ASSESSMENT OF ARSENIC, CADMIUM AND LEAD IN SNUFF IN THE ASHANTI REGION OF GHANA

Owusu-Asante, O. J.[1], Mensah, P.[1] Duah-Gyamfi, A.[1], Owusu, J.[1], Minkah, E.[1], Wumbeidow, H.[1], Ibrahim, S.[1], Ofori, E.[1], Nyarko, D. E[1].
[1]CSIR-Forestry Research Institute of Ghana, P. O. Box UP 63, Kumasi, Ghana
Corresponding Author Email: jowusu-asante@csir-forig.org.gh

ABSTRACT

Tobacco snuff usually comes from *Nicotiana tabacum* in the wet or the dry form. The leaves are finely ground either by pounding or crushing and sometimes may be contaminated with heavy metals such as Lead (Pb), Cadmium (Cd) and Arsenic (As) which can accumulate in the body following prolonged use resulting in adverse health conditions. The objectives of this study are to determine the presence of heavy metals (Pb, Cd and As) in different tobacco snuff from selected transport terminals in the Ashanti Region of Ghana and to compare the acceptable daily intake with permissible limits by the Food and Agricultural Organization/World Health Organization (FAO/WHO) recommendation. Three transport terminals (the Kumasi Metropolis, Ejisu and Offinso districts) in the Ashanti Region were randomly sampled for tobacco snuff. Snuff samples/brands from the different stations were subsequently sent to the laboratory for analysis. The wet acid digestion method was used to determine the concentration of Pb, Cd and As of each sample using the atomic absorption spectrophotometer. The study found 18 snuff samples common among the transport terminals within the Ashanti Region. The 18 snuff samples had significant levels of metals in the range: Lead (0.298- 0.533 µg/g), Cadmium (0.977- 1.947 µg/g) and Arsenic (0.071 - 1.011 µg/g). The acceptable daily intake of heavy metals from most of the samples was above the maximum permissible limit accepted by the Food and Agriculture Organization and World Health Organization. The results of this study showed that snuff contains toxic heavy metals that may cause health problems. Therefore, urgent regulation of the product is needed alongside an educative campaign to create awareness about the health implications associated with snuff. *[African Journal of Chemical Education—AJCE 12(2), July 2022]*

INTRODUCTION

Nicotiana tabacum is commonly referred to as tobacco. The leaves are smoked, chewed or sniffed either as a depressant or to induce sneezing during periods of lightheadedness or slight dizziness. *Nicotiana tabacum* is also popular for its cultural and traditional purposes [1]; [2]. Tobacco snuff usually comes in the wet or the dry form. Based on the quantity of the leaves and preference, the leaves are finely ground either by using the local way of pounding (mortar and pestle) or the grinding stone [3]. Thereafter, it is mixed with additives like flavors such as peppermint and eucalyptus oil, menthol or fruit extracts [4]. In Ghana, the main additive used is the saltpetre. In the dry form, snuff is sniffed or swallowed whereas in the wet form, the snuff is placed between the lower gum and the cheeks and mixed with saliva to aid in the release of nicotine into the mouth and body [5]. Through these assimilation processes, toxic metals may enter the body.

Research has shown that carcinogenic agents and toxic heavy metals such as Cadmium (Cd), Lead (Pb), Arsenic (As) could be present in the tobacco snuff as they are absorbed by the leaves during growth [6]. Some of these heavy metals are required in minute quantities in the function of plants and animals [7]. In higher concentrations, these heavy metals bioaccumulate and pose severe health effects such as anemia, kidney failure, irritability, coma and death in humans [8]. Heavy metals such as As, Cd and Pb are carcinogenic and may pose severe health risks in tobacco products [9]. As and Cd are known for causing lung injury [10]. Cd and Pb are of concern since they can be stored in the body for long and stimulate tissues to immune response, cell injury and tissue repair processes [11]. Cd exposure can cause cataract in the lens of the eye upon accumulation, can affect the kidney, the nervous system and the reproductive system [12]; [2]. Lead is particularly known to decrease the intelligence quotient levels especially in younger children and can cause an irreversible brain damage [1]; [13]. Aside being carcinogenic, Arsenic in the human body can cause skin pigmentation problems, ulcerations of the mouth, low haemoglobin, leukemia, acute renal failure, seizures and nerve damage [14]; [1].

