VARIATIONS IN SOME MINERALS AND NUTRIENTS LEVELS IN SELECTED AFRICAN LEAFY VEGETABLES DUE TO HARVESTING STAGES AND LOCATIONS OF PRODUCTION IN WESTERN KENYA

By

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ABSTRACT

The nutrition transition in developing countries has resulted in changes in diets and lifestyle habits. Such changes have led to paradoxical phenomenon where under nutrition and over nutrition coexist. This double burden is reflected in Kenyan children and adults. The Kenyan poverty incidence showed a large variation by province, with Western and Nyanza all above 45% of the population. Consequently, many people living in western Kenya suffer from nutrient deficiency diseases. Proper nutrition contributes to declines in under-five mortality rates and also improves the productivity of adults. Addressing nutritional problems requires adequate information on the diets of individuals and populations. This in turn requires reliable data on both the consumption of foods and their nutrients contents. African leafy vegetables (ALVs) are widely consumed and are harvested at different stages after planting by communities in different geographical locations. The ALVs can be a major source of the nutrients. The nutritional values of ALVs may vary depending on harvesting stage and location of production. Four ALVs (*Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*) are commonly grown in western Kenya, yet the potential of these ALVs have not been evaluated as possible remedy to malnutrition. The intention of this study was to assess the levels of some essential nutrients, the effect of harvesting stages and site of production on selected ALVs commonly grown in western Kenya, to guide consumption levels to supply recommended dietary allowances (RDAs) of the relevant nutrients. The trials were laid out in a randomized complete block design in three replications at each site in Busia, Kisumu and Lela. Leaves were sampled and analysed for N, P, K, Na, Ca, Mn, Mg, Fe and Zn levels. Soil pH and nutrients analyses were done to ascertain their initial levels. The levels of the nutrients significantly ($P \leq 0.05$) varied with species, location of production and harvesting stages. *Amaranthus hybridus* had higher ($P \leq 0.05$) levels of P, Ca, Zn, Mn and Na. The ALVs were significant contributors of Fe and Mg. Consumption of 61-118 g dry weight (DW) of each of the four ALVs for the supply of Fe and 144-472 g of *Solanum scabrum* for the supply of Mg is recommended to supply RDAs. The ALVs from Kisumu site had the higher ($P \leq 0.05$) levels of N, P, K, Ca, Mg and Zn, while higher ($P \leq 0.05$) levels of Fe; Mn and Na were reported in ALVs from Busia and Lela sites, respectively. The N, P, K, Ca and Zn levels significantly ($P \leq 0.05$) increased from 4 to 6 weeks after seed emergence (WAE) then decreased from 6 to 10 WAE, Fe levels increased from 4 to 6 WAE while the increase from 6 to 10 WAE was not significant. The Mg levels significantly ($P \leq 0.05$) increased from 4 to 8 WAE then decreased from 8 to 10 WAE, while Mn and Na levels did not vary with harvesting stage. Harvesting the ALVs from 4 to 6 WAE for the supply of P, K, Ca and Zn, 4 to 8 WAE for the supply of Mg and 4 to 10 WAE for the supply of Na and Mn is recommended to yield highest levels. There is need to establish the bioavailability of the nutrients in ALVs.

CHAPTER ONE

INTRODUCTION

1.1: Background information

Essential elements in humans can be classified into macro elements like sodium, potassium, magnesium, calcium, sulphur and chlorine micro elements like iron, zinc, copper and manganese, among others. Each element in both classes is essential, and each has its indispensable functions in the human body (Charlette and Allred, 1992). The deficiency or excess of any of the element may cause diseases and/or be deleterious to human health (O'Dell and Sunde, 1997).

The nutrition transition in developing countries has significantly resulted in changes in diet and lifestyle habits (Hawkes, 2006). Such changes have led to paradoxical phenomenon seen in developing countries where under nutrition and over nutrition coexist (Hawkes, 2006). Kenya, like other developing countries in sub-Saharan Africa, is faced with challenges of both under-and over nutrition (Swedberg, 2000). Under nutrition comprise of a number of nutritionally related conditions such as protein-energy malnutrition and micronutrient deficiencies, including those of vitamin A, iron, and iodine. Chronic energy deficiency is a risk factor for adult low productivity, morbidity and mortality (Hosegood and Campbell, 2003; Thomas and Frankenberg, 2002; WHO, 1995; Ferro-Luzzi *et al.*, 1992), with chronic under nutrition among women additionally being a major risk factor for adverse birth outcomes for their children (Kusin *et al.*, 1994). Approximately 30-40% of Kenyan children under-five years of age are stunted, 10-20% are underweight while 6-10% are wasted (UNICEF/WHO, 2012).

Globally, an estimated 43 million children under-five years of age, or 7%, were overweight in 2011, a 54% increase from an estimated 28 million in 1990 (UNICEF/WHO, 2012). Increasing trends in child overweight have been noted in most world regions, not only

developed countries, where prevalence is highest (15% in 2011). In Africa, the estimated prevalence of under-five overweight increased from 4% in 1990 to 7% in 2011 (UNICEF/WHO, 2012).

Obesity is one of the most significant contributors to increased prevalence of diabetes, leading to the use of the word "diabesity (Hossain *et al.*, 2007) both in rural and urban areas. Official statistics in Kenya note a diabetes prevalence of 3.5%, but it is believed the rate is much higher, about 10% of the population (Mario and Sridevi, 2008). In Kenya, alarming rates of child malnutrition emphasize the still urgent need to address malnutrition, while simultaneously concentrating on the documented rise of pediatric over nutrition (Mendez *et al.*, 2005; De Onis and Blossner, 2000).

Many Kenyans living in western Kenya suffer from nutrient deficiency diseases (Arthur *et al.*, 2003). The 2008-2009 malnutrition survey of Kenyan children and women showed major disparities across the country, reflecting the considerable variability in environmental and socioeconomic risk factors (NCPD, 1998), with under five mortality rate of 206 per 1000 live births in Nyanza province (Kenya National Bureau of Statistics, 2010). Poverty normally manifests itself mainly through malnutrition and poor health. The poverty incidence showed a large variation by province, with Western and Nyanza all above 45% of the population (Poverty Reduction and Economic Management Unit, Africa Region, 2008).

Proper nutrition contributes significantly to declines in under-five mortality rates (UNICEF/WHO, 2012) and also improves the productivity of adults. The problems of micronutrient malnutrition and over nutrition cannot be addressed without adequate information on the diets of individuals and populations. This in turn requires reliable data on both the consumption of foods and their nutrients contents. Food composition tables are an essential resource for understanding and analyzing dietary intake data for both individuals and communities, and for developing healthy recipes and food products.

The nutrient content of locally produced foods may vary significantly from region to region even within one country (Barikmo *et al.*, 2007). It is essential that efforts to analyse the nutrient content of foods are continued in African countries. Up-dated information is needed for all types of foods including ALVs. Foods, being biological materials, exhibit variations in nutrient levels due to factors such as season, geography, cultivar and husbandry (Liisa *et al.*, 2011). The African countries with food composition tables include Tanzania, Mozambique, South Africa, Mali and the recently published table for West Africa. There is need to establish a comprehensive food composition tables for Kenyan foods. The ALVs composition table developed by Sehmi (1993) for Kenyan ALVs is questionable as samples were bought from different markets.

With the publication of the Dietary Reference Intakes (DRI) between 1997 and 2005, the emphasis in the field of nutrition moved from preventing nutrient deficiencies toward a goal of optimal nutrition (Institute of Medicine, Food and Nutrition Board, 2006). The Recommended Dietary Allowances (RDAs) were expanded from the single RDA value to four reference values. The 2005 revision of the USDA My Pyramid Food Guidance System (USDA, 2008) changed guidelines to provide a range of intakes according to additional factors such as gender, age, body mass index, and activity levels.

Kenya is endowed with many varieties of indigenous food plants (WWF, 1993) like ALVs that can have outstanding potential to supply RDAs of relevant nutrients. Many Kenyans especially people from western regions consume ALVs mostly (*Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*) together with other starchy food stuff and other proteins for general meals. It is their general believes that the vegetables are important sources of vitamins and minerals (Fe and Ca) (ICRAF, 2004; Abukutsa-Onyango, 2003; Maccalla, 1994). However, the levels of beneficial nutrients in the ALVs grown in western Kenya have not been documented to guide consumption levels to supply

RDAs for relevant nutrients. Generally, poor communities that cannot afford expensive nutrient dense animal source foods consume a lot of ALVs which are freely available or are cheap (Modi *et al.*, 2006; Labadaries and Steyn, 1999). Many affluent adults are also now resolving to indigenous foods (Kimiywe *et al.*, 2006), as they are believed to be nutritious.

The nutrient content of *Cleome gynandra*, *Solanum scabrum* and *Amaranthus hybridus* leaves from same location of production significantly varied (Lyimo *et al.*, 2003) in Tanzania. The variations may have been due to differences in genetic constitution which resulted in varying abilities to extract soil available nutrients. Variations with species have also been reported in different ALVs from different markets (Sehmi, 1993). The variations may have been due to difference in genetic constitution or collection sites.

Nutrient content in vegetables vary according to their availability in the soil at different collection sites and plant uptake (Ogle and Grivetti, 1985). The intra-species and geographical area of production variations have been reported in Iringa and Morogoro districts in Tanzania (Kinabo *et al.*, 2003). The nutritional value of different amaranths, african eggplant and african nightshade differed according to species, variety and geographical area of production (Weinberger and Msuya, 2004). Western Kenya is known to produce *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* almost throughout the year due to favourable soils and climatic conditions, which vary slightly across the region spanning from the Lake Victoria to high equatorial rainy regions (Jaetzold *et al.*, 2005). The region receives a bimodal rainfall distribution pattern, with long rains falling between March and June and short rains are received from September to December (Jaetzold and Schmidt, 1983). The rainfall has marked peaks in March-May when half the annual precipitation may be expected and short rains between November and December (Jaetzold and Schmidt, 1983). These variations may lead to variations in nutrients

uptake by plants. Studies have been conducted on several food and cash crops except for the naturally occurring ALVs.

Exotic vegetables are normally harvested and consumed at a known stage of plant development. Unlike exotic vegetables, there is no documented information about the stage of plant development to define harvest maturity for ALVs. Hence data on their nutritional value is likely to vary widely (Jansen-Van-Rensburg *et al.*, 2004; Kruger *et al.*, 1998; Guarino, 1997), due to influences of plant age and the environmental conditions during plant growth. The yields and nutritive quality of four variants of black nightshades (*Solanum nigrum* L.) were significantly affected by plant age (Abukutsa-Onyango, 1993; Mwafusi, 1992). The yields, beta-carotene and total phenolic contents increased then decreased while crude proteins levels did not change significantly with age. Higher harvesting frequency resulted in a higher leaf yield than a single harvest. However, it is not known how harvesting stage affects the distribution of elements in ALVs. It is necessary to assess the levels of nutrients in ALVs, establish influence of plant maturity and geographical area of production on the nutrient content.

1.2: Statement of the problem

Many nutrients macro or micro play vital roles in metabolic processes and are essential for the general well being of humans. The deficiency or excess of any of the elements may cause diseases and/or be deleterious to human health. Widespread malnutrition still prevails in majority of people living in western Kenya. The double burden of under nutrition and over nutrition is also on the rise. The ALVs are compatible in use with starchy staples and are widely acceptable to both rural poor and urban communities. The ALVs could offer a sustainable solution to the problem of micronutrient malnutrition and over nutrition if they contain substantial amounts of beneficial nutrients. However, a suitable species with optimal amounts of the nutrients has not been identified to guide consumption levels to supply RDAs

of relevant nutrients. Besides, the impact of the different geographical locations within western Kenya to nutrients availability in the selected ALVs is not documented. The ALVs are harvested at different stages after seed emergence, however, nutrients availability in *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* at different harvesting stages have never been documented. It is necessary to establish the suitable stage for harvesting that would provide optimum nutrients for human benefit.

1.3: Objectives

1.3.1: Main objective

This study aims at assessing the total amounts of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and sodium (Na) in selected ALVs (*Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*) at different harvesting stages and from different locations of production within western Kenya regions to provide nutrient levels for selected ALVs.

1.3.2: Specific objectives

1. To assess the variations of the nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Na) in selected ALVs when planted in a single location.
2. To assess the variations of the nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Na) in selected ALVs in different locations of Busia, Kisumu and Lela.
3. To assess the variations of the nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn, and Na) in selected ALVs at different harvesting stages.

1.4: Null hypotheses H_0:

1. The levels of nutrients in different ALVs grown in a single location do not vary.
2. The levels of nutrients in ALVs do not vary with location of production.

3. The levels of nutrients in ALVs do not vary with the stage of harvesting from planting time.

1.5: Justification of the research

The integration of foods rich in macro and micronutrients into the diet is a sustainable way to improve nutrient status in the human body (Ali and Tsou, 1997). The ALVs are easily available and may be a major source of macro and micronutrients, and could offer practical and sustainable way to ensure nutrients adequately supplied through the diet would supplement alleviation of micronutrient malnutrition and over nutrition by providing data to guide consumption levels to supply RDAs of relevant nutrients. Identification of the species with optimum nutrients will provide a guide to consumption levels to supply RDAs of relevant nutrients would validate the fact that adequate consumption of such vegetable species is an integral effort towards alleviating micronutrient malnutrition and over nutrition. Food composition data is essential in nutritional research for assessing and planning interventions involving national food and nutrition policies, and prescribing therapeutic and institutional diets for individuals. Nutrient content in vegetables vary according to their availability in the soil at different collection sites and plant uptake (Ogle and Grivetti, 1985). Since harvesting stage and environmental conditions (Jansen-Van-Rensburg *et al.*, 2004; Kruger *et al.*, 1998; Guarino, 1997) have been established to influence nutritional value in ALVs, it is therefore important to determine the optimal harvesting stage for which the selected ALVs would exhibit optimal nutrients for each location of production.

1.6: Significance of the study

This study may identify the vegetable species, appropriate harvesting stage and location of production with optimum nutrients. This may provide information on ALVs nutrients levels for nutritionists and individuals. The study may also provide selected ALVs nutrient levels to guide consumption levels to supply RDAs. This may be incorporated into food composition

tables. The data may be highly useful not only to nutritionists, but for all individuals interested in knowing the nutrient content of a single vegetable. The food industry may also find this information valuable in developing healthy vegetable products.

CHAPTER TWO

LITERATURE REVIEW

2.1: Over and under nutrition

Over the past several decades, sub-Saharan Africa has been experiencing a nutrition transition in which traditional foods and food habits have been progressively replaced by the globalized food culture of the multinational corporations (Zimmert, 2000). The nutrition transition has significantly resulted in changes in diet and lifestyle habits (Hawkes, 2006). On a global scale such changes have led to paradoxical phenomenon seen in developing countries where under nutrition and over nutrition coexist (Hawkes, 2006). In Kenya, alarming rates of child malnutrition emphasize the still urgent need to address malnutrition, while simultaneously concentrating on the documented rise of paediatric over nutrition (Mendez *et al.*, 2005; De Onis and Blossner, 2000).

Kenya, like other developing countries in sub-Saharan Africa, is faced with challenges of both under and over nutrition. Under nutrition comprise of a number of nutritionally related conditions such as protein-energy malnutrition and micronutrient deficiencies, including those of vitamin A, iron, and iodine. Micronutrients are the essential vitamins and minerals required in low amounts by human beings to stimulate cellular growth and metabolism (FAO, 2003). The malnutrition of micronutrients refers to lack of adequate supply of essential vitamins and minerals leading to nutritional deficiency diseases (FAO, 1997). Malnutrition is a problem affecting primarily the poor in both developing and developed countries and is more widespread than hunger, affecting an estimated one in three people worldwide (WHO, 2001). However, most malnutrition is hidden and is primarily due to a lack of vital micronutrients. Micronutrient deficiencies can exist in populations even where the food supply is adequate to meet energy requirements, as the foods may be grossly deficient in one or more micronutrients (FAO, 2003). Although the major malnutrition

problems are in developing countries, inhabitants of developed countries also suffer from various forms of micronutrient malnutrition (FAO, 1997). Malnutrition is still unacceptably high and progress to reduce it in most regions of the world is slow (ACC/SCN, 2000). Low fruit and vegetable intake is the main contributor of micronutrient deficiencies in developing world, especially with low intakes of nutrient-dense animal source foods such as meat and dairy products (Blankhart, 1971)

Iron, vitamin A, zinc and iodine are nutrients whose deficiencies have the highest global prevalence (UNICEF, 2004). and have helped to bring into limelight the "hidden hunger" problem. However, they represent only a fraction of the problem, and subclinical deficiencies afflict a much larger proportion of the population (FAO, 2003). Iron deficiency anaemia affects more than 3.5 billion people in the developing world of which about 40% live in the sub-Saharan Africa (FAO, 2003). The substantial prevalence of under nutrition has resulted in chronic energy deficiency (Swedberg, 2000), a risk factor for adult low productivity, morbidity and mortality (Hosegood and Campbell, 2003; Thomas and Frankenberg, 2002; WHO, 1995; Ferro-Luzzi *et al.*, 1992), with chronic under nutrition among women additionally being a major risk factor for adverse birth outcomes for their children (Kusin *et al.*, 1994). The nutritional status of adults is vital, considering their role in the economic support system. Under nutrition has critical consequences including reduced productivity and increased disease susceptibility, while the results of over nutrition in the form of chronic disease can be just as deleterious (Kikafunda *et al.*, 2005; James *et al.*, 1994; Wolgenmuth *et al.*, 1982). This double burden is reflected in Kenyan adults (Jayne *et al.*, 2011). Protein-energy deficiencies and micronutrient malnutrition increase the risk of illness or death from infectious diseases and leads children not to develop to their full physical or mental potential (UNICEF, 2009). Approximately 30-40% of Kenyan children under-five

years of age are stunted, 10-20% are underweight while 6-10% are wasted (UNICEF/WHO, 2012).

Over nutrition cannot be considered only as a disease of the materially advantaged groups and that the burden of obesity generally shifts toward poorer groups as countries improve their level of economic development (Monteiro *et al.*, 2004; Sobal and Stunkard, 1989). Globally, an estimated 43 million children under-five years of age, or 7%, are overweight (UNICEF/WHO, 2012), a 54% increase from an estimated 28 million in 1990. Increasing trends in child overweight have been noted in most world regions, not only developed countries, where prevalence is highest (15% in 2011). In Africa, the estimated prevalence of under-five overweight increased from 4% in 1990 to 7% in 2011 (UNICEF/WHO, 2012).

