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Determination of heavy metals in medicinal plants from the wild and cultivated garden in Wilberforce Island, Niger Delta region, Nigeria

[Determinación de metales pesados en plantas medicinales silvestres y cultivadas en jardín en la isla de Wilberforce, región del delta del Níger, Nigeria]

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Abstract

Context: Adverse effects from herbal medicines may be partly due to the association of heavy metals with medicinal plants.

Aims: To determine residual levels of Ni, Cr, Pb and Cd in nine selected medicinal plant species and the surrounding soils collected from the Faculty of Pharmacy medicinal garden and College of Health Sciences residential quarters, Amassoma, Bayelsa state, Nigeria.

Methods: Nine plant species: *Jatropha tanjorensis, Ipomoea batatas, Celosia argentea, Zea mays, Colocasia esculenta, Corchorus olitorius, Vernonia amygdalina, Ocimum gratissimum* and *Talinum triangulare* were collected with their surrounding soil samples. The samples were dried and subjected to atomic absorption spectrophotometry (AAS) to determine the heavy metal concentrations.

Results: The detection frequencies of heavy metals in medicinal plants were: Cd – 100%, Pb - 11%, Ni – 0% and Cr – 0%. The residential quarter was more contaminated than cultivated medicinal garden. Order of residual concentration in bulk soils was Cr > Cd > Ni > Pb. Bioaccumulation factor ranged from 0 – 25.93 for foliar tissues. Cadmium in plant species ranged from 0.23 to 2.44 µg/g with > 88% exceeding the WHO maximum limit for medicinal plant materials.

Conclusions: The heavy metal concentrations in medicinal plants were dependent on the collection sites, plant species and physico-chemical properties of soil. Cd exhibited the greatest bioavailability in the investigated plants and soils. Cd and Pb found in plant foliage were due to uptake from soil and aerial deposition, respectively.

Keywords: contamination; heavy metals; medicinal plants; soil.

Resumen

Contexto: Los efectos adversos de las hierbas medicinales pudieran ser parcialmente debidos a la asociación de éstas con metales pesados.

Objetivos: Determinar los niveles residuales de Ni, Cr, Pb y Cd de nueve plantas medicinales seleccionadas y los suelos circundantes recogidos del jardín medicinal de la Facultad de Farmacia y el barrio residencial del Colegio de Ciencias de la Salud, estado de Amassoma, Bayelsa, Nigeria.

Métodos: Nueve especies vegetales: Se recogieron *Jatropha tanjorensis, Ipomoea batatas*, *Celosia argentea*, *Zea mays, Colocasia esculenta, Corchorus olitorius, Vernonia amygdalina, Ocimum gratissimum* and *Talinum triangulare* con sus muestras de suelo circundantes. Las muestras se secaron y se sometieron a espectrofotometría de absorción atómica (AAS) para determinar las concentraciones de metales pesados.

Resultados: Las frecuencias de detección de metales pesados en las plantas medicinales fueron: Cd - 100%, Pb - 11%, Ni - 0% y Cr - 0%. El barrio residencial estaba más contaminado que el jardín medicinal cultivado. El orden de la concentración residual en los suelos a granel fue Cr > Cd > Ni > Pb. El factor de bioacumulación osciló entre 0 - 25,93 para los tejidos foliares. El cadmio en las especies de plantas varió de 0,23 a 2,44 µg/g con > 88% excediendo el límite máximo de la OMS para los materiales vegetales.

Conclusiones: Las concentraciones de metales pesados en plantas medicinales dependieron de los sitios de recolección, las especies de plantas y las propiedades físico-químicas del suelo. El Cd mostró la mayor biodisponibilidad en las plantas y suelos investigados. Cd y Pb que se encontraron en el follaje de las plantas se debieron a la absorción del suelo y la deposición aérea, respectivamente.

Palabras Clave: contaminación; metales pesados; plantas medicinales; suelo.

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INTRODUCTION

Herbal self-medication is presently prevalent. This therefore calls for better and robusted regulation by local regulation agencies. A survey by the World Health Organization showed that about 80% of the world's population depend on indigenous medicinal plants for the treatment of various ailments (Gopal et al., 2014). More than a quarter of all prescription drugs in the Organization for Economic Co-operation and Development (OECD) countries, and approximately 60% of drugs dispensed in Eastern Europe, comprise plant or its products (The Lancet, 1994). In addition, modern pharmacopoeia have been found to contain at least 25% drugs of plant derivatives, while many others are synthesized analogues built on prototype structures of compounds isolated from plants (Fabricant and Farnsworth, 2001). Slightly above 35000 species of plant generally employed ethnomedicinally worldwide and a vast number of this are marketed uncontrollably (Lewington, 1993). Till date, only 40 secondary metabolites from less than 40 plant species from the tropics have been incorporated into orthodox drugs (van Seters, 1997).

Attraction towards herbal medicines may be due to the erroneous perception of low recorded incidences of serious adverse effects or absence of proper documentation of adverse effects of traditional/indigenous herbal medicines amongst other factors. But safety of herbal medicines does not only depend on the presence of relatively inherent toxic metabolites of medicinal plants but on the overall quality of the plants. For instance, accumulation of toxic industrial effluents in soil, air and water is continuously increasing due to fast urbanization and intensive environmental pollution (Huo et al., 2013). Consumption of medicinal plant products contaminated with toxic substances like heavy metals, have been reported to elicit deleterious effect on living organisms (Sethy and Ghosh, 2013). Generally, plants have been known to be extremely sensitive to environmental conditions and are able to accumulate heavy metals in their harvestable parts (Tangahu et al., 2011). Their introduction into plants can be through root uptake, foliar absorption and deposition of specific elements in leaves. The intensity of their uptake can change the overall elemental composition of the plants in its entirety. They in turn affect the growth and development and eventually their yield at the long run. Aside this negative effect on plants, there is the possibility that these potential toxic heavy metals can be transmitted to humans and animals along the food chain. Also, the direct use of these herbs grown in polluted areas by man is a serious concern for herbal medicine. Several studies have shown differentials in metal bioaccumulation between different plant species, between sampling locations and distribution in various parts of the same plant species (Stoltz and Greger, 2002; Baldantoni et al., 2004; Yang et al., 2008; Guala et al., 2010). Heavy metals such as Fe, Cu, Zn, Mn and Ni are essential nutrients at trace levels, however, at high concentrations beyond stipulated levels could be toxic and harmful. Metals such as Pb, Cd and Cr are reported to be non-essential to man (Serafim et al., 2012). Due to their very high potential toxic effects to human – as end user; accurate quantitative analysis of the residual levels of these heavy metals in medicinal plants is imperative (Vaikosen and Alade, 2011). In line with the aforementioned, the WHO has therefore as a matter of necessity required the qualitative and quantitative determination of heavy metal in plant species applied for medicinal use and diet (WHO, 2005; FAO/WHO, 2011).

