Monitoring of toxic heavy metals contamination in commonly used Chinese materia medica

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Abstract

Monitoring of toxic heavy metals (THMs) contamination, including lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg), for the quality control in Chinese materia medica (CMM) is critical for ensuring safe medication use in the pharmaceutical industry. A validated inductively coupled plasma–mass spectrometry method was used to determine THMs content in 1887 samples for 27 selected types of commonly used CMM. The correlation coefficient of the calibration curves for four THMs was higher than 0.995, indicating that the linearity is fine. The recovery rate was ranged in 82.6–105.4% for four THMs. The percentage of RSD in inter-day and intra-day precision test for four THMs was all lower than 10%. The limit of quantitation in test solution for Pb, As, Cd and Hg was 0.212, 0.500, 0.038, 0.044 µg/L, indicating that this validated ICP-MS method exhibited excellent sensitivity. Among these THMs, Pb and Cd are the most abundant contaminants in 27 selected types of CMM. In diverse permissible limits (PL) established by different countries/organizations, *Cinnamomi ramulus* and *Eucommiae cortex* usually exceeded the PL of 0.3 µg/g for Cd and occasionally exceeded the PL of 5.0 µg/g for Pb. *Chuanxiong rhizome* seldom exceeded the PL of 1.0 µg/g for Cd. *Eucommiae cortex*, *Raphani semen*, *Atractylodis macrocephalae rhizoma* and *Bupleuri radix* occasionally exceeded the PL of 0.3 µg/g for Cd. In conclusion, we hope to raise awareness of THMs contamination in commonly used CMM that affects safe medication use among farmers, manufacturer, and consumers.

Keywords Chinese materia medica · Inductively coupled plasma-mass spectrometry · Method validation · Permissible limit · Toxic heavy metals

Abbreviations

JF	Jujubae fructus	KF	Kochiae fructus
AR	Astragali radix	AF	Arctii fructus
HR	Hedysari radix	CAS	Cassiae semen
GRER	Glycyrrhizae radix et rhizome	PS	Plantaginis semen
CIR	Cinnamomi ramulus	BS	Benincasae semen
ASR	Angelicae sinensis radix	CHR	Chuanxiong rhizome
CC	Cornu cervi	PAR	Paeoniae alba radix
RR	Rehmanniae radix	GR	Ginseng radix
P	Poria	COR	Codonopsis radix
RS	Raphani semen	TCEP	Testudinis carapax et plastrum
CUS	Cuscutae semen	CRP	Citri reticulatae pericarpium
		EC	Eucommiae cortex

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AMR	Atractylodis macrocephalae rhizome
BR	Bupleuri radix
SR	Scutellariae radix
AAS	Armeniacae amarum semen
THMs	Toxic heavy metals
Pb	Lead
As	Arsenic
Cd	Cadmium
Hg	Mercury
CMM	Chinese materia medica
PL	Permissible limit
THP	Taiwan herbal pharmacopeia
HKCMMS	Hong Kong Chinese materia medica standards
ICP-MS	Inductively coupled plasma mass spectrometry
ChP	Chinese pharmacopoeia
LOQ	Limit of quantitation
TFDA	Taiwan food and drug administration

1 Introduction

Heavy metals are naturally existing elements widely distributed in our living environment and therefore can be absorbed into the human body through external sources, such as water, food, air, industrial waste, and chemical pollutants. Some toxic heavy metals (THMs), including lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg), can cause harmful health effects with extremely small amounts. As recommended by the Joint FAO/WHO Expert Committee for Food Additives (JECFA), the provisional tolerable weekly intake (PTWI) of Pb, inorganic As, Cd and Hg were set as 25 µg/kg body weight (BW) [1], 15 µg/kg BW [2], 7 µg/kg BW [3], and 5 µg/kg BW [1], respectively. The maximum tolerable intake for a 60-kg healthy adult is 214 µg/d for Pb, 129 µg/d for inorganic As, 60 µg/d for Cd and 43 µg/d for Hg by calculation. THMs contamination in Chinese materia medica (CMM) can be attributed to its own properties, agricultural environment, and the processing method. For example, Cinnabaris and Realgar are red mercuric sulfate and arsenic sulfide mineral containing Hg and As, respectively. In addition, contamination in soil and water and improper use of pesticides and fertilizers during cultivation as well as the use of containers or additives containing THMs during the processing step contributed to THMs contamination.

