Full Length Research Paper

Heavy metal contamination in *Thelesperma megapotamicum*

Christine Samuel-Nakamura[1]*, Felicia S. Hodge[2], Jane L. Valentine[3], and Wendie A. Robbins[4]

[1]Interdepartmental Program in American Indian Studies, University of California, Los Angeles, 3220 Campbell Hall, Mailcode 154802, Los Angeles, CA 90095-1548, USA.
[2]School of Nursing, University of California, Los Angeles, 5-940 Factor Bldg., Mailcode 691921, Los Angeles, CA 90095-6919, USA.
[3]School of Public Health, Environmental Health Sciences, University of California, Los Angeles, 66-062 CHS., Mailcode 177220, Los Angeles, CA 90095-1772, USA.
[4]Center for Occupational and Environmental Health Fielding School of Public Health, Environmental Health Sciences, University of California, Los Angeles, 5-254 Factor Bldg., Mailcode 956919, Los Angeles, CA 90095-6919, USA.

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

*Corresponding author. E-mail: csamnak@ucla.edu.

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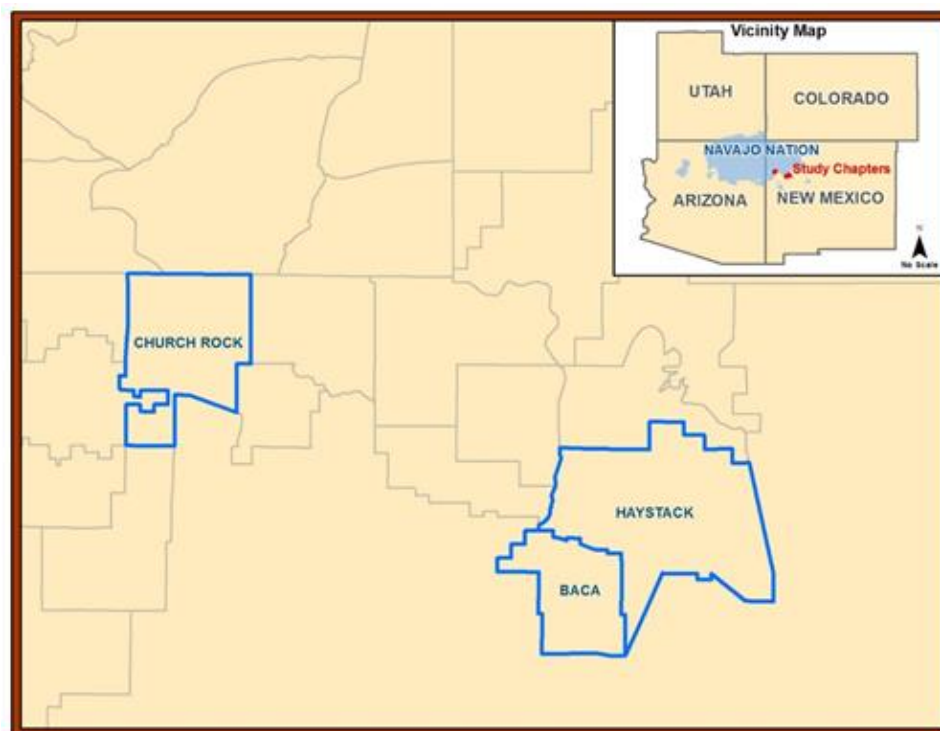
Figure 3. Study area map (depiction of the Navajo Nation in the Four Corners region of the U.S. Southwest). New Mexico Chapters or communities: Baca/Haystack and Churchrock Chapters provided tea samples.

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 ≥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.

ACKNOWLEDGEMENTS

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1990). Toxicological profile for thorium. ASTDR in collaboration with the U.S. Environmental Protection Agency, October 1990. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp147.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR) (2004). Toxicological profile for cesium.U.S. Department of Health and Human Services. April 2004. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp157.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR) (2012). Toxicological profile for vanadium. U.S. Department of Health and Human Services. September 2012. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp58.pdf.

Anke M, Seeber O, Muller R, Schafer U, Zerull J (2009). Uranium transfer in the food chain from soil to plants, animals, and man. Chemie der Erde-Geochem. 69:75-90.

Apeagyei E, Bank MS, Spengler, JD (2011). Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. Atmos. Environ. 45:2310-2323.

