ASSESSMENT OF TRACE METALS COMPOSITION OF VEGETABLE *AMARANTHUS SPINOSUS* (LINN SPECIES) GROWN ALONG URBAN MOTORWAYS USING TXRF TECHNIQUE

O.K. OWOADE, F.S. OLISE+ and H.B. OLANIYI
Department of Physics, Obafemi Awolowo University, Ile-Ife, Nigeria.

(*Submitted*: 18 February 2004, *Accepted*: 15 June 2004)

Abstract

Vegetables of *Amaranthus Spinosus Linn* species, commonly grown along urban motorways, were sampled in three different locations along motorways in Ile Ife, Nigeria. The washed and unwashed leaves, stems and roots portions of the vegetable samples as well as the soil samples from each site were separated for independent analysis. All samples were digested using analytical grade acids and subsequently analysed using Total Reflection X-ray Fluorescence (TXRF) technique. Elements identified mainly in the samples are K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Rb, Sr and Pb. Enrichment factors and soil-to-plant transfer ratios (TR_{s-p}) of the metals for each vegetable sample were determined. The enrichment factors reveal that anthropogenic contributions increased the levels of some elements: Cr, Mn, Br, Sr and Pb, in the vegetable samples.

Keywords: TXRF, *Amaranthus Spinosus Linn*, soil-to-plant transfer ratio, toxicity, enrichment factor

1. Introduction

Macronutrients and micronutrients required for plant growth are derived from the soil. These macronutrients include Ca, K, Mg and Fe. The micronutrients which are also known as the minor or trace elements include Mn, Zn, and Cu (Delaune *et al*., 1986). Nutritional elements widely required by human body structure and functions are derived essentially from the plants consumed as food. *Amaranthus Spinosus Linn* vegetable is widely consumed in the western part of Nigeria because of its nutrients composition, cheapness and availability. These nutritional elements are Ca, K, Fe, Cu, Mn, and Zn. Calcium and K occur in the greatest quantity in all parts of the plant. Magnesium and Ca play important roles in the glycolytic activities in cells (Chu-Fang, 1996). Magnesium occurs in teeth and bones and acts as a cofactor to certain enzymes. Iron is essential to haemoglobin formation and is part of cytochrome molecule (Hay, 1984). Of the essential elements, Cu, Mn and Zn are required in minute traces only (Hay, 1984). Copper deficiency leads to anaemia since Fe cannot be used without it. Manganese is an activator of enzymes and Zn is associated with proper growth of hair (Chu-Fang, 1996). It is also very important to mention that some elements such as As, Br and Pb are known to be toxic to human bio-system if they exceed the threshold limit (Baker, 1983). Although it has been discovered that high proportion of ingested toxic elements are excreted, the populace still face serious health risk from vegetable poisoning as large portions of the consumed vegetables are grown on contaminated plots along motorway within the city (Loppi *et al*., 1998).

Naturally, only very small concentrations of these elements, particularly, Pb are present in soils and plants, but as a result of anthropogenic activities the concentrations of these elements in plant increase (Chu-Fang, 1996). Swaine (1955) estimated the average content of lead in the Earth's crust to be 16 ppm and gave a range of total lead in agricultural soils of 2 to 200 ppm. Connor (1961) found a range of 16 to 710 ppm lead in some surface soils from New Jersey, Pennsylvania, and Maryland.

Obviously when the soil is impacted, the plant will also be impacted but the level of impaction of the plant from the soil depends on the soluble portion of the pollutants. It has been observed that the accumulation of heavy metals in plants depends upon many factors. These are the availability of the elements in soluble form to the plants, the characteristics of the plants- species, age, state of health, type of reproduction, etc; and other such parameters as temperature, available moisture, substratum characteristics, etc. (Conti and Cecchetti, 2001). Plant uptake of pollutant from soil will be better related to soluble than to total pollutant in soils. Concentrations of the pollutants in plants also depend on the part of the plant being analyzed.