Obesity is one of the most significant contributors to increased prevalence of diabetes, leading to the use of the word "diabesity (Hossain *et al.*, 2007) both in rural and urban areas. The nutrition transition has been directly implicated in the recent upsurge of Non-Communicable Diseases (NCDs) which account for 40% of deaths in developing countries, and this proportion is expected to increase significantly (WHO, 2001). Within the next 20 years, sub-Saharan Africa can expect a three-fold increase in deaths due to Cardiovascular Diseases (CVDs) (Leeder *et al.*, 2004), and a near three-fold increase in the incidence of type 2 diabetes (Wild *et al.*, 2004). Official statistics in Kenya note a diabetes prevalence of 3.5%, but it is believed the rate is much higher, about 10% of the population (Mario and Sridevi, 2008).

Nutritional problems in Kenya were first reported over forty five-years ago (Bohdal *et al.*, 1968). Many countries such as Ethiopia, Uganda, and Tanzania significantly reduced the levels of stunting in young children since the 1990s while Kenya registered an increase in stunting rates during the same period (UNICEF, 2009). Estimates of the prevalence of

malnutrition in children less than five years of age and women of reproductive age showed a large variation by province (Kenya National Bureau of Statistics, 2010), reflecting the considerable variability in environmental and socioeconomic risk factors (National Council for Population Development, 1998). The 2008-2009 malnutrition survey of Kenyan children and women showed under five mortality rate ranging from 54 per 1000 live births in Central province to 163 per 1000 live births in North Eastern province and 206 per 1000 live births in Nyanza province (Kenya National Bureau of Statistics, 2010). Poverty normally manifests itself mainly through malnutrition and poor health. The poverty incidence showed a large variation by province, with highest incidence reported in North-Eastern; Coast, Western, Nyanza and Eastern all above 45% of the population (Poverty Reduction and Economic Management Unit, Africa Region, 2008).

Proper nutrition contributes significantly to declines in under-five mortality rates (UNICEF/WHO, 2012) and also improves the productivity of adults. The problems of micronutrient malnutrition and over nutrition cannot be addressed without adequate information on the diets of individuals and populations. This in turn requires reliable data on both the consumption of foods and their nutrients content. Food composition tables are an essential resource for understanding and analyzing dietary intake data for both individuals and communities, and for developing healthy recipes and food products.

The nutrient content of locally produced foods may vary significantly from region to region even within one country (Barikmo *et al.*, 2007). Thus it is essential that efforts to analyse the nutrient content of foods are continued in African countries. Up-dated information is needed for all types of foods including ALVs. Foods, being biological materials, exhibit variations in composition due to factors such as season, geography, cultivar and husbandry (Liisa *et al.*, 2011). Many Kenyans especially people from western regions consume ALVs mostly (*Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and

Solanum scabrum) together with other starchy food stuff and other proteins for general meals. It is their general believes that the vegetables are important sources of vitamins and minerals (Fe and Ca) (ICRAF, 2004; Abukutsa-Onyango, 2003; Maccalla, 1994). However, the levels of beneficial nutrients in ALVs grown in western Kenya have not been documented to guide consumption levels to supply RDAs of relevant nutrients.

2.2: African Leafy Vegetables macro and micronutrient supply

African Leafy Vegetables (ALVs) originate from Africa (Maundu, 1997), and are plants whose young shoots, leaves, seeds and fruits are palatable and acceptable for consumption through custom, habit and tradition (FAO, 1988). There are more than 45,000 species of plants in sub-Saharan Africa of which about 1,000 can be eaten as green leafy vegetables, the mainstay of traditional African diets (Maccalla, 1994). The nutrients contents of these ALVs are superior to the popular exotic vegetables (Sehmi, 1993), but factors influencing their nutrient levels especially in western Kenya have not been established.

2.2.1: Nutrients content in different ALVs

Most common ALVs grown in western Kenya include; spider plant (*Cleome gynandra*), african nightshades (*Solanum scabrum*), vegetable amaranth (*Amaranthus hybridus*), slender leaf (*Crotalaria brevidens*), jute mallow (*Corchorus Olitorius*), vegetable cowpea (*Vigna unguiculata*), pumpkin leaves (*Curcubita muschata*) and african kale (*Brassica carinata*) (Abukutsa-Onyango *et al.*, 2006). Amaranth, pumpkin leaves, cowpea leaves and african nightshade are among the well known cultivated ALVs for their high contents of minerals (Fe and Ca) and vitamins (Weinberger and Msuya, 2004; Lyimo *et al.*, 2003; Schippers, 2000; Latham, 1997; Raja *et al.*, 1997; Ogle and Grivetti, 1985). Genotypical differences in a species brings about differences in the utilization of a nutrient and hence the demand and uptake of such mineral nutrient (Marschner, 1995).

In various parts of East Africa, the micronutrient content varied in different species (Habwe *et al.*, 2009; Lyimo *et al.*, 2003; Tayie and Asibey-Berko, 2003; Raja *et al.*, 1997; Sehmi, 1993). The nutrients content of cooked spider plant, nightshade and amaranths leaves significantly (Lyimo *et al.*, 2003) varied in Tanzania. The inter-species variations were also observed in Iringa and Morogoro districts in Tanzania (Kinabo *et al.*, 2003). Agronomic practices such as thinning, frequency of removal of tender leaves (Maundu *et al.*, 1999; Abukutsa-Onyango, 1993), deflowering and use of manure (Abukutsa-Onyango and Karimi, 2005) greatly influences nutrients content in ALVs. Biweekly removal of tender leaves allows regeneration of branches (Maundu *et al.*, 1999). It is not known how nutrient levels in *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* vary under similar agronomic practices in western Kenya.

2.2.1.1: *Solanum scabrum* M. (african nightshade)

Solanum scabrum (african nightshade) (English), "mnavu" (Swahili), "lisutsa" (Luhya), "osuga" (Luo), "rinagu" (Kisii) (Figure 1) belong to the family Solanaceae and genus Solanum. African nightshade is a branched annual herb that grows to a height of 1 metre. It has large round leaves and big fruits which turn shiny purple-black on ripening. Berries remain on the plant after ripening (Schippers *et al.*, 2002; Maundu *et al.*, 1999). *Solanum scabrum* is the most preferred species because of its mild taste as compared to the other solanum species (Flyman and Afolayan, 2006).

In nature *Solanum scabrum* species are mainly found in fairly humid environments with at least 500 mm of rain per annum (Edmonds and Chweya, 1997). They prefer fertile soils with high N and P contents (Van Averbeke and Juma, 2006). The optimal temperature for growth ranges between 20 °C and 30 °C, but most species will tolerate a temperature range of 15 °C to 35 °C. *Solanum scabrum* can grow in regions from 0-2500 m above mean sea level (Grubben, 2004; Maundu *et al*, 1999) and can do well in a variety of soils from

agro-ecological zones I-VI which supports rain-fed agriculture in Kenya (Jaetzold and Schmidt, 1983). It exhibits a C_4 photosynthetic pathway (Jones, 1992).

Leaves harvested fortnightly gives higher yields than those harvested weekly and its nutritive quality and leaf yield are significantly affected by plant age (Abukutsa-Onyango, 1993). Iron and Ca significantly increase with an increase in plant age (Mwafusi, 1992). Leaves harvested during vegetative stage have higher protein content than those harvested from the flowering stage onwards (Abukutsa-Onyango, 1993). *Solanum scabrum* has high levels of P, Ca, and Fe at a single harvest (Edmonds and Chweya, 1997). No study has been done to assess the distribution of P, K, Ca, Mg, Fe, Zn, Mn and Na at different harvesting stages and geographical locations in western Kenya.

Figure 1: *Solanum scabrum*

2.2.1.2: *Cleome gynandra* L. (spider plant)

Cleome gynandra (spider plant) (English) also known by local names such as: "mgangani" (Swahili), "dek" (Luo), "tsisaka" (Luhya), and "chinsaga" (Kisii) (Figure 2) is widespread in

western and coastal areas (Maundu *et al.*, 1999; Nekesa and Meso, 1997). The plant belongs to the family Capparaceae (formerly Capparidaceae) and genus *Cleome*. *Cleome gynandra* is an erect annual herb that grows to a height of between 0.5 m to1.3 m. The plant is strongly branched, with long taproot and few secondary roots (Chweya and Mnzava, 1997). The long tap roots make it survive even where surface water deficiency may be high. Stems and leaves are covered with glandular hair. Pigmentation on the stems varies from green to pink and purple. Purple stem cultivars are reported to be more nutritious than those with green stems and more resistant to insects infestation but more susceptible to diseases (Schippers, 2002).

Figure 2: *Cleome gynandra*

Application of fertilisers containing appreciable amounts of nitrogen delays flowering and increases the number and size of leaves. It does well on a wide range of soils, mostly on

sandy to clay loam, provided they are deep and well drained with pH 5.5-7.0 and soils with high organic matter and adequate mineral reserves (Chweya and Mnzava, 1997). *Cleome gynandra* contain phenolic compounds which give many medicinal and insecticidal properties (Malonza *et al.*, 1992; Verma and Pandey, 1987). The plant has a C_4 photosynthetic pathway (Tieszen *et al.*, 1979; Imbamba, 1976). The nutritive value of *Cleome gynandra* depends on factors such as soil fertility, age of the plant and agronomic practices used (Schippers, 2002; Chweya and Mnzava, 1997). The leaf yields, beta-carotene and total phenolic levels increase then decrease with age while leaf ascorbic acid significantly increase with an increase in plant age (Maumba, 1993). The vegetable is a rich source of nutrients, especially vitamins (A and C) and minerals (Ca and Fe). It also contains some protein, and the leaves contain over and above the normal recommended adult daily allowance of vitamins A and C and the minerals Ca and Fe (Arnold *et al.*, 1985). However, it is not known how other nutrients vary at different harvesting stages of plant growth and geographical locations of production in western Kenya.

2.2.1.3: *Vigna unguiculata* L. (Cowpea)

Vigna unguiculata (cowpea) (English), "kunde" (Swahili), "likhubi" (Luhya), "a lot-boo" (Luo), egesare (Kisii) (Figure 3) is a climbing, spreading, or erect annual herb with a well developed taproot with many lateral and adventitious roots (Ehlers and Hall, 1997).

Figure 3: *Vigna unguiculata*

Cowpea is believed to have originated from tropical Africa (Tindall, 1983) and has been cultivated for a long time on the continent (Vorster *et al.*, 2002; Schippers, 2000). It is an annual or perennial leaf and pulse crop that belongs to the Leguminosae family. *Vigna unguiculata* subspecies *unguiculata* is the most commonly found (Vorster *et al.*, 2002). The plant is a heat-loving, drought tolerant crop that has lower soil fertility requirements with unique ability to fix N even in very poor soils (Singh, 2006) and serves as a cheap source of plant proteins. It can be grown on a wide range of soil types with pH 5.5-7.5, provided they are well drained (Pandey and Westphal, 1989). The plant uses the C_3 photosynthetic pathway (Jones, 1992).

Leaves collected during the vegetative stage have higher protein content than those harvested from the flowering stage onwards (Schippers, 2002). The levels of minerals significantly varied with varieties. The cowpea varieties with highest iron levels were IT99K-7212-2-1 (23.8 mg/kg) and IT 96D-733 (21.2 mg/kg). Varieties with lowest Fe included Fahari (9.24 mg/kg) and TZA 263 (9.9 mg/kg). For Ca, IT99K-7-21-2-2-1 (1112.9, 32.2 mg/kg), IT97K499-8 (684.8 mg/kg) and IT96D-733 (630 mg/kg) had the highest levels,

whereas TZA 263 (320.5 mg/kg) and IT89KD-288(363 mg/kg) had the lowest levels. IT99K-7-21-2-2-1 (32.2 mg/kg), IT97K499-38 (28.3 mg/kg), B301 (26.9 mg/kg) and IT97K819-118 (26.1 mg/kg) had the highest Zn levels. The lowest Zn levels were reported in Fahari (17.1 mg/kg) and VULI1 (19.6 mg/kg) varieties. The variety that was best with respect to overall mineral content was IT99K-7-21-2-2-1 as reported in Iringa and Dodoma areas in Tanzania (Mamiro *et al.*, 2011). Similarly Zn levels in the range 1501-2071 µg/g were reported in different cowpea varieties (Asante *et al.*, 2006). The variation of K, Na, Mg, Fe, Zn, and Mn at different harvesting stages and locations of production has not been established.

2.2.1.4: *Amaranthus hybridus* L. (vegetable amaranth)

Amaranthus hybridus (amaranths) (English), "mchicha" (Swahili), "tsimboka" (Luhya), "omboga" (Luo) "emboga" (Kisii) (Figure 4) belongs to the family Amaranthaceae, genus Amaranthus.

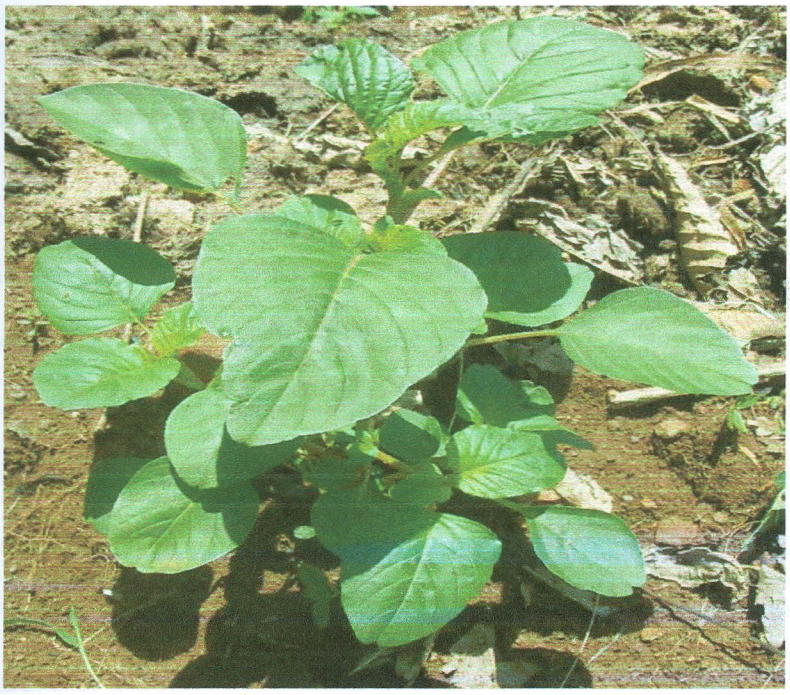

Figure 4: *Amaranthus hybridus*

Amaranthus hybridus is a wide spread vegetable in all countries of tropical Africa (Grubben, 2004). It is probably the most widely occurring leafy vegetable in Africa (Jansen-Van-Rensburg *et al.*, 2004; Guarino, 1997). Amaranths grows optimally under warm conditions (day temperatures above 25 °C and night temperatures not lower than 15 °C), bright light and adequate soil nutrients (Schippers, 2000; Dever *et al.*, 1997; Van den Heever and Coertze, 1996). *Amaranthus hybridus* is mainly used as a leaf vegetable; however, it has also been used as an alternative to drug therapy in people with hypertension and cardiovascular disease (Martirosyan and Miroshnichenko, 2007).

The nutritional value of *Amaranthus* leaves is significantly influenced by the growth temperature and stage of development at which the plant is harvested (Modi, 2007). Leaves collected during the vegetative stage have higher protein content than those harvested from the flowering stage onwards (Schippers, 2002). However, mineral nutrients, Ca and Fe increase in the leaves in response to increasing growth temperature and plant age (Modi, 2007). It is not known how the levels of P, Na, K, Mg, Zn, and Mn vary with plant age at different geographical locations of western Kenya.

2.2.2: Influence of maturity stage on nutrient content in ALVs

The physiological age of a plant or its part is the most important factor affecting mineral nutrients content in the plant dry matter. With the exception of Ca, sometimes Fe (Sanchez-Alonso and Lachica, 1987) and boron (B), there is usually a fairly clear decline in mineral nutrients content in the dry matter as plants and organs age. This decline is caused mainly by a relative increase in the proportion of structural material (cell walls and lignin) and of storage compounds (e.g., starch) in the dry matter. In addition, as the plants become older there is a decrease in demand for nutrients for new growth (Marschner, 1995). Mineral nutrients content corresponding to the adequate or critical deficiency range are therefore lower in old than in young plants. In grain sorghum, the P levels in the leaf dry matter

decreased from about 0.4% to 0.2% throughout the growing season (Myers *et al.*, 1987). In field-grown barley, the K content in shoot dry matter decreased from 5-6% in young plants to about 1% towards maturation, although the plants were well supplied with K (Leigh *et al.*, 1982).

Data on the nutritional levels of exotic vegetables are associated with a specific stage of plant development (Guarino, 1997). Unlike exotic vegetables, there is no documented information on the stage of plant development to define harvest maturity for ALVs (Guarino, 1997). Data on their nutritional values are likely to vary widely (Jansen-Van-Rensburg *et al.*, 2004; Kruger *et al.*, 1998; Guarino, 1997) due to influences of plant age and the environmental conditions during plant growth.

ALVs can be harvested at different stages of plant growth, ranging from young seedlings to the late juvenile stage (Modi, 2007), but data on the changes in leaf nutritional value with plant age are scanty. The nutritive qualities of ALVs are significantly affected by plant age (Abukutsa-Onyango, 1993; Mwafusi, 1992). The yields, beta-carotene and phenolic compounds increase then decrease (Maumba, 1993) while crude proteins levels do not change significantly with age. Usually higher harvesting frequency result in a higher leaf yield than a single harvest (Abukutsa-Onyango, 1993; Mwafusi, 1992) however, it's not known how this affects mineral distribution. In Zimbabwe, significant differences in the levels of mineral nutrients in amaranth leaves varied at different harvest stages (Makobo *et al.*, 2010). Different minerals in amaranth leaves reached their peak levels at different growth stages (Makobo *et al.*, 2010). The optimum levels of Ca and Zn was at 3, P at 4 and Cu, K and Na at 6 WAE (Makobo *et al.*, 2010). If amaranth is grown to meet the nutritional requirement for a particular mineral nutrient, it should be harvested at the stage it attains optimum levels for that particular mineral (Makobo *et al.*, 2010). If it has to meet the nutritional requirements of different minerals (Ca, K, P, Cu, Na, and Zn), the optimum

harvesting time is at 3 WAE in Zimbabwe (Makobo *et al.*, 2010). The harvesting stage with highest levels of these mineral nutrients in ALVs grown in western Kenya has not been established.

