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Bioavailability Study of Nine (Mn, Fe, Cu, Zn, Pb, Cr, Ni, Cd, Co) Heavy Metals in Four Edible Vegetables and a Crop Using AAS and EDS

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Abstract
Port Harcourt and its environs have been heavily impacted by environmental pollution and soil contamination. This study was carried out to investigate the bioavailability of heavy metals in some of the vegetables consumed daily by the indigenes of a village. These vegetables and the crop were digested with oxi-acidic mixture and analyzed with Atomic Absorption Spectrophotometer (AAS). The Energy Disperse x-ray Spectroscopy (EDS) was also used for further comparison. For all the samples, concentrations of Mn, Fe, Cu, Zn, Ni and Cd are highest during the dry spell period (Dec – Feb) for bitter leaf and during the less frequent rainfall (Sept – Nov) these metals, with the exception of Mn, are highest for the other vegetables and the crop. The AAS results show that Co has the highest concentration and is consistently high for all four vegetables followed by Fe while Pb has the least concentrations. The order of concentration is as follows: Co >Fe> Ni >Mn> Zn > Cd > Cu > Cr >Pb, while the trend for most metal concentrations in the samples studied is WL > SL > BL = FP > PF. Other metals and nonmetals detected using EDS are K, Ca, Mg, Al, Si, Mo, Cl, P, S and O in all the vegetable samples. All the above elements were also detected in the soil samples including Ti. Our results have shown the presence of the Heavy metals with some having concentrations above the FAO/WHO acceptable limits.

1. Introduction

Several indigenous African vegetables form the basis of most of our daily diet. They are used to increase the quality of our soups and also for dietary purposes (Sobukola et al., 2007). These are a rich source of vitamins, minerals and therapeutic resource. There are a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties (Kayode et al.,2011).Vegetables are an important ingredient of human diet that contains essential nutrients and trace elements (Abdulla et al., 1990)

Nutritionists recommend at least 7-8 servings of fruits and vegetable daily. These leafy vegetables constitute rich sources of essential nutrients and trace elements. They occupy a very important place in the human diets but unfortunately constitute a group of foods which contribute maximally to nitrate, other anions as well as heavy metals consumption (Akan et al., 2009).

Leafy vegetables accumulate higher metal contents than others (Al Jassir et al., 2005).

Rapid industrialization and urbanization with insufficient environmental monitoring has led to significant environmental pollution which has caused the contamination of soils.

If vegetables, which have taken up heavy metals decay and leached into the soil, these heavy metals are redistributed, thus bioaccumulation, geo accumulation and bio magnification of these heavy metals in the ecosystem may result (Asdeo et al, 2011).

Other sources of heavy metals uptake in vegetables are the use of fossil fuels (combination of coal and crude oil), fertilizers, rainfall in atmospheric polluted areas and exhausts of vehicles in from high traffic density areas.

Most heavy metals are not biodegradable; they have long half- lives and have the potential for accumulation in the different organs leading to unwanted side effects (Jarup, 2003, Sathawara et al., 2004) and health problems.

It is therefore imperative and important that the concentrations of these heavy metals be investigated to ascertain that their levels are not beyond acceptable (health) limits.

These vegetables were selected out of a host of variety available in our diet because they are the most frequently consumed on a daily basis in most homes.

The bitter leaf (*vernonia amygdalina)* is used for making "soup"(a slightly thick source) and some diabetics drink the juice collected during de-bittering.

The scent leaf (*ocimum gratissimum)* is a major component in the preparation of goat meat, chicken or fresh fish pepper soup. A delicacy widely enjoyed in the sub Saharan region of Nigeria. The scent leaf juice is often times applied to wounds as an antiseptic and also used for treating diarrhea. The fluted pumpkin *(telfairia occidentalis*) is widely used in making variety of "soups" and in the preparation of a special "vegetable soup" in combination with other vegetables. It is also an essential component in the diet of diabetics. Water leaf (*taliumt riangulare)* is soft, tender, succulent foliage which makes it most desired in the Southern parts of Nigeria in our "vegetable soup". The plantain fruit (*musa accuminata*) which is the crop in this study can either be eaten boiled, fried, roasted or ground into flour. It is a rich source of Fe and K and a major diet for diabetics in Nigeria and other West African countries. Reported sources of Heavy metals uptake in vegetables have been attributed to the use of fossil fuels (Kalagbor et al.,2014), fertilizers, rainfall in atmospheric polluted areas (Sobukola et al., 2010, Oti et al., 2013, Otitoju, et al., 2012) and high traffic density (Echem et al., 2012). Contamination of these vegetables by heavy metals can erase all the nutritional advantages associated with them and even trigger profound mental, health and cardiovascular problems.

2. Materials and Methods

2.1. Collection of Samples

The vegetable samples were collected at three different periods namely; at the dry spell period (before the onset of the rains), at the peak of the rains and during the less frequent rain period (just before the dry season).These were the months of February, June and September respectively.

The vegetables were collected randomly from a farm in triplicates and assigned the codes: BL (for bitter leaf), SL (scent leaf), FP (for fluted pumpkin), WL (for waterleaf) and PF (for plantain flour).The vegetable samples were not washed prior to digestion to avoid the removal of any heavy metals that may have been deposited on them.

Soil samples were also collected alongside the vegetable samples at a depth of 6 inches, 1ft and 2 ft taking into cognizance the average depth of the roots of these vegetables. These samples were given the code SS.

The samples were then transported to the laboratory for digestion and analysis using AAS and EDS.

2.2. Sample Preparation

The samples were air denied and ground in a blender. From the powder, 2g of each sample was weighed out and transferred into a flask. A mixture of trioxonitrate (V) acid, HNO_3 and tetraoxchlorate(VII) acid,$HClO_4$ in the ratio (4:1) was added. The contents of the flask were boiled for 4hrs.

After the vegetable samples were completely digested, they were allowed to cool, filtered and transferred into a 50ml volumetric flask and made up to mark with deionized water.

