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Full Length Research Paper

Heavy metal contamination in *Thelesperma megapotamicum*

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This study describes heavy metal (HM) contamination in the most commonly used herbal tea in several American Indian (AI) communities in northwestern New Mexico. The Navajo (Diné) reservation is located in an area that was heavily impacted by contamination associated with Uranium (U) mining that occurred from 1945 to 1988 and where more than 1,100 unreclaimed abandoned U mines and structures remain. The study objective was to establish the levels of HM contamination in this herb which is habitually and widely consumed in this reservation community. The study aims were to: (1) describe the dietary behavior in Diné residents related to ingestion of harvested tea *Thelesperma megapotamicum*; (2) compare U and other HMs in tea in high and low vehicle traffic areas; and (3) disseminate study findings to the leadership and Diné community. A descriptive comparative design was used to compare HMs in locally harvested herbs on the reservation. The plant specimens were paired with soil samples and analyzed utilizing ICP-MS. Samples were collected from areas spanning a 3.2 km radius from the central part of abandoned uranium mines and structures. Root samples of tea had higher concentrations of HMs than above ground plant parts for As, Cd, Cs, Mo, Pb, U and V ($p < 0.05$). Cadmium and Mo levels were greater in high traffic versus low traffic areas ($p < 0.001$). The Cd level (0.35 mg/kg) in this popular species of tea herb exceeded the World Health Organization medicinal plant maximum permissible level. Further research and monitoring is needed to identify factors that affect HM contamination in *T. megapotamicum* and other plant herbs used on the Navajo reservation as well as other U mining impacted areas.

Key words: American Indian, heavy metals, Diné/Navajo, *Thelesperma megapotamicum*, herbal tea, uranium, cadmium, molybdenum, mining.

INTRODUCTION

Tea is the second most common beverage consumed worldwide (Naithani and Kakkar, 2005). The use of herbs and herbal drinks is common among various communities, particularly American Indian (AI) populations. The use of medicinal plants and herbal teas are assumed to be safe, accessible, low cost and free of side effects. The vast majority of herbal teas and medicinal plants are not monitored for quality or safety. Multiple studies exist that have examined the bioaccumulation of heavy metals in herbal and medicinal plants (Arpadjan et al., 2008; Barthwal et al., 2008; Gomez et al., 2007; Mohammed and Sulaiman, 2009), although comparable data in North American Indian communities is absent. Reviews by Ernst (2002a, 2002b) report poisonings from Lead (Pb), Arsenic (As), Mercury (Hg), and Cadmium (Cd) from traditional Asian and Indian herbal products. The objective of this study was to examine the extent and impact of U and other heavy metal (HM) contamination of tea herbs that are locally harvested and used by the Diné, an AI community in northwestern New Mexico. Food-chain contamination from various elements can occur in or near industrially mined areas. A number of elements are associated with negative health effects if exposures are excessive. For instance, Uranium (U) is known to be a nephrotoxin (Gilman et al., 1998; Haley et al., 1982; Tracy et al., 1992). Inorganic As is known to arise from contaminated drinking water and is a teratogen (Eisler, 1988). Cadmium can accumulate in organs (Kirkam, 2006) and impair renal function (McLaughlin et al., 1999). Selenium (Se) toxicosis is rare but can cause hepatomegaly, neurological and gastrointestinal disruptions (McLaughlin et al., 1999). Lead is associated with adverse effects on nervous, renal, reproductive and developmental systems (Caldas and Machado, 2004). Animal studies have reported testis alteration and steroidogenesis upon chronic low dose exposures to Cesium (Cs) (Gridnard et al., 2008) and increased cancer risk related to high dose exposure (ATSDR, 2004). Thorium (Th) can be stored in the bone for a prolonged period of time and there is increased risk of developing lung disease and cancers of the pancreas and lung in chronic exposure (ATSDR, 1990). Molybdenum (Mo) is a suspected male reproductive toxicant in animals and humans (Meeker et al., 2010, 2008; Pandey and Singh, 2002; Vyskocil and Vau, 1999). Mammals exposed to high concentrations of vanadium (V) demonstrate adverse respiratory affects (ATSDR, 2012). The presence of heavy metal contamination in environmental air, soil, water, human and animal tissues is well documented; however, few studies have investigated the presence of heavy metals in herbal teas especially in North American Indian teas.

Thelesperma megapotamicum (Spreng.) Kuntze is an herbal plant commonly known as greenthread, Indian, Navajo or Hopi tea, or *cota*; it is of the sunflower family (Compositae). Greenthread is a perennial plant forb that typically flowers from July to October and has golden flowerheads. It is tall and slender and grows to about 61 cm tall (Figures 1 and 2). *T. megapotamicum* is a plant native to the Southwest Plains, and various parts of South America (Palacios et al., 2007) and is found widespread throughout these regions (Borneo et al., 2009). Dalgleish et al. (2010) found that the life span of *T. megapotamicum* was longer than one year but less than two years.

In one of the earliest publications on herbal plants Matthews (1886) explained that the Diné had several means of naming plants including nomenclatures for the medicinal properties or functions, the type of animals or insects attracted or resemblances. In the Southwest, there are several species of *Thelesperma*. For instance, *Thelesperma gracile* was identified by the Diné as "tooth medicine (*wo'tsin-i-a-zay*)" and was known to be a nervous stimulant and was a well-known beverage favored by the local American Indians and Mexicans (Matthews, 1886). In general, various parts of *T. megapotamicum* were used for multiple purposes, specifically the leaves and flowers were used to brew tea (Shemluck, 1982). In addition, the tea was used as a diuretic and for urinary or digestion problems (Dunmire and Tierney, 1997).

In South American traditional medicine, it is used as an anesthetic, for renal treatment (Figueroa et al., 2012), it is a digestive remedy and an antispasmodic (Borneo et al., 2009; Palacios et al., 2007). Contemporary studies have focused on *T. megapotamicum's* antioxidant capacity showing high antioxidant levels compared to its counterparts (Borneo et al., 2009), the plant flavonoid and phenylpropanoid compounds may demonstrate anticancer effects (Figueroa et al., 2012) and its antitumoral activities are being examined (Bongiovanni et al., 2006). Other species were used as a tea substitute (*Thelesperma longipes*), orange wood dye (*Thelesperma subnudum*), and beverage tea (*Thelesperma trifidum*). Darby et al. (1956) identified several "wild foods" including "*C'îl dehî*" or "wild mountain tea" as a plant used in the diet of the Diné community. In contemporary times, wild herbal tea was among several of the foodstuffs that supplied 41% of the food energy in the Diné community (Ballew et al., 1997). Aside from being a Diné food or medicine source, tea roots were used to dye wood an orange-yellow hue and to dye wool, baskets, artifacts and utensils a reddish-gold hue (Dunmire and Tierney, 1997).

