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Assessment of Some Toxic Metal Concentrations in Selected Ready-to-use Medicinal Plant Roots and Stem Barks in Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OIF and EEU designed the study. Author OIF wrote the protocol and the first draft of the manuscript. Author OIF managed the literature searches, analyses of the study and performed the spectroscopy analysis. Authors OIF and EEU managed the experimental process and identified the species of plant. Author ERC revised the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The aim of this study is to assess the concentrations of selected toxic metals (Cr, Ni, Cu, Zn and Cd) in some herbal plants sold in 3 markets within Ibadan, Nigeria. Sampling was done on a daily basis for 7 days in 2012. Blended plant samples were heated at 550ºC for 4 hours and digested using 2M HNO_3. The extracts were subjected to Flame Atomic Absorption Spectrometry (FAAS) to determine their toxic metal concentrations. Metal recovery study was used to validate the analytical method. The concentration of Cu ranged from 0.04mg/kg in *Olax subscorpioidea* to 9.44mg/kg in *Adansonia digitalia*. For Zn, the concentration was lowest (0.94mg/kg) and highest (35.40mg/kg) in *Senna podocarpa* and *Kigelia Africana* respectively. Pb concentration ranged from 1.6mg/kg in *Adansonia digitalia* to 6.15mg/kg in *Sphenocentrum jollyanum*. Cr, Cd and Ni levels were below the detection limits [Cr (0.04mg/L), Cd (0.01mg/L) and Ni (0.10mg/L] of the spectrophotometer used. The mean Zn (8.04mg/kg) and Cu (1.80mg/kg) concentrations

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in the plants were above the World Health Organization (WHO) limits for Cu (0.3-1.0mg/kg) and Zn (0.05-0.5mg/kg) respectively in the plant roots and stem barks. Spearman partial correlation showed variation in selected trace metal (Cu and Zn) concentrations in the plant samples. Recovery study for Zn in *Bridelia ferruginea* and *Adansonia digitalia* were (94.85%) and (101.40%) respectively. On the average, the concentrations of toxic metals in the herbal plants studied were within WHO acceptable limits.

Keywords: Toxic; metal; concentration; limit.

1. INTRODUCTION

In herbal medicine, the word herb applies to any plant or plant part used for its medicinal, flavoring or fragrant properties. Leaves, flowers, stems, roots, seeds, fruits and bark can all be constituents of herbal medicines. Medicinal or herbal plants can be identified by using their botanical names and/or native names that are more commonly known [1]. According to the World Health Organization (WHO), an estimated 4 billion people or 80% of the world population currently use herbal medicine for some aspect of primary health care [2]. Almost 80% of people in marginal communities use only medicinal plants for the treatment of various diseases [3]. For many decades, traditional remedies were empirically practiced in Nigeria, and indeed in Africa to treat various diseases. A good percentage of the population relies on herbal preparations for some aspect of primary health care [4]. Indigenous plants were used in the treatment of high blood pressure, diarrhea and fever [5].

The WHO has listed over 2000 plants that are known to perform one function or the other [2]. Plants accumulate a number of mineral elements that are essential to human nutrition, though they may equally accumulate other elements such as Cd, Co, Ag, etc. which have no direct use to the plants and animals that eat them but rather injurious to their health [6,7].

The past decade has seen a significant increase in the use of herbal medicine due to their minimal side effects, availability and acceptability to the majority of the populace of third world countries. Trace elements have both a curative and a preventive role in combating diseases. It is therefore imperative to establish the levels of some metallic elements in common herbal plants because, at elevated levels, these metals can also become dangerous, injurious and toxic [8].

Medicinal plants can be contaminated by several contaminants such as heavy metals, trace organics such as Polychlorinated biphenyls (PCB) and Poly Aromatic Hydrocarbons (PAH), chemical contaminants (e.g. agrochemical residues), biological contaminants (e.g. microbial growth), adulterated and undeclared substances and lastly radioactive contaminants. However, heavy metals serve as the most common and potent source of contaminants in medicinal plants. Several attempts have been made towards the determination of the metal content levels of herbal plants, both locally and internationally [9-14].

