# **Mineral profile of Ghanaian dried tobacco leaves and local snuff: A comparative study**

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The concentration of thirty-four elements each in Ghanaian dried tobacco leaves and snuff (powdered tobacco) have been determined using instrumental neutron activation analysis (INAA). The concentration of Hg, Cr, As and Cd in both set of samples were found to be in excess of WHO limits for drinking water, thus indicating potential toxicity of the samples. Cr, Cd, Sb and Cu were two to eight times high, whilst As and Hg were comparable in powdered tobacco and tobacco leaves. The aim of the study was to determine the pattern of elemental concentrations and the toxicological strengths in both tobacco leaves and tobacco powder. The results indicated that the toxicity of the snuff was higher than the tobacco. This indicated strongly that from the medical point of view, the level of toxic accumulation in users might be potentially high in the tobacco powder compared to the leaves. Thus, education of the Ghanaian public on the threshold value of toxic elements contained in both set of samples was suggested to safeguard users against these addictives.

#### **Introduction**

Tobacco may be used in different forms such as smoking, chewing, sniffing, etc. From research conducted it is known that the use of tobacco is associated with increase in heavy metals including cadmium and lead in the tissue of the consumers.1,2 Thus the use of tobacco, both smoking and smokeless, continues to be a major health problem in users and nonusers as well. All the various forms of the use of tobacco result in a number of negative side effects on human health that are well-documented. A complete list on the health effects from tobacco utilization can be found in the website of http://ash.org.uk/html/health/html/oral.html, as well as in Reference 3.

Many studies have been conducted so far to determine trace quantities of elements present in tobacco and tobacco products, especially is cigarettes. $4-6$ Therefore, knowledge of their potential toxicological effects can be understood and appreciated. Yet, only scanty information of the toxicological action of locally produced 'snuff' (tobacco powder) is available to the Ghanaian public since studies about its elemental contents have been scarcely researched. Snuff is locally referred to finely ground and smokeless tobacco intended for use by being sniffed or snorted into the nose. Snuff is also consumed by placing it between the cheek and gum, thereby enabling it to seep gradually into the mouth and body through mixing with saliva. In Ghana, snuff and tobacco leaves are used to induce sneezing to 'lighten" the head and also as a depressant

and stimulant. They are known to contain nicotine as active ingredient.7

In Ghana, local snuff is prepared by mixing the dried tobacco leave (*Nicotiana tabacum* L.) indigenous to the forestry areas with some chemicals (especially saltpetre) and then grinding it into fine powder. It is consumed mostly by the Ghanaian aging population. At present careful observation reveals that the attitude of the youth towards snuff consumption is on the increase. The trend is expected to show further appreciable level sooner. Thus, lack of knowledge of the mineral contents and concentration of locally produced snuff is very worrying. Hence, there is an urgent need to investigate this.

Information on the elemental profile of the dried tobacco leaves and the snuff would assist in knowing whether the toxicological strength of the tobacco reduces or increases as it is being converted into the snuff. Furthermore, with recurrent theme in research leading to the establishment of the fact that raw tobacco and its products elsewhere contain toxic elements like Cd and Pb should elicit concern. Thus, levels of all elements especially those of the toxic fraternity in local Ghanaian raw tobacco and snuff should therefore necessitate a study.

It may be of interest to know that after roasting the tobacco leaves, users, traditionally dip the roasted tobacco into the fly-ash of wood, before inserting it into the space between the lower gum and lip in order to release the nicotine which is accompanied by toxic elements.

