

ALUMINIUM SPECIES IN SOIL SOLUTION AND THEIR EFFECTS  
ON RHIZOBIUM INOCULATION AND ROOT GROWTH OF FIELD  
BEANS (PHASEOLUS VULGARIS L.) CV "ROSECOCO" //

By

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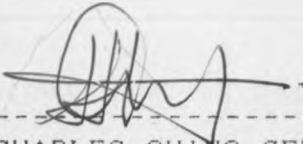
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- 11 -  
DECLARATION

I, Charles Owino-Gerroh, hereby declare that the work presented in this thesis is my original work and has not been submitted for a degree in this or any other University.

Signed



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Date

7-12-1988

This thesis has been submitted for examination with my approval as University Supervisor

Signed



DR. J.K.A. KETER

Date

7 Dec, 1988.

This work is dedicated to my parents Edward Francis Gero and Anna Celine Okech for the sacrifice they made to bring me up.

Table of Contents

		<u>Page</u>
	LIST OF FIGURES -----	
	LIST OF TABLES -----	
	LIST OF PLATES -----	
	ACKNOWLEDGEMENTS -----	
	ABSTRACT -----	
1	INTRODUCTION -----	1
2	LITERATURE REVIEW -----	11
	2.1 Soil Aluminium -----	11
	2.1.1 Occurrence and Importance -----	11
	2.2 Soil Factors Responsible for High Concentration of Aluminium in the Soil -----	13
	2.3 Soil pH and its Influence on the Aluminium Ion Species in Soil Solution -----	16
	2.4 Soil pH and Root Growth -----	20
	2.5 Hydrogen-ion Toxicity -----	24
	2.6 Aluminium and Plant Growth -----	25
	2.6.1 Beneficial Effects of Aluminium -	26
	2.6.2 Inhibitory Effects of Aluminium -	27
	2.7 Effect of pH and Aluminium on Rhizobia and Root Nodulation ----	33
3	MATERIALS AND METHODS -----	37
	3.1 Soils Investigated -----	37
	3.2 Soil Sampling and Sample Preparation -----	40

- v -

Table of Contents Cont'd

		<u>Page</u>
3.3	Soil Analysis -----	42
3.4	Greenhouse Studies -----	48
3.4.1	Nutrient Solution Experiments ---	49
3.4.1.1	Determination of the level of Aluminium Ion on nutrient Solution which is Toxic to the Growth of Field Beans -----	52
3.4.1.2	Determination of the Effects of Nutrient Solution Acidity (pH) and aluminium content on Root Growth and <u>Rhizobium</u> Inoculation of Field Beans -----	53
3.4.2	Experiments with Soils -----	57
3.4.2.1	Experiment I -----	57
3.4.2.2	Experiment II -----	60
4	RESULTS AND DISCUSSIONS -----	61
4.1	Soil Properties -----	61
4.1.1	Mechanical Analysis -----	61
4.1.2	Soil Reaction -----	64
4.1.3	Organic Carbon and Total Nitrogen -----	68
4.1.4	Phosphorus -----	70
4.1.5	Cation Exchange Capacity and Exchangeable Cations -----	71
4.1.6	Aluminium Saturation -----	76
4.1.7	Distribution of Aluminium Ion Species in the Soil -----	78
4.2	Nutrient Solution Experiments ---	87

Table of Contents Cont'd

	<u>Page</u>
4.2.1	Effect of Nutrient Solution Aluminium Content on the Growth of Field Beans ----- 87
4.2.2	Effect of Nutrient Solution Acidity (pH) and Aluminium Content on Taproot Elongation Root Growth, and <u>Rhizobium</u> Inoculation of Field Beans ----- 97
4.3	Soil Experiments ----- 107
4.3.1	Effects of Soil, Depth and <u>Rhizobium</u> inoculation on Taproot Length of Field Bean ----- 108
4.3.2	Effects of Soil, Depth and <u>Rhizobium</u> Inoculation on Root Growth of Field Beans ----- 115
4.3.3	Effects of Soil, Depth and <u>Rhizobium</u> Inoculation on the Number of Nodules formed on the Root of Field Beans ----- 124
4.4	Comparison of Nutrient Solution with Soil Experiment ---- 132
5	CONCLUSION ----- 134
	REFERENCES ----- 138
	APPENDICES ----- 172

List of Figures

<u>Figure</u>		<u>Page</u>
1	Diagram of Leonard jar -----	51
2a	Estimated linear regression between aluminium ion concentration in nutrient solution and taproot length (cm plant <sup>-1</sup> ) of field beans -----	94
2b	Estimated linear regression between aluminium ion concentration in nutrient solution and shoot dry matter weight (g plant <sup>-1</sup> ) of field beans -----	95
2c	Estimated linear regression between aluminium ion concentration in nutrient solution and pooled shoot and root dry matter weight (g plant <sup>-1</sup> ) of field beans -----	96

List of Tables

<u>Table</u>		<u>Page</u>
1	Treatments for nutrient solution experiment -----	55
2	Treatments for soil experiments -----	58
3	Some physical properties of three soils studied, Gituamba, Kitale and Kabete -----	63
4	Some chemical characteristics of the three soils studied, Gituamba, Kitale and Kabete -----	67
5	Concentration of Aluminium ion species in the three soils studied ----	82
6	Effects of nutrient solution aluminium content on the growth of field beans -----	90
7a	Effects of nutrient solution acidity and aluminium content on the length of the taproot of field beans -----	99
7b	Effects of nutrient solution acidity and aluminium content on the root dry matter weight of field beans -----	100
7c	Effect of nutrient solution acidity and aluminium content on the number of nodules formed on each root of field beans -----	101
8a	Effect of soil, depth and <u>Rhizobium</u> inoculation on the length of the taproot of field beans grown without fertilization -----	110
8b	Effect of soil, depth and <u>Rhizobium</u> inoculation on the length of the taproot of field beans grown when 69 kg P <sub>2</sub> O <sub>5</sub> /ha of fertilizer was added on the soil -----	111



Table of Contents Cont'd

<u>Table</u>		<u>Page</u>
9a	Effect of soil, depth and <u>Rhizobium</u> inoculation on the root dry matter weight of field bean grown without fertilizer -----	117
9b	Effect of soil, depth and <u>Rhizobium</u> inoculation on the root dry matter weight of field bean grown when 69 kg P <sub>2</sub> O <sub>5</sub> /ha fertilizer was added on the soil -----	118
10a	Effect of soil, depth and <u>Rhizobium</u> inoculation on the number of nodules formed on each root of the field bean grown without fertilization -----	127
10b	Effect of soil, depth and <u>Rhizobium</u> inoculation on the number of nodules formed on each root of the field bean when 69 kg P <sub>2</sub> O <sub>5</sub> was added on the soil -----	128
11	Total Concentration of aluminium in the three soils studied at different depths 28 days after 69 kg P <sub>2</sub> O <sub>5</sub> /ha fertilizer was added on the soil -----	131

List of Plates

Plate

Page

- 1      Effect of nutrient solution  
aluminium content on the growth  
of field beans (Phaseolus  
vulgaris L.) cv "Rosecoco" ----- 88

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ABSTRACT

Nutrient solution experiments were conducted in a greenhouse to determine the effect of aluminium and pH on root growth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Roseana". Nutrient solution pH's were adjusted to simulate the pH's of the three soils to be used to perform similar experiments, viz., Gituamba, Kitale and Kabete soils. Experiments were conducted in the greenhouse using the above named soils sampled at 0-15 and 15-30 cm depths. Gituamba soils had the lowest pH and lowest aluminium content and Kabete had the highest pH and content aluminium content. Kitale was intermediate in these properties. Aluminium content in the soils was determined colorimetrically using the aluminium method after leaching the soil with IN KCl solution.

The nutrient solution experiments were performed using Leonard jars. Some inoculated and non-inoculated pregerminated seeds were planted in the jars; after 14 days, the shoots were harvested and dried. The roots were removed and the nodules were counted; taproot length and the root dry matter weights determined. A high negative correlation was found between taproot length and aluminium content.

Above 10 ppm Al concentration was found to be detrimental to taproot elongation. Nutrient solution pH significantly reduced taproot elongation and root growth (dry matter weight). Both nutrient solution pH and high aluminium content significantly reduced nodule formation on the beans. The highest number of nodules was found on the roots at the highest pH (6.8) and 0 ppm aluminium. pH 5.0 was found to be the most detrimental pH for both taproot elongation, root growth and nodule formation.

Soil experiments were conducted using pots. Some inoculated and non-inoculated seeds were planted in the pots; after 28 days the shoots were harvested and dried. The roots were removed and the nodules counted; taproot length and the root dry matter weights were determined. Low phosphorus levels limited taproot length and root growth (root dry matter weight). Percent organic matter and nitrogen limited both taproot length and root growth (root dry matter weight) when phosphorus was not limiting. Regardless of phosphorus status in the soil, soil pH and aluminium content influenced the formation of nodules on the roots of the bean plants. The highest number of nodules was found in soils having lowest

aluminium content and highest soil pH (Kabete soil) and lowest in soils having the lowest pH and highest aluminium content (Gituamba soil).

A comparison between soil and nutrient solution experiment could only be made on the effect of pH and aluminium content on nodulation, with soils and nutrient solution having the highest pH and low aluminium concentration having the highest number of nodules.

## CHAPTER ONE

### INTRODUCTION

The wide variation in pH of soils in Kenya is attributed to the nature of their parent material and the intensity of leaching due to rainfall. The range of soils is also wide. This is attributed to the differences in their parent material, climate and topography. The altitude varies from sea level to 5200m (Mt. Kenya). The mean annual rainfall also varies from 255 to 2030mm (Climatological Statistics for East Africa, 1975). Therefore, there are marked differences in the organic matter contents of the soils, the rate of leaching and the rate of soil development from one location to another.

For a long time it has been realised that many crops do not grow well on acid soils. This poor growth has been attributed to some harmful effects of certain elements or soil conditions. These include the toxicities of aluminium and/or manganese, deficiencies of phosphorus, magnesium, calcium and various trace elements such as boron and molybdenum coupled with decrease in activities and population of soil organisms (von Uexkull, 1986).

Until the late 1950s hydrogen ion ( $H^+$ ) was generally believed to be the dominant cation in acid soils. The work of Coleman and other's (Coleman et al., 1958; Coleman, 1959; Lin and Coleman, 1960; Coleman and Thomas, 1967), proved that soluble aluminium ion rather than hydrogen ion was the dominant cation in the majority of soils with pH less than 5.

This soluble aluminium ion is usually toxic to many plant species at high concentration and is usually the universal infertility factor in acid soils (Adams, 1984). Usually, the main aim of liming operations is to increase the soil pH to a predetermined value and thereby improve the availability of minerals and decrease the concentration of toxic elements, such as heavy metals and aluminium, aluminium specifically being of special interest as it is a potentially toxic element and the main source of soil acidity (Hartwell and Pember, 1918; Hartwell et al., 1919; Mirasol, 1920; Nilsson et al., 1986).

Upto a point, a high hydrogen ion concentration (low pH) per se does not directly affect crop growth (Black, 1967). However, it favours weathering of soil minerals, resulting in the release of aluminium



ions and the leaching of ions such as potassium, magnesium, calcium and manganese. In most cases, crop growth is directly correlated with aluminium saturation or concentration in the soil solution. Aluminium saturation above 40% is usually considered toxic to many plants and poor growth results even for aluminium tolerant plant species. In less tolerant species, aluminium saturation of 30% may be considered toxic (von Uexkull, 1986; Monrique, 1986).

Rios and Pearson (1964), Adams and Lund (1966), Fleming and Foy (1968) and Lund (1970), have indicated that aluminium in soil solution affects crop growth. It is believed that high concentration of soluble aluminium may cause root injury and phosphorus starvation coupled with reduction in calcium uptake by plants (Wright, 1937; Kerridge et al., 1971).

The importance of field beans (Phaseolus vulgaris L.) the world over is now well established. Dry seeds and fresh or processed pods of beans are items of human diets both in developing and developed countries (Driifhouts, 1978). The success of the beans as a crop has been attributed to their increased importance (Smart, 1976). In terms of

- 4 -

Dry bean acreage yield and production in the world during 1978

Continent	Area harvested 1000' ha	Yield Kg/ha	Production 1000 MT
Africa	2131	603	1285
North and Central America	2767	794	2198
South America	5271	519	5736
Europe	1501	467	701
Australia	10	794	8
U.S.S.R	22	1865	94
World	25372	580	14202

Source: FAO Production yearbook, 1979

acreage, yields and production, the world production of beans in 1978 indicated some remarkable improvement.

In Kenya, field beans (Phaseolus vulgaris L.) are the most important grain legume (Floor, 1985) and serves as an important staple food crop for the majority of the population and also as the major source of cheap protein (C.I.A.T., 1981). In addition, beans are sold in considerable quantities for canning industries (van Eijnatten, 1975). Together with pulses, beans are the second most important group of crops in Kenya after maize (Mukunya and Keya, 1975). The total area devoted to legume production in Kenya is about 520,000 hectares annually. Of these, about 400,000 hectares are under beans alone. It has been reported that in 1984 bean production was as follows: Eastern, 30,977 tons (125,160 ha); Central, 61,231 tons (44,109 ha); Rift Valley, 27,623 tons (73,206 ha); Nyanza, 35,910 tons (50,180 ha) and Western, 49,180 tons (87,340 ha) (Anonymous, 1984).

In Kenya beans are cultivated in monoculture or in a mixture with other crops such as maize, cotton and banana in areas with annual rainfall of 900 - 2000mm (bimodal) and at altitude of 1250 - 2000m

above sea level. The most dominant soil types found in these areas are nitosols, derived from basic parent material and Acrisol derived from acid parent materials and at lower altitude Ferralsols and Luvisols derived from basement complex rocks, predominantly gneisses (Sombroek et al., 1982).

The soils differ in their characteristics, but generally at higher altitudes have an organic matter content ranging from about 2.0 to 3.5% while as a result of intense leaching their pH values and available nutrients are low. At lower altitudes, organic matter contents are generally lower, ranging from about 1.0 to 2.0%, but pH values and available nutrients tend to be higher than those of the soils of the higher altitude (Floor, 1985).

The field beans (Phaseolus vulgaris L.) are exposed to a large number of constraints such as unfavourable soil conditions, pests and diseases. These contribute to the large gap between the actual and potential yields. In Kenya, Mukunya and Keya (1975) gave an average yield of 500 Kg/ha but noted that the potential yield could be as high as 1500 Kg/ha. Average bean yields in Latin America are less than 600 Kg/ha compared to three to five tons obtained under experimental conditions in the same

countries. In the United States of America, monoculture yields of nearly 1400 Kg/ha have been reported (C.I.A.T., 1979; Schwatz and Galvez, 1980).

A major attribute of legume crops is their ability to use atmospheric nitrogen ( $N_2$ ) in symbiotic relationship with bacteria (Rice et al., 1977). To fully exploit this, legumes must be able to cover a wide range of soils and climatic conditions. Legume cultivation in tropical areas where shortages of food supplies are prevalent, is far below its potential. A primary reason for this being that early attempts to apply traditional knowledge of temperate rhizobium-legume systems to tropical situations met with discouraging failures. It is now apparent that temperate and tropical systems are quite different in many basic respects. Interaction between soil types, climatic factors and genetic capabilities of tropical rhizobia and legume symbiosis are more numerous and complex in tropical than temperate systems. When a substantial backlog of information on tropical systems is established it may be possible to use some generalization in cultivation of these legumes (Graham and Hubbell, 1975). Until then it is advisable to consider each legume trial a unique situation. Additional basic information on

tropical rhizobia and legumes and the influence of tropical soils and climate on their symbiotic association is a critical need.

In Kenya, nodulation of field beans (Phaseolus vulgaris L.) is erratic, but most soils are not devoid of Rhizobium phaseoli (Keya, 1977). In a survey carried out by the Department of Soil Science, University of Nairobi, less than 10% of the soils studied appeared to be lacking Rhizobium phaseoli (Keya, et al., 1982). Field experiments in East Africa have also shown inconsistent yield responses of beans to inoculation (De Souza, 1968; Stephen, 1969; Keya et al., 1981 and 1982). It has been suggested that environmental conditions such as adaptive factors may drastically affect nodulation of beans (Dobereiner and Campelo, 1977) and that understanding of the local conditions is therefore important (Keya et al., 1981).

The harmful effect of soil acidity on legumes which is mostly due to high levels of soil aluminium, has been recognized for many years (Barbers, 1967; Lund, 1970; Sartain and Kamprath, 1975). Recent investigations have also differentiated between soil pH (i.e. hydrogen ion activity) and factors related to acidity and between the effects of these factors.

such as aluminium ion in soil solution on plant growth, bacteria, nodule formation and function (Vincent, 1965; Munns, 1968; Franco and Munns, 1982; Lee et al., 1984 and Eaglesham et al., 1984).

It is therefore evident that the effect of soil pH (hydrogen ion activity) and its related factors, particularly the concentration of aluminium ion species in the soil solution on plant growth, bacteria, nodule formation and function in legumes is important, yet no work has been done on major acid soils in Kenya to systematically evaluate the extent to which this soil acidity-related limitation affects the above parameters.

The objectives of this study therefore, were:

1. To determine the concentration of aluminium ion species present in some Kenya soils in relation to soil pH.
2. To observe the effects of nutrient solution acidity (pH) and aluminium ion concentration on Rhizobium inoculation and root growth of Phaseolus vulgaris L.) cv "Rosecoco" grown in nutrient solution.

3. To observe the effect of soil pH and aluminium concentration on Rhizobium inoculation and root growth of field beans (Phaseolus vulgaris L.) cv "Rosecoco" grown on selected soils and compare the results with those obtained from nutrient solution (hydroponics).



## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Soil Aluminium

##### 2.1.1 Occurrence and Importance

Aluminium is the most abundant of the metallic elements in the earth's crust. It is next to oxygen and silicon as the most common element in the earth's crust, comprising more than 8% by weight. It is a major component of almost all common inorganic particles (exceptions being quartz, sand, chert fragments and ferromanganiferous concretions) (Mclean, 1965). It occurs most commonly in the primary minerals, micas, feldspars and cryolite ( $\text{Na}_3 \text{AlF}_6$ ), in the secondary clay minerals, and in ores such as bauxite ( $\text{AlOOH}$ ) (Mclean, 1976). It makes up 9.1, 8.2, 2.5, and 0.4% of igneous, shale, sandstone and limestone rocks, respectively and occurs also in many other forms in the soil (Coleman *et al.*, 1958; Rich, 1960; Rich *et al.*, 1960; Jackson, 1965 and Mclean, 1976).

Total aluminium contents of surface soils are generally of the same magnitude as that of the earth's crust (Jackson, 1964; Brady, 1974). However they are lower in cases where the soils have a predominance of sand due to the influence of sandy parent material or where the soil has lost much of its aluminium by intensive weathering.

Besides the structural role aluminium plays in various primary and secondary minerals, it may also exist and function in several other ways most of which adversely affect the soil as a plant growth environment. When aluminium is released from the structure of minerals by weathering processes, the  $Al^{3+}$  coordinates with 6  $OH_2$  groups. Each  $OH_2$  group dissociates a H ion in sequence as the pH increases. Some of the resulting  $Al^{3+}$ ,  $Al(OH)^{2+}$  ions and  $Al(OH)_2^+$  remain in the soil solution, more may be adsorbed as monomers to the exchange sites of the soil, and still more may be adsorbed and then complexed by soil organic matter. The implication of the roles these various forms of aluminium play in affecting the physio-chemical properties of soils and their effect on plant growth thereon are very important and are dealt with in the following sections.

## 2.2 Soil Factors Responsible for High Concentration of Aluminium in the soil

The solubility of aluminium and the severity of its toxicity to plants are affected by many soil factors, including soil pH, type of predominant clay mineral, concentration of other cations, total salt concentration and organic matter content (Foy, 1974). In general, aluminium toxicity does not occur in soils above pH 5.5 (McCart and Kamprath, 1965; Foy, 1974; Franco and Munns, 1982; Tisdale et al., 1985; Chong et al., 1987 and Monrique, 1986), but it is common in lower pH values and particularly severe below pH 5.0 where solubility of aluminium increases sharply and more than half the exchange sites may be occupied by aluminium (Evans and Kamprath, 1970, Mclean, 1976). For example, Foy (1974) reported that cotton (Gossypium sp) roots failed to proliferate in subsoil of pH 5.0 or below, growth was stunted and the plant wilted during midseason within three to four days after the rains. On the other hand, when sub-soils were within the pH range of 5.2 to 5.5, subsoil rooting occurred, yields were not reduced and plants could withstand drought periods of ten to fourteen days without wilting.

For a given acid soil, lime responses of crops are often well correlated with the KCl-exchangeable aluminium levels (Moschler *et al.*, 1960; Abruna-Rodriguez *et al.*, 1970; Kamprath, 1970; Monrique, 1986), but the soil pH at which aluminium becomes soluble in toxic concentration is different in different soils. Adams and Lund (1966) reported that the displaced solution of a Norfolk subsoil (Dominant clay - Kaolinite) contained toxic levels of aluminium for cotton at a soil pH of 5.40, but Bladen subsoil (Dominant clay - Montmorillonite) did not contain toxic levels above pH 4.9. The critical soil pH for primary cotton root penetration was about 5.5 for Norfolk subsoil but less than 5.0 for Dickson (Dominant clay - Vermiculite) and Bladen subsoils. Recent field studies by Monrique (1986) also demonstrated that certain soils of the tropical regions differ in critical pH value, i.e. the maximum pH at which a given crop responds to lime.