Studies made of transplanted *Evernia prunastri* highlighted the fact that the capacity for Pb accumulation after transplanting. It was found that the relationship between the concentration after the transplanting of *Evernia prunastri* and the initial concentration value is 10.2 in France, 3.7 for Germany and 4.4 for the city of Rome, Italy (Bartoli *et al*., 1994; Moriarty, 1999). In Italy, different biomonitoring studies carried out using lichens have shown that Pb is still very widespread in spite of the introduction of lead-free petrol. This indicates that high levels of this metal are still released (and/or re-suspended) by vehicle traffic (Cardarelli *et al*., 1993; Conti, 1996; Deruelle, 1996). Vehicular traffic seems to be the main source of atmospheric Cr, Cu, and Pb in the central Italian sites (Monaci *et al*., 1997). Some elements – Cd, Cu, Cr, Ni, Pb and Zn detected in the analysis of roadside

+ *corresponding author* (email: felix_rsh@yahoo.co.uk)

soils, vegetation and crops were found to have elevated concentrations attributable to traffic pollution (Ndiokwere, 1984). It has been reported that a considerable fraction of Pb particles are deposited on the soil and crops within a few hundred feet of the highways and that contact between Pb and the crops occurs both above and below ground. It was also reported that barley plants grown on soils treated with up to 800 ppm lead indicated less than 3 ppm in leaves but almost 800 ppm in the roots after analysis. (Schuck and Locke, 1970). They also reported as high as 890 ppm of lead in the roots of lemon cuttings grown in solution culture, whereas less than 3 ppm was found in the leaves. This means that automotive lead particulates exists mostly as a simple topical coating on vegetation and that the highest Pb concentrations are associated with those portions of the plant which have the greatest surface to volume ratio, e.g., leaves.

In this work, the urban grown *Amaranthus Spinosus Linn* vegetable is considered because of its availability and frequency of consumption among the food-stuff make-up of most people in the area. The trace elements in washed and unwashed *Amaranthus Spinosus Linn* vegetables, soil and water (used for wetting the vegetables) samples are presented. Elemental concentrations of the vegetable, soil and water samples were compared to know the source of increased level of some elements in the vegetable samples.

2. Materials and Methods

2.1 Site Location

This work was carried out in Ile-Ife, southwestern Nigeria. The vegetable plots on swampy lands are along motorways at about 10 and 100 meters from the motorways. Three sampling sites (which are totally free from tree cover) were chosen with samples collected randomly at the sites. Sample 1 means the samples from Site 1, etc.

2.2 Sampling

(a) Vegetables

The vegetable samples were randomly uprooted within an area of about 10m x 10m at each of the selected sites. Each sample was regarded as a composite sample. In the laboratory, the vegetables were grouped into two with one part washed and the other unwashed. The samples in each of the two groups were separated into leaves, stems and roots. The washing was carried out using double distilled water and all samples were dried at 60°C for 48 hours. The dried vegetable materials were homogenized in a 5% nitric acid-conditioned mortar and pestle.

Digestion of the vegetable samples was carried out according to Ogner *et al.* (1991). Sub-samples of 200mg were weighed into a 125ml Teflon bomb and 4ml of a 3:1 mixture of ultra-pure HNO_3 and H_2O_2 added. The Teflon bombs were then heated in a microwave oven at 300W, 450W and 600W, for 7 min. at each level. The solution was then analyzed after cooling.

(b) Soils

Ten samples of soils of approximately 50g were taken at each site. All vegetation, coarse rock fragments and roots were removed manually. The samples were dried and passed through a 2-mm sieve to remove stones prior to digestion. Each soil sample was homogenized and grounded with mortar and pestle. It was then digested as described above.

(c) Water

Irrigation water samples were collected from each of the sampling sites. The appropriate sample container was prepared in advance for actual sample collection for the analytes of interest. Water samples were collected directly into the containers from the stream in accordance with Quality Assurance Project Plan (QAPP) and Field Safety Plan (FSP) (USEPA, 1992). Three sub-samples of 1.0 ml were prepared, after thorough shaking, from each of the water samples and immediately analysed for the heavy metals.

2.3 Sample Analysis

A 5μl aliquot of each of the samples, with Gallium as internal standard, was pipetted on to a quartz sample carrier and dried using an infrared lamp. The dried samples were analyzed using a total reflection X-ray fluorescence spectrometer. The samples were irradiated with X-rays from a Mo-secondary target source operated at 40 kV and 20 mA. Fluorescence X-rays from the irradiated sample was collected and sorted using an X-ray spectrometer consisting of a Si(Li) detector, GENIE2K inspector hardware and GENIE2K software running on a PC. Each sample was analysed thrice and the spectra obtained were quantified using the QXAS software package. The data sets presented are the averages of the three measurements in respect of the spectra analyses and the averages for the soil and vegetable samples from a particular site.

