Determination of Calcium, Magnesium, Iron, Zinc and Chromium Contents in Wheatgrass Powder Sold in Nakuru Town, Thika Town and Nairobi City Regions, Kenya

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***Abstract:* - Wheatgrass is a food prepared from the cotyledons of the common wheat plant, *Triticum aestivum* (subspecies of the family *Poaceae*). It is sold either as a juice or powder concentrate . It contains a plethora of vitamins, minerals, amino acids and vital enzymes like superoxide dismutase and cytochrome oxidase. The vitamin content makes it an important adjuvant in anti-allergic and anti-asthmatic treatment; it has maximum health benefits like an advance therapy for cancer as well as thalassemia disease. Some of the essential elements in Wheatgrass are Manganese, Calcium, Selenium, Magnesium, Zinc and Iron. The study of essential nutritional elements (Calcium, Magnesium, Iron, Zinc and Chromium) in wheatgrass powder using Flame Atomic Absorption Spectroscopy revealed that indeed it contains a substantial amount of these elements; calibration curves of Calcium, Magnesium, Iron, Zinc and Chromium were drawn and had good positive linear regression coefficients of R^2 =0.9968, 0.9992, 0.9974, 0.9996 and 0.9990 respectively. The correlation graphs of Magnesium vs. Calcium and Iron vs. Zinc were drawn. Iron vs. Zinc pair had lower linear correlation coefficient (R^2=0.75220) as compared to Magnesium vs. Calcium (R^2=0.9295). This suggests that species in the same group of the Periodic Table exhibited much higher correlation than the ones in the same row of the periodic table**.

I. INTRODUCTION

Mineral salts are basic to all organic life (Both human and animal life), Organic minerals regulate the elimination and blood-building functions of the body. Magnesium and Calcium maintain the pH of the blood (Gibson, 2005). Calcium ions (Ca^{2+}) play a pivotal role in the physiology and biochemistry of organisms and the cell. They play an important role in signal transduction pathways, where they act as a second messenger, in neurotransmitter release from neurons, contraction of all muscle cell types, and fertilization. Extracellular Calcium is also important for maintaining the potential difference across excitable cell membranes and proper bone formation (Wilkinson, 1976), it is essential in maintaining total body health; about 99 % of Calcium in a person's body is found in bones and teeth and makes about two percent of body weight. Its deficiency causes depleted Calcium stores in the bones, thinning and weakening of the bones. Substantial decreases in extracellular Ca^{2+} ion concentrations result in hypocalcemic tetany (a condition characterized by muscle cramps, numbness and tingling in the arms and legs). It leads to porous and fragile bones thus fractures and breakage can occur (osteoporosis). Magnesium gives rigidity and flexibility to the bones, increases bioavailability of Calcium, regulates and normalizes blood pressure, prevents and reverses kidney stone formation, promotes restful sleep, helps prevent congestive heart failure, eases muscle cramps and spasms, lowers serum cholesterol levels and triglycerides, decreases insulin resistance, prevents atherosclerosis and stroke, end cluster and migraine headaches, enhances circulation, relieves fibromyalgia and chronic pain, treats asthma and emphysema, helps make proteins, prevents osteoporosis, proper Vitamin D absorption, protection from radiation, aids in weight loss, proper digestion of carbohydrates, (Wilkinson, 1976). Low intakes of Magnesium induce changes in biochemical pathways that can increase the risk of illness over time. A chronic lack of Magnesium in the body yields many consequences including low energy levels. Iron is an essential part of hemoglobin; the red coloring agent of the blood that transports oxygen through our bodies (Hoppe, 2005), Proper functioning of the immune system relies, in part, on sufficient Iron, Main causes of Iron deficiency are anemia dietary, body failing to utilize Iron stores adequately and increased losses. Large amount of ingested Iron can cause excessive level of Iron in the blood. High levels of free ferrous Iron in blood react with peroxides to produce free radicals which are highly reactive and can damage DNA, proteins, lipids and other cellular components. Iron typically damages cells in the heart and liver and this can cause coma, shock, liver failure and even death. Chromium plays a role in the body's use of energy-providing carbohydrates, protein and fat and, when in short supply, is associated with impaired glucose tolerance and diabetes-like symptoms (Nielsen, 1996), the first published case of a Chromium-diabetes link showed that the severe diabetic symptoms that developed in a woman while on long-term parental nutrition (intravenous feeding) were alleviated by supplemental Chromium (Glinsmann and Mertz, 1966). Chromium supplements may reduce blood sugar levels as well as the amount of insulin people with diabetes need; its deficiency impairs the body's ability to use glucose to meet its energy needs and raises insulin requirements. Zinc is an essential trace element, necessary for plants, animals, and microorganisms, it is "typically the second most abundant transition metal in organisms" after Iron and it is the only metal which appears in all enzyme classes (Bradley *et al*., 2007), it is very important in controlling prostate disorders, the appetite of a person, it is essential for the repair and functioning of DNA thus is necessary for quick growth of cells and promotes synthesis of collagen that helps in wound healing (Scoffern, 1861). Helps protect the skin and mucosal membranes and prevents cellular damage in the retina, which helps in delaying Age-related macular degeneration (AMD) and thus the progression of vision loss. Due to increased cases of diseases and conditions mainly caused by the deficiency of Calcium, Magnesium, Iron, Zinc and Chromium in human body, there is need for information to the public about their sources, especially the natural sources, this study is therefore important in establishing whether the Wheatgrass powder sold in selected supermarkets and shops in Nakuru, Thika and Nairobi contains the right nutritional content of these vital elements required by the human body for development and normal body metabolic processes.

II. METHODOLOGY

Sampling

Random sampling was carried out. In each of the three towns, supermarkets were selected at random and in each of these supermarkets, samples were obtained from them. The samples were transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT) laboratories for analysis, they were labeled appropriately according to the point of purchase and stored ready for analysis.

