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Heavy Metal Concentrations in Some Common Medicinal Plants from Different Geographical Locations in Douala, Cameroon

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Abstract

This study was aimed at investigating and comparing the levels of five heavy metals; Pb, Cd, Cr, Zn, and Cu in both leaves, shoot and root samples of six common medicinal plants: *Aloe vera*, *Ageratum conyzoides*, *Cleome ciliata*, *Cymbopogon citratus*, *Eremomastax speciosa*, and *Justicia secunda* collected from refuse dumps, roadsides and residential areas in Douala. The plant samples collected were divided into different parts. Concentrations of heavy metals were determined using Atomic Absorption Spectrometry. Results revealed that the selected medicinal plants accumulate heavy metals at different concentrations. Heavy metals repartition decreased in the trend roots>shoots>leaves. There was a significant difference ($p<0.0001$) in the content of metals between plants from different collection points. Samples collected from refuse dumps showed significantly higher concentrations ($p<0.0001$) of heavy metals followed by roadsides, then residential areas. Comparison of results with defined permissible limits led to the conclusion that the levels of all five heavy metals were beyond the permissible limits in the majority of medicinal plants analyzed. Only *Ageratum conyzoides* was below the permissible limit for Cu. High heavy metal concentrations in plants indicate high level of contamination and this raises consumers' health risk concerns.

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Heavy metals
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Health risk

Introduction

Medicinal plants (MP) have been playing a key role in world health for ages. People around the globe use MP in relation to their culture and historical behaviors (Rates, 2001; WHO, 2005). Though distributed worldwide, MP are most abundant in tropical countries. Approximately, 25 000 plant-based formulations are available in the indigenous medical texts (Gupta et al., 2004). Nowadays, many African countries are concerned by the overwhelming urbanization, ever-growing population and pollution due to high industrialization, dense traffic, large-scale agriculture, etc. The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metallic ferrous ores, municipal wastes, fertilizers, pesticides, and sewage. Consequently, with various pollutants gradually increasing, soil and plant contamination are becoming a serious concern with typical and significant causes being anthropogenic activities (agricultural, chemical and industrial), vehicle emissions, and improper waste disposal (Krishna and Govil, 2007). Among these toxic elements, presence of heavy metals which constitute an important group of hazardous substances, are ubiquitous in nature and cause serious harmful effects on living organisms (Nies, 1999; Lee and Lee, 2002). Medicinal herbs are easily contaminated during growth, development and processing. After collection and transformation into dosage form, the heavy metals confined in plants finally enter the human body and may disturb the normal functions of vital organs. Main heavy metals like cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and chromium (Cr) have been reported to be more toxic to plants than the others and have no other reported biological functions to plants (Fauziah et al., 2010; Ji et al., 2012).

Nonetheless, some heavy metals like copper (Cu), zinc (Zn) and magnesium (Mg) are essential and required by the human body in trace amounts. When these metals exceed the acceptable limits in the body, they become toxic (at very low concentrations) and cause serious harmful effects on living organisms (Nies, 1999; Lee and Lee, 2002; Adepoju-Bello et al., 2014). They may cause damage to vital organs of the body like the central nervous system, liver, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers (Uddin et al., 2012; Fernández-Luqueño et al., 2013).

The concentration of heavy metals in medicinal plants beyond permissible limits is an issue of great concern to public safety all over the world (Calixto, 2000). The problem is rather more serious in Cameroon, because medicinal plants which form the raw materials for the finished products are neither controlled nor properly regulated by quality assurance parameters. WHO recommends that medicinal plants which form the raw materials for the finished products should be checked for the presence of heavy metals. The WHO further regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead at 1.0, 0.3 and 10 ppm, respectively (WHO, 1989 and 1998).

Due to poverty and limited access to modern medicine, many people and traditional healers of large cities in particular harvest plants for healing in nature (regardless of location) without considering their quality and sustainable management of this heritage. Besides, this is an issue reflected by the proliferation of traditional healers in search of patients in urban centers. Therefore, the raw materials that go into making their recipe are unreliable because of a polluted and corrupt environment of the cities and their outskirts. This poses a public health problem in African cities since the majority of people remain bound to traditional medicine because of its easy access and their culture, accentuated by poverty. These medicinal plants are valuable resources for health care. Even though many studies have been carried out across the world (Abou-Arab and Abou Donia, 2000; Haider et al., 2004; Shad Ali Khan et al., 2008; Ayari et al., 2010; Maharia et al., 2012; Kulhari et al., 2013; Tshibangu et al., 2014; Nawaba et al., 2015; Lion et al., 2016), data on the accumulation of heavy metals in medicinal plants harvested in the wild in Cameroon are very rare. There is increasing interest in the study of the distribution of heavy metals in medicinal plants, not because these plants are an alternative but because this accumulation constitutes a health risk for the population. Moreover, it has been reported that traditional knowledge of plants is being implemented and followed by the inhabitants of different areas of Douala (Mpondo et al., 2012; Dibong et al., 2012). Our study aims to deepen the knowledge of plants used for medicinal purposes in the Douala region of Cameroon which is densely populated, urbanized, industrialized and polluted). This city, the economic capital of Cameroon, has experienced rapid urbanization and industrialization in recent years with associated soil and air pollution enhancing pollutant accumulation in medicinal plants, thereby increasing potential human health risks.