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Instrumental neutron activation analysis (INAA) has previously been shown to be feasible for multi-elemental determination in biological samples.<sup>8</sup> High accuracy, minimum sample handling, no added reagent, multielemental capability and low detection limits are among the advantages of this analytical technique. As a result of such numerous advantages it has been widely used in various elemental investigations.<sup>9–11</sup>

The present investigation was undertaken, principally to determine the elemental profile of locally produced snuff and dried tobacco leaves in Ghana. Furthermore, this study makes comparative analysis of the two varieties of locally processed tobacco, paying particular attention to toxic levels. In order to study simultaneously a large number of the elements in the samples, INAA was carried out at the Miniature Neutron Source Reactor of the GHARR-1 Centre of the Ghana Atomic Energy Commission in Ghana which operates at a high maximal flux of  $1.0 \cdot 10^{12}$  n·cm<sup>-2</sup>·s<sup>-1</sup>.

# **Experimental**

### *Sample preparation*

Samples of the snuff and dried tobacco leaves available in the country were randomly purchased from local retailers in multiple locations in the Accra-Tema Metropolis, Ghana. Eighteen samples from various locations were acquired for the tobacco leaves whilst twenty-five different purchasing points were contacted for the snuff samples. In the laboratory, the tobacco leaves gathered together by means of light strings were pulverized in a blender and the snuffs mostly wrapped in paper (usually used for previous packaging) were turned into separate composite samples.

Between 150 and 200 mg of each sample was enveloped via thermal sealing inside  $5\times5$  cm<sup>2</sup> polyethylene thin film which were heat-sealed in  $8.9 \text{ cm}^3$  rabbit capsule for irradiation. Initially, the polyethylene film and rabbit capsules were cleaned by soaking then into dilute nitric acid for three days and washed with de-ionized water.

To assess the analytical process and make a comparative analysis, a series of biological standard reference materials (SRM) were prepared in the same manner as the analytical samples. The standards used were: Orchard Leaves (SRM-1571); Peach Leaves (SRM-1575); and Oyster Tissue SRM, all from the National Institute of Standards and Technology (NIST). In addition was Hay Powder SRM obtained from the International Atomic Energy Agency (IAEA). All the analytical and standard samples were herein prepared in five replicates and analyzed accordingly. The standards were used to validate the accuracy of the analytical procedure employed for this work.

# *Irradiation and counting*

Both the analytical samples and the SRMs were analyzed by INAA. The neutron flux used for the irradiation was approximately  $5.0 \cdot 10^{11}$  n·cm<sup>-2</sup>·s<sup>-1</sup>. The samples were sent into the Miniature Neutron Source Reactor (MNSR) by means of a pneumatic transfer system operating at a pressure of 25 atmospheres. The scheme of the irradiation was chosen so as to take into account the half-lives of the radionuclides under investigation. In that regard, the following irradiation times were selected: 10 seconds for the short-lived radionuclides; 3600 seconds for the intermediate radionuclides; and 14400 seconds for long-lived elements.

After a short decay period the activity of the gammaray emitting radionuclides with short half-lives were measured. Similarly the gamma spectral intensities for medium and long half-life radionuclides were also measured after 2 days and between 2–4 weeks decay period, respectively.

The measurements of the gamma-ray spectral intensities were made using a spectroscopy system of high purity germanium (HPGe) N-type coaxial detector Model GR 2518; high voltage power supply Model 3105; and a spectroscopy amplifier Model 2020 (all manufactured by Canberra Industries, Inc.). The detector system at fixed geometry was coupled to an 8k Ortec multichannel analyzer (MCA) emulation card and a 486 microcomputer. The resolution of the detector system which operates at a bias voltage of  $-3000$  V full width at half maximum (FWHM) was 1.8 keV for <sup>60</sup>Co 1332 keV gamma-ray with 25% relative efficiency.

The output spectral intensities of both the analytical samples and the standards were processed and stored in the microcomputer software by means of the MCA card. Qualitative analysis of the radioisotopes was achieved by means of identifying their spectral intensities. The evaluations of the areas through integration under the photo peaks of the identified elements were converted into their concentrations using the comparator method.<sup>12</sup>

The accuracy of the INAA method was validated using NIST Peach Leaves (RSM-1575) SRM against IAEA Hay Powder SRM; NIST Orchard Leaves (SRM-1571) and NIST Oyster Tissue SRM. All measurements were analyzed both qualitatively and quantitatively.

