NUTRITIVE VALUE AND LEVELS OF HEAVY METALS IN MARKETED VEGETABLE AMARANTH GROWN WITH EFFLUENT WATER: A CASE OF RUAI AND NJIRU IN NAIROBI

By

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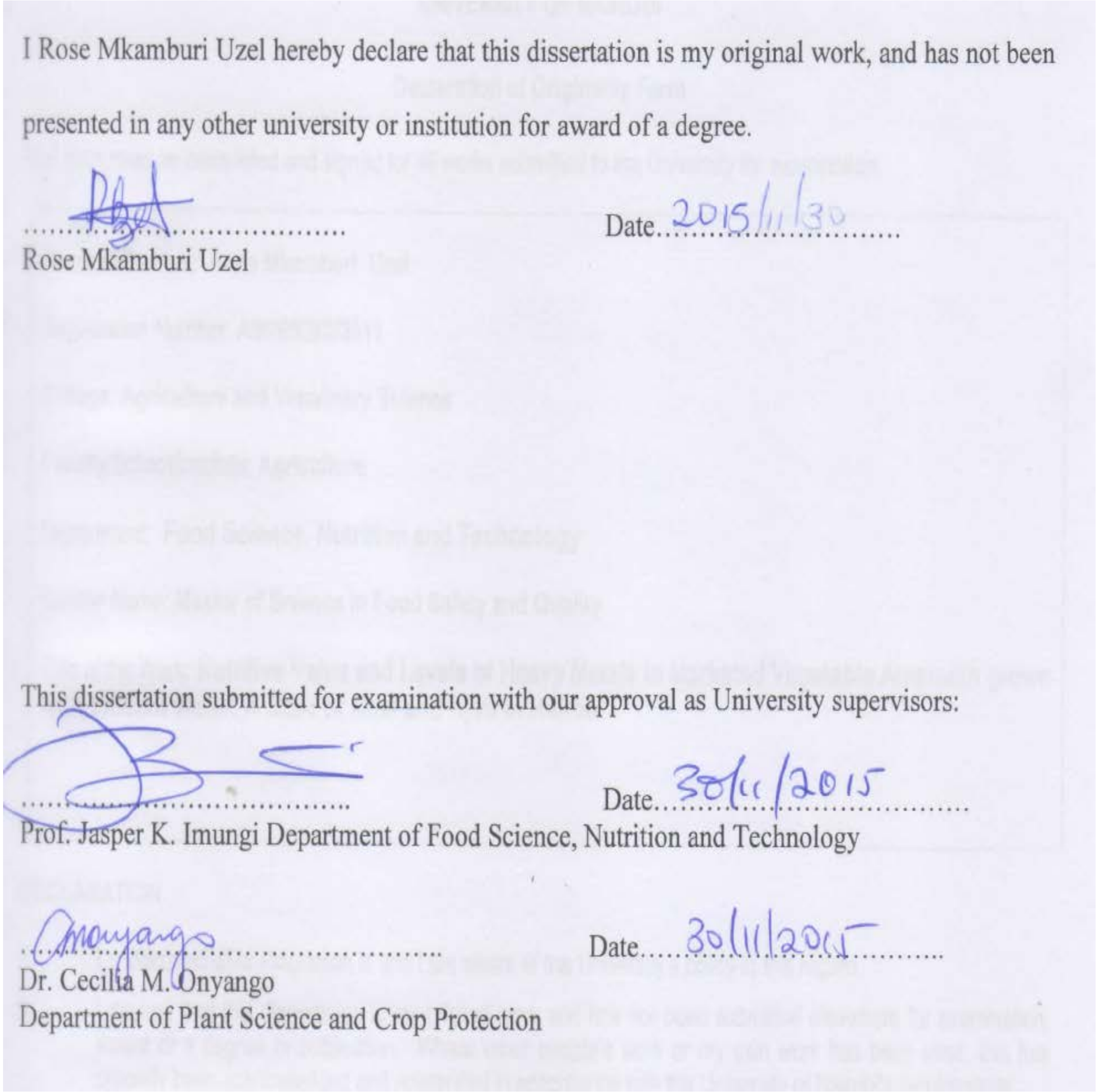
BSc (Chemistry and Biochemistry)

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SAFETY AND QUALITY OF THE UNIVERSITY OF NAIROBI

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY

2015

DECLARATION



I Rose Mkamburi Uzel hereby declare that this dissertation is my original work, and has not been presented in any other university or institution for award of a degree.

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DEDICATION

This dissertation is dedicated to my family especially baby Grace who has been very patient, cooperative and supportive; my husband Lucas for his love and financial support and my children Philicia, Florence and Zakaria for their understanding and support throughout the entire period.

ACKNOWLEDGEMENTS

To almighty God be all the glory, praise and honour for enabling me undertake and accomplish my studies. I am very grateful to several people for the effort and their input in the research leading to this dissertation. In particular, my deepest appreciation goes to my supervisors: Prof. J. K. Imungi and Dr. Cecilia M. Onyango for their unwavering support, willingness to help during the planning and execution of the project. Without their commitment, constant encouragement, honest criticism and generous input this work, would not have been complete. Many thanks to my employer, Kenya Bureau of Standards for granting me study leave and allowing me to use their accredited laboratories and facilities to conduct various tests and for the financial support during the entire period. I am greatly indebted to Tom Odour, KEBS Biochemical laboratory manager together with Nehemiah Achesa, Isaac Mugenya, Dr. Geoffrey Muriira, Caleb Luvonga, Jacqueline Kangiri and Charles Njenga for their technical support while carrying out analyses in the Kenya Bureau of Standards laboratories. I would also like to appreciate the support I received for the Njiru and Ruai farmers during collection of the samples especially from Sarah and Boniface and Jacqueline Ogolla for her technical guidance and support during data analysis. Finally I would like to thank my family and friends for their encouragement and unfailing support throughout my studies.

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LIST OF ABBREVIATIONS

AA:	Ascorbic acid
ADI:	Acceptable Daily Intake
ARs:	Average Requirements
DAP:	Di-ammonium Phosphate
DF:	Dilution factor
DNA	Deoxy-Ribonucleic Acid
DW	Dry weight
IPGRI	International Plant Genetic Resource Institute
EC	European Council
EFSA	European Food Safety Agency
EPA	Environmental protection Agency
FAO:	Food Agricultural Organization
IARC	International Agriculture Research Centre
IOSHI	International Occupational Safety and Health Information Centre
IQ	Intelligent Quotient
JECFA	Joint Expert Committee on Food Additives
LoD:	Limit of Detection
LoQ:	Limit of Quantification

ROS	Reactive oxygen Series
RUAF	Resource Centre on Urban Agriculture and Food Security
PMTDI	Provisional Monthly tolerable Intake
SBP	Systolic Blood Pressure
PRIs:	Population Reference Intakes
TLVs:	Traditional leafy vegetables
UL:	Upper Intake Level
ppm:	Parts per million
ppb:	Parts per billion
UPA	Urban and Peri-urban Agriculture
USFNB	United states Food and Nutrition Board
Vol-	Volume of the sample solution used
WHO	World Health Organization

ABSTRACT

The urban areas release enormous volumes of wastewater into the sewerage system. This water serves for irrigation in urban agriculture. However, the water contain large amounts of nutrients, but also toxic constituents including heavy metals, such as lead and cadmium that could get into the plant tissues meant for food .This study was designed to assess the nutritional value and the levels of the heavy metals lead and cadmium in vegetable amaranth grown with effluent water. The study was in two phases. The first phase was a baseline survey to assess the production, marketing and consumption practices, organized with 91 as farmers and market intermediaries using pretested, structured questionnaires. The second phase consisted of laboratory analyses of proximate composition, vitamin A and C, and the minerals; zinc, calcium and iron, the anti-nutrients; nitrates and oxalates and the levels of lead and cadmium in both raw and cooked vegetables.

The study established that 66% of the respondents ranged between 40 to 50 years, and 83% of them were females in full-time vegetable farming and 98 % of them trading. On average 70% of the respondents earned below KES 200, 000 per annum from sale of vegetable amaranth. Up to 77% of the respondents were semi-illiterate. Then 87% of the farmers practiced subsistence farming in less than 3 hectares of land and sold vegetable amaranths to 41% of intermediaries. The study established that 53% of the intermediaries made a profit of between KES.300-400 per 50kg bag of amaranth. Up to 70% of the respondents indicated cooking the vegetable by direct stewing. About 20% of farmers agreed that the waste water come into contact with the leaves, 45% admitted using raw animal manure/municipal/effluent biosolids.

The Moisture content of vegetables ranged from 81.03% to 84.69% crude protein from 22.33% to 36.46%, Total ash ranged from 21.23% to 9.75%, crude fibre ranged from 29.03% to 11.57%. The crude fat and soluble carbohydrate ranged between 0.36% to 1.23% and 6.55% to 36.86% respectively.

The β-carotene and ascorbic acid contents ranged between10.8 mg/100g to 53.8 mg/100g and from not detectable to 98.5 mg/100g, respectively. There was no significant difference in the levels of the β-carotene content of vegetables. B-carotene increased with increase in cooking time and highest values were obtained when the cooking water was discarded. Increase in cooking time resulted in increased loss of ascorbic acid. The oxalate and nitrates ranged between 1649.3 mg/100g to 2452.4 mg/100g and 1856.6 mg/100g to 3080 mg/100g, respectively. Boiling the vegetable and discarding the cooking water caused significant reduction in the oxalate and nitrate contents. Cadmium was not detected in the vegetables and in irrigating water, but in soil the values ranged from 62.3 ppb to 97.85 ppb. Lead in vegetables was between 2.8 ppm to 5.1ppm, in irrigation water 2.0 ppm to 4.5 ppm and in soil 3.0 ppm to 34.1 ppm. Cooking did not affect the levels of lead in vegetables from Njiru significantly, but had a significant effect on the levels in vegetables from Ruai. Minerals concentration was as follows: iron 87.5 mg/kg to 443mg/kg and zinc 23.4 ppm to 54.6 ppm. Cooking in large amount of water which was discarded resulted in higher increase in mineral contents than direct stewing. The study concluded that the vegetable amaranth grown in the two places with effluent water contains appreciable levels of nutrients and moderate levels of anti-nutrients. The levels of cadmium were undetectable. However, the levels of lead in the fresh vegetables were above the maximum limits allowed in Kenya, but considering the amounts of vegetables consumed, the amounts of the element ingested would not pose serious public health concerns.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND INFORMATION

Amaranth also called African spinach or pigweed is plant that is abundant globally and grows 90 to 150 centimeters tall. It is a diverse weed with about 60 Amaranthus species, most of which are cultivated as leafy vegetables, grains or ornamental plants in many countries in the world. The main species grown as vegetables are *A. tricolor, A. dubius, A. lividus, A. cruentus, A. palmeri* and *A. hybridus* while *A. hypochondriacus, A. cruentus* and *A. caudatus* are mainly grown for grain (Teutonico and Knorr, 1985) although *Amaranthus caudatus and A. hypochondriacus* are also grown as ornamental plants. They produce large amount of biomass in a short period of time thus the young plants can be uprooted or the leaves can be picked continuously from growing plants 3-5 weeks after sowing. They are important traditional food plants in Kenya. Amaranth is often eaten as side dish for Ugali (a paste made from maize meal) (Imungi, 1984).

The leaves are highly nutritious with high levels of protein, iron and vitamin A, vitamin C and folate. The levels of other vitamins including thiamine, niacin, and riboflavin, plus some dietary minerals including calcium, iron, potassium, zinc, copper, and manganese cannot be ignored. The grains are also rich in vitamins including thiamine, niacin, riboflavin, and folate, and dietary minerals including calcium, iron, magnesium, phosphorus, zinc, copper, and manganese-comparable to common grains such as wheat germ and oats. Amaranth therefore, has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care and as popular vegetable in the diets of Kenyans to contribute significantly to provision of micronutrients (Onyango *et al.,* 2008). Although amaranth is a valuable source of nutrients its consumption may pose some health risks due to accumulation of anti-nutritional factors such as nitrates and oxalates, and possible contamination with toxic materials such as lead due to poor cultivation practices and/or environment (Onyango *et al*., 2008). Besides, the use of high levels of synthetic fertilizers to achieve high yields may lead to accumulation of nitrates and oxalates as reported by (Onyango *et al*., 2008).

The feasibility of commercial production of amaranth (*Amaranthus hypochondriacus*) leaf vegetable by small-scale farmers in Kenya was assessed by Onyango *et al*., (2008) and was found to be a viable business thus, many entrepreneurs especially women grow and trade in the vegetables (Onyango and Imungi, 2007) hence amaranth is found selling regularly in local markets and in the major cities in Kenya.

The cities are however supplied by urban and peri-urban production. Urban and peri-urban agriculture is an important source of food and income generation for many low income households. It has been argued that the main reason why people are engaging in it is in response to inadequate, unreliable and irregular access to food supplies. However, in these cities and towns, the most common growing sites are the road sides and river valleys where it’s grown in small gardens with irrigation from raw/untreated sewage. Besides in these places, the vegetables are exposed to exhaust fumes from automobiles and to effluent from formal and cottage industries which often dump their effluent waste into the rivers. The major contaminants of food supply and environment are the heavy metals (Abdollatif *et al*., 2009).

The fumes from automobiles contain lead (Pb), cadmium (Cd), zinc (Zn), and nickel (Ni) which are normally added to fuels as anti-knock agents and these results in contamination of air, soils and crops which are close to motorways (Ikeda *et al.*, 2000). These heavy metals have been detected in vegetables grown under effluent conditions and with increased/continuous consumption of such vegetables there is likelihood of accumulation in the body, which results in chronic toxicity in the long run (Ferner, 2001; Ma *et al.*, 2006).

The growth of leafy vegetable in sewerage water in Kenya is widespread and municipal authorities, government ministries, along with the local population and media have raised concern regarding the potential health threats posed by use of polluted waters to crop irrigators and consumers alike and as such the growers are harassed and not recognized as evidenced in dangers of sewer vegetable video uploaded by Kenya citizen TV on 3rd October 2011. (www.youtube.com*/watch?v=_QJDORKdvqI).*Although metals are essential in nutrition; most of them are toxic or poisonous even when ingested in very small quantities. Heavy metals such as copper, selenium, zinc are essential nutrients in very small quantities .For example according to US FNB (2001) the PMTDI is 0.3-1mg/kg bw/day and are therefore categorized as trace elements. However, at higher concentrations they can be toxic to human body. The biological half-lives of these heavy metals are long, they are extremely persistent in the environment; non-biodegradable and non-thermo-degradable and can easily accumulate in the body (Bohn *et al.*, 1985).

According to Sajjad *et al*. (2009) and Oliver, (1997), Lead and Cadmium are the most toxic and the most abundant heavy metals in food. Their accumulation in human body is likely to results in serious systemic problems like cardiovascular, kidney, nervous and bone diseases. Therefore, heavy metals accumulations in food have implication in nutrition and health; hence acceptable safe levels of these heavy metals are of great concern in food production (Mohammad and Ahmed, 2006). Singh and Kumar, (2006) concluded that soil, irrigation water and some vegetables from peri-urban sites are significantly contaminated by the heavy metals including lead and cadmium.

Amaranth are consumed cooked, often by boiling in large volume of water which is thereafter discarded, then stewed in onion, tomato, and oil or by direct stewing with onion, tomato and oil. The time it takes to cook the vegetable varies depending on the part of Kenya it is consumed and the age of amaranth.

The processing and preparation methods of amaranth vegetables reduce the composition, including the nutritional quality. For instance, water-soluble vitamins such as vitamin B and C leach into the cooking water at high rates during cooking and are therefore lost if the cooking water is discarded. For example Mathooko and Imungi, (1994) have reported loss of up to 80% ascorbic acid in cooked drained *Amaranthus hybridus* while Yadav and Sehgal, (1995) reported ascorbic acid losses of up to 93% and β-carotene losses of 1.3% in *Amaranthus tricolor.* Ogbadoyi *et al*., (2011) reported that the amount of β-carotene in *Amaranthus cruentus* leaves boiled for 5 minutes was higher than in fresh.

This finding was also observed by Van Het Hof *et al*. (2000) in tomatoes. The USDA, (1998) also reported that moderate cooking increases the availability of β-carotene in the vegetables as a result of breakdown of plant cell walls. Rickman *et al*. (2007) further added that loss of soluble solids and the release of protein-bound β-carotenes that occurred during boiling may equally contribute to the observed increase in the pro-vitamin content. Likewise, Imungi and Potter,

(1983) reported losses of about 12% of the protein, an apparent increase of about 13% of the iron and a loss of less than 1% of the zinc in cooked and drained cowpea leaves. Sreeramulu et al., 1983 reported Vitamin C losses in cooked and drained up to 98.5% in *Moringa oleifera* to nil in *Emilia javani*ca. Imungi and Potter, (1983) have reported loss of up to 30% lead during cooking of cowpea leaves by boiling in water. An observable decrease in the concentration of the heavy metals chromium and cadmium with increased cooking duration has been demonstrated elsewhere (Joshua *et al*., 2012). Processing the samples has also been associated with a significantly decline in the nitrate content from the fresh sample (Abakr and Ragaa, 1996; Anjana *et al.*, 2007; Anjana and Muhammed, 2006; Waclaw and Stefan, 2004). Higher levels of oxalate in the fresh *Amaranthus cruentus* leaves than in the boiled leaves have also been reported (Adeboye and Babajide, 2007; Antia *et al*., 2006; Ogbodoyi *et al*., 2006). Similarly, boiling/cooking of vegetables before consumption significantly reduces the intake of oxalate (Ojiako and Igwe, 2008). This therefore would mean that cooking amaranth in whichever method results in losses of water soluble nutrients, anti-nutrients, and metals due to leaching into the cooking water.

1.2 PROBLEM STATEMENT

Food safety is a major public concern worldwide including Kenya. The increasing demands for food coupled with high levels of unemployment has forced people especially of the low income to utilize areas that were not meant for food production such as the road sides, sewer lines and improper use of synthetic fertilizers and pesticides.

Agricultural production in the urban centres is now widely accepted as an urban livelihood strategy (Rakodi and Lloyd-Jones, 2002; Dreschel *et al.,* 2008). In Kenya 30% of Nairobi residents practice urban agriculture with a majority of the farmers using untreated sewage to irrigate crop and fodder (Karanja *et al.*, 2010**)**. Despite the fact that wastewater re-use is not permitted in Kenya, it is widely used as a low-cost alternative to conventional irrigation water in the irrigation of urban farms (Qadir *et al.*, 2010). This exposes the public to health and environmental risks (Kaluli *et al*., 2011**)**. Heavy metals enter the food chain through irrigation with contaminated water, use of synthetic fertilizers and metal-based pesticides, industrial emissions, automobile fumes during harvesting, storage and sale. In Nairobi, vegetables grown with waste water and /or effluent conditions in various parts of the city are thought of being contaminated with heavy metals beyond toxic levels. However, there is no data to ascertain the validity of these claims. Therefore this study investigated the levels of heavy metals contamination, anti-nutrients and macro-nutrients in vegetables grown with effluent waters in Ruai and Njiru, both peri-urban regions of Nairobi County.

1.3 JUSTIFICATION

The growth of leafy vegetable in sewerage water in Kenya is widespread and municipal authorities, government ministries, along with the local population and media have raised concern regarding the potential health threats posed by use of polluted waters to crop irrigators and consumers alike and as such the growers are harassed and not recognized as evidenced in dangers of sewer vegetable video uploaded by Kenya citizen TV on 3rd October 2011. (www.youtube.com*/watch?v=_QJDORKdvqI).*Reports indicate that 30% of residents in Nairobi practice urban agriculture and supplies more than 90% of the vegetables amaranth to various outlets in Nairobi with a majority of the farmers using untreated sewage to irrigate crop and fodder (Karanja *et al.*, 2010**;** Onyango *et al*., 2008). It is feared that these vegetables are heavily contaminated with heavy metals and the consumers and government agencies in horticultural industries and those involved in quality standard regulations have, through design or default, failed to offer support to farmers' initiative of using waste water in the production of vegetables by resolving the quality and safety issues (Onyango and Kwarteng, 2011).

Therefore, study was designed to generate information about the levels of lead and cadmium, oxalates and nitrates, zinc, iron, vitamins A and C in vegetable amaranth grown with effluent conditions. The study also will provide insight into the effect of cooking method and time on these levels in amaranth. This information is useful to both the farmers in the trade with the vegetables and the consumer in making informed choices about the safe use of vegetable amaranth grown under effluent conditions. The information from this study will help with policy formulations on production of vegetables using sewerage water to promote urban and peri-urban agriculture to supplement the scarce income levels in these areas.

1.4 OBJECTIVES

1.4.1 Broad Objective

To evaluate the production, markets, nutritional values and the levels of cadmium and lead in raw and cooked vegetable amaranth grown in effluent water for the purpose of determining the safety of vegetable.

1.4.2 Specific objectives

1. To carry out a baseline survey on production practices, marketing, and methods of preparation for consumption of vegetable amaranth grown under sewerage conditions.

2. To determine the proximate composition, vitamin A and C and minerals: zinc, calcium and iron in raw and cooked vegetable Amaranth.
3. To determine the levels of the heavy metals; lead and cadmium in the raw and cooked vegetable Amaranth.
4. To determine the levels of the anti-nutrients; nitrates and oxalates in raw and cooked vegetable Amaranth.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

Land is the main asset in agricultural production and generally, limited availability of productive land in Kenya is a major constraint to increased agricultural production. There has been a decline to more than 100 millimeters in long rains in central Kenya since the mid-1970s; this has threatened the critical surplus crop growing areas making Kenya a food insecure country (Ngigi and Macharia, 2006). It has also been estimated that the current water availability stands at 650 m^3/year per capita, and could drop to about 350 m^3/year by 2020 (Ngigi and Macharia, 2006). This means that Kenya is becoming drier each passing day and as such the amount of prime arable land has also diminished substantially hence any available land is now being utilized for agriculture. Therefore, with decline in per capita freshwater availability, there is increase in dominance of wastewater in the water balance and this is making wastewater a very important source of irrigation water for urban agriculture (Githuku, 2009).

The current Kenyan population of about 38.6 million coupled with high prevailing birthrate of about 4% per year (Kenya National Bureau of Statistic, 2010) and majority of Kenyans estimated living on less than 1 US$ per day fresh water scarcity is getting worse each passing day and is becoming unaffordable to many urban poor dwellers hence majority are utilizing the abundant, low cost and readily available effluent water that could probably be heavily contaminated with heavy metals for food production regardless the concerns and harassment from the media, government ministries and municipal authorities.

The use of waste water poses a threat to quality and safety of vegetables. This is because excessive heavy metals contents in foods has been associated with cause of a number of diseases, especially cardiovascular, kidney, nervous system and bone diseases. This has drawn the attention of researchers to the risks associated with consumption of contaminated foodstuffs with pesticides residues, heavy metals and or toxins in vegetables (Mello, 2003).