This study was designed to determine the concentration of five heavy metals (Cr, Pb, Cd, Zn, Cu) in leaves, shoots and root samples of six common medicinal plants (*Cleome ciliata*, Schum., *Ageratum conyzoides* Linn., *Aloe vera* Linn., *Cymbopogon citratus* Stapf., *Eremomastax speciosa* (Hochst.) Cufod. and *Justicia secunda* Vahl.) used in the treatment, prevention, and management of diseases. Samples were collected from specified locations in Douala and their contamination status was evaluated with respect to established safe limits of the WHO, China and Canadian standard guidelines (WHO, 1998). This study may help understand the importance of location and ultimately heavy metal toxicity of medicinal plants. General description of medicinal plants undertaken in the present study are given in Table 1.

Table 1. Some medicinal plants commonly used in Douala.

Scientific names (Family)	Traditional medicinal use	Part used	Preparation	References
Aloe vera Linn. (Aloaceae)	Colic and cleaning of internal organs, injuries, stomach ache, blood purification, malaria, typhoid, poison, ovarian cysts, skin disease.	- Leaves - Shoots	- Maceration - Decoction - Extracting the sap - Friction	Dibong et al. (2011) Ngene et al. (2015) Jiofack et al. (2010)
Ageratum conyzoides Linn. (Asteraceae)	Wound, stomach whitlow, typhoid; evil kidney, skin diseases, analgesic, anti-inflammatory, diarrhea, nervous disorders.	- Whole plant - Leaves - Shoots - Roots	- Decoction - Infusion - Maceration	Ngene et al. (2015) Jiofack et al. (2010)
Cymbopogon Citratus Stapf. (Poaceae)	Malaria, jaundice, grippe, fever, typhoid, cough, Hypotension, dysentery, Diabetes, weight loss, digestive and refreshing drink, Sudorific, Useful in catarrhal.	- Whole plant - Leaves - Shoots	- Decoction - Infusion - Maceration	Ngene et al. (2015) Jiofack et al. (2010) Dibong et al. (2011)
Cleome ciliata Schum. (Capparidaceae)	Gastric ulcers, oral pharyngeal diseases, anti-inflammatory, Cleansing, hypoglycemia, diuretic, intestinal worms, sore throat, sore throat, cough, gingivitis, diabetes, boils and abscesses, Malaria.	- Whole plant - Leaves - Shoots	- Decoction - Infusion - Maceration	Ngene et al. (2015) Jiofack et al. (2010)
Eremomastax speciosa (Hochst.) (Acanthaceae)	Diabetes, chronic ulcer, pile, malaria, typhoid, itching, yellow fever, nettle rash, Eczema, Rheumatic pain, hypertension, eye infection, irregular and painful menstruation, herpes, zoster dysentery and diarrhea, hemorrhoids, antimicrobial, antidiarrhoeal activity, haematopoietic	- Leaves - Shoots - Whole plant	- Decoction - Infusion - Maceration	Oben et al. (2006) Okokon et al. (2007) Ndem et al. (2013) Dibong et al. (2011) Fongod et al. (2013)
Justicia secunda Vahl. (Acanthaceae)	Shortage of blood treatment of pharyngitis, gingivitis, bronchitis, infected wounds, topical ulcers, and as antiparasitic agents.		- Decoction - Infusion - Maceration	Fongod et al. (2013)

Materials and methods

Description of study sites

This study was conducted from December 2015 to February 2016 in some localities in Douala, Cameroon (Fig. 1) selected on the basis of their higher number of traditional healers, plant diversity of the region and the ancestral tradition related to traditional plant usage (Dibong et al., 2012; Priso et al., 2001). Three collection localities [residential areas (RA), roadsides (RS) and refuse dumps (RD)] were selected on the basis of survey results obtained from traditional practitioners and people concerned, presence of contaminated soil and atmospheric polluting units (presence of industries, heavy traffic and sites located near waste heaps) (Fig. 1).

The six aforementioned medicinal plant species were selected due to their significant importance in the Littoral region and their frequent utilization in novel pharmaceutical preparations, traditional drug formulations in the region, food supplements and their diverse medicinal properties. These plants which are common in most communities were selected because they are found in most herbal remedies on the Cameroonian markets and are widely used by individuals and families.