According to the World Health Organization (WHO) International Agency for Research in Cancer (IARC), Smokeless Tobacco Products (STPs) such as snuff has been classified as Group 1, Group 2A and Group 2B carcinogens. Group 1 carcinogens are definitely carcinogenic to humans while Group 2A and Group 2B are probably carcinogenic and possibly carcinogenic to humans, respectively [12]; [15].

In Ghana over the past few years, local snuff use has received high patronage despite the health risks posed to sniffers. This patronage is common around transport terminals where different snuff brands are sold to commuters travelling to different parts of the country. Smokers or sniffers have been shown to be more highly exposed to As, Cd and Pb [11] however, Pb, Cd and As are more poisonous even at lower levels than Ni [9].

The overall goal of this research is to create awareness among those who patronize snuff and the general public at large about the dangers posed by the presence of potential heavy metals in snuff. Therefore, in this study, we determined the levels of Cd, Pb and As as well as the acceptable daily intake of these toxic metals in snuff obtained from transport terminals in the Ashanti Region of Ghana.

METHODOLOGY

Sample Collection

Three transport terminals (Kumasi Metropolis (6.7252° N, 1.6566° W); (Ejisu District (6.7151° N, 1.5091° W) and Offinso districts (7.3049° N, 1.7928° W) in the Ashanti region of Ghana were randomly selected for this study (Figure 1). The terminals are among the busiest and serve as the exit terminals between the Ashanti region and other regions of Ghana. Eighteen samples of tobacco snuff were randomly obtained from the transport terminals in November 2019. The names of the local manufacturer or snuff brand were noted and coded for further analysis.