2.2.3: Influence of geographical area of production on nutrient content in ALVs

Minerals uptake in plants is through the root system from the soil solution (Claassen and Steingrobe, 1999; Black, 1968). The sources of these soluble nutrients include: decomposition of plant and animal residues, weathering of soil minerals, organic and inorganic fertilizer application among others. Thus, soil compositions tend to vary widely as evidenced by their texture and colour (Jaetzold *et al.*, 2005). Fluctuations in environmental factors such as temperature and soil moisture can affect the mineral nutrient content of plant leaves considerably (Maschner, 1995). These factors influence both the availability and uptake of nutrients by the roots and the shoot growth rate. Their effects are more distinct in shallow-rooted annual species than in deep-rooted perennial species, which have a higher nutrient buffer capacity within the shoot.

Minerals content in vegetables vary according to their availability in the soil at different growth sites (Ogle and Grivetti, 1985). The nutritional values of vegetables differ not only according to type of vegetable but also according to the geographical area of production (Weinberger and Msuya, 2004). Zinc and Fe levels in *Cleome gynandra* and *Amaranthus hybridus* varied in Kongwa, Muheza and Arumeru districts in Tanzania (Weinberger and Msuya, 2004). Iron and Zn levels in *Corchorus olitorius* and *Corchorus trilocularis* in Muheza district were lower than those in Morogoro district in Tanzania (Kinabo *et al.*, 2003). No study has been done to establish variations in nutrient levels in ALVs in different locations of western Kenya.

2.3: Mineral nutrients, the human and plant health

The human body is 3 to 4% minerals by weight (Charlette and Allred, 1992). The major minerals in the body in decreasing order are: Ca, P, K, S, Na, Mg, Fe, Zn (Charlette and Allred, 1992). The minerals are classified as either macro or micro minerals (Kasper *et al.*, 2005). Macro minerals are required in the diet in relatively large amounts and these include Ca, P, Fe, and Na among others while micronutrients are required in the diet in relatively low amounts, these include Mg, Zn and Mn among others. Heavy metals like Fe, Zn, and Mn are essential for the human health within certain permissible limits beyond which they can bio accumulate to toxic levels (Ansaric *et al.*, 2007). Such micronutrients can be adequately supplied through human diet (Eileen e*t al.,* 1996).

Every essential element follows a dose response curve where at lowest dosages the organism does not survive (Survival region) and in a deficiency region the organism exists with less than optimal function, increasing nutrient supply increases growth rate of the organism (NAS, 1980). In the third region, the growth rate reaches a maximum and remains unaffected by nutrient supply and after optimal dosage region there are high dosages which cause toxicity in organisms and eventually leads to lethality (NAS, 1980). This can be elaborated using the dose-response curve below (Figure 5).

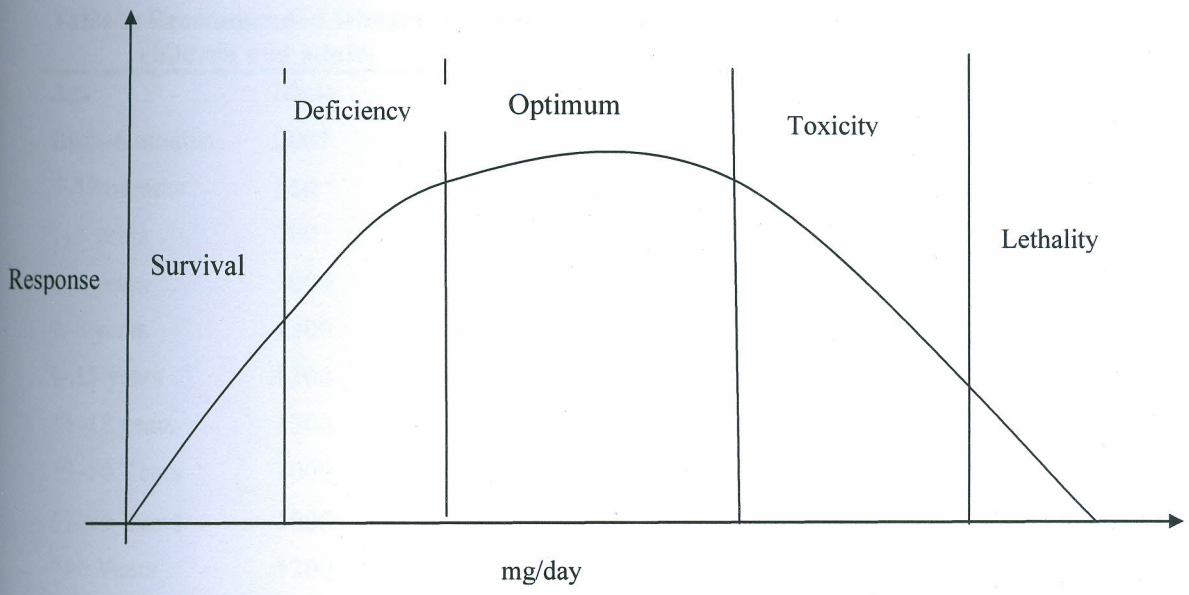

Figure 5: General dose-response curve for essential elements (Source: NAS, 1980)

2.3.1: Calcium

Calcium is an important element which remarkably contributes to body structure especially in the formation of bones and teeth (Charlette and Allred, 1992). The bones have living tissues and secrete collagen, which forms the framework of connected tissues and bones. Calcium, with other elements, is involved in nerves transmission and blood clotting. Calcium deficiency leads to stunted growth due to softening/weakening of bones and causes diseases like rickets, osteomalacia and osteoporosis (Walden, 1989). Phytates interfere with intestinal resorption of Ca thereby causing nutritional deficiencies in both monogastric animals (Welch *et al.*, 1974) and humans (Welch and House, 1984), especially children (Hambidge and Walravens, 1976). Institute of Medicine, Food and Nutrition Board (1997) established Recommended Dietary Allowances (RDAs) for the amounts of Ca required for bone health and to maintain adequate rates of Ca in healthy people (Table 1). RDAs have been used in the past to establish the minimal amounts of nutrients needed to be protective against possible nutrient deficiency.

Table 1: Recommended Dietary Allowances (mg/day) for calcium in healthy children and adults

Age	Male	Female	Pregnancies	Lactation
Birth–6 months	200*	200*	–	–
7–12 months	260	260	–	–
1–3 years	700	700	–	–
4–8 years	1000	1000	–	–
9–8 years	1300	1300	–	–
9–13 years	1300	1300	–	–
14–18 years	1300	1300	1300	1300
19–50 Years	1000	1000	1000	1000
51–70 Years	1000	1200	–	–
71+ Years	1200	1200	–	–

*The indicated Adequate Intake (AI)
Source: Institute of Medicine, Food and Nutrition Board (1997); NAS (1980)

Excessive intake of Ca impairs Fe absorption (Kasper *et al.*, 2005). Therefore, the Institute of Medicine, Food and Nutrition Board (1997) established tolerable upper intake level (ULs) for Ca. Long term intakes above the upper limits increase the risk of adverse health effects such as nausea, vomiting, muscle weakness, excessive drowsiness and kidney stones (Kasper *et al.*, 2005). Table 2 gives the tolerable upper intake levels for Ca in healthy children and adults.

Table 2: Tolerable upper intake levels (ULs) for calcium (mg/day) in healthy children and adults

Age	Male	Female	Pregnant	Lactating
0–6 months	1000	1000	–	–
7–12 months	1500	1500	–	–
1–8 Years	2500	2500	–	–
9–18 Years	3000	3000	3000	3000
19–50 Years	2500	2500	2500	2500
51+Years	2000	2000	–	

Source: Institute of Medicine, Food and Nutrition Board (1997); NAS (1980)

Variations in Ca levels in different ALVs have been reported in East Africa (Sehmi, 1993; FAO, 1988) and Nigeria (Iheanacho and Udebuani, 2009). Highest Ca levels were reported in *Cleome gynandra* (Sehmi, 1993) and *Solanum scabrum* (FAO, 1988) and in *Curcubita pepo* in Nigeria. The Ca levels significantly varied at different harvesting stages (Makobo *et al.*, 2010) in Zimbabwe. Highest Ca levels in *Amaranthus cruentus* were attained at 3 WAE (Makobo *et al.*, 2010). Similarly, the Ca levels in amaranth leaves significantly increased with plant age from 3 WAE to 9 WAE (Modi, 2007) in South Africa. Variation in the levels of Ca with species, harvesting stages and locations of production has not been established in western Kenya.

2.3.2: Phosphorus

Phosphorus is the second most abundant mineral in the body which together with Ca have common or supplement one another in their functions. These two important nutrients work closely together. Calcium and P are associated with each other for growth and maintenance of bones, teeth and muscles (Turan *et al.*, 2003; Dosunmu, 1997). Approximately 85% of the human body's P content is found in the bone and teeth but it is also present in cells and tissues throughout the body (Anderson, 1996). Energy production and storage in the body depend upon phosphorylated compounds such as adenosine tri-phosphate (ATP). Other P compounds impact genetic information as well as the functioning of various enzymes and hormones (Anderson, 1996). Phosphate compounds are also important body buffers for controlling acid-base balance (pH).

Phosphorus helps filter out waste in the kidneys and plays an essential role in how the body stores and uses energy (Breuer *et al.*, 1998). More important than the total P content of a food is the Ca: P ratio. Delicate balance between Ca and P is necessary for proper bone density and prevention of osteoporosis (Guil-Guerrero *et al.*, 1998; Berner and Shike, 1988). To maintain proper Ca balance, the dietary Ca: P ratio should be close to 1:0.5 (Berner and

Shike, 1988). Therefore, the Food and Nutrition Board (1997) established RDAs for the amounts of P required for bone health and to maintain adequate rates of P in healthy people (Table 3).

Table 3: Recommended Dietary Allowances for phosphorus (mg/day) in healthy children and adults

Age	Male	Female	Pregnancies	Lactation
Birth–5 months	300	300	–	–
6–12 months	500	500	–	–
1–3 years	800	800	–	–
4–10 years	800	800	–	–
11–14 years	1200	1200	–	–
15–18 years	1200	1200	–	–
19–24 Years	1200	1200	–	–
25–50 Years	800	800	–	–
51+ Years	1200	1200	–	–

Source: Institute of Medicine, Food and Nutrition Board (1997); NAS (1980)

Phosphorus is taken up by plants as either $H_2PO_4^-$ or HPO_4^{2-}. The uptake of $H_2PO_4^-$ is more rapid than HPO_4^{2-} (Tisdale and Ruker, 1964). The concentration of $H_2PO_4^-$ and HPO_4^{2-} in the soil solution is the single most important factor governing the availability of P to plants (Tisdale and Ruker, 1964). In the pH range of 5-8 two ionic species occur together (Equation 1)

$$H_2PO_4^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} HPO_4^{2-} \qquad \text{(Equation 1)}$$

Consequently, at low pH, $H_2PO_4^-$ dominates, whereas the reverse is true at high pH. Maximum availability of P generally occurs in the pH range of 6.0-7.0. Low root zone temperatures depress P uptake (Engels and Marschner, 1992a, 1992b). Retranslocation of P from shoots to roots occurs in P deficient soils (Maas *et al.*, 1988).

The P levels in ALVs vary with species (Birnin-Yauri *et al.*, 2011; Sehmi, 1993; FAO, 1988). Highest P levels were reported in vegetable amaranth (Sehmi, 1993), african nightshades (FAO, 1988) in ALVs from different East African markets, and in *Curcubita pepo* (Iheanacho and Udebuani, 2009) in Nigeria. The P levels in *Amaranthus cruentus* significantly varied with harvesting stages, with highest levels reported at 4 WAE (Makobo *et al.*, 2010) in Zimbabwe. However, the variation in P levels at different harvesting stages was not significant (Modi, 2007) in South Africa. No study has been done to establish the variation in levels of P in ALVs at different harvesting stages and locations of production in western Kenya.

2.3.3: Magnesium

Magnesium is an important mineral element which is a component of bones and many enzymes (Shills, 1999). It plays an important role in the regulation of blood sugar levels and blood pressure. It is necessary for the transmission of nerve impulses, which affects contraction and relaxation of muscles (Shills, 1999). Spontaneous deficiency of Mg in humans is unlikely. However, its' prolonged deficiency causes neurological disturbances (Rude, 1998). Intake recommendations for Mg are provided by the Institute of Medicine, Food and Nutrition Board (1997). Table 4 gives RDA for Mg in healthy children and adults.

Table 4: Recommended Dietary Allowances for magnesium (mg/day) in healthy children and adults

Age	Male	Female	Pregnancies	Lactation
Birth–6 months	–	–	–	–
7–12 months	–	–	–	–
1–3 years	65	65	–	–
4–8 years	110	110	–	–
9–13 years	350	350	–	–
14–18 years	350	350	350	350
19–50 Years	350	350	350	350
51–70 Years	350	350	–	–
71+ Years	350	350	–	–

Source: Institute of Medicine, Food and Nutrition Board (1997); NAS (1980)

Variations in Mg levels in ALVs have been reported in Kenya (Sehmi, 1993) and Nigeria (Bernin-Yauri *et al.*, 2011; Iheanacho and Udebuani, 2009). African nightshade had the highest levels of Mg (Sehmi, 1993) in Kenya and amaranths in Nigeria (Iheanacho and Udebuani, 2009; Bernin-Yauri *et al.*, 2011). There is no documented information on variation in levels of Mg in ALVs at different harvesting stages and locations of production in western Kenya.

2.3.4: Iron

Iron is a component of many enzymes. It is a major control element and many enzymes that are involved in secondary metabolism require it for their activity (Da Silva and Williams, 1991). Iron is virtually found in every food, with higher concentrations in animal tissues than in plant tissues (Hammound and Beliles, 1980). Iron is an essential part of haemoglobin (blood protein) and myoglobin (muscle protein) (Janet and Greger, 1988) and its main role in the body is in the red blood cells where it combines with a protein to form haemoglobin. Oxygen in our lungs is attracted to the Fe in haemoglobin and combines with it to form oxyhaemoglobin. This is then transported around the body by the blood cells released whenever it is needed to allow the conversion of carbohydrates into energy (Stokinger, 1981).

Lack of Fe in the body known as Fe deficiency can be due to inadequate amounts of Fe in the diet or insufficient number of blood cells caused by blood loss leading to anaemia. Phytates interfere with intestinal resorption of Fe. These cause nutritional deficiencies in both monogastric animals (Welch *et al.*, 1974) and humans (Welch and House, 1984), especially children (Hambidge and Walravens, 1976). Table 5 provides the Recommended Dietary Intake levels (RDI) showing the RDA, AI and UL of the Fe for healthy children and adults.

Table 5: Recommended dietary allowance for iron (mg/day) in healthy children and adults

Age	Males and females (RDA)		Males and females (UL)	Pregnancy	Lactation
Birth–6 Months	0.27*	0.27*	40	–	–
7–12 months	11	11	40	–	–
1–3 Years	7	7	40	–	–
4–8 Years	10	10	40	–	–
9–13 Years	8	8	40	–	–
14–18 Years	11	15	40	27	10
19–30 Years	8	18	40	27	9
31–50 Years	8	18	45	27	9
50+ Years	8	18	45	27	9

* Adequate Intake (AI) for iron in infants below 7 months
Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

Iron is the fourth most abundant element in the earth crust (5%) and is unavailable to plants because of its ability to become insoluble (Lepp, 1981). Iron solubility is largely determined by the pH with high pH making Fe less available and giving rise to chlorosis (Hamilton *et al.*, 1991) and oxidation-reduction potential of the substratum (Snowden and Wheeler, 1993).

The Fe levels significantly varied in different species in Kenyan ALVs (Habwe *et al.*, 2009; Sehmi, 1993) and Nigeria (Iheanacho and Udebuani, 2009). Cowpea had the highest levels of Fe (24.1 mg/g) while nightshades had the least (17.3 mg/g) (Habwe *et al.*, 2009) in Maseno, Kenya. The Fe levels in amaranths were higher than levels in nightshades in Tanzania (Weinberger and Msuya, 2004). Such variation has also been recently observed in Pakistan (Javid *et al.*, 2011). The Fe levels also varied with location of production (Weinberger and Msuya, 2004). The Fe levels in amaranths, nightshades and cowpea significantly varied in Kongwa, Singida and Muheza (Weinberger and Msuya, 2004) and Iringa and Morogoro regions in Tanzania (Kinabo *et al.*, 2003). Similar variations were observed in ALVs sold in Dar es Salaam markets (Raja *et al.*, 1997). The levels of Fe in amaranths significantly varied with harvesting stages (Modi, 2007) in KwaZulu-Natal, South Africa. The Fe levels significantly increased between 3 and 6 WAE while the increase between 6 and 9 WAE was not significant. There is no documented information on variation

in levels of Fe in ALVs at different harvesting stages and locations of production in western Kenya.

2.3.5: Zinc

Zinc is a mineral vital to healthy living, as deficiency can cause health problems. It is required for catalytic activity of approximately 100 enzymes (Institute of Medicine, Food and Nutrition Board, 2001) and it plays a role in immune function and cell division (Prasad, 1995); wound healing (Heyneman, 1996) and DNA synthesis (Prasad, 1995). Zinc also supports normal growth and development during pregnancy, childhood and adolescent (Manet and Sandstead, 2006; Simmer and Thompson, 1985) and is required for proper sense of taste and smell (Prasad *et al.*, 1997). A daily intake of Zn is required to maintain a steady state because the body has no specialized Zn storage system (Rink and Gabriel, 2000). Intake recommendations for Zn are provided in the RDI (Table 6) developed by Food and Nutrition Board, at the Institute of Medicine Food and Nutritional Board (2001).

Table 6: Recommended Dietary Allowances for zinc (mg/day) in healthy children and adults

Age	Male	Female	Pregnancy	Lactation
Birth–6 months	2*	2*	–	–
7 months – 3yrs	3	3	–	–
4–8 Years	5	5	–	–
9–13 Years	8	8	–	–
14–18 Years	11	9	–	–
19+ Years	11	8	–	–

*The indicated Adequate Intake (AI)
Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

Phytates interfere with intestinal resorption of Zn, such that the amount of Zn resorbed by the intestine is determined by the zinc/phytate ratio in the diet (Lantzsch *et al.*, 1980). Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired

immune function (Shankar and Prasad, 1998). In more severe cases, Zn deficiency causes hair loss, diarrhoea, delayed sexual maturation, impotence, eye and skin lesions (Wang and Busbey, 2005; Prasad, 2004). Weight loss, delayed healing of wounds, taste abnormalities and mental lethargy can also occur (Prasad *et al.*, 1997; Heyneman, 1996). Other diseases associated with Zn deficiency include malabsorption syndrome, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy and other chronic illness (Prasad, 2003). Chronic diarrhoea leads to excessive loss of zinc (Prasad, 2004). The Zn deficiency can lead to common cold and has the ability to prevent or shorten the duration of the colds (Caruso *et al.*, 2007). High Zn intakes can inhibit copper absorption, sometimes producing copper deficiency and associated anaemia (Willis *et al.*, 2005). Acute adverse effects of high Zn intake include nausea, vomiting, loss of appetite, abnormal cramps, diarrhoea and headaches (Kasper *et al.*, 2005). The Institute of Medicine, Food and Nutrition Board (2001) established ULs for Zn (Table 7).

