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Determination of Cr, Cd, Sn, and Pb in Selected Herbal Products Available in Philippine Markets

Joan S. De Vera
University of the Philippines Diliman

Leni B. Quirit
University of the Philippines Diliman

Irene B. Rodriguez*
University of the Philippines Diliman
Academia Sinica, Taiwan

ABSTRACT

The growing popularity of herbal products in the Philippines makes it imperative to monitor and ensure safety of consumers from metal contaminants. In this study, trace concentrations of Cr, Cd, Sn, and Pb in herbal products were simultaneously measured using a microwave-assisted digestion as sample pre-treatment and inductively coupled plasma mass spectrometry (ICP-MS) for elemental detection. Using the optimized method, recoveries of ERM CD281, the primary certified reference material (CRM) used, were found to be between 80-89%, and the method detection limits (MDL) for Cr, Cd, Sn, and Pb were 0.15, 0.07, 0.3, and 0.14 µg/L, respectively. The linear ranges for Cr and other elements (Cd, Sn, and Pb) were 0.01-500 and 0.01-50 µg/L, respectively. All correlation coefficients were 0.9999 or better. Most of the products tested had measurable trace metal concentrations, which were below the suggested maximum limits in herbal products. However, one product derived from mangosteen exceeded the limit for Cd (0.42 mg/kg). Subsequent analysis of metal content in tea infusions showed that only a small fraction of metals may leach out, suggesting that consumption of tea infusions pose lesser risks. The order of abundance of metals found in herbal products was Cr>Pb>Cd>Sn. The variability of metal concentrations in herbal products underlines the fact that many plant ingredients are susceptible to contamination, and quality control during processing must be improved to minimize the possibility of

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*Corresponding Author

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contamination. The results of this study suggest that vigilant monitoring of herbal products is imperative to avoid exposure to trace metal contaminants.

Keywords: Trace metal, ICP-MS, microwave digestion, herbal product contamination, herbal product safety

INTRODUCTION

The growing concerns on the possibility of trace metal contamination in plants and herbal products have initiated a number of research studies that measure such toxicants in various countries, such as in Bulgaria (Arpadjan et al. 2008), Italy (Mazzanti et al. 2008), Malaysia (Ang 2008), Nigeria (Ajasa et al. 2004), Pakistan (Mahmood et al. 2013), Poland (Leśniewicz et al. 2006), and Turkey (Başgel and Erdemoğlu 2006). In most cases, the studies focused on endemic plants and herbal products popular in the country where the studies were conducted. The Philippines is also known for its active market for herbal products and widespread use of traditional medicine, yet only a few studies have attempted to measure metal contaminants in herbal products (e.g., Solidum et al. 2012; Solidum 2014). A survey conducted by the National Integrated Research Program on Medicinal Plants in the University of the Philippines Los Baños estimated that around 1687 plant species are being used by traditional healers, implying the diversity of species that could possibly be used for medicinal purposes (Department of Agriculture 1995). Apart from the abundance of herbal products, the reliance of Filipinos on medicinal plants and products is viewed to be rooted to its culture, which fundamentally combines both western and traditional approaches to healing (Tan 1987; Sy 1997). The role of non-allopathic therapy, which includes the use of herbal products, is deeply ingrained in the health care system of the country and is recognized by the local government (Traditional and Alternative Medicine Act of 1997).

In the Philippines, the regulation of herbal products is under the supervision of Food and Drugs Administration. An FDA circular released in 2004 provides guidelines on how to register herbal products, which are defined as any preparations derived from plant materials, such as flowers, roots and leaves (FDA 2004). In the FDA guidelines, the maximum allowable limits of Pb and Cd in plant ingredients were set at 10 mg/kg and 0.3 mg/kg, respectively. The limits for Pb and Cd follow the guidelines set by the World Health Organization (WHO 1999). The low permissible values for Pb and Cd are instituted to ensure protection against known health risks from prolonged exposure to these metals. Chronic exposure to both Cd and Pb, even at low levels, can cause chronic kidney disease because of their tendency to accumulate in this organ (Hutton 1987; Satarug and Moore 2004; Navas-Acien et al. 2009). Pb also accumulates and affects the bones and liver (Hutton 1987). In addition, persistent low-level exposure to Pb can cause neurotoxic effects to children because of the permeability of their blood brain barrier to Pb (Jarup 2003). No guideline values have been set for Cr and Sn despite the known serious health conditions associated with these two metals. Some forms of Cr and Sn, such as Cr (VI) and organotin compounds, are considered toxic for humans (Kimbrough 1976; Kotaœ and Stasicka 2000; Yu et al. 2008).

