CORRELATION OF LEAF GLUCOSE CONTENT WITH MINERAL NUTRIENTS UPTAKE OF SOUTH AFRICAN GERANIUM *(Pelargonium sidoides)*

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A Research thesis submitted to the Graduate School in partial fulfillment of the requirements for the Master of Science degree in Chemistry of Egerton University

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DECLARATION AND RECOMMENDATION

DECLARATION

I James Kibiru Kinyua, hereby declare that this thesis is my original work and has not been submitted for an award of any degree in any other institution of learning to the best of my knowledge.

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RECOMMENDATION

We wish to confirm that this thesis was done under our supervision and has our approval to be presented for examination as per the Egerton University regulations.

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DEDICATION

To my wife and children for their prayers and encouragement, without whose, the products of this work would never have been realised.

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ABSTRACT

Pelargonium sidoides is a medicinal plant grown in Kenya for export to Germany where it is used in the manufacture of drugs for lung related diseases. The farm contributes to the well being of the local community by providing permanent employment to for over 80 persons, with 80 percent being women. It also offers free pre-primary education to their children including the ones from the neighbourhood. The broad spectrum of activity against viruses and bacteria may be caused by the coumarins and phenolic acids from a root of a healthy plant. Health of *P. sidoides* is controlled by appropriate mineral uptake and glucose concentration in the leaves. This concentration is governed by soil nutrients concentration and plant uptake capacity. Inorganic and organic fertilizers are normally applied to the crop. The relationship between nutrients uptake, glucose, and chlorophyll contents has not been established.

This study aimed at finding out the correlation between *P. sidoides* leaf glucose, chlorophyll contents and plant mineral nutrients uptake. *P. sidoides* plants from a selected field site, where the soil N, P, K and Ca contents had prior been analysed, were dressed with both inorganic and organic fertilizers (worm leachate) of known nutrient levels. Leaf glucose contents before and after application of the mineral nutrients was measured by colorimetry and results verified by high performance liquid chromatography fitted with a refractive index detector (HPLC). Mehlich Double Acid Extraction Method was used for the determination of P, Mg, Mn, Ca, K and Na on soil samples. P was determined colorimetrically, K and Na by flame photometer, and Mg, Mn and Ca by FAAS and N by Kjeldahl Method. Predictions for nutrients in the soil and plant tissues were done by DRIFT FTIR. FTIR predictions closely matched the soil's wet chemistry data. Leaf chlorophyll measurements were done by SPAD.

The data obtained was subjected to GENSTAT software for the ANOVA to find out whether there were any statistical significant differences in the means. Uptake of calcium fertilizer reflected the highest increase of glucose while that of worm leachate had the least effect. Chlorophyll content was also found to increase with nutrients uptake. Change in glucose level was evident within an hour after application, suggesting the possibility of predicting nutrients uptake within a short period of time. The study opened way for real time analysis of plant status and allowed for real time interventions where necessary.

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LIST OF ABREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometer
ANOVA	Analysis Of Variance
ATP	Adenosine Triphosphate
CRBD	Complete Random Block Design
DNS	Dinitrosalicyclic acid
DRIFT	Diffuse Reflectance Infrared Fourier Transform
EPs	Ethanolic *Pelargonium. sidoides*
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatograph-Mass Spectrometer
HPLC	High Performance Liquid Chromatograph
ICP-MS	Induced Coupled Plasma-Mass Spectrometer
KARI	Kenya Agricultural Research Institute
KASAL	Kenya Arid & Semi-Arid Lands Project
LC-MS	Liquid Chromatograph-Mass Spectrometer
meq	milliequivalent
MIR	Mid Infrared
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NIR	Near Infrared
non-GABH*S*	Non-Group A Beta Hemolytic *Streptococcus*
PC	Principal Components
PCA	Principal Components Analysis
OC	Organic Carbon
OPUS	Optical User Software
RID	Refractive Index Detector
RP	Reversed Phase
SSN	Sample Serial Number
SPAD	Soil Plant Analysis Development
Spc	Spectroscopic
TLC	Thin Layer Chromatography
UV/Vis	Ultra violet/Visible

CHAPTER ONE
INTRODUCTION

1.1 Background information

Most farmers and growers, have land whose soil has been in use for many years. On such lands, fertilizers applied over the years, have been nitrogen-phosphorus-potassium (N-P-K) and lime (Kinsey, 2001). Sometimes farmers have reported that in spite of the use of new seed varieties and other innovations, the yields or quality of their crops have either stagnated or begun to drop. This is in spite of using as much fertilizer as before. It is, however, important to note that plants will most likely take up nutrients from the soil in a given chemical form. The nutrient uptake depends on such parameters like soil pH and chemical structure of such plant nutrients (Fox, 2003). It is the expectations of any farmer to increase yields whenever fertilizers are applied. Good results are however achieved, when soil analysis has been carried out, correct fertilizer recommendations given and the necessary nutrients applied (Johnston, 2006).

Uptake of most plant mineral nutrients has the effect of altering leaf glucose contents in plants during photosynthesis. For instance, intake of CO_2, which is vital for glucose formation, is affected by changes in concentration of NO_3^-, PO_4^{3-}, and K^+ for plants like cotton. Studies on tomatoes showed correlation between leaf glucose concentrations and nutrients uptake as well as the intensity of the radiation applied (Claussen *et al*., 2006).

Mineral nutrients have a strong effect on photosynthates and their utilization in the roots (sink organs or storage sites). During photosynthesis, light energy is absorbed by chlorophyll and other pigments. It then converted into chemical energy in the form of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). Conversion of the light energy into chemical energy involves an electron flow between photosystem I (PS I) and photosystem II (PS II). This electron transport system is associated with regeneration of NADPH and establishment of an electrochemical gradient across the thylakoid membranes to produce ATP. NADPH and ATP are used as electron and energy sources at various steps of CO_2 reduction and carbohydrate synthesis. Mineral nutrients influence photosynthetic electron flow in various ways, either as constituents of the light-harvesting complex or as ions facilitating electron flow (Rengel, 1999).

Pelargonium sidoides (Umckaloabo, South African Geranium) is a perennial medicinal plant native to South Africa (plate1). The name is *Geraniaceae*, genius is *pelargonium* and the species is *sidoides* (Angiosperm Phylogeny Group, 2009).

It *P. sidoides* forms a rosette-like plant with crowded leaves. It is very similar to some forms of *P. reniforme,* but is easily distinguished by its blackish, rather than pink petals. The distinctive dark, reddish-purple (almost black) flowers are present almost throughout the year (Van der Walt and Vorster, 1988). The genus name *Pelargonium* is derived from the Greek word *Pelargos* which means stork. This refers to the rostrum of the schicocarb (seed capsule) which resembles the bill of a stock. The species name *sidoides* reflects the resemblance of the foliage to that of a European plant *Sida rhombifolia*. *P. sidoides* has a wide distribution. It occurs throughout the Eastern Cape, Lesotho, Free State and southern and south-western Guateng in South Africa. It grows in short grasslands and sometimes with occasional shrubs and trees on stony soil varying from sand to clay-loam. *P. sidoides* is found at altitudes ranging from near sea level to 2300 m. It is found in areas which receive rainfall varying from 200-280 mm per annum (http://www.plantzafrica, 2012).

Plate 1: Photograph of *Pelargonium sidoides* at a Rumuruti Farm

Cheap cold and flu medicines of various brands are widely available in the market under the Umcka brand name ("Umca" in Turkey). Studies have suggested that extracts from the plant could be used in treating acute bronchitis (Matthys *et al*., 2003, Chuchalin *et al*., 2005), acute non-Group A Beta Hemolytic *Streptococcus* (non-GABH), tonsillopharyngitis (sore throat) in children (Bereznoy *et al*., 2003) and the common cold (Lizogub *et al*., 2007). A systematic

review of these findings by the Cochrane Collaboration (an international network of people helping healthcare providers, policy makers, patients and their advocates) concluded that extracts of the plant might be effective in treating adults for acute rhinosinusitis and the common cold. The Cochrane Collaboration also noted that it might be effective in relieving the symptoms of acute bronchitis in adults and children, and also the symptoms of sinusitis (Timmer *et al*., 2008). In February 2009, the United Kingdom's largest pharmaceutical retailer, Boots Chemist, ran glossy magazine adverts offering *P. sidoides* drops as a traditional herbal remedy under the brand name "Kaloba". The plant is now important as foreign exchange earner, being exported to Germany, where it is used in the manufacture of chest related drugs. It grows in the wild in South Africa with some being cultivated as cash crop. The plant is currently being grown in Kenya at Rumuruti, Laikipia West District.

Chemical analysis of *P. sidoides* has led to characterization of about 65 metabolites including phenolic and cinnamic acids, tannins, flavonoids and coumarins (Kolodziej, 2000, Seidel and Taylor, 2004). Coumarins, glycosides and sulphates have been isolated from *P. sidoides* (Kayer and Tan, 2002). The broad spectrum of activity against viruses and bacteria may be caused by the coumarins and phenolic acids (Kolodziej, 2007).

Glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule. The carbohydrate part (glycone) is attached by an acetal linkage at carbon atom 1 to a non-carbohydrate residue called the aglycone which in most cases is non-sugar. Where the glycone is glucose, then the compound is called a glucoside (Brito-Arias and Marco, 2007). Some glycosides have antibacterial activity, which protect the plants from bacterial diseases (Bowyer *et al*., 1995).

A rapid TLC method, a HPLC-fingerprint analysis and HPLC-quantitative estimation were developed for coumarins containing the roots of *Pelargonium* species (Bladt and Wagner, 2007). Franco and de Oliveira (2010) presented a new, validated HPLC method for quality control of plant extracts and phytopharmaceuticals containing *P. sidoides*, using umckalin as chemical markers. TLC is a chromtographic technique of fractionating crude mixtures into their components by the movements of a solvent on a thin layer of suitable adsorbant. It has the advantage of speed, low cost and simplicity (Jork *et al*.,1990).

The total mineral content of EPs® 7630 (special aqueoues ethanolic *P. sidoides* roots extract) was found to be 10-12%. The cations were detected by ICP-MS: potassium (4%),

sodium (1.2%) and magnesium (0.4%). Anions were quantified by ion chromatography giving sulfate (4.5%), phosphate (2%) and chloride (1%) (Schoetz *et al*., 2008).

According to European Pharmacopoeia, *Pelargonium sidoides* root has to contain not less than 2.0% of tannis expressed as pyrogallol. The identification method of European Pharmacopoeia is thin layer chromatography of methanol root extract, but HPLC fingerprint analysis of *P. sidoides* extract have already been achieved (Bladt and Wagner, 2007). Schnitzer *et al*. (2008) analyzed the compounds of aqueous root extract of *P. sidoides* by LC-MS spectroscopy. Predominant coumarins, simple phenolic structure as well as flavonoid and catechin derivatives were identified as major constituents in *P. sidoides* extract.

According to a document on Biodiversity Management Plan for Pelargonium sidoides DC from South Africa (2013), due to lack of monitoring, the price paid to each trade chain is currently not know. The report suggests that prices be widely publicized and be published in government gazettes. The amount foreign exchange the plant earns for in Kenya was not available (David *et al.*, 2013).

1.2 Statement of the problem

Health of *P. Sidoides* is controlled by appropriate soil minerals uptake and glucose concentration in the leaves. This concentration is governed by soil nutrients concentration and plant uptake capacity. The nutrients are normally applied as commercial inorganic and organic fertilizers. Application of unnecessary and excessive fertilizers for *P. sidoides* production, leads to wastage of financial resources, and endangers the environment. There is need to establish the relationship between plant mineral nutrients uptake, glucose and chlorophyll contents in healthy plants. This way the correct type and form of fertilizer will be applied when needed.

1.3 Objectives

1.3.1 General objective

Determine the correlation between leaf glucose and chlorophyll contents with uptake of inorganic and organic fertilizers on *P. sidoides.*

1.3.2 Specific objectives

1. To determine available N, P, K and Ca in the soil in the experimental plot before application of recommended inorganic and organic fertilizers using both wet chemistry and FTIR.

2. To determine the available amount of N, P, K and Ca in organic fertilizer (worm juice).
3. To determine leaf glucose and chlorophyll contents on *P. sidoides* leaves before and after application of inorganic and organic fertilizers.
4. To determine Ca, N, P, K, Mg, Mn, and OC contents in the leaves *P. sidoides* before and after application of inorganic and organic fertilizers..

1.4 Hypotheses

1. There is no significant difference in values of N, P, K and Ca content in the soil analysed by wet chemistry from those analysed by FTIR.
2. Organic fertilizer (worm juice) does not contain significant levels of N, P, K and Ca.
3. There is no significant difference in *P. sidoides* leaf glucose and chlorophyll contents before and after the application of organic and inorganic fertilizers.
4. There is no significant difference between mineral nutrient contents in the leaves of *P. sidoides* before and after application of fertilizers.

1.5 Justification

P. sidoides is an important foreign exchange earner for Kenya. The quality of the root tuber extracts (the useful portion of the plant), determines commercial value of the exported produce. The quality of roots is determined by the importing agent and communicated to the farmer far much later after the produce has been processed. The relationship between fertilizers uptake, leaf glucose content and chlorophyll is not established. Leaf glucose is an indication of uptake of nutrients by the plants and imparts on the health of a *P. sidoides* plant. A healthy plant may correlate to high quality of root extract. Real time analysis of the plant status will allow for real time interventions where necessary. Determination of the appropriate fertilizers and uptake ability will also help growers to minimize fertilizer wastage, protect environment, and improve crop quality.

1.6 Expected outputs

1. Provide data for further research on the optimum soil nutrients requirement for a healthy and high yielding *P. sidoides* crop.
2. Obtain data on comparison of N P K and Ca availability between inorganic and organic (worm) fertilizers.
3. Publication in referred journal.
4. Be awarded a Master of Science degree in Chemistry.

CHAPTER TWO
LITERATURE REVIEW

2.1 Chemical process of photosynthesis

Photosynthesis is the process by which plants, some bacteria and some protists use the energy from sunlight to produce glucose from carbon dioxide and water (Smith, 1997). In photosynthesis water is the electron donor and, since its hydrolysis releases oxygen, the equation for this process is:

$$2n\ CO_2 + 4n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2 + 2n\ H_2O \quad (1)$$

Carbon dioxide + water + light energy → carbohydrate + oxygen + water

The 2n water molecules are cancelled on both sides, yielding:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2 \quad (2)$$

Carbon dioxide + water + light energy → carbohydrate + oxygen (Bryant and Frigaard 2006).

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs both light supply, as a source of energy to drive this process, and the electrons needed to convert carbon dioxide into a carbohydrate, which is a reduction reaction (Squire, 1990). In general, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy (Buick, 2008). Photosynthesis process leading to formation of glucose may be summarised by the equation;

$$6CO_2 + 6H_2O\ (+ \text{light energy}) \rightarrow C_6H_{12}O_6 + 6O_2 \quad (3)$$

This glucose can be converted into pyruvate which releases adenosine triphosphate (ATP) by cellular respiration while forming oxygen. The first product of photosynthesis is a three-carbon compound called glyceraldehydes 3-phosphate. Almost immediately, two of these join to form a glucose molecule (Bryant and Frigaard, 2006).

2.2 Effect of Photosynthesis and nutients concentration on plant uptake

Uptake of most plant mineral nutrients has the effect of altering leaf glucose contents in plants especially during photosynthesis. For instance, intake of CO_2, which is vital for glucose formation, is affected by changes in concentration of NO_3^-, PO_4^{3-}, and K^+ for plants like cotton (Radin *et al.*, 1978). Longstreth and Nobel (1980), also using cotton plants (*Gossypiun hirsutum*), studied the effect of nutrients on photosynthesis. They found out that the rate of CO_2 net uptake on cotton leaves reduced when the plants were grown at low concentrations of nitrogen (NO_3^-), potassium (K^+), or phosphorous (PO_4^{3-}). The reduction was primarily due to a decrease in CO_2 mesophyll conductance (Longstreth and Nobel, 1980).

Similar studies on tomatoes showed positive correlation between leaf glucose concentrations and nutrients (Claussen *et al*., 2006).

Mineral nutrients have a strong effect on photosynthates and their utilization in the roots (sink organs or storage sites). In photosynthesis process, light energy is absorbed by chlorophyll and other pigments and converted into chemical energy in the form of ATP (adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate). The light phase of photosynthesis requires the cooperation of two different chlorophyll assemblies and photochemical reactions, known as photosystem I (PS-I) and photosystem II (PS-II) (Vitousek *et al* 1986). Conversion of the light energy into chemical energy involves an electron flow between photosystem I (PS I) and photosystem II (PS II). This electron transport system is associated with regeneration of NADPH and establishment of an electrochemical gradient across the thylakoid membranes to produce ATP (Rojstaczer *et al*., 2001). NADPH and ATP are used as electron and energy sources at various steps of CO_2 reduction and carbohydrate synthesis. Mineral nutrients influence photosynthetic electron flow in various ways, either as constituents of the light-harvesting complex or as ions facilitating electron flow. Magnesium being a central part of chlorophyll molecules PS I and PS II plays an important role in absorption of light energy (Rengel, 1999).

2.3 Effect of mineral nutrients on plant sugar

In a study, where tomatoes were grown hydroponically at constant or changing nutrient concentration, changes in leaf proline content, reducing sugars, titratable acids, and osmolytes in the fruits were determined. Increase in radiation intensity had little effect on sugar contents at high nutrients concentration (Claussen *et al*., 2006).

A multiple regression analysis in leaves of beech revealed that magnesium, potassium and copper were closely correlated with total concentrations of sugars and phenols. The concentrations of sugars and phenols were also highly correlated. Thus a decline in leaf concentrations of mineral nutrients seems to be related to an increase in the concentrations of carbohydrates and secondary metabolites, including phenolic compounds (López-Granados *et al*., 2002). Leaf mineral nutrient concentrations were negatively related to leaf content of carbohydrates and phenols, suggesting that mineral nutrient deficiency leads to reduced growth and, hence, the retention in leaves of carbohydrates, which may be converted to secondary metabolites (Pai-ilsson, 1988).

Study in an olive orchard, showed that the sugars content in the leaves seems to be dependent of the spatial distribution of some soil properties. The highest leaf sugar contents had been observed in the area with better K contents. On the other hand, it was found that wood sugar content was low in areas with high Na content. This phenomenon may be caused by the concentration of these assimilates in leaves to balance the stress caused by the presence of Na (Gargouri *et al*., 2006).

2.4 Essential nutrients

In addition to carbon, hydrogen and oxygen which form the basis of all organic compounds, healthy plants requires sufficient amounts of 14 essential nutrient elements. These essential elements are divided into *macronutrients* (required in larger quantities because of their structural roles in the plant) and *micronutrients* (required in smaller quantities because they tend to be involved in regulatory roles in the plant) (Baker and Amacher, 1981). Carbon (C), Hydrogen (H), and Oxygen (O) form the bases of all organic compounds. Nitrogen (N), phosphorus (P) and potassium (K) are the primary macronutrients, and the ones most often in short supply in soils. The elements N, P and K are therefore the most likely to require replenishment in the form of applied fertilizer. Deficiencies of the secondary macronutrients, i.e. calcium (Ca), magnesium (Mg) and sulphur (S), are less commonly encountered (Baker and Eldershaw, 1993). The micronutrients required are iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), boron (B), chlorine (Cl) and nickel (Ni). Any of the above essential elements may also be present in excessive amounts, which can result in toxic effects (e.g. B and Mn). Other elements or groups of elements (e.g. sodium, bicarbonate) may also contribute to the toxic effects seen, for example, in saline or sodic soils. Sodium (Na) has been demonstrated to be

an essential element for some plants with a special photosynthetic pathway, but in practice, problems results from excessive amounts of Na, and not its deficiencies (Carrow *et al*., 2001).

Studies have also been carried out on the levels of sugar in sap and root for boron deficiency and toxicity results in both cases have made the assumption that boron plays a role in the photosynthesis or in one of the initial stages of sugar metabolism as well as in sugar transport (Raza *et al.,* 2002). Some work has been done on the effect of boron on the metabolism of nucleic acids and on photosynthesis (Dugger, 1973).

2.4.1 Production of compost tea

Compost tea or worm juice (leachate) is a by-product of composting. The leachate is collected from a filtration system. Normally in a compost bin, there are holes at the bottom of the bin to enable worm juice to be collected from the system. The compost worms consume organic matter which passes through the gut system which is later converted into worm castings. These castings are then brewed in highly oxygenated water to accelerate growth of beneficial microorganisms to further enhance degradation. The leachate is then collected at the bottom of the tray and then poured back into the compost bin to refine the process. It is the enzyme in the worm's digestive systems that allows all the nutrients and trace elements to become water soluble, therefore readily available for plant life (Shuster *et al.,* 2004).

Plate 2 shows worm tanks used for making leachate for this project. Manure compost tea is effective in the control of many pests because of certain microorganisms that exist in it naturally such as black spot on roses and early blight on tomatoes (Brinton, 2004). The primary benefit of the compost tea is supply of soluble nutrients, which can be used as liquid fertilizer. Compost tea has resistance-inducing properties and functions as a fertilizer that inhibits the development of plant pathogens (Siddiqui *et al*., 2009).

Plate 2: Photograph of biodegradation tanks for earthworm leachate production

2.5 Soil properties and productive capacity

The productive capacity of a soil often depends on complex interactions between the biological, chemical and physical properties of the soil. Good farm practice aims to manage the various factors that make up each of these three properties to optimise the yields of crops in environmentally friendly ways (http://www.hydroponicsearch.com, 2009). Soil analysis is an aid to managing soil nutrients efficiently to maintain soil fertility for those nutrients like phosphorous, potassium, nitrogen and magnesium that are retained in soil in plant available forms. If the amount of any of these nutrients in such forms in soil is too low, the yield is jeopardised, whereas increasing reserves in agricultural soils to very high levels is an unnecessary expense (http://www.eldoradochemical.com/fertiliz1.htm, 2009). The essential feature in crop nutrition is that there must be sufficient P, K, N, and Mg in the soil solution and readily available pool to meet both maximum daily demands for each nutrient in the early stages of growth and the maximum uptake to achieve optimum yield (Johnston, 2006).

2.6 The Importance of soil organic matter or humus

The terms soil organic matter and humus are frequently used interchangeably and have essentially the same meaning. Humus levels are not given as indexes because the level depends on the soil and farming system and critical values have rarely been determined. Humus itself is largely the end product of the microbial breakdown of organic matter added to soil. The amount of humus in soil cannot be measured directly but is estimated by measuring the percentage carbon (C) and multiplying this by 1.72 to give percentage soil organic matter or humus

(Fageria, 2002). Humus is important in maintaining soil structure, in slightly increasing the soil's water holding capacity and holding a small store of nitrogen, potassium, sulphur and trace elements in organic forms. These cannot be taken up directly by plant roots but have first to be converted by soil microbes to inorganic (ionic) forms identical to those supplied in fertilizers (Johnston**,** 2006).

2.7 Soil nutrients and factors influencing their availability.

The availability of nutrients, in particular of nitrogen (N), is one of the most important determinants of vegetation composition and N is the limiting resource for plant growth in many ecosystems (Vitousek, 1982). It has been shown by previous researches that the concentrations of soil nutrients (e.g., organic C, N, P, and K) are good indicators of soil quality and productivity because of their favorable effects on the physical, chemical, and biological properties of soil (Cao *et al.*,2011).

Soil pH affects the chemical reactions in soil (Zhao *et al*., 2011). Extremes of pH in soils, will lead to a rapid increase in net negative surface charge and thus increases the soil's affinity for metal ions (Wu *et al.,* 2003, Yang *et al.,* 2006). Soil organic components, such as soil organic carbon or total nitrogen are the most critical indices of soil fertility (Liu *et al*., 2011). Dynamics of soil organic carbon and total nitrogen storage in agricultural soils drives microbial activity and nutrient cycles, promotes soil physical properties and water retention capacity, and reduces erosion (Manna *et al*., 2007). It has been found that soil available nutrients (including N, P and K), coming from mineralization and available components of fertilizer, can be directly absorbed by plants, contributing greatly to the soil fertility (Vogeler *et al*., 2009).

Plant roots absorb phosphorus from the soil solution. In comparison to other macronutrients, the phosphorus concentration in the soil solution is much lower and ranges from 0.001 mg/Kg to 1 mg/Kg (Brady and Weil, 2002). Roots absorb phosphorus in the form of orthophosphate, but can also absorb certain forms of organic phosphorus (Brady and Weil, 2002). Phosphorus moves to the root surface through diffusion. Since phosphate is an anion, particles that generate an anion exchange capacity forms strong bonds with phosphate.

Nitrogen in soils can be in various different forms. Nitrogen is very dynamic and is constantly changing chemical species and concentrations. In most soils, nitrate is the common ionic form of plant-available nitrogen, but this element may also exist as ammonium (NH_4^+) or nitrite (NO_2^-) as well as other ions. Nitrogen is also incorporated in organic matter and microbes.

When organic matter decomposes, nitrogen is released in various forms into soil solution (Brady and Weil, 1999).

The original sources of potassium are the primary minerals, such as micas (biotite and muscovite) and potassium feldspar (orthoclase and microcline). As these minerals weather, the potassium becomes more available as readily exchangeable and soluble potassium which can be absorbed by the plant roots. At any one time, most soil potassium is in primary minerals and non-exchangeable and soil solution forms that plants can use directly, may be sufficiently rapid to keep plants supplied with enough potassium for optimum growth. Conversely, in relatively non-fertile soils, the levels of exchangeable and solution potassium may have to be supplemented by outside sources, such as chemical fertilizers, poultry manure, or wood ashes (Brady and Weil, 1999).

With the development of agricultural production, fertilization has been widely used as a common management practice to maintain soil fertility and crop yields (Shen *et al*., 2010).
Studies have been documented showing that use of fertilizers is necessary, and that continuous fertilizer application increases the concentrations of soil organic carbon, total nitrogen and other nutrients in the soil compared with the initial value before applications (Whitbread *et al*., 2003, Huang *et al*., 2010). However, other studies have shown that the continued use of fertilizers may result in the decline of soil quality and productivity (Yang *et al*, 2006).

2.8 Real-time analysis of plant nutritional status

Real-time analysis provides a constant stream of data which reflects a changing situation in real time. This is where changes in plant nutritional status is studied as these changes occur. Real-time diagnosis was first used by Roppongi (1991) in Japan using the fluid from plant tissues. He showed the relationship between the level of fertilizer applications and the nitrate concentration in the fluid of cucumbers. The ideal nitrogen concentration was determined according to the fruit yield of plant nutrition. Real-time analysis monitors the nutritional status of the plant. The samples of plant tissue used for analysis are taken from the living plant (Roppongi, 1991).

Further work has since been done to validate the accuracy of conventional analytical tools, and their use to measure nutrient levels in the fluids of living crop plants (Hiraoka *et al.*, 1990, Takebe, 2000, Kamiyama *et al.*, 2000, Morita and Ohta, 2001). In addition to nitrate and potassium, the applicability of these tools to phosphate, borate and organic components as

ascorbate or oxalate have been examined, and the results reported (Hiraoka *et al.,* 1990, Takebe, 2000, Kamiyama *et al.,* 2000, Morita and Ohta, 2001).

