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SAFETY EVALUATION OF SOME READY-TO-USE HERBAL CONCOCTIONS SOLD WITHIN ILORIN METROPOLIS

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ABSTRACT
Safety and quality of indigenous herbal concoctions in Nigeria is of public health concern as the consumption is on the increase. Hence, this study was conducted to evaluate microbial and heavy metals contaminants in selected ready –to- use herbal concoctions within Ilorin metropolis. The heterotrophic microbial counts using pour plate method were assessed and the contaminations of selected heavy metals were analyzed using Atomic Absorption spectrophotometer. The viable plate count for bacteria and fungi ranged between 2.0×10^4 - 10.5×10^5 CFU/ml and 2.0×10^4- 8.3×10^5 respectively. Bacteria and fungi isolated include *Pseudomonas aeruginosa, Micrococcus luteus, Citrobacter freundii, Staphylococcus aureus, Klebsiellia pneumoniae, Corynebacterium kutscheri, Aeromonas hydrophilia, Staphylococcus epidermis, S. cerevisae, Gliocladium* sp., *Penicillum* sp., *A. alternata, Aspergillus niger, C. albicans, R. stolonifer, Geotrichum* sp *and A. flavus* respectively. Pb and Cu level concentrations were found to be in the range of 0.1-0.3mg/L and 0.01- 074 mg/L respectively. The concentration of Pb and Cu were generally high and above the safe limits set by WHO/FAO while Cd was not detected in any of the samples. Generally, the consumers of these herbal products are unwittingly exposed to heavy metal poisoning and microbial contaminants. It is suggested that regulatory bodies should intensify efforts to minimize human exposure risk.
KEY WORDS: Heavy metals, herbal concoctions, microbial contamination, health implication

INTRODUCTION
Herbal preparations are often perceived as being natural and therefore safe, but they are not free from adverse effects which may be due to factors such as adulteration, substitution, and misidentification, lack of standardization, incorrect preparations and contamination with pathogenic bacteria that present serious health hazards (Arias *et al*., 1999). Although the World Health Organization advocated for the integration of herbal medicinal products into the primary health care system of developing countries (WHO, 1999), safety issues related to herbal drugs continue to be ignored by the producers whose methods of concocting herbal preparations for the public are usually unhygienic with microbiological hazards (Oyetayo, 2008). As plants are commonly consumed in daily food basket, the everyday increase in public's interest in taking herbal medicine and treatments instead of those of chemicals with destructive side effects they bring along has concerned the safety of herbal products. Researchers have also demonstrated that plants are polluted with copper, cadmium and lead toxic metallic elements that are stable in environment and disintegrated difficultly (Amanlou *et al*., 2015).

The government of Nigeria promulgates the Medical and Dental Practitioners (Amendment) Decree No. 78 in September 30, 1992, which placed natural medicine (traditional and alternative medicine) side by side with the orthodox system. Since then, there has been a high level of rivalry and advocacy against orthodox medicine by the traditional/herbal medical practitioners. Advertising in various forms by the herbal practitioners is beyond compare in Nigeria (Adenike *et al.*, 2006).

Unbridled hawking of herbal products in containers especially in motor parks, public places, artisan work areas and the unrestricted manners in which people consume them has arouse interest on their safety on consumption. The work is therefore aimed at investigating the level of heavy metals and microbial contaminants in some of the commercially available herbal concoctions in Ilorin, Kwara State.

MATERIALS AND METHOD
Sample collection
Total of 39 samples of herbal concoctions which were acclaimed to be against pile, typhoid, sexually transmitted infection were purchased within Ilorin metropolis (Oja Oba, Oja titun, Maraba, Ipata and different Motor parks) in a sterile sample bottle and taken to Microbiology laboratory for immediate analysis.