In consideration of the known risks of these metals, they should be monitored in herbal products, especially because these products are sold as supplements and are regularly consumed for a long period of time. This work is aimed towards the development and optimization of a method suitable for the determination of metal concentrations in herbal products commonly sold and consumed in the Philippines. An analytical method was optimized and validated using the certified reference materials (CRM) and spiked samples prior to the application of the method to actual samples. The data from this study may serve as a background on the extent of metal contamination in herbal products in the country since publications regarding metal levels are scant.

MATERIALS AND METHODS

The following CRM were used for the optimization and validation of the sample preparation and analytical method: ERM-CD281 (rye grass obtained from Institute for Reference Materials and Measurements, Belgium), BCR 150 (skim milk powder from Institute for Reference Materials and Measurements, Belgium), DORM-3 (fish protein from National Research Council, Canada), and NIST 1643e (Trace elements in water, National Institute of Standards and Technology, USA). ERM-CD281 was the primary CRM used because it has the closest matrix as the samples. BCR 150 and DORM-3 were analyzed to check the applicability of the method to more complex sample matrices. NIST 1643e was used as an additional CRM for tea infusions because it was the available reference material that has the closest matrix to liquid samples. For further validation, the applicability of the sample pre-treatment scheme for liquid samples was evaluated following the standard addition method on one of the herbal liquid samples (Noni plant juice), which was spiked with known analyte concentrations between 0.67 µg/L and 13.33 µg/L.

A total of 33 commonly available herbal products were purchased from local drug stores and supermarkets in Quezon City, Philippines between September 2011 and

November 2011. All products analyzed were manufactured in the Philippines and made from plant ingredients that are endemic to the country except for the Noni juice sample, which was included in the study to serve as the matrix for standard addition validation. The products were chosen according to their availability and whether consumers can easily acquire them through over-the-counter purchases and without prescription. The plant ingredients, formulations, and purported health benefits of the herbal products included in the study are summarized in Table 1. Of the samples analyzed, four plants, namely lagundi, sambong, amplaya and garlic, are listed in the circular of the Department of Health (1995) as safe, effective and scientifically-validated herbal medicinal plants. Malunggay, ginger, mangosteen, coconut and papaya plants are listed as medicinal plants with folkloric basis, and thereby require further scientific validation (Department of Health 1995). Other plants that were not mentioned in the list are also believed to have medicinal values based on folkloric evidences. Herbal products made from lagundi were already approved as drugs to cure cough, while other lagundi-derived products are marketed as dietary supplements.

Table 1. Plant ingredients, formulations and common medicinal benefits of the herbal products included in this study

Plant Ingredient (Scientific Name)	Formulations	Common Medicinal Benefits
Ampalaya (*Momordica charantia*)	capsule, tea	anti-diabetes
Banaba (*Lagerstroemia speciosa*)	capsule, tea	good remedy for diabetes, high blood pressure and kidney problems
Coconut (*Cocos nucifera*)	Oil	fights viral, bacterial, fungal and protozoan infections; aids in digestion and nutrient absorption
Garlic (*Allium sativa*)	capsule	anti-cholesterol
Ginger (*Zingiber officinale*)	tea	relief for gaseous distention, cough, arthritis and wounds
Lagundi (*Vitex negundo*)	capsule, syrup, tea	anti-cough, asthma relief
Malunggay (*Moringa oleifera*)	capsule, coffee	relief for arthritis, scabies, wounds and constipation
Mangosteen (*Garcinia mangostana*)	capsule, tea	relief for diarrhea and stomach pain; contains anti-oxidant
Narra (*Pterocarpus indicus*)	capsule	immune system support
Noni (*Morinda citrifolia*)	syrup	anti-oxidant; supports immune system; has tumor-fighting properties
Sambong (*Blumea balsamifera*)	capsule, tea	diuretic
Spirulina (*Arthrospira*)	capsule	antioxidant, anti-inflammatory and detoxifier.
Turmeric (*Curcuma longa*)	capsule	relief for digestive disorders
Papaya (*Carica papaya*)	syrup	relief for constipation and wounds

