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POTENTIAL HEALTH RISK ASSESSMENT OF SELECTED HEAVY METALS, NITRATE AND NITRITE, IN SNUFF INHALED IN AFIKPO-NORTH OF EBONYI STATE, NIGERIA

Ajayi Adekunle Adebo[1*], Ngobidi Kerian Chigozie[2], Ezem Stella Nwakego[3],

Vining-Ogu Ibukun Caroline[4], Aja Oti Agha[5] and Danladi Garba Jeremiah[6]

[1]Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[2]Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[3]Chemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[4]Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[5]Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[6]Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[*]Correspondence to lead author: ajayiadekunlea@gmail.com

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ABSTRACT: *The analyses of the concentrations of selected heavy metals, nitrate and nitrite in smokeless tobacco were carried out to determine the potential health risk of their exposure. The pulverized snuff samples (prepared by unknown methods) were obtained randomly from vendors from Afikpo, Amasiri and Unwana, and then analyzed using standard analytical procedures. The results showed concentrations of Zn, Cu and Fe having significant ($p<0.05$) increase in snuff obtained from Afikpo, Amasiri and Unwana respectively compared to other metals. The concentration of nitrate showed a significant ($p<0.05$) increase in snuff obtained from the Afikpo compared to nitrite. All the values were lower than the maximum permissible limit and the THQ of these substances were less than 1. However, target hazard quotients of Cd were greater than 1 and higher than other metals. Therefore, these findings suggest that exposure to Cadmium in snuff (smokeless tobacco) might pose noncarcinogenic health risk.*

KEYWORDS: Tobacco, Nitrates, Snuff, Tobacco, Metals, Noncarcinogenic.

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INTRODUCTION

Smokeless tobacco (SLT) is a type of tobacco that is not burnt but consumed in raw form. It is also known as chewing tobacco, oral tobacco, or inhaled as snuff. It contains substances such as nicotine and other harmful cancer-causing chemicals (National Cancer Institute, 2010). The tobacco plant (*Nicotiana tabacum*) is widely known for its leaves, which are smoked, chewed, or sniffed for various effects. It is well documented that the addition of tobacco comes with the chemical nicotine, which can be harmful to humans. Tobacco contains reportedly about 19 carcinogens and at least 30 metallic compounds, comprising heavy metals (Atrens, 2001).[2]

Harmful effects on human health are associated with exposure to the heavy metals: Lead (Pb), Cadmium (Cd), Cobalt (Co) and Chromium (Cr) (Jarup, 2003), and the effects of these metals on humans have been reviewed regularly by international bodies, such as the World Health Organization (WHO). These heavy metals are found in the atmosphere as well as many man-made sources and mostly do not have any metabolic function, as such, in the body (Golia *et al*., 2007). In addition, the major groups of carcinogens in smokeless tobacco products (STPs) include nonvolatile tobacco-specific nitrosamines (TSNA), N-nitrosamine acids and other constituents (Shaikh *et al*., 2002). Nitrates have been known as a precursor for the formation of N-nitroso compounds (Mensinga *et al*., 2003). Nitrogen trioxide (N_2O_3), or in other words, a powerful factor of nitrous, is able to add nitrogen oxide (NO) to secondary and tertiary amines for the potential formation of carcinogenic nitrosamine compounds (Knekt *et al*., 1999).

Marketed STPs vary considerably in form and content of toxicants, including nicotine, and thereby present associated health effects, such as increased heart rate, an increased risk in pregnancy, increased premature foetal death, and SIDS (Sudden Infant Death Syndrome) (Rodu & Jansson, 2004).

The extent to which smokeless tobacco endangers human health is an ongoing subject of debate with prevailing health concerns. Thus, this study aims to analyze the presence of some heavy metals and some nitrogen compounds in smokeless tobacco in 3 locations in Afikpo North Local Government, Ebonyi State, in order to assess the possible health risk that could arise from its exposure.

MATERIALS AND METHOD

Samples Collection and Preparation

The smokeless tobacco analysed were the leaves of *Nicotiana tabacum* which were already pulverised and prepared by unknown methods by the vendors. About 50g of smokeless pulverised samples were randomly obtained from different locations, namely Eke Market, Afikpo; Orie Market, Amasiri; and Afor Market, Unwana. The samples were labelled and packaged in a tight container and stored for analysis in the biochemistry laboratory in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, South East Nigeria.

