



Copper, Iron and Zinc concentrations of tobacco leaves and ready-to-use snuff products on sale in Imo State Southeastern Nigeria.

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ABSTRACT: This paper reports the results of the determination of the concentrations of Zn, Cu, and Fe in tobacco leaves and snuff products on sale in some regional markets in Imo State, Southeastern Nigeria. The samples were ashed at 550°C, mineralized with acid and the metal concentrations measured using flame atomic absorption spectrophotometer. The mean metal concentrations are $843 \pm 46 \text{ mg kg}^{-1}$ for Fe, $79 \pm 4 \text{ mg kg}^{-1}$ for Zn, and $22 \pm 2 \text{ mg kg}^{-1}$ for Cu in snuff samples and; $951 \pm 78 \text{ mg kg}^{-1}$ for Fe, $83 \pm 2 \text{ mg kg}^{-1}$ for Zn, and $24 \pm 2 \text{ mg kg}^{-1}$ for Cu in tobacco leaves. Comparably, higher metal concentrations were obtained in tobacco leaves than in the snuff products, and the metal concentrations varied according to the regional markets from where they were purchased. The results of this study indicate that snuff products could constitute trace metal exposure routes to users. © JASEM

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KEYWORDS: snuff; trace metals; tobacco; toxicology; Nigeria

INTRODUCTION

The global consumption of tobacco products, especially cigarette, has been on the increase. Tobacco is an herbaceous plant that contains nicotine and is native to North and South America, Australia, southwest Africa, and the South Pacific. Tobacco stands for any of various plants of the genus *Nicotiana* of the nightshade family (Solanaceae) as well as the leaves of several of these plants, which are processed for smoking, chewing or snuffing (Ali et al., 2006). *Nicotiana tabacum* is the cultivated tobacco plant most commonly used to make commercial tobacco products. Nicotine [3-(1-methyl-2-pyrrolidiny)-pyridine, $C_{10}H_{14}N_2$] is a highly toxic liquid alkaloid found naturally in several plant species, including *N. tabacum*, and is the key addictive component of tobacco products.

Nicotine is used to make consumer products such as cigarettes, cigars, pipe tobacco, chewing tobacco, and snuff. Cigarettes represent small rolls of cut tobacco wrapped in paper for smoking while cigars consist of rolls of tobacco leaves for smoking. The use of oral and nasal Smokeless Tobacco Products (STPs) including snuff, is common worldwide, but is highest on the Indian subcontinent (Ayo-Yusuf and Connolly, 2011).

STPs are pulverized or shredded tobacco which are chewed or placed between cheek and gum or inhaled in small pinches of about 0.1 g amounts through the nostrils. That means that STPs do not have to be lit as

opposed to other tobacco products (Ali et al., 2006). Snuff usually consists of tobacco that is ground and mixed with salts, flavors (such as peppermint oil, eucalyptus oil, menthol, or fruit extracts) and moisturizing agents. Snuff is "snuffed" or inhaled (insufflated) through the nose either directly from the fingers or by using specially made aids referred to as "snuffing" devices.

The role of tobacco and tobacco smoke as causative agents for a wide variety of cancers, cardiovascular and respiratory diseases has been well established (IARC, 1986). To date, more than 3000 chemicals have been identified and isolated from tobacco (Roberts, 1998; WHO, 2004). More than 1000 of the chemical constituents present in unburnt "whole" tobacco are also present as emissions in tobacco smoke (Hammond and O'Connor, 2008). The extent to which smokeless tobacco endangers human health is an ongoing subject of debate. There has been increasing health concern over the consumption of STPs (Evangelou et al., 2006). The most common adverse effect of snuff is the lesion in the oral cavity. Snuff with high pH and nicotine content produce more damage. The lesions are mostly reversible when snuff use is stopped.

To a small extent, heavy metals enter the body through food, drinking water and air. As trace elements, some heavy metals e.g. Cu, Zn, etc are essential to human body. However, at higher concentrations, they can lead to poisoning. Heavy

metals are dangerous because they tend to bioaccumulate. Tobacco products including snuff has been shown to contain some heavy metals and Interest in the trace metal concentrations of tobacco products have been on the increase (Mishra and Shaikh, 1985, 1986; Mishra et al., 1986; Shaikh et al. 1992; Shaikh et al., 2002; Addo et al. 2008; Dhaware et al. 2009; Pappas et al. (2008). Other studies have also investigated other toxic components of tobacco especially the tobacco specific nitrosamines (TSNA) (McNeill et al., 2006; Pakhale and Maru, 1998). Toxic metals in smokeless tobacco products have been less extensively studied (Pappas et al., 2008).

