Nig J. Biotech. Vol. 20 (2009) 21 – 27
ISSN: 0189 17131
Available online at http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Lead, Zinc and Nitrite Levels of Staple Crop Cultivars in Ameka and Abakaliki, Ebonyi State, Nigeria

***Nweke, F. N., Offor, E.C., Afiukwa, C.A and Oko, A.O.**

Department of Biochemistry/Biotechnology, Ebonyi State University,
P.M.B 053 Abakaliki, Nigeria,

Abstract

The levels of Lead (Pb) and Zzinc (Zn) were quantitatively determined in staple crop cultivars in Ameka using atomic absorption spectrophotometry. The survey was conducted to establish a base line pollution index for lead and zinc in Ameka environment and also to evaluate the role of foods as an exogenous source of these metals among the inhabitants. Crops harvested from the area were classified as cereals, tubers, leafy-vegetables, and fruits and analyzed. The recorded metallic mean values were Pb:0.03±0.01mg/g; 0.05±0.01mg/g; 0.17±0.03mg/g; 0.27±0.04mg/g and Zn: 0.38±0.05mg/g; 0.54±0.11mg/g; 0.80±0.21mg/g; 0.58±0.14mg/g respectively. Similarly, nitrite levels in vegetables and corn (maize) were determined spectrophotometrically in Abakaliki . The vegetables analyzed were spinach, garden egg leaf, bitter leaf, pumpkin and fluted-pumpkin leaf, while corn (maize) included yellow-corn, white-corn and pop-corn. Nitrite levels showed a decline from bitter leaf > pumpkin > garden egg leaf > spinach >fluted-pumpkin ($P<0.05$) with the highest and lowest concentrations of 4.92mg/g and 2.82mg/g found in bitter leaf and fluted-pumpkin, respectively. Yellow-corn and white-corn gave nitrite mean concentration of 3.52±0.30ppm and 2.80±1.50ppm, while 1.17±0.90ppm was obtained in pop-corn. Exceedingly low level of these metals characterized the staple crops. The concentration of lead in fruits and leafy-vegetables were below the 1ppm tolerable limit in plants.

Key words: Lead, Zinc, Nitrite, Crop Cultivars, Cereals, Tubers, Leafy vegetables and Fruits

**Correspondence: frinwalo2007@yahoo.com*

Introduction

Important staple foods worldwide include wheat, rice, maize, yam, cassava and potatoes. As staple foods, they serve in addition to other staple foods such as milk, to provide the minimum human requirements for nutrients, vitamins and minerals. Recently, people have been exposed to heavy metals in the environment. In areas with high lead and zinc contaminations of food and water, metallic poisoning usually occur. Metals leached from eating utensils and cookwares have contributed to this poisonings (Marschner, 1995).

The emergence of the industrial age and large-scale mining brought occupational diseases caused by various toxic metals (Orazulike, 1994). One of the main dietary sources of lead is from grains; whole grains contain high lead since fibrous seed coat retains minerals. Other dietary sources of lead are vegetables and meats. Lead enters the food chain from contaminated soil and Lead dust which accumulates on the plants. The Lead taken up by plant materials is eaten by animals which are indirect sources of lead uptake by humans (Watanbe *et al.*, 1989). Lead from atmosphere that settles on soil has low mobility and tend to stay in the top inch of the soil. Therefore shallow rooted plants such as grasses and common vegetable are particularly vulnerable to picking lead contamination that originated in the atmosphere (Watanbe *et al.*, 1989). In developing countries such as Nigeria, India, Pakistan, and China, the need to maximize food production is very essential. Therefore factors such as metal deficiency or toxicity that can prevent crops from attaining their potential yield need to be addressed (Stivastava and Gupta, 1996).

Nitrite is present naturally in soils, meats, plants and drinking water. However, under unfavorable conditions, nitrite may enter the food chain via microbial reduction of nitrate thereby endangering human health (Sebecic and Vedrina, 1998). Nitrate may be reduced to nitrite when cooking is carried out in aluminum utensils. This observation appears to be significant since some countries use aluminum utensils for boiling milk and water, a practice, which could lead to formation of sizeable quantities of nitrite (Corre and Breimer, 1979). Studies in Europe and in the Untied States have identified major factors in nitrite accumulation to include retard nitrate utilization by crop growth process and heavy application of nitrogen fertilizers. In either case, excess nitrate accumulates in the leaves and stems (Maynard *et al.,* 976).