Table 7: Tolerable upper intake levels (ULs) for zinc (mg/day) in healthy Children and adults

Age	Male	Female	Pregnancy	Lactating
0–6 months	4	4	–	–
7–12 months	5	5	–	–
1–3 Years	7	7	–	–
4–8 Years	12	12	–	–
9–13 Years	23	23	–	–
14–18 Years	34	34	34	34
19+ Years	40	40	40	40

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

In plants, Zn is taken up predominantly as a divalent cation (Zn^{2+}). Zinc availability is greatly affected by soil pH (Van Lierop, 1990). As soil pH increases, its availability decreases and vice versa. Availability of Zn to plants is lower in organic soils, and in mineral soils with significant amounts of organic matter (Kabata-Pendias and Pendias, 1984).

The Zn levels in amaranths and african nightshades significantly varied in Maseno, Kenya (Habwe *et al.*, 2009; Sehmi, 1993) and Swaziland (Ogle and Grivetti, 1985). The Zn levels in amaranths and nightshades grown in Swaziland were 1.20 ± 0.44 and 1.34 ± 0.45 mg/100 g, respectively. Significant variations were also observed in Tanzania, amaranths had the highest Zn levels (as high as 0.885 mg/100 g edible portion) followed by african nightshades while african eggplant had the least, having as low as 0.120 mg/100 g (Weinberger and Msuya, 2004). The Zn levels in ALVs also varied with location of production, with significant variations reported in Kongwa, Singida and Muheza (Weinberger and Msuya, 2004) and Iringa and Morogoro (Kinabo *et al.*, 2003) regions in Tanzania. The Zn levels in amaranths significantly varied at different harvesting stages (Makobo *et al.*, 2010; Modi, 2007), with highest levels reported at 3 WAE in Zimbabwe (Makobo *et al.*, 2010) and South Africa (Modi, 2007). No study has been done to establish the variation of Zn levels in ALVs at different harvesting stages and locations of production in western Kenya.

2.3.6: Manganese

Manganese is an essential element that is present in very small amounts in the human body. The body contains about 20 mg of Mn mostly concentrated in the bones, kidneys, liver and pancreas (Institute of Medicine, Food and Nutrition Board, 2001). Manganese primarily works as a coenzyme that facilitates various metabolic processes in the body (Willis *et al.*, 2005). It is involved in bone formation of connective tissues, sex hormone function, calcium absorption, blood sugar regulation, immune function and in fat and carbohydrate metabolism (NAS, 1980). To maintain Mn health one needs to maintain good sense of Mn nutrition. The element is abundant in natural sources but the Mn levels in ALVs grown in different locations of western Kenya remains unknown. Table 8 gives the recommended dietary allowances for manganese according to gender and different age limits.

Table 8: Recommended dietary allowances for manganese (mg/day) in healthy children and adults

Age	Males	Females	Males and Females (UL)	Pregnancy	Lactation
0–6 Months	0.003	0.003	N/A	–	–
7–12 Months	0.6	0.6	N/A	–	–
1–3 years	1.9	1.6	2	–	–
4–8 years	2.2	1.6	3	–	–
9–13 years	2.3	1.8	6	–	–
14–18 years	2.3	1.8	9	9	9
19+ years	2.3	1.8	11	11	11

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

Manganese deficiency leads to bone malformation, eye and hearing problems, high cholesterol levels, hypertension, infertility, weaknesses, heart disorders, memory loss, muscle contraction, tremors and seizures (NAS, 1980). Deficiencies are rare considering that they are naturally abundant in foods, but research has estimated about 37% of the population to be deficient (Institute of Medicine, Food and Nutrition Board, 2001) caused by improper diet and eating habits. Manganese has variable oxidation states. In biological systems, however, it mainly occurs in oxidation states II, III, and IV with Mn (I) and Mn (IV) being fairly stable (Hughes and Williams, 1988). In plants, Mn (II) is by far the dominant form, but it can readily be oxidized to Mn (III) and Mn (IV), it therefore plays an important role in redox processes. Soil pH plays a vital role in the uptake of Mn. With decreasing pH, the amount of exchangeable Mn increases, the increase is a function of the redox potential (Sanders, 1983) (Equation 2)

$$MnO_2 + 4\,H^+ + 2e^- \rightleftharpoons Mn^{2+} + 2H_2O \quad \text{(Equation 2)}$$

The levels of Mn in *Solanum nigrum* and *Fleurya aestuans* significantly varied with highest levels reported in *Solanum nigrum* (Glew *et al.*, 2009) in Ghana. The Mn levels significantly varied in different amaranth varieties in South Africa (Mnkeni *et al.*, 2007). Significant variation in Mn levels between two cowpea varieties were also observed in

Nigeria (Alayande *et al.*, 2012). The concentration of Mn for the two varieties was found to be 14.27 and 15.83 mg/100 g with white seeds having the higher value. Different vegetable species had varying levels of Mn in India (Arora *et al.*, 2008). No study has been done to establish the variation in levels of Mn in ALVs at different harvesting stages and locations of production in western Kenya.

2.3.7: Potassium

Potassium is essential for regulating water and mineral balance, normalises heart rhythms, aids in transporting oxygen to the brain and promotes healthy skin. Potassium together with Na is involved in nerve function and muscle contraction. New RDI values have been published by Institute of Medicine, Food and Nutrition Board (2004) (Table 9).

Table 9: Recommended Dietary Allowances for potassium (mg/day) in healthy children and adults

Age	Male	Female	Pregnancy	Lactation
Birth–6 months	500*	500*	–	–
7–12 months	600*	600*	–	–
1–2 years	1000	1000	–	–
2–5 Years	1400	1400	–	–
6–9 Years	1600	1600	–	–
10–18 Years	2000	2000	2000	2000
19+ Years	2000	2000	2000	2000

*The indicated Adequate Intake (AI)
Source: Institute of Medicine, Food and Nutrition Board (2004); NAS (1980)

Potassium is required by plants in much greater amounts than other mineral nutrients, with the exception of N. Potassium uptake by plants from soil solution is influenced by: developmental stage of the plant, concentration in the external solution, soil moisture conditions, pH, texture, aeration and root zone temperature. More K^+ is taken up during the vegetative growth stages when roots are actively growing than in fruit growth (reproductive) stages when root growth is inactive (Beringer *et al.*, 1986). Developing fruits are stronger

sinks for photoassimilates than roots and other vegetative tissues. This competition for photoassimilates reduces root growth and energy supply for nutrient uptake (Marschner, 1995). Thus, during reproductive development soil K^+ supply may not be adequate to support crucial processes that ultimately determine yield and quality.

Competition for binding sites between ions of the same electrical charge and ion diameter, for example, between K^+ and rubidium (Rb^+). Since the radius of hydrated Rb^+ is similar to that of hydrated K^+, the binding sites at the plasma membrane of root cells do not seem to distinguish between these two cations (Erdei and Trivedi, 1991), compared with K^+ and Rb^+, the affinity of the binding sites for caesium (Cs^+) is low and thus, in general Cs^+ uptake is distinctly depressed by K^+ (Erdei and Trivedi, 1991). In contrast, uptake of K^+ is hardly affected by Cs^+. Competition between K^+ and ammonium ion (NH_4^+) is difficult to explain simply by competition for binding sites at the plasma membrane. Whereas NH_4^+ is quite effective in competing with K^+ the converse (inhibition of NH_4^+ uptake by K^+) is not observed. This seems quite surprising, but similar results were obtained with rice (Mengel *et al.*, 1976). This is because a substantial proportion of ammonium nitrogen is not taken up in the form of NH_4^+, but that NH_3 also permeates the plasma membrane after deprotonation, leaving H^+ in the external solution (Bertl *et al.*, 1984).

The pH of external soil solution has a considerable influence on the uptake of K^+. As the H^+ concentration increases (i.e. the pH falls), in the absence of Ca^{2+} the net uptake of K^+ sharply declines below pH 5; below pH 4 there is a considerable net efflux of K^+ from the roots (Marschner, 1995). However, optimum absorption occurs at pH 5.5 as observed in *Phaseolus vulgaris* (Marschner, 1995; Islam *et al.*, 1980).

Different ALVs were reported to have varying levels of K (Birnin-Yauri *et al.*, 2011; Iheanacho and Udebuani, 2009; Sehmi, 1993). Amaranths had the highest K levels in Kenya (Sehmi, 1993) and Nigeria (Iheanacho and Udebuani, 2009; Birnin-Yauri *et al.*, 2011).

Significant variations in two cowpea varieties were reported in Nigeria with brown cowpea seeds showing higher levels of K than white cowpea seeds (Alayande, *et al.*, 2012). Variations in K levels were also reported at different harvesting stages with highest levels in *Amaranthus cruentus* reported at 6 WAE in Zimbabwe (Makobo *et al.*, 2010) and South Africa (Modi, 2007). Variations in K levels at different locations of production have not been established. In addition, no study has been done to establish the variation of K in various ALVs at different harvesting stages in western Kenya.

2.3.8: Sodium

Sodium is essential for regulating water and mineral balance, normalises heart rhythms (Charllete and Allred, 1992), aids in transporting oxygen to the brain (Kasper *et al.*, 2005) and promotes healthy skin. It is involved in nerve function and muscle contraction. The Institute of Medicine, Food and Nutrition Board (1997) provided the RDI showing the RDA for Na for healthy children and adults.

Table 10: Recommended dietary allowance for sodium (mg/day) in healthy children and adults

Age	Males and females	Pregnancy	Lactation
Birth–6 Months	–	–	–
7–12 months	–	–	–
1–3 Years	1500	–	–
4–8 Years	1900	–	–
9–13 Years	2200	–	–
14–18 Years	2300	2300	2300
19–30 Years	2300	2300	2300
31–50 Years	2300	2300	2300
50 + Years	2300	–	–

Source: Institute of Medicine, Food and Nutrition Board (1997); NAS (1980)

Sodium is a beneficial element in plants. Beneficial elements are elements which either stimulate growth but are not essential or which are essential only for certain plant species, or under specific conditions. Sodium may be classified as a mineral nutrient for at

least some of the C_4 species in the family's *Amaranthaceae*, *Chenopodiaceae* and *Cyperaceae* (Brownell, 1979), the amounts of Na required by these plant species being more typical of those for a micronutrient rather than a macronutrient. Many halophytes, whether C_3 or C_4 species are distinctly enhanced in growth by high Na concentrations (Munns and Termatt, 1986). However, Na does not function as a macronutrient (Munns and Termatt, 1986) but for osmotic adjustment (Eshel, 1985). In contrast to K^+, the uptake rate of Na^+ is much more concentration dependent, reflecting less specific binding sites in the plasma membrane or a higher efficiency of a K^+-Na^+ coupled Na^+ efflux pump (Binzel *et al.*, 1988). Although the mechanisms for Na^+ influx across the plant plasma membranes have not yet been established, it is evident that Na^+ can be transported into the cell through K^+ carriers.

Variations in Na levels were reported in different ALVs (Birnin-Yauri *et al.*, 2011; Iheanacho and Udebuani, 2009; Sehmi, 1993). Amaranths had the highest Na levels in Kenya (Sehmi, 1993) and Nigeria (Birnin-Yauri *et al.*, 2011; Iheanacho and Udebuani, 2009). Significant variations in Na levels in two cowpea varieties were also reported in Nigeria (Alayande *et al.*, 2012) with white cowpea seeds showing higher levels than brown cowpea seeds (Alayande *et al.*, 2012). The Na levels also varied with harvesting stage, with highest Na levels in *Amaranthus cruenthus* reported at 6 WAE in Zimbabwe (Makobo *et al.*, 2010) and 7 WAE in South Africa (Modi, 2007). There is no documented information on variation in levels of Na at different locations of production. Similarly, no study has been done to establish the variation of Na levels in ALVs at different harvesting stages and locations in western Kenya.

2.3.9: Nitrogen

Molecular nitrogen is converted to a reduced state to be useful to higher plants and animals. Nitrogen fixation is carried out by bacterial nitrogenises forming reduced N, NH_4^+ which can then be used by all organisms to form amino acids, the building block for proteins. It is also a

major part of the chemical structure of many compounds including Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA) and choline. Dietary protein consists of both essential and non essential amino acids that are required for protein synthesis. Protein intake is an indispensable requirement for the growth of any living organism. Every cell in our body needs protein to carry out any metabolism (Kasper *et al.*, 2005). The Institute of Medicine Food and Nutrition Board came up with RDA for proteins for healthy children and adults (Table 11).

Table 11: Recommended dietary allowances for proteins (g/day) for healthy children and adults

Age	Males	Females	Pregnancy	Lactation
Birth–6 Months	9.1	9.1	–	–
7–12 months	13.5	13.5	–	–
1–3 Years	13	13	–	–
4–8 Years	19	19	–	–
9–13 Years	34	34	–	–
14–18 Years	52	46	71	71
19–30 Years	56	46	71	71
31–50 Years	56	46	71	71
50 + Years	56	46	–	–

Source: Institute of Medicine, Food and Nutrition Board (2004); NAS (1980)

The conversion of the inert N_2 molecule into combined nitrogen (NH_3; NO_3^-, etc.) which can be utilized as a mineral nutrient is brought about either by reduction to ammonia (NH_3) or oxidation to nitrate (NO_3^-). This conversion is highly energy consuming, the principal reaction for dinitrogen reduction (Bergersen, 1991) (Equation 3).

$$N_2 + 8\,H^+ + 8e^- + 16Mg.\,ATP \rightarrow 2\,NH_3 + H_2 + 16Mg.\,ADP + 16Pi \qquad \text{(Equation 3)}$$

Nitrate (NO_3^-) and ammonium (NH_4^+) are the major sources of inorganic N taken up by the roots of higher plants. Most of the NH_4^+ has to be incorporated into organic compounds in the roots, whereas nitrate is readily mobile in the xylem and can also be stored

in the vacuoles of roots, shoots, and storage organs. Whether NH_4^+ or NO_3^- as sole source of N supply is better for plant growth and yield formation depends on many factors (Kirkby, 1981). Highest growth rates and plant yields are obtained by combined supply of NH_4^+ and NO_3^-. Depending on the plant species, development stage, and organ, the N content required for optimal growth varies between 2 and 5% of the plant dry weight. When the supply is sub optimal, growth is retarded; N is mobilized in mature leaves and re translocated to areas of new growth. Low external pH increases the uptake of NO_3^- but decreases uptake of NH_4^+ (Zsoldos and Haunold, 1982). At high external pH the uptake of NH_4^+ increases sharply, probably owing to an increase in the proportion of the molecular species (NH_3 and NH_4OH). Competition between chloride (Cl^-) and NO_3^-, NH_4^+ and NO_3^- affects uptake of NH_4^+ and NO_3^-. In almost all cases external NH_4^+ strongly suppresses net uptake of NO_3^-, in contrast, externally supplied NO_3^- has little or no effect on net uptake of NH_4^+ (Breteler and Siegerist, 1984).

Different varieties of amaranth had varying levels of N in South Africa (Mnkeni *et al.*, 2007). The N content significantly varied at different harvesting stages (Masinde and Agong, 2011) in Nairobi, Kenya The leaf N content declined in mature tomatoes (Schoelberg *et al.*, 2000; De Pinheiro and Marcelis, 2000) but remained unchanged in spider plant at different harvesting stages (Masinde and Agong, 2011). The variation in leaf N content in various ALVs at different harvesting stages and locations of production has not been established in western Kenya.

CHAPTER THREE

METHODOLOGY

3.1: Area of study

Western Kenya is known to produce *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* almost throughout the year due to favourable soils and climatic conditions, which vary slightly across the region spanning from the Lake Victoria to high equatorial rainy regions (Jaetzold *et al.*, 2005). The region receives a bimodal rainfall distribution pattern, with long rains falling between March and June and short rains are received from September to December (Jaetzold and Schmidt, 1983). The rainfall has marked peaks in March-May when half the annual precipitation may be expected and short rains between November and December (Jaetzold and Schmidt, 1983). This variation may cause large changes in the nutrients availability and absorption by ALVs. Various studies have been done on ALVs grown in Maseno University. The choice of Kisumu and Lela sites was meant to establish variations in the nutrients uptake by ALVs despite their close proximity while the choice of Busia site was to establish variations with levels from Kisumu and Lela and also to compare with findings from Maseno.

The trials were laid down in three geographical locations of Busia, Kisumu and Lela in western Kenya from April to September, 2009. The trial in Kisumu was laid at an altitude of 1132 metres above mean sea level latitude 0°03′S and longitude 34°45′E (http://www.mapsofworld.com/lat-long/kenya-lat-long.html). The area has a mean annual rainfall which range from 1100 mm to 1500 mm. The trial in Busia was set at 1350 metres above mean sea level and the district falls within the Lake Victoria basin with an a latitude of 0°28′N and longitude 34°6′E (http://www.fallingrain.com/world/KE/9/Busia.html). Lela is located near the Equator, 30 km north west of Kisumu city in Nyanza province at an altitude of 1560 metres above sea level, latitude of 0°1′S and longitude 34°36′E (http://www.fallingrain.com/world/KE/7/Lela.html). The trial sites are shown in Figure 6.