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Analytical Procedure

Heavy Metal Determination

Exactly 10g of each of the samples was weighed and transferred into 3 different digestion tubes. Then 10ml of the mixture of HCL and HNO_3 in the ratio 3:1 (i.e., 75ml of HCl and 25ml of HNO_3) was added into the digestion tubes and heated at 95°C for 4 hours till it turned colourless; then 50ml of deionized water was added to the solution, and then stirred and filtered. The filtrates however were used for the analysis and the acid mixture was used as blank. The determinations for Cadmium (Cd), Chromium (Cr), Zinc (Zn), Lead (Pb), Copper (Cu), Iron (Fe), and Manganese (Mn) were done using atomic absorption spectrophotometer (AAS) at respective wavelengths. Blank sample (made of acid mixture only) was prepared to zero the AAS, then series of samples were run on the instrument. Standards consisting of 2,4,6,8 ppm were prepared from 1000 ppm stock solution of the metals and values gotten were used to plot the calibration curve automatically by AAS. Concentration of each metal was then determined from the curve (AOAC, 2003).

Nitrate and Nitrite Determinations

The analysis for nitrate and nitrite were done spectrophotometrically according to the modified methods described by Ajebe and Bahiru (2018).

Determination of Estimated Daily Intake

The estimated daily intakes or the analysed metals were calculated by multiplying the respective mean concentration of the metal determined in each sample consumed by average individual in Nigeria.

$$EDI = \frac{\text{Concentration of metal in sample}}{\text{Body weight (70 kg)}} \times \text{Daily sample consumption}$$

Determination of Non Carcinogenic Risk (as described by Johann *et al.*, 2017)

Non carcinogenic risk assessments are typically conducted to estimate the potential health risk of pollutants using the target hazard quotient (THQ). TH values for the consumption of "food" by local inhabitants can therefore be assessed for each heavy metals. Calculations were made using standard assumptions for risk analysis.

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times BW \times TA} \times (10\ E\text{-}3)$$

EF = Exposure frequency (365 day/year). ED = Exposure duration (70 years), FIR = Food Ingestion rate (g/person./day) , C = metal concentration in samples (mg/kg), BW = Average body weight (adult – 70kg), TA = Average time for non carcinogens (365 day/year x 70 years), RFD = Inhalation reference dose

Statistical Analysis

The results obtained from the analysis were in triplicate and expressed as mean ± standard error of mean analyzed using International Business Machines Corporation (IBM) Statistical Product

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and Service Solution (SPSS) version 25.0. Analysis of Variance was used to determine multiple comparison and LSD post Hoc test. Mean was taken to be statistically significant at P<0.05.

RESULTS

The concentrations of selected heavy metals is shown in Table 1. Cadmium, Chromium and Copper have more concentration in samples obtained from Amasiri. Lead, Iron and Manganese were more abundant in samples that came from Unwana. However, Zinc occurred more in samples from Afikpo.

Table 1: Mean result of heavy metals in smokeless tobacco from different locations

Heavy metals (ppm)	Afikpo	Amasiri	Unwana
Cadmium	0.057±0.003	0.082±0.023	0.050±0.001
Chromium	0.014±0.009	0.023±0.004	0.022±0.006
Zinc	3.822±0.009*	2.050±0.013	2.359±0.033
Lead	0.022±0.009	0.012±0.007	0.108±0.012
Copper	2.311±0.011	4.096±0.015*	3.411±0.022
Iron	2.597±0.011	3.411±0.011	3.515±0.020*
Manganese	0.058±0.005	0.057±0.008	0.060±0.007

* significant P < 0.05

The concentration of Nitrate in the selected samples showed a reduction in the following order: Afikpo > Amasiri > Unwana, while Nitrite concentration is Unwana > Amasiri > Afikpo (Table 2).

Table 2: Mean result of nitrate and nitrate in smokeless tobacco from different locations

Locations	**Nitrate (Mg/l)**	**Nitrite (Mg/l)**
Afikpo	21.21±0.010*	7.71±0.023
Amasiri	17.50±0.010	12.76±0.014
Unwana	11.50±0.023	14.540±0.015

* significant P < 0.05

The THQ for Cadmium from the samples analyzed from the 3 different locations showed that the values were all >1, while that of Cadmium gave THQ=1 in Amasiri and Unwana. However, the THQ of other metals and those of nitrate and nitrite were all <1 as shown in Table 3.0

Table 3: Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of selected heavy metals in smokeless tobacco from different locations

Heavy metals	**Afikpo**		**Amasiri**		**Unwana**	
	EDI	**THQ**	**EDI**	**THQ**	**EDI**	**THQ**
Cadmium	0.008	1.400	0.012	2.100	0.007	1.200
Chromium	0.002	0.666	0.003	1.000	0.003	1.000
Zinc	0.546	0.015	0.292	0.008	0.337	0.009
Lead	0.003	0.008	0.002	0.005	0.015	0.042
Copper	0.330	0.073	0.585	0.130	0.487	0.108