Arpadjan S, Celik G, Taskesen S, Gucer S (2008). Arsenic, cadmium and lead in medicinal herbs and their fractionation. Food Chem. Toxicol. 46:2871-2875.

Ballew C, White LL, Strauss KF, Benson LJ, Mendlein JM, Mokdad H (1997). Intake of nutrients and food sources of nutrition among the Navajo: Findings from the Navajo health and nutrition survey. J. Nutr. 127:2085S-2095S.

Barthwal J, Nair S, Kakkar P (2008). Heavy metal accumulation in medicinal plants collected from environmentally different sites. Biomed. Environ. Sci. 21:319-324.

Basgel S, Erdemoglu SB (2006). Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. Sci. Total Environ. 359:82-89.

Bellis D, Ma R, Bramall N, McLeod CW, Chapman N, Satake K (2001). Airborne uranium contamination-as revealed through elemental and isotopic analysis of tree bark. Environ. Pollut. 114:383-387.

Bin C, Xiaoru W, Lee FSC (2001). Pyrolysis coupled with atomic absorption spectrometry for the determination of mercury in Chinese medicinal materials. Anal. Chim. Acta 447:161-169.

Bongiovanni G, Luchino N, Palacio L, Eynard AR, Cantero JJ, Goleniowski ME (2006). In vitro antitumoral activity determination of native plant extracts of the central region of Argentina. Mole. Med. Chem. 10:22-23.

Borneo R, Leon AE, Aguirre A, Ribotta P, Cantero JJ (2009). Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their in vitro testing in a model food system. Food Chem. 112:664-670.

Caldas ED, Machado LL (2004). Cadmium, mercury and lead in medicinal herbs in Brazil.Food Chem. Toxicol. 42:599-603.

Chambers JC, Sidle RC (1991). Fate of heavy metals in an abandoned lead-zinc tailings pond: I. Vegetation. J. Environ. Qual. 20:745-751.

Chan K (2003). Some aspects of toxic contaminants in herbal medicines. Chemosphere 52:1361-1371.

Dalgleish HJ, Koons DN, Adler PB (2010). Can life-history traits predict the response of forb populations to changes in climate variability? J. Ecol. 98:209-217.

Darby WJ, Adams CM, Pollard M, Dalton E, McKinley P (1956). A study of the dietary background and nutriture of the Navajo Indian: II. dietary pattern. J. Nutr. 60:19-34.

deLemos JL, Brugge D, Cajero M, Downs M, Durant JL, George CM, Henio-Adeky S, Nez T, Manning T, Rock T, Seshchillie B, Shuey C, Lewis J (2009). Development of risk maps to minimize uranium exposures in the Navajo Churchrock mining district. Environ. Health 8(1):29.

Dunmire WW, Tierney GD (1997). Wild plants and native peoples of the four corners. Santa Fe, New Mexico: Museum of New Mexico Press.

Duong TTT, Lee BK (2011). Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. J. Environ. Manage. 92:554-562.

Eisler R (1988). Arsenic hazards to fish, wildlife, and invertebrates: A synoptic view. U.S. Fish and Wildlife Service Biological Report. 85(1):12.

Ernst E (2002a). Heavy metals in traditional Indian remedies. Eur. J. Clin. Pharmacol. 57:891-896.

Ernst E (2002b). Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharmacol. Sci. 23:136-139.

Figueroa AC, Soria EA, Cantero JJ, Sanchez MS, Goleniowski ME (2012). Cytotoxic activity of Thelesperma megapotamicum organic fractions against MCF-7 human breast cancer cell line. J. Cancer Ther. 3:103-109.

Gilman AP, Villenueve DC, Secours VE, Yagminas AP, Tracy BL, Quinn JM, Valli VE, Willes RJ, Moss MA (1998). Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley rat. Toxicol. Sci. 41:117-128.

Gomez MR, Cerutti S, Sombra LL, Silva MF, Martinez LD (2007). Determination of heavy metals for the quality control in Argentinian herbal medicines by ETAAS and ICP-OES. Food Chem. Toxicol. 45:1060-1064.

Gridnard E, Guéguen Y, Grison S, Lobaccaro JMA, Gourmelon P, Souidi M (2008). In vivo effects of chronic contamination with 137 cesium on testicular and adrenal steroidogenesis. Arch. Toxicol. 82:583-589.

