for (n-1) degrees of freedom and at α =0.05 level of significance, t_x is taken as 1.96.
s - Standard error of the mean
d - Margin of error (the acceptable difference between the sample mean and population mean), here, taken as 0.04
N - Population size

If *N* is sufficiently large relative to n, Equation 3.1 reduces to Equation 3.2

$$n = \frac{s^2 t_x^2}{d^2} \quad \text{.....Eq.3.2}$$

Systematic sampling was done to identify the farms where the plant species were to be collected. After this, random sampling was done to identify six plants of every selected plant species used in this study. The leaves were then picked from six different plants of the same selected plant species. The leaves picked from different plants of the same species were mixed thoroughly, sun dried, put in clean plastic bags, sealed and labelled appropriately. The leaves in the plastic bags were then put into nylon sack and transported to the Kenyatta university research laboratory.

Soil sampling was done in the months of February and March in Nyandarua County. In these months (which are just before long rains) soil is ready for planting after the long dry spell from December to end of March. Systematic sampling was done to identify the farms where soil samples were to be collected. There are two soil sampling methods, these are traverse and zigzag methods. In this study, traverse method was applied. Four corners of the field were determined and sampling was done diagonally. Twenty soil samples were collected from each farm identified using soil auger to scoop out the soil. The soil samples were then mixed thoroughly and the sub-sample of the mixture was put in clean polythene bag for further analysis in the laboratory for pH tests. The remaining soil sample was reserved for monitoring the growth rate of the selected non-leguminous plants.In the laboratory, dry leaves were washed with distilled water and sun dried for three days after which they were ground into fine powder determining the levels of macronutrients. The same powder was used in treatment of acidic soil in which non-leguminous plants were grown.

3.6 Sample preparation

3.6.1 Preparation of leaf samples for analysis of N, P, K, Ca and Mg

A mass of 300 mg was oven dried at 70 ℃ for 4 hour. Leaves were ground placed a dry

clean dry 125 ml “Pyrex” conical flask. A 4 ml aliquot of concentrated sulphuric acid was added and the flask swirled to ensure that the entire sample was wetted. The flask and contents were heated on an electric hot plate set at “medium” heating for 10 minutes. The flask was removed and allowed to cool and 10 drops of 30 % hydrogen peroxide was added, 3-4 drops at a time to avoid vigorous reaction. The flask was swirled keeping contents at the bottom of the flask to avoid excessive heating. The flask was allowed to cool and six drops of hydrogen peroxide added carefully and reheated cooled and adding six drops of hydrogen peroxide added and repeated until the solution turned colourless. The digest was transferred into 100 ml volumetric flask and made up to 100 ml mark. It was then transferred into clean labelled plastic container and stored, awaiting analysis.



Plate 3.1: Samples being digested in the laboratory

3.6.2 Preparation of leaf samples for analysis of sulphur

A mass of 300 mg (oven dried at 70 ℃ for 4 hours) ground leaves were weighed into a dry clean dry 125 ml “Pyrex” conical flask. A 4 ml aliquot of concentrated nitric acid was added and the flask swirled to ensure that the entire sample was wetted. The flask

and contents were heated on an electric hot plate set at “medium” heating for 10 minutes. The flask was removed and allowed to cool and 10 drops of 30 % hydrogen peroxide added, 3-4 drops at a time to avoid vigorous reaction. The flask was swirled keeping contents at the bottom of the flask to avoid excessive heating. The flask was allowed to cool and six drops of hydrogen peroxide were added carefully and reheated. The cooling and adding six drops of hydrogen peroxide was continued until the solution turned colourless. The digest was transferred into 100 ml volumetric flask and made to 100 ml mark. The digest solution was then transferred into clean labelled plastic container and stored awaiting analysis.

3.6.3 Preparation of stock and standard solutions

Stock solutions for different elements were prepared from standards solutions just before analysis. All reagents used in this study were analytical grade. Concentrated nitric acid, sulphuric acid, hydrogen peroxide, potassium nitrate and hydrochloric acid were sourced from Thomas Baker Chemicals ltd Mumbai India.

During dilutions, the final acid concentration was maintained at about 1% to keep the metal in free ionic state. The stock solutions were stored in plastic bottles and labelled appropriately. Working standards were freshly prepared from stock solutions each time an analysis was carried out, using serial dilution Equation 3.3

$$C_1V_1 = C_2V_2 \quad \text{.....Eq.3.3}$$

Where:
C_1= original concentration
V_1= original volume
C_2= new concentration
V_2= new volume

In order to account for the background effects from the acids and to correct changes resulting from digestion procedures, six blank samples were digested following the same

procedure as the samples and each of the blank samples were determined for the elements of interest (K, Ca and Mg) by atomic absorption spectrophotometer. Their absorbances were recorded and their means and standard deviations calculated and used for calculating limit of detection using the formula below as described by (Christian, 2005):

$$\text{limit of detection} = \frac{3\text{x standard deviation of blank reading}}{\text{Absorbance of standard} - \text{mean absorbanceof blanks}} \ldots\ldots \text{Eq 3.4}$$

3.6.3.1 Magnesium stock and standard solutions

Magnesium stock solution (1000 µg/g) was prepared by dissolving 1.00 g of magnesium ribbon in 300 ml of 1:1 nitric acid then made to a litre with distilled water. A working magnesium standard solution (50 µ/g) made by diluting 10 ml of the stock solution to 200 ml. The calibration graph was made using solution having the following concentrations 0, 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 µ/g of magnesium.

3.6.3.2 Calcium stock and standard solutions

The stock solution for calcium (1000 µg/g) was prepared by dissolving 2.50 g of dry calcium carbonate in 30 ml of 1M hydrochloric acid and adjusting the volume to 1 litre with distilled water at 100 °C for two hours. Calibration curve was prepared from standard solution containing 0, 5, 15, 20, 30 and 40 µg/g calcium made by serial dilution of stock solution.

3.6.3.3 Potassium stock standard solutions

Stock solution for potassium (1000 µg/g) was prepared by dissolving 1.91 g of dry potassium chloride in about 100 ml of distilled water at 100 °C for two hours and making volume to one litre. The calibration curve was made from 0, 1, 2, 4, 6, 8 and 10 µg/g standards solutions of potassium.

3.6.3.4 Nitrogen stock and standard solutions

Stock solution for nitrogen (2500 µg/g) was prepared by dissolving 1.179 g of ammonium sulphate in water and volume adjusted to one litre with distilled water. The calibration was made from 0, 4, 8, 12, 16, 20, and 24 µg/g standard solution of nitrogen.

3.6.3.5 Phosphorous stock and standard solutions

Stock solution of phosphorous (1000 µg/g) was prepared by dissolving 1.10 g of oven dried potassium orthophosphate in a 250 ml volumetric flask and then the volume adjusted to 250 ml mark with distilled water. The calibration curve was made from 0, 1, 2, 3, 4, 5, 6, 7, and 8 µg/g standard solutions of phosphorous.

3.6.3.6 Sulphur stock and standard solutions

Stock solution of sulphur (1000 µg/g) was prepared by dissolving 2.72 g of potassium sulphate and volume made to 500 ml with distilled water. The calibration curve was made from 0, 5, 10, 20, 30, 40 and 50 µg/g standard solution of sulphur.

3.6.3.7 Sulphate stock and standard solutions

The working standard stock solution for determining mineralization of sulphates in a leaf water extract was made by dissolving 0.13 g of oven dried sodium sulphate in distilled water and making to one litre with distilled water. This solution contains 90 µg/g sulphates the calibration curve was made from 0, 9, 18, 27, 36, 45 and 54 µg/g standard solutions of sulphates.

3.7 Sample analysis

3.7.1 Analytical performance of the various instruments to various elements

Analytical performance of the various instruments that were used was done. The

absorbance of different sets of standards was used to plot the calibration curves presented in Appendix I-VI. The sensitivity of the analysis method was taken as the slope of the calibration curves. The detection limit was determined as the lowest mean levels obtained by the instrumental signal equal to the blank signal plus three times the standard deviation of blank using the regression equation. The results are summarised in Tables 3.1, 3.2 and 4.3.

3.7.1.1 Analytical performance of AAS (Varian spectra AA10) (2005)

Analytical performance of performance of AAS and the analytical wavelengths, the detection limits, the correlation coefficients, and the equations of the calibration curves for the determination of metals in leaves and leaf extracts are shown in Table 3.1.

Table 3.1: Analytical performance of AAS for the analytes

Analyte	Wavelength (nm)	Range of Standards (µg/g)	Sensitivity	Linearity (r^2)	Detection Limit(µg/g)	Equations for calibration Curve
Ca	422.7	0-10	0.0024	0.996	0.038	y=0.01x+0.003
Mg	285.2	0-10	0.0512	0.999	0.032	y=0.084x+0.038

y: absorbance; x: concentration

Regression analysis was used to evaluate the linearity of AAS using the established calibration curves. The absorbance readings and concentration of standards were used to calculate the correlation coefficients (r). The method detection limits were calculated as the concentration of the given signals equal to four times the standard deviations of the six blanks as described in section 3.8. The calibration curves were established by a plot of absorbance readings against the corresponding concentration of standards with optimized instrumental conditions.

3.7.1.2 Analytical performance of the flame photometry

Analytical performance of the flame photometry is shown in Table 3.2

Table 3.2: Analytical performance of flame photometry for analytes

Analyte	Range of Standards (μg/g)	Sensitivity	Linearity(r^2)	Detection Limit(μg/g)	Equations for calibration Curve
K	0-10	0.7718	0.996	0.041	y = 0.081x + 0.032

y: absorbance; x: concentration

Regression analysis was used to evaluate the linearity of flame photometry using the established calibration curves. The absorbance readings and concentration of ideal standards were used to calculate the correlation coefficients (r). The method detection limits were calculated as the concentration of the given signals equal to four times the standard deviations of the six blanks as described in Section 3.8. The calibration curves were established by a plot of absorbance readings against the corresponding concentration of ideal standards with optimized instrumental conditions.

3.7.1.3 Analytical performance of T80+ UV/Visible spectrophotometer

Analytical performance of UV/visible spectrophotometry is shown in table 3.3

Table 3.3: Analytical performance of UV/visible spectrophotometry

Analyte	Range of standards(μg/g)	Sensitivity	Linearity(r^2)	Detection limit(μg/)	Equations for calibration Curve
Sulphate	0-10	0.0671	0.996	0.038	y = 0.084x + 0.038
Phosphate	0-10	0.5684	0.990	0.003	y = 0.269x + 0.048
Nitrates	0-5	0.2406	0.990	0.048	y = 0.269x + 0.048

y: absorbance; x: concentration

Regression analysis was used to evaluate the linearity of the established calibration curves. The absorbances and concentrations of ideal standards were used to plot the calibration curves. The experimental detection limits were calculated as the lowest concentration obtained by the instrumental signal equal to the blank signal plus three times the standard deviation of the blank using the regression equation. The results are presented in Table 3.3. The standard solutions prepared from stock solutions were used to establish UV/visible calibration curves and in turn used to determine the concentrations of selected analyte in leaves and leaf extracts. From the results presented in Table 3.3, it can be concluded that the linearity of the established calibration curves are good, hence accurate measurement were guaranteed.

3.7.1.4 Calibration of pH meter

The pH meter was calibrated using solutions made from pH 4 and 7 tablets.

3.7.2 Determination of magnesium by AAS (Varian spectra AA10)

A 5 ml aliquot of wet digested sample was pipetted into a 50 ml volumetric flask then filled with distilled water. The standards, samples and blank were nebulised into the flame of atomic absorption spectrophotometer at wavelength of 285 nm. A calibration curve was plotted and concentrations of sample and the blanks read (Okalebo *et al*., 2002)

3.7.3 Determination of calcium by AAS (Varian spectra AA10) (2005)

A volume of 10 ml of wet digested sample solution was pipetted into a 50 ml volumetric flask then filled with distilled water. The standards, samples and blank were nebulised into the flame of atomic absorption spectrophotometer at wavelength of 422 nm. A

calibration curve was plotted and concentrations of sample and the blanks read (Okalebo *et al.,* 2002)

3.7.4 Determination of potassium by flame photometry Varian spectra (AA10) (2005)

A 5 ml aliquot of wet digested sample was pipetted into a 50 ml volumetric flask then filled to the mark with distilled water. A volume of 10 ml of the diluted sample and blank were diluted further in a ratio of 1:2. The standards, samples and blank were nebulised into the flame photometer randomly (Okalebo *et al*., 2002).