Studies into the trace metal contents of tobacco products have been focusing more on cigarettes (Zawadska et al. 1989; Jung et al., 1998; Spiff et al., 1999; Shaikh et a., 2002; Nnorom et al., 2005; Pappas et al., 2007) and cigarette/tobacco smoke (Mishra and Shaikh, 1985; Mishra et al., 1986; Schneider and Krivan, 1993; Shaikh et al. 1992; Shaikh et al, 2002; Pakhale and Maru, 1998; Pappas et al., 2007; Hammond and O'Connor 2008). Studies of snuff products have also been increasing in recent times (Mishra and Shaikh 1986; Brunnemann and Hoffmann, 2002; Addo et al., 2008; Pappas et al., 2008). Toxic metals in tobacco products are either from the tobacco leaves or as contaminants introduced during either the pre-harvesting treatment (e.g. from fertilizers and pesticides) or from the post-harvest additives (e.g. from preservatives) (Iskander, 1986).

Relevant data about trace metal contamination of tobacco leaves and the processed snuff products from Nigeria and the attendant health implications of human exposure to metal contaminants in these products are still limited and further studies need to be conducted. The objective of this study is to determine the concentrations of Fe, Zn and Cu in tobacco leaves and locally processed snuff products marketed in Imo State, southeastern Nigeria.

MATERIALS AND METHODS

Sample collection: Thirty samples of tobacco leaves and thirty locally processed snuff samples marketed in some regional markets in six Local Government Areas (LGAs) of Imo State, Southeastern Nigeria were used in this study. The tobacco leaves and snuff samples were purchased from local snuff sellers in different market locations in six LGAs in Imo State in July 2008. The LGAs are Owerri Municipal LGA, Owerri North LGA, Owerri West LGA, Okigwe LGA, Ikeduru LGA, and Mbanjo LGA. The markets are New Market Owerri, Afor Obazu Mbieri, Ekenta Ikeduru, Nkwo Market Nekede, Afor Ogbe Market,

Abakpa Market Okigwe, Orié Mbieri Market, Eke Obinze Market, Eke-Atta Ikeduru, Afor Egbu Market, Afor Emmi Market, Eke Okigwe Market, Eke Onunwa Market, Nkwo Irete Market, Nkwo Orodo Market Owerri, Oriekwu Umuguma Market, Avefe Market Owerri West, and Oriéagu Mbanjo.

One foreign brand of processed snuff was also purchased and investigated. On purchase, the samples were adequately labeled and transported to the laboratory.

Sample analysis: 1.0g of the sample was placed in a porcelain crucible and dry-ashed in a muffle furnace at 550°C for about 8 hours (until a white or grey ash residue was obtained), cooled in a desiccator and then wet digested. The wet digestion was performed by adding 5ml of conc. HNO₃ (69%, BDH Laboratory Supplies, Poole, England) to each sample and heating to near dryness on a heating mantle. Where necessary, additional acid was added, and heating continued until evolution of white fumes. The solution was allowed to cool, then filtered into a calibrated flask (10ml) and made up to volume with deionized water. Blanks were inserted at 10% insertion rate. The Fe, Zn, and Cu concentrations were determined using Buck 211 Atomic Absorption Spectrophotometer. The following quality assurance procedures and precautions were applied to ensure reliability of results: Samples were carefully handled to avoid contamination; all chemicals used were of analytical grade; all plastic-and-glassware were washed, rinsed severally with tap water, and then soaked in 5% HNO₃ (69%, BDH, Poole, England) solution for a minimum of 24 hours. They were rinsed severally with deionized water before use.