In Kenya, the most commonly consumed traditional leafy vegetables (TLVs) include the *Amaranthus* spp. (Pig weed) among *Vigna unguiculata* (Cowpea leaves), *Solanum nigrum* (Black nightshade), *Cleome gynandra* (Cat's whiskers), *Cucurbita* spp. (Pumpkin leaves) and *Corchorus* spp. (Jute) (Onyango *et al.*, 2008). Reports indicate that 30% of Nairobi residents practice urban agriculture with a majority of the farmers using untreated sewage to irrigate crop and fodder (Karanja *et al.*, 2010). About 30% of the residents are involved in urban agriculture supplies more than 90% of the vegetables amaranth to various outlets in Nairobi (Onyango *et al*, 2008), hence very little amaranth is supplied by the rural agriculture. There is limited documented information on vegetable amaranth grown with effluent conditions in urban set up. It is on this back drop that the nutritive value, the levels of lead and cadmium in the amaranth grown with effluent conditions are determined in order to add onto the existing knowledge.

The WHO recommends a minimum daily intake of 400g of fruits and vegetables (WHO, 2003). Although, it is not clear from the report what proportion of this total daily intake should go to vegetables, the FAO report has substantiated that the total daily intake is equivalent to five (5) servings of 80g each of fruits and vegetables (FAO/WHO 2004, FAO, 2003).This is because vegetable is an important contributor of human well-being and helps in prevention of diet-related chronic diseases.

Amaranth constitutes an important part of the diet for the Kenyan population. It grows quickly, requires little input and can be harvested within a short period of time between 6-10 weeks after planting. In Kenya, vegetable amaranth was found to contain moisture content of 85.5%, total ash 19.2%, crude protein 26.1% and the crude fiber 14.7% while the ascorbic acid content was 627 mg/100g, zinc 5.5 mg/100g and iron 18 mg/100g dry weight hence it is importance in alleviating poverty and nutritional related problems among the poor (Onyango *et al*., 2008). The production of, and the trade in, vegetable amaranth has remarkably increased in recent years due to increase in urban demand (Onyango and Imungi, 2007) as such vegetable amaranth is found selling in many supermarkets and green grocers' stores in the urban centres of Kenya.

2.2 NUTRITIVE VALUE OF VEGETABLE AMARANTH

2.2.1 Micronutrients

2.2.1.1 Vitamins A and C

Vegetable crops, especially African indigenous vegetables have been found to contain high levels of micronutrients and antioxidants (Yang and Keding, 2009), this means that indigenous vegetables provide higher amounts of provitamin A, vitamin C and several minerals than exotic vegetables both on a fresh weight basis and after preparation. Leafy vegetables have been cited as a potential source of micronutrients (Ejoh *et al*., 2005). In fact, amaranth is said to be rich in vitamin A and iron but it's yet to be documented for amaranth grown with effluent water, the two important nutrients are currently believed to be deficient in the diet of people in many countries.

Kenya’s main nutritional problems are the same as those of other developing countries, and include protein and vitamin A deficiencies which affect a large proportion of the poor urban and rural populations (Onyango *et al*., 2008). Vitamin A describes a group of lipid soluble compounds related metabolically to all-*trans*-retinol. Some Carotenoids such as α- and β-carotenes and β-cryptoxanthine can be cleaved into retinol, via an enzymatic process, which occurs mainly in the small intestine. Vitamin A and C are classified as antioxidants. Antioxidants are important because they quench free radicals and reactive oxygen species (ROS) in the body which are produced as part of normal cellular metabolism and in response to environmental factors such as ultraviolet irradiation (Halliwell, 2006). Dietary antioxidants may protect cell damage caused by free radicals by acting as radical scavengers, reducing agents and quenchers of singlet oxygen formation, and by forming complexes with pro-oxidant metals (Adedayo *et al*., 2010).

Carotenoids impart orange and red colors in vegetables. Perera and Yen, (2007) reported that consumption of carotenoids-rich foods reduces the incidence of several disorders such as cancers, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to compromised immune function, and other degenerative diseases. It has been reported that pro-vitamin A (β-carotene) deficiency in the human body results in blindness in thousands of children each year. It has been estimated that in 2004, 0.6million children died from vitamin A deficiency (Black *et al*., 2008) and that 190 million are Vitamin A deficient (WHO,2009). Different studies on fruits and vegetables have shown that bio-accessibility of carotenoids is enhanced by thermal and mechanical processing (Courraud *et al*., 2013).

The ministry of health in Kenya has specified the levels and made it mandatory the fortification with vitamin A for fast moving processed foods like flours, edible vegetable fats and oils and margarines (*Legal Notice* no. 62 of 15th June 2012) although the long term solution lies with dietary diversification. A number of adverse effects have been reported on intakes of preformed vitamin A above the population reference intake. The lowest doses reported to produce the different effects are:

Bulging fontanelle- 7500 µg RE (as a single dose in infants)

Hepatotoxicity- 7500 µg RE/day for 6 years

Bone density/fracture -1500 µg RE/day (trend analyses do not show a threshold)

Lipid metabolism- 7500 µg RE/day for 4 years (but a minor change only)

Teratogenicity >3000 µg RE/day (based on Rothman *et al*, 1995)

The risk for hip fracture in Swedish women is doubled for retinol intake greater than 1500 µg RE/day as compared to intakes less than 480 µg RE/day (Melhus *et al*, 1998). There is no documented information on the levels of vitamin A in amaranth grown with waste water in Kenya and whether the levels are within the safe limits. The tolerable upper levels apply to both dietary and supplemental intakes of vitamin A and are given in Table 1 (EFSA, 2006).

Table 1: The tolerable upper level for both dietary and supplemental intakes of vitamin A

Age (Years)	**Tolerable upper intake for preformed vitamin A (retinol and retinyl esters) µg RE/day**
1- 3	800
4 – 6	1100
7-10	1500
11-14	2000
15-17	2600
18 years and above	3000

Vitamin C (or L-ascorbic acid, AA) is a water-soluble organic compound that is needed for normal metabolic functioning of the body. It is an essential component of the diet, as humans and other primates have lost the ability to synthesize via the glucuronic acid pathway (Wheeler et al., 1998) hence information on the levels of vitamin C in this study amaranth will add to existing knowledge. It functions primarily to prevent common degenerative processes. The derivatives of ascorbic acid have been tested on cancer cells and showed that ascorbic acid esters have promising anticancer activity (Naidu, 2003). Ascorbic acid as found in most fruits and vegetables also protects against heart disease, high cholesterol, high blood pressure and cancer (Antonious *et al*., 2009). It has been demonstrated that 500 mg of vitamin C taken every 12 hours have a lot of benefits in the reversion of endothelial dysfunction, generation of new cardiac myocytes, reduction of mutations causing metastases, reduction of cataract odds by 64% and aging decline, among others (Ordman, 2010). The recommended intake for smokers is 35 mg/day higher than for non-smokers according to Food and Nutrition Board, Institute of Medicine, USA. This is because smokers are under increased oxidative stress from the toxins in cigarette smoke and generally have lower blood levels of vitamin C. A recent study found that vitamin C regenerated vitamin E from its oxidized form in cigarette smokers (Carr and Frei, 1999).

The safety and benefit of vitamin C supplements is of critical importance, especially for those in war zones at risk for brain trauma or of advancing years at risk for chronic disease and stroke. This is because vitamin C may reduce many major causes of chronic diseases. EFSA has not set any Tolerable Upper Intake Level (UL) for vitamin C. The limited available data from studies in animals and humans were considered to suggest a low acute toxicity of vitamin C (EFSA, 2004). According to (EFSA, 2013) a mean vitamin C intake of 91mg/day rounded to 90 mg/day was estimated to be required to balance daily losses in adults. The detailed vitamin C requirements are shown in table 2.

Table 2: Reference body weights, Average Requirements (ARs) and Population Reference Intakes (PRIs) of vitamin C for children, adolescents and adults

Age	**Reference body weight (kg)**		**AR (mg/day)$^{(f)}$**		**PRI (mg/day)$^{(g)}$**	
	Boys	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**
1-3 years	12.2$^{(a)}$	11.5$^{(a)}$	15	15	20	20
4-6 years	19.2$^{(b)}$	18.7$^{(b)}$	25	25	30	30
7-10 years	29.0$^{(c)}$	28.4$^{(c)}$	40	40	45	45
11-14 years	44.0$^{(d)}$	45.1$^{(d)}$	60	60	70	70
15-17 years	64.1$^{(e)}$	56.4$^{(e)}$	85	75	100	90
Age	**Reference body weight (kg)^{a}**		**AR (mg/day**		**PRI (mg/day)**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
18-79 years	68.1	58.5	90	80	110	95

Source: EFSA (2013)

2.2.1.2 Minerals

Iron is required for a number of vital functions, including growth, reproduction, healing, and immune function. Iron-deficiency anemia results when there is inadequate iron to support normal red blood cell formation (WHO, 2000). The anemia of iron deficiency is characterized as microcytic and hypochromic, meaning red blood cells are measurably smaller than normal and their hemoglobin content is decreased. Iron-deficiency induced anemia has also been reported to be prevalent among the general population, especially among children under five years of age and pregnant women (WHO, 2000). Iron deficiency may increase the risk of lead poisoning in children because iron deficiency and lead poisoning share common environmental risk factors and both are causes of neurocognitive toxicity. However, iron deficiency has been found to increase the intestinal absorption of lead in humans and animals (Wright, 1999). Information on the levels of iron in amaranth vegetable grown with waste water is scanty and as such intestinal lead absorption has not been determined.

On the other hand vitamin C strongly enhances the absorption of non-heme iron by reducing dietary ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and forming an absorbable, iron-ascorbic acid complex. *Amaranthus tricolor* contain 26.8 mg/100g dry weight of total iron and 629 mg/100g dry weight of ascorbic acid (Singh *et al*., 2001). It was found that mean ascorbic acid for vegetable amaranth was 627mg/100g while iron was 18 mg/100g, hence with such combination it makes iron more absorbable (Onyango *et al*., 2008).

The observed levels of iron in vegetable amaranth is within the provisional maximum tolerable daily intake of 0.8 mg/kg body weight per day according to JEFCA/61/2003 for adults assuming an average adult weighs 60kg. Iron accumulation in the liver and other body tissues may lead to cirrhosis of the liver, diabetes, heart muscle damage, and joint problems (Janssen and Swinkels, 2009).

Zinc is a nutritionally essential mineral needed for catalytic, structural, and regulatory functions in the body. Severe zinc deficiency is a rare, genetic or acquired condition. Dietary zinc deficiency, often called marginal zinc deficiency, is quite common in the developing world, affecting an estimated 2 billion people (Prasad, 1998). Zinc and vitamin A interact in several ways. Zinc is a component of retinol-binding protein, a protein necessary for transporting vitamin A in the blood. Zinc is also required for the enzyme that converts retinol to retinal. This latter form of vitamin A is necessary for the synthesis of rhodopsin, a protein in the eye that absorbs light and thus is involved in dark adaptation. Zinc deficiency is associated with decreased release of vitamin A from the liver, which may contribute to symptoms of night blindness that are seen with zinc deficiency (Boron *et, al*., 1988; Christian and West, 1998).

Isolated outbreaks of acute zinc toxicity have occurred as a result of the consumption of food or beverages contaminated with zinc released from galvanized containers.

Signs of acute zinc toxicity are abdominal pain, diarrhea, nausea, and vomiting. Single doses of 225 to 450 mg of zinc usually induce vomiting. Milder gastrointestinal distress has been reported at doses of 50 to 150 mg/day of supplemental zinc. Metal fume fever has been reported after the inhalation of zinc oxide fumes. Specifically, profuse sweating, weakness, and rapid breathing may develop within eight hours of zinc oxide inhalation and persist 12-24 hours after exposure is terminated (U.S. FNB, 2001, King and Cousins, 2006). High intake of zinc induces the intestinal synthesis of a copper-binding protein called metallothionein which traps copper within intestinal cells and prevents its systemic absorption although high copper intakes do not affect zinc absorption.

It has also been reported elsewhere that zinc has an inhibitory effect on iron absorption that lasts less than 30minutes when zinc is administered with iron in aqueous solution (Olivares *et al.*, 2007). This inhibitory effect can be attributed to the fact that transferrin, the main iron plasma transporter, has also been shown to bind zinc (Harris, 1983, King and Cousins, 2006). (Onyango *et al*. 2008) reported that vegetable amaranth contains 5.5 mg/100g. This is within the recommended levels of 15 mg/day for adult humans as per (JECFA, 1982) and of 8 mg/day for adult women and 11 mg/day for adult men according to U.S. FNB recommendations and appears sufficient to prevent deficiency in most individuals hence the PMTDI is 0.3-1 mg/kg body weight/day. Therefore, the consumption of vegetable amaranth helps balance vitamin and mineral intake (FAO, 1988; IPGRI, 2003; Shukla *et al*., 2005).However, the levels of zinc in vegetable amaranth grown with effluent water has not been documented in Nairobi.

Calcium is the most common mineral in the human body. It’s required for the healthy maintenance of skeleton and to prevent demineralization of bones (Wood and Ronnenberg, 2006). Increased calcium intake has strongly been associated with decreased colorectal cancer risk in men with higher circulating levels of Insulin-like Growth Factor-1 (IGF-1) (MaJ *et al*., 2001), in the treatment of osteoporosis (Heaney, 2000; U.S National Institute of Health, 2000), prevention of exposure to lead mobilized from the skeleton during bone demineralization (Hertz-Picciotto *et al*., 2000), decrease the severity of Premenstrual Syndrome (PMS) (Brown
, 2002).

Vegetable amaranth has been reported to contain an appreciable amount of calcium. Akubugwo *et al*. (2007) reported calcium content of 44.15 mg/100g dry weight in *Amaranthus hybridus*. It is important to note that abnormally elevated blood calcium or hypercalcemia due to over consumption of calcium has never been documented to occur from foods, only from calcium supplements. Generally, compared to spinach, *Amaranthus* contains three times more vitamin C, calcium and niacin and compared to lettuce, *Amaranthus* contains 18 times more vitamin A, 13 times more vitamin C, 20 times more calcium and 7 times more iron (Guillet, 2004).However, the levels of Calcium in amaranth grown with waste water has not been documented in Kenya.

2.2.2 Anti-nutrients

2.2.2.1 Heavy Metals

Heavy metals are those metals with an atomic weight greater than that of sodium and with specific gravity greater than 5 (Wood and Goldberly, 1977). Heavy metals include a group of 70 metals. Cr, Mn, Zn, Cu, and Fe are considered essential components of biological activities in the body, however, in excess are reported to cause problem to the human body (Lokeshwari and Chandrappa, 2006). On the other hand, Pb, Cd, and As have no important functions in human body, rather they play toxic role to living organism (Lokeshwari and Chandrappa, 2006). Heavy metals are toxic because they react with body's biomolecules, clog up receptor sites, break and bend sulfur bonds in important enzymes such as insulin, and damage the DNA (Arora *et al.*, 2008, Sharma *et al.*, 2007).

Cadmium in particular is an Environmental Protection Agency (EPA) regulated heavy metal that is used as anti-corrosion and decorative coatings on metal alloys. It's a soft, silvery white, ductile metal with a faint bluish tinge. It has an atomic weight of 112.40, which is derived from a mixture of eight isotopes. Other uses of cadmium include; plating, in the manufacture of pigments such as cadmium pigments used in plastics that are molded at high temperature, plastic stabilizers, nickel-cadmium batteries and phosphate fertilizers. It can therefore enter waterways through industrial discharges and galvanized pipe breakdown. It is a non-essential metal to living organisms and can become toxic by displacing zinc, because it has chemical characteristics similar with those of zinc and occurs in nature wherever zinc is found.

It is also produced as a by-product in the mining of zinc and lead. Low exposures may result in kidney damage (IOSHIC, 1999). In addition, epidemiological studies have revealed that cadmium may be a contributing factor in some forms of cancer in humans (IARC, 1998).There is little information of the level of cadmium in amaranth grown with waste water from domestic and industrial use in Nairobi.

On the other hand, lead is relatively abundant in nature and occurs primarily in the inorganic form in the environment and in association with zinc. Human exposure to heavy metals is mainly through food and water although to a minimal levels from air, dust and soil. Therefore, consumption of vegetables is one of the most important pathways by which heavy metals enter the food chain (Wang *et al*. 2004). Lead is absorbed more in children than in adults and accumulates in soft tissues and over time in bones. This is because children have high gastrointestinal uptake and the permeable blood-barrier that makes them susceptible to exposure than adults. Children exposed to high concentrations of lead may develop behavioral disturbances as well as learning and concentration difficulties (Jarup, 2003). The half-lives of lead in blood and bones are approximately 30 days and 10-30 years, respectively (EFSA, 2010). For instance, most of the accumulated Pb in a body is sequestered in skeleton, where half-life is 20 to 30 years (WHO, 1995).

Elementary lead is used in the manufacture of storage batteries, cables, various alloys, protection cloth against radiation and tetraethyl lead production for use as antiknock in petrol. Lead pigments are used in the manufacture of glass enamels and in the rubber industry while lead iodide is used as a seeding agent for rainfall enhancement and in fuel. Lead and Cadmium are the most toxic and the most abundant heavy metals in food (Sajjad *et al.*, 2009 and Oliver, 1997).

Their accumulation in human body is likely to results in serious systemic problems like cardiovascular, kidney, nervous and bone diseases.

It has been reported that vegetable amaranths has a way of concentrating metals in their tissues and or that aerial deposition may be a major source of contamination (Atayese *et al*., 2009.) Heavy metal concentrations in *Amaranthus* cultivated on soils characterized by heavy traffic were significantly higher ($P \leq 0.05$) than those cultivated on the reference soil which had been collected from the rural communities (Ateyese *et al*., 2009). (Othman, 2001) collaborated this finding and studied five varieties of green vegetables namely amaranth, Chinese cabbage, cowpeas, leafy cabbage and pumpkin leaves. He found out that Amaranth and pumpkin leaves had more than 60% higher content of heavy metals especially copper, chromium and lead than the other vegetables. The study also noted that the levels of zinc and lead in vegetable amaranth were higher than the maximum level recommended by World Health Organization, however there is no sufficient data supporting this claim in Kenya.

High levels of heavy metals in soil have been documented in kibera and Maili Saba in Nairobi where heavy metals accumulation in soils were found as follows: Cd (14.3 mg kg-1), Cr (9.7 mg kg-1) and Pb (1.7 mg kg-1) and Cd (98.7 mg kg-1), Cr (4.0 mg kg-1) and Pb (74.3 mg kg-1), respectively. This led to high phyto-accumulation of Cd, Cr and Pb in the crops that exceeded the maximum permissible limits (Karanja *et al.*, 2010). These findings in general indicated that while the levels of metals in soil were within the critical limits proposed by (Kabata-Pendias and Pendias, 1984), the range within the plant leaves were above the normal limit for plants.

(JECFA, 2011) withdrew the 25 µg/kg body weight Provisional Tolerable Weekly Intake (PTWI) established in 2010 that lead could no longer be considered health protective and as such the committee concluded that it was not possible to establish a new PTWI that would be considered health protective. This is because the previously established PTWI of 25 µg/kg body weight is associated with a decrease of at least 3 IQ points in children and an increase in SBP of approximately 3 mmHg. These changes are important when viewed as a shift in the distribution of IQ or blood pressure within a population. However, Cadmium Provisional Monthly intake was set at 25µg/kg body weight /month by JECFA, (2013). Information on the levels of heavy metals in vegetable amaranth, compared with the JECFA tolerable intake in Kenya is not available.

It has been reported that the lead content of vegetable amaranth collected from twenty one supermarkets in Kenya averaged 1.03 mg/100g dry weight (Onyango *et al*., 2008). However it has been assumed that under normal circumstances, the amounts of cooked vegetables consumed by individuals rarely exceed 100g of fresh weight per day (FAO, 1988).

2.2.2.2 Nitrates and Oxalates

Some leafy vegetables have been shown to have relatively high levels of nitrates (Maff, 1987). This is because green plants utilize nitrogen in protein synthesis. The level of nitrates in plants is influenced by a number of factors such as plant species and variety, geographical region, light intensity, air temperature, duration of growth period, harvesting period and the use of nitrogen containing fertilizer. Increase in light intensity decreases the accumulation of nitrates (Walter, 1999: Onyango *et al*., 2008; Gonzalez *et al*., 2010, Amelin *et al.*, 2002). Nitrate significance to human health is due to the fact that it can be converted to nitrite, nitric oxide and N-Nitroso compounds.

The most serious danger comes from nitrite that is produced by nitrate reduction and which can lead to methaemoglobinemia or form nitrosamines and nitrosamides by reacting with amines and amides, whose carcinogenic action is well documented (Walker, 1990, Gangolli *et al*., 1994). Methaemoglobinemia is reduced ability of blood to carry vital oxygen around the body due to reduced levels of haemoglobin thus young infant whose capacity to secrete acid in the stomach is lower than that of adult are more susceptible and show signs of blueness around the mouth, hands and feet hence the common name is “Blue baby syndrome”. On the other hand, it has been reported that dietary intake of nitrates and nitrites may help heart attack survival and recovery (Bryan *et al*., 2007).

The (EC, 2007), scientific commission on food had previously evaluated the safety of nitrites and nitrates (opinions expressed in 1990 and 1995). It established an ADI of 0.06 mg/kg body weight expressed as nitrite ion and for nitrate an ADI of 3.7 mg/kg body weight expressed as nitrate ion. The Panel confirmed that nitrite contributes to microbiological safety by providing direct protection against the growth of *Clostridium botulinum* in most meat products and also to the flavour, colour and anti-oxidative stability of meat products. (JECFA, 1974) retained the previously estimated ADI (0-5 mg/kg) but lowered the ADI for nitrites to 0.2 mg/kg body weight.

The leafy vegetables are the major vehicle for the entry of nitrates into the human system. (Onyango *et al*., 2008) reported that the nitrate contents in vegetable amaranth ranged between

505 mg/100 g and 1056 mg/100g; this translated to about 73.2 mg to 153.1 mg/100 g fresh weight though it must be observed that some components of vegetables such as ascorbic acid and phenols have been reported to inhibit the toxic effects of nitrites (Walker, 1990; Gangolli *et al*., 1994). Therefore, according to (FAO, 1988), Nitrate levels normally found in vegetable amaranth do not present a serious health problem to reasonably healthy individuals if consumption does not exceed 100g of leaf per day however this is has not been documented in vegetable amaranth grown with effluent water.