Takebe compared the levels of nitrate and phosphate both in fluids extruded from the crushed petiole, and water extract from crushed petioles of potato. The nitrate concentration was similar in samples from both extraction methods, except when the concentrations were very low. However, phosphate levels in the fluid were lower by 5 to 40 percent than in the water extracts. In spite of this, the level of inorganic P in the fluid showed a high correlation with the total P in the above-ground portion of the plant. These findings suggest that N and P levels in the fluid reflect the status of the whole plant (Takebe, 2000).

2.9 Fertilizer use and the environment

One of the main aims of plant nutrient analysis is to adopt fertilizer applications that match plant demand. This can give a high level of fertilizer availability, and helps reduce the leaching of fertilizer elements, especially nitrate which is a common contaminant of groundwater (Maeda *et al.,* 2003). It is also generally believed that nitrous oxide, a gas which is emitted by vegetable or fruit gardens when too much nitrogen fertilizer is added, has a strong effect on global warming (Maeda *et al.,* 2003). Ammonium (NH_4^+) and nitrate (NO_3^-) are the most important inorganic nitrogen sources in soils readily available to plants. NH_4^+, when supplied solely at high concentrations, is toxic and impairs plant growth (Gerendas *et al*., 1997, Britto and Kronzucker, 2002). Phosphates are a major source of pollution in lakes and streams, and high phosphate levels support over-production of algae and water weeds (Glasser, 2002).

2.10 Prediction of soil mineral nutrient by FTIR

An FT-IR (Fourier Transform InfraRed) Spectrometer is an instrument which acquires broadband NIR to FIR spectra. Unlike a dispersive instrument, i.e. grating monochromator or spectrograph, FT-IR Spectrometers collect all wavelengths simultaneously. FT-IR is a method of obtaining infrared spectra by first collecting an interferogram of a sample signal using an interferometer, and then performing a Fourier Transform (FT) on the interferogram to obtain the spectrum. An FT-IR Spectrometer collects and digitizes the interferogram, performs the FT function, and displays the spectrum (http://www.newport.com, 2014).

In spectroscopic analysis, visible (Vis), near infrared (NIR) and mid infrared (MIR) ranges are often used as they provide plenty of information on physical, chemical and biological

properties of objects. Wavelength ranges are 350 to 760 nm for Vis, 760-2500 nm for NIR, and 2500 to 25000 nm for MIR (often used in its wavenumber form 4000 to 400 cm-1). Frequencies in the Vis are due to electronic transition while those in the NIR are generally overtones and combination bands from the fundamental vibrations occur in the MIR, mainly O-H, N-H, and C-H bonds (Viscarra *et al.*, 2006).

When NIR and MIR radiations are focused onto a sample, the molecules in the sample will increase their vibration energy by absorbing energy at specific frequencies depending on the molecular geometry, bond strengths and atomic masses. The resulting Vis, NIR and MIR lights are thus modified, creating a spectrum or 'signatures' of the targeted object with peaks at the absorbing frequencies. The combined contributions from the various soil components can result in a very complex spectrum, difficult to analyze visually, but multivariate calibration models can be built to derive useful qualitative and quantitative relationships or models between the spectral signatures and many soil properties (Viscarra *et al*., 2010). Spectrometry is the combination of spectroscopy and chemometric (multivariate statistical) methods. The Vis-NIR-MIR spectrometry technique can predict multiple soil properties simultaneously (Mouazen *et al.,* 2006).

Numerous analyses of soil N and C have been conducted during the past decades using this technique, for examples, to predict the soil C and N mineralization rates (Fystro, 2002, Mutuo *et al.*, 2006).

2.10.1 Principles of DRIFT

Diffuse reflectance occurs when light impinges on the surface of a material and is partially reflected and transmitted. Light that passes into the material may be absorbed or reflected out again. Hence, the radiation that reflects from an absorbing material is composed of surface-reflected and bulk re-emitted components, which summed are the diffuse 190 Infrared Spectroscopy – Life and Biomedical Sciences reflectance of the sample (http://www.uksaf.org, 2012). In practice, DRIFT is used for soil analysis in diffuse reflection mode, where the incoming radiation is focused onto the soil sample surface, often in the form of a dry powder or <1 mm micro-aggregates, and the reflected radiation is passed back into the spectrophotometer (http://www.clw.csiro.au, 2012).

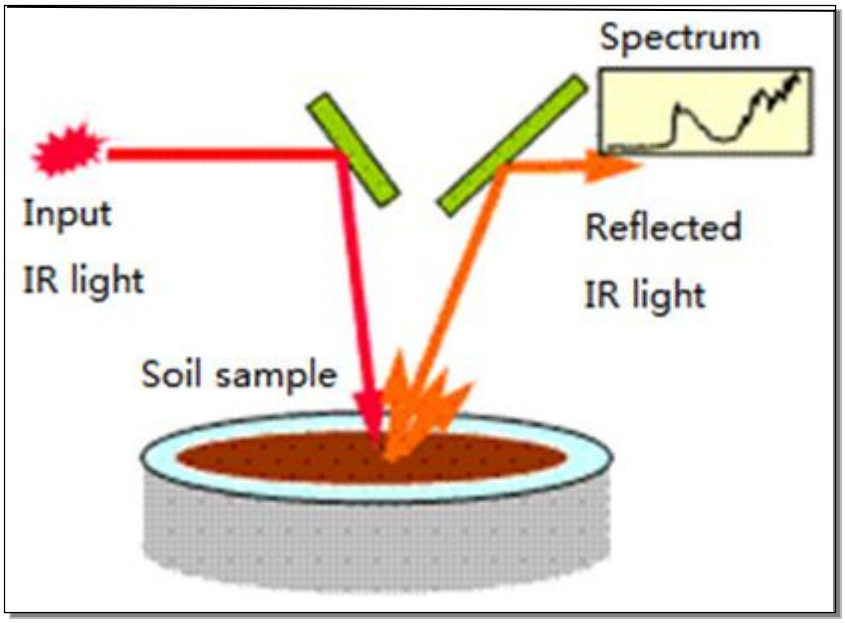


Figure 1: Description of the method for acquiring a DRIFT spectrum (www.intechopen.com, 2012)

2.10.2 Use of principal components analysis (PCA)

PCA is a data compression process (i.e. a bilinear modeling process), which can be used to reduce a complex multidimensional data (e.g. spectra) into a smaller number of principal components (PCs) which reflect the underling structure of the original dataset. The first principal component typically explains most of the variation in the dataset with further principal components being orthogonal to the preceding PC and explaining less variation in the dataset. By plotting the PCs in two or three dimensional data space, interrelationships between the samples and variables, can be examined (http://www.clw.csiro.au, 2012).

2.11 Principles of a chlorophyll meter

A chlorophyll meter (Minolta™ SPAD-502) has been developed to estimate the nitrogen status of crops. The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values the instrument calculates a SPAD (Soil Plant Analysis Development) value that is quite well correlated with chlorophyll content (Wood *et al.,* 1993, Markwell *et al.,* 1995).

2.12 Flame Atomic Absorption Spectrometry (FAAS)

A Flame Atomic Absorption Spectrophotometer (FAAS) is an instrument for the determination of metal elements. It is widely applied for samples such as, agricultural chemicals, clinical and biochemistry, minerals, food, drugs and environmental. Atomisation is achieved by spraying the analyte into to the flame by a nebulizer. An atomised element absorbs energy of a wavelength that is characteristic to that element (Skoog *et al.,* 1998). The method uses a hollow cathode lamp (HCL) as its light source. The HCL emits light of a wavelength that is characteristic the element of interest. Elements in a solution are atomised by heat from an air/acetylene or nitrous oxide/acetylene flame (2000K to 3000K). The amount of radiation absorbed is directly proportional to the atom concentration. When radiation of a certain wavelength is passed through the atomic vapour in ground state, part of this radiation is absorbed by atoms and which are then raised to the first excited electronic level (Willard *et al.,* 1986).

Most FAAS instruments are designed for both absorption and emission spectroscopy. In atomic emission sepectroscopy (AES), the sample is sprayed into the flame by the nebulizer where it is atomised. The atoms absorbs the energy from the flame and becomes excited to higher engergy levels in their atomic form. As the atoms fall back to their ground state, they emit the absorbed radiation at their characteristic wavelengths. The emitted radiation, which is proportional to the concentration of the analyte, is measured. (Fifield and Kealey, 2000).

2.13 Principles of High-performance liquid chromatography

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column (Skoog *et al*., 1998).

The sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (stationary phase). The velocity of

each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte (Skoog *et al*., 1998).

The refractive index detector (RI) is constructed as a flow-through differential refractometer which continuously measures the difference ∆n of the refractive index of the eluate and the pure eluent. The ability of a compound or solvent to deflect light provides a way to detect it. The RI is a measure of molecule's ability to deflect light in a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference flow cell. The amount of deflection is proportional to concentration. The RI detector is considered to be a universal detector but it is not very sensitive (Phyllis *et al.*, 1997).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Selection of project site, plant materials and soil sampling

The experimental field was in Laikipia West district near Rumuruti, 0.41^0 North, 36.6^0 East (Figure 2). The field was part of a large commercial plantation of *P. sidoides* plants and consent was sought from the owner for the project to be carried out there.

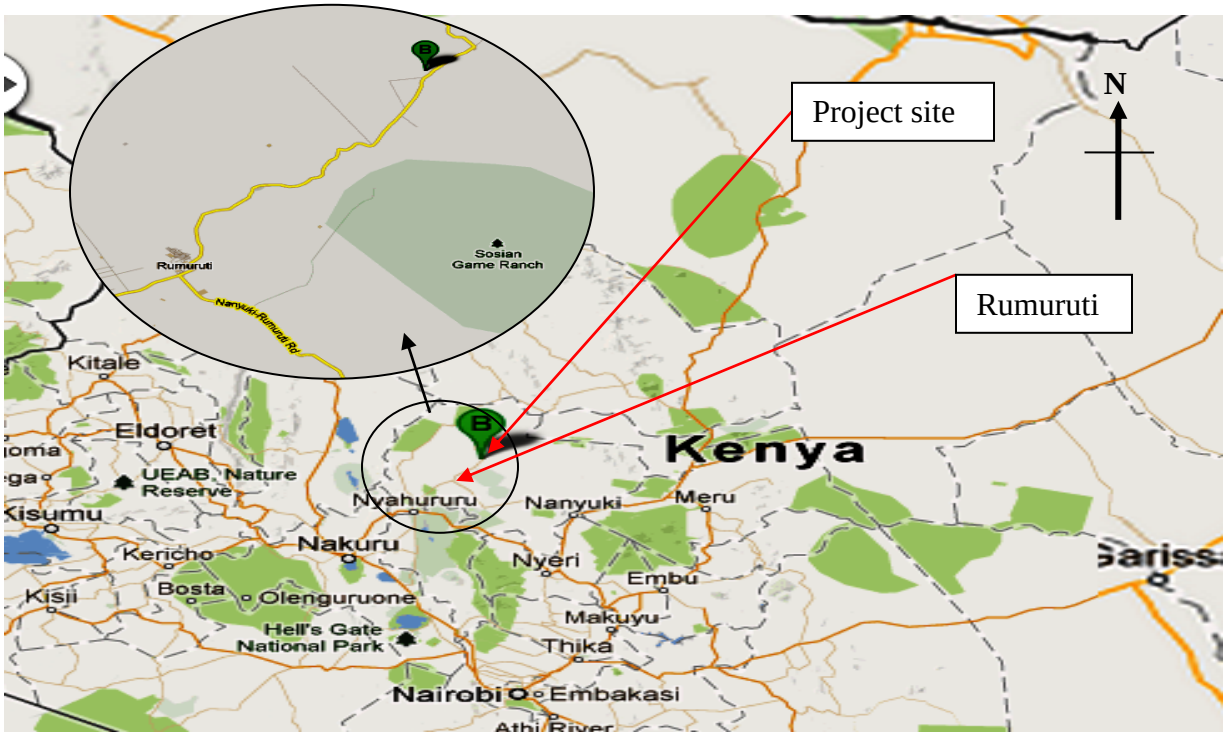


Figure 2: Project site at Rumuruti- 0.41^0 North, 36.6^0 East

A portion of the *P. sidoides* plantation was selected. Sampling, fertilizers application and sample analysis was carried out between January 2011 and December 2011. Soil and plant leaf samples were collected for analysis before the study began, so as to get baseline levels of Nitrogen, Phosphorous, Potassium, Magnesium, and Calcium in the soil and the initial levels of leaf glucose and chlorophyll. The plants were randomly divided into ten blocks of 3 rows each. Each row was 40 metres long and contained 160 plants. The blocks were divided into 3 replicates plus a control for each application to facilitate application of both commercial inorganic and organic fertilizers as shown on Table 1. Five grammes of each nutrient was applied to each plant in the rows while worm juice in ratio 1:1 water was applied with a watering can,

directing the solution into the roots. This was a complete randomized block design (CRBD) statistical experimental design (Clewer *et al.,* 2001).

Table 1: Details for fertilizers application used in the project

Block No.	Plot Name	Treatment	No. of Reps + Control
1	Worm Juice (WJ)	1:1 $^{v}/_{v}$ Worm Leachate :Water	4
2	Calcium Ammonium Nitrate (CAN)	Nitrogen Fertilizer	4
3	Triple Super Phosphate (TSP)	Phosphate Fertilizer (0: 40:0 +3S)	4
4	Potassium Nitrate (KNO_3)	Potassium Fertilizer (13:0:46)	4
5	Calcium Carbonate ($CaCO_3$	Calcium Fertilizer (26:0:0 +20Ca)	4
6	WJ+CAN	1:1$^{v}/_{v}$ Worm Juice : Water + N Fertilizer	4
7	WJ+TSP	1:1 $^{v}/_{v}$ Worm Juice : Water + P Fertilizer	4
8	WJ+KNO_3	1:1 $^{v}/_{v}$ Worm Juice : Water + K Fertilizer	4
9	WJ+CaCO3	1:1$^{v}/_{v}$ Worm Juice : + Ca Fertilizer	4
10	WJ+CAN+TSP+KNO_3+$CaCO_3$	1:1 $^{v}/_{v}$ Worm Juice : Water + N, K & P + Ca Fertilizers	4

Worm juice was prepared by sprinkling water on top of large worm tank and collecting the effluent at the bottom of the tank. The effluent was collected into a 200 litres drum. It was then 'brewed' for 48 hours by introducing oxygen (air) through the effluent using an aquarium pump. The highly oxygenated water assisted to accelerate growth of beneficial microorganisms to further enhance degradation. This solution was applied to the plant as organic fertilizer or worm juice (plate 2).

The soil samples were taken with a 10 mm diameter auger to a depth of 60 cm. This depth was chosen because this was the depth where most of the plant's roots grew. Since the soil and plot topography were evenly distributed, a shape of letter 'W' was used to mark the sampling locations (Hue *et al.,* 1997). Six locations within the field were sampled as shown on Figure 3 below. Three adjacent holes were sampled from each location, mixed well to form a

composite sample, and then the final 1kg portion was taken. The samples were placed in paper bags, labeled and then transported to laboratory.

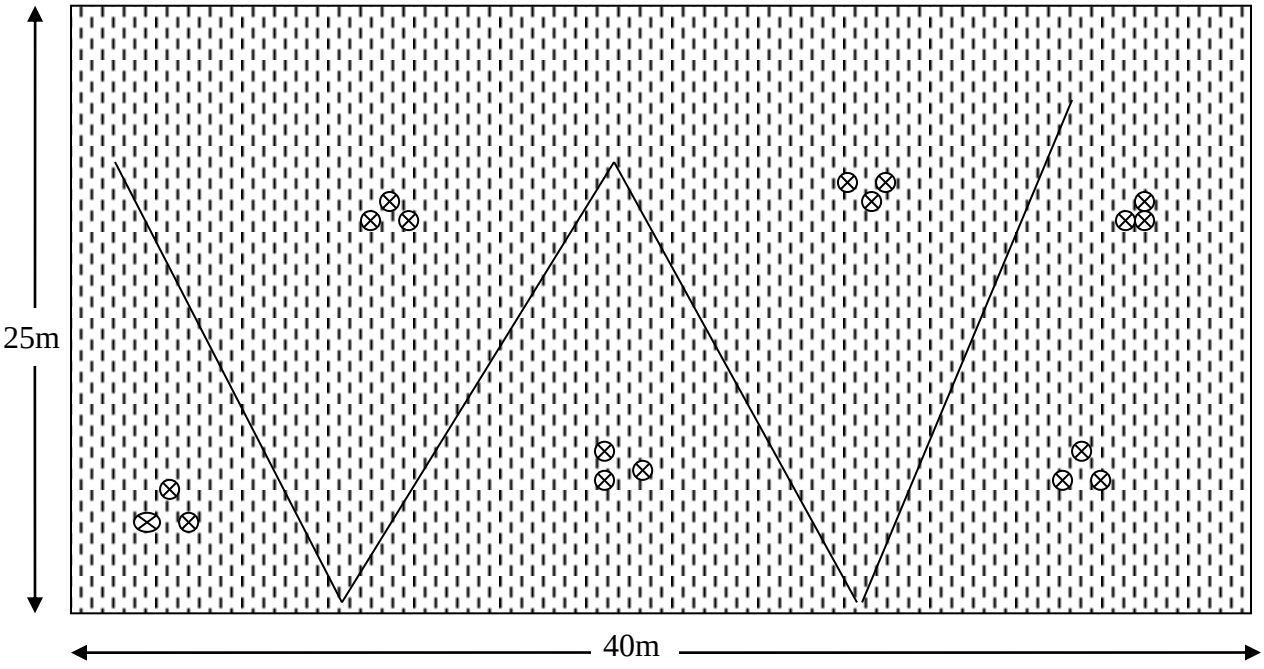


Figure 3: Layout of the plot showing the soil sampling spots

3.2 Application of fertilizers and sampling of leaves

Commercial inorganic N, P, K, Mg, Ca-based and organic fertilizers were applied to blocks each. Commercial fertilizers were purchased from Yara East Africa Ltd. Fertilizer applications was done only once during the three months of study. The biodegradation tanks (Plate 2) for earthworm leachate production were sprinkled with water and the leachate collected through drain taps at the bottom of the tanks. The leachate was then poured into a 200 litres tank, aerated with an air pump for 48 hours, to increase an aerobic (oxygenated) environment for enhanced microorganisms' activities. The resultant product was used on the plants as Worm Juice (WJ). The study site was a 25 m x 40 m plot of land containing 22 rows of *P. sidoides* plants and 160 plants per row. Leaves were randomly sampled in a cross-sectional pattern from the top of the plant, picking 10 leaves from each plant. A total of 45 plants were randomly sampled in each block. The samples were then combined to form three composite samples for each row. Sampling was carried out at intervals of 1, 2, 3, 6, 12, and 24 hours. Untreated plants were used as control samples. The control samples were taken from the untreated rows left

within any three replicates. Sampling was done between 10.00am and 4.00pm time of the day. Glucose was extracted in the field to an aqueous solution which was carried in a cold box to the laboratory.

3.3 Preparation of glucose extracts

Two grammes of leaves were weighed out and crushed in a pestle and mortar and then 10 ml of acetone were added as the crushing continued to extract the glucose. Acetone was used as the extracting solvent because it extracts more components and partitions well in hexane/water mixture. The extracts were filtered through a Whatman No 1 filter paper and the fluid collected in 100 ml test tubes before 10 ml n-hexane were added and mixed well. This was followed by10 ml of distilled water before mixing again to form both the aqueous and organic layers. The n-hexane organic layer being lighter than water separated on the top of the aqueous layer. The aqueous layer was collected into 100 ml conical flasks, corked and labeled. The same was repeated for all leaves samples collected at the respective intervals. Distilled water (3 ml) was added to each tube to dilute the samples before glucose determination.

3.3.1 Measurement of leaf glucose content

Glucose analysis was carried out in the laboratory at KARI after every sampling trip throughout the study. The analytical method as outlined by Miller, (1979) was followed. To prepare standard calibration curve, a set of five standards of glucose of concentrations: 1%, 0.5%, 0.1%, 0.05%, and 0% w/v were diluted in distilled water. (Glucose standard and other reagents were obtained from PROLABO™). To the standards, 3 ml of 1% Dinitrosalicylic acid solution were added to 3 ml of each glucose solution. (1% DNS solution was prepared by mixing 2.50 g of DNS with 0.125 g of sodium sulfite and 2.5 g of sodium hydroxide in 200 ml of distilled water and then topped up to 250 ml with distilled water. The solution was then topped up to 250 ml). The test tubes were covered with glass caps to avoid evaporation. The mixture was heated at 90 °C for 10 minutes (until a red-brown colour was obtained). One milliltre of 40% potassium sodium tartrate solution was added to stabilize the colour. The solutions were then cooled to room temperature in a cold water bath, before addition of 1 ml of phenol solution to intensify the color. The standards absorbancies were then read on a colorimeter at 540 nm to obtain a calibration curve. The samples were then run on the colorimeter and their glucose

concentration calculated from the calibration curve. The overall reaction could be summarized as in equation (4);

$C_7H_4N_2O_7$ + $C_6H_{12}O_6$ ⟶ $C_7H_6N_2O_5$ (4)

(3, 5-Dinitrosalicyclic acid) (Glucose) (3-Amino-5-nitrosalicyclic acid)

The method involved the reduction of 3, 5-dinitrosalicylic acid to 3- amino, 5-nitrosalicyclic acid by the free carbonyl group (-C= O) in the glucose molecule under alkaline conditions. Dissolved oxygen interfered with the oxidation of glucose. The sulphate added to the DNS mixture, which itself was not involved in the colour formation, absorbed the dissolved oxygen. In the reaction, one mole of glucose reacted with one mole of 3, 5-dinitrosalicylic acid (Miller, 1979).

3.3.2 Analysis of glucose by HPLC

The glucose samples were re-diluted in a 25:75 water **:** acetonitrile mixture mobile phase and analysed in HPLC equipment (Shimadzu model, SCL -10A) fitted with a refractive index detector (model RID - 6A). An EC 250/4 Nucleodur 100-5 NH_2 –RP (250 mm and 4 mm internal diameter colum) at 35^oC was used for the separation. A manual injection valve (Rheodyne 7125) fitted with a 20 micro litres sample loop was used. The sample injection volume was 20 micro litres. The mobile phase for eluting the sample through the analytical column, was a 25:75 water **:** acetonitrile mixture at flow rate of 0.80ml/min. Glucose standards in ranges of 1%, 0.5%, 0.1% and 0.05% w/v, were prepared, injected into the HPLC column and peak areas recorded in the HPLC software and stored in the computer. The peak areas were used to plot a standard calibration curve. The procedure was repeated for the unknowns to get their peak areas. Using peak areas obtained for the unknowns, the concentrations of glucose in the samples were interpolated from the curve.

3.4 Chlorophyll measurements by SPAD meter

Six young fully expanded leaves were randomly selected and measured from each of the 6 plants selected in each block for SPAD measurement. A photograph of the meter (Minolta™, model SPAD-502) used is shown on Plate 3. The initial chlorophyll measurements were done when the plants were twenty four weeks old from day of planting. Triplicate readings were

taken; on one side of the midrib of each single leaf blade, midway between the leaf base and tip and then averaged. Chlorophyll measurements were carried on week two and three months (12 weeks) after application of fertilizers followed by sampling on the leaves for glucose analysis.

Plate 3: Photograph of chlorophyll measurements SPAD meter

3.5 Mid Infrared (MIR) spectra of soil samples

Soil samples were collected with an auger to a depth of 60 cm because this is depth within which most of the plant's roots grows. The soil samples were oven dried at 40℃ for 48 hours and then crushed on a pestle and mortar. After that, the samples were sieved through a 2 mm mesh to remove large particles and sieved again though a 0.09 mm mesh sieve. The samples were placed in stainless steel cups using the sample filling appliances. The sample cup fitted into the sample carrier which held both the sample cup and an empty gold cup (which was used for background correction since gold does not absorb IR radiation).

MIR spectra were collected in an ALPHA™ Fourier Transform Infrared (FTIR) spectrometer (Bruker Optics, USA) with wavelength ranging from 4000-400 cm^{-1}, and equipped with DRIFT (diffuse reflectance infrared Fourier Transform) sampling modules. The Bruker ALPHA™ FTIR spectrometer, the sample filling appliances and the sample rack are shown on plates 4, 5 and 6 respectively.

Plate 4: Photograph of Bruker Alpha FTIR spectrometer

Plate 5: Photograph of the sample filling appliance

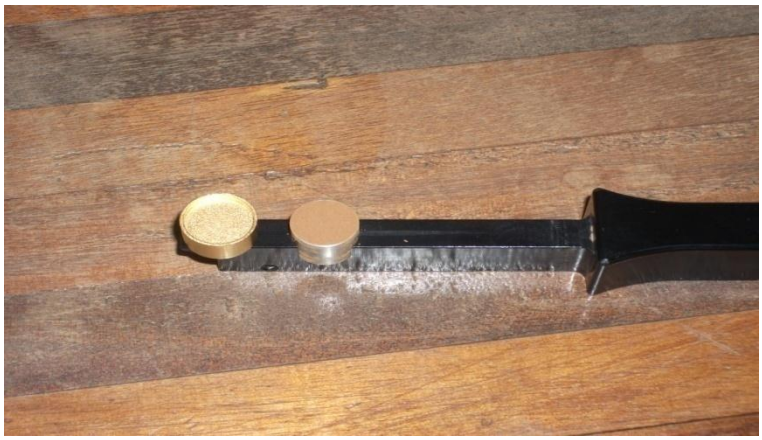


Plate 6: Photograph showing the sample rack, sample cup and the gold cup

The sample carrier once fitted in the FTIR equipment, was pushed in or pulled out to select the sample cup or the gold cup for measurement respectfully. The gold cup was used for

background signal correction. The spectrums obtained were in the OPUS 6.0 (Optical User Software) format and needed to be converted to spc files (file format in which all kinds of spectroscopic data, including amongst others infrared spectra, Raman spectra and UV/VIS spectra can be stored) so that they could be subjected to 'R' statistical software. The spc files were converted to alpha MIR file format before the software was used to plot the calibrations for each variable and the predictions. To achieve this, a script was prior prepared in 'R' statistical software. The spectral information in the spc files and the alpha MIR files format was too bulky to be easily handled by Microsoft excel and therefore the necessity of using 'R' statistical software.

3.6 Mehlich double acid extraction method for soil samples

The method involved the extraction of a soil samples in a ratio of 1:5 w/v (weight/volume) with a mixture of 0.05 N HCl and 0.025N H_2SO_4 solution. This extracting mixture was prepared by diluting 4ml concentrated HCl and 0.7 ml H_2SO_4 acids to 1litre of distilled water. The HCl assists to replace the bulk of the exchangeable metal cations, while the sulphuric acid's sulphate anions replaces the available soluble phosphorous which is held in the soil in an exchangeable form. Decolourising charcoal was added to absorb organic matter so as to obtain a clear filtrate. Calcium, potassium and sodium were analysed using flame photometry while phosphorous, magnesium and manganese were analysed by FAAS.

3.6.1 Extraction procedure

Within an accuracy of 0.01 g, 5 g of dried soil sample was weighed and transferred onto a 100 ml extraction bottle. About 0.5 g of activated decolourising charcoal was then added to remove organic matter followed by 25 ml of the 0.05 N HCl/0.025 N H_2SO_4 extraction mixture. The bottles were stoppered and shaken at room temperature for one hour on a reciprocating shaker at 120 oscillations per min. The suspension was filtered through Whatman No 1 filter paper into clean 100 ml test tubes. A blank sample was included for each extract as a control (Mehlich, 1953).