Preparation and Inoculation of Sample
One milliliters of sample was serially dispensed into sterile distilled water in test tubes and properly mixed up to 10^{-5} dilution. Aliquot was transferred from 10^{-3} and 10^{-5} dilutions onto Nutrient agar, Potato dextrose agar, Mannitol salt agar and Macconkey agar in sterile petri dishes respectively. The plates were swirled properly, allowed to solidify and were incubated at 37ºC for 24 hours for bacterial growth and at 25ºC for 48-72 hours for fungal growth.

Enumeration and Characterization of isolates
Colonies observed were counted and repeatedly sub cultured for purity of all the isolates. Thereafter, the pure cultures were characterized using standard methods (Cowan, 1974; Fawole and Oso, 2007). Gram negative bacteria were characterized using Microbact identification kit.

Preparation of solution for heavy metals determination
The level of selected heavy metal contaminants in the samples were assessed using atomic absorption spectrophotometer (model: ACCUSYS 211). The method of Etuk *et al.* (2008) and Amanlou *et al.* (2015) were adopted with slight modifications. The samples were analyzed in triplicates and the values expressed as mean ± standard deviation.

RESULTS AND DISCUSSION.
The total viable count of the herbal samples ranged from 1.0×10^4 to 10.2×10^7, the lowest bacteria count was from sample C_3 while the highest bacteria count from sample B_1. The fungal count of the herbal samples ranged from 5.0×10^4 to 8.3×10^5, the lowest fungal count was from sample M_1 while the highest fungal count was from sample I_2 (Table 1).

A large percentage of Nigerians do take herbal remedies as a ready therapy for all forms of diseases, however, they are unconsciously exposed to microbial contaminants and high concentrations of toxic metals. Occurrence of *Staphylococcus aureus* (22%), *Staphylococcus epidermis* (6%) and *Micrococcus luteus* (16%) from this study was also reported by Kolajo (2000) and remarked that the presence of these microorganisms in herbal medicines signifies the substandard production processing of the herbs.

Other enterotoxigenic gastroenteritis-causing organisms such as *Pseudomonas, Enterobacter, Aeromonas hydrophila* and *Klebsiella* (Figure 1) isolated from herbal samples in this study have also been previously reported by Adenike *et al.,* (2006); Araujo *et al.* (2002) in infantile gastroenteritis. *Pseudomonas aeruginosa* was similarly indicated in infantile gastroenteritis transmitted through water and foods by Adenike *et al*. (2006). *Klebsiella pneumoniae* is also recognized as being opportunistic pathogens and have become of increasing health importance.

Corynebacterium kutscheri isolated from herbal concoction during the study has not been reported by any authors. Research shows that the *Corynebacterium kutscheri* was first isolated from rats and mice. Transmission is primarily through faecal-oral route (Charles, 2009). This pathogen however, may be suspended in the air and travel over a metre or more from the source to the host. Also, another reason that can be adduce for the presence of this organism is mishandling by the herb sellers whereby mice or rat in house or environment where the concoction is prepared may come in direct contact.

The presence of *Aspergillus niger, Aspergillus flavus, Penicillium* sp. and *Rhizopus stolonifer* in this study is corroborated by the reports of Lee and Jo (2000). These authors noted that *Aspergillus niger, A. flavus* and *Rhizopus stolonifer* are common air contaminants probably present in the drying and packaging areas. *Aspergillus flavus* isolated is of highest concern as it is known to produce aflatoxin (Riba *et al.,* 2008).

Penicillium sp. and *Rhizopus stolonifer* isolates in this study were also isolated in the study conducted by Braide *et al.* (2013).

Odedara and Memuletiwon, (2014) reported that the microbiological background of herbal medicines depends on several environmental factors and exerts an important impact on the overall quality of the herbal products and preparations. Generally, microbial contamination of these herbal concoctions under study may be due to the materials used and personnel involved in the process of production as some of the organisms isolated are normal flora of soil, water and human as also reported by Braide *et al.* (2013).

Contamination of traditional medicines by heavy metals is of major concern because of the toxicity, persistence and bioaccumulative nature of such metals (Dzombu *et al.,* 2012). The results obtained shows that lead and copper was detected in 20 of 39 samples while cadmium was not detected in any of the sample. Figures 2, 3 and 4 showed heavy metals contaminants in herbal concoctions acclaimed for treatment of pile, typhoid fever and sexually acquired infections.

