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Variation of Mineral Micronutrient Elements in Robusta Coffee (*Coffea canephora* Pierre ex A. Froehner) As Measured by Energy Dispersive X -Ray Fluorescence

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Authors' contributions

This work was carried out in collaboration among all authors. Author PA collected study samples, EDXRF data, analyzed and wrote the paper. Author KN directed the research and edited the paper. Author DM supported EDXRF data collection and analyses. All authors read and approved the manuscript.

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ABSTRACT

Aim: Robusta coffee growing countries of the world such as Uganda, have experienced a decline in market prices since 2002 due to changed consumer preferences for other beverages. In Uganda, where Robusta coffee is the major income earner, one practical way of ensuring that coffee prices remain competitive is to enhance quality. The aim of this study was to analyze the extent of macro and microelement densities in the soils of Robusta coffee growing Ugandan farms, their

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relationships with density levels in the leaf and in the green bean and their ultimate contribution in the determination of cupping and marketing qualities. This information would enable coffee producers in Uganda to make decisions on which organic and inorganic fertilizer applications are needed to alleviate coffee quality which in turn is the key determinant of world prices.

Study Design: Soil, leaf and ripe cherry samples were derived from sixty seven tagged trees in twenty three on farm locations at Kawanda and Entebbe in fields that had no fertilizer application history.

Place and Duration of the Study: This work was conducted at the National Agricultural Coffee Research Institute (NaCORRI), Uganda and the Institute of Nuclear Science and Technology, University of Nairobi, Kenya, between January 2010 and December 2013.

Methodology: Nutrient densities of potassium (K), calcium (Ca), iron (Fe), titanium (Ti), zinc (Zn), copper (Cu), boron (B), lead (Pb) and manganese (Mn) of sixty seven soil, leaf and green bean samples collected from 23 districts were measured with Energy dispersive x-ray fluorescence (EDXRF). All the samples were oven-dried at 80^0C for 18-20 hours and ground to about 50 mm sieve-size. Three pellets of 2.5 cm in diameter were made and irradiated with a Cd-109 radioactive source. The X-rays emitted by the elements were detected by liquid nitrogen cooled Si (Li) detector and data analyzed with the Quantitative X-ray Analysis System (XQAS/AXIL) software.

Results: The results showed that the mean concentrations of Ca, Mn, Fe and Zn were highest in the soil but lowest in green beans. K concentrations were higher in green beans than in the leaf and were lowest in the soil. In most cases, the concentrations of microelements found in the soil determined the levels in the leaf and in the green bean. Microelement availability and concentrations were influenced by the interaction between the various elements, tree age, elevation, soil texture and location.

Conclusions: Mineral element K and microelement Cu were found in their highest concentration in the green bean. Mn, Zn and B were important elements in the determination of organoleptic cup attributes whereas soil, leaf and green bean nutrient concentrations were important in determining the medium size of the bean.

Keywords: EDXRF; green bean; microelements; robusta; soil.

1. INTRODUCTION

In many parts of the world, *Coffea* grows between latitude 25°N and 25°S and requires specific climatic conditions to produce high bean yield and quality. In Uganda, Robusta coffee is grown in a diversity of soils that range from red sandy clay or gravelly loam to soft laterite [1]. High soil organic content (6.66-17.8%) and acidic soils of pH 5.1-6.8 may promote Robusta coffee growing but where nitrogen, phosphorus, calcium and magnesium concentrations are low, crop yields may also remain low [2]. In some coffee growing areas, where soil acidity is high due to hydrolysis of aluminum, lime is added while soil fertility is normally amended using organic or inorganic fertilizer or in combination. Fertile soils contribute to the size, weight and quality of the coffee bean [3]. Various coffee species especially when grown under diverse environments may assimilate different levels of macro and micro-elements and this would lead to varying production capacity, bean quality and disease tolerance. Concentrations of macro and micronutrients in soils are strongly related to biological and geochemical cycles and to anthropogenic factors, such as deforestation and management [4].

Trace mineral content of plant tissue or foods reflect the trace element concentration in the soil in which they are grown. For example, soils rich in zinc ions enable the plant to have more zinc compared to the zinc-deficient soils depending on the species and plant genotype [5]. However, this ability to take-up ions from the soil is not only limited to the amounts present in the soil, but also to the inherent ability of certain crops to actively absorb certain ions. To track and quantify nutrient elements in coffee, instrumental methods such as Flame Atomic Absorption Spectrometry (FAAS) have been used to measure concentrations of major and minor elements. However because FAAS is not sensitive enough, to measure some trace elements, inductively coupled plasma optical emission spectrometry (ICP-OES), methods have been used to measure trace elements. Although, ICP-OES method has been used extensively to assess the nutrient elements in coffee, X-ray methods have been more preferred to ICP-OES techniques, because they are not as expensive, do not require extensive sample preparations, are non-destructive and can analyze many samples within a short period [6].