3. Results and Discussion

Table 1. *Concentration Of the Metals for Bitter leaf, BL, (mg/kg).*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	114.00	307.50	10.75	59.25	1.25	4.75	89.00	13.75	712.50
Pr	87.50	199.00	8.25	41.00	1.50	7.50	124.75	8.25	742.50
lf	71.75	77.50	6.50	34.75	1.25	2.25	69.75	5.50	752.50

Key: ds =dry Spell
pr =peak of rains
lf = less frequent rains

Table 2. *Concentration Of The Metals For Scent Leaf, Sl, (mg/Kg).*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	13.25	191.00	10.25	38.50	1.25	3.50	112.75	24.50	712.50
Pr	11.75	190.50	14.25	43.75	3.00	3.50	124.75	11.25	752.50
Lf	11.50	329.50	8.25	52.00	3.25	3.50	129.50	19.50	767.50

Table 3. *Concentration Of The Metals For Fluted Pumpkin, FP, (mg/Kg.)*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	82.75	236.25	11.25	52.75	0.50	4.00	98.50	21.50	687.50
Pr	49.25	217.50	10.75	38.25	1.50	5.00	112.75	8.25	752.50
Lf	33.25	530.00	12.75	62.75	1.25	5.75	120.00	14.25	753.00

Table 4. *Concentration Of The Metals For Water Leaf, WL (mg/Kg).*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	84.25	165.25	8.50	119.25	1.50	4.75	167.25	18.50	727.50
Pr	82.50	520.00	19.75	123.75	2.00	12.25	112.75	20.50	762.50
Lf	71.75	130.00	10.25	115.00	2.50	9.25	141.25	22.00	775.00

Table 5. *Concentration Of The Metals For Plantain Flour, PF, (mg/kg).*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	4.50	26.50	3.00	4.50	0.00	1.25	0.00	6.75	422.50
Pr	3.00	25.25	1.50	2.75	0.50	1.00	5.25	7.50	502.50
Lf	2.00	14.00	4.25	5.50	0.50	2.75	7.25	8.25	582.50

Table 6. *Concentration Of The Metals For Soil Samples,SS, (mg/Kg).*

	Mn	Fe	Cu	Zn	Pb	Cr	Ni	Cd	Co
Ds	9.75	26.50	1.25	24.00	0.25	2.25	26.5	6.25	462.50
Pr	67.00	1055	18.50	106.75	3.00	14.00	181.50	7.25	705.00
Lf	17.50	725.00	1.00	25.25	0.25	1.75	31.00	6.50	412.50

Table 7. *Concentration Ranges of the metals in the vegetables and crop samples vsSoil sample concentration (mg/kg).*

	Metals	Highest	Lowest	Soil Sample
1	Mn	114.00BL	2.00PF	9.75 – 67.00
2	Fe	530.00 UL	14.00 PF	26.5 – 1055
3	Cu	19.75WL	1.50PF	1.0 – 18.50
4	Zn	123.75WL	2.75 PF	24 – 106.75
5	Pb	3.25 SL	0.00 PF	0.25 – 3.00
6	Cr	12.25WL	1.00 PF	1.75 – 14.00
7	Ni	167.25 WL	0.00 PF	26.5 – 181.50
8	Cd	24.50 SL	5.50 BL	6.25 – 7.25
9	Co	775.00WL	422.50PF	412.50– 705.00