Cleaning of Apparatus

All the glassware and plastic-ware were thoroughly cleaned with soap and rinsed with tap water. Plastic-ware was air-dried afterwards and glassware was soaked in 10%v/v nitric acid for twelve to forty eight hours then removed and thoroughly rinsed with distilled water and placed in an oven at 105°C for at least two hours. This was done to remove any traces of adsorbed metal ions on the side walls of glassware. Volumetric glassware was kept on a bench to dry at room temperature (Stewart, 1989).

Sample Preparation

Since the sample was already in powder form, digestion was the first step in preparation of the samples, 1g of sample was accurately weighed and placed in a beaker, a little distilled water was added to moisten the sample, 6mls of a mixture of nitric acid and perchloric acid in the ratio 5:1 (v/v) were added and the resulting solution was heated for 45 minutes, brown fumes were evolved and a colorless solution formed. The stop of brown fumes evolution followed by colorless fumes for about 10 minutes indicated the end of extraction and heating was stopped. The samples were cooled and diluted with 25ml of 12% (v/v) nitric acid and filtered using Whatman filter paper no. 40. The filtrate was collected in a 50ml volumetric flask and diluted to the mark with distilled water (AOAC, 1997).

Preparation of Standards

Calcium

2.7693g of Calcium chloride ($CaCl_2$) salt was weighed and transferred into a 1000ml volumetric flask and dissolved with 100ml distilled water. The solution was topped up to the mark with distilled water, thus giving a 1000 mg/L of Calcium stock solution. 10mls of this solution was pipetted into a 100ml volumetric flask and then topped up with distilled water to make 100mg/L solution. Working standard solutions of 0, 1, 2, 3, 4, 5, 10 and 15 mg/L in 50ml volumetric flasks were prepared using serial dilution. 0.882% (mass of the anhydrous lanthanum chloride per volume) was prepared by accurately weighing 1.337g of hydrated lanthanum chloride ($LaCl_3.7H_2O$) in 100mls volumetric flask and topping up the mark using distilled water. 2mls of this solution was added to each of the working standards and diluted to the mark, before aspirating them in the Flame Atomic Absorption Spectrometer (FAAS). The samples were also treated the same way as the standards (addition of 2mls of 0.882% (m/v) of the above lanthanum chloride solution) before analysis with FAAS.

Magnesium

In a 1000-mL volumetric flask, 8.3632g of Magnesium chloride ($MgCl_2.6H_2O$) was dissolved and made to the mark with distilled water. This was done to prepare 1000 mg/L of Magnesium stock solution. 10mls of this solution was pipetted into a 100ml volumetric flask and then topped up with distilled water to make 100mg/L solution. Working standard solutions of 0, 1, 2 3, 4, 5 and 10 mg/L in 50ml volumetric flasks were prepared using serial dilution and 2mls of 0.882% (m/v) lanthanum chloride solution was added to each of the working standards (as described for the Calcium standards above) and the sample solutions before aspirating them in the FAAS.

Iron

7.0714g of Ammonium Iron (II) sulphate $[(NH_4)_2Fe(SO_4)_2.6H_2O]$ salt was weighed and dissolved in distilled water, the solution was put in 1000ml volumetric flask and topped up to the mark with distilled water. This was done to prepare 1000 mg/L of Iron stock solution. 10mls of this solution was pipetted into a 100ml volumetric flask and diluted to mark with distilled water to make 100mg/L solution. Working standard solutions of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 mg/L in 50ml volumetric flasks were prepared using serial dilution and the aspirated into the FAAS for analysis.

Chromium

0.3019g of Chromium (III) chloride ($CrCl_3$) salt was weighed and dissolved in distilled water, the solution was then put in

100ml volumetric flask and topped up to the mark with distilled. This was done to prepare 1000 mg/L of Chromium stock solution. 10mls of this solution was pipetted into a 100ml volumetric flask and diluted to mark with distilled water to make 100mg/L solution. Working standard solutions of 0, 0.6, 0.8, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L in 50ml volumetric flasks were prepared using serial dilution (AOAC, 1997). These solutions were then aspirated in the FAAS for analysis.

Zinc

0.4547g of Zinc nitrate [$Zn(NO_3)_2.6H_2O$] salt was weighed and dissolved in distilled water, the solution was then put in 100ml volumetric flask and topped up to the mark with distilled water. This was done to prepare 1000 mg/L of Zinc stock solution. 10mls of this solution was pipetted into a 100ml volumetric flask and diluted to mark with distilled water to make 100mg/L solution. Working standard solutions of 0, 0.6, 0.8, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L in 50ml volumetric flasks were prepared using serial dilution (AOAC, 1997). These solutions were then aspirated in the FAAS for analysis.

Instrumental Analysis

Flame Atomic Absorption Spectrometer with deuterium lamp for background correction was used. The working standards and the samples were aspirated sequentially into the Flame Atomic Absorption Spectrophotometer. The instrumental conditions applied for each element are shown in the following table.

Table 1: *AAS Instrumental Conditions Employed*

METAL ANALYTE AND SYMBOL	WAVELENGTH, λ, in nm	BAND PASS, nm	CALIBRATION Standard Solution Concentrations, mg/L
CALCIUM, Ca	422.7	0.7	0, 1, 2 3, 4, 5, 10 and 15
MAGNESIUM, Mg	285.2	0.7	0, 1, 2 3, 4, 5, and 10
IRON, Fe	248.3	0.2	0, 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0
ZINC, Zn	213.9	0.2	0, 0.6, 0.8, 2.0, 4.0, 6.0, 8.0 and 10.0
CHROMIUM, Cr	357.9	0.7	0, 0.6, 0.8, 2.0, 4.0, 6.0, 8.0 and 10.0

N.B. Appropriate dilutions were made from the Standard stock solution to obtain the calibration standard solutions required.