In this study, EDXRF was used to evaluate the element composition and concentration of soil, leaf and green beans. The principle behind EDXRF is that all elements emit secondary fluorescent X-rays when exposed to higher X-ray energy source and that the emitted X-ray fluorescence is specific and defines the characteristics of that element. Heavier elements require heavier energy levels to emit fluorescence and are easier to measure [6]. Macro-elements such as K, Ca and Na though detectable through EDXRF are not as heavy as trace elements such as Fe, Zn, Cu, Mn, a factor that makes the micro-elements more amenable to EDXRF measurement.

The EDXRF spectrometer instrument has a semi conductor detector that measures the entire energy spectrum when placed closer to the sample to minimize loss of energy by the fluorescent X-ray. Unlike X-ray tubes that generate high power monochromatic beams, the EDXRF instrument is capable of generating gamma or proton rays at high speed [7,8]. The detectors in EDXRF are able to discriminate the various X-ray intensities and therefore many samples can be analyzed simultaneously. The spectrometer maintains the detector at low temperature, reduces multi-proton pace and sets low conductivity giving more time for pulse shaping and better resolution [8]. Analyzing samples with well characterized shape and surface maintains uniformity in density, roughness, and thickness and helps to reduce matrix effects [8].

Robusta coffee is Uganda's major foreign exchange earner. In recent years, although coffee production has increased globally and reached about 120 million 60 kg bags in 2002, in the same period, there has been a dramatic decrease in the average price paid for coffee falling from USD 120 cents per pound in the 1980's to 47 cents per pound in 2002 [2]. In Uganda, it is estimated that in 2001/02 when coffee prices were at their lowest, farmers earned USD 5.50 from 650 kg un-hulled coffee per acre [2]. For Ugandan producers to remain competitive in the global markets, there is need to improve cupping quality. This study analyzed the relationships of major and trace elements in the soil, leaf and green bean in order to understand their ultimate influence on coffee quality.

2. MATERIALS AND METHODS

2.1 Soil, Robusta Coffee Leaf and Green Bean Sample Collection

Harvested ripe cherry (the ripe coffee fruit that contain the green bean) was poured in a bucket of water and beans and debris were removed before being sun dried in wire mesh boxes. The ripe cherry, with an estimated initial 50% moisture content [9]; http://www.ico.org/ecology.asp) was spread to a maximum of 1.5 cm layer thickness to avoid formation of mold or bean deterioration. The coffee was regularly turned until it achieved 12.5% moisture content. Drying lasted for about a month and the dry coffee bean was stored in a dry aerated room.

Leaf and soil samples were collected from sixty seven tagged trees whose ripe cherry had been picked for bean quality analysis in twenty three on farm locations at Kawanda and Entebbe (Table 1). The cherries were harvested when they were physiologically mature and the subsequent leaf and soil samples were collected from the same tree bearing the cherries. The soil was sampled from fields that did not have a previous two-year fertilizer application history and the surface litter was removed without scraping off top soil. An auger was used to drill up to a depth of 30 cm in three triangular spots located 30 cm- 150 cm tree radius and was cleaned after each sampling. Soil samples were placed in polythene bags and labeled. About six to ten physiologically mature leaves close to or within the ripe cherry clusters were picked using clean and gloved hands. Leaves were packed in paper sample bags, labeled and left aerated until they dried. Wet soil samples were initially sun dried and later air dried and then mixed together to constitute one sample for each tree.

2.2 Energy Dispersive X-Ray Fluorescence Measurement

About 20 grams of frozen green bean were initially ground using a nitrogen pre-cooled motorized grinder. Dried soil and leaf were ground to fine particle size using a motorized grinder in the University of Nairobi, Department of Nuclear Science and Technology. The pre-ground coffee green powder was further crushed to finer particles using a hand motor and pestle. Ground samples were sieved using 0.1 mm screen size and fine powder of 0.3-0.5 grams was weighed with AT460 Delta-Range balance. A compacting machine pressed fine powder to homogenous flat circular discs (pellets) of diameter 20-50 mm with sufficient thickness to absorb the entire X-ray florescence primary beam, including lighter elements which omit fluorescence of a few micrometers sample depth. The X-ray was calibrated using a representative soil, leaf and green bean sample of accurately known element concentration to enable calculation of the investigated sample element concentration. Sample pellets were randomly picked and geometrically placed at a standardized small distance on a high precision motorized sample stage located in a containment box that acted like a vacuum by sustaining X-ray energy. The sample stage was positioned accurately beneath the X-ray tube window in line with the detector assembly connected to liquid nitrogen (-197°C). Liquid nitrogen limited lithium atoms from drifting or migrating. To reduce the effect of sample irregularities, the sample was spanned at 5-20 rotations per minute (rpm). Individual pellets were then scanned by bombarding with X-rays from a Cd-109 (cadmium) radioisotope source. Canberra Multi-Channel Analyzer and spectral data processing software unit, MCA (S-100) was linked to a personal computer. Sample spectra multiple peaks for different energy intensities were visualized on the computer monitor as a plot of specific element distribution/composition and concentration was measured by an in built color video camera. Multiple elements in the sample were imaged concurrently and information from all detectable elements was captured simultaneously. The data generated was pre-amplified, amplified, stored and analyzed using XQAS/AXIL computer software. In pre-amplification, the burst of electrons were converted into signals of elements which were further amplified and transformed from analog to quantified values.

