DETERMINATION OF HEAVY METAL CONTENTS IN FLUTED PUMPKIN LEAVES (*TELFAIRIA OCCIDENTALIS*) ALONG ROADSIDES IN CALABAR, NIGERIA

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Abstract

Particle Induced X-ray Emission (PIXE) spectrometry was used for the analysis of elemental contents in the leaves of fluted pumpkin (Telfairia Occidentalis) grown along roadsides in Calabar for the purpose of ascertaining the levels of heavy metals. Seventeen elements, viz., Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr with concentration ranging from 1.2±3.3 (Zn) to 56562.8±118.8 ppm (K) were detected from ten different sample locations. Concentrations of these elements reveals that areas with the highest number of vehicles plying sample locations recorded highest levels of heavy metals compared to areas with low traffic density. Elements such as Mn/Cu (0.754), Cu/Zn (0.820), Al/Mn (0.658), Al/Cu (0.640), and Ti/Fe (0.653) had strong, positive, and significant correlation corroborating the results of the enrichment factor, cluster analysis, and vehicular count. This confirmed that the presence of heavy metals in fluted pumpkin leaves on roadsides in Calabar is associated with vehicular emission.

Keywords: Heavy metal, Fluted Pumpkin, Elemental Concentration, Roadside, Calabar

Introduction

Fluted pumpkin is a tropical vine plants. Its origin can be traced to the family of *Curcubitaceae* with botanical name known as *Telfairia Occidentalis* (Odiaka and Odiaka, 2011). It is cultivated across the low-land areas rich in mineral elements. Fluted pumpkin is found in abundance between 6 – 10 months in areas with high rainfall such as West Africa. During the dry season, the plant is watered frequently for the production of good quality leaves and fruits. The two main **varieties of *Telfairia Occidentalis* in** Nigeria **are male and female varieties. The** female plant produces big leaves than the male plant (Asiegbu, 1987). It is commonly known as ubong in Efik/Ibibio, mfang ubre in Oron, Ugu in Igbo, iroko or aporoko in Yoruba, umee in Urhobo, and umeke in Edo (Kayode and Kayode, 2011).

It is widely cultivated because of its palatable and nutritious leaves which are used mainly as vegetables. Because of its affordability, availability and the mineral elements it contains, it has become a popular vegetable consumed more than other vegetables in the population diet (Nwosu et al., 2012). The leaves of this vegetables are used in the preparation of several delicacies in southern Nigeria; one of which is Edikang ikong soup, a popular delicacy of Efik/Ibibio in Cross River and Akwa Ibom States in Nigeria (Ikhajiagbe et al., 2013). The leaves is very nutritious and contains important mineral elements such as K, Ca, Mg, P, Fe etc. "It is a good source of vitamin A, vitamin C, and vitamin E which is required for the production of blood, adequate supply of oxygen, building and maintenance of bones, cartilage and connecting tissues, and the maintenance of an healthier skin which in turn slows down ageing (Ikpe, 2013; Effiong, 2009). Furthermore, the consumption of vegetable can help prevent heart diseases, stroke, and high blood pressure. It also lowers the chances of cardiovascular diseases. According to FAO, the recommended daily intake is 200g. It is believed that this vegetable will provide balance diet to consumers who eat food containing little or no meat and other animal protein (Effiong, 2009). Many findings on the effect of consuming unsaved foodstuff were carried out. Vegetables grown along roadsides are contaminated by heavy metals as a result of vehicular emissions, re-suspension of road dust, use of oil or fossil fuel for heating, irrigation with contaminated water, addition of fertilizers and metal-based pesticides, industrial emissions, and other anthropogenic activities (Khan, 2009; Mansour, 2014; Inioti, 2012).