Oxalate is a common plant component considered to be an anti-nutrients as well as a toxin (Libert and Franceschi, 1987). Some plants are well known to contain oxalates and these tend to occur in higher concentrations in the leafy parts of vegetables rather than in the roots or stalks (Savage *et al*., 2000; Noonan and Savage, 1999). The distribution of oxalates in plants is also very variable. Some green leafy vegetables such as spinach, purple and green amaranth and colocasia contain very high oxalate levels (Brogren and Savage, 2003). There is high levels of between 8% and 10% dry weight basis in *Amaranthus cruentus* and *Amaranthus viridis*, respectively, while *Amaranthus spp* contained 1.1% to 7.9% oxalic acid contents in the dry weight (Radek and Savage, 2008; Ricardo, 1993). Oxalate accumulation in the leaves is affected by different factors such as soil conditions, climate, species or cultivar, and maturity hence the need to document the levels of oxalate in vegetable amaranth grown with effluent water.

Oxalic acid and its salts occur as end products of metabolism in a number of plant tissues. Oxalic acid forms water-soluble salts with Na^+, K^+ and NH_4^+ ions but can also form insoluble oxalates by binding with Ca^{2+}, Fe^{2+} and Mg^{2+} rendering these minerals unavailable for absorption by the intestine (Savage *et al*., 2000). Therefore it can result in calcium deficiency even when the diet is otherwise sufficient in calcium. Infants and children who are experiencing active bone and teeth formation and older persons who can suffer calcium resorption from bones leading to osteoporosis are more vulnerable (Harper *et al*, 1979). The consumption of high oxalate containing foods promotes oxaluria which leads to an increased risk of calcium oxalate kidney stones (Massey, 2003). The total oxalate content of vegetable amaranths collected from the market and from experimental plot ranged between 3700 - 6800 mg/100g while that of soluble oxalate ranged between 2800 - 4200 mg/100 g dry weight, and noted that the oxalate contents decreased with maturity of the vegetables in *A. hypochondriacus* (Onyango *et al*., 2008).

Unacceptable levels to humans have been suggested to be 2 to 5 g of oxalic acid per day for populations consuming low levels of calcium (Ricardo, 1993). High oxalate foods should always be cooked to reduce the oxalate content; soaking a food prior to cooking will also reduce the oxalate contents by leaching (Noonan and Savage, 1999). There is little data published on the level of anti-nutrients and the effect of cooking in vegetable amaranth in Kenya, therefore, this present study was designed to fill these gaps.

2.3 PRODUCTION OF AMARANTH

2.3.1 Influence of Production Environment on Leafy Vegetables

Rapid urban and industrial growths have resulted to release of enormous volumes of wastewater, which is increasingly utilized as a valuable resource for irrigation in UPA agri-horticultural practices. Besides being the source of irrigation water, this wastewater contains appreciable amounts of plant nutrients and toxic heavy metals, which are creating opportunities and problems for agricultural production, respectively (Singh *et al*., 2004).There is little information on the levels of heavy metals in waste water in Kenya.

Heavy metals are generally not removable even after the treatment of wastewater at sewage treatment plants, and thus cause risk of contamination of the soil and subsequently the food chain (Fytianos *et al*., 2001). A number of studies show an elevated level of heavy metals in vegetables grown in areas having long-term uses of treated or untreated wastewater (Sharma *et al*., 2007; Sharma *et al*., 2006; Singh *et al*., 2004). It has been found that heavy metals accumulate in the upper part of the soil due to strong adsorption and precipitation phenomena (Qadir *et al.*, 2010). Thus, introducing these pollutants into the plants growing there in through roots, which are translocated to foliage and even to edible fruit parts (Zayed *et al*., 1998; Barman *et al*., 2000; Fytianos *et al*., 2001)

The uptake and accumulation of mineral elements by plants may follow two different paths. That is, through the roots and foliar surfaces (Sawidis *et al*., 2001). As the plants age, die and decay, heavy metals and other organic compounds in their matrices are released back to the soil enriching it with pollutants. There are several environmental factors that impact on accumulation of nutrients/anti-nutrients in plants, thereby influencing nutritional quality of the vegetables. Such factors include altitude, soil quality, soil pH and salinity, production practices, insect injury and plant diseases. These growth and environmental factors influence the water and nutrient supply to the plant (Goldman *et al.,* 1999) which in turn influences the yield and quality of the material produced. These factors also affect the composition and sensory quality (appearance, texture, taste and aroma) of harvested plant parts (Goldman *et al*., 1999).

Various reports (Singh *et al*., 2004; Barman *et al*., 2000), also show that when wastewater is used for the irrigation of edible plants for prolonged period, soil health is affected and toxic metals apparently accumulate in soil. There is an ongoing buildup of heavy metals in soil at the waste dumps and concentrations are higher than established limits for some metals (Adelekan and Alawode, 2011).

In Dandora waste dump area in Nairobi indicated high levels of heavy metals, in particular lead, mercury, cadmium, copper and chromium in the soil samples obtained from the site. Similarly, medical examination of the children and adolescents living and schooling near the dump site indicated a high incidence of diseases that are associated with high exposure levels to these metal pollutants (Kimani, 2010). These findings demonstrate the severe risks associated with municipal waste dumps.

However, the uptake of heavy metal varies widely depending on the plant species being studied. Plant species have a different mechanisms and capabilities of removing and accumulating heavy metals. The vegetable amaranths have a way of concentrating metals in their tissues and /or those aerial parts (Atayese *et al*., 2009) may be a major source of contamination while Othman, (2001) reported that amaranth and pumpkin leaves had more than 60% higher content of heavy metals especially copper, chromium and lead than the Chinese cabbage, cowpeas and leafy cabbage. The uptake of metals from the soil depends on different factors such as their soluble content in it, soil pH, organic matter content, soil type, and plant growth stages, types of species, fertilizers and soil (Sharma *et al*., 2006; Ismail, *et al*., 2005; Fleming and Parle, 1977). Most of heavy metals are less available to plants under alkaline conditions, than under acid conditions (Hess, 1971). There is little information on the uptake of heavy metals by vegetable amaranth and the levels of these heavy metals in the soil in Kenya and yet More than 90% of the supply of the vegetable amaranth to various outlets in Nairobi is from farms that are within the environs of the urban and have been cultivated using untreated waste water (Onyango *et al.*, 2008).These vegetable is being grown in all types of available land including abandoned waste dumping sites, banks of polluted rivers, alongside roads, areas enclosing waste water ponds in Kenya. Singh and (Kumar, 2006) concluded that soil, irrigation water and some vegetables from UPA sites are significantly contaminated by the heavy metals and that lead and Cadmium were of more concern than copper and Zinc but this has not been documented in vegetable amaranth grown in Ruai and Njiru in Nairobi County.

2.3.2 Influence of Cooking on Nutritive and Antinutritive Levels of Leafy Vegetables

The processing and preparation methods of amaranth vegetable reduce the amounts of chemical constituents, including the nutritional quality of vegetables. For instance, water-soluble vitamins such as vitamin B and C leach into the cooking water at high rates during cooking and are therefore lost if the cooking water is discarded. Up to 80% of ascorbic acid in cooked drained *Amaranthus hybridus* is lost (Mathooko and Imungi, 1994), while 1.3% of β-carotene is lost from *Amaranthus tricolor* (Yadav and Sehgal, 1995).It has also been reported that the amount of β-carotene in *Amaranthus cruentus* leaves boiled for 5 minutes was higher than in fresh (Ogbagoyi *et al.*, 2011) and similarly in tomatoes (Van Het Hof *et al.*, 2000, USDA, 1998). The loss of soluble solids and the release of protein-bound β-carotenes that occurred during boiling may equally contribute to the observed increase in the pro-vitamin content (Rickman *et al.*, 2007).

Another possible explanation can be due to the fact that high-carotenoids foods typically have the vast majority of their carotenoids occurring in *all-trans* form. While this form can provide excellent health support, it is not as readily available to the bloodstream or to our cells as another form called the *cis* form. The cooking of a plant food decreases the total amount of *all-trans* Carotenoids found in the food, but it also increases conversion of many *all-trans* Carotenoids into their more available *cis* form. As a net result, some studies like that of (Van Het Hof et al., 2000) showed better support of Carotenoids levels in the blood after consumption of a cooked plant food product (like tomato paste) than a non-cooked plant food product (like fresh tomatoes). Imungi and Potter, (1983) reported losses of about 12% of the protein, an apparent increase of about 13% of the iron and a loss of less than 1% of the zinc in cooked and drained cowpea leaves. It was reported that vitamin C is lost during cooking and drainage of cooking water up to 98.5% in *Moringa oleifera* to nil in *Emilia javani*ca (Sreeramulu *et al*., 1983).There is little information on the effect of cooking on the levels of vitamins and minerals in vegetable amaranth grown with effluent water in Kenya.

The dietary intake of these nutrients from the cooked Amaranth vegetable is therefore expected to be substantially varied in raw and cooked vegetable. Generally and according to Funke (2011), long cooking or keeping vegetables hot for a long time has a destructive effect on the vitamins.

Accumulation of heavy metals differ according to plant species (Hooda *et al.*, 1997), the age of the plant, the environmental conditions in which the plant is grown and the part of the plant analyzed (Isabel and Concepcion, 1997).It is therefore necessary to determine the amaranth species grown in Kenya, the age and the prevailing environmental conditions for precise documentation of accumulation of heavy metals. It has also been reported that leafy crops are most susceptible to contamination from atmospheric deposition of lead from industrial and automotive sources (Atayese *et al., 2009*). But if the cooking is done by discarding the water, then the amount of lead is lowered by leaching into the cooking water. (Imungi and Potter, 1983) have reported loss of up to 30% lead during cooking of cowpea leaves by boiling in water.

A study conducted by Joshua *et al*., (2012) showed that there was decrease in the concentration of the heavy metals (chromium and cadmium) with increased cooking duration and greater losses were observed when the vegetables was boiled for 20, 25 and 30 minutes. The study recommended that vegetables should not be subjected to cooking beyond 15 minutes to maintain good nutritional value. Similarly, increased time of boiling amaranth was noted to significantly reduce the nitrate content (Ogbadoyi *et al*., 2011; Abakr and Ragaa, 1996; Anjana *et al.*, 2007, Anjana and Muhammed, 2006 and Waclaw and Stefan, 2004). The reduction in nitrate content of vegetables as a result of heat treatment such as boiling, cooking could have been perhaps due to degradation or conversion of nitrate to other compounds needs to be documented in vegetable amaranth grown with effluent water.

Similarly, the content of oxalate was found to significantly decrease in boiled or cooked *Amaranthus cruentus* leaves compared to the fresh ones (Adeboye and Babajide, 2007; Antia *et al*., 2006; Ogbodoyi *et al*., 2006; Ojiako and Igwe, 2008). However, these losses have not been quantified in vegetable amaranth grown with effluent and /or waste water in Kenya and there is possibility that, the levels found in cooked vegetables might not be high enough to warrant public health concern.

Lead is a naturally occurring element found in the earth’s crust and normally found at only low levels in plant-based food stuffs unless they are grown in sewerage or industrial effluents (David and Craig, 1994).The majority of dietary lead results from environmental pollution due to exhaust gases from automobiles especially if vegetables are grown near roads and from processing equipment and storage containers fabricated using lead containing materials such as solder (Pyke, 1981; David and Craig, 1994). Other sources of heavy metal pollution includes the increased use of synthetic fertilizers, waste water and other chemicals to meet the ever increasing demands for food production for humans (Sajjad *et al*., 2009).Therefore, the study was also designed to add onto the existing knowledge on the effect of cooking on the nutritive and antinutritive value of vegetable amaranth grown with effluent water in Kenya.

CHAPTER THREE

Production Practices, Marketing and Consumption of Vegetable Amaranth Grown with effluent water :A Case for Njiru and Ruai in Nairobi

Amaranths are increasingly becoming an important traditional food plants in Kenya. The farming and marketing practices of the vegetable amaranths varies from one place to another. Most of the vegetables in Nairobi are produced in effluent water. The vegetables are consumed cooked mainly as accompaniment for ugali (a paste made from maize meal). The methods of cooking vary but direct stewing seemed to be the most popular. The current study investigated the production practices, marketing and consumption of vegetable amaranth grown using effluent water a case study in Njiru and Ruai locations in Embakasi district, Nairobi County. The study sites were purposively selected because of their long tradition in producing the vegetables with effluent water. Data was collected using a pre-tested and validated questionnaire targeting 20 farmers from Njiru (Silancia) and 29 farmers from Ruai (Kwa Maji) and from 42 intermediaries (20 from Njiru and 22 from Ruai, who were purposively selected. Data was entered into Statistical Package for Social Sciences (SPSS, version 16.0), edited and then analyzed using descriptive and inferential statistics. Results showed that 66% of the farmers and intermediaries were between the ages of 40 and 50 years, and 83% of the respondents were females. Those on full time farming and trading made 98% and they make an on average annual income of between Kes.1000 to Kes.200, 000. On the education level, 70% were semi-illiterate then 87% practice subsistence farming on less than 3 hectares of land. 41% sell to intermediaries. Furthermore, 53% of the intermediaries made a profit of between KES.300-400 per 50kg sack of amaranth. Up to 70% of the respondents indicated cooking the vegetable by direct stewing. About 20% of farmers agreed that the waste water come into contact with the leaves, 45% admitted using raw

animal manure/municipal/effluent biosolids. The results show that semi-illiterate women dominate this subsistence profitable enterprise and the vegetable amaranth is predominately grown with effluent conditions. From the study, vegetable production in urban areas is dynamic, viable and largely a sustainable livelihood strategy, especially for poor urban dwellers in active labour force hence the government needs to institutionalize urban agriculture and educate the growers on good agricultural practices to enhance biological safety of these vegetables.

3.1 INTRODUCTION

Amaranths (African spinach or pigweed) are plants that grow 90 to 150 centimeters tall and are abundant weeds in many parts of the world. They are important traditional food plants in Kenya. They produce large amount of biomass within a short period thus the whole young plants can be harvested 3-5 weeks after sowing or the leaves can be picked continuously from growing plants. There are about 60 amaranth species, several of which are cultivated for leaf vegetables, grains or as ornamental plants in many countries in the world (Mbwambo *et al*., 2015). The species can be distinguished by either the size, the color of the leaves, flowers or the presence or absence of spines.

The main species grown as vegetables are *A. tricolor, A. dubius, A. lividus, A. cruentus, A. palmeri* and *A. hybridus* while *A. hypochondriacus, A. cruentus* and *A. caudatus* are the mainly grown for grain (Teutonico and Knorr, 1985). The economic importance of vegetable amaranth cannot be over-emphasized. The leaves are highly nutritious with high levels of protein, iron and vitamin A, vitamin C and folate. The levels of other vitamins including thiamine, niacin, and riboflavin and dietary minerals including calcium, iron, potassium, zinc, copper, and manganese cannot be ignored. Amaranth therefore, has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care and as popular vegetable in the diets of Kenyan (Onyango *et al.,* 2008). Increasing demands for food coupled with high levels of unemployment has forced people especially of the low incomes to utilize areas that were not meant for food production like along the roads, sewer lines and intensive use of synthetic fertilizers and pesticides.

Agricultural production in urban centers is now widely accepted as an urban livelihood strategy (Rakodi and Lloyd-Jones, 2002; Dreschel, *et al.*, 2008). Despite the fact that wastewater re-use is not permitted in Kenya, it is widely used in the irrigation of urban farms possibly increasing the exposure of the public to health and environmental risks (Kaluli *et al*., 2011**)**.

The commercial production of amaranth (*amaranthus hypochondriacus*) leaf vegetable by small-scale farmers in Kenya is a viable business attracting many enterprenuers especially women (Onyango *et al*., 2008; Onyango and Imungi, 2007).

The cities are however supplied by urban and peri-urban production. In fact, it has again been established that 30% of residents in Nairobi practice urban agriculture with a majority of the farmers using untreated sewage to irrigate crop and fodder (Karanja *et al.*, 2010**)**. These farmers supplies urban and peri-urban parts of Nairobi.

Wastewater is widely being used as a low-cost alternative to conventional irrigation water (Qadir *et al.,* 2010). In these cities and towns, the most common growing sites are the road sides and river valleys in small gardens with or without irrigation as is the case in Ruai and Njiru. The vegetables are exposed to exhaust fumes from automobiles and to effluent from formal and cottage industries which often dump their effluent waste into the Nairobi River. Urban and peri-urban agriculture is an important source of food and income generation for many low income households in fact; it has been argued that the main reason why people are engaging in it is in response to inadequate, unreliable and irregular access to food supplies.

High levels of urban poverty paired with rising food prices, makes the formal urban food supply system unaffordable and inaccessible to the urban poor. The link between urban agriculture and food security has been recognized for many years (Atkinson, 1994; Gutman, 1987; Sanyal, 1987). Urban agriculture can contribute significantly in combating urban hunger and malnutrition by providing increased and more consistent access to fresh, nutritional food at lower cost than market purchases (The UNDP, 1996: 162). This has been observed in Nanyuki town in Kenya where the prices of horticultural crops have stabilized due to reclamations of garbage dumpsite by a farmer using wastewater to irrigate 50 acres of land and growing various horticultural crops and fodder, thus improving the access to fresh vegetables in the town (Kenya daily nation newspaper dated 8th July 2012). However, urban agriculture has its own constraints among which are poor ratings as an authentic urban land use, high biological, chemical and biochemical contaminants.

Cooking methods for traditional leafy vegetables in Kenya are varied. Amaranth are consumed cooked, often by boiling in large volume of water which is thereafter discarded, then stewed in onion, tomato, and oil or by direct stewing in onion, tomato and oil. The time it takes to cook the vegetable is also varied depending on the age of amaranth.

It has become apparently clear that gender analysis is important because productivity and efficiency are enhanced when interventions are targeted towards the actual users (Nyakudya *et al*., 2006; Muthoni and Nyamongo, 2009). There is a general conception that women are always at disadvantage in terms of access to productive resources, extension services, marketing information and credit and that they are not capable of doing similar farming activities as men but this need to be tested on case by case basis.

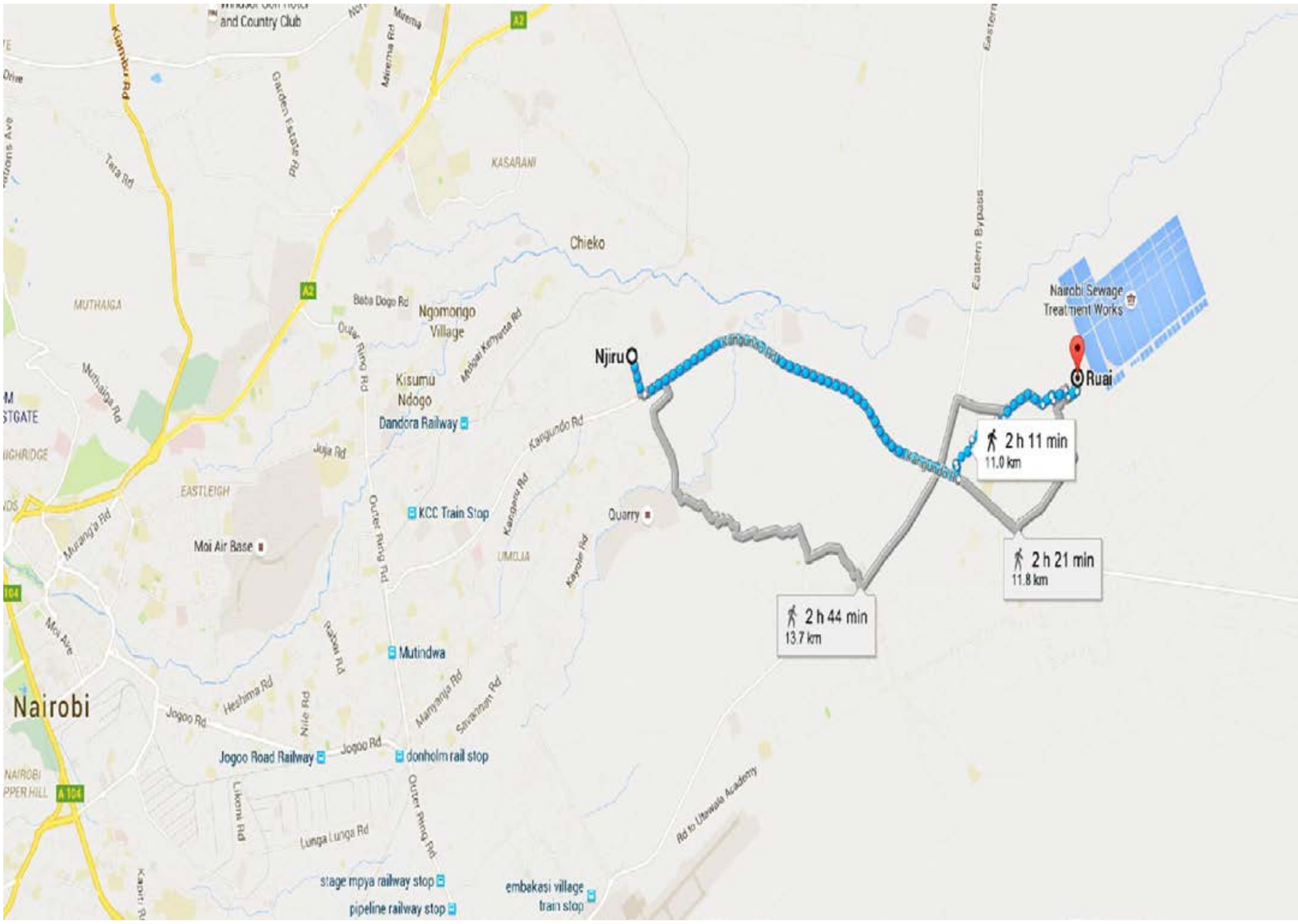
Gender analysis in urban agriculture is essential for policy formulation and programme planning to ensure equity in resource allocation and a balanced development that benefits both male and female urban dwellers (Danso *et al*., 2002). Female farmers in female headed households tend to limit their labour input in farm activities because of heavy commitment to reproductive roles such as nurturing and caring for children and attending to elderly members of the household. It turns out that in many cases, women use their land primarily for subsistence crops to feed their families, while men cultivates cash crops and keep the income (Kamara and Denkabe, 1993).

Therefore, urban agriculture seems to be dominated by females. There is little information on the pattern of gender-based occupational participation in urban agriculture and livelihood outcomes in Kenya hence this has made it difficult to recognize the importance of urban agriculture as a viable enterprise to be institutionalized and integrated into the urban planning, policy making, and programming agendas thus the objective of this study was to determine the socio-demographic characteristics of farmers and intermediaries, growing practice, markets and marketing channels and the methods of preparation for consumption of vegetable amaranth grown with sewerage conditions in Njiru and Ruai.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The study was carried out in Nairobi County, Kenya, in which Njiru and Ruai were purposively selected for this study because residents grow vegetables using effluent waters from residential and industrial places respectively and also because these two places are close to Nairobi market. They are among the eight locations in Embakasi division and its where all the industrial and domestic effluent in Nairobi is channeled to for recycling. Ruai coordinates are 1°15'10.25"S 37°00'24.46"E and is located about 25 Km East of Nairobi city centre while Njiru's coordinates are1°15'01.07"S 36°55'37.97"E. A map showing the location of these two sites from each other is as shown below.