3.7.5 Determination of nitrogen by T80+ UV/Visible spectrometer

Reagents N1and N2 were prepared for determination of nitrogen. N1 was prepared by 34.00 g of sodium salicylate, 25.00 g of sodium citrate, 25.00 g of sodium tartrate and 0.12 of sodium nitroprusside were dissolved and made to one litre. To prepare N2, 30.00 g of sodium hydroxide was dissolved and made to 1 litre and cooled. A 5 ml aliquot of sodium hypochlorite was added, shaken and adjusted to one litre. The two reagents were used in sample treatment.

A 5 ml of wet digested samples and blanks were diluted to ratio of 1:9 (v/v) with distilled water. With a micropipette, 0.2 ml of diluted wet sample digests; standards and blanks were pipetted in separate well labelled test tubes. To each of these test tubes 5.0 ml of reagents N1 was added and vortexed, then 5 ml of the reagent N2 added and vortexed. The test tubes were allowed to stand for two hours and absorbance measured at 650 nm using T80+ UV/Vis spectrometer PG instruments Ltd (Okalebo *et al.*, 2002).

3.7.6 Determination of phosphorous by T80+ UV/Visible spectrometer

A 12.00 g of ammonium molybdate was dissolved into a 250 ml of warm water ($50\ ^{\circ}C$) distilled water. Separately 0.29 g of antimony potassium tartrate was dissolved in 100 ml of distilled water. Both solutions were added to 100 ml of 2.5 M sulphuric acid, mixed thoroughly and diluted to two litres with distilled water. This resulting solution was ammonium molybdate/antimony potassium tartrate. To prepare ascorbic acid reducing agent, 2.11 g of ascorbic acid was dissolved in 400 ml of ammonium molybdnate/antimony potassium tartrate solution and mixed well. A 5 ml of wet digested sample was pipetted into 50 ml volumetric flask. 20 ml of distilled water was added, then 10 ml of ascorbic acid reducing agent and made to 50 ml with distilled water. They were then allowed to stand for 1 hour to permit full colour development and absorbance was measured at 880 nm using UV/visible spectrophotometer (Okalebo *et al*., 2002). Blanks and standards were also treated the same way.

3.7.7 Determination of sulphur by UV/ visible spectrophotometry

First, gelatine-barium chloride solution was made by dissolving 0.60 g of gelatin in 200 ml of distilled water at $60 - 70\ ^{\circ}C$ and allowed to stand in a refrigerator at 4 $^{\circ}C$ for 16 hours. After this the semi gelatinous fluid was brought to room temperature, 2.00 g of barium chloride added and mixed well until it dissolved. A 10 ml volume of digest was pipetted into 50 ml volumetric flask; 2 ml of gelatin- barium chloride solution was added and made to the mark with distilled water. After 30 minutes the absorbance was measured at 420 nm using UV/visible spectrophotometer (Okalebo *et al.*, 2002). Blanks and standards were also treated the same way.

3.7.8 Determination of mineralisation

On 20^{th}, 40^{th} and 60^{th} day a volume of 100 ml of deionised water was filtered from the containers where 100.00 g of leaves were soaked. This water was stored in a freezer and analysed for amount of potassium, calcium, magnesium, nitrates, phosphates and sulphates.

3.7.8.1 Determination of mineralisation of K^+, Ca^{2+}, Mg^{2+}, NO_3^- and PO_4^{2-}

The amount of K^+, $Ca^{2+}$and Mg^{2+} in leaf extracts were analysed the same way as for wet digested samples as in section **3.7.1**, **3.7.2** and **3.7.3.** The amounts of nitrates and phosphates in leaf extracts were determined the same way as for wet digested sample in section **3.7.4** and **3.7.5** but with standards for calibration curve varying between 0-8 µg/g for nitrates and 0 - 3.2 µg/g for phosphate as shown in Appendix I to VI.

3.7.8.2 Determination of mineralisation of sulphates by tubidmetry

The amount of sulphate in leaf water was determined using turbidmetric method as follows.

The following mixtures were made;

i) Glycerol-ethanol mixture was made by adding one volume of water white glycerol and two volumes of 95 % ethanol and mixed.

ii) Sodium chloride-hydrochloric acid solution was made by adding 67.0000 g of sodium chloride and 8 ml of concentrated hydrochloric acid to 200 ml of water and shaken well.

A 10 ml of volume of leaf extract was pipetted into 50 ml volumetric flask and made to the mark with distilled water. Standards, samples and blanks (50 ml of each after preparation) were put into well labelled beakers. 10 ml of glycerol–ethanol mixture and 5

ml of sodium chloride-hydrochloric acid mixture were added. A string bar was inserted and a beaker placed on a magnetic stirrer and stirred at a moderate rate. A stopwatch was used and a 0.02 g of barium chloride added. A stirrer was stopped at 60 seconds and absorbance was measured using UV/visible spectrophotometer at 420 nm. Two samples or standards were taken through this process to completion before beginning the next and absorbance was measured within 3-6 minutes after starting the stop watch (Okalebo *et al.*, 2002).

3.7.9 Calculation of concentrations of elements in the samples

The concentration of essential elements in the samples was worked out from the obtained AAS analytical results (readout) using the Equation 3.6.

$$\text{Actual concentration } (\mu g/g) = \frac{\text{Concentration } (\mu g/ml) \text{x Volume digested (ml)}}{\text{Weight of dried sample taken (g)}} \ldots \text{Eq. 3.6}$$

In cases of dilution, the actual weight was obtained by multiplying the read out results with the dilution factor. The means of the replicate measurements were then calculated from the actual concentration obtained. The concentrations of Ca and Mg in the samples were worked out by calculating their means±SD.

In cases where the sample readings were below the optimum working range, standards of known concentrations were added to bring the sample reading to this range. The absorbancies values were recorded for the original sample and after the addition of the standard. Equation 3.7 described by (Skoog *et al*., 1992) was used to calculate the actual weight of the sample.

$$Cx = \frac{A1CsVs}{(A2 - A1)Vx} \ldots\ldots\ldots\

3.11 The analysis of growth rate of non-leguminous plants

The combined leaves and leaf extract mixture was based on levels the of macronutrients and effect on pH of soil from this study and ealier study (Njagi 2008; Murungi, 1990). To maximise the plant nutrients leaves of *J. mimosifolia* (E1), *M. esculenta* (F1), *C. papaya* (F2), *H. annuus* (F3), *C. africana* (L1) and *T. diversfolia* (L4) were mixed in the ratio 3:3:2:2:3:2 of dry mass respectively. In addition to treating the acidic soil with combined leaves, 10 ml of *C. macrostachyus* (L2) and *V. keniensis* (L3) extracts of were added in intervals of 10 days for a period of 60 days for their high buffering capacity. Wheat, kales and corianders were grown in acidic soil treated with combined leaves in the ratio of 1850:150 of mass. In another set the same plants were grown in acidic soil treated with NPK in the ratio 1870: 130 NPK and in the third set plants were grown in untreated acidic soil.

For each type of nonleguminous plant there were 9 replicates labelled Le with 2.5 kg capacity for acidic soil treated with combined leaves, 9 replicates labelled FS with 2.5 kg capacity for soil treated with NPK and 9 containers labelled Us with 2.5 kg capacity of untreated soil making a total of 27 sets of replicates. In each of container 30 seeds of each plant were planted on the same day 1 cm apart. From each container 5 seedlings of each plant were uprooted for every set of the replicates (Le, Cf and Us) after every 14 days from the date of germination for a period of 60 days. The growth rate of wheat, kales and corianders based on dry mass and height was determined by using analytical balance for dry mass and metre rule for height. The seedlings from each set were washed with tap water and dried under the same conditions and the average dry mass (g) obtained for every set of experiment. The experiment was replicated after every 14 days for a period of 60 days.

3.12 Data analysis

The data was subjected to statistical analysis including the mean, one way ANOVA, standard deviation and Student-Newman-Keuls (SNK). The methods are useful in providing interdependence of the variables and significant differences in stations (Miller and Miller, 1988).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Introduction

In this study, the mean levels of the macronutrients (dry mass) in leaves and mineralisation of leaf extracts were determined. The results are presented in subsections 4.1 to 4.14.

4.2 Levels of potassium in leaves and leaf extracts

The mean levels of potassium in leaves and leaf extracts were determined by flame photometry.Results are given in Table 4.1

Table 4.1: Mean levels (µg/g) of K^+ in leaves and in leaf extracts

Plant species	**Leaves** K^+(mean±SE)	**Leaf extracts**		
		20^{th} day K^+(mean±SE)	40^{th} day K^+(mean±SE)	60^{th} day K^+(mean±SE)
J. mimosifolia (E1)	71.50 ± 2.29^{a}	17.32 ± 0.36^{f}	17.96 ± 0.18^{c}	19.89 ± 0.31^{b}
M. esculenta (F1)	181.67 ± 4.17^{e}	11.20 ± 0.38^{d}	18.28 ± 0.36^{c}	18.93 ± 0.15^{b}
C. papaya (F2)	243.50 ± 0.87^{f}	13.13 ± 0.36^{e}	16.99 ± 0.35^{c}	28.76 ± 0.36^{f}
H. annuus (F3)	76.23 ± 4.29^{a}	18.22 ± 0.38^{e}	23.43 ± 0.15^{e}	25.04 ± 0.18^{d}
C. africana (L1)	189.95 ± 5.17^{e}	13.13 ± 0.28^{e}	21.12 ± 0.38^{d}	22.79 ± 0.23^{c}
C. macrostachyus (L2)	170.83 ± 0.60^{d}	8.95 ± 0.28^{c}	16.35 ± 3.33^{c}	19.25 ± 0.11^{b}
V. keniensis (L3)	143.83 ± 0.33^{c}	3.80 ± 0.30^{a}	7.02 ± 1.35^{a}	10.22 ± 2.38^{a}
T. diversifolia (L4)	106.50 ± 0.29^{b}	6.05 ± 0.37^{b}	11.52 ± 0.35^{b}	27.29 ± 0.37^{e}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test).

The mean levels of potassium were highest in *C. papaya* (F2) 243.50±0.87 µg/g and lowest in *J. mimosifolia* (E1) 71.50±2.29 µg/g (Table 4.1). The highest rate of mineralisation by 20th day was shown by leaf extract of *H. annuus* (F3) 18.22±0.38 and lowest *V. keniensis* (L3) 3.80±0.30. On 40th day, the highest mean levels of potassium was found in leaf extract of *H. annuus* (F3) 23.43±0.15 and lowest *V. keniensis* (L3) 37.02±1.35 (Table 4.1). On 60th day *H. annuus* (F3) 28.76±0.36 had highest and *V. keniensis* (L3) 10.22±2.38 had lowest ($p > 0.05$, SNK test) Table 4.1. Mineralisation of potassium increased with time of soaking in all cases (Table 4.1).

The level of potassium in leaves were previously found to be high in *M. esculenta* (F1) 181.67±4.17, *C.papaya* (F2) 243.50±0.87, and *C. africana* (L1) 189.95±5 (Njagi, 2008; Lawrence, 1990 and Njoroge, 1999), which concur with this sudy. Other leaves that had high levels of potassium were *C. africana* (L1) 4725 µg/kg, *M. esculenta* (F1) 4541 µg/kg and *C. macrostachyus* (L2) (4270 µg/kg). This implies that the leaves of plant species with high mean levels of potassium can be used as an alternative source of potassium to bagged expensive inorganic fertilizers. The recommended levels of potassium enriched soil are 10000-24000 µg/kg (Lawrence, 1990) and since leaves of *C. papaya* (F2) have 6087 µg/kg, it is suitable for enriching the soil with potassium. The annual uptake of potassium by plant is 100 µg/kg (Lawrence, 1990). Potassium helps in the building of protein, photosynthesis, fruit quality and reduction of diseases. Potassium is supplied to plants by soil minerals, organic materials and fertilizers; however, from the results the levels of potassium were high in the composite mixture of combined leaves and leaf extracts and hence can be used as soil supplement for potassium.