For the solid samples, tea capsules/tea bags/coffee sachets were pooled and mixed to achieve sample homogeneity. From the pooled sample, triplicate samples (0.2500 g weighed to the nearest 0.0001 g) were obtained and digested using a Multiwave 3000 microwave system (Anton Paar, Austria). For samples in liquid form, triplicate aliquots (1 mL) were obtained for digestion. The digestion mixture is composed of 2 mL concentrated superspure HNO_3, 1 mL concentrated superspure HCl, and 5 mL ultrapure water. The digestion program for the complete mineralization of samples was as follows: digestion was initially carried out at 250 W for 1 minute; then ramped to 400 W for 5 minutes; 600 W for 5 minutes; and, 800 W for 20 minutes. The digestion system was allowed to cool before the digested samples were transferred to polyethylene containers, bulked to 15 mL with ultrapure water, and subsequently filtered through 0.45 µm Nylon filters prior to elemental analysis.

The concentrations of metals in tea infusions were also measured. For every brand of tea, three infusions prepared from different tea bags obtained from the same batch were analyzed. Tea infusion was made by adding 40 mL boiled (~100 ^{o}C) ultrapure water to a tea bag. The infusion was kept for 3 minutes, which is the commonly suggested length of infusion when preparing tea drinks. The infusion was filtered through a 0.45 µm Nylon filter, mixed with 0.5 mL concentrated HNO_3, and bulked to 50 mL with ultrapure water. Because of the formation of precipitates upon acidification, the infusions were filtered again with new Nylon filters prior to elemental analysis.

Simultaneous analyses of Cr, Cd, Sn and Pb in acid-digested sample solutions and tea infusions were performed using an Agilent 7500cx ICP-MS (Agilent, Germany) equipped with a MicroMist glass concentric nebulizer and an integrated autosampler (I-AS with type A vials, 89 x 6 mL capacity). Calibration standards ranging between 0.01 µg/L and 500 µg/L were prepared from stock standard solutions of Cr, Cd, Sn and Pb (10000 µg/mL, CPI Technology, USA). For Cr, the linear range was established from 0.01 µg/L to 500 µg/L. A wider range of concentration was necessary since ERM CD281 and the samples contain relatively higher amount of Cr. The linearity for Cd, Sn and Pb were set at a much narrower range of concentration (0.01 µg/L - 50 µg/L). The correlation coefficients (r^2) for all the calibration curves were 0.9999 or better. All solutions analyzed were spiked on-line with internal standard mixture containing Ge (1 mg/L), In (10 mg/L), and Re (10 mg/L) for signal drift correction. In addition, intra- and inter-day variabilities of the method were assessed by repeated measurements of a solution containing a final concentration equivalent to 1 ppb of each analyte and ERM CD281-digested solutions, respectively. The detection limit was estimated by analyzing seven individually prepared solutions containing 1 µg/L of each analyte in 1% HNO_3, which were subjected to digestion and subsequent

ICPMS analysis. The detection limit was only estimated for aqueous digests and not for solid samples because of the difficulty of ensuring homogeneity of spiked standards in solid samples. The standard deviations of the seven measurements multiplied with the student's t-value (i.e., student's t-value for n = 7 at 99% confidence level is 3.143) represents the estimated detection limit described in EPA document 40 CFR part 136.

