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Assessment of carcinogenic and toxic substances in *'Insunko'* herb

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ABSTRACT

There has emerged a herb in Zambia called *'insunko'* which has unknown chemical composition. The use of *'insunko'* herb with unknown chemical composition has brought mixed feelings among many Zambians. This study, therefore, aimed to assess the toxic and carcinogenic substances in*'insunko'* herb. *'Insunko'* herb was purchased from Chipata, Lusaka, Mpika, Mwense, Kitwe, and Solwezi. 5 samples were collected from each of these districts and were thoroughly mixed to give 6 consolidated samples (n = 6). Nicotine and, nitrosamines were analysed using UV spectrometer lambda 35 Perkin Elmer while trace metals were analysed using ICP-MS Inductively Coupled Plasma Mass Spectroscopy (Agilent Technologies, Santa Clara, CA, USA). Nicotine, nitrosamines, and trace metals were detected in high concentrations. The concentrations ranged from 3.87 to 9.83 mg/kg for nitrosamines and 10.94–34.01 mg/kg for nicotine. Hazard Indexes for arsenic, cadmium, chromium, manganese, and copper were greater than one (HI > 1). *'Insunko'* herb is a potentially toxic and carcinogenic substance because it contains toxic and carcinogenic constituents in high concentrations. These toxic and carcinogenic constituents have been confirmed to cause gastrointestinal disorders, cancers, degenerative, cardiovascular, hematopoietic, neurologic and cognitive problems as well as male infertility.

1. Introduction

There has emerged a herb in Zambia called *'insunko'* which is composed of powdered tobacco, ash, soda and other ingredients. *'Insunko'* herb is widely used in Zambia for sniffing while females also use it vaginally to make them tight and warm during sexual intercourse, thus increasing the sexual pleasure of the male partner [1]. There have been reports of vaginal drying agents in South Africa [2], Senegal [3], Malawi, Zimbabwe, Saudi Arabia, Democratic Republic of Congo (DRC), Haiti, Costa Rica, Cameroon, and Kenya [4].

Although *'insunko'* herb is widely used in Zambia, its chemical composition is not clearly understood. However, its main constituent is smashed tobacco and ash, therefore, can be classified as Smokeless Tobacco Product (STP). STPs ingredients have been associated with various health problems [5] like hypertension, cardiovascular diseases, diabetes, compromised platelet function and oxidative stress as well as oral and oesophageal cancers [6–13]. The World Health Organization (WHO) International Agency for Research on Cancer (IARC) has classified STPs as Group One carcinogens because they are confirmed human carcinogenic substances [14]. Group Two A substances are classified as probably carcinogenic to humans while those in Group Two B are possibly carcinogenic [14].

The main groups of carcinogens in STPs include *N*-nitrosamine acids, non-volatile tobacco-specific nitrosamines (TSNA) and some trace metals while nicotine remains the addictive constituent [15–17]. Trace metals are found in tobacco leaves and in processed tobacco products such as STPs and cigarettes due to the fact that ash is the binding factor for the other agents while lime is used to alkalinize the product [18]. Other trace metals that are detected in polluted air are absorbed from the soil [19,20]. The major health concerns in STPs come from Arsenic (As^{+3}), Cadmium (Cd^{+2}), Chromium (Cr^{+2}) and Nickel (Ni^{+2}) which are Group One carcinogens and Lead (Pb^{+2}) which is a Group Two A probable human carcinogen according to the declaration by IARC [21].

Consuming *'insunko'* herb, which has unknown chemical composition has brought mixed feelings among many Zambians more especially health service providers who highly suspect it to be carcinogenic while consumers are enjoying the traditional myths behind its ability to make the vagina tight and warm during sexual intercourse. Cancers have been on the increase in Zambia more especially cervical cancer. This study, therefore, aimed to assess toxic and carcinogenic substances in *'insunko'* herb.

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Fig. 1. Physical appearance of 'insunko' herb.

2. Materials and methods

2.1. Study design

This study was conducted in Zambia between 4th December 2017 and 24th June 2019 after ethical clearance from the National Health Research Authority of Zambia. Five (5) samples were collected from each of the six provinces of Zambia namely; Eastern, Lusaka, Luapula, Muchinga, Copperbelt and North Western. One representative district from each province was selected namely: - Chipata, Lusaka, Mpika, Kitwe, Solwezi, and Mwense. A total of 30 samples of *'Insunko'* herb was purchased and thoroughly mixed to have 6 consolidated samples (n = 6) according to the districts. Sellers were blinded whilst buying *'insunko'* herb to ensure that only what was being consumed by the general public was bought. *'Insunko'* herb is a dark grey substance made by way of roasting the powdered ingredients on a pan. Fig. 1 shows the physical appearance of *'insunko'* herb.