Lead and copper were found in 51.3% of the assessed samples. The minimum concentration of Pb was 0.100 mg/L while the highest Pb and Cu concentration were 0.300 and 0.740 mg/L respectively, all exceeding safe standard limit as set by Food and agricultural organization/ World health organization provisional tolerable intake for Pb, Cd and Cu to be 0.005 mg/L, 0.031 mg/L and 0.110 mg/L, respectively (Table 2). However, 97% of the samples were below the WHO safe limit for Cu. Table 3 represents a summary of these parameters for the metals of interest in this study.

Even though WHO has formulated guidelines for quality assurance and control of herbal medicine, traditional practitioners lack enough knowledge which may results in medication with various types of heavy metal contamination. Chris *et al.* (2011) reported several possibilities responsible for presence of heavy metals in herbal remedies; presence of heavy metals may be the result of accidental contamination during the manufacturing process, grinding weights, lead-releasing containers or manufacturing utensil. Also, these medicinal plants may grow in seriously polluted soil, water or air weight for humans. High levels of toxic metals can be found when fertilizers, organic mercury, or lead-based pesticides or fumigants contaminate irrigation water. Alwakeel (2008) reported that plant do not absorb or accumulate lead however, in soil with high lead content; it is possible for some lead to be taken up by the plant tissues. The greater the amount of heavy metals in the soil the increased chance of the metal to accumulate in plant tissues Therefore, there is a need to control the amount of Pb in these products to avoid cumulative toxicity. It is well known for its adverse effects on many parts of the body in humans. Once in the bloodstream, lead is primarily distributed among blood, soft tissue, and mineralizing tissue. High level exposure in men can damage the organs responsible for sperm production (Sabine and Wendy, 2009).

However, this study showed that 3% of Cu in the herbal samples is in high quantities (0.74) as compared to WHO safe limit (0.110). Excessive intake of copper can cause dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhea, vomiting, and liver damage (Rania, 2015). It is possible that some amounts can be taken up by the body system and accumulates for years of use. Thus, the issue of safety and vigilance on its serious adverse effects is of concern.

It can be shown that majority of the samples contained detectable amounts of one or more of the metals of interest (lead and copper). These explain the potential health risk that could be associated with the use of these herbal remedies which are contaminated with toxic metals. The salient question now is "how exposed is the populace to this menace?"

There were indications that the herbal concoctions being consumed by most dwellers in Ilorin, Kwara State, Nigeria are contaminated, hence have public health implications on the consumers.

CONCLUSION

Since applications of herbal medicines for curative purposes is on increase, there is a need for risk assessment of microbial load of the medicinal plants at critical control points during processing. Nigerian government also needs to introduce some standards that must be met by every herbal processor and` seller. There is a need for good sanitation training of all herbal processors so as to safeguard the health of herbal consumers.

REFERENCES

Adenike, A.O., Egunyomi, A. and Taiye, R. F. (2006). Bacterial profiles and consumer preference of some indigenous orally consumed herbal medications in Nigeria. *Journal of rural and tropical public health*, 5:27-33.

Alwakeel, S.S. (2008). Microbial and heavy metals contamination of herbal medicines. *Research of Journal of microbiology,* 3(12):683-691.

Amanlou, M., Maherniaa, S.H., Bagherzadeha, K., Ghorban Dadrassb, O. and Nasim Zinsaza, B. (2015). Determination of heavy metals (cadmium, lead, copper) in herbal syrups by polarography. *Journal of Chemical and Pharmaceutical Research*, 7(8):28-31.

Araujo, S.A., Pagliares, L.Q. and Fritas-Almedia, A.(2002). Occurrence of staphylococcus and entheropathogens in soft cheese commercial in the city of Riode Janeiro

Brazil. *Journal of Applied Microbiology,* 92:1172-1177.