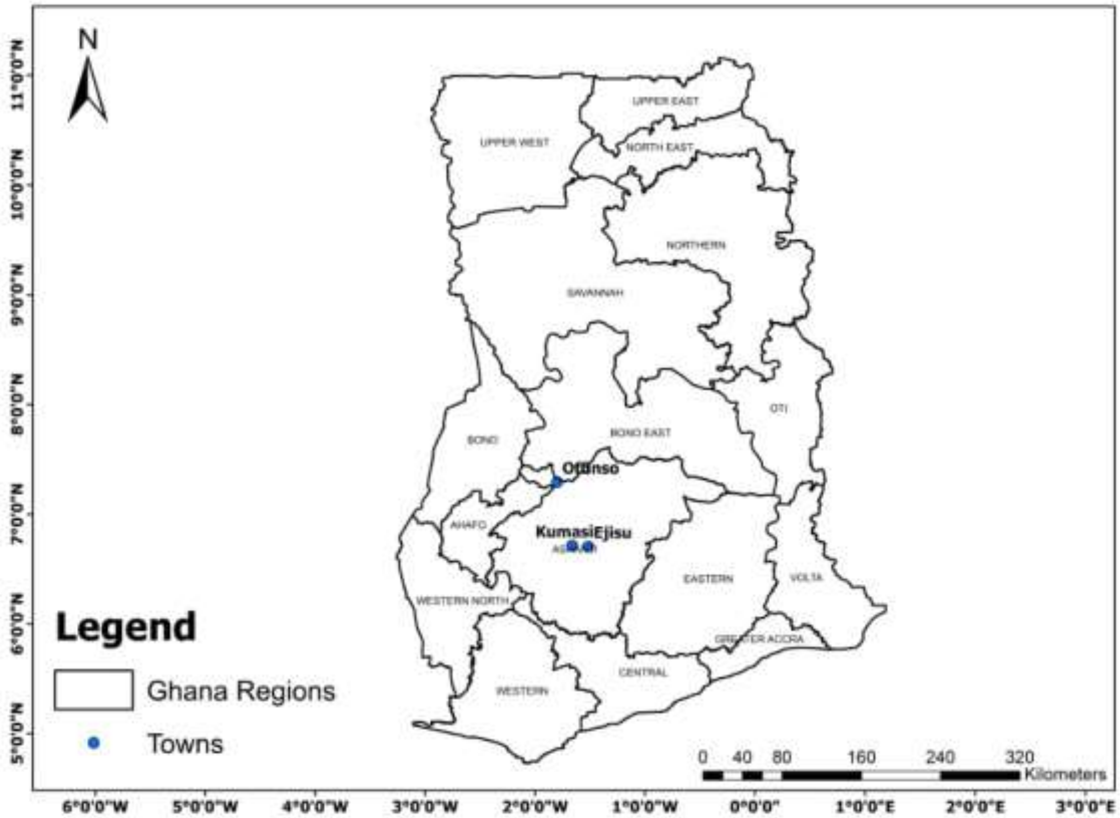

Figure 1: Ghana Regions

Sample Pretreatment

All samples purchased were kept in separate zip lock bags, sealed, and subsequently sent to the laboratory for analysis. Samples not in powdery form were ground, sieved and stored. One gram of each sample was weighed using an electronic balance (Mettler-Toledo Ltd, PM 4000) and used for further analysis as per the standard procedures given below for the different metals.

Sample Digestion

Each of the 1g snuff sample was poured into a Kjeldahl digestion tube. Perchloric ($HClO_4$), Nitric acid (HNO_3) and Hydrochloric acid (HCl) were added in the ratio of 1:2:3. The mixture was then digested at a temperature of 450 ^{0}C until complete digestion for about 30 to 60 minutes [16].

Sample Analysis

After digestion was complete, the mixture was decanted into a 100 ml volumetric flask and the solution was topped up to the 100 ml mark. This digest was used to analyze all the metals (Pb, As, Cd). The unknown concentration of this solution was then read using an atomic absorption spectrophotometer (AAS) (Buck Scientific Inc, 210 VGP) for the various metals at specified wavelengths [16]; [17].

Instrumentation

The basic setup (air pressure,50 – 60 psi; acetylene pressure, 10 -15 psi; voltage, 208 – 240V) of the AAS was ensured. The file for the type of analysis and hollow cathode lamps were selected with appropriate wavelengths – Pb at 283.31 nm, Cd at 228.80 nm, As at 193.70 nm. A calibration curve was plotted for each of the elements to be analyzed from the stock standards (Buck Scientific).

The prepared sample solution digest was analyzed for the elements. The total concentration of the element in the sample solution (100 ml) was calculated by multiplying the concentration in mg/L by 0.1L. This gave the total mass of the element in solution [17]**.** The percentage amount of the element was found by dividing the mass of the element in solution by initial amount of sample taken followed by a multiplication by 100 (Eqn 1)

$$\text{Conc. }(H_m)\text{ (mg/kg)} = \frac{\text{Conc. from AAS X Nominal volume}}{\text{Sample weight (g)}} \qquad (1)$$

Where, H_m = heavy metal, Nominal volume =100 ml, Sample weight = 1.00g

Data Analysis

A one-way Analysis of Variance (ANOVA) was used to examine differences in concentration of each of the heavy metals among the snuff brands. Prior to ANOVA, data was examined for normality using the Kolmogorov Smirnov test, and homogeneity of variance using the Levene test. Microsoft excel was used to generate graphs of means concentrations of heavy metals among the snuff brands.

RESULTS AND DISCUSSION

The concentration of each heavy metal in the snuff samples was shown in figures 1, 2 and 3. The results were reported as the mean ± standard deviation for each metal. Based on figure 1, the highest concentration of Pb was found in sample SPS at 0.533 ± 0.017 µg/g. Lead is known to be one of the non-essential metals that are highly toxic and can cause cancer even in small concentrations [18]. The highest concentration of As and Cd in snuff samples were also shown in figures 2 and 3 as 1.011 ± 0.053 µg/g and 1.947 ± 0.100 µg/g respectively. Cadmium was shown at the highest concentration at 1.947 ± 0.100 µg/g in sample OPS whilst Arsenic occurred in sample HAS at the highest concentration of 1.011 ± 0.053 µg/g.