Western Nigeria has a rich history and culture which includes the use of herbal medicine in treating diseases and other ailments. Majority of the rural dwellers in the rural areas of Oyo, Ogun, Osun, Ekiti and Lagos states rely heavily on native herbal remedies like ‘Dongoyaro’, ‘Ore-eefon’, ‘Kaju’, ‘Taju-taju’ among others in order to solve their health problems. The factors responsible for the preference of trado-medicine over orthodox medicine by these

rural dwellers can be attributed to poverty, superstitious beliefs and inaccessibility to good medical facilities.

A variety of analytical techniques, including Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma- Mass Spectrometry (ICP-MS), High Performance Liquid Chromatography (HPLC) and Energy-dispersive X-Ray fluorescence (EDXRF) have been employed to examine herbal extracts as well as clinical samples in suspected cases of heavy metal toxicity and contamination [15].

Flame atomic absorption spectrometer (FAAS) has been used to determine the concentrations of metals (Fe, Mn, Cu, Zn, and Pb) and macronutrients (Na, Ca, Mg, K and P) in eight useful herbal plants of South-Western Nigeria [10]. In addition, FAAS has been used to determine the concentration of selected locally ready-to-use herbal remedies in South-Eastern Nigeria [9]. The EDXRF technique has been used for the determination of essential and trace elements' content of some twenty Nigerian medicinal plants [16]. FAAS was however chosen for this study because of its availability, robustness, sensitivity and relative affordability in terms of costs.

Relevant research reports are scanty with respect to herbal plants endemic to Nigeria despite the heavy dependence on these plants as home remedies. The objectives of the present study were thus, to assess the heavy metal composition of local herbal plants using FAAS and to subsequently ascertain whether the toxic metal concentrations are comparatively high or low.

2. MATERIALS AND METHODS

2.1 Sample Collection/Identification

A total of thirty different medicinal plant roots and stem barks were purchased from three different local markets in Ibadan (Bode, Oje and Oja-Oba markets) after an initial market basket survey. . The market basket sampling was done on a daily basis consecutively for one week in March, 2012. The collected plant samples were kept in plastic bags pending their analysis. The samples were identified and characterized at the herbarium section of Botany Department of the University of Ibadan. The herbal plants' pharmacognostic properties such as its identity, local, botanical names as well as medicinal uses are shown in Table 1.

2.2 Reagents

Concentrated Nitric acid (BDH Laboratories Supplies, England) was used for sample digestion. Zinc Sulphate standard reagent (BDH Laboratories Supplies, England) was used for recovery study. It was of analytical grade.

2.3 Standards

Stock standard solutions of Zn, Cu, Cr, Cd and Ni containing 1000ppm of each metal were used. These standard solutions of heavy metals were provided by Merck (Darmstadt, Germany). Calibration standards of each element were obtained by appropriate dilution of the stock solutions. The standards were prepared from the individual 1000mg/L standards (Merck) in 0.1N HNO_3.Working standards were prepared from the previous stock solutions.