MATERIALS AND METHODS

This study was conducted in 2012 and examined food grown and harvested in a semi-arid to arid region of northwestern New Mexico on Diné reservation lands (Figure 3). Two chapters or communities agreed to participate in the study. The largest Chapter is 531 km^2 of land mass and the smallest Chapter is 233 km^2 in size. In total, the study area encompassed 764 km^2. The average precipitation was less than 25 cm per year according to the monthly climate

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Figure 1. Photograph of *Thelesperma megapotamicum*.

Figure 2. Photograph of *T. megapotamicum*.

meteorological data in New Mexico listed with the Western Regional Climate Center Western U.S. Climatic Historic Summaries (January 1, 2011 - September 2012).The study was reviewed and approved by the University of California, Los Angeles (UCLA) Institutional Review Board and the Navajo Nation Human Research Review Board.

Community residents who harvested food on the reservation were recruited if they met the following criteria: (a) 18 years of age and older, (b) not pregnant, (c) greater than 10 years of community residency, (d) consumed food grown and harvested locally, and (e) were willing to participate in the study. Participants in the herb study completed questionnaires on their harvesting practices, dietary intake and agreed to allow researchers to collect herb and soil specimens on their land. All study samples collected were from two harvesting seasons from August 01, 2012 to October 02, 2012. Tea herb or *T. megapotamicum* samples were collected at a mean elevation of 2,108.6 m (Standard Deviation [SD] = 43.1).

Herb and coupled soil samples were collected and placed immediately on dry ice and shipped to the University of New Mexico (UNM) Analytical Chemistry Laboratory Earth and Planetary Sciences Department where they were prepared for digestion and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis.

Herb samples

All herb samples were collected from wild, non-cultivated sources. The entire live plant (infused portion and roots) was obtained and stored in polyethylene (PE) plastic bags. The above ground portion of each plant was separated from the root. The plant roots were gently rinsed with deionized ASTM II heavy metal grade water. The samples were weighed, photographed, bagged, and placed on dry ice for shipment to UNM. Samples collected from areas ≤0.8 km from major vehicle roadways are defined as High Traffic Areas (HTA) and those collected from areas ≥2 km from major vehicle roadways are Low Traffic Areas (LTA).

Soil samples

Soil samples were collected using a stainless steel hand auger with a Teflon® coated-core sampler to avoid cross contamination. A PE liner was used as a core liner. Soil samples were obtained by utilizing a topographic soil zone sampling pattern using a random pattern. Soil samples were obtained from the topsoil (0 to 15 cm) and weighed at 100 g. Composited topsoils were collected from within a 1 m radius of the tea plant. Two non-root topsoil samples were composited and analyzed in duplicate. Single core root soil samples were obtained for each tea plant.

Plant identification and nomenclature

The live plants were placed between newspapers and cardboard then placed in a plant press for several weeks with daily press tightening. For excessively thick or moist plants, the plants were removed from the press for one to two hours and repressed. The dried samples were sent to the UNM Herbarium for identification and archiving. All samples were marked utilizing Global Positioning System (GPS) and Geographic Information Systems (GIS) analysis and mapping were completed.

Herb sample analysis

All samples were stored in a -20°C freezer until sample preparation and analysis. Samples were prepared by weighing about 2 g dry mass (based on availability and amount of submitted sample) into the digestion tube. 5 ml nitric acid (HNO_3) and 2 ml hydrogen

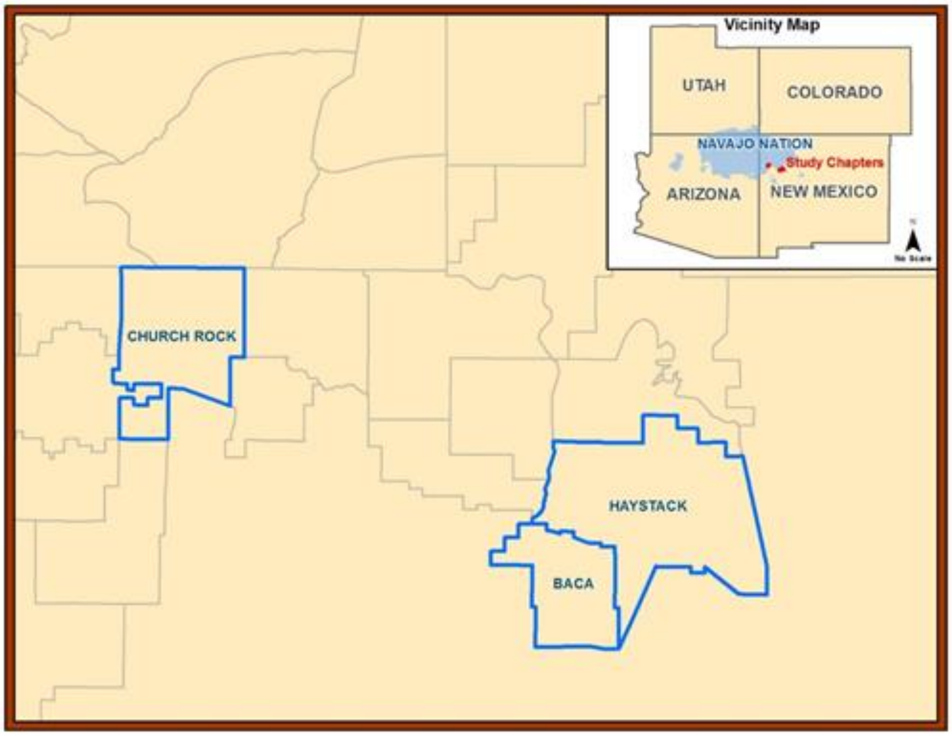

Figure 3. Study area map (depiction of the Navajo Nation in the Four Corners region of the U.S. Southwest). New Mexico Chapters or communities: Baca/Haystack and Churchrock Chapters provided tea samples.

peroxide (H_2O_2) were added and samples were heated gradually up to 95°C and digested for 2 h. Digested samples were transferred into 50 ml volume metric flasks and brought to volume using 18 mega ohm water. A reagent blank (3 ml HNO_3) was run with each batch of samples.

