DETERMINATION OF NITRATES AND NITRITES IN SOME EDIBLE VEGETABLES OBTAINED IN MUBI METROPOLIS, ADAMAWA STATE, NIGERIA

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Abstract:
Concentrations of nitrate and nitrite were determined in edible portions of some leafy vegetables okra (*Abelmos-chusesculent L.*) Sorrel (*herbiscus saderifa*), spinach (*Amaranthus caudatus*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) and water leaf (*Talinum triagulare*) obtained along the bank of river Yadzerem in Mubi metropolis. Nitrate and nitrite in the vegetable samples were determined using UV/Visible Spectrophotometric method. Levels of the nitrates in the vegetables generally ranged between 80.05 ± 1.50 to 121.40 ±1.80 for spinach, 63.00 ± 2.86 to103.90 ±1.73 for lettuce, 64.25 ± 2.20 to 113.20 ± 2.29 for cabbage, 60.85± 3.05 to 90.05± 0.59 water leave, 67.96 ± 2.74 to 99.89± 3.13 for sorrel and 56.93± 3.28 to 88.79±2.19 for okra. Nitrite ranged between 39.89 ± 2.13 to 53.10±2.29 for cabbage and 49.45 ±2.00 to 81.12±1.80 for spinach. Generally the levels of nitrate were observed to be higher than that of nitrite in each of the vegetable sample analysed. The results obtained in this study agree with the published maximum permissible content of nitrate and nitrite in some vegetables and fruits. Therefore consumption of these vegetables as food may not pose any possible health hazards to humans and animals at the time of the study.

Keywords: nitrate, nitrite, vegetables, concentration

Introduction

Vegetables are leafy outgrowth of plants used as food and include those parts used in making soup or served as integral parts of our daily meals. Consumption of vegetables and fruits as food offers the most rapid and lowest cost means of providing adequate supplies of vitamins, minerals and fibres to the people who live in the tropics. Vegetables are the major sources of the daily intake of nitrate by human beings, supplying about 72 to 94% of the total intake (Dich et *al*, 1996). Nitrate and nitrite are essential nutrients for plants protein synthesis and play a major role in nitrogen cycle in the ecosystem (Uwah *et al*, 2009). Nitrate is a naturally occurring form of nitrogen and nitrate is formed from fertilizers, decaying plants, manure and other organic residues. It is found in the air, soil, water and food (especially in vegetables) and is produced naturally within the human body by some metabolic functions. It is also used as a food additive mainly as a preservative and anti microbial agent (Walker, 1990). Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture; soil, vegetables and drinking water may contain higher concentrations of nitrate now than in the past (Santamaria, 2006). Nitrogen is the main limiting factor for most field crops, and nitrate is the major form of nitrogen absorbed by must crop plants. Farmers often use nitrogen fertilizers to increase crops and vegetable yields. Consequently, many vegetables and forage crops accumulate high levels of nitrate and nitrite than required. Vegetables such as spinach, lettuce and celery do contain nitrate at significant levels (Zhou *et al,* 2000 and

Hunt and Turner, 1994). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers, and growing conditions, especially the soil temperature and daylight intensity (Anjana et *al,* 2006). Vegetables such as cabbage, lettuce, radish and spinach often contain nitrate concentrations above 2500mg/kg especially when they are cultivated in green houses. Nitrite content in vegetables is usually very low compared to nitrates (Hunt and Turner, 1994). Nitrogen fixation makes nitrogen available for use by organism such as vegetable crops. The nitrogen in the atmosphere (N_2) is fixed by conversation to inorganic nitrogen in the form of nitrate or nitrite.

$$N_{2\,(g)} + 3H_{2\,(g)} \longrightarrow 2NH_{3(g)}$$

This dissolved in the soil and plant take it as nutrient in form of inorganic nitrogen. Animals then get their nitrogen from plants in the food chain to form amino acids, through a process called assimilation where the inorganic nitrogen is converted to organic nitrogen:

$$CO_{2\,(g)} + H_2O_{(l)} + NH_{3\,(g)} \longrightarrow$$

$CH_3NH_2COOH_{(aq)}$ (amino acid) which is an essential monomer of proteins in plants and animals. Nitrate is potentially reduced to nitrite which is known to cause adverse effect on human and animals' health. Nitrite in the body may react with secondary amines to form toxic and carcinogenic nitrosamine compounds. Nitrate is known to cause oxygen deficiency in infants called methemoglobinemia (Muramoto, 1999). Farmers' uses excessive amount of nitrogenous fertilizers so as to meet up the demand of vegetable in the market without considering the health implication of excess fertilizers applied to vegetable crops. Considering that they are reasonable insurances against yield loss 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 (Nosengo, 2003).This causes imbalance of nutrients in the soil and increases the nitrate level in the ground water supplies. These imbalances influence the nitrate content of plants, especially the leafy edible vegetables.