The concentration of heavy metals in brand MS ranged from 1.540 ± 0.069 µg/g to 0.459 ± 0.008 µg/g. Cd recorded the highest concentration followed by As and then Pb. OPS had concentrations of the heavy metals ranging from 1.947 ± 0.100 µg/g for Cd to 0.367 ± 0.017 µg/g for Pb. Cd>As> Pb was the observed pattern. The concentration of heavy metals in brand HMS ranged from 1.347 ± 0.108 µg/g to 0.292 ± 0.032 µg/g. Cd recorded the highest concentration followed by As and then Pb. The three heavy metals occurred in the order Cd>Pb>As. The concentration of heavy metals in brand AMS ranged from 1.353 ± 0.190 µg/g to 0.367 ± 0.008 µg/g. Cd recorded the highest concentration followed by As and then Pb. The three heavy metals occurred in the order Cd>As>Pb. The concentration of heavy metals in brand NMS ranged from Cd (1.253 ± 0.072) µg/g. to As (0.298 ± 0.013) µg/g.

The concentration of heavy metals in brand HAS ranged from 1.200 ± 0.132 µg/g to 0.362 ± 0.017 µg/g Cd recorded the highest concentration followed by As and then Pb. The concentration of heavy metals in brand KSS ranged from 1.370 ± 0.098 µg/g for Cd to 0.436 ± 0.013 µg/g for Pb.

The concentration of heavy metals in brand TKS ranged from 1.530 ± 0.203 µg/g to 0.115 ± 0.068 µg/g. Cd recorded the highest concentration followed by Pb and then As. The three heavy metals occurred in the order Cd>Pb>As. SMS had concentration of metals in the range from 1.180 ± 0.253 µg/g to 0.426 ± 0.024 µg/g. Cd recorded the highest concentration followed by As and then Pb. The concentration of heavy metals in brand NRS ranged from 1.003 ± 0.159 µg/g to 0.381 ± 0.025 µg/g Cd recorded the highest concentration followed by As and then Pb. KS had concentration of heavy metals in the range from 1.317 ± 0.234 µg/g to 0.445 ± 0.013 µg/g Cd recorded the highest concentration followed by As and then Pb.

The concentration of heavy metals in brand VS ranged from 1.343 ± 0.372 µg/g to 0.309 ± 0.017 µg/g Cd recorded the highest concentration followed by As and then Pb. The concentration of heavy metals in brand UYS ranged from 1.473 ± 0.493 µg/g to 0.370 ± 0.021 µg/g Cd recorded the highest concentration followed by As and then Pb. The three heavy metals occurred in the order Cd>As>Pb.

The concentration of heavy metals in brand UBWS ranged from 1.220 ± 0.306 µg/g to 0.434 ± 0.017 µg/g. The three heavy metals occurred in the order Cd>As>Pb. The concentration of heavy metals in brand UABS ranged from 0.977 ± 0.099 µg/g to 0.365 ± 0.013 µg/g. Cd recorded the highest concentration followed by As and then Pb. The three heavy metals occurred in the order Cd>As>Pb. The concentration of heavy metals in brand SPS ranged from 1.480 ± 0.207 µg/g to 0.071 ± 0.011 µg/g. Cd recorded the highest concentration followed by Pb and then As. Brand PS concentration of heavy metals in brand PS ranged from 1.233 ± 0.085 µg/g to 0.456 ± 0.025 µg/g Cd recorded the highest concentration followed by As and then Pb. Finally, the concentration of heavy metals in brand OS which is the last brand ranged from 0.599 ± 0.027 µg/g to 0.396 ± 0.004 µg/g This brand followed a usual pattern unlike the rest. Arsenic recorded the highest concentration followed by Cd and then Pb. The three heavy metals occurred in the order As>Cd>Pb.