Soil contamination by heavy metals is mainly due to impact from anthropogenic activities in the environment. Metal contaminants (such as Cd, Cr, Pb) in vegetative materials that are toxic can be attributed to many causes - these include environmental pollution such as contaminated waste emissions (gaseous, solid and liquid) from factories, solid waste dumpsites or refuse (and contaminated runoff water, which finds its way into farmlands), soil composition and fertilizers. These impacts have led to serious environmental problem with deleterious implications to human health (Moore et al., 2009). Soil-to-plant transfer and air-to-foliar absorption /diffusion or precipitation of substance are key processes of plant contamination by HMs and human exposure is through the food chain and herbal medication. Uptake via the roots from contaminated soils and direct deposition of contaminants from the atmosphere onto plant surfaces can lead to plant contamination by HMs (Zhuang et al., 2009).

Heavy metal contamination may alter the chemical composition of plants and thereby seriously affect the quality and efficacy of the natural products produced by medicinal plants species. The bulk of medicinal plants used for remedies are still harvested from the wild on forest land, only an insignificant percentage is cultivated (Schippmann et al., 2002; Gopal et al., 2014). It is pertinent to mention here that the guiding principles for assessing safety of herbal medicines with respect to contaminants and residues are based on the provisional tolerable intake (PTI) of individual contaminant (or sum of similar group of contaminants) and maximum residual limits (MRLs) of these contaminants. This study is therefore aimed at the evaluation of the foliar of nine medicinal plants from the Faculty of Pharmacy medicinal plant Garden at the College of Health Sciences (CHS), Niger Delta University and the wild around the CHS Housing Quarters, Wilberforce Island, Bayelsa, Nigeria, with their immediate surrounding soils on which they grow; these plant leaves are used in the treatment, prevention and management of different ailments in the Niger Delta region of Nigeria. In addition to determining the probable medium of introduction of found heavy metals – if through root uptake or foliar absorption by precipitation pathways; and also assess if residual metal levels are within or above maximum allowable regulation limits.

MATERIAL AND METHODS

Reagents and materials

Chemicals and reagents

Concentrated nitric acid and perchloric acid used were of analytical grade. They were manufactured by Merck KGa A of Germany and BDH Limited Poole England respectively. Distilled water used was double distilled (DD).

Instrumentation and measurements

Heavy metal measurements with a Varian Atomic Absorption Spectrophotometer (AAS), model Spectra AA 600 (Varian, California, USA) with flame system inter-phased to a computer and printer. Instrument was calibrated before use.

Sampling locations

The study area was in Amassoma, Wilberforce Island, Bayelsa State, Nigeria. It is located at latitude N 04° 58' and longitude E 006° 05' of the Greenwich meridian (Fig. 1). Herbal plant foliage used for the study were collected from the Faculty of Pharmacy (FoP) medicinal plant garden located within the premises of the College of Health Sciences (CHS) of the Niger Delta University and from the wild around the CHS residential quarters, Amassoma. The plants were identified at site and thereafter authenticated by Dr. A. T. Oladele of Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria and voucher specimen (Table 1) were deposited in her Herbarium.

Sample collection and pre-treatment

Nine selected different herbal plant species - *Vernonia amygdalina* Delile (Compositae), *Talinum triangulare* (Jacq.) Willd (Portulacaceae), *Jatropha tanjorensis* J. L. Ellis & Saroja (Euphorbiaceae), *Ocimum gratissimum* L. (Lamiaceae), *Corchorus olitorius* L. (Tiliaceae), *Celosia argentea* L. (Amaranthaceae), *Colocasia esculenta* (L.) Schott (Araceae), *Ipomoea batatas* (L.) Lam. (Convolvulaceae) and *Zea mays* L. (Poaceae) were used for this study (Table 1). At each sampling location for medicinal plants, fresh leaves were collected randomly with the aid of gloves, while soils at 0 - 15 and 15 – 30 cm depths were sampled at the base of the plant towards the root using a soil augur. Both samples were collected into aluminum foil and later placed into a cellophane bag. Leaves were dried at 30°C in an oven for 4 – 5 days, while the soil samples were air dried at room temperature (25°C). Each dried leaf tissue was carefully homogenized with a blender machine, while all lumpy air-dried soil samples were properly disaggregated and sieved (mesh size 2 mm) to remove extraneous matter. Each depth was homogenized before being analyzed.