In view of the importance of vegetables to health and fact that many people have now resulted to eating vegetables for the well being of their health, there is need to study the level of nitrate and nitrite cultivated in Mubi metropolis. This research work is aimed at determining the level of nitrate and nitrite concentrations in edible leafy vegetables cultivated along the banks of river Yadzerem in Mubi metropolis.

Materials and Methods

Study area: River Yadzerem passes through the heart of Mubi metropolis. The banks of the river serve as one of the major areas used for irrigational purposes. Most of the people in Mubi metropolis obtained their water supply from the river. It provides irrigation land which supplies most of the vegetable consumed in Mubi metropolis as shown in figure1.

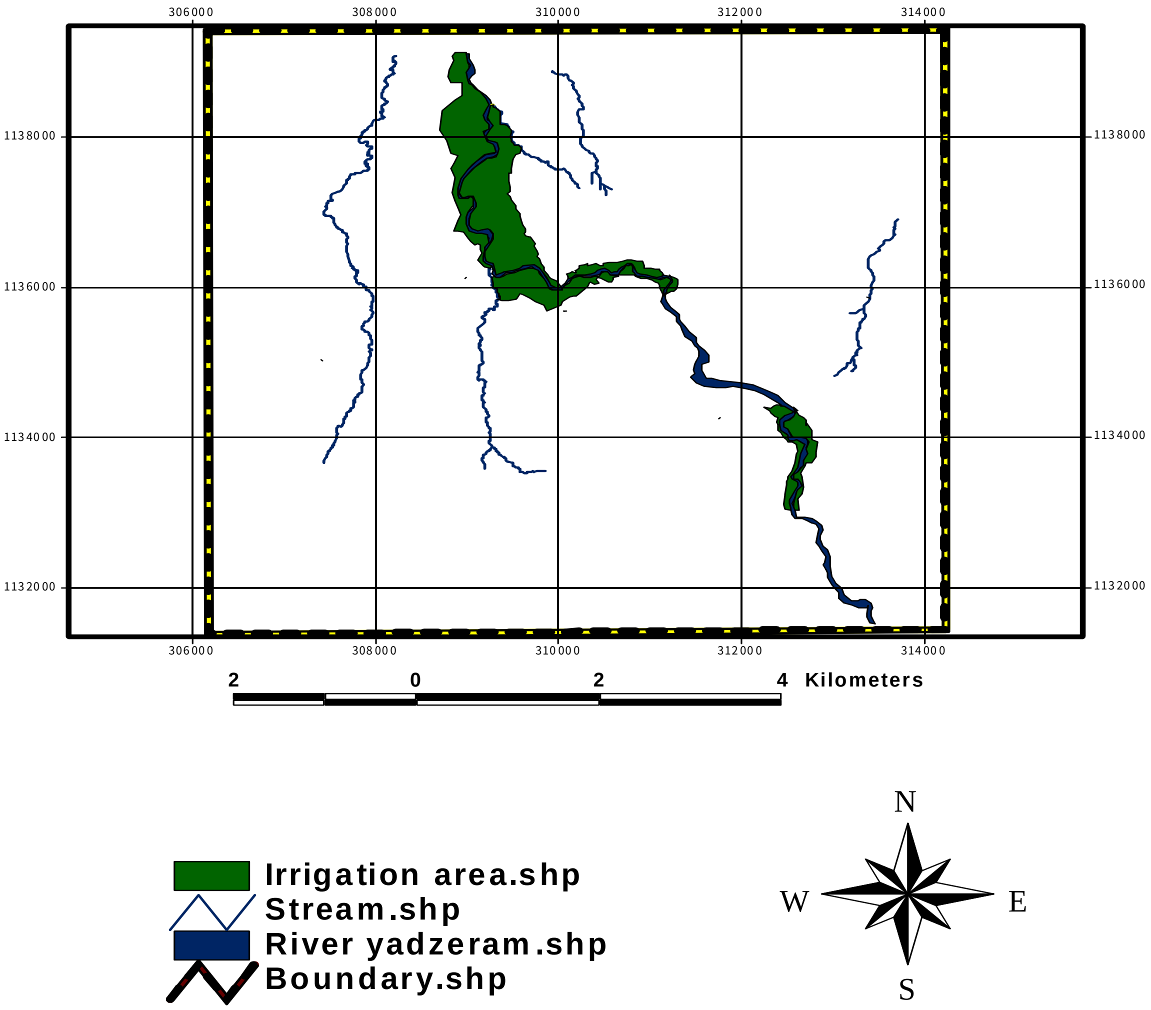

Figure 1: River Yadzeram Showing Irrigation Area

Sample and sampling

Sample Collection:

The study area supplies most of the vegetables consumed in Mubi metropolis and surrounding villages. Edible portions of okra (*Abelmos-chusesculent L.*) spinach (*Amaranthus caudatus*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) sorrel (herbiscus saderifa), and waterleaf (*Talinum triagulare*) leafy vegetables were collected from the vegetable farms cultivated along the banks of river Yadzerem. Samples were also collected from experimental farm Department of Crop Science, Adamawa State University, Mubi. Gardens cultivated on a piece of land, irrigated with unpolluted water and without the applications of fertilizers, manures, herbicides and pesticides, to serve as the controls. Vegetable samples were collected from December, 2009 to May, 2010. During each collection, samples were randomly collected from Different plots and homogenized into composite samples in the three sample areas. Sample collections were carried out according to the methods described by (Radojevic and Bashkin, 1999) into cleaned new polyethylene bags and transported to the laboratory for analysis.

Vegetable samples preparations for Nitrate and Nitrite analyses:

Vegetable samples were cleaned to remove visible soil and then washed with tap water, and with distilled water several times, the vegetable was then cut into pieces of nearly uniform sizes to facilitate drying at the same rate. The sliced samples were then dried in an oven at104°C for 24 hours (12hours for two days) until they were brittle and crisp. The dried samples were mechanically ground into fine particles using clean mortar and pestle and sieved to obtain <2mm fractions. A portion (1g) of each of the sieved samples was taken separately in100ml polyethylene or glass bottles, 50mL of distilled water were added, capped and shaken for 60 minutes. The solutions were filtered and the filtrates made up to the marks in100mLvolumetric flasks (Radojevic and Bashkin,1999)

Determination of Nitrate (NO_3^-) and Nitrite (NO_2^-) Concentrations in the Vegetable Samples.

The determination of nitrate in each of the vegetable sample solutions was performed by using digital spectrophotometer (model 2000) at a wavelength of 543nm. The programme number selected for nitrate was (64Nitrate/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).The concentration levels of nitrate vegetable dry weight (μgg^{-1}) in the samples were calculated from: NO_3^- (μgg^{-1}) = C x V/W . Where; C is the concentration of NO_3^- in the sample (ppm), V is the total volume of the sample Solution (100ml) and W is the weight of the sample (1g). Nitrite concentration in the digested sample were similarly determined using the programme number for nitrite was 67Nitrite/N and different reagent .The reaction time was five minutes as against ten minutes in the case of former. Nitrite/Nitrogen (NO_2^-/N) was converted to ppm Nitrite (NO_2^-) by multiplying by 3.3 (conversion factor) (LaMotte, 2000). The concentration of nitrite (μgg^{-1}) in the samples were calculated using the relation NO_2^- (μgg^{-1}) = C x V/W

Data Analyses:

Data obtained were subjected to statistical tests of significance using the Student t- test and Analysis of Variance (ANOVA) at $p<0.05$ to test the pairs results in the okra (*Abelmos-chusesculent L.*) spinach (Amaranthus caudatus), lettuce (Lactuca sativa), cabbage (Brassica oleracea), sorrel (*herbiscus saderifa*) and water leaf (*Talinum triagulare*). That is, to assess significant variation in the levels of nitrate and nitrite in the vegetables. Probabilities less than ($p < 0.05$) were considered statistically significant.

All statistical analyses were done by SPSS software.

Results

The mean concentrations of nitrate and nitrite for all the different vegetable samples cultivated along the banks of river Yadzerem are presented in Tables 1and 2. Higher values of nitrate and nitrite were observed in spinach which range between 80.05 ± 1.50 - 121.40 ±1.80 and 49.45±2.00 - 81.12±1.80 respectively. Lower values of nitrate/nitrite were observed in okra.

Table 1: Show the Concentration (μgg^{-1}) of Nitrates in edible vegetable and cultivated along the banks of river Yadzerem, Mubi metropolis.

Vegetable samples	*Concentration of nitrate(NO_3^-) in µgg^{-1}*			
	Sample Site A	*Sample Site B*	*Sample Site C*	*Control Sample*
Spinach	$96.34^a \pm 4.20$	$109.90^a \pm 3.00$	$121.40^b \pm 1.80$	$80.05^c \pm 1.50$
Lettuce	$95.00^a \pm 2.28$	$93.00^a \pm 3.40$	$103.90^b \pm 1.73$	$63.00^c \pm 2.86$
Cabbage	$87.50^a \pm 2.66$	$85.30^a \pm 3.06$	$113.20^b \pm 2.29$	$64.25^c \pm 2.20$
Water leaf	$88.12^a \pm 1.12$	$86.15^a \pm 0.72$	$90.05^a \pm 0.59$	$60.85^b \pm 3.05$
Sorrel	$80.00^a \pm 4.42$	$86.00^a \pm 3.1$	$99.89^a \pm 3.13$	$67.96^b \pm 2.74$
Okro	$79.00^a \pm 1.20$	$86.00^a \pm 1.50$	$88.79^a \pm 2.19$	$56.93^b \pm 3.28$

The above values are means of replicate values (n = 5). Across a row, means with different Alphabets are statistically different (p<0.05)

Table 2: Show the Concentration (µgg^{-1}) of Nitrites in edible vegetable and cultivated along the bank of River Yadzerem, Mubi metropolis.