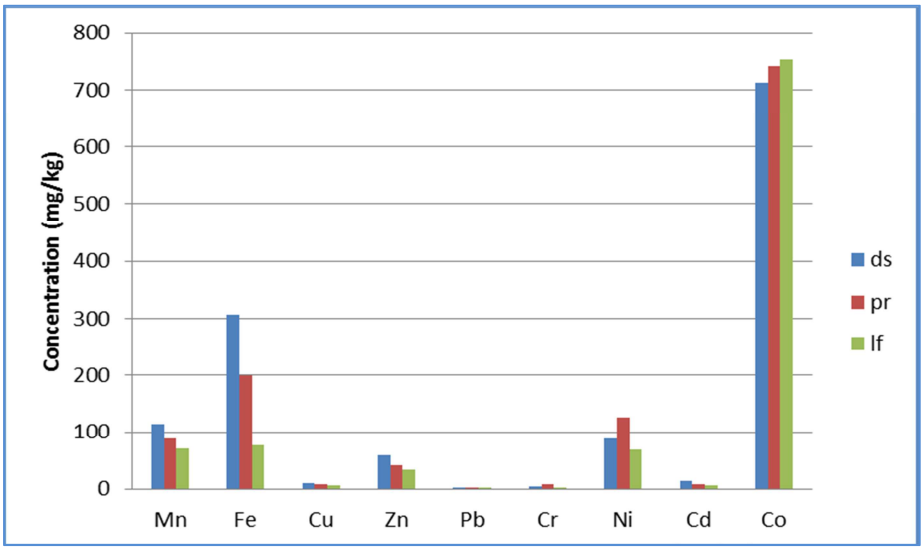

Fig 1. *Concentrations of the metals for Bitter leaf (mg/Kg).*

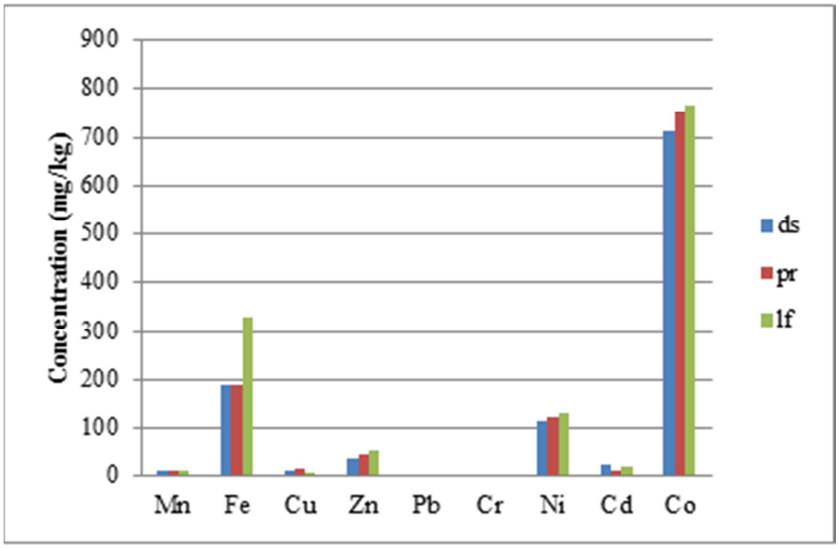

Fig 2. *Concentrations of the metals for Scentleaf (mg/Kg).*

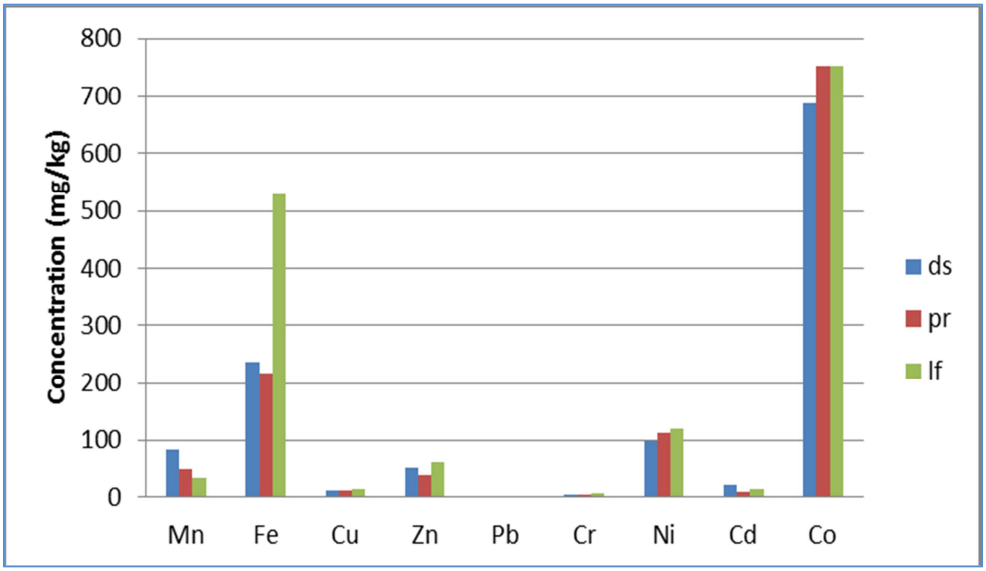

Fig 3. *Concentrations of the metals for Fluted pumpkin (mg/Kg).*

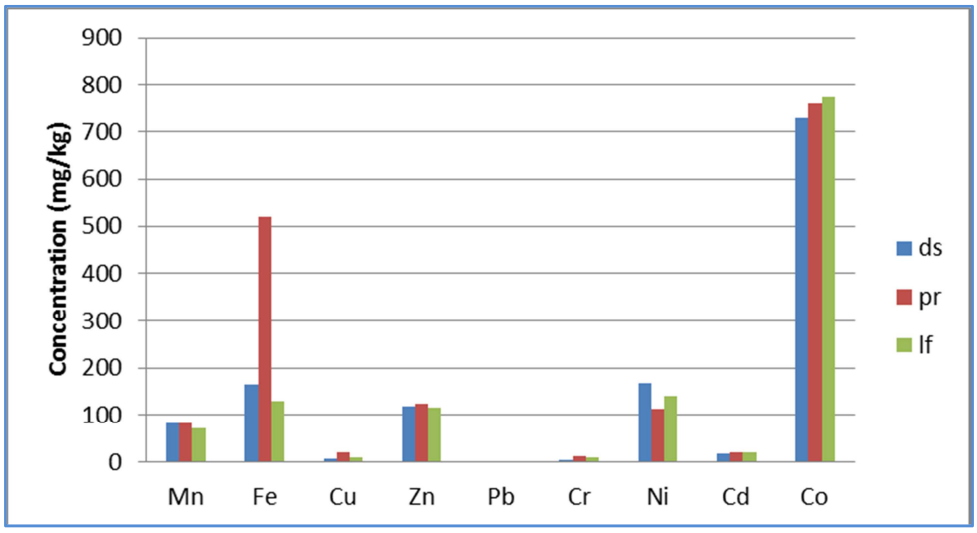

Fig 4. *Concentrations of the metals for Waterleaf (mg/Kg).*

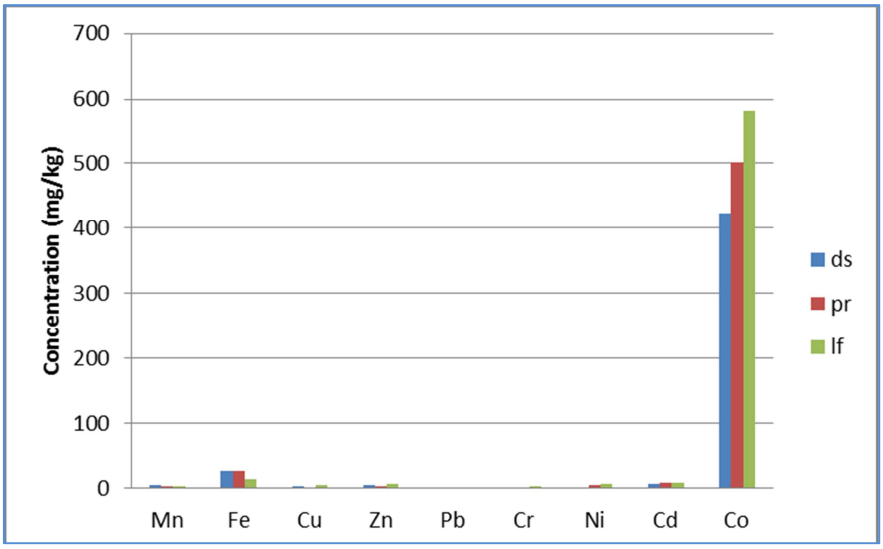

Fig 5. *Concentrations of the metals for Plantain Flour (mg/Kg).*

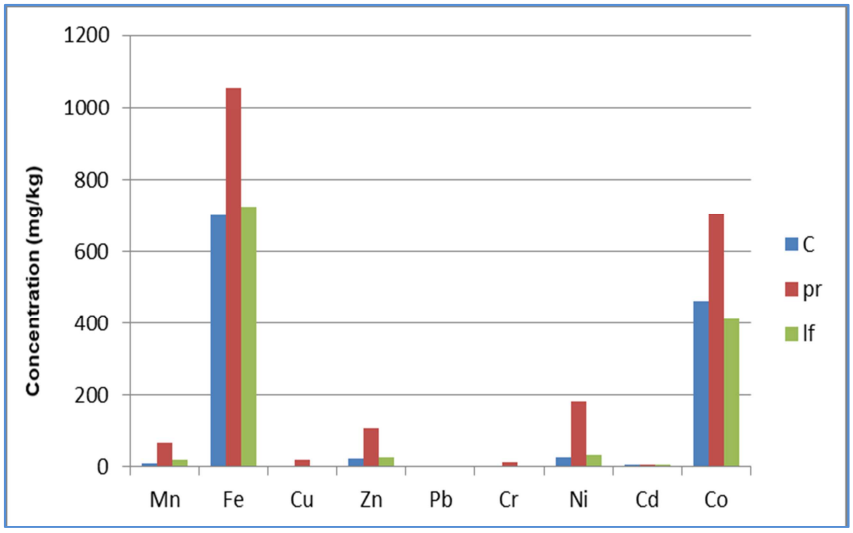

Fig 6. *Concentrations of the metals for Soil Samples (mg/Kg).*

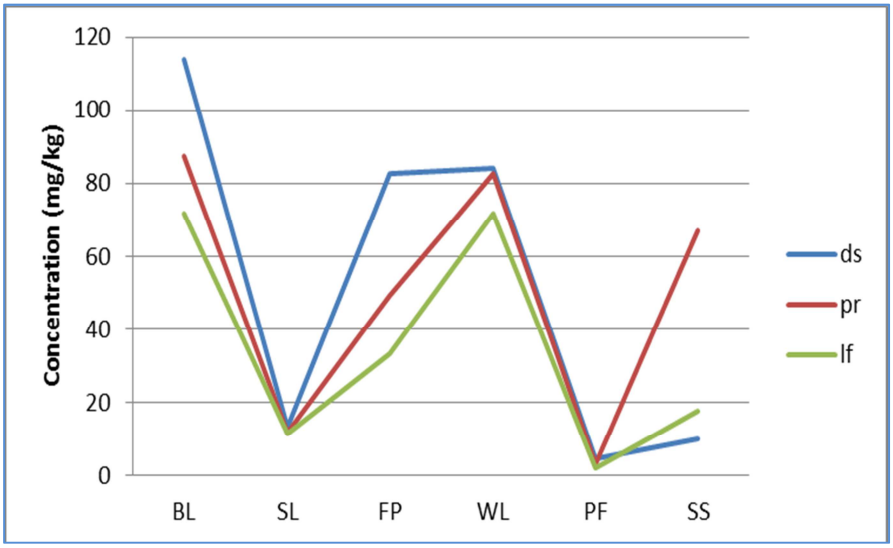

Fig 7. *Concentrations of Mn for the three sampling seasons.*

