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Evaluation of Nitrate and Nitrite Contents of Vegetables and Soils from Irrigated Farmlands within Makurdi metropolis, Benue State, Nigeria

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ABSTRACT

Common vegetables *(Amaranthus hybridus, Capsicum annuum, Telferia occidentalis, Talinum triangulare, Abelmoschus esculentus, Hibiscus sabdariffa, Corchorus olitorius, and Ocimum basilicum)* and soil samples were collected from irrigated farmlands along the bank of river Benue and analyzed for their nitrate and nitrite contents using UV- spectrophotometer. This was aimed at assessing the level of contamination of these vegetables by these anions. The results of the study indicate nitrates concentrations in the vegetables to range from 1829.54mg/kg to 5114.16mg/kg, while nitrite content of the vegetables was also found to range from 459.80 mg/kg to 718.63 mg/kg. Soil nitrate ranged from 1882.13 mg/kg to 4004.20 mg/kg, while the nitrite ranged from 244.20 mg/kg to 1232.70 mg/kg. Some of the vegetables were found to accumulate nitrate and nitrite contents higher than the permissible limits prescribed by WHO/FAO. The higher content of nitrate and nitrites in these vegetables and soil could be attributed to excessive use of fertilizers, animal manures, and other agro-chemicals as well as the wastewater used in irrigation. Both nitrate and nitrite transfer factors were above 0.5 which implies that the vegetable have high tendency to accumulate the anions, therefore, continuous consumption of these vegetable could be detrimental to health.

Keywords: Irrigated farmlands, Nitrate, Nitrite, Soil, Vegetables

INTRODUCTION

Vegetables are generally classified into three main types; earth, herbage and fruit vegetables, and are known to be important crops for human nutrition because they contain bioactive nutrient compounds such as dietary fiber, antioxidant vitamins and minerals (Anna *et al.*, 2018). They are also known to contained phytochemicals (phenolic compounds, flavonoids, peptides, etc) which are non-nutrient molecules. These nutrient and non-nutrient compounds aid the body in reducing the risk of chronic diseases such as cardiovascular diseases, diabetes, cancers and obesity (Joane and Beate, 2012).

Despite the importance of vegetables to the human body, they are known to be the major sources of nitrate and nitrite in diet. According Rasn and Rauji (2018), though, nitrates have relatively low toxicity; their conversion to nitrites in human body may lead to several adverse conditions such as methemoglobinemia. Also, the conversion of nitrites to N-nitroso compounds in human body may cause cancer to certain organs of the body.

Danijel *et al.* (2017) reported that the absorption and accumulation of nitrate by vegetable crops occur most often from natural sources and from nitrogen-based fertilizers which are used for faster and bigger growth. The constant use of most farmlands has led to the infertility of such lands and as such, farmers apply fertilizers on crops for maximum yield without considering the health implications.

The excessive use of nitrogenous fertilizers usually increases the acidity, nitrates and nitrites of the soil and consequently bioaccumulation of these chemicals in vegetables and crops that are grown on such lands. Other sources of nitrates and nitrites in vegetables could be light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period or season and species variation (Ajon *et al*., 2021). Studies have shown that leafy vegetables, such as lettuce or spinach, contain the highest concentrations of nitrate (Iammarino, 2014). Matthew *et al*, (2019), reported the concentrations of nitrates and nitrites in vegetables and fruits in Lafia, Nigeria, to be significantly high. Other researchers have reported varying concentrations of nitrates in vegetables from various parts of Nigeria and found to be lower than the maximum permissible limits (< 200 mg/kg), low (200-500 mg/kg), medium (500-1000 mg/kg), high (1000-2500 mg/kg) and very high (>2500 mg/kg) (Butu *et al.*, 2020; Ziarati *et al.*, 2018; Schrenk *et al.*, 2020; Oladeji 2021, Oladeji 2017).

Dietary nitrate and nitrite are known to be beneficial to the body system. For example, Linsha *et al.* (2018), reported that dietary nitrate and nitrite act as exogenous donors for biological nitrogen (II) oxide (NO), which plays an important role in some physiological activity in the body. They also have potential protective effect on body balance, improvement of some disorders like stroke, myocardial infarction, systemic and pulmonary hypertension, etc. Dietary nitrate and nitrite is also known to alleviate gastric ulcers.