Samples were then prepared for analysis using PerkinElmer NexION 300D ICP/MS by diluting 100 times (100X D.F.) in glass culture tubes. Mixed standards (V, Cs, Pb, Th, U, Se, Mo, As, and Cd) were prepared using single element standards. Calibration standards range was 5, 10, 25, and 50 µg/L (ppb). Quality control (QC) samples included: Initial Calibration Blank Verification (ICBV), Initial Calibration Verification (ICV), Continuing Calibration Verification (CCV), and Matrix Spike (MS), Matrix Spike Duplicate (MSD), and Matrix Spike Replicate (MSR).

A mixed internal standard Scandium, Yttrium, Indium, and Bismuth (Sc, Y, In, and Bi) was used to match analyte mass range. Two percent HNO_3 was used as a carrier and rinse solution. Elements were analyzed in three modes to minimize interferences, standard, dynamic reaction cell gas A (Anhydrous Ammonia), and dynamic reaction cell gas B (Oxygen) in groups. After analysis was completed, data were revised, validated, tabulated and concentrations were converted into mg/kg material using instrument corrected concentration reading, sample digest final volume, and sample weight.

Three replicates of each sample were measured. The accuracy of the method was assessed via the analysis of the certified reference materials NIST SRM 1573a (Tomato Leaves) (NIST, Gaithersburg, Maryland) and NIST SRM 2709a San Joaquin Soil (NIST, Gaithersburg, Maryland) yielding the following values Cd: 1.474±0.107 (Certified tomato leaves value: 1.52±0.04), V: 0.938±0.067 (Certified tomato leaves value: 0.835±0.010), Cd: 0.644±0.089 (Certified soil value: 0.371±0.02), and V: 83.204±7.699 (Certified soil value: 110±11). The precision of the results was satisfactory with relative standard deviations varying from 7.1 to 13.8%. Some elements determined in these references are not certified.

Statistical analysis

SPSS V. 21 (Ireland, IBM Corp.) was utilized for statistical analysis. Concentrations of HMs found in plant and soil samples are reported in mg/kg. Descriptive statistics (percentages, range, mean, SD, median) were used to summarize the data. Student's *t*-tests were used to compare HMs in root versus above ground plant, root soil and topsoil, high and low vehicle traffic areas, and plant height differences.

RESULTS

Tea harvester questionnaires

The tea harvesters were all female, age 43 to 78 years (Mean [M] = 61 years) having resided in or near the harvesting area for 43 to 61 years (M = 54 years). All boiled the tea in water as a dry bundle. One harvester reported that they did not wash or rinse their herbs before boiling the herbal teas for ingestion. One harvester reported using juniper ash as a dry cleanse to remove impurities from the freshly harvested tea plants. Herb selling and sharing was a common practice among

Table 1. Heavy metal concentrations in *T. megapotamicum* (Spreng.) Kuntze plant parts.

Metal	Tea Plant Parts and Soil Type			
	Herb^{a} (n=14)	Root^{a} (n=14)	Root Soil (n=2)	Topsoil (n=12)
As (mg/kg)	0.423±0.103^{b,d}	0.758±0.238^{b}	2.315±1.168	1.844±0.496^{d}
Cd (mg/kg)	0.346±0.311^{b,d}	0.634±0.658^{b}	0.050±0.029	0.512±0.228^{d}
Cs (mg/kg)	0.063±0.074^{b,e}	0.213±0.169^{b}	0.488±0.264	1.082±0.357^{e}
Pb (mg/kg)	0.304±0.731^{b,d}	0.809±0.286^{b}	5.783±3.704^{c}	5.207±1.266^{c,d}
Mo (mg/kg)	7.916±9.291^{b,e}	18.304±21.563^{b}	^{f}	10.562±5.232^{e}
Se (mg/kg)	0.738±0.393^{d}	1.242±0.557	^{f}	1.122±0.113^{d}
Th (mg/kg)	0.200±0.248	0.268±0.163	2.642±1.946	2.940±0.815
U (mg/kg)	0.019±0.012^{b,d}	0.114±0.041^{b}	0.829±0.420	1.290±0.224^{d}
V (mg/kg)	0.244±0.096^{b,e}	2.343±1.589^{b}	9.203±4.008	16.297±5.799^{e}

Data are expressed as mean ± standard deviation (SD). ^{a}*T. megapotamicum* (Spreng.) Kuntze. ^{b}Infused herb compared to root $p < 0.05$. ^{c}Root soil compared to topsoil $p < 0.05$. ^{d}Tea herb compared to topsoil $p < 0.05$. ^{e}Tea herb compared to topsoil $p < 0.001$. ^{f}Negligible.

participants in the study with one participant selling their herbal tea and all sharing free herbs with neighboring families across the reservation and out-of-state sites. Among the tea drinking harvesters, two reported consuming *T. megapotamicum* once to twice a week. The mean number of years of consumption by tea drinkers was 35 years (SD = 26) with a minimum of six years and a maximum of 58 years. Two thirds of study tea drinkers drank tea for non-therapeutic reasons (beverage and thirst quencher). One participant drank tea to "sooth the stomach." No tea drinkers were under the prescription or direction of a traditional practitioner for their tea consumption. One third of study tea herb harvesters reported past use of tea for wool textile pigment. Tea harvesters were representative of other types of harvesters in similar mining impacted areas in the region.

Heavy metals in plant tissue and soil

The heavy metals with greatest concentration in the above ground portion of the herb were: Mo (7.916±9.291), Se (0.738±0.393), and As (0.423±0.103). In herb root, metals with greatest concentration were Mo (18.304±21.563), V (2.342±1.589), and Se (1.242±0.557). In all the herb samples, heavy metal concentrations were higher in the roots compared to the above ground infused portion of the plant (Table 1). The majority of HMs for the above ground portion of the plant was greater than the root portion. Significance was found for V (t (26) = -4.93; $p < 0.05$), Cs (t (26) = -3.03; $p < 0.05$), Pb (t (26) = -6.40; $p < 0.05$), U (t (26) = -8.20; $p < 0.05$), Mo (t (25) = -1.64; $p < 0.05$), As (t (26) = -4.84; $p < 0.05$), and Cd (t (26) = 1.47; $p < 0.05$). For example, V was 9.6 times higher in was six times greater and Cs was 3.4 times greater in the root than the above part of the herb. The heavy metals with highest concentration in root soil were V (9.203±4.008) followed by Pb (5.783±3.704) then Th (2.642±1.946). In topsoil, V (16.297±5.799) was followed by Mo (10.562±5.232) then Pb (5.207±1.266). The HM concentrations in root soil was greater than tea topsoil for Pb (t (12) =0 .46; $p < 0.05$). The mean pH was 6.5±0.6 for all soil samples. Based on these findings and using a metal bio-accumulation factor (BF) which was calculated as follows:

BF = Metal concentration in plant (MC_{plant})/Metal concentration in soil (MC_{soil})

The metal accumulation factors in *T. megapotamicum* were 0.01, 0.01, 0.06, 0.06, 0.07, 0.23, 0.66, 0.68, and 0.75 for U, V, Cs, Pb, Th, As, Se, Cd, and Mo, respectively.