Zhang et al. (2012) opines that “the device of heavy metals emission from vehicles consists of exhaust fumes, fuel and engine oil consumption, tyre and brake wear, and road abrasion. Engine oil consumption is responsible for the largest emission for Cd; tyre wear contributes to the most important emission for Zn, and brake wear is the most important source of emissions for Cu and Pb”. Heavy metals constitute an important contaminant of fluted pumpkin, because this pumpkin absorbs metals from the polluted soil and also from the deposit on vegetative part of the plant that comes in contact with polluted air. Fluted pumpkin cultivated on these types of environment can contain high level of heavy metals which have side effect on consumers (Khan, 2009; Mansour, 2014; Inioti, 2012). Roadside pumpkins are also exposed to disease causing micro-organisms, resulting in epidemics of disease such as cholera, dysentery, typhoid fever, etc. Therefore, this study is aimed at determining heavy metal concentrations in fluted pumpkin leaves grown along traffic routes in Calabar, Nigeria.

Methods

Study Area

The study area is located between latitude $8^{0}15^{1}$E to $8^{0}25^{1}$E and longitude $4^{0}50^{1}$N to $5^{0}05^{1}$N as shown in Figure 1. Ten roadsides and a farmland in Eastern highway lane were chosen for this study within Calabar metropolis in Cross River State, Nigeria. Sample locations 1 to 11in the figure represents Marian road, Ikot Ishie road, Odukpani road, Atimbo road, New Airport road, Eneobong street, Atakpa street, Edet Essien street, Home farm estate, Satellite town, and Eastern highway lane respectively. However, the first five locations are areas of high traffic density, while the other five are of low traffic density. In this location, eleven stands, each of fluted pumpkin seed were planted 1.5 m away from the end of the road. Out of these, five were planted in a high traffic density location, another five were planted in a low traffic density, and one was planted in a farm that is 3 km away from the road to act as control experiment. The plants were watered, well-monitored, and were left to grow for three months until the leaves were matured enough for collection.

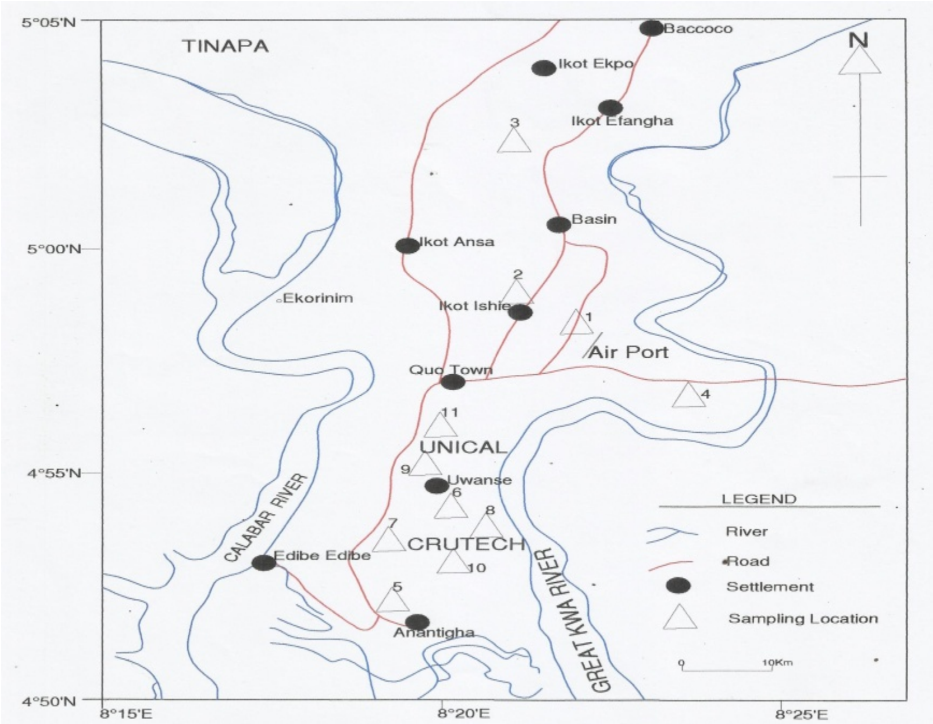

Figure 1: Map of sample locations in Calabar

Sampling of Pumpkin Leaves

The samples of vegetable leaves were collected from eleven locations using hand gloves. After each location, the gloves were properly washed and dried to avoid contaminating the samples before they were used in other locations. They were labeled and stored in a polythene bag accordingly to avoid being mixed up.

