IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 9, Issue 10 Ver. II (October. 2016), PP 97-104
www.iosrjournals.org

Levels of Iodide, fluoride and Chloride in Amaranth grain in Kenya

Tonui M. Kipngetich[1*], Ruth N. Wanjau[1], Nicholas K. Gikonyo[2], Jane Murungi[3]
[1]*Department of Chemistry, School of Pure and applied Science, Kenyatta University P.O. BOX 43844 00100 GPO Nairobi Kenya*
[2]*Department of Pharmacy and Complementary/Alternative Medicine P.O. BOX 43844 00100 GPO Nairobi Kenya*

Abstract: *The levels of chloride (Cl^-), fluoride (F^-) and iodide (I^-), in amaranth (Amaranthus hypochondriacus and Amaranthus cruentus) leaves at different stages of maturity (25 days, 50 days and 75 days) and seeds from four counties (Kiambu, Kericho, Bungoma and Kisii) of Kenya were quantitatively determined. The levels were determined using potentiometric and titrimetric methods. The mean levels of Cl^-, F^- and I^- in leaves of A. hypochondriacus and A. cruentus were not significantly different. The mean levels in leaves of the two species indicated that amaranthus grown in Kiambu had Cl^- and F^- at 673.81 and 7.61 mg/100 g respectively while I^- was at 4.29 mg/ kg. Leaves from amaranthus grown in Kericho county had mean levels of Cl^- and F^- of 635.24 and 7.22 mg/100 g dry weight, respectively while I^- was 3.41 mg/kg. The mean I^- level in amaranth leaves grown in Bungoma was 2.54 mg/kg while Cl^- and F^- were 503.74 and 9.67 mg/100 g respectively. The mean Cl^- and F^- level in leaves from Kisii were 595.54 and 8.96 mg/100 g of dry weight respectively, while the mean I^- level was 5.26 mg/kg dry weight. Samples of leaves collected in the dry season and in dry season were also accessedThe levels of all inorganic anions determined were found to be within the allowed daily intake (ADI) values. Based on the results of this study, it is recommended that leaves and grains from both species of amaranthus grown in most regions in Kenya may be consumed for nutritional requirements. The consumption of between 250 g and 300 g of fresh amaranthus leaves is sufficient to provide the required daily intake of all the anions considered in this study for all healthy individuals.*

Keywords: *Tonui , Amaranth, Iodine, Fluorine, Chlorine*

I. Introduction

In Kenya, child mortality rate and malnutrition remain high in spite of the government's commitment to create an enabling environment for the provision of quality health care and reduction of mortality and malnutrition levels, which is critical in rural areas and among poor households [1]. Stunting and underweight in children are key indicators of these deficiencies. In addition, the World Health Organization and World Bank have estimated that between 18-25 % of the developing countries burden of child disease is caused by under nutrition [2]. About 47% of Kenyans lack adequate food to meet nutritional requirements [3] . The unprecedented HIV/AIDS scourge has aggravated this. Millions of people are HIV positive and many of these die every year despite the fact that in developed countries prognosis of HIV/AIDS infected individual has shifted positively [2,4].

Research has shown that there is more HIV related deaths among the poor who cannot afford nutritious food [2]. In sub- Saharan Africa, the HIV prevalence ranges between (25-37 %) in child bearing age [4]. This has resulted in many babies being born to HIV positive mothers. There is need to improve protein, vitamins and important minerals in the diets.

Grain amaranth can provide a solution to nutrition related problems because they are rich in immune boosting essential trace elements such as selenium, zinc, iodine and fluoride. *Amaranthus* species of plants can be a cheap and affordable source of trace elements [5].

Currently, amaranth is gaining worldwide acceptance as a crop rich in high quality protein, due to its remarkable amino acid balance and excellent source of micronutrients [2]. Nutritionally, amaranth is better than milk in protein quality; it scores highly as a source of vitamins C and E as well as a source of calcium, iron and phosphorous. It also has medicinal qualities (contains lysine, methionine and tryptophan) that help prevent cold sores, reduce disease infection and boost the body's immune system. From the economic stand point, amaranth has the capacity to play four main roles in improving the rural farmer's welfare: First, it may provide impoverished households with an alternative source of income. Secondly, it could improve the nutritional content of food consumed by the household members especially vulnerable groups. Thirdly, amaranth could reduce unemployment rates through cultivation, processing and marketing activities and lastly, by lowering the relative price of cereals through increased output, amaranth could make livestock products and fruits more affordable. In Kenya, amaranth is grown in areas such as: Bondo, Bureti, Nakuru, Nyeri, Machakos, Meru,

Kitale, Migori and Kisumu among others. Amaranth grains are sold in some supermarkets in Nairobi and Western Kenya, but in very small quantities. It is consumed in hospitals and children's homes. In Kenyatta National Hospital, it is recommended for patients on special diet.