2.4 Statistical Analysis

The soil-to-plant transfer ratio (TR_{s-p}) and the enrichment factor (EF) can be used as means of inferring the relationship between the soil and the vegetables. The TR_{s-p}, defined as the ratio of the concentrations of element x in vegetable and soil samples, is given by:

$$TR_{s-p} = \frac{(C_x)_{veg}}{(C_x)_{soil}} \quad (1)$$

where C_x is the concentration of element *x*. The EF is the quotient of the ratio of the concentration of element *x* to the concentration of reference element *f* in the vegetable sample to the same ratio in the reference soil. It is expressed as:

$$EF = \left(C_x / C_f\right)_{veg} \Big/ \left(C_x / C_f\right)_{ref.soil} \quad (2)$$

where C_x and C_f are the concentrations of element *x* and reference element *f* in vegetable and reference soil samples.

Table 1: Average elemental concentration *(µg/g)* of unwashed and washed vegetable samples

Sampling Site 1 (10 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	45764.3	36601.6	45554.8	41031.2	32280.7	31224.5
Ca	10219.7	7483.0	10214.7	9857.4	16819.9	15362.9
Ti	160.7	92.5	84.3	37.0	141.1	73.0
Cr	35.0	14.0	74.8	37.4	118.8	17.8
Mn	93.9	36.7	30.5	29.6	91.6	81.1
Fe	2010.5	515.9	1085.5	366.3	1361.6	804.9
Cu	27.2	19.6	14.3	14.0	25.1	24.4
Zn	49.5	41.7	41.7	40.5	78.5	54.6
Br	10.4	6.7	25.3	24.7	20.9	17.0
Rb	98.0	77.6	35.9	33.3	57.9	41.8
Sr	124.6	94.8	115.6	105.5	135.7	128.0
Pb	95.7	31.6	270.9	112.5	138.7	113.5

Sampling Site 2 (100 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	20325.9	19016.9	46223.3	40005.9	38020.3	24194.8
Ca	7077.6	6502.2	10474.7	9853.3	15447.1	14586.4
Ti	92.9	47.2	35.9	16.9	55.6	41.2
Cr	71.5	60.7	72.5	55.6	104.1	29.2
Mn	36.4	28.1	21.4	15.8	54.3	42.8
Fe	1440.6	801.2	457.7	306.2	744.1	513.7
Cu	13.7	13.2	11.2	5.5	25.9	21.6
Zn	45.3	41.2	40.3	35.3	87.6	77.9
Br	4.5	3.2	7.2	7.1	12.1	6.3
Rb	31.0	28.8	72.7	62.2	71.1	38.7
Sr	67.0	66.2	106.9	99.2	87.8	70.4
Pb	138.8	126.2	78.6	58.1	50.7	48.3

Sampling Site 3 (10 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	39417.4	38228.3	38188.4	35765.1	26850.3	21021.3
Ca	9436.6	8830.6	10972.7	9601.6	21811.7	21717.6
Ti	195.4	87.6	44.3	25.7	120.6	37.0
Cr	43.9	40.5	35.4	28.4	28.0	14.0
Mn	43.7	38.9	14.8	11.4	46.6	36.2
Fe	2493.1	1298.9	434.6	374.2	1068.3	592.0
Cu	17.6	15.7	13.9	6.6	25.5	23.5
Zn	71.8	68.8	64.4	59.3	114.9	94.1
Br	5.2	4.3	4.4	3.4	5.0	4.3
Rb	85.5	81.5	68.2	57.2	84.0	72.1
Sr	84.3	82.9	91.2	86.8	122.0	116.5
Pb	49.6	45.7	39.2	33.1	54.5	51.5

Table 2: Elemental enrichment factor of the vegetable samples

Sampling Site 1					
	Cr	Mn	Br	Sr	Pb
Leaf	13.7	6.6	3517.8	26.6	622.2
Stem	56.6	4.7	10107.8	43.2	2928.7
Root	8.5	2.3	1089.6	15.5	136.6
Sampling Site 2					
	Cr	Mn	Br	Sr	Pb
Leaf	39.6	6.1	2297.1	25.9	468.7
Stem	184.4	5.5	6322.7	88.8	1374.7
Root	72.1	3.5	1014.4	21.2	1070.6
Sampling Site 3					
	Cr	Mn	Br	Sr	Pb
Leaf	21.2	5.8	1765.2	47.6	556.5
Stem	61.9	2.6	2008.2	51.1	514.7
Root	25.9	2.6	748.1	14.3	208.7