The primary sources of this nitrite pollution are agricultural fertilizers. These are applied in a reckless and widespread manner as the rural dwellers and amateurish farmers are encouraged by governments to use fertilizer extensively in order to produce more food. It is believed that over 50% of applied fertilizers used by ignorant farmers must have been washed away to pollute farmlands (Schippers, 2000). Nitrite can be absorbed by various routes which include oral foods and drinking water; the body can inhale nitrite from dust and fertilizers. Regardless of route of exposure, nitrite is rapidly transferred into the blood where they exert their toxic effect like methaemoglobinaemia, tachycardia, cynosis, and anoxia. (Shokrzadeh *et al.,* 2007) The aims of this study were:

(a) To assess the extent of lead and zinc accumulation in crop cultivars in Ameka which is a mining environment.

(b) To assess the nitrite level of corn-maize and vegetables in Abakaliki.

Materials and methods

Collection of Crop Sample and Treatment: The food crops were harvested fresh from farmlands in Ameka village. The crop samples collected included tubers (yam and cassava), cereal (rice), leafy-vegetable (fluted-pumpkin) and fruits (orange). Ten (10) different samples of each cultivar were randomly collected. Food crops like yam, cassava, and unshelled rice, which have coats, were peeled or unshelled. All samples were subsequently dried in electric oven at 110 ^{0}C for 12 hours. The dried food samples were then ground into powder in a mortar. About 0.5g of each ground sample was weighed into a boiling tube and made up with 50ml of a digestion mixture (which comprised perchloric acid and concentrated nitric acid, in a ratio of 1:2). It was swirled and kept in the fume cupboard overnight. Samples were further digested at the temperature of 150 ^{o}C on a hot plate for 2 hours or until frothing ceased, and cooled for 10 minutes. Approximately 30ml of 6.0M hydrochloric acid was added to the cooled tubes and subsequently digested for 1.5 hour. The tubes were then removed from the hot plate, allowed to cool and the contents of each made up to 250ml with distilled water. The resulting solution was analyzed with atomic absorption spectrophotometer (AAS).

Collection of Corn-maize and Vegetable Sample: Vegetables which include Bitter-leaf, Spinach, Garden-egg leaf, Fluted pumpkin leaf, and Pumpkin leaf and three (3) species of corn-maize, White-corn, Yellow-corn, and Pop-corn were collected from local farmland along Ebonyi River in Abakaliki, Ebonyi State. Ten (10) samples of each type were collected giving a total of eighty (80) samples

Preparation of Vegetable and Corn-maize Samples: All the vegetables were washed in clean water and oven dried at 20°C for 30mins and homogenized. Well homogenized sample (3g) were weighed; 60ml of distilled water was added and heated on a water bath for 15mins at 60-70°C.It was allowed to cool at 15-25°C, filtered and clarified using activated charcoal. The corn-maize were ground into powder after drying, 12.5g of each sample was suspended in 150ml of distilled water and filtered.

Principle and Analysis of Nitrite: Nitrite reacts with sulphanilamide and N-(1-naphthyl)-ethylene-diamine dihydrochloride to give a red-violet diazo dye. The diazo dye is measured on the basis of it's absorbance in the visible light range at 540nm. For determination of nitrite, 5ml of each sample were pipetted and 5 drops of reagent A and B of the nitrite kit was added, shaken and allowed to stand for 20mins. The absorbance was read at 540nm in spectrophotometer. Nitrite concentration was extrapolated from the nitrite standard curve.