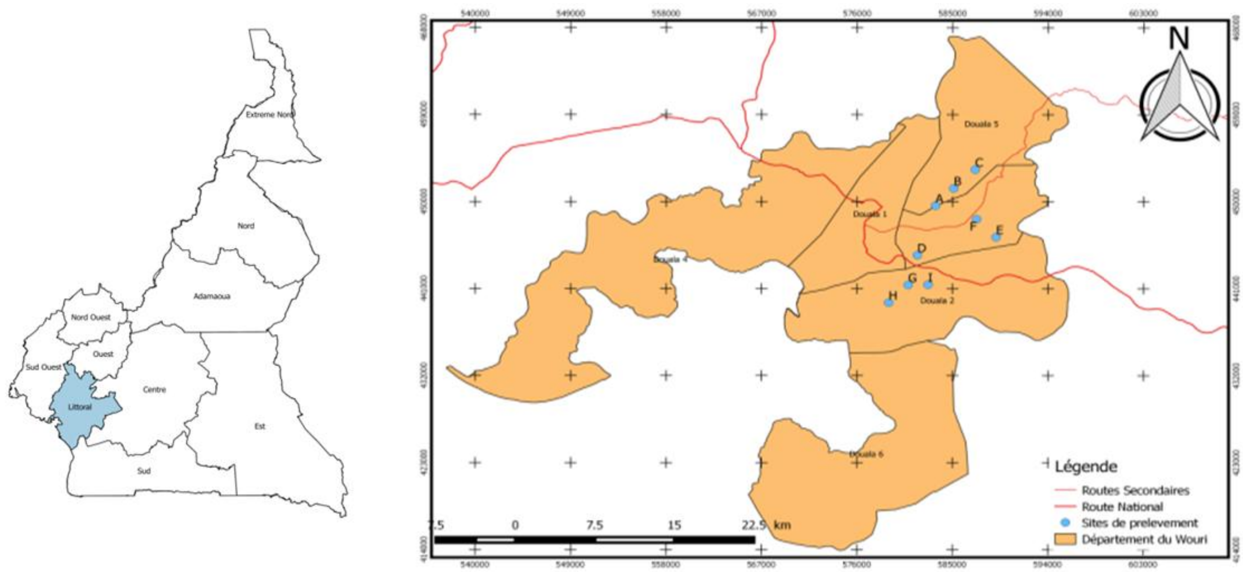

Fig. 1: Heavy metals' contents (mg/kg) in the medicinal plant samples from different geographical locations in Douala.

Douala is the largest metropolis of the coast of Cameroon with a climate that belongs to the equatorial area of a particular type called "Cameronian" characterized by two seasons. There is a long and stable rainy season with at least 9 months of heavy precipitation (about 4000 mm per year) and high temperatures (26.7°C). The minimum average temperature in Douala for 30 years (1961-1990) was 22.6°C in July and the average maximum temperature was 32.3°C in February. The relative humidity remains high and close to 100% all year round (Dibong et al., 2012; Mpondo et al., 2012). The population of the city is estimated at 2.5 million. The intensification of urban, industrial and agricultural activities, accelerated population growth have led to increased degradation of ecosystems in that locality (Priso et al., 2011).

Reagents and equipment

Analytical grade chemicals purchased from Hach Company and doubly de-ionized distilled water were used throughout the study. 65% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2), and 70% perchloric acid ($HClO_4$) were used for digestion purpose, while multi-element standard solution was used as a reference.

The Digester for Heavy Metal SH 230N was used to mineralize all samples and the spectrophotometer HACH DR 3900 was used for analyzing the aforementioned heavy metals in the laboratory of Biotechnology and environment of the University of Yaounde I. All glassware were soaked for 48 h in 10% HNO_3 and all the containers were rinsed repeatedly and successively in HNO_3/H_2O (1:1) and HCl/H_2O (1:1) before use. All apparatus were thoroughly washed with detergent solution followed by tap water and rinsed with, distilled water (Rashid et al., 2016).

Collection of samples

The selected medicinal plant samples were randomly collected in transects of 25 m^2 (5 m x 5 m), from three different sampling points (RA, RS and RD) in Douala. At each sampling site (Fig. 1), two samples of each plant were collected from six different points and mixed together. All the sample sites were far away (approximately 500-1000 m) from industrial pollution sources (such as landfill regions, gas stations and factories) and line pollution sources (about 200 meters away from houses and roads). All sites were located with GPS so as to facilitate any further investigations. *Cleome ciliata* Schum. (Fig. 4) and *Ageratum conyzoides* Linn. (Fig. 2) were harvested along RS; *Aloe vera* Linn. (Fig. 3) and *Cymbopogon citratus* Stapf. (Fig. 5) around RA; *Eremomastax speciosa* (Hochst.) Cufod. (Fig. 6) and *Justicia secunda* Vahl. (Fig. 7) in RD.

Preparations of samples

Whole plants collected from different locations were divided into different edible parts: roots, shoots, leaves. These plants parts were washed thoroughly with running tap water followed by washing with deionized autoclaved water to remove the dirt, dust particles, possible parasites. They were further rinsed again with deionized autoclaved water (Abou-Arab and Abou Donia, 2000). The plant samples were shade dried at

180°C in an auto-clave, crushed, powdered and homogenized using a laboratory blender. The powdered samples were put in plastic bags, stored in closed air tight bottles and transported to the laboratory of Biotechnology and Environment of the University of Yaounde I for further analysis.

Fig. 2: *Ageratum conyzoides* L. (Asteraceae)
Local name: Ewuda nyo na nyo

Fig.3: *Aloe vera* L. (Aloaceae)
Local name: Aloe vera

Fig. 4: *Cleome ciliata* L. (Capparidaceae)
Local name: Mbango

Fig. 5: *Cymbopogon citratus* Stapf (Poaceae)
Local name: Bekoko ba ti (citronnelle, fever grass)

Fig. 6: *Eremomastax speciosa* (Hochst.) Cufod (Acanthaceae)
Local name: Dibokuboku di mole

Fig. 7: *Justicia secunda* Vahl. (Acanthaceae)

Sample digestions using wet digestion procedure (HNO_3–$HClO_4$ digestion)

For heavy metals assessment, the digestion was carried out according to the methods reported by Hseu (2004) and Rashid et al. (2016). Pre-weighed 1 g each of different parts of each plant were placed into borosilicate digester tubes and 10 ml of concentrated HNO_3 were added in each tube. The mixture was placed on a hot plate (heavy metal digester SH 230N) for 30–45 min to allow for oxidation. After cooling, 5 ml of $HClO_4$ (70%) was added, and the mixture was reheated on a hot plate until the digest became clear and semi-dried. Then, the samples were cooled, reconstituted to 70 ml volume with deionized autoclaved water, filtered through Whatman No. 42 filter paper and stored at ambient temperature, in closed acid-washed glass vials. All experiments were done in triplicate for precision and accuracy of the results.