Adams and Lund (1966) found that the levels of exchangeable aluminium which are toxic to plants were also different in different soils being 0.1 m.e/100g for Norfolk and 1.5 m.e/100g for Dickson and 2.5 m.e/100g for Bladen. Thus, aluminium is toxic at a higher soil pH level and at lower level of

exchangeable aluminium in Norfolk subsoil, whose predominant clay mineral is Kaolinite than in Dickson and Bladen whose major clay mineral component are Vermiculite and Montmorillonite respectively. These investigators concluded that soil pH, exchangeable aluminium and the degree of aluminium saturation were not satisfactory indicators of root growth inhibition in the different soils studied. Studies by Monrique (1986) supported this conclusion. Richburg and Adams (1970) concluded that differences in critical pH values of soils were caused by difference in  $Al^{3+}$  ion activity and by differences in the relationship between the pH of soil-water suspension and the pH of displaced soil solution.

Organic soils are also known to have lower critical pH values for good crop growth than mineral soils (Welch and Nelson, 1950). The addition of humic acid lowers the pH at which plants are injured in certain acid soils (Mattson and Hester, 1933) and prevents aluminium toxicity of alfalfa (Medicago sativa) in nutrient solution (Brogan, 1967). Hester (1935) found that the detoxification of aluminium by the addition of organic matter to acid soils was associated with decreased aluminium solubility. Evans (1968) reported that aluminium solubility is very low at pH 5.0 in organic soils. Bhumbra and

Mclean (1965) suggested that the exchange acidity displaced at high soil pH values in some soils is due to organic-complexed aluminium or hydroxy-aluminium polymers. The evidence indicates that the lower critical pH values for plant growth in organic soils compared with mineral soils is due, at least in part, to the formation of aluminium - organic matter complexes of lower solubilities (Schnitzer and Skinner, 1963; Greene, 1963; Evans, 1968). However, there is also the possibility that aluminium is detoxified by chelation in water-soluble forms (Mclean, 1965; Coleman and Thomas, 1967; Coultier, 1969).

### 2.3 Soil pH and its Influence on the Aluminium Ion Species in Soil Solution

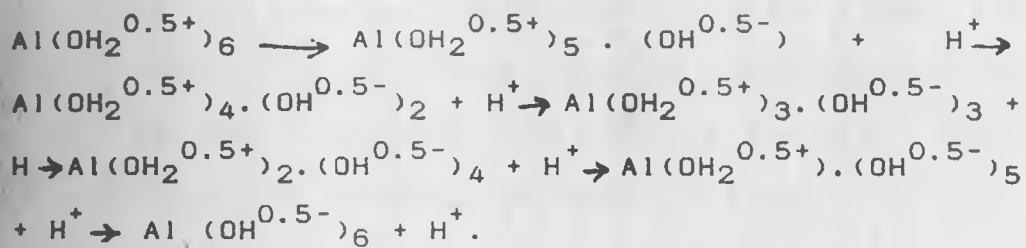
Soil pH is a measure of the activity of ionized hydrogen ( $H^+$ ) in the soil solution. It is one of the most indicative measurements of the chemical properties of a soil. Whether a soil is acidic, neutral or basic has much to do with the solubility of various compounds, the relative bondings of ions to exchange sites, and the activity of various microorganisms. Three pH ranges are particularly informative: a pH < 5.5 suggests the likely occurrence

of exchangeable aluminium; and a pH from 7.8 to 8.2 indicates the presence of calcium carbonate (Mclean, 1982).

The significance of soil pH is that it can indicate something about the percentage base saturation, depending on the predominant clay type. It can also indicate something about the degree of dissociation of  $H^+$  ion formation by hydrolysis of aluminium. Since the availability of most plant-essential elements depend on soil pH, it is an indication of the relative availability of the plant nutrients. Thus, soil pH is generally both a symptom of the soil's condition and a cause of many of the reactions that occur (Mclean, 1982).

Soil pH predominantly affects or influences the aluminium ion species present in the soil solution and the information on the reaction at the exchange site and on the solubility of aluminium in the soil solution and nutrient solution at different pH values are given by Mclean (1976) and Pillair-Nair (1978) respectively. According to Mclean, when  $H^+$  ion concentration in the soil solution increases to a pH of 4 or below, the hydronium ions ( $H_3O^+$ ) formed cause the dissolution of  $Al^{3+}$  from the edges of the mineral structure. Upon release, aluminium ions become

sixfold coordinated with oxygen in OH<sub>2</sub> groups i.e. Al(OH<sub>2</sub><sup>0.5+</sup>)<sub>6</sub>. These OH<sub>2</sub> groups are essentially aluminium substituted hydronium ions, and aluminium having replaced one hydrogen from each of six hydronium ions (OH<sub>3</sub><sup>+</sup>). The aluminium substituted hydronium ions, called aluminohexahydronium ions, are often designated as Al.6H<sub>2</sub>O<sup>3+</sup>, or simply as Al<sup>3+</sup> without the (-OH<sub>2</sub>)<sub>6</sub>. These aluminohydronium ions sequentially dissociate hydrogen ions as base is added (pH increases) leaving OH ions in place of the OH<sub>2</sub> groups.



The aluminohexahydronium is a weak acid comparable in strength to acetic acid, the dissociation constant of the former being  $1.08 \times 10^{-5}$  compared to  $1.8 \times 10^{-5}$  for the latter.

Some of the aluminohexahydronium ions may remain in solution, but most of them are adsorbed on soil cation exchange sites from which they are easily displaced with ordinary unbuffered salt solution such as 1 N KCl, if the pH is below 5.00. If the pH is



higher.  $\text{Al}(\text{OH})_2^{2+}$  or  $\text{Al}(\text{OH})_2^+$  is formed either before or after the ions are adsorbed to the soil cation exchange sites. These ions polymerize as continuous layers or discontinuous islands on the interlayer surfaces of clay minerals, or they complex with reactive groups of soil organic matter, neither of which are exchangeable with unbuffered salt solution. Since these ions both as monomers and as polymers are only partially neutralized, they are acid and hence require a base such as lime for neutralization. Also, when polymerized on the surface of clay minerals or complexed with organic matter, they are less accessible for being quickly neutralized when lime is added, and they obstruct the exchange sites of the soil for exchange of other cations.

According to Pillair-Nair (1978), the hydrolysis products of aluminium present in the pH range of 4 to 9 in simple systems such as aluminium hydroxide-water is still uncertain, but it is generally agreed that below pH 4, all the aluminium exists as  $\text{Al}^{3+}$  and that above this pH, hydroxy-Al complexes are expected to be formed. Both mono- and polynuclear complexes are expected to be formed, and mono and polynuclear species have also been reported and the equilibrium

constant ( $K_n$ ) of some of these species determined. However, there is little agreement on the nature of the aluminium species existing above pH 4.

Tisdale et al. (1985) reported that in soil solution at pH value below 4.7 the  $Al^{3+}$  is the predominant species. At pH values of 4.7 to 6.5 the predominant species is  $Al(OH)_2^+$  and, at pH of 6.5 to 8.0,  $Al(OH)_3^0$  is the principal species present in the soil solution. Above the pH 8,  $Al(OH)_4^-$  species are predominant. This supports earlier findings of Marion et al. (1976) and Franco and Munns (1982).

#### 2.4 Soil pH and Root Growth

Plant roots are subjected to a wide variation in the pH of the medium within the normal physiological pH range. Inherent soil pH is a result of the factors that determine soil development, and therefore the effects of pH on plant roots are confounded with other chemical properties of the soil. For this reason, most studies of pH effects on root growth have been done in nutrient solutions where pH and other variables can be more precisely established and maintained (Foy, 1974).

A direct effect of pH on root growth was illustrated in experiments conducted by Arnon and Johnson (1942). Three plant species, bermudagrass (Cynadon dactylon), tomato (Lycopersicon esculentum Mill) and lettuce (Lactuca sativa), were grown in nutrient solutions in which the pH was varied from 3 to 9. The pH of each treatment was maintained within  $\pm 0.2$  of a unit by daily adjustment, frequent solution changes and the use of large volumes of solution per plant. At pH 3, all the species showed a complete lack of root growth. The roots of the seedlings were severely damaged and collapsed soon after exposure to this pH. Substantial root growth occurred at pH 4. Root growth of tomatoes and lettuce were about half of that obtained at higher pH values, with a marked reduction in root growth occurring at pH 9; nevertheless, there was still a modest amount of growth. It is not clear to what extent reduced availability of the metal micronutrients may have been responsible for the reduced growth at high pH values since there was a steady decline in growth at pH values above 5. At pH values of 4 and 5 additional calcium in the nutrient solution resulted in a substantial improvement in the growth. This enhancement by calcium was not obtained at pH 6, suggesting that calcium may offset the harmful effect of  $H^+$ -ion (Foy, 1974; Franco and Munns, 1982).

Sutton and Hallsworth (1958) also reported that calcium decreases the toxicity of  $H^+$ -ion in nutrient solutions. Their results showed that  $H^+$ -ion was much more toxic where there was a rapid renewal of the solution immediately adjacent to the roots. Calcium was not as effective in preventing the  $H^+$ -ion damage as it was when the roots were grown in sand culture or in agar at comparable pH. Jackson (1967) suggested that this may be due to an increased in the pH immediately adjacent to the roots as a result of greater anion uptake. In contrast to a nutrient solution, this layer of high pH would be readily dissipated in sand culture or agar. Greater anion uptake as compared to cation uptake in acid solutions has been reported by Jacobson et al., (1957) for excised barley (Hordeum vulgare) roots.

Ekdahl (1957) reported that growth of root hairs was most sensitive to pH changes than was root elongation. Increasing the pH from 5.5 up to 7.2 resulted in only a ten percent increase in the rate of elongation of wheat (Triticum aestivum) roots. However, it should be noted that pH 5.5 is not an especially harsh  $H^+$ -ion environment (Foy, 1974; Franco and Munns, 1982). In contrast, root hair length was decreased by over forty percent over the same pH range. Root diameter was not affected. The

Distance back of the root tip to the first root hair  
is also affected by the pH of the solution. At pH  
5 the length of the hairless tip zone was 2.6 mm;  
at pH 7.2 it was 3.5 mm.

Kerridge (1969) reported that a small reduction  
in root elongation and root yields occurred due to H<sup>+</sup>-  
ion. He grew wheat plants in nutrient solutions and  
rigidly maintained the pH at 4.0 and 5.0. The plant  
showed only a negligible difference in dry weight or  
roots produced in 26 days. Root length was slightly  
longer at pH 5.0 than at pH 4.0. Burnstrom (1952)  
and Chong et al. (1987) also reported a small  
reduction in primary root elongation due to increase  
in H<sup>+</sup>-ion concentration. Although data show that  
root growth is affected by extremes of pH, root  
growth is only negligibly affected by pH in the range  
of 4.0 to 8.0 if significant amount of calcium ions  
are available and if excess toxic ions such as  
aluminium and manganese are not present (Chong et  
al., 1987). It is widely recognized that excess  
levels of aluminium and manganese are the major  
controlling factor in poor plant growth in acid soils  
(Jackson, 1967; Lie, 1971; Munns, 1976 and Andrew,  
1978).

## 2.5 Hydrogen-ion Toxicity

In addition to its competitive effects in ion absorption,  $H^+$ -ion can be damaging to roots (Foy, 1974). At pH values below about 4,  $H^+$ -ion causes a loss of previously absorbed ions from the root tissue. Sizeable losses of potassium from roots exposed to low pH in short-term experiments have been reported (Fawzy et al., 1954; Jacobson et al., 1950, 1957, 1960; Nielsen and Overstreet, 1955). Similar results were reported for magnesium (Moore et al., 1961b) and calcium (Jacobson et al., 1950; Moore et al., 1961b). Low pH also caused a loss of inorganic phosphorus, organic phosphorus and soluble nitrogen from barley roots which suggests that  $H^+$ -ions generally increase permeability of the cell membrane and allows cell constituents to leak out. At high temperature,  $H^+$ -ion was much more damaging to the tissue than at low temperatures (Jacobson et al., 1957).

Calcium and other polyvalent cations have been shown to protect the root tissue somewhat from the injurious effects of low pH (Fawzy et al., 1954; Jacobson, 1960). Apparently  $H^+$ -ion damage to the root in the absence of calcium is partially

reversible (Rains et al., 1964). Thus it appears that calcium is probably indispensable not only as a regulator of selective ion transport but indeed in maintaining the integrity of the membranes.  $H^+$ -ion adversely affects both the ion transport mechanism and the permeability of cell membranes, and the strong interaction between calcium and  $H^+$ -ion suggests a common site of action (Foy, 1974).

## 2.6 Aluminium and Plant Growth

The presence of aluminium in acid soils in relatively high concentrations and its deleterious effects on plant roots have been thoroughly established (Howard and Coleman, 1954; Coleman et al., 1958; Coleman et al., 1959; Lin and Coleman, 1960; Shroop et al., 1961; Coleman and Thomas, 1967; Sartain and Kamprath, 1975; Adam, 1984; Alva et al., 1986 and von Uexkull, 1986). However, neither the nature of its reaction with root tissue nor the levels required for toxicity levels have been clearly defined (Foy, 1974). Low aluminium concentrations appear to give a beneficial effect on the growth of most plants while some can tolerate very high concentration in their tissues (Mengel and Kirby, 1982). The effects of high concentrations of aluminium in the soil on plants appears to be limited

to the roots (Adams and Lund, 1966; Brenes and Pearson, 1973); subsequent effect on plant growth are as a result of reduced water and nutrient uptake. Characteristic symptoms of aluminium toxicity on roots include appearance of a brownish discolouration, loss of turgidity, thickening and distortion of main roots and development of short, stubby lateral buds and few fine feeder roots (von Vexkull, 1986).

#### 2.6.1 Beneficial Effects of Aluminium

Although aluminium is generally regarded as a non-essential element, during the past sixty years various claims have been made for its beneficial effects on plants when used at low concentrations. Higher plants usually contain about 200 ppm aluminium in their dry matter, (Mengel and Kirby, 1982). Chenery (1955) found that aluminium is required for healthy growth of tea and upto 5000 ppm of aluminium may be found on the tea leaves without any ill effects. Mcleod and Jackson (1965) found that aluminium concentration of 0.1 to 0.2 ppm in nutrient solution increased the growth of alfalfa and red clover (Trifolium pratense) seedlings. Aluminium at 5.0 ppm stimulate the root growth of Deschampia flexuosa, Alopecurus pratensis, Festuca pratensis



and Lolium perenne (Hackett, 1962; 1967). Lee (1971b) found that aluminium at 1.0 to 5.0 ppm (pH 3.7) stimulates vegetative growth and in some cases the uptake of magnesium and potassium by Irish potatoes (Solanum tubersum). In another study, Lee (1971a) found that addition of 20 ppm aluminium (pH 3.5) decreases overall yield of potato tubers. All the decreased yield was in the small and knobby potatoes; yields of the larger tubers and the specific gravity of the tubers increased. Aluminium added at 2.5 ppm (pH 3.5) stimulates root growth of aluminium - tolerant cranberry (Vaccinium macrocarpon) (Medappa and Dana, 1968).

The mechanism by which small quantities of aluminium benefit plant growth are not clear. A possible explanation is the increased iron solubility in the growth medium resulting from aluminium hydrolysis at a lower pH and also it may act as a catalytic agent in photosynthesis (Foy, 1974).

#### 2.6.2 Inhibitory Effects of Aluminium

Soluble aluminium is toxic to many plants and is a growth-limiting factor in many acid soils (Adams and Pearson, 1967). The problem is particularly serious in strongly acid sub-soils that are

difficult to lime (Adams and Lund, 1966; Adams, 1968; and 1969), and it is being intensified by the heavy use of acid-forming nitrogen fertilizers such as sulphate of Ammonia fertilizer (Abruna et al., 1958, Pearson et al. 1962; Wolcott et at., 1965; Pierre et al., 1971), and also by addition of non-nitrogenous fertilizers that displace exchangeable aluminium into the soil solution and lower soil pH even more (Ragland and Coleman, 1962). Strong sub-soil acidity, with aluminium at toxic levels, reduces root penetration and increases the probability of injury by drought, a frequent growth-limiting factor for crops. In acid soils aluminium is toxic as a cation but aluminium (anion) toxicity has also been reported in alkaline deposits by Jones (1961).

The damaging effects of aluminium on plants has been widely attributed to interference with phosphate uptake. Co-precipitation of aluminium and phosphorus in compounds of very low solubility certainly would reduce phosphorus availability. Similarly, phosphorus may be immobilized in root tissue after absorption. Under some condition aluminium may actually stimulate phosphorus uptake by roots (Wright, 1943; Ragland and Coleman, 1962), possibly

through precipitation in root cells, thus creating concentration gradient favourable to movement of phosphorus from external solution into the roots.

Although aluminium may be intimately related to phosphorus uptake and translocation in plants, its toxic effect on root is independent of this relation. Experiments by (1968), Willihan (1958) and Jones (1961) supported this view. Results of split-root experiments (Rios and Pearson, 1964) also confirm this independence of aluminium-toxicity and phosphorus-uptake. Even so, there is ample evidence in the literature (Foy and Brown, 1963; 1964; Chiasson, 1964; Munns, 1965b and von Uexkull, 1986) that phosphorus-uptake is usually depressed by aluminium when all, or a significant portion of the root system is exposed to aluminium. In addition, the possibility that absorbed aluminium prevents normal metabolism of phosphorus-translocation from other parts of the plant cannot be discounted. Wright and Donahue (1952) showed that absorbed phosphorus accumulate in roots exposed to soluble aluminium, but was translocated normally in the absence of aluminium. A further bit of evidence is found in a report by Sampson et al. (1965) who

discovered that exposure of roots of barley to aluminium interfered with normal DNA synthesis in the roots.

There has been considerable variation in the reported concentration of solution aluminium or level of exchangeable aluminium required to cause toxicity. Certainly, this is partly because of genetic differences among test plants in tolerance to this element (Foy, 1974). Also, difficulty in characterization of the state in which aluminium exists in the nutrient solution or soil solution is frequently a factor. In general, solution culture experiments designed to measure the direct effects of aluminium on root growth have shown that even in relatively tolerant species toxic symptoms appear at very low concentrations. For example, aluminium present in culture solutions in concentrations as low as 1 ppm retarded root growth of corn, sorghum and barley in experiments of Ligon and Pierre (1932). In another experiment by Rios and Pearson, (1964) on cotton, definite damage occurred at 0.5 ppm aluminium and the root quickly died at 1.0 ppm.

Frequently, aluminium concentration in the soil solution has been found to far exceed the levels known to depress root growth in solution culture

♦

(Ragland and Coleman, 1959; Brenes and Pearson, 1973; Nuwamanya, 1984; Chong et al., 1987). Yet critical levels seem to vary from soil to soil. Pierre (1931) for example, noted that soil solution aluminium concentration varied among different soils at the same pH, but that there was no clear relationship between plant growth and aluminium content of the different soil solutions. Even poor relationships may occur between exchangeable aluminium and root growth. Adams and Lund (1966) found normal cotton penetration of a soil at pH 4.9 having 2.55 milliequivalent exchangeable aluminium per 100g, whereas a significant depression in growth occurred in Norfolk subsoils at pH 5.4 with only 0.13 milliequivalent exchangeable aluminium per 100g. Although root penetration increased progressively as exchangeable aluminium decreased in a given soil, no relationship could be discerned when all three soils were considered. Neither was there a clear relationship between root penetration and percent saturation of the cation-exchange capacity with aluminium. Even when aluminium concentration in the soil solution was considered, no critical level could be identified that held for all the soils.

Reasoning that toxic effects of aluminium would be modified by the presence of other ions in solution, Adams and Lund (1966) calculated the molar activity of aluminium in the displaced soil solution and found the value increased in a reasonable consistent manner with decreasing root growth regardless of the soil. Similar results were demonstrated by Brenes and Pearson (1973) and Alva et al. (1986). Alva et al. (1986) observed that the normal aluminium concentration in solution is of little value as an index of aluminium toxicity of the nutrient or soil solution; second, that where considerable quantities of polymeric aluminium species are present in the soil, total aluminium concentration in nutrient solution is of little value as an index of aluminium toxicity. Thirdly, where the effects of aluminium studied in solutions of differing ionic strength, the concentration of monomeric aluminium in solution is a poor index of aluminium toxicity. The best index of aluminium toxicity, as measured by root growth, proved to be the sum of activities of monomeric aluminium which takes account of aluminium precipitation and polymerization as well as the effects of ionic strength in assessing aluminium toxicity.