2.3 Measurement of Organoleptic Attributes, Biochemical Compounds and Roast Bean Physical Characteristics

Organoleptic sensory attributes were evaluated in form of a descriptive manner as recommended by [10] and as described by [11] where a team of three professional Robusta coffee organoleptic cup testers from Uganda Coffee Development Authority (UCDA) used the International Coffee Organization (ICO, 2004) protocols to subjectively detect and quantify aroma, fragrance, flavor, aftertaste, mouth feel and bitterness /sweetness and balance cupping abilities.

Caffeine, chlorogenic acid, trigonelline, sucrose, fats and dry matter green bean compounds were measured with the Near Infra Red Spectroscopy (NIRS) using Nirsystem 6500 Foss (Denmark) Spectrometer that utilized computer Software ISI NIRS 2 version 4.11 (Infra Soft International, Port Matilda, USA) to analyze ground samples ranging from 400 nm to 2500 nm wavelengths [12-14]. The scored data was analyzed with XLSTAT version 2011.2.05 (Addinsoft, Paris, France). Physical bean characteristics measured included, size as graded by three screens, namely, large (A) ≥18 (7.0 mm), medium (B) ≥15-<17 (6.0–< 6.75 mm) and small size 15 (6.0 mm) described by [11]. Other characteristics measured included, roast time per gram, percentage weight decrease and percentage volume increase [11].

2.4 Statistical Analyses

2.4.1 Correlation coefficients and PCA

ANOVA was calculated with the XLSTAT version 2011.2.05 and the t-test was used to identify significant differences between the means as the sampled population was assumed not to be normally distributed. Pearson product moment correlation coefficient was used to calculate the correlation coefficient, r and the coefficient of determination R^2. The percentages of variance in the PCA analysis representing the eigen-values was calculated according to [15] and factorial discriminant analysis subsequently used to distribute the K means.

2.4.2 Multiple regression analysis

Multivariate Analysis of Variance (MANOVA) described in the Palaeontological statistics 2.14 package was used to calculate the effects of a dependent variable from the regressions coefficients of many independent variables. Data sets of various nutrient elements were independently regressed on organoleptic cup traits, biochemical compounds and on bean physical characters using the multiple linear regression relationship model; $Y=a + b_1X_1 + b_2X_2 + b_3X_3 \ldots + b_kX_k + I$, in the GenStat 12[th] edition, (where Y= response/dependent variable being predicted and X_1 is the first independent or predictor factor; a= alpha, the constant while b_1, b_2 , b_k are regression coefficients for X_1. In the first multiple regression, nutrient elements as independent variables were regressed on organoleptic attributes as dependent variable, where the fitted terms were as follows; Constant + MN(S) + PB(B) + CA(B) + FE(B) + ZN(S) + K(S) + K(L)+ CA(L) + B(S). The S, L and B letters refer to soil, leaf and bean elements respectively. In the second equation, nutrient elements were regressed on biochemical compounds, namely caffeine and trigonelline and the fitted terms were: Constant + PB(S) + CA(S) + FE(B) + CU(S)+ MN(S) + MN(L) + B(B) + ZN(B); and in the third equation, nutrient elements were regressed on roast bean physical characteristics and screen size 15 as; Constant + ZN(S) + CA(S) + MN(S) + CU(S) + PB(S) + K(L)+ MN(L) + B(L) + K(B) + CA(B) + CU(B) + ZN(B) + PB(B).

3. RESULTS

3.1 Nutrient Element Concentrations in Soil, Leaf and Green Bean

Nine nutrient elements consistently detected in the soil, leaf and green beans were potassium (K), calcium (Ca), titanium (Ti), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), lead (Pb) and boron (B) (Table 1). Manganese and iron values were minimal in the soil. Ca and Fe had minimal values in leaf samples but K values were highest in green beans. K, Ca, Mn, Fe, Cu, Zn, Pb and B were detected in the soil, in the leaf and in the green bean. The concentrations of Mn, Fe, Zn and Pb decreased progressively in the soil, in the leaf and were lowest in the green beans. The concentration of K in the soil was 8206.2 ppm compared to that of 911716.4 ppm in the leaves and 12904.4 ppm in the green bean. Ca in the soil had a mean value of 4939.3 ppm but the value increased to 13343.9 ppm in leaves and reduced to 12904.4 ppm in the green bean. Cu and B concentrations in the green beans were 12.8, 1.7 ppm respectively but were 10.0, 1.5 ppm respectively in the leaves.