Atomic Absorption Spectrometry (AAS): Procedure for herbal sample analysis

The stored samples were used for the analysis of heavy metals. Total content of Cd, Cr, Cu, Pb, and Zn in the edible parts of the six selected medicinal plants was determined according to the dithizone method (AOAC, 2005) with the spectrophotometer HACH DR 3900 in the laboratory of Biotechnology and Environment of the University of Yaounde I. All experiments were done in triplicate for precision and accuracy of the results. Concentration of each metal was determined from absorbance value of each replicate and articulated in mg/kg on a dry-weight basis of the plant sample. Concentrations obtained in samples from different plant parts were compared with established safe limits of the WHO for Cd and Pb, China for Cu and Canadian standard guidelines for Cr (1998).

Statistical analysis

The data, based on triplicate analysis were subjected to a two-way analysis of variance to bring out the effects of the plant's location on their mineral content as well as the effect the plant itself had on its mineral content. Variations among the locations, and plant samples were evaluated by means of least significance difference (LSD) at 5% level of probability ($p \leq 0.05$). Data analysis was conducted using R statistical software.

Results

The results obtained (Table 2) show that all heavy metals studied in Douala were present to greater or lesser extents in different organs and collection sites of the selected medicinal plant species. Heavy metals were recorded in this range: roots>leaves>shoots. Sites were affected ($p<0.05$) by the concentration of all the metals with highest level observed in RS and RD, and the lowest in RA.

Concentrations of heavy metals

Lead (Pb)

The concentration of Pb in plants from Douala varied from 3.02±0.02 mg/kg to 40.00±0.03 mg/kg, with a mean value of 13.95±8.73 mg/kg. Generally for all plant species, Pb contents ranged as follows: *Cleome ciliata*>*Ageratum conyzoides*>*Eremomastax speciosa*> *Justicia secunda*> *Cymbopogon citratus*>*Aloe vera*. The roots of *Cleome ciliata* from RS contained the highest level (40.00±0.03 mg/kg) of Pb, and the shoots of *Aloe vera* from RA had the least level (13.95±8.73 mg/kg). There very highly ($p<0.0001$) significant difference of Pb concentrations between plant parts studied in Douala. There was a highly significant difference ($p<0.0001$) in the data of roots and leaves, shoots and leaves, and a very highly significant difference ($p<0.0001$) in the data of root and shoots. From all the plants in all locations, the mean concentrations recorded from the roots were more than those recorded from the leaves followed by shoots. Among locations, Pb mean content was 7.42±2.77 mg/kg from RA, 21.72±10.23 mg/kg from RS and 12.72±3.79 mg/kg from RD. Generally, very highly significant difference ($p<0.0001$) of Pb concentrations were observed between all the three locations in Douala city. They were higher ($p<0.05$) Pb concentration from RS than RD and RA. However, the decreasing order of Pb samples in all sites was RS>RD>RA. Plants harvested in RD had very higher ($p<0.001$) Pb values than RA and those of RD were lower ($p<0.05$) as compared with those from RS (Fig. 8).

Plant samples collected from RA and RD contained Pb and Cr concentrations that exceeded the maximum permissible limits defined by the WHO (1998). However *Cymbopogon citratus* and *Aloe vera* samples harvested from RA were below the permissible limits. The prescribed limit for Pb contents in herbal medicine is 10 mg/kg dry weight while the dietary intake limit for Pb is 3 mg/week. Naithani and Kakkar (2006) in India found that the mean of Pb in the 5 medicinal plants studied was 6,39 mg/kg which is below the permissible limit of Pb. Maximum levels of 14.4 mg/kg and 21.7 mg/kg of lead were also reported in Egyptian and Iranian spices and medicinal plants, respectively (Li et al., 2012; Ziarati, 2012).

Lead is a non-essential heavy metal and is known to be one of the highly toxic environmental pollutants. The main source of Pb in plant parts is the soil on which they grow, which can be contaminated from several sources. The industrial (smelting process), gas emission from heavy traffic on roads (Atayese et al., 2009), agricultural (application of insecticides) and urban activities (combustion gasoline) can contribute to lead levels in soil (Falahi et al., 2013; Nookabkaew et al., 2006). It can complex with various biomolecules and adversely affect their functions. Lead exposure at low doses in the body causes colic, brain and kidneys damage, headache, anemia, miscarriage, lower sperm count, convulsions, chronic nephritis of the kidneys, central nervous system disorder, immune, renal, muscular and gastro-intestinal symptoms. Exposures to lead at prenatal and early childhood are associated with slowed cognitive development, learning deficits, and many other effects (Rehman et al., 2013).

Table 2. Mean and standard deviation concentrations of trace metals in plant parts (leaves, shoots and roots) collected from Douala.

