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Measurement of Nitrate and Nitrite Contents in Soils and Some Leguminous Vegetables Cultivated in Maiduguri, Nigeria

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Abstract: Edible portions of some leguminous vegetable samples [beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*) and peas (*Pisum sativum*)] as well as the cultivated soil samples were analyzed for their nitrate and nitrite contents, using UV-Visible Spectrophotometric method. Results were noticed that nitrate contents in the vegetable and soil samples were generally higher than the nitrite contents. Nitrate (NO_3^-) were ranged from 400.68±4.43 μg g^{-1} in peas to 980.60±11.83 μg g^{-1} in groundnut obtained in the two sample areas of Alau dam and Gongulon. Nitrite (NO_2^-) were ranged from 110.37±7.82 μg g^{-1} in beans to 190.75±3.60 μg g^{-1} in ground nut. Data were analyzed with t-test and ANOVA. The results in general recorded higher levels of the anions in soils and vegetables obtained in the sample areas than their corresponding levels in samples obtained from the control sites. According to the results, the nitrate and nitrite content in all samples were less than the standard limits.

Key words: Concentration · Nitrate · Nitrite · Soils · Vegetables · Fertilizers · Wastewater

INTRODUCTION

Vegetables are the leafy outgrowth of plants used as food and include those plants and parts of plants used in making soup or served as integral parts of the main sources of our meal [1]. Vegetables especially the leafy ones are the major source of nitrate and nitrite in human diet, supplying about 72 to 94% of the total intake. Due to the potential health hazards resulting from high intake of these anions, the determination of their concentration contents in vegetables has been considered and measured in many countries [2, 3]. Nitrate is potentially reduced to nitrite, which is known to cause adverse effects on human and animal health. Nitrite may react with secondary amines to form toxic and carcinogenic nitrosamine compounds. Accordingly, nitrite is also known to cause methemoglobinemia (oxygen deficiency) in infants [4, 5]. Nitrite content in vegetables is usually very low as compared to nitrate [6, 7]. Nitrogen fixation makes nitrogen available for use by organism such as vegetables. The nitrogen in the atmosphere (N_2) is fixed by conversation to inorganic nitrogen [8].

Excessive amount of nitrogenous fertilizers are applied to crops, considering that they are reasonable insurances against yield losses and their economic consequences. When the input of nitrogen exceeds the demand, plants are no longer able to absorb it and nitrogen then builds up in the soil, mostly as nitrates [9]. Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate now than in the past [10]. Nitrogen fertilization facilitates accumulation of nitrate in plant tissues as a result of an excess nitrogen uptake over its reduction. When taken up in excess of immediate requirement, it is stored as free nitrate in the vacuole and can be remobilized subsequently to meet the demand [11]. Nitrate accumulation in vegetables often depends on the amount and kinds of nutrients present in the soil and is closely related to the time of application and the amount and composition of the fertilizers applied [12]. An adequate fertilization programme may ensure sufficient plant growth without any risk of plant nitrate levels going too high [13]. Plants accumulate more nitrate as the nitrogen fertilization level increases [14]. Applying nitrogen once at the beginning of the cropping cycle is effective at controlling nitrate accumulation, since the plant and soil-nitrate concentrations decrease as plants reach a marketable size [13].

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It has been reported that vegetables that are consumed with their roots, stems and leaves have a high nitrate accumulation, whereas melons and those vegetables with only fruits as consumable parts have a low nitrate accumulation [12]. In their study; "Nitrate accumulation in vegetables", [15] noted that plants that develop fruits or storage organs, such as potato and tomato, usually have low nitrate-N concentrations. Accordingly, they noted that vegetables that do not develop storage organs or fruits have a different pattern of nitrate accumulation where nitrate often continues accumulating with the growing plant's age.

The European Commission (EC)'s Scientific Committee for Food (SCF) established, in 1995, the Acceptable Daily Intake (ADI) of nitrate ion as 3.65 mg kg^{-1} body weight (equivalent to 219 mg day^{-1} for a person weighing 60 kg). Similarly, it had been established that the criteria values of nitrate content in the same kind of vegetable may vary broadly from country to country owing to differences in vegetable consumption and vegetable production practices [16, 17].

This study is aim at determining the nitrate and nitrite contents in soils and some leguminous vegetables. This was carried out by analyzing spectrophotometrically the levels of nitrate and nitrite in samples of beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*), peas (*Pisum sativum*) and soils collected from vegetable farms of Alau dam and Gongulon which supply most of the vegetables consumed in Maiduguri.