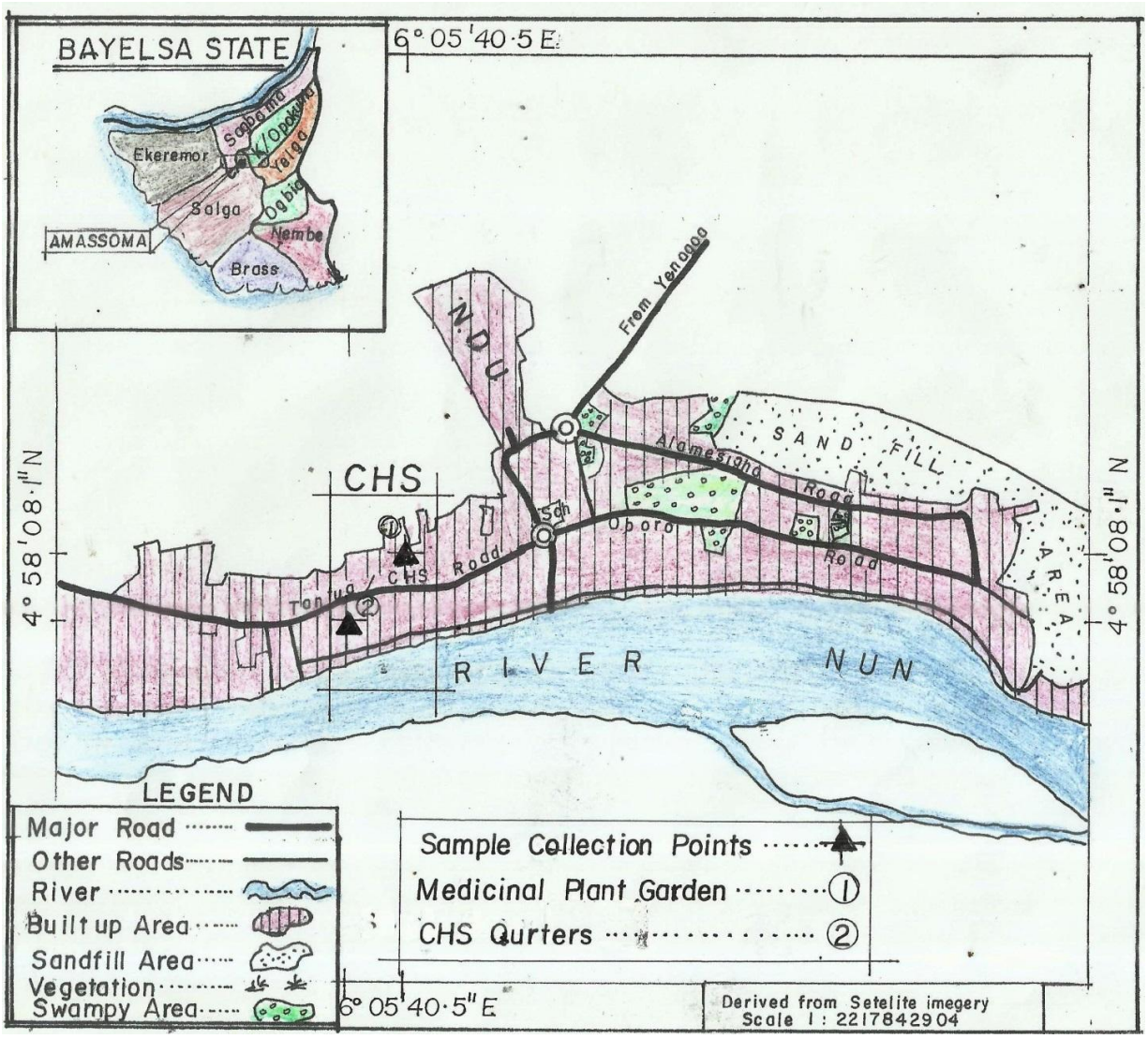

Figure 1. Map of Amassoma Town showing the sample collection points.

Sample preparation

Medicinal plant

The WHO quality control methods for medicinal plant materials were adopted for sample preparation (WHO, 2005).

Exactly 2.0 g of dried homogenized leaf tissues was weighed into a pre-tarred cleaned vitreous silica crucible of 25 mL, with same cover. Tarred sample was moistened with 1.0 mL of digestion mixture containing concentrated HNO_3 (70%) and $HClO_4$ (60%) in 1:1 ratio, covered and transferred into an oven without exerting pressure. The crucible was heated at 100°C for 3 hours; then increased to 120°C and maintained for 2 hours. Oven temperature was slowly and gradually increased to 240°C (to prevent losses that may result from possible violent reactions especially at the temperature range of 160-200°C). The temperature was further maintained for 4 hours. Resultant dry digest was allowed to cool and re-dissolved with 2.5 mL of concentrated nitric acid and transferred carefully into a 100 mL volumetric flask containing 20 mL of distilled water, the crucible was rinsed three times with 5 mL of distilled water, made to mark and used for the determination of the heavy metals by Atomic Absorption Spectrophotometry (AAS) (Mendhan et al., 2006; EDQM, 2007). Samples were analyzed for each metal in triplicates.

Table 1. Medicinal plant species, common uses and sampling location.

Plant species / voucher number	Family	Common uses	Sampling coordinates	Collection area
Vernonia amygdalina Delile / NDU160	Compositae	Fever, digestive tonic, laxative, purgative, expectorant, itching, parasitic infections, ringworm, anthelmintic, enteritis, GIT troubles, stomachic (Burkill, 1994a)	N04° 58' 08.4" E 006° 05' 50.9"	Medicinal plant garden
Talinium triangulare (Jacq.) Willd / NDU161	Portulacaceae	Inflammations, diuretic, stomach troubles, sore throat (Burkill, 1994d)	N 04° 58' 24.6" E 006° 05' 40.7"	CHS quarters
Jatropha tanjorensis J.L.Ellis & Saroja / NDU162	Euphorbiaceae	Antidiabetics, hypertension (Omobuwajo et al., 2011)	N 04° 58' 08.1" E 006° 05' 50.3"	Medicinal plant garden
Ocimum gratissimum L. / NDU163	Lamiaceae	Malaria, jaundice, vomiting, stomachic, dysentery, colds, bronchitis, sinusitis, antimicrobial, anthelmintic, rheumatism, lumbago, leprosy, headache, sedative, cough, sore throat, eye problem, vaginitis, nose troubles (Burkill, 1994c)	N 04° 58' 08.5" E 006° 05' 50.8"	Medicinal plant garden
Corchorus olitorius L. / NDU164	Tiliaceae	Fever, purgative, intestinal obstruction, tonic in malnutrition, heart troubles (Burkill, 1994e)	N 04° 58' 24.5" E 006° 05' 40.6"	CHS quarters
Celosia argentea L. / NDU165	Amaranthaceae	Diuretic, sores, wounds, skin eruptions, abscesses, antidote for snake bite, colic, gonorrhea, eczema, convalescents, dysentery, muscular troubles, antiscorbutic, anthelmintic (Burkill, 1994a)	N 04° 58' 24.8" E 006° 05' 40.5"	CHS quarters
Ipomea batata (L.) Lam. / NDU166	Convolvulaceae	Miscarriage, intercostal pain, mouth wash, toothache (Burkill, 1994a). Antidiabetic, antiscorbutic, abscesses, burn, miscarriage, mouth wash	N 04° 58' 08.2" E 006° 05' 50.9"	Medicinal plant garden
Zea mays L. / NDU167	Poaceae	Diuretic, urinary problem, anticancer, hypoglycemic, nausea, vomiting, stimulant, cystitis, Kidney stones, gonorrhea, mucal diarrhea, urogenital problems, bladder problems, osteomyelitis, warts (Burkill, 1994b)	N 04° 58' 24.7" E 006° 05' 40.7"	CHS quarters

Soil sample

The EPA 3050B method was adopted for the digestion of soil samples (USEPA, 1996).