High and low traffic areas

The mean levels of Mo (18.042±3.011) and Cd (0.681±0.106) were greater in tea plants sampled near busy roadways (HTA) than less busy roads (LTA) for Mo (t (11) = - 4.70; $p < 0.001$) and Cd (t (12) = -5.18; $p < 0.001$ (Table 2). The mean levels were greater in plants that were located in LTAs for V (t (12) = 1.20; $p < 0.05$), Cs (t (12) = 2.44; $p < 0.05$), and U (t (12) = 0.74; $p < 0.05$). Overall, the topsoil heavy metals levels were higher than the plant levels for V, Cs, Mo ($p < 0.001$), As, Cd, Pb, Se, and U ($p<0.05$; Table 1).

Road data was classified in accordance with the New Mexico Department of Transportation functional classification system (NMDOT, 2014). The classification reflects traffic volume, speed, and number of lanes. The classification ranges from one to seven (1=highest traffic the root than the above ground portion of the plant, U volumes and speeds, 7=lowest traffic volumes and speeds). The HTA tea samples were collected near roadways < 0.8 km and were classified between 1 and 4. The mean distance between HTA samples and from the

Table 2. Heavy metal concentrations in *T. megapotamicum* (Spreng.) Kuntze in high and low vehicle traffic areas.

Metal	Plant[a] location	
	High traffic areas (HTA n=6)	**Low traffic area (LTA n=8)**
As (mg/kg)	0.360±0.093	0.470±0.086
Cd (mg/kg)	0.681±0.106[c]	0.095±0.062[c]
Cs (mg/kg)	0.021±0.008[b]	0.095±0.085[b]
Pb (mg/kg)	0.320±0.030	0.292±0.094
Mo (mg/kg	18.042±3.011[c]	0.321±0.171[c]
Se (mg/kg)	0.996±0.246	0.544±0.379
Th (mg/kg)	0.501±0.264	0.087±0.121
U (mg/kg)	0.029±0.013[b]	0.013±0.005[b]
V (mg/kg)	0.306±0.119[b]	0.198±0.035[b]

Data are expressed as mean±standard deviation (SD). [a]*T. megapotamicum* (Spreng.) Kuntze. [b]LTA compared to HTA $p < 0.05$. [c]HTA compared to LTA $p < 0.001$

edge of busy roadways was 0.637 km (SD=0.055, range 0.581-0.712). Low traffic area samples were collected near roads $\geq$2 km and were classified 5 to 7. The mean distance between LTA samples and from the edge of busy roadways were 6.008 km (SD=4.398, range 2.121 to 11.550).

Tea height comparison

There were greater amounts of various metals in the above ground portions of smaller (30 to 37 cm) tea plants than the taller (40 to 47 cm) plants in V (t (12) = 2.20; $p < 0.05$), Th (t (9) = 3.34; $p < 0.05$), Mo (t (12) = 3.71; $p < 0.05$), and Cd (t (12) = 3.49; $p < 0.05$). There were greater heavy metal concentrations in taller than smaller tea plants for Cs (t (12) = -3.43; $p < 0.05$). For roots, the taller tea plants contained higher levels of HMs (V, Cs, Pb, U, As); they were not found to be significant. The Se (t (9) = 3.36; $p < 0.05$), Mo (t (11) = 2.91; $p < 0.05$), and Cd (t (12) = 3.08; $p < 0.05$) levels in tea root were greater in smaller plants than taller plants. There were not sufficient amounts of data to perform an adequate comparison between the root soil of small and tall tea plants. There were greater HM levels in the soil of smaller plants than the taller plants in Cs (t (10) = 0.123; $p < 0.05$) and Mo (t (10) = 2.10; $p < 0.05$). In contrast, there were more HMs in the soil of taller plants for Th (t (10) = -2.13; $p < 0.05$).

DISCUSSION

Contamination of *T. megapotamicum* (Spreng.) Kuntze with heavy metals has not been previously published in the literature. In the present study, HM concentrations were found to be higher in root than in the above ground portion of tea plants. This is consistent with published literature examining other tea species and plants (Anke et al., 2009; Shahandeh and Hossner, 2002). Participants in the current study reported using only tea leaves, stem and flowers to concoct tea but not the root. However, in the infused part of *T. megapotamicum* tea, the mean for Cd (0.35±0.31, range 0.04 to 0.84 mg/kg) exceeded the maximum permissible level set for raw medicinal plants by the World Health Organization (WHO, 1998): 0.3 mg/kg. Arsenic (1.0 mg/kg) and Pb levels (10 mg/kg) were not exceeded in this study. The majority of tea harvesters reported consuming tea once to twice per week. Consequently, consumption of tea more than twice a week would place one above the WHO level (more so if tea was collected from HTA). The consumption of water for drinking purposes and cooking were not included in this study nor those related to dermal exposure.

Cadmium was readily taken up by plants similar to those reported by McLaughlin (1999). The bioaccumulation factor for Cd in the current study was 0.68 and exceeded all other metals tested except Mo (0.75). Wu et al. (2008) found Cd transfer and accumulation in plants was facilitated by low pH of soil as was done by Singh and Mhyr (1998) who reported soil pH of 5.5 and 6.5. The mean soil pH in the present study was 6.5±0.6. Findings in this study suggest that heavy metal contamination of *T. megapotamicum* used for herbal tea is plausible and warrant further research to explore factors that may influence heavy metal uptake and other sources of contamination and the potential for hazard to human health. Other factors that influence HM uptake are many and include geochemical makeup of soil (Bin et al., 2001), the plants organic matrix (Basquel and Erdemoglu, 2006), solubility of the HM (Arpadjan et al., 2008), and pH of the infusion water (Arpadjan et al., 2008; Basguel and Erdemoglu, 2008).