After thorough mixing, each of the six samples was divided into two parts. Two experiments were conducted to assess the presence of carcinogenic substances in *'insunko'* herb. Nitrosamines and Nicotine were assessed using UV spectrometer lambda 35 Perkin Elmer. Trace metals were assessed using ICP-MS Inductively Coupled Plasma Mass Spectroscopy (Agilent Technologies, Santa Clara, CA, USA).

2.2. Sample preparation for nicotine and nitrosamines

Prior to use, all the containers were soaked in 10 % (v/v) HNO_3 and rinsed with ultra-pure water. 10 g of *'insunko'* herb sample from each district was weighed and put in the containers where a 100 ml NaOH solution was added. The mixture was stirred for 15 min followed by filtration. 30 ml of distilled water was then added to the filtrate to ensure the removal of all impurities during the second stage filtration. The filtrate was transferred to the separating funnel and extract by 25 ml of ether. The extraction procedure was repeated 3 times and filtrates were gathered in 4 conical flasks followed by drying using 1.0 teaspoon anhydrous potassium carbonate and filtering again. Diethyl ether was evaporated on the water bath and 4 ml of methanol was added to dissolve the resulting oil. Avoid direct heating as nicotine is hydrolyzed by extreme heating. 14 ml of 1.0 Mol/L HCL was then added and made up to 100 ml using distilled water [22].

2.3. Working standard and instrument calibration

A series of Quality Control (QC) samples were prepared to contain the standard N'-nitrosonornicotine (NNN) and 4-methylnitrosamino (NNK). The sequentially diluted quality control samples and standards solution were run to get a calibration. All known standard calculated values were within ± 3% of their true values or 0.003 g/dl. The prepared internal standard solution was evaluated by preparing and analyzing a blank sample at 206 nm for nicotine and 258 for nitrosamines. That was in demonstrating the absence of any interfering compounds and an area count within ± 20 % of the reference value.

The function group of nitrosamines is the amine group. Since nitrosamines are formed from nicotine, an acidic media was provided in order to detect the amines in *'insunko'* herb. The media provided better quantitation accuracy and precision. The matrix effect was observed by comparing the slope of the samples and the calibration graphs obtained for the QC and standards [23].

2.4. Sample preparation for trace metals

Before use, all the containers were soaked in 10 % (v/v) HNO_3 overnight and repeatedly rinsed with ultra-pure water, obtained from a Milli-Q system (Millipore Corporation, Billerica, MA, USA). We weighed all the samples and digested them overnight with 1 ml GR grade 65 % (v/v) HNO_3 (CNW Corporation, Shanghai, China), and then the following day with 1 ml GR grade 30 % (v/v) H_2O_2 (Sinopharm Chemical Reagent Co., Ltd, Beijing, China). The samples were then mixed, sealed in Teflon microwave digestion tubes, and digested in an accelerated microwave digestion system (Mars CEM, CEM Corporation, Matthews NC, USA) at 800 W, 120 C for 10 min and then 800 W, 170 C for 30 min. All digested samples were filtered by using 0.22 lm nylon membrane and finally, the volume was raised to 10 ml using ultra-pure water [24].