A mass of 1 g of previously dried, pulverized, sieved and homogenized soil samples were weighed into digestion vessels. To soil sample, 10 mL of 1:1 (v/v) ratio of HNO_3 to distilled water was added; the slurry was mixed, covered with a watch glass and heated to 95 ± 5°C for 15 min without boiling. The sample was allowed to cool and 5 mL of concentrated HNO_3 was added. The solution mixture was then refluxed for 30 min, with the glass cover replaced. This step was repeated several times until no brown fume was generated (ensuring complete oxidation of the sample by HNO_3). Sample mixture was further heated at 95 ± 5°C without boiling for 2 h with covering of reaction vessel maintained.

The sample mixture was allowed to cool, followed by addition of 2 mL of distilled water and 3 mL of 30% H_2O_2. The vessel was then covered with a watch glass and warmed gently with care to prevent excessive vigorous effervescence. The gentle heating of vessel was continued until the effervescence subsided and allowed to cool. After cooling 1 mL of 30% H_2O_2 was added intermittently, with gentle warming continued until effervescence is minimal or until the general sample appearance is unchanged (total added volume of 30% H_2O_2 < 10 mL). The acid-peroxide digestate vessel was then covered using a ribbed watch glass, with heating continued and maintained at 95 ± 5°C for 2 h until the volume has been reduced to approximately 5 mL. A 10 mL conc. HCl was added to the digestate and covered with a watch glass and further refluxed for 15 min at 95 ± 5°C. The digestate was allowed to cool and diluted with distilled water to 100 mL in a volumetric flask. The digestate solution was filtered through Whatman No 41 filter paper to remove particulates and analyzed for Cd, Ni, Cr and Pb using AAS.

Blank digest for correction

To correct that may have arisen from reagent used during sample preparation, separate blank analysis for medicinal leaf tissues and soil samples were carried out by following the same procedures for digestion enumerated for plant species and soil, without the test samples. Analyte metal concentrations in blank assay were subtracted from corresponding test assay to obtained actual concentrations of analyte metals in plant species and soil.

Evaluation of analytical methodology

To validate digestion procedure used for this study, weighed portions of each medicinal plant and composited soil (0 - 15 cm and 15 - 30 cm) samples were fortified with standard solution of known concentration of analyte metals. The fortified plant and soil samples were digested in triplicate following the same procedure employed for digestion of the plant and soil samples. The filtered digestate of the spiked samples were then analyzed for analyte metal. Percentage recoveries for medicinal plants and soils were calculated using the expression:

$$\%R = \frac{\text{Concentration of analyte metal recovered}}{\text{Concentration of analyte metal added}} \times 100$$

All soil samples collected at the CHS quarters and Medicinal garden at depths 0 - 15 cm and 15 - 30 cm were composited separately. The amount of standard analyte solution added to samples were as follows: Cd (0.5 µg/g), Pb (1.0 µg/g), Cr (10 µg/g), and Ni (2.0 µg/g).

Statistical analysis

Values were represented as mean ± standard deviation (SD) and bar charts were plotted using OriginPro8-Data Analysis and Graphing working space version 8E software, China.

RESULTS AND DISCUSSION

Physico-chemical properties of soil

Some selected soil properties of FoP medicinal plant garden and CHS housing quarters are presented in Table 2. Samples at both locations were composited. The pHs of soils were predominately acidic; values ranged between 5.41 ± 0.01 and 6.43 ± 0.02 for top and bottom soils respectively. The Total organic carbon (TOC) ranged from 0.80 – 2.43%. The top soils had higher TOC content than the bottom soils. The total organic matter (TOM) also followed the same trend, with values ranging from

1.42% to 4.33%. This was considered significantly high to influence physico-chemical properties of most soils. The Carbon:Nitrogen ratio for soils ranged between 10:1 and 13:1, which was within the normal C:N ratio range of 8:1 to 15:1 for most soils. The cation exchange capacity (CEC) ranged 19.81 ± 1.19 to 30.59 ± 0.10 meq/100g. Particle size distribution for soils showed that they are loamy in texture (Table 2).

Evaluation of analytical methodology

The recovery for Cd, Pb, Cr and Ni metals after fortification of medicinal leaves ranged from 94.6 ± 2.1% (Ni, *Ocimum gratissimum*) to 103.4 ± 5.8% (Cd, *Ipomoea batatas*), while for soils (0 - 15 cm, 15 - 30 cm) recovery values ranged from 95.6 ± 2.4 (Ni, 0 - 15 cm, Medicinal garden) to 99.0 ± 1.3% (Pb, 0 - 15 cm, CHS quarters) (Table 3). The calculated %RSD for the four metals were less than 10 for all replicate matrixes (n = 132). The %RSD ranged from 0.8 to 6.0 for both plant leaves and soils, while 63.9% and 68.8% of the replicate measurements in plant leaves and soils respectively had their %RSD ≤ 3.6%. These values indicated a reasonable variability and high precision of the analytical methods adopted.

The %RSD ranged from 0.8 to 6.0 for both plant leaves and soils, while 63.9, and 68.8% of the replicate measurements in plant leaves and soils respectively had their %RSD ≤ 3.6%.

Percentage detection frequency

In plant leaves, the detection frequency was Cd (100%), Pb (11%), Ni (0%), and Cr (0%) (Fig. 2a). Pb was found only in *Corchorus olitorius* (CHS quarters) (Fig. 2a). However, in soil (0 - 15 and 15 - 30 cm depths), all metals except Pb were detected. In soil, Cd recorded the highest detection frequencies of 100% and 70% at CHS residential quarters and FoP medicinal plant garden, respectively (Fig. 2a). Others were Cr: 25% (CHS quarters) and 0% (FoP garden); Ni: 25% (CHS quarters) and 10% (FoP garden). These detection frequencies portrayed the CHS residential environment to be more contaminated than the cultivated medicinal plant garden.

Heavy metals distribution in plant species and soils

Lead (Pb)

Lead (Pb) was not detected (ND) in the leaf tissues of any of the medicinal plants except in *Corchorus olitorius* with a concentration of 4.31 ± 0.15 µg/g, while it was not detected in any of the soils samples (Tables 4 and 5). Value recorded for *Corchorus olitorius* leaf was lower than levels obtained at two sites along the Nigeria-Benin Republic towards Seme town (Osundiya et al., 2014). Also, level of Pb found in *Corchorus olitorius* was significantly below the maximum residual limit (MRL) of 10 µg/g stipulated by the WHO and Canadian Health Authority for raw medicinal plants (WHO, 2005).