3.6.2 Determination of Phosphorus

A series of 0, 10, 20, 30, 50 ppm phosphorous standard solutions (diluted from 500 ppm stock solution obtained from Sherwood Scientific Ltd, UK) were prepared in 100 ml volumetric

flasks. Using a pipette, 5 ml of working standards, the soil extract and the blank sample were placed into clean test tubes before addition of 1 ml ammonium vanadate-molybdate mixture. Under acidic conditions, the orthophosphate ion, PO_4^{3-}, reacted with ammonium molybdate to form molybdophosphoric acid. A yellow compound vanadomolybdophosphoric acid, was formed in the presence of vanadium. The color intensity produced depended on the amount of phosphate present, and the acidity of the solution. By maintaining the same acidity in all the solutions and by adding the same large excess of vanadate/molybdate reagent to each solution, it was ensured that the amount of phosphate present controlled the intensity of color developed. The solution was mixed well, put in a 10 mm curvette and absorbance read at 430 nm on a Scanning UV/ VIS Spectrophotometer (PHILIPS™ PU 8730).

3.6.3 Determination of Calcium, Potassium and Sodium

The standard mixtures were prepared as follows; 0, 5, 10, 15, 20 meq Ca/100 ml and 0, 0.1, 0.2, 0.3, 0.4 meq K and Na/100 ml in 100 ml volumetric flasks (diluted from 1000 ppm stock solutions obtained from Sherwood Scientific Ltd, UK) both for potassium and sodium. Using pipettes, 2 ml of working standard, soil extract and reagent blank were placed into 25 ml vials. To each vial, 1 ml of 2% lanthanum solution and 14 ml of distilled water were added. The lanthanum prevented the calcium from forming complexes with phosphates which would hinder its atomization in the flame. The samples were then analysed by aspirating them into a flame photometer (CORNING 400™) using the respective optical filters. A standard calibration curve was obtained by plotting the emission reading versus the concentration of the standard solutions. Using the calibration curve, the sample concentrations were intrapolated from the emission readings.

3.6.4 Optimization of the AANALYST™ 100 AAS

The Atomic absorption spectrometer (PERKIN ELMER™ AANALYST™ 100) was switched on followed by the computer and allowed to warm up for 10 minutes. The instrument's software was opened up on the computer. The lamp for the element of interest was installed into turret lamp holder. The lamp details were selected on the software. The correct lamp current was selected. The lamp was aligned manually (using a white piece of paper) to get the light beam just above the burner head, and then left to warm up for 5 minutes. The fuel (acetylene) and oxidant (compressed air) pressures were set to 12 psi and 50 psi respectively, as per the instrument users'

manual (AAnalyst™ 100 manual). The lamp energy was adjusted to maximum using the gain control. The flame was the ignited and distilled water aspirated throughout and all the time between samples. Standard series and samples are then aspirated, the absorption read and recorded.

3.6.5 Determination of Magnesium and Manganese

The working standard series for the analysis of Mg were 0, 0.4, 0.8, 1.2, 1.6, 2.0 meq Mg/100 ml and 0, 0.5, 1.0, 1.5, 2.0 meq Mn/100 ml soil in 100 ml volumetric flasks (diluted from 1000 ppm stock solution obtained from Sherwood Scientific Ltd, UK). The samples absorptions were read using a flame atomic absorption spectrometer (PERKIN ELMER™ AANALYST™ 100) at 422.7 nm for magnesium and 279.5 nm for manganese. Standard calibration curves (absorbance versus the concentrations) for each element were then plotted. The sample concentrations were intrapolated from the curves.

3.7 Determination of total Organic Carbon by colorimetric method

In this method, the soil organic carbon was oxidised by acidified dichromate at 150^{o} C for 30 minutes. The oxidation by acidified dichromate follows the reaction (Anderson and Ingram, 1993).

$$2Cr_2O_7^{2-} + 3C + 16\,H^+ = 4Cr^{3+} + 3CO_2 + 8H_2O \qquad (6)$$

Fifteen grammes (15 g) of sucrose were weighed in a 250 ml beaker and heated at 105^{0} C for 2 hours in an oven. It was then cooled in a desiccator. Into a 100 ml volumetric flask, 11.877 g of the dried sucrose were weighed and filled to the mark with distilled water. This solution contained 50 mg/ml of carbon. The solution was then diluted accordingly to make serial working standards containing 0, 2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml C. Two ml of each working standard was transferred into 100 ml digestion tubes and heated to dryness at 105^{0} C. The tubes now contained 0, 5, 10, 15, 20, and 25 mg C respectively.

One gramme of ground soil was weighed (accuracy of 0.001 g) into 100 ml digestion tubes. It was diluted with 2 ml of distilled water. Ten millilitres of 5% potassium dichromate were then added to each sample tube including the tubes containing the standards and a reagent blank. The solution was allowed to completely wet the soil and the standards. This was followed by addition 10 ml of concentrated sulphuric acid to each tube. The samples were digested at 150^{o}

C, for 30 minutes in a digestion block, allowed to cool and then 50 ml of 0.4% barium chloride solution added. The mixtures were then shaken for 30 min and then allowed to stand overnight to attain clear supernatant solutions. Using colorimeter cuvettes, the absorbance of the standards and the samples were read on Scanning UV/VIS spectrophotometer (PHILIPS™ PU 8730) at 600 nm. A standard calibration curve (absorbance against concentration) was then plotted. The concentrations of the unknown and the blank were determined by interpolating from the standard calibration curve. The blank value was subtracted from the sample concentrations to obtain the corrected concentration values, K. The following equation was used to calculate percentage of organic carbon in the soil samples.

% organic carbon = (K x 0.1)/W Where; K = corrected concentration of unknown (mg)
W= Weight of the soil sample (g)

3.8 Determination of total Nitrogen by Kjeldahl method

Samples of 1.0 g of air dried soil were accurately weighed into 100 ml digestion tubes including a reagent blank solution. A portion of 0.5 g catalyst mixture of potassium sulphate, selenium powder and copper sulphate were added to the digestion tubes. Then 10 ml of 98% sulphuric acid as the digestion reagent were added before shaking the mixture and the tubes were placed into a 350^{o} C pre-heated digestion block for two hours. It was allowed to cool and transferred into 100 ml volumetric flasks and filled to the mark with distilled water after cooling. The samples were left to stand overnight before distillation followed by titration with standardized H_2SO_4 (Hinga *et al*., (1980). The percentage total nitrogen was calculated as follows;

$\%N = (a-b) \times V/W \times 10^2/10^6$

Where: a = concentration of N in the sample digest, ppm

b = concentration of N in the blank digest, ppm

V = total volume of digest at the end of the digestion procedure, ml

W = weight of plant material sample digested in grammes

$10^2/10^6$ = conversion factor from ppm to % (Lachat Instruments 1995).

3.9 Measurement of soil pH

Twenty grammes (20 ± 0.1 g) of soil were weighed into a 250 ml beaker. Fifty millilitres (50 ml) of distilled water were added to the beaker and the mixture stirred for 10 min. It was left to stand for 30 min before stirring again for 2min before reading the pH of the supernatant liquid (Anderson and Ingram, 1993). A SARTORIUS™ model PP-15 pH Meter used was calibrated using buffer solutions of pH 4, 7 and 10.

4.0 Data analysis

Analysis of variance (ANOVA) was done for the leaf glucose content data obtained to compare the amount of leaf glucose before and after nutrients application. The mean glucose contents **(** $\bar{x}_1$**),** determined before nutrients application, were compared with the mean glucose contents obtained after each nutrient had been applied **(** $\bar{x}_2$**).** Different $\bar{x}_1$ and $\bar{x}_2$, values were obtained for each application, e.g (***a*** $\bar{x}_1$**:** ***a*** $\bar{x}_2$**)**, (***b*** $\bar{x}_1$**:** ***b*** $\bar{x}_2$**)**, (***c*** $\bar{x}_1$**:** ***c*** $\bar{x}_2$**)**, (***d*** $\bar{x}_1$**:** ***d*** $\bar{x}_2$**)**, etc, where ***a***, ***b***, ***c***, ***d,*** *etc* was the different applications. These values were used to find out whether there was any significant difference between glucose levels before and after application of the nutrients studied. Statistical computer programs used to analyse the data, included Genstat, MS excel and 'R' statistical software. The correlation between the plant nutrients uptake and glucose contents was calculated on the data.

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Results for analysis of mineral nutrients in the soil samples

Analysis of soil mineral nutrients in the experimental field was carried out prior to treatments with the fertilizers so as to acquire baseline data. Six samples were collected by random sampling within the experimental plot and the samples analysed in triplicates. Tables 2 and 3 show the results of soil mineral nutrients analysis, for samples collected before application of fertilizers, by use of normal wet chemistry and Fourier Transform Infrared methods respectfully.

Results obtained showed that, the soil was deficient in most of the essential nutrients while a few were higher than recommended levels (Soil interpretive values are shown in Appendix 13.1). The results obtained by wet chemistry were verified using values obtained from the FTIR predictions. The soil pH lay in the neutral ranges between 7.00 and 7.13, which was neutral and suitable for most crops growth (Weast, 1981). The soil analytical results also showed that calcium was within sufficient levels, with results ranging between 10.0 and 12.8 meq per 100 grammes of soil against recommended values of 2.0-15 meq/100 g of soil (tables 1 and 2). Organic carbon was low. Manganese and magnesium were found to be a bit higher than adequate values. Manganese ranged between 1.49 and 2.85 meq % against a recommended range of 0.11 to 2.0 meq per 100 grammes of soil while magnesium was between 3.49 and 8.54 meq/100 grammes of soil. The recommended range for magnesium is between 1.0 and 3.0 meq/100 grammes of soil. Potassium was slightly in excess (above upper limit of 1.50 meq/100 grammes of soil, while phosphorous and nitrogen values were lower than recommended ranges of 30-80 ppm and 0.2-0.5% respectfully.

The FTIR predictions were obtained from calibration curves of 108 samples FTIR spectrums whose wet chemistry data were known. These were results from previous laboratory wet chemistry analysis, including some from inter-laboratory tests data. Figure 4 shows the raw spectra of the 108 wet chemistry samples including the unknowns, which were used for calibration and predictions. Results from the analysis of variance (ANOVA) for the two sets of data are shown in table 4

Table 2: Results for analysis of soil mineral nutrients by use of wet chemistry method.

SSN	Meq%					%		ppm	pH
	Ca	K	Mg	Mn	Na	N	OC	P	
kar000802	11.27	2.63	5.24	1.58	0.38	0.07	1.14	23.07	7.02
kar000803	11.67	2.97	4.08	1.72	0.41	0.12	1.56	22.91	7.10
kar000804	11.94	2.12	4.40	2.51	0.41	0.09	1.52	22.16	7.01
kar000805	12.01	2.35	4.49	2.35	0.44	0.11	1.72	22.98	7.11
kar000806	12.26	2.09	7.62	1.94	0.36	0.08	1.29	21.58	7.00
kar000807	12.35	2.29	4.00	2.80	0.40	0.10	1.34	20.87	7.11
Sufficient levels	**(2.0-15)**	**(0.2-1.5)**	**(1.0-3.0)**	**(0.1-2.0)**	**(0.0-2.0)**	**(0.2-0.5)**	**(2.7-5.3)**	**(30-80)**	**6.0-7.5**

Table 3: Results for analysis of soil mineral nutrients by use of FTIR predictions

SSN	Meq%					%		ppm	pH
	Ca	K	Mg	Mn	Na	N	OC	P	
kar000802	12.12	2.53	6.63	1.50	0.35	0.12	1.59	22.93	7.09
kar000803	12.02	2.34	4.40	1.95	0.46	0.18	1.51	23.01	7.13
kar000804	10.98	2.11	5.65	2.77	0.36	0.17	1.37	22.23	7.06
kar000805	12.73	2.35	4.45	2.60	0.47	0.18	1.40	21.80	7.09
kar000806	12.11	2.09	7.36	1.89	0.30	0.15	1.16	22.11	7.07
kar000807	12.07	2.29	4.96	2.83	0.45	0.18	0.82	20.11	7.08
Sufficient levels	**(2.0-15.)**	**(0.5-1.5)**	**(1.0-3.0)**	**(0.1-2.0)**	**(0.0-2.0)**	**(0.2-0.5)**	**(2.7-5.3)**	**(30-80)**	**6.0-7.5**

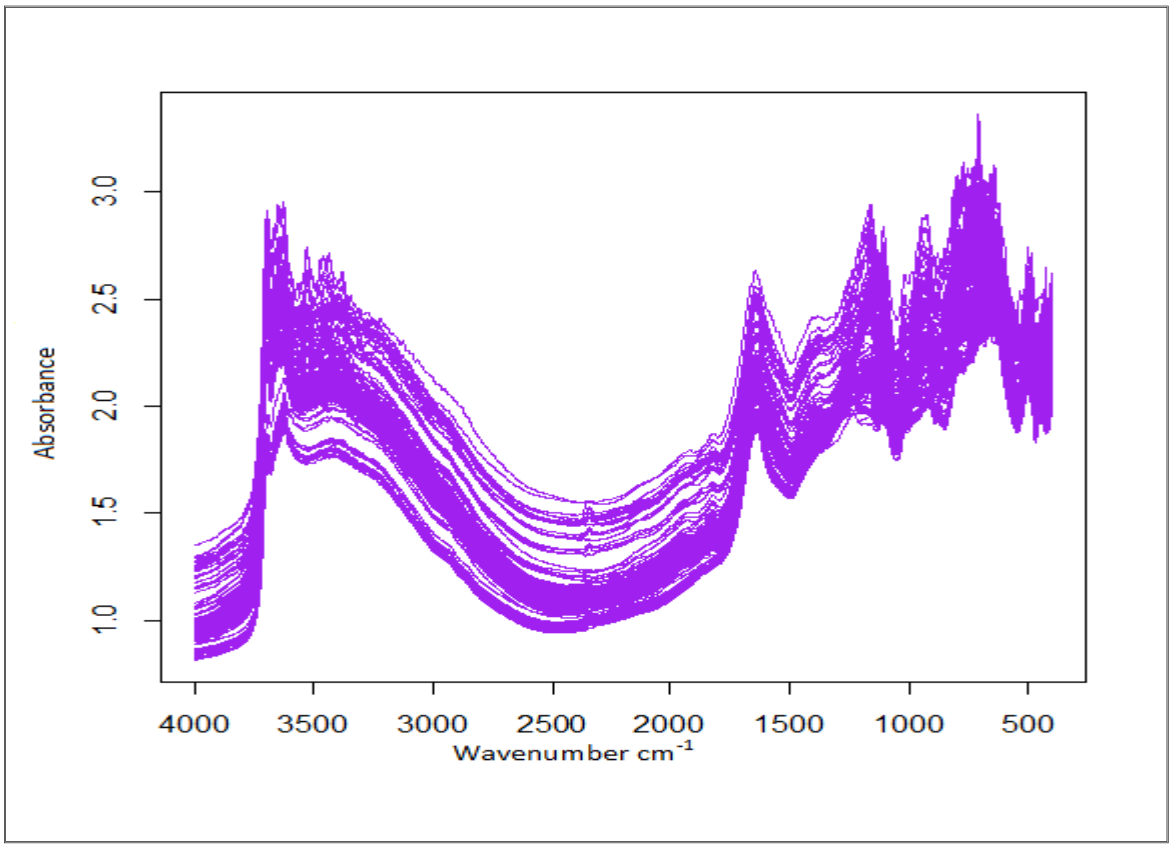


Figure 4: FTIR Spectrums overlays for wet chemistry samples used for calibration

4.2 Analysis of variance for wet chemistry and FTIR soil nutrients analytical data

The results on table 4 showed that there was no statistical significant difference in the results obtained from the two methods (ns) except for nitrogen and pH (s). The calibration curve for nitrogen showed the huge discrepancies between the measured and the predicted values (figure 5. The low nitrogen values obtained through wet chemistry analysis may have been as a result by loss of N during sample digestion. Soil analysis by FTIR requires minimal sample preparation, involves non-destructive process (Puckrin *et al.,* 1996). Though the pH values from the two methods were statistically significantly different, both showed neutral pH values of around 7.

Table 4: Results from ANOVA for FTIR versus wet chemistry soil analytical data

	Parameters measured								
	Meq%					%		ppm	
Method	**Ca**	**K**	**Mg**	**Mn**	**Na**	**N**	**OC**	**P**	**pH**
Fourier TIR (Mean)	12.01	2.28	5.57	2.26	0.40	0.16	1.38	22.03	7.09
Wet Chemistry(Mean)	11.92	2.41	4.97	2.15	0.40	0.09	1.43	22.26	7.06
α-value	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated p-value	**0.746**	**0.326**	**0.173**	**0.528**	**0.950**	**<0.01**	**0.621**	**0.667**	**0.041**
Differences in the two methods	ns	ns	ns	ns	ns	s	ns	ns	s

The worm juice was analysed to determine its N, P, K, Ca, Mg and Mn contents. The juice was rich in phosphorus at 27.3%, calcium at 12.4%/ while nitrogen was not detected (table 5).

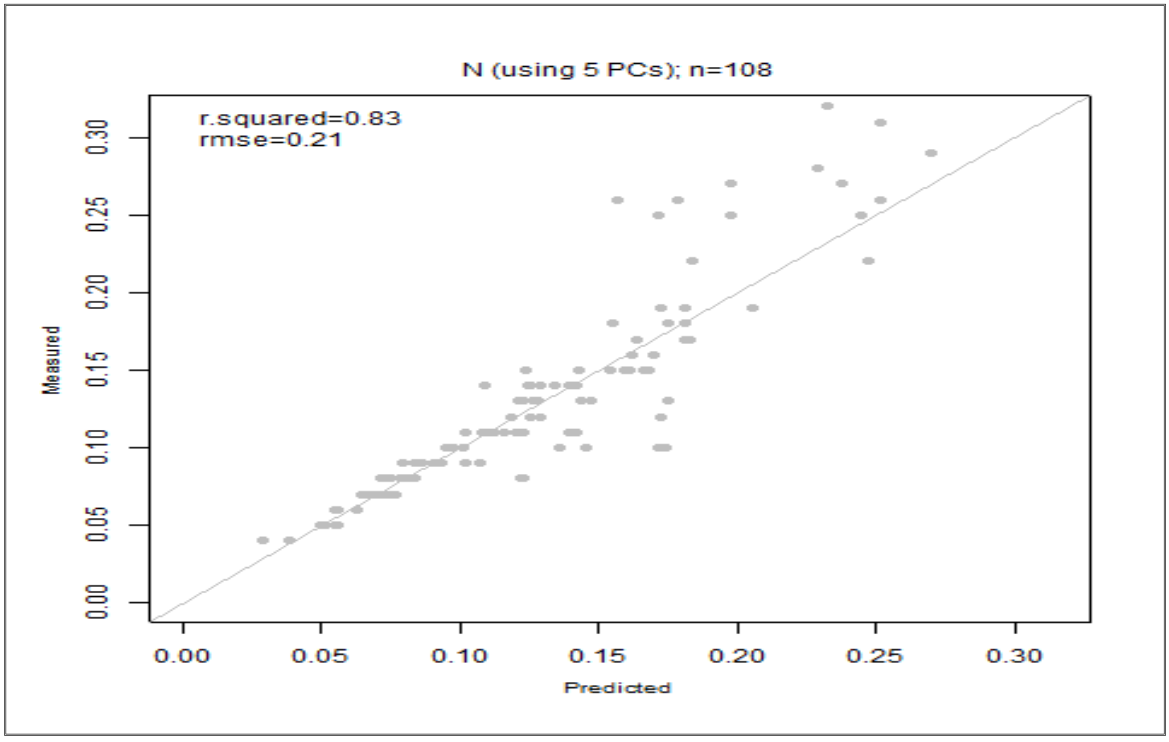


Figure 5: FTIR calibration curve for Nitrogen in soil samples

Table 5: Results for the analysis of mineral nutrients concentrations in worm juice

WORM JUICE	
Parameter tested	**Concentration**
Nitrogen (%)	Not Detected
Phosphorous (%)	27.30
Potassium (%)	0.21
Calcium (%)	12.40
Magnesium (%)	9.84
Manganese (%)	3.71

4.3 Effect of fertilizer applications on leaf glucose content

Table 6 shows the mean in glucose contents over time, after application of fertilizers. The untreated plants (controls) showed the minimum changes in glucose contents. Calcium Carbonate based fertilizer application displayed the highest amount of increase within the first hour's sampling at 2.50 mg/g of glucose. The lowest increase in glucose levels was observed at 0.77 mg/g for worm juice. It was however, noted that all applications yielded an increase in glucose levels. In all the treatments, the levels of glucose increased some within the first hour and decreased gradually with time except in the cases of potassium and worm juice where the highest increase was observed within the first six hours.

Table 6: Mean glucose levels after application of fertilizers

Treatments (N=6)	Mean glucose content (mg/g)				
	1 hour	6 hours	24 hours	2 weeks	12 weeks (3 months)
CAN	1.41	1.43	1.40	1.37	1.36
TSP	1.72	1.86	1.16	1.12	1/16
KNO_3	1.15	1.76	1.54	1.51	1.51
$CaCO_3$	2.50	1.69	1.42	1.43	1.32
WJ	0.77	1.09	1.06	1.13	1.12
CAN + WJ	1.28	1.33	1.52	1.38	1.38
TSP + WJ	1.61	1.74	1.86	1.92	2.06
KNO_3 +WJ	1.16	1.64	1.57	1.52	1.52
$CaCO_3$ + WJ	2.62	1.58	1.51	1.50	1.65
CAN+TSP+KNO3+CaCO3 +WJ	1.17	1.30	1.42	1.39	1.45

4.4 Results from the analysis of variance for changes in glucose contents

There was a statistical significant difference between initial and final glucose for each application (p<0.001). That showed that leaf glucose contents increased in the plants that were supplied with fertilizers with respect to the untreated one (table 7).

Table 7: Analysis of variance (variate = glucose content)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Time	4	0.392	0.099	19.01	<.001
Application	19	88.407	4.653	903.16	<.001
Time vis Application	76	18.320	0.241	46.79	<.001
Residual	500	2.576	0.005		
Total	599	109.695			

Figure 6 shows mean glucose contents versus time. The highest levels of glucose were observed within sixth hour after application of nutrients.

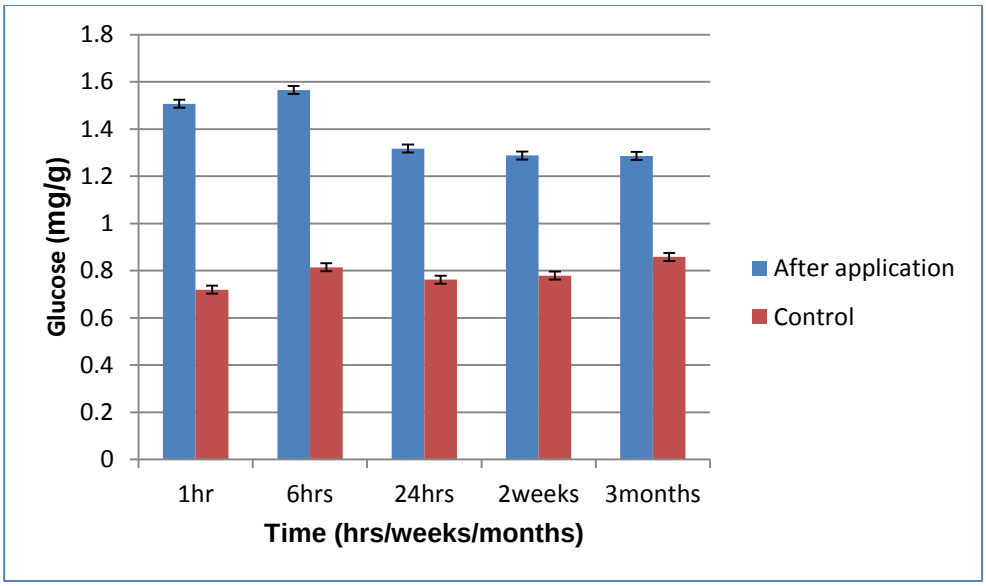


Figure 6: Variations in mean glucose contents with time

Figure 6 below shows the effect of mean leaf glucose content with each fertilizer application. The values shown were the mean glucose contents for each application for the period of the study. The highest changes in the mean glucose contents observed for an individual treatment, were those obtained after application of Calcium Carbonate based fertilizer. On the other hand, application of mixtures of Triple Super Phosphate and Worm Juice yielded the most significant changes in mean glucose contents during the study.

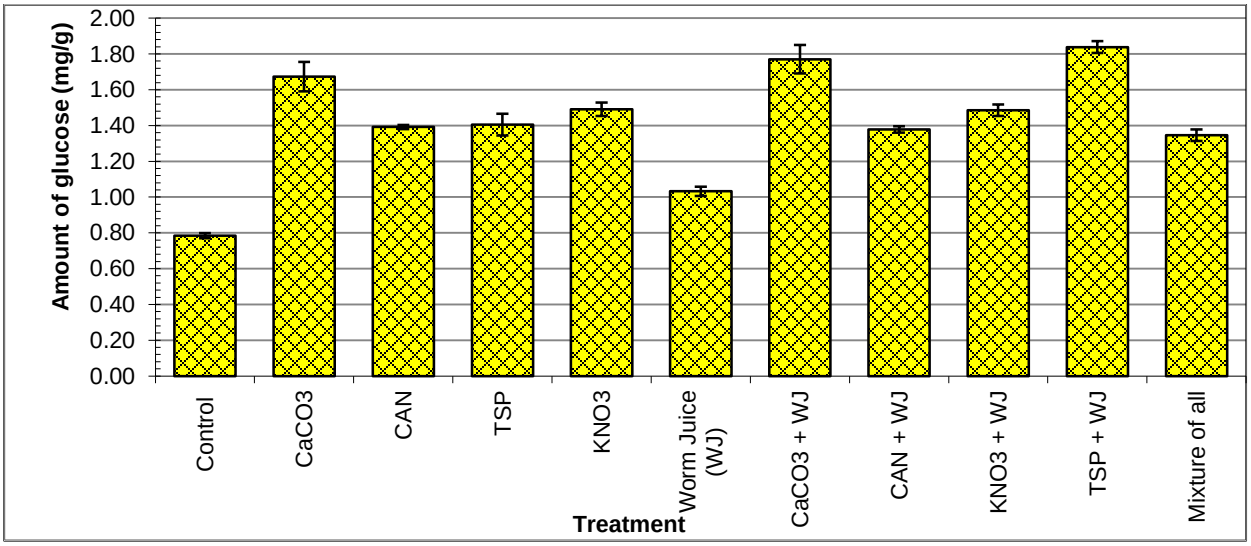


Figure 7: Changes in glucose contents (mg/g) after fertilizer application

Figure 7 below show the effect of application of each fertilizer on glucose contents, after a given period of time. The glucose contents for all untreated (control) plants, used alongside each fertilizer application, had significantly the same quantity of glucose. They also displayed the least changes in glucose contents throughout the time of study. Application of mixtures of $CaCO_3$ and worm juice ($CaCO_3$ + worm juice) yielded the highest changes in glucose content, one hour after the application of the mixture.