3.2 Principal Components Analysis; Relationships between Soil, Green Bean and Leaf Elements

The principal component analysis (PCA) in Fig1 shows the relationships between elements and Table 2 shows the associations within the soil elements. In Fig. 1, the total variance for principal component 1 and 2 was 51.29%. K concentration was not significantly different from Ca. Pb was inversely related to soil K and Ca but not to significant levels. In Table 2, K was significantly negatively correlated with Ti (r=-0.51) and Fe (r=-0.35) and the coefficient of determination R^2 value was higher with Ti (0.26) than with Fe (0.12). In both cases, the linear relationship between the variables was weak. Only 26% and 12% of the total variance could be explained by the linear relationships either between K and Ti and between K and Fe, respectively. Ca was significantly positively correlated with Mn and Zn but the correlation was strongest with Zn. Ti was positively significantly associated with Mn, Fe, Zn and B but the correlation was strongest with Fe (r=0.59) and B (r=0.4). About 35% of the total variance could be accounted for by the linear relationship between Ti and Fe. Mn had a significantly positive correlation with Fe and Zn whereas Fe was positively and significantly correlated with Cu and Zn.

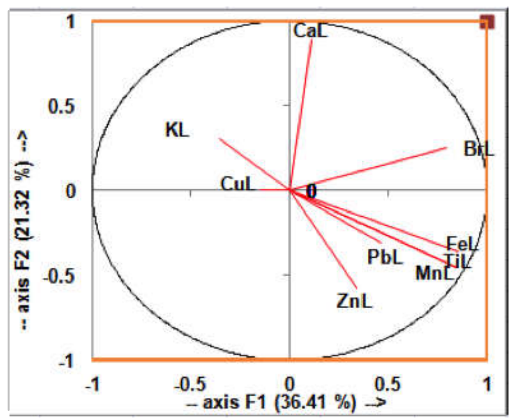

Fig. 1. PCA relationships among soil microelements

Fig. 2 and Table 3, show the PCA variances and correlation coefficients within leaf elements respectively. Fig. 2 shows that Zn, Mn, Pb, Ti and Fe were positively correlated to each other but were inversely related to K and Cu. From Table 3, K was significantly negatively correlated with Zn (r=-0.26) while Ca had a significant negative correlation with Ti (r=-0.31) and with Zn (-0.26) indicating little linear relationship between these variables. Ti was significantly positively correlated with Mn, Fe, Zn, Pb and B. The correlation was stronger with Zn (r-0.59) and with B (r=0.55) which explained only 35% and 30% respectively of the total variance between the elements. Mn was positively and significantly associated with Zn (r=0.28) and B (r=0.25) while Zn was positively significantly correlated with Pb (r=0.4) and with B (r=-.38). Only the regression between Ti and Zn and between Ti and Br appeared to be linear.

Table 1. Nutrient elements concentrations in ppm from soil, leaf and green bean samples from 67 farms in Uganda

Elmt	Soil				Leaf				Bean			
	Min	**Max**	**Mean**	**Sdev**	**Min**	**Max**	**Mean**	**Sdev**	**Min**	**Max**	**Mean**	**Sdev**
K	0.0	26750.	8206.2	6757.6	0.0	24000	11726.4	4004.6	6206.7	30666.7	12904.4	3746.4
Ca	0.0	22700	4939.3	4461.5	4220	24650	13343.9	4722.9	0.0	4281.3	1273.9	770.2
Ti	0.0	19950.	8744.6	2650.4	0.0	7370	212.3	1212.3	0.0	0.0	0.0	0.0
Mn	18.9	6846.7	1823.0	1063.1	0.0	1415	96.6	231.4	0.0	34.2	2.4	7.1
Fe	55.1	149000	55111.1	26062.1	81.8	40550	1444.1	6601.5	0.0	138.8	48.0	20.3
Cu	0.0	175.5	13.6	33.5	0.0	64.6	10.0	14.4	0.0	54.9	12.8	9.2
Zn	0.0	224.0	74.4	48.3	0.0	135.0	4.2	18.8	0.0	10.9	1.0	2.4
Pb	0.0	75.9	36.8	23.7	0.0	33.5	2.0	6.2	0.0	15.1	1.4	3.1
B	0.0	71.1	12.6	12.7	0.0	29.8	1.5	5.0	0.0	21.8	1.7	4.6

Key to Table 1: K=potassium; Ca=Calcium; Ti= Titanium; Mn=Manganese; Fe=Iron; Cu=Copper; Zn=Zinc; Pb=Lead; B=Boron

Table 2. Linear correlations (r) and correlations of determination (R^2) among soil micro-elements in 67 farms