The presence of Pb in *Corchorus olitorius* leaf tissues may have been through aerial deposition on the leaves, followed by diffusion/absorption through the stomata, since no Pb was detected in the soils. Pb is one of the most frequently encountered heavy metal in polluted environment and its accumulation in plant can be by up-take from surrounding soils through the root and/or aerial deposition on shoot (Smirjakova et al., 2005). Excess Pb can be toxic to most plants and it has the ability to cause morphological, physiological and biochemical dysfunctions in plants. Lead has been reported to be an abortifacient and can cause miscarriages and low birth weights in infants; it is also implicated in reduced sperm count and motility (Vaikosen and Alade 2011).

Nickel (Ni)

Ni was not found in any of the medicinal leaf tissues and as well at depth 0 - 15 cm, however, the residual concentrations at 15 - 30 cm depth, ranged from ND to 3.03 ± 0.06 µg/g. The highest residual value was found at the *Zea mays* sampling point, followed by *Talinum triangulare* and *Vernonia amygdalina* sampling locations, while other sampling locations were below Ni detection limit. The bottom soil from CHS residential quarters showed higher Ni contamination when compared to that of

the cultivated medicinal garden; both *Zea mays* and *Talinum triangulare* foliage were collected at the CHS residential quarters. The ratio of residual Ni concentration found in the CHS residential quarters to FoP medicinal plant garden was > 5. The non-detection of Ni at top soil may be due to leaching from run-off and its migration from top soil to bottom soil over time (for where they were found).

However, the presence of Ni in *Vernonia amygdalina* leaf tissues (3.5 ± 0.00 µg/g) has been reported in samples collected from the wild in Ghana, with non-detection in *Ocimum gratissimum* and *Jatropha gossypiifolia* foliage (Annan et al., 2010). The non-detection of Ni in *Vernonia amygdalina* and other foliage in spite of its presence in the bottom soil in the CHS environment may be due to geochemical properties such as pH, total organic carbon (TOC), clay content, among others. Geochemical properties of the soil and the selective inhibition of the accumulation of Ni by *Talinum triangulare* and *Zea mays* have been reported (Abu-Darwish, 2009). Clayey soils are reported to have high adsorption capacity and this would restrict desorption of bound metals in soil for up-take by plant through the root route. In addition, the uptake of heavy metals from soil by plants through the roots and subsequent translocation to the aerial parts of a plant is usually through the xylem or/and phloem in solubilized form. For example Ni would be easily absorbed as $Ni_2SO_4.7H_2O$ from the soil and translocated to the leaves.

Nickel is a constituent of the enzyme urease and small quantities are essential for some plant species (Takishima et al., 1988); it is an essential micronutrient, which is required by urease for hydrolyzing urea. High concentrations may be toxic to plants. Extremely high concentrations of nickel have left some farmland unsuitable for growing crops. Its toxic effects have been frequently reported, such as inhibition of mitotic activity of *Cajanus cajan*, reduction in germination of cabbage and adverse effects on fruit yield and quality of wheat.

Table 2. Physico-chemical characteristics of soils.

Parameters	CHS medicinal garden*		CHS Quarters*	
	0 – 15 cm	15 – 30 cm	0 – 15 cm	15 – 30 cm
pH	6.43 ± 0.02	6.01 ± 0.01	5.41 ± 0.01	5.47 ± 0.02
Conductivity (µs/cm)	184 ± 4.00	142 ± 4.00	116 ± 3.00	117 ± 2.00
Salinity (‰)	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Sulphate (mg/kg)	172 ± 4.00	305 ± 5.00	496 ± 6.00	381 ± 5.00
Total organic carbon (TOC) (%)	2.43 ± 0.12	1.09 ± 0.11	1.27 ± 0.09	0.80 ± 0.01
Total nitrogen (TN) (%)	0.19 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.00
C:N Ratio	13:1	11:1	11:1	10:1
Total organic matter (TOM) (%)	4.33 ± 0.21	1.94 ± 0.20	2.26 ± 0.16	1.42 ± 0.02
CEC (meq/100g)	30.59 ± 0.98	20.82 ± 1.31	23.74 ± 1.05	19.81 ± 1.19
Particle size distribution				
Sand (%)	58.0	59.5	63.1	61.3
Silt (%)	17.4	19.2	14.8	18.1
Clay (%)	24.6	21.3	22.1	20.6

*Composited soil samples. C:N – Carbon:Nitrogen ratio; CEC: Cation exchanging capacity; CHS: College of Health Sciences.
The soil samples are basically acidic.

Table 3. Percent recovery and relative standard deviation for analyte metals in matrixes.

Plant	**Cr**		**Cd**		**Pb**		**Ni**	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
Vernonia amygdalina	96.8 ± 2.2	2.3	93.6 ± 4.7	5.0	97.0 ± 2.0	2.0	93.0 ± 3.9	4.2
Talinum triangulare	99.8 ± 3.2	3.2	93.3 ± 1.9	2.1	97.0 ± 5.8	6.0	96.2 ± 3.2	3.4
Jatropha tanjorensis	94.7 ± 3.2	3.4	95.3 ± 3.9	4.1	93.2 ± 2.0	2.2	96.7 ± 4.7	4.9
Ocimum gratissimum	96.4 ± 3.4	3.6	94.9 ± 2.0	2.1	99.0 ± 4.0	4.0	94.6 ± 2.1	2.2
Celosia argentea	96.7 ± 1.6	1.7	98.4 ± 5.4	5.5	96.1 ± 1.2	1.3	100.2 ± 2.4	2.4
Corchorus olitorius	94.9 ± 4.1	4.3	97.3 ± 0.7	0.8	97.1 ± 2.0	2.1	101.5 ± 5.2	5.1
Colocasia esculenta	97.8 ± 2.4	2.4	95.8 ± 2.1	2.2	95.7 ± 0.8	0.8	96.5 ± 1.6	1.7
Ipomoea batatas	95.6 ± 3.8	4.0	103.4 ± 5.8	1.6	98.1 ± 1.1	1.1	98.0 ± 3.8	3.9
Zea mays	95.6 ± 1.6	1.7	98.1 ± 4.0	4.1	96.8 ± 3.2	3.3	98.6 ± 3.8	3.8
Soil								
Medicinal garden								
Top soil (0 - 15 cm)	98.7 ± 2.1	2.1	95.8 ± 4.1	4.3	95.8 ± 3.9	4.1	95.6 ± 2.4	2.5
Bottom soil (15 - 30 cm)	94.4 ± 3.4	3.6	95.4 ± 2.4	2.5	95.7 ± 2.2	2.3	97.3 ± 1.2	1.3
CHS quarters								
Top soil (0 - 15 cm)	96.6 ± 0.9	0.9	98.3 ± 2.2	2.2	99.0 ± 1.3	1.3	96.7 ± 2.8	2.9
Bottom soil (15 - 30 cm)	98.5 ± 4.4	4.5	95.8 ± 2.2	2.3	96.2 ± 6.1	6.3	96.1 ± 4.3	4.5

%R = Percent recovery; %RSD = Relative standard deviation.

