



Analysis of Heavy Metals Concentration in Kano Herbal Preparations for Major Disease Conditions

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ABSTRACT

The use of herbal medicines for treatment, management and prevention of different disease conditions has been as old as human history. These herbs usually contained some chemical substances which are considered as beneficial, while others were not. Study of the levels of some essential (Copper, Cadmium, Cobalt, Iron, Manganese, Nickel and zinc) and toxic (Cd and Pb) heavy metals content of herbal preparations sold in Kurmi market of Kano state, Nigeria was carried out. The aim of this study is to evaluate the levels of essential and toxic heavy metals in Kano herbal preparations used for commonly identified disease conditions. The samples were digested by wet digestion method with nitric acid and perchloric acids and analyzed using Flame Atomic Absorption Spectrometer (FAAS). The data obtained were statistically treated and the results presented as mean of three replicated. The range of mean concentrations of the essentials Cu, Co, Fe, Mn, Ni, Zn and toxic Cd, Pb heavy metals obtained were 4.94-10.03 $\mu\text{g/g}$, 3.33-3.80 $\mu\text{g/g}$, 163.04-350.95 $\mu\text{g/g}$, 57.57-65.05 $\mu\text{g/g}$, 9.94-15.63 $\mu\text{g/g}$, 6.86-66.59 $\mu\text{g/g}$ and 1.53-3.23 $\mu\text{g/g}$, 3.75-35.24 $\mu\text{g/g}$ respectively. The level of contamination of the herbal preparation with the toxic heavy metals indicated 100% of the samples analyzed contained cadmium above WHO permissible limits (0.3 $\mu\text{g/g}$) specified for herbal medicine and 33% of the samples contained lead (Pb) above WHO permissible limits (10 $\mu\text{g/g}$) specified for herbal medicine. The gross level of contamination of these herbal preparations with Pb and Cd metals could be associated to poor adherence to quality control requirements such as current/good manufacturing practice (CGMP) and effective surveillance/enforcement. There is need for commitment for immediate action by both the herbal medicine practitioners and the relevant stakeholders in order to avert impending health hazards due to the tendency of gradual accumulation of these toxic metals in the body system of the consumers of these herbal preparations in order to attain to safe and effective herbal medicine usage in Nigeria.

Keywords: Disease conditions, Heavy metals, Herbal preparations, Kano

INTRODUCTION

The use of plants-based in the traditional medicine systems play an important role in the healthcare of two-thirds of the world's inhabitants (IUPAC, 2008) according to documented literatures (API, 1989; But *et al.*, 1997; Chang and But, 2001) and many more information and knowledge are scattered around in communities, families, tribes, and with local herbal medicine practitioners. The diversity of the use of herbal medicine kept no region, culture or tribe completely ignorant of the use of herbal preparation for treating one disease ailment or the other. The World Bank estimates that trade in medicinal plants, botanical drug products, and raw materials are growing at an annual rate of between 5 and 15 % (Benedum, 1998; Patwardhan *et al.*, 2005). These have popularized herbal products throughout the world; but one of the impediments in its acceptance by all is lack of established standard quality control measures. Some of these

herbal medicines may contain elements of vital importance for human metabolism, disease prevention and healing (Obianjunwa *et al.*, 2004; Rajurkar and Damame, 1997), while on the other hand, they may as well contain non-beneficial elements which could pose health hazard to the body system due to unascertained safety and efficacy. Essential mineral element can also play a significant role in biological activity either with organic molecules such as metallo-enzymes or independently like redox or catalytic reaction (Adongo *et al.*, 2012). The importance of the knowledge of elemental contents of herbal preparations used for different medicinal purposes deserved more attention for screening than ever before (Arceusz *et al.*, 2010) due to high dependence on herbal preparation for medicinal purposes. The bio-toxic and poisoning effects of heavy metals occur when consumed above the bio-recommended limits and interfere with the normal body biochemistry in the normal metabolic