## 2.7 Effects of pH and Aluminium on Rhizobia and Root Nodulation

Legumes generally grow well at pH values which restrict the growth of Rhizobium (Loneragan and Dowling, 1958) and which restrict the infection and nodulation when supplied with combined nitrogen. But it appears that pH is less of a constraint to rhizobial survival in the soils than is desiccation or high temperatures (Eaglesham et al., 1984). In an experiment carried out by Mulongoy et al. (1981) in soils with low pH (acid soils) in Onne in Nigeria (pH 4.6, annual rainfall 2500mm) cowpea rhizobial count was  $4.3 \times 10^4$ /g soil, whereas at Maradi in the Sahel-Savannah in Niger Republic (pH 6.1, annual rainfall 600 mm) the count was  $4.9 \times 10^2$ /g soil. Laboratory studies of the effect of low pH on rhizobia from soils such as these have been based on growth in synthetic media. However, because rhizobia vary in their ability to withstand conditions associated with low pH, the acid tolerance of rhizobia cannot be predicted with the growth rate of acid production characteristics in liquid media at high pH (Munns et al., 1979). Slow-growing rhizobia generally tend to be more tolerant to low pH than fast growers though strain to strain differences exist (Graham and Parker, 1964). Some slow-growing

rhizobia native to acid soils are acid requiring and grow only at approximate pH 4.5 (Date and Halliday, 1979). On the other hand, in a survey of 65 strains of slow-growing rhizobia of mixed origin in liquid media acidity (pH 4.5 and 4.8) prevented the growth of 29 percent of the strains and slowed the growth of most of the rest. Low phosphate levels limited growth of some strains but with less severity than did acid. Aluminium (50  $\mu$ M) was the most severe stress factor, stopping the growth of 40 percent of the strains. Tolerance to acidity was not necessarily correlated with tolerance to aluminium, since aluminium increased the lag time or slowed the growth rate of almost all of the strains which were tolerant to low pH (Keyser and Munns, 1979). The adverse effects of acid and aluminium on rhizobial growth appears to be bacteriostatic rather than bacteriocidal (Munns and Keyser, 1981). There is little information available on the effect of high pH on rhizobial growth but the constraints to rhizobial survival, nodulation and legume growth pertaining to saline soils also apply to saline-alkaline soils (Eaglesham et al., 1984). When growing on mineral nitrogen, most legume species are only slightly adversely affected by acidity down to pH 4.0. Indeed some species actually grow better at pH 4.0 than in less acidic condition e.g. stylosanthes humilis



(Andrew, 1976). Legumes dependent on the root nodule symbiosis for nitrogen show a range of responses to low pH values below 5.0 (Andrew, 1976; Munns et al., 1977). In a survey carried out on the effect of liming eight acid soils of pH 3.4 to 4.2 the critical pH for nodule initiation and development in soyabean was in the range of 4.5 to 4.8 (Mengel and Kamprath, 1978). The inhibition of nodulation appears to result from a combination of low concentration of calcium and low pH since it is alleviated by increasing either the calcium concentration or the pH (Munns, 1977). The lesion in the infection process which is induced by calcium deficiency and acidity has not been identified (Munns, 1977). The nitrogen-fixing activity of nodules is also adversely affected by acidity in many species (Andrew, 1978; Munns et al., 1977).

The presence of available aluminium in acid soils inhibits nodulation directly (Franco and Munns, 1962) and indirectly by stunting root growth, reducing the potential number of sites where nodules may develop (Kamprath and Foy, 1971) and also tend to compound the effects of low levels of calcium by inhibiting its uptake (Andrew, 1978). Hallsworth (1958) showed that increased aluminium decreased nodulation markedly, while Dobereiner (1974)

indicated that aluminium-toxicity limits nodulation and N-fixation more than plant growth. Sartain and Kamprath (1975) found a small number of large and apparently ineffective nodules on soyabeans in a largely aluminium-saturated soils where under low aluminium saturation, a large number of small, more effective nodules were found. A correlation of the mean number of nodule with the levels of available aluminium was also observed on twelve soyabean cultivars by Andrew (1978). Exposure of nodulated roots of Phaseolus vulgaris to aluminium, however, had no effect on nodule development or function (Franco and Munns, 1982). The inhibitory effect of the aluminium-calcium interaction have been found to vary with soil type. In two soils of higher Ca:Al ratios, mean growth of thirteen soyabean cultivars at pH 4.5, although reduced in comparison with the plants at pH 6, was the same whether they were relying on mineral or nodule-fixed nitrogen. With  $2 \times 10^6$  rhizobia per seed as inoculum, nodule number and weight were the same at pH 4.5 as at pH 6 (Munns, 1981). These findings indicate that at least for soils of this type, improvement of aluminium tolerance is more likely to be achieved by manipulating the plant rather than the Rhizobium.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Soils Investigated

##### Gituamba Soils

The soils were collected at the Agricultural Research Sub-station - Gituamba, in Murang'a District, Central Province. The soils are derived from basalt and basaltic conglomerates of the Simbara series (KSS, 1977). The station is situated in an area with dissected foot ridges (Aberdares), rolling volcanic uplands, and hills with minor scarps. The altitude is 2130m above sea level and the soil moisture and temperature regimes are udic and mesic respectively. The area receives an annual rainfall of 2005mm (bimodal) with the main season falling from October to November; no month is completely dry. The mean annual temperature is 13°C with the hottest months occurring between December and February according to East African Meteorological Department (EAMD) (1975). The land is mainly used for growing tea and pyrethrum. The soil samples were collected from an area which had been uncultivated for sometime and was under grass. The soils are classified by

Kenya Soil Survey (KSS, 1977) as Humic Andosols according to FAO/Unesco and Oxic Dystrandept according to USDA Soil Taxonomy.

### Kitale Soils

The soils were collected at the National Agricultural Research Station - Kitale in Trans Nzoia District, Rift Valley Province. The soils are derived from basement system gneisses and schists rich in feldspar, biotite, hornblende and garnet. Minor exposures of granite and pegmatitic dykes are also found (KSS, 1977). The station is situated in an area with slightly undulating uplands (Kitale level), the altitude is 1860 m above sea level and the soil moisture and temperature regimes are ustic and isothermic respectively. The area has an annual rainfall of 1193 mm which is spread throughout the year but concentrated mainly between the months of April and September. The mean annual temperature is 18.2°C with the months of January, February and March being slightly hotter (EAMD, 1974). The vegetation originally was moist combretum woodland to bushland but is now mainly used for rainfed maize and pasture research. The soil samples were collected from an area which had been uncultivated for sometime but

was under pasture. The soils are classified (KSS, 1977) as orthic Ferralsols according to FAO/Unesco and Typic Haplustox according to USDA soil. Taxonomy.

#### Kabete Soils

The soils were collected at the Field Station of the Faculty of Agriculture, Kabete Campus, University of Nairobi. The soils are derived from Limuru and Quartz Trachyte. The station is situated on broad interfluves, part of a volcanic ridge landscape. The altitude is 1740 m above sea level and the soil moisture and temperature regimes are ustic and isothermic respectively. The area has an alternating dry and wet season and an absence of large seasonal changes of temperatures. The mean annual temperature is 18°C and the precipitation pattern is bimodal with annual rainfall of 973mm, the main season falling from mid-March to May and secondary one from mid-October to December. Between June and October, it is rather cool, cloudy and almost dry, while the warmest time of the year is encountered from mid-October to mid-March (EAMD, 1975). The vegetation and land use consist of experimental fields of maize, sunflower, pulses, tomatoes, Irish potatoes and flowers. The soil samples were collected from an area which had been uncultivated for sometime and was under grass.

The soils are classified (KSS, 1977) as Humic  
 Entosols according to FAO/Unesco and Oxic Paleustult  
 according to USDA Soil Taxonomy.

### 3.2 Soil Sampling and Sample Preparation

#### Soil Sampling

Bulk samples of soils (from depths 0 to 15 cm  
 and 15 to 30 cm) were collected from three sites in  
 Kenya. The criteria used to select the three soils  
 were: their soil order, varying pH values, contents  
 of aluminium, organic matter and cation exchange  
 capacity. The soil samples were taken after clearing  
 vegetation above, from the top of one wall, at  
 intervals of 15 cm from a soil profile pit measuring  
 100cm by 200cm and 120cm deep.

The first set of soil samples was taken from a  
 profile pit at Gituamba Agricultural Research Sub-  
 station. A site had been chosen on the basis that  
 it had been uncultivated for a considerable period  
 and was less susceptible to erosion and surface  
 drainage. The second set of soil sample was taken  
 from a profile pit at National Agricultural Research  
 Station - Kitale. A site was chosen on the basis  
 that it had been under pasture for quite sometime and

therefore was less disturbed compared to other sites previously marked. The third set of soil samples was taken from a profile pit at the Field Station of the Faculty of Agriculture, Kabete Campus, University of Nairobi. A site had been chosen on the basis that it had been uncultivated for a considerable period of time and was under grass.

#### Sample Preparation

The soils (except for the pot experiments which were carried out in the greenhouse) were air-dried, ground in a mortar with a pestle and sieved to pass through a 2.00mm sieve. Some of the soil was further ground and sieved to pass a 0.5mm sieve. The soils were then used for the analysis of soil pH, percent organic carbon, percent nitrogen, phosphorus, cation exchange capacity (CEC), exchange acidity, exchangeable aluminium, exchangeable manganese, total aluminium, monomeric and polymeric aluminium concentration in the soil solution and texture. Soils for the pot experiment were air-dried and ground but not sieved.

### 3.3 Soil Analysis

#### Soil Reaction (pH)

Ten grams of soil sieved through a 2.00 mm sieve was mixed with 25 ml of water and 1N KCl solution respectively (soil/water and soil/1N KCl ratios: 1:2.5) and shaken for 30 minutes using a mechanical shaker. It was then left to stand for 30 minutes. Soil pH was then determined electrometrically using a pH meter with a glass electrode at soil/water and soil/1N KCl as described by Black (1965).

#### Organic Carbon

Organic carbon was determined according to the Walkley-Black method (1934) as outlined in the International Institute of Tropical Agriculture Manual (IITA, 1974). The organic matter in 0.5g of soil sieved through a 0.5mm sieve was initially oxidized using potassium dichromate in excess concentrated sulphuric acid ( $H_2SO_4$ ). The excess chromic acid was titrated with Ferrous sulphate solution. For "correction" of percent organic carbon, assuming that the recovery was 77% as found by Walkley-Black, the results were multiplied by a



conventional factor of 1.33. The percent organic matter was calculated by multiplying the "corrected" percentage of organic carbon by a factor 1.72 (58% carbon occurs in soil organic matter).

### Total Nitrogen

Total Nitrogen was determined using Kjeldhal method as outlined by Jackson (1958). Total Nitrogen in 1.0g dried soil sieved through a 2.00 mm sieve was extracted by using 3.5 ml phenol-sulphuric acid. 0.5 g of sodium thiosulphate was added to the mixture after 15 minutes. After another 15 minutes, 0.5 g of selenium mixture, 0.5 g potassium sulphate and 3.5 ml of concentrated sulphuric was added. After the mixture had been digested and cooled, it was transferred to a distillation flask. 40 ml of saturated sodium hydroxide was then added quickly to the distillation flask. The distillate was collected in 1% boric acid and then titrated with 0.01 IN  $H_2SO_4$  using a mixed indicator.

### Phosphorus

Using a 5.0 g soil sample sieved through a 2.00 mm sieve, soil phosphorus was extracted using 50 ml of Mehlich 1 extractant (Double acid composed of 0.05

$\text{NHCl}$  and  $0.025 \text{ NH}_2\text{SO}_4$ ). The soil was extracted by shaking the soil in the extractant for 15 minutes using a mechanical reciprocal shaker. The colour of the filtrate was developed using 8 ml of ascorbic acid dissolved in 200 ml of ammonium molybdate, potassium antimony tartrate and  $5\text{N}$  sulphuric acid solution (McLean, 1982). The amount of phosphorus in the soil extract was determined colorimetrically using SP 500 spectrophotometer as outlined by Kamprath and Watson (1980).

#### Cation Exchange Capacity (CEC) and Exchangeable Cations

(a) Five grams of air dried soil sieved through a 2.00mm sieve was leached with four, 25 ml portions of  $1.0 \text{ NH}_4\text{OAc}$  (ammonium acetate) at pH 7.0. The soil was then washed with ethyl alcohol to remove excess  $\text{NH}_4\text{OAc}$  solution. The soil containing the adsorbed ammonium ion was then leached with four, 25ml portions of  $1\text{N KCl}$  solution. The ammonium contained in the leachate was determined by adding magnesium oxide to the leachate and then distilled. The distillate was adsorbed by boric acid to form ammonium borate. The cation exchange capacity was then determined by titrating the borate against  $0.1\text{N HCl}$  (Legger, 1978).

(b) Exchangeable calcium and magnesium were determined from the one normal ammonium acetate leachate by titrating with EDTA (Ethylene-diamine tetraacetic acid). The presence of interfering  $Al^{3+}$  and  $Fe^{3+}$  ions were removed by adding triethanolamine solution and  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Ni^{2+}$  and  $Fe^{2+}$  ions by adding potassium cyanide solution. The exchangeable calcium ions were determined first by titrating the leachate with EDTA using calcon indicator and the exchangeable calcium and magnesium ion were determined from second titration using Eriochrome Black indicator. The exchangeable magnesium ions were obtained by the difference between the two titrations (Ahn, 1975).

Exchangeable potassium and sodium were determined from the one normal ammonium acetate leachate directly using a flame photometer as outlined by Jackson (1958). Exchangeable manganese was determined from the one normal ammonium acetate leachate by flame emission as outlined by Black (1965) using an atomic absorption spectrophotometer.

#### Exchange Acidity

Exchangeable acidity was determined by leaching 5.0 g of soil sample sieved through a 2.00 mm sieve with four 25 ml portions of 1N KCl solution as

outlined by Ahn (1975). The exchangeable acidity was then determined by titrating the leachate with 0.05N NaOH.

### Exchangeable Aluminium

Exchangeable aluminium was extracted from the soil by leaching 5.0g of soil sieved through a 2.00 mm sieve with four, 25-ml portions of 1N KCl solution and then determined by titrating the leachate with 0.05N NaOH. After the end point was reached, one drop of 0.05N HCl was added bringing back the solution to the original colourless. 10 ml of Sodium Fluoride was then added. The final solution was then titrated with 0.05N HCl (Ahn, 1975).

### Aluminium Saturation

Aluminium saturation was determined by calculating the sum of exchangeable bases (Ca, Mg, K, Na and Mn) and exchangeable acidity, and then getting a percentage of aluminium saturation as follows:

$$\% \text{ Aluminium Saturation} = \frac{S}{T} \times 100$$

Where  $S$  = Exchangeable aluminium in m.e/100g and

$T$  = Total sum of exchangeable bases and acidity

Total aluminium, monomeric and polymeric aluminium concentration in soil solution

Total aluminium was extracted from the soil by leaching 5.0g of soil sample sieved through a 2.00 mm sieve with four, 25-ml portions of 1N KCl solution. The concentration of total aluminium species in the leachate was determined colorimetrically using the aluminon method as described by Hsu (1963) and Jayman et al. (1974) on a Beckman Du-8B-spectrophotometer.

For the determination of the concentration of monomeric and polymeric aluminium species in the soil, a method developed by Blamey et al. (1984) for measuring the concentration of aluminium monomers in solution based on the lack of a rapid equilibrium between monomeric and polymeric aluminium (Hsu, 1963) and the slow increase in colour intensity (over a period of days) as the aluminium reacts with polymeric aluminium species in solution was used. The method uses the same procedure as that of Hsu (1963) and Jayman et al. (1974) but omits the steps involving

the addition of HCl and heating for 30 minutes at 80°C. Colour intensity was read 30 minutes after addition of the aluminon buffer solution.

Polymeric aluminium concentration in the soil solution was determined by subtracting the monomeric aluminium species concentration from the total aluminium species concentration in the soil as described by Blamey et al. (1984).  
Polymeric aluminium concentration = Total aluminium concentration - monomeric aluminium concentration

#### Soil Texture

The soil texture was determined using the Bouyoucos Hydrometer method (1957). Hydrogen peroxide ( $H_2O_2$ ) was used to destroy the organic matter present in the soil.

#### 3.4 Greenhouse Studies

The experiments in the greenhouse were conducted at the Field Station of the Faculty of Agriculture; Kabete Campus, University of Nairobi. The greenhouse has a north - south orientation and is partially whitewashed to increase reflection of the sun's

radiation. - thereby reducing day temperature fluctuations. Temperature in the greenhouse was thermostatically controlled using an air conditioner and ranged between 20°C at night and 25°C during the day. This was monitored daily using a thermometer. The pots and Leonard Jars used in the study were placed on a Table about one metre above the ground to avoid draft of air coming through the lower parts of the greenhouse. Field beans (Phaseolus vulgaris L.) cv "Rosecoco" was used as the test crop. Rhizobia phaseoli inoculant (Multistrain 445 and 446) used was prepared by the Nairobi Microbial Resource Centre (MIRCEN) project and was recommended for field beans. Its effectiveness was assured through quality controlled tests. Several experiments which are described below were carried out in the greenhouse.

#### 3.4.1 Nutrient Solution Experiments

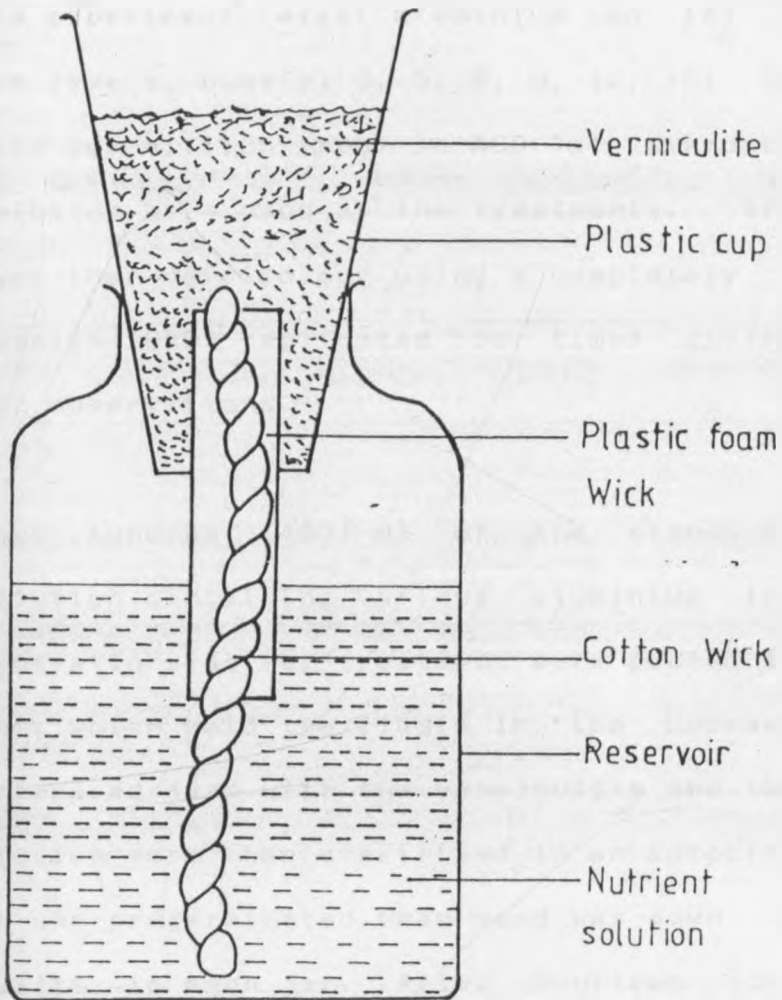
In these studies, a bulk nitrogen-free nutrient solution (Hydroponic) was prepared consisting of 100  $\mu\text{M}$  Ca ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ); 500  $\mu\text{M}$  P ( $\text{KH}_2\text{PO}_4$ ); 10  $\mu\text{M}$  Fe (Fe Citrate); 250  $\mu\text{M}$  Mg ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ); 1500  $\mu\text{M}$  K ( $\text{K}_2\text{SO}_4$ ); 500  $\mu\text{M}$  S (Sulphur) 1  $\mu\text{M}$  Mn ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ); 2  $\mu\text{M}$  B ( $\text{H}_3\text{BO}_3$ ); 0.5  $\mu\text{M}$  Zn ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ); 0.2  $\mu\text{M}$  Cu

( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ):  $0.1 \mu\text{M}$  Co ( $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ ) and  $0.1 \mu\text{M}$  Mo ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) as outlined by Samasegaran et al. (1985).

A standard solution containing 500 ppm  $\text{Al}^{3+}$  ion was prepared by dissolving 0.5g of electrically prepared metallic aluminium sheet in 15 ml of 6N HCl in one litre volumetric flask. After the aluminium sheet had dissolved the solution was diluted with distilled water to one litre volume and mixed thoroughly (I.I.T.A. Manual, 1974).

Leonard jars (Fig. 1) used in these studies were constructed as recommended for the Nairobi Microbial Resource Centre (MIRCEN) project. Vermiculite grade A was used as the medium for sowing the pregerminated seeds of field beans (Phaseolus vulgaris L.) cv "Rosecoco". The Leonard jars for each treatment were sterilized in an autoclave for 30 minutes before the pregerminated seeds were sown as recommended for the MIRCEN project. In these studies, two experiments were carried out as described below:





9 1 Diagram of modified Leonard Jar

3.4.1.1 Determination of the level of Aluminium Ion in Nutrient Solution which is Toxic to Root Growth of Field Bean (Phaseolus vulgaris L.) cv "Rosecoco"

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In this experiment, eight aluminium ion ( $Al^{3+}$ ) concentration levels, namely: 0, 3, 6, 9, 12, 15, 18 and 21 parts per million (ppm) in 400 ml standard nutrient solution were used as the treatments. The experiment was then carried out using a completely randomized design (CRD) replicated four times giving a total of 32 observations.

The four hundred (400) ml of the standard nutrient solution containing various aluminium ion ( $Al^{3+}$ ) concentrations as per treatment were poured in the container which held the liquid in the Leonard jars. The prepared jars with the vermiculite and the nutrient solution were then sterilized in an autoclave, after which one pregerminated bean seed was sown on the vermiculite in each jar. After fourteen (14) days, the bean plant shoot was harvested by cutting at the base with a sharp razor blade. The roots were then uprooted carefully and the vermiculite washed off with tap water. The taproot (primary root) length was then measured using a string and a tape measure and then recorded. The shoots and the roots were then dried at  $70^{\circ}C$  for 24 hours in an oven after which their weights were recorded.