Several analytical techniques, including flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, cold vapor atomic absorption spectrometry, atomic fluorescence spectrometry, high-performance liquid chromatography, inductively coupled plasma (ICP) optical emission spectrometry or mass spectrometry

(MS), X-ray fluorescence spectrometry, differential pulse polarography, neutron activation analysis, and anodic stripping voltammetry, have been used to determine heavy metals content in natural products or herbal products [4]. ICP-MS is one of the most sophisticated analytical instrumentation with the advantage of multi-elemental character, low limits of detection, wide dynamic range, etc. Several national pharmacopeias, including Taiwan Herbal Pharmacopeia (THP), Hong Kong Chinese Materia Medica Standards (HKCMMS) and Chinese Pharmacopoeia (ChP), have recommended ICP-MS as the analytical method to determine THMs content in CMM for ensuring the safety of pharmaceutical products. In this study, THMs content, including Pb, As, Cd, and Hg, was determined in 1887 samples for 27 selected types of CMM using nitric acid and microwave digestion followed by a validated ICP-MS method. The microwave was used for the CMM samples digestion because it exhibited better accuracy in relation to both time and recovery rate [5]. Based on the ICH Q2(R1), the validation items included linearity, accuracy (recovery rate), inter-day and intra-day precision, method detection limit (MDL) and limit of quantitation (LOQ) [6].

2 Materials and methods

2.1 CMM samples collection

A total of 1,887 samples for the 27 types of selected CMM were purchased from different provinces of China and chosen according to the top 20 imported CMM between 2016 and 2018. The imported quantities of the CMM were retrieved by the department of Chinese Medicine and Pharmacy, Ministry of Health and Welfare (Taipei, Taiwan), as indicated in Table 1. It should be noted that the sum of imported quantities of Astragali radix (AR) and Hedysari radix (HR) is ranked second. In addition, the sum of imported quantities of seven CMM, including Raphani semen (RS), Cuscutae semen (CUS), Kochiae fructus (KF), Arctii fructus (AF), Cassiae semen (CAS), Plantaginis semen (PS) and Benincasae semen (BS), is ranked ninth.

2.2 Preparation of analytical sample solution

All CMM were ground into powder using pulverizer followed by passing through a 40-mesh filter. Briefly, 0.2 g of each sample powder was placed into a microwave digestion tube with 6 mL of concentrated nitric acid and 1.5 mL of hydrogen peroxide solution for 20 min. The sample solution was digested using MARS microwave-accelerated reaction system (MARS Xpress, CEM, USA). Digestion parameters for the microwave system were set as 1,600 W for microwave, 30 min for ramp time, 205 °C

Table 1 Toxic heavy metals contamination in 27 selected Chinese materia medica in our factory