Table 3: Elemental enrichment factor of soil samples

	Cr	Mn	Sr	Pb
Site 1	-	2.1	13.4	46.2
Site 2	2.8	1.4	12.9	37.9
Site 3	2.8	1.2	14.2	182.5

Table 4: Average elemental concentration (*µg/g*) of soil samples

	Sample-1	Sample-2	Sample-3
K	3517.0	4654.4	5774.9
Ca	8414.4	6446.9	11557.2
Ti	5600.1	3411.9	4106.5
Cr	0.0	409.0	329.0
Mn	2122.4	1706.3	1132.2
Fe	46436.9	56271.4	44614.4
Ni	37.3	38.3	0.0
Cu	50.1	30.5	29.3
Zn	281.6	213.9	390.2
Rb	44.3	48.3	83.5
Sr	139.9	184.7	161.9
Ba	2808.6	1296.1	2542.6
Pb	564.2	560.9	2137.3

Table 5: Average elemental concentration (µg/g) of water samples

	Sample-1	Sample-2	Sample-3
K	128.5	600.5	329.9
Ca	666.6	1171.1	541.7
Ti	12.8	19.9	8.2
Mn	48.4	18.4	16.3
Fe	89.8	109.6	101.2
Ni	6.5	6.4	0.0
Cu	8.2	4.9	1.8
Zn	14.7	10.5	12.8
In	300.4	525.9	209.4
Ba	36.9	62.0	24.0

Table 6: Soil-to-plant transfer ratio for washed parts of *Amaranthus* vegetable

Sample-1	Ti	Cr	Mn	Sr	Pb
Leaf	0.01	-	0.04	0.92	0.20
Stem	0.01	-	0.01	0.75	0.48
Root	0.02	-	0.02	0.68	0.06
Sample-2					
Leaf	0.01	0.07	0.03	0.38	0.09
Stem	0.01	0.14	0.01	0.54	0.10
Root	0.01	0.15	0.02	0.36	0.22
Sample-3					
Leaf	0.01	0.04	0.03	0.72	0.02
Stem	0.01	0.09	0.01	0.54	0.02
Root	0.02	0.12	0.03	0.51	0.02

It is agreed in principle that if the value of TR_{s-p} is greater than 0.9, the vegetables possessed high uptake ability for the element from sources other than soil or indicate that the soil is not the only source of contribution of the element (Jenne, 1968). It is to be noted, however, that large proportions of the pollutants are washed to the soil from the atmosphere and get leached to various levels of the soil profile.

3. Results and Discussion

In Table 1, the average elemental concentrations of twelve major, minor and trace elements in *Amaranthus Spinosus Linn* (green variant) for both washed and unwashed samples from each of the sample sites are reported. For lithophilic elements like Ti, Mn and Fe, elemental concentrations were higher for the unwashed samples than the washed samples. The difference in elemental concentrations between the washed and the unwashed samples are however minimal for Cu, Zn, Br, Rb Sr and Pb. This calls for serious concern in view of the immediate toxic nature of these heavy metals at high concentrations, apart from the long time accumulation effects. It was observed that Br, Sr and Pb were highly enriched and Cr and Mn moderately enriched, taking EF > 5 as indication of possible anthropogenic influence (Tables 2 and 3). The vegetable plots are strongly impacted from wind and automobiles related re-entrained particulates that settle soon after re-entrainment as a result of their closeness to highways since. Plots far from highways or totally outside urban centers would be less impacted especially from automotive related sources. It is interesting to note from Tables 4 and 5 that Ba was detected in soil samples and In and Ba in water samples but the two elements were not detected in the vegetable samples. It could therefore be stated that the uptake level for these elements is low. The soil-to-plant transfer ratio, TR_{s-p}, (Table 6) showed that the elements Ti, Cr, Mn and Sr were majorly taken up from the soil. The observed increased levels of Cu, Zn, Br and Pb detected in the leaves as compared to the stem and root parts of the vegetable samples could be attributed to strong contributions from surface contaminations from anthropogenic sources especially road traffic. This is because of the closeness of the sampling sites to the motorways and the established fact of the said elements being source finger-print elements for road traffic (Monaci *et al.*, 1997).

