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Influence of Soil and Rain on the Levels of Inorganic Anions in Amaranth Leaves from Selected Parts of Kenya

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Abstract
The aim of this work was to determine the level of inorganic anions (NO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}, F^- and I^-) in the leaves of two species of amaranth (*Amaranthus hypochondriacus* and *Amaranthus cruentus*) planted in Kenya. The mean levels of inorganic anions, nitrate (NO_3^-), chloride (Cl^-), sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), fluoride (F^-) and iodide (I^-), in the soil from four study regions (Kenyatta University (KU), Bureti, Kisii and Elgon) of Kenya were quantitatively determined. The levels were determined using spectrophotometric, potentiometric and titrimetric methods. The mean levels of Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, F^- and I^- in soils ranged from 1146.54 to 2733.31 mg/100 g, 1821.60 to 2185.33 mg/100 g, 828.54 to 1111.36 mg/100 g, 1015.55 to 1910.66 mg/100 g, 66.95 to 79.77 mg/100 g and 8.00 to 12.57 mg/kg respectively. The levels of most anions in leaves of *A. hypochondriacus* and *A. cruentus* were not significantly different. The means levels of the anions in leaves of the two species indicated that Cl^- ion had range of 503.74 to 673.81 mg/100 g, SO_4^{2-} 701.61 to 955.17 mg/100 g, PO_4^3 532.36 to 629.46 mg/100 g, NO_3^- 495.79 to 880.99 mg/100 g, F^- 7.22 to 9.67 mg/100 g while I^- ranged from 2.54 mg/kg to 5.26 mg/kg dry weight. The 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: Amaranth, Anions, Amaranthus, Tonui

1. Introduction

The consumption of vegetables and fruits as food offers a means of providing adequate vitamins supplies, minerals and fibre. Vegetables used as food include those used in making soups or served as integral parts of the main meal (Ihekeronye and Ngoddy, 1985). Each plant species has its nutritive value that may differ from others. Thus, different plants growing under similar conditions may contain varying concentrations of minor and macro elements. Leafy vegetables occupy a very important place in the human diet hence the need to determine their nutritional value.

Kenyans have traditionally made use of edible leaves of some plant species for health, nutrition, income generation and food security. These indigenous vegetables are nutritionally equal or superior to exotic vegetables. Researchers, agriculturalists, policy makers and granting agencies, have neglected indigenous vegetables and yet most people in the region live below the poverty line that is manifested in malnutrition and poor health. Limited research has identified some of the indigenous leafy vegetables that could be developed as commercial horticultural crops and these include grain amaranth, spider plant and African nightshades. The major constraints of production include lack of good quality seed and lack of technical information on their production. Nitrogen and phosphorus are key elements in the production of leafy vegetables as they enhance leafy yield by promoting cell division and expansion in leaves and root development. Studies to improve production will help raise the livelihood of people in Kenya by improving health through consumption and income generation.

Too much emphasis has been laid on crops that require a lot of inorganic fertilizers that are expensive for the rural poor. The use of organic manure has not been maximized to help the farmer cultivate indigenous vegetables for their household use and/or for sale as one way of income generation. Such income sources are often important for women and children in poor rural areas. Indigenous food crops such as grain amaranth, African nightshade and spider plant can provide food security.

Amaranth is highly nutritious, and is extremely attractive and useful. (Mosyakin and Robertson, 1996). In Africa, this vegetable has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care. It can be easily harvested, produces a lot of fruits (and thus seeds) which are used as grain, are highly tolerant of arid environments which are typical of most subtropical and some tropical regions, and due to large amounts of protein and essential amino acids, such as lysine (De Macvean and Poll,

1997). Amaranth grains grow very rapidly and their large seed heads can weigh up to 1 kilogram and contain a half-million seeds (Tucker, 1986). *Amaranthus* species are reported to have a 30% higher protein value than other cereals, such as rice, wheat flour, oats, and rye (De Macvean and Poll, 1997).

Besides protein, amaranth grain provides a good source of dietary fiber and dietary minerals such as iron, magnesium, phosphorus, copper, and especially manganese (Makus and Davis, 1984; Willis *et al.,* 1984). Metals are found in plants combined with inorganic anions or complexed with organic molecules. These anions play an important role in the body and their level in amaranth need to be investigated. Amaranth contains tannin and is astringent (Bown, 1995; Chevallier, 1996). It is used internally in the treatment of diarrhea and excessive menstruation (Bown, 1990, Chevallier, 1996). It can be used as a gargle to soothe inflammation of the pharynx and to hasten the healing of ulcerated mouths (Chevallier, 1996), whilst it can also be applied externally to treat vaginal discharges, nosebleeds and wounds (Bown, 1995). The plant can be used fresh or it can also be harvested when coming into flower and dried for later use (Bown, 1995).