MATERIALS AND METHODS

Study Area: Maiduguri, capital of Borno State and a commercial nerve center in the Northeastern Region of Northern Nigeria, lies between latitude 11° 51'N and longitude 30° 05'E at an altitude of 345 meters above sea level [18]. This area is known for its dryness, with Sudan type of Climate, Savanna or Tropical grasslands Vegetation, light annual rainfall of about 864mm (34 inches) and the temperature ranging from 22-31°C, with mean of the daily maximum exceeding 40°C between March and May before the onset of the rains in June [19]. In this area, vegetables are irrigated with dam waters and all kinds of available waste and polluted waters. Similarly, to enhance the yield of these vegetables, fertilizers and manures are occasionally added to the soil. There are the possibilities of over applications of these fertilizers and manures. Hence, the uptake and storage of nitrate and nitrite from these water, fertilizers and manures by these vegetables are very likely since these salts are soluble and mobile in ground water.

Sample and Sampling: Edible portions of beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*) and peas (*Pisum sativum*) vegetables as well as top or surface soil (0-20 cm) samples were collected from the vegetable farms of Alau dam and Gongulon, irrigated with the Alau dam water, sewage and all kinds of available wastewater and cultivated with the applications of fertilizers, manures, herbicides and pesticides. Samples were also collected from experimental gardens cultivated on a piece of virgin land (left uncultivated for about 5 years), irrigated with unpolluted water and without the applications of fertilizers, manures, herbicides and pesticides, to serve as the controls. Collections were made from December, 2007 to May, 2008. Samples collections were made six (6) times during the period. During each collection, samples were randomly collected from different plots and homogenized into two composite samples in the two sample areas. Sample collections were carried out according to the methods described by [17] into pre-cleaned polyethylene bags and transported to the laboratory.

Vegetable Samples Preparations for Nitrate and Nitrite Analyses: Samples were cleaned to remove visible soil and then washed with tap water, thereafter with distilled water several times and then sliced into nearly uniform sizes to facilitate drying at the same rate. The sliced samples were then dried in an oven at 105°C for 24 hours until they were brittle and crisp. At this stage no micro organism can grow and care was taken to avoid any source of contamination. The dried samples were mechanically ground into fine particles using clean mortar and pestle and sieved to obtain <2 mm fractions. A portion (1g) of each of the sieved samples was taken separately in 100ml polyethylene or glass bottles, 40mL of distilled w ater were added, capped and shaken for 30 minutes. The solutions were filtered and the filtrates made up to the marks in 100mL volumetric flasks [17].

Digestion of Soil Samples: Soil samples were then dried in an oven at 105°C for 24 hours until they were brittle and crisp. A portion (1g) of dried, disaggregated and sieved soil sample were placed in 50 mL, Teflon beakers and then digested with 10 mL of HNO_3-$HCLO_4$-HF to near dryness at 80-90°C on a hot plate. The digests were filtered into a 50 mL volumetric flask using Whatman No. 42 filter paper, the soil distillates [17].

Determination of Nitrate (NO_3^-) and Nitrite (NO_2^-) Concentration Levels in the Vegetable Samples: The determination of nitrate in each of the vegetable sample solutions was performed by using spectrophotometer (model 2000) at a wavelength of 543 nm. The equipment was scrolled to select the stored programme number for nitrate (64 Nitrate-N). The result, which was obtained as Nitrate-Nitrogen (NO_3^--N) was converted to ppm Nitrate (NO_3^-) by multiplying by 4.4 (conversion factor) [20]. The concentration levels of nitrate ($\mu g\ g^{-1}$) in the samples were calculated from:

$$NO_3^-(\mu g\ g^{-1}) = C \times V/M \qquad (1)$$

Where; C is the concentration of NO_3^- in the sample (ppm), V is the total volume of the sample solution (100ml) and M is the weight of the sample (1g) [20].

Nitrite levels in the sample solutions were similarly determined except that in this case, different reagents were used. The programme number for nitrite was 67 Nitrite-N and the reaction period was five minutes as against ten minutes in the case of nitrate. Nitrite-Nitrogen (NO_2^--N) was converted to ppm Nitrite (NO_2^-) by multiplying by 3.3 (conversion factor) [20]. The concentration levels of nitrite ($\mu g\ g^{-1}$) in the samples were calculated from:

$$NO_2^-(\mu g\ g^{-1}) = C \times V/M \qquad (2)$$

Where; C is the concentration of NO_2-in the sample (ppm), V is the total volume of the sample solution (100mL) and M is the weight of the sample (1g) [17].