RESULTS AND DISCUSSION

A prerequisite to trace metal analysis in actual samples is the existence of an analytical method that is optimized for the specific measurement and duly validated to be suitable for the deemed purpose. Prior to the analysis of herbal products, the reliability of the method was established by analyzing CRM and spiked liquid herbal product. The results from the analysis of the different CRM are summarized in Table 2. Results from the analysis of the primary CRM, ERM-CD281 (n = 15), revealed that the recoveries for Cr, Cd, Sn and Pb were at least 80%. Results from other solid reference materials DORM-3 and BCR 150 showed that measured values approximate well the certified values of the analytes, indicating that the mineralization procedure and subsequent elemental determination were robust for the determination of more complex sample matrices. This is particularly advantageous for the samples in this study which were of complex nature. Results of the analysis of NIST 1643e revealed that the optimized method was suitable for the analysis of Cr, Cd and Pb as reflected by the agreement between certified and measured values.

To further validate the suitability of the method for liquid samples, as tea infusions may have more complex matrix than water, we also carried out the standard addition

Table 2. Validation of digestion method and ICPMS detection in CRM (mean ± SD, n = 15 for ERM-CD281, n =3 for other CRM)

Certified Reference Material		Cr mg/kg	Cd mg/kg	Sn mg/kg	Pb mg/kg
ERM-CD281	Certified Value	24.8 ± 1.3	0.120 ± 0.072	0.062 ± 0.011	1.67 ± 0.11
	Measured Value	19.8 ± 0.5	0.101 ± 0.003	0.067 ± 0.003	1.46 ± 0.03
DORM-3	Certified Value	1.89 ± 0.17	0.290 ± 0.020	0.066 ± 0.012	0.39 ± 0.05
	Measured Value	2.08 ± 0.10	0.27 ± 0.01	0.08 ± 0.03	0.33 ± 0.01
BCR 150	Certified Value	not certified	0.0218 ± 0.014	not certified	1.00 ± 0.04
	Measured Value		0.018 ± 0.001		0.76 ± 0.01
Certified Reference Material		**Crμg/L**	**Cdμg/L**	**Snμg/L**	**Pbμg/L**
NIST	Certified Value	20.4 ± 0.24	6.568 ± 0.073	not certified	19.63±0.21
	Measured Value	21.4 ± 0.4	6.7 ± 0.1		20.2 ± 0.7

method (Table 3). One of the liquid samples, Noni juice, was spiked with mixtures containing different concentrations of the target analytes. Except for Cr at the lowest spiked concentration, most of the analytes at different concentrations resulted in acceptable recoveries within the range of 90% to 120%. The high standard deviation observed in the values obtained for Cr at the lowest spiked concentration may have been due to the difficulty in completely mineralizing Cr after it has equilibrated with the Noni juice. Evaluation of the method detection limits (MDL) following the suggested protocol by the US Environmental Protection Agency resulted in the following values: 0.15, 0.07, 0.3, and 0.14 µg/L for Cr, Cd, Sn and Pb, respectively. These values were low enough to allow trace determination of the analytes should they be present as contaminants in the target samples. Digested samples and tea infusions with metal concentrations below MDL were reported as <MDL.

Table 3. Validation of digestion method and ICPMS detection by standard addition. Various concentrations of Cr, Cd, Sn and Pb standards were spiked in Noni juice

Concentrations of the spiked standards, µg/L	% Recovery (n = 3)			
	Cr	Cd	Sn	Pb
0.67	163 ± 20	109 ± 1	116 ± 8	120 ± 20
3.33	95 ± 4	100 ± 1	111 ± 3	100 ± 1
6.67	99 ± 4	100 ± 1	112 ± 1	103 ± 3
13.33	109 ± 3	97 ± 2	111 ± 2	93 ± 1
33.33	99 ± 3	92 ± 1	105 ± 1	91 ± 1

The precision of the method was further assessed by measuring intra-batch and inter-day repeatability. Intra-batch repeatability was determined by monitoring the response of a 1.0 µg/L standard solution. During an entire ICP-MS run, the same solution was read at regular intervals to check if the instrument gave stable signals. From the response of the instrument, %RSD was calculated for every ICP-MS run. The calculated %RSD obtained for Cr, Cd, Sn and Pb during the entire course of the study (6 different ICP-MS runs were performed) were within 5% and were generally within 2% for each analysis day. Inter-batch repeatability was evaluated by analyzing ERM CD281 samples in triplicates for five different days. The concentrations measured for Cr, Cd, Sn and Pb are shown in Table 4. The method was suitable for the determination of the target elements whose measured concentrations were comparable to certified values. Overall, the results obtained on different days of analyses suggest that the optimized method is reliable and robust enough to be used over time.