The Fisher's protected least significant difference test on table 8, shows that there was no significant difference between the mean glucose content in the first 6 hours and the content 3 months later, at 95% confidence level and p=0.05. This may be attributed to the reduced effects of the fertilizers on the crop with time. Where the means share the same alphabets, it implies that there was no significant difference between the means.

Table 8: Fisher's protected least significant difference test

Time after application of fertilizers	**Mean glucose content (mg/g)**	**Difference**
6 hours	1.164	**a**
3months	1.158	**a**
1hour	1.119	**b**
24hours	1.108	**b**
2weeks	1.104	**b**

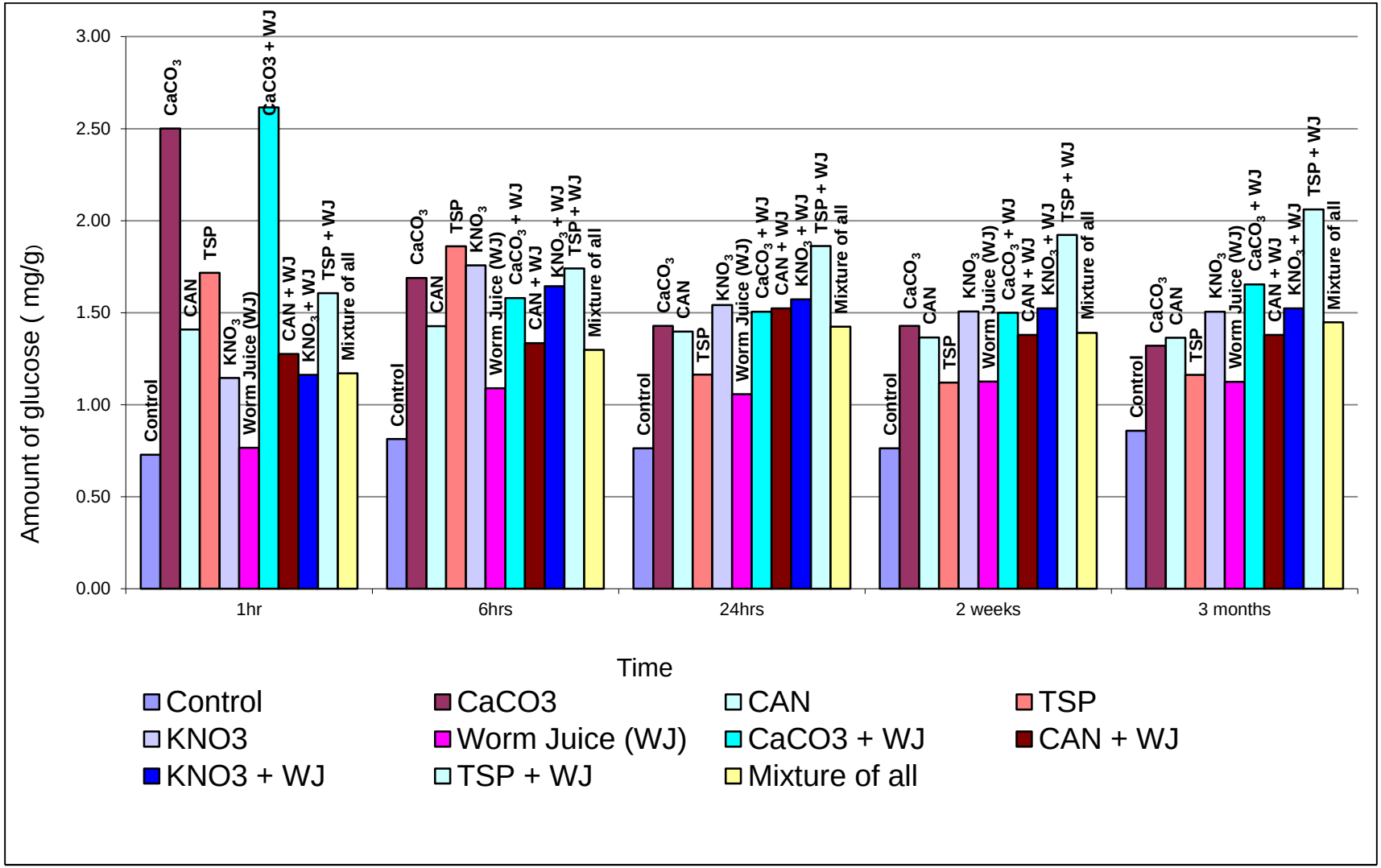


Figure 8: Changes in glucose contents versus all applications after a given time

4.5 Verification of results from DNS method with those from HPLC

Results obtained from the analysis of leaf glucose using the Dinitrosalicyclic Acid (DNS) method, were verified by use of High Performance Liquid Chromatography (HPLC). Five samples of leaf glucose extracts were randomly selected (to represent both time and applications) for the verification. The results were then subjected to statistical t-test to find out whether there was real significant statistical difference between the two methods.

It was noted that the concentration of glucose was slightly higher in the DNS compared to the HPLC method. This is because HPLC analysis is highly specific. However, in the DNS method there are other side reactions such as the decomposition of sugar, which competes for the availability of 3,5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color (Miller, 1979).

However, there was no significant statistical difference between the results obtained by the DNS method from those from HPLC analysis for the 5 samples that were used for verifications (tables 9 and 10).

Table 9: Results of glucose contents obtained by DNS and HPLC methods

Application	**HPLC method Glucose (mg/g)**	**DNS method Glucose (mg/g)**
Calcium Carbonate (1hour after application)	2.31	2.50
Triple Super Phosphate (1 hour after application)	1.56	1.72
Triple Super Phosphate (24hours after application)	1.09	1.16
WJ (24hours after application)	0.82	1.06
Control (24hours after application)	0.68	0.79

Table 10: Analysis of t-test for results by DNS versus these by HPLC

Sample	**Size**	**Mean**	**Variance**	**Std deviation**	**Std error of the mean**
Glucose DNS versus Glucose HPLC	5	0	0.0125	0.1118	0.05
t-value ($\alpha = 0.05$ and 4d.f.) = **2.132**				**Calculated t = 0.00**	

4.6 Effect of nutrients uptake on leaf chlorophyll levels

Table 14 below shows the changes in chlorophyll contents (SPAD) two weeks and three months after application of fertilizers. The t-test analysis for the results for chlorophyll SPAD measurements are shown on Table 15.

Table 11: Changes in leaf chlorophyll with time after nutrients application

Treatments	**Chlorophyll contents (SPAD)**			% SPAD increase
	Initial	After 2 weeks	After 3 months	
$CaCO_3$	43.1	43.3	44.2.	3.9
CAN	42.5	52.1	56.4	13.9
TSP	42.6	46.7	51.2	8.6
KNO_3	40.8	49.9	51.9	11.1
Worm Juice	42.9	44.3	45.9	3.0
$CaCO_3$ + WJ	40.1	41.4	42.8	2.7
CAN + WJ	41.9	52.1	59.2	17.3
TSP + WJ	42.7	46.6	52.5	9.8
KNO_3 +WJ	41.8	52.1	56.9	15.1
Mixture of All	42.1	50.9	52.9	10.8
Control	42.1	42.4	42.3	0.5

4.6.1 t-test analysis for initial and final chlorophyll contents

Table 12: t-test for the changes in leaf chlorophyll three months after applications

Sample	**size**	**Mean**	**Variance**	**Standard Deviation**	**Standard error of mean**
Initial (SPAD)	10	42.05	0.91	0.955	0.302
After 3 months(SPAD)	10	51.39	30.65	5.537	1.751

Difference of means	-9.340
Standard error of difference	1.777
95% confidence interval for difference in means	(-13.33, -5.355)
t-value ($\alpha = 0.05$, 9d.f.) = **1.833**	Test statistic t = **5.26**

There was a significant difference between chlorophyll contents before fertilizers application and the contents three months later (table 12). The changes in the levels of

chlorophyll were more pronounced on applications of nitrogen based fertilizers. The highest percentage increase on chlorophyll contents were observed after an application of calcium ammonium nitrate (CAN) and potassium nitrate (KNO_3), and were higher where the two were mixed with worm juice (WJ). There is a very close link between chlorophyll and nitrogen content as many investigators have shown (Evans, 1983, Field and Mooney, 1986, Amaliotis *et al.*, 2004). This is understandable because, nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Tucker, 2004, Daughtry, 2000). Application of triple super phosphate fertilizer (TSP) also had an effect on chlorophyll levels. Calcium carbonate ($CaCO_3$) had the least observed variations.

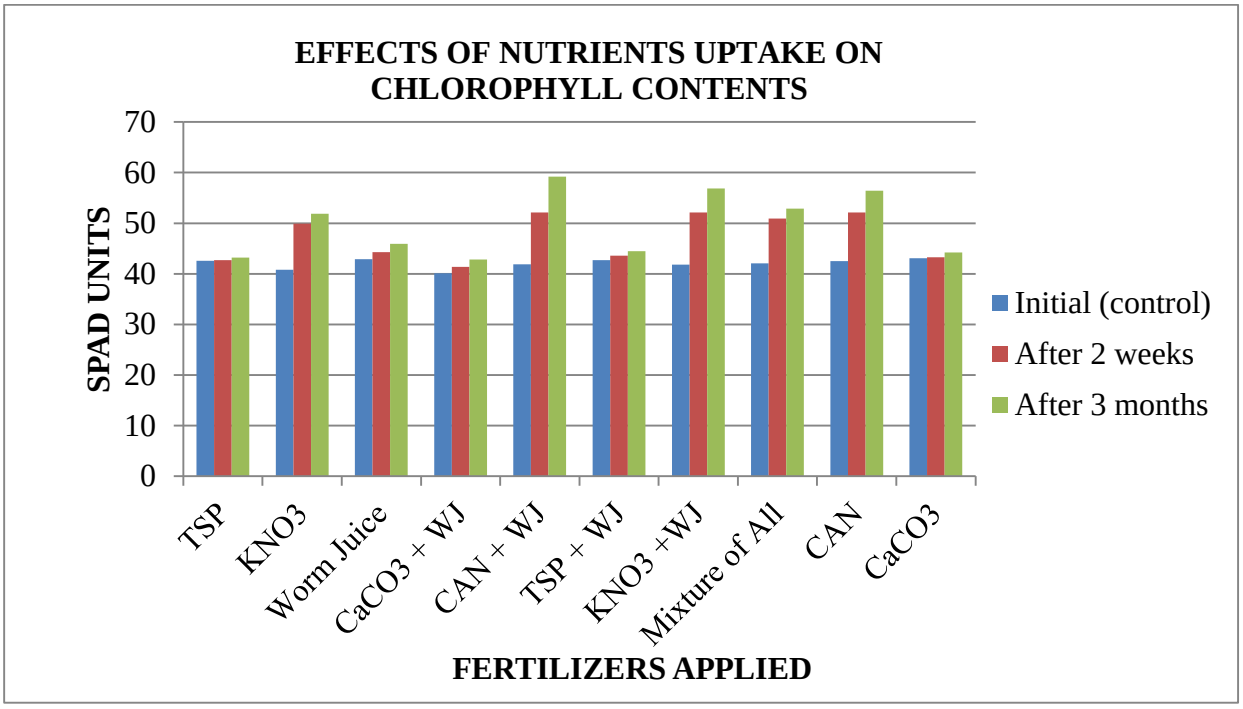


Figure 9: Effect of nutrients application on leaf chlorophyll content

4.7 FTIR predictions of leaf nutrient contents

Leaves were analysed for mineral nutrient contents before and three months after application of fertilizers. Analysis was done by FTIR using predictions based on plant tissue samples' wet chemistry data. Calibration data used were results from previous wet chemistry plant tissues analysis. Tables 13 and 14, shows leaf mineral nutrient contents before and 3 months after applications, respectively. The results were compared with data from the table for Geranium (*Pelargonium*) interpretative values on appendix 13.2 (Reuter and Robinson, 1986). The leaf nutrients were found to be below the recommended sufficient levels for N, P and K but higher for Ca while Mg and Mn were sufficient. The results in shows the comparison between the recommended plant nutrient ranges with the values obtained from analytical tests.

Table 13 shows the results of analysis by FTIR, of plant tissue samples before fertilizer application, while in table 14 are the results obtained 3 months after application. Figure 10 shows the spectrum overlays for the plant tissue wet chemistry, used for FTIR calibrations and production of the nutrients prediction data. ANOVA was used to find out whether there were significant statistical differences in the leaf nutrients before and after application fertilizers.

Table 13: FTIR predictions of leaf nutrients before application of fertilizers

SSN	Treatments	%						ppm
		Ca	**K**	**Mg**	**N**	**P**	**Fe**	**Mn**
kar000054	$CaCO_3$	1.26	1.23	0.41	2.22	0.17	742	60.4
kar000055	CAN	1.14	1.16	0.67	2.15	0.22	596	67.2
kar000056	TSP	1.23	1.52	0.40	2.05	0.28	814	66.4
kar000057	KNO_3	1.31	1.23	0.39	2.29	0.20	705	59.0
kar000061	WJ	1.25	1/23	0.35	2.24	0.18	659	63.4
Sufficient levels		**0.80-1.20**	**2.50-4.30**	**0.20-0.50**	**3.50-4.80**	**0.40-0.70**	**100-250**	**40-200**

Table 14: FTIR predictions of leaf nutrients 3 months application of fertilizers

SSN	Treatments	%					ppm	
		Ca	**K**	**Mg**	**N**	**P**	**Fe**	**Mn**
kar000054	$CaCO_3$	2.00	1.58	0.41	2.19	0.19	614	49.3
kar000055	CAN	1.67	1.32	0.39	2.40	0.20	609	56.3
kar000056	TSP	1.23	1.34	0.51	2.18	0.38	689	60.7
kar000057	KNO_3	1.29	1.41	0.61	2.48	0.16	938	72.0
kar000061	WJ	1.27	1.23	0.54	2.18	0.21	660	60.5
Sufficient levels		**0.80-1.20**	**2.50-4.30**	**0.20-0.50**	**3.50-4.80**	**0.40-0.70**	**100-250**	**40-200**

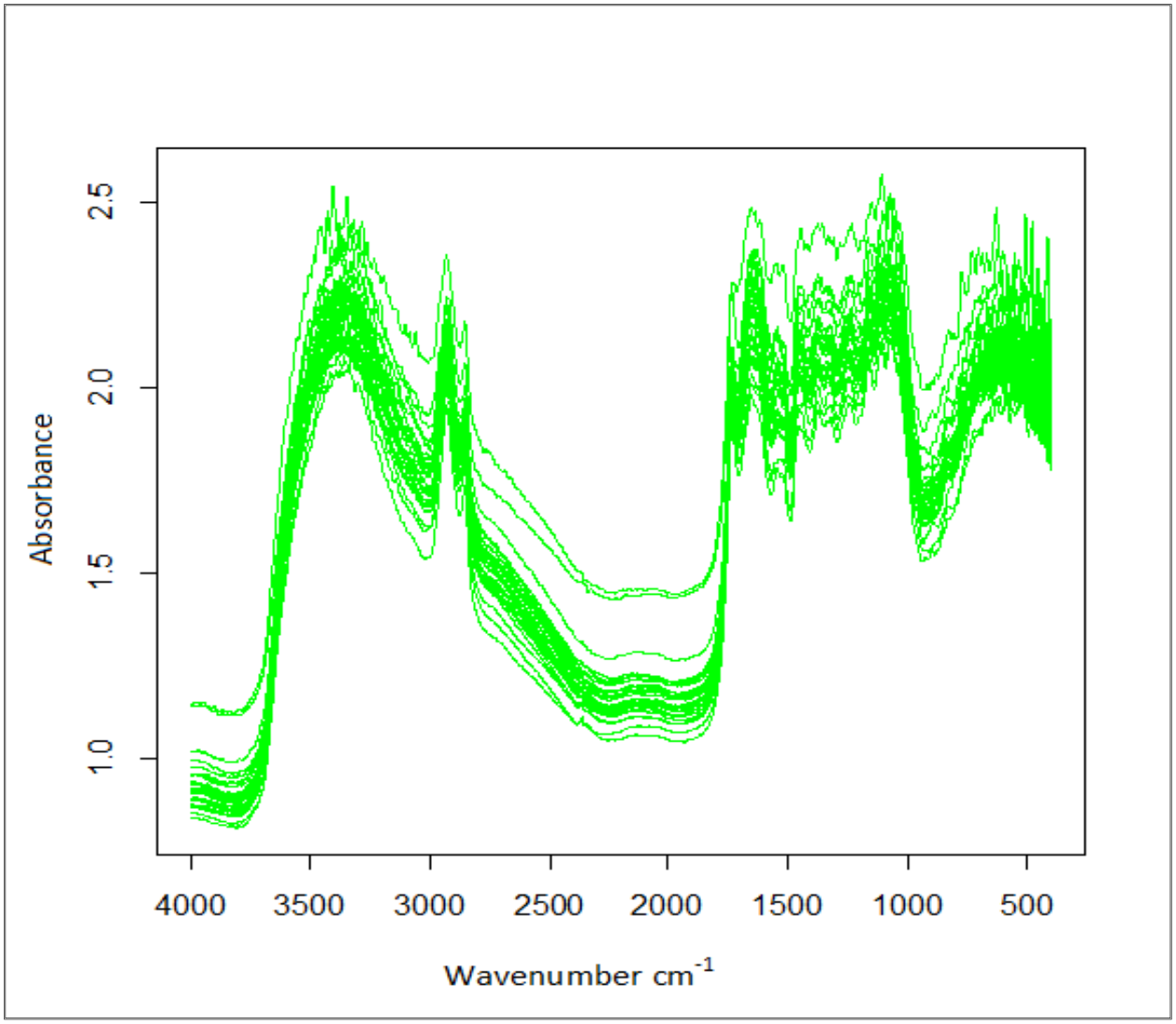


Figure 10: FTIR spectrums overlays for plant wet chemistry samples used for calibration

T-test was used to compare the differences in mineral contents in the leaves before and three months after application as shown on Table 15. Results indicated a significant increase in leaf calcium after application of $CaCO_3$ and CAN fertilizers (P= <0.001 at 95% confidence interval). Equally, nitrogen increased significantly three months after application of CAN fertilizers. There were significant changes in P three months after addition of TSP and worm juice fertilizers. The significant increase in P in the leaves after only worm juice was applied may be as result of high concentration of P in the worm juice at 273 mg/l (table 5).

Table 15: t-test for the changes in leaf nutrients 3 months after application of fertilizers

Treatment	**Time**	%						ppm	
		Ca	**K**	**Mg**	**Na**	**N**	P	**Fe**	**Mn**
$CaCO_3$	Before (mean)	1.26	1.23	0.41	59.82	2.22	017	742.3	60.4
	After (mean)	2.00	1.58	0.41	59.90	2.19	0.19	613.7	49.3
p-value		**<0.001**	**0.108**	**0.935**	**0.995**	**0.641**	**0.519**	**0.509**	**0.348**
CAN	Before (mean)	1.14	1.16	0.67	45.53	2.15	0.22	596.3	67.2
	After (mean)	1.673	1.32	0.39	48.37	2.50	0.20	609.0	56.3
p-value		**<0.001**	**0.336**	**0.009**	**0.888**	**0.044**	**0.386**	**0.888**	**0.116**
TSP	Before (mean)	1.23	1.52	0.40	48.85	2.05	1.18	813.7	66.43
	After (mean)	1.23	1.34	0.51	51.11	2.18	1.38	689.0	60.67
p-value		**0.840**	**0.540**	**0.242**	**0.862**	**0.536**	**0.001**	**0.092**	**0.343**
KNO_3	Before (mean)	1.31	1.23	0.39	49.67	2.29	0.197	705.3	58.97
	After (mean)	1.29	1.41	0.61	48.67	2.48	0.163	938.3	71.97
p-value		**0.633**	**0.210**	**0.125**	**0.956**	**0.208**	**0.152**	**0.020**	**0.056**
Wj	Before (mean)	1.25	1.23	0.53	48.67	2.24	0.18	659.0	63.4
	After (mean)	1.27	1.23	0.54	48.73	2.18	0.21	695.7	60.5
p-value		**0.189**	**0.927**	**0.798**	**0.981**	**0.304**	**0.018**	**0.988**	**0.646**

The soil was low in N and P, but sufficient with Ca and high in K levels (compared to the optimum levels shown in Soil interpretative values table in appendix 21.2). However, the glucose increases were observed for Ca and P applications more than in others. Calcium forms insoluble compounds with other elements in soil, such as phosphorous, and calcium in such insoluble form is not available to plants (Bruce *et al.,* 1989). Calcium-phosphorous precipitation occurs when free calcium accumulates in the soil solution, and calcium tends to form insoluble compounds

with phosphorous. Consequently, phosphorous availability is also significantly decreased (Brady and Weil, 2002). Therefore application of these two nutrients into the soil may have increased the availabilities of Ca and P (in readily plant absorbable form) and hence an increase in glucose levels. Low soil Ca may have been as result of the presence of competing ions. Calcium competes with other positively charged ions, such as sodium (Na^{+}), potassium (K^{+}), and magnesium (Mg^{+2}) which in this case were found to be high in the soil.

There exists a strong interaction between N and K where a crop must also have access to, and take up, an adequate amount of potassium from the plant-available (exchangeable K) pool of K in the soil (Johnson and Milford, 2012)**.** For that reason, glucose levels for KNO_3 treatments increased with time and then stabilized. This could have been attributed to the low K found in the plant tissues analysis although there was more than sufficient K in the soil.

The slow mobility of phosphorous in the first, sixth and twenty fourth hours after application of fertilizers, was attributed to the fact that phosphorous, is only slightly soluble in the soil and enters the plant by diffusion. P-sorption occurs when the orthophosphates, $H_2PO_4^{-}$ and HPO_4^{2-}, bind tightly to soil particles (Scachtman *et al.,* 1998). The solubility of phosphate minerals is very dependent upon soil pH. The soil pH for optimum phosphorus availability is 6.5 (Brady and Weil, 2002). At higher or neutral pH, phosphate reacts with calcium to form minerals. The pH of this soil was neutral which could have led to low P availability.

Glucose increases were observed on the application of CAN and KNO_3 fertilizers. Plants can take up N in the ammonium (NH_4^{+}) or nitrate ($N0_3^{-}$) form **(**Schwoerbel and Tillmaernns, 1977). At pH values near neutral (pH 7), the microbial conversion of NH_4^{+} to nitrate (nitrification) is rapid, and crops generally take up nitrate **(**Brown, 1982). This was observed by the evidence of an increase in the chlorophyll levels in the leaves.

Verification of glucose contents analysis by reduction of 3, 5-dinitrosalicylic acid to 3-amino, 5-nitrosalicyclic acid by the free carbonyl group in the glucose molecule, with the HPLC with Refractive Index detection, showed that the two results were comparable. Worm juice had the least effect on glucose levels due to absence of or very low mineral nutrients except for P. Worm juice had a high P content of 27.3%.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From initial soil analytical results, the nutrients, N, P, K, Ca, Mg, and Mn ranged variously between sufficient, low and high levels. Soil calcium appeared to be sufficiently available, but carbon and nitrogen were lower while magnesium and manganese were found to be slightly higher than recommended. Though soil analysis indicated that calcium was sufficient, it showed only partial availability to the plant. This was confirmed by the high rate of glucose increases on addition of calcium based fertilizers which suggest that soil calcium may have been unavailable to the plant. There exist a strong calcium-phosphate bond ($Ca_3(PO_4)_2$) formed between soil calcium ions (Ca^{2+}) and orthophosphates (PO_4^{3-}). This is the bond that is broken after introduction of 2% lanthanum chloride into the samples during the analysis of soil nutrients by FAAS. There was a significant increase in leaf glucose levels after application of calcium based fertilizers. This was despite soil test results indicating adequate availabilities of calcium in the soil. The effective increase in glucose levels after calcium based fertilizers application may be attributed to initial lack of plant available calcium in the soil.

Application of worm juice alone had little effect on glucose contents. The juice contained low levels in most nutrients but rich in phosphorous with nitrogen being undetected. It was however observed, that leaf glucose levels increased when worm juice was applied alongside inorganic fertilizers especially $CaCO_3$. The leaves were chosen for the study because it was easier to monitor real plant status through glucose analysis rather than the roots.

The results showed a correlation between soil mineral nutrients uptakes and leaf glucose level in the *P. sidoides*. Changes in glucose contents were observable as fast as within an hour after fertilizer application as observed in the $CaCO_3$ and worm juice mixture. Both HPLC and DNS methods were found to give comparable in glucose results.

Correlations were also observed between leaf chlorophyll and nitrogen fertilizers application. The amount of chlorophyll, in SPAD, increased significantly three months after application of CAN and KNO_3 fertilizers.

Leaf tissue samples analysed for nutrients before and three months after application of fertilizers, showed an increase in mineral nutrients. Calcium increased in the leaves where

$CaCO_3$ and CAN were applied with notable increase in leaf nitrogen in cases of CAN and KNO_3 applications. There were observable increases in P where TSP and worm juice were applied.

FTIR, being fast and requiring minimal sample preparation and practically none use of chemical reagents, was chosen for this study. It is equally becoming popular as the future method for soil analysis. The method was found useful for soil and plant tissue nutrients analysis. Equipped with an FTIR spectrometer, wet chemistry analysis need only be employed for calibration and confirmation purposes where doubtful results are encountered.

The farm contributes to the well being of the local community by providing permanent employment to for over 80 persons, with 80 percent being women. It also offers free pre-primary education to their children including the ones from the neighbourhood. An improved and sustained crop will be indirectly beneficial to local community.

5.2 Recommendations

1. The analysis on the effects of soil nutrients applications on the *P. sidoides* active ingredients could not be carried out. This was because of unavailability of materials such as standard root glycosides and standard coumarins (umckalin). Further studies ought to be carried out to find out the effect of nutrients uptake on the root glycosides and coumarins (umckalin).
2. There is also need to find out whether the nutrients in the worm juice effluent maybe improved by extending biodegradation period. This could be done by collecting the effluent as was done in the study and storing it for specified period in a biodegradable tank before use. Only five grammes of each fertilizer were applied.
3. Study should be done to determine whether at higher application quantities of fertilizers or applications at constant and regular interval, will affect both the glucose levels and the plant tissues nutrient contents.
4. The worm juice could be improved by looking for worm feeding materials that can enhance the production of more nutrients that are useful to the plant, e.g. calcium, nitrogen etc.
5. Effort should be put to introduce and promote farming of the plant by more farmers in other areas of the country.

REFERENCES

Angiosperm Phylogeny Group. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III *Botanical Journal of the Linnean Society* **161** (**2**): 105–121. (10th Jan 2012 at 9.40am).

Amaliotis D., Therios I. and Karatissiou M. (2004). Effect of nitrogen fertilization on growth leaf nutrient concentration and photosynthesis in three peach cultivars. ISHS, *Acta Horticulturae* **449:** 36-42.

Anderson J.M. and Ingram J.S.I. (1993). Tropical Soil Biology and Fertility. A handbook of methods. CAB international, Wallingford, Oxon, UK **2:** 63-65

Baker D. E. and Amacher, M. C. (1981). The development and interpretation of a diagnostic soil-testing program. *Pennsylvania State University Agricultural Experiment Station Bulletin **826***. State College, PA.