processes most especially when ingested in the acid medium of the stomach, they are converted to their stable oxidation states and combine with the body's bio-molecules such as proteins and enzymes to form strong and stable chemical bonds (Duruibe *et al.*, 2007) which results to bio-accumulation through biological chains over the years due to their persistence and non-biodegradability. Human health therefore is at risk and can be grossly affected by heavy metal intoxication if concurrent ingestion of contaminated/polluted crude herbal products are not continuously monitored and suggest for remediation strategies (Das and Singh, 2011). The tendency of developing disease ailment instead of treating existing ones are eminent if continual consumption of contaminated herbal preparations with heavy metals are used (Ernst. and Coon 2001; Robert *et al.*, 2008; Martena *et al.*, 2010). Several studies have been conducted on heavy metals and macronutrient status of herbal plants however, limited and scanty information are available with respect to Kano herbal preparations despite the fact Kano state is one of the biggest commercial cities in Nigeria which has a large market for traditional herbal medicine which serve as an outlet for distribution of herbal preparations to other states. These therefore, necessitate the need for the present study. Several analytical techniques have been used to determine heavy metals in various samples of different matrices (Helaluddin *et al.*, 2016). In the present study, the heavy metal content of the herbal preparations were determined using Atomic Absorption Spectrometer (AAS) based on the principle of absorption of UV-visible radiation at specific frequency (Skoog *et al.*, 2007).

Statistical Data Analysis

All the data obtained were subjected to statistical analysis using Microsoft Excel 2010 for processing and the results were reported as mean, standard deviation and range as calculated (Rao, 1996).

Materials and Methods

Sample Collection and Preparation

The study was carried out on Kano herbal preparations used for six (6) popular disease conditions which included sexual-drive (SD), anti-malaria (AM), anti-sickel-cell (ASC), anti-ulcer

(AU), anti-hypertension (AH) and anti-diabetes (AD). The herbal preparations were examined at the point of sales (Kurmi markets–Kano state), purchased, properly packaged in polyethene bags and coded as Kn1(AD), Kn2(AH), Kn3(AM), Kn4(AU), Kn5(ASC) and Kn6 (SD), taken to laboratory, shed-dried and finely powdered using mortar and pestle.

Sample Digestion

1.0 g of each sample was placed into beakers and added 20 cm³ of concentrated nitric acid and kept for 24 hours after which they were carefully heated on a powered electrically connected sand-bath in fume hood chamber with periodic addition of 10-20 cm³ concentrated nitric acid until the production of red nitrous oxide (NO₂) ceased. The samples were cooled and 2-4 cm³ of perchloric acid (HClO₄) was added and heated until the cleared sample solution was obtained. The samples were diluted with deionized water, filtered into 100cm³ volumetric flasks, made up to mark and transferred into a capped labeled plastic bottle and kept in fridge prior to analysis. The ratio of the acids; HNO₃:HClO₄ used for the digestion was 5:1 (Sneddon *et al.* 2006; Hseu, 2004). Blank sample was also prepared by digesting the same proportion of the reagents used in the sample digestion under the same experimental condition without the sample.

Preparation of Standard Solutions

Stock standards solution of the metals of interest were prepared by weighing out accurately 1.000g of the pure metal and dissolved in concentrated nitric acid and made up to mark in 1 dm³ volumetric flask with deionized water or the appropriate salt of the metal weighed out as calculated in relation to the formula weight, dissolved in 1 dm³ volumetric flask and made up to mark with deionized water (Dhanlal, 2000). The working standards of appropriate concentrations were prepared from the stock solutions by diluting appropriate volume with deionized water and made up to mark in 100cm³ volumetric flask and used for calibrating the AAS equipment after optimization according to the standard conditions specified in Table 1.0.