Good nutrition is important to all but more so to those who are either with medical or surgical conditions and also those on treatment. The relationship between nutrition and immunity has long been established (WHO and FAO, 2002). Good nutrition is the key to the overall management and control of most diseases. Poor nutrition lowers a person's immune system and leads to lower T- cells count, which are part of the protective system of the body against attack by any invading germs (WHO and FAO, 2002).

When food intake is low, multivitamins and mineral supplements often in form of pills can help to increase requirements. This is the case where the body is suffering from certain diseases including HIV and AIDS, cancer, diabetes and osteoporosis. These supplements are not always readily available since they are expensive and the majority of the Kenyan population cannot afford to buy them. Even if micronutrient supplements are available and useful, they cannot replace eating a balanced diet. Taking high doses of some of these supplements causes nausea, vomiting, decreased appetite, kidney and liver problems as well as interfering with the immune system (WHO and FAO, 2002). It is therefore important to take these nutrients in their natural form.

This study investigated the levels of some selected anions in two species of grain amaranth grown in four regions of Kenya.

2. 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. Soil was sampled in the farms in the four counties were the plants were planted and levels of Chloride, Sulphate, Phosphate , Nitrate, Fluoride and the Iodide were determined

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. Sampling procedure

The leaves of *Amaranthus hypochondriacus* and *Amaranthus cruentus* were sampled and pruned. The samples were placed in clean mini-grip polythene bags, labelled and transported to the laboratory where they were stored in dust free environment prior to oven drying. Before planting, soil at a depth of between 0-30 cm was sampled using an auger at 5 random positions within the farms where amaranth samples were collected. The combined soil sample was mixed thoroughly and sub-sampled by the quartering procedure (Hesse, 1971). The soils were spread uniformly over a sheet of polythene and divided into 4 equal portions numbered 1 to 4. Portions 1 and 4 were discarded and the remaining portions 2 and 3 were further spread and reduced by half using the same procedure. The process was repeated to retain a sample of 1 Kg. The sample was labelled and stored in clean paper bags. The procedure ensured that a representative sample was obtained.

2.4. Soil pre-treatment

Soil samples were air-dried at room temperature for one week. Using porcelain pestle and mortar, they were ground and screened through 2 mm (80 mesh) nylon sieve. One portion was oven dried at 105 ^{o}C for 48 hours

and kept in polythene bags.

2.5. Extraction and determination of phosphate

Vegetable samples were chopped into small pieces and then air-dried. The dried samples were ground and sieved in a sieve of mesh 1 mm. A 1.0000 g portion of each of the ground and sieved samples was weighed into acid-washed porcelain crucibles. The crucibles were labeled and 5.0 mL of 20 % (weight per volume) magnesium acetate added and evaporated to dryness. The crucibles were then transferred into the furnace and the temperature raised to 500 °C. The samples were ashed at this temperature for 4 hours then removed and cooled in desiccators. Exactly 10.0 mL of 6.0 M HCl were then added to each of the crucible and covered, then heated on a steam bath for 15 minutes. The contents of each crucible were transferred into different evaporating basins and 1.0 mL of concentrated HNO_3 was added. The heating was continued for 1 hour to dehydrate silica. A 1.0 mL of 6.0 M HCl was then added, swirled and then followed by the addition of 10.0 mL of distilled water and again heated on the steam bath to complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No. 1 filter paper into 50.0 mL volumetric flasks and the volumes made up to the marks with distilled water (Radojevic and Bashkin, 1999). Phosphate ion concentration in the samples was determined using the molybdenum blue method (Dick and Tabatabai, 1977).

