ASSESSMENT OF THE CONCENTRATIONS OF SOME ANIONS IN SOILS AND SELECTED COMMON VEGETABLES GROWN AND CONSUMED IN DELTA STATE, NIGERIA

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ABSTRACT

The study determined the levels of some anions (NO_3^-, NO_2^-, SO_4^-, PO_4^- and SO_4^-) in some leafy vegetables common in Delta State, Nigeria. Three farms well spread across Delta State were taken as sampling sites. There are from Agbor, which is from the northern part of the state, Abraka, from the central and Ozoro from the south. Top soil samples as well as vegetable samples were collected from these farms. Vegetables selected were; Telfairia occidentalis, Talinium triangulare, Verononia amygdalina, Amaranthus hybridus and Ocimum gratissimum. Samples were collected for wet and dry seasons, representing the two seasons in Nigeria. The concentrations of the anions were determined using UV-Visible Spectrophotometry. Results of the concentrations of anions for all the anions showed that Ocimum grattissimum had the highest concentration and Amaranthus hybridus had the lowest concentration. The order is as follows; O. gratissimum > T. occidentalis > T. triangulare > V. amygdalina > A. hybridus. The transfer factor from soil to vegetable also followed the order. Statistical analysis (Students't-test) shows that there is no significant difference between the concentrations of the anions in the two seasons. ANOVA analysis also showed no significant difference in the levels of the anions from the three sites, which is an indication of similar agricultural practices. The concentrations of the anions are all below the World Health Organisation (WHO) tolerable limits. It therefore implies that these vegetables do not pose any health risk to humans as a result of these anions content.

Keywords: *Anions, nitrates, nitrites, phosphates, sulphates, transfer factor*

INTRODUCTION

The edible part of plants that are consumed wholly or parts either cooked or raw as part of salads, are known as vegetables. Vegetables are rich carbohydrates, vitamins and minerals (Fasuyi, 2006; Ihekoronye and Ngoddy, 1985). They constitute a very important part of human diet since most of the vitamins needed by man come from the vegetables. It is important to note that minerals and vitamins cannot be synthesized by man and so must come from plants (vegetables) sources. Vitamins and minerals are important for the general well being of humans. They are in fact indispensable. It is believed generally that when vegetables are adequately added to the diet of postpartum women, it aids in the contraction of the uterus (Achikanu *et. al.*, 2013). Nitrate is the naturally occurring form of Nitrogen. It is an important part of the nitrogen cycle. Nitrate itself is not toxic but the product, nitrite is very toxic.

Nitrate is rapidly converted to nitrite by bacteria occurring naturally on the tongue and intestine of human. Vegetables are the main sources of nitrate in humans (Dich *et al.*, 1996).

The nitrite is then absorbed into the blood stream. Nitrite reacts with the haemoglobin displacing the oxygen in the molecule to form methaemoglobin, thus depriving the organism of adequate oxygen (Knobecloth *et al.*, 2000; Mensinga *et al.*, 2003). Once the Methaemoglobin in humans reaches 10 % of normal Oxyhaemoglobin levels, clinical symptoms start showing (Santamaria, 2006). This condition which is potentially fetal is known as "Methaemoglobinaemia" or "blue baby syndrome" (Knobcloth *et al.*, 2000; Walker, 1990). It has been reported that this condition is more common with infants less than three months old (Santamaria, 2006). Older children and adults are less susceptible to this disease condition because of the induction of methaemoglobin reductase which occurs during the period of physiological post-weaning .This study is important to both the public as well as nutritionist since result obtained would act as a guide for choice of vegetable to consume. This study was aimed at determining the concentrations of nitrate, nitrite, phosphate and sulphate in some leafy vegetables grown and consumed and also soil supporting these vegetables in Delta State, Nigeria.

MATERIALS AND METHODS

Sampling sites and sampling

Vegetables sampled are Pumpkin leaf (*Telfairia occidentalis*); Water leaf (*Talinum triangulare*); Bitter leaf (*Verononia amygdalina*); Green leaf (*Amaranthus hybridus*); Scent leaf (*Ocimum gratissimum*). Three major farms spread across the state were chosen. The selected cities were Agbor $6^0 16^1 0^{11}$ N $6^0 9^1 0^{11}$ E (Delta North); Abraka $5^0 47^1 0^{11}$ N $6^0 6^1 0^{11}$ E (Delta Central) and Ozoro $5^0 32^1 18^{11}$ N $6^0 12^1 58^{11}$ E (Delta South).