4.3 Levels of calcium in leaves and leaf extracts

The mean levels of calcium were determined are given in Table 4.2.

Table 4.2: Mean levels (µg/g) of Ca^{2+} in leaves and leaf extracts

Plant species	**Leaves** Ca^{2+}(mean±SE)	**Leaf extracts**		
		20th day Ca^{2+}(mean±SE	**40th day** Ca^{2+}(mean±SE)	**60th day** Ca^{2+}(mean±E)
J. mimosifolia (E1)	49.33±0.17[a]	4.87±0.32[b]	5.23±0.27[b]	7.57±0.21[b]
M. esculenta (F1)	230.83±0.93[d]	24.22±0.15[e]	29.66±0.30[d]	37.53±0.18[d]
C. papaya (F2)	185.50±0.58[c]	3.66±0.27[b]	5.07±0.27[b]	8.74±0.27[c]
H. annuus (F3)	316.50±0.50[e]	17.16±0.21[d]	34.06±0.37[f]	39.99±0.18[f]
C. africana (L1)	367.33±0.67[e]	2.34±0.30[a]	2.45±0.21[a]	3.42±0.30[a]
C. macrostachyus (L2)	193.83±0.73[c]	13.86±0.18[c]	21.76±0.40[c]	38.26±0.27[e]
V. keniensis (L3)	114.83±0.67[b]	14.21±0.32[c]	28.26±0.27[d]	38.91±0.40[e]
T. diversifolia (L4)	138.00±0.58[b]	28.01±0.24[f]	31.12±0.24[e]	38.33±0.24[e]

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean levels of calcium in leaves ranged from 49.33±0.17 µg/g in *J. mimosifolia* (E1) to 367.33±0.67 µg/g *C. africana* (L1). Leaf extracts of *M. esculenta* (F1) 24.22±0.15, *H. annuus* (F3) 17.16±0.21 and *T. diversifolia* (L4) 28.01±0.24 had the highest rate of mineralisation of calcium by the 20th day. On 40th and 60th days *H. annuus* (F3) had highest level while *C. africana* (L1) had the lowest (Table 4.2). The general trend for mineralisation for calcium increased with time of soaking (Table 4.2).

The recommended levels of calcium enriched soil are 1700-24000 µg/kg while the annual plant uptake of calcium is 170 µg/kg (Groot, 1991). The calcium supply in the soil was found to be 9183.25 µg/kg for *C. africana* (L1), 7912.50 µg/kg for *V. keniensis* (L3) and 5770.75 µg/kg for *M. esculenta* (F1) which implies that they are good organic sources of calcium. The leaves therefore can be used in soils with low calcium to meet the annual plant uptake rather than using other sources of calcium such as dolomitic lime, gypsum and superphosphate which are expensive hence an ordinary farmer may not be able to access. The results of this study agree with what is reported by (Njagi, 2008) where *H. annuus* (F3) highest and *C. africana* (L1) had lowest concentration of Ca^{2+}.

4.4 Levels of magnesium in leaves and leaf extracts

The mean levels of magnesium were determined by AAS for all leaves and leaf extracts and results are given in Table 4.3.

Table 4.3: Levels of Mg^{2+} (µg/g) in leaves and leaf extracts (20^{th}, 40^{th} and 60^{th})

Plant species	**Leaves** Mg^{2+}(m±SE**)**	**Leaf extracts**		
		20^{th} day Mg^{2+}(m±SE)	**40^{th} day** Mg^{2+}(m±SE)	**60^{th} day** Mg^{2+}(m±SE)
J. mimosifolia (E1)	12.67 ± 1.33^{a}	3.18 ± 0.20^{b}	3.33 ± 0.25^{b}	3.35 ± 0.11^{b}
M. esculenta (F1)	30.17 ± 1.17^{c}	6.04 ± 0.20^{d}	6.09 ± 0.33^{d}	6.12 ± 0.37^{d}
C. papaya (F2)	41.00 ± 0.17^{d}	3.75 ± 0.29^{b}	4.21 ± 0.38^{c}	4.29 ± 0.20^{c}
H. annuus (F3)	114.33 ± 0.33^{e}	12.34 ± 0.10^{f}	12.38 ± 0.33^{f}	12.58 ± 0.25^{f}
C. africana (L1)	28.17 ± 0.17^{b}	2.23 ± 0.29^{a}	2.33 ± 0.26^{a}	2.43 ± 0.10^{a}
C. macrostachyus (L2)	30.33 ± 1.17^{c}	5.02 ± 0.26^{c}	12.23 ± 0.20^{f}	12.22 ± 0.26^{f}
V. keniensis (L3)	29.67 ± 0.17^{c}	11.84 ± 0.11^{f}	12.69 ± 0.31^{f}	12.78 ± 0.25^{f}
T. diversifolia (L4)	11.50 ± 0.17^{a}	9.17 ± 0.26^{e}	9.67 ± 0.26^{e}	9.90 ± 2.33^{e}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean levels of Mg^{2+} in leaves ranged from 11.50±0.17 µg/g for *T. diversifolia* (L4) to 114.33±0.33 µg/g *H. annuus* (F3). The leaf extract for *C. africana* (L1) 2.23±0.29 was lowest while *H. annuus* (F3) 12.34±0.10 was highest on 20th day. On 60th day the Mg^{2+} levels ranged from 2.43±0.10 for *C. africana* (L1) to 12.78±0.25 for *V. keniensis* (L3) (Table 4.3)..

The best trend for Mg^{2+} were found in *H. annuus* (F3) and *Vitex keniensis* (L3) throughout the study period and can be used as soil supplement for Mg^{2+} (Table 4.3). The results of this study agree with what is reported by (Njagi, 2008) where *H. annuus* (F3) and *C. papaya* (F2) had the highest concentration of Mg^{2+}. They were found to be good supplement for soils where deficiency of Mg^{2+} is detected. The levels of Mg^{2+} were almost the same in leaf extracts since it is the only metal.in chlorophyll (Table 4.3).

4.5 Levels of nitrate in leaves and leaf extracts

Mean levels of nitrate in leaves and leaf extracts were determined by UV/visible spectrophotometers and the results are given Mean.

Table 4.4: Mean levels of NO_3^- (µg/g) in leaves and leaf extracts

Plant species	**Leaves** NO_3^-(m±SE)	**Leaf extracts**		
		20th day NO_3^-(m±SE)	**40th day** NO_3^-(m±SE)	**60th day** NO_3^-(m±SE)
J. mimosifolia (E1)	93.06±0.59^{e}	0.31±0.22^{a}	0.42±0.12^{a}	2.12±0.16^{d}
M. esculenta (F1)	68.17±0.21^{d}	1.37±0.39^{b}	1.39±0.21^{b}	1.84±0.12^{c}
C. papaya (F2)	71.58±0.11^{e}	0.40±0.19^{a}	0.69±0.20^{a}	0.89±0.13^{a}
H. annuus (F3)	26.40±0.35^{a}	0.23±0.11^{a}	0.41±0.18^{a}	1.25±0.17^{b}
C. africana (L1)	40.25±0.02^{b}	0.22±0.22^{a}	0.43±0.19^{a}	2.22±0.27^{d}
C. macrostachyus (L2)	30.47±0.08^{b}	0.21±0.16^{a}	0.43±0.33^{a}	1.23±0.17^{b}
V. keniensis (L3)	50.65±0.24^{c}	0.19±0.20^{a}	0.58±0.23^{a}	0.89±0.19^{a}
T. diversifolia (L4)	322.25±0.40^{f}	0.23±0.26^{a}	0.61±0.16^{a}	2.75±0.29^{e}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean levels of nitrates in leaves ranged from 26.40±0.35 µg/g *H. annuus* (F3) to 322.25±0.40 µg/g *T. diversifolia* (L4) (Table 4.4). The results showed that *T. diversifolia* (L4) had high levels of nitrate–nitrogen 322.25±0.40 while leaves for *H. annuus* (F3) 26.40±0.35 had lowest (Table 4). On 20th day the mean levels leaf extract raged from 0.19±0.20 *V. keniensis* (L3) to1.37±0.39 *M. esculenta* (F1) (Table 4.4). On the 40th day the mean levels of nitrate-nitrogen ranged from 0.41±0.18 µg/g *H. annuus* (F3) to 1.39±0.21 (µg/g) *M. esculenta* (F1) (Table 4.4).

The nitrates-nitrogen content in *T. diversifolia* (L4) was found to be within the recommended levels of soil enriched with nitrogen 2000-10000 µg/kg since it had 8056.25 µg/kg. and was found suitable supplement for nitrogen in soil. The annual plant uptake of nitrogen is 100 µg/kg which could be supplied by *T. diversifolia* (L4) which

was a good organic source of nitrogen from the results of this study. This concurs with what was reported by (Njagi, 2008; Ayuke, 2000; Gachengo *et al*., 1999 and Nziguheba *et al*., 1998). These finding also agree with recommendations to use *T diversifolia* (L4) for top dressing because it decomposes fast and releases mineral in water (Nancy and Mary, 1990).

4.6 Levels of phosphate-phosphorous in leaves and leaf extracts

Mean levels of phosphate-phosphorous in leaves and leaf extracts were determined by UV/visible spectrometry and the results are given in Table 4.5.

Table 4.5: Mean levels (µg/g) of PO_4^{3-} in leaves and leaf extracts

Plant species	**Leaves** PO_4^{3-} (m±SE)	**Leaf extracts**		
		20th day PO_4^{3}(m±SE)	**40th day** PO_4^{3-}(m±SE)	**60th day** PO_4^{3-}(m±SE)
J. mimosifolia (E1)	38.90±0.31^{d}	1.42±0.33^{a}	1.83±0.26^{a}	2.08±0.30^{a}
M. esculenta (F1)	49.47±0.29^{e}	1.28±0.14^{a}	1.79±0.17^{a}	2.03±0.11^{a}
C. papaya (F2)	32.37±0.31^{c}	3.05±0.20^{d}	3.45±0.15^{c}	3.72±0.15^{c}
H. annuus (F3)	10.60±4.31^{a}	3.14±0.33^{d}	3.59±0.14^{c}	4.35±0.32^{d}
C. africana (L1)	28.02±1.54^{c}	1.92±0.26^{c}	3.28±0.20^{c}	3.83±0.17^{c}
C. macrostachyus (L2)	18.38±0.31^{b}	1.96±0.30^{c}	2.69±0.33^{b}	3.53±0.14^{c}
V. keniensis (L3)	37.97±0.31^{d}	1.97±0.14^{c}	2.89±0.33^{b}	2.98±0.30^{b}
T. diversifolia (L4)	55.38±0.31^{f}	2.35±0.30^{b}	2.89±0.20^{b}	3.56±0.33^{c}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean levels of phosphate-phosphorous in leaves ranged from 10.60±4.31 µg/g *H. annuus* (F3) to 55.38±0.31 µg/g *T. diversifolia* (L4) as in (Table 4.5). The best leaf

extract in the mineralisation of phosphate- phosphorous 20th day were those of *C. papaya* (F2) 3.05±0.20 µg/g and *H. annuus* (F3) 3.14±0.33 µg/g. Leave extracts of *C. papaya* (F2) 3.59±0.14 µg/g.and *H. annuus* (F3) 3.14±0.33 µg/g had the highest mean levels of phosphate- phosphorous on 40th day (Table 4.5). On 60th day the mean levels of leaf extracts ranged from 2.03±0.11 for *M. esculenta* (F1) to 4.35±0.32 µg/g for *H. annuus* (F3) (Table 4.5). The concentration of in leaf extract increased with increase of length of time of soaking (Table 4.5).

From results of this study, phosphorous can be obtained from leaves of *T. diversifolia* (L4) and *M. esculenta* (F1) which had relatively high levels and are readily available as food crops and as fodder crops. The levels of phosphorous were found to agree closely with those of (Njagi, 2008) and (Njoroge, 1999).

4.7 Level of sulphate-sulphur in leaves and leaf extracts

The mean levels of sulphate-sulphur in leaves and leaf extracts were determined using turbidmetric method. The results are given in Table 4.6.

