Borneo Journal of Resource Science and Technology (2023), 13(2): 142-150
DOI: https:doi.org/10.33736/bjrst.5530.2023

Assessment of Toxic Metals in Commonly Used Energy-Stimulating Herbal Drugs Manufactured in Rajshahi City, Bangladesh

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Received: 16 March 2023 Accepted: 20 June 2023 Published: 31 December 2023

ABSTRACT

Having been a common healthcare distress, erectile dysfunction is upsetting the quality of life of men from all walks of life. Along with allopathic therapy, a sizable number of patients opt for energy-stimulating herbal drugs (ESHDs) to treat the ailment. However, a global threat vestige for metal contamination in plant-based drugs at above threshold concentrations. Investigation into metal toxicity through samples from Bangladesh is scarce. Six metals: chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), and zinc (Zn) were probed in 25 energy stimulating herbal drug samples by flame atomic absorption spectrophotometer (F-AAS). Metal content was below detection limit, 51.86 ± 0.07 ppm for Cr, below detection limit, 7.26 ± 0.07 ppm for Cu, 3.41 ± 0.14 – 59.00 ± 0.09 ppm for Fe, 3.54 ± 0.09 – 26.16 ± 0.04 ppm for Mn, below detection limit to 67.34 ± 0.58 ppm for Pb, and below detection limit 27.79 ± 0.07 ppm for Zn. Exposure assessment found Cr and Pb concentrations in the objectionable limits in 12% and 20% of the samples, respectively. This study signifies concern metal toxicity in ESHDs which demands additional probes in the future for other samples to guarantee safe consumption of the drugs.

Keywords: Drug safety, energy stimulating herbal drugs (ESHDs), erectile dysfunction, exposure assessment, metal contamination

INTRODUCTION

Erectile dysfunction is a quite a common health care difficulty, distressing the quality of life of men of all ages. The prevalence of erectile dysfunction varies significantly across nations (Selvin *et al*., 2007). It is estimated that by 2025, 320 million people will be suffering from erectile dysfunction (Abolfotouh & Al- Helali, 2001). Hindering of a stable erection by vascular abnormalities of the penile blood supply and erectile tissue causes erectile dysfunction (ED) (Jeremy *et al*., 1997; Melman *et al*., 2004; Sexual Function Health Council, 2004; Selvin *et al*., 2007). Lack of sexual desire (libido), ejaculation complications, and premature ejaculation in people suffering from the disease are also observed (Pamplona-Roger, 2000; Roper, 2001). Diseases like cardiovascular diseases and hypertension, and some drugs (tranquilizers, appetite suppressants, antihistamines, antidepressants, etc.) can trigger erectile dysfunction (Bener, 2007; Lim, 2017).

When patients with erectile dysfunction visit mainstream doctors, synthetic drugs are suggested. These drugs include oral testosterone, which is found to be potent in lessening the dysfunction in some men who have lower levels of natural testosterone. However, the drug is found to be ineffective for most patients and those who are taking the medication risk liver damage (Sexual Function Health Council, 2004). Other drugs (phentolamine, papaverine, etc.) pose side effects like a sudden rise in blood pressure. Moreover, the majority of patients in the developing world lack health insurance. Managing money to treat diseases is an extra burden to the people living in the region. Conventional drugs are available by visiting practitioners after paying for consultation. Patients are also offered a diagnosis, if needed. All of these costs are added to the price of the synthetic drug. However, energy stimulating herbal drug (ESHD) practitioners neither charge a consulting fee nor offer clinical tests to

patients. The patients needed to pay for only the drugs. Considering these events, the prices of herbal medicines are deemed lower than their allopathic counterparts. As a result, a fraction of frustrated patients searches for natural remedies to treat this ailment (Zamir *et al*., 2019).