Samples of the leaves of the vegetables and soils were collected over six months period (three dry and three wet seasons). These were packed and well labeled in paper bags and taken to the laboratory for analyses.

Determination of Some Anions

Sample Preparation: The vegetables were washed with tap water and severally with distilled water. They were then sliced into approximately equal shred to facilitate uniform and quick drying. The drying was carried out in an oven at 105° C for 24 hours. The dried samples were then ground mechanically and sieved to obtain < 2 mm fractions. One (1) g each of the samples was extracted in distilled water. This was done by putting the 1 g of the sample into a 100 ml bottle and 40 ml of distilled water added, shaken for 30 minutes and filtered into a 100 ml volumetric flask (Radojevic and Bashkin, 1999).

Preparation of soil samples.

Soil samples were digested with the 10 mL of the triacid mixture after the soils had been thoroughly dried in an oven at 105° C for 24 hours. 1 g of the dried, sieved soil was placed in a 50 mL Teflon beaker and digested with 10 mL of the triacid (HNO_3-$HClO_4$-HF) to close to dryness at between 80-90 °C on a hot plate. The digest was then filtered with a No. 42 Whatman filter paper into a 50 ml volumetric flask (Radojevic and Bashkin, 1999)

Determination of nitrate and nitrite in the vegetables:

These were carried out spectroscopically (Model 2000 spectrophotometer) at a wavelength of 543 nm and the result was obtained as Nitrate-Nitrogen and was converted to Nitrate by multiplying with a conversion factor of 4.4 (LaMotte, 2000).

Nitrite determination was carried with the same procedure as that for nitrate except that the reaction time was for five minutes as against ten minutes for nitrates. Result was obtained as Nitrite-Nitrogen which was then converted to Nitrite by multiplying with the conversion factor of 3.3 (LaMotte, 2000).

Determination of phosphate:

Phosphate was determined according to the procedure of Radojevic and Bashkin, 1999. Phosphate was then assessed using a Hach direct reading 2000 spectrophotometer.

Determination of sulphate:

Sulphate was evaluated according to the method of Radojevic and Bashkin, 1999. Sulphate concentration was determined spectrophotometrically using the Smart Spectrophotometer 2000.

Determination of Anions in soil:

Soil samples were air dried, homogenize and sieve through a 2 mm sieve. One (1) g of prepared soil sample was put in a Teflon beaker and was digested using 10 mL of HNO_3 – $HClO_4$ –HF on a hot plate at 80- 90° C until near to dryness. It was then filtered through a No. 42 Whatman filter paper into a 50 mL volumetric flask (Radojevic and Bashkin, 1999). The various anions were then determined spectrophotometrically as previously described as for the vegetables.

Transfer factor for anions from soil to vegetables:

Transfer factor was computed according to the method described by Harrison and Chirgawi, (1989)

TF = Pa ($\mu g g^{-1}$ dry wt.)/Sa ($\mu g g^{-1}$ dry wt.)

TF = transfer factor

Pa = Plant anions

Sa = Soil anions

Data analyses:

ANOVA analysis was used to determine the difference in the level of anions from the three sites, Student t- test was used to evaluate seasonal variations among the anions while Pearson Correlation was used to determine relationship among the anions. Transfer Factor was also used to evaluate the uptake efficiency of the various vegetables for the anions from soil. $P < 0.05$ was considered to be statistically significant. All statistical analyses were done using the SPSS software for windows.

RESULTS AND DISCUSSION

Tables 1 and 2 show the concentrations of the studied anions in vegetables in the dry and wet seasons. The mean concentrations of nitrate in the dry season ranged from 121.60±71.80 µg/g in *A. hybridus* to 266.70±160.61 µg/g in *O. gratissinum*. In the wet season, it followed the same pattern with mean range of 116.50±53.77 µg/g in *A. Hybridus*, to 250.10± 22.07 µg/g in *O. gratissinum* (Table 4). The levels of anions in the soil samples in all the sites and in all seasons were higher than those in the vegetables. The levels of anions in the vegetables were low compared with other workers (Uwah *et al.*, 2009ab; Akan *et al.*, 2010). The fact that the level of anions was generally low as compared with literature is an indication of little or pollution or moderate usage of nitrogenous fertilizer. The levels of nitrate and nitrites in the vegetables studied were lower than those obtained by other workers (Uwah *et al.*, 2009ab, Akan *et al.*, 2010) while results were comparable with some results obtained by Uwah, *et al.*, 2007).