However, exposure to high levels of nitrate and nitrite in foods and vegetables has adverse effects on those who consume such foods. The consumption of nitrate and nitrite could result to a disease condition known as methemoglobinemia or blue baby syndrome. This occurs through the conversion of nitrate to nitrite in the body (Tamme *et al.*, 2010). Parvizishad *et al* (2017) observed that high concentrations of nitrate and nitrite in foods causes the enlargement of the thyroid gland and affect people with diabetes mellitus. Nitrate and nitrite concentration is also an indicator of fodder quality. Studies have shown that cattle that consume nitrates at sub-toxic levels suffer from milk production, vitamin A deficiency, abortions, stillbirths and even poisoning leading to loss of many herds of cattle (Schrenk *et al.*, 2020). This study therefore, considers the assessment of nitrates and nitrite levels in vegetables cultivated on irrigated farmlands along the south bank of river Benue within Makurdi metropolis pertinent, since most of the residents depend on these vegetables for their dietary fiber, antioxidant vitamins and minerals and other nutrients contained by vegetables for their balance diet. The research also examined the characteristic risks related to high uptake of nitrates and nitrites.

MATERIALS AND METHODS

The study area

Makurdi is the capital of Benue State, Nigeria which is usually referred to as the food basket of the nation. The town is located on latitude 7^{o}44'N and longitude 8^{o}32'E of the Equator. It is located in a valley in North Central Nigeria with an elevation of 100 m above sea level. It is characterized with a tropical savannah climate with an average annual temperature of 36 °C (Ajon and Anjembe, 2018). Makurdi is traversed by the second largest river in the country, the river Benue. The river divides Makurdi town into north and south Banks, which are connected by two bridges. The presence of the river has made it possible for farmer to engage in irrigated farming on the banks of the river. Various vegetables are cultivated during the dry season farming. According to Anhwange *et al.* (2013), the most common vegetables cultivated on the river banks are: *Telfairia occidentalis* (Fluted pumpkin), *Amaranthus hybridus* (Spinach) and *Abelmoschus esculentus* (Okra), *Talinum triangulare* (water leaf) and *Capsicum annum* (Pepper).