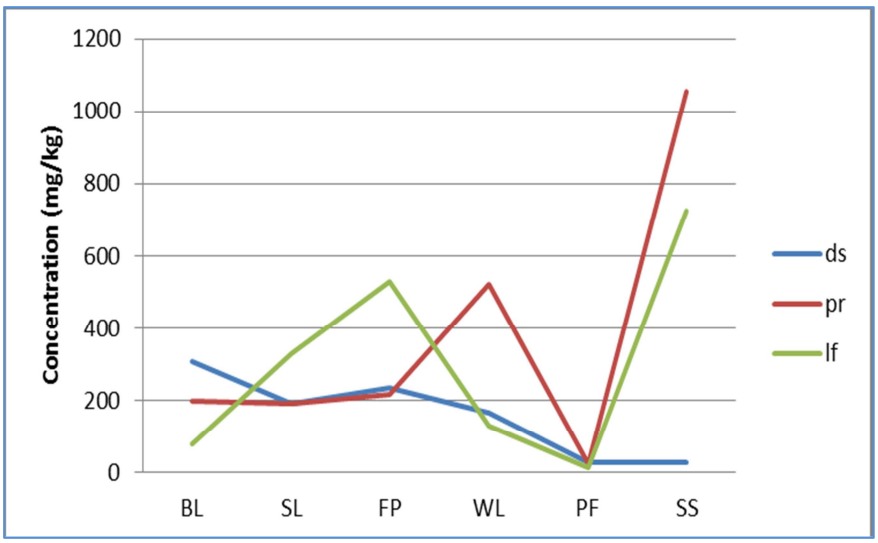

Fig 8. *Concentrations of Fe for the three sampling seasons.*

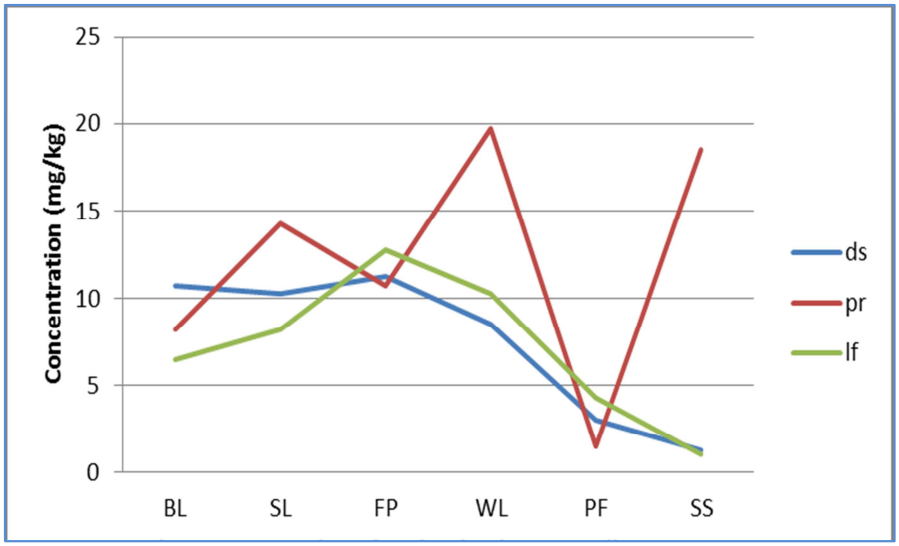

Fig 9. *Concentrations of Cu for the three sampling seasons.*

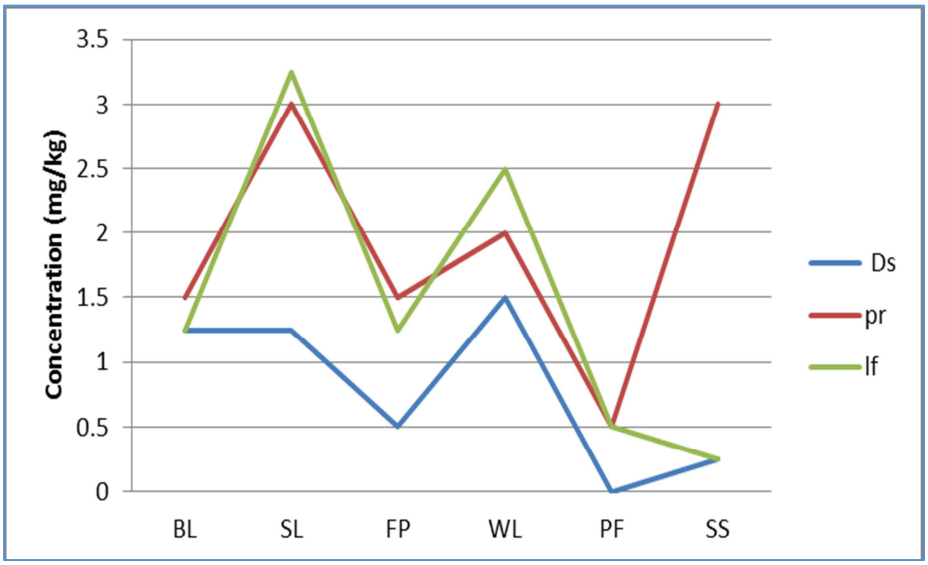

Fig 10. *Concentrations of Pb for the three sampling seasons.*

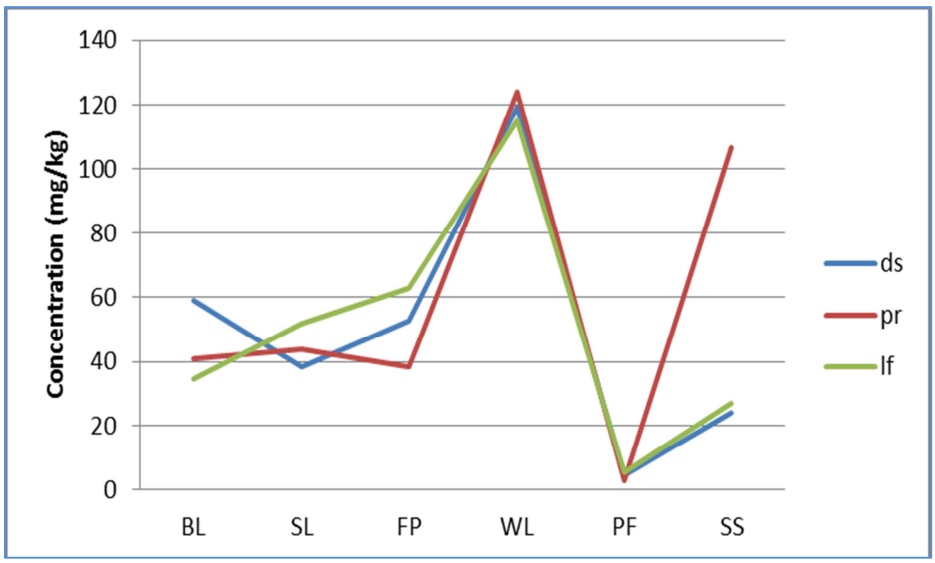

Fig 11. *Concentrations of Zn for the three sampling seasons.*

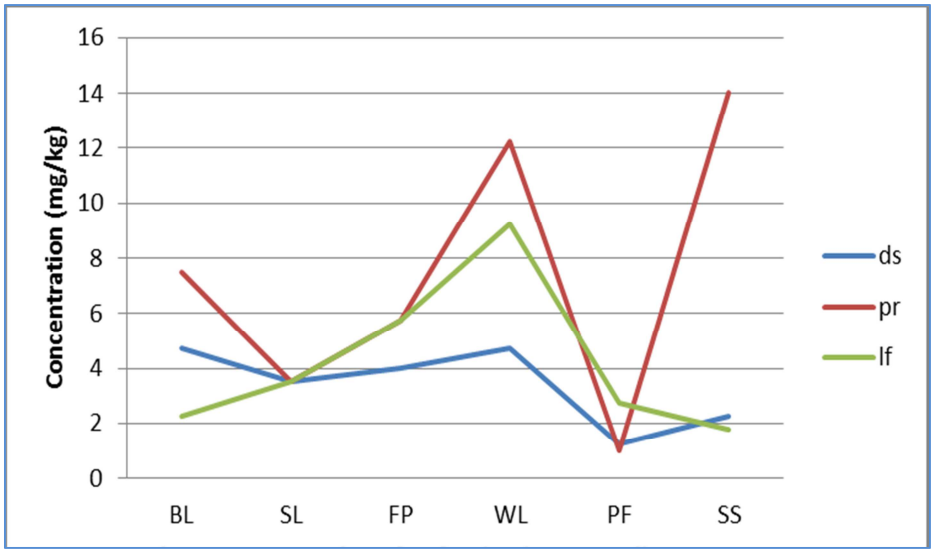

Fig 12. *Concentrations of Cr for the three sampling seasons.*

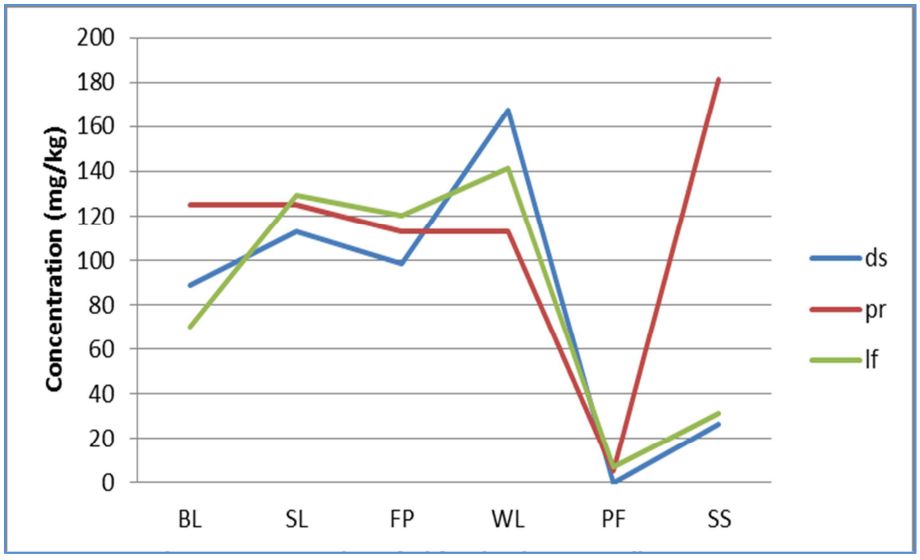

Fig 13. *Concentrations of Ni for the three sampling seasons.*

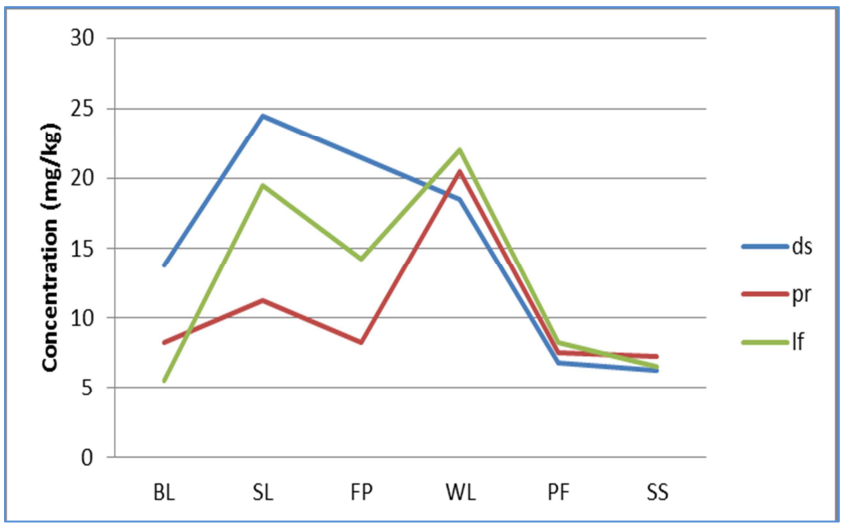

Fig 14. *Concentrations of Cd for the three sampling seasons.*