Washing and Drying

The leaves of the sampled vegetable were properly separated to remove rotten vegetables, and were washed with water to remove dirt. It was dried in an oven at the Chemistry Department, University of Calabar, at a temperature of 40^0C for two days. However, this was done before they were packaged and properly labeled, and before being taken to the Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife for further preparation before analysis.

Grinding and Pelletizing of Samples

Each of the dried samples of pumpkin leaf was ground and pulverized to fine powder by SPEX mixer/mill, model S100 agate mortar. After each sample was ground into powder, the mortar and piston were properly cleaned with acetone solution before the procedure was repeated for the next sample. This was done to avoid cross-sample contaminations. Each of the samples was then mixed with 20% ultra-fine carbon in the mixer to reduce charging effects in the sample (Chaudnuri and Crawford, 1981). Furthermore, they were pressed into pellets of 13 mm diameter using a pelletizing machine.

Calibration of Detector

The detector was calibrated before the analysis to ensure accuracy and quality assurance. This was done by analyzing a standard reference material from the National Institute of Standards and Technology (NIST) 1515 Apple Leaves. The values obtained from this analysis were then compared with the certified values, and were found to be in good agreements.

Analysis of Samples using PIXE Technique

A Pelletron accelerator, model 5SDH, installed at the Centre for Energy Research and Development (CERD) Obafemi Awolowo University, Ile-Ife, Nigeria was used for the analysis. Eleven samples of vegetable leaves were loaded in a sample ladder, and were placed in an irradiation chamber. A proton of 4 mm wide with 2.5 MeV, and a charge of 0.5 μC and a beam current which varies between 5.3 –7.5 nA were directed on the sample. Canberra Si(Li) detector was used in order to obtain a suitably high sensitivity characteristic X-ray emitted by each sample for each element.

Results and Discussion

The results of the analysis of NIST standard reference material - APL1515C, Apple leaves and control sample are presented in Table 1. The results were in good agreement with the certified values thereby leading to confidence in the reliability of the data obtained in this study.

The results of the sample locations and concentrations of all the elements in fluted pumpkin leaves are presented in Table 2. Nineteen elements Viz., Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and their respective concentrations (in ppm) were determined. It was observed that K with the range of 20066.4 ±78.3 to 56562.8±118.8 ppm had the highest concentrations, while Zn with the range of 1.2±3.3 to 41.5±4.5 had the least concentrations.

Table 1: NIST standard reference material- APL1515C and control sample

Elements	Concentration (ppm) ± Error	Certified value (ppm)	Concentration (ppm) ± Error (Control)
Mg	[b]	[b]	1225.2 ±629.1
Al	285.8 ±80.9	286	826.9 ±108.3
Si	368.3 ±67.1	[b]	1448.3 ±97.0
P	[b]	[b]	13957.4 ±1480.9
S	1807.1 ±108.9	1,800	5135.2 ±186.9
Cl	571.2 ±56.8	579	113.9±98.4
K	16147.1 ±51.7	16,100	41737.6±960
Ca	15215.0 ±65.4	15,260	16378.1 ±91.7
Ti	[b]	[b]	71.1 ±7.7
Mn	54.4 ±6.1	54	9.3 ±3.6
Fe	85.5 ±4.0	83	597.7 ±7.1
Ni	[b]	[b]	19.2 ±2.6
Cu	[b]	[b]	2.9±1.9
Zn	12.4 ±5.3	12.5	10.0 ±2.9
Rb	[b]	[b]	13.7 ±6.1
Sc	170.7 ±64.0	[b]	[b]