RESULTS AND DISCUSSION

The Fe, Zn and Cu concentrations of the tobacco leaves and snuff samples investigated are presented in Figures 1-3 respectively. The only foreign brand studied is indicated as FB (Foreign Brand) in Figures 1-3. Comparisons of the mean metal concentrations of the samples according to the regional markets are presented in Figure 4 for Fe, Figure 5 for Zn and Figure 6 for the Cu concentrations. The summary of metal concentrations for samples investigated is presented in Table 1. Comparably, higher metal concentrations were obtained in tobacco leaves than in the processed snuff products (Figure 1-3; Table 1). In the production of nasal snuffs, tobaccos are blended and milled in a high speed mill to produce very fine powder. The powder is moistened, fermented and matured in a cool storage room for about three to four weeks. After final blending, flavorings are added as well as moistening agents

such as glycerol or paraffin oil (Ali et al., 2006). Consequently the use of these additives which naturally do not contain significant levels of these metals in the snuff products may contribute to the lowering of the metal concentrations in the finished product.

The Fe concentration of the only foreign brand (FB) investigated (1961 mg kg^{-1}) was more than twice the mean Fe concentration of the locally processed snuff (843 mg kg^{-1} ; range, $678\text{-}919 \text{ mg kg}^{-1}$). The Zn and Cu concentrations of the FB (indicated as FB on Figures 2 and 3 respectively) were comparable to the data obtained for the locally processed snuff products. The Zn concentration of the FB was however moderately lower (67 mg kg^{-1}) than the mean value for the locally processed snuff product (79 mg kg^{-1} ; range, $67\text{-}84 \text{ mg kg}^{-1}$) whereas the Cu concentration of the FB was slightly higher (25 mg kg^{-1} for FB and 22 mg kg^{-1} for local snuff products).

Wider variations were obtained in the Fe concentrations of both the tobacco leaves and the finished snuff products as indicated by the very wide range and high standard deviation for Fe compared to the other metals (Table 1).

As shown in Figures 4-6, moderate variations exist in the contents of trace metals for both tobacco leaves and snuff samples according to the regional markets sampled. The mean metal concentrations obtained in samples from different regions (LGAs) vary thus: the mean metal concentration of tobacco leaves according to LGAs ranged from $906 \pm 58 \text{ mg kg}^{-1}$ (Mbano LGA) to $1008 \pm 36 \text{ mg kg}^{-1}$ (Okigwe LGA) for Fe; $23 \pm 3 \text{ mg kg}^{-1}$ (Ikeduru LGA) to $26 \pm 3 \text{ mg kg}^{-1}$ (Owerri Municipal LGA) for Cu and $82 \pm 1 \text{ mg kg}^{-1}$ (Owerri West LGA) to $84 \pm 2 \text{ mg kg}^{-1}$ (Owerri Municipal LGA) for Zn (Figures 3-5).

For snuff products, the mean concentration according to LGAs ranged from $818 \pm 96 \text{ mg kg}^{-1}$ (Mbano LGA) to $855 \pm 39 \text{ mg kg}^{-1}$ (Owerri West LGA) for Fe; $20 \pm 4 \text{ mg kg}^{-1}$ (Ikeduru LGA) to $23 \pm 2 \text{ mg kg}^{-1}$ (Owerri Municipal LGA) for Cu and $76 \pm 3 \text{ mg kg}^{-1}$ (Ikeduru LGA) to $81 \pm 2 \text{ mg kg}^{-1}$ (Okigwe LGA) for Zn (Figures 3-5). The Fe, Zn and Cu concentrations in the foreign brand (English snuff) investigated were 1961 mg kg^{-1} , 67 mg kg^{-1} and 25 mg kg^{-1} respectively (Figures 1-3: indicated as FB). This indicates that the foreign brand contained much higher Fe concentration but lower Zn concentration. Various studies have shown tobacco to be a rich source of heavy metals. The occurrence of metals in tobacco is largely attributed to the growth and cultivation of tobacco, as tobacco is known to readily accumulate

metals from underlying soil (Schneider and Krivan, 1993; Golia et al., 2007; Tso, 2011). Consequently, the metal content of tobacco reflects the metal contents of the soil and therefore the mineral composition of the bedrock from which the soil was derived. The use of chemicals in the form of fertilizers, herbicides etc in the cultivation of tobacco may also contribute to the metal load of the soil and subsequently, the tobacco plant. Pakhale and Maru (1998) observed that plant variety, cultivation, curing methods, and designs of the smoking products (including wrapper and the presence/absence of filters differing in efficiencies) are factors that influence the formation and yields/levels of toxic chemicals in tobacco and tobacco smoke (both mainstream smoke (MS) as well as side-stream smoke).