Due to their natural origin, herbal drugs are assumed to be safe. Investigations by the scientific community didn't find the traditional belief entirely true (Ernst, 2002; Street, 2012; Ulla *et al*., 2012). The presence of toxic metals was evident to varying degrees in herbal drugs (Boraa *et al*., 2014; Zamir *et al*., 2019; Luo *et al*., 2021). One of the main concerns associated with these therapeutics is contamination with toxic metals. Prolong lead intake causes an imbalance in free radical production and antioxidant levels. As a result, conditions like ionic and oxidative stress arise. Lead toxicity causes cellular damage by means of reactive oxygen species production due to free radical and antioxidant level misbalancing. The ionic mechanism of lead toxicity can also negatively affect inter- and intracellular signaling, the release of neurotransmitters, and protein folding and maturation which result in different diseases (Lynch & Berry, 2007; Kim *et al*., 2014). Copper toxicity causes irritation of the upper respiratory tract, nausea, and hair and skin discoloration dermatitis (Greenwood & Earnshaw, 1984; Martin & Griswold, 2009; Ulla *et al*., 2012; Dghaim *et al*., 2015). Liver damage, renal failure, lung cancer, and massive gastrointestinal conditions set in due to chromium toxicity (McGrath & Smith, 1990; Woods *et al*., 1990). Manganese over uptake is associated with mood swings, skin rashes, upset stomach, nose irritation, etc (Tan *et al*., 2006). Prolong the intake of zinc at high concentrations can cause liver failure, kidney failure, and anemia (Steenkamp *et al*., 2000). Overexposure to iron causes hemosiderosis, organ damage, and colon cancer (Lund *et al*., 2001).

Medicinal plants pick up metal from soil in which they grow and metal contamination is possible from contaminated irrigation water and storage of plants (Islam *et al*., 2020; Karahan *et al*., 2023). The formulation of herbs into finished drugs also contributes to metal contamination, both unintentionally and intentionally, assuming synergistic effects (Gogtay *et al*., 2002). The metals, upon exceeding their safety limits, lead to malfunction and malformation of organs, abdominal pain, vomiting, severe anemia, hemoglobulinuria with dark colored stools, etc. The regulations for metal contaminants have been settled in different countries (USP, 2021). The World Health Organization (WHO) advocates safety assessment of herbal drugs for contaminants like toxic metals to secure consumer safety due to the ingestion of drugs (WHO, 2004). WHO also suggests using appropriate and best available pharmaceutical limit tests to be performed on the contaminants to satisfy national and international regulatory authorities.

Erectile dysfunction is a common non-communicable disease among Bangladeshi adults (Mahbub *et al*., 2019). It affects sexual intercourse, which is needed for sexual pleasure, reproduction, or both purposes. The disease is taboo, and due to the nature of the disease, anxiety builds up among the sufferers. This atrocious setting is utilised by manufacturers of herbal medicines in both electronic and print media, by offering patients an easy, safe, and effective way of therapy than allopathic alternatives. Through this process, a sizable number of patients turn to ESHDs. However, no investigation into metal contaminants is available on frequently consumed ESHDs in this region. Therefore, distrust towards the premarketing quality checks of the drugs remains. This increases the possibility of risking public health safety by affecting drug safety. The determination of the level of toxic metals copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), lead (Pb), and zinc (Zn) in ESHDs and assessing their safety was intended as the objective of the study. The metal concentrations in herbal drugs for treating erectile dysfunction were compared with international regulations from the World Health Organization (WHO), jointly, World Health Organization and Food and Agricultural Organization (WHO/ FAO), United States Pharmacopoeia (USP) etc. It is hoped all stakeholders, local people, regulatory authorities, and scientific community would benefit from the investigation.

MATERIALS AND METHODS

Sample Collection

All investigated samples were commercially finished packs bearing Directorate of Drug

administration (DGDA) ID's. Samples were collected from the Rajshahi city corporation area. The city is surrounded by two satellite towns, Nowhata and Katakhali, in its close vicinity. The conjunction creates an urban cluster of about ten lac population, making the city the fourth most populous city in Bangladesh after Dhaka, Chattogram, and Khulna city (populationstat.com/Bangladesh/Rajshahi).

Treatment, prevention and management of different ailments are satisfied by herbal drugs, and a large share of the intake goes to sex-stimulating therapeutics. Drugs were procured from different manufacturers, where each manufacturer was found to sell several drugs of the same or similar therapeutic classes. Drug selling outlets pose the similarity, densely populated and mass transaction of people taken from the selected places, making them drug selling hotspots (Figure 1).

After procurement, samples were preserved as per the written information on the packaging wall or package inserts. Prior to analysis, samples were blindfolded for coding (Table 1).