Table 1.0: Standard Atomic Absorption Condition for Determination of the Heavy Metals

Element	wavelength	Detection limit (µg/ml)	Slit width (nm)	Lamp Current (mA)	Nebulizer uptake	Gas flow (Air/acetylene)
Cu	324.7	0.02	0.5	3.0	5ml/min	13.5/2.0
Cd	228.8	0.002	0.5	3.0	5ml/min	13.5/2.0
Co	240.7	0.005	0.2	7.0	5ml/min	13.5/2.0
Fe	372.0	0.45	0.2	7.0	5ml/min	13.5/2.0
Mn	279.5	0.03	0.2	5.0	5ml/min	13.5/2.0
Ni	232.0	0.04	0.2	4.0	5ml/min	13.5/2.0
Pb	217.0	0.06	1.0	5.0	5ml/min	13.5/2.0
Zn	213.9	0.008	0.5	5.0	5ml/min	13.5/2.0

Equipment Suitability Test

Equipment suitability and method sensitivity were determined by using quality calibration standards from which calibration curves of acceptable weighted-linear correlation coefficients (≥ 0.995) were generated before proceeding for sample analysis.

Sample Analysis

SpectrAA 240 Model of Fast Sequential Atomic Absorption Spectrometer was used for the sample analysis where Cu, Co, Cd, Fe, Mn, Ni, Pb and Zn

were determined in the digested herbal preparations according to the operating conditions specified in Table 1.0 and calibrated using the prepared standard solutions of the metals of interest followed by the blank solution run and the sample analyte. The data obtained were processed by calculating the actual concentrations in the samples analyzed using the relation:

$$\text{Metal } (\mu \frac{g}{g}) = \frac{C \times V \times d.f}{W}$$

Where C is the concentration of the sample solution in mg/L; V is the volume of the sample solutions in mL; W is the sample's weight in grams and d.f is the dilution factor, if used (Chionyedua *et al.*, 2015; AAS Manual, 1996).

Replicate of samples of herbal preparations used for six (6) commonly identified diseases conditions were analyzed for essential and toxic heavy metals and the results were presented in Table 2.0 (Essential metals) and Table 3.0 (Toxic metals) as mean \pm SD, mean and range after statistical treatment of the data.

Results and Discussion**Table 2.0:** Essential Metals in Kano Herbal Preparations

Disease	Mean Concentration (µg/g)					
	Cu	Co	Fe	Mn	Ni	Zn
Kn1(AD)	4.94 \pm 0.58	3.77 \pm 0.02	350.95 \pm 0.52	ND	9.94 \pm 0.05	66.59 \pm 0.80
Kn2(AH)	5.52 \pm 0.03	3.33 \pm 0.02	164.45 \pm 0.35	ND	12.04 \pm 0.06	49.38 \pm 0.55
Kn3(AM)	10.03 \pm 0.53	3.60 \pm 0.01	216.49 \pm 0.30	65.05 \pm 0.55	11.57 \pm 0.10	16.83 \pm 0.60
Kn4(AU)	5.76 \pm 0.42	3.70 \pm 0.02	163.04 \pm 0.30	57.57 \pm 1.20	14.48 \pm 0.01	21.34 \pm 0.16
Kn5(ASC)	6.94 \pm 0.01	3.80 \pm 0.02	315.63 \pm 0.45	ND	15.63 \pm 0.03	18.04 \pm 0.50
Kn6(SD)	8.79 \pm 0.08	ND	281.19 \pm 0.25	ND	14.27 \pm 0.01	6.86 \pm 0.65
Mean	7.00	3.64	248.60	61.31	12.91	30.00
Range	4.94-10.03	3.33-3.80	163.04-350.95	57.57-65.05	9.94-15.63	6.86-66.59

Note: ND means not detected

Table 3.0: Toxic Heavy Metals in Kano Herbal Preparations

Sample code	Concentrations ($\mu\text{g/g}$)	
	Pb	Cd
Kn1(AD)	35.24 \pm 5.22	2.53 \pm 1.26
Kn2(AH)	ND	1.83 \pm 0.66
Kn3(AM)	9.79 \pm 0.10	2.79 \pm 0.30
Kn4(AU)	3.75 \pm 0.15	1.53 \pm 1.38
Kn5(ASC)	ND	3.23 \pm 1.00
Kn6(SD)	5.78 \pm 0.44	2.2 \pm 0.56
Mean	6.44	2.35
Range	3.75-35.24	1.53-3.23