Table 4. Inter-batch repeatability study using ERM CD281 measured during five different analyses days (n = 3 per day of analysis)

Element	Certified Values	Measured concentration (mg/kg) and standard deviation of measurements (*SD*)				
		Day 1	Day 2	Day 3	Day 4	Day 5
Cr	24.8 (*1.3*)	24.9 (*0.9*)	19.4 (*0.6*)	22.8 (*0.3*)	19.6 (*0.2*)	19.8 (*0.5*)
Cd	0.12 (*0.007*)	0.098 (*0.008*)	0.086 (*0.003*)	0.099 (*0.001*)	0.096 (*0.002*)	0.101 (*0.003*)
Sn	0.062 (*0.011*)	0.071 (*0.004*)	0.055 (*0.009*)	0.04 (*0.01*)	0.049 (*0.008*)	0.067 (*0.003*)
Pb	1.67 (*0.11*)	1.38 (*0.01*)	1.18 (*0.05*)	1.42 (*0.02*)	1.24 (*0.04*)	1.46 (*0.03*)

The mean concentrations of Cr, Cd, Sn and Pb from triplicate measurements of solid and liquid herbal supplement and products are summarized in Tables 5 and 6, respectively. Generally, the order of abundance of metals found in herbal products was Cr > Pb > Cd > Sn. For Cr, four samples obtained concentrations that were below the MDL. In solid samples, including tea leaves, coffee powder, and capsules, Cr was determined to be within the range of 0.14 to 2.6 mg/kg. In liquid samples, including juice and oils, the values ranged from 50 to 1100 µg/L. We observed that the values obtained for Cr are relatively higher than what Solidum et al. (2012) measured in Philippine herbal products, wherein only 3 out of 10 products have measurable Cr concentrations that range between 0.0084 and 0.0578 mg/kg. The Cr concentrations measured in this study are comparable to values (0.34–2.12 mg/kg in herbs) reported in a study performed in Turkey (Baºgel and Erdemoğlu 2006). However, compared to the results obtained in studies done in Pakistan (11.4–66.6 mg/kg) and Poland (0.3–63.1 mg/kg), the samples in the present study have much lower concentrations of Cr (Leœniewicz et al. 2006; Mahmood et al. 2013). The detection of Cr in most samples warrants further studies that focus on speciation studies that attempt to find out whether Cr is present in these products as Cr (VI), which poses a higher health risk compared to Cr (III).

Pb, another ubiquitous contaminant in the environment, was determined in 30 samples and was below the detection limit in three samples. The concentrations were within the range 0.02–1.2 mg/kg and 5–51 µg/L in solid and liquid products, respectively. Although none was found to exceed the 10 mg/kg guideline value, the determined values suggest that some products are susceptible to Pb contamination at significant concentrations. Pb is known to be bio-accumulated, and since most of these products are taken regularly, presence of Pb at trace concentrations may lead to health risks. In the measurements made by Solidum (2014) for Pb, medicinal plants and herbal tea ingredients in the Philippines were in the range of 0.006 – 0.873 mg/kg, which is comparable to this study. Compared to herbal products from other countries, the levels of Pb measured in the Philippines (e.g., this study and Solidum 2014) were lower. For instance, some samples exceeded the 10 mg/kg limit in studies performed in Pakistan (0.5 – 41.01 mg/kg) by Mahmood et al. (2013) and in Malaysia (2.34 – 13.2 mg/kg) by Ang (2008). The coffee in this study was also found to have lower Pb concentration (0.02 mg/kg) than that of the study made by Kapur and West (1974) in England (0.45 – 1.65 mg/kg).