III. RESULTS AND DISCUSSION

Calibration Curves

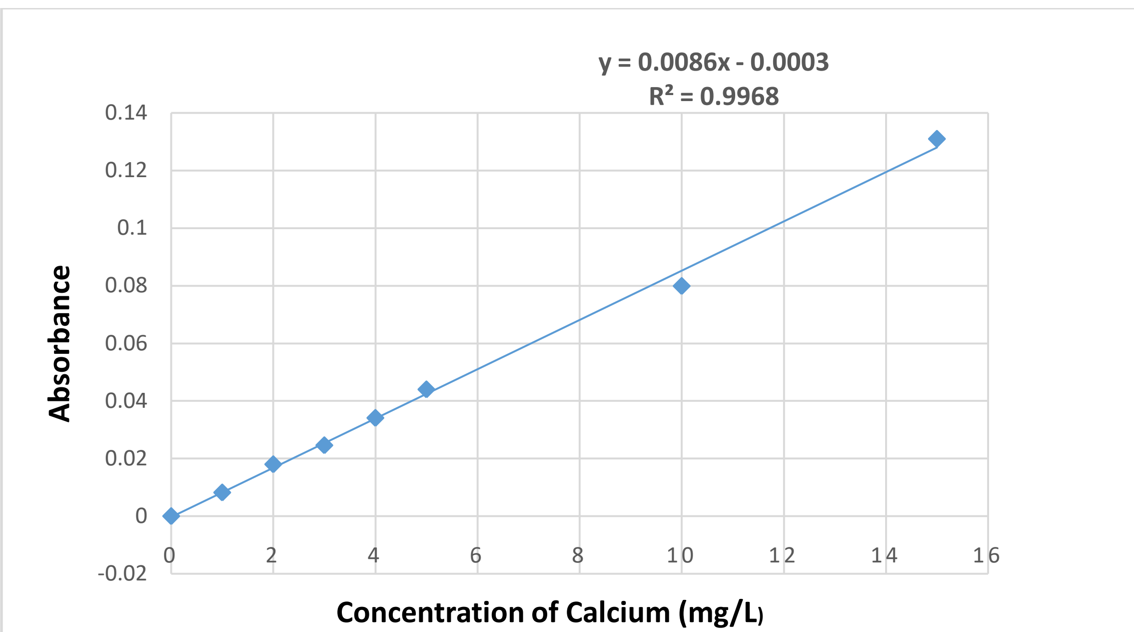

Figure 1: Calibration Curve of Absorbance against Concentration of Calcium

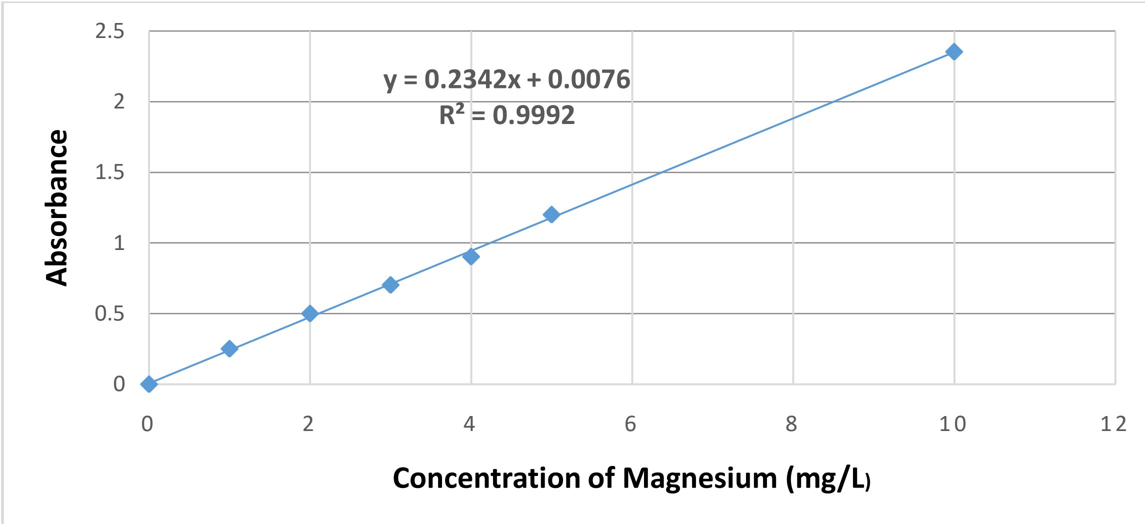

Figure 2: Calibration Curve of Absorbance against Concentration of Magnesium

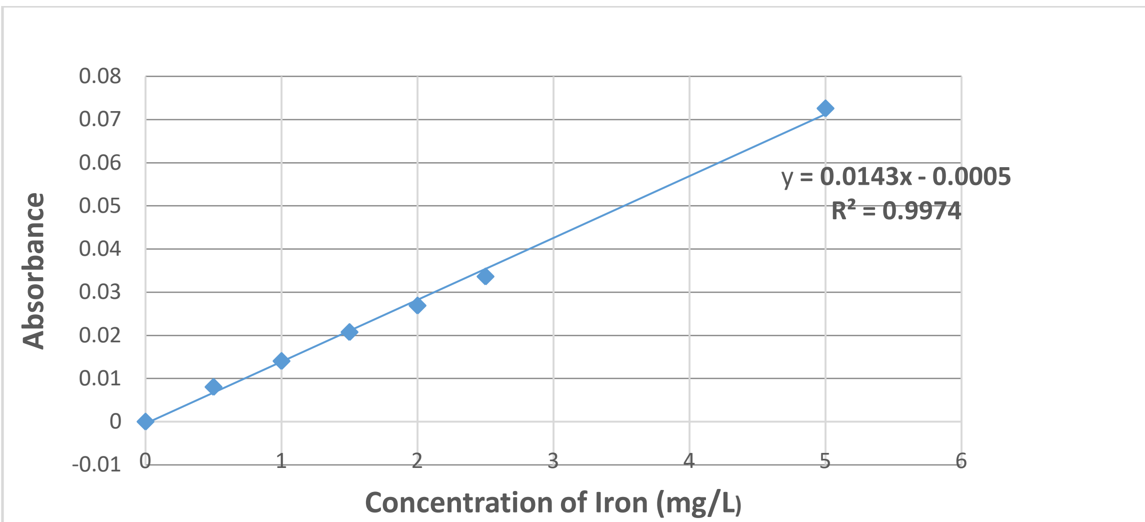

Figure 3: Calibration Curve of Absorbance against Concentration of Iron

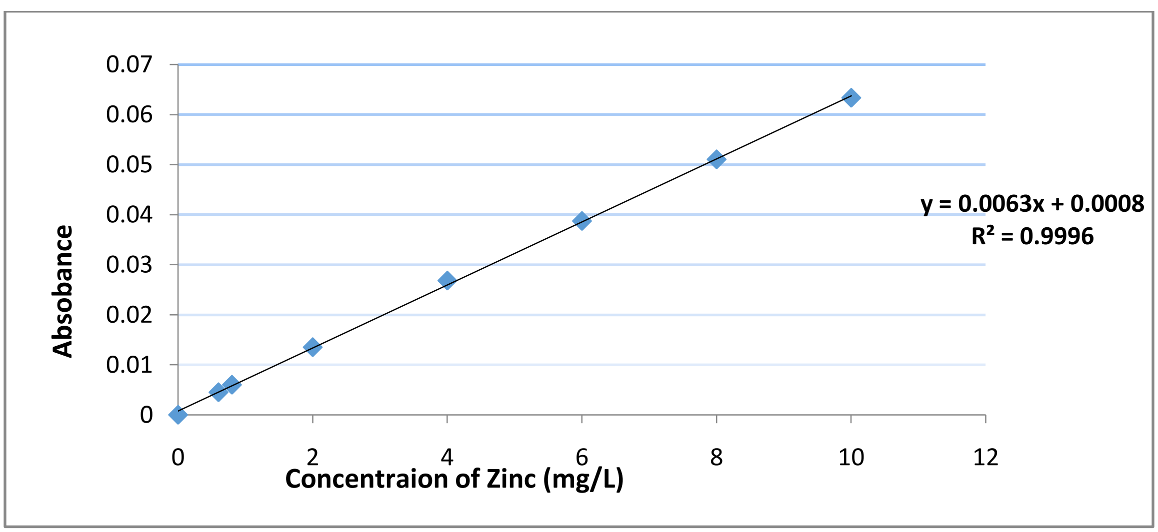

Figure 4: Calibration Curve of Concentration against Absorbance of Zinc