Tobacco constituents are also modified during the curing process. For instance, tobacco specific nitrosamines (TSNAs) form primarily during the curing process and levels vary according to the curing method (Hammond and O'Connor, 2008). In fact, Pakhale and Maru (1998) observed that besides differences in plant variety and cultivation, a possible reason for significant difference in the levels of alkaloids in tobacco may be curing methods, such as sun-/air-drying of bidi/chutta tobacco while flue-curing for cigarette tobacco. N-nitrosamines are considered major contributors to the carcinogenic activity of oral snuff (Brunnemann et al., 2002). Studies also noted that flue-cured tobacco contains relatively high levels of sugars naturally present in the tobacco, produced from tobacco starch during the flue-curing process (Leffingwell, 1999; Baker et al., 2004).

The summary of metal concentrations in the tobacco leaves studied was $951 \pm 78 \text{ mg kg}^{-1}$ (range, $834 - 1164 \text{ mg kg}^{-1}$; median 961 mg kg^{-1}) for Fe; $83 \pm 2 \text{ mg kg}^{-1}$ (range, $79 - 87 \text{ mg kg}^{-1}$; median 83 mg kg^{-1}) for Zn; and $24 \pm 2 \text{ mg kg}^{-1}$ (range, $21 - 29 \text{ mg kg}^{-1}$; median 24 mg kg^{-1}) for Cu (Table 1). The summary of metal concentrations in the snuff samples was $843 \pm 46 \text{ mg kg}^{-1}$ ($678 - 919 \text{ mg kg}^{-1}$; median 843 mg kg^{-1}) for Fe; $79 \pm 4 \text{ mg kg}^{-1}$ ($67 - 84 \text{ mg kg}^{-1}$; median 79 mg kg^{-1}) for Zn; and $22 \pm 2 \text{ mg kg}^{-1}$ ($16 - 27 \text{ mg kg}^{-1}$; median 22 mg kg^{-1}) for Cu (Table 1). Based on the results of this study as reflected in the mean concentrations (Table 1), the trace metals contents of tobacco leaves and snuff products were arranged in the following decreasing order: $\text{Fe} \gg \text{Zn} > \text{Cu}$.

Toxicological Concerns of metals in tobacco: Literature abound with studies showing that the consumption of tobacco products by both smoking

and non-smoking ways affect the health of users directly as well as non-smokers via passive smoking (Stephens et al., 2005; Galazyn-Sidorczuk et al., 2008). A significantly increased risk for oesophageal and pancreatic cancer has been associated with oral snuff use in studies undertaken in the USA and northern Europe (Boffetta et al. 2008). Snuff inhalation has also been reported to be associated with nasal sinus and nasopharyngeal cancer in some parts of Africa (Feng et al. 2009). Tobacco use kills more than 5 million people per year and it is responsible for 1 in 10 adult deaths (WHO 2011). Among the five greatest risk factors for mortality, it is the single most preventable cause of death (WHO 2011). Studies have identified tobacco smoking as one of the significant sources of toxic metals in both human body and environment (Schneider and Krivan 1993; Verma et al., 2010). While the harmful health effects of carbon monoxide, nicotine, tar, irritants and other noxious gases that are present in tobacco smoke are well known, those due to heavy metals and other toxic mineral elements in tobacco smoke are not sufficiently emphasized (Chiba and Masironi, 1992). Studies have observed that users of STPs including snuff face no known cancer risk to the lungs but more of a risk in the oral region than smokers, and have a greater cancer risk than people who do not use any tobacco products (Boffetta et al., 2008). This is because the primary harm from smoking comes from the smoke itself (Phillips and Heavner, 2009). Consequently, snuff has been proposed as a way of reducing harm from tobacco (Phillips and Heavner, 2009). Unlike tobacco smoke, snuff is free of tar and harmful gases such as carbon monoxide and nitrogen oxides. Since it cannot be inhaled into the lungs, there is no risk of lung cancer, bronchitis, and emphysema (Russell et al. 1981).