Association		R	R^2	P value
K	Ti	-0.51	0.26	0.0001***
K	Fe	-0.35	0.12	0.005**
Ca	Mn	0.24	0.06	0.05*
Ca	Zn	0.40	0.16	0.001**
Ti	Mn	0.34	0.12	0.005**
Ti	Fe	0.59	0.35	0.0001***
Ti	Zn	0.25	0.06	0.05*
Ti	B	0.40	0.16	0.001**
Mn	Fe	0.37	0.14	0.001**
Mn	Zn	0.47	0.22	0.0001**
Fe	Cu	0.24	0.06	0.05*
Fe	Zn	0.24	0.07	0.05*

*Key to Table 2; P values of *, **, ***significant at, 0.05, 0.001 and 0.0001 probability levels respectively*

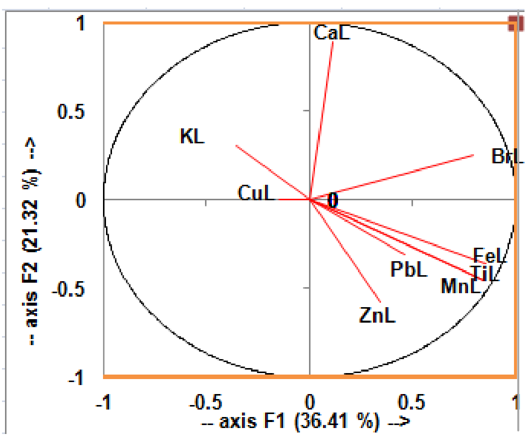

Fig 2. PCA relationships among leaf micro-elements

Table 3. Linear correlations (r) and coefficients of determination (R^2) among leaf microelements in 67 farms

Association		R	R^2	P value
K	Zn	-0.26	0.07	0.05*
Ca	Ti	-0.31	0.09	0.01*
Ca	Zn	-0.26	0.07	0.05*
Ti	Mn	0.25	0.06	0.05*
Ti	Fe	0.26	0.07	0.05*
Ti	Zn	0.59	0.35	0.0001***
Ti	Pb	0.37	0.13	0.005**
Ti	B	0.55	0.30	0.0001***
Mn	Zn	0.28	0.08	0.05*
Mn	B	0.25	0.06	0.05*
Zn	Pb	0.40	0.16	0.001**
Zn	B	0.38	0.14	0.001**

*Key to Table 3; P values of *, **,*** significant at 0.5, 0.001 and 0.0001 probability levels respectively*

Fig. 3 shows PCA relationships between green bean elements whereas Table 4 gives the correlation coefficients. As shown in Fig. 3, Ca had an inverse insignificant correlation with Pb, Zn and Mn while K had a significant positive correlation with Ca, Fe and Cu. In Table 4, K was positively significantly associated with Fe (r=0.54) and with Cu (r=0.41) with 29% and 17% of the total variance respectively being explained by their linear relationships. Mn was significantly and positively related to Zn (r=0.46), to Pb (r=0.40) and to B (r=0.38) (Table 4). Fe was significantly positively correlated to Cu (r=0.39) while Zn was positively significantly correlated with Pb (r=0.47) and with B (r=0.26), the linear relationship being stronger between Zn and Pb.

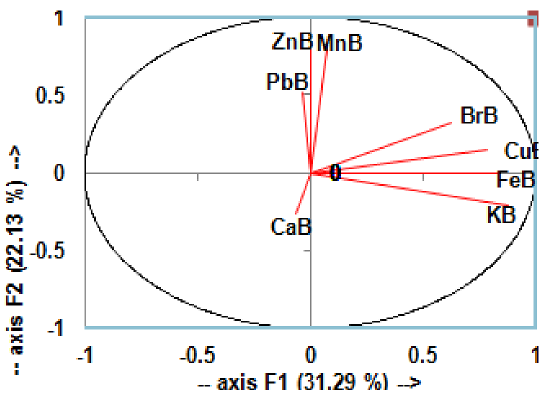

Fig. 3. PCA relationships in Green Bean Microelements

Table 4. Linear correlations (r) and coefficients of determination (R^2) among green-bean microelements in 67 farms

Association		R	R^2	P value
K	Ca	0.34	0.11	0.005**
K	Fe	0.54	0.29	0.0001***
K	Cu	0.41	0.17	0.001**
Mn	Zn	0.46	0.21	0.001**
Mn	Pb	0.40	0.16	0.001**
Mn	B	0.38	0.14	0.002**
Fe	Cu	0.39	0.15	0.001**
Zn	Pb	0.43	0.19	0.001**
Zn	B	0.26	0.07	0.03*
Pb	B	0.47	0.22	0.0001***

*Key to Table 4; P values of *, **,*** significant at 0.5, 0.001 and 0.0001 probability levels respectively*

Table 5 shows regression effects of soil elements on green bean elements. The only significant linear regression equations are those of green-bean Fe on soil B, green bean Fe on soil Mn, and green bean Mn on soil Ti as indicted by their significant p values. Their respective R^2 values of 10.9%, 10.9% and 10.7% gives the amount of variation accounted for by their linear relationships.