In view of the above mentioned importance of essential trace elements; Chloride, Fluorine and Iodine study was done to ascertain the levels of these elements in *Amaranthus cruentus* and *Amaranthus hypochondriacus*.

1.1 Chloride ion

Chloride is an essential element and is the main extracellular anion in the body. Chloride ions are found throughout the body; in the blood, in the fluid inside cells and in the fluid between cells along with sodium and potassium [6]. A highly mobile ion easily crosses cell membranes and is involved in maintaining proper osmotic pressure, water balance and acid-base balance.

Chloride works with potassium and sodium to regulate the amount of fluids in the body and to regulate pH in the body. This vital element helps muscles flex and relax normally. Chloride combines with hydrogen in the stomach to form hydrochloric acid, a powerful digestive substance that is responsible for the breakdown of proteins, absorption of other metallic minerals and activation of intrinsic factor, which in turn absorbs vitamin B12. Chloride is especially transported into the gastric lumen in exchange for bicarbornate that maintains electric neutrality across the stomach membrane. Chloride ion may play a more active and independent role in renal function [7, 8], neurophysiology [9] and nutrition [10]. The amount of chloride in the intestinal contents declines as the contents move along the gastrointestinal tract. Typically, 540 mg of chloride enter the duodenum each day[11]. Chloride is absorbed in the jejunum by "solvent drag" and in the ileum and colon by active chloride transport coupled to bicarbonate secretion [11, 12]. Both of these processes are linked to sodium-based co-transport mechanisms that create the necessary osmotic and electrochemical gradients. Human body contains 0.15% chloride or 105 g/70 kg body weight [13]. Most of this chloride is extracellular, as shown by serum levels of 98 to 106 meq/L, compared with the approximate 1 meq/L for tissue cells [14]. Stomach secretions are high in chloride, with concentrations between 45 and 155 meq/L in gastric residues. All body chloride is considered to belong to an exchangeable pool [15].

Body chloride concentrations are regulated by excretions, primarily via the kidneys. Balance studies in young men have shown that 92% of the ingested chloride is excreted in the urine [16]. Both chloride and sodium are regulated by aldosterone (Tortora and Anagnostakos, 1983). The urinary excretion of chloride for adults is about 4.4 g/d, with a range of 2.2 to 13 g/d; the amount excreted is closely related to the amount of salt in the diet [17]. Chloride loss in the faeces is low, with 14 to 110 mg excreted daily by this route. Significant additional daily losses of chloride occur in perspiration [16]. Deficiency of chloride results in life threatening condition known as alkalosis, in which the blood becomes overly alkaline [18]. Excessive intake of chloride leads to fluid retention and high blood pressure. The recommended daily intake is 2300 mg/day for adults [19].

1.2 Fluoride ion

Fluoride is present in the teeth, bones, thyroid gland and skin of animals. It plays an important role in the formation of dental enamel and normal mineralization in bones. About 95% of the total body fluoride is found in bones and teeth [20]. Fluoride's primary function in the human body is to strengthen the bone and it is known to prevent tooth decay [20]. Fluoride strengthens the teeth's enamel by strengthening the mineral composition of the teeth themselves. Fluoride stimulates bone formation [21] and small concentration of fluorides has beneficial effects on the teeth by hardening the enamel and reducing the incidence of caries [22]. At low concentrations (<2 mg/ml) soluble fluoride in the drinking water may cause mottled enamel during the formation of teeth, but at higher levels other toxic effects may be observed [23]. Excessive intake of fluoride results in skeletal and dental fluorosis [24]. Severe symptoms lead to death when fluoride doses reach 250-450 mg/ml [25]. Fluoride content is high in edible parts of all vegetables. Fluoride contents of 3.88 – 20.29 mg/kg dry wt in amaranth, spinach (0.71 – 1.70 mg/kg dry weight), cabbage (0.12 to 0.17 mg/kg dry weight), woman's finger (0.14 to 0.43 mg/kg dry weight) and 0.12 to 2.0 mg/kg of dry weight for tomatoes, were reported in Andhra Pradesh, India [26].

1.3 Iodide ion

The iodine content of food depends on the iodine content of the soil in which it is grown. The iodine present in the upper crust of the earth is leached by glaciations and repeated flooding, and carried to the sea. Seawater is, therefore, a rich source of iodine [27]. Iodine is absolutely vital for the formation of thyroid hormone in the human gland. Lack of iodine can cause changes to thyroid gland directly leading to poor functioning of metabolism and immunity. Iodine deficiency promotes free radical damage in the thyroid gland, directly stressing the health of the gland. Free iodine is in high concentration in the ovaries and breast tissues, acting as a protective buffer. Adequate iodine is also needed to block various compounds from accumulating in

the thyroid gland. Iodine also assists in the proper functioning of numerous hormone receptors throughout the body, thus helping hormones in communicating more efficiently [28]. Iodine is also essential for brain function and intelligence [29]. Human body requires iodine content of 150 mg to 200 mg per day [30]. Iodine concentrations of 0.15–4.59 mg g−1 dry matter [31] have been reported in different foods.