3.2.2 Sample Size Determination and Sampling Procedure for the Baseline Survey-Farmers and Intermediaries

This study employed purposive sampling and simple random sampling techniques. Out of the nine districts in Nairobi County, Embakasi district was purposively sampled. Out of the eight locations in Embakasi district, two locations (Njiru and Ruai) were purposively selected because they grow vegetable amaranth. A baseline purposive field survey was conducted targeting the amaranth farmers and intermediaries in Njiru and Ruai of Nairobi County. A total of 49 farmers and 42 intermediaries from the two study areas were interviewed using a pretested, validated questionnaire with both open ended and close ended questions. These farmers were 20, and 29 from Njiru, Silancia area and Ruai, Kwa Maji area, respectively. The intermediaries were 20 and 22 from Njiru, and Ruai, respectively. The questionnaires sought information primarily on the socio-demographics, growing conditions of amaranth, markets and marketing channels of vegetable amaranth, harvesting and cooking method of vegetable amaranth. Also, field observations were made in the study areas.

Data collected was coded into Statistical Package for Social Sciences (SPSS, version 16.0), edited and then analyzed using descriptive and inferential statistics. The analyses results are presented in tables and figures.

3.3 RESULTS AND DISCUSSION

3.3.1 Socio-demographic Characteristics of Farmers and Intermediaries

As depicted in Table 3, majority of the farmers were women making 85% and 82% in Njiru and Ruai, respectively. Men made a proportion of 15% and 18%, in Njiru and Ruai, respectively. On the basis of age, 67.35% of the respondents are in the age of 40-50 years followed by 14.29% at 30-40 years and the least were those above 60 years making a 5%. Generally, 83.5% of the farmers are women while, 16.5% are men from both areas of the study.This is shown in Table 3.

For the intermediaries, 65% in Njiru and 68% in Ruai were of the age between 40-50 years followed by 20% and 27% of the age between 30-40 years and 5% in both Njiru and Ruai of the age above 60years. Most of the intermediaries are women, making 85% and 82% for Njiru and Ruai, respectively.

In terms of the years of formal education among vegetable producers and intermediaries in Njiru and Ruai, 78% have the basic education and only 2% have tertiary education. This implies that literacy level is low among producers and intermediaries. It is however a reflection of the general literacy rates of the populace in the study area, and these findings are consistent with those by (Idowu *et al*., 2012).

87% of the respondents are subsistence farmers cultivating between 1–3 hectares of land, and these makes 98% of the respondents who have no other source of income for their livelihood.

Table 3: Socio-demographic Characteristics of farmers and intermediaries

Variables	Njiru Farmers (%) n=20	Ruai Farmers (%) n=29	Njiru Intermediaries (%) n=20	Ruai Intermediaries (%) n=22
Age of Respondents				
Less Than 30	10	0	10	0
30-40	20	27	20	27
40-50	65	68	65	68
Above 60	5	5	5	5
Gender				
Male	15	18	15	18
Female	85	82	85	82
Educational Status				
Illiterate	15	0	0	0
Primary	55	69	100	86
Secondary	25	28	0	14
Tertiary	5	3	0	0
Size of Land in Hectares				
Less Than 3	80	93	N/A	N/A
3-6	20	7	N/A	N/A
Annual Income from the sale of amaranth				
Below 200,000	75	76	70	59
200,000-500,000	25	17	30	41
500,000-1,000,000	0	7	0	0
Other Sources of Income				
Yes	0	7	0	0
No	100	93	100	100
Consumption of amaranth grown/sold by themselves				
Yes	100	59	60	59
No	0	41	40	41

Our study findings are consistent with findings by (Idowu *et al*., 2012), who reported that 67% female and 33% male practice urban agriculture in Freetown in Sierra Leone, and in fact it was reported that urban vegetable production is dominated by women (Winnebah *et al*., 2006). This may be attributed to the profitability of vegetable production thus providing this gender with a degree of financial independence (IPGRI, 2003) relative to other income generating activities.

Majority of respondents (67%) are between the ages of 40-50 years. This age category represents the active labour force that is exploring urban agriculture as a livelihood option. Likewise the increase in demand has stimulated many entrepreneurs, especially women, to grow and trade in these vegetables on a small scale as shown in table 3**.** Therefore, it is evident that traditional vegetables play an increasingly important role in income generation, especially among small scale women farmers (Onyango and Imungi, 2007).

However, there are more respondents in the age categories of 40-50 years involved in urban farming than all the other age brackets as shown in Table 3**.** Possibly because of the profitability of vegetable production compared to other income generating activities and the demand effect of urban vegetable production for meeting household food security.

Open-space vegetable production in urban areas is dynamic, viable and largely a sustainable livelihood strategy, especially for poor urban dwellers (Dreschsel and Dongus, 2010), and there is continued realization of the importance of urban food production despite growing pressures on open space in the city (Hampwaye, 2008; Hampwaye *et al*., 2007). it is noted that an average household land holding size ranged from 0.125 to 3 hectares and a mean of 1.2 hectares in

Nyahururu town, Kenya down from 2.5 hectares reported by (Mandere *et al*., 2009).The same household land holding size was observed in this study.

On economic benefits of urban agriculture, 70% respondents earning ranged between Kes.1000 to Kes. 200, 000 per annum. This is perhaps because these vegetables are widely grown for subsistence and can offer a significant opportunity for poor households to generate income through commercial production. A similar observation was reported by (Onyango *et al*., 2008), and (Hillman, 2003) who concurs that subsistence agriculture confines its practitioners to grinding poverty and little dignity.

It was further found that 70% of the respondents consume amaranth growth in Njiru and Ruai even though they are aware that it is grown with effluent water and poses a health risk. A combination of factors including high levels of food insecurity in the cities and a change in official attitudes from intolerance to indifference may explain the high levels of urban household food production and consumption recorded (Crush *et al*., 2011). These findings concur with what was reported by (Frayne *et al*., 2010) that 70% of households obtain their food from informal sector and 68% from small retail and fast food outlets. However, it was found that 30% do not consume the vegetable, and this could be due to the facts that a third of a group of urban cultivators has emerged as small-scale entrepreneurs who engage in urban food production explicitly for sale rather than home consumption (Nugent, 2003).

3.3.2: Duration of Vegetable Amaranth Growing and Trading among the Farmers and Intermediaries in Njiru and Ruai.

66% of the respondents have been growing and trading in vegetable amaranth for between 10 to 30 years and specifically this comprised of 80% of the Njiru farmers (Table 4). 83% of the intermediaries have been trading in vegetable amaranth for between 5-10 years however, it was observed that in Ruai the oldest farmers have been farming for 13 years in contrast with Njiru where the oldest farmers has been farming for 30 years. This could be due to the cost of pumping water from the Nairobi River to the farms which is done in Ruai unlike in Njiru where irrigation is done by flooding hence rendering the amaranth production expensive and uneconomical. These findings concur with what was observed by (Drechsel and Dongus, 2010) that spatio-temporal analysis of urban agriculture in some West African cities shows that urban agriculture is not a short-lived or transitional phenomenon-probably as long as it can maintain its comparative market advantage, and the fact that vegetable farming along lowlands is the most prominent among producers (Idowu *et al*., 2012).

Table 4: Percentage response of farmers and intermediaries on the duration of growing and/or trading in amaranth

Duration of Growing/Trading in Amaranth (in Years)	Njiru farmers	Ruai farmers	Njiru Intermediaries	Ruai Intermediaries
1	0	4	0	9
2	0	18	0	5
3	0	14	10	0
4	0	3	0	0
5	5	4	30	9
6	0	29	10	27
7	10	0	10	9
8	5	7	10	18
9	0	0	5	0
10	25	14	15	23
12	0	3	5	0
13	0	4	0	0
15	15	0	5	0
17	5	0	0	0
18	5	0	0	0
20	25	0	0	0
30	5	0	0	0

3.3.3 Growing Conditions and Harvesting of Vegetable Amaranth in Njiru and Ruai

As indicated in table 5, all the farmers in Njiru use sewage or drain to irrigate their farms while all the farmers in Ruai use waste water from Nairobi River. The findings are consistent with observations by (Kaluli *et al.*, 2011; Karanja *et al.*, 2010; Sunday Daily nation 8th July 2012), that waste water is widely used in Kenya for irrigation of urban farms despite the health and environmental risks. Access to quality water, manure and compost of good quality is imperative to urban farmers. These are difficult to obtain although readily available in many rural set ups (RUAF Foundation, 2010).

Farmers in Njiru use channels or flooding method of irrigation, while 3% of farmers in Ruai use sprinkler irrigation and 97% use manual sprinkler methods of irrigation. On whether agricultural water comes in contact with the leaves, 66% of the Ruai farmers agreed with the statement compared to 5% of the Njiru farmers. This shows that a significant proportion of vegetable amaranth leaves in Njiru do not come into contact with water compared to those of Ruai. This is supported by the fact that in Ruai, they use sprinkler and manual sprinkler irrigation method unlike Njiru where flooding of the farms is done. On the soil type, 80% and 90%, in Njiru and Ruai respectively indicated that the soil is medium followed by medium fine. Synthetic/non-organic fertilizers are the commonly used by 83% of the farmers in Ruai followed by municipal biosolids which is used by 65% of the farmers in Njiru. On the susceptibility to of amaranth to diseases, 30% and 69% of the farmers in Njiru and Ruai respectively indicated that they are more susceptible than other conventional vegetables hence farmers in Ruai were found with more acreage under conventional vegetable than amaranth compared with the Njiru.

100% of farmers in Njiru concurred that the vegetable amaranth becomes ready for harvesting at the age of four weeks compared to 83% in Ruai. This information is summarized in the table 5.

From this study it was observed that the preferred method of harvesting vegetable amaranth was by picking leaves and shoots 100% and 93% and putting in sacks 75% and 100% in Njiru and Ruai respectively. This information has been summarized in the table 5.

Table 5: Growing Conditions and Harvesting of Amaranth in Njiru and Ruai

Variable	% Response in Njiru	% Response in Ruai
Source of Irrigation water		
Sewage/Drain	100	0
Nairobi River	0	100
Method of irrigation		
Channels/Flooding	100	0
Sprinkler	0	3
Pump/overhead	0	97
Agricultural Water Come in Contact with Amaranth Leaves		
Yes	5	66
No	95	34
Predominant soil Type		
Very Fine	5	0
Medium	80	90
Medium Fine	5	10
Coarse	5	0
No Predominant	5	0
Type of Fertilizer used		
Raw Animal Manure	25	0
Aged Animal manure	0	10
Municipal biosolids	65	0
Compost	10	4
None	0	3
Non-Organic/Synthetic	0	83
Overall Amaranth Disease Susceptibility		
As Usual	35	7
More Susceptible	30	69
Less Susceptible	35	24
Age of Vegetable Amaranth when is ready for harvesting		
3 Weeks	0	7
4 Weeks	100	83
5 Weeks	0	3
6 Weeks	0	7
Method of Harvesting		
Uproot/Cut the Plant	0	7
Pick Leaves and shoots	100	93
Packaging Container		
Polythene	25	0
Sacks	75	100

Amaranth grows quickly, require little input such as herbicides, and can be harvested within a short period of time after planting (Onyango *et al*., 2008). The method of harvesting ensures continuous supply of vegetable in the market hence making them useful in nutrition-intervention programmes (Okigbo, 1977) and contribution to food security and poverty reduction (Onyango *et al*, 2008) as was observed in this study. It is has also been recommended elsewhere that for greater nutritional benefit, *Amaranthus* should be grown under warm conditions and that leaves should preferably be harvested at young growth stages, 20 days after sowing (Modi, 2007) and that the nutrient contents of vegetables differ not only between species or cultivars but are also affected by stage of harvesting (Olsson *et al*., 2004).

3.3.4 Major Markets for Vegetable Amaranth based on the gender of the farmers and Intermediaries

The major markets of vegetable amaranth depend on the gender of the farmers and intermediaries as indicated in Figures 1 and 2.

There are more men who sell their vegetable to intermediaries than women and most of the intermediaries are female as shown in Figure 2 hence the number of female intermediaries selling amaranth to Kiosk/retail grocers and local individuals is higher than the males. This may be due to the fact that in developing countries, TLVs cultivation and trading is highly dominated by women and thus considered women entrepreneurship and as such men shy away from actively participating in trade in vegetable and prefer selling to intermediaries (Onyango *et al*., 2008).

The increase in entrepreneurs is due to a continued increase in demand of amaranth vegetables. The female farmers sell slightly more to local individuals (16%) than intermediaries and/or Kiosk/retail grocers (15%). This could be because local markets are the best option for the marginal and small farmers to dispose their perishable surplus to get quick returns (Khan and Khan, 2012). Inadequate transport and infrastructural facilities, makes most of the farmers prefer local markets instead of going to the specialized markets or near-by town area.

As presented in Figure 1 and 2**,** urban markets is also a system of direct marketing, which is essentially economical for both producer, sellers or intermediaries, and consumers in rural markets (Khan and Khan, 2012). The farmers get higher price for their vegetables realizing middlemen's or intermediaries' profit, sell relatively at lower price than the retail price prevailing in near-by markets. The intermediaries/kiosk/retail grocers, though not getting similar profit as the producer, also get handsome profit because they buy the vegetables from these urban farms at lower price.

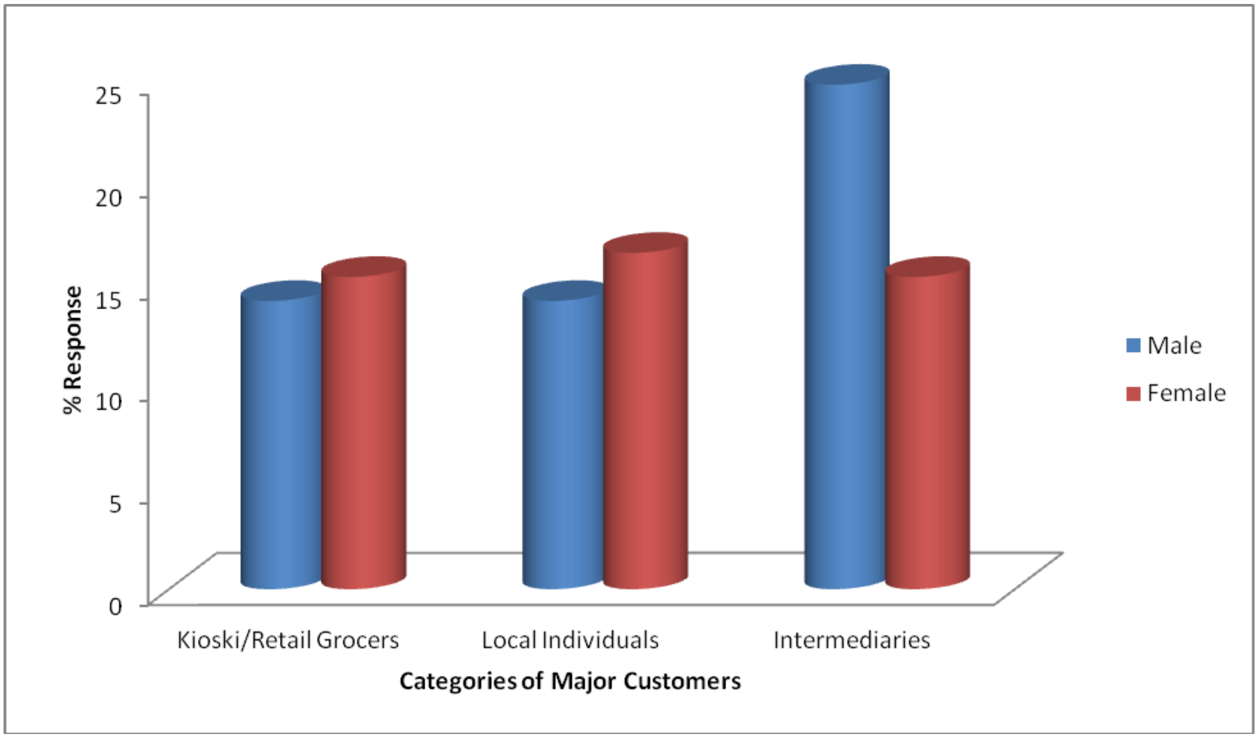


Figure 1: Major markets of vegetable amaranth for the farmers

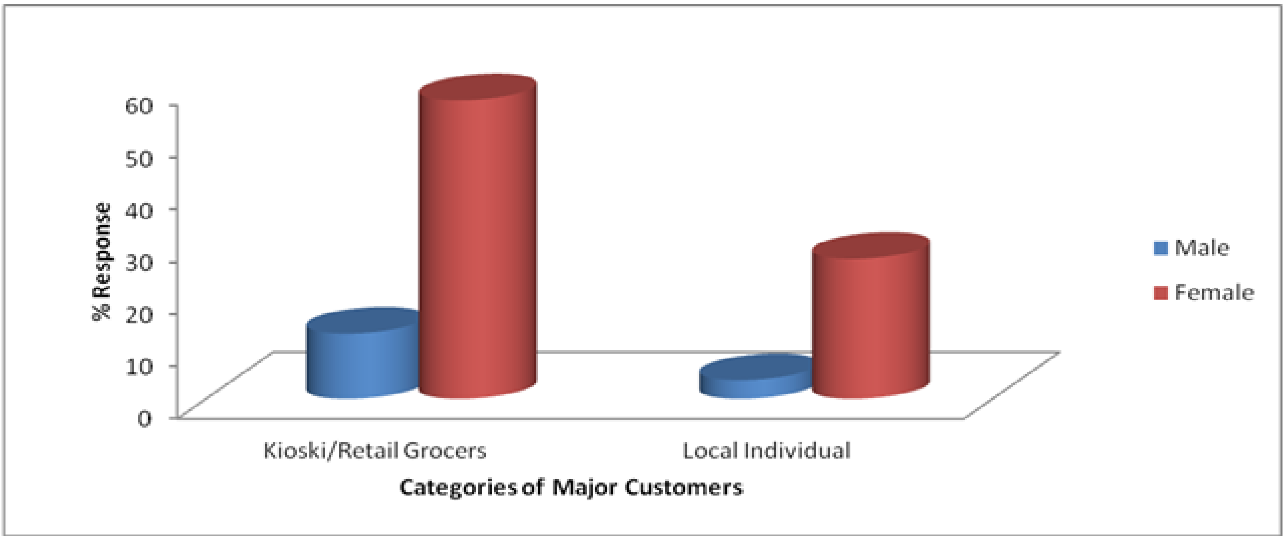


Figure 2: Gender Analysis of Intermediaries on Markets and Marketing Channels of Vegetable Amaranth.

3.3.5: Cooking Method and Time for Vegetable Amaranth by Farmers and Intermediaries

The most popular way of cooking vegetable amaranth among farmers is by direct stewing in oil, onions, and Tomatoes for 15 minutes according to 55% of the farmers as shown in Figure 3, while 36% of the intermediaries prefer direct stewing in oil, onions and tomatoes for five minutes and boiling without discarding the water then stewing for 5 minutes. Only 12% of the intermediaries prefer the farmers method of cooking that is prefer stewing for 15 minutes as shown in Figure 4.

Few farmers (12%) and intermediaries (4%) boil the vegetable in unspecified amount of water and discard the water then stew in oil, onions and tomatoes for 15 minutes (Onyango *et al.*, 2008; Imungi and Potter, 1983). Direct stewing or cooking of vegetables for 15 minutes has been recommended as a way to maintaining a good nutritional value (Joshua *et al.*, 2012). Therefore, with careful selection of good choice of cooking method, the nutritional potential of leafy vegetable can be fully harnessed. In all time durations of cooking, the method of cooking and discarding the water is practiced by the least number of the farmers and intermediaries.

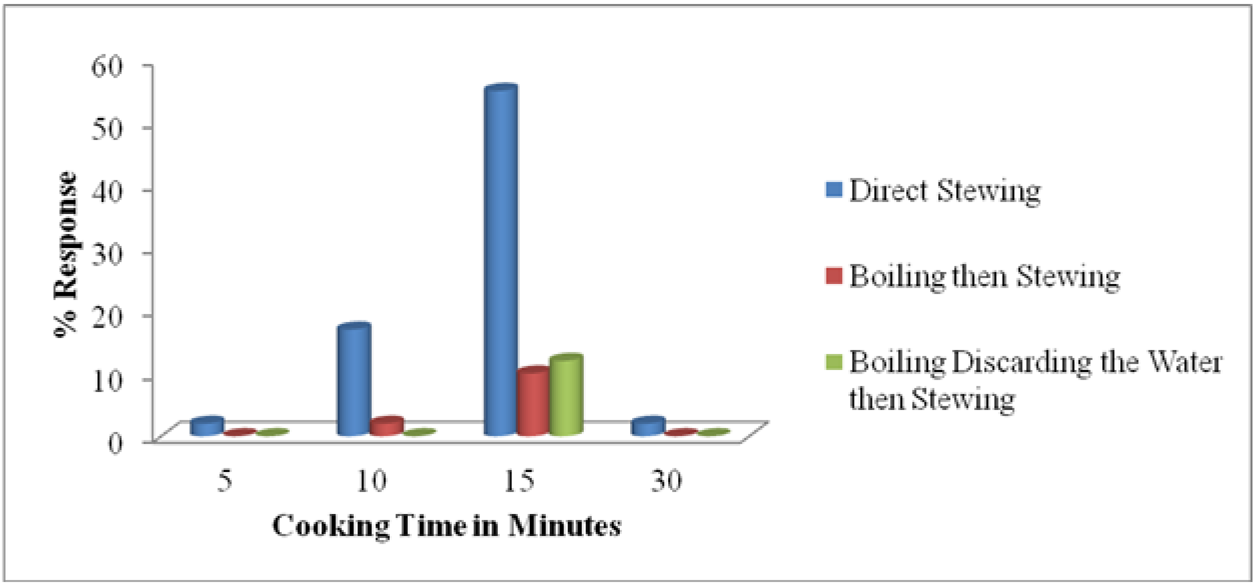


Figure 3.Cooking Method and Time for Vegetable Amaranth by Farmers

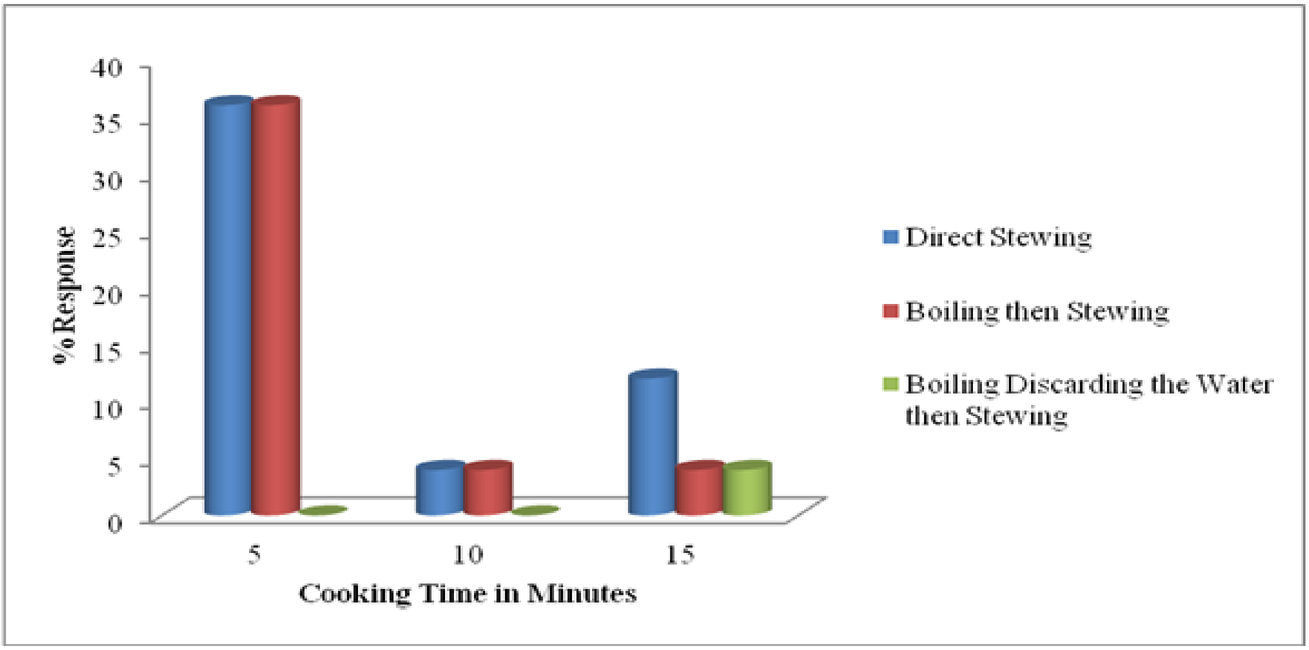


Figure 4: Cooking method and time for vegetable amaranth by intermediaries

3.4 CONCLUSION

In conclusion, this study shows that vegetable amaranth is a very important traditional food plants in Kenya. It can be harvested 3-5 weeks after sowing or the leaves can be picked continuously from growing plants and it is not affected by the waste water. Women in the age categories of 40-50 years in the active labour force are more involved in urban agriculture than men giving them a degree of financial independence and as such amaranth farming is a source of food security, income and employment for the people in urban and peri-urban locations of Nairobi.