For both seasons, for all sites and for all the vegetables, it showed that the levels of nitrates were consistently higher than those of nitrites. This observation is consistent with results in literature (Aworh *et al.*, 1980, Hunt and Turner, 1994, Akan *et al.*, 2009, Uwah *et al.*, 2007, Uwah, *et al.*, 2009ab). This is expected since nitrates are usually formed before nitrites. Nitrates are in fact the precursor to nitrites. When the concentration of nitrate in vegetable is high, it is an indication of over application of nitrogen containing fertilizer on the farmland (Hardisson *et al.*, 1996). It is a known and acceptable fact the levels of nitrates and nitrite vary from place to place and from country to country due to the fact that agricultural practices also differ from country to country and place to place.

The Acceptable Dietary Intake (ADI) which is a measure of the amount of a specific substance that can be ingested daily throughout one's lifetime without any appreciable health risk may vary from place to place and from country to country because of differing consumption pattern and agricultural practices. For example, Radojevic and Bashkin (1999) gave the maximum permissible nitrate levels (mg/kg) of spinach as: USA, 3600; Russia, 2100; Czech Republic, 730 and the Netherlands as 400. Results obtained from this study showed that the levels of nitrate and nitrite were within the Daily Dietary Intake and so are safe for consumption.

The general trend observed in the anion concentration is as follows; *O. gratissinum* > *T. occidentalis* > *T. triangulare* > *V. amygdalina* > *A. hybridus*. Comparing the levels of the various anions from the various sites indicated that there was no significant difference. ANOVA revealed that there were no significant differences between the anions from the different sites as well as within the individual anions. This thus gives an indication that these places have similar agricultural practices as well as anthropogenic activities. Pearson correlation analysis revealed high positive correlation between nitrate and nitrite and between phosphate and sulphate. This indicates similar origin/source. It also showed that they are influenced by similar anthropogenic sources in the same way. Transfer factor which is used to assess the efficiency of vegetable specie to accumulate a given anion was calculated for the vegetable. This is to assess the bioavailability of anions to plant or to identify of the vegetables with high efficiency to bioaccumulation of the anions. The transfer factors were found to be generally low (< 1). The following order was found: *O. gratissinum* > *T. occidentalis* > *T. triangulare* > *V. amygdalina* > *A. hybridus*.

Table 1: Concentrations of anions in vegetables (μg/g dry weight) Dry Season.

Samples	Locations	Nitrates	Nitrites	Phosphates	Sulphates
A. hybridus (green leaf)	Agbor	204.30± 2.00	58.50± 4.20	125.00 ± 2.20	94.00 ± 1.50
	Abraka	75.20 ± 1.06	23.10 ± 1.40	134.20 ± 1.00	122.20 ± 0.00
	Ozoro	85.30 ± 0.10	46.30 ± 0.30	205.20 ± 0.00	135.40 ± 2.10
V. amygdalina (bitter leaf)	Agbor	225.80 ± 1.50	64.80 ± 1.00	134.10 ± 2.80	99.10 ± 1.70
	Abraka	119. 00 ± 2.10	34.30 ± 1.50	140.80 ± 0.30	120.80 ± 1.20
	Ozoro	96.10 ± 0.10	42.80 ± 0.50	232.30 ± 0.00	180.00 ± 1.10
T. triangulare (Water leaf)	Agbor	306.00 ± 3.00	64.80 ± 2.60	156.80 ± 0.30	163.80 ± 1.60
	Abraka	154.80 ± 0.00	36.30 ± 4.50	151.30 ± 1.00	126.30 ± 0.40
	Ozoro	105.30 ± 0.10	54.90± 0.50	246.00 ± 2.00	241.00 ± 0.10
T. occidentalis (Pumpkin leaf)	Agbor	327.10 ± 2.10	66.80 ± 1.50	166.20 ± 1.30	184.50 ± 0.60
	Abraka	187.30 ± 2.60	69.30 ± 2.10	162.00 ± 1.20	130.20 ± 0.80
	Ozoro	120.10 ± 0.30	57.30 ± 0.40	246.40 ± 0.10	248.50 ± 0.10
O. gratissimum (Scent leaf)	Agbor	443.70 ±3.10	97.70 ± 2.50	218.00 ± 0.30	194.50 ± 1.20
	Abraka	226.00 ± 4.20	72.50 ± 9.10	164.20 ± 0.20	143.50 ± 2.50
	Ozoro	130.30 ± 0.10	72.60 ± 0.30	257.60 ± 0.30	255.00 ± 1.10
Soil	Agbor	450.00 ± 2.50	150.00 ± 2.30	223.00 ± 1.20	216.00 ± 2.30
	Abraka	300.30 ± 1.40	126.50 ± 1.10	170.50 ± 1.40	155.00 ± 2.30
	Ozoro	150.30 ± 0.10	95.50 ± 1.10	300.10 ± 0.30	260.30 ± 1.00