4. Conclusion

This study has shown that contamination of vegetables grown within urban centers could be significant. Though research has shown that high proportions of ingested toxic elements are excreted, the populace still face serious health risks from food poisoning in respect of heavy metals like Pb, Cu, Zn and Cr. This is because of the almost daily consumption of this food item leading to accumulation of such toxic metals over time. The cultivation of such food items in urban centers should be discouraged in view of its contamination from anthropogenic sources. It has been observed that thorough washing removes a lot of the lithophilic elemental contaminants so that clean cooking habits at homes should be emphasized.

REFERENCES

Baker, D.A., 1983. Uptake of cations and their transport within the plants. In: Robb, D.A., Pierpoint, W.S. (Eds.), Metals and micronutrients: uptake and utilization by plants. Academic Press, London, pp.3-9.

Bartoli, A., Cardarelli, E., Achilli, M., Campanella, L., Massari, G., 1994. Biomonitoraggio dell'aria di Roma: accumulo di metallic pesanti in trapianti di lichens. Allionia (Turin), 35, 69-85.

Cardarelli, E., Achilli, M., Campanella, C., Bartoli, A., 1993. Monitoraggio dell'inquinamentoda metallic pesanti mediante l'uso di licheni nella citta di Roma. Inquinamento, 6, 56-63.

Chu-Fang, W., 1996. Essential and toxic trace elements in the Chinese medicine. Journal of Radio. Nuc. Chemistry, 211, 2, 333-347.

Connor, J., 1961. Lead in Soils and Plants: Its relationship to traffic Volume and Proximity to Highways. Ph.D. Thesis, Rutgers University. 117pp.

Conti, M.E., 1996. The pollution of the Adriatic sea: Scientific knowledge and policy actions. Int. J. Environment and Pollution, 6, 2/3, 113-130.

Conti, M. E., and Cecchetti G., 2001. Biological monitoring: lichens as bioindicator of air pollution assessment – A review. Environmental Pollution, 114, 471-492.

Delaune, R.D., Jones, G.L., Smith, C. J., 1986. Radionuclide concentration in Louisiana soils and sediments. Health Physics, 51, 2, 239-244.

Deruelle, S., 1996. The reliability of lichens as biomonitors of lead pollution. Ecologie (Brunoy), 27, 4, 285-290.

Hay, R. W., 1984. Bio-inorganic Chemistry. Ellis Horwood Ltd, Chichester. pp. 207-229.

Jenne, E.A., 1968. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentration in soils and water: the significant role of hydrous Mn and Fe oxides. Advances in Chemistry, Series 73, 337-387.

Loppi, S., Pacioni, G., Olivieri, N., Di Giacomo, 1998. Accumulation of trace metals in the lichen *Evernia prunastri* transplanted at biomonitoring sites in central Italy. Bryologist, 101, 3, 451-454.

Monaci, F., Bargagli, R. and Gasparo, D., 1997. Air pollution monitoring by lichens in a small medieval town of central Italy. Acta Botanica Neerlandica, 46 (4), 403-412.

Moriarty, F., 1999. Ecotoxicology: The study of pollutants in Ecosystems. Academic Press, London.

Ndiokwere, C. L., 1984. A study of heavy metal pollution from motor vehicle emissions and its effect on roadside soil, vegetation and crops in Nigeria. Environmental Pollution, B, 7, 35-42.

Ogner, G., Opem, M., Remedios, G., Sjorlie, B., 1991. The chemical analysis program of the Norwegian Forest Research Institute. ÅS, Norway. pp. 224-330.

Schuck, E.A. and Locke, J. K., 1970. Relationship of automotive lead particulates to certain consumer crops. Environmental Science and Technology, 4, 4, 324-330.

Swaine, D.J., 1955. The Trace Element Content of Soils, Commonwealth Bur. Soil Sci. Tech. Comm. No. 48, Hearld Printing Works, York (England). pp. 231-237.

United States Environmental Protection Agency (USEPA), 1992. Quality Assurance Project Plan (QAPP) and Field Safety Plan (FSP) on Ground Water Sampling Procedures.