In Table 6, the most significant regression effects were those of Cu leaf on soil Pb, K and Mn with R^2 values of 17.1 %. There was some significant regression of leaf K on soil B with an R^2 value of 11.5%. An increase in soil Cu concentration caused an increase in leaf Pb but decreases in soil K concentration and Mn caused increases in leaf Cu concentration. High concentrations of soil B significantly reduced leaf K. Increasing soil Ti significantly increased leaf Zn. Even with insignificant fitted terms, soil Pb, Ti and Cu elements had significant effects on leaf micro-elements. Leaf micro-element variability explained by soil elements ranged from 3-17.1%.

The effect of increasing coffee tree age and elevation on green beans micro-element concentrations and quantity is shown in Table 7. Green bean concentrations of Mn and Zn increased significantly with tree age and with altitude respectively as shown in Table 7. Green bean Mn concentration increased with altitude up to 1300 metres above sea level (m a s l) but declined thereafter, while green bean Zn concentration on the other hand increased from 0.5 ppm at 13000 m a s l to reach 3.5 ppm at higher elevation of 1500 m a s l and declined afterwards (Fig. 4). Altitude accounted for 11.8% in green bean Zn variability while age of trees accounted for only 5.5% in green bean Mn variability (Table 7).

Table 5. Regression effects of soil elements on green bean elements in 67 farms

Bean elements	Soil elements	Vr	Pvalue	T-test	R^2 (%)
B	Ti	1.75	Ns	**	7.4
Ca	Ca	1.55	Ns	**	5.5
Fe	B	2.15	**	**	10.9
Fe	Mn	2.15	**	**	10.9
K	B	1,66	Ns	*	6.6
Mn	Ti	2.13	**	**	10.7
Fstat, 7, 59					

*Key to Table 5; P values significant at *, **, *** at 0.05, 0.001, 00001 levels of probability d.f=degrees of freedom; v.r=variance ratio; F value=Fisher probability test; t-test=Student t test; R^2= coefficient of determination ns=not significant; Fstat; F7, 59= Fisher statistics; 7 factors and 59 variable degrees of freedom*

Table 6. Regression effects of soil elements on leaf nutrient elements in 67 farms

Leaf elements	Soil elements	Vr	P value	T-test	R^2%
B	Pb	1.62	Ns	**	5.3
Cu	Pb	3.26	***	**	17.1
Cu	K	3.26	***	**	17.1
Cu	Mn	3.26	***	**	17.1
Fe	Ti	1.29	Ns	**	2.6
K	B	2.43	**	***	11.5
Mn	Ti	1.76	Ns	***	6.5
Mn	Cu	1.76	Ns	*	6.5
Ti	Ti	1.34	Ns	**	3.0
Zn	Ti	2.15	*	**	9.5
Fstat: 6, 60					

*Key to Table 6; P values significant at *, **, ***, **** significant at 0.05, 0.001, 0.0001 levels of probability respectively; Fstat; F6, 60= Fisher statistics; seven factors, 59 variable degrees of freedom*

Table 7. Effects of tree age and altitude on Mn and Zn green bean elements

Green bean element	Predictor	Coefficient	s.e	vr	Fp	Tp	R^2(%)
Mn	Age	0.10	0.04	2.94	**	**	5.5
Zn	Altitude	0.06	0.002	5.42	***	***	11.8
Fstat; F2, 64							

*Key to Table 7; P value, *, **, *** significant at 0.06, 0.05 and 0.009 levels of probability*

3.3 Multiple Regression Analysis of Nutrient Elements on Organoleptic Attributes, Biochemical Compounds and Physical Characteristics

Table 8 shows significant multiple effects of soil, leaf and bean elements on organoleptic cup. High concentrations of soil Mn increased cup fragrance and aroma. 19.7% of the variability in flavor was influenced by the high amounts of Pb and Fe in the bean. High concentrations of Fe in the bean enhanced aftertaste. High concentrations of leaf K accounted for 6.7% of cup bitterness/sweetness. High concentrations of Pb and Fe in the bean, influenced mouth feel by 17.7% and soil Zn content accounted for only 6.7% of cup acidity. Overall, 22.1% of organoleptic cup highest variability was explained by the positive effect of soil Mn on fragrance/aroma.