Table 1. Pharmacognostic features of plant samples investigated

Code	English name	Botanical name	Local name	Parts	Uses
P1	Ira	*Bridelia ferruginea*	Ira	Bark	Sore Throat
P2	Sesame	*Sesamum indicum*	Okuku	Bark	Purgative
P3	West African Laburnum	*Senna sieberiana*	Aidan Toro	Root	Purgative
P4	Monkey cola	*Carapa procera*	Oganwo	Bark	Purgative
P5	Raisin	*Grewia pubescens*	Olora Igbo	Root	Haemorrhoid
P6	Gum Arabic	*Senna arabica*	Kasia	Root	Haemorrhoid
P7	Combretum	*Conbretum mucronatum*	Ogbolo	Root	Low Sperm Count
P8	Sausage Tree	*Kigelia Africana*	Pandoro	Bark	High Blood Pressure
P9	Corpse Awakener	*Calliandra portocensis*	Tude	Root	High Blood Pressure
P10	Baobab	*Adansonia digitala*	Ose	Root	Increase Body Mass
P11	Persian lilac	*Melia Azdarach*	Afoforo	Bark	Body Rashes
P12	Cluster Leaf	*Terminalia macroptera*	Ponpola	Bark	Body Rashes
P13	Cabbage Tree	*Anthocleista djalonensis*	Shapo	Bark	Body Rashes
P14	Wild African black plum	*Vitex doniana*	Oori	Bark	Body Rashes
P15	Yellow Mombin	*Spondias mombin*	Iyeye	Bark	Body Rashes
P16	Cup of Water	*Tetracera alnifolia*	Opon	Bark	Body Rashes
P17	Brimstone tree	*Morinda lucida*	Oruwo	Root	Typhoid
P18	Crimson Thyme	*Brysocarpus coccineus*	Amuje	Bark	Blood Tonic
P19	Cocoa	*Theobroma cacao*	Koko	Bark	Blood Tonic
P20	Phragma tree	*Entandrophragma utile*	Jebo	Bark	Blood Tonic
P21	Napoleona	*Napoleona vogelii*	Atoo	Root	Cough
P22	African Parqutina	*Parquetina nigrescens*	Ogbo	Root	Cough
P23	Sand-paper Plant	*Ficus asperifolia*	Ipin	Root	Cough
P24	Stool Wood	*Alstonia congensis*	Ahun	Bark	Head Ache
P25	Pricalima	*Pricalima nitride*	Erin	Bark	Stomach disorder
P26	Nuclear African Peach	*Nauclea latifolia*	Egbesi	Bark	Head Ache
P27	Senna	*Senna podocarpa*	Asuwon	Root	Gonorrhoea
P28	Yellow mombin	*Spondias mombin*	Iyeye	Bark	Body rashes
P29	Lead tree	*Lophira lanceolata*	Pahan	Root	Stomach disorder
P30	Olax	*Olax subscorpioidea*	Ifon	Bark	Gonorrhoa

2.4 Sample Pretreatment

The plant samples were dried in open air for 24 hours and a sizable quantity of each sample was chopped into smaller parts using a sharp machete. The chopped plant parts were further reduced in size by pounding them in a wooden mortar and pestle before grinding them to fine powder using a portable blending machine. The powdered samples were later sieved using a mechanical sieve shaker (EW-59986-60) with particle size (OD) 8 and a speed of rpm 1750 to remove particulates and stored in sample paper bags, prior to analysis. 5g of each of the blended plant samples was weighed out into precleaned porcelain crucibles. The samples were then heated to ashes in a muffle furnace at $550^{O}C$ for 4 hours. After complete dry ashing, the samples were dissolved in 20mL of 2M HNO_3 and filtered using 11cm whatman filter paper.

2.5 Heavy Metals Analysis

Analysis for the heavy metals of interest was performed using a Thermo-elemental type SOLAAR atomic absorption spectrometer (Fischer Scientific, USA). The filtered extracts were then transferred into precleaned Erlenmeyer flasks and passed through a precalibrated atomic absorption spectrometer for analysis. Measurements were made using the hollow cathode lamps for Cd, Cr, Cu, Zn and Ni according to the method specified in USEPA, 1983 [17]. The slit width was adjusted for all metals at 0.7 nm, except for Ni which was adjusted at 0.2 nm. The detection limit (DL) of the analytical method for each metal was calculated as double the standard deviation of a series of measurements of a solution, the concentration of which is distinctly detectable above, but close to blank absorbance measurement [17]. These detection limit values were 0.001, 0.004, 0.005, 0.05 and 0.10 ppm for Cd, Cr, Cu, Zn and Ni, respectively. For the determination of these metals, two solutions were prepared for each sample and three separate readings were made for each solution. The means of these figures were used to calculate the concentrations.