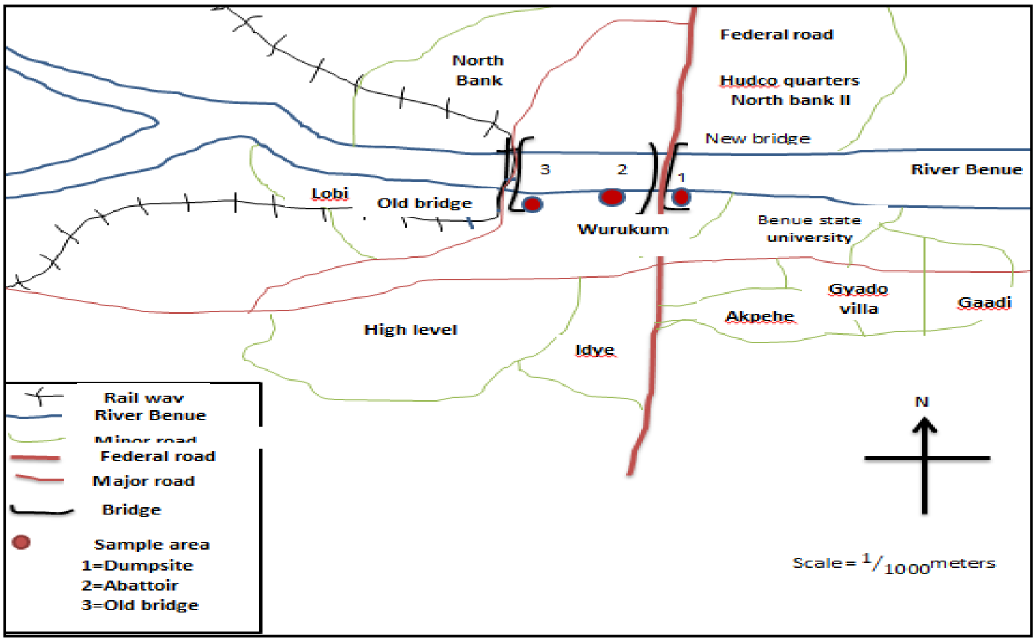

Fig 1: A sketch showing the sampling areas

Sample collection

Whole vegetable samples; spinach (*Amaranthus hybridus*), pepper (*Capsicum annuum*), fluted pumpkin (*Telfairia occidentalis*), waterleaf (*Talinum triangulare*), okra (*Abelmoschus esculentus*), roselle (*Hibiscus sabbdariffa*), jute mallow (*Corchorus olitorius*), and sweet basil (curry) (*Ocimum basilicum*) were collected from these locations (New bridge area, Makurdi main Abattoir and the Old bridge area) on irrigated farmlands along the bank of river Benue. Top-soil (0–20 cm) samples were also collected from these farmlands. The samples were collected in polyethylene bags, labeled and grouped into three according to the different locations. The fresh vegetable samples were harvested from irrigated farmlands along the River Benue and properly identified at the herbarium unit of the Department of Biological Sciences of the Benue State University, Nigeria. They were then transported to the laboratory for analysis.

Sample pre-treatment

The vegetable samples were properly washed under running tap water and rinsed with distilled water to remove all environmental residues from the surfaces. The edible parts of the vegetables were separated from the non-edible parts and were stored in a fridge below 4 ^{o}C until analysis. All the soil samples were dried in a preheated oven at 110 ^{o}C for 10 minutes. The samples were ground using a porcelain mortar and pestle and then sieved with a 1 mm sieve to obtain fine particles and stored at room temperature for until analysis.

Extraction of nitrate in vegetables and soil

Exactly 5 g of vegetable samples were weighed and placed in petri dishes and allowed to freeze in the freezer at -8 ^{o}C for 20 minutes. The samples were then crushed with a porcelain mortar and pestle. The crushed samples were transferred into separate 20 mL centrifuge tubes and 10 mL of distilled water was added into each centrifuge tube. The samples were placed in a water bath at 100°C for 20 minutes with the lid closed to reduce nitrate reductase from converting the nitrate to nitrite. The samples were allowed to cool for 15 minutes and a vortex mixer was used to homogenize the mixture for 5 minutes. The samples were then centrifuged at 3000 rpm for 1 h to ensure complete extraction of nitrate from the sample. (Mathew *et al*., 2019; Rezaei *et al*., 2014).The same procedure was repeated using the soil samples and the supernatants were used for nitrate analysis.

Extraction of nitrite in vegetables and soil

Exactly 5 g of each vegetable samples were weighed and placed in a separate petri dish. The samples were allowed to freeze at -8 ^{o}C for 20 minutes and then crushed using a porcelain mortar and pestle. The crushed samples were transferred into 20 mL centrifuge tubes and 10 mL of extraction reagent (5 % KOH, 1 % EDTA and 7 % cysteine) was added. The samples were homogenised using a vortex mixer for 2 minutes and were then centrifuged at 3000 rpm for 1 hour to separate the residue from the supernatant (Takushi. and Yuki, 2017, Rezaei *et al*., 2014). The same procedure was repeated using soil samples and the supernatants were used for analyses.

Preparation of nitrate calibration curve

A nitrate dilution series was prepared using potassium nitrate (0, 2.5, 5.0, 7.5, 10.0, 12.5, 25.0, and 50.0 mg/mL) in distilled water. In each case, 1 mL of KNO_3 standard solution was pipetted and transferred into the test tubes respectively. Into each of the test tubes, 4 mL of reagent 1(0.05% (w/v) salicylic acid in sulfuric acid) was added and they were vortexed for 2 minutes. The test tubes were then incubated at room temperature for 20 minutes. After incubation, 1 mL of reagent 2 (8% (w/v) NaOH in distilled water) was gently added and vortexed again until the solution became clear. Absorbance was then measured at 410 nm using the spectrophotometer (Shimadzu, Model: 1650PC) and a standard curve was plotted (Takushi and Yuki, 2017, Alexander *et al*., 2016).

Preparation of nitrite calibration curve

A nitrite dilution series was prepared using sodium nitrite (0, 5, 10, 20, and 40 µg/mL) in the extraction reagent 1 (50 mMKOH (pH 7.6), 1 mM EDTA, and 7 mM cysteine freshly prepared). The standard solutions were then mixed with equal volumes of reaction reagent 3 [1% (w/v) sulfanilamide in 1 mol/L hydrochloric acid] and reaction reagent 4 [0.02% (w/v) N-1-naphthylethylenediamine dihydrochloride in distilled water] in the ratio 1:1:1 (v/v/v) in respective test tubes. The solutions were incubated at room temperature for 15 minutes and the absorbance was taken at 540 nm using a UV-spectrophotometer (Shimadzu, Model: 1650PC) and a standard curve was plotted (Takushi and Yuki, 2017).