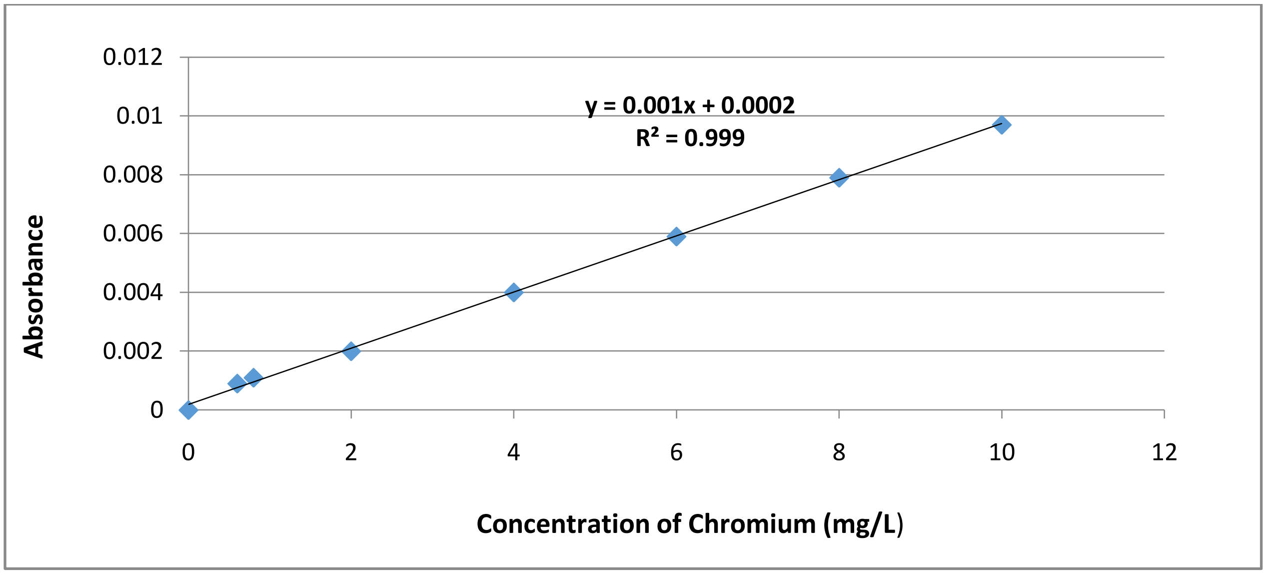

Figure 5: Calibration Curve for Concentration against Absorbance of Chromium

Good calibration curves with R^2 =0.9968, 0.9992, 0.9974, 0.9996 and 0.9990, and were obtained for Calcium, Magnesium, Iron, Zinc and Chromium respectively.

This shows that Beer's law was closely followed and therefore reliable sample results obtained.

Table below gives the Limits of Detection for Iron, Calcium and Magnesium were calculated statistically at 95% confidence level.