II. Material and methods

Two species of amaranth, *Amaranthus cruentus* and *Amaranthus hypochondriacus* were found to be grown by farmers and their seeds sold in agro- veterinary stores across Kenya. The two species of amaranth, were grown in different farms in kenya.

2.1 Equipments and reagents

The equipments used in this study included UV/Visible spectrometer, Cecil CE 2041 2000 series and the analytical balance, AAA model from Britain. High quality analytical grade reagents were used. These chemicals were supplied by Loba Chemie from India. Water distillation was done using distillation machine, model WSB/4 and water deionization done using Elegastat Micromeg 1190.

2.2 Reagents and glassware

All glassware were cleaned in a non-ionic liquid soap (Laser clean from Laser Chemicals International) and soaked in 5 % nitric acid for 24 hours. They were then rinsed in distilled de-ionized water before drying in the oven at 105 ^{o}C. Dry apparatus were cold and safely kept in clean drawers away from dust

2.3 Extraction and determination of iodine

Iodine was extracted using acid digestion method [32]. A dry sample of mass 2.0000 g was placed in Kjeldahl flask then 2.0 mL of water was added. Ten millilitres of concentrated nitric acid was added and the flask covered and allowed to stand overnight. Concentrated sulphuric acid (5.5 mL) and perchloric acid (20.0 mL) were added. A condenser was inserted into the flask, and the flask heated to reflux the mixture. The condenser was removed after 30 minutes and the heating was continued until the colour of the solution changed from yellow to colourless and back to yellow. Heating was increased to boil off the remaining perchloric acid. The sample was allowed to cool then diluted to 100.0 mL with water. A 1 mL of 2.0 M H2SO4 was added followed by 5.0 mL 10 % potassium iodide solution. A solution of M sodium thiosulphate was titrated against the sample using starch indicator solution.

2.4 Extraction and determination of fluoride ion

For measurement of the water-soluble fluoride in soil, a 0.1000 g portion of each soil sample, which had been dried in an oven and passed through a 0.45 mm sieve, was put into a 125.0 mL shaking bottles containing 25.0 mL of boiling distilled and deionized water. After mixing for 30 minutes in an oscillator, the mixture was filtered to obtain a water filtrate. Fluoride levels were measured by fluoride ion selective electrode potentiometer. For measurement of fluoride in vegetable and grains, each sample was weighed dry after being dried in an oven at 80 °C. A 0.1000 g portion of each vegetable sample was passed through a 0.45 mm sieve, put into 125.0 mL shaking bottles with 25.0mL of 0.05 M sulphuric acid, mixed by gentle shaking for 30 min and then extracted at 60 °C for 30 min. Fluoride content was measured at room temperature using a fluoride ion selective electrode. An ORION Model 818 (USA) instrument was used for all measurements of fluoride.

2.5 Extraction and determination of chloride

Chloride was extracted by shaking 300 mg of dried plant material with 30 mL of 0.01M CaSO4 solution for 30 minutes. A 0.85 g of pre-washed charcoal was added to each sample and shaking was continued for 5 additional minutes. The mixture was centrifuge for 20 minutes and filtered using whatman No. 42. The aliquot was analyzed colorimetrically by the mercury (II) thiocyanate method. In this method, a reddish yellow-colored complex of ferric thiocyanate was formed when chloride ions sequester the mercury ions of mercuric thiocyanate (thereby freeing the thiocyanate) in the presence of excess ferric nitrate. The color was measured on a Cecil CE 2041 2000 series spectrophotometric instrument.

III. Results and discussion

3.1 Comparison of the mean Fluoride, Chloride and Iodide in *A. hypochondriacus* and *A. cruentus* leaves

Table 1: The mean fluoride, chloride and Iodide ion level in *A. hypochondriacus* and *A. cruentus* leaves grown in selected counties of Kenya