STUDY AREAS (BUSIA, LELA, KISUMU)

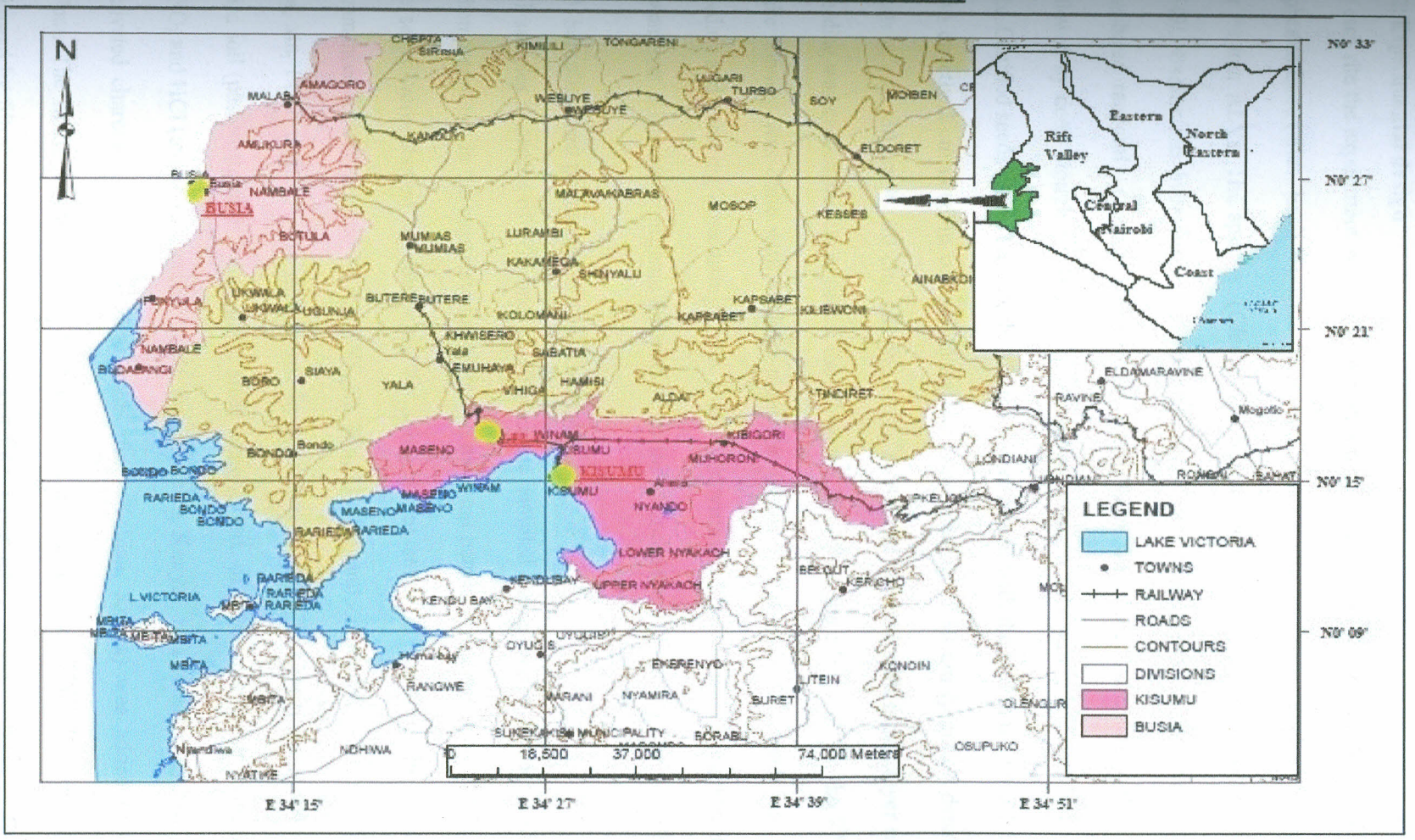

Figure 6: Map showing sites of ALVs production trials.

3.2: Experimental design

At each site the experiment was laid down in a randomized complete block design (RCBD) replicated three times. Each replicate (4 m^2) was divided into four plots (each 1 m^2) to cater for the four ALVs, The species: (*Cleome gynandra,* variety PS, *Solanum scabrum,* variety SS49*), Amaranthus hybridus,* amfune variety and *Vigna unguiculata,* fahari variety) were distributed randomly (Appendix 1). Land preparation was done by clearing the weeds followed by deep ploughing. Harrowing was done using a folk jembe and levelled with a rake. Certified seeds (from Lagrotech, Kisumu) were mixed with the soil in the ratio 1:10 and sawn directly 2 cm deep in rows with an inter-row spacing of 30 cm (planting density 11 plants m^{-2}, i.e. 30 cm by 30 cm) (Kagho *et al.*, 1990) and covered with a thin layer of soil. Weeding was carried out three times; the 1st on the 3rd, 2nd on the 5th and 3rd on the 7th week while thinning was done with an inter-plant spacing of 30 cm on the 3rd week just before weeding and spraying done using Ortus 5 SC (fenpyroximate as the active ingredient from Mwangavet, Kisumu) an organic insecticide for insect and pathogen control after weeding.

3.3: Sampling and preparation of soil samples

Soil samples at depths of 0-15 and 15-30 cm were sampled using Johnson Bucket soil auger (Thompson type) in the experimental plots in a diagonal pattern (Okalebo *et al.*, 2002) before the seeds were planted to determine the soil nutrient status at the time the experiment commenced for soil pH and N, P, K, Na, Ca, Mn, Mg, Fe, and Zn analyses. Total K, Na, Ca, Mg, Mn, Fe, P and Zn were determined by digestion of 5.0 g (composite samples) of the dried soil (passed through 2 mm mesh size) using 25 mL of a 3:1 mixture of concentrated HNO_3 and HCl (analytical reagent grade, from Kobian, Kenya), in a 50 mL polythene bottle. Activated charcoal (0.5 g, analytical grade, from Kobian, Kenya) was added to remove colour. The mixture was stoppered, mixed well and shaken mechanically for six hours followed by filtration through medium speed filter paper into a 50 mL volumetric flask and

made to mark with 0.1 N HCl. The metal elements (K, Na and Ca) were analysed using flame photometer while Mg, Zn, Fe and Mn analyses using Atomic absorption spectrophotometer (AAS) as explained in section 3.5.1.

3.4: Sampling and determination of moisture content in leaf samples

The upper palatable aerial parts of individual plants (2^{nd} and 3^{rd} leaves) were sampled fortnightly four times, after 4 WAE. The moisture content was determined according to the method of AOAC (1990). Porcelain crucibles were properly washed and allowed to dry in an air-oven at 110 °C for 10 min to a constant weight. The crucibles were allowed to cool in a dessicator for 30 min, then labeled and weighed (W_1). 2.0 g of each sample were accurately weighed into the crucibles and reweighed (W_2). The crucibles containing the samples were placed in an oven maintained at 105 °C for 14 h. They were removed and transferred to desiccators to cool, finally weighed (W_3). The moisture content was then calculated.

3.5: Preparation of leaf samples

The Association of Official Analytical Chemists (AOAC, 1990) method of sample preparation was used. Leaf samples were washed in deionised water three times to remove soil particles, oven-dried at 60 °C then crushed into fine powder using a mill. Dried and ground leaves (0.5 g) in porcelain crucible were ashed in a muffle furnace at 450 °C until greyish white ash was obtained. This was done to remove all organic matter. The samples were cooled on top of asbestos sheet and 5 mL analytical grade 1 N HNO_3 (from Kobian, Kenya) solution added to each sample for digestion. This was then evaporated to dryness on a steam bath to ensure complete digestion. The samples were returned to the furnace for a further 15 minutes until a perfect grey ash was obtained. The grey ash was cooled on asbestos sheet and 10 mL of analytical grade 1 N HCl (from Kobian, Kenya) added for mineral extraction then filtered into 50 mL volumetric flask. The crucible and filter paper were rinsed three times and the aliquots added to the flask before making to the mark with 0.1 N HCl.

The samples were analysed for K, Na and Ca using flame photometer, Mg, Mn, Fe and Zn analysis using atomic absorption spectrophotometer (AAS) and P using electrophotocolorimeter as explained in section 3.5.1 and 3.6.1, respectively.

3.5.1: Determination of K, Na, Ca, Mg, Mn, Fe, and Zn in leaf and soil samples

The leaf and soil samples were pipetted (0.5 mL) into 50 mL volumetric flasks and made to the mark with double deionised water after addition of 1 mL strontium chloride (analytical grade, $SrCl_2.6\ H_2O$ from Kobian, Kenya). The purpose of strontium chloride was to remove interference in the absorption of the specific metal by other metals at the same wavelength by acting as a buffer hence preventing further ionization of the ions (Ikuo *et al.*, 1965). The extract was analysed for K, Na and Ca using flame photometer (Jenway Model, PFP 7) and Mg, Mn, Fe, and Zn using atomic absorption spectrometer, AAS, Series 712013v1.26. Wavelengths of 285.2 nm, 279.5 nm 248.3 nm, and 213.9 nm were used to measure absorbance of Mg, Mn, Fe, and Zn, respectively. Analytical grade salts of potassium chloride, sodium chloride, calcium carbonate, potassium permanganate and metals: iron, magnesium, and zinc (from Kobian, Kenya) were used to prepare known concentrations per salt in 100 mL volumetric flasks after addition of 1 mL strontium chloride. These salts were used as standards and a calibration curve was drawn which was used to determine the concentration of the analyte.

3.6.1: Preparation and determination of total phosphates in soil samples

A method described by Okalebo (1985) was adapted with slight modifications, 1.0 g of oven dried (60 ^{o}C) ground soil (passed through 0.2 mm mesh size) were weighed into a labelled dry and clean 500 mL digestion tube. The samples were macro-KjeldahL digested using 25 mL of digestion mixture [analytical grade reagents, 3.2 g salicylic acid in 100 mL of sulphuric acid-selenium mixture (3.5 g of selenium in one litre of sulphuric acid heated to 300 ^{o}C while covering the beaker with a watch glass)], to each tube and the reagent blanks for

each batch of samples was digested at 110 °C for one hour, removed, cooled and three successive 1 mL portions of 30% analytical grade, hydrogen peroxide added. Digestion temperature was raised to 330 °C as heating was continued until the solution became clear and remaining sand white. After cooling, 25 mL of distilled water was added, mixed well until no more sediments dissolved, cooled again and made up to 50 mL with distilled water and allowed to settle. The digested sample (10 mL) was pipetted into a 50 mL volumetric flask and 0.2 mL of 0.5%, analytical grade para-nitro phenol indicator solution added. The solution was made just alkaline (yellow colour) with 6 N NH_3 (analytical grade) solutions with drop-wise addition with gently shaking. Drop wise addition of 1 N HNO_3 with shaking was done until the solution turned colourless. A volume of 5 mL analytical grade ammonium molybdate, $(NH_4)_6MO_7O_{24}.4H_2O$ and ammonium vanadate mixed reagent was added. The mixture was diluted to the mark with deionised water and left to cool for 10 minutes. Absorbance was measured using PU-8625 UV-Vis spectrophotometer of the yellow reaction product, vanadomolybdophosphate, at 460 nm. Absorbance of a blank and standards that were prepared from solutions of phosphate, 1.0982 g of oven-dried analytical grade, KH_2PO_4 was dissolved in distilled water and the volume made to 250 mL, carried through the same steps were measured. A calibration curve was prepared by plotting optical density of standard solution against parts per million (0, 50, 100, 150, 200, 250 ppm) P and total concentrations of phosphates were obtained.

3.6.2: Determination of total phosphates in leaf samples

A method described by AOAC (1970) was used. Leaf sample (from dry ashing) solution (5 mL) was pipetted into 100 mL volumetric flask and 45 mL deionised water added. Within 5 minutes, 20 mL of vanado-molybdate reagent was added and the volume made to mark with deionised water. The contents were mixed and allowed to stand for 10 minutes. Absorbance was measured using an ultra violet spectroscopy (PU-8625 UV-Vis spectrophotometer) of the

yellow reaction product, vanadomolybdophosphate, at 460 nm. Absorbance of a blank and standards that were prepared from solutions of phosphate, 1.0982 g of oven-dried KH_2PO_4 was dissolved in distilled water and the volume made to 250 mL, carried through the same steps were measured. A calibration curve was prepared by plotting optical density of standard solutions against parts per million (0, 50, 100, 150, 200 and 250 ppm) P and total concentrations of phosphates were obtained.

3.7: Preparation and determination of total nitrogen in soil samples

Total N was determined using a method described by AOAC (1965) with slight modifications, 2 g of air-dried ground soil sample (passed through 0.2 mm mesh size) was weighed in a dry 500 mL macro-KjeldahL flask and 88 mL of deionised water was added. The flask was swirled for five minutes then allowed to stand for 30 minutes. One tablet of analytical grade mercury catalyst and 10 g of K_2SO_4 (added to raise the boiling point in order to achieve the needed 360 °C digestion temperature) were added followed by 30 mL of analytical grade concentrated H_2SO_4 (wet oxidation of organic matter) through an automatic pipette. The flask was heated cautiously at low heat on the digestion stand. When all the water had been removed and frothing ceased, heating was increased until the digest cleared. The mixture was boiled for 5 hours (the heating was regulated during boiling so that the H_2SO_4 condensed about half way up the neck of the flask). After cooling, the digested samples were filtered into 100 mL volumetric flask (to retain all sand particles in the original digestion flask because sand can cause severe bumping during KjeldahL distillation), the sand residue was rinsed four times and the aliquot transferred into the same flask and the volume made to mark with deionised water. From the solutions in the volumetric flask, 10 mL samples were used for distillation using 82 mL 40% analytical grade NaOH (free ammonia was liberated from digested solution by steam distillation in presence of excess NaOH). The distillate was collected in a receiver containing 72 mL boric acid (1%, analytical

grade H_3BO_3) mixed with four drops of analytical grade mixed indicator (0.099 g bromocresol green, 0.066 g methyl red, and 0.011 g thymol blue all dissolved with shaking in 100 mL ethanol 96% (v/v)). Distillation was continued for two minutes from the time the indicator turned green. The distilled sample was titrated against 0.1 N HCl and the volume of HCl used up noted, the end point being reached when the indicator changed from green through grey to a definite pink. Percent nitrogen was then calculated using the expression:

$$\%N = [(T-B) \times N \times 1400]/S$$

Where: T= volume of sample titre

B= volume of reagent blank titre

N= normality of HCl

S= sample weight

Steam was passed through the apparatus for 30 minutes and the steam blank was checked by collecting 50 mL distillate which was titrated with 0.1 N HCl. Occasionally, standard ammonium sulphate solution was used in place of the sample to check if the distillation recovery was satisfactory. The dilution factor was taken into consideration in the calculation.

3.8: Preparation and determination of total nitrogen in leaf samples

Total N was determined using a method described by AOAC (1995) with slight modifications. Samples (50 mg) were weighed into a 500 mL KjeldahL flask. Deionised water (88 mL) was added and the flask left to stand for 30 minutes. With sample in the flask, 3 mL salicylic-sulphuric acid mixture was added and allowed to stand with occasional swirling for two hours. Salicylic acid was used to allow recovery of nitrate-nitrogen which is not uniformly recovered by the standard KjeldahL procedure (Okalebo, 1985). Through a dry thistle tube funnel, 0.5 g powdered analytical grade, $Na_2S_2O_3.5H_2O$ was added. The mixture was cooled and 1.33 g K_2SO_4 catalyst mixture added followed by 20 mL of deionised water with swirling. The mixture was heated cautiously on the digestion rack until frothing stopped

(exhaust fumes draining through aspirator). Heating was increased to gently boil so that H_2SO_4 condensed about 1/3 way up neck of flask. Boiling was continued for 30 minutes longer after the digest cleared. The mixture was allowed to cool and 10 mL of deionised water was slowly added with swirling until undissolved materials remained in suspension. Steam was flushed into the distillation apparatus for 5 minutes to clean and bring it up to temperature. To the sample in the flask, 82 mL of 40% NaOH was added before distillation using a vapodest 50 S automatic distiller into a receiver containing 72 mL 1% H_3BO_3 with drops of mixed indicator (0.099 g bromocresol green, 0.066 g methyl red and 0.011 g thymol blue dissolved all with shaking in 100 mL ethanol). Free NH_3 was liberated from solution by steam distillation in presence of excess NaOH. The distilled sample was then titrated against 0.1 N HCl and the volume of HCl used up noted, the end point being reached when the indicator changed from green through grey to a definite pink. Percent nitrogen was then calculated using the expression:

$$\%N = [(T-B) \times N \times 1400]/S$$

Where: T= volume of sample titre

B= volume of reagent blank titre

N= normality of H_2SO_4

S= sample weight

Steam was passed through the apparatus for 30 minutes and the steam blank was checked by collecting 50 mL distillate which was then titrated with 0.1 N HCl. Occasionally, standard ammonium sulphate solution was used in place of the sample to check if the distillation recovery was satisfactory.

3.9: Determination of soil pH

Soil pH in water (2.5:1 water to soil ratio) was determined using glass-electrode pH meter (3071 Jenway) according to Rhoades (1982). The pH meter was calibrated with pH 7.0, pH

4.0 buffer before use. In this method, 50 mL of deionised water was added to 20 g of air-dried soil (passed through 2 mm mesh size) into a 50 mL beaker. The mixture was stirred for 10 minutes and allowed to stand for 30 minutes. The mixture was again stirred for 2 minutes and the pH reading of the partly settled suspension was taken. The pH electrode was rinsed with deionised water and wiped dry with filter paper after each reading.

3.9.1: Data analysis

The data for ALVs from Busia, Lela and Kisumu at different harvesting stages was analysed using RCBD in a 3-factorial arrangement with species as the main treatment, location as sub-treatment, and harvesting stage as sub-sub-treatment. On the other hand, data for ALVs at different harvesting stages in a single location was analyzed using RCBD in a 2-factorial arrangement, with species as the main treatment and harvesting stage as sub-treatment, soil data was analysed using RCBD in a 1-factorial arrangement using MSTAT-C statistical package (Michigan State University, MI) for ANOVA.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Variation in levels of nutrients in ALVs grown in a single location with respect to stage of harvesting

The WHO panel on diet, nutrition, and prevention of chronic diseases recommends an individual intake of at least 400 g of fruits and vegetables a day-the equivalent of five servings of 80 g each (WHO, 1990; WHO/FAO, 2003). The levels of nutrients in this study were measured in mg/g DW. The mean leaf moisture content in different ALVs and locations of production was determined and may be used to convert dry weight to fresh weight (FW) (Table 12).

Table 12: Leaf moisture content (g/100 g) in ALVs by locations

		Species			
Loc	H. stage (wks)	*V.unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*
Busia	4	84.73±0.25	81.21±0.19	83.40±0.18	84.41±0.27
	6	84.82±0.17	81.55±0.22	83.62±0.23	83.68±0.46
	8	84.85±0.13	81.51±0.20	83.59±0.29	84.27±0.11
	10	84.82±0.12	81.36±0.14	83.55±0.09	84.26±0.18
Kisumu	4	79.91±0.10	76.28±0.31	76.63±0.20	78.64±0.17
	6	79.91±0.08	73.81±0.05	76.72±0.19	76.79±0.23
	8	79.75±0.12	74.15±0.16	76.25±0.40	77.89±0.07
	10	79.57±0.30	73.88±0.15	76.83±0.21	77.87±0.11
Lela	4	85.72±0.42	83.72±0.23	84.35±0.37	85.53±0.28
	6	85.63±0.32	83.87±0.11	84.07±0.26	85.28±0.10
	8	85.68±0.33	83.57±0.06	83.60±0.18	85.48±0.13
	10	85.86±0.18	83.80±0.15	83.89±0.09	85.56±0.28

± indicates standard errors of the mean

4.1.1: Variation in levels of nutrients in ALVs in Busia with respect to stage of harvesting

The levels of nutrients significantly ($P \leq 0.05$) varied in the four species studied. *Amaranthus hybridus* had significantly ($P \leq 0.05$) higher levels of P, K, Ca, Mg, Zn, Mn and Na. *Vigna unguiculata* had higher levels of N, *Solanum scabrum* had higher levels of Mg while *Cleome gynandra* had higher levels of Fe (Table 13 and 14).