In this study, anti-diabetic (AD) herbal preparations analyzed indicated the concentrations of essential metals Fe, Zn, Cu, Co and Ni as 350.95 \pm 0.52 $\mu\text{g/g}$, 66.59 \pm 0.80 $\mu\text{g/g}$, 4.94 \pm 0.58 $\mu\text{g/g}$, and 3.77 \pm 0.02 and 9.94 \pm 0.05 $\mu\text{g/g}$ (Table 2.0) respectively. The presence of cobalt and zinc in the anti-diabetic herbal preparation is evidence that, it has potential to handle diabetic disease condition based on the facts reported by Sakurai *et al.*(2010) who claimed that, mechanisms involve with these metals which included, stimulation of the activities of the gluconeogenic enzymes, antioxidant properties (cobalt and zinc), stimulation of glucose uptake, glycogen and lipid synthesis in muscle, adipose and hepatic tissues and inhibition of gluconeogenesis (cobalt) handle the challenges of diabetic disease condition. Nomura *et al.* (2005) also reported significant decline in blood glucose of diabetic rats when treated with cobalt chloride, without any effect on plasma insulin. Higher zinc intake has also been associated with a slightly lower risk of type II diabetes according to Sun *et al.* (2008). However, imbalance of some essential metals could affect pancreatic islet and cause development of diabetes due to production of reactive oxygen species according to Khan and Awan (2014) this could also further decrease the insulin gene promoter activity and mRNA expression in pancreatic islet cells due to hyperglycemic condition (Valiko *et al.*, 2005; Galhardi *et al.*, 2004; Jiang *et al.*, 2004; Guidotti *et al.*, 2010).

The anti-malarial (AM) herbal preparations analyzed indicated the concentrations of Fe, Zn, Cu, Co and Ni as 216.49 \pm 0.30, 16.83 \pm 0.60 $\mu\text{g/g}$, 10.03 \pm 0.53 $\mu\text{g/g}$, 3.60 \pm 0.01 $\mu\text{g/g}$ and 11.57 \pm 0.10 $\mu\text{g/g}$ (Table 2.0). In malaria disease condition, the role of iron is a vital requirement for optimum production of hemoglobin in order to overcome anemic condition resulted from infected red blood cell by malaria parasite (*Plasmodium falciparum*) and anemia in which the condition is considered as measure of the cumulative impact of malaria on the individual patient (Ekvall, 2003), in such situation iron is necessarily required for boosting the red blood cell in order to avert the anemic condition and other hematological changes

such as decrease in platelet count due to the high level of *Plasmodium falciparum* in patient with malaria (Kotepui *et al.*, 2015). High level of iron in the anti-malarial herbal preparations analyzed boost optimum hemoglobin supply which suppress severity of malaria condition by meeting the body requirement for all age and sex groups (Kumar *et al.*, 2008; Bashawri *et al.*, 2002).

The anti-sickle cell (ASC) herbal preparations analyzed indicated the concentrations of Fe, Zn, Cu, Co and Ni as 315.63 \pm 0.45 $\mu\text{g/g}$, 18.04 \pm 0.50 $\mu\text{g/g}$, 6.94 \pm 0.01 $\mu\text{g/g}$, 3.80 \pm 0.02 $\mu\text{g/g}$ and 15.63 \pm 0.03 $\mu\text{g/g}$ (Table 2.0). Sickle cell which is also characterized by anemia and immunological disturbances are linked to essential metal deficiency, due to increased needs of many of these essential metals in the sickle cell patients according to Idonije *et al.* (2011) and Tagney *et al.* (1989). The presence of iron, zinc, copper in the analyzed anti-sickle cell herbal preparations analyzed indicated their role alleviating the crises that may result due to the disease condition, the likely presence of antioxidant in these herbal preparations (Okpuzor and Okochi, 2009) protect and enhances the red blood cell membrane for optimum functioning in sickle cell patient against generated free radicals in the body system according to Idonije *et al.* (2011).