3. RESULTS AND DISCUSSION

A total of 5 elements (Cu, Zn, Cr, Cd and Ni) were determined in the powdered medicinal plant samples using FAAS. The data in Table 2 showed that most of the metals were accumulated to some extent by all the plant species investigated. Copper and Zinc were found to be present in all the plant samples studied in the mean concentrations of 1.83mg/kg and 8.04mg/kg respectively; which is to be expected since Cu and Zn are essential nutrients absorbed by the plant roots and subsequently transported to other parts of the plant like the stem (bark), leaves and seeds. Thus, as a whole, these medicinal plants were found to be good accumulators for Cu and Zn metals. Chromium, Cadmium and Nickel concentrations were in all cases found to be below their respective detection limits for all the thirty different plant samples investigated.

Concentration of Cu ranged from 0.04mg/kg in *Olax subscorpioidea* to 9.44mg/kg in *Adansonia digitalia* However, the mean Cu concentration recorded in this study was 1.83mg/kg, which is above the 0.3-0.1.0mg/kg range set for Cu by the WHO permissible limit for crude herbal drugs [12]. Furthermore, the mean Cu concentration (1.83mg/kg) observed was also lower than the Cu concentration (2.68mg/kg) recorded by Nwosu et al. [10] in a similar study. Copper performs a very useful function in animals as it increases their rates of Fe absorption [18].

Table 2. Heavy metal Concentration in plant material (in mg/kg)

Code	Botanical name	Cu	Zn	Cr	Cd	Ni
P1	*B. ferruginea*	4.680±0.03	8.545±0.02	BDL	BDL	BDL
P2	*S. indicum*	0.300±0.05	1.480±0.06	BDL	BDL	BDL
P3	*S. sieberiana*	0.685±0.01	1.965±0.05	BDL	BDL	BDL
P4	*C. procera*	3.300±0.02	4.590±0.04	BDL	BDL	BDL
P5	*G. pubescens*	0.330±0.04	5.035±0.01	BDL	BDL	BDL
P6	*S. arabica*	0.945±0.03	4.660±0.03	BDL	BDL	BDL
P7	*C. mucronatum*	0.330±0.02	5.035±0.04	BDL	BDL	BDL
P8	*K. Africana*	1.700±0.02	35.400±0.03	BDL	BDL	BDL
P9	*C. portocensis*	1.765±0.03	23.600±0.04	BDL	BDL	BDL
P10	*A. digitala*	9.440±0.07	8.395±0.05	BDL	BDL	BDL
P11	*M. Azdarach*	3.930±0.03	5.870±0.04	BDL	BDL	BDL
P12	*T. macroptera*	1.290±0.07	3.135±0.05	BDL	BDL	BDL
P13	*A. djalonensis*	1.765±0.03	23.600±0.04	BDL	BDL	BDL
P14	*V. doniana*	0.525±0.01	5.765±0.01	BDL	BDL	BDL
P15	*S. jollyanum*	1.230±0.04	6.475±0.04	BDL	BDL	BDL
P16	*T. alnifolia*	1.640±0.05	4.715±0.02	BDL	BDL	BDL
P17	*M. lucida*	2.845±0.04	7.210±0.05	BDL	BDL	BDL
P18	*B. coccineus*	0.560±0.02	1.280±0.03	BDL	BDL	BDL
P19	*T. cacao*	1.425±0.06	7.345±0.03	BDL	BDL	BDL
P20	*E. utile*	1.225±0.01	4.150±0.01	BDL	BDL	BDL
P21	*N. vogelii*	1.005±0.01	2.580±0.04	BDL	BDL	BDL
P22	*P. nigrescens*	2.840±0.06	24.900±0.03	BDL	BDL	BDL
P23	*F. asperifolia*	0.700±0.02	2.425±0.02	BDL	BDL	BDL
P24	*A. congensis*	1.010±0.03	5.035±0.06	BDL	BDL	BDL
P25	*P. nitride*	1.255±0.02	2.565±0.01	BDL	BDL	BDL
P26	*N. latifolia*	1.168±0.05	7.350±0.03	BDL	BDL	BDL
P27	*S. podocarpa*	0.355±0.02	0.935±0.04	BDL	BDL	BDL
P28	*S. mombin*	1.445±0.01	21.950±0.03	BDL	BDL	BDL
P29	*L. lanceolata*	0.035±0.03	8.380±0.04	BDL	BDL	BDL
P30	*O. subscorpioidea*	0.035±0.03	8.380±0.04	BDL	BDL	BDL