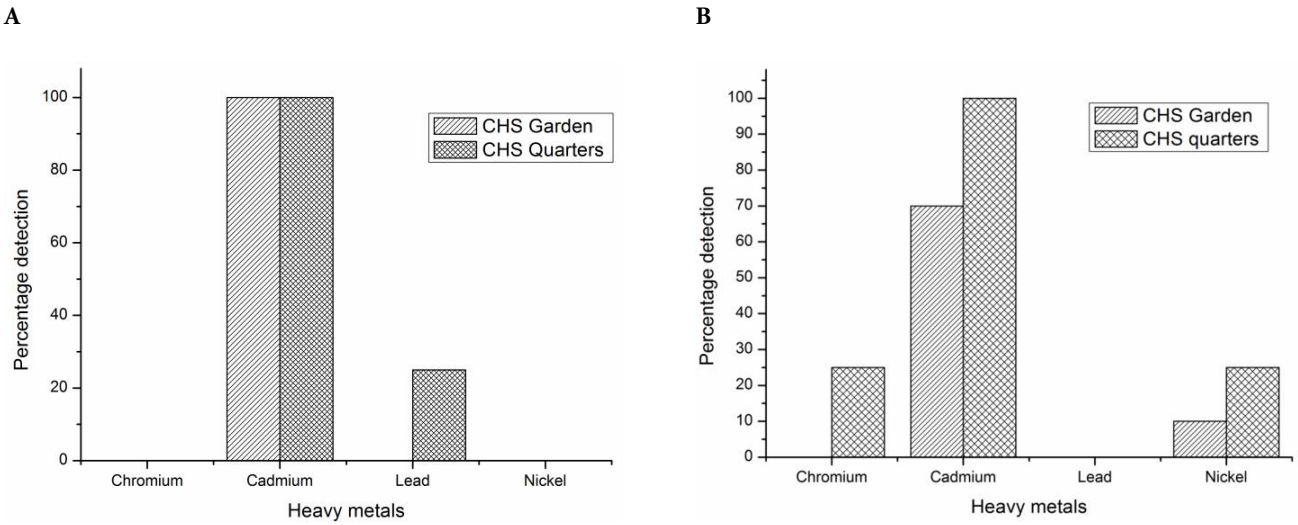

Figure 2. Percentage detection frequency of heavy metals in **(A)** herbal plants and **(B)** soils.

The number of times each of the heavy metals were detected in herbal plants and in soils at the CHS quarters and FoP (CHS) garden were computed into percentage detection frequency (i.e. ratio of number of times metal was detected to total no. of samples analyzed per location multiplied by 100). Cd had the highest detection frequency in both soil and leaf samples.

Table 4. Levels of heavy metals in the leaves of plant species.

Plant species	Concentration (µg/g)			
	Cr	Cd	Pb	Ni
Vernonia amygdalina	ND	0.54 ± 0.01	ND	ND
Talinum triangulare	ND	0.23 ± 0.01	ND	ND
Jatropha tanjorensis	ND	2.44 ± 0.08	ND	ND
Ocimum gratissimum	ND	0.41 ± 0.01	ND	ND
Celosia argenta	ND	0.66 ± 0.02	ND	ND
Corchorus olitorius	ND	1.23 ± 0.03	4.31 ± 0.15	ND
Colocasia esculenta	ND	0.87 ± 0.02	ND	ND
Ipomoea batata	ND	1.72 ± 0.03	ND	ND
Zea mays	ND	0.92 ± 0.02	ND	ND

ND (Not detected) < 0.001 µg/g.

Table 5. Distribution of heavy metals in soils.

Medicinal plant Species	Concentration in soil profiles (µg/g)							
	Top soil (0 – 15 cm)				Bottom soil (15 – 30 cm)			
	Cr	Cd	Pb	Ni	Cr	Cd	Pb	Ni
Vernonia amygdalina	ND	0.95 ± 0.08	ND	ND	ND	0.26 ± 0.01	ND	0.38 ± 0.01
Talinum triangulare	ND	0.20 ± 0.02	ND	ND	3.90 ± 0.11	0.19 ± 0.02	ND	2.04 ± 0.05
Jatropha tanjorensis	ND	0.00 ± 0.00	ND	ND	ND	0.19 ± 0.01	ND	ND
Ocimum gratissimum	ND	0.00 ± 0.00	ND	ND	ND	ND	ND	ND
Corchorus olitorius	ND	0.21 ± 0.01	ND	ND	ND	0.47 ± 0.04	ND	ND
Celosia argentea	ND	0.89 ± 0.04	1.63 ± 0.09	ND	ND	0.22 ± 0.01	ND	ND
Colocasia esculenta	ND	0.50 ± 0.02	ND	ND	ND	0.57 ± 0.02	ND	ND
Ipomoea batata	ND	0.39 ± 0.01	ND	ND	ND	0.68 ± 0.02	ND	ND
Zea mays	ND	0.37 ± 0.01	ND	ND	5.32 ± 0.09	0.80 ± 0.04	ND	3.03 ± 0.06

Cadmium (Cd)

Cadmium was found in all medicinal plant foliar tissues, with residual levels ranging from 0.23 ± 0.01 µg/g (*Talinum triangulare*) to 2.44 ± 0.08 µg/g (*Jatropha tanjorensis*) (Table 2). The order of residual concentration was *Jatropha tanjorensis* > *Ipomea batatas* > *Celosia argenta* > *Zea mays* > *Colocasia esculenta* > *Corchorus olitorius* > *Vernonia amygdalina* > *Ocimum gratissimum* > *Talinum triangulare*. Sobukola et al. (2010) reported much lower concentrations for bitter leaf (*Vernonia amygdalina)* and water leaf (*Talinum triangulare)* purchased from selected markets in Lagos, Nigeria. Also, residual values reported in foliar part for *Ocimum gratissimum* and *Vernonia amygdalina* sampled from Road Side and Physique Garden in Kwame Nkrumah University of Science and Technology and natural habitat respectively in Ghana (Annan et al., 2010; 2013), were significantly higher than level found in the same plant species cultivated in FoP medicinal plant garden. All plant species except *Talinium triangulare* exceeded the 0.3 µg/g maxi-

mum allowable limit stipulated by WHO for raw medicinal plant material (WHO/FAO, 2011).