2.6 Extraction and determination of nitrate ion

Vegetable sample solutions were prepared by chopping the leaves into smaller sizes. The chopped samples were air-dried. The air-dried samples were ground and sieved with a sieve of mesh 1.0 mm. Nitrates was extracted by shaking 300 mg of dried plant material with 30 mL 0.1 M CaSO4 solution for 30 min. A 0.85 g of prewashed charcoal was added to each sample and shaking was continued for 5 additional minutes. Samples was centrifuged and filtered through a Whatman No. 42 filter paper (Radojevic and Bashkin, 1999).. Nitrate concentrations in the filtrate are determined colorimetrically by the cadmium reduction method. In this method, nitrate is reduced to nitrite in a copperized cadmium column. The nitrite ions react with sulfanilamide under acidic conditions to form a diazo compound. This couples with N-1-Napthylethylenediamine dihydrochloride to form a reddish purple azo dye which is measured at 520 nm on an Cecil CE 2041 2000 series (Wood *et al.*, 1967).

2.7 Extraction and determination of iodine

Iodine was extracted using acid digestion method (Bahman, 1944). 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 H_2SO_4 was added followed by 5.0 mL 10 % potassium iodide solution. A solution of 5×10^{-6} M sodium thiosulphate was titrated against the sample using starch indicator solution.

2.8 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 in the plant materials was extracted by shaking 0.50 g of dried plant material in 20 mL of 0.05 *M* H_2SO4 for 15 minutes. Then 20 mL of 0.01 *M* NaOH was added followed by an additional 15 minutes of shaking. Potential interference from Al, Si, and Fe are reduced by adding 5 mL of 3 M NaOAc and 10 mL of 0.5 *M* Sodium Citrate buffers before analysis. Fluoride was measured with a fluoride ion selective electrode (An ORION Model 818, USA) under constant stirring and temperature.

2.9 Extraction and determination of chloride

Chloride was extracted by shaking 300 mg of dried plant material with 30 mL of 0.01M $CaSO_4$ 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

2.10 Extraction and determination of sulphate

To extract sulphate, 5.0 mL of magnesium nitrate solutions were added to each of the ground and sieved samples in the crucibles. These were then heated to 180 °C on a hot plate (Huart SB162). The heating process was allowed to continue until the colour of the samples changed from brown to yellow (Helrich, 1990). The samples were then transferred to the furnace at a temperature of 500 °C for four hours. Magnesium nitrate was added to prevent loss of sulphur. The contents of each crucible were carefully transferred to different evaporating basins. A 10.0 mL portion of concentrated HCl was added to each of them and covered with watch glasses. They were boiled on a steam bath for 3 minutes. On cooling, 10.0 mL of distilled water were added to each of the basins and the contents of each were filtered into 50.0 mL volumetric flasks and the volumes made up to the marks with distilled water (Radojevic and Bashkin, 1999). Sulphate was determined using turbidmetric method (Massuomi and cornfield, 1963).

Data Analysis

The mean levels of inorganic anions were determine for the four Counties (Bomet, Kiambu, Kisii and Bungoma) in Kenya. The SPSS version 11.5 program was run to give the mean levels of inorganic anions, analysis of variance which was to check variations in inorganic anions between the varieties from the same location and different locations. Duncan tests were performed to establish varieties which were high in the tested inorganic anions.

3. Results and Discussion

The levels of Chloride, Sulphate, Phosphate, Nitrate, Fluoride and the Iodide in the soil and the amaranth samples in the area of study were determined. This was with a view of establishing any differences in their levels in the species within and between locations (Table 1).

3.1.1 Chloride ion

The mean chloride level in the wet season ranged from 1148 to 4189 mg/100 g with a range of 970 to 1831 mg/100 g in the dry season. This difference was significant (P>0.05) in the level of chloride ion in the soil in the dry season from the wet season. There was a wider range of chloride level in the soil during the wet season. This may have been brought about as a result of leaching in soil in some areas. Regional variation was observed with Kenyatta University showing the highest mean level of chloride in *A. hypochondriacus* of 755 mg/100 g followed by Bureti, Kisii and Mt. Elgon with 742, 699 and 585 mg/100 g respectively. Levels in *Amaruntus cruentus* were 728, 639, 585 and 733 mg/100 g in Kenyatta University, Bureti, Elgon and Kisii respectively. The reported values for the different species in Kenyatta University, Bureti, Elgon and Kisii were 0.631, 0.145, 0.971 and 0.681 respectively (table 2). The mean values of chloride ion from all the regions for the two species had no significant difference (P>0.05). The recommendated daily intake for chloride is 2300 mg/day for adults (Osiecki, 2005). The levels in *A. hypochondriacus* and *A. cruentus* were lower than the recommended daily intake (RDI) of 2300 mg/day for adults (Osiecki, 2005), however they were more than those reported in cabbage, pumpkins, tomatoes, spinach, pepper, onions and carrots (Osiecki, 2005). On average if one relies solely on amaranth as a source of chloride ions, 1kg per day will meet the daily requirement. Kisii region recorded the highest (869 mg/100 g dry weight) chloride level in the dry season while Elgon recorded the lowest (442). Kenyatta University 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, Kenyatta University and Kisii had the higher means than sample from Bureti and Elgon during dry season. The mean difference in chloride ion levels in the wet and dry seasons was significant between Elgon and Kisii but insignificant between Kenyatta University and Bureti. The chloride levels in all the regions were higher than those in pumpkins, pepper and carrots. Therefore, amaranth is a better source of chloride ion than these kinds of foods.