Baker D. E. and Eldershaw, V. J. (1993). Interpreting soil analyses – for agricultural land use in Queensland. *Department of Primary Industries Project Report Series QO93014***.** DIP, Brisbane.

Bereznoy V. V., Riley D. S., Wassmer G. and Heger M. (2003). Efficacy of extract of *Pelargonium sidoides* in children with acute non-group A beta-hemolytic streptococcus tonsillopharyngitis: a randomized, double-blind, placebo-controlled trial. *Alternative therapies in health and medicine* **9:** 68–79.

Bladt S, and Wagner H. (2007). From the Zulu medicine to the European phytomedicine Umckaloabo. *Phytomedicine* **14:** 2-4.

Bowyer P., Clarke B. R., Lunness P., Daniels M. J. and Osbourn A. E. (1995). Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* **267:** 371-374.

Brady N.C. and Weil R.R. (1999). The Nature and Properties of Soils. 12th edition. Pearson Education, Inc, New Jersey 880-881.

Brady N.C. and Weil R.R. (2002). The Nature and Properties of Soils. 13th edition. Pearson Education, Inc, New Jersey 267-268.

Brinton W. (2004). Compost teas: Microbial hygiene and quality in relation to method of preparation. *Biodynamics* **4:** 36–45.

Brito-Arias and Marco A. (2007). *Synthesis and characterization of glycosides*. Springer edition 2007. National Polytechnic Institute, Mexico: 12-14

Britto D. T. and Kronzucker H. J. (2002). Ammonium ion toxicity in higher plants: a critical review. *Journal of Plant Physiology* **159:** 567–584.

Brown D. H. (1982). Mineral nutrition. In: Smith, A. J. E. (ed.). *Bryophyte Ecology*, Chapman & Hall, London, 383-444.

Bruce R.C., Warrell L.A., Bell L.C. and Edwards D.G. (1989). Chemical attributes of some Queensland acid soils. I. Solid and solution phase compositions. *Australian Journal of Soil Research* **27:** 333-51.

Buick R. (2008). When did oxygenic photosynthesis evolve? *Biological Science* **363:** 2731–43.

Bryant D.A. and Frigaard N.U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. *Trends Microbiology* **14:** 488–496.

Cao C., Jiang S., Ying Z., Zhang F. and Han X., (2011). Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub Caragana microphylla Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China. *Ecological Engineering* **37:** 1467–1475.

Carrow R. N., Waddington D. V. and Rieke P. E. (2001). Turfgrass Soil Fertility and Chemical Problems: Assessment and Management, Ann Arbor Press, Chelsea. Michigan 212-215.

Clewer A.G. and Scarisbrick D. H. (2001) *Practical Statistics and Experimental Design for Plant and Crop Science*. John Wiley & Sons. 58-60.

Chuchalin A. G., Berman B. and Lehmacher W. (2005). Treatment of acute bronchitis in adults with a *Pelargonium sidoides* preparation (EPs 7630): a randomized, double-blind, placebo-controlled trial. *The Journal of Science and Healing* **1**: 437–445.

Claussen W., Bernhard B., Krumbein A. and Lenz F. (2006). Long-term response of tomato plants to changing nutrient concentration in the root environment - the role of proline as an indicator of sensory fruit quality. *Plant Science* **171:** 323-331.

Daughtry C. S. T., Walthall C. I., Kim M. S., Brown de Colstoun E. and Mcmurtrey J. E. (2000). Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment* **74:** 229-239.

David N., Domitilla R., Lisebo M. and Christine L. (2013). Biodiversity management plan for *pelargonium sidoides* in South African 2011-2020.

Dugger W. M. (1973). Functional aspects of boron in plants as a trace element in the environment. *Advances in Chemistry, American Chemical Society*. Washington D.C. **123:** 112-129.

Evans J. R. (1983). Nitrogen and photosynthesis in the flag leaf of wheat *(Triticum aestivum L.). Plant Physiology* **72:** 297-302.

Fageria N. K. (2002). Soil quality vs. environmentally-based agricultural management practices. *Communications in Soil Science and Plant Analysis* **33:** *2301-2329*

Field C. and Mooney H. A. (1986). The photosynthesis – nitrogen relationship in wild plants. - In: *On the economy of plant form* (GIVNISH T. J., Ed.). Cambridge, University Press, 25-53.

Fox T. C. (2003). pH as the Master Variable. *Handbook of Plant Growth.* Department of Biology University of West Florida 11000 University Parkway Pensacola **43:** 1575-1576.

Franco L, de Oliveira B.H. (2010). Determination of umckalin in commercial tincture and phytopreparations containing *Pelargonium sidoides* by HPLC: Comparison of sample preparation procedure. *Talanta* **81**:1368-1372.

Fystro G. (2002). The prediction of C and N content and their potential mineralisation inheterogeneous soil samples using Vis-NIR spectroscopy and comparative methods. *Plant and Soil* **246:** 139-149.

Gargouri K., Sarbeji M. and Barone E. (2006). Assessment of soil fertility variation in an olive orchard and its influence on olive tree nutrition. Second International Seminar "Biotechnology and Quality of Olive Tree Products Around the Mediterranean Basin" 5-10 November 2006 Marsala-Mazara del Vallo, Italy.

Gerendás J., Zhu Z., Bendixen R., Ratcliffe R. G. and Sattelmacher B. (1997). Physiological and biochemical processes related to ammonium toxicity in higher plants. *Journal of Plant Nutrition and Soil Science* **160:** 239–251.

Glasser G. (2002). Death in the Air: Air Pollution from Phosphate Fertilizer Production. *Synthesis/Regeneration* **8**: 29.

Hinga G., Muchena, F. N. and Njihia, C. N. (1980). Physical and chemical methods of soil analysis. National Agric. Laboratories, Nairobi. Laboratory manual of Total Nitrogen Analysis **2:** 16-15

Hiraoka K., Matsuoka T. and Yoneyama T. (1990). Analysis of nitrate and potassium in soil extract and crop exudates by portable ion meter. *Japanese Journal of Soil Science and Plant Nutrition* **61:** 638-640.

Huang S., Zhang W., Yu X. and Huang Q. (2010). Effects of long-term fertilization on corn productivity and its sustainability in an Ultisol of southern China. *Agriculture, Ecosystems & Environment* **138:** 44–50.

Hue N. V., Uchida R. and Ho M. C. (1997). Testing Your Soil. Why and How to Take a Soil-Test Sample. Department of Agronomy and Soil Science 1910 East-West Rd., Honolulu, HI 96822.

http://www.clw.csiro.au, (13th Feb 2012 at 11:45am)

http://www.eldoradochemical.com/fertiliz1.htm. (6th Dec 2009 11:10pm) Roles of the 16 essential nutrientsin crop development.

http://www.hydroponicsearch.com. (5th Dec 2009 at 11:23pm). Plant Nutrients Primary secondary and micro nutrients.

http://www.newport.com/Introduction-to-FTIR-Spectroscopy/405840/1033/content.aspx. (20th April 2014 at 11.34 am**)**

http://www.plantzafrica.com/plantnop/pelargsidoid.htm. (10th Jan 2012 at 10:45am)

http://www.uksaf.org, ((13th Feb 2012 at 12:10pm)

Johnson A. E. and Milford G. F. J. (2012). Potassium and nitrogen interactions in crops. *Potash Development Association*. Rothamsted Research, Harpenden, Hertfordshire AL5 2JG.

Johnston J. (2006). *Assessing soil fertility*; the importance of soil analysis and its interpretation. Lawes Trust Senior Fellow, Rothamsted Research, Harpenden, AL5 2JQ, UK. RB 209. *The Potash Development Association* **11:** 2-9.

Jork H., Funk W., Fisher W., and Wimmer H. (1990). Thin-Layer Chromatography: Reagents and Methods. VCH Weinheim **1:** 23-24

Kamiyama K., Ohwaki Y. and Yoneyama T. (2000). Establishment of diagnosis of boron status in crop with easy analytical tool. *Japanese Journal of Soil Science and Plant Nutrition* **44:** 253.

Kayer O. and Tan N. (2002). Novel coumarin sulphates from *Pelargonium sidoides:* isolation, structure and synthetic approach. *Proceedings of the Phytochemical Society of Europe* **47**: 59-64

Kinsey N. (2001). Rebuilding Soil Fertility: Greater Yields through the Albrecht Method of Soil Management. *A Voice for Eco- Agriculture* **31:** 2-4.

Kolodziej H. (2000). Traditionally used *Pelargonium species:* Chemistry and biological activity of umckaloabo extracts and their constituents. *Current Topics in Phytochemistry* **3:** 77-93.

Kolodziej H. (2007). Fascinating metabolic pools of *Pelargonium sidoides and Pelargonium reniforme,* traditional and phytomedical sources of the herbal medicine Umckaloabo. *Phytomedicine* **6:** 9-17.

Lachat Instruments. (1995). Total Kjeldahl Nitrogen in Soil/Plant. *Quick Chemistry Method* **13**: 2-3.

Liu M., Li Z.P., Zhang T.L., Jiang C.Y. and Che Y.P. (2011). Discrepancy in response of rice yield and soil fertility to long-term chemical fertilization and organic amendments in paddy soils cultivated from infertile upland in subtropical China. *Agricultural Sciences in China* **10:** 259–266.

Lizogub V. G., Riley D. S. and Heger M. (2007). Efficacy of a *Pelargonium sidoides* preparation in patients with the common cold: a randomized, double blind, placebo-controlled clinical trial. *The Journal of Science and Healing* **3:** 573–584.

Longstreth D. J. and Nobel P. S. (1980). Nutrient Influences on Leaf Photosynthesis. *Plant Physiology* **65:** 541-543.

López-Granados F., Jurado-Expósito M., Atenciano S., Garc´ıa-Ferrer A., Sánchez de la Orden M. and Garc´ıa-Torres L. (2002). Spatial variability of agricultural soil parameters in Southern Spain. *Plant and Soil* **246:** 97–105.

Maeda M., Ozaki Y. and Yoneyama T. (2003). Nitrate leaching in an Andisol treated with different types of fertilizers. *Environmental Pollution* **121**: 477-487.

Manna M., Swarup A., Wanjari R., Mishra B. and Shahi D. (2007). Long-term fertilization, manure and liming effects on soil organic matter and crop yields. *Soil and Tillage Research* **94:** 397–409.

Matthys H., Eisebitt R., Seith B. and Heger M. (2003). Efficacy and safety of an extract of *Pelargonium sidoides* (EPs 7630) in adults with acute bronchitis. A randomised, double-blind, placebo-controlled trial. *Phytomedicine* **4:** 7-17.

Mehlich A. (1953). Determination of P, Ca, Mg, K, Na, and NH_4. North Carolina Soil Test Division (Mimeo 1953).

Miller G. L., (1979). Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Analytical. Chemistry* **31:** 426.

Morita A. and Ohta M. (2001). An analysis of oxalic acid in tea extract using a simple reflection photometer system. *Japanese Journal of Soil Science and Plant Nutrition* **72:** 274-276.

Mutuo P. K., Shepherd K. D., Albrecht A. and Cadisch G. (2006). Prediction of carbon mineralization rates from different soil physical fractions using diffuse reflectance spectroscopy. *Soil Biology and Biochemistry*, **38:** 1658-1664.

Mouazen A. M., Baerdemaeker, J. D. and Ramon, H. (2006). Effect of wavelength range on the measurement accuracy of some selected soil constituents using visual-near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, **14**, No.3, 189-199, ISSN 0967-0335.

Pai-ilsson B. A. M. (1988). Mineral nutrients, carbohydrates and phenolic compounds in leaves of beech in southern Sweden as related to environmental factors. Department of Ecology, *Plant Ecology*, University of Lund **6:** 22-35.

Phyllis B., Kathryn D. and Prentice H. (1997). Handbook of Instrumental Techniques for Analytical Chemistry" Frank Settle, Editor: "High Performance Liquid Chromatography" 147-164.

Puckrin E., Evans W.F.J. and Adamson T.A.B. (1996). Measurement of tropospheric ozone by thermal emission spectroscopy. *Atmospheric Environment* 30(4): 563-568

Radin J. W., Parker L. L. and Sell C. R. (1978). Partitioning of sugar between growth and nitrate reduction in cotton roots. *Plants physiology* **62:** 550-553

Raza M., Mermut A.R., Schoenau J.J. and Malhi S.S. (2002). Boron fractionation in some Saskatchewan soils. *Canadian Journal of Soil Science* **82:** 173-179.

Rengel Z. (1999). Mineral nutrition of crops: fundamental mechanisms and implications. *Technology & Engineering, Food product Press* **6**: 121-123.

Reuter D. J. and Robinson J B. (1986). The plant analysis. An interpretation manual. Inkata Press Pty Ltd., Victoria, Australia**:** 140-141

Rojstaczer S., Sterling S.M. and Moore N. J. (2001). Human appropriation of photosynthetic products. *Science* **294**: 2549-2552.

Roppongi K. (1991). Studies on nutritional diagnosis in fruit vegetables. The diagnosis of nitrogen nutrition in cucumber on nitrate density of petiole juice. *Research Bulletin of the Saitama Horticultural Research Center* **18:** 1-15.

Scachtman D. P., Reid R. J. and Ayling S. M. (1998). Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiology* **116:** 447-453.

Schnitzer P., Schneider S., Stintzing F.C., Carle R. and Reichling J. (2008). Efficacy of an aqueous *Pelargonium sidoides* extract against herpes virus. *Phytomedicine* **9:** 734-740.

Schoetz K., Erdelmeier C., Germer S. and Hauer H. (2008). A Detailed View on the Constituents of EPs® 7630. *Planta Medica.* **74:** 667-674.

Schwoerbel J. and Tillmanns G. C. (1977). Uptake of nitrate from the water and activity of nitrate reductase by Fontinalis antipyretica L. under light and dark conditions. *Archives of Hydrobiology (Supplement.)* **48:** 412-423.

Seidel V. and Taylor P.W. (2004). In vitro activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. *International Journal of Antimicrobial Agents* **23:** 613-619.

Siddiqui Y., Meon S., Ismail R., and Rahmani M. **(**2009). Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. *Biological Control* **49:** 38-44.

Shen J.P., Zhang L.M., Guo J.F., Ray J.L. and He J.Z. (2010). Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Applied Soil Ecology* **46:** 119–124.

Shuster W. D., Shipitalo M. J., Subler S., Aref S., McCoy E. L. and McKeegan C. (2004). Earthworm additions affect leachate production and nitrogen losses in typical midwestern agroecosystems. *Journal of Environmental Quality* **32:** 2132-2139.

Skoog D. A., Holler F. J. and Nieman T. A. (1998). Principles of Instrumental Analysis, fifth edition, Saunders College Publishing 673-697, 725-766.

Smith A. L. (1997). Photosynthesis – the synthesis by organisms of organic chemical compounds, esp. carbohydrates, from carbon dioxide using energy obtained from light rather than the oxidation of chemical compounds. *Oxford dictionary of biochemistry and molecular biology* **1:** 508.

Squire G.R. (1990). *The physiology of tropical crop production.* CAB International.Wallingford Oxon, UK 236.

Takebe M. (2000). The latest research of diagnosis plant nutrition and the problem on determination of diagnostic critical value *Bulletin of the National Agricultural Research Center* **46:** 57-71.

Timmer A., Günther J., Rücker G., Motschall E., Antes G. and Kern W. V. (2008). *Pelargonium sidoides* extract for acute respiratory tract infections. *Phytomedicine* **15:** 378-385.

Tucker M. (2004). Primary Nutrients and Plant Growth. - In: *Essential Plant Nutrients.* North Carolina Department of Agriculture

Van der Walt J.J.A. and Vorster P. J. (1988). *Pelargoniums* of South Africa. Kirstenboschi, National Botanical Gardens. South African National Biodiversity Institute **1:** 20-24.

Vitousek P. (1982). Nutrient Cycling and Nutrient Use Efficiency**.** *The American Naturalist* **553:** 553-572.

Viscarra R. R. A., Walvoort D. J. J., McBratney A. B.. Janik L. J. and Skjemstad J. O. (2006). Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. *Geoderma* **131:** 59-75.

Viscarra R. R. A. and Behrens T. (2010). Using data mining to model and interpret soil diffuse reflectance spectra. *Geoderma*, **158:** 46-54.

Vitousek P., Ehrlich P. R., Ehrlich A. H. and Matson P. (1986). Human appropriation of the products of photosynthesis. *BioScience* **36**: 368-373.

Vogeler I., Rogasik J., Funder U., Panten K., and Schnug E. (2009). Effect of tillage systems and P-fertilization on soil physical and chemical properties, crop yield and nutrient uptake. *Soil and Tillage Research* **103:** 137–143

Weast R.C. (1981). Handbook of chemistry and physics: 150-151.CRC Press, Boca Raton, FL.

Whitbread A., Blair G., Konboon Y., Lefroy R. and Naklang K. (2003). Managing crop residues, fertilizers and leaf litters to improve soil C, nutrient balances, and the grain yield of rice and wheat cropping systems in Thailand and Australia. *Agriculture, Ecosystems & Environment* **100:** 251–263.

Wood C.W., Reeves D.W., Himelrick D.G. (1993). Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield : A review. *Proceedings Agronomy Society of New Zealand* **23**: 1–9.

Wu Z., Gu Z., Wang X., Evans L., and Guo H. (2003). Effects of organic acids on adsorption of lead onto montmorillonite, goethite and humic acid. *Environmental Pollution* **121:** 469–475.

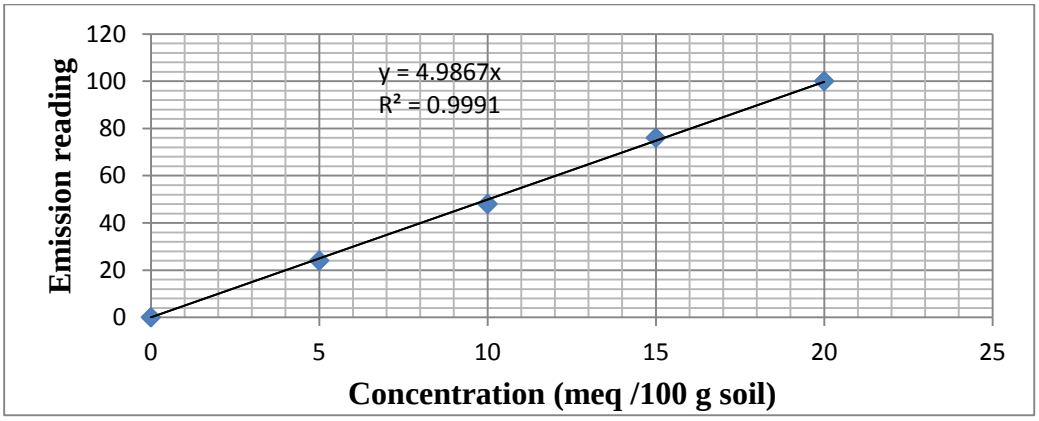
Yang J.Y., Yang X.E., He Z.L., Li T.Q., and Shentu J.L. (2006). Effects of pH, organic acids, and inorganic ions on lead desorption from soils. *Environmental Pollution* 1**43**: 9–15.

Zhao J., Dong Y., Xie X., Li X., and Zhang X. (2011). Effect of annual variation in soil pH on available soil nutrients in pear orchards. *Acta Ecologica Sinica* **31:** 212–216.

APPENDICES

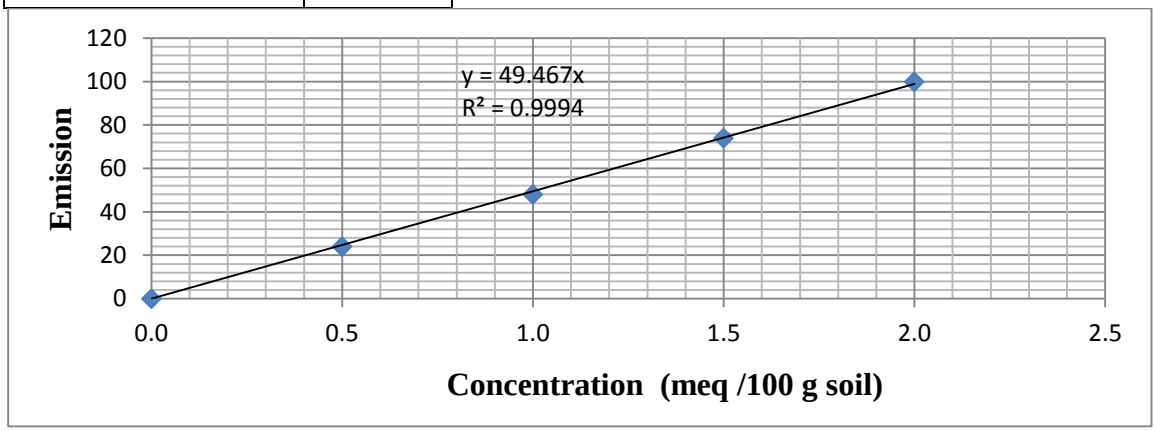
APPENDIX 1: STANDARD CALIBRATION CURVE FOR **Ca** IN SOIL SAMPLES

Calcium	
Conc. (meq%)	Emission
0	0
5	24
10	48
15	76
20	100



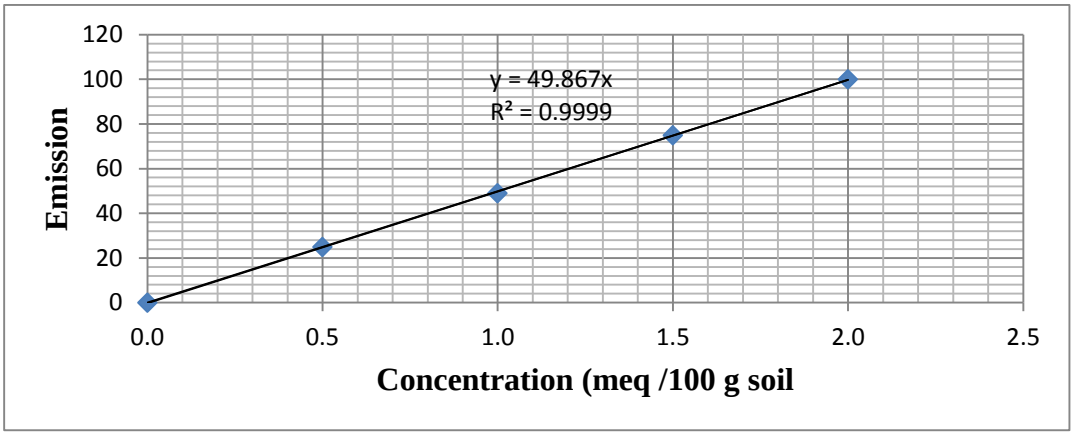
APPENDIX 2: STANDARD CALIBRATION CURVE FOR **K** IN SOIL SAMPLES

Potassium	
Conc. (meq%)	Emission
0.0	0
0.5	24
1.0	48
1.5	74
2.0	100



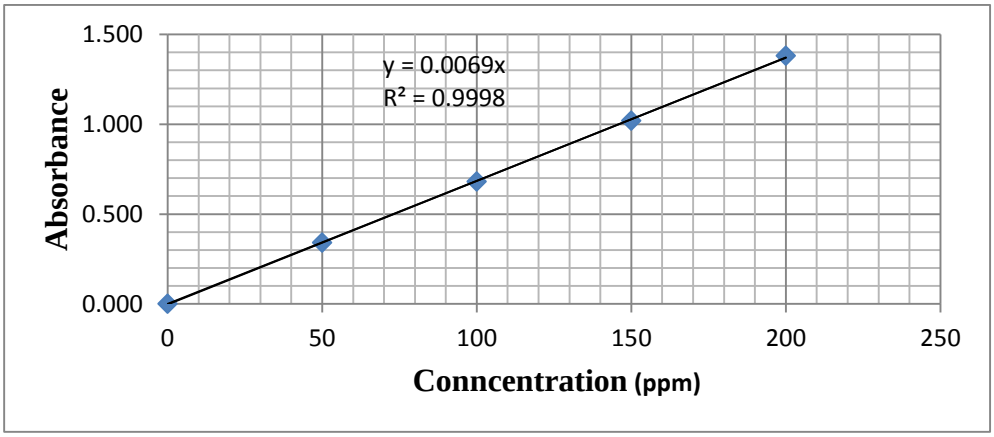
APPENDIX 3: STANDARD CALIBRATION CURVE FOR Na IN SOIL SAMPLES

Sodium	
Conc. (meq%)	Emission
0.0	0
0.5	25
1.0	49
1.5	75
2.0	100



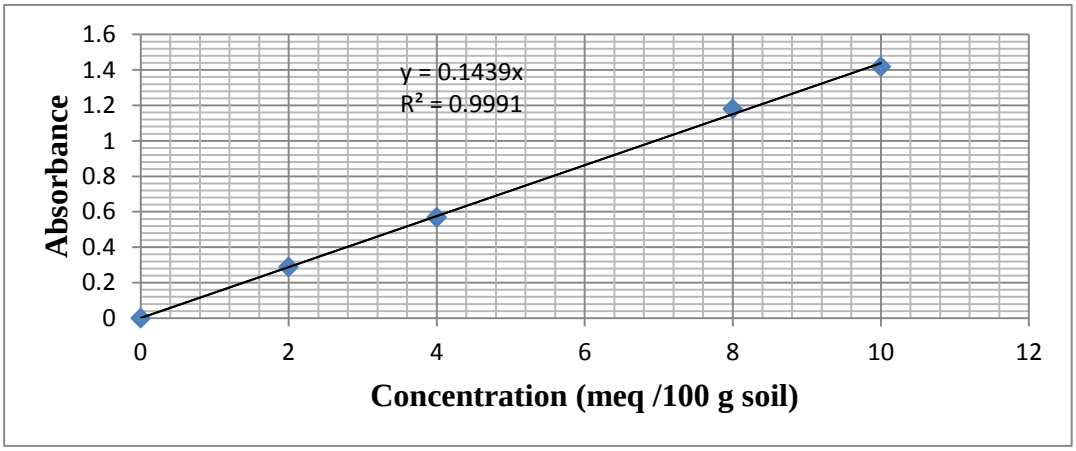
APPENDIX 4: STANDARD CALIBRATION CURVE FOR **P** IN SOIL SAMPLES

Phosphorous	
Conc (ppm)	Abs
0	0.000
50	0.341
100	0.680
150	1.020
200	1.380



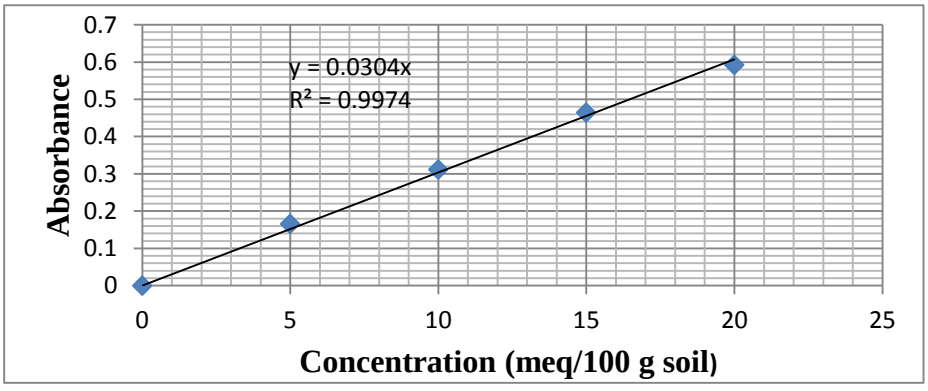
APPENDIX 5: STANDARD CALIBRATION CURVE FOR **Mg** IN SOIL SAMPLES