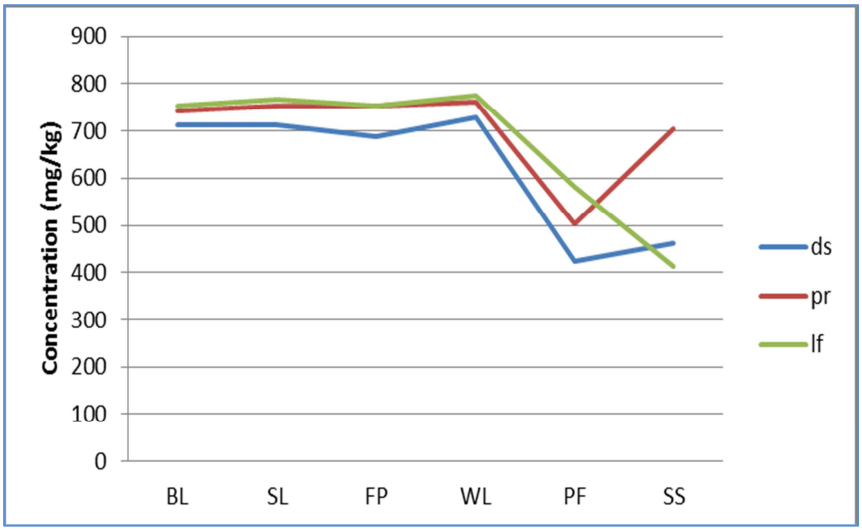

Fig 15. *Concentrations of Co for the three sampling seasons.*

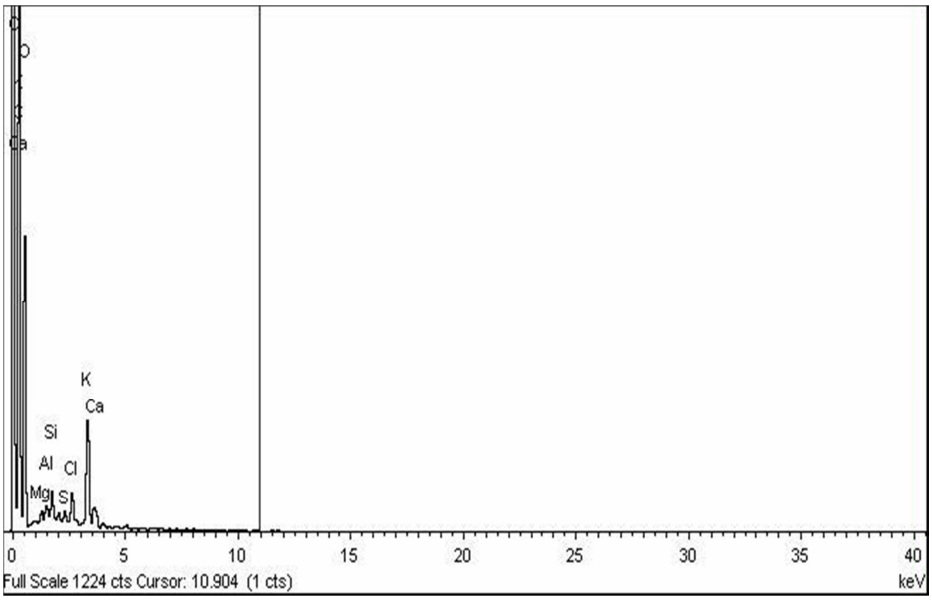

Fig 16. *EDS Bitter leaf Metal Concentration.*

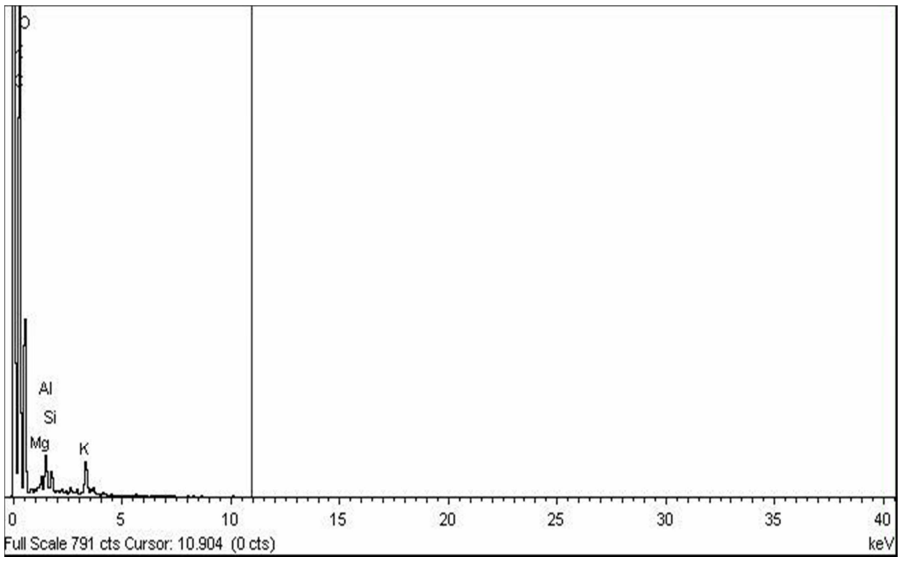

Fig 17. *EDS Scent leaf Metal Concentration.*

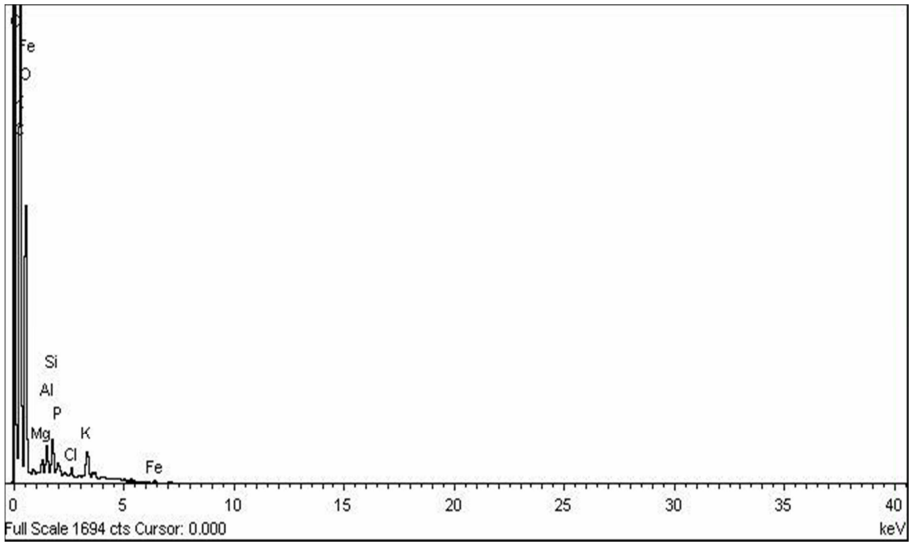

Fig 18. *EDS Fluted pumpkin Metal Concentration.*

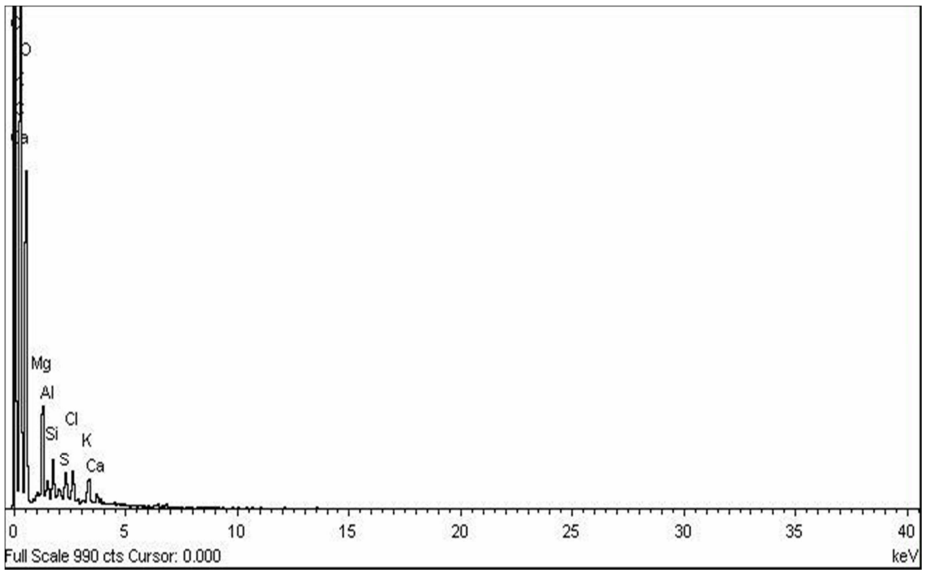

Fig 19. *EDS Water leaf Metal Concentration.*

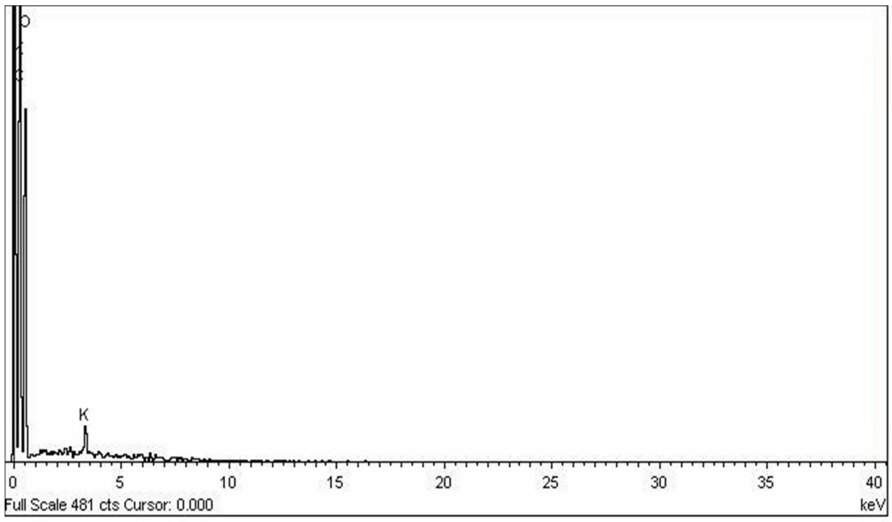

Fig 20. *EDS Plantain flour metals concentrations.*

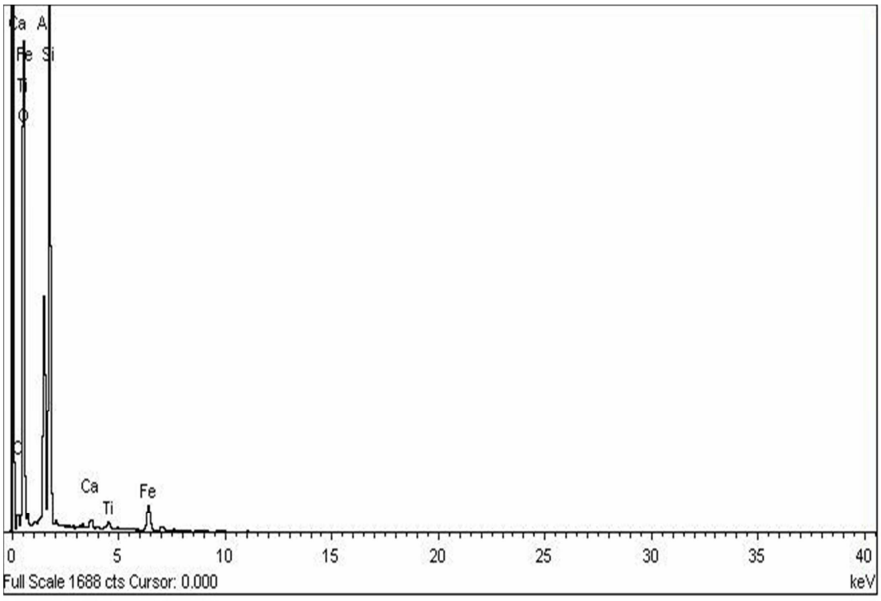

Fig 21. *EDS Soil samples metal Concentration.*

4. Discussion