3.1.2 Sulphate

Soil had the mean sulphate ion of 1863 mg/100 g of dry weight in the wet season and 2155 mg/100 g in the dry season. The mean concentration of sulphate in the soil during the dry season was significantly higher than in the wet season. The soil sulphate ions level has a range of 1467 mg/100 g to 2259 mg/100 g dry weight. This big range is an indication that soil in the wet season has uneven distribution of sulphate. Soil had high mean sulphate levels in the dry season than in the wet season. This big difference may be attributed to the amount of fertilizer used. The mean sulphate ion level in *A. hypochondriacus* varied from 919 mg/100 g in KU to 1186 mg/100 g in Kisii while in *A. cruentus* it varied from 740 mg/100 g in KU to 1109 mg/100 g in Kisii. The mean sulphate ion level in *A. hypochondriacus* was higher than the mean sulphate ion in *A. cruentus* in all the four regions of the study. The mean sulphate level in *A. hypochondriacus* in Bureti was significantly higher than in *A. cruentus* (P<0.05). The mean sulphate ion amount in *A. hypochondriacus* in KU, Elgon and Kisii were not significantly different (P>0.05) from those in *A. cruentus*. From this result, it can be concluded that the species does not

influence the level of sulphate in amaranth. Kenyatta University and Elgon had their mean levels of sulphate being higher in the wet season than in the dry season while Bureti and Kisii had higher levels of sulphate in the wet season than in the dry season. The mean sulphate levels in leaves from Bureti and Kisii were significantly different in the two seasons ($P<0.05$). The sulphate concentration in spinach, lettuce, carrots, onions, tomatoes, groundnuts, beans and garlic in Maiduguri, Borno state, Nigeria range from 17000 µg g^{-1} to 12600 µg g^{-1} (Uwah *et al*., 2007) in fresh vegetables. By calculation, the sulphate range was 456 mg/100g to 37500 mg/100 g dry weight. The levels of sulphate ion in *A. hypochondriacus* and *A. cruentus* from the four regions under study were within this range.

3.1.3 Phosphate

The mean level of phosphate in the soil in the dry season was 358 mg/100g while in the dry season was 1300 mg/100 g dry weight. This difference is significant ($P<0.05$) Phosphate levels in the soil differ from one season to another. During the dry season, there was a big range (1300 - 1920 mg/100 g dry weight) with the mean being 1300 mg/100 g. Levels of phosphate ion in leaves in the wet season were significantly higher than levels in the dry season. The high levels of phosphate in the wet season can be attributed to more dissolved phosphate in the wet season available for assimilation and hence increasing the amount available for absorption. *Amaranthus Hypochondriacus* recorded slightly higher mean levels of phosphate than *A. cruentus* in each of the four regions under study. One way ANOVA reveals the mean levels in *A. hypochondriacus* was not statistically higher than levels in *A. cruentus* from the same region ($P>0.05$). The levels of phosphate anion in fresh vegetables was 1100 µg g^{-1} in spinach and 3300 µg g^{-1} in beans. By calculation, this translates to about 330 mg/100 g of phosphate in spinach and 990 mg/100 g in beans. The levels of phosphate in leaves of *A. cruentus* and *A. hypochondriacus* are comparable to those in other vegetables. The levels of phosphate in amaranth were lower than those reported in other vegetables (Uwah *et al*., 2007). Based on a phosphate allowance of 1000 mg a day, the mean levels in all the regions where samples were grown was adequate for human consumption.