Results

The results obtained from the analysis of lead (Pb) in crop cultivars are shown in the figure 1

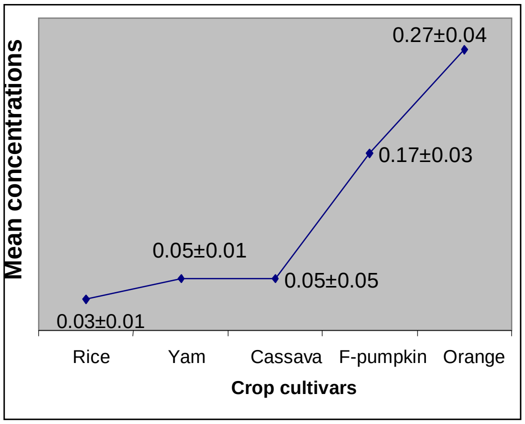

Fig. 1. Mean values of lead in crop cultivars

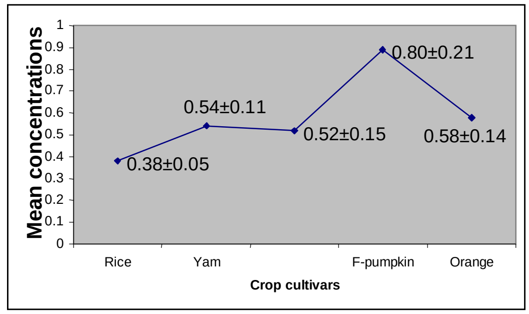

Fig 2 Mean values of zinc in crop cultivars.

The cereal (rice) and tuber (yam) recorded mean concentrations of 0.03±0.01mg/g and 0.05±0.01mg/g respectively, while leafy-vegetable (fluted-pumpkin) and fruits (orange) gave 0.17±0.03mg/g and 0.27±0.04mg/g. The mean concentrations of zinc (Zn) obtained are shown in figure 2. The cereal (rice) and tuber (yam) gave 0.38±0.05mg/g and 0.54±0.11mg/g, while 0.80±0.21mg/g and 0.58±0.14mg/g were recorded in leafy-vegetable (fluted-pumpkin) and fruit (orange), respectively.

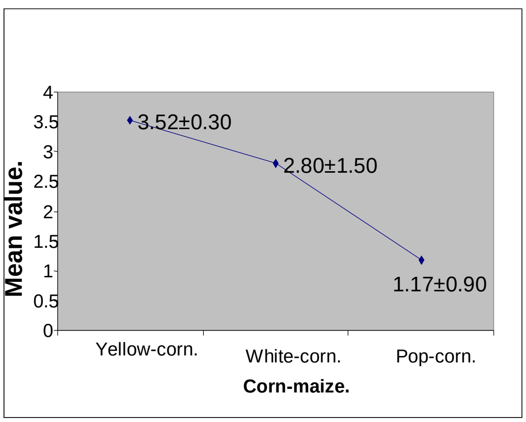

Fig. 3. Mean valves of nitrite in corn-maize

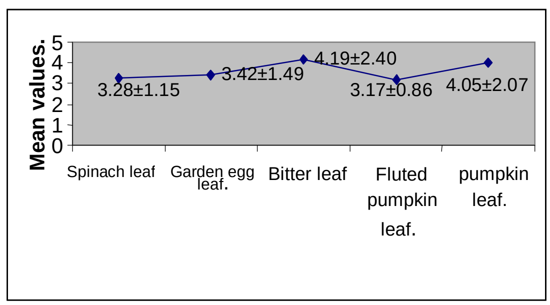

Fig.4.Mean values of nitrite in vegetables

Figure 3 represents the mean value of nitrite in corn-maize. It showed a decline in order yellow corn > white corn > pop-corn with highest and lowest mean values of 3.52±0.30 mg/g and 1.17±0.90mg/g obtained in yellow-corn and pop-corn, respectively ($p < 0.05$).

The mean concentrations of nitrite obtained in vegetables are shown in figure 4. Occurrence of nitrite in vegetables showed a decline in the following order bitter leaf > pumpkin leaf > garden egg leaf > spinach > fluted pumpkin. The highest and lowest mean concentrations of 4.19±2.40mg/g and 3.17±0.86mg/g were found in bitter leaf and fluted pumpkin ($p<0.05$), respectively.