Table 2: Total elemental concentrations (ppm) in pumpkin leaves and vehicular movement

Locations	Mg	Al	Si	P	S
Atimbo	2205.6 ±540.2	1115.9 ±85.8	3511.9±104.7	11532.4±1592.6	6871.1 ±191.0
Atakpa	2757.2 ±582.3	578.8 ±91.1	3244.7 ±101.6	24799.6±1505.3	8598.5 ±220.9
Edet Essien	[b]	616.4 ±94.9	1723.9 ±86.4	34650.8±1261.3	6409.8 ±216.7
Eneobong	1840.7 ±510.1	595.1 ±86.2	1461.3 ±80.2	23918.9±1188.8	6126.7 ±195.4
Home farm	[b]	515.8 ±89.8	950.0 ±73.9	19490.0 ±1114.8	5736.0 ±186.4
Ikot Ishie	[b]	508.2 ±90.1	1965.2 ±86.7	45272.6±1421.6	5817.6 ±228.6
Marian	2225.5 ±500.1	683.4 ±86.6	1757.2 ±86.8	21452.5±1188.5	5642.7 ±196.4
New Airport	2580.1 ±533.6	644.6 ±83.3	2190.7 ±87.2	23289.2±1336.8	7325.0 ±205.8
Odukpani	[b]	600.4 ±105.9	2127.3 ±95.5	20340.3±1450.5	6710.9 ±240.3
Satellites T.	[b]	472.3 ±77.7	1601.7 ±90.3	[b]	3714.8 ±175.3

Locations	Cl	K	Ca	Ti	Cr	Mn
Atimbo	403.7±125.5	20512.9±75.9	17479.7±61.2	148.2±7.2	10.4±3.2	58.6 ±3.8
Atakpa	322.1±138.7	33630.2 ±94.2	14948.6±68.8	203.2±6.9	17.0±3.1	20.0 ±2.9
Edet Essien	269.0 ±117.8	43996.8±105.6	5921.2 ±69.3	64.6 ±6.2	16.9 ±2.8	8.7 ±2.6
Eneobong	195.3 ±115.8	37475.2 ±97.4	5109.7 ±62.9	115.7 ±6.4	21.0±2.9	14.4 ±2.6
Home farm	192.3 ±105.7	42141.7 ±101.1	5683.9 ±74.5	113.0±6.7	8.5 ±2.9	11.2 ±2.7
Ikot Ishie	369.9±140.6	43153.6 ±107.9	11121.0±72.3	123.1±6.1	[b]	31.3 ±2.9
Marian	193.6 ±123.2	37475.5 ±97.4	3871.8 ±60.8	175.9 ±7.2	[b]	31.6 ±3.5
New Airport	384.3±131.7	30326.4 ±90.9	12192.0±63.4	130.6 ±6.7	[b]	24.0 ±2.8
Odukpani	227.9 ±141.8	56562.8±118.8	9229.0 ±84.9	75.7 ±5.9	[b]	24.8 ±2.7
Satellites T.	234.6±134.9	20066.4 ±78.3	20511.3±65.6	74.3 ±7.4	19.6 ±3.8	40.0 ±3.8