#### **Results and discussion**

To confirm the accuracy of the method of the analysis used, comparison was made in respect of some elements which were well resolved in the standard reference materials (SRM) namely: Orchard Leaves (RSM-1575), Oyster Tissue SRM and Hay Powder SRM, and the results of this work. As seen from Table 1, the results are generally in good agreement with the certified values.

A total of 35 major, minor and trace elements were determined in the analysis. The results of the mean

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concentration  $(mg \cdot kg^{-1})$ , median and range of elements in both tobacco leaves and snuff are shown in Tables 2 and 3, respectively, where they are arranged in alphabetical order. The concentration values were obtained from five replicate measurements.

*Table 1.* Comparison of concentrations in various standard reference materials and local laboratory values

Element	Reference material	No. of measurement	Reported value	This work	Unit	Ratio
Al	Oyster Tissue	5	$197.2 \pm 6.0$	$170.32 \pm 18.74$	mg/kg	0.86
Ba	Oyster Tissue	5	$8.6 \pm 0.3$	$8.65 \pm 0.74$	mg/kg	1.01
C <sub>d</sub>	Oyster Tissue	5	$2.48 \pm 0.08$	$2.40 \pm 0.19$	mg/kg	0.97
C <sub>1</sub>	Oyster Tissue	5	$0.514 \pm 0.010$	$0.511 \pm 0.007$	$\frac{0}{0}$	0.99
Cu	Oyster Tissue	5	$71.6 \pm 1.6$	$70.74 \pm 9.42$	mg/kg	0.99
Ni	Oyster Tissue	5	$1.04 \pm 0.09$	$1.11 \pm 0.08$	mg/kg	1.07
Rb	Oyster Tissue	5	$3.262 \pm 0.145$	$3.07 \pm 0.29$	mg/kg	0.94
Ca	Orchard Leaves	5	$2.09 \pm 0.03$	$2.131 \pm 0.044$	$\%$	1.02
Cr	Orchard Leaves	5	2.30	$1.74 \pm 0.16$	mg/kg	0.76
Fe	Orchard Leaves	5	270.0	$301.07 \pm 24.09$	mg/kg	1.12
Co	Hay Powder	5	0.13	$0.125 \pm 0.008$	mg/kg	0.96
Hg	Hay Powder	5	0.013	$0.015 \pm 0.001$	mg/kg	1.15
K	Hay Powder	5	$2.10 \pm 0.03$	$2.059 \pm 0.023$	$\frac{0}{0}$	0.98

*Table 2*. The mean, median and ranged concentrations (in mg·kg<sup>-1</sup>) of elemental profile for dried Ghanaian tobacco leaves



Element	No. of	Mean	Median	Range
	measurement			
Ag	5	1.600	1.497	1.485-1.812
As	5	0.21	0.243	$0.108 - 0.256$
Al	5	4075.01	4007.33	3006.20-5166.51
Ba	5	166.09	170.60	110.15-202.57
Br	5	57.73	51.77	41.22-78.56
Ca	5	13323.11	14231.13	10289.57-16083.54
Cd	5	1.079	1.078	$1.056 - 1.105$
Ce	5	6.58	6.85	$3.67 - 8.69$
Cl	5	14345.92	15054.29	13060.60-15095.24
Co	5	0.182	0.179	$0.165 - 0.201$
Cr	5	1.148	1.084	$0.945 - 1.405$
Cs	5	0.081	0.085	$0.067 - 0.093$
Cu	5	22.39	21.18	18.50-27.65
Eu	5	0.172	0.176	$0.143 - 0.205$
Fe	5	3156.78	3365.87	2433.20-5981.77
Hg	5	0.009	0.0082	$0.0074 - 0.0115$
I	5	0.515	0.501	$0.410 - 0.626$
K	5	32333	31233.00	28900.61-61755.42
La	5	13.20	12.50	$9.44 - 16.03$
Mg	5	39300.51	39803.15	38331.30-40757.81
Mn	5	130.09	132.90	120.70-138.85
Na	5	8484.94	8489.44	8438.01-8529.17
Nd	5	2.52	2.57	$2.16 - 2.81$
Rb	5	6.00	6.12	$4.69 - 7.11$
Sb	5	0.074	0.017	$0.065 - 0.086$
Sc	5	0.592	0.571	$0.520 - 0.722$
Se	5	4.25	4.46	$3.75 - 4.59$
Sm	5	0.083	0.089	$0.067 - 0.094$
Sn	5	< 0.090	0.096	$0.068 - 0.109$
Sr	5	3180.66	2908.66	2341.30-4047.02
Th	5	0.457	0.484	$0.401 - 0.492$
U	5	0.022	0.022	$0.018 - 0.026$
$\mathbf{V}$	5	8.43	8.78	$6.56 - 10.57$
Yb	5	0.630	0.68	$0.46 - 0.751$