Discussion

Staple foods of this nation are made from legumes, cereals and tubers. Maize of the genus Zea, especially the *Zeamays* sub-species described as corn in many countries of the word is important cereal grain, providing nutrients for humans and animals and serving as a basic raw material for industries. This work was done at Ameka village and Abakaliki local government area to assess the extent of contamination of staple crop cultivars. A total of one hundred and thirty (130) samples were analyzed for the presences of nitrite and some heavy metals using spectrophotometry and atomic absorption spectrophotometry (AAS), respectively.

The results obtained supports the submission of Igwegbe *et al* (1992) to the effect that metals uptake by leafy-vegetables are usually more than those of tubers, legumes and grains. This observation justifies the recommendations that fresh vegetables should be used for anaemic patients who require metal supplement (Brown *et al*., 1993). The 0.17mg/g and 0.27mg/g concentrations of lead obtained in leafy-vegetable and fruit in these food crops did not exceed the 1ppm plants tolerable limit proposed for 2007-2011 (WHO, 2000). Higher concentrations of lead and zinc were found in fruits (orange), leafy-vegetables (fluted-pumpkin) and on the surface of root crops (Figs.1&2)

Generally, it has been considered safe to use garden produce grown in soils with total lead level less than 150mg/g. However, Enyigba soil was reported to contain between 205 and 251mg/g of lead (Nweke *et al.,*2007). The risk of lead poisoning through the food chain increases as the soil lead level rises above this concentration (Chaneny, 1980). Even at soil levels above 150mg/g, most of the risk is from lead contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant. All the plants analyzed recorded 0.03-0.27mg/g and 0.38-0.80mg/g mean concentrations of lead (Pb) and zinc (Zn) respectively. The study confirmed that Enyigba village had high lead (Pb) and zinc (Zn) because of the lead-zinc ore deposits and the on-going mining activity. Plants are capable of absorbing extra metal; some plants like fruits and leafy-vegetables naturally absorb far more metal than others.

Similarly, some of the corn-maize and vegetables grown within Abakaliki are mainly near Ebonyi River, streams or in swampy areas. The vegetables and corn-maize were collected at their various stages of life cycle and dried to ease analysis. Nitrite concentrations of corn-maize and vegetables analyzed from the local farms in Abakaliki, Ebonyi State were found to be high. The joint Expert committee on food additives (JECFA) of the Food and Agricultural Organization of the United Nations/World Health Organization and the European Commission's Scientific Committee on food has set an acceptable daily intake (ADI) for nitrite of 0-0.07mg nitrite ion/kg body weight. EPA has set an RFD of 0.1mg nitrite nitrogen/kg body weight per day (equivalent to 0.33mg nitrite ion/kg body weight per day)(Agency for Toxic substances and Disease,2007).

The 3.52±0.30mg/g and 4.19±2.40mg/g found in corn-maize and vegetables may accumulate in the body of the consumers above the recommended limit depending on the rate of consumption and accumulation. These values were also higher than the 0.33-3.48mg/g found in fruits and vegetables in Onitsha metropolis (Ezeonu and Okaka, 1992). Excess dietary nitrite may cause a life-threatening heamoglobin malfunction in infants, and may be converted to carcinogenic nitrosamine during digestion, posing a possible cancer risk for all ages. Although the extent of this cancer risk is unknown, vegetables are the main source of dietary nitrite and researchers agree that efforts should be made to lower nitrite levels in leafy vegetables (Mark, 1988).

Intensive agricultural activities with fertilizer applications above the aquifer area practiced annually were responsible for high nitrite level in these staple crops. During precipitation, some of the fertilizers are washed off as part of runoff into streams, lakes, rivers and farmland. Their NO_2 concentrations are thereby raised to pollution levels. The rural poor people also consume these waters. Some of the NO_3 is leached into the soil zone where it may be taken up by plants through soil water. Even if the excess water may be evaportranspired, it is possible that the vegetables or fruits trees may concentrate the NO_3 in their system to be consumed later by humans.

Conclusion

Heavy metal status of crops is easily assessed by plant analysis and it is important for farmers to investigate crops in areas with highly susceptible soil especially where poor yields are obtained without other obvious explanations such as drought or disease. The crops cultivated at Ameka had some amount of these trace metals which may be unhealthy for the inhabitant of the area in future. The high nitrite level obtained may be as a result of the following:

Vegetable and corn-maize nitrite levels are due to the rate and type of nitrogen fertilizers applied and by soil nitrification activity, soil texture and harvest time. Water that was utilized by plants in this area was from the flowing Ebonyi River unlike other areas that use well water for irrigation.