Also, in soil, cadmium was found at all corresponding locations where plant species were collected, except for *Ocimum gratissimum*, and *Jatropha tanjorensis* at both depths and 0 - 15 cm depth, respectively. The level of residual Cd concentration ranged from ND (*Ocimum gratissimum* and *Jatropha tanjorensis*) to 0.95 ± 0.08 µg/g (*Vernonia amygdalina*) at depth 0 – 15 cm, while depth 15 - 30 cm ranged from ND (*Ocimum gratissimum*) to 0.80 ± 0.04 µg/g (*Zea mays*). These concentration ranges were comparable to values reported in soils on which four medicinal plant species were collected in Karak District, Khyber Pakhtunkhwa, Pakistan (Shah et al., 2013), but significantly lower than residual concentrations in Haridwar, India (Maharia et al., 2010) and high traffic urban areas in Aba city, Nigeria, where *Ocimum gratissimum* and *Vernonia amygdalina* foliage were harvested (Princewill-Ogbonna and Ogbonna, 2011). The presence of Cd on *Ocimum gratissimum* leaf tissues and its non-detection in surrounding soils (top and bottom profiles) implied that the Cd found on *Ocimum gratissimum* foliage may have been through aerial deposition and not by uptake from the soil through the root via translocation. However, the residual concentrations of Cd in all soils were below the stipulated permissible limit of 3 µg/g by FAO/WHO (2011).

Cadmium has been reported to have adverse effect on sperm count and motility (Kumar et al., 2005; Ige et al., 2012) and also implicated in renal dysfunction and anaemia. Xu et al. (2001) reported the significant reduction in sperm count and motility seven days after administration in rats. In plants, it has caused profound reduction in the production yield at concentrations ≥ 5 mg/kg (Hussain et al., 2005). Cd as a potential toxic metal has been found widely in water, soil, milk, dietary products, medicinal plants and herbal products.

The major sources leading to accumulation of cadmium in soil and plants are phosphate fertilizers, non-ferrous smelters, lead mines, sewage sludge application and combination of fossil fields.

Cadmium is used as a catalyst in the manufacturing of products such as margarine and peanut butter; wrapping papers and plastics in packaging refined foods; as plating on equipment or inks, dyes and lubricants used on and around food processing equipment (Wilson, 2012).

Chromium (Cr)

Residual Cr was not detected in any of the medicinal plant leaf tissues and soils at 0 -15 cm depth. However, at depth 15 – 30 cm, residual concentrations were 1.95 ± 0.05 µg/g and 2.66 ± 0.06 µg/g at *Talinum triangulare* and *Zea mays* sampling locations respectively, while other sampling location were below detection limit. The non-detection of Cr in the leaf tissues of *Talinum triangulare* and *Zea mays* may be due to the non-availability of the Cr metal in solubilized form for easy uptake from the soil. In addition, the non-availability of Cr may be due to its adsorption to the surrounding soil particulate. The interaction between heavy metals and soil particulates are influenced by variables such as organic carbon (OC), clay, temperature and pH have been reported (Basta et al., 2005). The presence of high percentage clay and OC tends to enhance adsorption of metals such as Cr to soil particulates; thereby making it unavailable for uptake by plant species. The concentrations of Cr in all soils were significantly below the level of maximum permissible level of 50 µg/g for agricultural soil (MAFF, 1992). Sources of contamination may be due to solid waste disposal of items such as plastic materials, paint containers, polythene bags and paper waste at the residential area of the CHS. Chromium is used on a large scale in industries such as metallurgical, electroplating, paints and pigments, tanning, wood preservation, pulp and paper (Zayed and Tery, 2003). The maximum residue level (MRL) in raw medicinal plant stipulated by WHO is 2 µg/g (WHO, 2005).

Chromium has been reported to elicit reduction in motility and sperm morphology (Kamel, 2011) caused 75% reduction in sperm count at 10 mg/kg in rats (Li et al., 2001). Chromium is regarded as one of the most toxic pollutants in the world and is released by tanneries, steel industries and sewage sludge applications.

The order of bulk soil contamination for total metal [i.e sum (∑) of Cr, Cd, Ni, Pb] at sampling locations were as follows: *Zea mays* > *Talinum triangulare* > *Vernonia amygdalina* > *Celosia argenta* > *Colocasia esculenta* > *Ipomea batatas* > *Corchorus*

olitorius > *Jatropha tanjorensis* > *Ocimum gratissimum* (Fig. 3); while the order of residual concentration of heavy metals in bulk soils was Cr > Cd > Ni > Pb.

Bioaccumulation factor

Calculated bioaccumulation factor (BCF) for Cr, Cd, Pb and Ni in nine medicinal plants is presented in Fig. 4 (values were computed as ratio of metal concentrations in leaf tissue to bulk soil – average of both depths). BCF values ranged from 0 – 25.93 for all foliar tissues of medicinal plants and heavy metals. BCF value was 0 (zero) for Cr, Pb and Ni in all medicinal leaf tissues, except for *Celosia argenta*, which recorded a value of 0.19 for Pb, while values for Cd were as follows: *Jatropha tanjorensis* (25.93), *Ipomea batatas* (3.23), *Celosia argenta* (2.21), *Corchorus olitorius* (1.94), *Colocasia esculenta* (1.62), *Zea mays* (1.57), *Talinum triangulare* (1.17), *Vernonia amygdalina* (0.89) and *Ocimum gratissimum* (0) in decreasing order.