3.4.1.2 Determination of the Effects of Nutrient Solution pH and Aluminium Content on Root Growth and Rhizobium inoculation on Field Bean (Phaseolus vulgaris L.) cv "Rosecoco"

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A three factor randomized complete block design (RCBD) in four (4) replicates was used in this experiment. The first factor consisted of the pH's of the nutrient solutions (pH 4.0, 5.0 and 6.8). The second factor consisted of aluminium concentrations in nutrient solution (0, 5, 10, 15 and 20 ppm Al) and the third factor consisted of whether the beans were inoculated or not inoculated. These gave a factorial combination of 30 treatments (Table 1). The pH's were selected to simulate the pH of the soils being studied i.e Gituamba, Kitale and Kabete soils respectively in nutrient solution. The aluminium concentration levels were arrived at from observations of the experiment in Section 3.4.1.1.

Four hundred (400) millilitres of nutrient solution having different pH values and aluminium concentration as per above treatment were poured into Leonard jar. The jars were sterilized in an autoclave for 30 minutes and then the seeds, which had been pregerminated for one day, were sown on the vermiculite at the rate of one seed per Leonard jar. Prior to sowing some of the pregerminated seeds were

inoculated at a rate of 8g of the Rhizobium inoculant per 100g seeds in one millilitre of gum arabic. The Rhizobium inoculant (Multistrain 445 and 446) used was the one recommended for use on field beans in the MIRCEN project. After fourteen (14) days, the shoot of each bean plant was harvested by cutting at the base using a sharp razor blade. The roots were then carefully removed by first washing off the vermiculite with tap water and then pulling them gently from the Leonard jars. The number of nodules on the root was noted. The taproot length was measured using a string and a tape measure and then recorded. The shoots and the roots were dried in the oven at 70°C for 24 hours and their weights recorded.

Table 1: Treatments

1	pH4/0ppmAl/noninoculated seed
2	pH4/0ppmAl/inoculated seed
3	pH4/5ppmAl/noninoculated seed
4	pH4/5ppmAl/inoculated seed
5	pH4/10ppmAl/noninoculated seed
6	pH4/10ppmAl/inoculated seed
7	pH4/15ppmAl/noninoculated seed
8	pH4/15ppmAl/inoculated seed
9	pH4/20ppmAl/noninoculated seed
10	pH4/20ppmAl/inoculated seed
11	pH5/0ppmAl/noninoculated seed
12	pH5/0ppmAl/inoculated seed
13	pH5/5ppmAl/noninoculated seed
14	pH5/5ppmAl/inoculated seed
15	pH5/10ppmAl/noninoculated seed
16	pH5/10ppmAl/inoculated seed
17	pH5/15ppmAl/noninoculated seed
18	pH5/15ppmAl/inoculated seed
19	pH5/20ppmAl/noninoculated seed
20	pH5/20ppmAl/inoculated seed
21	pH6.8/0ppmAl/noninoculated seed
22	pH6.8/0ppmAl/inoculated seed
23	pH6.8/5ppmAl/noninoculated seed
24	pH6.8/5ppmAl/inoculated seed
25	pH6.8/10ppmAl/noninoculated seed

- 26 pH6.8/10ppmAl/inoculated seed
  - 27 pH6.8/15ppmAl/noninoculated seed
  - 28 pH6.8/15ppmAl/inoculated seed
  - 29 pH6.8/20ppmAl/noninoculated seed
  - 30 pH6.8/20ppmAl/inoculated seed
- 

N.B: ppm - parts per million of Al<sup>3+</sup> in nutrient solution

### 3.4.2 Experiment with Soil

Three soil samples from 0 to 15cm and 15 to 30cm depths were used in the studies. Round plastic pots with a top diameter of 20cm and capacity of 2.5 litres were used for growing beans. Two kilogram of each soil sample (ground but not sieved) were placed in the pots. In these studies two experiments were carried out as described below:

#### 3.4.2.1 Experiment 1

A randomized complete block design with four replicates was used in this study. There were twelve (12) treatments (Table 2) consisting of a combination of soil, depth and whether the seeds were inoculated or not inoculated. Prior to sowing, some of the seeds were inoculated with Rhizobium phaseoli inoculant (Multistrain 445 and 446) at the rate of 8g of the inoculant per 100g of seeds in one millilitre of gum arabic. The seeds were sown as per treatment at a rate of three seeds per pot on the soils wetted with distilled water and was thinned to one plant per pot just after emergence (or germination). No fertilizer was applied in the soils in this experiment. The plants were regularly watered using distilled water. Just enough water was added to the

soil to wet it adequately. This was done to avoid waterlogging and water stress. Pests and diseases were controlled by using Rogor E and Dithane M45, insecticide and fungicide, respectively.

Four weeks after germination of the seed, the shoot was harvested by cutting at the base using a sharp razor blade. The soil was then carefully washed off the root and then later removed from the plastic pot, care being taken to avoid any root breakages or detachment of nodules from the roots. The number of nodules on the root on each root was counted and recorded. The tap root length was then measured using a string and a tape measure and then recorded. The shoots and the roots were dried in the oven at 70°C for 24 hours and their weights recorded.



Table 2: Treatments

1	Gituamba Soil/0-15cm/noninoculated seed
2	Gituamba Soil/0-15cm/inoculated seed
3	Gituamba Soil/15-30cm/noninoculated seed
4	Gituamba Soil/15-30cm/inoculated seed
5	Kitale Soil/0-15cm/noninoculated seed
6	Kitale Soil/0-15cm/inoculated seed
7	Kitale Soil/15-30cm/noninoculated seed
8	Kitale Soil/15-30cm/inoculated seed
9	Kabete Soil/0-15cm/noninoculated seed
10	Kabete Soil/0-15cm/inoculated seed
11	Kabete Soil/15-30cm/noninoculated seed
12	Kabete Soil/15-30cm/inoculated seed

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### 3.4.2.2 Experiment II

The same operations were carried out in this experiment and the same amounts of soil were used as those in Experiment I (Section 3.4.2.1) except that one day before sowing the seeds, the soils were fertilized with triple superphosphate (TSP) fertilizer at a rate of 69 Kg  $P_2O_5$ /ha. The fertilizer was thoroughly mixed with the soil before sowing the seeds. After the harvest of the shoots and washing and removal of the roots, nodule numbers were recorded, tap root length measured and the shoots and roots dried in an oven at 70°C for 24 hours and their weights recorded.

## CHAPTER FOUR

## RESULTS AND DISCUSSIONS

4.1 Soil Properties4.1.1 Mechanical Analysis

Table 3 shows some physical properties of the three soils studied. The depths of the three soils appear to be uniform with the topsoil (0 - 15cm) being slightly different from the subsoil (15 - 30cm) particularly with regard to particle size distribution. In Gituamba soils (Andosols), sand is the predominant texture at both depths (40.4 and 42.4% at depth 0 - 15cm and 15-30cm respectively) followed by clay (29.6 and 32.0% at depths 0-15 and 15-30cm respectively). In Kitale and Kabete soils, Ferrasols and Nitosols respectively, clay texture is predominant at both depths. There is more silt in Gituamba soils than in Kitale and Kabete soils respectively. Because of high percent sand and silt in Gituamba soils, it is slightly coarse textured as compared to Kitale and Kabete soils which have a fine texture. Gituamba soils are clay loam, whereas Kitale and Kabete soils are both clayey in texture.

The bulk densities of the three soils are also shown in Table 3. Apart from Gituamba soils, the variability of the bulk densities within the soils is small. This is because the other soils (Kitale and Kabete) usually have a deep even profile and variabilities are less expected upto the depths studied. In the case of Gituamba soils, high organic matter contents in the topsoil could be a contributing factor to the observed low bulk density as compared to that of the subsoil. In general, the observed particle size distribution, and bulk densities are consistent with the properties of the three types of soils reported by other workers.

Table 3: Some Physical Properties of the three Soils, Gituamba, Kitale and Kabete

Soil	Classification (FAO/UNESCO)	Depth (cm)	Mechanical Analysis % of Soil			Texture	Bulk Density (gm/cm <sup>3</sup> )
			Sand	Silt	Clay		
Gituamba	Humic Andosol	0-15	40.4	30.3	29.6	Clay loam	0.65
		15-30	42.4	25.6	32.0	Clay loam	0.79
Kitale	Orthic Ferrasol <sup>L</sup>	0-15	36.4	4.0	59.6	Clay	0.88
		15-30	42.4	2.0	55.6	Clay	0.82
Kabete	Humic Nitosol	0-15	20.8	19.6	59.6	Clay	1.10
		15-30	16.4	24.0	59.6	Clay	1.01

<sup>L</sup> Hydrometer Method

#### 4.1.2 Soil Reaction

Table 4 shows the pH of the three soils studied. All the soils exhibit acidic reaction, with Gituamba soil, pH 5.08 and 4.78 in water and 3.90 for both depths in 1N KCl solution for depths 0-15 and 15-30 cm respectively, exhibiting the most acidic reaction (strongly acid). This is followed by Kitale soil which is moderately acid with pH 5.50 for both depths in water and 4.40 for both depths in 1N KCl solution for depths 0-15 and 15-30 cm respectively. Kabete soils, show the least acidic reaction (weakly acid) with pH 6.15 and 6.80 in water and 4.70 and 5.20 in 1N KCl solution for depths 0-15 and 15-30 cm respectively. The variability of soil pH in water is evident in Gituamba and Kabete Soils and also in 1N KCl solution in Kabete soils.

The difference between the pH/water and pH 1N KCl averages about one unit. This difference is expected because the measurement of pH of soil-water suspension is influenced by the presence of soluble salts. Use of a salt, such as 1N KCl or 0.01 M CaCl<sub>2</sub> tends to mask the variability of the pH caused by differences in the salt concentration of the soil solution and give a more precise estimate of the activity status of the soil than that measured in

soil-water suspension. The concentration of soluble salts which can influence measurement of pH in soil-water is assumed to be negligible with respect to the amount of salt added in the solution.

The pH differences observed between the soils was expected and was the basis of choosing the three soils. They may be attributed to the difference in organic matter contents in the soils (Gituamba, 7.90 and 6.24%C, Kitale 2.19 and 1.32%C and Kabete 2.8 and 1.47%C for depths 0-15 and 15-30 cm respectively), parent material and/or degree of weathering and leaching losses due to rain. Rainfall (mean annual, 2005, 1193 and 973 mm for Gituamba, Kitale and Kabete respectively) and organic matter content play a more important role in that they contributed more to leaching losses of bases and an increase in aluminium ion saturation by lowering of soil pH, due to the decomposition of organic matter in the soil.

The differences in soil pH between the two horizons sampled was low except for Kabete soils where slight differences were observed (pH 6.15 and 6.80 in water and 4.70 and 5.28 in 1N KCl solution for depths 0-15 and 15-30 cm respectively). The differences observed for the soil pH between the

topsoils and the subsoil could not be actually pinned on one factor but could probably be due mainly to high organic matter in the topsoil (2.87 and 1.47% C for depth 0-15 and 15-30 cm respectively) which after decomposition increased the concentration of  $H^+$  ions in the soil, as such lower pH for the topsoil. Percentage base saturation could also be a contributing factor. Due to leaching of bases from the topsoil to the subsoils, the subsoils have a higher base saturation (91.42%) than the topsoil soil (86.82) and for a soil of any organic or mineral composition, the pH level increases with an increase in the degree of base saturation (Tisdale et. al., 1985). Base saturation also reflects the degree of leaching. The higher <sup>the</sup> the base saturation, lower <sup>is</sup> the degree of leaching. So it is apparent from the above base saturation values, that the topsoils are more leached than the subsoils.



Table 4: Some Chemical Characteristics of the three Soils, Gituamba, Kitale and Kabete

Soil	Depth (cm)	Soil pH (1:2.5)		Organic Carbon	Total Nitrogen	Phosphorus <sup>††</sup> (ppm)	Exchangeable cations (m.e/100g) <sup>†††</sup>					ECEC (m.e/100g)	CEC <sup>†††</sup> (m.e/100g)	% Base Saturation	% Al Saturation
		Soil H <sub>2</sub> O	Soil/KCl				Ca	Mg	Na	K	Al				
Gituamba	0-15	5.08	3.90	7.97	0.65	0.60	0.55	0.50	0.50	0.70	3.62	11.11	31.90	7.05	35.58
Gituamba	15-30	4.78	3.90	6.24	0.54	0.35	0.55	0.45	0.60	0.40	2.25	9.10	27.60	7.25	35.71
Kitale	0-15	5.50	4.40	2.19	0.15	3.67	6.40	0.20	0.27	0.47	0.90	9.50	14.00	52.43	9.36
Kitale	15-30	5.50	4.40	1.32	0.11	1.00	5.20	0.80	0.74	0.74	0.79	9.10	11.00	63.34	8.68
Kabete	0-15	6.15	4.70	2.87	0.36	2.33	14.70	3.00	0.55	1.59	0.28	20.25	23.00	66.28	1.38
Kabete	15.30	6.80	5.26	1.47	0.25	1.80	14.60	2.20	0.69	1.20	0.30	19.29	21.00	96.89	1.53

<sup>†</sup> Extractant used was double acid (0.05N HCl + 0.025NH<sub>2</sub>SO<sub>4</sub>)

<sup>††</sup> Extractant used was one normal ammonium acetate (NH<sub>4</sub>OAc) at pH 7.0

<sup>†††</sup> Extractant used was one normal ammonium acetate (NH<sub>4</sub>OAc) at pH 7.0

#### 4.1.3 Organic Carbon and Total Nitrogen

Table 4 shows the percentages of organic matter and total nitrogen in the three soils and within their depths. The carbon figures are corrected Walkley-Black values. The topsoils (0-15 cm) show higher percentage of organic matter than the subsoils (15-30 cm) i.e. 7.97 and 6.24, 2.19 and 1.32 and 2.87 and 1.47% C for depths 0-15 and 15-30 cm for Gituamba, Kitale and Kabete soils respectively. This is undoubtedly due to addition of organic matter mainly at the top. The variability of percentages of organic matter between the soils is possibly due to unequal amount of plant material added at the top of the soil and their varying degree of decay and ease of incorporation with the soil. The soil depths give a different but expected pattern of distribution of organic matter and total nitrogen (C:N Ratio:Gituamba, 12.26:1 and 11.55:1; Kitale 14.6:1 and 12.1 and Kabete 7.55:1 and 5.88:1 for depths 0-15 and 15-30 cm respectively). As the percentage organic matter in the soil decreases, the percentage total nitrogen in the soil also decreases because for the undisturbed soils, the amount of nitrogen present in the soil depends on the mineralization of organic matter for C:N ratios less 20 (Tisdale and Nelson, 1971). Therefore the higher the amount of organic matter in the soil, the higher is the amount of

nitrogen present in the soil. For the undisturbed soils, the C:N ratios is usually high for the topsoil than the subsoil. This in many cases is partly due to high concentration of  $\text{NH}_4^+$  nitrogen and the general lower amount of carbon (Tisdale and Nelson, 1971). Otherwise, between the three soils total nitrogen is deficient in Kitale soils, medium in Kabete soils and high in Gituamba soils at both depths according to National Agricultural Laboratories deficiency levels of greater than 1% N (of soil by weight) for very high, 0.5 to 1.0%N for high, 0.2 to 0.5%N for medium, 0.1 to 0.2% N for low and less than 0.1%N for very low.

The major factors influencing the organic matter content in East African soils are elevation, climate and biotic conditions, whereas the minor factors include type of clay, depth and degree of development of the profile (Birch and Friend, 1956). Gituamba, Kitale and Kabete have altitudes, 2130 m, 1860 m and 1980 m respectively above sea level. Gituamba, Kitale and Kabete also receive mean annual rainfalls of 2005, 1193 and 973 mm respectively and the mean annual temperatures for Gituamba, Kitale and Kabete are 13.0°, 18.2° and 18.0°C respectively. As a rule, the organic matter content is higher in cooler climate than in warmer ones. Furthermore, for any

Given level of mean annual temperature and type of vegetation, the content of organic matter rises with an increase in effective precipitation. All these factors favour Gituamba soils as such, the higher organic matter content than Kitale and Kabete soils. The difference in organic matter content in Kitale and Kabete soils is small and may be due to the minor factor such as the depth and degree of development of the soil profile between the two soils. The same factors which influence the level of organic matter in these soils apparently also play a major role in determining the total nitrogen content in the soils.

#### 4.1.4 Phosphorus

Table 4 shows also the phosphorus contents of the three soils studied. The soil phosphorus was extracted using Mehlich 1 extractant (Double acid composed of 0.05 N HCl in 0.025 N H<sub>2</sub>SO<sub>4</sub>). The phosphorus levels are low and deficient i.e. 0.60 and 0.35; 3.67 and 1.00; and 2.33 and 1.80 ppm P for Gituamba, Kitale and Kabete soils and at depths 0-15 cm and 15-30 cm respectively when compared with values obtained for similar soils by Dr. Keter of the Department of Soil Science, University of Nairobi (personal communication) Gituamba soils exhibit the lowest phosphorus levels among the three soils. This

is probably due to high percentage aluminium saturation in Gituamba soils when compared to Kitale and Kabete soils. Solubility of aluminium phosphate is very low (solubility product  $p'K_{sp}$  lies between 28 and 32) (Chang and Jackson, 1957). Therefore any presence of soluble aluminium in the soil solution will considerably reduce the concentration of phosphate in the soil solution, by reacting with it to form an insoluble compound which is not available in the soil solution as such will not be available to plants. The variability of phosphorus content within the soil depths which show a downward trend i.e. from topsoil to the subsoil could be due to decrease in organic matter content because according to Tisdale *et al.* (1985) there is a positive correlation between the organic matter content and the amount of phosphorus present in the soil.

#### 4.1.5 Cation Exchange Capacity and Exchangeable Cations

The cation exchange capacity (CEC) and exchangeable cation of the three soils and within their sampling depths are shown in Table 4. Gituamba soils have the highest cation exchange capacity namely 31.90 and 27.60 m.e/100g for depths 0-15 and 15-30 cm respectively. They are followed by Kabete soils (23.00 and 21.00 m.e/100g for depths 0-15 and

15-30 cm respectively) and then Kitale soils (14.50 and 13.50 m.e/100g for depths 0-15 and 15-30 cm respectively). The cation exchange capacity for Gituamba and Kabete soils compare well with those of Nuwamanya (1984) for similar soils of 32.00 m.e/100g for depth 0-30 cm for Gituamba and 21.00 m.e/100g for depths 0-23 and 23-83 cm respectively for Kabete soils. Kitale soils being Ferral soils, are expected to have a CEC value of less than 16.00 m.e/100g according to von Uexkull (1986) and this agrees well with the values obtained here for similar soils.

The topsoils have a high cation exchange capacity than the subsoils for all the three soils, although the percentage base saturation for the subsoils are higher than those of the topsoils (7.05 and 7.25% for Gituamba, 52.43 and 63.34% for Kitale and 86.26 and 96.89% for Kabete for depths 0-15 and 15.30 cm respectively). The only explanation for the higher CEC observed for the topsoils could be due to the organic matter content in the three soils. The topsoils of all the three soils have a higher organic matter content than the subsoils i.e. 7.97 and 6.24% C for Gituamba, 2.19 and 1.32% C for Kitale and 2.87 and 1.47% C for Kabete for depths 0-15 and 15-30 cm respectively.

The magnitude of the cation exchange capacity of the three soils, namely, Gituamba, Kitale and Kabete soils is mainly controlled by the organic matter content, the clay mineralogy and the soil pH. The higher the organic matter content, the higher the cation exchange capacity (see Table 4), since organic matter content supplies most of the cation exchange capacity (CEC) of acid and highly weathered soils and a rapid decrease in organic matter content results in a marked reduction in CEC and nutrient holding capacity (von Uexkull, 1986). This is very evident among the three soils (see Table 4). The clay mineralogy of the three soils also contribute to the difference in the cation exchange capacity (CEC). This is more evident between Kitale and Kabete soils, where we observe that both soils have more or else the same amount of clay content (see Table 3) but Kabete soils have higher CEC than Kitale soils. This is because though both soils have 1:1 type of clays, Kabete soils have clays which are more crystalline and have lower content of sesquioxide and hydrous oxides of Al and Fe than Kitale soils (Sombroek et al., 1982).

The magnitude of the cation exchange capacity of these soils namely, Gituamba, Kitale and Kabete soils is also controlled by the soil pH. The lower the

soil pH, the lower the CEC (von Uexkull, 1986). This is because the CEC of these soils is pH dependent since the factors which contribute the CEC i.e. the clay type and organic matter content all have surface charges which are pH dependent. This is less evident for Gituamba soils due to the high organic matter content but is well exhibited in Kitale and Kabete soils where lower CEC in Kitale than in Kabete soils were observed (see Table 4).