The concentrations of the nine metals studied in the four vegetables, the crop and the soil samples are presented in Tables 1 to 6. Presented in Table 7 are the concentration ranges of the nine metals; Mn, Cu, Zn, Fe,Pb, Cr, Ni, Cd and Co as well as the values for these metals obtained from the soil samples. From Fig. 1, results for bitter leaf show that during the dry spell, concentrations of Mn, Fe, Cu, Zn and Cd, were high and they are low as the rains became less frequent. Cr and Ni concentrations were higher at the peak of the rains when compared to data from the other two seasons. Co remained high all through the three sampling seasons.

For scent leaf, the concentrations of Fe, Zn, Pb, Ni, and Co are highest as the rains become less frequent, while concentrations of Mn and Cd are highest during the dry spell. This is presented in Fig.2. Concentrations of Co remained high all through the three seasons while concentrations of Cr were unchanged. Fig. 3 shows the concentrations of the metals for fluted pumpkin. Fe, Cu, Zn, Cr, Ni, and Co concentrations were highest as the rains became less frequent with Fe concentrations being significantly high. Cd concentrations were highest during the dry spell. Co concentrations remained the highest for all three seasons. Results for water leaf presented in Fig. 4 shows that Fe, Cu, Zn and Cr have the highest concentrations at the peak of the rains with Fe being significantly high. Mn and Ni have the highest concentrations during the dry spell periods while Pb, Cd and Co concentrations are high during the less frequent rains.