	Fe	Ni	Cu	Zn	Rb	Sr	Vehicular

Locations							counts
Atimbo	450.3±6.1	[b]	16.2 ±1.2	9.2±2.3	[b]	14.1±5.6	30254
Atakpa	559.7±6.6	[b]	4.6 ±1.5	23.2±3.2	16.8±4.7	26.4 ±6.4	12706
Edet Essien	365.9±5.3	[b]	4.3 ±1.5	20.2±2.5	25.2 ±5.3	[b]	4767
Eneobong	352.1±5.2	[b]	4.2 ±1.2	6.0±2.8	31.6 ±5.2	[b]	14858
Home farm	315.2±5.0	3.5±1.1	6.2 ±1.5	3.1±3.3	11.6±5.6	[b]	4185
Ikot Ishie	390.5±5.4	4.4±1.2	7.2 ±1.4	5.8±3.2	20.8 ±4.3	5.1 ±5.8	35842
Marian	403.4±5.8	4.6±2.1	13.2±12.3	41.5±4.5	23.2 ±6.9	[b]	88020
New Airport	440.3±5.8	[b]	9.3 ±1.3	7.9±2.9	11.6±4.2	15.0 ±4.9	59259
Odukpani	312.1±4.9	5.1±1.2	15.1 ±1.5	1.2±3.3	20.2 ±5.3	10.0 ±5.5	71203
Satellites T.	445.2±6.3	5.8±1.4	7.9 ±1.7	12.1±2.1	[b]	18.3±6.3	9636

[b] not detected.

Heavy metals such as Al, Cr, Mn, Fe, Cu and Zn with concentrations ranging from (472.3 – 1115.9) ppm; (8.5 – 21.0) ppm; (8.7 – 58.6) ppm; (312.1 – 559.7) ppm; (4.2 – 7.9) ppm and (12.1 – 41.2) ppm present in this study were reported by Zhang et al. (2012) as elements associated with vehicular emissions. Also, the concentrations found in this study were in good agreement with the study by Inioti et al (2012) and the investigation of Ikhajiagbe et al. (2013). Nevertheless, the concentrations and number of elements found in their studies were less than the present study. Further investigation by Echem and Kabari (2013) reveal that Fe, Pb, Cr, and Zn were at high level. This was same for Fe and Zn in the present study although Pb was not detected in the present study. It should be noted that Ikhajiagbe et al. (2013), Inioti et al. (2012), and Echem and Kabari (2013) employs Atomic Absorption Spectroscopy. AAS is less sensitive than PIXE. Besides, it is destructive, non-multi-elemental in nature, and can analyze metals only, provided the cathode lamp for the analyte element is available.

In studying the rate of traffic density along sample locations, counting statistics was carried out on the number of vehicles plying each sample location per week. This was done in order to study if there is any relationship between the number of vehicles plying these routes and their influence on the pumpkin by the roadsides. As shown in Table 2, heavy metal concentrations in this study were found to be higher in areas of high traffic density (major roads) than low traffic density areas (street).

The results of enrichment factor (EF) are presented in Table 3 for some of the elements detected in the leaves in each sample location. Significant enrichment were obtained in the leaves for Mn (8.4, 5.1, 5.0, 5.1 and 5.8) at Atimbo road, Ikot Ishie road, Marian road, Odukpani road, and Satellite town; Cu (7.4, 6.7 and 10.0) at Atimbo road, Marian road and Odukpani Road; Zn (5.2, 5.5, 6.1 and 7.9) at Atimbo road, Ikot Ishie road, Marian road and Odukpani road respectively. The significant enrichment obtained for this study are mostly found in areas with high traffic density and should be the basis of worry because of their plausible unfavorable consequences.