Determination of Nitrate (NO_3^-) and Nitrite (NO_2^-) in the Soil Distillates: Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in the soil distillates were then determined by using spectrophotometer (model 2000) at a wavelength of 543 nm, similar to those previously described for the vegetable samples.

Determination of Some Physical and Chemical Parameters and Particle Fractions of the Soils: Organic carbon was determined by means of a potassium dichromate back titration method as described by [21]. Cation exchange capacity (CEC) was determined by the silver thiourea method as described by [22]. The soil pH (1:5 soil water extract), Electrical conductivity (EC) (1:5 soil water extract) and the soil particle size fractions were equally determined using standard laboratory methods as described by [22].

Transfer Factors (TF) for Heavy Metals from Soils to Vegetables: Transfer factor (TF) is the ratio of the concentration of nitrate or nitrite in a plant to the concentration of nitrate or nitrite in soil. TF for nitrate and nitrite were computed based on the method described by Harrison and Chirgawi, 1989[23], according to the following formula:

$$TF = Ps\ (\mu g\ g^{-1} \text{dry wt})/St\ (\mu g\ g^{-1} \text{dry wt}) \qquad (3)$$

Where Ps is the plant nitrate or nitrite content originating from the soil and St is the total nitrate or nitrite contents in the soil.

Data Analyses: Data collected were subjected to statistical tests of significance using the Student t-test and Analysis of Variance (ANOVA) at $p<0.05$ to assess pairs results in the beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*) and peas (*Pisum sativum*) as well as in the soil samples. That is, to assess significant variation in the concentration levels of nitrate and nitrite in the vegetables as well as in soils. Probabilities less than 0.05 ($p < 0.05$) were considered statistically significant. Correlation coefficient was used to determine the association between the nitrate and nitrite in beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*) and peas (*Pisum sativum*) at $p = 0.05$. All statistical analyses were done by SPSS software for windows.

RESULTS AND DISCUSSION

Nitrate (NO_3^-) and Nitrite (NO_2^-) Concentrations in the Vegetable Samples: Table 1 presents the concentration levels of nitrate and nitrite in leguminous vegetable samples and equally compares the concentration levels of the anions in the vegetables obtained in Alau dam and Gongulon with their corresponding levels in the control samples. Nitrate levels were ranged from $400.68 \pm 4.43\ \mu g\ g^{-1}$ in peas to $980.60 \pm 11.83\ \mu g\ g^{-1}$ in ground nut obtained in the two sample areas. Accordingly nitrite levels ranged from $110.37 \pm 7.82\ \mu g\ g^{-1}$ in beans to $190.75 \pm 3.60\ \mu g\ g^{-1}$ in groundnut. The concentration levels of nitrite in the leguminous vegetables were generally low compared to the nitrate contents. These were in agreement with the fact that nitrite contents in vegetables are usually very low compared to nitrate [6, 7]. The trend of nitrate and nitrite variations in the vegetables were in the order groundnut > beans > peas. The results show high nitrate and nitrite concentration levels in the leguminous vegetables when compared with a similar study carried out for the fruit vegetables, carrot, as well as the onion and melon families of vegetables. These could be attributed to the fact that the leguminous vegetables belong to the nitrogen-fixing family (*Leguminosae*), which contain the nitrogen-fixing bacteria in their root nodules.

Table 1: Concentrations in μg g^{-1} of nitrate and nitrite in leguminous vegetables obtained in Alau dam, Gongulon and the control sites

Vegetables	Locations	Nitrate (NO_3-)	Nitrite (NO_2^-)
Ground nut	Alau dam	$825.40^a \pm 8.93$	$151.52^a \pm 3.62$
	Gongulon	$980.60^b \pm 11.83$	$190.75^b \pm 3.60$
	Control	$650.10^c \pm 2.40$	$110.24^c \pm 1.70$
Beans	Alau dam	$620.10^a \pm 34.13$	$110.37^a \pm 7.82$
	Gongulon	$730.70^b \pm 9.65$	$160.24^b \pm 18.60$
	Control	$510.30^c \pm 3.50$	$90.50^c \pm 4.40$
Peas	Alau dam	$400.68^a \pm 4.43$	$151.62^a \pm 3.57$
	Gongulon	$420.30^a \pm 7.19$	$159.37^a \pm 0.95$
	Control	$320.36^b \pm 1.84$	$100.00^b \pm 1.34$

The above values are means of replicate values (n = 6). Within column, means with different alphabets are statistically different ($p<0.05$)