2.5. Analytical aspects

Trace metals (As^{+3}, Cd^{+2}, Cu^{+2}, Co^{+2}, Cr^{+2}, Ni^{+2}, Mn^{+2}, and Pb^{+2}) were analysed using ICP-MS Inductively Coupled Plasma Mass Spectroscopy (Agilent Technologies, Santa Clara, CA, USA). Operating parameters were set as RF power 1510 W, carrier gas 1.1 L min1, makeup gas 0.10 L min1, helium gas 3.5 ml min1 and nebulizer pump 0.1 reps. We obtained standard stock solution mixed with Cr^{+2}, Ni^{+2}, Cd^{+2}, As^{+3}, Cu^{+2}, Co^{+2}, Mn^{+2} and Pb^{+2} (100 lg mL1, GSB 04-1767-2004) from the National Centre of Analysis and Testing for Nonferrous Metals and Electronic Materials (NCATN), China. Quality Control (QC) sample was then prepared by mixing aliquots of each sample in a composite broadly representative of the whole sample set. The mixture of internal standards (Sc^{+3}, Ge^{+4}, Rh^{+3}, Tb^{+3}, Lu^{+3}) was used in order to check the stability and sensitivity of the instrument, and the mean and %RSD values of these elements were also calculated. The working solutions were prepared on a daily basis by appropriate dilutions of standard stock solution using a mixture of 65 % (v/v) HNO3, 30 % (v/v) H_2O_2 and H_2O (v/v/v = 1:1:3). We used a randomized fashion to run all the samples in order to reduce the possible uncertainty from the artifacts related to the injection order and instrumental sensitivity change. The sampler probe was washed between two samples injections, in three steps: rinsing by Milli-Q water for 30 s; washing with 5% HNO3 for 30 s; washing again by Milli-Q water for 30 s. After the three washing steps, the instrument would run the next sample [25,26].

3. Results

3.1. Observed levels of nicotine and nitrosamines in 'insunko' herb

High levels of nicotine and nitrosamines were detected in *'insunko'* herb. All the samples indicated that *'insunko'* herb has high levels of nicotine more especially the herb from Mpika, Mwense and Solwezi districts. Nitrosamines were more concentrated in *'insunko'* herb from Mwense and Chipata districts. Table 1 shows the details.

In this study, we compared the observed levels of nicotine and nitrosamines in *'insunko'* herb with the concentration ranges of Nicotine-

Table 1
Concentration of Nitrosamines and Nicotine in *'Insunko'* herb.

District	Nitrosamines Concentration (mg/kg) & SD	Nicotine Concentration (mg/kg) & SD
Chipata	9.58 ± 1.46	19.29 ± 0.62
Solwezi	6.39 ± 3.89	22.22 ± 0.16
Lusaka	3.87 ± 0.32	10.94 ± 0.29
Kitwe	6.93 ± 1.65	17.27 ± 0.19
Mpika	5.81 ± 0.78	34.01 ± 0.27
Mwense	9.83 ± 0.95	23.18 ± 0.23

Table 2
International comparison of the concentration ranges of NNN and NNK in smokeless tobacco products.

			Concentration (mg/kg) & SD	
Country	Product	Reported as	NNN	NNK
Belgium	CT	Dry	7.38 ± 1.25	0.13 ± 0.01
Canada	CT	Dry	2.09 ± 0.05	0.24 ± 0.02
Denmark	CT	Wet	0.84 ± 0.76	0.96 ± 0.94
Germany	CT	Dry	1.85 ± 0.45	0.03 ± 0.001
	DS	Dry	10.6 ± 8.2	3.47 ± 2.93
India	MS	Wet	0.56 ± 0.01	0.24 ± 0.01
	CT	Dry	0.66 ± 0.19	0.37 ± 0.24
	Cutka	Wet	0.59 ± 0.52	0.24 ± 0.2
	Mishri	Dry	3.65 ± 3.35	0.7 ± 0.41
Sweden	MS	Wet	2.45 ± 1.96	0.75 ± 0.56
UK	CT	Dry	0.9 ± 0.01	0.3 ± 0.01
	DS	Wet	1.8 ± 0.01	0.26 ± 0.01
USA	CT	Dry	3.59 ± 2.92	1.05 ± 0.01

CT- chewing tobacco, MS-moist snuff, DS- dry snuff.

derived nitrosamine ketone (NNK) and N-Nitrosonornicotine (NNN) in smokeless tobacco products according to IARC Monograph volume 89. The concentration of nicotine and nitrosamines in *'insunko'* herb was much higher than the concentration of NNK and NNN in smokeless tobacco products from European Countries, North America, and India as shown in Table 2.

3.2. Observed levels of trace metals in 'insunko' herb

Eight trace metals were detected in *'insunko'* herb namely, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, and nickel. Fig. 2 shows the levels of arsenic, lead, cobalt, and cadmium in *'insunko'* herb in milligrams per kilogram (mg/kg) by district. *'Insunko'* herb from Kitwe and Solwezi had high levels of arsenic followed by Chipata and Mpika. The detected level of Lead in *'insunko'* herb from Chipata was very high compared with other districts.