From toxicological and environmental point of view, the determination of toxic metals in consumer products such as snuff and the raw materials has promoted interest. This is because the use of these products may be a route for human exposure to potentially toxic metals. Zinc is essential for biological functions of all living matter. Zinc is necessary for growth, appetite, testicular maturation, skin integrity, mental activity, wound healing and immunocompetence. Zinc is required for the metabolic activities of over 70 metalloenzymes (Abdel-Mageed and Oehme, 1990). Even though zinc is an essential requirement for a healthy body, excess zinc can be harmful, and cause zinc toxicity. Excessive absorption of zinc can suppress copper and iron absorption (Fosmire, 1990). Zinc is considered to be relatively nontoxic, particularly if taken orally. However, manifestations of overt toxicity symptoms

(nausea, vomiting, epigastric pain, lethargy, and fatigue) will occur with extremely high zinc intakes (Fosmire, 1990).

Copper is known to be an essential nutrient for human beings, but a Recommended Dietary Allowance has not yet been established (Turnlund, 1988). A safe and adequate range of intake was established in 1980 for copper with a range of 2 to 3 mg/day (Turnlund, 1988). Again, measurements of the dietary requirements for copper in adult men have shown the requirement to range from about 1.5 to 2.0 mg daily (Sandstead, 1982). Persons who consume diets high in zinc and low in protein are at risk of copper deficiency. High intakes of sources of dietary fiber apparently increase the dietary requirement for copper (Sandstead, 1982). Dietary factors that may affect the bioavailability of copper include the levels of copper, zinc, and molybdenum in the diet; iron deficiency; ascorbic acid intake; intake of carbohydrates, including fructose, glucose, and starch; and fiber and phytate intakes (Turnlund, 1988).

Comparison with Literature and review of Toxicological Concerns: Though studies of metal concentrations in snuff are limited, various studies of other tobacco related products have reported wide variation of heavy metals in cigarettes and other tobacco products (Elinder et al., 1983; Zawadska et al., 1989; Jung et al., 1998; Spiff et al., 1999; Ebisike et al., 2004; Nnorom et al., 2005; Pappas et al., 2007; 2008; Ajab et al., 2008; Iwegbue et al., 2009). Available literature indicates a detailed study of metal concentrations in tobacco products in use in India, including cigarette, cigars, biri, chewing tobacco, and snuff (Mishra and Shaikh, 1983; 1986; Mishra et al., 1986; Shaikh et al., 2002; Verma et al., 2010). Compared with documented studies of heavy metals in snuff products (Table 2), it was shown that the metal concentrations in the present study were similar to values reported in literature for tobacco and snuff products (Mishra and Shaikh, 1983; Mishra and Shaikh, 1986; Mishra et al., 1986; Shaikh et al., 2002; Verma et al., 2010). However, Jung et al., (1998) reported an average values of 7.7 mg kg⁻¹ for Cu, and 39 mg kg⁻¹ (range, 35 to 46 mg kg⁻¹) for Zn in Korean cigarettes; and 13 mg kg⁻¹ for Cu, and 31.9 mg kg⁻¹ (27 to 37 mg kg⁻¹ Zn) for Zn in cigarettes from the United Kingdom. Mussalo-Rauhamaa et al., (1986) reported that Cu in filter cigarette tobacco sampled from Finland between 1960 - 1980s averaged 16 mg kg⁻¹, with a range of 9.2 - 18 mg kg⁻¹. Other studies have also reported elevated Zn concentrations in tobacco from India (77-180 mg kg⁻¹) and America (15-31 mg kg⁻¹) (Iskander, 1992) (4.1-54 mg kg⁻¹). Nada et al. (1999) studied cigarette components

(tobacco, paper, ash) of two brands of cigarette and reported in the tobacco samples elevated Fe (range: $7859 \pm 79 \text{ mg kg}^{-1}$ - $2864 \pm 29 \text{ mg kg}^{-1}$) and Zn ($180 \pm 5 \text{ mg kg}^{-1}$ - $77 \pm 2 \text{ mg kg}^{-1}$) concentrations. Nada et al. (1999) suggested that the high concentrations of Fe and Zn among other metals determined may suggest that cigarette components are contaminated by dust and air particulate during shipping and aging of the raw materials or during the manufacturing process.