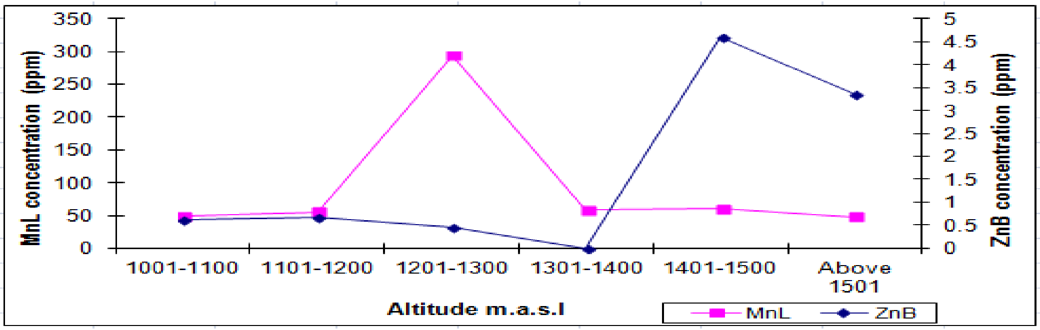

Fig. 4. Comparing mean leaf manganese and bean zinc nutrient elements at altitude ranges

Table 8. Multiple regression effects of nutrient elements on organoleptic cup attributes, biochemical compounds and bean physical characters

Cupping traits	**Nutrient element**	**v.r**	**t pr**	**R^2 (%)**
Aroma	MnS	1.95	**	22.1
Flavour	PbB	1.82	**	19.7
	FeB	1.82	**	19.7
Aftertaste	FeB	1.66	**	16.5
Bitter/Sweet	KL	1.24	**	6.7
Mouthfeel	PbB	1.72	**	17.7
Salt acidity	ZnS	1.22	**	6.3
Fstat: F9, 21				
Biochemical compounds	**Nutrient element**	**v.r**	**t pr**	**R^2 (%)**
Caffeine	BrB	1.34	**	8.4
	ZnB	1.34	**	8.4
Trigonelline	PbS	2.70	**	31.2
	BB	2.70	**	31.2
Fstat:F8, 22				
Physical character	**Nutrient element**	**v.r**	**t pr**	**R^2 (%)**
Bean weight loss	BrL	3.39	***	50.9
Rb Vol Inc	MnL	3.19	**	48.7
	KB	3.19	**	48.7
	CuB	3.19	***	48.7
	ZnB	3.19	***	48.7
	PbB	3.19	**	48.7
Screen Size 15	PbS	1.74	**	24.3
	KL	1.74	**	24.3
	ZnB	1.74	**	24.3
Fstat: F13, 17				

*Key to Table 8; **, P, significant at 0.05 levels of probability; S, L, B =soil, leaf and bean elements; Mn=Manganese, Pb=Lead Ca=Calcium; Fe=Iron, Zn=Zinc; K=Potassium, B=Boron, Cu=Copper*

Significant multiple effects of soil and bean elements on biochemical compounds are shown in Table 8. Trigonelline content variability of 31.2% was explained by high soil lead and high bean B concentrations but 8.4% of variability in caffeine was explained by concentrations of B and zinc in the green bean.

Table 8 also shows significant effects of elements on green bean physical characters. High leaf B explained 50.9% variability in green bean weight loss. High concentrations of green bean K and Zn, low amounts of leaf Mn and low contents of green bean Cu and Pb produced beans with a high roast volume increase. Low concentrations of soil Pb, leaf K and bean Zn produced beans of screen size 15.

4. DISCUSSION

From Table 1, soil K concentrations increased progressively in the leaf and were highest in the green bean while Ca levels in the leaf increased threefold of those in the soil but reduced drastically in the green bean. K like other macro-elements such as nitrogen, phosphorus, is a primary requirement by the coffee plant for growth, development and yield [10,12]. However, it is important to note that the ratio between K and Ca cations in the soil is antagonistic and does not contribute to coffee quality characteristic such as aroma [13]. Nevertheless, K together with Zn have been used in organoleptic analyses to describe and classify coffee according to type either as roasted or instant soluble [16].

Except for Ti, all the macro or micro elements measured and detected in the soil were also found in the leaves and green beans, but at varying amounts (Table 1), implying that they had vital physiological roles to contribute in these two organs. In the leaf, the major activities at play include photosynthesis, transpiration and gaseous exchange. Trace elements, namely, Mn, Fe Zn and Pb concentrations were significantly higher in the leaves than in the green beans probably because they contribute to the functions of several biochemical reactions and are involved in plant, growth, development, and reproduction. Fe is a critical element in the photosynthetic system 1 both in the leaf and during green bean development. The presence of Fe in the green bean was influenced by microelements in the soil such as B and Mn that accounted for 10.7% of the total variability (Table 2).

Trace minerals such as Cu, Zn, Mn, and Fe combine with vitamins to form enzymes and are involved in almost every physiological and metabolic processes [17]. Leaf trace elements would be expected to support photosynthesis, transpiration and gaseous exchange processes. The results reported in this study, indicated that Mn, Fe and Zn were found in high concentrations in the leaf at 96.6, 1444.1, 4.2 ppm respectively but were in lower concentrations in the green bean at 2.4, 48.0, 1.0 ppm respectively confirming their crucial presence. Cu and B, concentrations, however, were higher in the green bean (12.8 ppm, 1.7 ppm) than in the leaf (10.0 ppm, 1.5 ppm) respectively (Table 1). Cu levels in the leaf were influenced positively by the levels of Pb, Mn and K and its relationship with these three elements contributed about 17% of the total variation in each case as shown in Table 5. The higher concentration of Cu in the bean than in the leaf is an indication that Cu is not only involved in protein synthesis and carbohydrate metabolic activities in the fruit but it is also required during photosynthetic and cell wall elongation stages in the leaf. Cu is known to be a contributor to photosynthesis and respiration processes during leaf growth and fruit development and together with Zn it is involved in the synthesis of the growth hormone Indole Acetic Acid (IAA) [18].