Table 2: Concentrations of anions in vegetables (μg/g dry weight) Wet Season.

Samples	locations	Nitrates	Nitrites	Phosphates	Sulphates
A. hybridus (green leaf)	Agbor	177.50 ±3.60	55.00 ± 2.50	188.20 ±1.00	166.20 ± 0.40
	Abraka	76.00 ± 5.00	18.00 ± 2.10	147.00 ± 1.00	126.00 ± 1.20
	Ozoro	96.00 ± 5.00	17.95 ± 2.10	250.10 ± 1.10	192.10 ± 0.10
V. amygdalina (bitter leaf)	Agbor	223.00 ± 3.00	54.40 ± 3.00	125.00 ± 0.30	110.00 ± 3.20
	Abraka	142.80 ± 6.00	18.40 ± 2.70	152.50 ± 0.20	128.00 ± 1.00
	Ozoro	102.80 ± 6.00	18.40 ± 2.70	322.40 ± 0.20	230.10 ± 0.10
T. triangulare (Water leaf)	Agbor	255.00 ± 1.00	60.00 ± 1.50	138.20 ± 0.90	135.10 ± 2.90
	Abraka	174.10 ± 2.30	36.30 ± 4.50	154.80 ± 0.20	130.60 ± 0.20
	Ozoro	114.10 ± 2.30	36.80 ± 2.00	333.10 ± 1.20	243.00 ± 2.00
T. occidentalis (Pumpkin leaf)	Agbor	254.30 ± 2.00	60.65 ± 1.00	146.00 ± 2.00	136.50 ± 2.90
	Abraka	216.80 ± 0.50	66.50 ± 4.90	166.10 ± 1.00	152.40 ± 0.20
	Ozoro	200.80 ± 0.50	57.30 ± 0.40	325.20 ± 0.20	265.10 ± 1.00
O. gratissimum (Scent leaf)	Agbor	269.80 ± 2.00	90.80 ± 2.70	188.20 ± 1.00	166.20 ± 0.40
	Abraka	255.20 ± 2.50	86.60 ± 2.50	176.00 ± 1.20	152.50 ± 1.20
	Ozoro	225.20 ± 2.50	86.60 ± 2.50	343.80 ± 0.10	265.20 ± 0.00
Soil	Agbor	360.10 ± 1.80	132.80 ± 1.50	205.00 ± 1.30	196.00 ± 1.00
	Abraka	328.50 ± 2.60	175.5 0± 3.80	185.50 ± 1.30	164.90 ± 2.00
	Ozoro	230.50 ± 2.60	105.30 ± 3.80	380.50 ± 0.30	282.40 ± 0.10