Effect of pH, clay and organic matter contents on metal availability

The behavior of metals in soils are affected by principal factors such as pH, texture and quantity of clays with the oxides present, organic matter and the type of humic substances (Basta et al., 2005; Wahba and Zagloul, 2007). Cr, Cd and Ni were detected in soils at *Vernonia amygdalina, Talinium triangulare* and *Zea mays* sampling locations. Order of heavy metal residual concentrations was Ni > Cd (*Vernonia amygdalina)*; Cr > Ni > Cd (*Talinium triangulare* and *Zea mays*) (Table 4). Only Cd was found in all three plant species. This implies that Cd was most available for uptake. Higher Cd residual levels relative to other metals in *Theobroma cocao* nibs have been reported for contaminated soil (Nartey et al., 2012). At low pH, Cd exhibits higher mobility in soils relative to other heavy metals (Kabata-Pendias and Pendias, 1992; Alloway, 1995); thus greater availability in soil solution for plant uptake. The pH of soils in this study ranged from 5.41 - 6.43. This was acidic enough for its preferential up-take to Ni and Cr.

The presence of Cd and non-detection of Ni and Cr in corresponding plant species with respect to sampling locations may also be due to metal-soil properties relationship (Tables 2 - 5). Clay is usually nutrient rich and are too tightly bound to be easily released and absorbed by plant roots (Kerrigan and Nagel, 1998) for subsequent translocation to the shoots and leaves. Soil properties such as clay and organic matter contents have been reported to favor greater affinity for Ni than Cd (Chorom et al., 2013), hence stronger adsorption of Ni to soil and non-availability for plant uptake. These phenomena may have contributed largely to the uptake of Cd by all plant species investigated in this study.

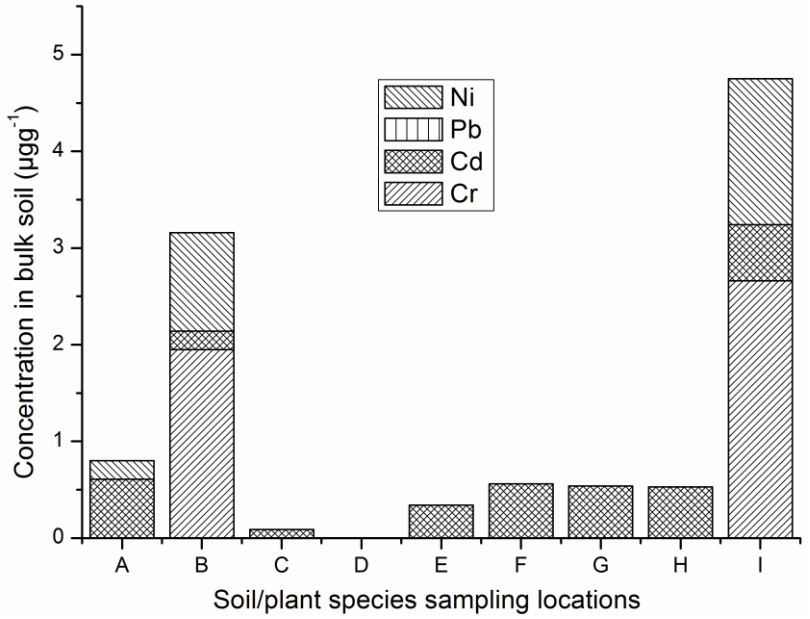

Figure 3. Heavy metal distribution in bulk soil. Average concentration of each heavy metals in top soil (0 -15 cm depth) and sub-soil (15 -30 cm) were computed to give concentration of heavy metals in bulk soil for sampling point where each of the nine herbal plants were collected. *Zea mays* had the highest bulk soil contamination.

A:*Vernonia amygdalina;* B:*Talinum triangulare;* C: *Jatropha tanjorensis;* D: *Ocimum gratissimum;* E: *Corchorus olitorius*; F: *Celosia argenta*; G: *Colocasia esculenta*; H: *Ipomea batata*; I: *Zea mays*

Phyto-toxic assessment of plant species

The potential risk of residual heavy metals in leafy medicinal plant species was evaluated by comparing residual levels in medicinal plant foliage, with stipulated limits for heavy metals in raw medicinal plant materials or/and leafy vegetables by the WHO and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The WHO maximum residue limits (MRL) for cadmium in raw medicinal plant is 0.3 µg/g, while for leafy vegetables, stipulated JECFA maximum permissible limit is 0.2 µg/g (WHO, 2005; FAO/WHO, 2011). The residual Cd concentrations in all nine medicinal plant species investigated in this study ranged from 0.23 to 2.44 µg/g. This implies that 100% of the plant species exceeded the JECFA maximum limit, while > 88% exceeded the WHO limit for medicinal plant species. Residual Pb was found only in *Corchorus olitorius*, with a mean concentration of 4.31 µg/g. This value exceeded the JECFA permissible maximum limit of 0.3 µg/g for Pb when *Corchorus olitorius* is consumed as vegetable (FAO/WHO, 2011). However, the WHO maximum limit as medicinal plant is 10 µg/g (WHO, 2005).

The provisional tolerable monthly intake (PTMI) of Cd for adult is 25 µg/kg body weight (FAO/WHO, 2011). The predicted quantity of plant species that would be equivalent to the PTMI of Cd for adult with body weight of 60 kg are as follows; *Vernonia amygdalina* (2.79 kg), *Talinium triangulare* (6.67 kg), *Jatropha tanjorensis* (0.62 kg), *Ocimum gratissimum* (3.64 kg), *Ipomea batatas* (0.87 kg), *Celosia argenta* (2.26 kg), *Corchorus olitorius* (1.22 kg), *Colocasia esculenta* (1.73 kg), *Zea mays* (1.63 kg). This depicts that the consumption of these plant species above the predicted PTMI in our diets or herbal medications is likely to be harmful.

CONCLUSIONS

The present study has demonstrated that the variation in heavy metal concentrations in medicinal plants is dependent on the collection sites, plant species and physico-chemical properties of soil such as pH, clay and organic matter contents. Cd exhibited the greatest bioavailability in the investigated soils and also recorded the highest rate of phyto-toxicity assessment. Medicinal plants growing within the CHS residential areas in the wild were more contaminated due to the loitering of solid waste in this environment. Cd and Pb found in plant foliage were due to uptake from soil and aerial deposition respectively, while > 88% exceeded the WHO limit for medicinal plant species.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Vaikosen EO	Alade GO
Concepts or Ideas	X	X
Design	X	X
Definition of intellectual content	X	X
Literature search	X	X
Experimental studies	X	X
Data acquisition	X	X
Data analysis	X	X
Statistical analysis	X	X
Manuscript preparation	X	X
Manuscript editing	X	X
Manuscript review	X	X

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