Table 4.6: Mean levels (µg/g) of sulphate-sulphur in leaf extracts

Plant species	**Leaves** SO_4^{2-}(m±SE)	**Leaf extract**		
		20th day SO_4^{2-} (m±SE)	**40th day** SO_4^{2-} (m±SE)	**60th day** SO_4^{2-}(m±SE)
J. mimosifolia (E1)	56.48±0.23^{e}	1.37±0.23^{a}	1.49±0.29^{d}	1.94±0.38^{d}
M. esculenta (F1)	12.75±0.40^{a}	0.97±0.40^{a}	1.31±0.33^{c}	1.49±0.18^{c}
C. papaya (F2)	30.49±0.21^{d}	0.68±0.11^{a}	1.33±0.30^{c}	1.94±0.36^{d}
H. annuus (F3)	31.14±0.11^{d}	0.17±0.23^{a}	0.27±0.35^{a}	0.52±0.36^{a}
C. africana (L1)	19.14±0.08^{b}	0.74±0.38^{a}	1.15±0.23^{b}	1.64±0.15^{c}
C. macrostachyus (L2)	19.64±0.24^{b}	0.99±0.11^{a}	1.12±0.29^{b}	1.99±0.33^{d}
V. keniensis (L3)	19.74±0.40^{b}	0.13±0.24^{a}	0.35±0.29^{a}	1.29±0.40^{b}
T. diversifolia (L4)	23.52±0.35^{c}	0.74±0.38^{a}	0.97±0.35^{b}	1.48±0.20^{b}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean levels of sulphate-sulphur in leaves ranged from 12.75±0.40 µg/g *M. esculenta* (F1) to 56.48±0.23 µg/g in *J. mimosifolia* (E1) (Table 4.6). The mean levels of sulphate-sulphur in leaf extracts ranged from 0.13±0.24 µg/g *Vitex keniensis*(L3) to 1.37±0.23 µg/g *J. mimosifolia* (E1) by 20th day (Table 4.6). By 40th day, ranged from 0.27±0.35 µg/g *H. annuus* (F3) to 1.49±0.29 µg/g in *J. mimosifolia* (E1) and 0.52±0.36 µg/g *H. annuus* (F3) to 1.99±0.33 µg/g for *C. macrostachyus* L2 by 60th day (Table 4.6).

The supply of sulphur by *H. annuus* (F3) leaves was 778 µg/kg and 762 µg/kg for *C. papaya* (F2). Since this supply is below the recommended levels of sulphur (1500-1600 µg/kg) then high supply of sulphur require higher application of leaves to meet soil demand. The leaf extracts can be supplied as foliar feed for plant suspected for sulphur deficiency (Table 4.6). The concentration of in leaf extract increased with increase of length of time of soaking Table 4.6. From the results of this study the leaves of *H.*

annuus (F3) and *C. papaya* (F2) can be used as an alternative organic source of sulphur.The level of sulphur in this study was found to be very low for all species which concurs with Njagi (2008) and Murungi (1990).

4.8 Recommended levels of the plant nutrients and plant leaves that are most suitable

A summary of macronutrient requirements for plant growth and suitable plant leaves are presented in the Table 4.7

Table 4.7: Macronutrients and the best sources from the plants studied

Macronutrients	**Common range levels in soil µg/kg**	**Recommended range enriched soil µg/kg**	**Annual uptake µg/kg**	**Best leaves for macronutrients**
N	2000-4000	2000-10000	100	*T. diversifolia* L4
P	100-5000	400-2000	20	*T. diversifolia* L4, *M. esculenta* F1, *J. mimosifolia* E1 and *V. keniensis* L3
K	400-5000	10000-15000	100	*C. papaya* F2, *C. africana* L1, *M. esculenta* F1, and *C. macrostachyus* L2
Ca	400-35000	1700-24000	170	*C. africana* L1, *H. annuus* F3 and *M. esculenta*
Mg	600-15000	9000-15000	15	*H. annuus* F3 and *C. papaya* F2
S	100-10000	1500-1600	15	*H. annuus* F3 and *C. papaya* F2

Source: (Groot, 1991).

M. esculenta (F1), *C. papaya* (F2) and *H. annuus* (F3) had the highest occurrence in the supply of macronutrients (P, K and Ca), (Ca, Mg and S) and (K, Mg, and S) respectively (Table 4.7). *C. africana* (L1) and *T. diversifolia* (L4) have good levels of (K and Ca) and (N and P) respectively. The best composition for good supply of macronutrients is *C.*

papaya (F2) for potassium, *H. annuus* (F3) for magnesium and sulphur, *C. africana* (L1) for calcium and *T. diversifolia* (L4) for nitrogen and phosphorous (Table 4.7).

4.9 The pH leaf extracts and acidic soils

The pH of the leaf extracts and the soils were determined by the pH meter within a period of 60 days and results are discussed in the following sub-sections 4.4.1 and 4.4.2

4.10 The pH of leaf extracts

The pH values of leaf extracts of plant species were determined and results are given in Table 4.8.

Table 4.8: The pH of leaf extracts on days 0, 15, 30, 45 and 60

Plant species	**Days**				
	0	15	30	45	60
J. mimosifolia (E1)	6.67±0.04^{a}	4.58±0.27^{a}	4.60±0.24^{a}	4.97±0.13^{a}	5.38±0.04^{a}
M. esculenta (F1)	6.67±0.03^{a}	5.40±0.13^{b}	4.87±0.28^{a}	4.90±0.06^{a}	5.12±0.03^{a}
C. papaya (F2)	6.67±0.07^{a}	5.34±0.15^{b}	5.50±0.28^{b}	5.49±0.23^{b}	5.26±0.07^{a}
H. annuus (F3)	6.67±0.08^{a}	6.19±0.32^{d}	6.25±0.16^{c}	5.78±0.06^{c}	6.42±0.08^{c}
C. africana (L1)	6.67±0.04^{a}	6.21±0.23^{d}	7.04±0.25^{d}	7.28±0.05^{f}	7.44±0.04^{d}
C. macrostachyus (L2)	6.67±0.18^{a}	5.49±0.15^{b}	5.85±0.47^{b}	6.27±0.08^{d}	6.00±0.18^{b}
V. keniensis (L3)	6.67±0.05^{a}	5.78±0.24^{b}	5.66±0.06^{b}	5.78±0.28^{c}	6.45±0.05^{c}
T. diversifolia (L4)	6.67±0.20^{a}	6.07±0.23^{d}	6.32±0.47^{c}	6.67±0.12^{e}	6.79±0.20^{c}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The pH of leaf extract day 0 had no significant difference ($p > 0.05$, SNK test). However by day 15 there was drop from 6.67±0.04 on day to 4.58±0.27 on day 15. On day 15 leaf

extracts of *C. africana* (L1) 6.21±0.23, *T. diversifolia* (L4) 6.07±0.23 and *H. annuus* (F3) 6.19±0.32 (Table 4.8). The same trend was observved on days 30th and 45th (Table 4.8). There was a rise in pH from 6.25±0.16 for *H. annuus* (F3) to 7.44±0.04 *C. africana* **(**L1). The pH for *C. africana* (L1) ranged from 6.21±0.23 to 7.44±0.04 (Table 4.8) which imply that its leaf extracts would be best for treatment of acidic soil. The pH of *J. mimosifolia* (E1) *C. papaya* (F2) and *M. esculenta* (F1) ranged 4.58±0.27 to 5.49±0.23 within the 60 days (Table 4.8).)

The highest pH reached by 60th day was 7.44±0.04 *C. africana* (L1) and the lowest was 4.58±0.27 *J. mimosifolia* (E1). The pH of leaf extract for all the species dropped from initial 6.67 to below 6.00 except for *H. annuus* (F3), *V. keniensis* (L3) and *T. diversifolia* (L4) by the 50th day (Table 4.8). The trend for leaf extracts in this study agrees with those of (Murungi, 1990; Njagi, 2008) where pH dropped and then increased or decreased depending on the type of leaves and the length of soaking in distilled water.

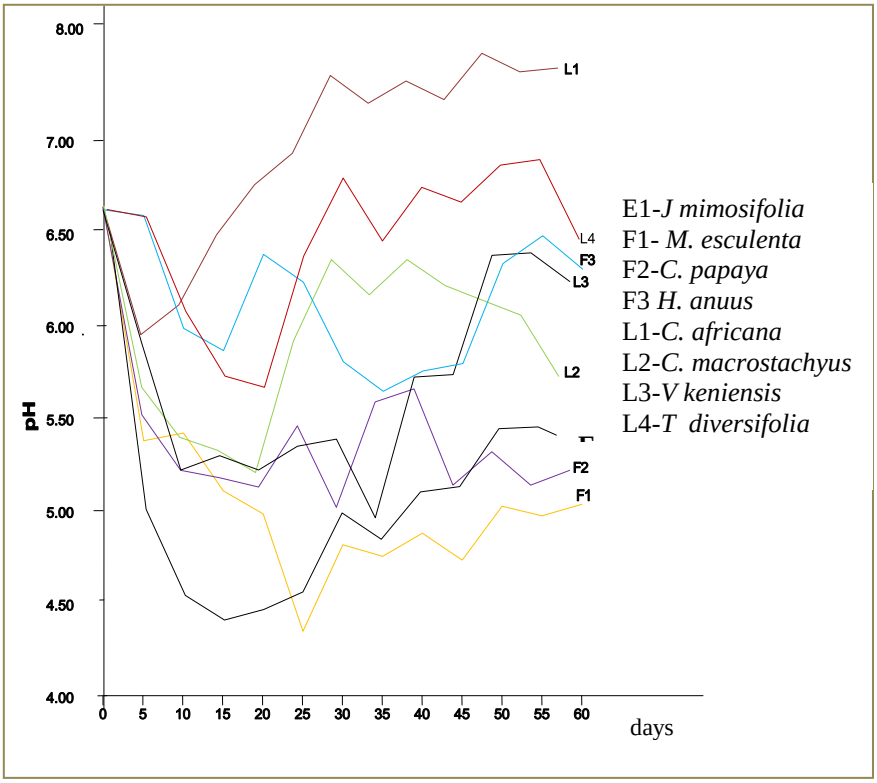


Figure 4.1: pH trends for leaf extracts

Within 60 days period the pH of *C. africana* (L1) rose above 7.00 (Figure 4.1). The pH did not rise above 5.50 after the initial fall for *C. africana* (L1), *J. mimosifolia* (E1), *C. papaya* (F2) and *M. esculenta* (F1) as shown in figure 4.1. Some species such as *J. mimosifolia* (E1), *C. africana* (L1), *H. annuus* (F3), *V. keniensis* (L3), *T. diversifolia* (L4), and *C. macrostachyus* (L2) .had pH rising above 5.50 after initial fall (Figure 4.1)

4.11 Changes in Soil pH with type of soil treatment

The pH values of acidic soil sample before treatment was 4.75 which was taken as control and did not change significantly with time. The pH values before and after treatment of acidic soil with leaves and leaf extracts, inorganic fertilizer and untreated soil were determined for a period of 60 days by pH meter. The pH values of the soils are given in Table 4.9.

Table 4.9: pH of; untreated soil, soil treated with NPK and soil treated with leaves and leaf extracts

Soils	Days						
	0	10	20	30	40	50	60
(Us)	4.75±0.02^{a}	4.45±0.01^{b}	4.42±0.01^{a}	4.21±0.01^{a}	4.12±0.01^{a}	4.02±0.02^{b}	4.12±0.01^{a}
(Le)	4.75±0.01^{a}	4.34±0.02^{a}	4.73±0.01^{c}	5.67±0.02^{c}	5.67±0.02^{c}	6.75±0.01^{c}	6.69±0.01^{b}
(Cf)	4.75±0.01^{a}	4.43±0.02^{b}	4.45±0.01^{b}	4.42±0.02^{b}	4.42±0.01^{b}	3.98±0.01^{a}	4.12±0.01^{a}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The pH of the untreated soil (Us) ranged from 4.75±0.02 to 4.02±0.02 by the 50th day from the Table 4.9. However the pH was found to rise from 4.02±0.02 to 4.12±0.01 by 60th day. The pH of the soil treated with leaves and leaf extracts (Le) ranged from 4.75±0.01 on the 10th day to 6.69±0.01 by the 60th days (Table 4.9). The pH of the soil treated with NPK dropped from 4.75±0.01 to 4.43±0.02 by the 10th day and then rose to 4.45±0.01 by the 20th day and significantly different ($p > 0.05$, SNK test). Its pH also dropped to 4.42±0.01 by 30th to 4.42±0.01 by 40th days and finally dropped to 3.98±0.01 by 50th day and then rose to 4.12±0.01 by the 60th day. In general the initial soil pH dropped for all soils by the 10th day the Table 4.9.