Determination of nitrate in vegetables and soils

The method prescribed by Takushi and Yuki (2017), was adopted for the analysis. Exactly 4 mL of the supernatant was taken from each sample solutions and transferred into separate test tubes. Into each test tube, 1 mL of 1% w/v salicylic acid in H_2SO_4 was added. The solutions were thoroughly homogenized using a vortex mixer for 2 minutes and then incubated at room temperature for 20 minutes (the incubation temperature was maintained at 20 - 25 °C) in order to ensure the formation of a yellow precipitate. Lastly, 2 mL of 8% (w/v) NaOH in distilled water was gently added and mixed until the contents became clear (Anna *et al*., 2018). The same procedure was repeated using soil samples and the absorbance was measured at 410 nm using a UV-spectrophotometer (Shimadzu, Model: 1650PC). The nitrate concentration was calculated using equation 1;

$$[NO_3^-]_{sample} = \frac{(\text{True } [NO_3^-](\text{mg/mL}) \times \text{Volume extracted (mL)})}{\text{Slope}} \quad (1)$$

Determination of nitrite in vegetables and soils

The method described by Takushi and Yuki (2017) and Anna *et al*, (2018), was adopted for the analysis. Exactly 100 µL of the supernatant of each vegetable sample was transferred to separate 10 mL test tubes. Into each of the test tubes, 100 µL of 1 % (w/v) sulfanilamide in 5 % H_2SO_4 and 100 µL of 0.02 % (w/v) N-1-naphthylethylenediamine dihydrochloride in distilled water were added. The solutions were incubated at room temperature for 15 minutes until the formation of a pink colour. The same procedure was repeated using soil samples and absorbance was measured at 540 nm using a UV-spectrophotometer (Shimadzu, Model: 1650PC). The nitrite concentration was calculated using equation 2;

$$[NO_2^-]_{(\mu g/g\ f.w)} = \frac{\text{True } [NO_2^-]\ (\mu g/mL) \times \text{Extracted volume (mL)}}{\text{Fresh weight (g)}} \quad (2)$$

Transfer factors (TF) for nitrate and nitrite from soils to vegetables

Transfer factor (TF), or transfer coefficient, is the ratio of the concentration of nitrate or nitrite in a plant to the concentration of nitrate or nitrite in soil (Uwah *et al*., 2009). TF for nitrate and nitrite were computed according to equation 3;

$$TF = \frac{NO_3^- \text{ or } NO_2^- \text{ contents in vegetable}}{NO_3^- \text{ or } NO_2^- \text{ contents in soil}} \quad (3)$$

Data analysis

The results of nitrate and nitrite in vegetable and soil samples analyzed were expressed in form of tables. The results obtained were subjected to one way Analysis of Variance (ANOVA) and the post hoc test was used to separate means by Duncan multiple range test at 95% confidence level to check if there was significant difference in the concentrations of nitrate and nitrite analyzed.

RESULTS AND DISCUSSIONS

Nitrate contents in the vegetables and soil

The results of nitrate concentrations of the vegetables *H. sabbdariffa, A. hybridus, C. annum. A. esculentus, A. officcinalis, T, triangulare. O. basilicum and T. occidentalis* were found to range between 2685.03 – 2788.03 mg/kg, 2627.30 - 3121.70 mg/kg, 3633.90 – 5114.16 mg/kg, 1829.53 – 3802.06 mg/kg, 3302.40 – 4548.10 mg/kg, 3576.90 – 5024.36 mg/kg, 3564.93 – 4792.90 mg/kg, 2081.70 - 4231.46 mg/kg, 1882.13 – 4004.20 mg/kg respectively (Table 1). There was significant difference at $p < 0.5$ in nitrate contents of all the vegetables in the three sites. The values of nitrates recorded in this study are comparatively higher than those reported by Onyesom and Okoh (2006), Henni *et al*. (2016) and Roshna *et al*. (2021) which may be attributed to environmental factors. However the nitrates contents of *A. hybridus* (spinach) were above the values (4259 mg/kg) reported by Rasn and Marapana (2018), for the same plant. The river bank is usually over flooded by water from the river and runoff waters from the metropolis during rainy season. This runoff carries all kinds of wastes which eventually deposit on the bank of the river. Also the using of nitrogen fertilizers and other agrochemicals on both the soil and vegetable could be responsible for the higher content of the nitrate in the vegetables. Among the vegetables, waterleaf (*Talinum triangulare*) had the highest content of nitrates in all the three sites, while Roselle (*H. sabbdariffa)* was found to accumulate the lowest content of nitrate. The amount of nitrate absorbed by the vegetable has been found to be function of soil nitrate.