County	Ion	*A. hypochondriacus* mean levels in mg/100 g		*A. cruentus* mean levels in mg/100 g		P value
		Mean ± SE (n=18)	Range	Mean ± SE (n=18)	Range	
Kiambu	Iodide	3.80 ± 0.88	0.32-11.37	4.49 ± 1.01	0.44 - 10.76	0.611
	Fluoride	9.09 ± 0.28	7.18-10.70	6.94 ± 0.43	3.24-8.29	0.000
	Chloride	755 ± 40	463-1053	728 ± 39	442-1090	0.631
Kericho	Iodide	3.84 ± 0.80	0.30-14.33	2.98 ± 0.42	0.24-5.62	0.347
	Fluoride	6.09 ± 0.54	3.80-13.80	8.46 ± 0.46	5.89-12.50	0.002
	Chloride	742 ± 49	289-1124	639 ± 48	236-905	0.145
Bungoma	Iodide	2.45 ± 0.31	0.81-5.32	2.35 ± 0.39	0.58-4.92	0.846
	Fluoride	11.97 ± 0.62	7.93-16.30	11.33 ± 0.72	6.24-15.10	0.512
	Chloride	585 ± 68	273-1000	282 ± 6	322-889	0.971
Kisii County	Iodide	5.12 ± 1.14	0.60-13.53	4.79 ± 1.04	0.53-12.35	0.782
	Fluoride	11.88 ± 0.96	6.68-21.20	10.45 ± 0.66	4.98-21.20	0.027
	Chloride	699 ± 54	490-1325	733 ± 61	450-1356	0.681

The *A. hypochondriacus* had the mean fluoride ions level in Kiambu, Kericho, Bungoma and Kisii counties being 9.09, 6.09, 11.97 and 11.88 mg/100 g respectively. The *A. cruentus* had the mean fluoride level of 6.94, 8.46, 11.33 and 10.45 mg/100 g respectively. The difference was significant in Kiambu, Kericho and and Kisii ($P<0.05$). Comparing the two species of amaranth, *A. hypochondriacus* had significally higher levels of fluoride than *A. cruentus*. This can be attributable to the difference in the levels of fluoride in the soil in the different counties. The reported values in other vegetables are 0.79 mg/100 g in kahuku (*cucubita*) and 5.93 mg/100 g in *A. hybridus* [33]. This study found the levels of fluoride in amaranth being significantly higher than the reported values.

Kisii County recorded the highest levels of Iodide ions (5.12 mg/kg) in *A. hypochondriacus* and 4.79 mg/kg in *A. cruentus*. The lowest level was recorded in Bungoma in the two species. However, the levels of I^- in the two species of amaranth were not significantly different.

Data from Kiambu County showed the highest mean level of chloride in *A. hypochondriacus* of 755 mg/100 g followed by Kericho, Kisii and Bungoma Counties with 742, 699 and 585 mg/100 g respectively. Levels in *A. cruentus* were 728, 639, 585 and 733 for Kiambu, Kericho, Bungoma and Kisii respectively. The reported values for the different species in Kiambu, Kericho, Bungoma and Kisii were 0.631, 0.145, 0.971 and 0.681 respectively. The mean values of chloride ion from all the regions for the two species had no significant difference ($P>0.05$). The recommended daily intake for chloride is 2300 mg/day for adults (Osiecki, 2005). The levels in *A. hypochondriacus* and *A. cruentus* were lower than the RDI, however they were more than those reported in cabbage (40 mg/100 g), pumpkins (40 mg/100 g), tomatoes (41.67 mg/100 g), spinach (100 mg/100 g), pepper (133.33 mg/100 g), onions (100 mg/100 g) and carrots140 mg/100 g) as reported by Osiecki, 2005. On average if one relies solely on amaranth as a source of chloride ions, 1kg per day will meet the daily requirement.

3.2 Comparison of the mean Fluoride, Iodide and Chloride level in amaranth leaves at different stages

The mean levels of Fluoride, Iodide and Chloride ions at different stages of the two species are tabulated in Table 2.

Table 2: The mean fluoride ion levels in *A. hypochondriacus* and *A. cruentus* leaves at different stages

Maturity	Ions	*A. hypochondriacus* mean fluoride levels in mg/100 g		*A. cruentus* mean fluoride levels in mg/100 g		P value
		Mean ± SE (n=24)	Range	Mean ± SE (n=24)	Range	
25 days	Iodide	1.66 ± 0.34	0.30-6.59	1.22 ± 0.26	0.24-5.47	0.325
	Fluoride	11.00^a ± 0.79	6.42-21.20	10.41^a ± 0.56	6.18-15.30	0.544
	Chloride	697 ± 58^a	289- 1325	672 ± 63^b	236-1356	0.769
50 days	Iodide	3.57 ± 0.58	0.76-10.89	4.04 ± 0.70	0.76-10.76	0.565
	Fluoride	10.57^a ± 0.75	4.80-16.30	9.22^a ± 0.58	6.17-15.10	0.165
	Chloride	652 ± 42^a	273- 1000	635 ± 35^b	322-889	0.775
75 days	Iodide	6.31 ± 0.86	1.99-14.33	5.70 ± 0.70	2.32-12.35	0.583
	Fluoride	7.71^b ± 0.50	3.80-13.80	7.19^b ± 0.51	3.24-12.50	0.467
	Chloride	737 ± 40^a	385- 1124	704 ± 36^b	343-905	0.546

Mean values with the same lower case letter (a or b) within the same column are not significantly different at 95% confidence level

The mean levels of Iodide in *A. hypochondriacus* were 1.66, 3.54 and 6.31 mg/kg dry weight at 25 days, 50 days and 75 days respectively. The same trend was revealed in *A. cruentus*, it was lowest at 25 days and highest at 75 days. Different species had significantly different levels of anion at the same stage of maturity.