	Plants	Plant parts	Heavy metals (mg/kg)				
			Pb	Cd	Cr	Zn	Cu
RA	*Aloe vera* Linn.	Leaves	6.79±0.06^{a}	6.03±0.05^{a}	0.12±0.02^{a}	4.28±0.03^{a}	20.06±0.08^{a}
		Roots	8.64±0.04^{a}	7.10±0.07^{a}	1.83±0.03^{a}	8.16±0.01^{a}	24.43±0.05^{a}
		Shoots	3.20±0.04^{a}	6.36±0.05^{a}	0.05±0.01^{a}	2.29±0.02^{a}	14.39±0.05^{a}
	Cymbopogon citratus Stapf.	Leaves	8.65±0.10^{b}	6.14±0.03^{b}	0.09±0.03^{b}	4.38±0.02^{b}	19.58±0.04^{b}
		Roots	11.73±0.05^{b}	7.37±0.03^{b}	2.35±0.05^{b}	9.46±0.03^{b}	28.43±0.03^{b}
		Shoots	5.50±0.04^{b}	6.44±0.06^{b}	0.03±0.00^{b}	1.60±0.55^{b}	13.09±0.05^{b}
	Ageratum conyzoides Linn.	Leaves	14.90±0.10^{c}	6.63±0.02^{ab}	11.70±0.04^{c}	8.52±0.03^{c}	44.13±0.04^{c}
		Roots	27.50±0.08^{c}	7.91±0.06^{c}	16.90±0.035^{c}	14.97±0.04^{c}	51.86±0.01^{c}
		Shoots	10.18±0.09^{c}	5.52±0.05^{c}	6.08±0.04^{c}	9.28±0.04^{c}	30.20±0.04^{c}
RS	*Cleome ciliata* Schum.	Leaves	22.70±0.06^{d}	6.27±0.03^{ab}	8.16±0.08^{bd}	9.10±0.05^{d}	35.19±0.04^{d}
		Roots	40.00±0.06^{d}	8.70±0.03^{b}	13.28±0.05^{b}	12.22±0.03^{b}	49.96±0.03^{d}
		Shoots	15.07±0.06^{d}	3.16±0.07^{d}	5.59±0.02^{d}	4.59±0.03^{d}	27.09±0.02^{d}
	Eremomastax speciosa	Leaves	11.28±0.04^{e}	7.27±0.04^{c}	4.25±0.01^{e}	4.59±0.02^{e}	44.31±0.02^{a}
		Roots	19.83±0.0^{a}	9.22±0.03^{d}	7.40±0.02^{d}	7.36±0.06^{b}	60.30±0.04^{e}
		Shoots	10.40±0.06^{e}	5.73±0.03^{e}	2.52±0.03^{e}	2.34±0.52^{e}	37.25±0.04^{e}
RD	*Justicia secunda* Vahl.	Leaves	10.45±0.03^{f}	8.61±0.59^{d}	2.46±0.02^{f}	5.26±0.03^{f}	66.46±0.04^{e}
		Roots	15.13±0.08^{e}	10.32±0.03^{e}	5.07±0.03^{e}	9.93±0.03^{bcd}	71.19±0.03^{f}
		Shoots	9.22±0.09^{f}	7.10±0.04^{a}	1.26±0.04^{f}	3.36±0.03^{f}	41.33±0.04^{f}
Permissible limits			10	0.3	2	27.4	20

The heavy metal concentrations are means ±SD. Means with the same alphabetic letter and corresponding to the same organ of different species are not significantly different between each other ($p<0.05$) according to Student's t test. WHO permissible limits for Pb: 10 mg/kg; Cd: 0.3 mg/kg (WHO, 1998); CANADA permissible limits for Cr: 2 mg/kg (WHO, 2005); CHINA permissible limits for Cu: 20 mg/kg (WHO, 2005).

Cadmium

The cadmium (Cd) concentrations in Douala (Table 2) were in the range from 3.16±0.07 mg/kg to 10.32±0.03 mg/kg for all the samples, with a mean value of 6.99±1.58 mg/kg. Of all the selected plant species, the highest concentrations of Cd were noticed in *Justicia secunda* followed by *Eremomastax speciosa*, *Ageratum conyzoides*, *Cymbopogon citratus*, *Aloe vera* and *Cleome ciliata* in decreasing order of concentrations. Among the plant parts, the highest concentration was recorded from the roots of *Justicia secunda* (10.32±0.03 mg/kg) harvested from RD and the lowest mean value was recorded from the shoots of *Cleome ciliata* (3.16±0.07 mg/kg) harvested from RD. Cd concentrations showed very highly ($p<0.0001$) significant difference between plant parts studied in Douala. Thus there was a highly significant difference ($p<0.0001$) between roots and leaves, shoots and leaves, and a light significant difference ($p<0.05$) in the data of roots and shoots. Overall in all the studied plants, Cd concentration was recorded in this range: roots>leaves>shoots. Amongst locations, Cd mean content was 6.57±0.51 mg/kg from RA, 6.36±1.83 mg/kg from RS and 8.04±1.57 mg/kg from RD. The trend of Cr concentration in plants from RA and RS were similar but lower ($p<0.05$) than in those from RD. Cd repartition based on the collection site was as follows: RD>RA ≥ RS. Plants harvested at RD had very higher ($p<0.05$) Cd values than those collected from RA and RS (Fig. 8). The experimental results showed that 100 % of the samples analyzed had Cadmium concentrations that exceeded the permissible limit defined by the WHO (2005); prescribed limit for Cd contents in medicinal plant is 0.3 mg/kg and the maximum acceptable concentration for food stuff is around 1 ppm. Similar results of high levels of cadmium in Egyptian, Nigerian, Eritrean and Sud African medicinal herbs and plants have been reported in earlier studies (Abou Arab and Abou Donia, 2000; Yusufa et al., 2003; Sium et al., 2016; Lion et al., 2016).