Fig. 5 shows that the concentrations of Mn and Fe in plantain flour were highest during the dry spell period while concentrations for Cu, Zn, Cr, Ni, Cd, and Co were highest during the less frequent rains. Concentrations of the metals in the soil samples are highest during the peak of the rains as can

be seen from Fig. 6. Fe is the highest (1055 mg/kg) followed by Co (705.00 mg/kg) and Ni (181.50 mg/kg). In Fig. 7, bitter leaf showed the highest concentrations for Mn (114.00 mg/kg) out of all the samples studied and also for the sampling seasons, followed by water leaf (84.25 mg/kg) and fluted pumpkin (82.75 mg/kg). Fe concentrations recorded in this study were very high and this is presented in Fig. 8. The soil samples collected at the peak of the rains gave the highest concentration of Fe (1055 mg/kg). Fluted pumpkin and water leaf had concentrations of 530.00 mg/kg and 520.00 mg/kg for two different seasons(less frequent rains and peak of the rains) respectively. Fig 9 presents the concentration of Cu for the three sampling seasons with the highest concentration recorded for waterleaf (19.75 mg/kg) followed by the soil sample (18.50 mg/kg). These concentrations were recorded for the peak of the rains sampling for both samples. Pb concentrations are the lowest of all the metals studied. Fig. 10 shows that these low concentrations are recorded for all the samples and through the sampling seasons. This notwithstanding, the values exceed the WHO acceptable limit. In Fig. 11, waterleaf has the highest concentration of Zn for the three sampling seasons in comparison to the other samples followed by soil sample (10.75 mg/kg) for peak of the rains samples. The concentrations of Cr in all the samples studied for the three sampling seasons are presented in Fig 12. Waterleaf showed high concentrations of 4.75 mg/kg, 12.25 mg/kg and 9.25 mg/kg for all the seasons.

Fig 13 shows the concentrations of Ni for all the samples. From the results obtained, only plantain flour had concentrations below 10.0mg/kg. The soil sample had values of 26.50 mg/kg (for dry spell) and 31.00 mg/kg (less frequent rains). The rest of the samples had values ranging from 69.75 mg/kg to 167.25 mg/kg and the soil sample recording the highest value (181.50 mg/kg) at the peak of the rains. The concentrations of Cd for the samples in all the three sampling seasons do not follow any specific pattern. There is a lot of fluctuation between the highs and lows with respect to seasons. This is presented in Fig. 14. However, plantain flour and the soil samples have values below 9.00 mg/kg, while scent leaf and waterleaf have the highest values (18.50 mg/kg to 24.50 mg/kg). Co concentrations are presented in Fig 15. As was earlier noted, out of the nine metals investigated, this metal has the highest concentration (412.50mg/kg to 775.00mg/kg). An average concentration value of 742.00mg/kg was recorded for all the vegetables studied. This is a source of concern as high levels of Co are associated with problems of the organs such as the heart, liver and the kidneys.

The results of the elemental analysis of the samples from the EDS are presented in Figs. 16 – 21. These were compared with those obtained from AAS and none of the metals under study were detected except Fe in fluted pumpkin, waterleaf and the soil samples. The range (520 -1055 mg/kg) detected in the EDS is likely due the sensitivity of the EDS instrument. From the EDS analysis, lower concentrations (<500 mg/kg) of the metals under study were not detected. The EDS reported other important elements which were not included in this work. They are C, K, Ca, Mg, Al, Si, Fe, Mo and Ti. The nonmetals Cl, P, S and O were also recorded. This shows that in future studies, the EDS can be utilized to investigate and quantify some of these elements. The concentrations of Fe (520 – 1055 mg/kg) recorded for these samples are within the concentrations found for Co (422.50 – 775.00 mg/kg). The Co peak did not show on the EDS spectrum for the samples due to the presence of overlapping peak location it has with the Fe peak. This is in agreement with the results reported by Greene et al (2014). Most of the metals were detected using the 1st voltage (Kα) which is the energy level at the K shell. For Fe, this value is 6.3996 while for Co it is 6.9254. The Fe shows peaks caused by x-rays given off as electron return to the K electron shell (Kα) and another from the L shell (Lα).

5. Conclusion

The concentrations of most of the metals under study were highest when the rains were less frequent or gone (as in the dry spell period). This was the pattern observed. Mn and Cd were outstandingly high during the dry spell period while Fe, Zn, Pb, Cu, Ni, and Cr were highest during the periods the rains were less frequent. This is attributable to less moisture in the atmosphere causing the metals to accumulate. This is in agreement with results obtained by Akan et al., 2009.

The dry spell samples were recorded as having the highest concentrations of Mn, Cd, Ni and Fe. Scent leaf and fluted pumpkin recorded Mn and Cd as the highest, water leaf recorded Mn and Fe, while bitter leaf had high concentrations recorded for Mn, Fe, Cu, Zn and Cd .During the less frequent rains, Fe, Zn, Ni, Pb and Co were also found to be high in scent leaf, while Fe,Cu, Zn,Cr, Ni, and Co concentrations were high in fluted pumpkin. Pb, Cd and Co concentrations were high in water leaf during the less frequent rains also. At the peak of the rains only a few of the metals studied were present in high concentrations. These were Cr and Ni for bitter leaf and Fe, Cu, Zn and Cr for water leaf. However, the soil samples showed the highest concentrations for all the metals investigated except Cd, at the peak of the rains.

Plantain recorded the least values for all the metals. Though these values are low, they exceed the WHO acceptable limits in vegetables and crops and therefore pose health risks when consumed.

Fe is the only metal that has high concentration for the soil sample from the peak of rains sampling. All other metals concentrations were low. It is therefore probable that the high concentrations in the vegetables and low concentrations in the plantain flour would not be from the soil rather from atmospheric deposition which subsequently is transported into the leaves via the stomata. This phenomenon explains why waterleaf has the highest concentration of the nine metals studied. For the plantain fruit, the peels are removed to reveal the fruit which is either processed into flour, cooked, fried or roasted. This may explain the reason for the low concentrations of these metals recorded for plantain flour in comparison to the other samples. It has the least concentration for all the metals except for Cd which indicates that there is no uptake of these metals from the soil when compared with the

values from the soil samples. The EDS confirmed the high concentrations of Fe recorded in this study for fluted pumpkin, waterleaf and the soil samples.

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