Recommendations

(1) Vegetables and maize known to have very high nitrate/nitrite content should be avoided in baby food preparation.

(2) Fertilizer and irrigation practice that produce vegetable with low nitrite content compatible with optimum yield must be developed

(3) A more comprehensive environmental health examination, screening and monitoring will be required to reveal the occupational and environmental hazard to which these communities will be exposed in future.

The State Government is hereby advised to undertake a comprehensive survey on the health status of the occupationally exposed inhabitants of the state.

References

Agency for Toxic substances and Diseases (2007). Nitrate/nitrite toxicity C.D.C Revised Edition GA 30341 Atlanta. 1-3.

Angelone, M. and Bini, C. (1992). Trace element concentrations in soils and plants of Western Europe. Biogeochemistry of trace metals 1st Edition, Lewis publishers, Boca Raton, 20-24.

Brown, P. H., Cakmak, I. andZhang, O. (1993). Form and function of zinc in plants, 1st Edition, Kluwer Academic publishers, Dordrecht, 96-100.

Chaneny, R. C. (1980). Spring seminar on sludge management, Washington, D.C. 19:28-30.

Choudlary, M.., Balley, L. D., Grant, C. A. and Leisle, D. (1995). Effect of zinc on the concentration of Cd and Zn in plant tissue of two durum wheat lines. *Canadian Journal of plant sciences*, **75**:445-448.

Corre, W. J .and Breimer, T. (1979). Nitrate and nitrite in vegetables 2nd Edition Center for Agricultural publication and documentation, Wageningen,Netherlands, 430-445.

Ezeonu, F. C. and Okaka, A. N. C.(1992). Nitrate and nitrite in fruits and vegetables. *An international journal of environmental toxicology* **11**:1-5.

Igwegebe, A..O.,Belhaai, H. M. and Gibali, A. S. (1992). Effect of a highways traffic on the level of lead and Cadmium in fruits and vegetables grown along road sides, *Journal of food safety*, **13**: 7-10.

Mark, S. (1988). Nitrate in winter greenhouse leafy vegetables New Alchemy quarterly, newly Alchemy Institute Inc. **33**:1-4.

Marschner, H. (1995). Mineral nutrition of higher plants, 2nd Edition, London, 887-889.

Maynard, D. N..,Baker, P. L. and Minotti, N. H. (1976). Nitrite accumulation in vegetables, *Advances in Agronomy, **28**:30-35.*

Nweke , F. N., Okaka, A. N. C. and Offor, E. C. (2007). Lead, Zinc and pH concentrations of Enyigba soils in Abakaliki local Government Area of Ebonyi State, Nigeria*, African Journal of biotechnology,* **6**: 622-625.

Orazulike, D.M. (1994). The mineralogy and texture of lead-zinc-copper ores of Enyigba lodge, Abakaliki, *Journal of mining and geology*, **30**: 25-32.

Schippers, R. R. (2000). African indigenous vegetables An overview of the cultivated species, 1st Edition WRI Catalogue services, UK, 9-11.

Sebecic, B. and Vedrina I. D .(1998). Nitrate and nitrite in vegetables from areas affected by war-time operations in Croatia, *Journal of foods and drug,* **3:1**-4.

Shokrzadeh, M., Shokrvie, M., Ebadi, A, G., Babaee, Z. and Tarighati, F. (2007). The measurement of nitrate and nitrite content in leek and spinach and corn-maize sampled from central cities of mazandara state of Iran, *Journal of world Applied Sciences,* **2**:121-124.

Stivastava, P. C. and Gupta, U. C. (1996). Trace element in crop production, 1st Edition, Science publishers, Lebanon, 355-556.

Watanbe, J., Nakatuka, H. and Ikeda, M. (1989). Cadmium and lead contents in rice available in various areas of Asia, *Science of total environment,* **80**:175-184.

WHO. (2000). Effects of organo-halogen compound. Plenum press New York, 234-255, 258-269.