The sex-drive (SD) herbal preparations analyzed indicated the concentrations of Fe, Zn, Cu, Co and Ni as 281.19 \pm 0.25 $\mu\text{g/g}$, 6.86 \pm 0.65 $\mu\text{g/g}$, 8.79 \pm 0.08 $\mu\text{g/g}$, ND and 14.27 \pm 0.01 $\mu\text{g/g}$ (Table 2.0). Sex-drive is a biological need or craving that inspires individuals to seek out and become receptive to sexual experiences and sexual pleasure (Baumeister *et al.*, 2001). Chronic illness/disorder such as cardiovascular disease, diabetes, arthritis, high-blood pressure, enlarge prostates (in men), Parkinson's disease and cancer can have serious effect on biological drive and psychological motivation for sexual desire, functioning and response of an individual (Levine, 2003; Regan and Atkins, 2006). According to DeLamater and Sill, (2005), the use of anticoagulants, cardiovascular medications, medications to control cholesterol, and medications for hypertension by women contributed to low

levels of desire, while in men, only the use of anticoagulants and medications for hypertension was related to low levels of desire. High level of Fe obtained in the analyzed sex-drive herbal preparations are required to increase blood volume for optimal sex performance, while Zn increases semen volume and testosterone level for male sexual vitality, Zn deficiency in other hand can result to infertility, impotency, or poor reproductive system development. The recommended level of zinc for male infertility is 30-60 mg per day (Rungby, 2010). Copper plays a similar role with Zn therefore its presence in these herbal preparations contributes to effective sexual activities.

The results of toxic heavy metals content of the herbal preparations analyzed indicated thirty three percent (33%) of the samples contained lead (Pb) and 100% had cadmium (Cd) above WHO (2007) permissible limits of lead (10 μ g/g) and cadmium (0.3 μ g/g) in herbal medicine. This could pose serious health hazard to human health as these metals are not friendly to most of the major organs such as kidney, liver, renal tract, cardiovascular, reproductive and others (Maobe *et al.*, 2012; Martin and Griswold, 2009; Johnson, 1998). The uptake and bioaccumulation of heavy metals in herbs and other plants materials are influenced by a number of factors such as climate, atmospheric deposition, concentration in the soil, the nature of the soil on which the herbs are grown and the degree of maturity of the plant at the time of harvest (Lake *et al.*, 1984). Long-term uses of treated or untreated wastewater, plants grown along heavy traffic ways, and previous dumpsites and other anthropogenic source of heavy metals which includes the addition of organic manures waste sludge, fertilizers and pesticides which may affect the uptake of heavy metals by modifying the physicochemical properties of the soil such as pH, organic matter and bioavailability of the heavy metals in the soil are also other factors. Farmlands near heavy traffic high-ways are exposed to atmospheric pollution in the form of metal containing aerosols. These aerosols can be deposited on soil and are absorbed by plants leaves, barks and fruits (Nwachukwu *et al.*, 2010; Liu *et al.*, 2007; Sharma *et al.*, 2006; Remon *et al.*, 2005). The observed variation in concentrations of the heavy metals among the samples analyzed may be attributed to several factors such as different absorption and accumulation capacities of the toxic and essential heavy metals by the herbal materials as suggested by Singh *et al.* (2011) and Prasad, (2007). The use of smelting, industrial waste, processing, commercial and other anthropogenic activities could also be the contributing factors (Bergeson, 2008, ATSDR, 2005) in a commercialized city as Kano.

CONCLUSION

The outcome of the study revealed that, the herbal preparations used for common disease conditions in Kano states contained essential metals which play vital role in handling the specified disease conditions and result also indicated gross level of contaminations of these herbal preparations with Lead (Pb) and cadmium (Cd). This indicated that a potential health risk await the consumers of these herbal preparations over a period of time due to the tendency of gradual accumulation in the body system. The study therefore suggest that the Traditional Medicine Practitioners monitor their process chain from harvest, processing, storage to the sell-point in order to ensure no unforeseen sources of heavy metal contamination of their products occur and on part of government, an enforcement of regulations on herbal preparations through monitoring and evaluation surveillance on the quality of the herbal preparations are required with urgency in order to protect the health of the citizens.