Arias, M. L., Chaves, C. and Alfaro, D. (1999). Microbiological analysis of some herbal infusions used as medicines. *Rev Biomed*., 10 (1): 1-6.

Braide W., Oranusi, S. U., Nwaoguikpe, R.N., Offor-Emenike, I. U., Nwosu, I.L., Akonbodu, C.I., Chike-Reginald C. and Popgbara, L.B. (2013). Evaluation of the Microbiological status and Antibacterial Susceptibility Pattern of some Herbal Remedies administered orally in Nigeria. Research *Journal in Engineering and Applied Sciences*, 2(1):35-42.

Charles, R. (2009). *Corynebacterium kutscheri* (Pseudotuberculosis). *Research model and services,* askcharlesrivers@crl.com

Chris, O. N. and Leo M. (2011). Heavy metal contamination of Ready-to-use Herbal Remedies in South Eastern Nigeria. *Pakistan Journal of Nutrition,* 10(10): 959-964.

Cowan, S. T. (1974). *Manual for the identification of medical bacteria*. 2d Ed. London: Cambridge University Press, pp. 45-122.

Dzombu, P., Chayamiti, T. and Togarepi, E. (2012). Heavy metal content of selected Raw medicinal Plant Materials: Implication for Patient Health. *Bulletin of Environment, pharmacology and life sciences,* 1(10): 28-33.

Etuk, E.U, Igbokwe, V., Ajagbonna, O. P and Egua, M.O. (2008). Heavy Metals and Microbial Contaminants in a Commercial Polyherbal Product in Nigeria. *Nigeria Journal of Basic and Applied Sciences,* 16(2):197-202.

Fawole, M. O. and Oso, B. A. (2007). *Laboratory manual of microbiology. Nigeria*. Spectrum Books Limited, pp. 15-35.

Kolajo, T. T. (2000). The Study of ecology and microbial flora of some locally prepared herbal medicines in Abeokuta. National Diploma project, pp. 20-45.

Lee, J. H. and Jo, W. K. (2000). Characteristics of indoor and outdoor biaerosols at Korean high-rise apartment buildings. *Environmental research,* 101(1):11-17.

Odedara, O. O. and Memuletiwon, E. J. (2014). Microbial quantity of some locally consumed Herbal Concoctions in Abeokuta Metropolis, Nigeria. *Journal of natural science, Engineering and Technology,* 13: 58-66.

Oyetayo, V. O. (2008). Microbial load and antimicrobial property of two Nigerian herbal remedies. *Afr. J. Traditional, Complementary and Alternative Medicines,* 5(1): 74 – 78.

Rania Dghaim,Safa Al Khatib,HUsna Rasool, & Munawwar Ali khan (2015). Determination of heavy metals concentration in traditional herbs commonly consumed in the united Arab Emirates. *Journal of Environmental and public health,* pp 1-6.

Riba, A., Mokrane, S. and Mathieu, F. (2008). Mycoflora & Ochratoxin. A producing strain of *Aspergillus* in Algerian wheat. *International Journals of food Microbiology,* 122(12): 85-92.

Sabine, P.G. & Wendy, (2009). Human Health Effects of Heavy Metals. *Cente* World Health Organization, WHO (2002). Global situation report on traditional medicine. WHO executive board (111th Session). Provisional agenda item 5-7. World Health Organization Secretariat. *Journal for Hazardous Substance Research,* pp 1-7.

World Health Organization, WHO (1999). Making a difference. The World Health Report. World Health Organization, Geneva.

Table 1: Total microbial count of herbal samples hawked within Ilorin metropolis.