The concentrations of non-essential elements, Ti and Pb were found to be highest in the soil but were almost negligible in the green bean as would be expected. K, Ca, Zn and Cu concentrations accounted for most of the macro and micro-element variation in the soil but an in increase in K concentration in the soil led to decreases in Ti and Fe. Ti was heavily associated with almost all the trace elements in the soil. It is interesting that Ti which plays no part in plant metabolism was found in high levels in all the farms surveyed and was inextricably associated with almost all trace elements in the leaf. In the green-bean the association between Zn and Mn and between K and Fe caused most of the variation, while an increase in K resulted into a decrease in Fe and Cu.

The higher Ca concentrations in the leaf is a pointer to the role the element plays in supporting transpiration uptake, promoting cell elongation, and in protecting the plant against biotic and abiotic stresses. The non-significant regression between soil Ca and green bean Ca in Table 5 confirms that the element is not critical during green bean development. K levels were higher in the coffee beans because, K+ ions are principally required for maintenance of turgor and cytosol ionic balance during photosynthesis. Developing green coffee fruits stomata represent 20-30% of the total photosynthetic surface on heavily bearing trees [19] and account for about 30 % of daily respiration needs, 30% of total growth and 12% of total carbon requirement [20]. The positive significant correlation between K and Fe and Cu signified the critical role the three elements play in enhancing photosynthetic and hormonal metabolic activities during this stage. Mn and Zn were also indispensable during fruit formation as indicated by the positive significant correlations between the variables. During bean filling developmental stage, high levels of K were found because at this stage 70% of the endosperm dry matter is being formed [21].

Coffee organoleptic cup qualities have been shown to be influenced by factors such as genotype, age, altitude, and soil. Elements such as Zn and Mn have been shown to be important organoleptic components that affect caffeine content, taste, acidity and aroma and as implied earlier these microelements are essential during fruit development. [21] reported that increased concentrations of Mn and Zn influenced aroma positively in *C. arabica* wild types. As indicated in Table 7 and in Fig 4, these two trace elements increased with age but at higher elevations where Robusta coffee is hardly grown, Zn was still critical in the determination of these organoleptic qualities. In sensory testing, Cu and Mn concentrations have been effective in discriminating roasted Robusta types because their variation is related to point of origin [16]. In this study, soil Mn accounted for most the variability in aroma as shown by Table 8, concurring with the findings of [20] but flavor was more under the influence of Fe and Pb in the green bean. Zn in the soil and in the green bean positively influenced the concentrations of caffeine and trigonelline as shown in Table 8 but it was B that caused the highest variability in the trigonelline. Trigonelline, a pyridine alkaloid is reported to be involved in flavor formation [22,23]. These results would appear to suggest that Fe and B in the bean are important elements that affect the concentration of trigonelline in unexplainable way and which in turn influences flavor. Leaf B was responsible for most of the variability in bean weight loss (Table 8) whereas screen size 15 was influenced by the concentrations of Pb soil, K in the leaf and Zn in the bean. Soil, leaf and green bean elements explained 24.3-50.9% of the bean physical character variability. In interpreting the overall results, it is recognized that a number of external factors such as fertilizer, farm yard manure and coffee residue (husks and mulch) application would affect the composition of several elements that finally determine the quality of coffee. Although in Uganda, few farmers apply commercial fertilizers and organic manures which may be the sources of K, N, P, and S and Cu, Zn, Mn respectively, the analyzed samples in this study as mentioned earlier can be claimed to have been obtained from an original organic farming setting.

5. CONCLUSION

EDXRF is an effective tool in the measurement of micronutrient concentrations of Robusta coffee growing soils, leaves and green beans. K concentrations were highest in the green bean and lowest in the soil, whereas Ca concentrations were higher in the leaf than in the green-bean. Cu was an essential microelement of physiological and metabolic processes in both leaves and in the green bean whereas Mn, Zn and Fe are critical microelements during photosynthesis. While all the trace elements measured, namely, Mn, Cu, Fe and Zn were crucial determining factors in bean and cup qualities of Robusta coffee, Zn and Mn were the more important microelements during fruit development. Soil Mn positively influenced flavor and aroma cup attributes whereas green bean amounts of B and Zn positively influenced trigonelline and caffeine concentrations. All microelements measured were important in determining the size of the bean.

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COMPETING INTERESTS

The authors declare that in publishing this paper, there is no conflict of interest with other person(s) or organization (s).

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