There was no significant difference in levels of fluoride in *A. hypochondriacus* and *A. cruentus* of the same stage of maturity. The *post hoc* analysis of homogeneous subsets reveals that fluoride ion level was significantly higher than at 25 and at 50 days in the two species. *A. hypochondriacus* have slightly elevated levels of fluoride than *A. cruentus*. This fluoride levels were higher than those reported in other food [33]. The RDI varies from one country to another with tolerable upper limit >30 mg per day [34]. The levels revealed in this study are below the tolerable limits.

The levels of chloride in *A. hypochondriacus* at 25 days, 50 days and 75 days old were 697 mg/100 g, 652 mg/100 g and 737 mg/100 g, respectively. The mean chloride ion level in *A. cruentus* at 25 days, 50 days and 75 days old were found to be 672, 635 and 704 mg/100 g of dry weight, respectively. The P values were 0.769, 0.775 and 0.546 at 25 day, 50 days and 75 days respectively. Comparison of the mean chloride ion levels of the *A. cruentus* and *A. hypochondriacus* using one way ANOVA showed no statistical difference (P>0.05) between the means at the different stages of maturity. Levels of chloride were higher in spinach (100 mg/100 g fresh weight), pepper (100 mg/100 g fresh weight) and carrots (100 mg/100 g fresh weight) than in amaranth.

3.3 Comparison of the mean levels of fluoride, iodide and chloride ion in leaves in the try and wet season

Table 3: The mean fluoride, Iodide and Chloride ion levels in leaves *A. hypochondriacus* and *A. cruentus* for two seasons

County		Dry season	Wet season	P values
		Mean ± SE in mg/100 g	Mean ± SE in mg/100 g	
Kiambu	Iodide	6.30 ± 1.09	2.00 ± 0.30	0.001
	Fluoride	8.02 ± 0.45	8.32 ± 0.45	1.000
	Chloride	726 ± 52	757 ± 20	0.580
Kericho	Iodide	5.36 ± 0.58	1.45 ± 0.23	0.001
	Fluoride	7.56 ± 0.61	7.00 ± 0.53	0.497
	Chloride	631 ± 60	751 ± 31	0.087
Bungoma	Iodide	2.15 ± 0.27	2.66 ± 0.41	0.306
	Fluoride	11.65 ± 0.68	11.60 ± 0.68	1.000
	Chloride	442 ± 44	725 ± 62	0.001
Kisii	Iodide	7.67 ± 1.24	2.33 ± 0.18	0.001
	Fluoride	10.45 ± 0.94	10.35 ± 0.94	1.000
	Chloride	869 ± 60	563 ± 18	0.001

The mean iodide ions level in the dry season was higher than the wet season in Kiambu, Kericho and Kisii Counties. These differences were significant in Kiambu, Kericho and Kisii. This shows that seasonal variation has an effect on the levels of iodide in leaves. Iodide ion accumulate in the leaves in the dry season. These mean iodide values can be compared to those in shellfish [27]. The recommended daily intake (RDI) for iodide for adult is 150 to 200 µg/day and infants 90 µg/day [35]. *A. hypochondriacus* and *A. cruentus* is therefore a good source of iodine.

The mean fluoride ions level in leaves of amaranth grown in Kiambu, Kericho, Bungoma and Kisii were 8.02, 7.56, 11.65 and 10.45 mg/100 g respectively in the dry season. In the wet season Kiambu, Kericho, Bungoma and Kisii recorded 8.32, 7.00, 11.60 and 10.35 mg/100 g dry weight respectively. The differences in the mean levels of fluoride in leaves in the dry and wet seasons were not significantly different.

The Adequate Intake (AI), in the case of fluoride is the daily intake level required to reduce tooth decay without causing moderate dental fluorosis. The AI for fluoride from all sources is set at 0.05mg/kg/day. Calculations from these results shows that, an adult with body weight 60 kg can eat 100 g of amaranth without passing the AI of fluoride per day. Amaranth is a good source of fluoride, which can maximize the benefit and with minimize the harmful effect. People should therefore be encouraged to eat amaranth vegetable.