Calculated Concentrations

The value of n i.e. Sample size was 3.

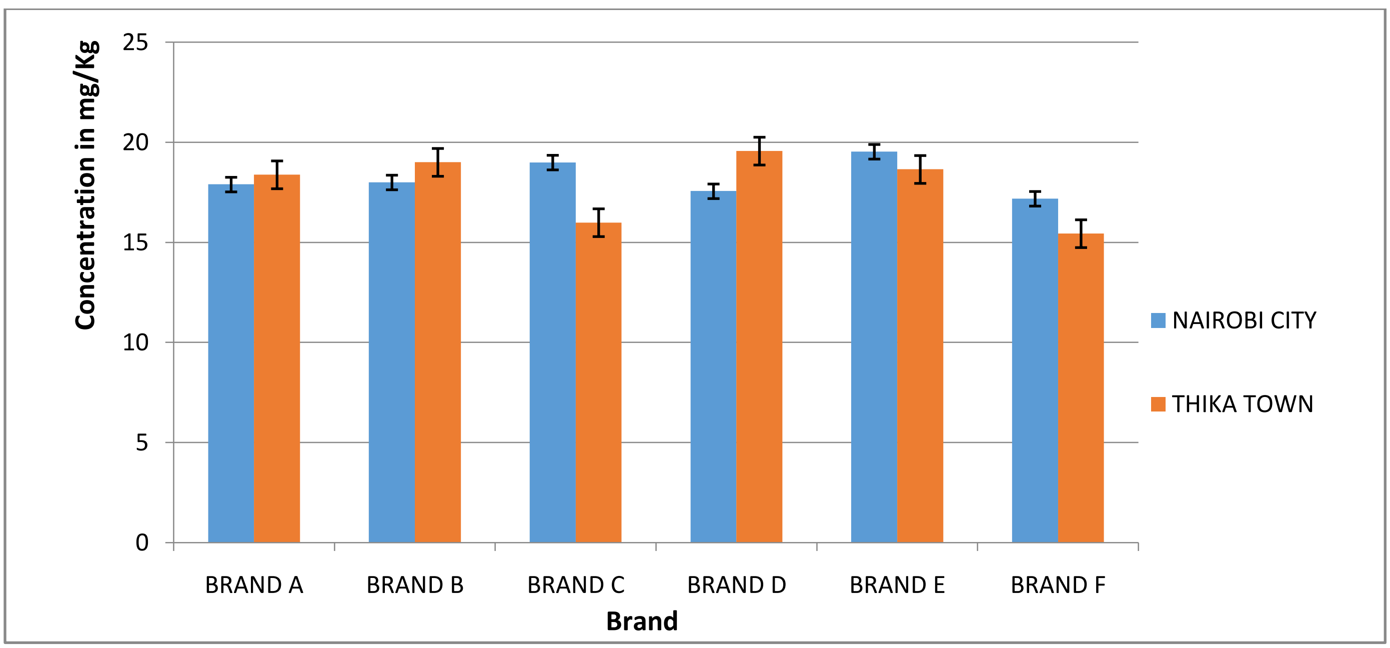

FIGURE 6: Column Chart relating Calcium content in different Brands of Wheatgrass.

Wheatgrass had a relatively higher concentration of Calcium as compared to Magnesium, Iron, Zinc and Chromium. This is because naturally, the earth's crust is rich in Calcium (5^{th} most abundant element by mass), and Calcium is one of the major components of most plants. Apparently, levels of Calcium in each of the Brands had the following trend E>D>B>A>C>F, however based on statistical test at 95% confidence level there were no significance difference in Calcium contents between E and D, D and B, B and A, A and C, C and F brands.