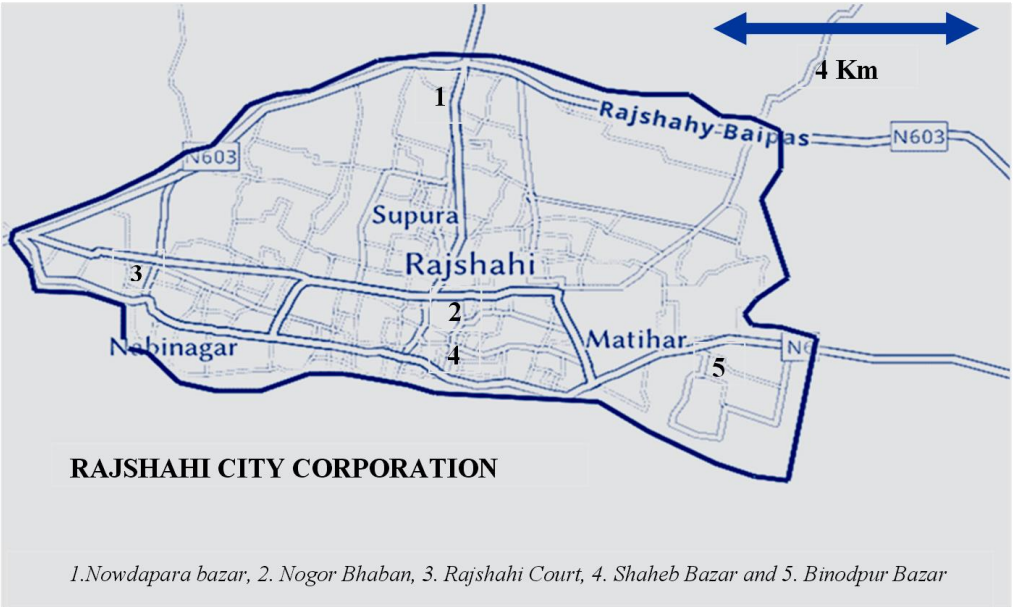

Figure 1. Sampling locations in Rajshahi City Corporation Area (Figure credit Nat Geo software)

Table 1. Sample description in this study

Sample ID	Batch No	Producer ID	Dosage	Mfg. Date	Expire Date
S 1	4	M 1	2 capsules to be taken 1-2 times daily	Nov.21	Nov.22
S 2	2	M 1	1-2 tablets 1-2 times daily	May.21	May.24
S 3	2	M 1	1-2 capsule to be taken with milk	July.21	July.23
S 4	11	M 1	1 capsule 2 times daily	July.21	July.23
S 5	3	M 1	2 tablets to be taken with a cup of milk	May.21	May.24
S 6	2	M 1	1 capsule twice daily	Dec.20	Dec.22
S 7	9	M 1	2 tablets 2 times daily	Sep.21	Sep.23
S 8	1	M 1	1 tablet 2 times daily	March.21	March.24
S 9	4	M 1	2-3 tea spoonful 1-2 times daily	April.21	April.23
S 10	14	M 1	1/2 tea Spoon 1-2 time daily	Dec.20	Dec.22
S 11	B09M21E23	M 2	1-2 capsule 1 time daily after meal	Sep.21	Aug.23
S 12	B08M21E23555	M 2	1-2 tablets to be taken with milk	Aug.21	July.23
S 13	B08M21E23	M 2	1tablet 1-2 times daily	Aug.21	July.23
S 14	B020721	M 2	1-2 capsule 1 time daily after meal	Jul.21	Jun.21
S 15	310M21E23	M 2	1 tablet 1 time daily	Oct.21	Oct.23
S 16	5	M 3	1 tablet 2 times daily	Aug.21	July.24
S 17	3	M 3	1-2 capsule 1-2 times daily	Apr.21	March.24
S 18	4	M 3	1-2 tablet 2-3 times daily	July.21	June.24
S 19	3	M 3	3-5 tablets 3-2 times daily	April.21	March.24
S 20	10	M 3	3-4 tablets 2-3 times daily	Oct.21	Sep.24
S 21	4	M 3	1 capsule 2-3 times daily	July.21	Jun.24
S 22	7	M 3	1 capsule 2-3 times daily	Oct.21	Oct.24
S 23	1	M 4	1-2 tablets 1 time	Sep.20	Sep.23
S 24	20	M 4	1-2 tablets 1 time	Mat.21	May.24
S 25	101	M 4	1-2 tea spoonful 2 times	Mar.21	Mar.24