Table 5. Concentrations of Cr, Cd, Sn and Pb in solid herbal products. Values represent mean ± SD of triplicate samples

Sample ID	Herbal Ingredient	Form	mg/kg ± Standard Deviation (n = 3)			
			Cr	Cd	Sn	Pb
A1	Ampalaya	Capsule	2.6 ± 0.2	0.127 ± 0.002	< MDL	0.33 ± 0.02
A2	Ampalaya	Capsule	0.98 ± 0.04	0.059 ± 0.001	< MDL	0.6 ± 0.1
A3	Ampalaya	Tea leaves	0.8 ± 0.2	0.052 ± 0.001	0.059 ± 0.003	0.58 ± 0.03
A4	Ampalaya	Tea leaves	0.25 ± 0.01	0.013 ± 0.001	0.010 ± 0.003	0.13 ± 0.02
B1	Banaba	Capsule	0.9 ± 0.1	0.187 ± 0.004	< MDL	1.0 ± 0.3
B2	Banaba	Tea leaves	0.8 ± 0.1	0.223 ± 0.008	0.054 ± 0.004	0.19 ± 0.01
B3	Banaba	Tea leaves	0.7 ± 0.1	0.187 ± 0.003	0.027 ± 0.003	0.19 ± 0.02
G1	Garlic	Capsule	0.43 ± 0.05	0.074 ± 0.004	< MDL	0.2 ± 0.1
R1	Ginger	Tea leaves	4.2 ± 0.2	0.057 ± 0.001	0.013 ± 0.004	0.11 ± 0.02
L1	Lagundi	Capsule	1.1 ± 0.3	0.011 ± 0.001	< MDL	0.5 ± 0.1
L2	Lagundi	Capsule	0.6 ± 0.1	0.11 ± 0.01	< MDL	0.20 ± 0.03
L3	Lagundi	Tea leaves	0.58 ± 0.04	0.010 ± 0.002	0.035 ± 0.001	0.19 ± 0.01
L4	Lagundi	Tea leaves	0.6 ± 0.1	0.014 ± 0.002	0.028 ± 0.001	0.15 ± 0.01
M1	Malunggay	Capsule	0.5 ± 0.1	0.03 ± 0.01	0.04 ± 0.01	0.30 ± 0.03
M2	Malunggay	Capsule	< MDL	0.025 ± 0.001	0.07 ± 0.01	0.71 ± 0.01
M3	Malunggay	Coffee	0.16 ± 0.04	< MDL	0.003 ± 0.001	0.02 ± 0.01
M4	Malunggay	Tea leaves	0.8 ± 0.1	0.007 ± 0.001	0.02 ± 0.01	0.12 ± 0.04
M5	Malunggay	Tea leaves	0.14 ± 0.02	0.012 ± 0.001	0.029 ± 0.003	0.15 ± 0.02
T1	Mangosteen	Capsule	0.21 ± 0.03	0.083 ± 0.001	<MDL	0.07 ± 0.03
T2	Mangosteen	Capsule	< MDL	0.29 ± 0.01	0.02 ± 0.01	0.10 ± 0.01
T3	Mangosteen	Tea leaves	0.4 ± 0.1	0.43 ± 0.02	0.02 ± 0.01	0.09 ± 0.01
N1	Narra	Capsule	< MDL	0.010 ± 0.001	0.036 ± 0.002	0.44 ± 0.02
S1	Sambong	Capsule	2.8 ± 0.1	0.216 ± 0.002	0.20 ± 0.02	1.2 ± 0.2
S2	Sambong	Tea leaves	0.23 ± 0.04	0.22 ± 0.01	0.012 ± 0.002	0.3 ± 0.1
S3	Sambong	Tea leaves	0.89 ± 0.01	0.18 ± 0.01	0.03 ± 0.01	0.3 ± 0.1
P1	Spirulina	Capsule	0.6 ± 0.1	0.018 ± 0.001	0.013 ± 0.001	< MDL

The detection limits for Cr, Cd, Sn, and Pb were 0.15, 0.07, 0.3, and 0.14 μg/L, respectively. The solutions of digested samples with concentrations below the detection limits were reported as <MDL. Tea samples from which infusions were prepared from are indicated. The trace metals in the infusions are summarized in Table 7.