The nitrate content of the soil was found to range between 1882.13 - 4004.20mg/kg (Table 1) across the study sites. The amount of soil nitrate at the old bridge was 4004.20 mg/kg, and this is reflected in the amount absorbed by the vegetables. The high content of soil nitrate at the old bridge could also be linked to the use of wastewater from the abattoir and also wastes produced from the commercial activities at the area. According to Rezaei *et al*. (2014), the application of animal manure, fertilizers coupled with uses of wastewater could lead to high levels of nitrates in the soil. The deposition of ammonia, nitrate and nitrite containing substances in wastewater used for irrigation which flows from the abattoir through a drainage channel to the old bridge could also leached nutrients to soil. This flow of polluted water to the old bridge farmlands subsequently raised the level of ground water, which contained a substantial amount of pollutants such as nitrate and nitrite (Uwah *et al.*, 2009).

Table 1: Nitrate contents (mg/kg) in vegetables and soils

S/N	Sample	Nitrate		
		New bridge	Abattoir	Old bridge
1	*Hibiscus sabbdariffa*	2685.03^{e} ± 0.95	2788.03^{b} ± 0.64	2736.53^{c} ± 1.03
2	*Amaranthus hybridus*	2627.30^{d} ± 0.27	3121.70^{d} ± 1.27	3016.40^{a} ± 1.19
3	*Capsicum annuum*	3633.90^{h} ± 0.13	4033.60^{g} ± 1.49	5114.16^{i} ± 1.67
4	*Abelmoschus esculentus*	1829.53^{a} ± 0.66	2734.66^{b} ± 1.62	3802.06^{c} ± 1.11
5	*Corchorus olitorius*	3302.40^{f} ± 0.85	4075.90^{h} ± 0.45	4548.10^{f} ± 1.13
6	*Talinum triangulare*	3576.90^{g} ± 0.07	4235.63^{i} ± 2.51	5024.36^{h} ± 1.84
7	*Ocimum basilicum*	3564.93^{g} ± 0.92	3921.03^{f} ± 1.19	4792.90^{g} ± 2.36
8	*Telfairia occidentalis*	2081.70^{cb}± 0.30	2831.13^{c} ± 2.11	4231.46^{e} ± 2.34
9	Soil	1882.13^{b} ± 0.78	3573.93^{e} ± 3.25	4004.20^{d} ± 2.94
	WHO/FAO	2700	2700	2700

Values are mean ± standard deviation of two replicates
Means in a row with the same superscript were not significantly different at $p<0.05$

Nitrite contents in vegetables and soils

Nitrite contents of the vegetables (Table 2) were found to follow similar trends with nitrates. *T. triangulare* recorded the highest content of nitrites in the three sites (506.40 – 1232.70) mg/kg, while the nitrite content of *H. sabbdariffa* was found to be lowest (329.66 – 401.96) mg/kg. The nitrite contents of *A. hybridus*, *C. annuum*, *A. esculentus*, *C. olitorius*, *O. basilicum* and *T. occidentalis* were found to range as follows; 286.46 – 700.96 mg/kg, 244.20 – 772.50 mg/kg, 276.80 – 878.16 mg/kg, 300.86 – 946.20 mg/kg, 325.00 – 1104.73 mg/kg and 430.78 – 1087.56 mg/kg respectively. There was significant difference at $p<0.05$ in the content of nitrites absorbed by the vegetables. Here also, it was observed that the nitrite content of the vegetables was relatively high compared to other literature values. However, the observation is consistent with the nitrite values of the soil (459.80 – 718.63) mg/kg. The consistency in the values of nitrate/nitrite of soil to that of nitrate/nitrates of the vegetables agreed with other literature valves as reported by Onyesom and Okoh (2006), Henni *et al*. (2016) and Roshna *et al*. (2021). Most of the values recorded were found to be below the 700 mg/kg prescribed by WHO/FAO for leafy vegetables. The higher levels of nitrites in the vegetables and soil could be also be linked to the activities of microorganism from the wastewater used for irrigation of the vegetables which promote the nitrate reductase activity as well as denitrification (Uwah *et al*., 2007, Ebong and Etuk, 2017). Conversely, Matthew *et al*. (2019) reported lower values for similar plants.