Exchangeable calcium is markedly low for only Gituamba soils (0.55 m.e/100g for both depths of 0-15 and 15-30 cm) but agrees with values obtained for similar soils by Kenya Soil Survey (KSS, 1977) of 0.40 m.e/100g for depths 0-60 cm. Values for Kitale (6.40 and 5.2 m.e/100g) and Kabete soils (14.70 and 14.80 m.e/100g) for depths 0-15 and 15-30 cm respectively are higher when compared to values obtained for similar soils by Kenya Soil Survey (KSS, 1977) of 3.60 and 2.20 m.e./100g for depths 0-23 and 23-83 cm in Kitale respectively and 3.0 and 4.0 m.e/100g for depths 0-18 and 18-37 cm in Kabete respectively although similar methods were used during the analysis. Exchangeable magnesium is low in Gituamba and Kabete soils (see Table 4). Exchangeable potassium is not deficient in all the three soils and reflects the fact that East African rocks are often high in this element. Exchangeable



manganese levels are low and are not toxic according to the National Agricultural Laboratories deficiency levels (1.00 m.e/100g). Exchangeable aluminium, concentration is markedly high in Gituamba soils (3.62 and 3.25 m.e/100g) for depths 0-15 and 15-30 cm respectively.

Within the soil depths, the variability of exchangeable cation is small with exchangeable Ca, Na, K and Al being higher in some topsoils than in subsoils. Exchangeable Mn is higher in all the topsoils than in the subsoils. The variability of exchangeable cations between the soil could be due to the difference in the soil pH which affect the weathering of rocks and leaching losses due to different amount of rainfall received in each area where the soils were collected. The areas from where the soils were collected received a mean annual rainfall as follows: 2005, 1193 and 973mm for Gituamba, Kitale and Kabete respectively. The variability of the cations within the soil depths could be probably due to the activities of various ions in the soils which in turn also reflect soil pH, drainage and mineralogical nature.▼

#### 4.1.6 Aluminium Saturation

Percentage aluminium saturation is also shown in Table 4 for the three soils and within their sampling depths. Highest percentage aluminium saturation (32.58 and 35.71% Al for depths 0-15 cm and 15-30 cm respectively) are observed in Gituamba soils followed by Kitale (9.38 and 8.68% Al for depths 0-15 cm and 15-30 cm respectively). Very low values are observed for Kabete soils (1.38 and 1.55% Al for depths 0-15 cm and 15-30 cm respectively). The availability within the soil depths is small and percentage saturation is high in the top soils for Gituamba and Kitale soils.

Percentage aluminium saturation indicates the present day leaching and is a very useful differentiating parameter between soil types of humid Tropics (Young, 1980). High percentage aluminium saturation also indicates high degree of leaching of bases as such, highly leached soils have low base saturation. The difference in percentage aluminium saturation between the three soil could be explained by this fact. The areas from which the three soils were collected received different amount of rainfall with Gituamba receiving the highest annual rainfall (2005 mm) followed by Kitale (mean annual rainfall

1993) and Kabete (mean annual rainfall 973). So rainfall which is responsible for leaching of bases could be an answer for the large difference in percentage aluminium saturation between the three soils. The type of clay present in the three soils could also explain the large difference in percentage aluminium saturation. Gituamba soils is mainly composed of volcanic ash (allophane) and amorphous hydrous oxide of aluminium and iron. Kitale soils is composed of Kaolinite, sesquioxide and hydrous oxides of aluminium and iron. Kabete soil is composed mainly of kaolinite though there are traces of hydrous oxide of aluminium and iron. Gituamba soil therefore contains a higher concentration of aluminium and iron oxide than either Kitale or Kabete soil because of the nature of the type of clay mineral present and the concentration of aluminium oxide present. Kitale soils have higher concentration of aluminium oxide than Kabete soils since it has high concentration of sesquioxides and aluminium oxide than Kabete soil. The pH of the soils could also be responsible (von Vexkull, 1986) for the difference in percentage aluminium saturation. The lower the soil pH, the more soluble is aluminium in the soil solution, therefore, since Gituamba soils have lower pH than Kitale and Kabete soils it should have high percentage aluminium

saturation as indicated in Table 4. Kitale soils also having lower pH than Kabete soils is expected to have a high aluminium saturation than Kabete soil and this agree with the values obtained in Table 4. The variability of percentage aluminium saturation within the soil depths could probably be due to activities of various ions in the soils which in turn also reflect soil pH, drainage and mineralogical nature.

#### 4.1.7 Distribution of Aluminium ion species in the Soil

The distribution of total, monomeric and polymeric aluminium ion species among the three soils and within their depths is shown in Table 5. The distribution of Exchangeable aluminium ion species in the soils and within their depths is shown on Table 4. Total aluminium ion species is the sum of polymeric and monomeric aluminium ion species in the soil and was extracted by using 1N KCl solution.

The monomeric aluminium ion species are the substituted aluminium hydronium ions called aluminohexahydronium ions often designated as  $Al.H_2O^{+++}$  or simple as  $Al^{+++}$  (Mclean, 1976). These aluminohydronium ions sequentially dissociate H ion as base is added (pH increase) leaving OH ion in place of the OH<sub>2</sub> group. Some of the alumino-hexahyd-

ronium ions may remain in solution, but most of them are adsorbed on the soil cation exchange sites from which they are easily displaced with ordinary unbuffered salt solution such as 1N KCl if the pH is below 5. If the pH is higher,  $\text{OH-Al}^{++}$  or  $(\text{OH})_2\text{Al}^+$  is formed either before or after ions are adsorbed to the soil cation exchange site. These ions polymerize as continuous layers or continuous islands on the surfaces of clay minerals. These polymerised aluminohydronium ions constitute the polymeric aluminium ion species (Mclean, 1976). The polymeric aluminium ion species is determined by determining first the total aluminium and monomeric aluminium ion concentration as described in section 3.3. The difference between the total concentration of aluminium and monomeric aluminium ion concentration constitutes the concentration of polymeric aluminium species. Exchangeable aluminium ion species is the aluminium ion which is adsorbed on the soil cation exchange sites and which is easily displaced by unbuffered salts such as 1N KCl and determined by titrating the extract with 0.05N NaOH and then 0.05 HCl as described in section 3.3.

Gituamba soil pH (in water) of 5.08 and 4.78 at 0-15 and 15-30 cm depths respectively and pH (in 1N KCl) of 3.90 at each of the depths, have the highest

concentration of total, monomeric and polymeric ion species among the three soils followed by Kitale soil with a pH (in water) of 5.50 and pH (in 1N KCl) of 4.40 at each of the depths i.e. 0-15 and 15-30 cm respectively. Kabete soils have the least concentration of both total, monomeric, polymeric and exchangeable aluminium ion species. Within the soil depths, the variability of total, monomeric and exchangeable aluminium ion concentration is small for all the soils. Slightly higher variabilities are only observed for the polymeric aluminium ion species within the soil depths of Gituamba and Kabete soils. In Gituamba, the topsoil has 0.48 m.e/100g polymeric aluminium ion species concentration whereas the subsoils has 0.06 m.e/100g. For Kabete, the topsoil has 0.18 m.e/100g whereas the subsoil has 0.28 m.e/100g of polymeric aluminium ion species concentration. Although the variability of total monomeric and exchangeable aluminium ion species is small i.e. total aluminium: 4.32 and 4.06 m.e/100g for Gituamba, 0.59 and 0.63 m.e/100g for Kitale and 0.26 and 0.30 m.e/100g for Kabete for depths 0-15 and 15-30 cm respectively; monomeric aluminium: 3.84 and 4.00 m.e/100g for Gituamba, 0.35 and 0.32 m.e/100g for Kitale and 0.08 and 0.02 m.e/100g for Kabete at depths 0-15 and 15-30 cm respectively; Exchangeable aluminium: 3.65 and 3.25 m.e/100g for Gituamba, 0.90

and 0.79 m.e./100g for Kitale and 0.28 and 0.30 m.e./100g for Kabete for depths 0-15 and 15-30 cm respectively, it can be seen that for the total aluminium ion species concentration, the topsoil of Gituamba soil has higher concentration than the subsoil whereas for Kitale and Kabete, the reverse is true. For the monomeric aluminium ion species, the concentration is higher for the subsoil in Gituamba soils but higher in the topsoils for Kitale and Kabete soils. Concentration of exchangeable aluminium is higher in the topsoils for all the three soils studied.

Table 5: Concentration of aluminium ion species in the three soils, Gituamba, Kitale and Kabete

Soil	Depth (cm)	<u>Aluminium ion species (m.e/100g)</u>		
		Total Al*	Monomeric Al	Polymeric Al
Gituamba	0-15	4.32	3.84	0.48
	15-30	4.06	4.00	0.06
Kitale	0-15	0.59	0.35	0.24
	15-30	0.63	0.32	0.30
Kabete	0-15	0.26	0.08	0.18
	15-30	0.30	0.02	0.28

NB\* Total aluminium was determined by extracting the soil with 1N KCl followed by colorimetric measurement using the aluminon method



The variability observed of total, monomeric, polymeric and exchangeable aluminium ion species among the three soils could be attributed to the soil pH, organic matter content and the type of predominant clay present in each of the soils. Leaching losses due to different amounts of rainfall received in each area where the soils were collected could also be a contributing factor. According to Foy (1974), Juo and Kamprath (1979) and von Uexkull (1986), the solubility of aluminium in soil solution is controlled by the soil pH, the type of predominant clay, organic matter content, the concentration of other cation and the total salt concentration. According to Mclean (1976), Sartain and Kamprath (1975), Alva et al. (1986) and von Uexkull (1986), at high or intermediate pH, exchangeable aluminium is held tightly to the negative charges of the layer silicates and sesquioxides coated systems as  $Al(OH)^{++}$  or  $Al(OH)^{+}_2$ , but as pH drops below 5.0 exchangeable aluminium ion increased markedly as does the aluminium ion concentration in the soil solution. This could be one of the explanations for the differences in the concentration of aluminium ion species in the soil solution between the three soils as shown in Table 5. The type of predominant clay mineral present in the three soils could also be a contributing factor for the differences in aluminium

ion concentration between the soils. Gituamba soils, an Andosol are mainly composed of volcanic ash which is made up of amorphous hydrous oxides of aluminium and iron. Kitale soils, a Ferrasol are composed mainly of 1:1 type of clay which is less crystalline, sesquioxides and hydrous oxides of aluminium and iron. Kabete soils, a Nitosol are composed mainly of 1:1 type of clay which is more crystalline. Therefore, because of the nature of the type of clay mineral present in the three soils, Gituamba soils are expected to have a high concentration of aluminium ion species followed by Kitale soils. Very little aluminium ion species is expected to be found in Kabete soil. This agrees with the results found in this study (see Table 5) and also explains the differences in the concentration of aluminium ion species between the three soils.

The amount of organic matter present in the three soils (see Table 4) could also explain the difference observed in the concentration of aluminium ion species between the three soils. The addition of humic acid through the decay of organic matter lowers to an extent the soil pH and as a result may increase the solubility of aluminium ion species in the soil solution. The differences in the amount of rainfall

received in each area where the soils were collected (mean annual rainfall are 2005, 1193 and 973 mm for Gituamba, Kitale and Kabete, respectively) could also explain the differences observed in aluminium ion species concentration between the three soils. Aluminium ion species is less mobile when compared to other cations in the soil and is therefore usually not leached in the soils as much as other cations. So in areas with more rainfall aluminium ion species is usually found in considerable quantities in soil solution compared with areas with less rainfall and where leaching is less pronounced. Therefore, more aluminium ion species is expected to be found in Gituamba than in Kitale or Kabete soils. More aluminium is also expected to be found in Kitale than in Kabete soils. This agrees with the findings in this study (see Table 5). The concentration of other cations and the total salt concentration could also have contributed to the differences in the concentration of aluminium ion species in the soil solution of the three soils studied but their contributions were less compared to those discussed above.

The distribution of polymeric and monomeric aluminium ion species in the soil solution is predominantly affected by the soil pH (Mclean, 1976).

Upon release, aluminium ion becomes sixfold-coordinated with oxygen on  $\text{OH}_2$  groups, i.e. becomes  $\text{Al}(\text{OH}_2)_6^{+++}$ . These  $\text{OH}_2$  groups are essentially aluminium-substituted hydronium ion, the aluminium having replaced one hydrogen from each of six hydronium ions  $(\text{OH}^+)_3$ . The aluminium substituted hydronium ion, called aluminohydronium ions are often designated  $\text{Al}.6\text{H}_2\text{O}^{3+}$  or simply  $\text{Al}^{3+}$  without the  $(-\text{OH}_2)_6$ . The alumino-hydronium ions sequentially dissociate hydrogen as pH increases leaving OH ions in place of the  $\text{OH}_2$  groups. Some of these aluminohydronium ions may remain in solution but most of them are adsorbed at the soil cation exchange site from which they are easily displaced with ordinary unbuffered salt solution such as 1N KCl if the pH is below 5.00. If the pH is higher,  $\text{Al}(\text{OH})_2^+$  or  $\text{Al}(\text{OH})_2^+$  is formed either before or after the ions are adsorbed to the soil cation exchange sites. These ions polymerize as continuous layers or discontinuous islands on the interlayer surface of clay minerals, or they complex with reactive groups of soil organic matter, neither of which are exchangeable with unbuffered salt solution. Since these ions both as monomers and as polymers are only partially neutralized, they are acid and hence require a base, such as lime for neutralization. This could be the reason for the difference in the

concentration of monomeric and polymeric aluminium ion species within the soil depths and between the soils studied (see pH in Table 4). It should also be noted that according to Mclean (1975) the polymerized aluminohexahydronium ion species on the surface of the clay mineral or organic matter are much less accessible for neutralization with lime and obstruct exchange sites of the soil for exchange of other cations. The variability of concentration of aluminium ion species within the soil depths are small (although there are a few exceptions as shown above) and could probably be due to controlling factors which influence the activities of the various ions in the soil which in turn reflects, the soil pH, age, drainage and mineralogical nature.

#### 4.2 Nutrient Solution Experiments

##### 4.2.1 Effects of nutrient solution aluminium content on the growth of field beans (Phaseolus vulgaris) cv "Rosecoco"

Plate 1 shows the effect of increasing concentration of aluminium on the growth of field beans (Phaseolus vulgaris L.) cv "Rosecoco" grown in nutrient solutions pH 6.80. It is evident from Plate 1 that there was a reduction in root proliferation as

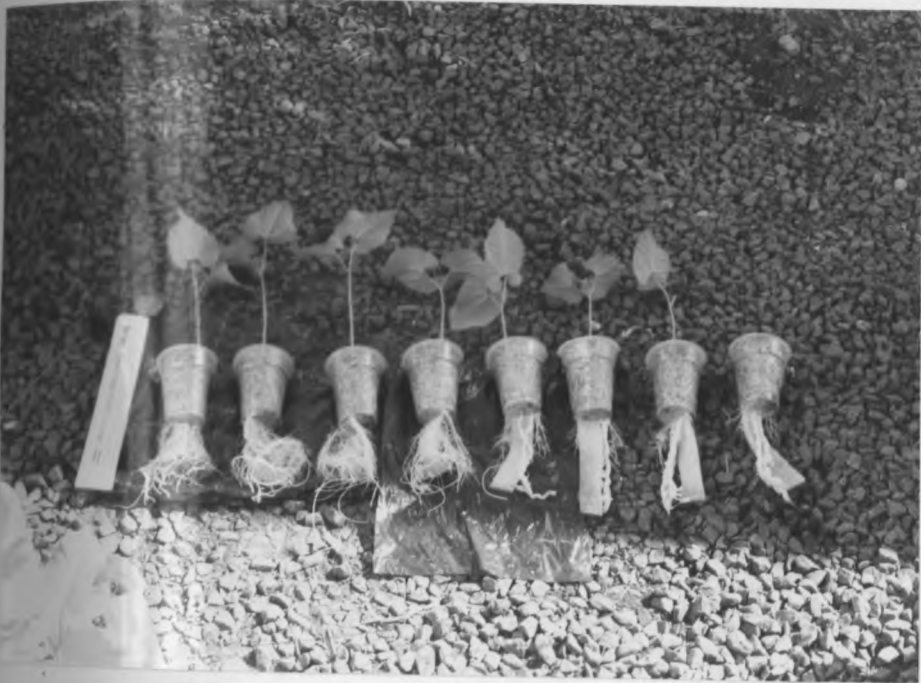


Plate 1: Effect of aluminium ion concentration in nutrient solution on the roots and shoot growth of field bean (Phaseolus vulgaris L.) cv "Rosecoco". The concentration of aluminium in nutrient solution i.e. 0, 3, 6, 9, 12, 15, 18 and 21 ppm increases from left to right

the concentration of aluminium in the nutrient solution increased. This is not very evident on the shoot but it can be seen that at the highest aluminium concentration of 21 ppm very little shoot growth was observed. Table 6 (see appendix 1) shows the effect of increasing aluminium concentration on the taproot length, root, shoot and pooled shoot and root dry matter weights of field beans (Phaseolus vulgaris L.) cv "Rosecoco" grown in nutrient solution. The length of the taproot, shoot and pooled shoot and root dry matter weights decreased significantly ( $P = 0.01$ ) as the concentration of aluminium in the nutrient solution increased. Fig. 2a, b, and c show the effect of nutrient solution aluminium content on the length of the taproot (taproot elongation), shoot and pooled shoot and root dry matter weights. There was a high negative correlation ( $r = -0.91$ ) between the aluminium content in the nutrient solution and the length of the taproot root. A negative correlation also existed between the aluminium content in nutrient solution and the shoot dry matter weight ( $r = -0.79$ ) and pooled shoot root dry matter weight ( $r = -0.76$ ), although this was not as high as was the case with the taproot length (taproot elongation). The high negative correlation between the aluminium content in

the nutrient solution and the length of the taproot shows that the taproot elongation is the

Table 6: Effect of Aluminium Content in Nutrient Solution on the Growth of Field Beans (*Phaesolus vulgaris* L.) cv "Rosecoco"

Concentration of Al (PPM) at pH 6.8	Mean Taproot length (cm plant <sup>-1</sup> )	Mean <sup>T</sup> Root dry matter weight (g plant <sup>-1</sup> )	Mean <sup>T</sup> Shoot dry matter weight (g plant <sup>-1</sup> )	Mean <sup>T</sup> Pooled shoot and dry matter weight (g plant <sup>-1</sup> )
0	34.25	0.54	0.79	1.34
3	32.75	0.29	0.57	0.87
6	32.50	0.47	0.47	0.94
9	30.75	0.40	0.56	0.95
12	25.75	0.56	0.57	1.03
15	19.50	0.35	0.57	0.92
18	18.50	0.37	0.44	0.82
21	1.75	0.17	0.11	0.27
S.E	1.890	-	-	-
C.V%	15.39	11.00	8.55	12.31
F	***	NS	***	**
r	-0.91	-0.50	-0.79	-0.76

N.B. 1 <sup>T</sup> implies the means in question were derived after  $(x + 0.5)^{1/2}$  transformation during the analysis of variance. What is presented is re-transformed data

2 \*\* Significant at P = 0.01

\*\*\* Significant at P = 0.001

NS = Not significant

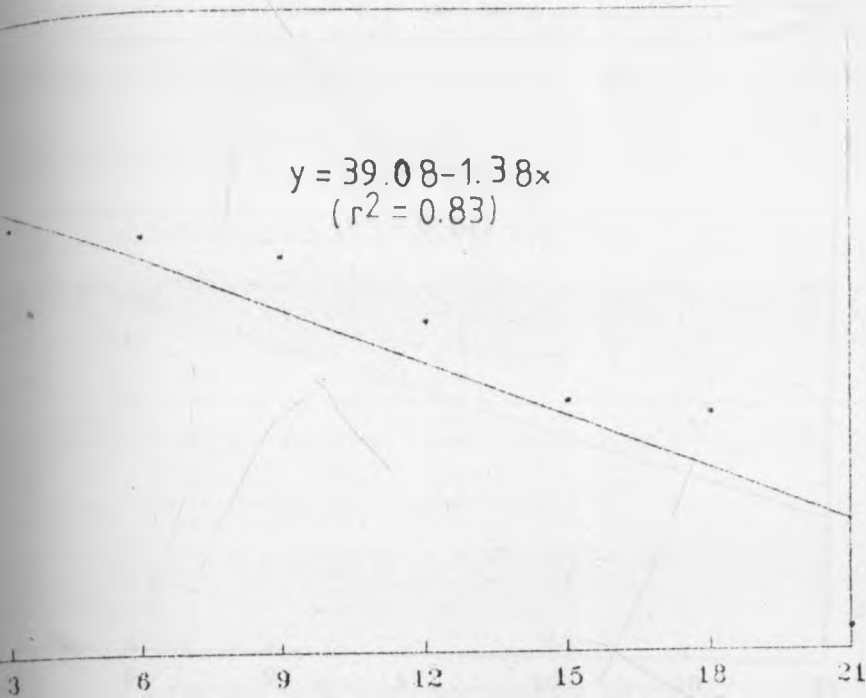


most affected part of the field beans (Phaseolus vulgaris L.) cv "Rosecoco" when the level of aluminium in the nutrient solution becomes toxic to the plant. This also means that it shows the highest negative response as the concentration of aluminium in the nutrient solution becomes toxic. The shoot and the pooled shoot and root dry matter were also affected negatively as the concentration of aluminium in the nutrient solution increased. This shows that the shoot and the whole field bean plant was affected negatively as the concentration of aluminium in the nutrient solution increases but not as much as the taproot elongation. In fact, above 12 ppm Al in the nutrient solution, there was an over 43.0% reduction on taproot length as compared to 28.8 and 41.0% for the shoot and pooled shoot and root dry matter weights at the same concentration respectively. From these observations it can be stated that for the field beans (Phaseolus vulgaris L.) cv "Rosecoco" at pH 6.8, aluminium concentration above 12 ppm would be considered toxic.