It is evident from the findings of this study that amaranth farming is not a short-lived or transitional phenomenon –probably as long as it can maintain its competitive market advantage it’s there to stay and that waste water is widely used in Nairobi for vegetable farming even though it’s illegal.

Amaranth farming enhances livelihoods sustainability and land use among the urban poor and has provided a source of household food security since it does not require synthetic fertilizers and matures very fast even under effluent conditions.

The preferred method of cooking among the amaranth farmers and intermediaries in Kenya was identified in this study as stewing the amaranth vegetable in oil, onions and tomatoes for 15 and 10 minutes respectively hence maintaining a good nutritional value as was reported by (Joshua *et al.*, 2012).

3.5 RECOMMENDATIONS

Urban agriculture has been a response to increasing urbanization and the worsening economic situation of the urban poor especially in Kenya and with an increasing migration to the city. Since urban agriculture is there to stay then the implications of urban agriculture practices are that programme and legislation can be introduced to institutionalize urban agriculture, reduce the contamination of wastes, modify agricultural practices, and educate cultivators.

From this study, urban agriculture contributes immensely in elevating poverty and food insecurity among the urban poor hence should be legalized and incorporated into city plans.
Urban agriculture as an informal safety net strategy for the urban poor should not be overlooked or discouraged, but rather be supported by the national government. Also, support services, such as agricultural extension services and microfinance should be included in the policy and directed to urban agriculture practitioners to enhance the biological safety of these vegetables.

Therefore, the Government should invest more on education of the farmers, intermediaries and the public since development in all spheres of life including urban agriculture hinges on education of the people.

The commercial production of vegetable amaranth by small-scale farmers in Kenya is a viable business attracting many entrepreneurs. Women involvement in agriculture is on the increase compared to men as depicted in the study; hence they should be given appropriate types of technology to cater for the labour intensive farm activities, good financial support and access to more farm land through appropriate land reforms.

CHAPTER FOUR

Nutritive Value and the Levels of Cadmium and Lead in Raw and Cooked Vegetable Amaranth Grown with Effluent Water: A case of Ruai and Njiru

ABSTRACT

The increase in demand for food and unemployment has forced people especially of the low incomes and dwelling in peri-urban and urban centers to utilize areas not meant for farming and untreated waste water in vegetable production. Vegetables grown with effluent conditions in various parts of Nairobi city are thought to be heavily contaminated with heavy metals. The current study investigated the nutritive value, and the levels of lead and cadmium in vegetable amaranth grown with effluent condition. On the nutritive value the proximate composition, anti-nutritive content, and micronutrients contents of raw and cooked *A. cruentus* and *A. lividus* was determined using standard methods. All data was treated by analysis of variance (ANOVA) using Genstat statistical software version 14.1.The treatment averages were compared using the least significance test (LSD) at the level of 5% of the probability. The Moisture content of vegetable amaranth ranged from 81.03% to 84.69% crude protein varied from 22.33% to 36.46%, Total ash ranged from 21.23% to 9.75%, crude fibre ranged from 29.03% to 11.57% .The crude fat and total soluble carbohydrate ranged between 0.36% to 1.23% and 6.55% to 36.86% respectively. Vitamin A amount ranged from 10.8 mg/100g to 53.8mg/100g; vitamin C was 98.5 mg/100g. The levels of oxalate and nitrates ranged between 1649.3 mg/100g to 2452.4 mg/100g and 1856.6 mg/100g to 3080 mg/100g, respectively. Cadmium was not detected in vegetables, however in the soil the levels ranged between 62.3 ppb to 97.85 ppb. Lead in vegetable was between 2.8 ppm to 5.1 ppm, in water 2 ppm to 4.5 ppm, and in soil 3 ppm to 34.1 ppm. Amaranth grown with effluent conditions is a good source of protein, vitamin A and

minerals especially calcium hence can contribute to alleviating nutritionally related problems in Kenya.

However, the levels of lead were above the maximum allowed limits of 2ppm in the Kenya standard specification for dehydrated vegetables (KS 435:2012) and cadmium was not detected. Cooking did not significantly affect the levels of nutrients and antinutrients and the levels of the heavy metals in vegetable amaranth hence with careful selection of good choice of cooking method and the growing environment; the nutritional potential of vegetable amaranth can be fully harnessed.

4.1 INTRODUCTION

Most of the developing nations, Kenya included depend on starch-based foods as the main staple food for the supply of both energy and protein. This accounts in part for protein deficiency which prevails among the population according to Food and Agricultural Organization (FAO) (Akubugwo *et al*., 2010). The WHO recommends a minimum daily intake of 400 g of fruits and vegetables (WHO, 2003). The recommended total daily intake is equivalent to five (5) servings of 80 g each of fruits and vegetables (FAO/WHO 2004, FAO, 2003).

Traditional vegetables have been reported to play a very important role in subsistence and generation of income (Schippers, 2000). They provide low-cost quality nutrients for large parts of the population in both rural and urban areas. Vegetables are the cheapest and most readily available source of important proteins, vitamins, minerals and essential amino acid (Akubugwo *et al*., 2010). One example is amaranth, which contains more of these nutrients compared to a typical exotic leafy vegetable e.g. white cabbage (Weinberger and Msuya, 2004). The leaves are highly nutritious with high levels of protein, iron, vitamin A and C among other nutrients. The levels of other vitamins including thiamine, niacin, and riboflavin, plus some dietary minerals such as calcium, iron, potassium, zinc, copper, and manganese cannot be ignored. Amaranth protect against several disorders such as defective vision, respiratory infections, recurrent colds, retarded growth, functional sterility, bleeding tendencies, leucorrhoea, and premature ageing (Bakhru, 2007). It is readily available during rainy and dry seasons, is affordable and accessible to the local population.

Amaranth requires little stress in preparation and can conveniently accompany any meals. It is eaten at any time of the day as an accompaniment for all carbohydrate dishes in Kenya. Amaranth therefore, has great potential to improve nutrition, boost food security, foster rural development and support sustainable land care and as popular vegetable in the diets of Kenyans to contribute significantly to provision of micronutrients (Onyango *et al.*, 2008).

Although amaranth is a valuable source of nutrients its consumption may pose some health risks due to accumulation of anti-nutrient factors such as nitrates and oxalates, and possible contamination with toxic materials such as lead due to poor cultivation practices and/or environment (Onyango *et al*., 2008). It has been reported that 90% of the supply of the vegetable amaranth to various outlets in Nairobi is from farms that are within the environs of the urban and have been cultivated using untreated waste water (Onyango *et al.*, 2008). These vegetable is being grown in all types of available land including abandoned waste dumping sites, banks of polluted rivers, alongside roads, areas enclosing waste water ponds in Kenya as with the case in Njiru and Ruai.

The use of waste water poses a threat to quality and safety of vegetables because excessive heavy metal contents in foods has been associated with etiology of a number of diseases, especially cardiovascular, kidney, nervous system and bone diseases. It has been reported that vegetable amaranth has a way of concentrating metals in their tissues and or that aerial deposition may be a major source of contamination (Atayese *et al.*, 2009). There are about two and one major commercial species in cultivation in Njiru and Ruai, respectively.

On the other hand, several vegetables cooking methods are used without taking into account their effect on the vitamins, anti-nutrients, heavy metals and minerals compositions. The amaranth leaves are a good source of protein, dietary fiber, iron, useful fats, among others and should contain no toxic elements such as lead and cadmium and anti-nutrients such as oxalates and nitrates. Many of the local vegetable are under-exploited because of inadequate scientific information on the nutrients and toxic elements present in their matrices. The current study evaluates the contents of nutrients, anti-nutrients, and heavy metals in raw and cooked amaranth leaves grown with effluent conditions in Njiru and Ruai. It further relates the levels of Pb and Cd with those in the water and soil in order to ascertain the relationship between bioaccumulation in amaranth. The information obtained from this study is used in ensuring that the quality and safety of the amaranth vegetables consumed by the residents of Nairobi and its environs is known and required legislation is formulated with regard to the usage of untreated waste water for growing crops.

4.2 MATERIALS AND METHODS

4.2.1. Sample Size Determination and Sampling Procedure for Soil, Water and Vegetable Amaranth Analyses

From Njiru and Ruai all the farms that are growing amaranth were selected. Sampling frame was obtained in which the number of farms growing the commercial species of the vegetable amaranth was established. Due to small farm sizes with mixed cropping it was not possible to obtain adequate samples for analysis from each individual farm. Therefore the sampling site in Njiru, Silancia was divided into two sites based on the geographical terrain. These two sites are; Njiru highlands as study site A and Njiru lowlands as study site B.

Two amaranth species which were predominately cultivated in Njiru were sampled. These were *Amaranthus cruentus* and *Amaranthus lividus.* In each study site three samples of each species was collected for analysis. In Ruai, (Kwa Maji site) it was found that only *Amaranthus lividus* was predominant and therefore selected for the study, the Kwa maji site was also divided into study site A and B based on the acreage. The farms were triangulated and from each farm three samples of 600-700 g of vegetable amaranth were picked for analysis.

Water samples in Njiru were collected from the source and along the channels by ensuring sampling bottled is submerged into the water. In Ruai, water sample was collected from Nairobi River; this was done by allowing the pumped water to irrigate the farm for 30 minutes after which a sample was taken.

Clean, dry, air tight plastic bottles were used in collection of water and soil samples. The samples were taken to the laboratory in a cooler box and stored at 4°C for analyses.

Soil samples we collected according to methods described by (Ordonez *et al*., 2003) with slight modifications. In summary, soil samples up to 15 cm deep from the ground surface were collected at several locations in a zig-zag pattern to ensure homogeneity. A spade was used in making a ‘V’ shaped cut to a depth of 15 cm then a thick slices of soil from top to bottom of exposed face of the ‘V’ shaped was cut and placed in a clean bucket, up to 16 samples were collected from each sampling unit. The samples were thoroughly mixed and foreign materials like roots, stones, pebbles and gravels removed. The bulkiness of the samples was reduced to 500g by dividing the thoroughly mixed soil samples into four equal parts. The two opposite quarters were discarded and the remaining two quarters were remixed and the process repeated until the desired amount was obtained. Soil from fields with similar physical appearance, production and past-management practices were grouped into a single sampling unit. Soil sampling was avoided in dead furrows, wet spots, areas near trees, manure heaps and irrigation channels the surface liter at the sampling spot was removed.

Total Number of samples collected for analysis is as follows: Three (3) samples of water was taken in Ruai while six (6) in Njiru because of the two sites and three (3) soil samples were collected from Ruai while in was six (6) making a total of 18. For the vegetable amaranth, a total of 18 vegetables were collected as shown in the sampling frame in figure 5.

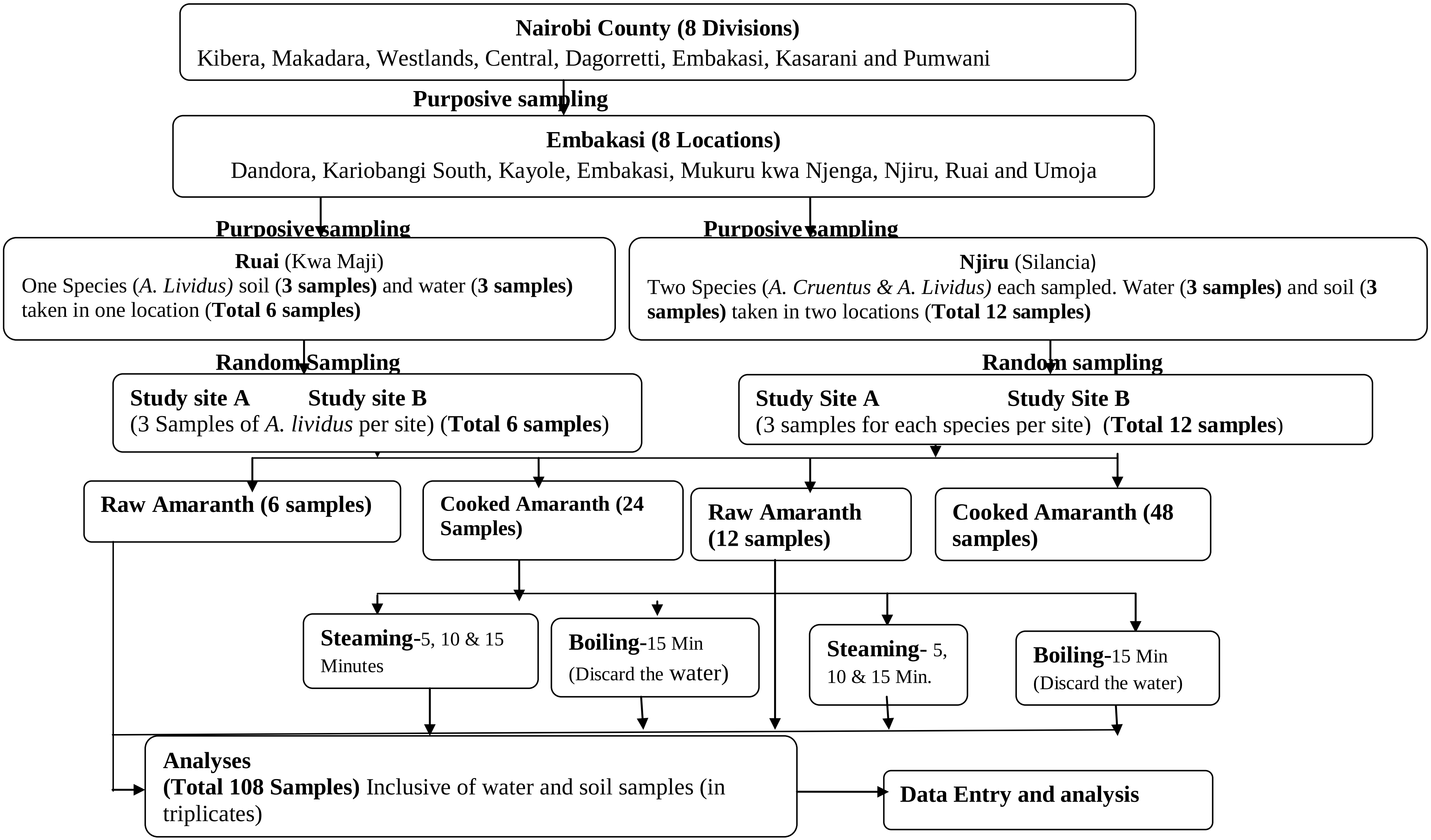


Figure 5: Schematic representation of the sampling procedure

4.3 COOKING OF THE VEGETABLES

Leaves of the amaranth which forms the edible part of each batch was quantitatively separated from the hard stalks and other inedible parts using bare hands. The samples were then coded as follows:

ACNA means *Amaranthus Cruentus* collected in Njiru site A

ACNB means *Amaranthus Cruentus* collected in Njiru site B

ALNA means *Amaranthus Lividus* collected in Njiru site A

ALNB means *Amaranthus Lividus* collected in Njiru site B

ALRA means *Amaranthus Lividus* collected in Ruai site A

ALRB means *Amaranthus Lividus* collected in Ruai site B.

Each fraction was thoroughly mixed, washed in running tap water, rinsed with distilled water and then samples prepared for analysis as follows:

The raw sample: The leaves were drained, analyzed for moisture content and then air dried in a relatively dark room for two weeks. No heating was done (raw).The leaves were then ground to powder using pestle and mortar and stored in clean oven dried air tight plastic bottles.

5 minutes: The leaves were drained and then steamed in a pressure cooker for 5 minutes using a gas burner. The leaves were then air dried in a relatively dark room for two weeks and prepared for analysis as the raw sample.

10 minutes: The leaves were drained and steamed in a pressure cooker for 10 minutes using a gas burner. The leaves were then air dried in a relatively dark room for two weeks and prepared for analysis as the raw sample.

15 minutes: The leaves were drained and steamed in a pressure cooker for 15 minutes using a gas burner. The leaves were then air dried in a relatively dark room for two weeks and prepared for analysis as the raw sample.

15 minutes and cooking water discarded: The leaves were drained and then submerged into water and boiled for 15 minutes using a gas burner, the cooking water was discarded, the leaves were again drained and air dried in a relatively dark room for two weeks and prepared for analysis as the raw sample. The moisture content of the dried samples was determined during the analysis for each sample. Each sample was analyzed in triplicate.

4.4 ANALYTICAL METHODS

4.4.1 Determination of Proximate Composition

4.4.1.1 Determination of moisture content

The moisture content was determined using (AOAC, 1999) method by drying in an air oven at 105^{o}C to constant weight.

4.4.1.2 Determination of total ash content

Total ash was determined by AOAC methods (AOAC, 1999).

4.4.1.3 Determination of crude fat

Crude fat was determined by AOAC methods (AOAC, 1999) using Soxlet extraction unit with reflux rate of about 10ml per minute.

4.4.1.4 Determination of crude fibre

Crude fiber was determined as loss on ignition of dried residue by incineration after acid and base digestion according to the AOAC method (AOAC, 1999).

4.4.1.5 Determination of crude protein

Crude protein was determined as total nitrogen by the semi-micro Kjeldahl method. The percent nitrogen was multiplied by an empirical factor of 6.25 to convert it to percent protein (AOAC, 1999).

4.4.1.6 Determination of total soluble carbohydrate

The total soluble carbohydrate content of the vegetable amaranth was calculated as the difference between 100 and the sum of the moisture, ash, crude fat and crude protein contents (Eyeson and Ankrah, 1975).

4.4.2 Determination of Iron, Zinc, Calcium, Lead and Cadmium in Amaranth Vegetable

The contents of iron, zinc, Calcium, lead and cadmium in vegetable amaranth was determined in all the samples by using microwave plasma atomic emission spectrometer (An Agilent model 4100 MP-AES) from Agilent technologies. The wavelengths were selected using the software, nebulizer pressures were optimized automatically using the Agilent MP Expert software and automatic sample introduction mode was used. For analysis approximately 0.5g of sample was weighed into a porcelain crucible. Then placed on a hot plate to char until all the organic material had burnt out, ashed at 600°C in a muffle furnace for five hours, removed and left to cool to room temperature. 2ml of conc. HNO_3 for extraction was then added cautiously, the sample was then filtered into 100ml volumetric flask: rinsed the filter paper (what man 540) and topped up to the mark with distilled water. Finally, an Agilent 4100 MP-AES was used for the determination of analytes in samples against their standards. The concentration of the metals in the plant samples was calculated as follows:

Formula for calculating (Zn, Fe, Ca, Pb, Cd concentrations) = (reading-blank) × Vol. × **DF**/weight of sample.

Where:

Reading- reading of the plant sample given by the instrument (MP-AES)
Blank - The reading of the blank that was determined just before the plant sample.
Vol-Volume of the sample solution used

DF-dilution factor

4.4.3 Determination of Cadmium and Lead in soil

The metals and minerals in soil were determined as in 4.4.2 by using microwave plasma atomic emission spectrometer (An Agilent model 4100 MP-AES). The sample was ground to fine powder and air dried in an oven at 100^{o}C for 1hr to remove moisture, then 1g of sample was weighed and 10 mL of extracting solution 1:1 HNO3: HOH was added in a 250 ml beaker. The sample was then heated to 95 ± 10 °C for about 15 minutes covered with a watch glass. The digest was allowed to cool, before 2 mL of deionized water and 3 mL of 30% H_2O_2 was added and heated to 95 ±5 °C. The digest was cooled again, another 1 mL f 30% H_2O_2 was added. Heating continued until the sample volume reduced to approximately 5 mL. The digest was then allowed to cool. Filtration was done using Whatman 540 filter paper then made to 100 ml using deionized water in a volumetric flask. Finally, an Agilent 4100 MP-AES was used for the determination of analytes in samples against their standards

4.4.4 Determination of Cadmium and Lead in water

Lead and cadmium in water was determined using microwave plasma atomic emission spectrometer (An Agilent model 4100 MP-AES). For analysis, the water sample was filtered with Whatman No.540 filter paper into 25 ml standard flask, the filtered water was then acidified with Concentrated HNO_3 to break down the matrix, and then analyzed.

4.4.5 Determination of Vitamin A

Vitamin A was determined using HPLC –photo diode array detector waters 2998 model from waters corporation, 34 Maple street, Milford MA 01757 USA by the measurement of all trans-retinol and 13-cis retinol using a method previously described by (Karau *et al*., 2012) with slight modification. 1 gram of the plant powder was dissolved in 50ml methanol in a 250ml ground-neck round bottom flask. To the mixture 0.25g of ascorbic acid was added and 5ml of 50% sodium hydroxide .The mixture was blanketed with nitrogen and saponified in water bath at 60^{o}C for 1 hour with intermittent shaking after every 20 minutes. After saponification, the flasks were cooled in running stream of cold water. Then 50 ml of NaOH was added to the sample to avoid emulsion and the mixture transferred into separating funnel.