'Insunko' herb from Chipata, Mpika, Kitwe, Solwezi, and Lusaka had high levels of cobalt in that order while *'insunko'* herb from Mwense had a moderate level. High level of cadmium was observed in *'insunko'* herb from Solwezi followed by Mpika, Kitwe, Mwense, Lusaka, and Chipata in that order.

Fig. 3 shows the levels of chromium, nickel, copper, and manganese in *'insunko'* herb. The herb from Chipata had a very high level of chromium compared with other districts. Other districts with high levels of chromium in *'insunko'* herb were Mpika, Kitwe, and Solwezi in that order. Nickel was highly concentrated in *'insunko'* herb from Mpika followed by Solwezi and Kitwe in that order.

Copper was the second most concentrated trace metal in *'insunko'* herb after manganese. By district however, copper was highly concentrated in *'insunko'* herb from Solwezi followed by Mpika, Kitwe, and Chipata in that order. The most concentrated trace metal in *'insunko'* herb was manganese. *'Insunko'* herb from Chipata District had the most concentrated manganese followed by Mpika and Solwezi. Generally, *'insunko'* herb from Chipata had high concentration of all the trace metals compared with other districts.

3.3. Potential carcinogenicity and toxicity of 'insunko' herb

After weighing the daily intake of *'insunko'* herb as guided by the users, the average daily use was 6 g vaginally and 3 g via sniffing and chewing. *'Insunko'* herb is placed under the tongue where it is mixed with saliva which is then swallowed. Women who use *'insunko'* herb vaginally do so in order to give maximum sexual pleasure to the male partner. *'Insunko'* herb is believed to tighten the vagina and increase vaginal temperature. Therefore, toxic substances from *'insunko'* herb can enter the human body through ingestion, inhalation, and vaginal route.

Daily intake of nicotine and nitrosamines in *'insunko'* herb is shown in Table 3.

Table 4 shows the intake of trace metals in *'insunko'* herb in mg per day/use. We further compared the daily intake of trace metals in *'insunko'* herb to their reference dose (RfD). The United States Environmental Protection Agency (US EPA), Risk Assessment Information System (RAIS) and the Agency for Toxic Substances and Disease Registry (ATSDR) have set a reference dose as a threshold for daily consumption of substances beyond which would be detrimental to human health. Hazard Index (HI) for cadmium, arsenic, chromium, copper, and manganese in *insunko'* herb is greater than 1 which means that *insunko'* herb is potentially carcinogenic to consumers.

Table 5 shows reference dose for the eight trace metals detected in *'insunko'* herb.

4. Discussion

'Insunko' herb is a complex substance with complex chemical composition. This study has established that *'insunko'* herb has a high concentration of nicotine and nitrosamines. Nicotine is a very addictive substance mostly found in both cigarettes and smokeless tobacco products [15–17]. Previous studies have shown that the uptake of approximately 2 mg of nicotine gives rise to average arterial plasma concentrations of about 0.03 mg/l equivalent to 30 ng/ml [27] and bioavailability of nicotine is 20 % orally [28]. The high rate of leukoplakia has also been observed at a place in the mouth where Smokeless Tobacco users place their chew or dip. Approximately 75 % of daily users of moist snuff and chewing tobacco had noncancerous or precancerous lesions in the mouth [29–31]. The prolonged use of *'insunko'* herb in the vagina could have similar adverse effects.

Nitrosamines pose the most dangerous health hazard and prove to be the most harmful carcinogens which are present in smokeless tobacco. An increasing body of knowledge about the carcinogenicity of nitrosamines indicate that most nitrosamines pertain to structure-activity relationships rather than to dose-response. Nitrosamines are responsible for liver, lung and kidney tumours when administered orally and through sniffing [32]. They also act as a trans-placental carcinogen when administered to pregnant rats, mice, and Syrian golden hamsters by several routes [33].

Our study has also detected eight trace metals in *'insunko'* herb namely, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, and nickel. These trace metals are highly concentrated in *'insunko'* herb hence posing high health risk to consumers.