BDL means below detection limit Detection Limit of Cd= 0.01mg/L, Detection limit of Cr=0.04mg/L, Detection limit of Cu=0.005, Detection limit of Zn=0.005, Detection limit of Ni=0.10.

However, Zn concentration ranged from 0.94mg/kg in *Senna podocarpa* to 35.40mg/kg in *Kigelia Africana*. Furthermore, the mean Zn concentration of 8.04mg/kg recorded is far above the 0.05-0.5mg/kg range set for Zn by the WHO [12]. In addition, the mean Zn concentration (8.04mg/kg) was also higher than that recorded by Nwosu et al. (6.65mg/kg) in a similar study conducted in 2005 [10]. Thus, we can conclude that the herbal plants investigated had Zn levels above the WHO permissible limit set for crude herbal drugs. Zinc has also been reported to function as a nucleic acid synthesis protein in animals [18].

On the other hand, Cr, Cd and Ni were not detected by the instrument used. This observation is expected, since Cr, Cd and Ni are micronutrients that are found only in minute quantities in the soil; hence their plant uptake is minimal. Table 3 compares the toxic metal levels (mg/kg) in present study with the permissible standards set by WHO and selected countries.

The results of this research study were also compared with other similar works reported on toxic levels of metals in plants [10,11,19] and presented in Table 4.

This comparison shows that the metals detected in this present study were comparatively low when compared to metal content levels of similar research (as indicated in Table 4). This further goes to show that the heavy metals in the medicinal plants studied does not pose any health threat because of their low toxic nature.

The average percentage recoveries of the plant samples were 95.50% for Zn in *B. ferruginea* and 101.40% for Zn in *A. digitalia* respectively. The recovery results were in agreement with expected values (Table 6).

3.1 Correlation Studies

A Spearman Zero-order partial correlation test was performed to investigate correlations between the metal contents in all tested medicinal samples. The entire data were subjected to a statistical analysis and correlation matrices were produced to examine the inter relationship between the investigated metal concentrations. After investigating the correlations when two variables (Cu and Zn) are free of any control, one variable was placed as control and its correlation with the other variable investigated. All statistical analysis was done using SPSS Software Version 16.

From Table 5, it can be seen that there exist a weak positive correlation between Zn-Cu with corresponding r value of 0.219. This shows that there are slight increases in the concentration values of Zn for slight increases in the concentration values of Cu.

For the case of Zn as control (depicted in Table 5), the positive correlation between Zn and Cu with corresponding coefficient of 0.265 shows that the herbal samples were probably harvested from the same or similar farmlands within Ibadan.

However, for the case of Cu as control (Table 5), the negative correlation between Cu and Zn with corresponding r value of -0.093 indicates that some of the herbal samples were harvested from dissimilar areas with varying soil types.

3.2 Recovery Studies

The validity of the procedures used for sample treatment and analysis was tested by spiking experiment. 10ppm (10µg/ml) $ZnSO_4$ standard was used to spike 5g each of both plant samples. For every 1ml of standard spiked, it introduces 2µg/g Zn into the sample matrix. Thus, expected concentration = original concentration (µg/g) + 2µg/g.