Magnesium	
Conc. (meq%)	Absorbance
0	0
2	0.288
4	0.569
8	1.180
10	1.418



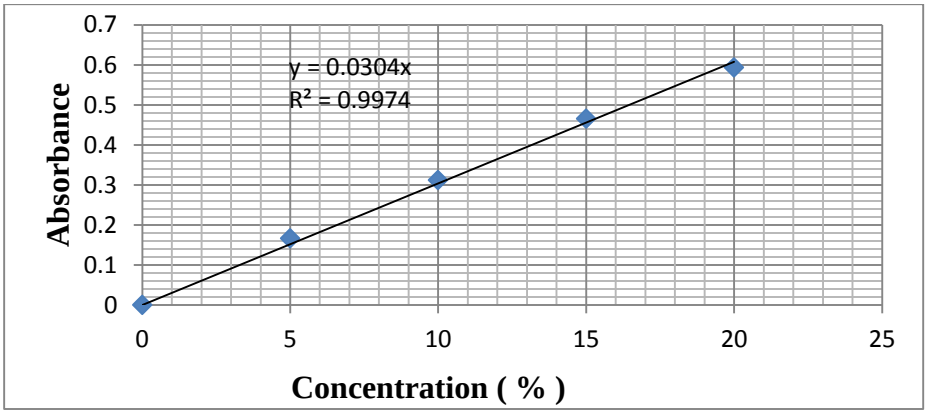
APPENDIX 6: STANDARD CALIBRATION CURVE FOR **Mn** IN SOIL SAMPLES

Manganese	
Conc. (meq%)	Abs
0	0
5	0.166
10	0.312
15	0.465
20	0.593

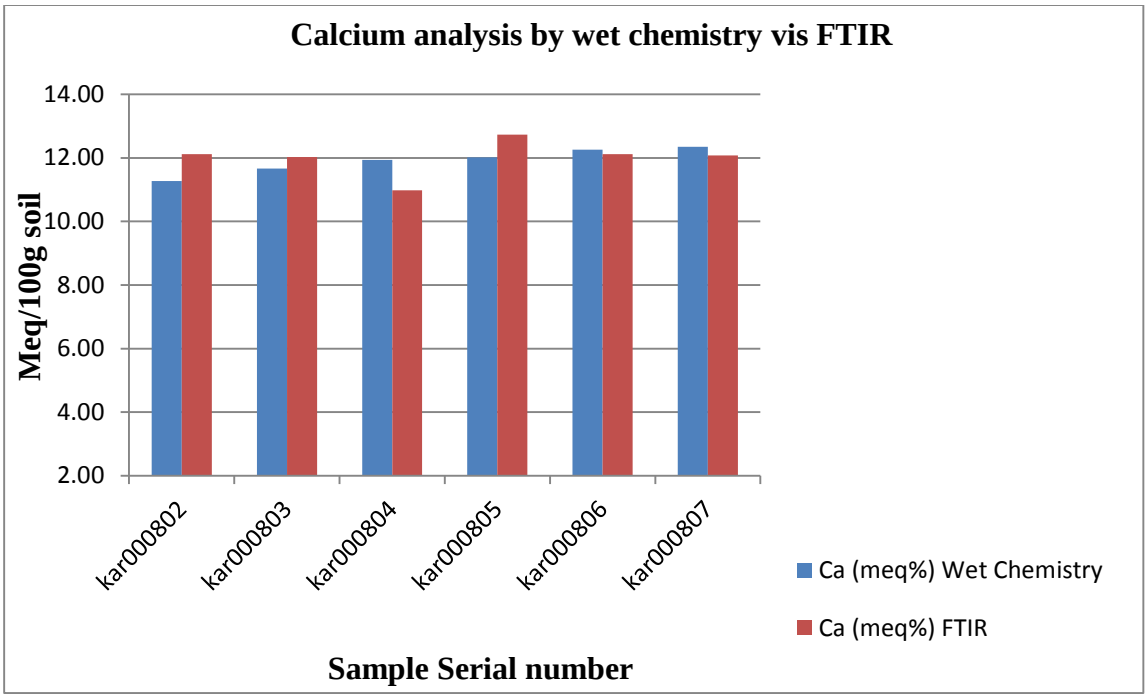


APPENDIX 7: STANDARD CALIBRATION CURVE FOR **OC** IN SOIL SAMPLES

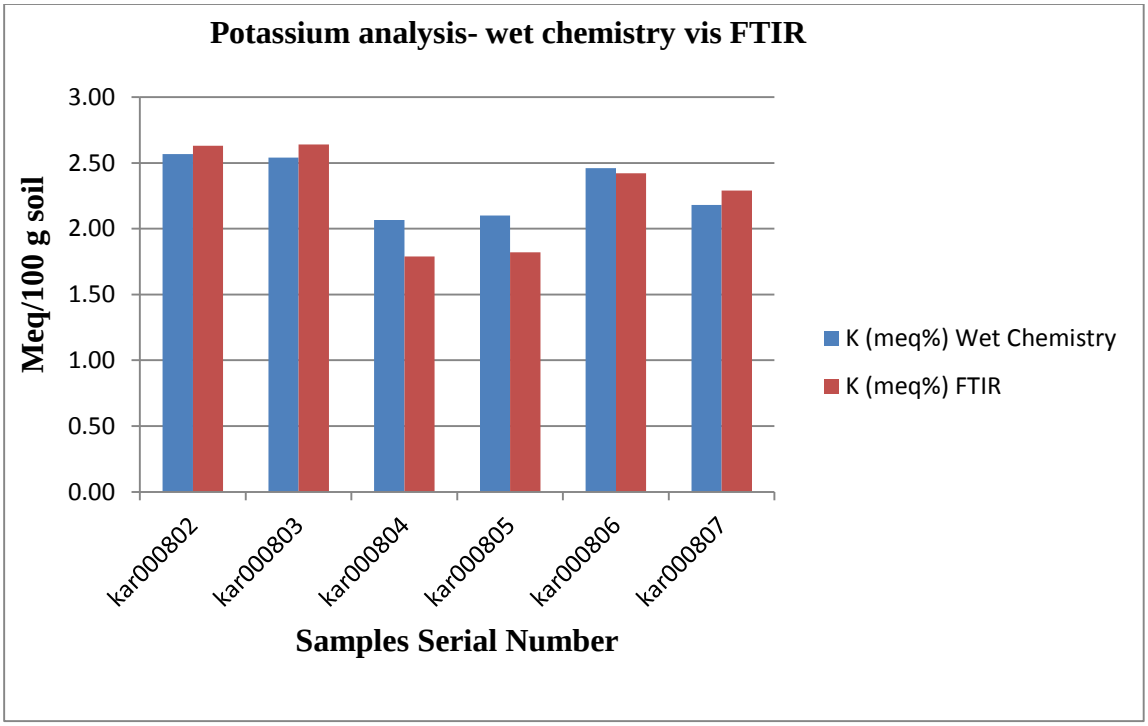
Organic Carbon	
% carbon	Abs
0	0
05	0.166
10	0.312
15	0.465
20	0.593



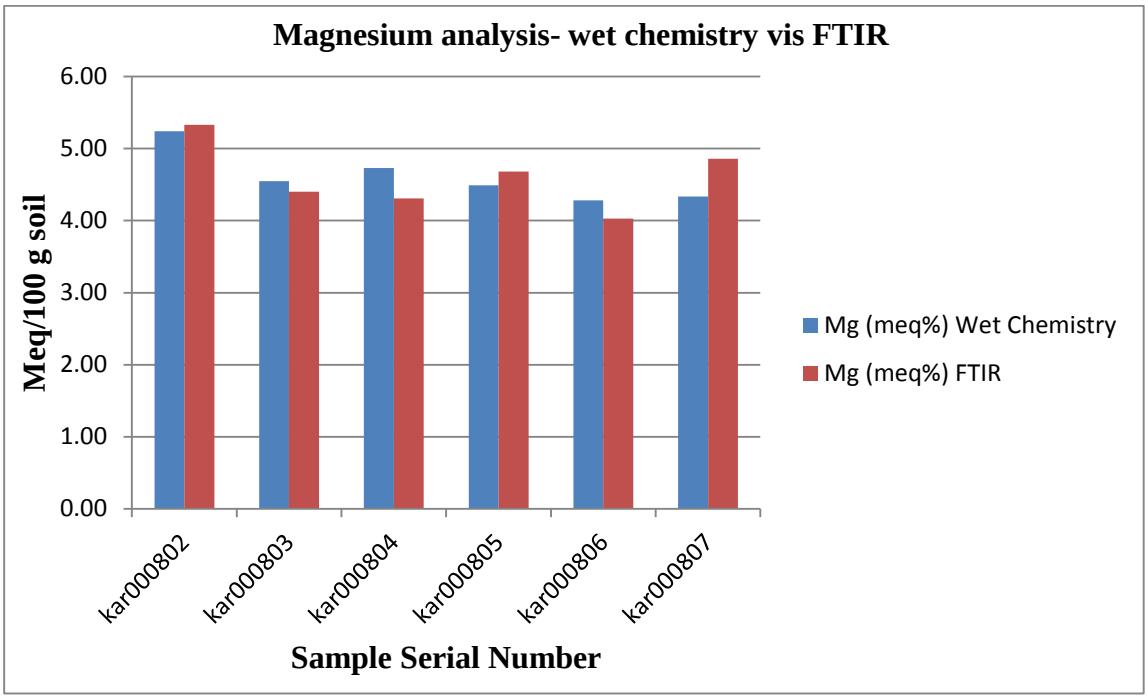
APPENDIX 8: SOIL ANALYSIS – WET CHEMISTRY VERSUS FTIR METHODS



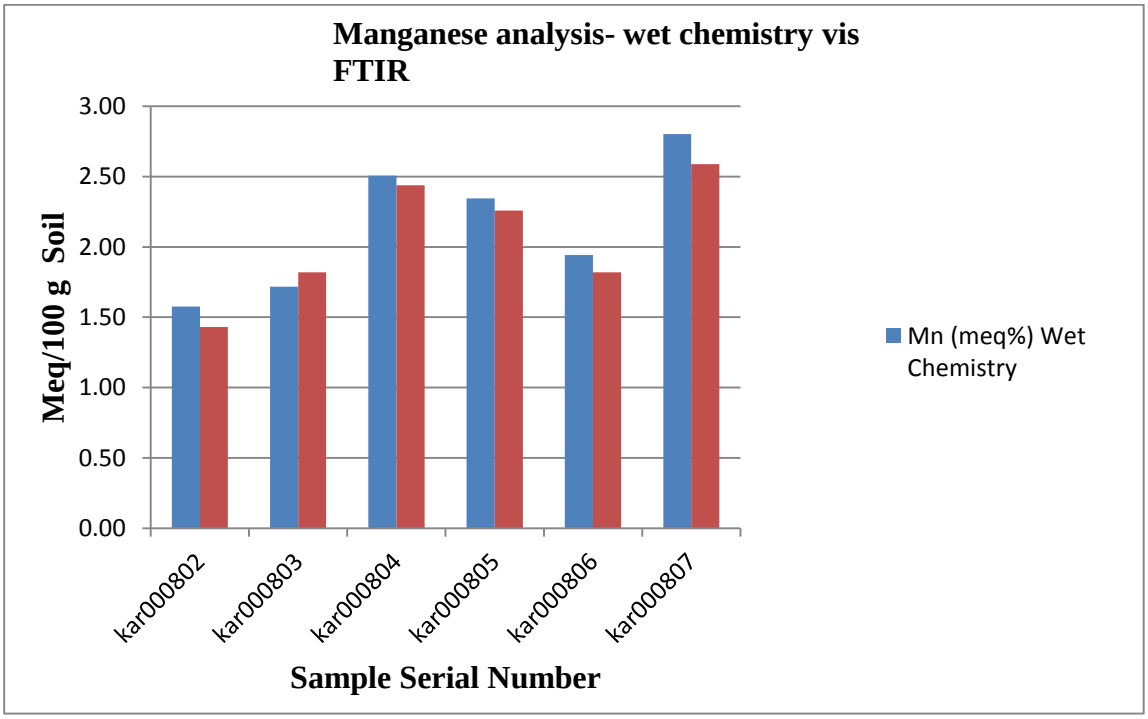
8.1 Concentration of Calcium in the soil - wet chemistry versus FTIR data



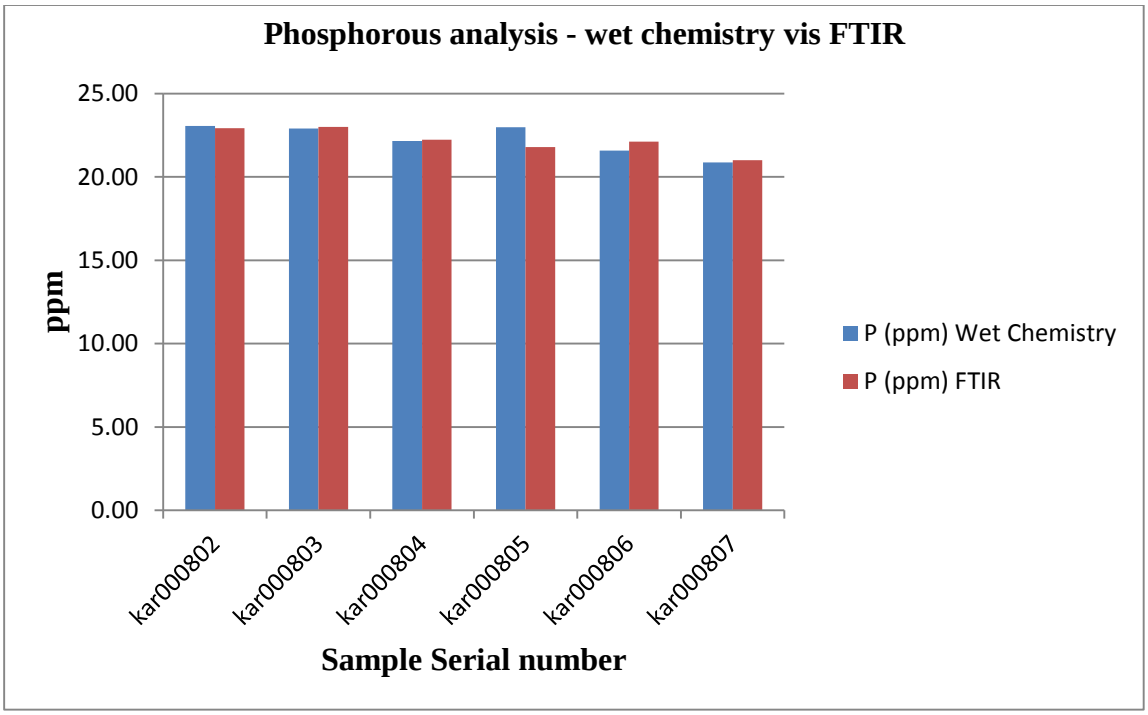
8.2 Concentration of Potassium in the soil -wet chemistry versus FTIR data



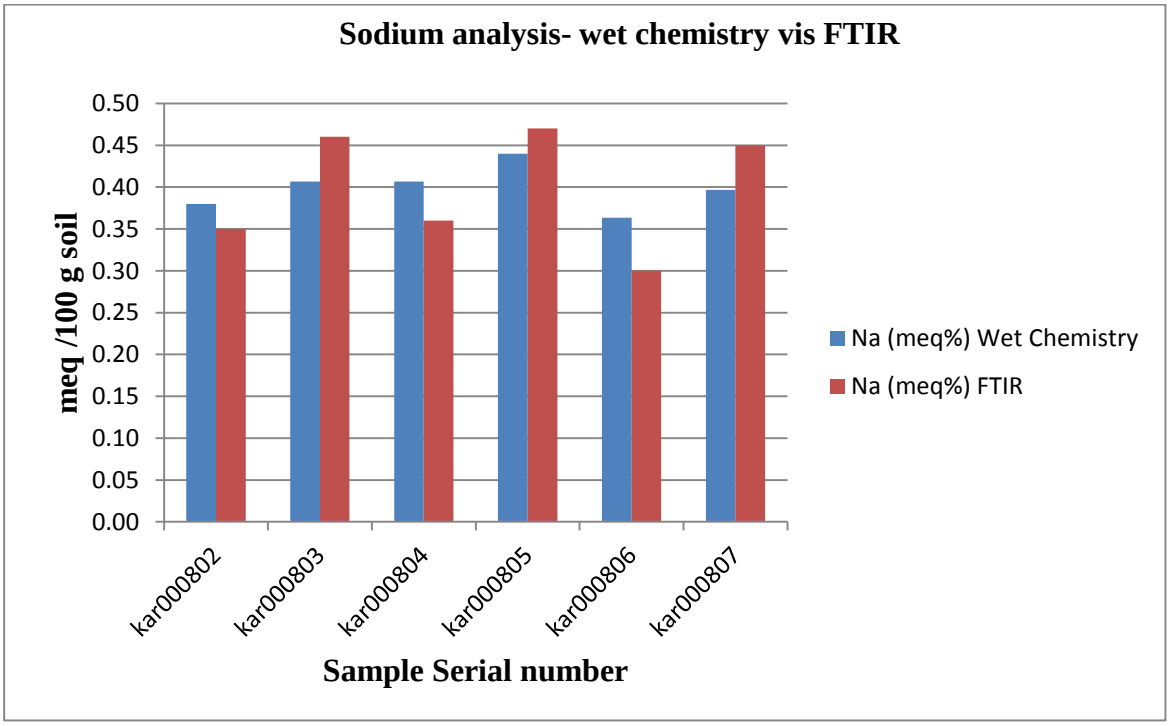
8.3 Concentration of Magnesium in the soil - wet chemistry versus FTIR data



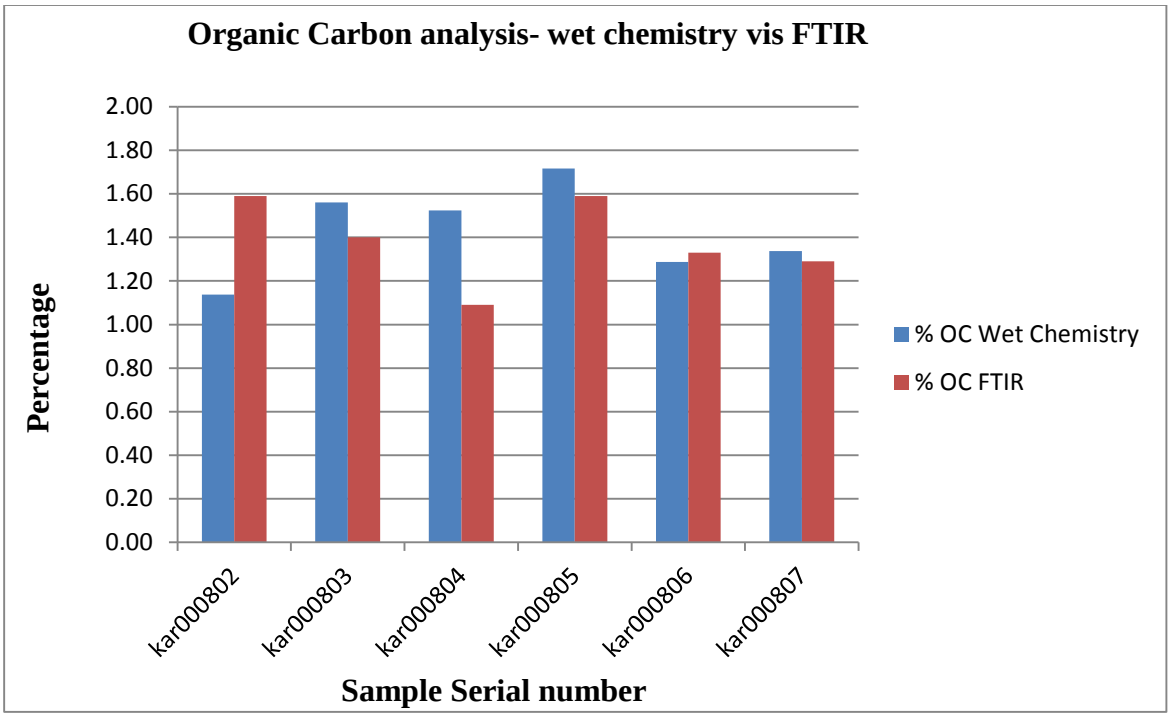
8.4 Concentration of Manganese in the soil - wet chemistry versus FTIR data



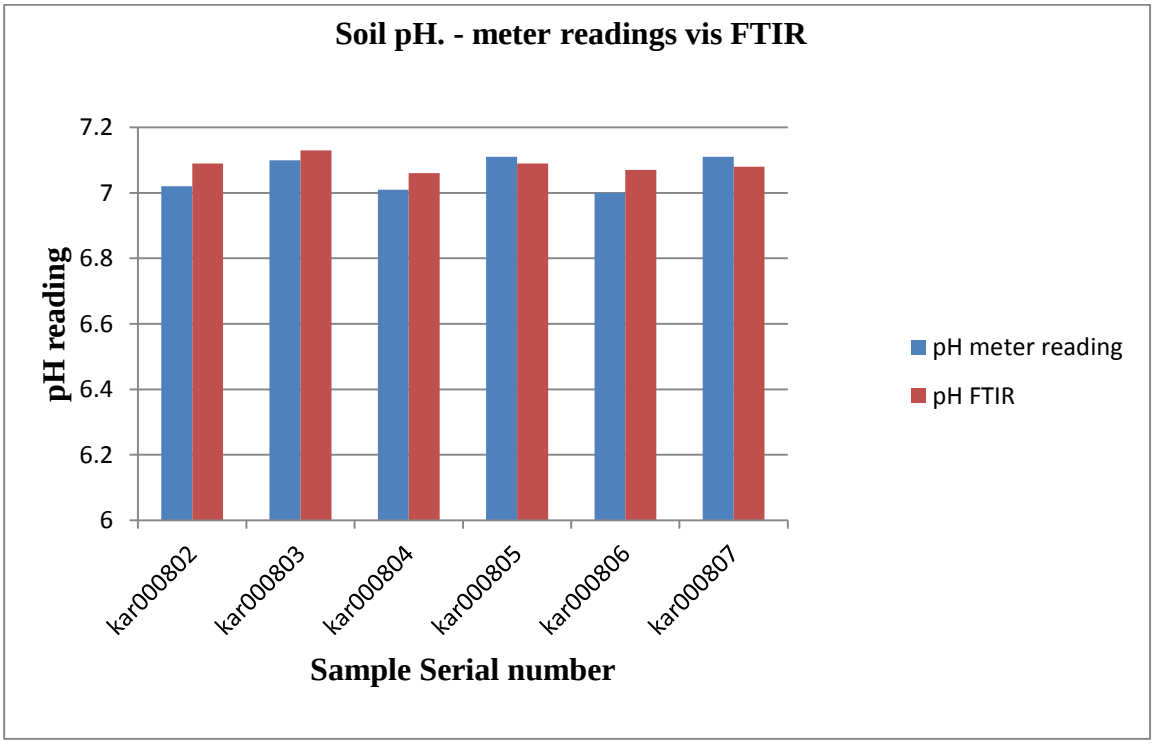
8.5 Concentration of Phosphorous in the soil - wet chemistry versus FTIR data



8.6 Concentration of Sodium in the soil - wet chemistry versus FTIR data



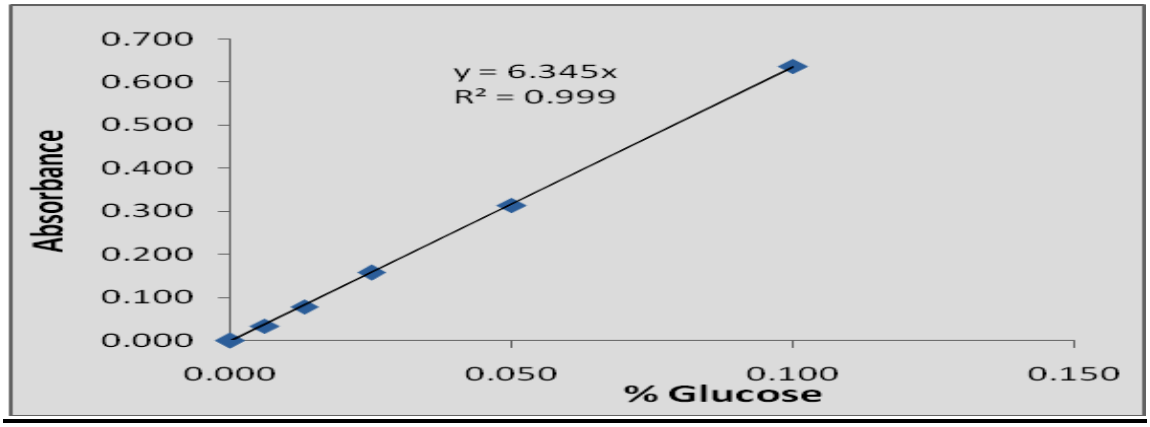
8.7 Concentration of Organic Carbon in the soil - wet chemistry versus FTIR data



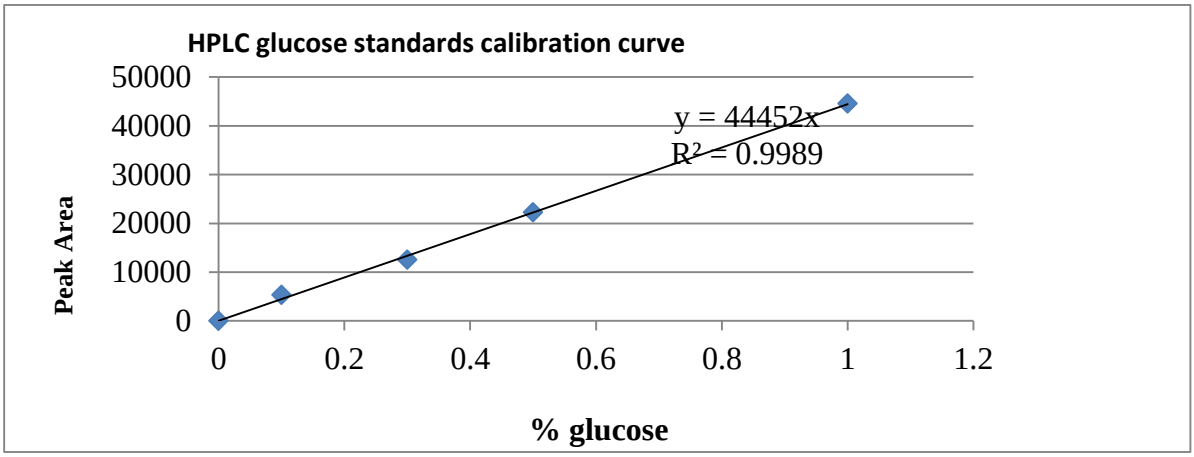
8.8 Soil pH - meter readings versus FTIR data