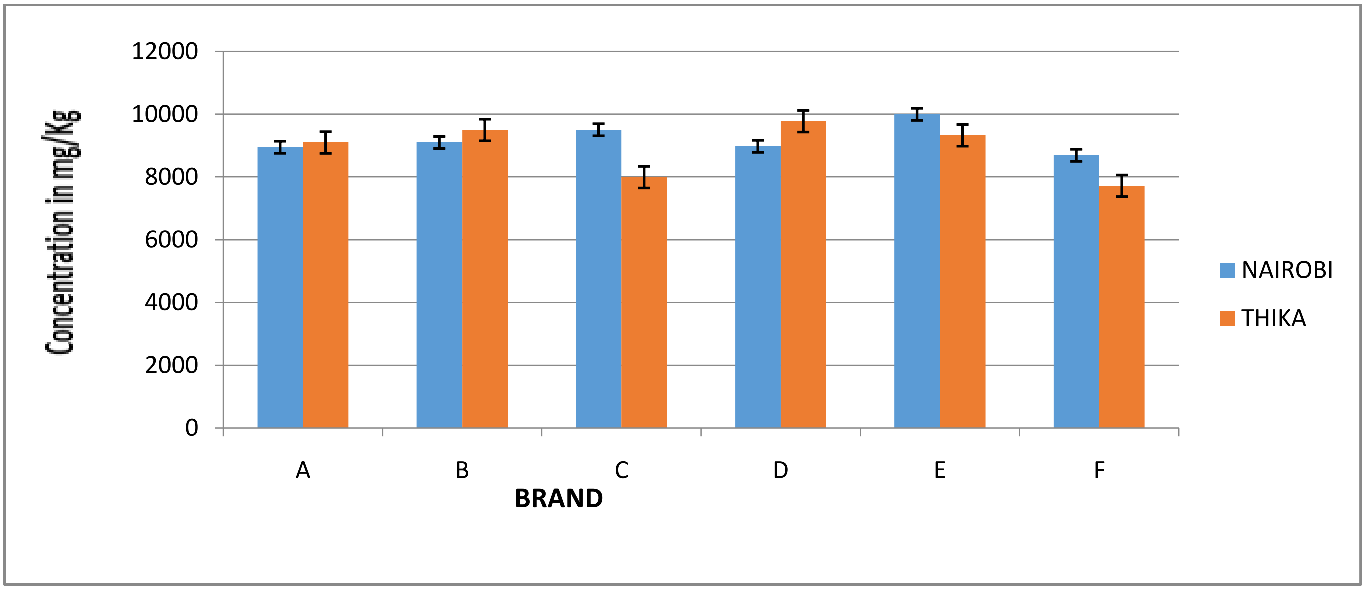

FIGURE 7: Column Chart relating Magnesium content in different Wheatgrass Brands

Magnesium concentrations in Wheatgrass resemble those of Calcium in abundance. Natures' Health Limited (Brand E) had the largest Magnesium concentration. This can be attributed to the soil type in which it was grown and also the harvesting time, weather conditions among other factors. Apparently Magnesium contents in each of the Brands following the E>D>B>A>C>F trend, however based on statistical test at 95% confidence level there were no significance difference in Magnesium contents between E and D, D and B, B and A, A and C, C and F brands.

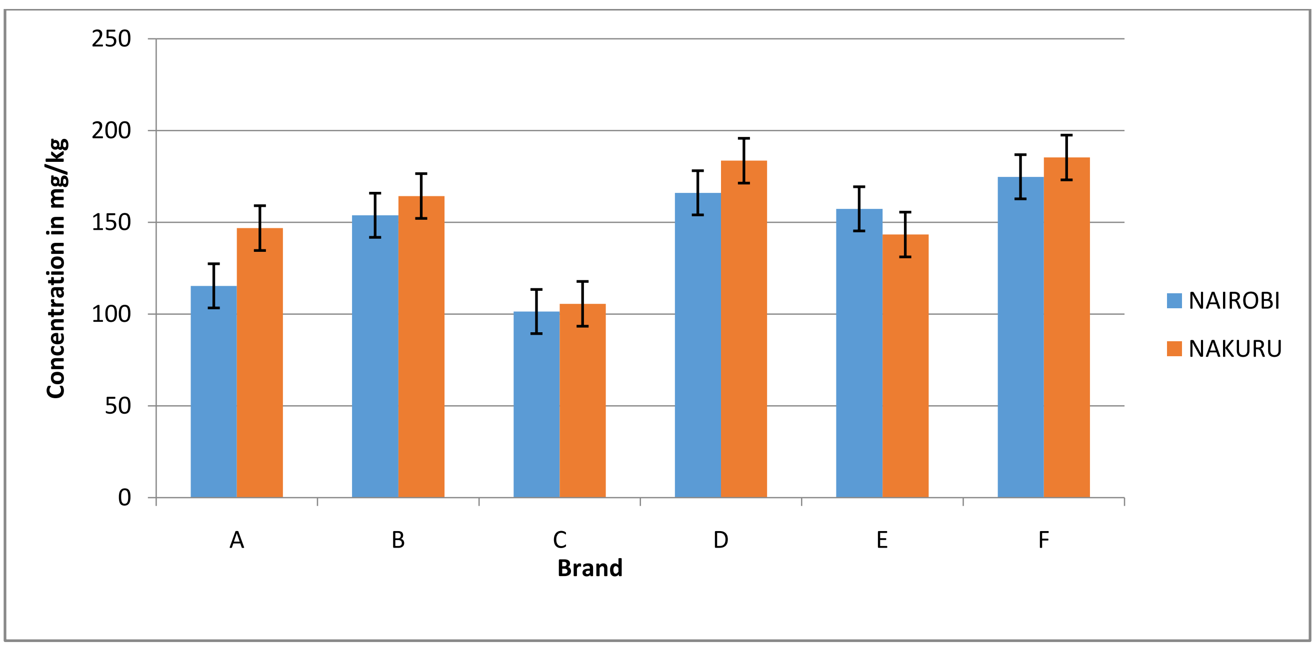

FIGURE 8: Column chart relating Iron content in different Brands of Wheatgrass

Iron levels in Wheatgrass were also considerably high as shown from the chart above. Brand E (Natures' Health Limited) had the highest Iron level; this can be attributed to the soil or region from which the raw wheatgrass was obtained from. Iron levels appeared to observe the E>D>C>B>F>A trend as per each of the available Brands. But based on statistical tests at 95% confidence level Iron had the trend of D>C>B>F>A, however there were no significance difference in Iron contents between E and D Brands at 95% confidence level.