Vegetable Samples	*Concentration of nitrite (NO_2^-) in µgg^{-1}*			
	Sample Site A	*Sample Site B*	*Sample Site C*	*Control Sample*
Spinach	$60.96^a \pm 4.20$	$69.09^a \pm 3.00$	$81.12^b \pm 1.80$	$49.45^c \pm 2.00$
Lettuce	$63.98^a \pm 2.28$	$65.70^a \pm 3.40$	$73.90^b \pm 1.73$	$52.34^c \pm 1.56$
Cabbage	$44.93^a \pm 2.66$	$47.20^a \pm 3.06$	$53.10^a \pm 2.29$	$39.89^a \pm 2.13$
Water leaf	$56.70^a \pm 1.12$	$58.90^a \pm 0.72$	$60.85^a \pm 0.59$	$40.97^b \pm 1.95$
Sorrel	$58.68^a \pm 4.42$	$66.20^a \pm 3.21$	$59.0^a \pm 3.13$	$41.30^b \pm 2.05$
Okro	$54.90^a \pm 1.20$	$66.87^b \pm 1.50$	$68.93^b \pm 2.19$	$40.18^c \pm 3.00$

The above values are means of replicate values (n = 5). Across a row, means with different Alphabets are statistically different (p<0.05).

Discussion:

Table1 and 2, presents the concentrations of nitrate and nitrite in edible vegetable samples and equally compares the levels of the anions in the edible vegetables obtained in three different vegetable farm location(A,B and C) along the banks of river Yadzerem with their corresponding levels in the control samples. The results obtained indicated higher levels of nitrate and nitrite in spinach in all the vegetable samples and sampling sites which range between 80.05 ±1.50 to 121.40 ±1.80 and 49.45 ±2.00 to 81.12±1.80 for nitrate and nitrite in spinach respectively. In all the vegetable analysed, nitrates and nitrite were found to be higher in sample site C as compared to all the sampling sites. Statistical test of significance using ANOVA revealed that significant differences ($P<0.05$) between anions concentration levels in the edible vegetables obtained in three different sampling sites with respect to their corresponding levels in samples obtained from control site. These could be attributed to possible pollution taking place in the sampling sites as a result of various anthropogenic activities like different farm practices such as excessive use of wastewater in irrigating the soil and large scale use of fertilizers, manure, herbicides and pesticides in the areas compared to the result obtained the control sample. Salts of various kinds are found in most soils and many are essential to plant growth. However, some soils contain excessive concentrations of certain salts. Nitrate and nitrite contents of the soil may have contribute the level of nitrate and nitrites in the vegetable samples analysed. The result level of nitrates and nitrites in this study is lower than other studies carried out in Kano and Maiduguri (Akan *et al*, 2009 and Uwah *et al*, 2009) respectively.

Zhou *et al* (2000), reported that vegetables that are consumed with their roots, stems and leaves have a high nitrate and nitrite accumulation, whereas okra and those vegetables with only fruits as consumable parts have a low nitrate accumulation. This study agrees with what was reported by (Zhou *et al*, 2000 and Maynard *et al*, 1978). This observation was also noted by (Santamaria *et al*, 1999) where leaf and stem accumulate the most nitrate, and nitrite followed by stem and roots. Maynard *et al* (1978), in their study showed that nitrate and nitrite accumulation in vegetables, such as spinach and lettuce contains nitrate and nitrite at significant levels. They noted that plants that develop fruits or storage organs, such as potato and tomato, usually have low nitrate and nitrite concentrations. Nitrite content in vegetables is usually very low compared nitrate (Aworh *et al*, 1980).

The result of this study showed that the concentration of nitrates and nitrite were lower than studies carried out Maiduguri and Kano (Uwah *et al*, 2009 and Akan *et al*, 2009) respectively. The concentration of nitrate and nitrite in the vegetable studied agrees with the minimum limit recommended by WHO.

Conclusion

This study was able to determine the levels of nitrate and nitrite in some leafy vegetables obtained in Mubi metropolis. The vegetables investigated are not polluted with nitrate and nitrite despite the usage of fertilizers, manures, pesticides, herbicides and other agro/chemicals as well as the use of wastewater in irrigating the soils. The lower level of nitrate and nitrite in the study area did not indicate pollution but nitrate/nitrite may accumulate in soil with time.

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