In assessing the potential health risk associated with the use of *'insunko'* herb, we considered parameters set by key global health bodies such as the United States Environmental Protection Agency (US EPA), Risk Assessment Information System (RAIS) and the Agency for Toxic Substances and Disease Registry (ATSDR) [34–37]. Based on this approach, this study has identified five toxic trace metals namely, arsenic, cadmium, chromium, copper, and manganese with Hazard Index greater than one (HI > 1) indicating high health risk to consumers of *'insunko'* herb. *'Insunko'* herb constituents can cause cancers, gastrointestinal disorders, degenerative, hematopoietic, cardiovascular,

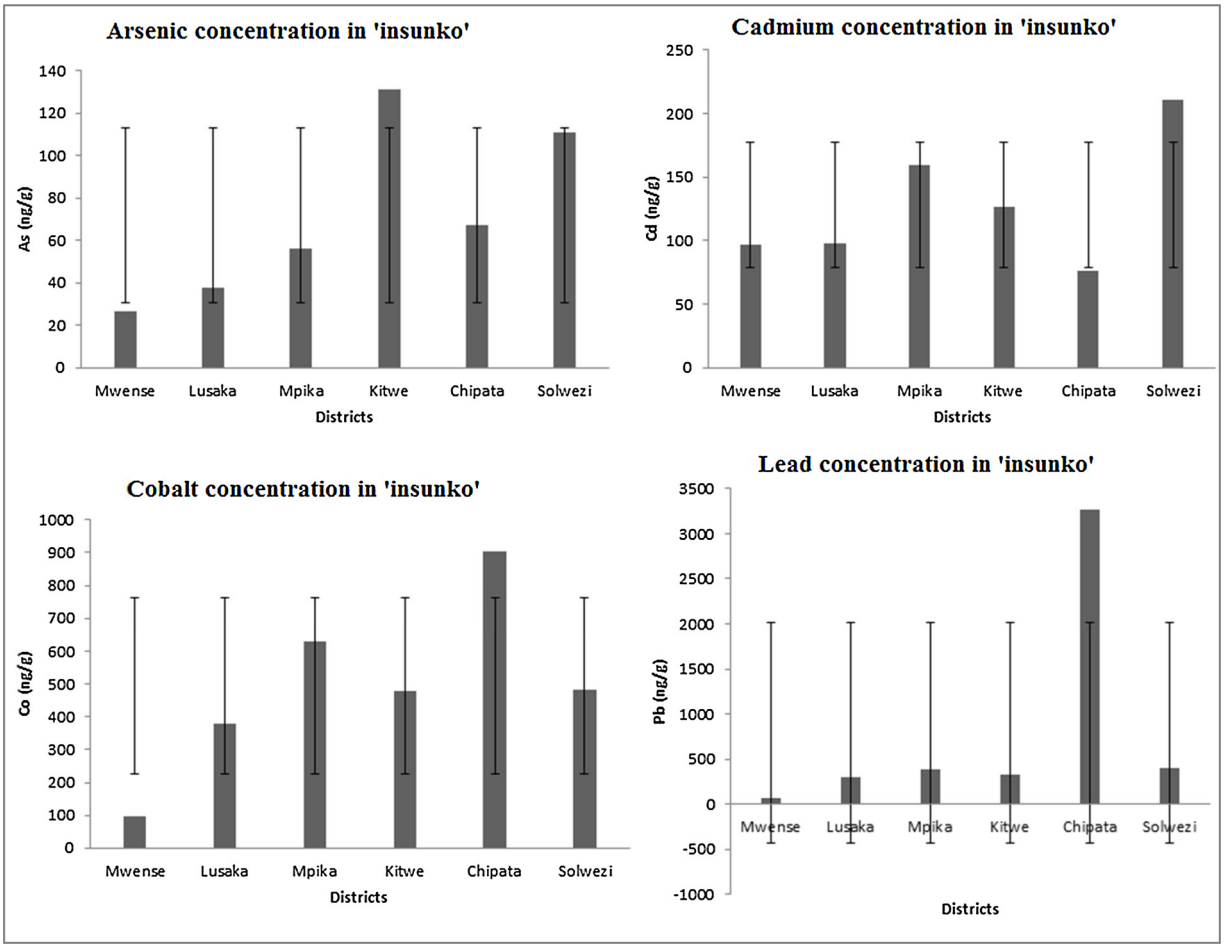

Fig. 2. Concentrations of arsenic, cadmium, cobalt and lead in 'insunko' herb.

neurologic and cognitive problems. They can also cause prenatal neurological and developmental disorders as well as male infertility [38]. Arsenic, cadmium and chromium belong to Group 1 carcinogen to humans [39].