Table 3: Total Anion concentrations in the various locations studied

	Nitrate		Nitrite		Phosphate		Suphate	
	Dry	Wet	Dry	Wet	dry	wet	dry	wet
Agbor	301.38 ± 94.97	235.92 ± 36.84	70.52 ± 15.51	64.17 ± 15.15	160.02 ± 36.43	157.12 ± 29.35	147.18 ± 47.56	142.8 ± 23.82
Range	204.30 - 443.7	177.5 - 269.8	58.5 - 97.7	54.4 - 90.8	125 - 218	125 - 188.2	94 - 194.5	110 -166.2
Abraka	152.46 ± 58.56	172.98 ± 68.92	47.1 ± 22.33	45.16 ± 30.43	150.5 ± 13.04	159.28 ± 11.65	128.6 ± 9.11	137.9 ± 13.38
Range	75.2 –226	76 - 255.2	23.1 - 72.5	18 - 86.6	134 2 - 164.2	147 - 176	120.8 - 143.5	126 - 152.5
Ozoro	107.42 ± 18.07	147.78 ± 60.51	54.78 ± 11.61	43.41 ± 29.05	237.5 ± 20.16	314.92 ± 37.17	211.98 ± 52.23	239.1 ± 30.26
Range	85.3 - 130.3	96 - 225.2	42.8 - 72.6	17.95 - 86.6	205.2 - 257.6	250.1 -343.8	135.4 - 255	192.1 - 265.2

Table 4: Concentration of Anions in the various Vegetables

	Nitrate		Nitrite		Phosphate		Sulphate	
	Dry	Wet	dry	Wet	dry	wet	dry	wet
A. hybridus	121.6 ± 71.80	116.50 ± 53.77	42.63 ± 17.98	30.32 ± 21.38	154.8 ± 43.89	195.10 ± 51.90	117.20 ± 21.15	161.43 ± 33.31
V. amygdalina	147.00 ± 69.23	156.20 ± 61.21	47.30 ± 15.74	30.40 ± 20.78	169.10 ± 54.86	199.97 ± 101.92	133.30 ± 41.87	156.03 ± 64.77
T. triangulare	188.4 ± 104.52	181.1 ± 70.70	52.00 ± 14.47	44.37 ± 13.54	184.70 ± 53.16	208.70 ± 108.05	177.00 ± 58.48	169.57 ± 63.63
T. occidentalis	211.50 ± 105.60	224.00 ± 27.46	64.47 ± 6.33	61.48 ± 4.66	191.50 ± 47.56	212.43 ± 98.17	187.70 ± 59.22	184.67 ± 70.11
O. gratissimum	266.70 ± 160.61	250.10 ± 22.07	80.93 ± 14.52	88.00 ± 2.42	213.30 ± 46.88	236.00 ± 93.56	197.67 ± 55.82	194.63 ± 61.50
soil	300.2 ± 149.85	306.37 ± 76.58	124.00 ± 27.34	137.87 ± 35.37	231.20 ± 65.19	257.00 ± 107.40	210.40 ± 52.87	214.43 ± 60.88

Table 5:Transfer Factor of Anions from soil to Vegetables

	Nitrate		Nitrite		Phosphate		Sulphate	
	Dry	wet	dry	Wet	Dry	wet	dry	wet
A. hybridus	0.41	0.38	0.34	0.22	0.67	0.76	0.56	0.75
V. amygdalina	0.49	0.51	0.38	0.22	0.73	0.78	0.63	0.73
T. triangulare	0.63	0.59	0.42	0.32	0.8	0.81	0.84	0.79
T. occidentalis	0.7	0.73	0.52	0.45	0.83	0.83	0.89	0.86
O. gratissimum	0.89	0.82	0.65	0.64	0.92	0.92	0.94	[illegible]

Table 6: Correlations Coefficient Among the Anions

		nitrates	nitrite	phosphate	sulphate
nitrates	Pearson Correlation	1	.	.	.
	Sig. (2-tailed)			.	.
	N	30			
nitrite	Pearson Correlation	.761**	1	.	.
	Sig. (2-tailed)	.000			.
	N	30	30		
phosphate	Pearson Correlation	-.206	.027	1	.
	Sig. (2-tailed)	.275	.886		.
	N	30	30	30	
sulphate	Pearson Correlation	-.074	.173	.918**	1
	Sig. (2-tailed)	.697	.361	.000	
	N	30	30	30	30

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

CONCLUSION

This study determined the levels of nitrate, nitrite, phosphate and sulphate in selected leafy vegetables grown and consumed in Delta State, Nigeria. The study revealed that the concentrations of these anions were within the maximum permissible limits (WHO). This therefore showed that the soil was not polluted from anion concentration and the there were no indication of excessive usage of nitrogenous fertilizers. The vegetables when consumed will not pose any health risk from anion concentration. It is however suggested that periodic monitoring be carried out to avoid anion accumulation vegetables in future.

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