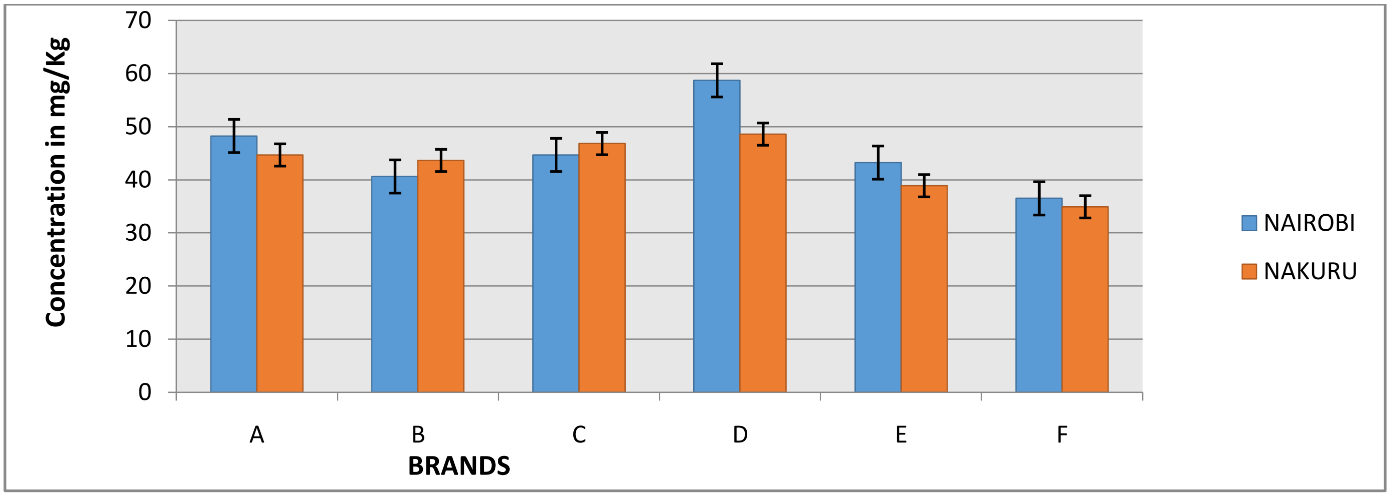

Figure 9: Column chart relating Zinc content in different Brands of Wheatgrass

Brand B (Green Era Foods) contained the highest amount of Zinc while Brand F (Fresh Food International) had the second highest amount of Zinc compared to other manufacturers, the apparent trend observed as per Brand was B>F>C>D>A>E; these can be attributed to among others soil type in which they were grown in and also the time at which these particular wheatgrass was grown and harvested. Based on statistical tests at 95% confidence level Zinc had the trend of F>C>D>A>E, however there were no significance difference in Zinc levels between B and F Brands.

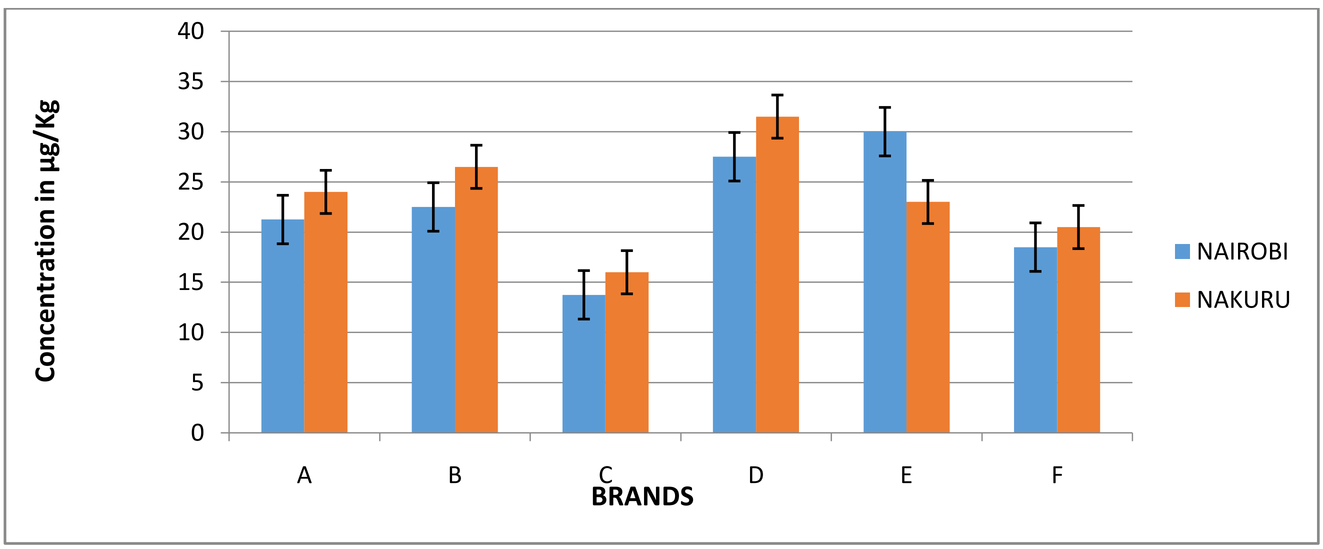

Figure 10: Column chart relating Chromium content in different Brands of Wheatgrass

Wheatgrass powder from Brand D (Winnie's Pure health) contained the highest amount of Chromium while that from Brand F (Fresh Food International) had the second highest amount of Chromium compared to other manufacturers, Chromium levels in each of the Brands appeared to follow D>F>B>E>A>C trend, based on statistical tests at 95% confidence level, Chromium levels had the D> F>B and A>C trend, however there was no significance difference in Chromium contents between Brands B and E and E and A at 95% confidence level. The trend observed can be attributed to soil type in which they were grown in and also the time at which these particular Wheatgrass were grown. Other factors include the storage duration and the process involved in the powder manufacture as some manufacturing processes may denature some of the mineral contents.

Key for the sample Brands above:

- Brand A – Sileo Company
- Brand B – Green Era Foods
- Brand C – Valley Feeds And Hay Limited
- Brand D – Winnie's Pure health

- Brand E –Natures' Health Limited
- Brand F – Fresh Food International

Correlation Curves for Magnesium vs. Calcium and Iron vs. Zinc Contents in Wheatgrass Powder

The results below show that on average, the pair Iron-Zinc had lower linear correlation coefficient as compared to Magnesium-Calcium. This evidently suggested that species in the same group of the periodic table exhibited much higher correlation than the ones in the same block of the periodic table. This was expected because the bonding and hence chemical characteristics between species of the same charge or group, say Magnesium and Calcium are relatively identical, compared to those pertaining to different groups or blocks (e.g. transition metals). Charges of a particular sign, such as Group I or II cations will have comparably higher interaction with the oppositely charged ions, such as chloride or sulphate respectively, than dissimilar ones.