Table 13: Levels of N, P, K, Ca and Mg (DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Busia site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage
N (%)	4	2.241	1.735	1.641	2.019	1.909
	6	2.570	1.743	1.644	2.125	2.020
	8	2.546	1.749	1.659	2.227	2.045
	10	2.290	1.757	1.664	2.365	2.019
	Mean species	2.412	1.746	1.652	2.184	
	CV (%)			4.98		
	LSD (P≤0.05)			0.129	0.129	
	Interaction			0.184		
P	4	18.679	48.370	26.726	37.363	32.784
(mg/100 g)	6	23.097	52.128	29.583	42.010	36.704
	8	19.642	48.340	25.293	36.798	32.518
	10	16.988	42.790	21.215	35.432	29.107
	Mean species	19.602	47.907	25.704	37.901	
	RDA (mg)			800.000		
	AVC	6.221	2.517	4.737	3.178	
	CV (%)			4.12		
	LSD (P≤0.05)			1.756	1.756	
K	4	69.932	80.402	69.337	65.480	71.288
(mg/100 g)	6	76.472	83.000	79.916	75.475	78.715
	8	65.550	80.747	76.190	70.085	73.143
	10	62.472	71.944	68.808	58.302	65.381
	Mean species	68.607	79.023	73.563	67.335	
	RDA (mg)			2000.000		
	AVC*	2.932*	2.537 *	2.714 *	2.996*	
	CV (%)			9.48		
	LSD (P≤0.05)			8.771	8.771	
Ca	4	178.370	239.350	180.327	185.643	195.923
(mg/100 g)	6	190.890	254.447	254.293	192.570	223.050
	8	155.707	208.777	170.740	175.203	177.607
	10	149.420	187.367	156.337	145.423	159.637
	Mean species	168.597	222.485	190.424	174.710	
	RDA (mg)			1000.000		
	AVC*	0.784*	0.592*	0.705*	0.752*	
	CV (%)			0.83		
	LSD (P≤0.05)			2.032		
	Interaction			2.886		
Mg	4	28.823	17.807	34.527	366.743	111.975
(mg/100 g)	6	31.523	19.990	37.397	390.283	119.798
	8	34.103	22.380	29.270	424.707	127.615
	10	32.920	22.690	26.747	434.347	129.176
	Mean species	31.842	20.717	31.985	404.020	
	RDA (mg)			350.000		
	AVC*	1.103*	1.705*	1.112*	0.087*	
	CV (%)			1.44		
	LSD (P≤0.05)			2.287	2.287	
	Interaction			3.251		

AVC *, amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 14: Levels of Fe, Zn, Mn and Na (mg/100 g DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Busia site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage
Fe	4	9.413	9.079	15.304	9.100	10.724
	6	10.022	10.562	15.894	9.248	11.432
	8	10.206	10.929	16.556	9.346	11.759
	10	10.454	11.406	15.499	9.436	11.699
	Mean species	10.024	10.494	15.813	9.282	
	RDA (mg)			18.000		
	AVC *	0.179 *	0.172 *	0.114*	0.193 *	
	CV (%)			5.40		
	LSD (P≤0.05)			0.799	0.799	
Zn	4	1.554	2.065	1.531	1.795	1.736
	6	1.715	2.266	1.800	1.963	1.936
	8	1.615	2.045	1.382	1.675	1.679
	10	1.368	1.922	1.032	1.624	1.486
	Mean species	1.563	2.075	1.436	1.764	
	RDA (mg)			9.000		
	AVC*	0.514 *	0.386*	0.579 *	0.455 *	
	CV (%)			3.78		
	LSD (P≤0.05)			0.084	0.084	
	Interaction			0.119		
Mn	4	0.018	0.055	0.026	0.028	0.032
	6	0.029	0.044	0.021	0.026	0.030
	8	0.038	0.045	0.031	0.016	0.032
	10	0.041	0.039	0.024	0.025	0.032
	Mean species	0.031	0.046	0.025	0.024	
	RDA (mg)			1.800		
	AVC *	4.573 *	2.885 *	5.199 *	5.742 *	
	CV (%)			22.91		
	LSD (P≤0.05)			0.009	NS	
	Interaction			0.013		
Na	4	25.621	195.150	13.483	64.047	74.621
	6	25.593	152.557	13.593	63.673	63.854
	8	24.697	151.447	13.463	64.757	63.591
	10	25.783	156.537	13.290	64.470	65.020
	Mean species	25.470	163.923	13.457	64.237	
	RDA (mg)			2300.000		
	AVC *	9.048 *	1.433 *	1.709 *	3.580 *	
	CV (%)			26.93		
	LSD (P≤0.05)			16.606	NS	

AVC*, amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Variations in levels of nutrients in ALVs had also been reported in Maseno (Habwe *et al.*, 2009), Nairobi (Mwajumwa *et al.*, 1991), ALVs from different Kenyan markets (Sehmi,

1993), Ghana (Adotey *et al.*, 2009) and Swaziland (Ogle and Grivetti, 1985). The observed variation in the current study is therefore not surprising. The P, K, Ca and Na levels reported in this study were lower than the levels reported by Sehmi (1993) but Fe levels (in all species) and Mg levels in *Solanum scabrum* were similar to the findings of Sehmi (1993) and FAO (1988). However, the P, K, Zn and Fe levels in *Amaranthus hybridus* were similar to the levels reported in Nigeria (Akubugwo *et al.*, 2007). Similarly, the Fe levels in amaranths reported by FAO (1988) were similar to the findings of this study but much lower in *Cleome gynandra*, *Solanum scabrum* and *Vigna unguiculata*.

The Fe levels in *Solanum scabrum*, *Vigna unguiculata* and *Amaranthus hybridus* were lower than the levels reported in Maseno (Habwe *et al.*, 2009). The leaf N content in *Cleome gynandra* was lower than the levels reported in Nairobi (Masinde and Agong, 2011). The Mn levels in this study were lower than the levels reported in Nigeria (Glew *et al.*, 2009). The observed differences in the levels reported in this study with previous studies could be due to variations in soil nutrient levels, agronomic practices and climatic conditions during growth.

The ALVs were significant contributors of Fe, Mg and Zn. The Fe (in all species), Mg in *Solanum scabrum* and Zn levels in *Amaranthus hybridus* were above the RDA. Consumption of 179 g, 172 g, 114 g and 193 g DW of *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*, respectively is recommended to supply RDAs of Fe, while consumption of 172 g, 386 g and 592 g DW of *Amaranthus hybridus* is recommended to supply RDAs of Fe, Zn and Ca, respectively and 87 g of *Solanum scabrum* to supply RDA of Mg. The ALVs were not significant contributors of P, K, Mn and Na as consumption of more than half a kilogram is recommended to supply RDAs of P, K, Mn and Na.

There was significant ($P \leq 0.05$) interaction between species and harvesting stages for N, Ca, Mg, Zn and Mn, suggesting the response patterns were different in each species and

harvesting stages. This implies that variations in the levels of these nutrients at different harvesting stages did not follow similar patterns in different species. This can be attributed to the differences in genetic constitution of different species and thus in the demand for each nutrient (Marschner, 1995).

4.1.2: Variation in levels of nutrients in ALVs in Kisumu with respect to stage of harvesting

The levels of nutrients significantly ($P \leq 0.05$) varied in the four species. *Amaranthus hybridus* had significantly ($P \leq 0.05$) higher levels of P, K, Ca, Zn, Na and Mn. *Vigna unguiculata* had higher levels of N and least K and P while higher levels of Mg and Fe were reported in *Solanum scabrum* and *Cleome gynandra*, respectively (Table 15 and 16).

Table 15: Levels of N, P, K, Ca and Mg (DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Kisumu site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. Stage
N (%)	4	2.365	1.775	1.766	2.214	2.030
	6	2.552	1.872	1.769	2.345	2.135
	8	2.576	1.878	1.778	2.431	2.166
	10	2.577	1.837	1.793	2.563	2.193
	Mean species	2.518	1.841	1.777	2.388	
	CV (%)			2.09		
	LSD (P≤0.05)			0.058		0.058
	Interaction			0.082		
P	4	35.057	71.794	41.292	60.946	52.356
(mg/100 g)	6	39.505	77.158	46.168	64.626	56.864
	8	33.255	73.958	41.292	58.370	51.719
	10	32.170	69.404	38.414	52.693	48.170
	Mean species	34.997	73.078	41.875	59.159	
	RDA (mg)			800.000		
	AVC *	2.299 *	1.095 *	1.922 *	1.359 *	
	CV (%)			6.92		
	LSD (P≤0.05)			4.696		4.696
	Interaction			6.677		
K	4	95.271	127.603	102.787	100.574	106.559
(mg/100 g)	6	102.453	131.017	112.647	109.503	113.905
	8	92.941	125.010	98.092	98.118	103.540
	10	91.227	118.077	89.693	92.176	97.793
	Mean species	95.473	125.427	100.804	100.093	
	RDA (mg)			2000.000		
	AVC *	2.101 *	1.596 *	1.996 *	2.005 *	
	CV (%)			1.36		
	LSD (P≤0.05)			1.87		1.87
	Interaction			2.66		
Ca	4	279.930	401.780	198.053	295.963	252.015
(mg/100 g)	6	287.653	435.767	227.993	315.327	408.048
	8	240.133	404.617	188.367	247.210	196.294
	10	200.343	390.027	170.763	211.883	267.596
	Mean species	252.015	408.048	196.294	267.596	
	RDA (mg)			1000.000		
	AVC *	0.404 *	0.245 *	0.514 *	0.426 *	
	CV (%)			1.36		
	LSD (P≤0.05)			49.391		49.391
	Interaction			70.222		
Mg	4	44.123	25.037	54.190	569.177	173.132
(mg/100 g)	6	44.780	25.993	56.103	577.193	176.018
	8	48.607	24.317	49.323	583.237	176.371
	10	46.160	23.403	45.700	573.010	172.068
	Mean species	45.917	24.687	51.329	575.654	
	RDA (mg)			350.000		
	AVC *	0.763 *	1.419 *	0.685 *	0.061 *	
	CV (%)			1.33		
	LSD (P≤0.05)			3.020		3.020
	Interaction			4.294		

AVC *, amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 16: Levels of Fe, Zn, Mn and Na (mg/100 g DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Kisumu site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. Stage
Fe	4	7.564	6.835	13.929	5.594	8.481
	6	8.052	7.674	15.764	6.298	9.447
	8	8.087	7.848	15.000	6.514	9.362
	10	8.102	10.518	16.040	6.691	10.338
	Mean species	7.951	8.218	15.183	6.274	
	RDA (mg)			18.000		
	AVC *	0.226 *	0.224 *	0.118 *	0.288 *	
	CV (%)			8.47		
	LSD (P≤0.05)			1.035		NS
	Interaction			1.472		
Zn	4	1.098	2.973	2.371	1.609	2.013
	6	1.177	3.871	2.676	1.883	2.402
	8	1.058	3.150	2.316	1.028	1.888
	10	0.910	2.887	1.926	0.882	1.651
	Mean species	1.061	3.220	2.322	1.350	
	RDA (mg)			9.000		
	AVC *	0.760 *	0.251 *	0.348 *	0.651 *	
	CV (%)			2.22		
	LSD (P≤0.05)			0.057		0.057
	Interaction			0.081		
Mn	4	0.021	0.065	0.046	0.045	0.044
	6	0.036	0.084	0.045	0.036	0.050
	8	0.047	0.084	0.034	0.033	0.050
	10	0.051	0.089	0.019	0.032	0.048
	Mean species	0.039	0.081	0.036	0.036	
	RDA (mg)			1.800		
	AVC *	5.232 *	2.268 *	5.670 *	5.020 *	
	CV (%)			28.18		
	LSD (P≤0.05)			0.018		0.018
	Interaction			0.025		
Na	4	22.137	171.840	16.307	64.910	68.798
	6	22.327	242.310	16.267	66.483	86.847
	8	22.247	180.210	16.620	65.360	71.109
	10	22.677	186.993	17.027	67.443	73.535
	Mean species	22.347	195.338	16.049	66.049	
	RDA (mg)			2300.000		
	AVC *	10.292 *	1.198*	13.897 *	3.482*	
	CV (%)			19.41		
	LSD (P≤0.05)			18.925		NS
	Interaction			26.907		

AVC *, amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

The K levels in *Amaranthus hybridus*, Ca in *Vigna unguiculata* and *Cleome gynandra* were lower than levels reported by Sehmi (1993) but P levels in *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum,* K levels in *Vigna unguiculata* and *Solanum scabrum* and Mg levels in *Vigna unguiculata* and *Cleome gynandra* were similar to levels reported by Sehmi (1993). The Fe and Na levels (four species) were similar to levels reported by Sehmi (1993) while P, K, Ca, Zn and Na levels in *Amaranthus hybridus* were similar to levels reported in Nigeria (Akubugwo *et al.*, 2007). The levels of Ca in *Vigna unguiculata* in this study were much higher than levels reported in Tanzania (Mamiro *et al.,* 2011) but similar to the levels reported by Edmonds and Chweya (1997). The Fe levels in *Solanum scabrum*, *Vigna unguiculata* and *Amaranthus hybridus* were lower than the levels reported in Maseno (Habwe *et al.*, 2009). While the leaf N content of spider plant in this study was lower than the levels reported in Nairobi (Masinde and Agong, 2011). The variations in the levels of nutrients with species may be attributed to the variations in genetic constitution of different species and thus in the demand of nutrients.

The ALVs were significant contributors of Ca, Mg, Fe and Zn. Consumption of 245 g, 251 g DW of *Amaranthus hybridus* is recommended to supply RDAs of Ca and Zn respectively, 61 g of *Solanum scabrum* is recommended to supply RDAs of Mg and 226 g, 224 g, 118 g and 288 g of *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*, respectively is recommended to supply RDAs of Fe. The leafy vegetables were not significant contributors of P, K, Mn and Na as consumption of more than one kilogram DW is recommended to supply RDAs P, K, Mn and Na.

There was significant ($P \leq 0.05$) interaction between species and harvesting stage for all the nutrients analysed suggesting the response patterns were different in each species and harvesting stages. This implies that variations in the levels of nutrients at different harvesting stages did not follow similar patterns in different species. This can be attributed to the

differences in genetic constitution of different species and thus in the demand for each nutrient (Marschner, 1995).

4.1.3: Variation in levels of nutrients in ALVs in Lela with respect to stage of harvesting

The levels of nutrients significantly ($P \leq 0.05$) varied in the four species except K, Mg, and N in *Cleome gynandra* and *Vigna unguiculata*, P in *Amaranthus hybridus* and *Solanum scabrum*, and Zn in *Cleome gynandra* and *Solanum scabrum* (Table 17 and 18). Nitrogen, P and K are required by plants in much greater amounts than other nutrients while Mg is required in relatively low amounts. The non significant variation in the levels of these nutrients may partly be attributed to the fact that nutrients may have been bound and therefore not readily available to the plants. In addition, the initial soil pH was not within optimal range required for most ALVs. The uptake of these nutrients may have been inhibited.

Table 17: Levels of N, P, K, Ca and Mg (DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Lela site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage
N (%)	4	1.538	1.591	1.554	1.786	1.617
	6	1.579	1.634	1.565	1.825	1.651
	8	1.585	1.642	1.571	1.768	1.641
	10	1.587	1.650	1.574	1.845	1.664
	Mean species	1.572	1.629	1.566	1.806	
	CV (%)			3.14		
	LSD (P≤0.05)			0.067		NS
P	4	12.473	18.876	16.760	17.002	16.278
(mg/100 g)	6	13.089	21.483	16.150	19.113	17.459
	8	10.331	16.821	13.697	16.658	14.377
	10	8.440	17.195	12.749	18.328	14.178
	Mean species	11.083	18.594	14.839	17.775	
	RDA (mg)			1000.000		
	AVC *	7.436 *	4.364 *	5.460 *	4.514 *	
	CV (%)			4.61		
	LSD (P≤0.05)			0.933	0.933	
	Interaction			1.327		
K	4	43.679	56.681	42.735	49.659	48.189
(mg/100 g)	6	47.607	59.085	47.297	53.162	51.788
	8	41.521	56.348	43.078	49.633	47.645
	10	35.480	52.679	39.967	43.049	42.794
	Mean species	42.072	56.198	43.269	48.876	
	RDA (mg)			2000.00		
	AVC *	4.807 *	3.564 *	4.638 *	4.115 *	
	CV (%)			3.13		
	LSD (P≤0.05)			1.933		1.933
	Interaction			2.749		
Ca	4	106.937	141.590	148.107	120.100	129.183
(mg/100 g)	6	116.290	156.247	173.353	133.480	144.842
	8	83.264	125.120	138.480	110.390	114.321
	10	72.890	100.038	122.030	104.967	99.981
	Mean species	94.845	130.749	145.500	117.234	
	RDA (mg)			1000.000		
	AVC *	1.091 *	0.786 *	0.698 *	0.859 *	
	CV (%)			2.31		
	LSD (P≤0.05)			3.668		3.668
	Interaction			5.215		
Mg	4	22.147	18.863	29.543	268.913	84.867
(mg/100 g)	6	26.413	17.467	32.277	281.163	89.330
	8	25.510	19.707	26.093	327.047	99.589
	10	22.487	18.623	22.470	309.103	93.171
	Mean species	24.139	18.665	27.596	296.557	
	RDA (mg)			350.000		
	AVC *	1.458 *	1.878 *	1.466 *	0.118*	
	CV (%)			4.29		
	LSD (P≤0.05)			5.114		7.272
	Interaction			5.114		

AVC *, amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 18: Levels of Fe, Zn, Mn and Na (mg/100 g DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Lela site

		Vegetable species				
Nutrient	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage
Fe	4	6.046	5.445	7.688	3.392	5.643
	6	6.517	5.555	9.491	3.741	6.326
	8	6.6222	5.641	9.367	3.931	6.390
	10	6.880	6.082	9.053	4.260	6.569
	Mean species	6.516	5.681	8.900	3.831	
	RDA (mg)			18.000		
	AVC *	0.276 *	0.317 *	0.203 *	0.472 *	
	CV (%)			7.12		
	LSD (P≤0.05)			0.576		0.576
Zn	4	0.636	1.192	0.887	0.946	0.915
	6	0.759	1.278	0.995	1.182	1.053
	8	0.612	1.221	0.830	0.806	0.867
	10	0.464	1.076	0.687	0.685	0.728
	Mean species	0.618	1.192	0.850	0.905	
	RDA (mg)			9.000		
	AVC *	0.133 *	0.673 *	0.958 *	0.920 *	
	CV (%)			5.06		
	LSD (P≤0.05)			0.058		0.058
	Interaction			0.083		
Mn	4	0.035	0.085	0.052	0.088	0.065
	6	0.042	0.089	0.051	0.094	0.069
	8	0.056	0.085	0.057	0.099	0.074
	10	0.065	0.085	0.062	0.100	0.078
	Mean species	0.050	0.086	0.056	0.095	
	RDA (mg)			1.800		
	AVC *	0.385 *	2.093 *	3.262 *	1.894 *	
	CV (%)			7.58		
	LSD (P≤0.05)			0.007		0.007
	Interaction			0.009		
Na	4	41.630	187.707	22.713	86.603	84.663
	6	43.193	187.990	23.143	88.313	85.660
	8	42.787	192.603	22.920	88.417	86.682
	10	42.973	195.037	23.353	87.683	87.262
	Mean species	42.646	190.834	23.032	87.754	
	RDA (mg)			2300.000		
	AVC *	5.393 *	1.205 *	9.986 *	2.620 *	
	CV (%)			1.22		
	LSD (P≤0.05)			1.359		1.359
	Interaction			1.932		

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Amaranthus hybridus had the highest (P≤0.05) levels of P, K, Zn, Na and Mn and lowest levels of Mg, *Vigna unguiculata* had the lowest levels of P, K, Ca, Zn and Mn while *Cleome gynandra* had the highest levels of Ca and Fe and *Solanum scabrum* had the highest levels of N and Mg. These results are similar to the findings of Sehmi (1993). However, the P, K, Mg, and Fe levels (four species) were lower than levels reported earlier but Na and Ca (*Solanum scabrum*) levels were similar to the levels reported by Sehmi (1993). The Zn levels in amaranths and african nightshades reported in this study were similar to the levels reported in Tanzania (Kinabo *et al.*, 2004; Weinberger and Msuya, 2004) but Fe levels were lower than the levels reported in different districts of Tanzania (Kinabo *et al.*, 2004; Weinberger and Msuya, 2004) and Swaziland (Ogle and Grivetti, 1985) but much lower than the levels reported in Dar es Salaam (Raja *et al.*, 1997).