The results agree with what was reported by (Njagi, 2008) that for the purpose of liming *C. africana* (L1), *C. macrostachyus* (L2), *T. diversifolia* (L4), and *V. keniensis* (L3) raised pH of soil to 6.0 and above. From this study leaves and leaf extracts of F3, *V. keniensis* (L3), *T. diversifolia* (L4) and *C. africana* (L1) are the best in supplementing acidic soil since their pH rose above 6.00 as shown in (Tables 4.9) and (Fig 4.2).

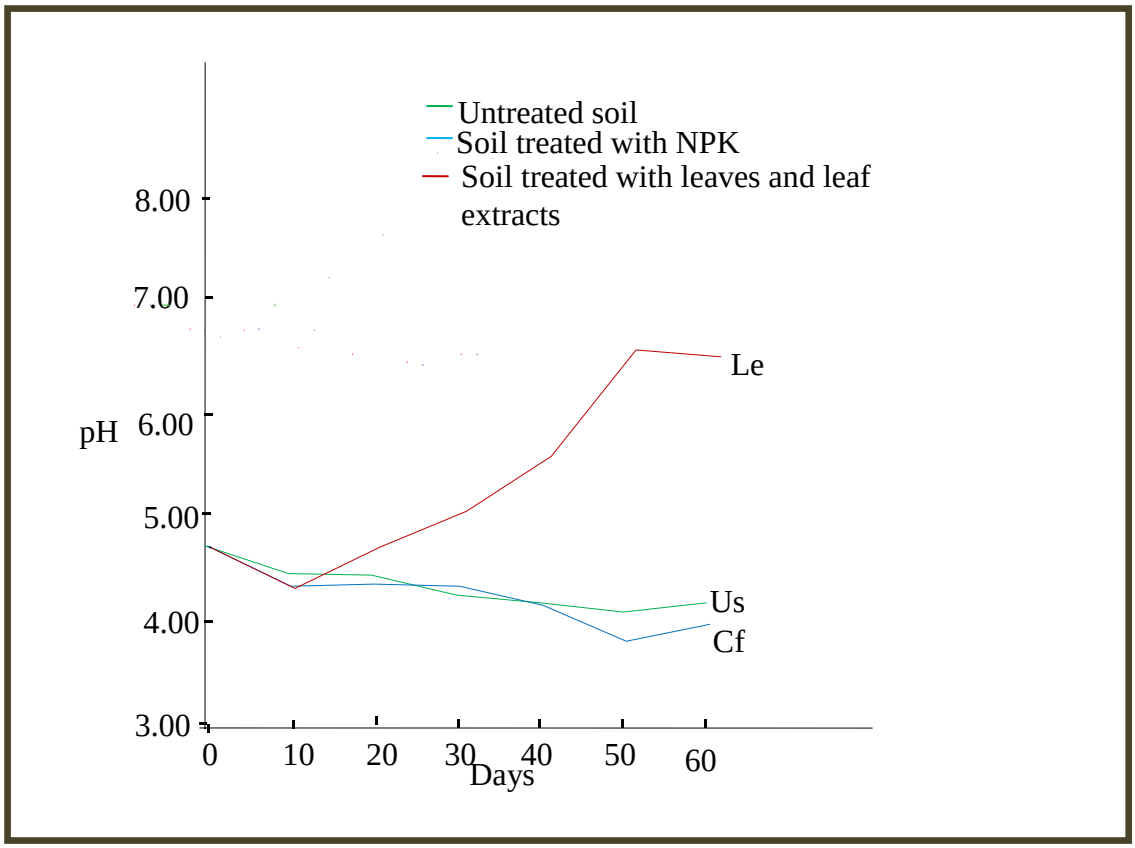


Figure 4.2: pH trends of soils Le, Cf and Us

The pH trend for soil treated with leaves and leaf extracts as indicated attained the highest pH value of 4.75±0.01 after treatment of acidic soil compared to both soil treated with NPK and untreated soil as presented in (Fig 4.2). The soil treated with NPK showed the lowest pH of 3.98±0.01 on the 50th day while the soil treated with combined leaves and leaf extract had the highest pH. The results shows that the leaves and leaf extracts had significant effect on the soil pH since on application there was rise in pH. On contrary to this, application of NPK, the pH dropped to the lowest pH value of 3.98±0.01 on 60th day (Tables 4.9) and (Fig 4.2) meaning that NPK was the possible cause for this drop since the untreated soil attained a slightly higher pH of 4.02±0.02 by 50th day.

4.12 The effect of combined leaves and leaf extracts on growth rate of *T. aestivum* (wheat)

The results of the growth rate of *T. aestivum* (wheat) grown in soils treated with leaves and leaf extracts (Le), soil treated with NPK fertiliser (Cf) and untreated soil (Us) are given in Table 4.10.

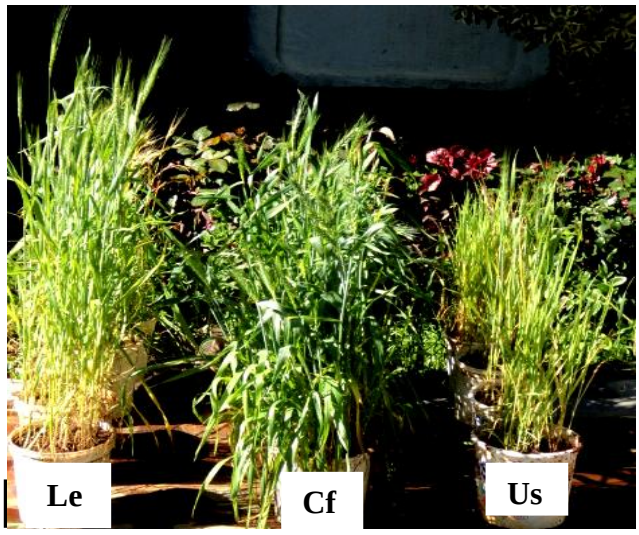
Table 4.10: Mean growth rate of *T. aestivum* (wheat) dry mass (g)

Soil type	**Dry mass** (Mean±SE) g/wk	**Height** (Mean±SE) cm/wk
(Us)	0.32 ± 0.02^{a}	3.98 ± 0.97^{a}
(Cf)	1.27 ± 0.13^{b}	6.20 ± 1.95^{b}
Le	7.29 ± 1.43^{b}	7.29 ± 1.43^{b}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean growth rate of *T. aestivum* (wheat) on the soil treated with leaves and leaf extracts was 1.27±0.13 g/wk dry mass and 7.29±1.43 cm/wk height while for soil treated with NPK fertiliser was 1.26±0.12 g/wk dry mass and 6.20±1.95 cm/wk height and untreated soil mean growth rate was 0.32±0.02 g/wk dry mass and 3.98±0.97 cm/wk height (Table 4.10).Using ($p > 0.05$, SNK test), the growth rate of *T. aestivum* (wheat) grown in untreated soil 0.32±0.02 g/wk was different from wheat grown in the soil treated with leaves and leaf extracts 1.27±0.13 g/wk and soil treated with NPK fertiliser 1.26±0.12 g/wk. However, *T. aestivum* (wheat) in the soil treated with leaves and leaf extracts NPK fertiliser did not show any significance difference (Table 4.10). The contributing factor to this observation could be that the macronutrients in the soil treated with leaves and leaf extracts and NPK fertiliser were almost equivalent or both combined were contributing adequate amount of macronutrients.

The growth rate of *T. aestivum* (wheat) height showed that the *T. aestivum* (wheat) grown in soil treated with leaves and leaf extracts (Le) had mean height of 7.29±1.43 cm/wk by eighth week and were taller than both *T. aestivum* (wheat) grown in untreated soil (Le) which had mean height of 3.98±0.97 cm/wk and the soil treated with NPK fertiliser (Cf) which had mean height of 6.20±1.95 cm/wk(Table 4.10). Using ($p > 0.05$, SNK test), the rates of growth of *T. aestivum* (wheat) grown in untreated soil (Us) 3.98±0.97 cm/wk, leaves and leaf extracts 7.29±1.43 cm/wk and NPK fertiliser 6.20±1.95 cm/wk showed significant growth rate. The contributing factor to this observation could be that the macronutrients levels in the three sets of experiments were different resulting in different height of *T. aestivum* (wheat). Results indicate that leaves and leaf extracts contained macronutrients that enhance height in *T. aestivum* (wheat). The *T. aestivum* (wheat) grown in soil treated with leaves and leaf extracts (Le), NPK fertiliser (Cf) and untreated soil (Us) is shown in the Plate 4.1



- Untreated soil
- Soil treated with commercial fertilizer
- Soil treated with leaves and leaf extracts

Plate 4.1: *T. aestivum* (wheat) grown in soil treated with Le, Cf and Us

4.13 The effect of combined leaves and leaf extract on growth rate *B. acephala* (kale)

The growth rate of *B. acephala* (kale) grown in soils treated with leaves and leaf extracts (Le), soil treated with NPK fertiliser (Cf) and untreated soil (Us) was monitored for a period of 60 days. The results are given Table 4.11

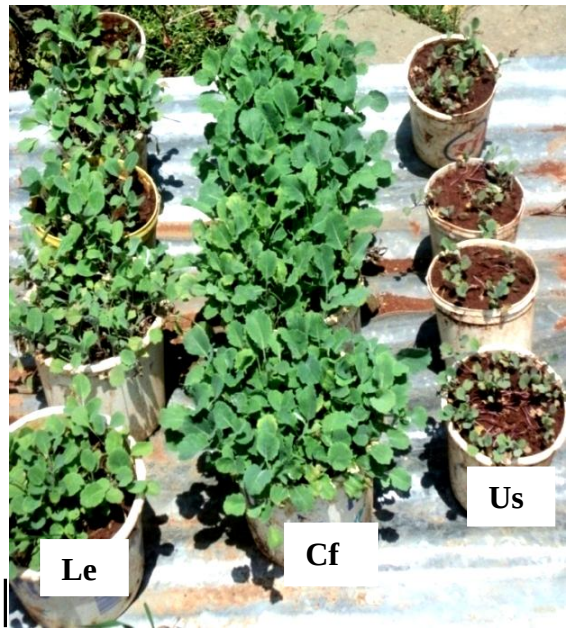
Table 4.11: Mean growth rate of *B. acephala* (kale) dry mass (g)

Soil type	**Dry mass** (Mean±SE) g/wk	**Height** (Mean±SE) cm/wk
Us	0.03 ± 0.01^{b}	1.04 ± 0.50^{a}
Cf	0.30 ± 0.02^{a}	2.57 ± 0.88^{b}
Le	0.20 ± 0.01^{a}	2.15 ± 0.85^{c}

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The growth rate of *B. acephala* (kale) dry mass was highest in NPK fertiliser (Cf) 0.30±0.02 g/wk, followed by leaves and leaf extracts (Le) while the untreated soil (Us) had the lowest 0.03±0.01 g/wk (Table 4.11). The growth rate of *B. acephala* (kale) in soil treated with leaves and leaf extracts (Le) was 0.20±0.01 g/wk, NPK fertiliser (Cf) 0.30±0.02 g/wk showed no significant difference ($p > 0.05$, SNK test). However the growth rate of *B. acephala* (kale) in the soil treated with soil treated with NPK fertiliser (Cf) was highest 2.57±0.88 cm/wk while growth rate was lowest in untreated soil (Us) 1.04±0.50 cm/wk. However, the growth rate of *B. acephala* (kale) in soil treated with leaves and leaf extracts (Le) was high 2.15±0.85 cm/wk (Table 4.11). This shows that the levels of macronutrients were high in both the soil treated with NPK fertiliser (Cf) and soil treated with leaves and leaf extracts (Le) to meet the plant demand. From the results of this study it was found that *B. acephala* (kale) responded well to the leaves and leaf extracts (Table 4.11), heights were comparable with those of NPK fertilizer. The *B.*

acephala (kale) grown in soil treated with leaves and leaf extracts (Le), untreated control soil (Us) and soil treated with NPK fertiliser (Cf) is shown in the Plate 4.2.