Retinol was extracted from the saponified sample solution using 100 ml of n-hexane containing 30 ppm butylated hydroxylated toluene (BHT). For optimal extraction, the separating funnel was gently shaken while avoiding pressure build up by releasing the stop cork. The two phases were allowed to separate and the aqueous phase drained to the round bottomed flask and the n-hexane layer into a conical flask covered with aluminium foil. The procedure was repeated two times with 50 ml of n-hexane containing 30 ppm BHT. The combined extracts were washed to neutral with distilled water and pH was determined with phenolphthalein indicator. The extract was evaporated in a rotary evaporator under pressure and temperature below 50^{o}C. The remaining extract was reconstituted in 10 ml of methanol, filtered with a 0.45 µm syringe filter and 20 µl injected into HPLC for determination of retinol. 100 mg of vitamin A-palmitate standard (Fluka, of 98% purity) was dissolved in absolute ethanol. The samples were analyzed for vitamin A with HPLC-Photo diode array detector. The mobile phase was prepared by mixing methanol and HPLC grade water in the ratio of 77:3. The mobile phase was sonicated and then filtered into a reservoir ready for use. The flow rate was set at 1.5 mL/min, column oven temperature at 20.01^{o}C and detector wavelength of 325 nm. Run time was 4.5 min and elusion at 3.15-3.6.the pressure was set at 208.3and Waters spherisorb ODS-1 column (RP C18) of particle size 5µ, 250mm long and internal diameter 4.6mm was used. The column was balanced with mobile phase until a good baseline suitable for analysis was obtained. Concentration of vitamin A in the plant samples was calculated based on the calibration graph over the linear range using the formula:

Total retinol (mg/Kg) =Cs× V_T**×DF/m**

Where: **Cs** is the concentration in µg/mL from the calibration curve.

V_T is the volume of the sample test solution in Ml

DF is the dilution factor

m is the mass of the test portion in grams

4.4.6 Determination of Ascorbic Acid

Ascorbic acid was determined in the extracts as total L+ and D+ ascorbic acid with HPLC-UV method using UV-VIS detector model SPD -20AU, Shimadzu, Japan and isocratic flow by a method described by (Karau *et al*., 2012). Phenomenex column (C_{18}) 175×3.20 mm× 5µ internal diameter at 265nm wavelength, flow rate of 2.0 ml/minute and oven temperature of 25^{o}C was used. Serial dilution of the ascorbic acid standard 99.7% pure (Pancreac, Spain) was prepared at a concentration range of 4.89-24.53mg/Kg and the linearity of the calibration curve, the limit of detection and quantification were determined from the standards (annexed).Then 5µL of the samples were injected into the HPLC and peak areas recorded for the determination of the concentrations.

4.4.7 Determination of Oxalates

The oxalate was determined by calcium oxalate precipitation method according to (Adeniyi *et al.*, 2009) with slight modifications. Briefly, 0.1 g of the sample was extracted with 30 ml 1N HCL by shaking in a water bath at 100°C for 30 minutes. The pH of the filtrate was adjusted to over 8 with 8M NH_4OH and adjusted back to pH of between 5.0-5.2 with 6N CH_3COOH. Precipitation was then carried out with 5% calcium chloride and allowed to settle at room temperature overnight.

The suspension was then centrifuged at 6000 rpm (centrifuge, model Hettich EBA 20; D78532, Tuttlingen, Germany) for 20 minutes. The supernatant was discarded and the pellet washed twice with 2 ml 0.35M NH_4OH then drip dried the cake and dissolved it in 0.5M H_2SO_4. The resultant solution was titrated with standardized ammonium oxalate solution 99.5% pure with 0.02M $KMnO_4$ in a water bath at 60°C to faint violet colour that was stable for at least 15 seconds after which the burette reading was taken. The oxalate content was evaluated from the titer value. The overall redox reaction is:

$$2MnO_4^{-} + 5C_2O_4^{2-} + 16H^{+} \longrightarrow 2Mn^{2+} + 8H_2O + 10CO_2$$

4.4.8 Spectrophotometric Determination of Nitrates

The samples were ground to fine powder, and then re-dried in an oven at 70°C overnight. Then 0.3g of the sample was weighed and suspended in 30 ml distilled water. The suspension was incubated at 45°C for 1 hour to allow complete leaching of the nitrate and then filtered through Whatman No. 540 filter paper. The filtrate was analyzed for nitrate-N according to the method described by (Cataldo *et al*., 1975). The concentration of nitrates-N was determined by reading the absorbency in a UV-VIS Spectrophotometer (model UV-1700 Phamaspec, Shimadzu, Japan) at 410nm using a 1 cm QS cell.

4.5 DATA ANALYSIS

All data was treated by analysis of variance (ANOVA) using Genstat statistical software version 14.1.The treatment averages were compared using the least significance test (LSD) at the level of 5% of the probability.

4.6 RESULTS AND DISCUSSIONS

4.6.1: Proximate Composition of Raw *Amaranthus cruentus* and *Amarantus lividus* Leaves

As depicted in table 6, the proximate composition parameters were found to significantly vary from one location to another. The parameters with low variations were the moisture content and crude fat, while the rest varied greatly. From all the locations the moisture content varied between 81.60 to 84%, an indication of high water content in the flesh of the vegetables.

Generally, vegetable amaranth in Njiru had higher moisture content than that from Ruai probably because it cost nothing to flood the farms in Njiru but cost money to pump water in Ruai.

The crude protein contents varied between 22.33% in *A. lividus* in Ruai site A to 36.46% in *A. lividus* in Njiru site B (Njiru lowlands). The two values are significantly different $p<0.05$ from each other, as shown in table 6. The values for *A. cruentus* in Njiru B was comparable to that of Ruai amaranth but was significantly different $p<0.05$ from that of A. *lividus* in Njiru A and B. Generally, amaranth from Njiru had higher protein content than that from Ruai probably because the effluent water used in irrigating farms in Njiru is from domestic household while in Ruai is mainly from industrial use. The total ash ranged between 21.23% in *A. cruentus* from Njiru A to

9.75% in *A. lividus* from Ruai B. The values for different locations were significantly different $p<0.05$ from each other. Since total ash is a measure of the total amount of minerals within a food when moisture content and organic component has been removed then this study shows that amaranth in Njiru had more minerals than in Ruai.

The crude fibre contents of the vegetables varied between 29.03% in *A. lividus* from Njiru B to 11.57% in A. cruentus from Njiru A. The values for Ruai A were not significantly different $p>0.05$ to those of Ruai B but were significantly different $p<0.05$ from the value for Njiru. It observed that amaranth from Njiru with low crude fibre reported higher values of soluble carbohydrates and vice versa except in Ruai. The values for crude fat ranged between 0.36 % in *A. cruentus* from Njiru A to 1.23 % in *A. lividus* from Njiru B. The crude fats content in Ruai A and B were comparable. The value for *A. cruentus* differed significantly $p<0.05$ with each other while the values for Njiru B were comparable $p>0.05$. The soluble carbohydrate contents of the amaranth leaves ranged from 6.55% to 36.86%. The values differed significantly $p<0.05$ in Ruai and Njiru and also from each other.

The results obtained in table 6 shows that location of production had significant effect on proximate chemical composition parameters except for moisture content and crude fat contents, consistent with findings by Mziray, (1999). There is a significance difference in percentage moisture content in amaranth grown in Ruai and similarly among those obtained from Njiru. The moisture content of the two amaranth species is very high above 80%. High moisture content of vegetable amaranth has been reported elsewhere (Imaobong, 2013; Kwenin *et al*., 2011; Onyango *et al*, 2008; Asffiev-Berko and Tavie, 1999; Mziray, 1999), and this indicates that the vegetables are prone to deterioration hence need care for appropriate preservation. The high water content of amaranth when eaten raw helps the body as the body does not need to use some of its own water to digest them. This means that the body uses less energy and resources to digest the greens and can then assimilate all the nutrients of the vegetables much faster. Less pressure is therefore put on the digestive system (Lussier, 2010).

The findings on crude protein are consistent with similar study findings on amaranth (Imaobong, 2013; Kwenin *et al*., 2011; Onyango *et al*., 2008; Asffiev-Berko and Tavie, 1999; Mziray 1999). However, our findings demonstrate lower crude protein compared to 49.02% reported elsewhere in *A. hybridus* (Asaolu *et al*., 2012). This makes the plant advantageous as a rich source of vegetable protein over some vegetables such as *Solanum nigrum* with 5.6% crude protein dry weight (Asffiev-Berko and Tavie, 1999).

The ash content values observed in this study are comparable to other study findings such as that of *Amaranthus hybridus* leaves that reported 19.61% (Nwaogu *et al*., 2000); 18.6 -20.9% *Amaranthus Hypochondriacus* leaves (Onyango *et al*., 2008); 14.4% *Amaranthus* incurvatus (Asffiev-Berko and Tavie, 1999; Kwenin *et al.*, 2011); 23.64% *Amaranthus hybridus* leaves (Imaobong, 2013) among others. The high ash content in is an indicator that the leaves are rich in mineral elements.

The crude fiber content in the amaranth is consistent with similar findings (Kwenin *et al*., 2011; Onyango *et al*., 2008; Asffiev-Berko and Tavie, 1999). Dietary fibre is important because it helps to reduce serum cholesterol level, risk of coronary meat disease, colon and breast cancer and hypertension (Gauong, 2003) and vegetable the amaranth is a good source.

Table 6: Proximate chemical composition of raw *Amaranthus cruentus* and *Amaranthus lividus* leaves[1] expressed as a percent of on dry matter basis

Location of Production	Moisture Content	Crude protein (N× 6.25)	Total ash	Crude fibre	Crude fat	Soluble Carbohydrates
ACNA	83.06±0.6b	32.85±0.60bc	21.23±0.03g	11.57±0.19b	0.36±0.01a	25.39±0.46d
ACNB	84.69±0.83b	30.06±0.08a	20.06±0.01e	26.51±0.12c	1.19±0.01c	10.48±1.02a
ALNA	81.83±1.0b	32±1.73b	15.44±0.00c	27.29±0.10d	1.21±0.02c	15.89±0.23b
ALNB	83.41±0.78b	36.46±0.03d	18.11±0.00d	29.03±0.08e	1.23±0.04c	6.55±0.89c
ALRA	81.03±0.4a	22.33±1.72a	10.19±0.58b	21.35±0.02a	0.41±0.02b	36.86±0.08e
ALRB	81.60±0.05a	23.06±0.93a	9.75±0.83a	21.33±0.05a	0.41±0.02b	36.25±0.12e

[1] Values are means of three determinations ± standard deviation

Values with the same letters in the same column are not significantly different at 5% level of significance $p>0.05$

The Crude fat value observed in this study is greater than 0.3% reported for *spinach* leaves and lower than1.60% reported in *Amaranthus hybrids* leaves (Nwaogu *et al*., 2000) and compares well with reported values in *Amaranthus* incurvatus by (Asibey-Berko and Tayie, 1999). Fat in food is the principal source of energy.

The soluble carbohydrates contents compares favorably with 23.7% reported in *Amaranthus* incurvatus (Asibey-Berko and Tayie, 1999); 10.40% in *A. cruentus* (Kwenin *et al*., 2011); 3.36% in *Amaranthus hybridus* (Asaolu *et al*., 2012).The low Carbohydrate value in the sample is due to high level of protein content (Asaolu *et al*., 2012). The values are lower than 54.20% reported for water spinach leaves (Asibey-Berko and Tayie, 1999). Carbohydrate is an important source of energy and contributes to the sweetness, appearance and textural characteristics of many food substrates and can be boosted with foods that are rich in carbohydrate.

4.6.2: Vitamins Contents of *Amaranthus cruentus* and *Amaranthus lividus* Leaves

The amounts of vitamin C was determined as ascorbic acid and vitamin A as retinol in the leaves of *amaranthus cruentus* and *amaranthus lividus*. The results of vitamin A are shown in table 7.

4.6.2.1: Amounts of vitamin A

As demonstrated in table 7, the highest levels of vitamin A in mg/100 g dry weight of the leaves were observed when the vegetables were boiled for 15 minutes and the cooking water was discarded. It was found that amounts of vitamin A were not significantly different $p>0.05$ for vegetables from Njiru and Ruai, however significant differences were observed with different cooking time at ($P<0.05$). It was established that regardless of the species and collection site, the levels of vitamin A increased with increase in cooking time.

The amounts of vitamin A from raw amaranth vegetables are consistent with those reported elsewhere (Mziray, 1999). However, a*maranthus cruentus* had higher levels of vitamin A in raw samples compared to *amaranthus lividus* but with small increment in percentages of 2-5% in uncooked and cooked vegetables. *Amaranthus Lividus* sampled from Ruai had the highest percentage increase in the concentration of vitamin A of about 157-166% in the uncooked and the cooked samples. These differences in the concentrations of vitamin A may be related to species, location, degree of maturity at harvest, cultivation, and post- harvest handling practices (Chen and Chen, 1992). The increase in the concentration of vitamin A in cooked samples has been reported by many studies (Sungpuag *et al*., 1999; Grimme and Brown, 1984; Braumann *et al*., 1982; Anderson *et al*., 1978), which demonstrated that carotenoids in plants are bound by protein, and heat treatments help to release bound carotenoids and enable them to be extracted easily.

As observed in this study increase in vitamin A with increase in cooking time could be attributed to the change in tissue morphology allowing greater penetration of organic solvents into the cells and enhances release of β-carotene. The high percentage increase in the levels of vitamin A observed from the vegetables collected from Ruai could be attributed to the system of irrigation. This is because waste water comes into contact with the leaves and as such the leaves become highly contaminated with microorganisms hence the cooking heat may not have destroyed all them therefore during drying, microorganisms may have aided in breaking down the plant matrix further by digestion, and thus more vitamin A was extracted.

Table 7: Mean levels of vitamin A (mg/100g dry weight) of *Amaranthus cruentus* and *amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	ACNA	ACNB	ALNA	ALNB	ALRA	ALRB
Raw	43.4±0.1d	43.8±1.3c	30.1±1.3a	29.8±0.8a	20.9±1.0bc	19.9±0.2b
5 Minutes	41.8±0.2c	44.1±0.8c	34.7±1.0b	34.7±0.7b	10.8±0.3a	11.9±1.8a
10 Minutes	27.2±0.1a	29.6±1.2a	41.3±1.0c	43.1±0.8c	22.0±0.0c	22.6±0.9cd
15 Minutes	35±0.1b	36.6±0.7b	45±0.5d	45.4±0.7d	22.7±1.0cd	22.0±0.6c
15 Min. Boiled & water discarded	44.7±0.5e	46.2±0.3d	47±0.1e	46.7±0.7de	53.8±0.4e	53.2±1.4e

[1] Values are means of three determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

Different studies on fruits and vegetables have also shown that bio-accessibility of carotenoids is enhanced by thermal and mechanical processing and/or moderate cooking is superior in conserving and improving bioavailability of vitamin A (Courraud *et al*., 2013; Musa and Ogbadoyi, 2012; Rickman *et al*., 2007; Ejoh *et al*.,2005; USDA, 1998). The first steps of carotenoid absorption include disruption of the food matrix, mechanically and by digestive enzymes, and the subsequent release of the carotenoids from this matrix and protein complexes (Britton, 1995). Homogenization and heat treatment disrupt cell membranes, whereas heat treatment has been suggested to disrupt further the protein-carotenoid complexes (Erdman *et al*., 1988). It may be assumed that heating caused both pigment degradation and an extractability increase due to the breaking of protein-carotenoid complexes (D'Evoli *et al*., 2013).The release of carotenoids from intact cells is thus indeed a limiting factor for carotenoids extractability and uptake (Van Het Hof *et al*., 2000).

Published data on the effects of cooking on carotenoids from vegetables are not consistent (D'Evoli *et al*., 2013) reported loses in lutein and β-carotene that is 0.15 mg/100 g and 0.75 mg/100 g in canned tomatoes *versus* 0.11 mg/100 g and 1.00 mg/100 g in raw tomatoes respectively which could account for the loses observed in A. *cruentus* after cooking for 10 minutes. (Ogbadoyi *et al.*, 2011), similarly reported that *A. cruentus* leaves boiled for 5 minutes although, not significantly different from fresh sample, contained more of the provitamin A than the fresh sample. Yadav and Sehgal, (1995) reported β-carotene losses of 1.3% in *Amaranthus tricolor. (*Ogbadoyi *et al.,* 2011) reported that the amount of β-carotene in *Amaranthus cruentus* leaves boiled for 5 minutes was higher than in fresh. (Rahman *et al*., 1990) reported losses of β-carotene of 31% of the initial content in *Amaranthus gangeticus* when cooked in water for 7 – 9 minutes followed by discarding the cooking water and then frying the vegetables in a small quantity of oil for 4-6 minutes.

(Dietz and Erdman, 1989) reported that steaming results in greater than 100% retention of β-carotene in vegetables, because denaturation of carotene binding proteins releases the carotenoids so that they can be extracted more easily. (Rickman *et al.*, 2007) further added that loss of soluble solids and the release of protein-bound β-carotenes that occurred during boiling may equally contribute to the observed increase in the pro-vitamin content especially when the cooking water was discarded. The levels of Vitamin A recorded in this study are adequate to meet the recommended daily intake of 3mg retinol equivalent per day for adult however there is need to for further study to determine the bioavailability of the same in human body.

4.6.2.2 Amounts of vitamin C

The vitamin C content results are presented in table 8.It was observed that increase in cooking time for amaranth vegetables resulted in greater losses of vitamin C. Vitamin C levels in amaranths collected from Ruai are lost immediately after cooking compared to those obtained from Njiru. The levels ranged between 98.5mg/100g dry weight non-detectable in cooked. The high loses observed could also be due to the fact that vitamin C is photo labile and thermo labile and that any delay in analysis and exposure to UV light may have greater loss.

There was significant difference in the level of vitamin C collected in different sites. These differences in the concentrations may be related to species, location, degree of maturity at harvest, cultivation, and post- harvest handling practices (Chen and Chen, 1992).

Table 8: Mean levels of vitamin C (mg/100g dry weight) of *Amaranthus cruentus* & *Amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	54.7±1.3b	79.3±1.5b	65.3±1b	98.5±1.3b	65.9±1.5a	44.4±1.3a
5 Minutes	21.9±0.00a	33.3± 1a	10.9±1a	32.8±0.00a	ND*	ND*
10 Minutes	ND*	ND*	ND*	ND*	ND*	ND*
15 Minutes	ND*	ND*	ND*	ND*	ND*	ND*
15 Min. boiled & water discarded	ND*	ND*	ND*	ND*	ND*	ND*

LoD = 0.49mg/Kg

LoQ=1.49mg/Kg

*Not detected

[1] Values are means of three determinations± standard deviation.

Values with the same letters in the same column are not significantly different at 5% level of significance.

Most of the work on the effects of processing amaranth deals with effect of various preparation methods on the vitamin C content of *amaranth spp* (Funke, 2011**).** It was observed that Vitamin C was not detected in cooked amaranth from Ruai probably because of the type of irrigation practiced which contaminated the leaves with microorganisms and it has been reported that the enzymatic oxidation of vitamin C is more important than non-enzymatic oxidation by oxygen, catalyzed by traces of metals such as copper and iron (Ajayi *et al*., 1980). *(*Mathooko and Imungi, 1994) reported loss of up to 80% ascorbic acid in cooked drained *Amaranthus hybridus* while (Yadav and Sehgal, 1995) reported ascorbic acid losses of up to 93%. (Funke, 2011) reported losses of up to 49.6% in steamed by pouring boiling water on the leaves for two minutes while (Keshinro and Ketiku, 1979) reported 80.3% loss of vitamin C from parboiling for 5 minutes, this increased to 91.5% after final cooking for another 5 minutes. (Sreeramulu *et al*., 1983) reported vitamin C losses in cooked and drained up to 98.5% in *Moringa oleifera* to nil (non-detectable) in *Emilia javani*ca. (Zeng, 2012) reported that raw spinach had 89mg/100g dry weight while boiled had 44mg/100g dry weight. Similarly, raw broccoli had106.4mg/100g while when boiled reduced 48.3mg/100g. All these findings collaborates the findings observed in this study that generally vitamin C concentrations are affected by temperature and prolonged heating. It recommends consumption of raw vegetables as the best way to obtain vitamin C (EFSA, 2013) and /or stewing for 5 minutes.

4.6.3: Anti-nutrients

The anti-nutrients analyzed included the oxalates and the nitrates.

4.6.3.1: The levels of oxalates in amaranthus cruentus and amaranthus lividus vegetables

The amount of oxalate in raw and cooked vegetable amaranth is presented in Table 9. It was observed that cooking caused significant reduction in the oxalate contents of vegetable amaranth in this study. For example, the oxalate content in raw *A. lividus* collected from Njiru A was reduced by 36.8% when boiled in water and the cooking water discarded. This trend is consistent with other findings (Ilelaboye *et al*., 2013; Ogbadoyi *et al*., 2011). Generally, the content of oxalate in the raw amaranth vegetables ranged between 2452.4mg/100g and 1649.3mg/100g compared with 1734mg/100g and 1311mg/100gm observed in vegetable that was boiled and cooking liqour discarded. The high levels of oxalate observed in this study agree with observation made by (Brogren and Savage, 2003) that some green leafy vegetables such as spinach, purple and green amaranth and colocasia contain very high oxalate levels. (Radek and Savage, 2008) reported high levels of between 8% and 10% dry weight basis in *Amaranthus cruentus* and *Amaranthus viridis,* respectively. (Ricardo, 1993) reported oxalic acid contents ranging from 1.1% to 7.9% dry weight *Amaranthus spp.* The values obtained in this study were close to oxalate concentration reported by (Muchoki *et al*., 2010; Savage *et al*., 2000)) for raw *V. unguiculata* L, and in spinach respectively. The levels are much lower than those reported by (Onyango *et al*., 2008) in *Amaranthus hypochondriacus*. The consumption of high oxalate containing foods promotes oxaluria which leads to an increased risk of kidney stone formation especially composed of mainly crystals of calcium oxalate (Massey, 2003). However, there is great variation in the amount of oxalate obtained in the different sites in this study and those obtained by other authors on similar vegetables.

Sources of variation in oxalate content of fresh fruits and vegetables include genetic, pre-harvest conditions, maturity of the edible product at harvest, post-harvest handling, storage and processing (Chen and Chen, 1992).