Table 2: Nitrite contents (mg/kg) in vegetables and soils

S/N	Sample	Nitrite		
		New bridge	Abattoir	Old bridge
1	*Hibiscus sabbdariffa*	329.66^{c} ± 1.84	401.96^{d} ± 1.62	365.81^{a} ± 0.50
2	*Amaranthus hybridus*	286.46^{b} ± 3.60	589.70^{f} ± 1.66	700.96^{b} ± 0.15
3	*Capsicum annuum*	244.20^{a} ± 1.60	367.43^{c} ± 0.11	772.50^{d} ± 1.83
4	*Abelmoschus esculentus*	276.80^{b} ± 0.60	331.86^{a} ± 1.06	878.16^{e} ± 0.65
5	*Corchorus olitorius*	300.86^{bc} ± 1.36	341.10^{b} ± 0.55	946.20^{f} ± 0.86
6	*Talinum triangulare*	506.40^{e} ± 4.95	618.23^{g} ± 0.66	1232.70^{i} ± 1.77
7	*Ocimum basilicum*	325.00^{c} ± 0.17	785.03^{i} ± 0.37	1104.73^{h} ± 4.82
8	*Telfairia occidentalis*	430.76^{d} ± 0.25	765.86^{h} ± 0.40	1087.56^{g} ± 1.71
9	Soil	459.80^{d} ± 0.51	540.80^{e} ± 1.82	718.63^{c} ± 1.33
	WHO/FAO	700	700	700

Values are mean ± standard deviation of two replicates
Means in a row with the same superscript were not significantly different at $p<0.05$

Tables 3 and 4 presents the transfer factors of nitrate and nitrite from soils to vegetables respectively. Transfer factors for the anions between the soils and vegetables indicate the effectiveness of a vegetable species to accumulate a given anion. Transfer factors were computed to quantify the relative differences in bioavailability of anions to plants 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 from the soil. If a transfer factor is ≥ 0.5, it implies that the plant has a greater tendency to absorb the anions or cations in question (Uwah *et al*., 2007; Etim *et al.*, 2015). The results of the study revealed all the vegetables investigated have higher tendency of accumulating nitrate and nitrite.

Table 3: Nitrate transfer factor

S/N	Sample	Nitrate transfer factor		
		New bridge	Abattoir	Old bridge
1	*Hibiscus sabbdariffa*	1.42	0.78	0.68
2	*Amaranthus hybridus*	1.39	0.87	0.75
3	*Capsicum annuum*	1.93	1.12	1.28
4	*Abelmoschus esculentus*	0.97	0.76	0.95
5	*Corchorus olitorius*	1.75	1.14	1.31
6	*Talinum triangulare*	1.90	1.18	1.25
7	*Ocimum basilicum*	1.89	1.09	1.91
8	*Telfairia occidentalis*	1.10	0.79	1.05

Table 4: **Nitrite transfer factor**

S/N	Sample	Nitrite transfer factor		
		New bridge	Abattoir	Old bridge
1	*Hibiscus sabbdariffa*	0.71	0.74	0.51
2	*Amaranthus hybridus*	0.62	1.09	0.97
3	*Capsicum annuum*	0.53	0.67	1.07
4	*Abelmoschus esculentus*	0.60	0.61	1.22
5	*Corchorus olitorius*	1.65	0.63	1.31
6	*Talinum triangulare*	1.10	1.14	1.71
7	*Ocimum basilicum*	0.70	1.45	1.53
8	*Telfairia occidentalis*	0.93	1.41	1.51

Transfer factor ≥ 0.5 implies the vegetable has a higher tendency of accumulating nitrate and nitrite

CONCLUSION

The study has been able to assess the nitrates and nitrites contents of soil and some vegetables grown on irrigated farmland on the south bank of river Benue. The presence of the nitrate and nitrites in these vegetables and plants could be due to the contamination of the area as a result of to excessive usage of nitrogenous fertilizers, animal manures, pesticides, herbicides and other agro-chemicals as well as the use of wastewater in irrigating the soils, and the other anthropogenic factor pertinent to the study area. The valves of nitrates and nitrites recorded in some of the vegetables were found to be higher than the permissible limits prescribed by WHO/FAO for leafy vegetable. This implies that continuous consumption of these vegetable could be detrimental to health.

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