The beneficial and inhibitory effects of aluminium on plant growth are reviewed in section 2.6. Symptoms of the beneficial effect of aluminium on plant growth are well discussed by Chenery (1955), Mengel and Kirby (1982), and Franco and Munns (1982). Symptoms of inhibitory effects of aluminium on plant

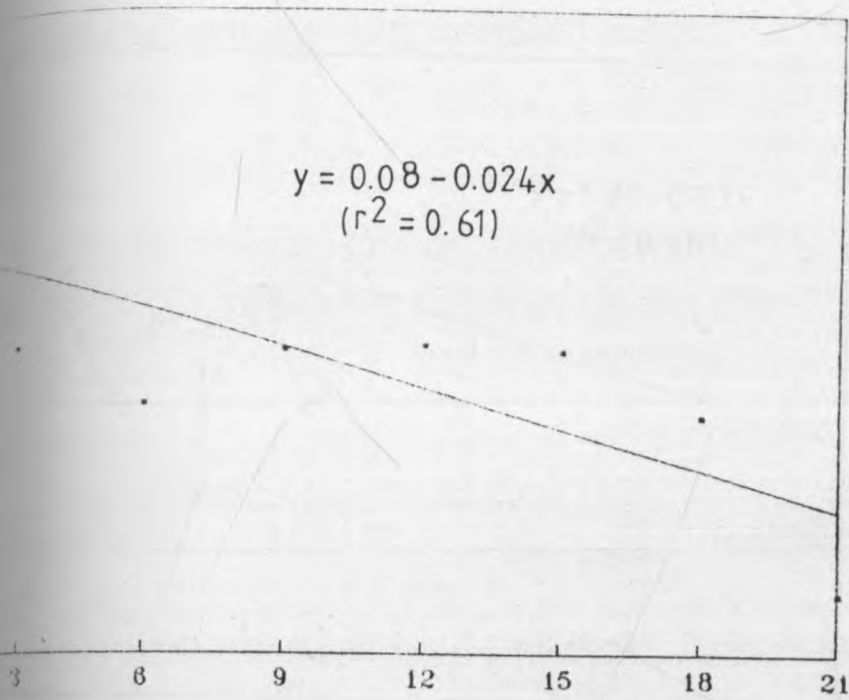
growth are also well discussed by Adams and Lunds (1966), Brenes and Pearson (1973), Foy (1974), Sartain and Kamprath (1975), Franco and Munns (1982), Adams (1984), Alva et al. (1986), and von Uexkull (1986). According to Franco and Munns (1982), in the presence of adequate amount of phosphorus in the nutrient solution, aluminium has no effect on root weight, but decrease taproot elongation and shoot growth of field beans (Phaseolus vulgaris L.) grown in nutrient solution. Franco and Munns (1982) in their study, observed that nodulated field beans (Phaseolus vulgaris L.) plant grew equally well at 10 and 1000 ppm phosphorus and aluminium had no effect on root weight but decreased taproot elongation and shoot growth and both shoot and root weights were greater at pH 4.8 than at pH 4.5. In addition, they found that low levels of aluminium in solution stimulated taproot elongation, there was a change in root morphology, taproot length and root weights increased but total root length decreased indicating that low levels of aluminium was on formation and/or elongation of laterals. They became stunted as observed in alfalfa (Helyer, 1978). At severely toxic aluminium concentration, even primary roots become stunted, and thickened, an observation which was made in this study when the concentration of aluminium exceeded 12 ppm Al in the nutrient

solution. Results obtained in this study agree with those of Franco and Munns (1982). The high significant difference ( $P = 0.01$ ) observed on the weights of pooled shoot and root dry matter weights as the concentration of aluminium in the nutrient solution increased could be due to the shoot dry matter weight since the pooled shoot and root dry matter weight is a summation of the two plant portions but it does give a tentative picture of the effect of different concentrations of aluminium in nutrient solution on the field bean plant which is also supported by Plate 1.



Aluminium concentration (in ppm).

Fig. 2a: Estimated linear regression between aluminium ion concentrates in nutrient solution and taproot length (cm plant<sup>-1</sup>) of field beans (Phaseolus vulgaris L.) cv "Rosecoco"



Aluminium concentration (in ppm).

Fig. 2b: Estimated linear regression between aluminium ion concentration in nutrient solution and shoot dry matter weight (g plant<sup>-1</sup>) of field beans (Phaseolus vulgaris L.) cv "Rosecoco"

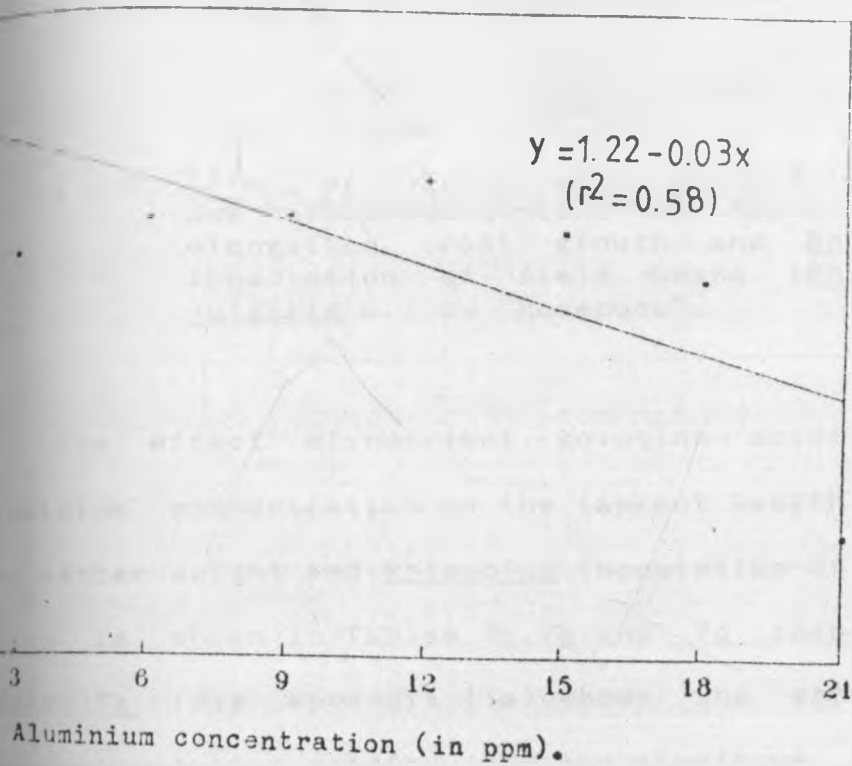


Fig. 2c: Estimated linear regression between aluminium ion concentration in nutrient solution and pooled shoot and root dry matter weight (g plant<sup>-1</sup>) of field beans (Phaseolus vulgaris L.) cv "Rosecoco"

4.2.2 Effect of nutrient solution acidity (pH) and aluminium content on taproot elongation, root growth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco"

The effect of nutrient solution acidity and aluminium concentration on the taproot length, root dry matter weight and Rhizobium inoculation of field beans is shown in Tables 7a, 7b and 7c respectively.

Table 7a (see appendix 11a) shows the effect of nutrient solution acidity (pH) and aluminium content on the length of taproot of the bean plant. From the table, it can be seen that aluminium concentration in the nutrient solution significantly ( $P = 0.001$ ) affected the length of the taproot since there was a decrease on the taproot length from 28.58 cm plant<sup>-1</sup> at 0 ppm Al to 13.81 cm at 10 ppm Al. Further decrease in length was observed after this concentration of aluminium (10 ppm Al). This represent an over 50 percent reduction in taproot length from the initial length of 28.58 at 0 ppm Al. This was consistent with results found in section 4.2.1 which show that above 12 ppm Al, there was over 43% reduction on the taproot length of the field beans. Inoculation of the field bean seed prior to sowing after pregerminating for 24 hours also significantly ( $P = 0.001$ ) affected the length of the

taproot since there was a decrease in taproot length on inoculation of the bean seed (18.68 and 16.07 cm plant<sup>-1</sup> for the non-inoculated and inoculated seeds respectively). Interaction of Rhizobium inoculation and aluminium concentration in the nutrient solution is also significant (P = 0.05).

The negative effect of inoculation on taproot length could be attributed to the fast growing Rhizobium Phaseoli inoculant used (Multistrain 445 and 446) which has an acidifying effect (MIRCEN, 1988) and this would decrease pH of nutrient solution within the vicinity of the pregerminated seeds and this would delay the germination of the bean plant and hence a decrease in the taproot length. This would also account for the observed significant interaction between aluminium content in the nutrient solution and Rhizobium inoculation. Nutrient solution acidity was also observed to have significantly (P = 0.05) affected the taproot length with the highest length being obtained at pH 4.0 (18.23 cm plant<sup>-1</sup>) followed by pH 6.8 (17.86 cm plant<sup>-1</sup>) and then pH 5.0 (16.03 cm plant<sup>-1</sup>). This could not be explained although it is known that aluminium ion is usually more toxic in certain forms which are usually controlled by the pH of the



solution.

Table 7a: Effects of nutrient solution acidity (pH), aluminium content and Rhizobium Inoculation on mean Taproot length (cm plant<sup>-1</sup>) of field beans (Phaseolus vulgaris) cv "Rosecoco"

Aluminium Content (ppm)	pH			Inoculation		
	4.0	5.0	6.8	N <sub>0</sub>	N <sub>1</sub>	Mean
	Taproot length (cm plant <sup>-1</sup> )					
0	29.75	27.63	28.63	30.75	26.42	28.58
5	24.25	17.00	26.75	24.17	21.17	22.67
10	16.19	13.25	12.00	16.63	11.00	13.81
15	10.88	11.63	12.13	11.67	11.42	11.54
20	10.06	12.13	10.06	10.17	10.33	10.25
				<u>Mean</u>		
Inoculation N <sub>0</sub>	20.55	16.90	18.58	18.68		
N <sub>1</sub>	15.90	15.15	17.15	16.07		
Mean	18.23	16.03	17.86			

C.V = 19.80%

S.E. ( $\bar{X}$ ) (Aluminium levels) = +0.72

S.E. ( $\bar{X}$ ) (pH levels) = +0.56

S.E. ( $\bar{X}$ ) (inoculation) = +0.46

S.E. ( $\bar{X}$ ) (Aluminium levels vs pH levels) = + 1.25

S.E. ( $\bar{X}$ ) (Aluminium levels vs Inoculation) = +1.02

S.E. ( $\bar{X}$ ) (pH level vs Inoculation) = +0.56

N.B N<sub>0</sub> = Non-inoculated

N<sub>1</sub> = Inoculated

Table 7b: Effect of nutrient solution acidity (pH), aluminium content and Rhizobium inoculation on the mean root dry matter weight (g plant<sup>-1</sup>) of field beans (Phaseolus vulgaris L.) cv "Rosecoco"

Aluminium Content (ppm)	pH			Inoculation		Mean
	4.0	5.0	6.8	N <sub>0</sub>	N <sub>1</sub>	
Root dry matter weight (g plant <sup>-1</sup> )						
0	0.26	0.20	0.27	0.25	0.23	0.24
5	0.20	0.21	0.26	0.25	0.20	0.22
10	0.21	0.19	0.24	0.21	0.21	0.21
15	0.24	0.19	0.22	0.24	0.20	0.22
20	0.23	0.21	0.25	0.23	0.23	0.23
				<u>Mean</u>		
Inoculation N <sub>0</sub>	0.23	0.23	0.25			0.24
N <sub>1</sub>	0.23	0.17	0.25			0.22
Mean	0.23	0.20	0.25			

C.V 27.20

S.E ( $\bar{X}$ ) (Aluminium levels) = 0.01

S.E ( $\bar{X}$ ) (pH levels) = 0.01

S.E ( $\bar{X}$ ) (inoculation) = 0.01

S.E ( $\bar{X}$ ) (Aluminium levels vs pH level) = 0.01

S.E ( $\bar{X}$ ) (pH levels vs inoculation) = 0.01

N.B N<sub>0</sub> = non-inoculated

N<sub>1</sub> = inoculated

Table 7c: Effect of nutrient solution acidity, aluminium content and Rhizobium inoculation on the mean number of nodules formed per plant on the root of field beans (Phaseolus vulgaris L.) cv "Rosecoco"

Aluminium Content (ppm)	pH			Inoculation		
	4.0	5.0	6.8	$N_0$ -1)	$N_1$	Mean
	No. of root nodules (Plant <sup>-1</sup> )					
0	8.5	0	13.12	0	14.42	7.20
5	5.5	0	6.62	0	8.08	4.04
10	1.87	0	2.75	0	3.08	1.54
15	2.12	7	4.37	0	9.00	4.50
20	2.50	1.75	4.00	0	5.5	2.75
				<u>Mean</u>		
Inoculation $N_0$	0	0	0	0		
$N_1$	8.2	3.5	12.35	8.01		
Mean	4.1	1.75	6.17			

S.E.  $(\bar{X})$  (Aluminium levels) = 0.22

S.E.  $(\bar{X})$  (pH levels) = 0.17

S.E.  $(\bar{X})$  (Inoculation) = 0.14

S.E.  $(\bar{X})$  (Aluminium levels vs Inoculation) = 0.34

S.E.  $(\bar{X})$  (pH levels vs Inoculation) = 0.24

S.E.  $(\bar{X})$  (Aluminium vs pH) = 0.38

N.B. 1)  $N_0$  = Non-inoculated

$N_1$  = Inoculated

2)

Table 7b (see appendix 11b) shows the effect of nutrient solution acidity and aluminium concentration on the root dry matter weight of the bean plant. From the table, it can be seen that the root dry matter weight increase significantly ( $P = 0.01$ ) from pH 5.00 ( $0.20\text{g plant}^{-1}$ ), followed by pH 4.0 ( $0.23\text{g plant}^{-1}$ ) and then to pH 6.8 ( $0.25\text{g plant}^{-1}$ ), but it can be generally stated from the above observation that as the nutrient solution acidity decrease, the root dry matter weight increase. This is consistent with findings of Foy (1974) and Franco and Munns (1982). According to Franco and Munns (1982), field beans grown in nutrient solution with adequate amounts of phosphorus i.e. between 10 and 1000 ppm, the shoot and root dry matter weights were greater at pH 4.8 than at pH 4.5. The significant difference ( $P = 0.05$ ) observed in the root dry matter weight when there was an interaction between soil acidity and inoculation could be attributed to the acidifying effect of the Rhizobium inoculant used (Multistrain, 445 and 446) which is acidifying and might have lowered the pH around the pregerminated seed thereby delaying its germination thus a reduction in root dry matter weight (i.e.  $0.22$  and  $0.24\text{g plant}^{-1}$  for the inoculated and non-inoculated seeds respectively).

Table 7c (see appendix 11c) shows the effect of nutrient solution acidity and aluminium content on the number of nodules formed on the root of each bean plant. Nutrient solution acidity affect significantly ( $P = 0.001$ ) the number of nodules formed on the root of each plant since different nodules number were obtained at different pH's. At pH 6.8, 5.0 and 4.0 the mean number of nodules on the roots per plant were 6.17, 1.75 and 4.10 respectively. The concentration of aluminium in the nutrient solution also affected significantly ( $P = 0.05$ ) the nodules formed on the root of the field bean plant since there was a reduction in the number of nodules formed on the root per plant as the concentration of aluminium increased. Inoculation of the bean seed also significantly ( $P = 0.001$ ) affected the number of nodules formed on the root per bean plant. No nodules were formed where the bean seeds were not inoculated with Rhizobium inoculant whereas on inoculation of the bean seed, the mean number of nodules formed on the roots per plant was 8.01.

Effectiveness of the nodules was not one of the factors considered in this study and the number of nodules presented here are for both effective and non-effective nodules. The highest mean number of

nodules per plant on the root of the bean plant was 13.12 at pH 6.8 and 0ppm Al. This was considerably low compared to 216 nodules per plant formed on the root of field bean grown under similar conditions by Anyango (1984), although she used a different field bean cultivar (Canadian Wonder). According to Anyango (1984) field bean (Phaseolus vulgaris L.) show a very poor response to inoculation. Results from this experiment therefore indicates that the field bean cultivar Rosecoco show a much poor response to inoculation than cultivar Canadian Wonder. This could be the reason for the low nodule counts on the root of field bean cultivar used.

Nutrient solution acidity could be the major factor inhibiting nodulation of the beans since it was observed that it has a high significant effect ( $P = 0.001$ ), (see appendix 11c) on the number of nodules formed on the roots per bean plant. At 0ppm Al, we observe a reduction in the mean number per plants from 13.12 at pH 6.8 to 0 at pH 5.0. An increase at pH 4.0 of 8.5 is also observed. This is consistent with findings of Franco and Munns (1982) who observed that a drop of pH 5.5 to 5.0 resulted in a decrease in the number of nodules from 60 nodules per plant to 10 and no further decrease was observed at pH 4.5. Even though aluminium in solution can

Inhibit formation of a few nodules formed at low pH, this effect would have little biological significance when compared to the effect of low pH, although according to Eaglesham et. al. (1984) aluminium above 50 m is the most severe stress strain factor stopping the growth of most strains of Rhizobium in acidic media. The inhibition of nodulation in the present study could not be explained by manganese toxicity, since more manganese was adsorbed from solution at high pH and the amount added to the nutrient solution (1 m) was below toxic levels according to Nairobi Microbial Resource Centre (MIRCEN). Manganese toxicity may explain failure of beans to nodulate in some acid soils (Dobereiner, 1966) but is less widespread than acidity and aluminium toxicity in tropical soils.

One sensitive step in inoculation is infection, but acidity can also inhibit root colonization (Graham and Parker, 1964; Vincent, 1965; Munns, 1968; and Munns and Keyser, 1981). The Rhizobium strain used (Multistrain 445 and 446) was able to grow well at pH 6.8 to 4.0. Quality control tests on the effectiveness of the inoculant used, Rhizobium strain (445 and 446 Multistrain) was also confirmed (Result not presented here) to ensure good nodulation. These observations imply that poor rhizobial growth itself

can not explain poor nodulation at low pH's (4.0 and 5.0). Nutrient solution acidity might merely delay nodulation since according to Munns and Keyser (1981) the adverse effect of soil acidity and aluminium on rhizobial growth appear to be bacteriostatic rather than bacteriocidal. With time more nodules might have been formed. This explain the high significant effect ( $P = 0.001$ ) observed between the interaction of nutrient solution acidity (pH) and inoculation. It may also explain the low nodule counts per plant root observed in this study contrary to that of Anyango (1984).

From Table 7c, it was observed that the effect of nutrient solution aluminium content on the number of nodules on the roots per plant was not as sensitive as the nutrient solution acidity although above 5ppm Al the number of nodules on the plant root was restricted. The presence of available aluminium in nutrient solution inhibit nodulation directly (Franco and Munns, 1982) through bacteriostatic effects (Munns and Keyser, 1981). This could be the reason for the significant effect ( $P = 0.05$ ) observed between the interaction of nutrient solution aluminium content and inoculation on the number of nodules formed on the root of each plant. The presence of available aluminium might also inhibit



nodulation indirectly by stunting root growth, reducing the potential number of sites where nodules may develop (Kamprath and Foy, 1971) and also tend to compound the effect of low levels of calcium by inhibiting its uptake (Andrew, 1978) and this could be the reason for the significant effect ( $P = 0.05$ ) observed between the nutrient solution aluminium content and the number of nodules per plant on the root of the beans.

Tolerance to acidity is not necessarily correlated with tolerance to aluminium, since aluminium increases the lag time or slows the growth rate of almost all of the strains of Rhizobium which are tolerant to low pH (Keyser and Munns, 1979). This could have been the reason why there was no significant difference between the interaction of nutrient solution acidity and aluminium content on the number of nodules formed per plant on the root of field bean (Phaseolus vulgaris L.) cv "Rosecoco".

#### 4.3 Soil Experiment

The effect of the three soil types, namely Gituamba (an Andosol), Kitale (Ferralsol) and Kabete (Nitosol) at 0-15 and 15-30 cm depths and Rhizobium

inoculation on the taproot length, root dry matter weight and the number of nodules formed on the roots per bean plant were studied.

#### 4.3.1 The Effects of Soil, Depth and Rhizobium Inoculation on Taproot Length of Field Beans

The effect of the soil, sampling depth and Rhizobium inoculation on the length of the taproot of the field bean cultivar is shown in Tables 8a and 8b (see appendices IIIa and IIIb). Tables 8a and 8b show the effect of these variables and their interactions on the length of the taproot when the soil was not fertilized and when fertilized with triple superphosphate (TSP) fertilizer at the rate of 59 kg P<sub>2</sub>O<sub>5</sub>/ha respectively. The increase in taproot length among the three soils was not significant (Table 8a). Within Gituamba soil, the taproot length increased significantly ( $P = 0.05$ ). High taproot lengths were obtained at the depth of 0-15 cm (3.31 cm plant<sup>-1</sup>) than at depth 15-30 cm (2.69 cm plant<sup>-1</sup>). After fertilization (Table 8b) the taproot length among the three soils increased significantly ( $P = 0.01$ ) with the highest length being obtained in Gituamba (3.24 cm plant<sup>-1</sup>) followed by Kabete (2.87 cm plant<sup>-1</sup>) and the Kitale (2.53 cm plant<sup>-1</sup>). After fertilization, inoculation of the bean seed increased

the length of the taproot significantly ( $P = 0.05$ ) in Kabete soils with highest length being obtained when the bean was inoculated (3.01 and 2.64 cm plant<sup>-1</sup> for inoculated and non-inoculated bean seed respectively). Looking at Table 8a and 8b we observe the effect of phosphate fertilizer on the length of taproot of the bean plant between the three soils, at different depths and Rhizobium

Table 8a: Effect of soil, depth and *Rhizobium* inoculation of field beans (*Phaseolus vulgaris* L.) cv "Rosecoco" on mean taproot length (cm plant<sup>-1</sup>) for three soils (No Fertilizer was added on the soil).