'Insunko' herb can cause tumors in many tissues including skin, liver, lung, kidney, and bladder, as well as prostate and uterus due to its arsenic constituent [40,41]. Acute exposure to *insunko'* herb can lead to gastrointestinal tract disorders [42], whereas chronic exposure can result in degenerative, inflammatory, and neoplastic changes of the respiratory, hematopoietic, cardiovascular, and the central nervous system [38]. *'Insunko'* herb can also damage testicular cells which can lead to male infertility [43–45] while exposure in utero can lead to spontaneous abortion and miscarriage as well as decreased birth weights [46–48].

The cadmium and chromium constituents in *insunko'* herb can lead to lung, liver, pancreas and stomach cancers and can also damage the male reproductive system [49–55] while the highly neurotoxic constituent of lead in the herb can lead to raised systemic blood pressure and reduced glomerular filtration rate among elderly people [56,57].

'Insunko' herb has also been found to contain high concentration of copper which can cause liver damage, gastrointestinal and renal diseases and can also affect the respiratory pathways leading to alveolar migration of macrophages, eosinophilia, formation of histiocytic and noncaseating granulomas among others [58].

Last but not the least; excess manganese in the herb can cause serious side effects, including symptoms resembling Parkinson's disease, such as shaking or tremors. Neurotoxicity symptoms do not manifest immediately but usually become clinically detectable with long-term exposure [59]. Compounds of manganese are suspected to induce or exacerbate asthma [60]. This is of great concern as *'insunko'* herb has been found to contain a very high concentration of manganese.

This study had some limitations; firstly, we could not detect specific nitrosamines such as non-volatile tobacco-specific nitrosamines (TSNA) and *N*-nitrosamine acids in *'insunko'* herb. Secondly, the study only assessed carcinogenic and toxic substances in *'insunko'* herb and did not involve animal model to detect carcinogenicity and toxicity of the herb. Future studies should, therefore, consider *in vivo* research on *'insunko'* herb to determine its carcinogenicity and toxicity.

In conclusion, this study has established that *'insunko'* herb is potentially carcinogenic and toxic as in contains nicotine, nitrosamines and toxic and carcinogenic trace metals (As^{+3}, Cd^{+2}, Cr^{+2}, Cu^{+2}, and Mn^{+2}) in high concentrations. *'Insunko'* herb constituents can cause cancers, gastrointestinal disorders, degenerative, hematopoietic, cardiovascular, neurologic and cognitive problems. They can also cause prenatal neurological and developmental disorders as well as male infertility. Consumers of the herb both vaginally and sniffing should, therefore, be wary of the consequences of using *'insunko'* herb.

Authors' contributions

MK designed the study, developed and programmed the model and drafted the manuscript. HS gave technical support and statistical analysis while TK did the chemical analysis. All authors read and approved the manuscript.