The ALVs are significant contributors of Mg, Fe, Zn and Mn. Consumption of 118 g of *Solanum scabrum*, 133 g and 385 g DW of *Vigna unguiculata* is recommended to attain RDAs of Mg, Zn and Mn, respectively. While consumption of 276 g, 317 g, 203 g and 472 g of *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*, respectively is recommended to attain RDAs of Fe. The ALVs are not significant contributors of P, K, Ca, Mn and Na as consumption of more than half a kilogram of the leafy vegetables is recommended to attain RDAs of P, K, Ca, Mn and Na.

There was significant (P≤0.05) interaction between species and harvesting stage for all the nutrients (except N and Fe) suggesting that the response patterns were different in each species. This can be attributed to the differences in genetic characteristics of the various species and in the uptake of the nutrients as mineral nutrition of plants is under genetic control (Marschner, 1995) indicated by the nutritional differences between species. Major nutritional features are under the control of a single gene pair or more complex genetic systems involved in acquisition and utilization of nutrients (Hoan *et al.*, 1992; Gerloff and

Gabelman, 1983; Graham, 1983). There are distinct differences among plant species in ion uptake characteristics. The variation in the uptake of nutrients by different species is due to differences in the plant metabolism and plant constitution. In addition, plants have binding sites in the plasma membrane of root cells which differ in affinity and probably in number for various mineral nutrients (Asher and Edwards, 1982). This explains the variation in nutrient uptake by different species.

4.2: Variation in levels of nutrients in ALVs with location of production

The levels of N, P, K, Ca, Mg, Fe, Zn and Mn significantly ($P \leq 0.05$) varied with location of production except Na that showed non significant variation. The ALVs from Kisumu site had significantly ($P \leq 0.05$) higher levels of N, K, P, Ca, Mg and Zn, ALVs from Lela site had higher levels of Mn and Na while ALVs from Busia site had higher levels of Fe (Tables 19-27).

Table 19: Variation in levels of N (%) in ALVs at different locations and harvesting stages

		Species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	2.241	1.735	1.641	2.019	1.909	
Busia	6	2.570	1.743	1.644	2.125	2.020	
	8	2.546	1.749	1.659	2.227	2.045	
	10	2.290	1.757	1.664	2.365	2.019	
	Mean spp	2.412	1.746	1.652	2.184		1.998
	CV (%)			4.98			
	LSD (P≤0.05)			0.129		0.129	
	Interaction			0.184			
Kisumu	4	2.365	1.775	1.766	2.214	2.030	
	6	2.552	1.872	1.769	2.345	2.135	
	8	2.576	1.878	1.778	2.431	2.166	
	10	2.577	1.837	1.793	2.563	2.193	
	Mean species	2.518	1.841	1.777	2.388		2.131
	CV (%)			2.09			
	LSD (P≤0.05)			0.058		0.058	
	Interaction			0.082			
Lela	4	1.538	1.591	1.554	1.786	1.617	
	6	1.579	1.634	1.565	1.825	1.651	
	8	1.585	1.642	1.571	1.768	1.641	
	10	1.587	1.650	1.574	1.845	1.664	
	Mean species	1.572	1.629	1.566	1.806		1.643
	CV (%)			3.14			
	LSD (P≤0.05)			0.067		NS	
all sites	4	2.048	1.700	1.654	2.006	1.852	
	6	2.234	1.750	1.660	2.098	1.935	
	8	2.236	1.756	1.669	2.142	1.951	
	10	2.156	1.748	1.677	2.258	1.959	
	Mean species	2.167	1.739	1.665	2.126	1.924	1.924
	CV (%)			3.62			
	LSD (P≤0.05)			0.052		0.052	0.061
	Interaction			0.120			

Table 20: Variation in levels of P (mg/100 g DW) in ALVs at different locations and harvesting stages

		Vegetable species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	18.679	48.370	26.726	37.363	32.784	
Busia	6	23.097	52.128	29.583	42.010	36.704	
	8	19.642	48.340	25.293	36.798	32.518	
	10	16.988	42.790	21.215	35.432	29.107	
	Mean spp	19.602	47.907	25.704	37.901		32.778
	CV (%)			4.12			
	LSD (P≤0.05)			1.756		1.756	
	Interaction			NS			
Kisumu	4	35.057	71.794	41.292	60.946	52.356	
	6	39.505	77.158	46.168	64.626	56.864	
	8	33.255	73.958	41.292	58.370	51.719	
	10	32.170	69.404	38.414	52.693	48.170	
	Mean spp	34.997	73.078	41.875	59.159		52.277
	CV (%)			6.92			
	LSD (P≤0.05)			4.696		4.696	
	Interaction			6.677			
Lela	4	12.473	18.876	16.760	17.002	16.278	
	6	13.089	21.483	16.150	19.113	17.459	
	8	10.331	16.821	13.697	16.658	14.377	
	10	8.440	17.195	12.749	18.328	14.178	
	Mean spp	11.083	18.594	14.839	17.775		15.573
	CV (%)			4.61			
	LSD (P≤0.05)			0.933		0.933	
	Interaction			1.327			
all sites	4	22.070	46.343	28.371	38.437	33.806	
	6	25.230	50.256	30.634	41.916	37.009	
	8	21.076	46.373	26.761	37.275	32.871	
	10	19.199	43.130	24.126	35.485	30.485	
	Mean spp	21.894	46.526	27.473	38.278		33.543
	RDA (mg)						1200.00
	AVC*						3.577*
	CV (%)			7.14			
	LSD (P≤0.05)			1.795		1.795	2.102
	Interaction			4.106			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 21: Variation in levels of K (mg/100 g DW) in ALVs at different locations and harvesting stages

		Species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	69.932	80.402	69.337	65.480	71.288	
Busia	6	76.472	83.000	79.916	75.475	78.715	
	8	65.550	80.747	76.190	70.085	73.143	
	10	62.472	71.944	68.808	58.303	65.381	
	Mean spp	68.607	79.023	73.563	67.335		71.236
	CV (%)			9.48			
	LSD (P≤0.05)			8.771		8.771	
	Interaction			NS			
Kisumu	4	95.271	127.603	102.787	100.574	106.559	
	6	102.453	131.017	112.647	109.503	113.905	
	8	92.941	125.010	98.092	98.118	103.540	
	10	91.227	118.077	89.693	92.176	97.793	
	Mean spp	95.493	125.427	100.804	100.093		105.449
	CV (%)			1.36			
	LSD (P≤0.05)			1.868		1.868	
	Interaction			2.655			
Lela	4	43.679	56.681	42.735	49.659	48.189	
	6	47.607	59.085	47.297	53.162	51.788	
	8	41.521	56.348	43.078	49.633	47.645	
	10	35.480	52.679	39.967	43.049	42.794	
	Mean spp	42.072	56.198	43.269	48.876		47.604
	CV (%)			3.13			
	LSD (P≤0.05)			1.933		1.933	
	Interaction			2.749			
all sites	4	69.627	88.229	71.620	71.904	75.345	
	6	75.511	86.253	79.953	79.380	80.274	
	8	66.670	87.368	72.453	72.612	74.776	
	10	63.060	80.900	66.156	64.509	68.656	
	Mean spp	68.717	85.687	72.546	72.101		74.763
	RDA (mg)						2000.00
	AVC*						2.675*
	CV (%)			5.50			
	LSD (P≤0.05)			3.081		3.081	3.608
	Interaction			NS			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 22: Variation in levels of Ca (mg/100 g DW) in ALVs at different locations and harvesting stages

		Species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
Busia	4	178.370	239.350	180.327	185.643	195.923	
	6	190.890	254.447	254.293	192.570	223.050	
	8	155.707	208.777	170.740	175.203	177.607	
	Mean species	168.597	222.485	190.424	174.710	159.637	189.054
	CV (%)			0.83			
	LSD (P≤0.05)			2.032		2.032	
	Interaction			2.886			
Kisumu	4	279.930	401.780	198.053	295.963	252.015	
	6	287.653	435.767	227.993	315.327	408.048	
	8	240.133	404.617	188.367	247.210	196.294	
	10	200.343	390.027	170.763	211.883	267.596	
	Mean species	252.015	408.048	196.294	267.596		280.988
	CV (%)			1.36			
	LSD (P≤0.05)			49.391		49.391	
	Interaction			70.222			
Lela	4	106.937	141.590	148.107	120.100	129.183	
	6	116.290	156.247	173.353	133.480	144.842	
	8	83.264	125.120	138.480	110.390	114.321	
	10	72.890	100.038	122.030	104.967	99.981	
	Mean species	94.845	130.749	145.500	117.234		122.082
	CV (%)			2.31			
	LSD (P≤0.05)			3.668		3.668	
	Interaction			5.215			
all sites	4	188.412	260.907	175.496	200.569	206.346	
	6	198.278	282.153	218.547	213.792	228.193	
	8	159.701	246.171	165.872	177.601	187.336	
	10	140.884	225.811	149.710	154.091	167.624	
	Mean species	171.819	253.760	177.406	186.513		197.375
	RDA (mg)						1000.000
	AVC*						0.506*
	CV (%)			12.14			
	LSD (P≤0.05)			17.968		17.968	21.043
	Interaction			NS			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 23: Variation in levels of Mg (mg/100 g DW) in ALVs at different locations and harvesting stages

		Vegetable species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	28.823	17.807	34.527	366.743	111.975	
Busia	6	31.523	19.990	37.397	390.283	119.798	
	8	34.103	22.380	29.270	424.707	127.615	
	10	32.920	22.690	26.747	434.347	129.176	
	Mean spp	31.842	20.717	31.985	404.020		122.141
	CV (%)			1.44			
	LSD (P≤0.05)			2.287		2.287	
	Interaction			3.251			
Kisumu	4	44.123	25.037	54.190	569.177	173.132	
	6	44.780	25.993	56.103	577.193	176.018	
	8	48.607	24.317	49.323	583.237	176.371	
	10	46.160	23.403	45.700	573.010	172.068	
	Mean spp	45.917	24.687	51.329	575.654		174.397
	CV (%)			1.33			
	LSD (P≤0.05)			3.020		3.020	
	Interaction			4.294			
Lela	4	22.147	18.863	29.543	268.913	84.867	
	6	26.413	17.467	32.277	281.163	89.330	
	8	25.510	19.707	26.093	327.047	99.589	
	10	22.487	18.623	22.470	309.103	93.171	
	Mean spp	24.139	18.665	27.596	296.557		91.739
	CV (%)			4.29			
	LSD (P≤0.05)			5.114		5.114	
	Interaction			7.272			
all sites	4	31.698	20.569	39.420	401.611	123.324	
	6	34.239	21.150	41.926	416.213	128.382	
	8	36.073	22.134	34.896	444.997	134.525	
	10	33.856	21.572	31.639	438.820	131.472	
	Mean spp		33.966	21.356	36.970	425.410	129.426
	RDA (mg)						350.000
	AVC*						0.270*
	CV (%)			2.16			
	LSD (P≤0.05)			2.099		2.099	2.458
	Interaction			4.802			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 24: Variation in levels of Fe (mg/100 g DW) in ALVs at different locations and harvesting stages

		Vegetable species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	9.413	9.079	15.304	9.100	10.724	
Busia	6	10.022	10.562	15.894	9.248	11.432	
	8	10.206	10.929	16.556	9.346	11.759	
	10	10.454	11.406	15.499	9.436	11.699	
	Mean species	10.024	10.494	15.813	9.282		11.403
	CV (%)			5.40			
	LSD (P≤0.05)			0.799		0.799	
	Interaction			NS			
Kisumu	4	7.564	6.835	13.929	5.594	8.481	
	6	8.052	7.674	15.764	6.298	9.447	
	8	8.087	7.848	15.000	6.514	9.362	
	10	8.102	10.518	16.040	6.691	10.338	
	Mean species	7.951	8.218	15.183	6.274		9.407
	CV (%)			8.47			
	LSD (P≤0.05)			1.035		NS	
	Interaction			1.472			
Lela	4	6.046	5.445	7.688	3.392	5.643	
	6	6.517	5.555	9.491	3.741	6.326	
	8	6.622	5.641	9.367	3.931	6.390	
	10	6.880	6.082	9.053	4.260	6.569	
	Mean species	6.516	5.681	8.900	3.831		6.232
	CV (%)			7.12			
	LSD (P≤0.05)			0.576		0.576	
	Interaction			NS			
all sites	4	7.675	7.119	12.307	6.029	8.282	
	6	8.197	7.930	13.716	6.429	9.068	
	8	8.305	8.139	13.641	6.597	9.170	
	10	8.479	9.335	13.531	6.796	9.535	
	Mean species	8.164	8.131	13.299	6.463		9.014
	RDA (mg)						18.000
	AVC*						0.199*
	CV (%)			7.09			
	LSD (P≤0.05)			0.480		0.480	0.562
	Interaction			NS			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 25: Variation in levels of Zn (mg/100 g DW) in ALVs at different locations and harvesting stages

		Species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	1.554	2.065	1.531	1.795	1.736	
Busia	6	1.715	2.266	1.800	1.963	1.936	
	8	1.615	2.045	1.382	1.675	1.679	
	10	1.368	1.922	1.032	1.624	1.486	
	Mean species	1.563	2.075	1.436	1.764		1.710
	CV (%)			3.78			
	LSD (P≤0.05)			0.084		0.084	
	Interaction			0.119			
Kisumu	4	1.098	2.973	2.371	1.609	2.013	
	6	1.177	3.871	2.676	1.883	2.402	
	8	1.058	3.150	2.316	1.028	1.888	
	10	0.910	2.887	1.926	0.882	1.651	
	Mean species	1.061	3.220	2.322	1.350		1.988
	CV (%)			2.22			
	LSD (P≤0.05)			0.057		0.057	
	Interaction			0.081			
Lela	4	0.636	1.192	0.887	0.946	0.915	
	6	0.759	1.278	0.995	1.182	1.053	
	8	0.612	1.221	0.830	0.806	0.867	
	10	0.464	1.076	0.687	0.685	0.728	
	Mean species	0.618	1.192	0.850	0.905		0.891
	CV (%)			5.06			
	LSD (P≤0.05)			0.058		0.058	
	Interaction			0.083			
all sites	4	1.096	2.077	1.596	1.450	1.555	
	6	1.217	2.472	1.824	1.676	1.797	
	8	1.095	2.139	1.509	1.169	1.478	
	10	0.914	1.962	1.215	1.064	1.289	
	Mean species	1.080	2.162	1.536	1.340		1.530
	RDA (mg)						9.000
	AVC*						0.588*
	CV (%)			3.47			
	LSD (P≤0.05)			0.037		0.037	0.047
	Interaction			0.091			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 26: Variation in levels of Mn (mg/100 g DW) in ALVs at different locations and harvesting stages

		Vegetable species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	0.018	0.055	0.026	0.028	0.032	
Busia	6	0.029	0.044	0.021	0.026	0.030	
	8	0.038	0.045	0.031	0.016	0.032	
	10	0.041	0.039	0.024	0.025	0.032	
	Mean species	0.031	0.046	0.025	0.024		0.032
	CV (%)			22.91			
	LSD (P≤0.05)			0.009		NS	
	Interaction			0.013			
Kisumu	4	0.021	0.065	0.046	0.045	0.044	
	6	0.036	0.084	0.045	0.036	0.050	
	8	0.047	0.084	0.034	0.033	0.050	
	10	0.051	0.089	0.019	0.032	0.048	
	Mean species	0.039	0.081	0.036	0.036		0.048
	CV (%)			28.18			
	LSD (P≤0.05)			0.018		0.018	
	Interaction			0.025			
Lela	4	0.035	0.085	0.052	0.088	0.065	
	6	0.042	0.089	0.051	0.094	0.069	
	8	0.056	0.085	0.057	0.099	0.074	
	10	0.065	0.085	0.062	0.100	0.078	
	Mean species	0.050	0.086	0.056	0.095		0.072
	CV (%)			7.58			
	LSD (P≤0.05)			0.007		0.007	
	Interaction			0.009			
all sites	4	0.025	0.068	0.041	0.054	0.047	
	6	0.036	0.072	0.039	0.052	0.050	
	8	0.047	0.071	0.041	0.049	0.052	
	10	0.052	0.071	0.035	0.053	0.053	
	Mean species	0.040	0.071	0.039	0.052		0.051
	RDA (mg)						1.800
	AVC*						3.529*
	CV (%)			18.43			
	LSD (P≤0.05)			0.007		0.007	0.008
	Interaction			0.016			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Table 27: Variation in levels of Na (mg/100 g DW) in ALVs at different locations and harvesting stages