	Gitumba Soil			Kitale Soil			Kabete Soil		
	Soil Depth (cm)			Soil Depth (cm)			Soil Depth (cm)		
	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean
Inoculation	Taproot length (cm plant <sup>-1</sup> )			Taproot length (cm plant <sup>-1</sup> )			Taproot length (cm plant <sup>-1</sup> )		
N <sub>0</sub>	3.54	2.46	3.00	3.15	3.42	3.28	3.21	3.14	3.18
N <sub>1</sub>	3.09	2.93	3.01	3.12	3.22	3.17	3.54	3.33	3.43
Mean for depth	3.31	2.69		3.13	3.32		3.38	3.23	
Mean for Soil	3.00			3.23			3.30		

1) Means of 4 plants each grown in a pot and harvested 14 days after planting

2 C.V = 9.84%

S.E ( $\bar{X}$ ) (between the soils) = 0.08

S.E ( $\bar{X}$ ) (inoculation within the soil) = 0.11

S.E ( $\bar{X}$ ) (Depth within the soil) = 0.11

S.E ( $\bar{X}$ ) (Inoculation and depth interaction with the soil) = 0.16

3) N<sub>0</sub> = Non-inoculated

N<sub>1</sub> = Inoculated

Table 8b: Effect of soil, depth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco" on mean taproot length (cm plant<sup>-1</sup>) for three soils.

(Triple Superphosphate (TSP) was added on the soil at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha

	Gituamba Soil			Kitale Soil		Kabete Soil			
	Soil Depth (cm)			Soil Depth (cm)		Soil Depth (cm)			
	0-15	15-30	Mean	0-15	15-30	0-15	15-30	Mean	Mean
	Taproot length (cm plant <sup>-1</sup> )			Taproot length (cm plant <sup>-1</sup> )		Taproot length (cm plant <sup>-1</sup> )			
Inoculation N <sub>0</sub>	3.27	3.42	3.35	2.66	2.67	2.66	2.59	2.68	2.64
N <sub>1</sub>	3.23	3.01	3.12	2.33	2.47	2.40	3.47	2.73	3.01
Mean for depth	3.25	3.22		2.50	2.57		3.03	2.70	
Mean for soil	3.24			2.53		2.87			

1) Means of 4 plants each grown in a pot and harvested 14 days after planting

2) C.V = 16.08%

S.E ( $\bar{X}$ ) (between soils) = 0.12

S.E ( $\bar{X}$ ) (inoculation within the soil) = 0.16

S.E ( $\bar{X}$ ) (depth and inoculation interaction within the soil) = 0.1

N<sub>0</sub> = Non-inoculated

N<sub>1</sub> = Inoculated

inoculation. The variability of the length of the taproot on fertilisation with TSP is small within the three soils. Increase in length is observed in Gituamba soils at both depths and on inoculation after fertilization whereas a decrease in length is observed in Kitale and Kabete soils at both depths and on inoculation after fertilization of the soil.

These observations made from Tables 8a and 8b can be explained by looking at the soil texture and bulk density in Table 3 and percent organic carbon, phosphorus concentration, cation exchange capacity, per cent soil Nitrogen in Table 4 and total, monomeric and polymeric aluminium ion concentration in Table 5. In Gituamba soils, a significant difference ( $P = 0.05$ ) is observed on the taproot length within the two soil depths before fertilization. On fertilization, the soil depths do not significantly affect the length of the taproot of the bean plant. From this observation it can be noted that the difference in taproot length observed before fertilization with phosphate fertilizer could be due to difference in phosphorus content in the two soil depths, but the amounts of phosphorus in the two soil depths i.e. 0-15 and 15-30 cm are 0.65 and 0.35 ppm P respectively which are very low. Therefore this significant effect cannot be explained. The

significant difference ( $P = 0.01$ ) observed on the length of the taproot between the three soils after fertilizer could be explained by looking at some of the parameters stated above in Tables 4 and 5 and not including the soil pH. The possible explanation for the difference in taproot length between Gituamba, e.g. Kabete soil on P-fertilization could be due to the difference in organic matter and soil percent nitrogen content (see Table 4). Before P-fertilization, there was no significant difference on taproot length between the three soils but after fertilization, the taproot length became longer in Gituamba soils than in Kabete and Kitale soils. The effect of addition of phosphate fertilizer in Gituamba soils could be the reduction of toxic levels of aluminium ion (see Table 4) through a process referred to as "liming with phosphate" in apparently acidic soils of Gituamba. Also the addition of phosphate fertilizer would increase nitrogen mineralization in organic matter and this would explain the taproot elongation in Gituamba soils since it has more organic matter than Kabete or Kitale soils. According to Munnvar and Wallum (1977) working on concepts in Columbia, addition of phosphate increased nitrogen mineralization and evolution of  $\text{CO}_2$  when compared to plots not treated with phosphate. They also observed significant mineralization of

carbon and Nitrogen interaction. They concluded that available phosphorus was a rate - limiting factor for organic matter mineralization in Andepts and this could affect plant performance. The difference in taproot length between Kitale and Kabete soils after P-fertilization could be due to the difference in their cation exchange capacity and available exchangeable bases. Apparently before P-fertilization, phosphorus is the limiting factor on taproot elongation but after its addition the taproot growth would be determined by cation exchange capacity and exchangeable bases available since from Table 4 most exchangeable bases are not deficient in both soils but are present in different concentration in both soils.

The decrease in taproot elongation or length on P-fertilization in Kabete and Kitale soils could be due to mineralization of organic matter on addition of phosphate which could result in a decrease in cation exchange capacity, since organic matter in the soil also contributes to the cation exchange capacity. Also during the mineralization of organic matter on addition of phosphate fertilizer according to Munnevar and Wallum (1977),  $\text{CO}_2$  is evolved. This will dissolve in soil solution to form carbonic acid. On ionization, it will release  $\text{H}^+$  ion and this will



result in a decrease in soil pH. A decrease in soil pH will affect the availability of some essential elements either through their precipitation or through the physiological effect of the pH on the bean plant root which will make them not absorbable into the roots.

#### 4.3.2 The Effects of Soil, Depth and Rhizobium Inoculation on the Root Dry Matter Weight of Field Beans (Phaseoli vulgaris L.) cv "Rosecoco"

The effect of the soil, sampling depth and Rhizobium inoculation on the root dry matter weight of field beans is shown in Tables 9a and 9b (see appendices IVa and IVb). Tables 9a and 9b show the effects of the three soil variables and their interaction on the root dry matter weight when the soil was not fertilized and when fertilized with TSP fertilizer at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha respectively. From Table 9a we observe that the increase in root dry matter weight among the three soils was significant (P = 0.01) with high root dry matter weight being obtained in Kitale (0.46g plant<sup>-1</sup>) followed by Gituamba (0.28g plant<sup>-1</sup>) and then Kabete (0.22g plant<sup>-1</sup>). Within Kitale soil, there was a significant (P = 0.01) effect of inoculation of the bean seed on root dry matter weight (0.56 and 0.36g plant<sup>-1</sup>) for the inoculated and non-inoculated bean

seeds respectively). An increase in root dry matter weight was also significant ( $P = 0.05$ ) within Kitale soil with highest root dry matter weight being obtained in the topsoil ( $0.55$  and  $0.37\text{g plant}^{-1}$ ) for depths  $0-15$  and  $15-30$  cm respectively). Interaction between the soil depth and inoculation within Kitale soil was also significant ( $P = 0.05$ ). Table 9b shows the effect of phosphate fertilization on the root matter dry weight. There was a significant ( $P = 0.01$ ) increase in root dry matter between the three soils with the highest root dry matter weight being obtained in Gituamba soils ( $3.37\text{g plant}^{-1}$ ) followed by Kabete ( $1.08\text{g plant}^{-1}$ ) and then Kitale ( $1.02\text{g plant}^{-1}$ ). After fertilization, there was also a significant ( $P = 0.01$ ) increase on the root dry matter weight within Gituamba soils with the highest root dry matter weight being obtained in the topsoil ( $3.97$  and  $2.77\text{g plant}$  for depths  $0-15$  and  $15-30$  cm respectively).

From Tables 9a and 9b we can see the effect of P-fertilization on the root growth of field bean plant. There is an obvious increase in root growth on P-fertilization since an increase in root dry matter weight is observed in all the soils with the maximum increase being observed in Gituamba soil (from  $0.28$

Table 9a: Effect of soil, depth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco" on the mean root dry matter weight (g plant<sup>-1</sup>) for three soils (The soil was not fertilized)

	Gitumba soil		Kitale soil		Kabete soil				Mean
	Soil depth (cm)		Soil depth (cm)		Soil depth (cm)				
	0-15	15-30	0-15	15-30	0-15	15-30	Root dry matter weight (g plant <sup>-1</sup> )		
	Root dry matter weight (g plant <sup>-1</sup> )		Root dry matter weight (g plant <sup>-1</sup> )		Root dry matter weight (g plant <sup>-1</sup> )				
			Mean	Mean	Mean	Mean			
Inoculation N <sub>0</sub>	0.23	0.34	0.28	0.37	0.35	0.36	0.17	0.25	0.21
N <sub>1</sub>	0.32	0.24	0.28	0.73	0.40	0.56	0.23	0.21	0.22
Mean for depth	0.28	0.29		0.55	0.37		0.20	0.23	
Mean for soil	0.28		0.46		0.22				

N.B 1) Means for 4 plants each grown in a pot; harvested 14 days after planting

S.E (X) (between the soils) = 0.4

S.E (X) (inoculation within the soil) = 0.06

S.E (X) (depth within the soil) = 0.06

S.E (X) (in interaction of inoculation and depth within the soil) = 0.09

N<sub>0</sub> = Non-inoculated

N<sub>1</sub> = Inoculated

Table 9b: Effect of soil, depth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco" on mean root dry matter weight ( $\text{g plant}^{-1}$ ) for three soils. The soils were fertilized with Triple Superphosphate (TSP) fertilizer at the rate of 69 kg  $\text{P}_2\text{O}_5/\text{ha}$

	Gituamba soil		Kitale soil		Kabete soil				
	Soil depth (cm)		Soil depth (cm)		Soil depth (cm)				
	0-15	15-30	0-15	15-30	0-15	15-30			
	Root dry matter weight ( $\text{g plant}^{-1}$ )		Root dry matter weight ( $\text{g plant}^{-1}$ )		Root dry matter weight ( $\text{g plant}^{-1}$ )				
			Mean	Mean	Mean	Mean			
Inoculation $N_0$	3.67	2.53	3.10	0.97	1.02	0.99	1.36	1.04	1.20
$N_1$	4.28	3.02	3.65	1.11	0.99	1.05	0.91	0.99	0.95
Mean for depth	3.97	2.77		1.04	1.00		1.14	1.02	
Mean for soils	3.37			1.02			1.08		

1.1) Means of 4 plants each grown in a pot; harvested 14 days after planting

S.E (X) (between the soils) = 0.23

S.E (X) (inoculation within the soils) = 0.32

S.E (X) (depths within the soils) = 0.32

S.E (X) (interaction of inoculation and depth within the soil) = 0.46

3)  $N_0$  = Non-Inoculated

$N_1$  = Inoculated

to 3.37g plant<sup>-1</sup>), Kabete (0.22 to 1.08g plant<sup>-1</sup>) and then Kitale (0.46 to 1.02g plant<sup>-1</sup>). The increase is considerable and supports a historical fact that good supply of phosphate always increases root growth (Tisdale and Nelson, 1971).

The observed significant difference on the root growth of the field bean plant when the soils were not fertilized can be explained by looking at the soil pH, which affect nutrient availability and solubility in soil solution; percent organic matter in the soil which contributes to cation exchange capacity as such soil fertility and water relations in the soil; soil Nitrogen which is an essential plant nutrient; cation exchange capacity, which affects the soil fertility; soil solution aluminium content which affect the soil pH, cation exchange capacity and is toxic in high concentration and last but not least, phosphorus concentration in the soil solution which is also an essential plant nutrient and which is important for the root growth (see Tables 4 and 5). The above mentioned parameters would also be the cause of the significant difference on the root growth observed within Kitale soils.

On P-fertilization, apart from the significant difference ( $P = 0.01$ ) observed on root dry matter weight among the three soils all the other significant differences observed become insignificant. From this observation and the concentration of phosphorus as shown in Table 4, it can be stated that phosphate is the main limiting factor on root growth among the three soils studied. Also from this observation it can be stated that the high root dry matter weight obtained in Kitale soil when the soil had not been fertilized was because it had a relatively high content of phosphorus (though relatively low) in its topsoil, (3.67 ppm P) than in all the other soils. This fact can also be elucidated by looking at the root dry matter weight within Kitale soils, where the topsoil had a root dry matter weight of 0.55 compared to 0.37g plant<sup>-1</sup> in the subsoil. The difference in the root growth between Gituamba and Kabete soils when the soil had not been fertilized could be due to the higher total soil nitrogen and organic matter content in Gituamba soils than in Kabete soils since all the other factors favour the Kabete soils than Gituamba soils and yet Gituamba soils had a high root dry matter weight than Kabete soils (0.28 and 0.22g plant<sup>-1</sup> for Gituamba and Kabete respectively) an indication that more root growth occurred in Gituamba soils than in

Kabete soils. The soil texture (clay loam and clay for Gituamba and Kabete respectively) could also account for the observed difference in root growth. The clay loam soils of Gituamba are more porous (low bulk density of 0.65 and 0.79g/cm for depths 0-15 and 15-30 cm respectively) as such do not restrict root growth much when compared to the clay soil of Kabete (bulk density 1.10 and 1.01g/cm<sup>-3</sup> for depths 0-15 and 15-30 cm respectively). The significant difference (P = 0.01) observed in root growth within Kitale soils on Rhizobium inoculation (0.36 and 0.56g plant<sup>-1</sup> for the non-inoculated and inoculated seed respectively) could be due to the effectiveness of the nodules formed on the root of the bean plant because when the nodules were separate from the roots when being counted, over 50% of the nodules on dissection, had a pinkish coloration, an indication that they could possibly fix nitrogen. The possible reason for the interaction observed within Kitale soil between the soil depths and inoculation on the root growth of field bean could be due to the phosphate content in the soil. According to van Schreven (1958), although species and cultivars differ in their nutritional needs, legumes have a relatively high phosphorus requirement for optimum growth. Some require significantly more phosphate to reach optimum yield when relying on symbiotically

fixed nitrogen in comparison to when supplied with fertilizer (Cassman et al., 1981). The high root dry matter weight (0.73 and 0.40g plant<sup>-1</sup> for depths 0-15 and 15-30 cm respectively) observed for the topsoil could be due to the relatively high phosphate content in the soil (3.67 and 1.00 ppm P for depths 0-15 and 15-30 respectively).

Addition of phosphate fertilizer has a significant influence on the root growth as stated earlier and this is supported by looking at Tables 9a and 9b. From Table 9a we can see that based on the information on Table 4 and 5 for Gituamba soils, the limiting factor on root growth is probably the soil pH and aluminium content in the soil solution. The same may apply for Kabete and Kitale but not as much as for Gituamba soils. Addition of Triple Superphosphate (TSP) fertilizer does not affect the soil pH, because TSP fertilizer is neither acidifying nor basic in character. But it is added to the three soils, it considerably reduce the concentration of total aluminium (see Table 11) in Gituamba soil than in Kitale and Kabete soils. It can be stated here that the process often referred to as "liming by phosphate" is more defined here than before.



The significant difference ( $P = 0.01$ ) observed in the root growth among the three soils on P-fertilization could therefore be explained by looking at the organic matter content in the three soils which is high in Gituamba soils than in Kitale and Kabete soils (see Table 4). According to Munevar and Wallum (1971) addition of phosphate onto the soil favours nitrogen mineralization and this could be good for the plant growth. Therefore the addition of phosphate probably favoured nitrogen mineralization from organic matter more in Gituamba soil and this was good for the root growth.

The difference in soil nitrogen content could also be a reason for the difference in root growth between the three soils but since Gituamba soils had more soil nitrogen (see Table 4) than Kitale and Kabete soils, more growth was obtained in the former soils. The porosity of the soil could also affect the rate of root growth. The higher is the soil bulk density, the more difficult it is for the root to penetrate and proliferate in the soil. Gituamba soil having a lower bulk density than Kitale and Kabete soils therefore did have more root growth and this too, could explain the difference in the root dry matter weight between the three soils when the soil was fertilized. Between Kitale and Kabete soils the

possible reason for the difference in the root growth i.e. root dry matter weight, could be due to the difference in the fertility of the two soils. Kabete soil is more fertile (higher CEC) than Kitale soil. Kabete soils also have higher organic matter, nitrogen content and exchangeable bases than Kitale soil (Table 4). This could account for the difference in root dry matter weight between the two soils. The significant difference in the root dry matter weight within Gituamba soils at the two soil depths could be due to the difference in organic matter content (7.97 and 6.24% C for depths 0-15 and 15-30 cm respectively) and the amount of soil nitrogen present in the soil (0.65 and 0.54% N for depths 0-15 and 15-30 cm respectively).

#### 4.3.3 The Effects of Soil, Depth and Rhizobium Inoculation on the Number of Nodules Formed on the Root of Field Beans (Phaseolus vulgaris L.) cv "Rosecoco"

The effects of the soil, sampling depth and Rhizobium inoculation on the number of nodules formed on the root per plant of the field beans (Phaseolus vulgaris L.) "Rosecoco" is shown on Tables 10a and 10b (see appendices Va and Vb). Tables 10a and 10b show the effects of these variables and their interaction on the number of nodules formed on the root per plant, when the soil was not fertilized and

when fertilized with Triple Superphosphate (TSP) at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha respectively. Table 10a shows that the number of nodules formed on the root per plant was affected significantly (P = 0.001) among the three soils since different numbers of nodules were formed on the root of each plant. Highest mean number of nodules was obtained in Kabete soils (9.94 nodules per plant<sup>-1</sup>) followed by Kitale soils (9.49 nodules per plant<sup>-1</sup>) and then Gituamba soils (8.00 nodules per plant<sup>-1</sup>). Within Kabete soils, the mean number of nodules per plant increased significantly (P = 0.01) from 9.08 before inoculation to 10.81 after inoculation of the beans during planting. Table 10b shows the effect of the three variables and their interaction when the soil had been fertilized prior to planting the seeds. The mean number of nodules on the roots per plant of the beans plant increased significantly (P = 0.01) from 26.20 in Gituamba to 102.02 in Kitale and then to 160.71 in Kabete soils. No significant difference was observed within the soils.

From Tables 10a and 10b we observe the effect of phosphate fertilization on the number of nodules formed on the roots per plant of the field bean cultivar. The increase in the mean number of nodules on the root per plant after phosphate fertilization

s.e. 8.00 to 26.00 in Gituamba, 9.49 to 102.02 in Kitale and 9.94 to 160.71 nodules per plant<sup>-1</sup> in Kabete soils before and after phosphate fertilization respectively show the effect of phosphorus on nodule formation. According to Stalder (1952) and Diener (1950) nodule development requires adequate phosphorus and nodules accumulate at high phosphorus content than root content (Mosse et al., 1976), although indications are that phosphorus definitely limits nodulation indirectly by limiting legume growth rather than the infection process per se (Andrew, 1978; Zaroug and Munns, 1979). So the low number of nodules formed on the root per plant of field bean before fertilization could be attributed to the low phosphorus content (see Table 4) in the three soils which is offset by addition of phosphate fertilizer and this is consistent with the findings of Franco and Munns (1982) who found that in solution culture at different phosphorus levels, highest number of nodules were produced on the root of common bean (vulgaris L.) at the highest phosphorus levels.

Table 10a: Effects of soil, depth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco" on mean number of nodules on the root per plant on the three soils (The soil not fertilized)

	Gituamba Soil		Kitale Soil		Kabete Soil				Mean
	Soil depth (cm)		Soil depth (cm)		Soil depth (cm)				
	0-15	15-30	0-15	15-30	0-15	15-30			
	Nodule Number (plant <sup>-1</sup> )	Mean	Nodule Number (plant <sup>-1</sup> )	Mean	Nodule Number (plant <sup>-1</sup> )	Mean			
Inoculation N <sub>0</sub>	11.53	5.05	8.31	8.92	10.70	9.81	9.30	8.86	9.08
N <sub>1</sub>	8.55	7.58	8.06	8.73	9.43	9.09	11.53	10.09	10.81
Mean for depth	10.04	6.32		8.82	10.16		10.41	9.47	
Mean for soils	8.00		9.49		9.94				

C.V = 25.87%

S.E ( $\bar{X}$ ) (between the soils) = 0.08

S.E ( $\bar{X}$ ) (inoculation within the soil) = 0.11

S.E ( $\bar{X}$ ) (depths within the soil) = 0.11

S.E ( $\bar{X}$ ) (interaction of inoculation and soil depth within the soil) = 0.16

1) The means are for 4 plants each growing in a pot and harvested 14 days after planting.

2) N<sub>0</sub> - non-inoculated

N<sub>1</sub> - inoculated

3) The data presented here was transformed during the analysis of variance using  $1/2$

(X + 1) transformation.