The concentration of Pb in the 18 snuff brands ranged from 0.533 ± 0.017µg/g to 0.298 ± 0.013 µg/g to Local snuff brand SPS recorded the highest whilst brand NMS recorded the least. Fig 1 gives the concentration of Pb in the 18 snuff brands There was a significant difference ($P < 0.05$) in the concentration of Pb among the 18 local snuff brands examined. This was similar to the lead concentration with a range from 0.28 ± 0.03 µg/g to 0.85 ± 0.01 µg/g [(Pappas *et al.*, 2008) during the analysis of toxic metals in commercial moist snuff and Alaskan iqmik. [8] published concentrations of lead in 30 samples of tobacco (*Nicotiana tabaccum*) snuff in Nigeria. The concentration of lead in the tobacco snuff samples in Nigeria ranged from 0.41 to 1.13 µg/g for north central, 0.45 to 2.48 µg/g for southwest, 0.01 to 1.48 µg/g for southeast, and 0.02 to 1.16 for northwest. These values were within the range of this study except the values were higher than that of the study. [1] had 0.03–68 µg/g at its range for Lead the study of toxic metals in snuff samples in India. The upper limit of 68 µg/g was far above that of the current investigation.

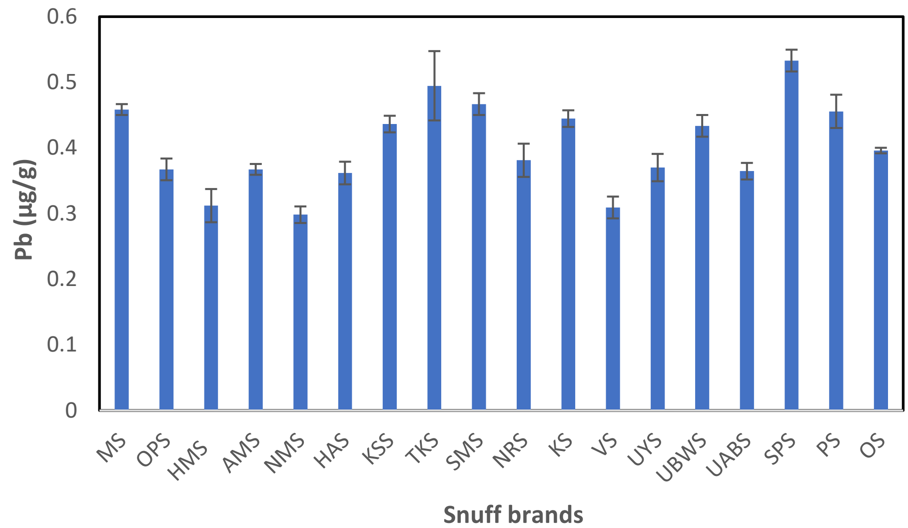

Fig 1: Concentration of Pb in the 18 snuff brands

As recorded its concentration in the range of 1.011 ± 0.053 µg/g to 0.071 ± 0.011 µg/g as shown in Fig 2. HAS had the highest concentration of As whilst SPS had the lowest concentration of As. There was a significant difference ($P < 0.05$) in the concentration of As among the 18 local snuff brands examined. These values were lower compared to what [19] reported for the mineral profiling in Ghanaian local snuff and imported snuff in the range of 0.862 µg/g to 1.93 µg/g.

[1] determined toxic metals in Indian smokeless tobacco products. Levels of Arsenic in the smokeless tobacco products were (0.1–3.5 µg/g) and these values were in the range of the lower limits and higher than the upper limits of the study. The levels of Arsenic recorded by [20] in the

analysis of commercial moist snuff were in the ranges 0.13 ± 0.01 µg/g and 0.36 ± 0.06 µg/g. The lower limits found in [20] was higher than that of the study but the upper limit was lower than that of the present study.