3.1.3 Nitrate ion

The mean nitrate level in the wet season was 1936 mg/100 g dry weight while in the dry season the mean nitrate level was 1206 mg/100 g dry weight. One way ANOVA reveals a significant difference in the nitrate levels in the wet and the dry season. The nitrate ion levels in the soil in the regions under study were comparable to the reported values in Ebony State, Nigeria. The level of nitrate in leaves in Bureti was 1044 mg/100 g dry weight in the dry season and 843 mg/100 g dry weight in the wet season. Elgon recorded the lowest level of nitrate in the dry season (483 mg/100 g) and wet season (478mg/100 g). The levels of nitrate in all leaves were higher during the dry season than the wet season in all areas. The difference in nitrate level in Kenyatta University, Bureti and Kisii regions were significant. The means nitrate in samples from Elgon was not significantly higher in the dry season than the wet season. The level of nitrate ion in *A. hypochondriacus* from KU, Bureti, Elgon and Kisii were,913., 908, 489 and 685 mg/100 g, respectively while the levels in *A. cruentus* were 903, 979, 481 and 636 mg/100 g ,respectively. In all the regions, difference in the mean concentration was not statistically significant. Species does not influence the level of nitrate in the leaves of the vegetable. These levels are comparable to the literature values. These means are comparable with the means of amaranth with a range 729 mg/100 g to 1474 mg/100 g reported in South Africa (Mnkeni *et al*., 2007). WHO and United Nations recommend an Acceptable Daily Intake (ADI) of nitrate of 0 to 3.7 mg nitrate ions per kg body weight (CSPH, 2005). By calculation a person of 60 kg body weight has an ADI of 222 mg. Amaranth grown in Kenya can be used as vegetable without causing danger to anaemic patients.

3.1.4 Fluoride ions

The mean fluoride ion level in the soil in the wet season was 70 mg/100 g dry weight and 70 mg/100 g dry weight in the dry season. This difference in the mean fluoride level in the wet and the dry seasons were not significant. The range of fluoride in the soil was 42mg/100 g to 100 mg/100 g dry weight. These values compares closely with those found in other areas. The *A. hypochondriacus* leaves had the mean fluoride ions level in Kenyatta Unuversity, Bureti, Elgon and Kisii regions being 9.09, 6.09, 11.97 and 11.88 mg/100 g respectively. The *A. cruentus* leaves had the mean fluoride level of 6.94, 8.46, 11.33 and 10.45 mg/100 g respectively. The difference was significant in Kenyatta University, Bureti and Kisii ($P<0.05$). Comparing the two species of amaranth, *A. hypochondriacus* had significally higher levels of fluoride than *A. cruentus*. The reported values are 0.79 mg/100 g in kahuku (*cucubita*) and 5.93 mg/100 g in *A. hybridus* (Kahama *et al,* 1997). The levels of fluoride in amaranth in this study were significantly higher than the reported values. This is attributable to the high levels of fluoride in the soil in this study. The mean fluoride ions level in leaves of amaranth grown in Kenyatta university, Bureti, Elgon and Kisii were 8.02, 7.56, 11.65 and 10.45 mg/100 g respectively in the dry season. In the wet season Kenyatta University, Bureti, Elgon and Kisii recorded 8.32, 7.00, 11.60 and 10.35 mg/100 g dry weight respectively. The difference in the mean levels of fluoride in leaves

and grains in the dry and wet seasons were no 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 show, 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 or use amaranth flour.

3.1.5 Iodine

The mean iodide ions level in the wet season was 13.80 mg/kg of dry weight while the mean iodide level in the dry season was 6.80 mg/kg. The levels of anions in soil in the wet season was significantly higher mean than levels the dry season. Kisii recorded the highest levels of Iodide ions (5.12 mg/kg) in *A. hypochondriacus* leaves and 4.79 mg/kg in *A. cruentus* leaves which was also the higher than in other regions. The lowest level was recorded in Elgon in the two species. The levels of I^- in the two species of amaranth were not significantly different. The mean iodide ions level in the dry season was higher than in the wet season in KU, Bureti and Kisii. The difference was significant in KU, Bureti and Kisii. The season influences the level 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 (Koutras *et al*., 1985). The recommended daily intake (RDI) for iodide for adult is 150 to 200 µg/day and infants 90 µg/day (WHO/NHD/01.1, 2001). *A. hypochondriacus* and *A. cruentus* is therefore a good source of iodine all age groups.