Table 6. Concentrations of Cr, Cd, Sn and Pb in juice and other liquid herbal products. Values represent mean ± SD of triplicate samples

Sample ID	Herbal Ingredient	Concentration, µg/L			
		Cr	Cd	Sn	Pb
C1	Coconut	60 ± 10	< MDL	< MDL	7 ± 2
C2	Coconut	< MDL	< MDL	< MDL	5 ± 1
L5	Lagundi	400 ± 100	< MDL	< MDL	22 ± 3
L6	Lagundi	50 ± 1	< MDL	< MDL	< MDL
L7	Lagundi	50 ± 1	< MDL	< MDL	< MDL
O1	Noni	60 ± 1	< MDL	11 ± 1	6 ± 1
P1	Papaya	1100 ± 300	2 ± 1	36 ± 1	51 ± 5

The method detection limits for Cr, Cd, Sn and Pb were 0.15, 0.07, 0.3, and 0.14 µg/L, respectively. The solutions of digested samples with concentrations below the detection limits were reported as <MDL.

In the case of Cd, one solid sample had a value below the detection limit, while only one liquid sample had a Cd level higher than the detection limit. Cd is another persistent contaminant in the environment, and the solid samples had Cd levels in the range of 0.0073–0.42 mg/kg. Two different mangosteen-based herbal products contained the highest Cd concentrations (0.29 and 0.42 mg/kg), with one exceeding the permissible limit of 0.3 mg/kg. In a study by Zein et al. (2010), they were able to establish the possibility of using mangosteen shell as a biosorbent for Cd, Pb, and Co because it contains functional groups (e.g., hydroxyl, amino, ester, carboxyl and ether) for metal binding. The findings in this study could perhaps explain the propensity of mangosteen to take up metals present in the environment where they were grown. However, analysis of more mangosteen products should be made in order to verify the uptake of metals by mangosteen. In the study made by Solidum et al. (2012) of 10 herbal products, including mangosteen, Cd was not measured. It is likely that the instrument was not sensitive enough to measure low-level Cd since they reported a detection limit of 0.050 mg/kg. Generally, the Cd concentrations found in the present study were comparable to those found in medicinal plants included in the studies carried out by Başgel and Erdemoğlu in 2006 (0.004–0.44 mg/kg) and Leśniewicz et al. in 2006 (0.09–0.42 mg/kg). The results from the study of Mahmood et al. (2013) in Pakistan (0.09–0.96 mg/kg) reflected slightly higher values than those obtained in this study.

Among the four elements, Sn is the least frequently studied trace metal, albeit being one of the most important targets for speciation studies due to the toxicity of different organotin compounds. In this study, Sn was found to be the least abundant, and was only quantified in 21 samples whose concentrations ranged between 0.003 – 0.20 mg/kg and 11 – 36 µg/L in solid and liquid products, respectively. The highest concentration of Sn (0.20 mg/kg) was measured in a sambong capsule. The results from the present study were much lower compared to the results observed by ª ahan et al. (2007), wherein the concentrations of Sn in olives were in the range of 14.40 – 53.62 mg/kg.

The results from the analysis of tea leaves showed that most tea samples have measurable concentrations of Cr, Cd, Sn and Pb (Table 5). However, these are not directly ingested like capsules and syrups, hence we deemed it necessary to analyze tea infusions. The amount of metals measured in tea infusions were normalized to the weight of the tea leaf powder used (Table 7). Only small fractions of metals leached out to the tea infusions. The metal concentrations measured in tea infusions were in the range of 0.006 – 0.40 mg/kg for Cr, Cd and Pb. Sn, which was the least abundant among the four metals, was not detected at all in any of the infusions, while Cr and Pb were measured in almost all samples. Cd was only measured in five products, which include the mangosteen tea leaves that exceeded the limit. The mean concentration of Cd in the infusion of mangosteen tea leaves that

Table 7. Amount of Cr, Cd and Pb that leached out in tea infusions (mg) normalized to the weight of tea leaves (kg)
Values represent the average concentration (± SD) or the range of metal concentrations determined in triplicate tea infusion preparations