Ranking	Imported quantity ^a	Items	No. of samples	Contaminated rate (%)				Maximal value (µg/g)				Mean value (µg/g)				Median value (µg/g)			
				Pb	As	Cd	Hg	Pb	As	Cd	Hg	Pb	As	Cd	Hg	Pb	As	Cd	Hg
1	7,655 Ton	JF	99	28	0	40	0	2.92	N.D	0.30	N.D	0.26	N.D	0.05	N.D	N.D	N.D	N.D	N.D
2	5,123 Ton	AR	125	48	3.2	53	0	1.73	0.42	0.19	N.D	0.34	N.D	0.07	N.D	N.D	N.D	0.10	N.D
		HR	38	50	11	95	2.6	1.66	0.56	0.39	0.33	0.44	N.D	0.13	N.D	N.D	N.D	0.14	N.D
3	3,762 Ton	GRER	140	61	11	31	2.1	2.10	1.02	0.20	1.25	0.41	N.D	0.04	N.D	0.22	N.D	N.D	N.D
4	3,473 Ton	CIR	84	100	1.2	96	0	10.98	0.55	0.90	N.D	4.23	N.D	0.52	N.D	3.62	N.D	0.51	N.D
5	3,226 Ton	ASR	133	74	20	59	0	3.22	1.37	0.25	N.D	0.78	N.D	0.07	N.D	0.69	N.D	0.10	N.D
6	3,016 Ton	CC	30	60	3.3	40	0	2.41	0.31	0.19	N.D	0.38	N.D	0.06	N.D	0.28	N.D	N.D	N.D
7	2,579 Ton	RR	124	65	9.7	63	0	2.17	4.05	0.19	N.D	0.59	N.D	0.08	N.D	0.28	N.D	0.06	N.D
8	2,324 Ton	P	125	27	0	34	1.6	1.54	N.D	0.22	0.07	0.14	N.D	0.04	N.D	N.D	N.D	N.D	N.D
		RS	13	85	31	100	0	1.17	0.57	0.08	N.D	0.37	N.D	0.25	N.D	0.26	N.D	0.28	N.D
		CUS	15	87	20	6.7	0	0.70	0.35	0.04	N.D	0.32	N.D	N.D	N.D	0.27	N.D	N.D	N.D
		KF	7	100	0	100	0	0.95	N.D	0.16	N.D	0.42	N.D	0.12	N.D	0.38	N.D	0.14	N.D
9	2,172 Ton	AF	12	58	33	83	0	0.63	0.39	0.13	N.D	0.29	N.D	0.07	N.D	0.39	N.D	0.08	N.D
		CAS	19	26	0	68	0	1.10	N.D	0.27	N.D	0.19	N.D	0.08	N.D	N.D	N.D	0.06	N.D
		PS	14	79	14	100	0	1.52	0.57	0.33	N.D	0.46	N.D	0.17	N.D	0.27	N.D	0.16	N.D
		BS	13	77	0	31	7.7	0.70	N.D	0.05	0.09	0.31	N.D	N.D	N.D	0.20	N.D	N.D	N.D
10	2,026 Ton	CHR	110	93	0.9	100	2.7	9.33	0.37	2.17	0.08	2.09	N.D	0.77	N.D	1.74	N.D	0.72	N.D
11	1,860 Ton	PAR	92	28	3.3	83	3.3	1.82	0.39	0.50	0.11	0.23	N.D	0.11	N.D	N.D	N.D	0.12	N.D
12	1,561 Ton	GR	131	52	2.3	96	0.8	3.54	0.93	0.44	0.20	0.43	N.D	0.18	N.D	0.16	N.D	0.17	N.D
13	1,304 Ton	COR	73	67	12	86	0	3.78	1.58	2.24	N.D	0.60	N.D	0.20	N.D	0.26	N.D	0.13	N.D
14	1,156 Ton	TCEP	23	70	8.7	48	0	1.44	1.20	0.27	N.D	0.60	N.D	0.08	N.D	0.66	N.D	N.D	N.D
15	1,068 Ton	CRP	69	88	4.3	35	0	3.61	1.60	0.19	N.D	0.99	N.D	0.05	N.D	0.59	N.D	N.D	N.D
16	1,051 Ton	EC	22	96	0	100	4.5	13.85	N.D	0.40	0.11	3.77	N.D	0.25	N.D	3.21	N.D	0.24	N.D
17	931 Ton	AMR	106	57	1.9	97	0	2.84	1.29	1.46	N.D	0.62	N.D	0.26	N.D	0.17	N.D	0.20	N.D
18	931 Ton	BR	114	91	32	92	1.8	8.43	1.63	2.26	0.14	1.83	N.D	0.22	N.D	1.70	N.D	0.18	N.D
19	870 Ton	SR	107	59	6.5	58	0	3.50	0.30	0.34	N.D	0.56	N.D	0.09	N.D	0.33	N.D	0.11	N.D
20	742 Ton	AAS	49	10	0	31	0	1.38	N.D	0.14	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Total	46,827 Ton	-	1887	61	7.6	67	0.9	-	-	-	-	-	-	-	-	-	-	-	-

^aThe imported quantities of the CMM were retrieved by the department of Chinese Medicine and Pharmacy, Ministry of Health and Welfare (Taipei, Taiwan)

N.D. is represented as the detected value below LOQ

for temperature, and 20 min for hold time. After digestion, 1 mL of internal stock standard solution was added to deionized water to produce a final volume of 100 mL followed by passing through a 0.45- μ m filter to obtain the analytical sample solution.