Cadmium is a non-essential trace element having functions neither in human body nor in plants. It is extremely toxic even at low concentration. The high levels of cadmium exercise a serious toxicological effect on human health. It causes learning disabilities and hyperactivity in children. Cadmium accumulates in human body and damages mainly the kidneys and liver. Cd intoxication can therefore lead to kidney, bone and pulmonary damages (Godt et al., 2006). At high concentrations, cadmium produces serious effects on the liver and vascular and immune systems (Neil, 1993).

Chromium

The mean concentration of Cr (Table 2) found in different samples of Douala was 4.95±4.84 mg/kg and Cr content varied from 0.03±0.00 mg/kg to 16.90±0.04 mg/kg in all plant species studied. The highest concentration of Cr contents was found in *Ageratum conyzoides* followed by *Cleome ciliata*, *Eremomastax speciosa*, *Justicia secunda*, *Cymbopogon citratus* and *Aloe vera*. High concentration of Cr occurred in the roots of *Ageratum conyzoides* (16.90±0.04 mg/kg) harvested from RS while low concentration was found in the shoots of *Cymbopogon citratus* (0.03±0.00 mg/kg) from RA. Cr concentrations showed highly ($p<0.05$) significant difference between plant parts, probably due to the absorption of Cr contents from the polluted air. There was no significant difference ($p>0.05$) between roots and leaves and between shoots and leaves. However, there was a highly significant difference ($p<0.05$) between the data from roots and shoots. From all the plants in all locations, the mean concentrations recorded from the roots were more than those recorded from the shoots followed by leaves.

The experimental data revealed that Cr mean content was 0.75±0.99 mg/kg in samples from RA, 10.28±4.18 mg/kg for RS and 3.83±2.09 mg/kg for RD. The Cr repartition based on collection sites was as follows: RS>RD>RA. There was a very highly ($p<0.0001$) significant difference in Cr contents between different habitats studied in Douala and the results showed very higher significant levels ($p<0.001$) of Cr concentrations in plant samples obtained from RS compared with those obtained from RD and RA. All plants collected from RD had higher ($p<0.05$) Cd values than those collected in RA (Fig. 8).

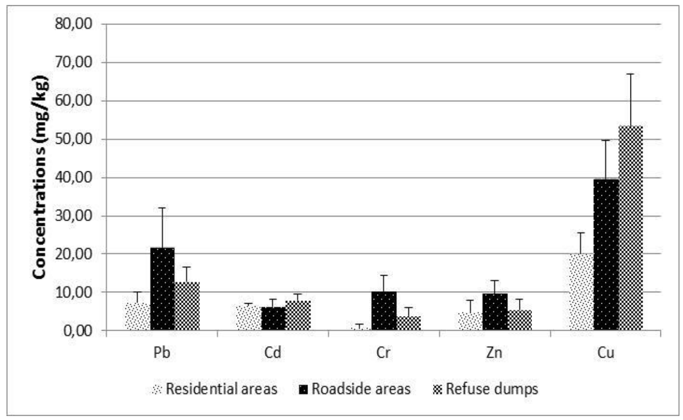

Fig. 8: Heavy metals' contents (mg/kg) in the medicinal plant samples from different geographical locations in Douala.

There are no established WHO concentration limits for Cr in medicinal plants. However, permissible limits for Cr in medicinal plants set by Canada are 2 mg/kg in raw medicinal plant materials (WHO, 2005) and the daily intake of Cr 50-200 µg has been recommended for adults by US National Academy of Sciences (Waston, 1993). Plants collected from RS and RD were higher than the permissible limit of 2 mg/kg as set by Canadian standards while those harvested from RA did not exceed that permissible limit. The toxic effects of Cr intake are skin rashes, nose irritation, bleeding, stomach ache, kidney and liver damage and cancer. Chromium deficiency is characterized by disturbance in glucose lipids and protein metabolism (McGrath & Smith, 1990; Khan et al., 2010). Sources of Cr in the deposited dust may be due to natural weathering and wind deflation or anthropogenic emission from the industries and vehicles.

Zinc

The range and mean concentrations of Zn found in samples during this study were 1.60±0.55 mg/kg – 14.97±0.04 mg/kg and 6.76±3.63 mg/kg DW respectively (Table 2). In all plant species, the Zn contents ranged as follows: *Ageratum conyzoides*> *Cleome ciliata*>*Justicia secunda*> *Cymbopogon citratus* >*Aloe vera*>*Eremomastax speciosa*. The roots of *Ageratum conyzoides* from RS contained the maximum amount (14.97±0.04 mg/kg) of Zn while the shoots of *Cymbopogon citratus* (1.60±0.55 mg/kg) from RA had the minimum amount of Zn (14.97±0.04 mg/kg). It may be due to the absorption of the metals from the polluted soils. Concentration of Zn varied in different plant organs and the differences obtained in leaves, shoots and roots of all the plants were highly significant (p<0.0001). So there were higher Zn concentrations (p<0.0001) in roots than leaves and shoots respectively. The transportation of Zn was found in the order roots>shoots>leaves which are in line with results from Das et al. (2001) and Bhattacharya et al. (2013). The mean content of Zn was 5.03±2.97 mg/kg in samples from RA, followed by 9.78±3.31 mg/kg for RS samples and then 5.47±2.61 mg/kg for RD. The repartition of Zn based on the collection sites was as follows: RS>RD>RA. There was a very highly significant difference of Zn between the different sites studied in Douala (p<0.0001). The trend of Zn concentration in samples from RD and RA were practically similar but lower (p<0.0001) than in those from RS (Fig. 8). RS plants had the highest (p<0.001) values of Zn possibly due to the high vehicle traffic.