Table 9: Levels of Oxalates (mg/100g dry weight) of *Amaranthus cruentus* and *Amaranthus lividus* grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	2057.9±14d	2452.4±55d	2075.6±51d	1846.1±26a	1649.3±49c	1711.4±51cd
5 Minutes	1735.3±15c	2130.7±16c	1874.1±60c	2003.7±14c	1601.0±21bc	1653.1±32bc
10 Minutes	1567.8±16b	1798.8±43b	1767.4±18c	1972.0±19b	1593.3±21b	1580.4±30b
15 Minutes	1449.2±14a	1750.9±17a	1515.8±84b	1871.3±11ab	1580.1±32b	1561.7±28ab
15 Minutes boiled & water discarded	1444.7±8a	1734.6±32a	1311.1±33a	1772.0±19b	1540.6±6a	1541.8±21a

[1] Values are means of three determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

The composition of nutrients and anti-nutrients in food plants may vary depending on the variety and growing conditions and that high oxalate foods should always be cooked to reduce the oxalate content (Noonan and Savage, 1999; Ruale and Nair, 1993), hence the levels observed in cooked samples in this study are below the unacceptable levels to humans of 2 to 5 g of oxalic acid per day for populations consuming low levels of calcium (Ricardo, 1993).

4.6.3.2: Nitrates levels in Amaranthus cruentus and Amaranthus lividus vegetables

As depicted in table 10, the amount of nitrate in raw amaranth vegetable varied from 1856.6 mg/100g in *A. cruentus* to 3080 mg/100g dry weight in *A. lividus*. A peculiar trend was observed in which the levels of nitrates reduced drastically after cooking for 5 minutes then increased after cooking for 10 and 15 minutes. Boiling and discarding the water caused huge reduction in the levels of nitrates. The values of nitrates differed significantly $p<0.05$ with cooking time and the sites of sampling. It was observed that cooking resulted in significant reduction of nitrates. This study observed that *A. lividus* accumulated higher levels of nitrates and registered higher loses than *A. cruentus.* Observed reduction in amounts of nitrate in boiled and cooking water discarded compared with raw amaranth ranged from 63.2% in *A. cruentus* in Njiru A to 83.1% in *A. lividus* in Ruai A. This could be explained by the fact that nitrates are water soluble and therefore some loss through leaching is expected, especially if the cooking water is discarded (Ezeagu, 2006; Onyango *et al*., 2008; Ilelaboye *et al*., 2013).

It was similarly observed that blanching caused 31.70% to 50.76% reduction in nitrates and 64.105% to 67.67% in nitrite contents of the vegetables (Muchoki *et al*., 2010). The high values of nitrates observed in this study may be due to the fact that nitrogen nutrition and maturity at harvest have greatest influence on yield and the contents of most nutrients in leafy vegetables and could be contributed by application of nitrogen fertilizers (Onyango *et al*., 2008).

The vegetable in this study are classified as high nitrate content vegetable because the nitrate value is between 1000-4000mg/kg (Anjana *et al*., 2007). However the levels do not warrant public concern especially after cooking for 15minutes without discarding the water because they are within the ADI of 3.7 mg/kg body weight which is equivalent to 222 mg of nitrate per person per day at a body weight of 60 kg and consuming 100g dry weight of vegetable.

Nitrate is highly variable and dietary exposure to nitrate from sources other than vegetables is estimated to be on average in the range of 35-44 mg/person/day of which some 20 mg/person/day is contributed by water (EFSA, 2008). The harmful effects of nitrate are related not so much to its toxicity, which is low, but to the dangerous compounds that are synthesized in the organism. The most serious danger comes from nitrite which is produced by nitrate reduction and which can lead to methaemoglobinemia or form nitrosamines and nitrosamides by reacting with amines and amides which are known potential carcinogens (Gangolli *et al*., 1994; Walker, 1990).

The findings in this study therefore need further verification by more detailed studies, which should include a wider volume of food materials since it must be observed that some components of vegetables such as ascorbic acid, phenols, etc. have been reported to inhibit the toxic effects of nitrites (Gangolli *et al*., 1994; Walker, 1990). Due to the high sensitivity of young infants, nitrates should be determined more often particularly in water and plant foods.

Table 10: Mean levels of nitrates (mg/kg dry weight) of *Amaranthus cruentus* and *Amaranthus lividus* grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	1958.4±10.1e	1856.6±3.1e	1961.2±3.2e	3080.5±11.1e	2847.4±1.8e	2829.6±4.2d
5 Minutes	872.4±7.2b	1015.3±4.0b	892.1±10.1b	882.6±9.2b	1221.5±7.2b	1328.5±7.0b
10 Minutes	1098.5±10.2c	1341.9±3.1c	1003.1±10.1c	1221.3±9.1c	2249.8±9.8d	2416.5±8.1e
15 Minutes	1572.6±1.9d	1642.9±6.1d	1899.6±7.1d	1656.3±9.0d	1818.5±3.8c	2015.9±9.2c
15 Minutes boiled & water discarded	721.3±1.2a	346.1±2.7a	397.8±2.3a	849.6±2.9a	482.4±1.7a	581.4±3.2a

[1] Values are means of three determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance p>0.05.

4.6.4 Heavy Metals

The heavy metals analyzed in this study included Cadmium and the lead in amaranth vegetables, soil and effluent water.

4.6.4.1 Cadmium levels in amaranth vegetables, water and soil

As demonstrated in table 11 and figure 8, cadmium was not detected in the vegetable. However, it was detected in the soil where the amounts ranged between 97.85 ppb in Njiru B, 92.35ppb in Njiru A to 62.3 ppb in Ruai. Furthermore, cadmium was not detectable in Nairobi river water.

Table 11: Levels of cadmium in ppb of *Amaranthus cruentus* and *Amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	ND*	ND*	ND*	ND*	ND*	ND*
5 Minutes	ND*	ND*	ND*	ND*	ND*	ND*
10 Minutes	ND*	ND*	ND*	ND*	ND*	ND*
15 Minutes	ND*	ND*	ND*	ND*	ND*	ND*
15 Min. boiled & water discarded	ND*	ND*	ND*	ND*	ND*	ND*

***ND-Not Detected**

The absence of cadmium in water could possibly be due to the Nairobi river basin programme which was started in 1999 by multi stake holder initiative comprising of the Government of Kenya, the UNEP, UN-habitat, UNDP, the private sector and civil society. The programme was a three phased programme that was to run up to 2008 with an objective of rehabilitating, restoring and managing Nairobi River in order to improve livelihood, enhance biodiversity and sustainable supply of water for domestic and industrial, recreational and emergency.

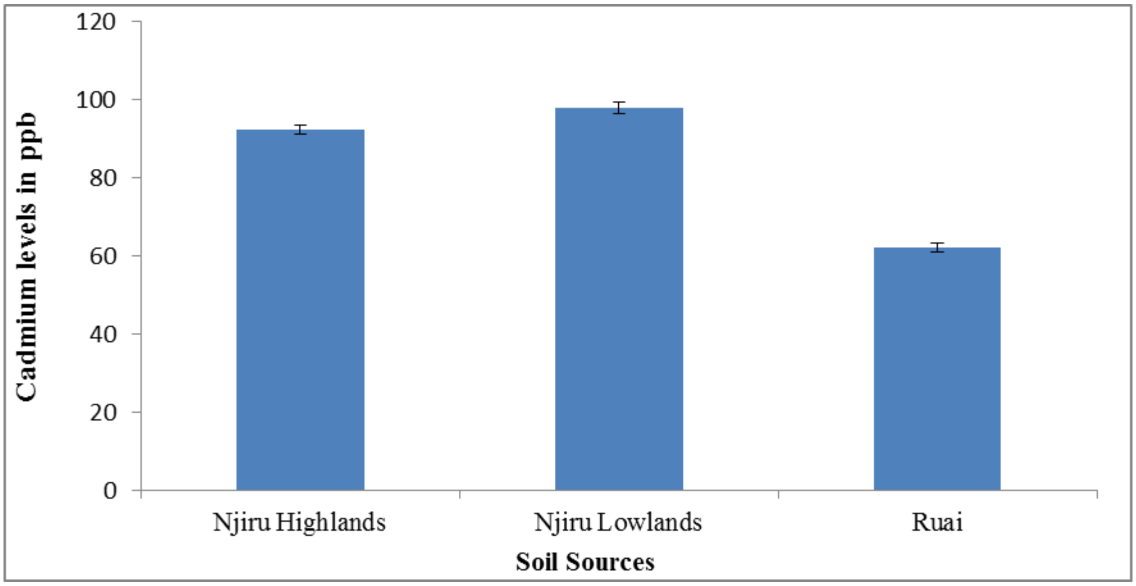


Figure 6: The levels of Cadmium in ppb in Soils from different sources. The errors bars represent standard deviations.

These finding agrees with other reports that when wastewater is used for the irrigation of edible plants for prolonged period, soil health is affected, toxic metals apparently accumulate in soils and as such there is an ongoing buildup of heavy metals in soil and concentrations are higher than established limits for some metals (Adelekan and Alawode, 2011; Singh *et al*., 2004; Barman *et al*., 2000). The findings also compares favorably with the findings of other authors that the uptake of metals from the soil depends on different factors such as their soluble content in it, soil pH, organic matter content, soil type, plant growth stages, types of species, fertilizers and soil (Sharma *et al*., 2006; Ismail *et al*., 2005; Fleming and Parle, 1977). Cadmium Provisional Monthly intake was set at 25µg/kg body weight /month by (JECFA, 2013) hence the vegetable is safe.

4.6.4.2 Lead levels in amaranth vegetables, water and soil

As depicted in the table 12, figures 8 and 9, the levels of lead in raw vegetable amaranth ranged between 2.8 ppm in *A. lividus* in Njiru B to 5.1ppm in *A. lividus* in Ruai A. The levels of lead in the soil ranged between 3ppm in Njiru A to 34.1 ppm in Ruai soil, the levels ranged between 0.2ppm in water that is flowing in channels in Njiru water to 4.5ppm in Ruai Nairobi river water. The amounts of lead in water at the water source in Njiru were significantly different $p<0.05$

from the values in the water channel. It was observed that as water flows through the water channels the concentration of lead decreases due to deposition of lead since it's a heavy metal and as such the levels at the source (purposively opened manhole) was higher than the levels in water flowing through the channels. The results show that cooking did not have significant effect $p<0.05$ on the levels of lead in Njiru consistent with the observations by (Mwaura, 2003) but had a significant effect on the levels in Ruai. The levels of lead were highest in Ruai vegetables as shown in figure 8.

Table 12: Levels of Lead (in ppm) dry weight of *Amaranthus cruentus* and *Amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	4.1±0.4a	4±0.4a	3.7±0.2a	2.8±0.2a	5.1±0.6b	4.8±0.6a
5 Minutes	5.1±0.7b	4.1±0.3a	3.9±0.3a	3±0.1a	9.7±1.0c	8.1±0.7b
10 Minutes	5.8±0.0b	4.4±0.0a	4.2±0.2a	3±0.1a	10.2±0.5c	10.6±0.2c
15 Minutes	6.9±0.2b	4.6±0.2a	4.4±0.2a	3.2±0.2a	14.4±0.8d	15.9±0.1d
15 Minutes boiled & water discarded	2.9±0.3a	3.8±0.4a	3.5±0.5a	1.2±0.0a	3.2±1.0a	4.2±0.2a

Values are means of three determinations± standard deviation.
Values with the same letters in the same column are not significantly different at 5% level of significance $p>0.05$.

Cooking and discarding the cooking water resulted to losses in lead ranging between 5% as was noted in *A. cruentus* grown in Njiru B to 57% in *A. lividus* grown in Njiru B. (Imungi and Potter, 1983) reported losses of up to 30% lead during cooking of cowpea leaves by boiling in water.

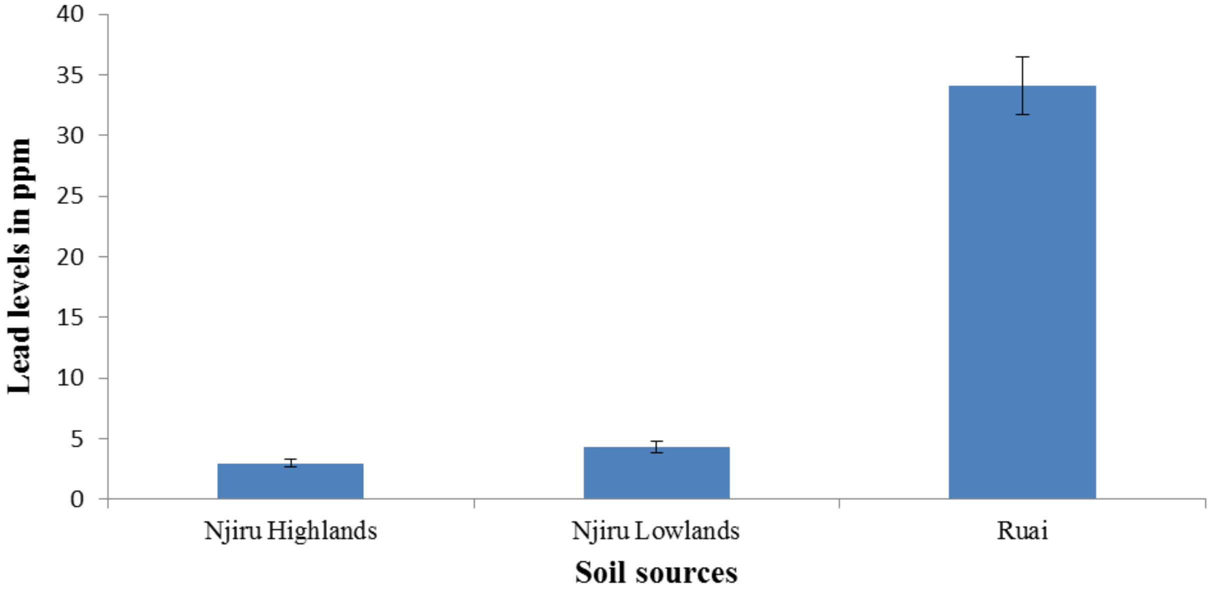


Figure 7: The levels of Lead in ppm in soils from Njiru and Ruai
The error bars represent standard deviations of the determinations.

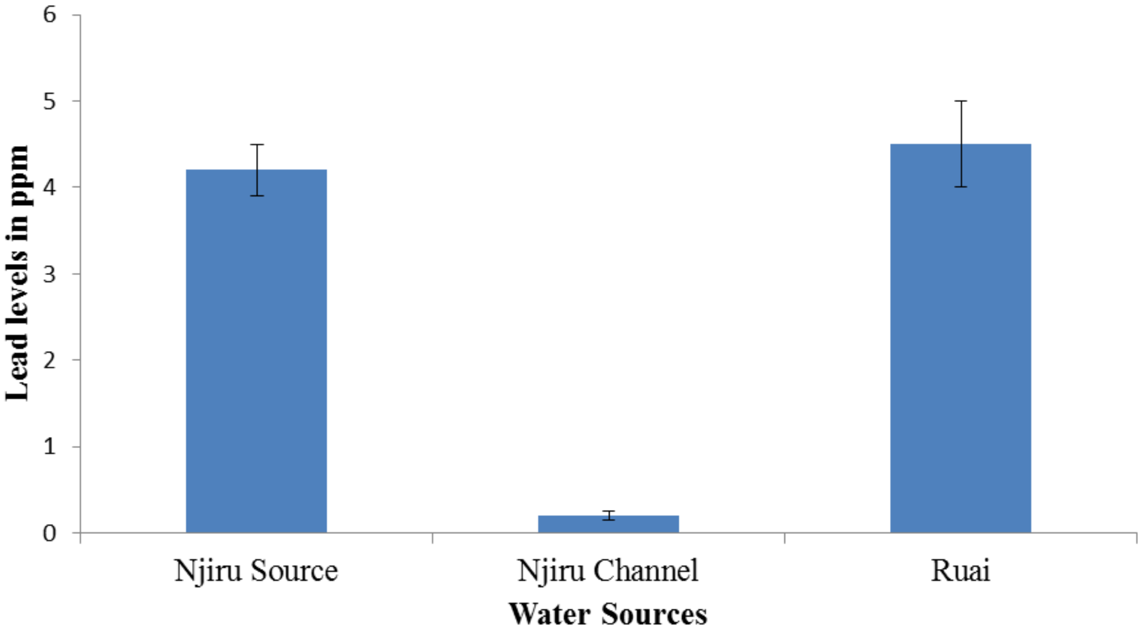


Figure 8: The levels of Lead in ppm from water sources in Njiru and Ruai
The standard error bars represent standard deviations.

It was observed that cooking without discarding the cooking water resulted to higher values of lead in vegetable and this observation is consistent with a study that observed that homogenization and heat treatment disrupt cell membranes extracting lead from the plant matrix (Erdman *et al*., 1988). The amounts of lead in this study are comparable to those reported in other studies (Karanja *et al.,* 2010; Atayese *et al*., 2009; Onyango *et al*., 2008), and values are within the range observed by (Fleming and Parle, 1977) that normally, plant metal levels for Pb vary in the range of 1 – 12 mg kg^{-1} dry weight.

Elementary lead has various uses such as in the manufacture of storage batteries, cables, various alloys, protection cloth against radiation and tetraethyl lead production for use as antiknock in petrol among other uses. Lead accumulation in in the human body is likely to results in serious systemic problems like cardiovascular, kidney, nervous and bone diseases. Children have high gastrointestinal uptake and the permeable blood-barrier makes them more susceptible to Pb exposure than adults. Children exposed to high concentrations of Pb may develop behavioral disturbances as well as learning and concentration difficulties (Jarup, 2003).

Therefore, the cumulative values in this study are higher than the withdrawn 25 μg/kg body weight Provisional Tolerable Weekly Intake (PTWI) by (JECFA, 2011) after it established that 25 μg/kg bw PTWI is associated with a decrease of at least 3 IQ points in children and an increase in SBP of approximately 3 mmHg. The values are also higher than the maximum limits in Kenya standard for dehydrated vegetable of 2 ppm.

There are no safe limits for lead hence the values in this study should warrant public concern especially if this vegetable is consumed on a daily basis considering its biological half-lives is long (10-30 years) in bones, is extremely persistent in the environment; non-biodegradable and non-thermo-degradable and can easily accumulate in the body (Bohn *et al*., 1985).

4.6.5: Minerals

The minerals analyzed in this study included Zinc, iron and calcium in amaranth vegetables

4.6.5.1 Iron levels in amaranth vegetables, water and soil

The levels of iron present in amaranth vegetables ***(****Amaranthus cruentus* & *Amaranthus lividus****)*** grown in Ruai and Njiru are indicated in table13.

Table 13: Levels of Iron in ppm dry weight of *Amaranthus cruentus* and *Amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	**ACNA**	**ACNB**	**ALNA**	**ALNB**	**ALRA**	**ALRB**
Raw	87.3±5.2c	97.1±8.4b	165.1±15a	152.6±4.8bc	218±12a	281.4±18a
5 Minutes	121±7.3b	106.6±6.3b	169.9±12a	163.1±2.6ab	328.1±18b	346.8±23b
10 Minutes	148.2±4.3a	165.2±5.3a	170.8±5a	165.6±2.6a	349.1±9c	388.3±9.1c
15 Minutes	159.7±2.8a	170.5±3.8a	174.8±9.2a	168.6±3.1a	428.2±28d	443.2±13d
15 Min. boiled & water discarded	78.6±2.2c	109.5±1.8b	164.2±11.2a	146.1±2.1c	393.4±21e	374.5±10.4e

Values are means of three determinations ± standard deviation, and the values with the same letters in the same column are not significantly different at 5% level of significance $p>0.05$

The amounts of iron in amaranth vegetable ranged between 87.5 mg/kg (lowest level) in *A. cruentus* in Njiru A to 443mg/kg (highest level) in *A. lividus* in Ruai B. *A. lividus* samples obtained from Ruai had higher levels of iron compared to *A. lividus* samples from Njiru. Increased cooking time resulted in increased concentration of iron, however discarding the cooking water resulted to loses in iron across all the amaranth species when compared with amount in vegetables stewed for 15 minutes without discarding the cooking water. There was no significant difference p>0.05 between the concentration of iron in raw and cooked samples when cooking was done by boiling and discarding the water in Njiru vegetable except for *A. lividus* in Njiru B while significant difference p<0.05 was observed with Ruai vegetable.

The values of iron in this study are within the range reported elsewhere in *A. hypochondriacus* (Onyango *et al*., 2008). Similarly, the levels compares well with values reported by other authors in vegetables (Carvalho *et al*., 2012; Musa and Ogbadoyi, 2012; Bains and Shruti, 2007; Singh *et al*., 2001) the values are within the normal range for iron from 18.8-82.4 mg/100g that has been reported (Costa *et al*., 2006). However, these levels may vary depending on various factors: species; variety; processing factors, such as storage time, temperature, and food preparation (Welch and Graham, 2002). The increase in amounts of iron with cooking has also been reported elsewhere. (Imungi and Potter, 1983) reported an apparent increase of about 13% of the iron in cooked and drained cowpea leaves. Iron is required for a number of vital functions, including growth, reproduction, healing, and immune function iron-deficiency induced anemia has also been reported to be prevalent among the general population, especially among children under five years of age and pregnant women (WHO, 2000).

Iron deficiency may increase the risk of lead poisoning in children however accumulation of iron may lead to cirrhosis of the liver, diabetes, heart muscle damage (cardiomyopathy), or joint problems (Janssen and swinkels, 2009). It has also been reported that iron from plants is less efficiently absorbed than that from animal sources, the U.S. Food and Nutrition Board (FNB) has estimated that the bioavailability of iron from a vegetarian diet to be only 10% but on the other hand vitamin C strongly enhances the absorption of non-heme iron by reducing dietary ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and forming an absorbable, iron-ascorbic acid complex hence consumption of vegetable amaranth helps balance vitamin and mineral intake (Shukla *et al*, 2005; IPGRI, 2003; FAO, 1988).

4.6.5.2 Zinc levels in amaranth vegetables

As shown in the table 14, the amaranth vegetables obtained from Njiru had higher levels of zinc compared to those from Ruai but statistically there was no significant difference p>0.05. The levels of zinc ranged from 23.4 ppm in raw *A. lividus* vegetables from Ruai to levels of 54.6 ppm in *A. lividus* from Njiru B that had been cooked for 15 minutes. Zinc levels increased with increased cooking time however statistically there was no significant difference p>0.05.The zinc levels decreased once the water was discarded in all the samples however statistically there was no significant difference p>0.05 on the level of zinc after cooking. This finding agrees with a study reported by (Joshua *et al*., 2012). The values in this study are within the range reported in other studies (Onyango *et al*., 2008; Singh *et al*., 2012; Joshua *et al*., 2012; Makobo *et al*., 2010).