REFERENCES

- AAS Manual, (1996). *Analytical Methods for Atomic Absorption Spectroscopy*, PerkinElmer Inc. Printed in United States of America Manual no.0303-0152 (D).pp.46-47
- Adongo, S.O., Murungi, J. and Wanjau, R.(2012).Determination of levels of selected essential elements in the medicinal plants used by Chuka community, Meru - Kenya using AAS.*International Journal of Physical and Social Sciences*; 2 (5), 2249-5894.<http://www.ijmra.us>
- Agency for Toxic Substances and Disease Registry (ATSDR, 2005).*Toxicological Concentration for Nickel*.US Public Health Service Atlanta, GA, U.S.A, 2005:3-55
- Ayurvedic Pharmacopoeia of India* (API, 1989). Department of Health, Ministry of Health and Family Welfare, Government of India, New Delhi, p. 45
- Arceusz, A., Radecka, I. and Wesolowski, M. (2010).Identification of diversity in elementscontent in medicinal plants belonging to different plant families. *Food Chemistry*; 120: 52-58.
- Bashawri, L.A., Mandil, A.A., Bahnassy, A.A. and Ahmed, M.A. (2002). Malaria: *hematological aspects*. *Ann Saudi Med.*; 22: 372–376
- Baumeister, R. F., Catanese, K. R. and Vohs, K. D.(2001)."Is There a Gender Difference in Strength of Sex Drive? Theoretical Views, Conceptual Distinctions, and a Review of Relevant Evidence".*Personality and Social Psychology Review*.5(3): 242.
- Benedum, J. (1998).*In Phytopharmaka IV*. Forschung und Klinische Anwendung , D. Loew, N. E. Rietbrock (Eds.), p. 3
- Bergeson, L. L. (2008). "The proposed lead NAAQS: Is consideration of cost in the clean

air act's future?" *Environmental Quality Management*; 18: 79–84.

But, P.P H., Kimura, T., Guo, T.J. X., Sung, C .K. and Han, B. H. (Eds.) (1997) International Collation of Traditional and Folk Medicine, *World Scientific*, Singapore, p. 232

Chang, H. M. and But, P. P. H. (2001). *Pharmacology and Applications of Chinese Materia Medica*, Vols. I and II, S.- C.Yao, L.-L. Wang, S. C.-S. Yeung (Translators), World Scientific, Philadelphia. pp.62-87

Chionyedua, T.O., Ngozi, A. and Isiaka, A.O (2015) Levels of Potentially Toxic Metals In Selected Herbal Medicines In Lagos, Nigeria, *Journal of Natural Sciences Research*; 5 (2): 2015

Das, A.P. and Singh, S.(2011) Occupational health assessment of chromite toxicity among Indian miners. *Indian J Occup Environ. Med.*; 15 (1):6-13

DeLamater, J.D. and Sill, M.(2005)."Sexual Desire in Later Life".*The Journal of Sex Research*.42(2): 138–149.

Dhanlal, D.L. (2000). Delloyd's Lab Tech chemistry resources: *The Lab Tech resources and techniques used in the analytical chemistry laboratory*. Pages 1-6 <http://delloyd.50megs.com> Retrieved 17th April, 2015

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N.(2007).Heavy metal pollution and human biotoxiceffects.*Int'l J. Physic. Sci.* 2(5):112-118

Ekvall, H. (2003). Malaria and anemia. *Curr. Opin. Hematol.*; 10: 108–114

Ernest, E. and Coon, J.T (2001). Heavy metals in traditional Chinese medicines: A systematic review. *Clin.Pharmacol.Ther.*; 70: 497-504.

Galhardi, C.M., Diniz, Y.S., Faine, L.A, Rodrigues, H.G., Burneiko, R.C., Ribas, B.O., Novelli, E.L. (2004): Toxicity of copper intake: lipid concentration, oxidative stress and susceptibility to renal dysfunction. *Food Chem. Toxicol.*; 42(12):2053–2060.