All harvestors in this study shared free tea and one also sold herbs. Emphasis should be placed on determining the incidence and frequency of food selling and sharing when assessing food chain or harvesting behaviors. Emphasis should be placed on assessing tea contamination levels in those who are biologically susceptible such as children and renal compromised individuals. The study population has a high incidence of chronic kidney disease and end-stage renal disease (Narva, 2003) whereby excessive or persistent exposure to heavy metals will further exacerbate preexisting renal conditions.Harvesting activities can overlap in and near mining impacted areas; it is important to consider consumption of contaminated food not only by individuals and their families, but potentially the whole community and beyond due to food sharing practices (Tsuji et al., 2007). This study community relies on plants for sustenance, medicinal purposes, and for use in cultural implements such as wool textiles, basketry, and tools. Assessing risk as related to a communities' collective ethnobotanical use of plants, their environment, and its related health impact on the community is important.

In the current study, tea samples were collected from high and low traffic areas. There were significantly greater Cd and Mo levels ($p < 0.001$) in HTA than LTA. Other tea and herb studies have found similar results. A medicinal herb and tea study (Barthwal et al., 2008) compared plant samples in high traffic, residential, and industrial areas in metropolitan India and demonstrated that heavy metal levels (Pb, Cd, Cr, and Ni) were greater in soil than plant parts (similar to this study for As, Cd, Cs, Mo, Pb, Se, U and V), heavy metal accumulation varied from plant to plant (even when the same plants were collected from three different locations), and the high traffic areas showed higher metal levels than the residential areas. Similarly, Jin et al. (2005) found that washed tea leaves near roadways exhibited higher levels of Pb in China. Dust transference and aerosolization of HMs associated to HTA is concerning as they pose risks of inhalation, ingestion, as well as dermal exposure (Bellis et al., 2001; Steenkamp et al., 2005). Studies have shown HM road emissions result from resuspended road dust, water runoff, tires, breaks, parts wear (Apeagyei et al., 2011) and vehicle emissions (Duong and Lee, 2011). Our results demonstrated that higher road volume correlated with higher HM concentrations, similar to other studies (Apeagyei et al., 2011; Duong and Lee, 2011). Future study efforts are needed to compare heavy metal concentrations in greenthread and other locally harvested plants in high and low traffic areas. Cadmium WHO levels were exceeded and collecting herbs in HTA (0.68±0.11) in U impacted areas appear to be a contributing factor and needs further exploration particularly in relation to Cd and Mo.

The majority of tea samples collected for our study were mature plants. Smaller plants tended to contain more V, Th, Mo, and Cd ($p < 0.05$) than taller plants. Similar findings occurred in the roots of smaller plants for Se, Mo, and Cd ($p < 0.05$). The species of plant and its surface area characteristics (leaf, flower, or stem capture), its maturation stage, and soil characteristics are only a few variables that may influence the uptake of heavy metals. For example, examining tea plants at various growth stages and harvesting seasons would allow their influences to be better explored and characterized. Anke et al. (2009) reported that younger black tea leaves contained higher levels of U. Laroche et al. (2005) also demonstrated that plant tissues in their seedling stages concentrated more U than in the flowery stage. It may be possible to tailor optimal harvesting time by better understanding plant uptake characteristics.

It is beyond the scope of this paper to discuss all the possible ways that herbal tea could be contaminated with HMs. Toxic conditions under which the drying and processing occur including the storage and transportation (Chan, 2003), geoclimatic factors (Haider et al., 2004) and rainfall (Basgel and Erdemoglu, 2006) are all seen as ways in which transfer of heavy metals to plants can occur. Contaminants in rinse and infusion water are a concern and need consideration. Study participants in the present study reported using both regulated and unregulated water for personal consumption. The extent to which tea rinse and infusion water is contaminated with heavy metals and utilized to concoct tea are unknown. Encouraging the continued utilization of Water Use Recommendation and Soil Restriction maps developed by deLemos et al. (2009) for the study area for harvesting activities is recommended.

Limitations

The herb samples were unwashed before analysis and the heavy metal concentrations obtained reflect both heavy metal plant uptake and surface contamination. As this was an examination of harvested food, we were interested in evaluating HM concentrations available to humans (including HMs taken up by tea plants plus surface contamination). Chambers and Sidle (1991) demonstrated that the difference of HMs paralleled those between a controlled environment study and a field study of unwashed plants.

The location, precision and accuracy of GIS was high but it was not found to be an adequate surrogate for evaluating contamination. For example, even though tea harvesters did not water their tea products, ephemeral water sources outside the defined proximity may have impacted the contamination levels. Less or more contaminated water may have passed through the grounds of tea herb harvesting areas.

Conclusions

This is the first time that heavy metal levels have been reported in *T. megapotamicum.* Heavy metals were greater in roots than the above ground infused portion of the tea plant and soil acidification was present. Mean Cd tea herb levels exceeded the WHO maximum permissible level (>0.3 mg/kg); Cadmium and Mo were demonstrated to exist in high traffic areas which warrants concern and emphasizes further study and continued monitoring. The findings emphasize on the need to evaluate other food and therapeutic plants utilized as there may be considerable differences of contamination level in various plant species. Areas for future research have been highlighted as well as ways to refine the work. The findings from this study and future research recommendations will be shared with the communities as well as their leaders. The research findings also have the capacity to reach other U mining impacted areas outside the study community.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Herbal teas heavy metal evaluation with renal function assessment in regular consumers in Benin

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Herbal teas are openly sold in markets and main streets of big cities like Cotonou in Benin. Most people treat themselves at low cost with wide variety of herbal plants with proven therapeutic properties. The purpose of this research was to evaluate the content of heavy metals in herbal teas sold daily in Cotonou. In addition, we would evaluate if this heavy metal content of herbal teas could affect the renal function of regular consumers. Therefore, herbal teas samples were collected from selected sellers at well-known places. Samples' analyses were done by reverse anodic stripping voltammetry with the Metalyser HM 3000 coupled to PC 101 NT pump. Biological markers of kidney failure in blood and urine of regular consumers were also assessed. Renal creatinine clearance and albuminuria were measured, the ratio of micro-albuminuria/creatininuria was calculated, and then red and white blood cells were counted. The results indicate that 7.69 and 30.77% of the herbal teas samples displayed an abnormal high concentration in cadmium (Cd) and lead (Pb) concentration, respectively. Statistical significant difference was found between the analyzed herbal tea samples in regard to Cd and Pb content ($P < 0.01$). No obvious biological sign of severe kidney damage has been noted among regular consumers using their blood and urine samples. Only 8.60% of them had clearances between 60 and 89 ml/min/1.73 m^2 with mild kidney failure. This study indicates that some herbal teas contain toxic chemicals such as Cd and Pb over recommended limits of 3 and 10 µg/L, respectively. The regular consumption of these herbal teas could be health threatening for the population.