Table14: Levels of Zinc in ppm dry weight of *Amaranthus cruentus and Amaranthus lividus* leaves grown in different sites in Njiru and Ruai

Treatment	ACNA	ACNB	ALNA	ALNB	ALRA	ALRB
Raw	47.1±1.7a	32.9±0.6	40.7±1.5a	35.7±0.9b	23.4±0.9a	28.2±1.4a
5 Minutes	48.8±2.1a	36.4±0.8a	42.6±0.7a	36.1±1.3b	26.9±2.6a	31.3±0.3a
10 Minutes	50.3±1.3a	38.9±1.6a	43.9±1.6a	44.7±0.9ab	28.8±0.0a	33.7±1.9a
15 Minutes	53.6±0.5a	40.3±1.3a	47.4±2.3ab	54.6±2.9a	31±1.2a	34.4±1.3a
15 Min. boiled & water discarded	45.3±1.7a	36.5±1.0a	42.9±0.5a	32.3±1.7b	25.5±1.5a	29.2±0.9a

Values are means of three determinations± standard deviation, and the values with the same letters in the same column are not significantly different at 5% level of significance p>0.05

Zinc is a nutritionally essential mineral needed for catalytic, structural and regulatory functions in the body. Severe zinc deficiency is a rare, genetic or acquired condition. Dietary zinc deficiency, often called marginal zinc deficiency, is quite common in the developing world, affecting an estimated 2 billion people (Prasad, 1998). One of the most important roles of zinc in human nutrition is to maintain the body's immune system (Oliver, 1997; Nauss and Newberne, 1982). The effects of zinc deficiency in this regard are 'profound' rendering the zinc-deficient person extremely vulnerable to a whole range of infections (Nauss and Newberne, 1982).

Zinc and vitamin A interact in several ways. Zinc is a component of retinol-binding protein, a protein necessary for transporting vitamin A in the blood. Zinc is also required for the enzyme that converts retinol (vitamin A) to retinal. It has also been reported elsewhere that zinc has an inhibitory effect on iron absorption but according to (Olivares *et al*., 2007) the inhibitory effect is temporary. This inhibitory effect can be attributed to the fact that transferrin, the main iron plasma transporter, has also been shown can also bind zinc (King and Cousins, 2006; Harris, 1983).

4.6.5.3: Calcium levels in amaranth vegetable

As depicted in the table 15, the levels of calcium ranged between 2157mg/100g (lowest value obtained in the study) in *Amaranthus lividus* in Njiru A to 5692mg/100g (highest value) in *Amaranthus cruentus* in Njiru A. Highest concentration of calcium was observed when the vegetable was stewed for 15 minutes without discarding the water as was also observed for zinc. Stewing vegetable had significant effect ($p<0.05$) on the concentration of calcium regardless of cooking time. The Levels of calcium increased with cooking and decreased below the levels in raw samples when the cooking water was discarded. *Amaranthus cruentus* recorded higher levels of calcium especially in Njiru highlands (A) than *Amaranthus lividus* in Njiru and Ruai. The effect of less water in highlands could have concentrated the mineral in the leaves. However,this could also mean that *Amaranthus cruentus* is more nutritious than *Amaranthu.s lividus*

The values are slightly higher than those reported by (Mziray *et al*,. 2001) that ranged between 2062 mg/100g to 2263 mg/100g dry weight and (Akubugwo *et al*., 2007) of 44.15mg/100g dry weight. The findings in this study agrees with report by (Maundu *et al.*, 1999) that cooked leaves (not mixed with other foods) are rich in calcium, iron and vitamins A and C. Calcium is the most common mineral in the human body. It's required for the healthy maintenance of skeleton (Wood and Ronnenberg, 2006) otherwise the body will demineralize bone to maintain normal blood calcium levels when calcium intake is inadequate for its own normal physiological functioning.

Increased calcium intake has strongly been associated with decreased colorectal cancer risk in men with higher circulating levels of Insulin-like Growth Factor-1(IGF-1) (MaJ *et al*., 2001), in the treatment of osteoporosis (Heaney, 2000; U.S National Institute of Health, 2000), prevention of exposure to lead mobilized from the skeleton during bone demineralization (Hertz-Picciotto *et al*., 2000) and decrease the severity of Premenstrual syndrome (PMS)

The levels of calcium in these (Brown, 2002) vegetables are higher than the levels of oxalates. This is important because According to (Noonan and Savage, 1999), the adverse effect of oxalates on calcium bioavailability may be pronounced when the oxalate/calcium mole ratio is 9: 4. Oxalic acid forms water-soluble salts with Na^+, K^+ and NH_4^+ ions but can also form insoluble oxalates by binding with Ca^{2+}, Fe^{2+} and Mg^{2+} rendering these minerals unavailable for absorption by the intestine (Savage *et al*., 2000), therefore it can result in calcium deficiency even when the diet is otherwise sufficient in calcium. Infants and children who are experiencing active bone and teeth formation and older persons who can suffer calcium resorption from bones leading to osteoporosis are more vulnerable (Harper *et al.*, 1979). However, oxalate content: calcium ratio in vegetable amaranth needs further investigation.

Table 15: Levels of Calcium in mg/100g dry weight of *Amaranthus cruentus* and *Amaranthus lividus* leaves[1] grown in different sites in Njiru and Ruai

Treatment	ACNA	ACNB	ALNA	ALNB	ALRA	ALRB
Raw	4454.2±11d	3203.2±15d	2886.4±18d	3762.9±.9a	2919.2±13d	3028.9±12d
5 Minutes	5128.9±08c	3642.4±23c	3017.2±9c	3908.4±.1b	3945.1±8c	3753.2±17c
10 Minutes	5328.1±10b	4028.8±7b	3103.6±19b	4431.5±.2d	4427.7±15b	4350.5±21b
15 Minutes	5692.2±14a	4376.5±9a	3109.8±12a	4668.5±.9e	4631.9±19a	4511.1±16a
15 Min. boiled & water discarded	4381.2±05e	3142.4±12e	2157.0± 12e	4053.9±.8c	2854.4±3e	3029.3±15d

Values are means of three determinations± standard deviation,

Values with the same letters in the same column are not significantly different at 5% level of significance $p>0.05$

4.7 CONCLUSION

Our current findings indicate that *Amaranthus cruentus* and *Amaranthus lividus* are a good source of protein, dietary fibre, carbohydrates, vitamin A, vitamin C and some essential mineral elements (zinc, iron and calcium). Cooking does not significantly influence the levels of minerals however the result of cooking has inevitable consequences on the nutritional values of the vegetables. The level of lead, nitrates and oxalates in the vegetable are high enough to induce toxicity in humans. With careful selection of good choice of processing method, the nutritional potential of this commonly consumed leafy vegetable can be fully harnessed. In this present study, we recommend stewing for 15 minutes without discarding the cooking water as a choice of processing method, as this method significantly increases the vegetable amaranth nutrients and slightly reduces anti-nutrients.

The high levels of calcium observed in this study are important especially because this vegetable contains appreciable levels of lead thus calcium will prevent exposure to lead mobilized from the skeleton during bone demineralization. The study indicate that vegetable amaranth indeed can help in alleviating nutritional deficiencies observed in Kenyan population and that fortification with Vitamin A is not necessary if the population is advised to incorporate this vegetable in their daily diets.

Cadmium was not detected in the vegetable amaranth probably because the uptake of minerals/metals depend on the plant species or the solubility of metals depends on the type of soil however the levels of lead were above the maximum allowable limits of 2ppm in Kenya Standard (KS 435:2012) specification for dehydrated vegetables hence as per the standard Act Chapter 496 of laws of Kenya this vegetable should not be found retailing in the market.

Lead is relatively abundant in nature and occurs primarily in the inorganic form in the environment and in association with zinc thus the high levels of lead observed in amaranth in this study does not necessarily mean it’s because the vegetable is grown with effluent water. There is need to conduct more research and compare with vegetable grown with conventional/potable water.

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The findings of this study show that urban agriculture is not a short-lived or transitional phenomenon – probably as long as it can maintain its comparative market advantage it’s going to exist regardless whether it’s illegal. It also shows that urban agriculture enhances livelihoods sustainability and land use among the urban poor especially women in their active labour years that is between 40-50 years. The study confirms that urban agriculture is practiced in effluent conditions characterized by the use of untreated waste water from domestic and industrial usage and the use of municipal untreated biosolids. The urban population is supplied with these vegetables and poses a considerable health risk in terms of microbiological contamination but not necessarily in terms of heavy metals (cadmium).

The findings of this provided insight on the different methods of cooking amaranth vegetable in Kenya and was able to identify the preferred method as stewing the vegetable in oil, onions and tomatoes for 15 minutes where it was also observed that this method of cooking preserved or in some cases increased the nutritional content of the vegetable. Generally, long cooking or keeping vegetables hot for a long time has a destructive effect on the vitamins.

There is a positive correlation between the levels of lead in soil and vegetable and that cooking does not influence the levels of minerals significantly however the result of Cooking has inevitable consequences on the nutritional values of the vegetables. For example, the minerals contained in the vegetables showed varying degrees of stability when the vegetables was cooked while vitamin A increased when the cooking water was discarded however water soluble vitamins were lost when cooking water was discarded. Cooking has also have been highlighted as possible means of reducing the anti-nutrient levels in plant food source to innocuous level that can be tolerated by monogastric animal including man.

The study showed that vegetable amaranth grown with effluent conditions is of good quality because it contained high protein, dietary fibre, Vitamin A and minerals contents especially calcium with no cadmium however it may not be safe because of the levels of lead and Njiru vegetable is slightly safer than Ruai because of the levels of lead and the mode of irrigation (the leaves of the amaranth do not come into contact with effluent water). Therefore domestic waste water is slightly safer for irrigating crops than industrial waste water in terms of heavy metal contamination.

The levels of lead were beyond the maximum limits of 2ppm allowed in Kenya as per KS 435:2012 – Kenya standard specification for dehydrated vegetable and it should also be noted that (JECFA, 2011) withdrew the 25 μg/kg body weight Provisional Tolerable Weekly Intake (PTWI) established in 2010 of lead because it could no longer be considered health protective and as such the committee concluded that it was not possible to establish a new PTWI that would be considered health protective therefore this vegetable should not be found retailing in the market.

However, the study also showed that the different species of vegetable amaranth contain appreciable amount of anti-nutrients; oxalates and nitrates. The levels of oxalates and nitrates are within the normal range found in vegetable grown with conventional conditions. The levels of anti-nutrients (oxalates and nitrates) found in this study do not warrant public concern especially when the cooking water is discarded.

5.2 RECOMMENDATIONS

It is recommended that few precautions help reduce the losses associated with cooking. Amaranth leaves should be washed in plenty of fresh water before cutting to reduce microbial and aerial heavy metal contamination and consumed quickly. Where possible, steam or stew the vegetable rather than boiling for 15minutes to get adequate nutrients without discarding the cooking water. The water from cooking should be used to make soup or sauce.

The implications of urban agriculture in terms of economic and health is enormous as observed in the study and we recommend that programme and legislation can be introduced to institutionalize urban agriculture, reduce the contamination of wastes with lead, modify agricultural practices, and educate cultivators and support services, such as agricultural extension services and microfinance should be included in the policy and directed to urban agriculture practitioners.

The study observed that vegetable amaranth is very nutritious and we recommend that public education on the nutritive value of traditional leafy vegetables should be undertaken so that there is dietary biodiversity instead of mandatory food fortification being undertaken in Kenya for wheat flour, maize flour and edible fats and oils.

We also recommend that further research should be conducted to determine the levels of lead in vegetable amaranth grown with conventional or potable water so as to conclusively declare that vegetable grown with effluent water has high levels of lead however to comprehensively determine the safety risk posed by this vegetable we recommend microbial analysis also be carried out.

It is also recommended that future studies on vegetables grown with effluent conditions should take into account the maturity of the leaves which was not possible in this study due to scarcity of samples of similar maturity age.

Likewise, more research on other traditional leafy vegetables grown with effluent conditions in Njiru and Ruai be undertaken in order to identify different accumulation levels of lead since lead accumulation in plant depends on plant species. We also recommend that lead exposure levels in children living in Nairobi be conducted so that adequate detoxification measures can be undertaken to reduce the effect of lead poisoning in children.

In light of the levels of lead in the soil, water and vegetable we suggest that the proposed banning of the use of lead in petroleum and gloss paint contained in the formulated pollution regulatory bill as reported by National Environment Management Authority of Kenya (NEMA) Chief Research Officer Mr. Isaac Elmi in the standard newspaper dated 23rd October 2013 page 13 should be made law and enforced immediately.

The governor of Nairobi County should embrace and allocate funds to facilitate the Nairobi river basin programme (which involved cleaning of the river and was initiated by the late honourable minister Mr. Muchuki) which has so far made an impact in the levels of cadmium in water and vegetable grown with waste water from Nairobi River and efforts be geared to identifying the sources of lead in waste water so that appropriate control measures are instituted.

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APPENDICES

APPENDIX-1

QUESTIONNAIRE FOR AMARANTH FARMERS

TITLE: NUTRITIVE VALUE AND THE LEVELS OF CADMIUM AND LEAD IN RAW AND COOKED VEGETABLE AMARANTH GROWN WITH EFFLUENT WATER

INTRODUCTION

This interview is geared at gathering information on the effect of growing environment on the nutritive value and the levels of cadmium and lead in raw and cooked vegetable amaranth. There is no right or wrong answers rather your opinion on practises of growing vegetable amaranth is required. The information obtained will assist in the formulation of policies, research and extension programs that are appropriate to your area. Your cooperation is highly appreciated.

GENERAL INFORMATION

Name of respondent:

Phone Number:

Location of farm:

Date:

PART A-FARMER'S CHARACTERISTICS

1. Sex of the respondent ☐ Male ☐ Female

2. How old are you? (in years)

☐ Less than 30 ☐ 30-40 ☐ 40-50 ☐ Above 60

3. What is your highest level of education? (kindly indicate the number of years)

☐No education ☐Primary education ☐ Secondary education ☐Tertiary education

☐Non formal (Adult education) indicate number of weeks

4. What area of farm (in acres) is used for growing amaranth?

☐ Less than 3 ☐ 3-6 ☐6-9 ☐ 9-12 ☐Greater than 12

5. For how long have you been growing amaranth?..

6. Who are your major customers? ☐Supermarkets ☐Restaurants/hotels

☐Kiosks/Retail groceries ☐Local Individuals ☐ Intermediaries ☐Other (specify)

7. During which period is the sale of vegetable amaranth highest?.....................................

8. Do you have other sources of income apart from vegetable amaranth?☐ Yes ☐No

If Yes kindly explain:………………………………………………………

9. What is your approximate total annual income from the sale of vegetable amaranth?

☐ Below Kes 200,000 ☐ Kes 200,000-500,000 ☐Kes.500, 000-1,000,000

☐ Above Kes.1, 000,000

PART B-GROWING ENVIRONMENT

10. What type of variety have you planted?...

11. When was it planted?...

12. What water is used for growing vegetable amaranth? ☐Rain ☐Irrigation

13. If it's by irrigation what is/are the sources of water? (Tick all the appropriate sources)

☐ Well ☐ City water supply ☐ open/surface ☐Rain ☐Sewerage/drain

☐Others (specify)

14. What are/is the method(s) of delivery and/or irrigation of water? ☐Gravity/channels

☐ Sprinkler ☐Pivot ☐ Drip ☐Watering Can ☐ Other (Specify)

15. Is the quality of your water source(s) /supplies tested? (If yes then indicate how often)

☐ Yes ☐No ☐Not applicable

16. Has the water been treated?☐ No ☐ Yes

If so explain:

17. Does the agricultural water come in contact with vegetable amaranth? ☐No ☐Yes

18. What is the predominant soil type of the amaranth grown area (soil texture)/Observe:

☐Very fine (clay) ☐fine (clay, sandy clay, silty clay)

☐Medium (sandy clay loam, clay loam, sandy silt)

☐Medium-fine (silty clay loam, silt loam) loam)

☐Coarse (sand, loamy sand, sandy loam)

☐No predominant soil type (too variable across the amaranth grown area on the farm)

19. Characterize soil quality of the Amaranth grown area (fertility):

☐Below average – poor ☐ average – normal ☐ above average -good

20. What do you use as fertilizer? (Tick all the appropriate fertilizers)

☐ Raw animal manure ☐ Aged animal manure ☐ Municipal Biosolids/Sludge

☐ Compost ☐ Non-Organic Fertilizer ☐ Others specify

21. Do you use pesticides? (If yes kindly specify the type used and frequency of application)

☐ Yes ☐ No

22. How many days prior to harvest were the chemicals/pesticides applied?............................

23. What is your overall assessment of disease susceptibility of amaranth compared to other traditional leafy v☐getables? ☐ usual More susc☐ptible Less susceptible

24. What method of harvesting do you u☐? Uproot/cut the plant ☐ Pick leaves & shoots

25. What do you use in harvesting vegetable amaran☐? Bare hand ☐ Gloved hand

☐ Automated/machine (no hand contact) ☐ bare hand with utensil (e.g.: knife)

☐ Gloved hand with utensil ☐ other (Specify)

26. How old was the crop when you first harvested the leaves?..

27. How often do you harvest the leaves?...

28. What type of container/packaging material is used for transporting vegetable amaranth from the farm? ☐ Polythene bags ☐ sacks ☐ cooler box ☐ cartons/box

☐ None ☐ other (specify)

29. In what quantities do you package vegetable amaranth?......................................

30. Do you consume/eat vegetable amarant☐? Yes ☐No

31. If yes how do you cook vegetable amaranth?

☐ Direct Stewing ☐ Steaming ☐ boiling ☐ Boiling then Stewing

☐ Boiling discarding the water then frying ☐ other (specify)

32. If cooking is by boiling, what quantity of water do you use?..

33. How long does it take to cook vegetable amaranth?

Thank you.

APPENDIX-11

QUESTIONNAIRE FOR AMARANTH INTERMEDIARIES

TITLE: NUTRITIVE VALUE AND THE LEVELS OF CADMIUM AND LEAD IN RAW AND COOKED VEGETABLE AMARANTH GROWN WITH EFFLUENT WATER

INTRODUCTION

This interview is geared at gathering information on the marketing channels and markets vegetable amaranth grown in Kenya. There is no right or wrong answers rather your opinion on consumption of vegetable amaranth is required. The information obtained will assist in the formulation of policies, research and educational programs that are appropriate to your market needs. Your cooperation is highly appreciated.

GENERAL INFORMATION

Name of respondent:

Phone Number:

Date:

PART A-INTERMEDIARIES'S CHARACTERISTICS

1. Sex of the respondent ☐ Male ☐ Female

2. How old are you? (in years)

☐ Less than 30 ☐ 30-40 ☐ 40-50 ☐ Above 60

3. What is your highest level of education? (kindly indicate the number of years)

☐ No education ☐ Primary education ☐ Secondary education

☐ Tertiary education ☐ Non formal (Adult education) indicate number of weeks

4. For how long have you been trading in vegetable amaranth?...

5. The amaranth market is very large in comparison to other traditional leafy vegetables, do you?

   Strongly ☐ agree ☐Agree ☐Disagree ☐Strongly disagree

6. Has the demand of vegetable amaranth risen in the past few years?☐Yes ☐No

7. Who are your major customers? ☐Supermarkets ☐Restaurants/hotels

   ☐ Kiosks/Retail groceries ☐Local Individuals ☐Other (specify)

8. During which period is the sale of vegetable amaranth highest?.....................................

9. What type of container/packaging material is used for transporting vegetable amaranth from the farm? ☐Polythene bags ☐sacks ☐cooler box

   ☐ Cartons/box ☐ None ☐other (specify)

10. In what quantities do you package vegetable amaranth?..

11. What is your profit margin per package?...

12. Do you have other sources of income apart from vegetable amaranth?☐ Yes ☐ No

    If yes please explain:……………………………………………………………………….

13. What is your approximate total annual income from the sale of vegetable amaranth?

    ☐ Below Kes.200, 000 ☐ Kes.200, 000-500,000

    ☐ Kes.500, 000-1,000,000 ☐ above Kes.1, 000,000

14. Do you consume/eat vegetable amaranth? ☐ Yes ☐ No

15. If yes how do you cook vegetable amaranth?

☐Direct Stewing ☐Steaming ☐ boiling ☐ boiling then Stewing

☐ Boiling discarding the water then frying ☐ other (specify)

16. If cooking is by boiling, what quantity of water do you use?..

17. How long does it take to cook vegetable amaranth?

18. Are there any customer complaint(s) about vegetable amaranth? ☐ Yes ☐ No

If yes then explain:

19. What is your overall impression of the quality and safety of vegetable amaranth that is grown in this area? ☐Excellent quality ☐Good ☐Average ☐ Poor

Thank you

APPENDIX III

Irrigation in Njiru

Effluent water flowing in dug channels by gravity in Njiru(Silancia site) from Manhole. The channels are blocked and the effluent water floods the farms**.**

APPENDIX IV

Irrigation in Njiru (Silancia Area)

The effluent water channel is blocked and effluent water floods the farm as shown in the two pictures below.

APPENDIX V

Irrigation in Njiru (Silancia Area)

The effluent water channel is blocked and effluent water floods the farm as shown in the two pictures below

APPENDIX VI

Manual Splinker Irrigation in Ruai

The farmers in Ruai prefer manual Splinker irrigation shown below over conventional sprinkler irigation because they believe its cost effective that is, less fuel is used by the water pump in pumping the water from Nairobi river to the high ridged farms.

APPENDIX VII

Amaranthus lividus in Njiru

Amaranthus lividus shown below is grown and sold by farmers in Njiru.

APPENDIX VIII

Amaranthus lividus in Ruai

Amaranthus lividus shown below is grown and sold by farmers in Ruai. The amaranth has smaller leaves than Njiru amaranth.

APPENDIX IX

Amaranthus cruentus in Njiru

*Amaranthus cruentus (*purple in colour) shown below is grown and sold by farmers in Njiru.

APPENDIX X

Vitamin C Calibration Curve

(Limits of detection and limits of quantification Calculations of Vitamin C)

Conc (mg/Kg)	Peak A	Peak B	Peak C	Avg Peak	STDev	%RSD
0.00	0	0	0	0	0	#DIV/0!
4.89	34476	33861	34641	34326	411	1.20
9.52	69240	70660	71538	70479	1160	1.65
14.41	106913	107712	107936	107520	538	0.50
19.31	142233	143253	142577	142688	519	0.36
24.53	180361	180024	180545	180310	264	0.15

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999894636
R Square	0.999789282
Adjusted R Square	0.999736603
Standard Error	1096.324348
Observations	6

STEYX	1096	
Slope	7378	
LoD	0.49	**mg/Kg**
LoQ	1.49	**mg/Kg**

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	22811046192	22811046192	18978.73	1.67E-08
Residual	4	4807708.303	1201927.076		
Total	5	22815853900			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-360.6805122	789.4000062	-0.456904623	0.671451849	-2552.406295	1831.045271	-2552.406295	1831.045271
Conc (mg/Kg)	7398.162659	53.70198426	137.7633017	1.66519E-08	7249.062047	7547.26327	7249.062047	7547.26327