Guidotti, T., McNamara, J. and Moses, M. (2010): The interpretation of trace element analysis in body fluids. *Indian J. Med. Res.*; 128(4):524

Helaluddin, A.B.M., Khalid, R.S., Alaama, M. and Abbas, S.A. (2016): Main Analytical Techniques Used for Elemental Analysis in Various Matrices, *Tropical Journal of Pharmaceutical Research*; 15 (2): 427-434

Hseu, Z.Y. (2004) Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresource Technol*; 5:53–9.

Idonije, B.O., Iribhogbe, O.I. and Okogun, G.R.A.(2011). Serum Trace Element Levels In Sick Cell Disease Patients In An Urban City In Nigeria. *Nature and Science* 2011;9(3):67-71. <http://www.sciencepub.net>

IUPAC (2008). Technical Report: Protocols on safety, efficacy, standardization, and documentation of herbal medicine, *Pure and Applied Chemistry* Vol. 80, No. 10, pp. 2195–2230

Jiang, R., Manson, J.E., Mei Jianggs, J.B., Ma, J., Rifai, N. and Hu, F.B. (2004): Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA*; 291(6):711–717.

Johnson, F.M (1998). "The genetic effects of environmental lead," *Mutation Research-Reviews in Mutation Research*; 410 (2): 123–140

Khan, A.R. and Awan, F.R. (2014). Metals in the pathogenesis of type 2 diabetes, *Journal of Diabetes & Metabolic Disorders*, 13:16

Kotepui, M., Piwkham, D., PhunPhuech, B., Phiwklam, N., Chupeerach, C. and Duangmano, S.(2015).Effects of Malaria Parasite Density on Blood Cell Parameters. *PLoS ONE*, 10(3): e0121057. doi:10.1371/journal.pone.0121057

Kumar, A., Rai, A.K., Basu, S., Dash, D. and Singh, J.S.(2008). Cord blood and breast milk iron status in maternal anemia. *Pediatrics*; 121:e673-e677.

Lake, D.L., Kirk, P.W.W. and Lester, J.N.(1984). The fractionation, characterization and speciation of heavy metals in sewage sludge and sewage sludge amended soils: *A review. J. Environ. Qual.*, 13: 175-183.

Levine, S. B. (2003). "The nature of sexual desire: A clinician's perspective".*Archives of Sexual Behavior*. 32(3): 279–285.

Liu, C., Zhang, Y., Zhang, F., Zhang, S., Yin, M., Hou, H. Ye., Dong, H., Zhang, M., Jiang, J. and Pei, L. (2007).Assessing pollutions of soil and plant by municipal waste dump.*Environ. Geol.*, 52: 641-651.

Martena, M.J., Van-Der Wielen, J.C., Rietjens, I.M., Klerx, W.N., De Groot, H.N., Konings, E.J.(2010).Monitoring of mercury, arsenic and lead in traditional Asian herbal preparations on the Dutch market and estimation of associated risks. *U.S Nat. Lib.Med.Contr. Expo. Risk*; 27: 190-205.

Martin, S. and Griswold, W.(2009). "Human health effects of heavy metals," in *Environmental Science and Technology Briefs forCitizens*, vol. 15, pp. 1–6, Center for Hazardous Substance Research, Manhattan, Kan, USA

Maobe, M. A. G., Gatebe, E., Gitu, L. and Rotich, H. (2012). "Concentration of heavymetals in selected medicinal plants used for the treatment of diabetes, malaria and pneumonia in Kisii Region southwest Kenya," *Global Journal of Pharmacology*, vol. 6, no. 3, pp. 245–251, 2012.

Nomura, Y., Okamoto, S., Sakamoto, M., Feng, Z. and Nakamura, T. (2005): Effect of cobalt on the liver glycogen content in the streptozotocin-induced diabetic rats. *Mol Cell Biochem*; 277(1-2): 127-130

Nwachukwu, M.A., Feng, H. and Alinor, J. (2010). Assessment of heavy metal pollution in

soil and their implication within and around mechanic villages. *Int. J. Environ. Sci.Tech.*, 7: 347-358.

Obianjunwa, E.I., Adeleke, C., Adebajo, O. and Omubuwojo, O. (2004). Essential and trace element contents of some Nigerian medicinal plants. *Journal of Radioanalytical and Nuclear Chemistry*; 252(3): 473-476.