-Untreated soil

-Soil treated with commercial fertilizer

-Soil treated with leaves and leaf extracts

Plate 4.2: *B. acephala* (kale) grown in soil treated with Le, Cf and Us

4.14 The effect of combined leaves and leaf extract on growth rate of *C. sativum* (coriander)

The results of the growth rate of *C. sativum* (coriander) grown in soils treated with leaves and leaf extracts (Le), soil treated with NPK fertiliser (Cf) and untreated soil (Us) are given in Table 4.12.

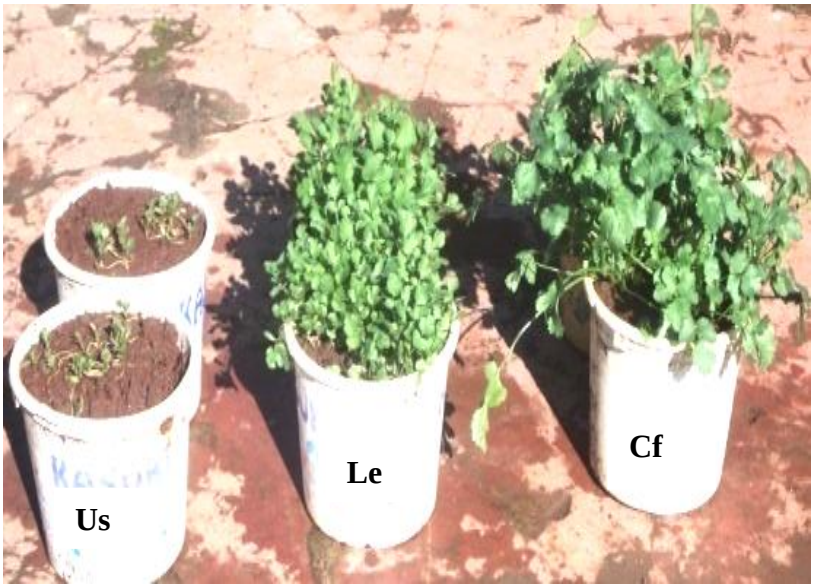
Table 4.12: Mean growth rate of *C. sativum* (coriander) in terms of dry mass (g)

Soil type	**Dry mass** (Mean±SE) g/wk	**Height** (Mean±SE) cm/wk
(Us)	0.01±0.01[a]	0.79±0.57[a]
(Cf)	0.17±0.02 [b]	1.86±0.58[b]
(Le)	0.06±0.01[a]	1.85±0.56[b]

Mean values with the same letters within the same column are not significantly different ($p > 0.05$, SNK test)

The mean growth rate of growth of *C. sativum* (coriander) dry mass grown in soil treated with combined leaves and leaf extract (Le) 0.06±0.01 g/wk soil treated with NPK (Cf) 0.17±0.02 g/wk and untreated soil (Us) 0.01±0.001 g/wk showed no significant difference ($p > 0.05$, SNK test) (Table 4.12). The highest growth rate was in soils treated with leaves and leaf extracts (Le) 1.86±0.58 cm/wk and the lowest growth rate was in untreated soil (Us) 0.79±0.57 cm/wk (Table 4.12).

It was observed that the rate of growth rate of *C. sativum* (coriander) dry mass was best in both soil treated with NPK fertiliser (Cf) and soil treated with combined leaves and leaf extracts (Le) higher than in untreated soil (Us). The difference in rate of growth of *C. sativum* (coriander) grown in soils treated with NPK fertiliser (Cf) and those grown in soil treated with combined leaves and leaf extracts (Le) was due to the fact that soil treated with combined leaves and leaf extracts took longer to avail macronutrients for the plants while NPK supplied them directly to the soil. The *C. sativum* (coriander) grown in untreated soil (Us), soil treated with leaves and leaf extracts (Le) and soil treated with NPK fertiliser (Cf) (Plate 4.3).



-Untreated soil

-Soil treated with commercial fertilizer

-Soil treated with leaves and leaf extracts

Plate 4.3: *C. sativum* (coriander) grown in soil treated with Le, Cf and Us

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMEDATIONS

5.1 CONCLUSION

The study showed that different leaves have different levels of macronutrient. The two species with highest levels of each element are *J. mimosifolia* (E1) 93.06±0.59 µg/g and *T. diversifolia* (L4) 322.25±0.40 µg/g (nitrogen), *M. esculenta* (F1) 49.47±0.29 µg/g and *Tithonia diversifolia* (L4) 55.38±0.31 µg/g (phosphorous), *C. papaya* (F2) 243.50±0.87 µg/g *C. macrostachyus* (L2) 189.95±5.17 µg/g (potassium), *H.annuus* (F3) 316.50±0.50 µg/g *C. africana* (L1) 367.33±0.67 µg/g (calcium), *H. annuus* (F3) 114.33±0.33 *Carica papaya* (F2) 41.00±0.17 µg/g (magnesium) and *J. mimosifolia* (E1) 56.48±0.23 µg/g, *H. annuus* (F3) 31.14±0.11 µg/g (sulphur).

The pH of water in which the leaves were soaked dropped and then rose after some time depending on the type of plant species and the length of time of soaking. Leaf extracts for *C. africana* (L1) ranged from 6.67±0.04 to 7.44±0.04 and *V. keniensis* (L3) ranged from 6.67±0.05 to 6.45±0.05 were settled for in this study due to their good pH characteristics as well as good supply of macronutrient. The pH of *H. annuus* (F3), *C.africana* (L1), *V. keniensis* (L3) and *T.diversifolia* (L4) was above 6.0 and could also be used in low acidic soil.

The growth rate of *T. aestivum* (wheat) in the acidic soil treated with combined leaves and leaf extracts of *J. mimosifolia* (E1), *M. esculenta* (F1), *C. papaya* (F2), *H. annuus* (F3), *C. africana* (L1) and *T. diversfolia* (L4) and leaf extracts of *C. macrostachyus* 9L2) and *V. keniensis* (L3) responded better than that *B. acephala* (kale) and *C. sativum* (coriander). This implies that the leaves and leaf extracts used in this study can be used to reduce cost of production of *T. aestivum* (wheat) in the area Nyandarua County. Leaves

J. mimosifolia (E1), *M. esculenta* (F1), *C. papaya* (F2), *H. annuus* (F3), *C. africana* (L1), *C. macrostachyus* (L2), *V. keniensis* (L3) and *T. diversfolia* (L4) used in this study have both economic value and authentic characteristic to human needs.

5.2 RECOMMENDATIONS

5.2.1 Recommendations from this study

The following recommendations from this research are made for farmers in the area of study.

i) Sun flower (*H.annuus* F3) farming should be encouraged due its high levels of Mg, Ca and S in the leaves to minimise the cost of crop production.

ii) Farmers should be sensitized about the value of indigenous trees such as *C. macrostychus, C. africana* (L1) and *J. mimosifolia* (E1) for their high contents of K, Ca and N, respectively.

iii) Farmers should be encouraged to grow *T. diversifolia* (L4) as live fence as well as farm input to improve soil fertility especially for nitrogen.

iv) Leaves of *C. africana* (L1) and *C. macrostachyus* (L2) should left in the farm for several days before planting since they lower pH in soil.

v) Farmers should be encouraged to grow *M. esculenta* (F1) and *C. papaya (*F2) due high nutritional value to human as well the high content of the elements K, P and S in the leaves.

vi) Since plant leaves studied were found to contain different amounts of macronutrients, they should be planted by farmers in agro-forests as sources of organic manure.

vii) The treatment of acidic soil with leaves and leaf extracts improved the soil fertility and raised pH. Planting these plant species together with food crops

would improve the forest cover which will take care of soil erosion and avail the leaves that contain high levels of macronutrients.

5.2.2 Suggestions for further studies

The following are recommendations for further studies

i) Pilot project based on this research recommended and plant leaves to be done in selected area of Kenya to investigate the amount of crop yields.

ii) Investigate why the organic fertilizer enhanced the height of wheat and coriander more than the inorganic fertilizer.

iii) Monitor the effect of combined leaves and leaf extracts used in this study on the growth rate of other plants in acidic soils.

iv) Investigate why the crops grown in soil treated with combined leaves and leaf extracts were more stable than crops grown in soil treated with inorganic fertilizer when exposed to sunny conditions.

v) Investigate the effect of applying the leaves of *T. diversifolia* L4 in top dressing of the actual crops.