Conclusion: The major objective of this study was to investigate the concentrations of Fe, Zn and Cu in tobacco leaves and snuff products. Elevated Fe, Zn and Cu concentrations were observed in tobacco leaves and snuff samples marketed in selected regional markets in Imo state Nigeria. Trace metal concentrations in tobacco and snuff are related to the source: the regional markets. Higher concentrations of these metals were found in tobacco leaves compared to the snuff samples. These data suggest that local processing activities which may include the addition of additives which may not contain these metals in significant concentrations results in the lowering of the metal concentrations in the finished snuff products. Knowledge of trace metal contents of

products such as tobacco and snuff which are regularly used by the aged and elderly in southeastern Nigeria provides important information to the evaluation of trace metals available to the users who are highly vulnerable.

This study shows that the use of snuff marketed in Imo State, Nigeria could constitute a significant contributor of heavy metals intake in the consumers. The present work has demonstrated the need of establishing a national regulatory framework for the levels of trace metals in these products. There is also need for further studies to ascertain the forms of these metals in the tobacco products and to evaluate the potential risk to human from the use of these products. In snuff, the main exposure routes are ingestion and inhalation depending on the mode of use: oral application or sniffing. There is also minute risk of passive exposure in family members including the vulnerable groups: children, the aged and pregnant women. It is hoped that these results could serve as a guide in decision making and formulation of policies on the local production/preparation and consumption of snuff.

Table 1. Summary of trace metal content of tobacco leaves and snuff products (mg kg^{-1})

	Tobacco leaves			Snuff		
	Fe	Zn	Cu	Fe	Zn	Cu
Minimum value	834	79	21	678	67	16
Maximum value	1164	87	29	919	84	27
Mean	951	83	24	843	79	22
Standard deviation	78	2	2	46	4	2
Geometric mean	946	83	24	840	78	21
Median	961	83	24	843	79	22
Standard error	14	0.3	0.4	8.4	0.7	0.4

Table 2. Comparison of results of present study with literature (mg kg^{-1})

	Fe	Zn	Cu	References
Tobacco	1565	19	-	Mishra and Shaikh, 1986
(chewing tobacco)	1050	20	11	Mishra et al, 1986
	110	28	12	Shaikh et al, 2002
	1686-1720	12-25	-	Mishra and Shaikh, 1983
	853	56	42	Verma et al, 2010
	951	83	24	Present study
Snuff	2323	46	-	Mishra and Shaikh, 1986
	2090	43	21	Mishra et al, 1986
	-	42	28	Shaikh et al, 2002
	2972	37	16	Verma et al, 2010
	843	79	22	Present study