The WHO limits are not yet established for Zn in medicinal plants. However, the permissible limit set by FAO/WHO (1989) in edible plants is 27.4 mg/kg. In this study, all harvested plants widely exceeded the permissible limit of Zn except for *Ageratum conyzoides* which was below that value. Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also lose the sense of touch and smell (Hunt, 1994).

Copper

As can be seen from the data (table 2), the mean concentration of Cu obtained in this study was 37.74±17.05 mg/kg DW and Cu varied between 13.09±0.05 mg/kg and 71.19±0.03 mg/kg. The average Cu concentration (mg/kg) trend for medicinal plants studied was *Justicia secunda*>*Eremomastax speciosa*> *Ageratum conyzoides*>*Cleome ciliata*> *Cymbopogon citratus*>*Aloe vera*. There was very highly significant difference (p<0.0001) of Cu between the plant species studied in Douala. The highest mean value was recorded from the roots of *Justicia secunda* (71.19±0.03 mg/kg) obtained from soil collected from RD. The lowest mean value was recorded from the shoots of *Cymbopogon citratus* (13.09±0.05 mg/kg) harvested from soil collected from RA. However, Cu concentrations showed highly significant difference (p<0.0001) between plant parts studied in Douala. There was a very highly significant difference (p<0.0001) between roots and shoots and a highly significant difference (p<0.05) in the roots and leaves, shoots and leaves. From all the plants in all locations, the mean concentrations recorded from the roots were more than those recorded from the shoots, followed by leaves.

Among locations, Cu mean content was 20.00±5.48 mg/kg from RA, 39.74±9.78 mg/kg from RS and 53.47±13.44 mg/kg from RD. The Cu repartition based on the collection site was as follows: RD>RS>RA. There was a very highly significant difference (p<0.0001) in Cu contents between different study sites in Douala. Plants collected from RD had higher (p<0.001) Cu values than those collected from RS and RA respectively. However, there was significant variation in the values of Cu obtained from RS and RA (p<0.05) (Fig. 8). The high concentrations of Cu in the roots of plant led to two hypotheses: (i) it may be due to higher absorption ability of the plants to get the trace heavy metals from the polluted soils, or (ii) it may be due to the presence of higher amounts of copper in the respective soil samples. In this light, *Justicia secunda* plants have the highest ability to absorb the metals from the soil.

Although Cu is an essential enzymatic element for normal plant growth and development, it can be toxic at excessive levels. High intakes of copper are detrimental to human health and may cause fever with flue like symptoms, hair and skin decoloration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea, and some other fatal diseases in human beings (Khan et al., 2008). Copper deficiency results in anemia and congenital inability to excrete copper resulting in Wilson's disease (Gupta. 1975). Therefore, Cu content in medicinal plants must be controlled on a daily basis (Karak and Bhagat, 2010). However for medicinal plants, the WHO limits are not yet established for copper. Although in medicinal plants, permissible limits for copper set by China and Singapore, are 20 ppm and 150 ppm respectively (WHO, 2005). In this study, all harvested plants widely exceeded this permissible limit of China (20.00 mg/kg), except for *Ageratum conyzoides* which was below that value. Maximum levels of Cu were also reported in Eritrea by Sium et al. (2016) and South African medicinal plants, respectively (Lion et al., 2016). High concentrations of Cu and the other trace heavy metals present in the underground parts of the plants may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils. Cu contamination could mainly originate from vehicles and fungicides (Han et al., 2006).

Discussion

Overall comparison of the metal contents in medicinal plants in some other parts of the world revealed that the levels are generally comparable in Cameroon and elsewhere. This trend could be ascribed to the high values of studied heavy metal (Pb, Cd, Cr, Zn and Cu) concentrations present in the studied plants. Six plant species were assessed for metal accumulation in their different parts in Douala where plant samples were collected from RA (*Cymbopogon citratus* and *Aloe vera*), RS (*Cleome ciliata* and *Ageratum conyzoides*) and RD (*Eremomastax speciosa* and *Justicia secunda*).

The results showed a significant difference in the contents of metals in all plants from different sites ($p<0.0001$). The study reveals that heavy metal pollution of the selected plants is significant in the Douala region. It also points to RD as the most important source of contamination. Samples collected from RS zones showed significantly higher concentration of heavy metals followed by RD and then RA. A study of Deepalakshmi et al. (2014), who evaluated the potential for accumulation of heavy metals in roadside plants in the city of Bangalore (India) found high values of Pb (84.98 ± 0.11 mg/g), Cu (55.64 ± 0.17 mg/g), Zn (80.34 ± 0.26 mg/g), Cd (9.56 ± 0.25 mg/g) in *Ageratum conyzoides*. Messou et al. (2013) obtained similar results in their research: Pb (48.98 ± 0.11 mg/g), Cr (6.56 ± 0.35 mg/g), Cu (59.94 ± 0.34 mg/g), Zn (67.34 ± 0.16 mg/g). Effiong (2013) in the southwestern region of Nigeria suggested that average levels of Cu and Pb at the refuse dump sites were significantly higher than other locations (industrial waste dump sites, industrial areas, residential areas, around an effluent channel). In this study, metal levels at the residential areas were about the levels in the control. Generally, the degree of contamination within the vicinity of the site was of the order Cu>Pb>Cd >Cr. It can be stated that the site of growth noticeably influences heavy metal uptake by the six plant varieties assessed in this study.