Sample number	Total viable count (CFU/ml)	Total Coliform count (CFU/ml)	Total Staphylococcal count (CFU/ml)	Total fungal count (CFU/ml)
A_1	1.6×10^5	0.0	2.5×10^4	0.0
$_2$	2.4×10^5	0.0	2.2×10^3	0.0
$_3$	$2.4. \times 10^4$	0.0	6.0×10^3	0.0
B_1	10.2×10^7	12.6×10^6	6.0×10^2	4.2×10^5
$_2$	3.2×10^7	1.8×10^7	1.0×10^3	5.5×10^5
$_3$	26×10^7	3.2×10^7	1.0×10^2	2.8×10^5
C1	1.5×10^5	4.3×10^5	0.0	3.6×10^5
2	1.3×10^5	2.3×10^5	$5 .0 \times 10^2$	4.8×10^5
3	1.0×10^4	0.0	0.0	1.0×10^5
D1	1.1×10^4	0.0	0.0	0.0
2	1.0×10^4	0.0	0.0	0.0
E1	5.0×10^4	0.0	2.4×10^3	0.0
2	2.5×10^5	1.2×10^5	1.6×10^3	0.0
F1	8.0×10^6	1.3×10^7	2.0×10^3	8.0×10^5
2	8.7×10^5	3.2×10^5	3.2×10^3	3.0×10^5
3	2.0×10^4	0.0	0.0	$2 .0 \times 10^5$
G1	3.2×10^4	0.0	3.0×10^2	3.2×10^5
2	2.0×10^4	0.0	1.8×10^3	0.0
3	6.0×10^4	0.0	5.0×10^2	2.6×10^5
H1	6.0×10^4	3.0×10^5	2.4×10^3	3.5×10^5

2	1.1×10^4	12×10^5	1.9×10^3	3.7×10^5
I1	8.0×10^4	0.7×10^5	2.2×10^3	1.8×10^5
2	2.0×10^5	2.5×10^5	1.4×10^3	8.3×10^5
J1	3.6×10^5	1.3×10^5	4.4×10^3	3.1×10^5
2	2.3×10^5	1.1×10^5	5.0×10^3	2.7×10^5
K1	2.2×10^4	0.0	9.0×10^2	0.0
2	1.9×10^5	8.6×10^5	0.0	0.0
L1	5.0×10^5	8.2×10^5	3.0×10^2	8.2×10^5
2	3.0×10^4	1.5×10^1	1.2×10^3	1.5×10^5
3	6.0×10^5	6.5×10^5	2.9×10^3	6.5×10^5
M1	1.8×10^4	0.0	7.0×10^2	5.0×10^4
2	7.3×10^5	4.0×10^5	6.7×10^3	5.3×10^5
3	6.8×10^5	1.24×10^6	4.9×10^3	2.5×10^5
N1	6.0×10^4	0.0	8.0×10^2	1.0×10^5
2	1.3×10^5	0.0	1.7×10^3	4.5×10^5
3	2.0×10^5	1.42×10^6	3.2×10^3	4.0×10^5
O$_1$	1.0×10^4	0.0	1.4×10^3	0.0
2	1.2×10^4	3.0×10^4	3.0×10^3	3.5×10^5
3	1.5×10^6	1.3×10^7	4.0×10^3	3.1×10^5

Key: A-O: sellers, 1= Pile concoction, 2= Typhoid concoction, 3= sexually acquired infection concoction