Kisii County recorded the highest (869 mg/100 g dry weight) chloride level in the dry season while Bungoma recorded the lowest (442). Kiambu recorded the highest (757 mg/100 g dry weight) in the dry season while Kisii recorded the least (563 mg/100 g dry weight). Comparing the levels of chloride ions in leaves within the same region, Kiambu and Kisii had the higher means than sample from Kericho and Bungoma during dry season. The mean difference in chloride ion levels in the wet and dry seasons was significant in Bungoma and Kisii but insignificant in Kiambu and Kericho. The chloride levels in all the regions were higher than those in pumpkins, pepper and carrots [20]. Therefore, amaranth is a better source of chloride ion than these kinds of foods.

3.4 Comparison of the mean levels of ions in seeds from different Counties

Table 4: The mean Fluoride, Iodide and Chloride ions levels in *A. hypochondriacus* and *A. cruentus* seeds from selected Counties of Kenya

County	Ions	*A. hypochondriacus* mean fluoride levels in mg/100 g		*A. cruentus* mean fluoride levels in mg/100 g		P value
		Mean ± SE	Range	Mean ± SE	Range	
Kiambu	Iodine	5.36 ± 1.90	1.50-13.36	4.05 ± 0.94	1.78-6.23	0.550
	Fluorine	7.27 ± 0.25	6.79-8.07	5.51 ± 0.41	4.42-6.68	0.005
	Chlorine	551 ± 61	324-688	387 ± 119	47-661	0.250
Kericho	Iodine	3.68 ± 0.77	1.94-5.46	3.15 ± 0.65	1.62-4.62	0.611
	Fluorine	5.00 ± 0.35	4.39-6.12	9.11 ± 0.01	9.08-9.13	0.000
	Chlorine	456± 112	203-710	476 ± 92	265-689	0.892
Bungoma	Iodine	3.28 ± 0.82	1.32-5.17	2.64 ± 0.70	0.98-4.52	0.571
	Fluorine	3.97 ± 0.43	2.80-5.14	3.43 ± 0.39	2.55-4.63	0.374
	Chlorine	257 ± 7	239-276	269 ± 25	262-277	0.185
Kisii	Iodine	6.13 ± 2.02	1.46-10.73	5.98 ± 1.84	1.84-10.19	0.958
	Fluorine	4.15 ± 0.16	3.88-4.65	4.79 ±0.56	3.10-6.14	0.306
	Chlorine	248 ± 39	161-396	216 ± 31	145- 290	0.537

The *A. hypochondriacus* had the mean level of fluoride in Kiambu and Bungoma being higher than the mean level in *A. cruentus*. One way ANOVA reveals the P values in Kiambu, Kericho, Bungoma and Kisii being 0.005, 0.000, 0.374 and 0.306 respectively. The difference in levels in the two species were significant ($P<0.05$) in Kiambu and Kericho. The difference between the levels in samples from Bungoma and Kisii was insignificant. Levels of fluoride in leaves of amaranth are comparable with the literature values [33].

Kisii recorded the highest level of 6.13 mg/kg with a range of 1.46 mg/kg to 10.73 mg/kg of dry weight in *A. hypochondriacus.* Bungoma recorded the lowest level of 3.28 mg/kg in *A. hypochondriacus* and 2.64 mg/kg in *A. cruentus*. The P values were 0.550, 0.611, 0.571 and 0.958 in Kiambu, Kericho, Bungoma and Kisii respectively. These values are more than 0.05, hence the difference in the mean levels of iodide in *A. hypochondriacus* and *A. Cruentus* are not statistically different. The mean levels of iodide in leaves of amaranth were close to those reported in the literature [27].

The levels of chloride ion in the grains range from 248 ± 39 mg/100 g to 551 ± 61 mg/100 g in grains. Levels of chloride ion in leaves from Kiambu county were highest in *A. hypochondriacus* while levels in *A. cruentus* from Kericho County were also highest. In Kiambu, Kericho, Bungoma and Kisii counties samples had $P > 0.05$ at 95% confidence level. The difference in levels of chloride in *A. hypochondriacus* and *A. cruentus* grains was insignificant. This implies that, according to this study the species do not influence the concentration of chloride ions.

3.5 Comparisons of the mean levels of ions in the seeds in the dry and wet season.

Table 5: The mean Iodine and Fluoride Chloride ion levels in grains of *A. hypochondriacus* and *A. cruentus* in the dry and wet Seasons