Sample ID	Herbal Ingredient	mg/kg ± SD		
		Cr	Cd	Pb
A3	Bitter Gourd	0.04 ± 0.02	< MDL	0.03 ± 0.01
A4	Bitter Gourd	0.012 ± 0.005	< MDL	0.01 ± 0.01
B2	Banaba	0.05 ± 0.02	< MDL to 0.004	0.02 ± 0.02
B3	Banaba	0.06 ± 0.01	< MDL to 0.003	0.02 ± 0.02
R1	Ginger	0.40 ± 0.03	< MDL	0.01 ± 0.01
L3	Lagundi	0.09 ± 0.05	< MDL	0.03 ± 0.01
L4	Lagundi	0.28 ± 0.06	< MDL	0.03 ± 0.01
M4	Malunggay	0.30 ± 0.04	< MDL	0.01 ± 0.01
M5	Malunggay	0.06 ± 0.02	< MDL	0.02 ± 0.02
T3	Mangosteen	< MDL to 0.006	0.005 ± 0.001	0.01 ± 0.01
S2	Sambong	0.08 ± 0.04	< MDL to 0.01	0.03 ± 0.03
S3	Sambong	0.110 ± 0.003	0.0034 ± 0.0003	0.01 ± 0.01

Sn was below MDL for all samples. The method detection limits for Cr, Cd, Sn and Pb were 0.15, 0.07, 0.3, and 0.14 µg/L, respectively. The solutions of digested samples with concentrations below the detection limits were reported as <MDL.

exceeded the limit was 0.005 mg/kg, which is about 80-fold lower than the Cd found in the actual tea leaves. From the results of triplicate analyses, it can be observed that some of the infused samples have varied concentrations of the metals (high SD especially for Pb). Although the temperature of the water used and the length of infusions were constant, minor variations during the actual preparation were inevitable. The actual level of metals in each tea bag may vary as well. During preparation, the colors of the resulting infusions were observed to be not similar even when the same product was being compared. These factors were considered as possible reasons as to why the results of the replicates vary in some samples.

Although the extent of metal contamination is minimal, with only one product exceeding the limit, vigilant monitoring is still crucial, since bioaccumulation from prolonged exposure is highly possible. Moreover, the existence of at least one product that exceeds the prescribed limit suggests that, despite the presence of regulatory bodies, there could still be products in the market that do not adhere to the guideline values. The high variability of metal concentrations of herbal products from the same plant ingredients underlines the fact that all plants are susceptible to contamination, and factors, such as the environment at which the plants were grown and the level of quality control employed during processing, may have influenced the variations observed. As for the analysis of tea infusions, the results show that the elemental impurities present in the tea leaves may not be completely transferred to infusions. Since we only sampled a small fraction of the herbal products available in the market, it is encouraged that more herbal products be tested for heavy metal contamination. Finally, the presence of Cr in almost all samples and Sn in some samples suggests that herbal products may possibly contain their harmful forms (e.g., Cr (VI) and organotin). It is therefore recommended to carry out speciation in order to determine the toxic forms of Cr and Sn, such as Cr (VI), trimethyltin, and triethyltin. The data provided in this study may be used as preliminary information for future speciation analysis of Cr and Sn.

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Joan S. De Vera <jsdevera.43@gmail.com> earned her BS and MS Chemistry degrees from the IC-UPD. This work comprised her MS thesis. JS De Vera is currently a Ph.D. candidate at the Department of Earth Sciences of the University of Toronto, Canada.

Leni L. Quirit <lquirit@yahoo.com> is a professor at the IC-UPD with specializations in analytical chemistry, radiochemistry, and nuclear chemistry. LL Quirit earned her Ph.D. Chemistry from the Ateneo De Manila University.

Irene B. Rodriguez <hanrodriguez@yahoo.com> is currently a postdoctoral fellow at the Academia Sinica in Taiwan. IB Rodriguez earned her doctoral degree in Chemistry from the Karl-Franzens University of Graz in Austria.