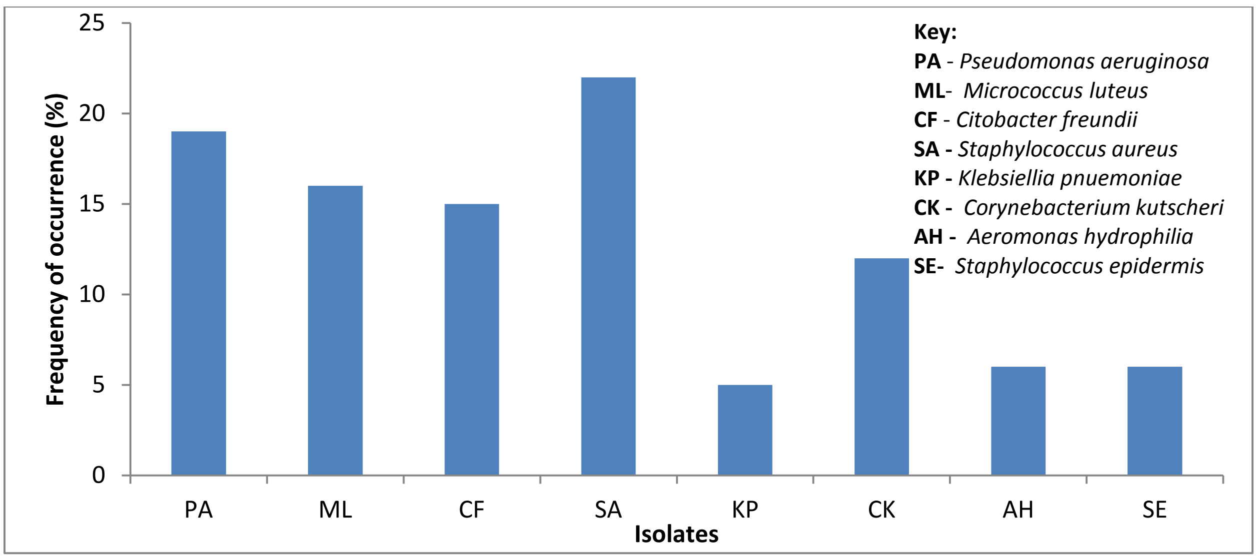

Figure 1: Frequency of occurrence of Bacterial isolates in the herbal samples Heavy metal analysis

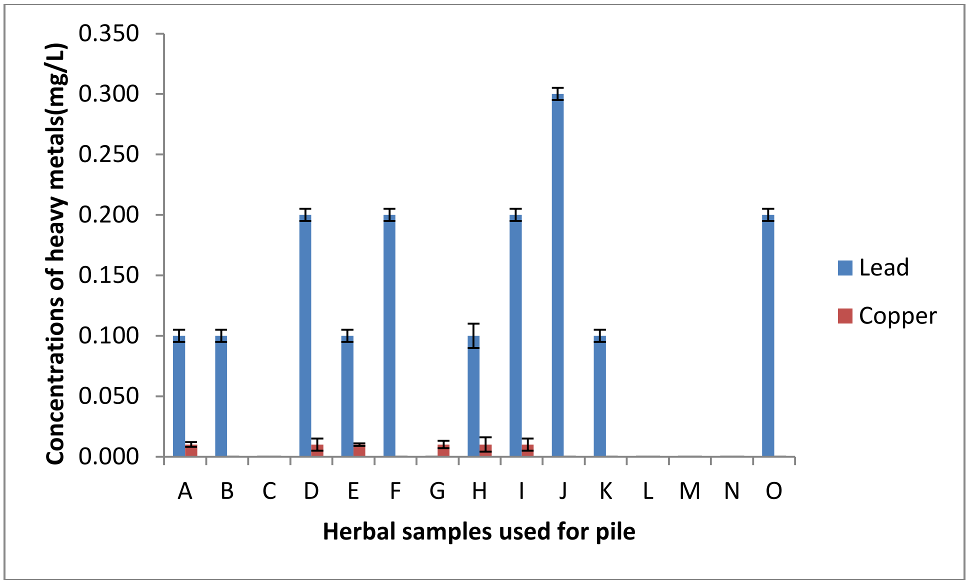

Figure 2: Available Lead and Copper concentration in commercial herbal concoctions
n=3 ± SD