Key words: Herbal teas, heavy metals, environmental pollution, human renal function, anodic stripping voltammetry.

INTRODUCTION

In Benin, traditional cure such as herbal teas are openly sold in markets and main streets of main cities like Cotonou. People consumed herbal teas for medical purposes and keeping their health. They treat themselves at lower cost from a large biodiversity of medicinal plants with proven therapeutic properties. However, medicinal

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plants can be easily contaminated by heavy metals during cultivation or later during the processing stage (Jabeen et al., 2010). Literature has described some toxic heavy metals in herbal teas (Samali et al., 2012). Along with other pollutants, heavy metals can be released into the environment through industrial activities, household wastes, engine exhaust, pesticides and fertilizers used in agriculture (Järup, 2003). Generally, the concentrations of heavy metals in herbal teas changed according to the types of herbal teas and geological conditions. Medicinal plants often used in Cotonou under maceration, decoction or infusion forms can be contaminated in their biosphere or during processing (Marcos et al., 1998). Lead (Pb) and cadmium (Cd) are trace metals which are not essential for either humans or plants, while could easily induce toxic effects in humans at low concentrations. For crops, the lowest range of Cd which can cause yield reduction is 5 to 30 µg/kg^{-1}, while its maximum allowable concentration in edible plants is as low as 1 µg kg^{-1} (Esetlili et al., 2014). Therefore, herbal tea safety has piqued great interest because contaminants threaten the life and health of humans, animals and the environment, leading to economic losses (Baranowska et al., 2002; Friberg and Lene, 1986). The genetic and epigenetic effects of dietary heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As) and selenium (Se) in the human body are associated with an increased risk of different cancers (Hunt, 2003). Thus, the harmful effects of medicinal plants can be linked to an indirect contamination of those plants by heavy metals and toxic or radioactive agrochemical residues (Zeggwagh et al., 2013). Prolonged consumption of heavy metals from food can lead to their accumulation in the kidney and liver, causing disruption of numerous biochemical processes and potentially causing cardiovascular, nervous, kidney and bone diseases (Jabeen et al., 2010). In order to protect human health from Cd and lead intoxication, a limit was set by the world health organization (WHO) to 3 µg/L for Cd and 10 µg/L for Pb (World Health Organization, 2011a, b). It has been reported that the average quantities of lead and cadmium measured in *Senna rotundifolia* L. extracts in Benin were well above recommended limits of the WHO (Montcho et al., 2014). In our knowledge, until now, no scientific work in Benin has tackled the possible high content of heavy metal in herbal teas commonly called “adômansin”. These herbal teas are frequently drunk by a large portion of the population especially in the population with poor and middle income. According to WHO, toxic heavy metals have to be controlled in herbal plants in order to assure their safety (World Health Organization, 1998; Hussain et al., 2011). Therefore, controlling the heavy metal concentrations in teas and biological fluids of regular consumers should be done to ensure the safety of herbal teas. The concentrations of heavy metals have not been reported in herbal teas available in Cotonou, Benin. This study was intended to determine the concentration of Cd and Pb in selected herbal teas daily sold in Cotonou and assess some of the biological markers of kidney failure among regular consumers. The data obtained could be used for the quality control process to ensure the purity and safety of medicinal plants used for herbal teas in Benin.

MATERIALS AND METHODS

Instrumentations

Anodic stripping voltammetric (ASV) determination of Cd and Pb were performed with trace metal analyzer (Metalyser HM 3000) with three-electrode system coupled to PC 101 NT pump.

Sample collection and preparation

Herbal tea selling was practiced along many streets in Cotonou. Thirteen sellers were randomly selected in well-known places and herbal tea samples were purchased from them. Each sample is a mixture of several medicinal plants. The content of these mixture changes from one seller to another. A volume of 10 ml of herbal tea samples were collected in volumetric flask for the determination of Cd and Pb and analyzed within the next 4 h.

Preparation of standard solution

Reference standards of Cd and Pb were purchased from Inorganic Ventures, Inc. Lakewood, NJ and used for the preparation of aqueous standard solutions of 1.000 g/L. Mixed working solution (1.000 mg/L) of Cd and Pb was prepared by appropriate dilution for the voltammetric analysis from stock solution of 1.000 g/L.

Dosage of cadmium and lead

Ultra-pure water (10 ml) and 1 ml of acetate buffer (pH 4.6) were taken in polarographic vessel, well mixed, and the voltammogram of the blank was recorded. Volume of 1 ml of herbal tea sample was added into the polarographic vessel and then voltammogram of the sample solution was recorded under the same conditions. After the sample voltammogram was recorded, 0.2 ml of 1 mg/L mixed standard of Cd and Pb was added and then voltammogram of the standard was recorded. The procedure was repeated three times and the current was measured. The concentrations’ values of Cd and Pb were directly displayed on the screen consecutively with the voltammogram. The limit of detection is 0.5 µg/L for both analytes.

Recruiting regular herbal tea consumers

A survey with the herbal tea sellers was conducted to identify regular consumers. Only clients who were faithful to the sellers, aged over 18 years, and who have been drinking herbal tea for more than three months, were included in the study. Consumers with medical background of diabetes, high blood pressure, obesity, chronic renal failure, urinary infection and other chronic pathology, were excluded. Subjects who did not give their informed consent were not included.

Renal function assessment of regular herbal teas consumers

Thirty five regular consumers were received at the office of the

Table 1. Cadmium and lead content of the herbal teas samples.