.

Haider S, Naithani V, Barthwal J, Kakkar P (2004). Heavy metal content in some therapeutically important medicinal plants. Bull. Environ. Contam. Toxicol. 72:119-127.

Haley DP, Bulger RE, Dobyan DC (1982).The long-term effects of uranyl nitrate in the structure and function of the rat kidney. Virchows Arch. 41:181-192.

Jin CW, He YF, Zhang K, Zhou GD, Shi JL, Zhang SJ (2005). Lead contamination in tea leaves and non-edaphic factor affecting it. Chemosphere 61:726-732.

Kirkam MB (2006). Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. Geoderma 137:19-32.

Laroche L, Henner P, Camilleri V, Morello M, Garnier-Laplace J (2005). Root uptake of uranium by a higher plant model (Phaseolus vulgaris)-bioavailability from soil solution. Radioprotection 40(S1):S33-S39.

Matthews W (1886). Navajo names for plants. Am. Nat. 20:767-777.

Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ (2008). Cadmium, lead, and other metals in relation to semen quality: Human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect. 116:1473-1479.

Meeker JD, Rossano MG, Protas B, Padmanahban V, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ (2010). Environmental exposure to metals and male reproductive hormones: Circulating testosterone is inversely associated with blood molybdenum. Fertil. Steril. 93(1):130-140.

McLaughlin MJ, Parker DR, Clarke JM (1999). Metals and micronutrients–food safety issues. Field crop Res. 60(1):143-163.

Mohammed MI, Sulaiman MA (2009). Analysis of some metals in some brands of tea sold in Kano, Nigeria. BaJPAS. 2:34-39.

Naithani V, Kakkar P (2005). Evaluation of heavy metals in Indian herbal teas. Bull. Environ. Contam. Toxicol. 75:197-203.

Narva AS (2003). The spectrum of kidney disease in American Indians. Kid. Int. 63:739-746.

New Mexico Department of Transportation (NMDOT) (2014). Functional Classification Guidance Manual. Available at: http://dot.state.nm.us/content/dam/nmdot/planning/NMDOT _FC_Guide.pdf

Palacios SM, Maggi ME, Bazan CM, Carpinella MC, Turco M, Munoz A, Alonso RA, Nunez C, Cantero JJ, Defago MT, Ferrayoli CG, Valladares GR (2007). Screening of Argentinian plants for pesticide activity. Fitogerapia 78:580-584.

Pandey R, Singh SP (2002). Effects of molybdenum on fertility of male rats. Biometals 15:65-72.

Shahandeh H, Hossner LR (2002). Role of soil properties in phytoaccumulation of uranium. Water Air Soil Pollut. 141:165-180.

Shemluck M (1982).Medicinal and other uses of the compositae by Indians in the United States and Canada. J. Ethnopharmacol. 5:303-358.

Singh BR, Mhyr K (1998). Cadmium uptake by barley affected by Cd sources and pH levels. Geoderma 84:185-194.

Steenkamp V, Stewart MJ, Chimuka L, Cukrowska E (2005). Uranium concentrations in South African herbal remedies. Healthy Phys. 89:679-683.

Tracy BL, Quinn JM, Lahey J, Gilman AP, Mancuso K, Yagminas AP, Villenueve DC (1992). Absorption and retention of uranium from drinking water by rats and rabbits. Health Phys. 62:65-73.

Tsuji LJS, Manson H, Wainman BC, Vanspronsen EP, Shecapio-Blacksmith J, Rabbitskin T (2007). Identifying potential receptors and routes of contaminant exposure in the traditional territory of the Ouje-Bougoumou Cree: Land use and the geographical information system. Environ. Monit. Assess. 127:293-306.

Vyskocil A, Viau C (1999). Assessment of molybdenum toxicity in humans. J. Appl. Toxicol. 19:185-192.

World Health Organization (WHO) (1998). Quality control methods for medicinal plant materials. Geneva Switzerland: WHO.

Wu J, Zou Y, Zhan X, Chen S, Lu G, Lai F (2008). Survey of heavy metal population in four Chinese crude drugs and their cultivated soils. Bull. Environ. Contam. Toxicol. 81:571-573.