4. Conclusion and recommendation

The results of this study showed that all the samples analyzed contained the selected six inorganic anions (NO_3^-, PO_4^{3-}, F^-, I^-, Cl^-, SO_4^{2-}) considered in the study. The levels of selected inorganic anions were higher in the soil than in leaves. *Amaranthus hypochondriacus* generally higher levels of the specified inorganic anions compared to *Amaranthus cruentus* although the difference is not significant. *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 nitrate and iodide. It was also concluded that different ecological regions have significantly different levels of anions in amaranth of the same species. Phosphate, sulphate and chloride were significantly high in ecological zones with high rainfall while nitrates was high in amaranth in zones with low rainfall. Fluoride did not show significant variation in different ecological zones. The samples studied had anions levels below the RDI except fluoride in Mt. Elgon which were high. *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.

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Table 1: The mean ± SD, range and P- values of anion in the soil in the wet and dry seasons

Anion (N=24)	Mean chloride levels in mg/100 g in the wet season		Mean chloride levels in mg/100 g in the dry season		P value
	Mean ± SE	Range	Mean ± SE	Range	
Chloride	2228 ± 241	1148-4189	1311 ± 67	970-1831	0.001
Sulphate	1863 ± 70	1467-2259	2155 ± 11	2023-2213	0.001
Phosphate	358 ± 13	305-481	1502 ± 51	1300 - 1920	0.001
Nitrate	1936 ± 82	1138-2395	1206 ± 76	716- 1611	0.000
Fluoride	70 ± 3.40	42-100	70 ± 3.40	42-100	0.972
Iodide *	13.80 ±0.47	11.64-18.86	6.80 ± 0.39	41.90-99.80	0.001

*Mean levels in mg/ kg

Table 2: The mean ± SD, range and P- values of chloride ion in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya in all seasons

Region	*A. hypochondriacus* mean chloride levels in mg/100 g		*A. cruentus* mean chloride levels in mg/100 g		P value
	Mean±SE (n=18)	Range	Mean ± SE (n=18)	Range	
KU	755 ± 40	463-1053	728 ± 39	442-1090	0.631
Bureti	742 ± 49	289-1124	639 ± 48	236-905	0.145
Elgon	585 ± 68	273-1000	282 ± 6	322-889	0.971
Kisii	699 ± 54	490-1325	733 ± 61	450-1356	0.681

Table 3 The mean ± SD, range and P- values of chloride ion in leaves *A. hypochondriacus* and *A. cruentus* for two seasons

Dry season		Wet season	P value
Region	Mean ± SE in mg/100 g	Mean ± SE in mg/100 g	
Kenyatta University	726 ± 52	757 ± 20	0.580
Bureti	631 ± 60	751 ± 31	0.087
Elgon	442 ± 44	725 ± 62	0.001
Kisii	869 ± 60	563 ± 18	0.001

Table 4 The mean ± SD, range and P- values of sulphate ions in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya

Region	*A. hypochondriacus* mean sulphate levels in mg/100 g (n=18)		*A. cruentus* mean sulphate levels in mg/100 g (n=18)		P value
	Mean ± SE	Range	Mean ± SE	Range	
KU	919 ± 109	571-1883	740 ± 53	486-1080	0.149
Bureti	1003 ± 82	280-1603	757 ± 86	180-1172	0.047
Elgon	1019 ± 79	600-1474	944 ± 71	567-1349	0.487
Kisii	1186 ± 85	652-1939	1109 ± 73	630-1570	0.500

Table 5 The mean ± SD, range and P- values of sulphate ion leaves of *A. hypochondriacus* and *A. cruentus* for two seasons

Dry season		Wet season	P value
Region	Mean ± SE in mg/100 g	Mean ± SE in mg/100 g	
KU	723 ± 45	937 ±111	0.083
Bureti	696 ± 108	1064 ± 21	0.002
Elgon	1035 ± 67	929 ± 81	0.320
Kisii	903 ± 71	1392 ± 29	0.001

Table 6 The mean ± SD, range and P- values of phosphate ions in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya

Region	*A. hypochondriacus* mean phosphate levels in mg/100 g (n=18)		*A. cruentus* mean phosphate levels in mg/100 g (n=180		P value
	Mean ± SE	Range	Mean ± SE	Range	
KU	588 ± 89	140-1034	543 ±84	137-978	0.715
Bureti	695 ± 92	276-1226	679 ± 94	255-1270	0.901
Elgon	680 ± 457	152-1460	5941 ± 106	147-1460	0.589
Kisii	638 ± 103	199-1220	609 ± 100	177-1196	0.836