Table 2: Concentrations in μg g^{-1} of nitrate and nitrite in soil samples obtained in Alau dam, Gongulon and the control sites

Sample areas	Nitrate (NO_3-)	Nitrite (NO_2^-)
Alau dam	$311.55^a \pm 0.75$	$203.33^a \pm 4.80$
Gongulon	$398.65^b \pm 2.56$	$253.93^b \pm 3.41$
Control	$198.52^c \pm 2.01$	$84.31^c \pm 0.03$

The above values are means of replicate values (n = 6). Within column, means with different alphabets are statistically different ($p<0.05$)

Statistical test of significance using ANOVA revealed that significant differences ($p < 0.05$) between the anions concentration levels in the leguminous vegetable samples obtained in the two sample areas with respect to their corresponding levels in samples obtained from the control sites. These could be attributed to possible pollution taking place in the sample areas as a result of various anthropogenic activities like different farming practices such as the excessive usage of wastewater in irrigating the soils and large-scale use of fertilizers, manures, herbicides and pesticides in the areas. These are in addition to a number of environmental factors such as drought, temperature, day light intensity and soil type. In addition the highest concentration levels of the nitrate and nitrite in the vegetable samples obtained in the Gongulon area could be attributed to a much higher possible pollution of the area as a result of the vast agricultural activities and the downstream deposition of fertilizers and other chemicals as the Alau dam water flows into the area.

Nitrate (NO_3^-) and Nitrite (NO_2^-) in the Soil: Table 2 presents the concentration levels of nitrate and nitrite in the soil samples and equally compares the concentration levels of the anions in the soil samples obtained in Alau dam and Gongulon with their corresponding levels in samples obtained from the control sites. Nitrate levels were relatively higher than those of nitrite in the two sample areas. Nitrate concentration levels were 311.55±0.75 μg g^{-1} and 398.65 ± 2.56 μg g^{-1} in soil samples obtained in Alau dam and Gongulon, respectively. The concentrations recorded for nitrite were 203.33±4.80 μg g^{-1} and 253.93 ± 3.41 μg g^{-1} in the two areas. Statistical test of significance using the ANOVA revealed that significant differences ($p < 0.05$) between the soil's anions concentration levels in each of the soil samples obtained in the two sample areas of Alau dam and Gongulon with their corresponding levels in samples obtained from the control sites. These might also be attributed to possible pollution of the soils as a result of excessive usage of fertilizers, herbicides and other agro-chemicals and as well as the use of wastewater in irrigating the soils and the environmental conditions pertinent in the areas. In addition the highest concentration levels of the anions in the soil samples obtained in the Gongulon area could also be attributed to a much higher possible pollution of the area as a result of the vast agricultural activities and the downstream deposition of fertilizers and other chemicals as the Alau dam water flows into the area.

Some Physical and Chemical Parameters of the Soils: The results for the determination of some *physical and chemical* 1 parameters in soil samples are shown in Table 3. The results showed low organic carbon (OC) and organic matter (OM) in the study area. The values of OC (%) were ranged from 0.40 and 0.74 in Alau dam and Gongulon, respectively. Those of OM (%) were 0.69 and 1.28 in the two areas. Similarly, cation exchange capacity (CEC) in meq 100 g^{-1} and the electrical conductivity (EC)

Table 3: Some physical and chemical properties parameters of the soils Sample Areas Parameters

	Areas Parameters				
Sample	OC (%)	OM (%)	CEC (meq100g^{-1})	EC (μmhocm^{-1})	pH
Alau dam	0.40[a]	0.69[a]	5.33[a] ± 0.01	0.22[a] ± 0.03	6.23[a] ± 0.20
Gongulon	0.74[b]	1.28[b]	5.43[a] ± 0.10	0.24[a] ± 0.05	6.69[a] ± 0.04
Control	0.22[c]	0.35[c]	5.04[a] ± 0.15	0.20[a] ± 0.12	6.67[a] ± 0.57

The above values are means of replicate values (n = 6). Within column, means with different alphabets are statistically different (p<0.05)

Table 4: Some particle size distribution of the investigated soils

	Particle size distribution		
Sample area	Clay (%)	Sand (%)	Silt (%)
Alau dam	9.00[a]	86.00[a]	5.00[a]
Gongulon	11.50[b]	87.00[a]	7.50[b]
Control	6.83[c]	84.00[a]	3.40[c]