Several factors control the selective accumulation of metals in plant and their bioavailability: soil and climatic conditions, plant genotype and agronomic techniques (Chunilall et al., 2005). The traffic flow also had a significant effect on the content of heavy metals in plants growing along the road. Further away from the road, and under lower traffic flow intensity, the amounts of contaminants originating from vehicle traffic decreased. Emissions of trace elements depend on the differences in the traffic flow intensity, number, type, and speed of vehicles and on the atmospheric conditions as well as the distribution of buildings (Modrzewska et al., 2014). Annan et al. (2013) reported that the 5 minerals namely: Pb, As, Hg, Cd and Al were present in 10 medicinal plants sampled from 5 different geographical locations in Ghana and a significant variation existed in mineral content for the various locations ($p\leq0.05$).

In the present study, most of the plant parts harvested from the refuse dump sites had significant and high values of all heavy metals studied. Ebong (2007) found that concentrations of the metals in the dump site soil and plant parts in Nigeria were higher and significant differences of heavy metals' (Cu. Zn. Pb. Cd) accumulation were observed between plant parts. This could be attributed to the high metal contents in the dump site soils which were eventually accumulated by plants grown on them. This also indicates that the concentrations of metals in plants are dependent upon their concentrations in the soil on which they grow.

Permissible limits of heavy metals

All the plants studied exceeded the different permissible limits of Pb, Cd, Cr, Zn and Cu except for *Ageratum conyzoides* which was below the Cu value. But since the metal accumulation and translocation potential varied between different metals and vegetables and did not follow any particular pattern, their impact on health needs should be evaluated based on the elements that surpassed the maximum limit (Manzoor et al., 2006; Tiwari et al., 2011; Nabulo et al., 2011; Weldegebriel et al., 2011). A similar investigation was conducted for plants obtained from Bahrain, and Nigeria. In Bahrain, Al-Saleh and Chudasama (1994) reported that a large portion of the plants examined contained high concentrations of toxic metals and some of them exceeded the limits of toxicity; the reported data indicate a potential health hazard. Nwoko et al., (2011) showed that the Zn, Cd and Pb contents of selected ready-to-use herbal remedies in South-east Nigeria were beyond WHO permissible limits. The health risk due to metal contamination, in general, depends on the average daily dietary intake (Dghaim et al., 2015).

Plant parts

The results obtained in this study show that concentrations of Cu and Zn were highest in all the plants studied and that the plants accumulated more of the trace metals in their roots than in shoots. The ability of plants to bio-accumulate more trace metals in their roots than the shoot has been reported in literature. The study of Lion et al. (2016) observed that from all the plants studied, the concentrations of trace metals in the roots were more than those recorded in the shoot with a significant difference (p<0.05). Hazrat et al. (2012) showed that the concentrations of trace metals were greater in roots than in shoots. A similar observation was also made by Peralta et al. (2001) where alfalfa plants accumulated more of the trace metals in the roots than any other part.

Different vegetable species accumulated different metals depending on environmental conditions, metal species and plant available forms of heavy metals. Plants have a natural propensity to take up and maintain metals at relatively low concentrations by avoiding excessive metal uptake and transport (McGrath et al., 2001). Some metals like Cu, Co, Fe, Mo, Mn, Ni and Zn, are essential mineral nutrients, while Cd and Pb, have no known physiological activity (McGrath et al., 2001).

Conclusion

Heavy metal contamination in the environment is of more concern worldwide. The results of the present assessment showed that the selected medicinal plants are good absorbers of metals; and concentration of minerals depends on the different geographical conditions as well as their components. This study proved that plants overgrowing in GS and RS soils accumulate much higher amounts of Cd, Pb, Cr, Cu, and Zn than the plants from RA. The highest contents of heavy metals were found in the roots, but their levels in the leaves and shoots were elevated as well, implying a risk of transport of toxicants along the food chain. It may be due to geological strata or pollution of the studied area. The assay of heavy metals varied from site to site, plant to plant, and also in different parts of each plant, depending upon the chemical composition of soil and absorption rate by plants. Thus, it is essentially required that every medicinal plant should be checked for contaminant load before processing for further pharmaceutical purposes or for local human consumption. This study also concluded that those plants can also be used for the removal of heavy metal from the soil and waste water because they have the capacity to absorb the metals from the soil.

Generally, the concentration of heavy metals in the different parts of *Eremomastax speciosa* followed the order Cu>Pb>Cd>Zn>Cr in shoots and roots compared to leaves. *Justicia secunda* accumulated Cu>Pb>Cd>Zn>Cr in roots, shoots and leaves. In *Cymbopogon citratus*, Cu > Pb > Zn > Cd > Cr accumulation was observed in roots, Cu>Pb>Cd>Zn>Cr in leaves and Cu>Pb>Cd>Zn>Cr in plant shoot. In *Aloe vera*, the concentration decreasing pattern of metals was Cu > Pb > Zn > Cd > Cr in roots, Cu > Pb > Cd > Zn > Cr in leaves and Cu > Cd > Pb > Zn > Cr in shoots. *Cleome ciliata* followed the order Cu > Pb > Cr > Zn > Cd in roots and shoots and Cu > Pb > Zn > Cr > Cd in leaves. *Ageratum conyzoides* followed the order Cu > Pb > Cr > Zn > Cd in roots and leaves and Cu > Pb > Zn > Cr > Cd in shoots.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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