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Iron	0.365	0.004	0.487	0.006	0.502	0.006
Manganese	0.008	0.001	0.008	0.001	0.009	0.001
Nitrate	3.029	0.018	2.500	0.002	1.643	0.010
Nitrite	1.102	0.068	1.823	0.115	2.077	0.129

DISCUSSION

The determination of Cadmium, Chromium and Copper (Table 1) showed a nonsignificant ($p>0.05$) increase in samples obtained from Amasiri compared to other two locations. The result of Cadmium is lower than that reported by Watanabe *et al. (2017)*, that of Chromium is lower than that obtained by Elinder (2010) and Eneji *et al*. (2013), while that of Copper is lower than that obtained by Hammond and O'connor (2008). Likewise, these results are lower than the maximum permissible value described by reports of joint FAO/WHO (1989). The high toxicity potentials of both Cadmium and Chromium have led to their classification as group 1 carcinogen by International Agency for Research on Cancer (IARC) (Kim *et al*., 2015), and Cadmium is capable of causing cell proliferation and apoptosis; it could bind to mitochondria to inhibit cellular respiration and oxidative phosphorylation at low concentration in humans (Guthrie *et al*., 2009). Chromium on the other hand is capable of causing skin irritation (Rodgman & Perfetti, 2009) while Copper is capable of causing liver diseases and neurological defects to the health (Hatsukami *et al*., 2011) The THQ of Cadmium from this study was greater than 1 in the three locations (Table 3); therefore, it is likely to cause adverse health hazard. The THQs of Chromium from samples from Amasiri and Unwana were equal to 1, while those of Copper were less than 1 and not likely to pose adverse health risks.

The analysis of the sample of SLT from Unwana showed a non-significant ($p>0.05$) increase in the level of Lead and Manganese compared to samples obtained from Afikpo. However, concentrations of Zn, Cu and Fe showed a significant ($p<0.05$) increase in snuff obtained from Afikpo, Amasiri and Unwana respectively. While Lead is a non-biological and non-essential heavy metal, Iron, Manganese and Zinc on the other hand are essential parts of biological functions but could be toxic at high concentrations. The concentrations of Lead, Iron, Manganese and Zinc in (Table 1) were lower than those reported by Massadeh *et al*. (2005), Elinder (2010), Hammond and O'connor (2008) and Golia *et al.* (2007) respectively. The concentrations of Lead, Iron, Manganese and Zinc from the SLT analyzed were lower than the maximum permissible value described by reports of FAO/WHO (1989). Lead is capable of causing impaired petal growth and brain development in infants and young children (Neuspiel *et al*., 2004). Iron toxicity could cause swollen tongue, difficulty in regulating body temperature, and sickle cell anaemia (Varma *et al*., 2001). Zinc consumption is beneficial but at high concentrations is capable of causing chronic renal diseases and sickle cell anaemia (McNeill *et al*., 2006). However, the THQs of lead, iron, manganese and zinc were less than 1; therefore, they are not likely to pose any health risk.

Nitrate and Nitrite concentrations in SLT could enhance the formation of nitrosamines that could predispose subjects to cancer especially when they react with secondary amines. Nitrate concentration was more in Amasiri while Nitrite was more abundant in samples from Unwana (Table 2). The values were lower than the maximum permissible value by WHO (2006). Nitrate can lead to methemoglobinemia and some carcinogenic effects (Weightman & Elizabeth,

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2013), and likewise, the ingestion of food containing Nitrites can expose an individual to nitrosamine which is a carcinogenic agent. The THQ results of Nitrate and Nitrite were less than 1; therefore, they might not cause health risk. The concentration of Nitrate showed a significant ($p<0.05$) increase in snuff obtained from the Afikpo compared to Nitrite.

CONCLUSION

Cadmium and Chromium are potentially toxic heavy metals which has led to their classification as group 1 carcinogen. Their concentrations, including that of Copper, were high in samples of smokeless tobacco obtained from Amasiri and consequently the THQs of Cadmium were greater than 1, which indicates that they might cause potential health risk. Heavy metals like Lead and biologically essential ones like Iron, Manganese and Zinc gave the highest concentrations in Afikpo samples compared to the other two locations. However, their THQs were less than 1 and might not cause any possible health risk. The two nitrosamine precursors, Nitrate and Nitrite, were more in samples of tobacco obtained from Amasiri and Unwana respectively. Their THQs were less than 1 and they might not cause any potential health risk. Therefore, from this study, the presence of Cadmium due to exposure to smokeless tobacco might pose a noncarcinogenic health risk.

Future Research: Future research study will look at the possibility of in vivo carcinogenicity animal study as a result of the exposure to snuff. Also, biomolecular and histopathological examination of tissues and organs of animals involved can be done.

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Conflicting Interest: None

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