Table 7 The mean ± SD, range and P- values of phosphate ion in the leaves of the two species of amaranth in two seasons

Dry season		Wet season	P value
Region	Mean ± SE in mg/100 g dry weight (n= 36)	Mean ± SE in mg/100 g dry weight (n=36)	
KU	223 ± 19	908 ± 27	0.001
Bureti	315 ± 12	1059 ± 30	0.001
Elgon	221 ± 12	1053 ± 70	0.001
Kisii	238 ± 12	1009 ± 53	0.001

Table 8 The mean ± SD, range and P- values of nitrate ion for two seasons in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya

Region	*A. hypochondriacus* mean nitrate levels in mg/100 g (n=18)		*A. cruentus* mean nitrate levels in mg/100 g (n=18)		
	Mean ± SE	Range	Mean ± SE	Range	P value
KU	913 ± 63	455-1339	903 ± 74	111-1208	0.920
Bureti	908 ± 56	580-1330	979 ± 37	779-1250	0.294
Elgon	489 ± 15	397-569	481 ± 12	372-688	0.512
Kisii	685 ± 30	519-890	636 ± 35	451-854	0.295

Table 9 The mean ± SD, range and P- values of nitrate ion in leaves of amaranth in two seasons

Dry season		Wet season	P value
Region	Mean ± SE in mg/100 g dry weight	Mean ± SE in mg/100 g of dry weight	
KU	1037 ± 67	779 ± 57	0.006
Bureti	1044 ± 48	843 ± 35	0.002
Elgon	483 ± 15	478 ± 21	0.834
Kisii	684 ± 37	638 ± 29	0.330

Table 10 The mean ± SD, range and P- values of fluoride ion in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya

Region	*A. hypochondriacus* mean fluoride levels in mg/100 g		*A. cruentus* mean fluoride levels in mg/100 g		P value
	Mean ± SE (n=18)	Range	Mean ± SE (n=18)	Range	
KU	9.09 ± 0.28	7.18-10.70	6.94 ± 0.43	3.24-8.29	0.000
Bureti	6.09 ± 0.54	3.80-13.80	8.46 ± 0.46	5.89-12.50	0.002
Elgon	11.97 ± 0.62	7.93-16.30	11.33 ± 0.72	6.24-15.10	0.512
Kisii	11.88 ± 0.96	6.68-21.20	10.45 ± 0.66	4.98-21.20	0.027

Table 11 The mean ± SD, range and P- values of fluoride ion in leaves for two seasons

Region	Dry season	Wet season	P values
	Mean ± SE in mg/100 g	Mean ± SE in mg/100 g	
Kenyatta University	8.02 ± 0.45	8.32 ± 0.45	1.000
Bureti	7.56 ± 0.61	7.00 ± 0.53	0.497
Elgon	11.65 ± 0.68	11.60 ± 0.68	1.000
Kisii	10.45 ± 0.94	10.35 ± 0.94	1.000

Table 12 The mean ± SD, range and P- values of iodide ion in *A. hypochondriacus* and *A. cruentus* leaves from different regions of Kenya

Region	*A. hypochondriacus* mean iodide levels in mg/kg		*A. cruentus* mean iodide levels in mg/kg		P value
	Mean ± SE (n=28)	Range	Mean ± SE (n=28)	Range	
KU	3.80 ± 0.88	0.32-11.37	4.49 ± 1.01	0.44 - 10.76	0.611
Bureti	3.84 ± 0.80	0.30-14.33	2.98 ± 0.42	0.24-5.62	0.347
Elgon	2.45 ± 0.31	0.81-5.32	2.35 ± 0.39	0.58-4.92	0.846
Kisii	5.12 ± 1.14	0.60-13.53	4.79 ± 1.04	0.53-12.35	0.782

Table 13 The mean ± SD, range and P- values of iodide ion in leaves of *A. hypochondriacus* and *A. cruentus* for two seasons

Region	Dry season	Wet season	P value
	Mean ± SE Mg/kg (n=36)	Mean ± SE Mg/kg (n=36)	
KU	6.30 ± 1.09	2.00 ± 0.30	0.001
Bureti	5.36 ± 0.58	1.45 ± 0.23	0.001
Elgon	2.15 ± 0.27	2.66 ± 0.41	0.306
Kisii	7.67 ± 1.24	2.33 ± 0.18	0.001

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