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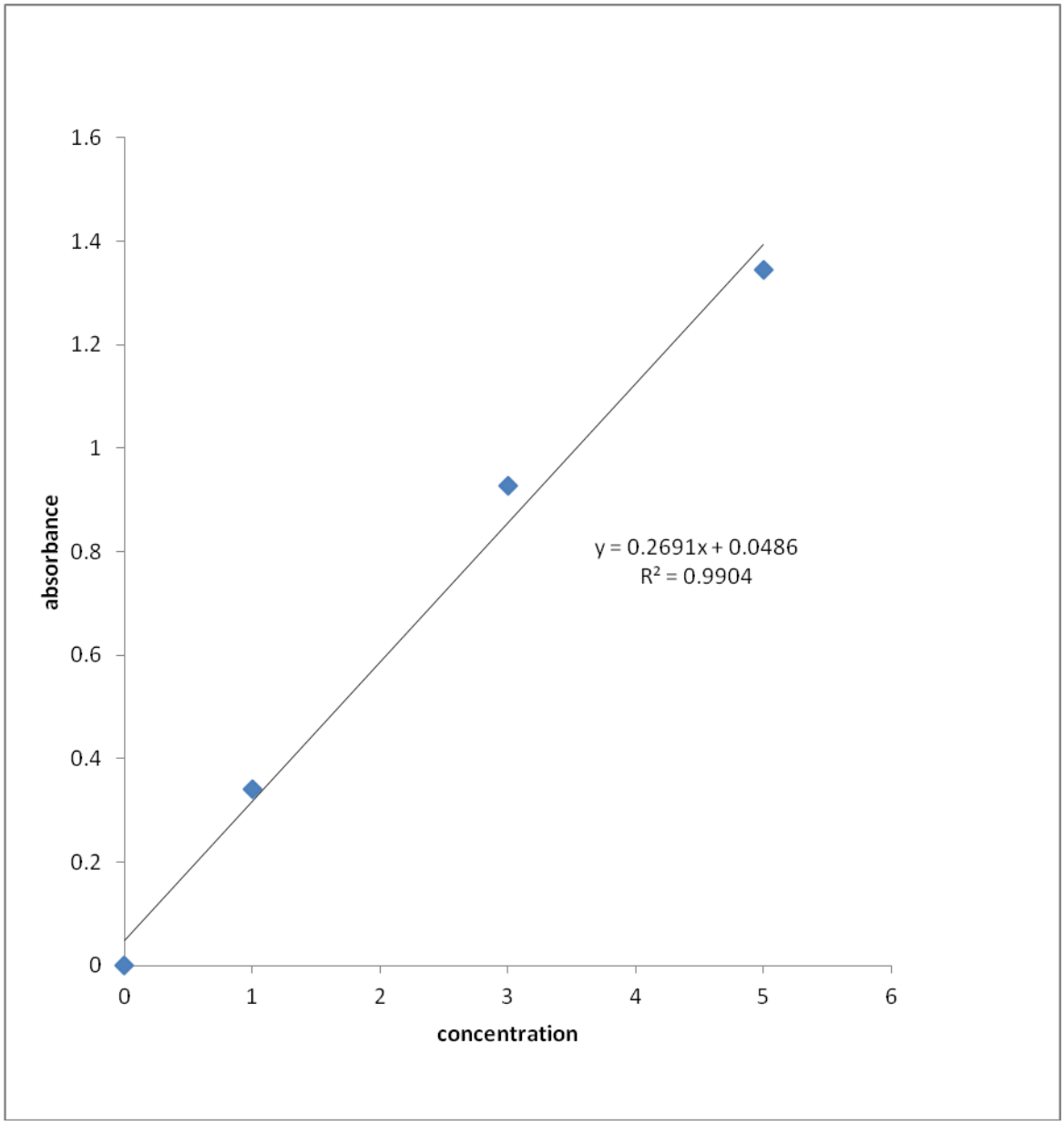
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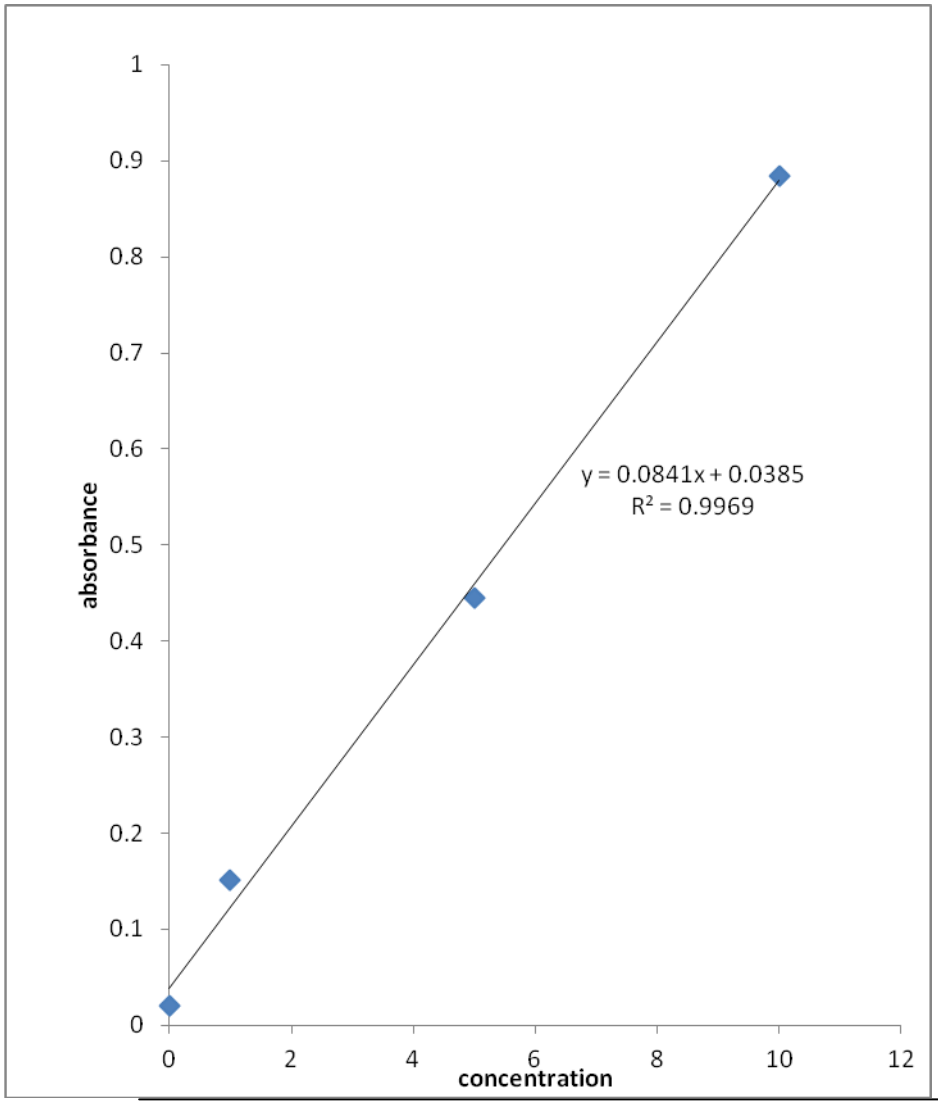
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APPENDIX I
Calibration curve for phosphate