Table 10b: Effect of soil, depth and Rhizobium inoculation of field beans (Phaseolus vulgaris L.) cv "Rosecoco" on the mean number of nodules on the roots per plant on the three soils (The soil was fertilized at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha)

	Gituamba Soil			Kitale Soil			Kabete Soil		
	Soil Depth (cm)			Soil Depth (cm)			Soil Depth (cm)		
	0-15	5-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean
Inoculation N <sub>0</sub>	36.94	23.70	30.32	86.57	114.78	90.67	192.82	133.80	162.31
N <sub>1</sub>	22.81	21.37	22.09	106.54	120.22	113.38	144.20	174.30	159.11
Mean for depth	29.87	22.53		86.55	117.50		167.51	153.91	
Mean for soils	26.20			102.02			160.71		

C.V = 20.93%

S.E ( $\bar{X}$ ) (between the soils) = 0.12

S.E ( $\bar{X}$ ) (inoculation within the soil) = 0.16

S.E ( $\bar{X}$ ) (depths within the soil) = 0.16

S.E ( $\bar{X}$ ) (interaction of inoculation and depth within the soil) = 0.23

1) Means for 4 plants each growing in a pot and harvested 14 days after planting

2) N<sub>0</sub> - non-inoculated

N<sub>1</sub> - inoculated

3) The data presented here was transformed during the analysis of variance using 1/2

(X+1) transformation.

Field beans (Phaseolus vulgaris L.) are extensively grown without N-fertilizer, where N-fixation is needed under adverse soil conditions. Response to inoculation in field trials has been extremely variable due to many poorly understood limiting factors, especially in acid soils (Tisdale et al 1985). The study of factors that limit nodulation and plant growth in soils is sometimes impeded by the complexity of soil. So, by looking at Table 10a and 10b, the significant difference observed in the number of nodules formed on the roots per plant of the field bean between the three soils can only be postulated. Soil pH could be a possible reason for the difference in the number of nodules formed on the roots per plant between the three soils. According to Graham and Parker (1964), slow growing rhizobia strains are in general more tolerant to low pH than fast growers, although strain to strain differences exist. Rhizobium inoculant used (Multistrain 445 and 446) is a fast-growing rhizobia and therefore might be less tolerant to low soil pH in Gituamba soils than in Kitale and Kabete soils. The same might apply for Kitale and Kabete soils, since they have different soil pH (see Table 4) and this might affect the Rhizobium inoculant used although it was able to grow well at pH 6.8 to 4.5 during the quality control tests. The bacteriostatic

effect of the soil acidity on the rhizobia could also be the possible reason for the difference in the number of nodules formed on the roots of the bean plant between the three soils. Aluminium concentration in the soil solution between the three soils (see Table 5) could also be the possible reason for the difference in the number of nodules formed on the roots between the soils because according to Munns and Keyser (1981) higher aluminium ion concentration adversely affects rhizobial growth through the bacteriostatic effect just like the soil acidity. Therefore the difference in aluminium content between the three soils could be the cause of the difference in number of nodules formed on roots per plant.

The significant difference ( $P = 0.01$ ) observed on the number of nodules formed on the root per plant within Kabete soil when no fertilizer was added could be due to Rhizobium inoculation (9.08 and 10.81 nodules plant<sup>-1</sup> for the non-inoculated and inoculated beans respectively).



Table 11: Total concentration of aluminium in the three soils and at different depths 28 days after fertilizing the soil with TSP (Triple Superphosphate) fertilizer at the rate of 150kg TSP/ha

Soil	Depth (cm)	Aluminium Concentration (m.e/100g soil)
Gituamba	0-15	2.20
Gituamba	15-30	2.60
Kitale	0-15	0.34
Kitale	15-30	0.20
Kabete	0-15	0.24
Kabete	15-30	0.30

4.4 Comparison of nutrient solution with soil experiments

Soil experiments involving plants are usually difficult to interpret due to the complex interactions which do occur within the soils. Nutrient solutions are usually prepared to simulate the soil conditions and these are used to explain some of the soil reactions. In the present study, pH and aluminium content in the soil were simulated in a nutrient solution study in order to explain their effects on taproot elongation, root growth and number of nodules formed on the root of the field beans (Phaseolus vulgaris L.) cv "Rosecoco".

It was difficult to observe the effect of pH and aluminium content in the soils before the addition of phosphate fertilizer since phosphorus was very limiting and masked any other effect of soil conditions on the above mentioned parameters except for the number of nodules formed on the roots of the bean plant. After the addition of phosphate fertilizer, the amount of organic matter and nitrogen in the soil became the most limiting factors and thus controlled the taproot elongation and root growth. This was not so with the number of nodules on the root of the field bean cultivar since before and

after the addition of phosphate fertilizer, the highest number of nodules were found in the soil with the highest pH and lowest aluminium concentration i.e. Kabete soil followed by Kitale and then Gituamba. Therefore, the only comparison which could be made in this study between the soil and nutrient solution experiments was on the effect of pH and aluminium content on the number of nodules formed on the root of field bean cultivar "Rosecoco" after Rhizobium inoculation. The number of nodules formed on the root of field beans cultivar is lower in the nutrient solution than in the soil experiment even though their pH values were similar i.e. at nutrient solution pH 6.8 and aluminium concentration is 0 ppm Al, the mean number of nodules formed on the root per bean plant was 13.12 whereas in Kabete soils at depth 15-30 cm and pH (water) 6.8 and total aluminium concentration of 27 ppm Al, the mean number of nodules on the root per plant was 153.91. Toxicity levels of aluminium in nutrient and the soil solution also vary. 5 ppm Al is considered toxic for nodule formation in nutrient solution whereas in the soil solution, 27 ppm Al (Kabete soils, depth 15-30 cm) does not appear to be toxic at all.

## CHAPTER FIVE

### CONCLUSIONS

All the three soils showed acidic reactions. Gituamba soils had the highest acidity followed by Kitale and then Kabete. The difference in acidity between the three soils was most likely due to weathering and/or leaching losses due to the rainfall received in the sites where the soils were collected, concentration of aluminium and the organic matter content in the soils. Distribution of all forms of aluminium ion species between and within the soils was pH-dependent, with the highest concentration being found where the pH was low.

Aluminium concentration in the nutrient solution suppressed taproot elongation whereas nutrient solution acidity suppressed both the taproot elongation and root growth as measured by root dry matter weight of field beans (Phaseolus Vulgaris L) cv "Rosecoco". Both nutrient solution acidity and aluminium content suppressed nodule formation as measured by the number of nodules formed on the roots of the bean plant. This was due to the bacteriostatic effect. Aluminium also suppressed

nodule formation by stunting root growth. Therefore, the highest number of nodules was formed where the pH was high and aluminium content low. Inoculation of the beans prior to sowing reduced the length of the taproot. This was most likely due to the acidifying effect of the Rhizobium inoculant used (Multistrains 445 and 446) which caused a delay in seed germination.

In all the three soils studied, phosphorus content was limiting and suppressed taproot elongation and root growth as measured by root dry matter weight. No difference was observed in the taproot length among the three soils before phosphate-fertilization whereas for the root growth, highest root dry matter weights were obtained in the soils having the highest phosphorus levels (Kitale soils). After phosphate-fertilization a general increase in taproot length and root growth was obtained for all the soils. A difference in growth among the soils was obtained for both the taproot length and root growth as measured by root dry matter weight, with the highest growth being obtained in soils having the highest organic matter and soil nitrogen content, namely, highest in Gitamba and lowest in Kitale soils (Kabete being intermediate). Therefore, after phosphate

fertilization, organic matter and soil nitrogen content became the limiting factors for both taproot and root growth.

Soil pH and aluminium content became limiting only in nodule formation, with the soils having the highest pH and low aluminium content (Kabete, Kitale and Gituamba soils respectively) having the highest number of nodules formed on the root of field beans (Phaseolus Vulgaris L) cv "Rosecoco" regardless of phosphate status of the soils. However, there was an increase in the number of nodules formed after phosphate fertilization in all the soils, (with highest still in Kabete and lowest in Gituamba) an indication that phosphorus is necessary for nodule formation.

Nutrient solution acidity suppressed taproot elongation and root growth as measured by root dry matter weight. Nutrient solution aluminium content suppressed taproot elongation and nodule formation. Nutrient solution acidity and aluminium content suppressed nodule formation as measured by the number of nodules formed on the root of the bean plant. No nodule was formed when the beans were not inoculated in the nutrient solution study. In the soil before phosphate fertilization phosphorus content is

limiting and suppresses both the taproot elongation and root growth. After P-fertilization organic matter and soil nitrogen content become limiting and determined both taproot elongation and root growth. Regardless of phosphate fertilization, soil pH and aluminium content determined the nodule formation with an increase in the number of nodules formed on the roots when phosphorus is added. Therefore, comparison could only be made between the soil and nutrient soil experiment on the effect of pH and aluminium content on nodule formation where it was observed that nodulation was poorer in the nutrient solution than in the soil.

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Appendix I

ANOVA: For the effect of nutrient solution aluminium content on the length of taproot (cm) of field beans (Phaseolus Vulgaris L) cv "Rosecoco"

Source of variation	df	SS	MS	F
Total	31	3667.22		
Treatment	7	3321.47	474.49	39.99**
Error	24	345.75	14.40	

CV = 15.39%

ANOVA: for the effect of nutrient solution aluminium concentration on the shoot dry weight (g) of field beans (Phaseolus Vulgaris L.) cv "Rosecoco".

Sources of variation	df	SS	MS	F
Total	31	0.46		
Treatment	7	0.28	0.04	5.63**
Error	23	0.17	0.007	

CV = 8.55%

ANOVA: For the effect of nutrient solution aluminium concentration on root dry weight (g) of field beans (Phaseolus Vulgaris L.) cv "Rosecoco"

Sources of variation	df	SS	MS	F
Total	31	1.39		
Treatment	7	0.45	0.064	0.17NS
Error	24	0.94	0.038	

ANOVA. For the effect of nutrient solution aluminium content on the pooled shoot and root dry matter weight of field beans (Phaseolus vulgaris L.) cv "Rosecoco".

(Transformation  $(g+0.5)^{1/2}$  was used during the analysis of the data)

Source of variation	df	SS	MS	F
Total	31	1.014		
Treatment	7	0.51	0.073	3.52**
Error	24	0.50	0.020	

CV =12.31%

NB: \*\* = significantly different at P=0.01

NS = Not significantly different

## Appendix 11a

ANOVA. For the effect of nutrient solution acidity (pH), aluminium content and Rhizobium inoculation on the Taproot length (cm) of field bean (Phaseolus Vulgaris L.) cv "Rosecoco".

source of variation	df	SS	MS	F
Total	119	8175.15		
Replicate	3	149.37	49.791	
A	4	6026.72	1506.679	131.83***
P	2	111.30	55.652	4.87**
AXP	8	399.57	49.946	4.37***
N	1	204.10	204.102	17.86***
AXN	4	152.95	38.237	3.34*
PXN	2	63.65	31.527	2.76NS
AXPXN	8	73.74	9.217	<INS
Error	87	994.34	11.430	

## NB:

1. A = Aluminium levels

P = pH levels

N = Inoculation

2. \* = Significantly different at P = 0.05

\*\*\* = Significantly different at P = 0.001

NS = Not significantly different

Appendix 11b

ANOVA : For the effect of nutrient solution acidity (pH), aluminium content and Rhizobium inoculation on root dry matter weight (g plant<sup>-1</sup>) of field beans (Phaseolus Vulgaris L) cv " Rosecoco".

Source of Variation	df	SS	MS	F
Total	119	0.54		
Replicate	3	0.07		
Treatment	29	0.14	0.005	1.31NS
A	4	0.01	0.003	<1NS
P	2	0.05	0.268	7.18**
AXP	8	0.02	0.002	<1NS
N	1	0.01	0.010	2.8NS
AXN	4	0.01	0.003	<1NS
PXN	2	0.03	0.137	3.66*
AXPXN	8	0.01	0.001	<1NS
Error	87	0.32	0.004	

NB:

1. A = Aluminium level  
P = pH level  
N = inoculation
2. \*\* = significantly different at P = 0.01  
\* = significantly different at P = 0.05  
NS = Not significantly different

Appendix 11c

ANOVA : For the effects of nutrient solution acidity (pH) aluminium content and Rhizobium inoculation on the number of nodules formed per plant on the root of field bean (Phaseolus Vulgaris L.) cv " Rosecoco"

Sources of variation	df	SS	MS	F
Total	119	247.81		
Replicates	3	6.53	2.176	
Treatments	29	140.97	4.861	4.22**
A	4	11.75	2.939	2.55*
P	2	14.08	7.040	6.11***
AXP	8	16.93	2.117	1.84NS
N	1	57.41	57.406	49.79***
AXN	4	11.75	2.939	2.55*
PXN	2	14.08	7.040	6.11***
AXPXN	8	14.96	1.869	1.62NS
Error	87	100.31	1.153	

CV = 62.59%

NB:

1. A = Aluminium levels
- P = pH levels
- N = inoculation

2. \* = significantly different at P = 0.05
- \*\* = significantly different at P = 0.01
- \*\*\* = significantly different at P = 0.001
- NS = not significantly different

3. Analysis of variance was done after the original data was transformed using  $(x + 1)^{1/2}$  transformation

Appendix IIIa

ANOVA For the effect of the soils, depth and Rhizobium inoculation on the length of the taproot (cm) of field beans (Phaseolus Vulgarius L.) cv "Rosecoco" in the three soils when no fertilizer was added on the soil.

Source of variation	df	SS	MS	F
Total	47			
Replicates	3	0.33	0.110	
Treatments	11	1.90	0.173	1.72NS
Between soils	2	0.29	0.144	1.43NS
Within soil S <sub>0</sub>	3	1.03	0.345	3.44*
N	1	0.19	0.189	1.88NS
D	1	0.62	0.621	6.19*
NXD	1	0.22	0.226	2.25NS
Within soil S <sub>1</sub>	3	0.21	0.072	<1NS
N	1	0.05	0.051	<1NS
D	1	0.14	0.138	1.38NS
NXD	1	0.25	0.258	<1NS
Within soil S <sub>2</sub>	3	0.36	0.121	1.20NS
N	1	0.26	0.263	2.63NS
D	1	0.08	0.080	<1NS
NXD	1	0.07	0.019	<1NS
Error	33	3.31	0.100	

NB:

1. S<sub>0</sub> = Gitumba soil
- S<sub>1</sub> = Kitale soil
- S<sub>2</sub> = Kabete soil
- N = inoculation
- D = soil depth
2. \* = significantly different at P=0.05
- NS = not significantly different

## Appendix IIIb

ANOVA: for the effect of the soil, depth and Rhizobium inoculation on the length of the taproot (cm) of field beans (Phaseolus Vulgaris L.) cv "Rosecoco" in the three soils when the soil was fertilized with TSP fertilizer at a rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha

source of variation	df	SS	MS	F
Total	47			
Replicates	3	0.56		
Treatment	11	6.75	0.614	2.88**
Between soils	2	7.44	3.721	17.47**
Within soils S <sub>0</sub>	3	0.30	0.101	<1NS
N	1	0.81	0.181	<1NS
D	1	0.00	0.002	<1NS
NXD	1	0.12	0.121	<1NS
Within soils S <sub>1</sub>	3	0.28	0.094	<1NS
N	1	0.25	0.255	1.20NS
D	1	0.16	0.162	<1NS
NXD	1	0.01	0.119	<1NS
Within soil S <sub>2</sub>	3	2.17	0.729	2.42*
N	1	1.10	1.103	5.18*
D	1	0.63	0.632	2.97ns
NXD	1	0.45	0.452	2.12ns
Error	33	7.03	0.213	

- NB:
- S<sub>0</sub> = Gituamba soil

S<sub>1</sub> = Kitale soil

S<sub>2</sub> = Kabete soil

N = inoculation

D = soil depth
  - \*\* = significantly different at p = 0.01

\* = significantly different at p = 0.05

NS = not significantly different



Appendix IVa

ANOVA for the effect of the soil depth and Rhizobium inoculation on root dry matter weight (g) of field beans (Phaseolus Vulgaris L.) cv "Rosecoco" for the three soils when no fertilizer was added on the soil.

Source of variation	df	SS	MS	F
Total	47			
Replicates	3	0.16		
Treatment	11	0.95	0.086	4.26**
Between soils	2	0.51	0.255	12.58**
Within soils S <sub>0</sub>	3	0.04	0.012	<1NS
N	1	0.00	0.000	<1NS
NXD	1	0.04	0.036	1.78NS
Within soils S <sub>1</sub>	3	0.39	0.129	6.38**
N	1	0.17	0.166	8.18**
D	1	0.13	0.128	6.30*
NXD	1	0.09	0.094	4.66*
Within soils S <sub>2</sub>	3	0.01	0.005	<1NS
N	1	0.00	0.000	<1NS
D	1	0.00	0.004	<1NS
NXD	1	0.01	0.010	<1NS
Error	33	0.64		

NB:

1. S<sub>0</sub> = Gituamba soil
- S<sub>1</sub> = Kitale soil
- S<sub>2</sub> = Kabete soil
- N = Inoculation
- D = soil depth
2. \*\* = significantly different at P = 0.01
- \* = significantly different at P = 0.05
- NS = not significantly different

## Appendix IVb

ANOVA: for the effect of the soil, Depth and Rhizobium inoculation on the root dry matter weight (g) of field beans (Phaseolus Vulgaris L.) cv "Rosecoco" for the three soils when TSP fertilizer was added on the soil at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha

Source of variation	df	SS	MS	F
Total	47			
Replicates	3	9.37		
Treatments	11	65.20	(5.927)	7.09**
Between soils	2	57.70	28.851	34.51**
Within soils S <sub>0</sub>	3	7.00	2.333	2.79NS
N	1	1.21	1.215	1.4NS
D	1	5.77	5.772	6.90**
NXD	1	0.01	0.013	<1NS
Within soils S <sub>1</sub>	3	0.05	0.017	<1NS
N	1	0.01	0.012	<1NS
D	1	0.00	0.006	<1NS
NXD	1	0.03	0.032	<1NS
Within soils S <sub>2</sub>	3	0.45	0.149	<1NS
N	1	0.24	0.242	<1NS
D	1	0.15	0.154	<1NS
NXD	1	0.15	0.154	<1NS
Error	33	27.59	0.836	

NB:

1. S<sub>0</sub> = Gituamba soilS<sub>1</sub> = Kitale soilS<sub>2</sub> = Kabete soil

N = inoculation

D = Soil depth

2. \*\* = significantly different at P = 0.01

\* = significantly different at P = 0.05

NS = not significantly different.

Appendix Va

ANOVA for the effect of the soil, depth and Rhizobium inoculation on the number of nodules formed on the root per plant of field beans (Phaseolus Vulgaris L) CV "Rosecoco" for the three soils when no fertilizer was added on the soil.

Source of variation	df	SS	MS	F
Total	47			
Replicates	3	0.04		
Treatments	11	464.82	42.256	5.51**
Between soils	2	300.15	150.077	19.60**
Within soil S <sub>0</sub>	3	0.000	0.000	<1NS
N	1	0.000	0.000	<1NS
D	1	0.00	0.000	<1NS
NXD	1	0.00	0.000	<1NS
Within soil S <sub>1</sub>	3	0.62	0.208	<1NS
N	1	0.17	0.166	<1NS
D	1	0.45	0.451	<1NS
NXD	1	0.00	0.006	<1NS
Within Soil S <sub>2</sub>	3	164.04	54.681	70.54**
N	1	163.88	163.881	211.41**
D	1	0.02	0.016	<1NS
NXD	1	0.14	0.145	<1NS
Error	33	25.58	0.775	

NB:

1. S<sub>0</sub> = Gituamba soil
- S<sub>1</sub> = Kitale soil
- S<sub>2</sub> = Kabete soil
- N = Inoculation
- D = soil depth

2. \*\*\* = significantly different at P = 0.01
- \* = significantly different at P = 0.05
- NS = not significantly different.

3. analysis of variance was done after the original data was transformed using  $(x + 1)^{1/2}$  transformation

## Appendix Vb

ANOVA : for the effect of the soils, depth and Rhizobium inoculation on the number of nodules on the root per plant of field beans (Phaseolus Vulgaris L) cv "Rosecoco" for the three soils when TSP fertilizer was added on the soil at the rate of 69kg P<sub>2</sub>O<sub>5</sub>/ha.

Source of variation	df	SS	MS	F
Total	47			
Replicate	3	26.57		
Treatment	11	515.34	46.849	7.93**
Between soils	2	472.02	236.009	39.96**
Within soils S <sub>0</sub>	3	5.01	1.669	<1NS
N	1	2.98	2.984	<1NS
D	1	1.32	1.324	<1NS
NXD	1	0.70	6.699	<1NS
Within soils S <sub>1</sub>	3	20.00	6.668	<1.13NS
N	1	6.71	6.711	1.14NS
D	1	8.86	8.862	1.50NS
NXD	1	4.43	4.432	<1NS
Within soil S <sub>2</sub>	3	18.31	6.103	1.03NS
N	1	0.47	0.466	<1NS
D	1	2.45	2.446	<1NS
NXD	1	15.40	15.396	2.61NS
Error	33	194.90	5.906	

## NB:

1. S<sub>0</sub> = Gituamba soil

S<sub>1</sub> = Kitale soil

S<sub>2</sub> = Kabete soil

N = inoculation

D = soil depth

2. \*\* = significantly different at P = 0.01

\* = significantly different at P = 0.05

NS = not significantly different.

3. Analysis of variance was done after the original data was transformed using (x)<sup>1/2</sup> transformation.