*Table 3*. The mean, median and range concentrations (in mg  $kg^{-1}$ ) of elemental profile for local snuff

All the elements displayed their presence in both the snuff and the raw tobacco leave samples, except Mo for the snuff and Nd for the tobacco leaves. In addition to these elements, spectral lines for Au, Dy, Ga, Ge, Hf, Si, Ti and Tm were observed. However, concentrations of these elements could not be evaluated either due to lack of SRMs containing the elements or their photo peaks could not be subtracted from the Region of Interest (ROI). The qualitative similarities between the two sets of samples demonstrated that dried tobacco leaves are the major raw materials for local snuff production.

As follows from Tables 2 and 3, the elements did not display any consistency in their concentration relationship. The mean concentration ratio of the elements between the two different samples ranged between 1.10 and 11.18. Exceptional to this range was sodium concentration in snuff samples which was 83 times higher than in the tobacco samples. It is evident that in general, the levels of elemental contents in the snuff were higher or comparable to concentrations obtained for the samples in the dried leaves. Exceptions are, Ba, Mn, Cu, Ca, Br, Se, Sc and Co which exhibited higher concentration in the case of the tobacco leaves.

However, these discrepancies may depend on several factors. Among these factor were: the chemicals (mainly saltpetre; a geologic material) used in the preparation of snuff; the availability of the tobacco leaves used in the snuff preparation coming from different regions of the country, hence, from different environments; difference in the mass ratio of the tobacco to other chemicals in the product; the source and type of elemental contaminants introduced during the preparation process; and finally the effect of packaging the products locally (e.g., Na from packing material may increase Na content in the product).

The presence of some heavy metals as Hg, Cd, As, and Cu in the samples under investigation testify the toxicological nature of tobacco and its products. For instance, research has shown that a significant flux of heavy metals, among other toxins, reaches the lungs through smoking.13,14 Other studies have found out that Cd contents in the livers and kidneys of smokers were higher than those of non-smokers.<sup>15,16</sup> Thus, the use of tobacco and its derivatives are undesirable to health from the medical point of view.