The above values are means of replicate values (n = 6). Within column, means with different alphabets are statistically different (p<0.05)

in (μmho cm^{-1}) values were generally low. The CEC (meq 100 g^{-1}) values for the two areas were 5.33 ± 0.01 and 5.43 ± 0.10 and those of EC (μmho cm^{-1}) were 0.22 ± 0.03 and 0.24 ± 0.05. The soil pH values in the two areas were as high as 6.23 ± 0.20 and 6.69 ± 0.04 respectively. As observed in Table 3, the high pH values of 6.23 ± 0.20 and 6.69 ± 0.04 in the two respective sample areas of Alau dam and Gongulon are indicative of slightly acidic environment. The highest pH values in the study area may be attributed mainly to the buffering effect of carbonate containing materials such as cement or bricks [24]. Statistical test of significance using the ANOVA, revealed that significant differences (p<0.05) between the values of organic carbon (OC) and organic matter (OM) in the soil samples obtained in the two areas with their corresponding values in the control samples. However, cation exchange capacity (CEC), electrical conductivity (EC) and pH values in samples obtained in the two areas did not show statistical differences (p > 0.05) with their corresponding values in the control samples.

Some Particle Size Distribution of the Soils: Table 4 presents the levels of some particle size distribution of the soils. Particle size analyses of the soils revealed the levels of clay (%) in the two respective areas were: 9.00 and 11.50; sand (%) as 86.00 and 87.00 and silt (%) as 5.00 and 7.50. In Table 4, there were significant differences (p>0.05) between the concentration levels of clay, silt in soil samples obtained in the two sample areas of Alau dam and Gongulon with their corresponding concentration levels in the control samples. In general, the results revealed the soils in the sample areas to be loamy sand in texture and slightly acidic with low organic matter contents. Organic matter may plays an important role in soil structure, aggregation, infiltration and retention of water and other physical characteristics. Furthermore, the adsorption complex (clay and humus) serves the soil as a store of nutrients and is a significant contributor to the buffering capacity of soils even as cation exchange capacity can be used to determine the amount of lime that needs to be applied to reduce acidification. The electrical conductivity of soil can be used to assess the viability of saltwater flooded soils, in monitoring surveys and in assessing irrigation and drainage needs. It is also useful in determination of heavily fertilized soils such as soils in green houses and vegetable gardens. Conductivity of soil has also been related to plant growth [17].

Transfer Factors (TF) for Nitrate and Nitrite from Soils to Leguminous Vegetables: Table 5 presents the transfer factors of nitrate and nitrite from soils to the leguminous vegetables. Transfer factors for the anions between the soils and vegetables identify the efficiency of a vegetable species to accumulate nitrate and nitrite. Transfer factors were computed to quantify the relative differences in bioavailability of the anions to the vegetables or to identify the efficiency of a plant species to accumulate a given anion. These factors were based on the root uptake of the anions [25]. The results indicated that the leguminous vegetables considered in this study have the potential of accumulating more nitrate as compared to nitrite.

Table 5: Transfer factors (TF) of nitrate and nitrite from soil to leguminous vegetable samples

Sample areas	Vegetables	Nitrate (NO_3-)	Nitrite (NO_2^-)
Alau dam	Ground nut	2.65	0.75
Gongulon	"	2.46	0.75
Alau dam	Beans	1.99	0.54
Gongulon	"	1.83	0.63
Alau dam	Peas	1.29	0.75
Gongulon	"	1.05	0.63

Relationships Between Nitrate and Nitrite in the Leguminous Vegetables: The relationships between nitrate and nitrite concentration levels in the leguminous vegetables were established using Pearson's correlation coefficient method [26]. Perfectly positive correlation values of 1 were observed between the two anions in each of the leguminous vegetables, indicating that they are influenced by similar anthropogenic sources in the same positive directions.

CONCLUSIONS

This study has been able to measured nitrate and nitrite contents in soils and some leguminous vegetables cultivated in Maiduguri, Nigeria and suggested possible pollution of the study area due to excessive usage of fertilizers, manures, pesticides, herbicides and other agro-chemicals as well as the use of wastewater in irrigating the soils and the environmental factors pertinent in the study area. The physical and chemical parameters and particle size distribution of the soils determined in the study revealed the soils to be loamy sand in texture, slightly acidic with low organic matter contents and small amount of clay. The results were below or within the published maximum permissible contents of nitrate and nitrite in some vegetables and fruits. Therefore consumption of these vegetables as food may not pose possible health hazards to humans and animals at the time of the study. The results in this study would go a long way in providing a baseline data for the measurement of nitrate and nitrite content in some leguminous vegetables and soils obtained in Maiduguri, Nigeria.

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