The formula used for calculating percentage recovery is:

$$A\text{-}O/E\text{-}O \times 100 \quad (1)$$

Where A = actual concentration of the spiked sample after re-analysis; E = expected concentration of sample after spiking and O = original concentration of the sample before spiking.

The average percentage recoveries of the plant sample were 95.50% for Zn in *B.ferruginea* and 101.40% for Zn in *A.digitalia* respectively. The recovery results were in agreement with expected values.

Table 3. Comparison of Toxic Metal levels (mg/kg) in Present Study with Permissible Standards by World Health Organization (WHO) and selected Countries (Kosalec et al. [12])

	Zn	Cu	Cd	Ni	Cr
Present study	8.04	1.83	NS	NS	NS
WHO standard	0.05-0.5	0.3-1.0	NS	NS	NS
Canada HD, HP	NS	NS	0.30	NS	2.00
China HD	NS	NS	1.00	NS	NS
Malaysia HP	NS	NS	NS	NS	0.50
Singapore HP	NS	NS	NS	NS	NS
Thailand HD	NS	NS	NS	NS	NS
USA Pharmacopeia	NS	NS	0.30	NS	NS
Ph.Eur.draft monograph	NS	NS	0.30	NS	NS
Regulation (EC) 629/2008	NS	NS	0.10	NS	NS

NS-Not Specified; WHO-World Health Organization; USP – United States Pharmacopoeia 29th revision and the National Formulary 24th edition, 2006; FUI – Farmacopea Ufficiale della Repubblica Italiana, 11th edition, 2002; HD - crude herbal drugs; HP - finished herbal products; HE – herbal extracts; FS - food supp.

Table 4. Comparison of Results of Present Study with other Relevant Work

	Present Study	Ogunwade et al. [11]	Nwosu et al. [10]	Shad Ali Khan et al. [19]
Zn (ppm)	8.0373	35.100±0.01	47.500±0.01	38.140±0.02
Cu (ppm)	1.8302	24.400±0.01	24.00	9.650±0.04

Table 5. Correlation Coefficients between pairs of heavy metals in selected Medicinal Plants

A	No Control	Zn as Control	Cu as Control
Cu and Zn	0.219*		
		0.265*	
			-0.093*

A: cells contain Zero order (Pearson) Correlations
**: Correlation is significant at the 0.05 level (2-tailed) for values≤0.31*

Table 6. Recovery Study for Zn in *B. ferruginea* and *A. digitalia*

Sample code	Volume spiked (ml)	Amount of Zn spiked (µg)	Initial amount of Zn present (mg/kg)	Expected Concentration (mg/kg)	Actual Concentration (mg/kg)	% Recovery
1	2.00	20.00	8.55	12.55	12.36	95.50
2	2.00	20.00	8.40	12.40	12.45	101.40

1 = B. ferruginea sample (2ml ZnSO4 standard spiked)
2 = A. digitalia sample (2ml ZnSO4 standard spiked)

4. CONCLUSION

Out of 5 elements (Cu, Zn, Cr, Cd and Ni) determined in the powdered medicinal plant samples using FAAS, investigations revealed that their concentration levels were appreciable to some extent. The heavy metal concentrations of medicinal plants investigated were in most cases found to be well below the critical limit, except for Cu and Zn whose average levels were appreciably higher than the recommended limit set for them by the WHO. Chromium, Cadmium and Nickel levels were below the detection limit and hence pose no immediate toxic challenges. The implications of this finding may be taken into consideration when using the plants for medicinal or nutritional purposes. The results suggests that medicinal plants used for human consumption or for preparation of herbal products should be collected from an unpolluted natural habitat since there is less risk of suffering ailments resulting from consumption of contaminated herbs grown in polluted industrial/residential areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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