Table 3: Enrichment factor of elements in fluted pumpkin leaves

Sample Locations	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Mn	Ni	Cu	Zn	Rb	Sr
Atimbo	2.4	3.5	3.2	1.1	1.8	4.7	0.7	1.4	2.8	8.4	[b]	7.4	5.2	[b]	2.0
Eneobong	2.6	1.2	1.7	2.9	2.0	4.1	1.3	0.1	2.8	2.6	[b]	2.5	2.7	2.8	[b]
Edet E.	[b]	1.2	1.9	4.1	2.0	3.9	1.7	0.6	1.5	1.5	[b]	2.4	2.4	3.0	[b]
Home F.	[b]	1.1	0.2	0.1	2.1	3.2	1.9	0.7	2.5	2.3	0.3	1.4	2.5	2.2	[b]
Ikot Ishie	[b]	1.2	2.1	4.9	1.7	4.9	1.6	0.3	2.7	5.1	0.4	3.8	5.5	2.3	4.2
Marian	2.7	1.2	1.8	2.4	1.6	2.5	1.3	0.1	3.7	5.0	0.4	6.7	6.1	2.5	[b]
New A.	2.9	1.1	2.1	2.3	1.9	4.6	1.0	1.0	2.5	3.5	[b]	4.4	2.8	1.1	2.2
Atakpa	2.4	0.7	2.4	1.9	1.8	3.0	0.9	1.0	3.1	2.3	[b]	1.7	2.5	1.3	3.1
Odukpani	[b]	1.4	2.8	2.8	2.4	3.8	2.6	1.1	2.0	5.1	0.5	10.0	7.9	2.8	2.1
Satellite T.	[b]	0.8	1.5	[b]	1.6	2.8	0.6	1.7	1.4	5.8	0.4	3.7	1.6	[b]	2.7

[b] not detected

Cluster analysis of elements in the leaves of fluted pumpkin using analyzed elements as variables is presented in Figure 2. The x-axis of the dendrogram contains the similarity matrix rescaled distance cluster combined, while the y-axis contains listed number of clusters which corresponds to the analyzed elements. From the clustering analysis results, four groups showed up. The first and second groups are K/P and Ca/Cl/S respectively. These two groups are associated with the remains of plants and animals since the soil type around the sample locations is alluvia in nature. Thus, these mineral elements are eroded and deposited at the sample locations. The third group consists of Cu/Ti/Al/Fe/Mg/Si, while the last group consists of Cr/Rb/Mn/Zn/Sr/Cu/Ti/Al/Fe. These two groups are associated with traffic-related cases including exhaust fumes, wear and tear of tyres and engine parts, and corrosion of metal parts as many vehicles are left on the road unsystematically (Ogunsola et al., 1994).