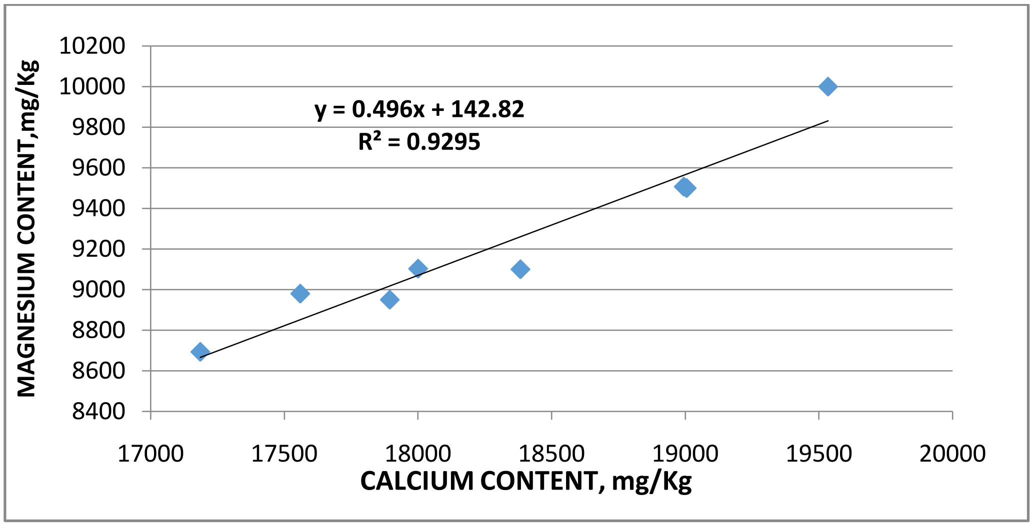

Figure 11: Correlation Graph of Magnesium vs. Calcium Content in Wheatgrass Powder

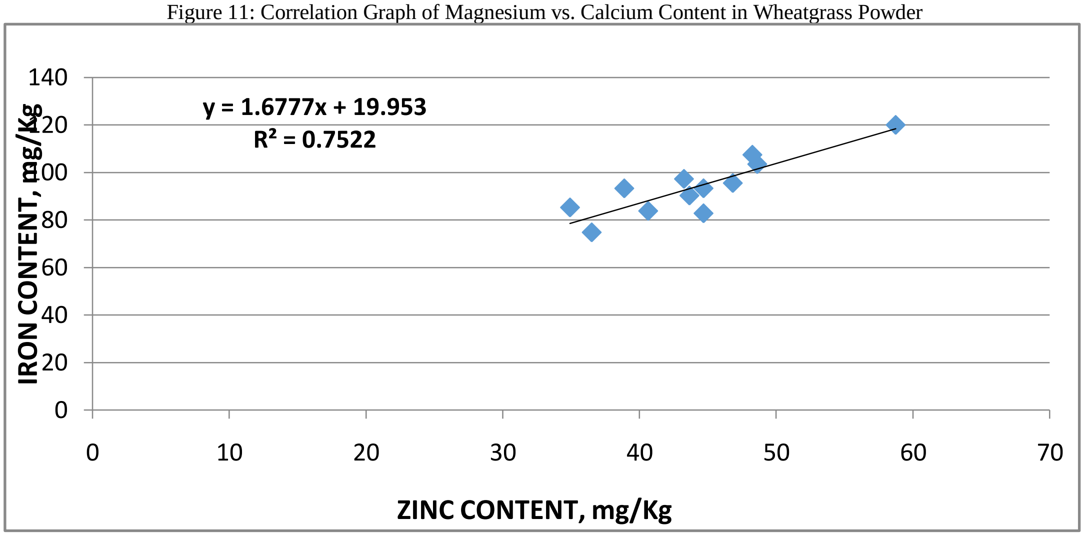

Figure 12: Correlation Graph of Iron vs. Zinc Content in Wheatgrass Powder

IV. CONCLUSIONS AND RECOMMENDATIONS

The research on wheatgrass powder as an alternative natural source of some essential mineral elements (Calcium, Magnesium, Iron, Zinc and Chromium) revealed that Calcium content was the highest of all the elements studied, followed by Magnesium , Iron, Zinc and Chromium in that order. From the results obtained, the mean mineral contents were 18017 mg/kg for Calcium, 9055 mg/kg for Magnesium, 149.825 mg/kg for Iron and 44.137 mg/kg for Zinc. On the other hand, the recommended mineral contents in Stinging Nettle are as follows: 36658.56 mg/kg for Calcium, 4349.56 mg/kg for Magnesium, 102.190 mg/kg for Iron and 47.932 mg/kg for

Zinc (Phillips *et al.*, 2014). It is evident that Magnesium and Iron contents in Wheatgrass were higher than that in Stinging Nettle as given in the above literature while the other metals' contents were lower in Wheatgrass powder currently reported. The WHO and FAO specifications for Chromium is not less than 30μg/kg but not more than 35μg/kg, wheatgrass Powder analyzed had 22.917 μg/kg of Chromium content. As discussed above its evident that wheat grass powder can be relied upon as the main source of natural Calcium and Magnesium, it can also be used as a source of natural Iron, Zinc and Chromium, the following pairs of elements in same group or block of the periodic table or similar physiological or environmental roles gave fairly good correlations ($R^2 > 0.5000$): Ca & Mg ($R^2 = 0.9295$), Fe & Zn ($R^2 = 0.7522$). This suggests that wheatgrass powder can be relied upon for these particular mineral nutrients. From the above results and discussion the authors would like to recommend that the public needs to be encouraged to consume Wheatgrass as it is rich in natural essential nutritional elements so as to avoid and prevent diseases like osteoporosis, hypocalcaemia, anemia, hypokalemia and diabetes which are caused by deficiency of these elements, more so over- reliance on laboratory synthesized supplements should be discouraged and the public encouraged taking more natural sources of nutrients. More research needs to be done on other food plants a part from Wheatgrass to establish which food crops are rich in these essential elements.

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