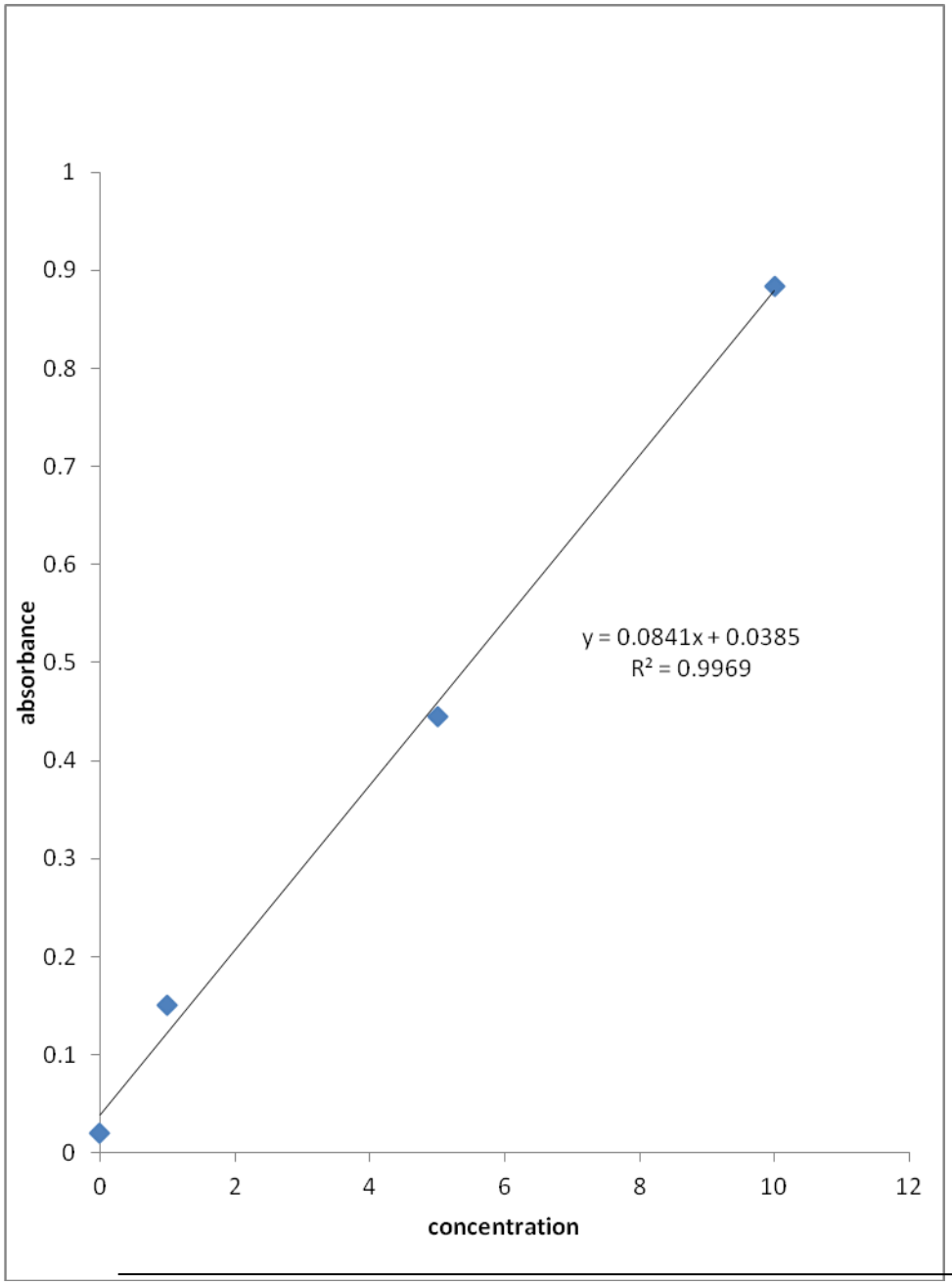
APPENDIX II

Calibration curve for sulphate



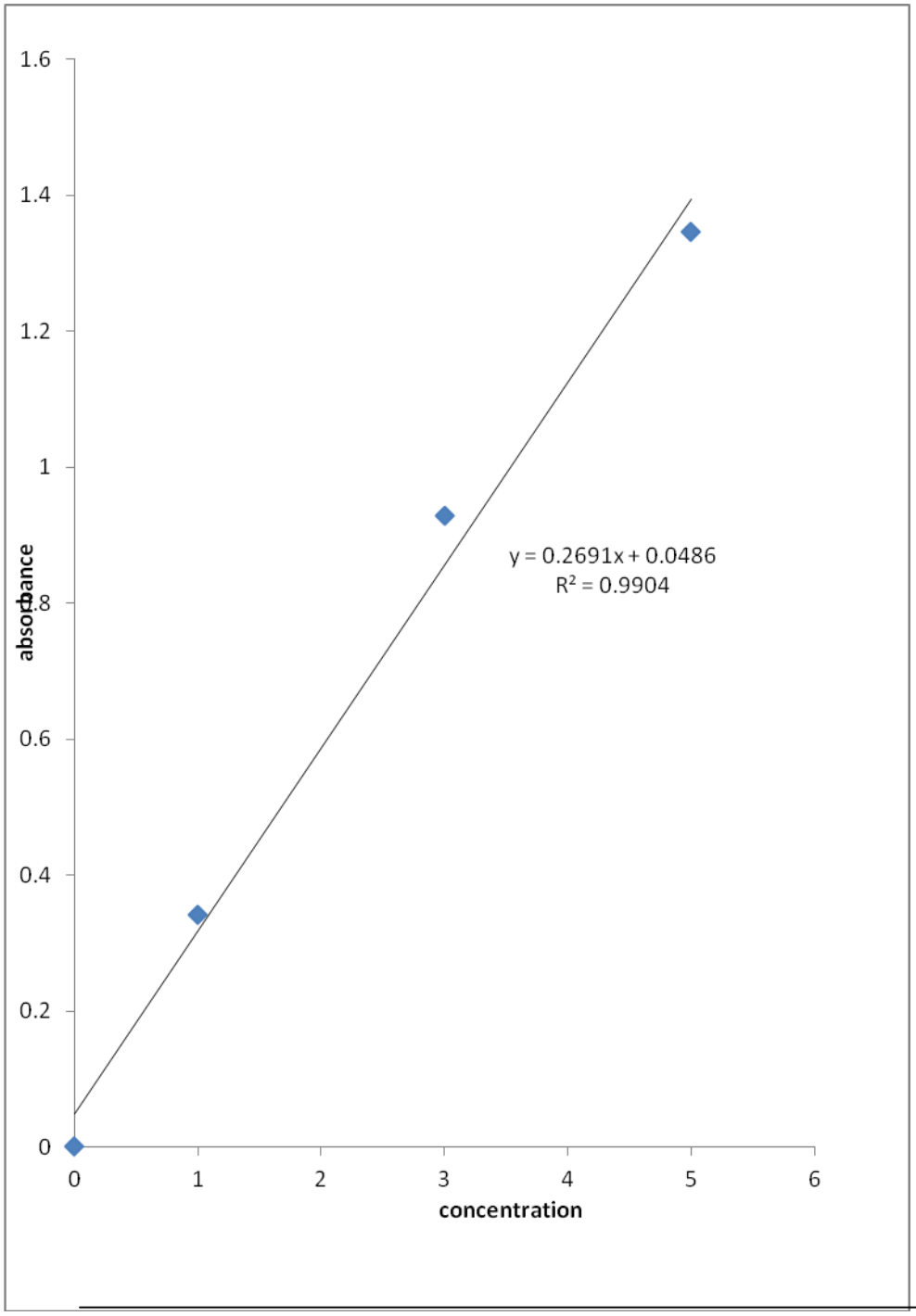
APPENDIX III

Calibration curve of calcium



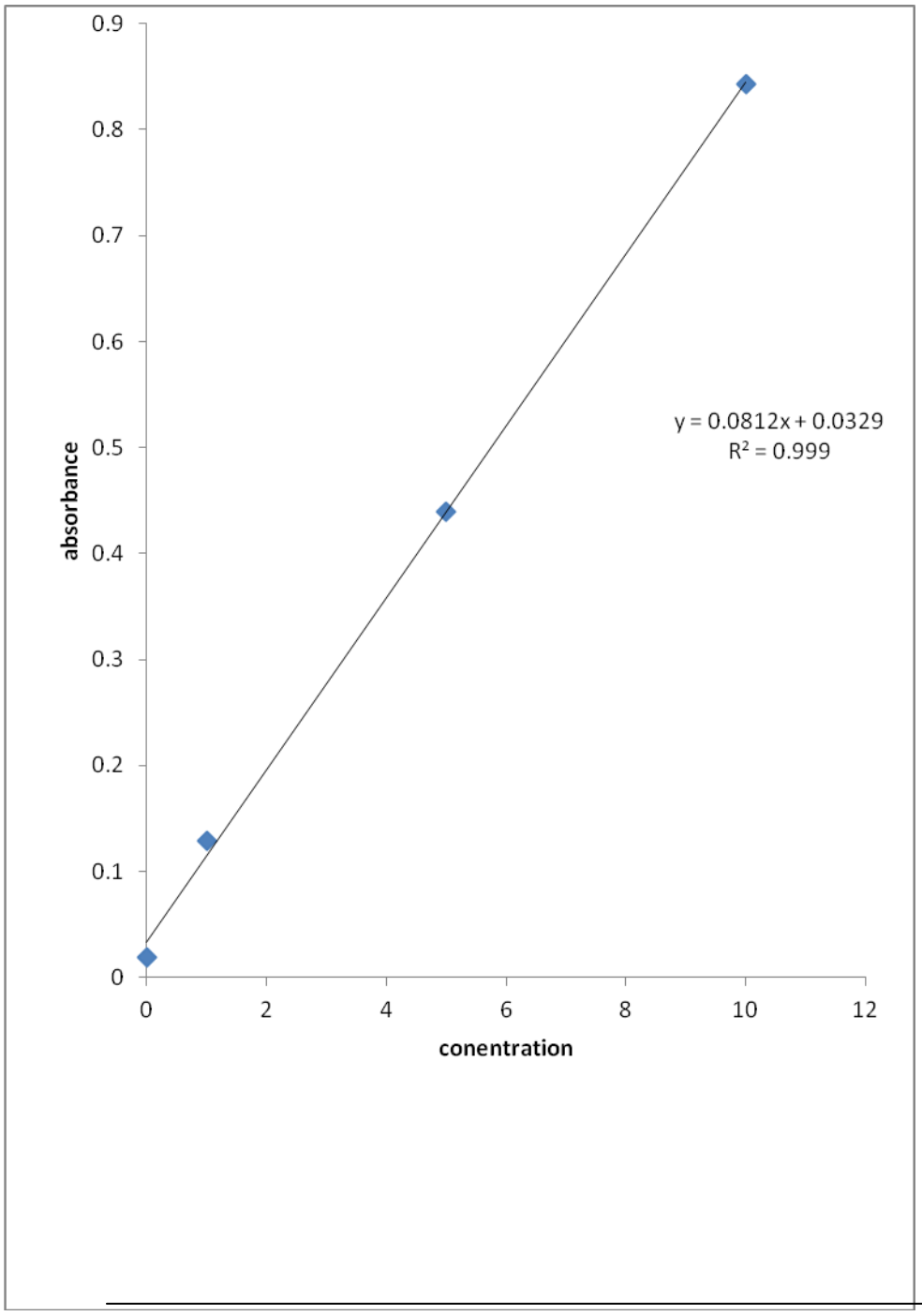
APPENDIX IV

Calibration curve for nitrates



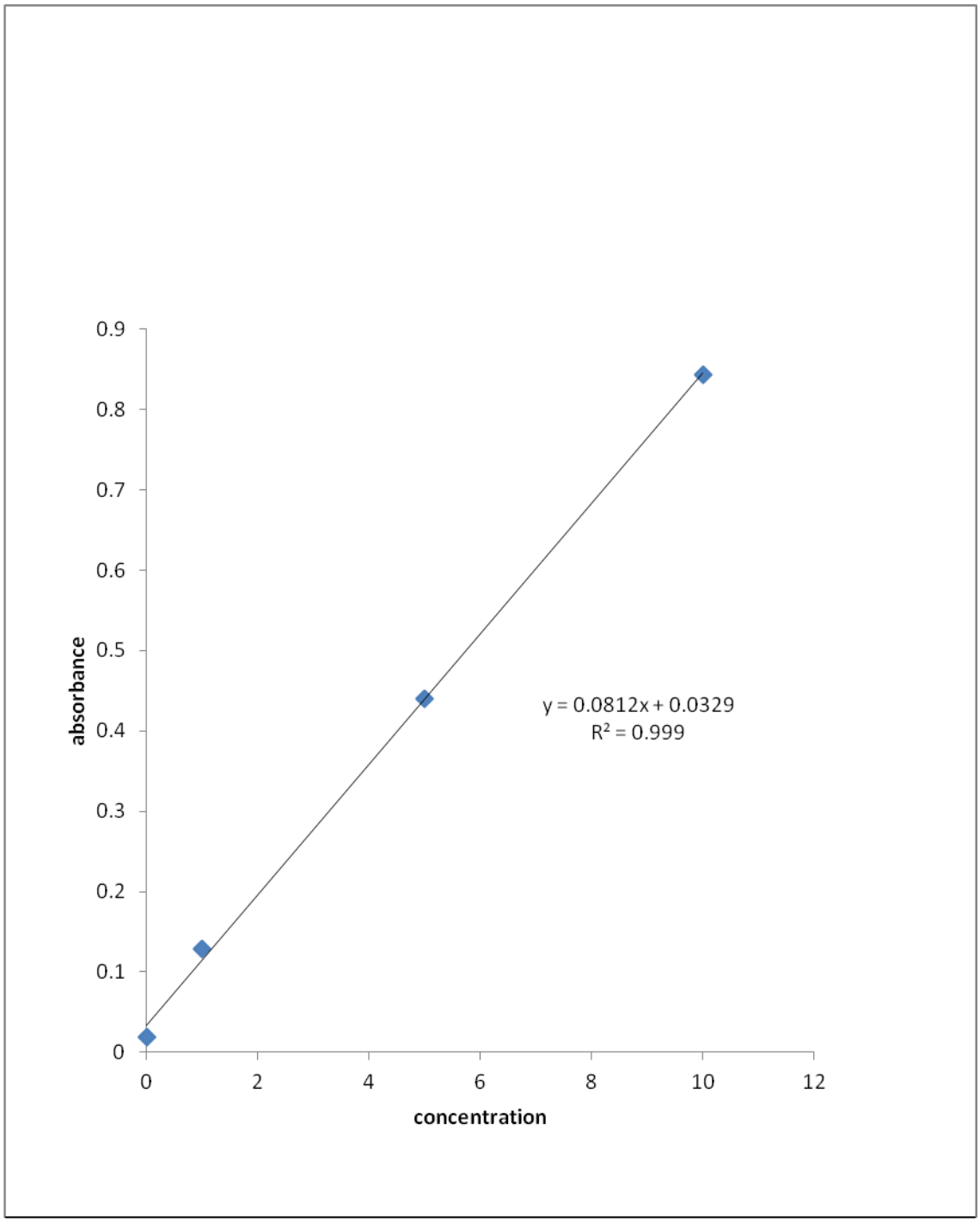
APPENDIX V

Calibration curve of magnesium



APPENDIX VI

Calibration curve of potassium



APPENDIX VII

pH of untreated soil (Us)

DAY	SAMPLE	SOIL pH	SOIL pH	SOIL pH
0	Us 0	4.75	4.75	4.75
10	Us 1	4.45	4.45	4.45
20	Us 2	4.42	4.42	4.42
30	Us 3	4.21	4.21	4.21
40	Us 4	4.12	4.12	4.12
50	Us 5	4.02	4.02	4.02
60	Us 6	4.12	4.12	4.12

Us –untreated soil

APPENDIX VIII

The pH of soil treated with NPK (Cf)

DAY	SAMPLE	SOIL pH	SOIL pH	SOIL pH
0	Cf 0	4.75	4.75	4.75
10	Cf 1	4.43	4.43	4.43
20	Cf 2	4.45	4.45	4.45
30	Cf 3	4.42	4.42	4.42
40	Cf 4	4..42	4.42	4.42
50	Cf 5	3.98	3.98	3.98
60	Cf 6	4.12	4.12	4.12

Cf Soil treated with NPK

APPENDIX IX

The of pH soil treated with leaves and leaf extracts (Le)

DAYS	SOIL pH	SOIL pH	SOIL pH	SOIL pH
0	Le 0	4.75	4.75	4.75
10	Le 1	4.34	4.34	4.34
20	Le 2	4.73	4.78	4.78
30	Le 3	5.12	5.12	5.12
40	Le 4	5.67	5.67	5.67
50	Le 5	6.75	6.75	6.75
60	Le 6	6.69	6.69	6.69

Le Soil treatedwithcombined leaf and leaf extract

APPENDIX X

The pH of leaves between 0 and 60 days

Days	0	15	30	45	60
J. mimosifolia (E1)	6.67±0.04[a]	4.58±0.27[a]	4.60±0.24[a]	4.97±0.13[a]	5.38±0.04[a]
M. esculenta (F1)	6.67±0.03[a]	5.40±0.13[b]	4.87±0.28[a]	4.90±0.06[a]	5.12±0.03[a]
C. papaya (F2)	6.67±0.07[a]	5.34±0.15[b]	5.50±0.28[b]	5.49±0.23[b]	5.26±0.07[a]
H. annuus (F3)	6.67±0.08[a]	6.19±0.32[d]	6.25±0.16[c]	5.78±0.06[c]	6.42±0.08[c]
C. africana (L1)	6.67±0.04[a]	6.21±0.23[d]	7.04±0.25[d]	7.28±0.05[f]	7.44±0.04[d]
C. macrostachyus (L2)	6.67±0.18[a]	5.49±0.15[b]	5.85±0.47[b]	6.27±0.08[d]	6.00±0.18[b]
V. keniensis (L3)	6.67±0.05[a]	5.78±0.24	5.66±0.06[b]	5.78±0.28[c]	6.45±0.05[c]
T. diversifolia (L4)	6.67±0.20[a]	6.07±0.23[d]	6.32±0.47[c]	6.67±0.12[e]	6.79±0.20[c]

APPENDIX XI

Concentration of K, Ca, Mg, P, N and S in leaves

Leaves	K^+ (m±SE)	Ca2+ (m±SE)	Mg^{2+} (m±SE)	PO_4^{3-} (m±SE)	NO_3^- (m±SE)	SO_4^{2-} (m±SE)
J. mimosifolia (E1)	71.50±2.29[a]	49.33±0.17[a]	12.67±1.33[a]	38.90±0.31[d]	93.06±0.59[e]	SO_4^{2-} (m±SE)
M. esculenta (F1)	181.67±4.17[e]	230.83±0.93[d]	30.17±1.17[c]	49.47±0.29[e]	68.17±0.21[d]	56.48±0.23[e]
C. papaya (F2)	243.50±0.87[f]	185.50±0.58[c]	41.00±0.17[d]	32.37±0.31[c]	71.58±0.11[d]	12.75±0.40[a]
H. annuus (F3)	76.23±4.29[a]	316.50±0.50[e]	114.33±0.33[e]	10.60±4.31[a]	26.40±0.35[a]	30.49±0.21[d]
C. africana (L1)	189.95±5.17[e]	367.33±0.67[e]	28.17±0.17[b]	28.02±1.54[c]	40.25±0.02[b]	31.14±0.11[d]
C. macrostachyus (L2)	170.83±0.60[d]	193.83±0.73[c]	30.33±1.17[c]	18.38±0.31[b]	30.47±0.08[b]	19.14±0.08[b]
V. keniensis (L3)	143.83±0.33[c]	114.83±0.67[b]	29.67±0.17[c]	37.97±0.31[d]	50.65±0.24[c]	19.64±0.24[b]
T. diversifolia (L4)	106.50±0.29[b]	138.00±0.58[b]	11.50±0.17[a]	55.38±0.31[f]	322.25±0.40[f]	19.74±0.40[b]

APPENDIX XII

Wheat samples and weighing of samples





APPENDIX XIII
Sample digestion and drying of samples





APPENDIX XIV
Sample preparation



APPENDIX XV

Map of Study Area