Sample Digestion

The collected ESHDs were positioned onto individual porcelain dishes distinctly, where each dish with the particular sample was placed in an oven. The temperature was programmed (70 °C) until a constant weight was obtained. Oven dried samples were pulverised to fine powder and preserved in a plastic vial inside a desiccator. The rest of the procedure was done in a fume hood with an exhaust system. About 1 g of homogeneous powder of ESHD was taken in a Teflon vessel, and 10 mL of HNO_3 acid was added to decompose and abolish the organic materials present in the drug matrix. After that, an acid mixture of 6 mL concentrated HNO_3 (Merck, Germany), 3 mL concentrated $HClO_4$ (Merck, Germany), and 10 mL HF (Wako, Japan) was used for sample digestion. The obtained solution was evaporated at 180 °C to dryness on a ceramic hot plate. The solid mass was dissolved in 5 mL of HF and 1 mL of $HClO_4$ acid and heated to near dryness. The procedure was repeated thrice. The presence of HF was removed by the addition of HNO_3 acid, and the solution was heated until it gave off white color fumes. At last, the residue was diluted with 0.1 N HNO_3 and the volume was made up to 25 mL (Rao *et al*., 2011).

Sample Analysis

The spectral band pass, wavelengths, and other instrumental conditions were set as per the requirements of the manufacturer (Table 2). Stock standard solutions (1000 mg/L) procured from Wako Chemicals, Japan, of each metal were diluted to the desired working standards, and the standards were added to the autosampler of Atomic Absorption Spectrophotometer (AAS-7000, Shimadzu, Japan). The flame atomizer was turned on, and proper fuel oxidant flow was controlled through a computer program. Samples were nebulized and atomized in the flame of a 10 cm air acetylene burner. The spectrophotometer was delivered with single element hollow cathode lamp. A list of instrumental operating conditions is provided below.

Table 2. Instrumental operating conditions for each metal

Metal	Digested sample flow, mL/ min	Spectral line			Entrance and exit slits, nm
		Source	Intensity, mA	Wavelength, nm	
Cr	5	HCL	7	357.9	0.2
Cu	5	HCL	3	324.7	0.5
Fe	5	HCL	7	372	0.2
Mn	5	HCL	5	279.5	0.2
Pb	5	HCL	5	217	1.0
Zn	5	HCL	5	232	0.5

HCL= Hollow Cathode Lamp

Linearity Study

A calibration curve linearity study was adopted to validate the data. Calibration standards for each metal were prepared, and absorbance values were obtained for the respective standards. Plots of calibration standard vs. absorbance provided a linearity curve equation, and coefficients (R^2) (Table 3).

Table 3. Linearity study

Metal	Regression Equation	Linearity Coefficient (R^2)
Cr	0.0638x+0.0034	0.9934
Cu	0.1155x-0.0033	0.9999
Fe	0.0863x+0.0028	0.9973
Mn	0.1741x-0.0091	0.9580
Pb	0.0139x+0.009	0.9397
Zn	0.3719x+0.002	0.9991

Recovery Study

The accuracy of F-AAS method was ascertained by spike and recovery experiments. A 1 ppm of each element was spiked into a natural sample matrix. Standard addition was recovered in the range of 82 – 112% (Table 4).

Table 4. Spike and recovery experiment

Analyte	Recovery (%)
Cr	82
Cu	89.8
Fe	95.6
Mn	112
Pb	98
Zn	108.5

Statistical Analysis

Research data were tabulated and compiled using statistical software (Microsoft Office Excel 2016), where they were processed, calculated, and stored in a personal computer. Triplicates of each concentration data point for individual metals in each sample were represented as their geometric mean and standard deviation.

RESULTS AND DISCUSSION

Different countries apply local or international regulatory authority safety limits to assess drug safety (Inada *et al*., 2023). Bangladeshi authority, DGDA relies on international regulations like the USP for drug safety assessment (Zamir *et al*., 2019). As part of surveillance, the authority conducts an inspection on suspected drugs where post-market drug withdrawal is common.

The consumption of energy stimulating herbal drugs has gained momentum in Bangladesh. However, strict quality assurance (QA) of remedies like their allopathic counterparts, is not being practiced to benefit patrons and the scientific community (Boraa *et al*., 2014).

Ten toxic metals were quantified using atomic absorption spectrometry coupled with a flame atomizer. The concentration data of different metals in each sample were compared with those established by the international regulatory authority, the WHO, WHO/FAO established data and with those established by the national authority, the USP and the Brazilian Pharmacopoeia established permissible safety concentration data for elemental impurities.