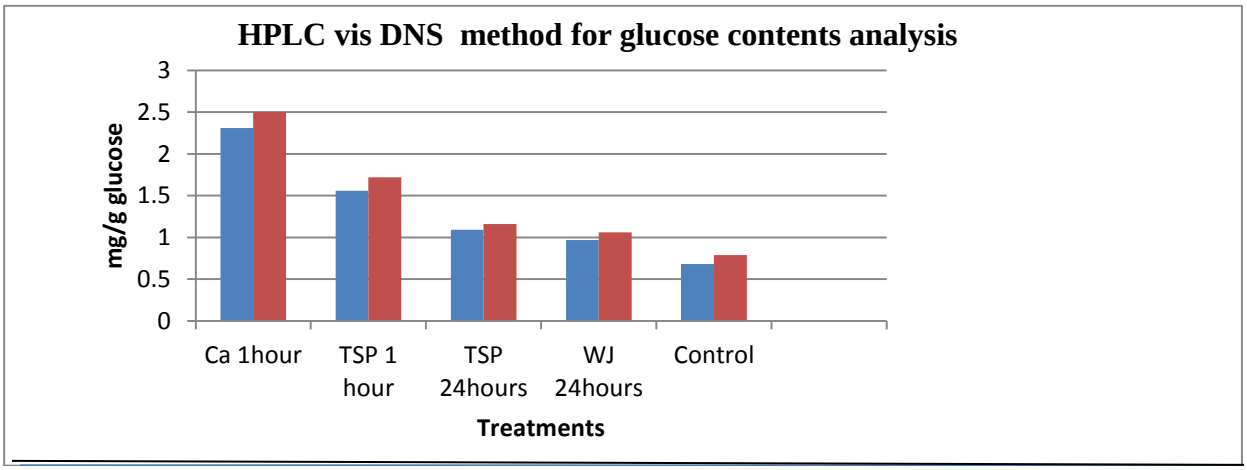
APPENDIX 9: STANDARD CALIBRATION CURVE FOR GLUCOSE ONE HOUR AFTER APPLICATION OF FERTILIZERS

Glucose Standards (%)	Absorbance
0.000	0.000
0.006	0.034
0.013	0.079
0.025	0.158
0.050	0.314
0.100	0.637

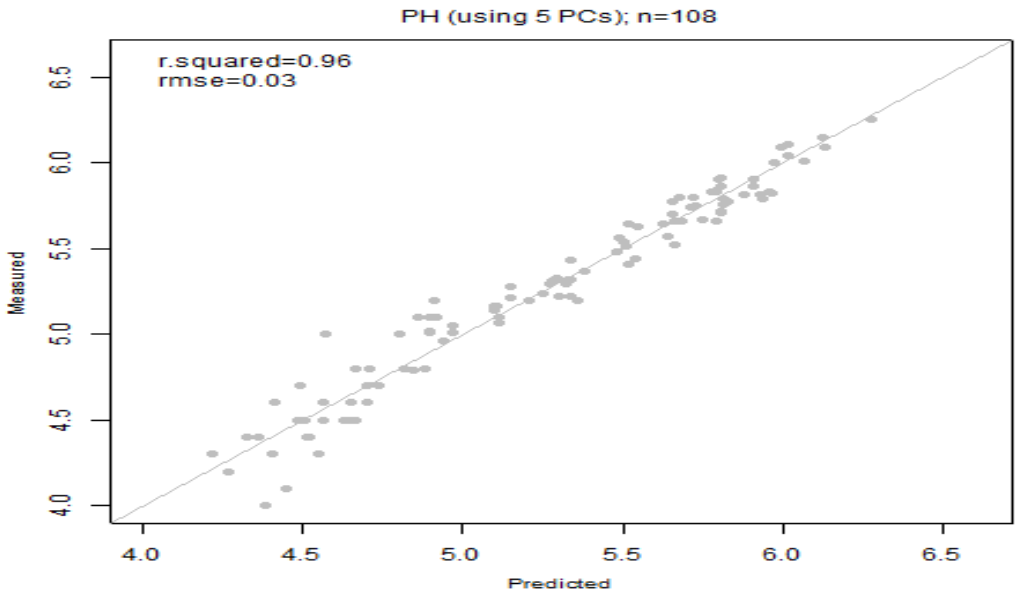


APPENDIX 10: CALCULATIONS FOR THE COMPARISON OF HPLC AND DNS DATA



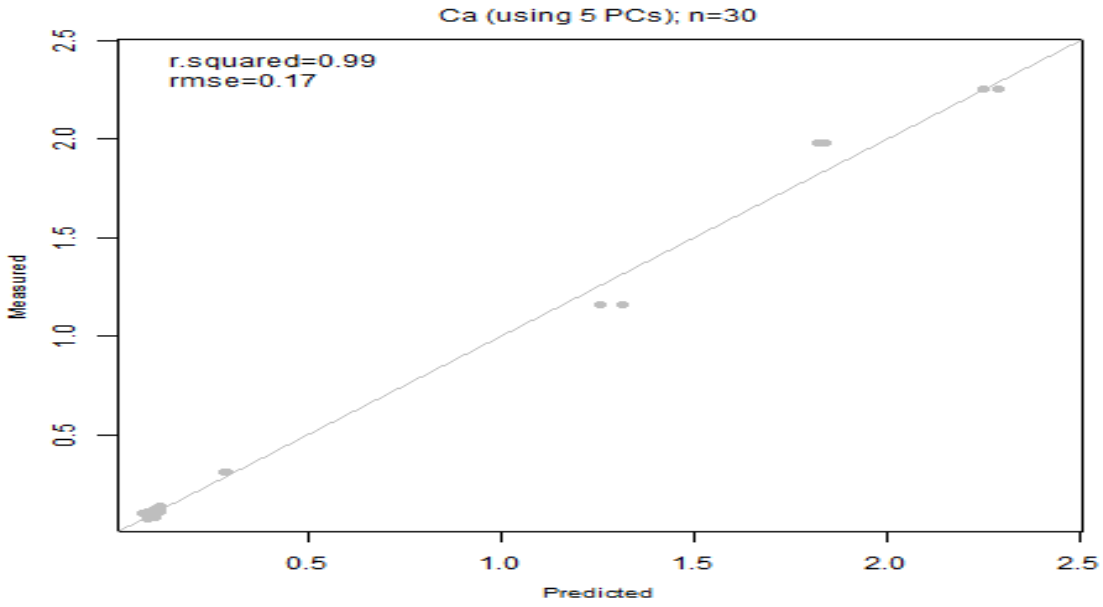


APPENDIX 11: CALIBRATION CURVE FOR FTIR PREDICTIONS FOR SOIL pH

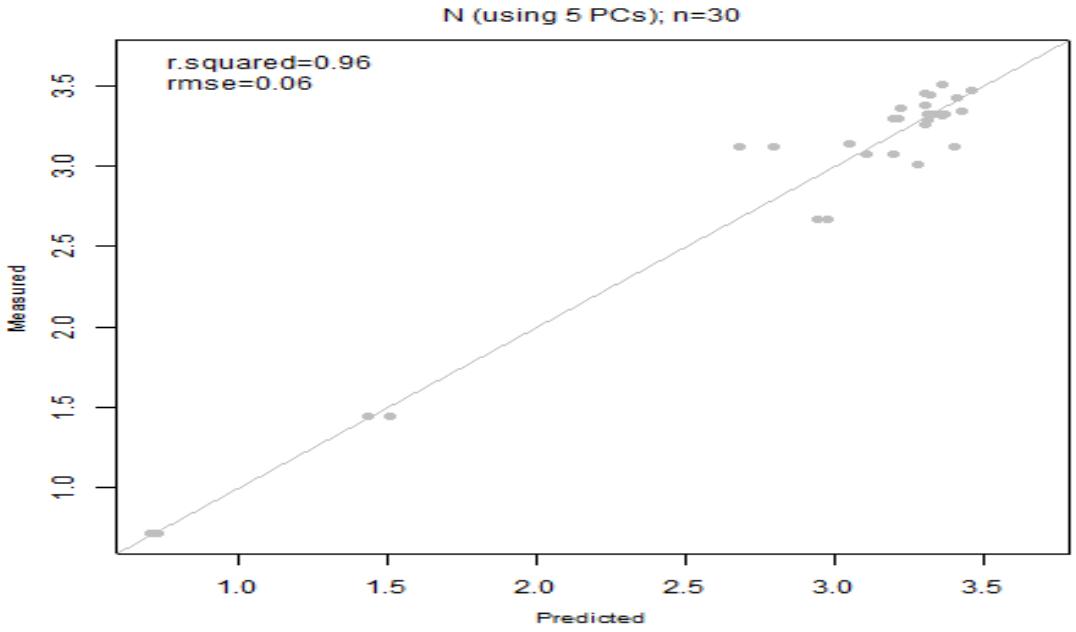


11.1 FTIR calibration curve for pH in soil samples

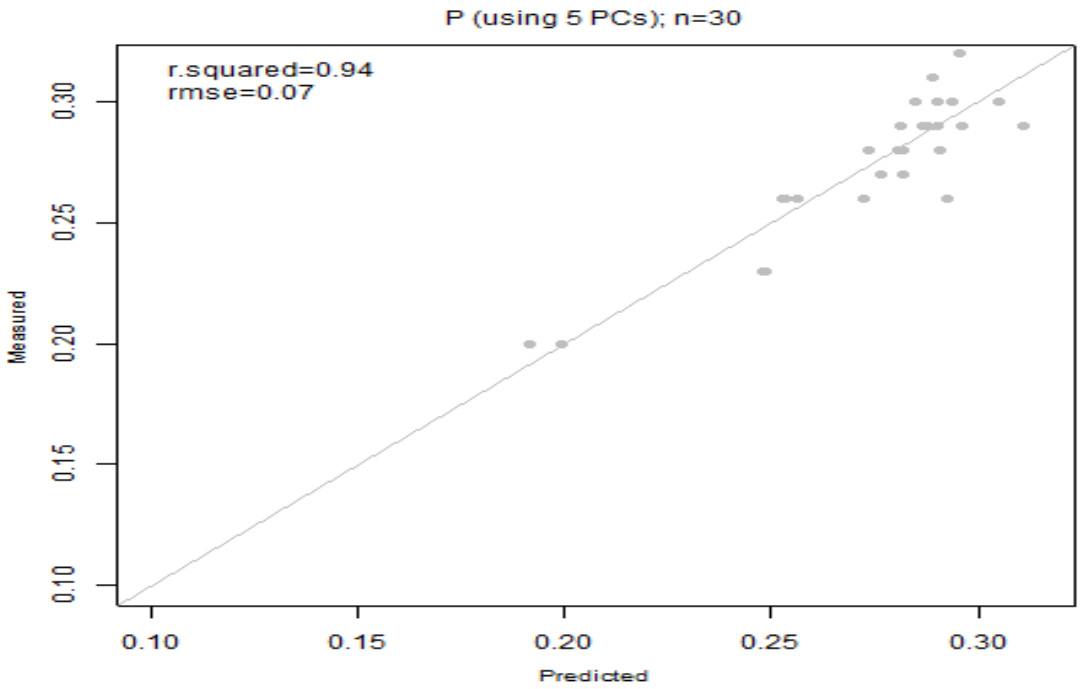
APPENDIX 12: CALIBRATION CURVE FOR LEAF NUTRIENTS USING FTIR PREDICTIONS



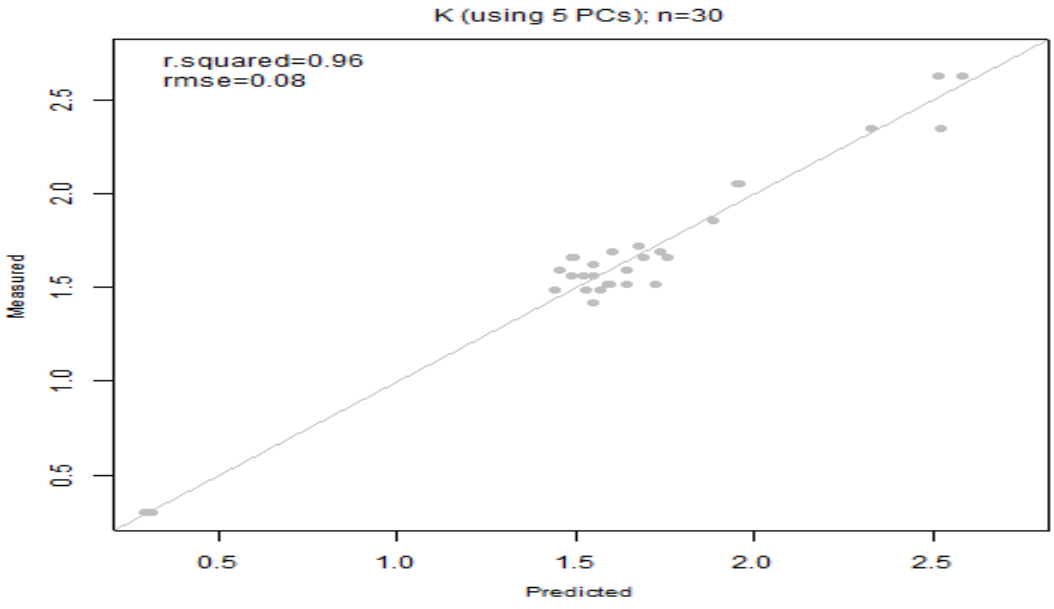
12.1 Calibration curve for leaf Calcium predictions using FTIR



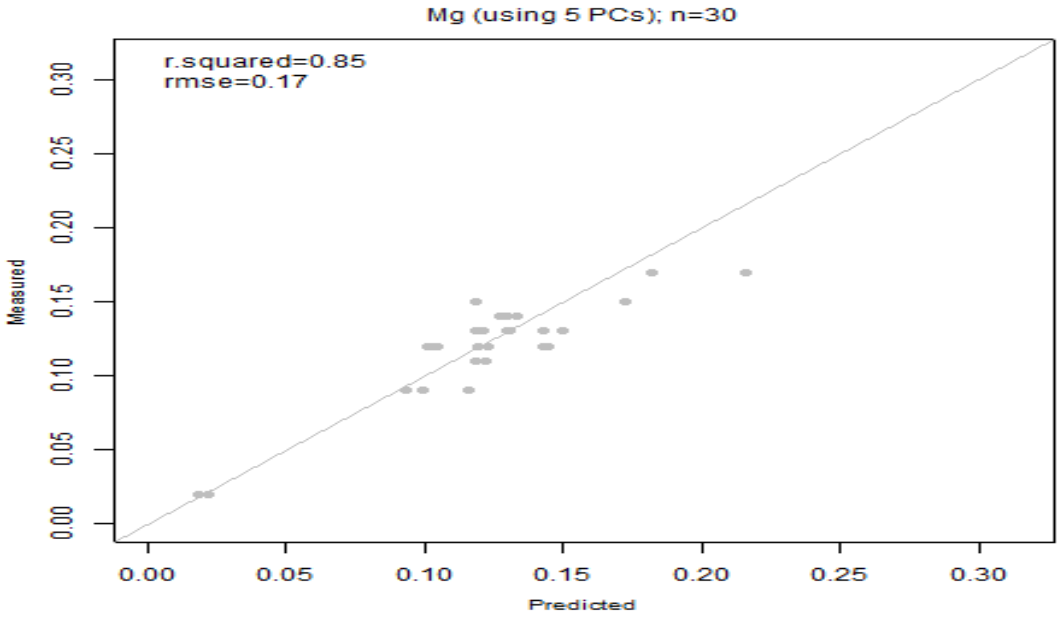
12.2 Calibration curve for leaf Nitrogen predictions using FTIR



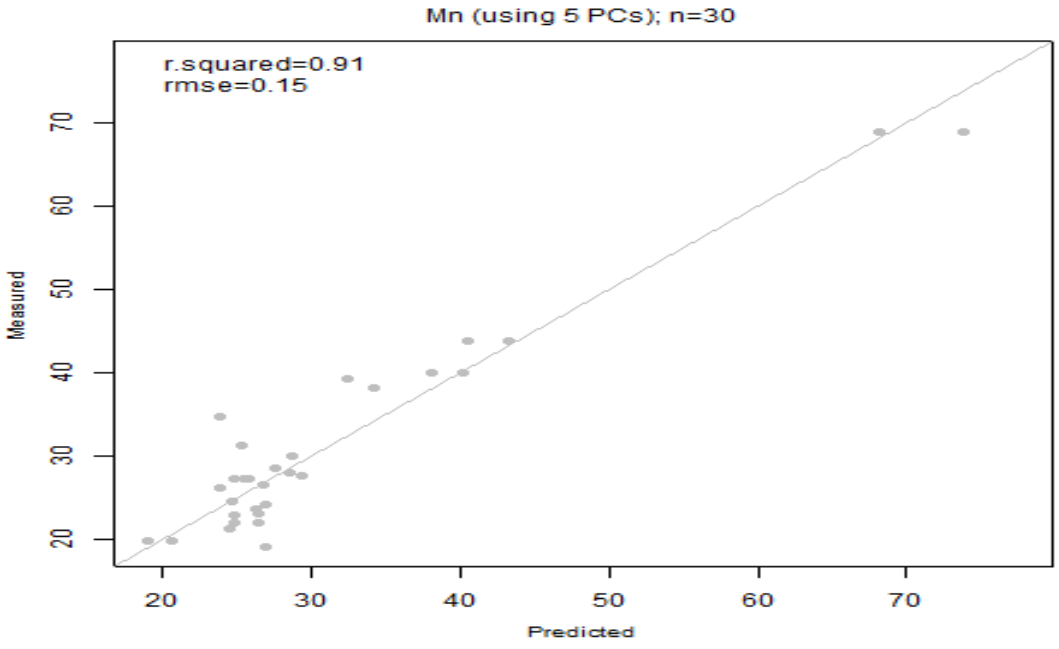
12.3 Calibration curve for leaf Phosphorous predictions using FTIR



12.4 Calibration curve for leaf Potassium predictions using FTIR



12.5 Calibration curve for leaf Magnesium predictions using FTIR



12.6: Calibration curve for leaf Manganese predictions using FTIR

APPENDIX 13: TABLES OF INTERPRETATIVE VALUES

13.1 Table for soil interpretative values

CLASSIFICATION OF AVAILABLE NUTRIENTS IN SOILS

Nutrient	Deficiency level	Adequate level	Excessive level	Remarks
MEHLICH METHOD				
Sodium, me%	seldom applies	0-2.0	> 2.0	excessive levels in saline & sodic soils
Potassium, me%	< 0.24	0.24-1.5	> 1.5	
Calcium, me%	< 2.0	2.0-15.0	> 15.0	
Magnesium, me%	< 1.0	1.0-3.0	> 3.0	
Phosphorus, ppm	< 30 < 80	30-80	> 80	for flowers
Manganese, me%	< 0.11	0.11-2.0	> 2.0	excessive levels in very acid or poorly drained soils
TOTAL NITROGEN & CARBON				
Total nitrogen, %	< 0.2	0.2-0.5	> 0.5	
Total organic carbon, %	< 1.33	2.66-5.32	> 5.32	1.33-2.65 moderate level
Extraction with 0.1 M HCl				
Copper, ppm	< 1.0			
Iron, ppm	< 10			
Zinc, ppm	< 5.0			
OLSEN METHOD				
Nutrient	Deficiency level	Adequate level	Excessive level	
Phosphorus, ppm	< 10	10.0-20.0	> 20.0	

13.2 Table for Geranium (Pelargonium) interpretative values

:00	>200		Zn	36	
:00	>200				

nia jasminoides)

developed

ICIENT	HIGH
%	
0-3.0	>3.0
6-0.4	>0.4
0-3.0	>3.0
0-1.3	>1.3
5-1.0	>1.0
0-0.4	>0.4
pm	
5-70	>70
5-40	>40
250	>250
250	>250
150	>150

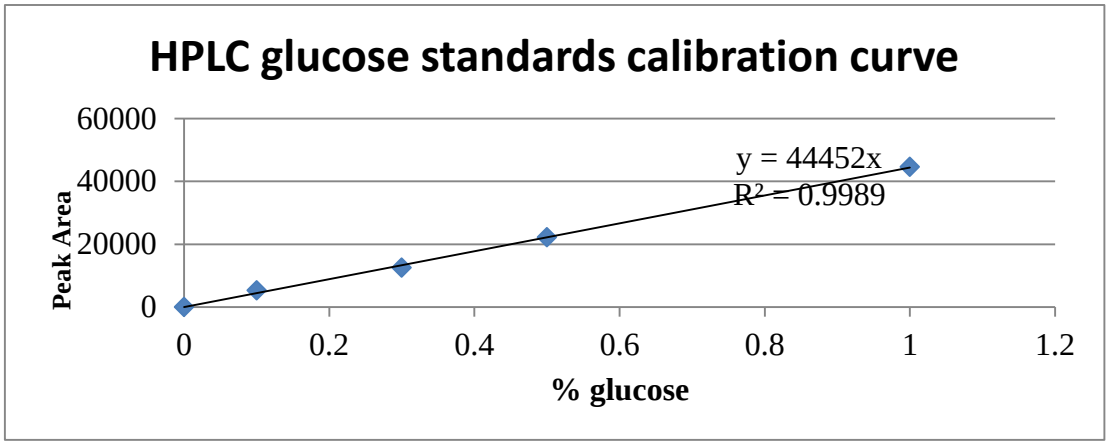
CROP	Geranium (*Pelargonium x hortorum*)
NUMBER	15
PLANT PART	Most recent fully developed leaf
TIME	none specified

ELEMENT	LOW	SUFFICIENT	HIGH
		%	
N	3.00-3.49	3.50-4.8	>4.8
P	0.30-0.39	0.40-0.7	>0.7
K	1.00-2.49	2.50-4.3	>4.3
Ca	0.60-0.79	0.80-1.2	>1.2
Mg	0.15-0.19	0.20-0.5	>0.5
S	0.20-0.24	0.25-0.7	>0.7
		ppm	
B	18-29	30-200	201-300
Cu	5-6	7-25	>25*
Fe	60-99	100-250	>250
Mn	25-39	40-200	>200
Zn	12-17	18-200	>200

(Reuter and Robinson, 1986)

APPENDIX 14: STANDARD CALIBRATION CURVE FOR THE ANALYSIS OF LEAF GLUCOSE using HPLC

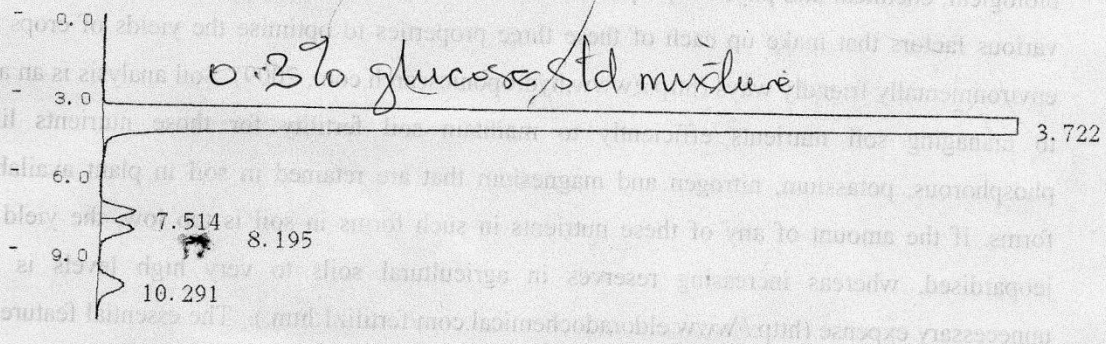
% glucose	Peak Area
0	0
0.1	5351
0.3	12575
0.5	22234
1	44586



APPENDIX 15: HPLC CHROMATOGRAMS FOR THE ANALYSIS OF LEAF GLUCOSE

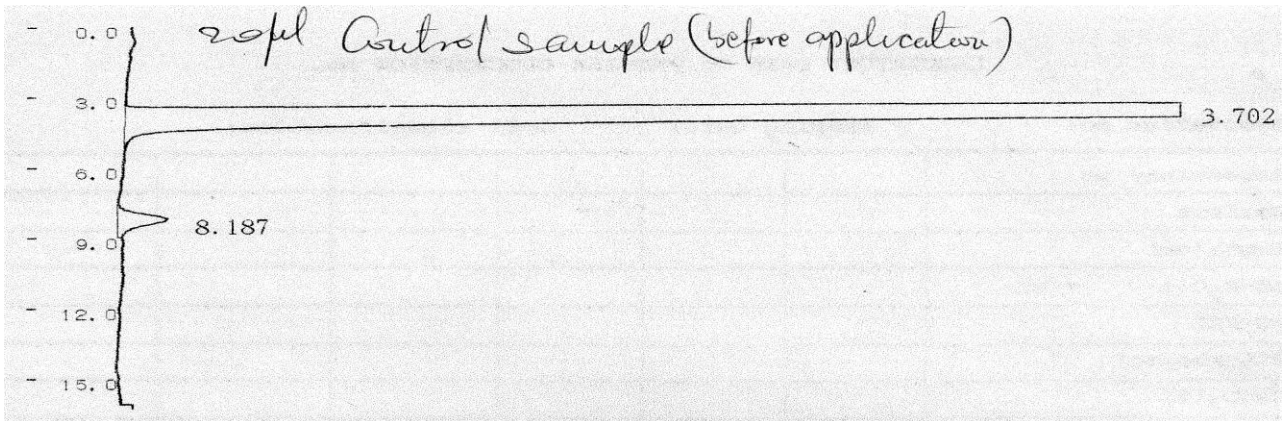
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	24	3.727	1927237	67203	SV		97.3198	
	28	7.522	17578	642			0.8876	
	29	8.204	18051	567	V		0.9115	
	31	10.299	17447	441			0.881	
		TOTAL	1980313	68853			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=34 DATA=1:@CHRM1.C00 12/07/19 17:36:10



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	26	3.722	1864392	63678	SV		97.2282	
	30	7.514	15717	615			0.8196	
	31	8.195	18953	589	V		0.9884	
	32	10.291	18480	457	S		0.9637	
		TOTAL	1917541	65339			100	

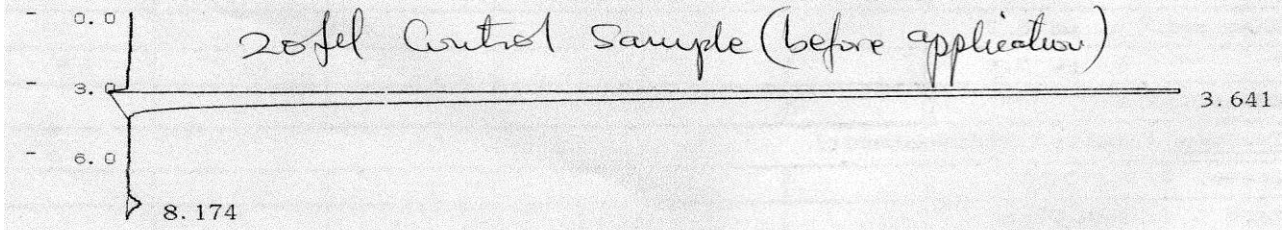


r26-1

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	23	3.702	1803346	68091	SV		98.5426	
	25	8.187	26670	764			1.4574	
		TOTAL	1830016	68854			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=27 DATA=1:@CHRM1.C00 12/07/19 16:06:12