County		Dry season	Wet season	P values
		Mean ± SE in mg/100 g	Mean ± SE in mg/100 g	
Kiambu	Iodide	7.58 ± 1.16	1.83 ± 0.10	0.001
	Fluoride	6.39 ± 0.52	6.30 ± 0.65	1.000
	Chloride	274 ± 73	664 ± 7.4	0.001
Kericho	Iodide	5.00 ± 0.18	1.87 ± 0.06	0.000
	Fluoride	7.06 ± 0.95	7.09 ± 0.90	1.000
	Chloride	238 ± 14	695 ± 5.8	0.001
Bungoma	Iodide	4.67 ± 0.22	1.26 ± 0.10	0.000
	Fluoride	3.70 ± 0.43	3.74 ± 0.33	1.000
	Chloride	256 ± 7.2	270 ± 2.2	0.094
Kisii	Iodide	10.36 ± 0.13	1.75 ± 0.82	0.000
	Fluoride	4.47 ± 0.44	4.37 ± 0.54	1.000
	Chloride	156 ± 4.9	308 ± 17	0.000

The levels of chloride in the seeds in the dry season varied from 274 mg/100 g in Kiambu to 156 mg/100 g in Kisii. The chloride ion levels in the wet season were highest in Kiambu with 664 mg/100 g and lowest in Kisii with 308 mg/100 g. The difference in the mean concentration in the wet season and dry season was very big in Kericho. The difference in means chloride ion between the dry season and wet season at Kiambu, Kericho and Kisii was significant. Thus, it can be concluded that seasonal variation influences the concentration of chloride in grains.

Kiambu had the highest mean level of fluoride in both the dry season and the wet season while Bungoma region had the lowest level in the dry season and wet season. The P value indicates the difference was significant between the seasons in all regions.

The mean iodide level in the dry season were 7.58, 5.00, 4.67 and 10.36 mg/kg of dry weight in Kiambu, Kericho, Bungoma and Kisii respectively. In the wet season the means were 1.83, 1.87, 1.26 and 1.75 mg/kg respectively in Kiambu, Kericho, Bungoma and Kisii respectively. The difference in means of the wet and dry seasons in the same region was significant. Climatic conditions influence the level of iodide in seeds.

IV. Conclusion

With respect to the results obtained from this study, the following conclusions can be made:-

i. All the samples analyzed contained the selected inorganic anions (F^-, I^-, Cl^-) considered in the study.
ii. *Amaranthus hypochondriacus* generally had higher levels of the specified inorganic anions compared to *Amaranthus cruentus* although the difference is not significant.
iii. Levels of inorganic anions in *Amaranthus hypochondriacus* and *Amaranthus cruentus* grown in different seasons were significantly different. Dry season (September to February) which had relative low rainfall than wet season (March to August) had high levels of iodide.
iv. Different ecological regions were found to have significantly different levels of anions in amaranth of the same species. Chloride was significantly high in ecological zones with high rainfall while Iodide was high in amaranth in Counties with low rainfall. Fluoride did not show significant variation in different ecological zones.
v. Levels of inorganic anions in amaranth leaves at different stages of growth differ significantly. Fluoride and chloride levels in leaves reduced as the plant matured while iodide increased with the maturity of the two species of amaranth. Levels of anions in grains were lower than levels in leaves at 75 days maturity.
vi. All the samples studied had anions levels below the RDI except fluoride in Bungoma which were high.
vii. *Amaranthus hypochondriacus* and *Amaranthus cruentus* if used as vegetable and the grain used to make flour would provide the body with the require daily intake of anions.