CRediT authorship contribution statement

Maybin Kalubula: Conceptualization, Methodology, Software,

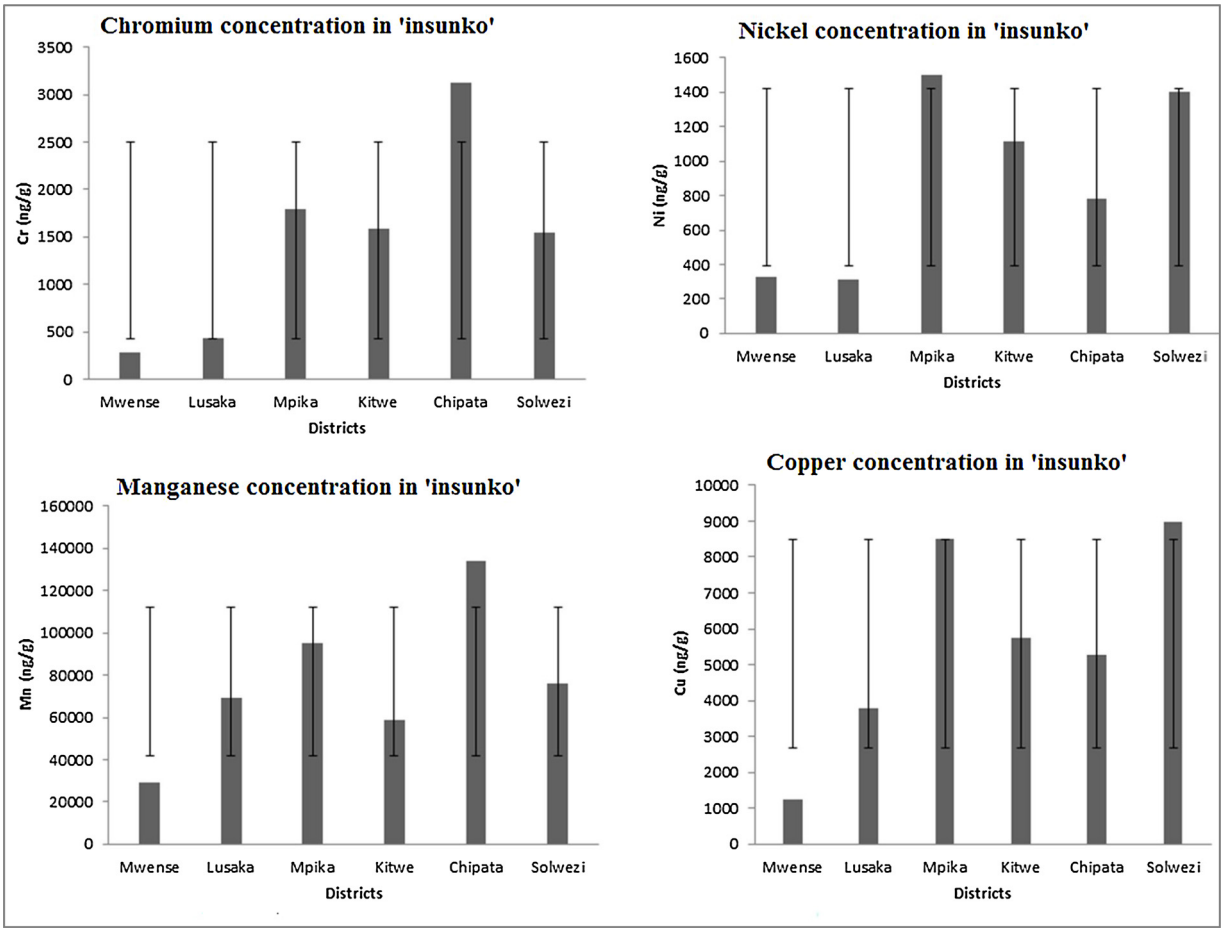

Fig. 3. Concentrations of chromium, nickel, manganese and copper in 'insunko' herb.

Table 3
Daily intake/ use of nicotine and nitrosamines in *'insunko'* herb.

District	Nitrosamines Vagina (mg/use) & SD	Oral/Sniff (mg/day) & SD	Nicotine Vagina (mg/use) & SD	Oral/Sniff (mg/use) & SD
Chipata	5.75E-2 ± 3E-4	2.87E-2 ± 3E-4	1.16E-1 ± 2E-3	5.79E-2 ± 2E-4
Solwezi	3.83E-2 ± 2E-4	1.92E-2 ± 2E-4	1.33E-1 ± 3E-3	6.67E-2 ± 3E-4
Lusaka	2.32E-2 ± 2E-4	1.16E-2 ± 2E-4	6.56E-2 ± 2E-4	3.28E-2 ± 2E-4
Kitwe	4.16E-2 ± 3E-4	2.08E-2 ± 3E-4	1.04E-1 ± 2E-3	5.18E-2 ± 2E-4
Mpika	3.49E-2 ± 2E-4	1.74E-2 ± 2E-4	2.04E-1 ± 3E-3	1.02E-1 ± 3E-3
Mwense	5.9E-2 ± 3E-4	2.95E-2 ± 3E-4	1.39E-1 ± 3E-3	6.95E-2 ± 3E-4

Table 4
Vaginal/ Oral intake of trace metals in *'insunko'* herb (mg/day/use).