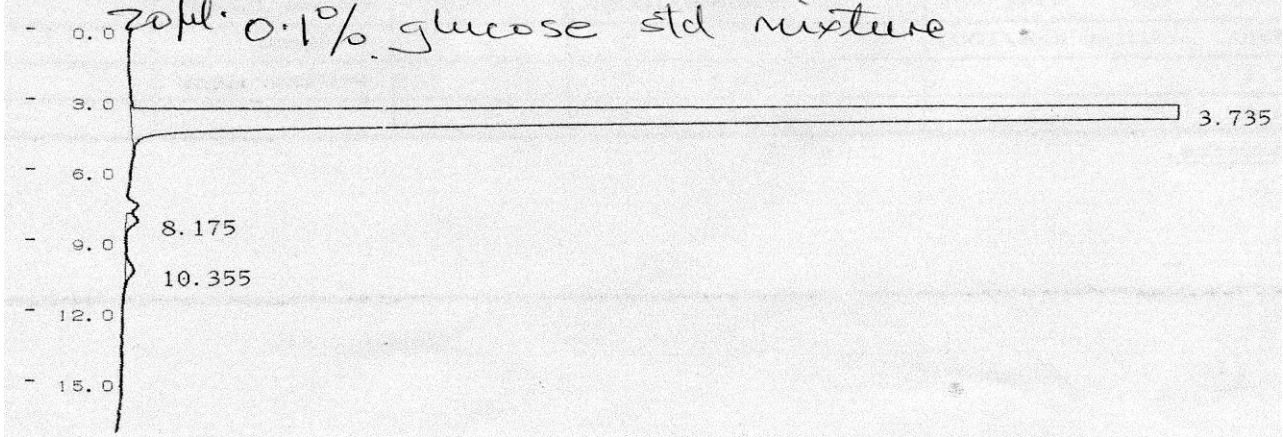


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	26	3.641	398056	19162			98.6931	
	27	8.174	5271	205			1.3069	
		TOTAL	403327	19367			100	

0.1mg/ml std mixture

C-R7A CHROMATOPAC CH=1 REPORT No.=28 DATA=1:@CHRM1.C00 12/07/19 16:16:40



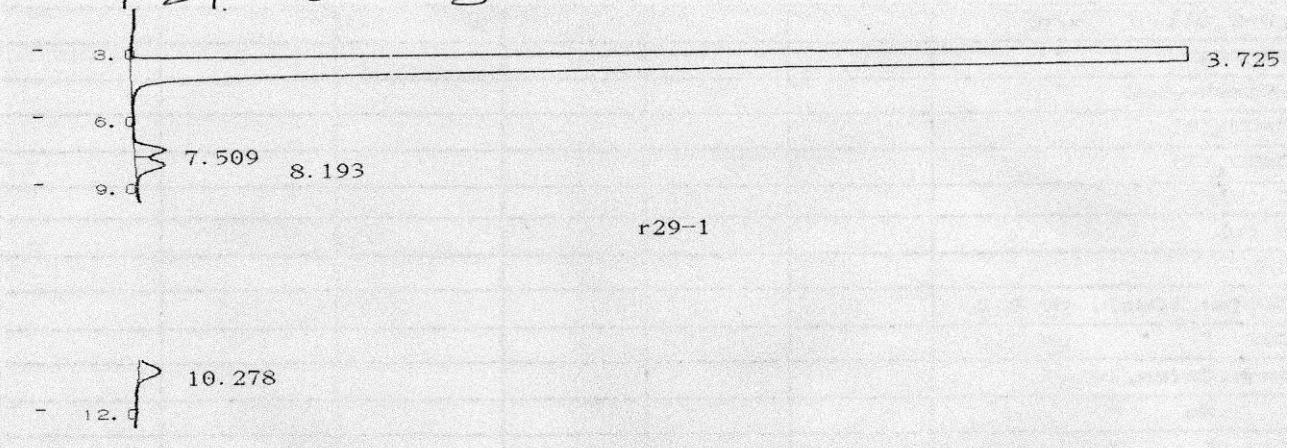
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	23	3.735	1817963	66539	SV		99.4273	
	26	8.175	5351	184	V		0.2927	
	28	10.355	5120	142	V		0.28	
		TOTAL	1828434	66865			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=29 DATA=1:@CHRM1.C00 12/07/19 16:34:08

0.3mg/ml

20µl 0.3% glucose Std mixture



r29-1

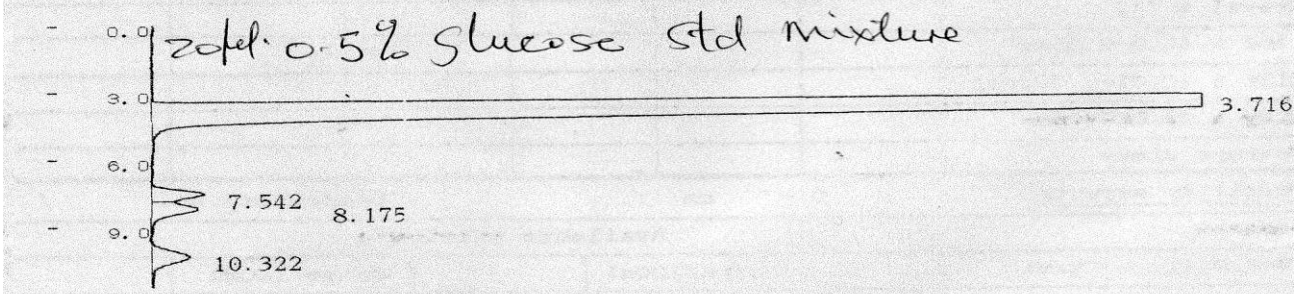
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	24	3.725	1891541	65133	SV		98.0052	
	29	7.509	12575	483			0.6515	
	30	8.193	14674	455	V		0.7603	
	33	10.278	11252	327			0.583	
		TOTAL	1930042	66398			100	

0.5mg/ml

C-R7A CHROMATOPAC CH=1 REPORT No.=30 DATA=1:@CHRM1.C00 12/07/19 16:49:38

20µl 0.5% glucose Std mixture

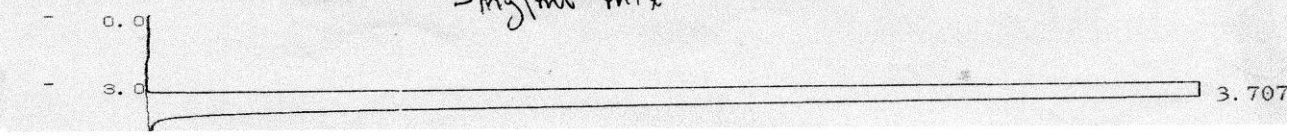


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	26	3.716	1842094	64535	V		96.4299	
	27	7.542	22234	813			1.1639	
	28	8.175	23420	712	SV		1.226	
	31	10.322	22547	582	S		1.1803	
		TOTAL	1910294	66642			100	

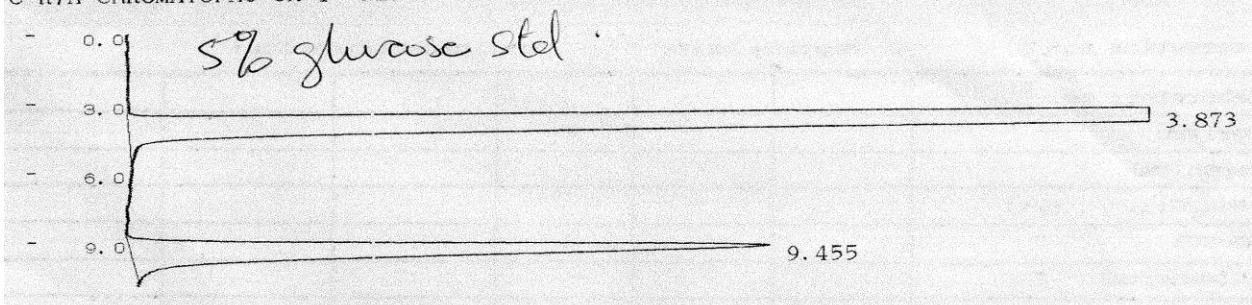
C-R7A CHROMATOPAC CH=1 REPORT No.=31 DATA=1:@CHRM1.C00 12/07/19 17:02:00

5mg/ml mix



TOTAL	2463881	83603	100

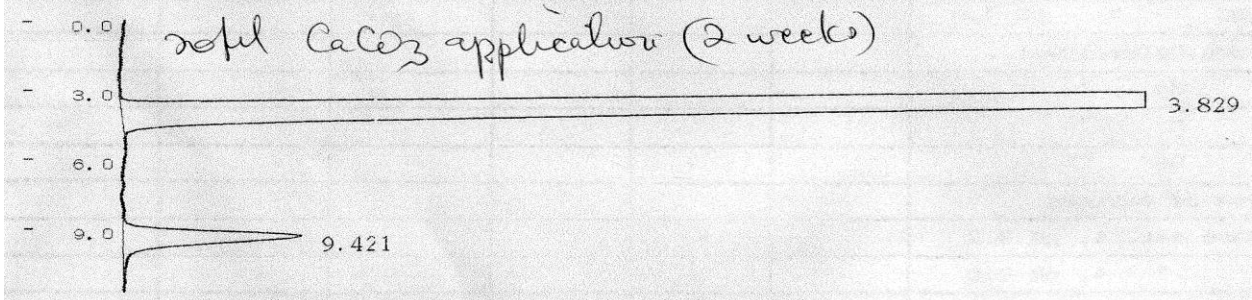
C-R7A CHROMATOPAC CH=1 REPORT No.=7 DATA=1:@CHRM1.C00 12/07/19 11:36:02



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	30	3.873	2167736	82835	SV		85.6087	
	39	9.455	364409	10003			14.3913	
		TOTAL	2532146	92838			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=8 DATA=1:@CHRM1.C00 12/07/19 11:47:14

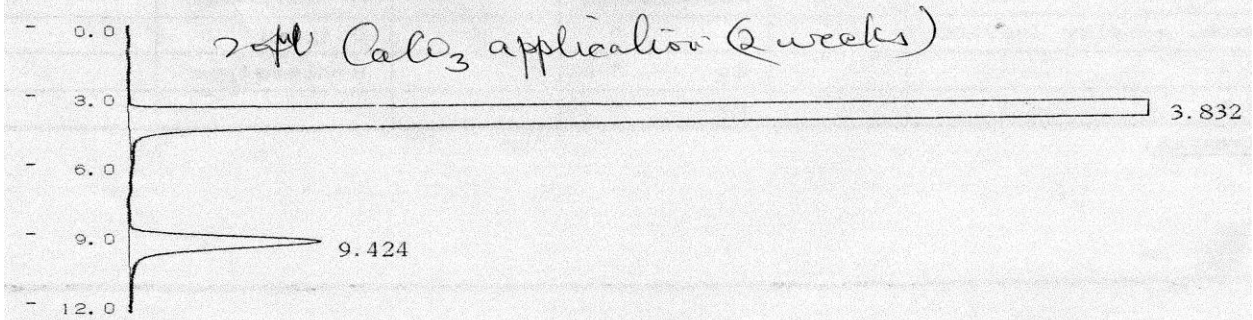


r8-1

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	27	3.829	2225477	72019	V		95.5913	
	36	9.421	102639	2794	S		4.4087	
		TOTAL	2328116	74813			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=9 DATA=1:@CHRM1.C00 12/07/19 12:00:42



** CALCULATION REPORT **

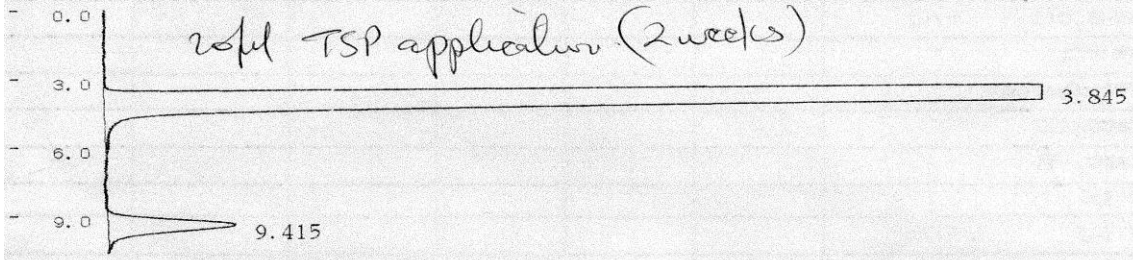
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	30	3.832	2334854	77606	SV		95.3421	

12.0

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	30	3.832	2334854	77606	SV		95.3421	
	39	9.424	114068	2990	S		4.6579	
		TOTAL	2448922	80596			100	

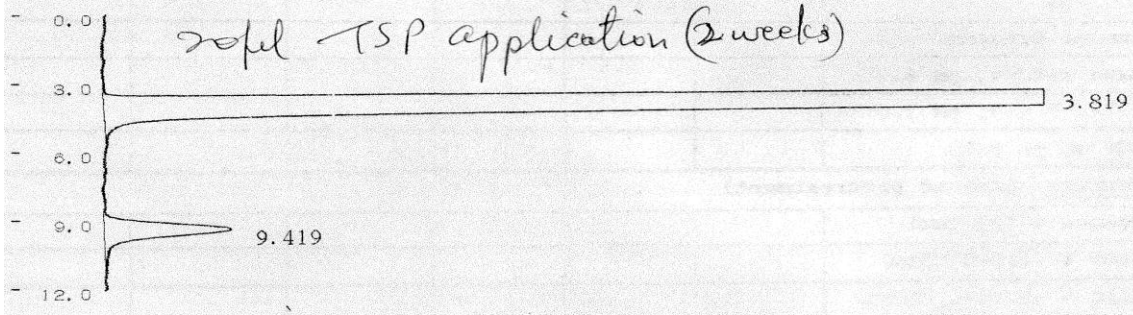
C-R7A CHROMATOPAC CH=1 REPORT No.=10 DATA=1:@CHRM1.C00 12/07/19 12:13:56



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	29	3.845	2320709	76147	SV		96.6959	
	40	9.415	79299	2170			3.3041	
		TOTAL	2400009	78317			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=11 DATA=1:@CHRM1.C00 12/07/19 12:24:56

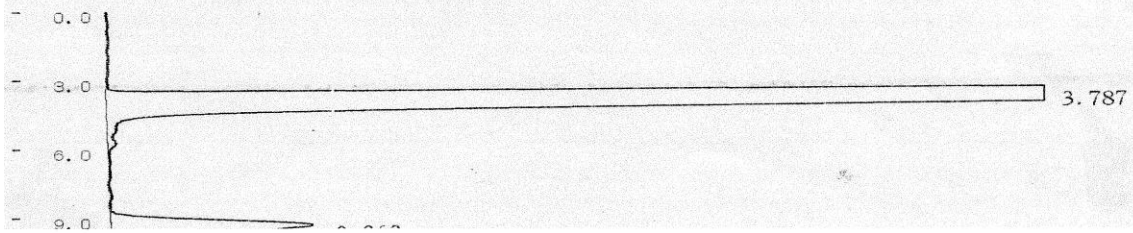


r11-1

** CALCULATION REPORT **

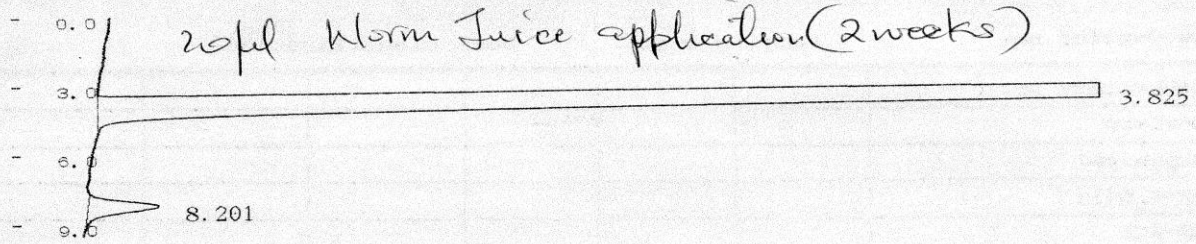
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	28	3.819	2438864	82169	V		96.6814	
	35	9.419	83713	2168	S		3.3186	
		TOTAL	2522577	84337			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=12 DATA=1:@CHRM1.C00 12/07/19 12:37:24



TOTAL	1957700	71036		100

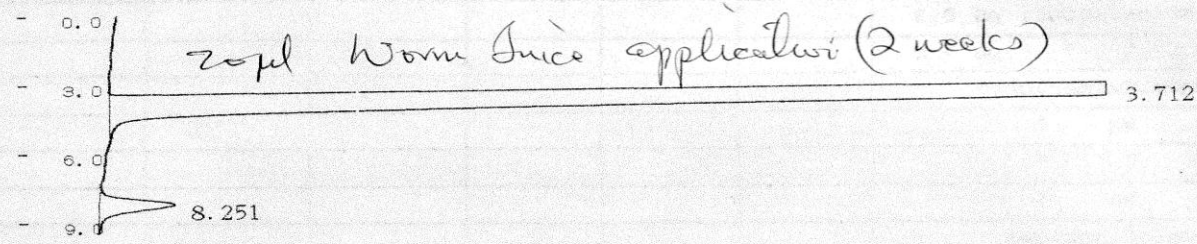
C-R7A CHROMATOPAC CH=1 REPORT No.=18 DATA=1:@CHRM1.C00 12/07/19 14:26:20



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	21	3.825	1834557	59839			97.9746	
	22	8.201	37925	1147			2.0254	
		TOTAL	1872482	60986			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=19 DATA=1:@CHRM1.C00 12/07/19 14:36:42



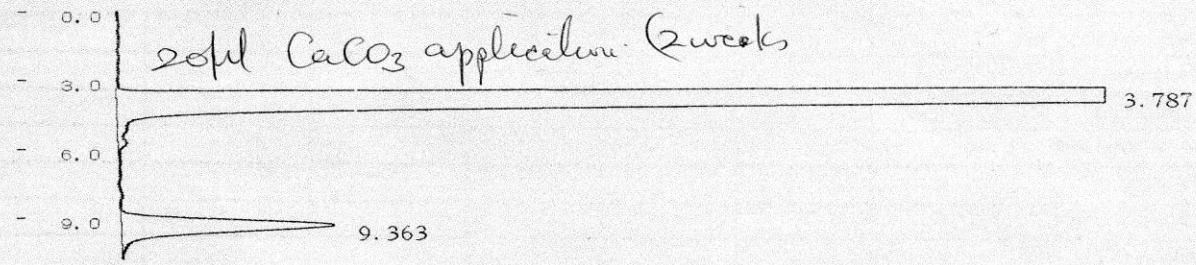
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	24	3.712	1829773	70187	S		97.8234	
	31	8.251	40712	1218	V		2.1766	
		TOTAL	1870485	71406			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=20 DATA=1:@CHRM1.C00 12/07/19 14:46:40 Blank

1	28	9.819	2438804	82109				
	35	9.419	83713	2168	S		3.3186	
		TOTAL	2522577	84337			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=12 DATA=1:@CHRM1.C00 12/07/19 12:37:24



** CALCULATION REPORT **

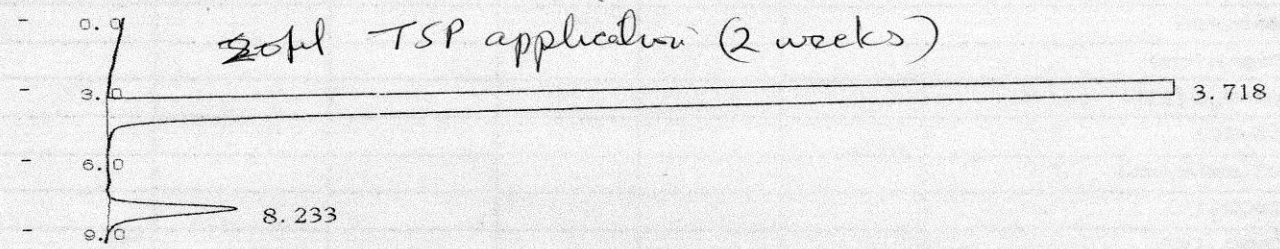
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	30	3.787	2277633	80810	SV		94.7297	
	42	9.363	126715	3439	S		5.2702	
		TOTAL	2404348	84249			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=13 DATA=1:@CHRM1.C00 12/07/19 12:58:10

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	26	3.755	1958499	74468	V		100	
		TOTAL	1958499	74468			100	

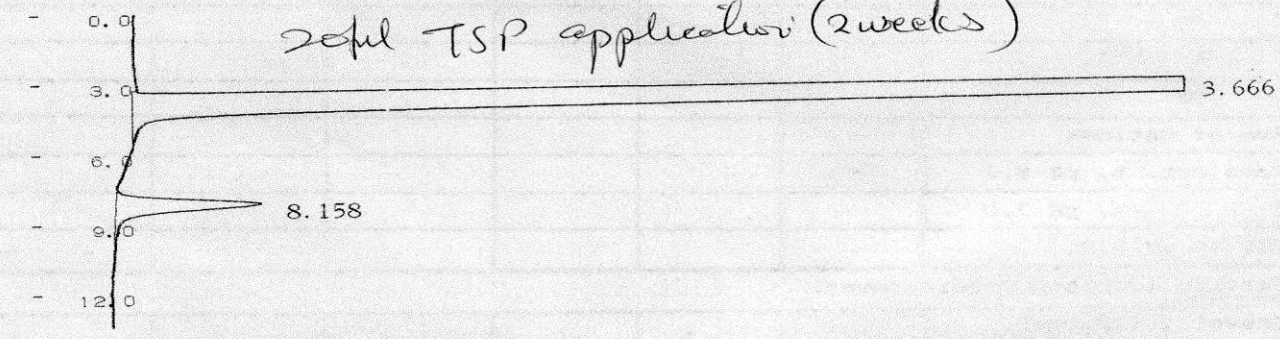
C-R7A CHROMATOPAC CH=1 REPORT No.=21 DATA=1:@CHRM1.C00 12/07/19 14:57:04



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	19	3.718	1846246	67332			96.7149	
	21	8.233	62710	1944			3.285	
		TOTAL	1908956	69276			100	

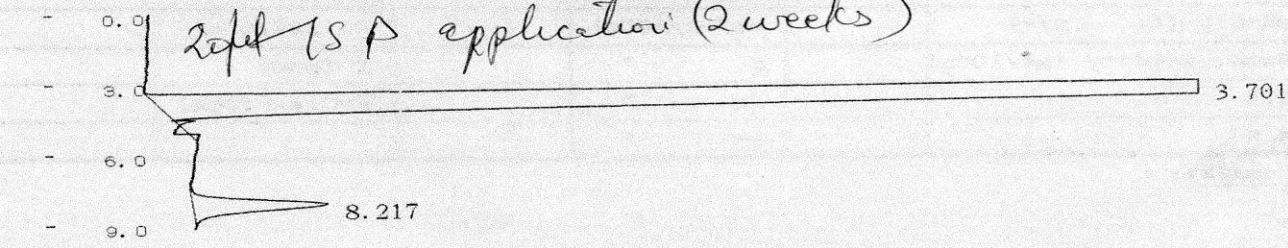
C-R7A CHROMATOPAC CH=1 REPORT No.=22 DATA=1:@CHRM1.C00 12/07/19 15:06:58



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	27	3.666	1847687	62827	SV		96.1878	
	33	8.158	73230	2206	SV		3.8122	
		TOTAL	1920917	65032			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=23 DATA=1:@CHRM1.C00 12/07/19 15:21:12

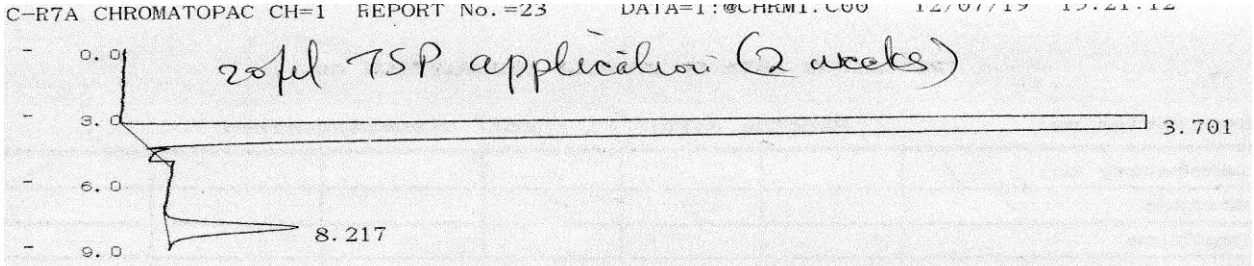


r23-1

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
							96.4191	

C-R7A CHROMATOPAC CH=1 REPORT No.=23 DATA=1:@CHRM1.C00 12/07/19 15:21:12

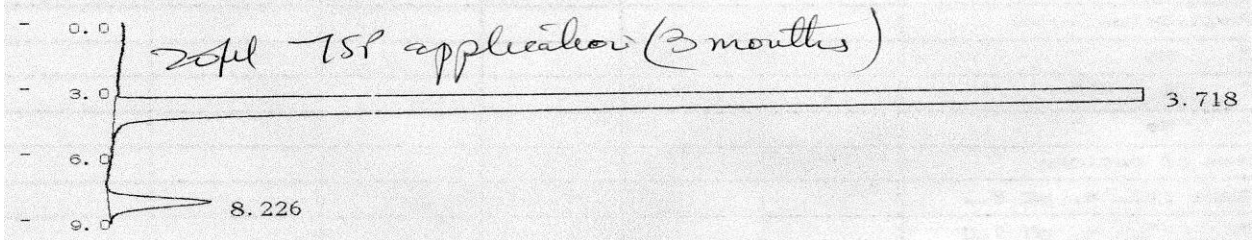


r23-1

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	25	3.701	1758300	63627	S		96.4191	
	30	8.217	65301	2038	V		3.5809	
		TOTAL	1823602	65665			100	

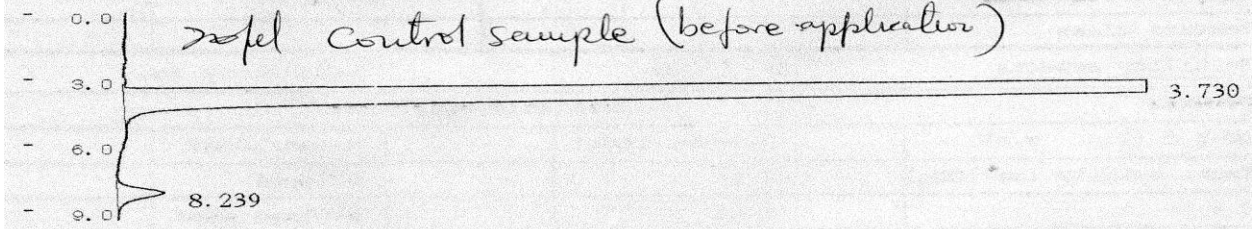
C-R7A CHROMATOPAC CH=1 REPORT No.=24 DATA=1:@CHRM1.C00 12/07/19 15:30:36



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	23	3.718	1747889	61031	V		97.222	
	24	8.226	49943	1606			2.778	
		TOTAL	1797833	62637			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=25 DATA=1:@CHRM1.C00 12/07/19 15:40:02



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	26	3.73	1620951	57592	SV		98.5069	
	31	8.239	24570	739	SV		1.4931	
		TOTAL	1645520	58331			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=26 DATA=1:@CHRM1.C00 12/07/19 15:49:46