Herbal tea sample	Cadmium [Mean±SD] (µg/L)	Lead [Mean±SD] (µg/L)
T1	0.853 ± 0.31	0.942 ± 0.25
T2	0.783 ± 0.45	4.073 ± 1.32
T3	1.977 ± 0.42	20.798 ± 2.35 *****
T4	1.103 ± 0.49	17.519 ± 2.99 *****
T5	0.864 ± 0.22	1.029 ± 0.32
T6	0.833 ± 0.25	1.036 ± 0.18
T7	1.389 ± 0.76	10.675 ± 2.25 *****
T8	1.192 ± 0.71	10.120 ± 2.35 *****
T9	0.945 ± 0.10	0.440 ± 2.21
T10	1.050 ± 0.22	0.967 ± 0.41
T11	0.937 ± 0.43	0.896 ± 0.22
T12	5.536 ± 1.63 *****	8.770 ± 3.03
T13	1.031 ± 0.24	0.892 ± 0.11
Average	1.423 ± 0.48	6.012 ± 2.01

*****High content of Cd or Pb.

National Laboratory of Narcotics and Toxicology (LNST) in Cotonou. The study was approved by the local ethical committee, and all volunteers gave their written consent. Blood (4 ml) was drawn from each volunteer into a dry test tube. The samples were centrifuged and serums were harvested for the creatinine level determination using JAFFE method. Urine samples were also collected from each subject. Before collecting urines, every participant was asked to empty his bladder. Then, urine of the following 3 h was collected into plastic bottles and the following analysis was performed: creatininuria, albuminuria, red and white blood cell count, and determination of the ratio micro-albuminuria/creatininuria.

Statistical analysis

Analysis of variance (ANOVA) was realized to verify the existence of significant difference between thirteen herbal teas studied for Cd and Pb contents. Data were processed with R software R 3.3.2 (R Development Core Team, 2016). At first, normality of data and equality of variances were verified, respectively with Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene (Zar, 1999) tests. Student test, Newman and Keuls tests were used for statistical analysis and comparison, and outcomes were represented by barplot with errors bars.

RESULTS

Heavy metals concentrations

The concentration of Cd and Pb contained in each herbal tea sample are recorded in Table 1. The normal values authorized for these two metals are respectively less than 3 and 10 µg/L. From our study, 7.69% of the herbal teas samples had a high concentration in cd, while 30.77% of those samples indicated high lead content. The statistical analysis showed statistical significant different between the analyzed samples in regard to Cd and Pb content. The results of Cd content showed that three groups were statistically different ($P < 0.01$), the first is constituted by T12 only, second is composed of T3 and third is constituted by T1, T10, T11, T13, T2, T4, T5, T6, T7, T8 and T9 (Figure 1). Meanwhile, the results of Pb content showed eight groups were statistically different with $P < 0.01$ (Figure 2).

Periods of herbal teas consumption

The volunteers enrolled in this study have several years' experience in drinking these herbal teas. The majority of the volunteers (43%) have been drinking these herbal teas for five to ten years, while 40% have been drinking over ten years.

Assessment of renal function

Socio-demographic characteristics

The socio-demographic data of the 35 volunteers were reviewed in this study. The median age of the volunteers was 42 (range 21 to 66) years, while the mean age was 40±12 years (M±SD). The socio-demographics of the volunteers are shown in Table 2.

Clearance of creatinine

The study found that 8.57% of the volunteers had a glomerular filtration rate between 60 and 89 ml/min/1.73 m^2 indicating that they were suffering from mild renal failure. Table 3 displays renal clearance values according to the Modification of Diet in Renal Disease (MDRD) method.

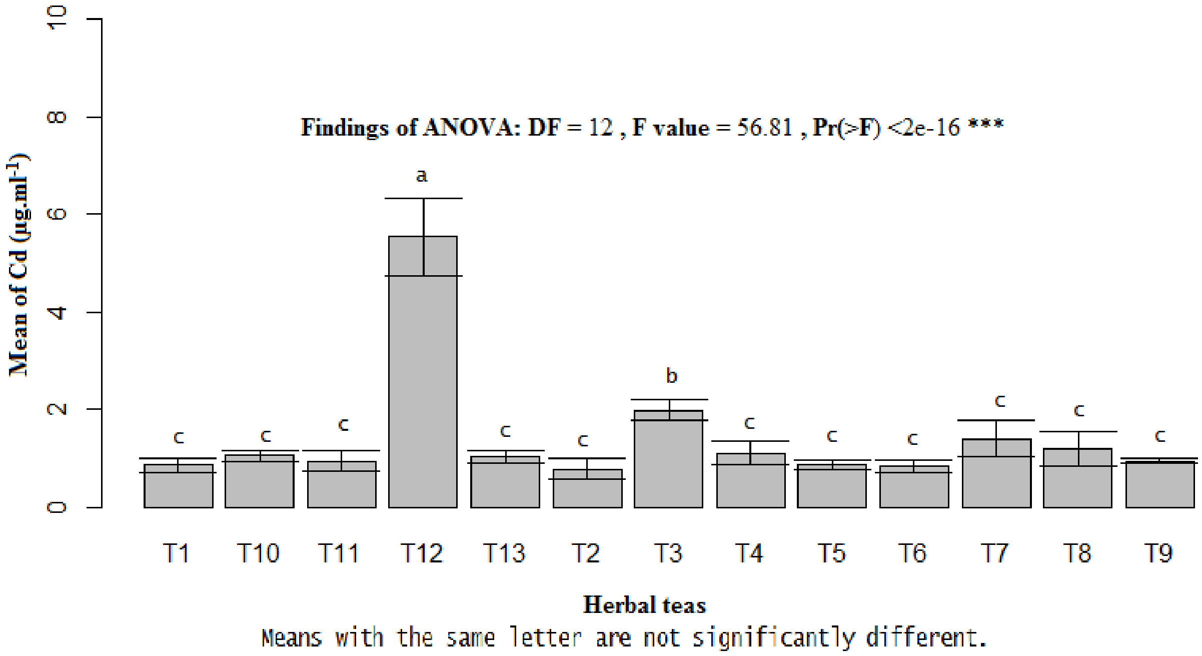

Figure 1. Comparison of cadmium content in herbal teas.