		Intake of trace metals in *'insunko'* (mg/day/use)							
District	Route	Cr	Mn	Cu	Ni	Co	As	Cd	Pb
Mwense	Vagina	1.66E-3 ± 3E-5	1.76E-1 ± 2E-3	7.43E-3 ± 2E-5	1.99E-3 ± 3E-5	5.88E-4 ± 2E-6	1.61E-4 ± 1E-6	5.83E-4 ± 3E-6	4.42E-4 ± 1E-6
	Oral/Sniff	8.32E-4 ± 3E-6	8.8E-2 ± 2E-4	3.72E-3 ± 2E-5	9.94E-4 ± 3E-6	2.94E-4 ± 2E-6	8.06E-5 ± 1E-6	2.92E-3 ± 3E-5	2.21E-3 ± 1E-6
Lusaka	Vagina	2.29E-3 ± 2E-5	4.17E-1 ± 2E-3	2.26E-3 ± 2E-5	1.99E-3 ± 2E-5	3.78E-3 ± 3E-5	2.27E-4 ± 1E-6	5.89E-4 ± 2E-6	1.84E-3 ± 2E-5
	Oral/Sniff	1.29E-3 ± 2E-5	2.08E-1 ± 2E-3	1.13E-2 ± 2E-4	9.45E-4 ± 2E-6	1.34E-3 ± 3E-5	1.14E-4 ± 1E-6	2.94E-4 ± 2E-6	9.22E-4 ± 2E-6
Mpika	Vagina	1.08E-2 ± 2E-4	5.7E-1 ± 3E-3	5.1E-2 ± 2E-4	8.98E-3 ± 3E-5	3.78E-3 ± 3E-5	3.37E-4 ± 1E-6	9.59E-4 ± 3E-6	2.28E-3 ± 3E-5
	Oral/Sniff	5.4E-3 ± 3E-5	2.85E-1 ± 3E-3	2.55E-2 ± 2E-4	4.49E-3 ± 3E-5	1.89E-3 ± 2E-5	1.69E-4 ± 1E-6	4.8E-4 ± 3E-6	1.14E-3 ± 3E-5
Kitwe	Vagina	9.52E-3 ± 2E-5	3.51E-1 ± 3E-3	3.44E-2 ± 2E-4	6.68E-3 ± 3E-5	2.87E-3 ± 2E-5	7.86E-4 ± 2E-6	7.57E-4 ± 2E-6	1.96E-3 ± 2E-5
	Oral/Sniff	4.76E-3 ± 2E-5	1.75E-1 ± 2E-3	1.72E-2 ± 2E-4	3.34E-3 ± 2E-5	1.43E-3 ± 3E-5	3.93E-4 ± 2E-6	3.79E-4 ± 2E-6	9.79E-4 ± 2E-5
Chipata	Vagina	1.88E-3 ± 2E-4	8.03E-1 ± 4E-3	3.17E-2 ± 2E-4	4.67E-3 ± 2E-5	5.42E-3 ± 3E-5	4.03E-4 ± 2E-6	4.58E-4 ± 3E-6	1.96E-2 ± 2E-4
	Oral/Sniff	9.38E-3 ± 3E-5	4.02E-1 ± 4E-3	1.58E-2 ± 2E-4	2.34E-3 ± 2E-5	2.71E-3 ± 2E-5	2.02E-4 ± 2E-6	2.29E-4 ± 3E-6	9.79E-3 ± 2E-5
Solwezi	Vagina	9.28E-3 ± 3E-5	4.57E-1 ± 3E-3	5.39E-2 ± 2E-4	8.4E-3 ± 2E-5	2.88E-3 ± 2E-5	6.65E-4 ± 2E-6	1.26E-3 ± 2E-5	2.39E-3 ± 3E-5
	Oral/Sniff	4.64E-3 ± 2E-5	2.29E-1 ± 3E-3	2.7E-2 ± 2E-4	4.2E-3 ± 2E-5	1.44E-3 ± 2E-5	3.33E-4 ± 2E-5	6.31E-4 ± 2E-6	1.2E-3 ± 3E-6

Table 5
Reference dose for trace metals.

Heavy metal	Reference dose (RfD)		REFERENCE
	Inhalation (mg/m^3)	Oral (mg/kg/ day)	
Chromium	1.00E-4	3.00E-3	EPA
Manganese	5.00E-5	1.40E-1	EPA
Copper	1.43E-1 ± 2.25E-2	4.00E-2	RAIS
Nickel	9.00E-5	2.00E-2	EPA
Cobalt	ND by EPA	3.00E-2	RTP
Arsenic	4.3E-6	3.00E-4	EPA
Cadmium	1.00E-5	1.00E-3	EPA/ATSDR
Lead	ND by EPA	3.5E-3	ND by EPA

*ND = Not Determined. RTP (see ref [31]).

Data curation, Writing - original draft. **Heqing Shen:** Supervision, Validation, Writing - review & editing. **Tasawar Khanam:** Visualization, Investigation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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