		Species					
Loc	H. stage (wks)	*V. unguiculata*	*A. hybridus*	*C. gynandra*	*S. scabrum*	M. stage	M. loc
	4	25.621	195.150	13.483	64.047	74.621	
Busia	6	25.593	152.557	13.593	63.673	63.854	
	8	24.697	151.447	13.463	64.757	63.591	
	10	25.783	156.537	13.290	64.470	65.020	
	Mean species	25.470	163.923	13.457	64.237		66.772
	CV (%)			26.93			
	LSD (P≤0.05)			16.606		NS	
	Interaction			NS			
Kisumu	4	22.137	171.840	16.307	64.910	68.798	
	6	22.327	242.310	16.267	66.483	86.847	
	8	22.247	180.210	16.620	65.360	71.109	
	10	22.677	186.993	17.027	67.443	73.535	
	Mean species	22.347	195.338	16.049	66.049		75.072
	CV (%)			19.41			
	LSD (P≤0.05)			18.925		NS	
	Interaction			26.907			
Lela	4	41.630	187.707	22.713	86.603	84.663	
	6	43.193	187.990	23.143	88.313	85.660	
	8	42.787	192.603	22.920	88.417	86.682	
	10	42.973	195.037	23.353	87.683	87.262	
	Mean species	42.646	190.834	23.032	87.754		86.067
	CV (%)			1.22			
	LSD (P≤0.05)			1.359		1.359	
	Interaction			1.932			
all sites	4	29.857	184.899	17.501	71.853	76.028	
	6	30.371	194.286	17.668	72.823	78.787	
	8	29.910	174.753	17.668	72.844	73.794	
	10	30.478	179.522	17.890	73.199	75.272	
	Mean species	30.154	183.365	17.682	72.680		75.970
	RDA (mg)						2300.00
	AVC*						1.747*
	CV (%)			17.46			
	LSD (P≤0.05)			9.946		9.946	11.647
	Interaction			22.748			

AVC *, average amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA

Variation in levels of nutrients with location of production had also been observed earlier in Tanzania (Weinberger and Msuya, 2004) in amaranths, african nightshades and

cowpea. The Fe and Zn levels in amaranths, african nightshades and cowpea significantly ($P \leq 0.05$) varied in Kongwa, Singida and Muheza (Weinberger and Msuya, 2004) and in Iringa and Morogoro districts in Tanzania (Kinabo *et al.*, 2003). The significant variation in the levels of nutrients in ALVs in this study is therefore not surprising. The variations in this study may be attributed to variations in initial soil pH and soil nutrient levels. Soil analysis was done to ascertain soil nutrient status especially N, P and K, primary nutrients required in relatively large amounts. This was done to establish if the levels of nutrients in the soil could support plant growth as most small holder farmers grow ALVs without fertilizer application. On the other hand, excess nutrients in the soil-crop system increases the risk of nutrient losses to the environment and the risk of impaired product quality (Santamaria, 2006; Belec *et al.*, 2001), whereas deficiencies reduce crop production. Initial soil nutrient levels and pH significantly ($P \leq 0.05$) varied in the three geographical locations (Table 28).

Table 28: Initial soil pH and total nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Na (DW) by locations

Nutrient/Location	Busia	Kisumu	Lela	CV%	LSD ($P \leq 0.05$)
pH	6.467	6.800	5.200	1.080	0.234
N (%)	0.453	0.600	0.277	16.900	0.263
P (mg/100 g)	124.299	202.039	63.534	10.780	49.199
K (mg/100 g)	272.385	379.088	203.088	5.530	55.395
Ca (mg/100 g)	848.974	1071.407	568.219	4.610	134.221
Mg (mg/100 g)	467.841	728.285	354.631	3.060	55.600
Fe (mg/100 g)	1210.713	1033.021	639.859	5.300	178.832
Zn (mg/100 g)	7.565	9.941	4.965	6.370	1.677
Mn (mg/100 g)	0.234	0.167	0.453	20.480	0.205
Na (mg/100 g)	252.449	387.668	512.948	5.750	77.700

Soils from Busia and Kisumu sites were nearly at neutral pH while those from Lela were slightly acidic. Low soil pH affects different physiological and biochemical processes, both in the soil and plant (Foy, 1984). For instance, low soil pH enhances the uptake of Mn, (Mullin and Reeves, 1996; Tisdale *et al.*, 1985) and Zn (Van Lierop, 1990) while high soil pH enhances the uptake of K, Na, Ca, and Mg (Tan *et al.*, 1991; Rengel, 1990) by plants. The

results of the current study were in agreement with the previous studies that the uptake of K, Ca, Mg and Zn by ALVs were enhanced with high soil pH in Kisumu and Busia sites but contrary for P and Na. However, the decrease in the uptake of Mn with enhanced pH in the current study was in agreement with previous studies (Mullin and Reeves, 1996; Tisdale *et al.*, 1985).

The soil nutrient levels significantly ($P \leq 0.05$) varied in the three sites except N that showed non significant variation. These findings were in agreement with observations made earlier in different locations of western Kenya (Jaetzold *et al.*, 2005). Soils from Kisumu site had significantly ($P \leq 0.05$) higher levels of N, P, K, Ca, Mg, and Zn; soils from Lela site had lower levels but with higher levels of Mn and Na while soils from Busia site had higher levels of Fe. The total N, K, Ca, and Mg levels in soils from the three locations were very high compared to (Tekalign *et al.*, 1991) ratings. However, ALVs from Lela site had significantly ($P \leq 0.05$) lower levels of N, P, K, Ca and Mg, in fact all below the RDA. The low levels could partly be attributed to the slightly acidic nature of Lela soils. In addition, the soil pH was below optimum range (5.5-7.5) for growing most ALVs (Grubben, 2004).

Low soil pH in soils from Lela site may have resulted in low P bioavailability due to high P fixation capacity; toxicities of Al, Fe, Mn (occasionally H); deficiencies of K, Ca, Mg, Zn, S and Mo; and low cation exchange capacity (CEC) (Clark *et al.*, 1988). These deficiencies or toxicities often act together to limit plant growth (Clark, 1982). This may have limited the uptake of P, K, Ca, Mg and Zn and enhanced the uptake of Mn by ALVs grown in Lela site. Similarly, the high levels of Na and Mn in soils from Lela site could have inhibited the uptake of other nutrients due to the antagonism between Na and K which may have resulted in direct osmotic gradient (Epstein, 1966). This may have resulted in high influx of Na into the root cells. Also, Na may have enhanced the efflux of K into the growth medium because of disturbed membrane integrity (Cramer *et al.*, 1985).

High Na levels in the external medium greatly reduces the activity of Ca in the solution and may result in a decrease in the amount of Ca available for uptake by the plants (Alam, 1994; Grattan and Grieve, 1992). Calcium is strongly competitive with Mg. The binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg than for Ca (Marschner, 1995) which explains the higher intake of Ca than Mg.

Temperature usually contributes economically towards the crop growth, development and productivity of a crop. The change from vegetative stage of a crop to reproductive phase may be influenced by temperature changes. The mean temperature of 25.7, 23.9, and 21.9 °C were reported in Busia, Kisumu, and Lela, respectively during the study period (Table 29).

Table 29: Maximum, minimum and mean temperatures (°C) during the study period (April-September, 2009)

		Months						
Temp.	**Location**	**April**	**May**	**June**	**July**	**August**	**Sept**	**Mean.loc**
Max. temp	Busia	38.5	38.3	38.3	38.0	30.0	38.0	36.9
Min. temp		14.0	14.0	11.7	11.0	13.0	12.0	12.6
Mean. temp		**26.3**	**26.0**	**25.0**	**25.0**	**26.0**	**26.0**	**25.7**
Max. temp	Kisumu	29.7	30.1	30.4	30.0	30.8	31.9	30.5
Min. temp		18.2	17.9	16.4	16.1	17.4	17.8	17.3
Mean. temp		**24.0**	**24.0**	**23.4**	**23.0**	**24.3**	**24.9**	**23.9**
Max. temp	Lela	29.1	30.5	29.8	28.7	29.9	30.0	29.7
Min. temp		13.7	14.0	13.6	13.5	13.8	14.5	13.9
Mean. temp		**21.4**	**22.3**	**21.7**	**21.1**	**21.9**	**22.3**	**21.9**

Source: KARI-Alupe, KARI-Kisumu and ATC-Maseno

The air temperature was within the optimal range (20-30 °C) for the growth of most vegetables (Modi, 2007; Dever *et al.*, 1997) in the three locations. The variation in the levels of nutrients in ALVs cannot be attributed to variation in air temperature. The rainfall data during the study period is presented in (Table 30). Busia had the highest amount of rainfall while Lela had the lowest.

Table 30: **Total and mean rainfall (mm) received during the study period April-September, 2009)**

		Months						
	Location	**April**	**May**	**June**	**July**	**August**	**Sept**	**Mean. loc**
Total	Busia	326.7	268.2	48.9	95.4	86.7	214.1	
Mean		**11.0**	**17.9**	**5.4**	**23.9**	**6.2**	**15.3**	**13.3**
Total	Kisumu	270.4	124.2	53.1	101.7	89.3	229.6	
Mean		**12.3**	**7.8**	**5.9**	**12.7**	**7.4**	**14.4**	**10.1**
Total	Lela	198.7	152.5	137.8	41.4	97.5	175.5	
Mean		**18.1**	**19.0**	**19.6**	**8.3**	**12.1**	**14.6**	**15.3**

Source: KARI, Alupe; KARI, Kibos and ATC, Maseno

During the study period, over half the annual precipitations of 1040.0, 868.3, and 803.4 mm were received in Busia, Kisumu, and Lela, respectively. April-May-June, 2009 showed deficient rainfall of 643.8, 447.7, and 489.0 mm and 396.2, 420.6, and 314.4 mm in July-August-September in Busia, Kisumu and Lela, respectively. The rainfall in the three locations was within the optimal range required for vegetable growing (Grubben, 2004).

There was significant ($P \leq 0.05$) interaction between species and location of production for all the nutrients suggesting the response patterns were different at each location. The ALVs from the three locations were significant contributors of Mg and Fe; ALVs from Kisumu site were significant contributors of Ca and Zn while ALVs from the three locations were not significant contributors of P, K, Mn and Na. When vegetables are bought from the market, their source is unknown therefore, on average, consumption of 3.577, 3.608, 0.506, 0.270, 0.199, 0.588, 3.529 and 1.747 kg DW of the four species is recommended to supply RDAs of P, K, Ca, Mg, Fe, Zn, Mn and Na, respectively.

4.3: Variation in levels of nutrients in ALVs at different harvesting stages

The levels of N, P, K, Ca, and Zn in ALVs significantly ($P \leq 0.05$) increased from 4 to 6 WAE then decreased from 6 to 10 WAE, Fe levels increased (from 4 to 6 WAE) while Mg levels increased (from 4 to 8 WAE) then decreased from 8 to 10 WAE. The Na and Mn levels did not vary significantly with harvesting stage (Tables 20-28). Higher levels of N, P, Ca, Fe and

Zn in *Amaranthus hybridus;* N, P, K, Zn and Mn in *Vigna unguiculata;* P, K and Zn in *Solanum scabrum and* P, Ca, Mg, Fe and Zn in *Cleome gynandra* were attained at 6 WAE. Generally, higher levels of N, P, K, Ca, Mg, Fe and Zn were reported at 6 WAE. Variations in the levels of nutrients in ALVs at different harvesting stages had been reported in Nairobi, Kenya (Masinde and Agong, 2011), Zimbabwe (Makobo *et al.*, 2010) and South Africa (Modi, 2007). Highest levels of Ca and Zn in *Amaranthus cruentus* were attained at 3 WAE, K at 6 WAE and P at 4 WAE in Zimbabwe (Makobo *et al.*, 2010) and South Africa (Modi, 2007).

However, highest levels of Na were reported at 6 WAE in Zimbabwe (Makobo *et al.*, 2010) and 7 WAE in South Africa (Modi, 2007). The levels of N significantly ($P \leq 0.05$) increased from 4 to 6 WAE contrary to the results reported in spider plant in Nairobi, Kenya (Masinde and Agong, 2011). Differences at the stage at which highest levels of nutrients were attained in the current study and the previous studies could be attributed to variations in the initial soil nutrient levels, agronomic practices, and environmental conditions of the study sites.

The uptake rate of many nutrients depend on the nutrients demand for growth (Clement *et al.*, 1978a, 1978b) determined by the role the nutrients play in plant growth. Most of the functions of Ca and P are as structural components of macromolecules, K is required for cell expansion, protein synthesis and in stomatal regulation, while Mg is the central atom in the chlorophyll molecule. The uptake of N, K, P, Ca, and Mg was high during the vegetative stage when roots were actively growing than in reproductive stages (Beringer *et al.*, 1986). This explains the significant increase in the levels of N, P, K, Ca, Mg, and Zn from 4 to 6 WAE. During the reproductive stage, nutrients are partitioned towards the reproductive organs (Schippers, 2000). For example, developing fruits are stronger sinks for photoassimilates (Marschner, 1995) and the relative increase in the proportion of structural

material (cell walls and lignin) and of storage compounds (e.g. starch) in the dry matter. In addition, retranslocation of nutrients P, K and N in the form of amino acids from shoots to roots in the absence of soil nutrient replenishment and a decrease in demand for nutrients for new growth as plants age accounts for the decrease in the levels of nutrients in ALVs with increasing plant age. The results of this study are in agreement with findings of Marschner (1986). The non significant increase in levels of Mn and Na in ALVs with increasing plant age may partly be attributed to the role the nutrients play in plant growth. Manganese plays an important role in redox processes and is required in very small amounts while Na is a beneficial element required for osmotic adjustment (Eshel, 1985). Its uptake is concentration dependent with no specific binding sites in the plasma membrane (Binzel *et al.*, 1988).

There was significant ($P \leq 0.05$) interaction between species and harvesting stage for all the nutrients suggesting the response patterns were different in each species and harvesting stage. Different nutrients reached their highest levels (except P and Zn) at different harvesting stages in all species. If the ALVs are grown to meet the nutritional requirement for a particular nutrient, harvesting should be done at the stage they reach their highest levels for that particular nutrient. If they are grown to meet the nutritional requirement for different nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Na) the average harvesting stage from 4 to 7 WAE for *Vigna unguiculata* and from 4 to 5 WAE for *Amaranthus hybridus, Cleome gynandra* and *Solanum scabrum* should be considered as the most appropriate. However, harvesting the four species from 4 to 6 WAE is recommended for the highest supply of P and Zn.

CHAPTER FIVE

SUMMARY, CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

5.1: Summary

1. The levels of nutrients in ALVs significantly ($p \leq 0.05$) varied with species. *Amaranthus hybridus* had significantly higher ($p \leq 0.05$) levels of P, K, Zn, Mn and Na, *Vigna unguiculata* had higher levels of N *and Cleome gynandra* had higher levels of Fe while *Solanum scabrum* had higher levels of Mg in ALVs from the three sites. There was significant ($P \leq 0.05$) interaction between species and harvesting stage for all the nutrients (except N and Fe).
2. The levels of N, P, K, Ca, Mg, Fe, Zn and Mn significantly ($P \leq 0.05$) varied with location of production. The ALVs from Kisumu site had higher levels of N, K, P, Ca, Mg and Zn; ALVs from Lela site had higher levels of Mn and Na while ALVs from Busia site had higher levels of Fe. There was significant ($P \leq 0.05$) interaction between species and location of production for all the nutrients.
3. The levels of N, P, K, Ca, and Zn in ALVs significantly ($P \leq 0.05$) increased from 4 to 6 WAE then decreased from 6 to 10 WAE, Fe levels increased from 4 to 6 WAE while the increase from 6 to 10 WAE was not significant. Magnesium levels increased from 4 to 8 WAE then decreased from 8 to 10 WAE. The Na and Mn levels did not vary significantly with harvesting stage.

5.2: Conclusion

1. The ALVs were not significant contributors of P, K, Ca, Zn, Mn and Na but contribute significantly to the supply of Mg (*Solanum scabrum*) and Fe (four species).
2. The ability of ALVs as contributors of nutrients varies with location of production. The ALVs from Busia site accumulated the higher levels of Fe; those from Kisumu

site accumulated the higher levels of P, K, Ca, Mg, and Zn while ALVs from Lela site accumulated higher levels of Mn and Na. The Mg and Fe levels in all the species and locations of production, Ca and Zn levels in ALVs from Kisumu site had values above the RDA.

3. Different nutrients reached their highest levels at different harvesting stages (except P and Zn). Highest levels of N, P, Ca, Fe and Zn in *Amaranthus hybridus;* N, P, K, Zn and Mn in *Vigna unguiculata;* P, K and Zn in *Solanum scabrum and* P, Ca, Mg, Fe and Zn in *Cleome gynandra* were attained at 6 WAE. If the vegetables are grown to meet the nutritional requirement for a particular nutrient, harvesting should be done at the stage they reach their highest levels for that particular nutrient. If they are grown to meet the nutritional requirement for different minerals (N, P, K, Ca, Mg, Fe, Zn, Mn and Na) the average harvesting stage should be considered as the most appropriate.

5.3: Recommendations

1. Consumption of 61-118 g DW of each of the four ALVs, 144-472 g of *Solanum scabrum* is recommended to supply RDA of Fe and Mg, respectively, while consumption of more than 1 kg of the ALVs is recommended to attain RDAs for K, P, Mn and Na in healthy adults.
2. On average, consumption of 3.577, 3.608, 0.506, 0.270, 0.199, 0.588, 3.529 and 1.747 kg DW of the four species from the three locations is recommended to supply RDAs of P, K, Ca, Mg, Fe, Zn, Mn and Na, respectively.
3. Harvesting the four species from 4 to 6 WAE for optimum supply of P and Zn, *Amaranthus hybridus* from 4 to 6 WAE for the supply of Ca and Fe, *Vigna unguiculata* from 4 to 6 WAE for the supply of K and Mn, *Solanum scabrum* from 4 to 6 WAE for the supply of K and *Cleome gynandra* from 4 to 6 WAE for the supply

of Ca, Mg and Fe is recommended. However, for the optimum supply of all the nutrients (P, K, Ca, Mg, Fe, Zn, Mn and Na), an average harvesting stage from 4 to 7 WAE for *Vigna unguiculata* and from 4 to 5 WAE for *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* is recommended.

5.4: Suggestion for further studies

1. A study to be done to establish the bioavailability of K, P, Ca, Mg, Fe, Zn, Mn and Na in ALVs.

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