Cadmium is one of the most toxic elements with reported carcinogenic effects in humans.17 It accumulates mainly in the kidney and liver and high concentrations have been found to lead to chronic kidney dysfunction. It also induces cell injury and death by interfering with Ca regulation in biological systems. The mean cadmium concentration in the snuff sample was  $1.079 \text{ mg} \cdot \text{kg}^{-1}$  and  $0.425 \text{ mg} \cdot \text{kg}^{-1}$  in the tobacco samples. The toxicological nature for As and Hg are also known. For instance, Hg is a neurotoxin in any form. Adults, children and developing fetuses are at risk from ingestion exposure to Hg.18 When methyl mercury (Me-Hg) enters the human system, it readily crosses the walls of the gastrointestinal tract, due to its fast transport through biological membranes, thus accumulating in the envelopes of the nerve cells causing neurological damages.19 In addition, new studies show that Hg also damage cardiovascular, immune and reproductive systems.21 It is interesting to note that there is good agreement between the results of Hg for the snuff  $(0.009 \text{ mg} \cdot \text{kg}^{-1})$  and tobacco  $(0.011 \text{ mg} \cdot \text{kg}^{-1})$ . On the part of As, especially in soluble organic form, can have immediate toxic effect. Ingestion of large amounts can lead to gastrointestinal symptoms such as severe vomiting, disturbances of the blood in circulation, damage to the nervous system; and eventually death.<sup>21</sup> This work recorded  $0.19 \text{ mg} \cdot \text{kg}^{-1}$  As for tobacco and  $0.21 \text{ mg} \cdot \text{kg}^{-1}$  for the snuff stuff. JUNG et al.<sup>4</sup> found a similar mean value of  $0.23 \text{ mg} \cdot \text{kg}^{-1}$  As in Korea cigarette. Meanwhile, the World Health Organisation's22 (WHO) recommended limits for Hg, As and Cd in drinking water are  $0.001$ ,  $0.05$  and  $0.003$  mg·kg<sup>-1</sup>, respectively. Locally, regulatory limits for these local substances do not exist. All the samples contained these elements in excess. Thus, the levels of the elements in both samples compared to limits for drinking water indicate potential toxicity.

Similarly, the mean levels of Cr in the snuff  $(1.148 \text{ mg} \cdot \text{kg}^{-1})$  and tobacco  $(0.517 \text{ mg} \cdot \text{kg}^{-1})$  are in excess of the recommended limit of  $0.05 \text{ mg} \cdot \text{kg}^{-1}$  for drinking water. However, the results are much less than what NADA et al. $8$  found in a popular brand of Egyptian cigarette tobacco. Chromium toxicity in man has been limited to haemorrhage, respiratory impairment and liver lesions.23

The presence of both uranium and thorium in both samples quickly brings attention to the effect of radiological hazard that can be derived from the use of tobacco. Radium and radon are both members of the uranium and thorium decay series. <sup>226</sup>Radium is a longlived,  $\alpha$ -emitter nuclide and its daughter is  $^{222}$ Rn, a short-lived (3.8 d)  $\alpha$ -emitter nuclide that has very shortlived daughters (from seconds to microseconds). The fact of the quasi-simultaneous emission of three  $\alpha$ -and two β-particles makes this radionuclide the most significant radiation hazard in majority of uranium

mines.<sup>24</sup> Thus, further indicating how undesirable cigarettes and other tobacco products could pose to human health.

Rn delivers the highest dose to man from all radiation sources and, therefore, has an increasing impact on the health of society. Health effects attributed to radon decay products inhalation are lung cancer, skin cancer and kidney diseases. In the UK, Rn decay products inhalation may account for up to 2,500 lung cancer per year out of a total of 41,000 cases. Similarly, in the U.S. and Europe it may account for six million of lung cancer cases in the future.25,26 Mean levels of uranium in tobacco and snuff samples were found to be 0.020 and  $0.022 \text{ mg} \cdot \text{kg}^{-1}$ , respectively. A significant difference of  $0.171 \text{ mg} \cdot \text{kg}^{-1}$  was registered for thorium in the tobacco  $(0.286 \text{ mg} \cdot \text{kg}^{-1})$  and snuff  $(0.457 \text{ mg} \cdot \text{kg}^{-1})$  samples.