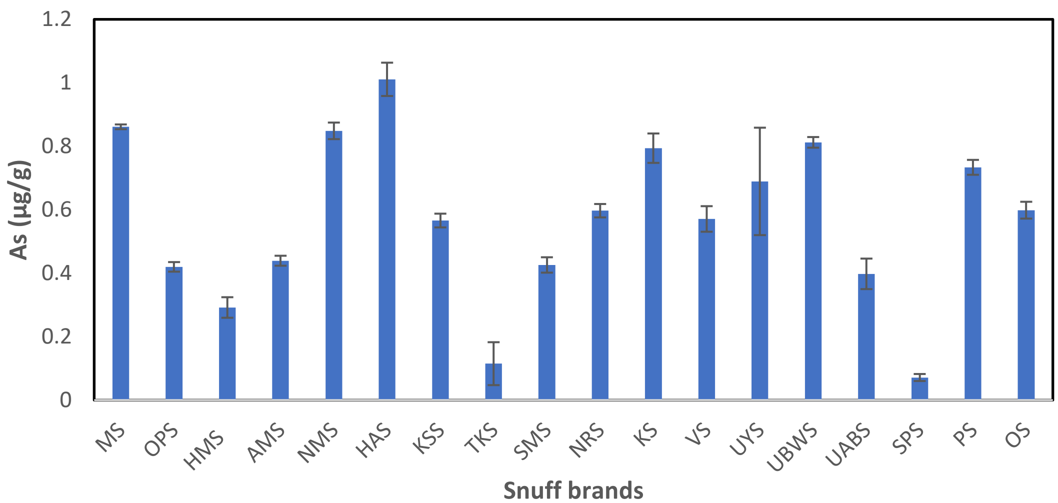

Fig 2: Concentration of As in the 18 snuff brands

The concentration of Cd in the 18 snuff brands ranged from 1.947 ± 0.100 µg/g to 0.417± 0.032 µg/g as shown in Fig 3. Local snuff brand OPS recorded the highest whilst brand OS recorded the least. There was a significant difference ($P < 0.05$) in the concentration of Cd among the 18 local snuff brands examined. [1] had 0.01–3.2 µg/g as its range for Cadmium during the study of toxic metals in snuff samples in India. The lower limit from [1] of 0.01 µg/g was lower than 0.977 µg/g. However, the upper limit of 3.2 µg/g from [1] was far higher than that of 1.947 µg/g obtained from the current study.

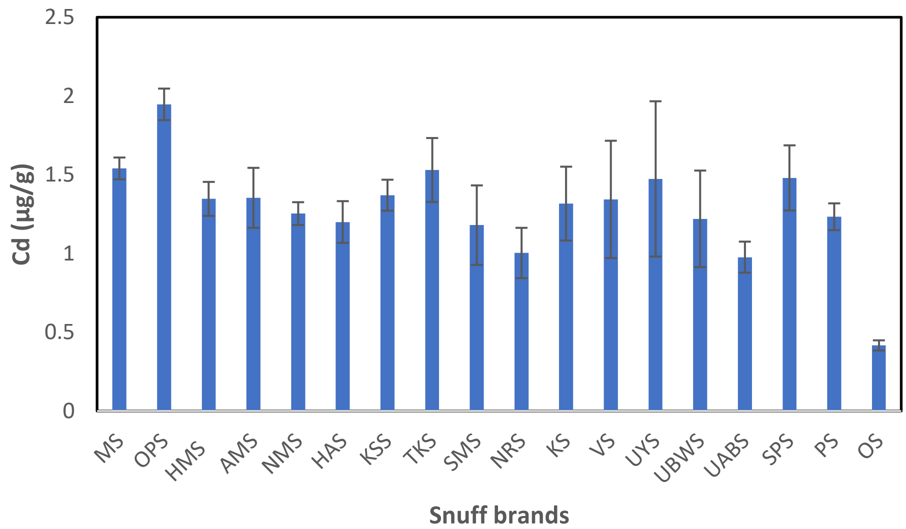

Fig 3: Concentration of Cd in the 18 snuff brands

Acceptable Daily Intake

Acceptable Daily Intake **(**ADI) is the amount of a substance, expressed on a body-mass basis, daily ingested in food or drinking water over lifetime without imposing any appreciable risk to human health [21]. Table 2 gives the permissible intake levels of Pb, Cd and As.

According to [1] (Dhaware *et al* ., 2009)**,** the daily intake was calculated by the equation below:

$$DI\ (\mu g/day) = C_{metal} \times W_{analyses} \times D_{intake} \qquad (2)$$

Where C_{metal} = concentration of the metal in the snuff sample taken for analysis.