Okpuzor, J. and Okochi, V.I. (2009). Micronutrients as therapeutic tools in the management of sickle cell anemia. *African Journal of Biotechnology*; 7(5):416-420

Patwardhan, B., Warude, D., Pushpangadan, P. and Bhatt, N. (2005). Evidence-Based Complem. *Altern.Med.* 2: 465

Prasad, M.N.V. (2007). Trace Element accumulation in medicinal and aromatic plants collected and cultivated in periurban area risk or remedy. In: Zhu, Y., Lepp, N., Naidu R. (eds). *Biochemistry of trace elements: Environmental Protection, Remediation and Human Health*. Tsinghua University Press, China, pp.105-105

Rajurkar, N.S. and Damame, M.M. (1997). Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. *Journal of Radioanalytical and Nuclear Chemistry*; 219 (1): 77-80

Rao, K.V. (1996). *Biostatistics: A Manual of Statistics Methods for Use in Health, Nutrition and Athropology*. Jaypee Brothers Medical Publishers Private Limited; 2nd ed. P754.

Regan, P.C. and Atkins, L. (2006). "Sex Differences and Similarities in Frequency and Intensity of Sexual Desire", *Social Behavior & Personality: An International Journal*. 34(1): 95–101.

Remon, E., Bouchardon, J.L., Cornier, B., Guy, J.C., Sinha, S., Pandey, K., Gupta, A.K., Bhatt, K. and Faure, O. (2005). Soil characteristics, Accumulation of metals in vegetables and crops heavy metal availability and vegetation recovery at a grown in the area irrigated with river water. *Bull. Former metallurgical landfill: Implications in risk Environ. Contam.Toxicol.*; 74: 210-218.

Robert, B.S., Russell, S.P., Anusha S., Nadia K., Roger, B.D., Janet, P., Venkatesh T., De Stefano, K. (2008). Lead, mercury and arsenic in

US and Indian-manufactured *Ayurvedic* medicines sold via the Internet. *JAMA*; 300 (8): 915-923.

Rungby, J. (2010). Zinc, zinc transporters and diabetes. *Diabetologia*, 53: 1549-51.

Sakurai, H., Katoh, A., Kiss, T., Jakusch, T. and Hattori, M. (2010). Metallo-allixinate complexes with anti-diabetic and anti-metabolic syndrome activities. *Metallomics*; 2:670–682.

Sharma, R.K., Agrawal, M. and Marshall, F.M. (2006). Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. *Bull. Environ. Contam. Toxicol.*, 77: 311-318. identity: Herbal medicine as a cause lead toxicity. *American. Journal of Medicine*; 20: 795-798.

Skoog, D.A., Holler, F.J. and Crouch, S.R. (2007). *Principles of Instrumental Analysis*, 6th edition, Published by Brooks Cole United State; pp.25-36

Singh, R., Gautam, N., Mishra, A. and Gupta, R. (2011). "Heavy metals and living systems: an overview," *Indian Journal of Pharmacology*, 43 (3): 246–253

Sneddon, J., Hardaway, C., Bobbadi, K.K. and Reddy, A.K. (2006). Sample Preparation of Solid samples for Metal Determination by Atomic Spectroscopy-An Overview and Selected Recent Applications. *Applied Spectroscopy Reviews*; 41 (1):1-14,

Sun, Q., Dam, R.M.V., Willett, W.C. and Hu, F.B. (2008): Prospective Study of Zinc Intake and Risk of Type II Diabetes in Women. *Diabetes Care*; 32(4): 629-634

Tagney, C.C., Phillips, G. and Bell, R.A. (1989). Selected indices of micronutrient status in adult patients with sickle cell anemia. *Am. J. Hematol.* 2:161-166

Valko, M., Morris, H. and Cronin, M.T. (2005): Metals, toxicity and oxidative stress. *Curr. Med. Chem.*; 12(10):1161–1208.

World Health Organization (WHO, 2007). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva, Switzerland. P.24