In the current study, the chromium concentration was found to be below the detection limit- 51.86 ± 0.07 ppm (Table 5). The highest concentration was observed in S17, whereas the lowest concentration was observed in S25. The regulatory authority sets a safety limit for Cr 25 ppm (Brazilian Pharmacopoeia, 2010). With this ceiling, three samples (S17, S23, and S24) were found unacceptable. The possibility of organ damage by means of hepatotoxicity and renal toxicity increases upon consumption of unacceptable samples for a long time (McGrath & Smith, 1990; Woods *et al*., 1990). However, two investigators, Jurowski (2019) and Tokalioglu (2012), found chromium concentrations ranging from 4.42 to 8.74 ppm and from 0.44 to 8.71 ppm, respectively, implying safe consumption for all samples from their manufacturers.

In the current study, copper concentration was found in the range of below detection limit (BDL), 7.26 ± 0.07 ppm. The highest and lowest contents of Cu were observed in S4 and S22, respectively. A small variation in sample concentrations is observed for Cu. The regulatory authority fixed the permissible concentration of elemental impurities for oral consumption considering individual metals at 300 ppm (USP, 2021). All samples were safe to consume for Cu. African investigations led to a similar conclusion on Cu content in samples (0.89 – 3.15 ppm) (Onwordi *et al*., 2015).

In the current investigation, iron concentration ranged as 3.41 ± 0.14 – 59.00 ± 0.09 ppm. A slight variation in Fe content was evident in the investigated samples. Highest and lowest Fe was found in S23 and S25, respectively. Another local probe found quite similar iron concentration in samples where the maximum Fe concentration was 10.73 ppm (Zamir *et al*., 2021).

Manganese concentration ranged as 3.54 ± 0.09 – 26.16 ± 0.04 ppm in ongoing study. A slight variation in Mn content was evident in the investigated samples. Highest and lowest Mn was found in S16, and S18 respectively. The regulatory authority establishes the safety endpoint for Mn as 320 ppm (FAO/WHO, 1984), which declares all samples acceptable for oral consumption. However, a sample exceeding the safety limits for Mn was observed in a national investigation (Zamir *et al*., 2019).

Ongoing study revealed the concentration of lead in herbal medicines ranged from detection limit to 67.34 ± 0.58 ppm. The highest concentration was observed in S8 and the lowest concentrations were observed in 14 samples. The regulatory authority established the safety endpoints for Pb as 10 ppm (FAO/WHO, 1984), which found 5 samples (S14, S15, S16, S17, and S23) unacceptable for oral consumption. Another local investigation found unacceptable levels of Pb in some samples (Zamir *et al*., 2019). Excess consumption of Pb is associated

with different reproductive dysfunctions by means of altered sperm morphology and decreased sperm count and disorganized epithelia (Ejidike & Orianwa, 2015).

Table 5. Concentration of quantified metals in energy stimulating herbal drug samples