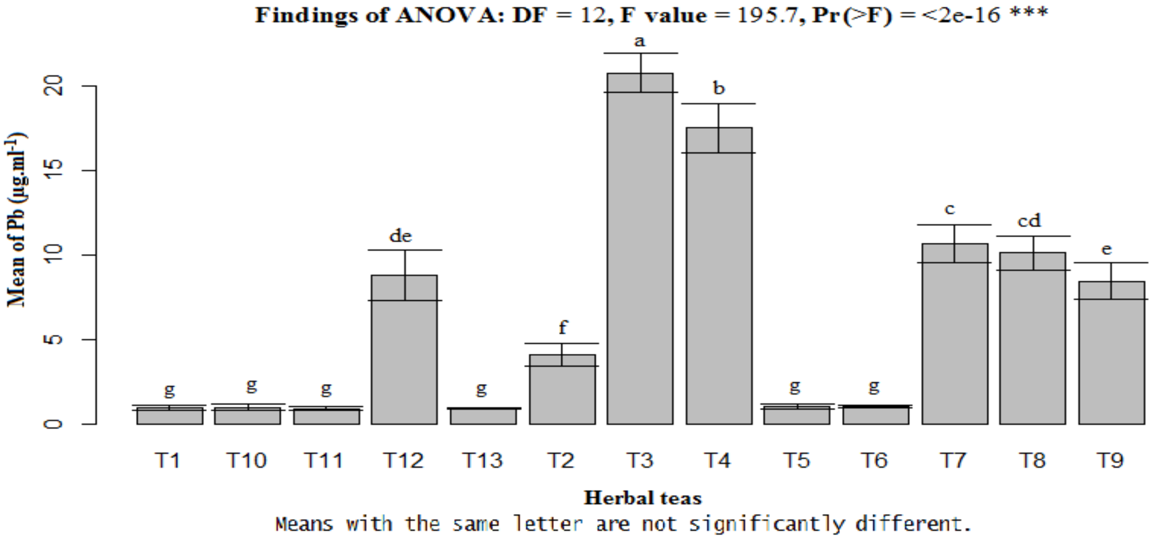

Figure 2. Comparison of lead content in herbal teas.

Micro-albuminuria/creatininuria ratio

All data were within the normal range values for micro-albuminuria/creatininuria ratio for each volunteer as shown in Table 4.

Number of red and white blood cells in urine

All participants had the red and white blood cells count inferior to 5000. No abnormality was found. Table 5 displays the numbers characterizing each volunteer.

DISCUSSION

Identification of heavy metals

In the recent years, human activities, such as industry

Table 2. Socio-demographic data of regular herbal teas consumers.

Socio-demographic data	Results	
Sex of the consumers	94% (n=33) of the regular herbal teas consumers enrolled were male	
Age of the consumers	Minimum	21
	Maximum	66
	Average	40.31
	Standard deviation	11.87
	Median	42
	Interquartile range	28-46
Place of residence of the consumers	51.43% (n=18) of regular consumers mainly come from the suburban area (51.43%)	
Professions of the consumers	40% of the participants were motor bike taxi drivers commonly called "*zémidjans".* Craftsmen occupy the second position (31.5%). No other profession reaches the percentage of 3% of the study population.	

Table 3. Renal creatinine clearances.

Clearance (ml/min/1.73 m^2)	Interpretations	Number of consumers (n=35)
≥90	No renal failure	32
60 – 89	Mild renal failure	03
30 – 59	Moderate chronic renal failure	00
15 – 29	Severe chronic renal failure	00
<15	Terminal chronic renal failure	00

Table 4. Micro-albuminuria/creatininuria ratio of the consumers.

Ratio (mg/g)	Number (n=35)
<30	35
≥30	00

Table 5. Red and white blood cells count in the urines of consumers.

Number of cells per minute	Number (n = 35)
Red blood cells <5000/min	35
Red blood cells ≥5000/min	00
White blood cells <5000/min	35
White blood cells ≥5000/min	00

and agriculture, promote heavy metal release into the environment. Thus, the analytical determination of metals in medicinal plants has become a part of quality control in order to establish their purity, safety and efficacy (Baranowska et al., 2002). In this study, 7.69% of the herbal teas samples showed a high Cd concentration with an average of 5.84 µg/L and 30.77% of the same samples showed a high Pb concentration. According to Montcho et al. (2014), heavy metals persist in the ground because they are not chemically or biologically deteriorated. Heavy metals could end up in herbal teas from waste products containing manufactured items such as batteries, electronic wastes which are thrown in the environment, and then pollute the rain water used by medicinal plants for their growth. The heavy metals are stocked in the leaves, roots, and barks of those plants. These heavy metals can also accumulate in clay or organic matter by complex connections or under ionic form in solution (Montcho et al., 2014). They can also form inorganic compounds or get fixed to the surface of particles by adsorption. According to Adam et al. (2010), norms about heavy metals, Cd and Pb concentrations of drinkable water should be respectively inferior to 3 and 10 µg/L.

In Montcho et al. (2014) study, samples of *Chamaecrista rotundifolia* (Pers.) (Greene.) collected on Dantokpa's, Vossa's and Godomey's markets in Benin had respectively high Pb concentrations of 2733, 1825, and 1902 ppb. The difference in Cd and Pb concentrations between our study and that of Montcho et al.

(2014), could be explained by the fact that this study quantified heavy metals in the entire decoction (drinkable solution) made of several medicinal plants, while Montcho et al. (2014) worked directly with *C. rotundifolia* (Pers.) (Greene.) plant. Heavy metals can seriously damage health. According to Stengel (1996), Cd causes proximal renal tubulopathy in the human and the main sign of the disease is a proteinuria of small molecular weight often associated to Fanconi' syndrome. The frequency of renal stones is increased in workers exposed to Cd. The tubular proteinuria observed is irreversible and is accompanied with a faster damage to the renal function with age. According to Bismuth et al. (2000), Pb can also induce a proximal tubulopathy observed when the contamination is important and when the damage of the kidney is chronic (after 10 to 30 years of exposure).

Renal function assessment

None of the participant was found to have chronic renal failure. Only 8.57% had a glomerular filtration rate between 60 and 89 ml/min/1.73 m^2 which indicate a mild renal failure. According to Vigan et al. (2013), the frequency of chronic renal failure (CRF) is 2.78% in hospital setting. This study has been the first to assess the content of heavy metals in herbal teas and the impact of their regular consumption on renal function in Beninese. The founding of this study suggests further study of epidemiological prevalence of chronic renal failure within the general population of Cotonou in order to conclude on the impact of heavy metals in regular herbal tea drinkers.

Conclusion

This study has measured heavy metals in 13 types of herbal teas sold in Cotonou as well as some biological markers of kidney failure in 35 regular consumers of herbal teas in Cotonou. The herbal tea samples collected in Cotonou, Benin, contained high concentrations of heavy metals such as Cadmium and Lead. It is urgent to get the attention of regular consumers on potential risks associated with high concentrations of heavy metals in herbal teas sold in Cotonou. No severe sign of kidney damage has been identified among participants. This study results recall the question about the safety of herbal teas widely used in Cotonou. This study could provide basis for further research to evaluate the global renal impact of such consumption on a long period between regular and non-regular consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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