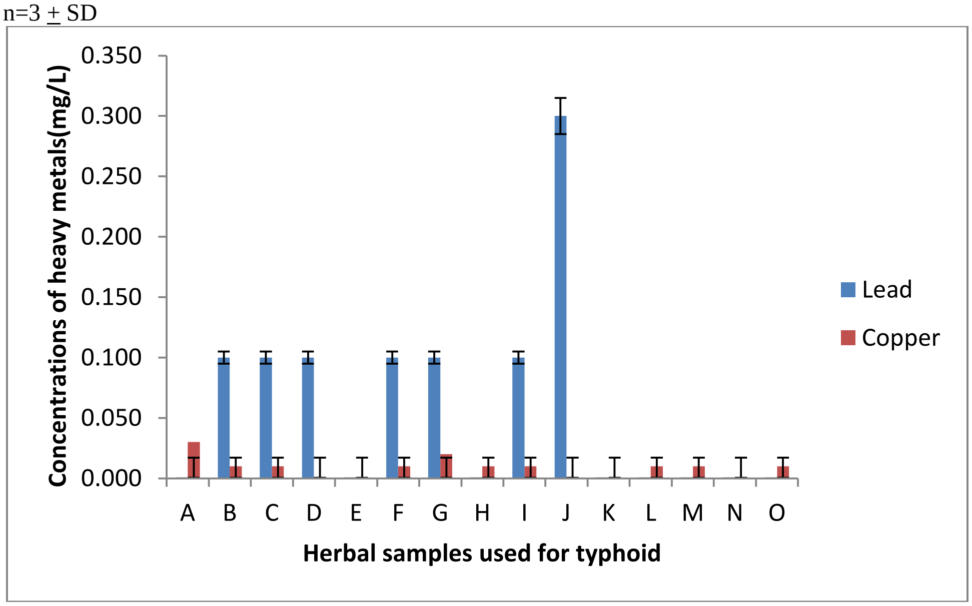

Figure 3: Available Lead and Copper concentration in commercial herbal concoctions
n=3 ± SD

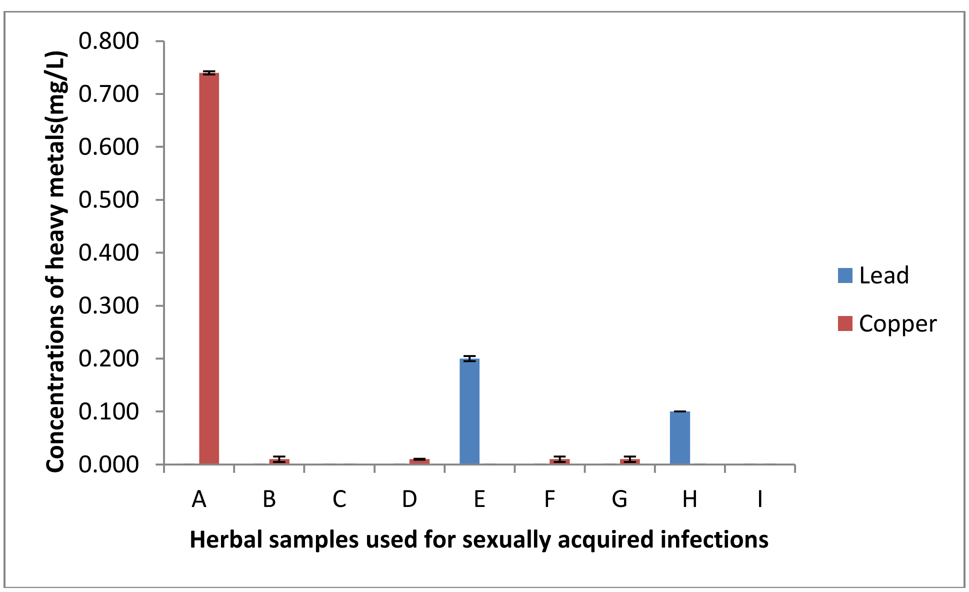

Figure 4: Available Lead and Copper concentration in commercial herbal concoctions
n=3 ± SD

Table 2: Summary of the atomic absorption of Lead, Cadmium and Copper metal ions.

Parameters	Pb	Cd	Cu
Number of samples	39	39	39
Number of samples with detectable metal ion	20	0	20
Percentage of samples with detectable metal ions	51.3%	0	51.3%
Minimum concentration of metal ion detected (mg/L)	0.100	0	0.010
Maximum concentration of metal ion detected (mg/L)	0.300	0	0.740
WHO safe limit (mg/L)	0.005	0.031	0.110
Number of samples above WHO limit	39	0	1
Percentage of sample above WHO limit	100%	0	3%