Sample ID	Metal concentrations (ppm)					
	Cr	Cu	Fe	Mn	Pb	Zn
S 1	8.38 ± 0.38	3.45 ± 0.08	41.45± 0.53	6.65 ± 0.05	1.13 ± 0.31	1.98 ± 0.04
S 2	6.32 ± 0.02	2.50 ± 0.03	25.08 ± 0.21	12.39±0.01	BDL	2.06 ± 0.03
S 3	5.25 ± 0.16	2.18 ± 0.07	35.97 ± 0.04	6.95 ± 0.03	1.40 ± 0.38	0.86 ± 0.06
S 4	3.14± 0.01	7.26± 0.07	55.48 ± 0.74	7.83 ± 0.07	0.50 ± 0.37	1.19 ±0.01
S 5	5.25 ± 0.06	2.09 ± 0.06	31.91 ± 0.03	3.79 ± 0.10	BDL	0.67 ±0.03
S 6	4.97 ± 0.12	3.16 ± 0.06	28.21± 0.47	14.05 ± 0.10	1.94 ± 0.43	2.30 ± 0.06
S 7	8.01 ± 0.04	2.91 ± 0.16	7.27 ± 0.16	6.52 ± 0.05	BDL	1.41 ±0.09
S 8	5.16 ± 0.03	4.19 ± 0.12	26.30 ± 0.31	24.14 ± 0.19	1.71 ± 0.12	8.51± 0.08
S 9	9.22 ± 0.06	2.77 ± 0.14	34.12 ± 0.06	8.88 ± 0.03	BDL	0.64 ± 0.01
S 10	5.60 ± 0.01	2.37 ± 0.02	12.32 ± 0.03	4.36 ± 0.08	BDL	0.97 ± 0.03
S 11	6.54 ± 0.03	1.72 ± 0.02	26.49± 0.02	11.47 ± 0.12	BDL	0.76 ± 0.08
S 12	5.34± 0.07	1.71 ± 0.06	27.95 ± 0.10	8.12 ± 0.06	BDL	0.65 ± 0.02
S 13	6.13 ± 0.03	4.40 ± 0.13	47.35 ± 0.72	8.16 ± 0.05	BDL	1.14 ± 0.09
S 14	7.31 ± 0.05	0.44 ± 0.10	23.21 ± 0.04	5.94 ± 0.02	13.88 ± 0.30	0.28 ± 0.04
S 15	6.14± 0.05	1.25 ± 0.03	20.35 ± 0.06	6.54 ± 0.08	34.54 ± 0.16	0.60 ± 0.09
S 16	5.79 ± 0.07	2.88 ± 0.07	44.42± 0.05	26.16 ± 0.04	23.45 ± 0.38	1.77 ± 0.03
S 17	51.86 ± 0.07	1.42 ± 0.02	36.53 ± 0.02	15.46 ± 0.12	12.50 ± 0.00	3.56± 0.09
S 18	1.62 ± 0.02	1.56 ± 0.04	36.66 ± 0.07	3.54 ± 0.09	BDL	2.09 ±0.02
S 19	7.93 ± 0.05	1.06 ± 0.13	27.00 ± 0.29	9.17 ± 0.13	BDL	BDL
S 20	5.05 ± 0.05	2.80 ± 0.20	28.26 ± 0.44	6.11± 0.16	0.99 ± 0.38	0.38 ±0.06
S 21	6.35 ± 0.08	4.60 ± 0.09	25.57 ± 0.06	14.58 ±0.14	BDL	1.23 ± 0.06
S 22	3.34 ± 0.09	BDL	7.34 ± 0.09	7.33 ± 0.06	BDL	1.11 ± 0.15
S 23	31.71 ± 0.08	6.20 ± 0.01	59.00 ± 0.09	24.94 ± 0.05	67.34 ± 0.58	27.79 ± 0.07
S 24	30.15 ± 0.05	0.95 ± 0.139	24.65 ± 0.12	11.42 ± 0.15	BDL	0.30 ±0.08
S 25	BDL	2.52 ± 0.02	3.41 ± 0.14	7.12 ± 0.04	BDL	1.04 ± 0.01

BDL= Below detection limit

The present investigation on samples found zinc concentration ranged from below the detection limit to 27.79 ± 0.07 ppm. Slight variation in Zn content was observed throughout the probed drugs. Highest and lowest Zn were found in S27 and S19, respectively. The regulatory authority establishes the safety endpoint for Zn as 50 ppm (FAO/WHO, 1984) which declares all samples acceptable for ingestion. In agreement with this investigation, a local study estimated Zn at a lower concentration where all samples were within the acceptable limit (Zamir *et al*., 2021).

Metals are widely distributed in nature. The last century witnessed rapid industrial development. Therefore, apart from the already existing background concentrations, human activities release metal into the environment which adds up and creates a burden for organisms. Plants pick up metals from contaminated soil and irrigation water during their development stages (Islam *et al*., 2020). The conversion of medicinal herbs into finished formulations consists of different manufacturing stages, where from atmosphere of the factory and processing utensils metal enters the formulations. There is also evidence of intentional addition of metals into herbal formulations in the belief that the metals would pose a synergistic effect, where metals are burnt down at high temperature and the remain is added to the formulation (Gogtay *et al*., 2002).

Table 6. Instrumental sensitivity

Metal	Detected samples	
	n	%
Cr	24	96
Cu	24	96
Fe	25	100
Mn	25	100
Pb	11	44
Zn	24	96

CONCLUSION

Summing up the metal concentrations of the current probe, we can conclude that lead and chromium were the toxic metals for which concern remains, as unacceptable concentrations of metals were found in 20% and 12% energy stimulating herbal drugs, respectively, in comparison with regulatory body safety limits. For the rest of the metals, the copper concentration was below the detection limit- 7.26 ± 0.07 ppm, iron concentration was 3.41 ± 0.14 – 59.00 ± 0.10 ppm, manganese concentration was 3.54 ± 0.09 – 26.16 ± 0.04

ppm, and zinc concentration was below detection limit, 27.79 ± 0.07 ppm. With uncontrolled dosing, the risk of adverse health effects from other samples also applies to the investigated metals. More lab-based work is needed to conduct investigations on other samples.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution of International Science Program (ISP), Uppsala University, Sweden, through the Swedish International Development Cooperation Agency (SIDA) for its financial support for carrying out this investigation.

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