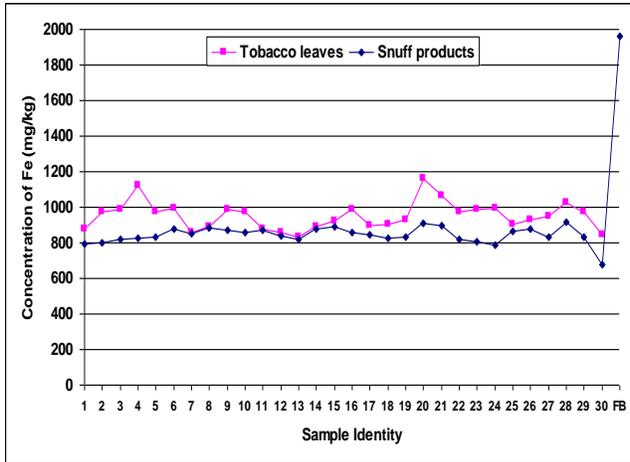


Fig 1. Fe concentrations in tobacco leaves and snuff samples

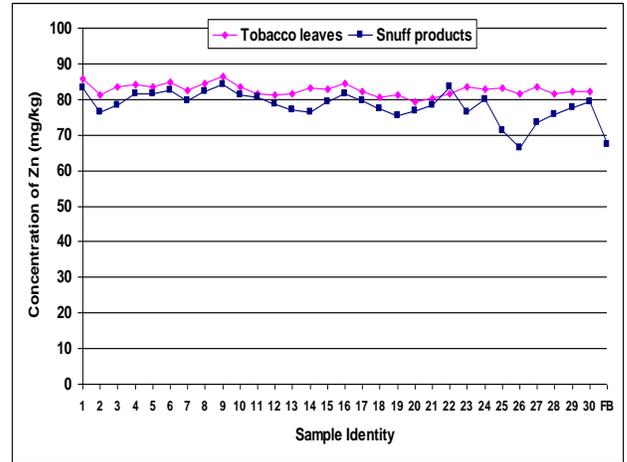


Fig 2. Zn concentrations in tobacco leaves and snuff samples

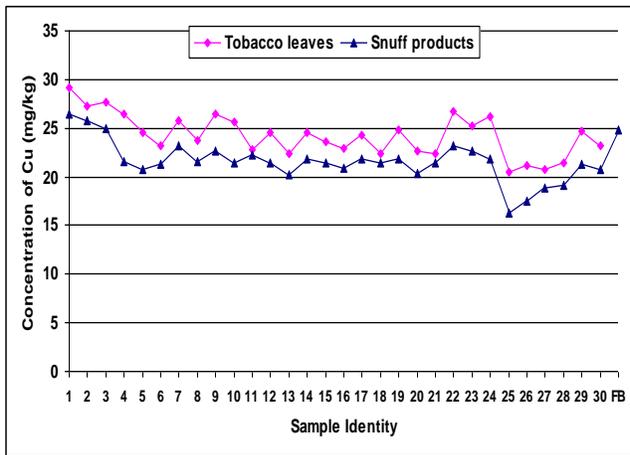


Fig 3. Cu concentrations in tobacco leaves and snuff samples

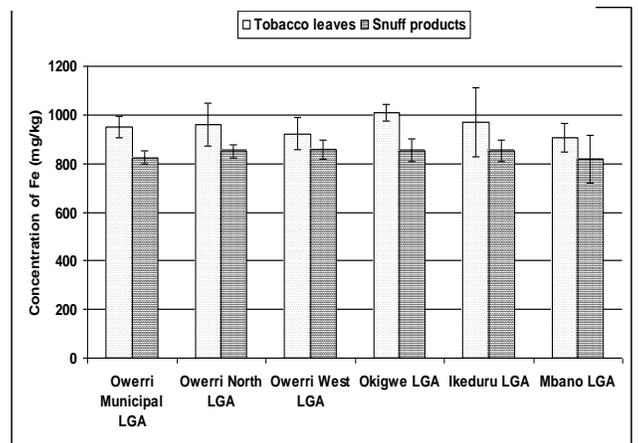


Fig 4. Comparison of Fe concentrations in tobacco leaves and snuffs

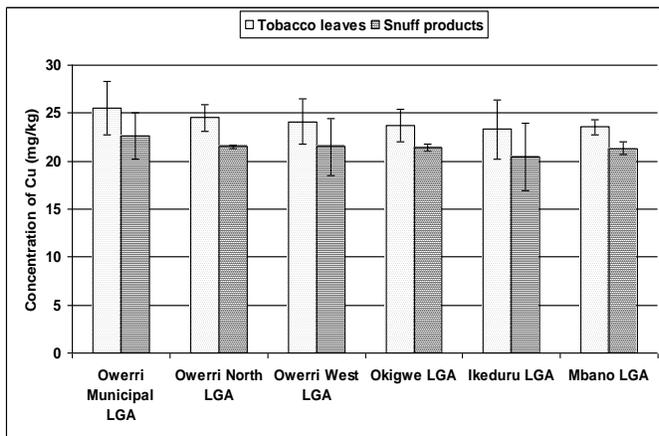


Fig 5. Comparison of Cu concentrations in tobacco leaves and snuffs

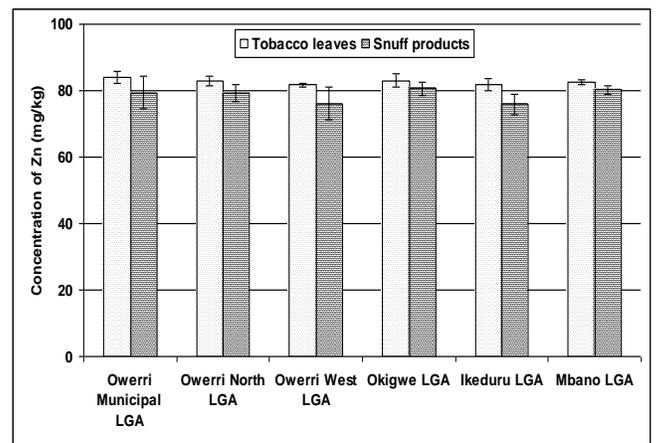


Fig 6. Comparison of Zn concentrations in tobacco leaves and snuffs

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