References

[1]. CBS/MOH/ORC (2004). Kenya demographic and health survey, Calverton, Maryland. Pp 114-168.
[2]. Tagwira, F. (2009). Why grain amaranth in Zimbabwe. Mutare: African University (pp 1-4).
[3]. GOK/MOH (2006). Kenya national guidelines on nutrition and HIV/AIDS, Nairobi; Pp 1-6.
[4]. Tagwira, M., Tagwira, F.D. and Okum, B. (2006). Using grain amaranth to fight malnutrition. *RUFORUM working document*. 1: 201- 206.
[5]. Kauffman, C.S. and Weber, L.E. (1990). Grain Amaranth file://C:/Documents%20and % settings/Jackie-Chan/Desktop/grain%20 Amaranth.htm. Accessed on 3rd July, 2008.
[6]. Weast, R.C., (1986). CRC handbook of chemistry and physics, 67th ed. Boca Raton, FL, CRC Press.
[7]. Jaina, A., Kapuler, S., Govendo, S., Serban, I. and Eliahou, H.E., (1980). Blood pressure and renin activity in Na^+, Cl^-, or NaCl loading in rats. Miner. *Electrolyte Metab*. **3**: 276.
[8]. Toto, R.D., Hulter, H.N., Mackie, S. and Sebastian, A., (1984). Renal tubular acidosis induced by dietary chloride. *Kidney Int.* **25**: 26.
[9]. Sackmann, B. and Neher, E., (1984). Patch clamp techniques for studying ionic channels in excitable membranes. *Annu. Rev. Physiol*., **46**: 455.
[10]. Honeyfield, D.C. and Froseth, J.A., (1985). Effects of dietary sodium and chloride on growth, efficiency of feed utilization, plasma electrolytes and plasma basic amino acids in young pigs. *J. Nutr.* **115**: 1366.
[11]. Weinberg, J.M., (1986). Fluid and electrolyte disorders and gastrointestinal diseases. In: Fluids and electrolytes. Pp 20.
[12]. Schultz, S.G., (1984). A cellular model for active sodium absorption by mammalian colon. Annu. *Rev. Physiol.*, **46**: 435.
[13]. National Academy of Sciences, (1980). Recommended dietary allowances. 9th edition. Washington, DC
[14]. Siggaard-Anderson, O., (1976). Blood gases and electrolytes. In: Fundamentals of clinical chemistry
[15]. Department of National Health and Welfare, (1983). Recommended nutrient intake for Canadians. Ottawa.
[16]. International Commission on Radiological Protection,(1975). Report of the task group on reference man, ICRP Publication No. 23, Pergamon Press, Oxford. p. 379
[17]. Tortora, G.J. and Anagnostakos, N.P., (1984). Principles of anatomy and physiology. 4th edition. Harper & Row, New York, NY
[18]. Kaleita, T.A., (1986). Neurologic/behavioral syndrome associated with ingestion of chloride-deficient infant formula. *Pediatrics* **78**: 714-715
[19]. Osiecki, H., (2002). The nutritional Bible. Bio- concepts publishing QLD.
[20]. Cerklewski, F.L., (1997). Fluoride bioavailability- nutritional and clinical aspects. *Nutri Res*. **17**: 907-929
[21]. Richards, A., Moskilder, L. and Sogaard, C.H., (1994). Normal age-related changes in fluoride content of vertebral trabecular bone-relation to bone quality. *Bone* **6:**15-21
[22]. Fung, K., Zahang, Z., Wong, J. and Wong, M., (1999). Fluoride content in tea and soil from tea plantations and release of fluoride into tea liquor during infusion. *Environ. Pollut*. **104**: 197-205
[23]. Weast, R.C. and Lide, D.R., (1990). Handbook of chemistry and physics, 70th edition, CRC Press, Boca Raton, Fluoride pp B-17
[24]. Czarnowski, W., Kerchniak, J., Urbanska, B., Stolarska, K., Taraszewska, M. and Muraszko, A., (1999). The impact of water borne fluoride on bone density. *Fluoride* **32**: 91-95
[25]. Luther, S., Poulsen, L., Dudas, M. and Rutherford, P., (1995). Fluoride absorption and mineral stability in an Alberta soil interacting with phosphogypsum Leachate. *Can. J. soil Sci*. **76**: 83-91
[26]. Khandare, A.L and Shanker G.R., (2006). Uptake of fluoride, aluminum and molybdenum by some vegetable from irrigation water. *J. Hum. Ecol.*, **19**: 283-288
[27]. Koutras, D.A., Matovinovic, J. and Vought, R., (1985). The ecology of iodine. In: Stanbury JB, Hetzel BS, eds. Endemic goitre and endemic cretinism. Iodine nutrition in health and disease. New Delhi, Wiley Eastern Limited 185–195
[28]. Utiger, R.D., Felig, P. and Frohman, L.A., (2001). Thyrotoxicosis, hypothyroidism and the painful thyroid in endocrinology & metabolism. Mcgraw-hill, Inc Medical Publishing Division pp275

[29]. Hollowell, J., Staehling, N., Hannon, W., Flanders, D., Gunter, E. and Maberly G., (1998). Iodine nutrition in the United States. Trends and public health implications: Iodine excretion data from national health and nutrition examination surveys I and III *J Clinical Endocrinology and Metabolism*, **83**: 3401-3408
[30]. Mahesh, D.L., Deosthale, Y.G., Narasinga, B.S., Karmarkar, M.G., Pandav, C.S. and Ahuja, M.M.S., (1990). Environment, genetics and thyroid disorders. New Delhi., pp 127-132
[31]. Larsen E.H and Ludwigsen, M.B., (1997). Determination of iodine in food-related certified reference materials using wet ashing and detection by inductively coupled plasma mass spectrometry. *J. Ana. Atomic Spectrometry*. **12**:1
[32]. Bahman, K.S., (1944). Modification of the chlorate digestion method for determination of iodine in biological materials. California, USA
[33]. Kahama, R.W., Kariuki, D.N., Kariuki, H.N. and Njenga, L.W. (1997). Fluorosis in children and sources of fluoride around lake Elementaita region of Kenya. *Fluoride* **30**: 19-25
[34]. Neurath, C., (2005). Tooth decay trends for 12 years old in non fluorinated and fluorinated countries. *Fluor.* **38**: 324-325
[35]. WHO/NHD/01.1 (2001).Assessment of the iodine deficiency disorders and monitoring their elimination.Geneva