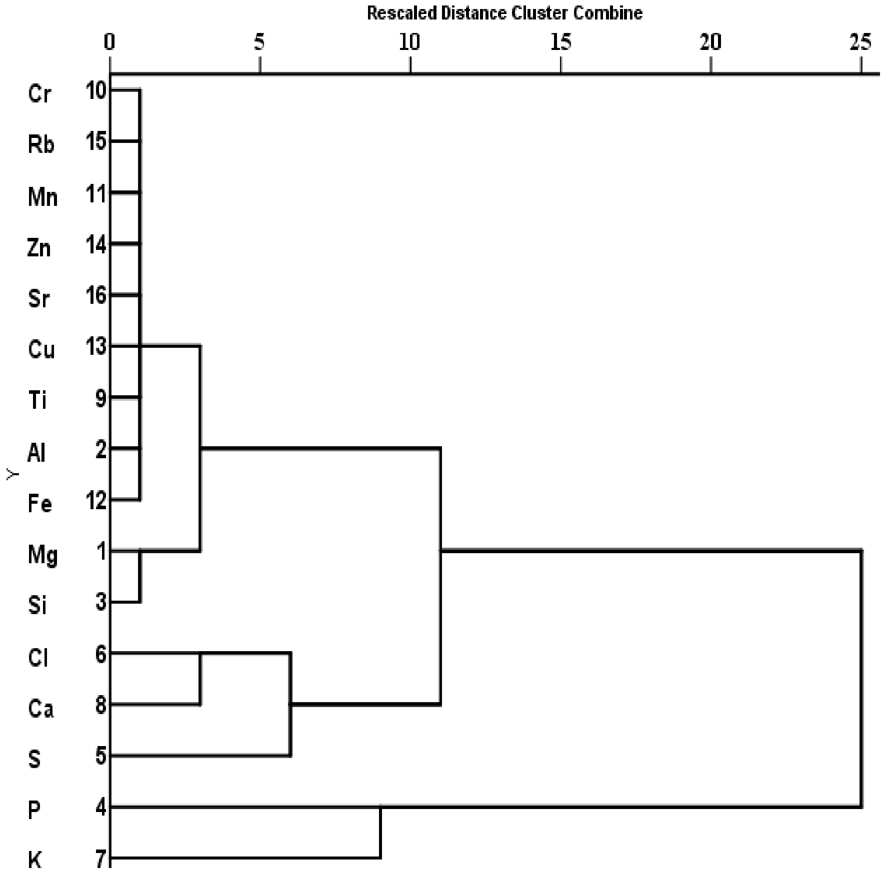

Figure 2: Cluster analysis of analyzed elements in fluted pumpkin leaves

The results of Pearson correlation matrices for the analyzed elements in the leaves of fluted pumpkin using their elemental concentration as variables are presented in Table 4. In order to determine which of the correlations were significant, multiple correlation coefficient *r* results, reveals that some of the elements were positively correlated, while others were negatively correlated. The correlation between Ti/Fe (0.653), Mn/Cu (0.754), Cu/Zn (0.820), Al/Mn (0.658), Al/Cu (0.640), Si/Fe (0.712), Mg/Fe (0.883), Ca/Mn (0.674), Ca/Fe (0.651), Mg/Cl (0.893), Ca/Cl (0.869), Al/Si (0.674), Si/S (0.650) and P/K (0.820), had strong, positive, and significant correlation. K/Ca, K/Fe and Cu/Sr with (0.694), (0.651) and (0.858) respectively had strong, negative, and significant correlation as this was in confirmation of the results of the enrichment factor and cluster analysis.

Table 4: Pearson correlation matrix of the analyzed elements in fluted pumpkin leaves

	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn	Rb	Sr
Mg	1.000															
Al	-.197	1.000														
Si	.539	.674*	1.000													
P	.244	-.571	-.221	1.000												
S	.822	.320	.650*	-.129	1.000											
Cl	.893*	-.041	.388	.080	-.224	1.000										
K	-.154	-.400	-.368	.820**	.176	-.561	1.000									
Ca	.559	.217	.573	-.167	-.050	.869**	-.694*	1.000								
Ti	.646	.306	.538	-.526	.546	.093	-.282	.050	1.000							
Cr	-.248	-.451	-.135	.626	-.186	.334	-.058	.071	-.214	1.000						
Mn	-.070	.658*	.576	-.296	-.181	.628	-.620	.674*	.203	-.201	1.000					
Fe	.883*	.208	.712*	-.360	.399	.604	-.651*	.651*	.653*	.207	.366	1.000				
Cu	-.073	.640*	.492	-.033	.034	.342	-.103	.267	.113	-.300	.754*	-034	1.000			
Zn	.063	.507	.476	.231	.282	.060	.232	-.059	.312	-.398	.494	.001	.820**	1.000		
Rb	-.912	.203	-.171	.184	-.319	-.279	.169	-.489	-.204	.877	-.101	-.225	-.057	.004	1.000	
Sr	.795	-.403	.086	.067	.122	.196	-.122	.127	.581	.532	-.285	.616	-.858*	-.375	.180	1.000

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Conclusion

Particle Induced X-ray emission (PIXE) spectrometry was employed in the analysis of heavy metal contents in pumpkin leaves grown along roadsides in Calabar metropolis in order to ascertain their elemental composition. The results of the analysis reveal that the samples were rich in mineral elements. Seventeen elements: Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr were detected in ten different sample locations. Results of counting statistics of number of vehicles plying sample locations corroborate with the enrichment factor results, cross plot results, and correlation matrices among the elements. Hence, heavy metals in this study were found to be associated with vehicular emissions.

Ogunsola et al. (1994) opines that the consumption of vegetables cultivated along roadsides will in the long run endanger consumer's health since the ingested heavy metals bioaccumulate in the human body. Hence, there is need for all relevant government and non-government agencies to bring this to the knowledge of the general public.

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