Furthermore, another important member of the Th and U decay series is Pb. Unfortunately, INAA is not a good method for the determination of this element. Its presence in both samples, however, cannot be denied owing to the presence of U and Th and also to the fact that Pb have been established in other related studies.1,2,4

The toxicity of Pb at high levels of exposure is well known, but the major concern of today is the possibility that continual exposure to relatively low level of Pb may entail adverse health effects.27,28 Lead impairs the renal, homopoietic and nervous system and reports of various surveys suggest that Pb is casually related to deficiency in cognitive functioning.29,30

Certain elements encountered in the study such as Cu and Co are classified as essential to life due to their involvement in certain physiological processes. Elevated levels of these elements, however, have been found to be toxic. Copper, Fe and Co form the essential group of metals required for some metabolic activities in organisms.31 Toxicological effects of large amounts of Co include vasodilation, flushing and cardiomyopathy in humans and animals.<sup>32</sup> Their significance to this study lies between essentiality and possible toxicity when present in elevated levels. However, in contrast to Pb, the definition of an exact toxicity limit to these elements could not be obtained for the study. The decisive point is whether absorption of the existing elements actually took place when the tobacco is in use.

From the results obtained in this study it is clear that except for Cu and Co, all the elements considered toxic in the analysis were higher or comparable in both the snuff and in the tobacco leaves. This strongly suggests that the level of toxic accumulation in the human system is potentially higher for the snuff than for the tobacco. This is even more so, considering the fact that snuff applications involve its total assimilation whereas accumulation in respect of the leaves is mostly through inhalation of smoke in which some fraction is lost through the smoke. The reverse is the case for nonsmokers regarding environmental smoking or secondhand smoking. Meanwhile, more work is needed to

determine what fraction of elements are eliminated or retained during smoking or what fraction enters the smoke into the respiratory system.



One of the common trends in studying elemental concentrations in tobacco leaves and their products is to compare the results of trace elements and heavy metals with other studies from other geographical regions. Table 4 represents some comparison of trace elements and heavy metals of tobacco leaves and published data from other countries. The table contains As, Br, Cd, Cr, Hg, Sb, and Se which are well known to be toxic and carcinogenic.

In Table 4, concentrations of Br, Cd, Cr, Hg, Sb, and Sm of dried tobacco leaves from Ghana are evidently much lower than those from other countries.<sup>1,3,6–9</sup> Thus, it can be said that tobacco leaves regarding these elements from Ghana can be considered to be relatively 'safe' or 'clean'. However, precise information on permissible limits in Ghana are not available to make such an inference.

Whereas in the concentration of As, Co, Fe, La and Sc, there were close similarities between the results of this work and the results of other published data, however, the upper limit of Fe is far less than the mean concentration obtained by NADA et al. $8$  in Egyptian cigarette tobacco. The mean concentration of La was reasonably similar to those found by GARG et al.<sup>34</sup> in Indian tobacco although the upper limit was less than that of the present study. The study recorded very high mean concentrations in Cu, Sr and Se which by far exceeded those found by GARG et al.<sup>34</sup> and ISKANDER et al.,33 a situation testifying that Ghanaian Tobacco leaves can be hazardous to the user.

# **Conclusions**

The assessment of the mineral profile of local Ghanaian dried tobacco leaves and snuff (a product of tobacco leaves) have indicated serious health problems for users in the country. The mean level of heavy metals like Hg, As, Cd and Cr found in them were in high excess compared to WHO limit for drinking water. This requires that the Food and Drugs Board (FDB) in conjunction with other research institutions should determine toxicity threshold for these substances to safeguard users. Education of parents and the youth regarding risks to these additives is very vital. Therefore, there is the need to incorporate tobacco and its related issues into health and healthcare framework system in Ghana or in other developing nations in Africa where the utilization of these additives is on the increase.

In conclusion, this study has revealed that continuous use of raw tobacco and its products could result in an increase in the heavy and trace metals in the human body beyond acceptable limits through accumulation.

Furthermore, the qualitative results obtained in the study will be of great interest for future work in developing a reference material for biologically based samples. This can be affirmed from the fact that as much as 35 minerals were qualitatively analyzed in addition to some more which could quantitatively not be determined due to assigned reasons discussed earlier.

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