$W_{analyses}$ = weight of the sample taken for analysis (1 g in this study)

D_{intake} = daily intake of pouch (10 pouches a day)

Table 2: Acceptable daily intake of Pb, As and Cd

Sample 1D	ADE of Pb/ (µg/day)	ADE of As/ (µg/day)	ADE of Cd/ (µg/day)
MS	4.59	8.61	15.40
OPS	3.67	4.20	19.47
HMS	3.12	2.92	13.47
AMS	3.67	4.39	13.53
NMS	2.98	8.49	12.53
HAS	3.62	10.11	12.00
KSS	4.36	5.66	13.70
TKS	4.94	1.15	15.30
SMS	4.67	4.26	11.80
NRS	3.81	5.97	10.03
KS	4.45	7.94	13.17
VS	3.09	5.71	13.43
UYS	3.70	6.89	14.73
UBWS	4.34	8.12	12.20
UABS	3.65	3.98	9.77
SPS	5.33	0.71	14.80
PS	4.56	7.33	12.33
OS	3.96	5.99	4.17

Comparison of the Acceptable daily intake of Pb, As and Cd to the WHO permissible limits

The ADI of all the metals detected in all brands (Table 2) were compared with Permissible Intake Levels as per FAO/WHO Recommendations (Table 3) to ensure the safety of the snuff for consumer use.

Table 3: Permissible Intake Levels as per FAO/WHO Recommendations [1]

Metal	**Provisional Tolerable Weekly Intake (µg/kg/week**	**Per Day Intake (µg/kg/day)**	**For a 60-kg Individual (µg/day)**	**Ref**
Pb	25	5	300	FAO/WHO
As	15	3	180	FAO/WHO
Cd	3.5	0.2-1	30	WHO/JECFA

From Table 3 the daily recommended intake for Lead is 5 µg/kg/day. 17 samples were within the permissible limit except for sample SPS which had an ADI of 5.33. However, a continuous use of the smokeless tobacco could result in bioaccumulation of lead with its negative health effects such as abdominal pain, headache, anemia, irritability, kidney failure [8] and negative effects on the hematic and the immune system [1]. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the accumulation of lead in the body was based on its net absorption which comprises of 40% from dietary sources, 10% from food and drinking water, and up to 50% from inhalation of lead compounds. This means that at an intake of 5 µg/kg bw/day, retention of leas in the body leads to an increased blood lead level [13].

In the case or Arsenic, most of the levels were above the permissible intake levels. Out of 18 snuff samples, only three samples (HMS, TKS and SPS) had permissible limits of 2.92 µg/day, 1.15 µg/day and 0.71 µg/day respectively that were lower than the FAO/WHO acceptable limit of 3 µg/kg/day. All the 15 samples were above the permissible limits.

From table 3, it was observed that all the 18 snuff samples were far above the permissible limits of **0.2**-1 µg/kg/day as set by the WHO/JECFA for Cadmium.

CONCLUSION

In summary, the 18 snuff brands tested had significant levels of metals as compared to other groups of smokeless tobacco in the range: Pb (0.298- 0.533 µg/g), Cd (0.977- 1.947 µg/g) and As (0.071 - 1.011 µg/g). This study emphasizes the acceptable daily intake of these toxic heavy metals. In the case of Pb, all 17 samples were within the permissible limits of the WHO with the exception of only 1 sample which was higher than the permissible levels. For Cd, almost all their samples were above the permissible limits of the WHO while As had only three samples in the permissible limits of WHO. Further, the acceptable daily intake of the tested metals exceeded the allowable safe limits recommended by the FAO and the WHO. This poses a high risk to consumers as they could develop harmful health effects. It is therefore recommended that the sale of the snuff be regulated in these transport terminals.

In addition, educative programs aimed at awareness creation among the youth and patronizes of snuff especially drivers be implemented to sensitize them on the dangers associated with snuff. Further research and screening of snuff and other local products (e.g., lipstick) displayed on the market for the presence of heavy metals and poisonous substances need urgent attention and consideration.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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