2.3 Preparation of standard solution

One thousand μ g/mL of Pb, As, Cd, Hg, rhodium (Rh), and gold (Au) standard solution was purchased from Sigma-Aldrich (St. Louis MO, USA). The external standard stock solution I containing 10 μ g/mL of Pb and As and 1 μ g/mL of Cd and Hg was prepared by adding 1 mL of 1,000 μ g/mL of Pb and As standard solution and 0.1 mL of 1,000 μ g/mL of Cd and Hg standard solution to 1% (v/v) nitric acid solution to produce a final volume of 100 mL. The external standard stock solution II containing 1 μ g/mL of Pb and As and 0.1 μ g/mL of Cd and Hg was prepared by adding 1 mL of external stock standard solution I to 1% (v/v) nitric acid solution to produce a final volume of 10 mL. The internal standard stock solution consisting 1 μ g/mL of Rh and 10 μ g/mL of Au was prepared by combining 0.1 mL of 1000 μ g/mL of Rh and 1 mL of 1000 μ g/mL of Au standard solution to 1% (v/v) nitric acid solution to produce a final volume of 100 mL. Standard working solution containing 0.2–50 μ g/L of Pb and As, 0.02–5 μ g/L of Cd and Hg, 10 μ g/L of Rh and 100 μ g/L of Au was made by combining different volumes (10, 50, 250, 1000 and 2500 μ L) of the external standard stock solution II to 500 μ L of internal standard stock solution diluted in 1% (v/v) nitric acid to a final volume of 50 mL.

2.4 ICP-MS condition

A PerkinElmerNexIONTM300X ICP-MS (MA, USA) was used for simultaneous detection of Pb, As, Cd and Hg content in CMM. The setting of the ICP-MS condition was established as the followings: plasma power 1500 W, carrier gas Ar, plasma gas flow 18 L/min, auxiliary gas flow 1.2 L/min and nebulizer gas flow 1.1 L/min. The m/z ratios for the four THMs were set as ^{208}Pb , ^{75}As , ^{111}Cd and ^{202}Hg .

2.5 Method validation

The linearity of the calibration curves for the four THMs was established using five different concentrations (0.2–50 μ g/L for Pb and As and 0.02–5 μ g/L for Cd and Hg) in triplicates for each concentration. The intercept, slope of the regression line and the correlation coefficient were recorded. The values of correlation coefficient for the four THMs should be higher than 0.995. For accuracy, free-THMs samples were spiked with low (4.0 μ g/mL for Pb and As and 0.4 μ g/mL for Cd and Hg), medium (5.0 μ g/mL for Pb

and As and 0.5 μ g/mL for Cd and Hg) and high (6.0 μ g/mL for Pb and As and 0.6 μ g/mL for Cd and Hg) concentrations of the four THMs followed by nitric acid and microwave digestion in triplicates. The recovery rate was calculated as (detection value $\div$ spike value) $\times$ 100%, and the detection value has been subtracted the elemental background from matrix. An acceptable quality level of recovery rate should be ranged in 75–120%. For inter-day and intra-day precision, free-THMs samples were spiked with 5.0 μ g/mL for Pb and As and 0.5 μ g/mL for Cd and Hg of the four THMs followed by nitric acid and microwave digestion in six times. The percentage of relative standard deviation (RSD) was calculated as follows: (SD value $\div$ mean value) $\times$ 100%, and the calculated value should be lower than 10%. For determining MDL and LOQ, the calibration curves for the four THMs were established using relative low different concentrations (0.01–1.0 μ g/L for Pb, 0.01–5.0 μ g/L for As and 0.01–2.0 μ g/L for Cd and Hg). Test solution containing 0.4 μ g/L for Pb, 1 μ g/L for As and 0.1 μ g/L for Cd and Hg was carried out in seven times. The slope of calibration curves for the four THMs was recorded. The standard deviation (SD) in ratio of intensities between standard and internal standard for seven times determination was calculated. The MDL and LOQ were calculated as $3.3 \times \text{SD} \div \text{slope}$ and $10 \times \text{SD} \div \text{slope}$, respectively.

3 Results and discussion

3.1 Method validation

The definition of validation is the action of checking or proving the validity or accuracy of developed experimental method. Evaluation by ICP-MS method was validated by assessing the correlation coefficient, accuracy (recovery rate), inter-day, and intra-day precision, MDL, and LOQ. The correlation coefficient of the calibration curves for the four THMs were all higher than 0.995, indicating that the linearity is fine (Table 2). THMs determination by ICP-MS is known to be interfered by spectral and non-spectral interferences. This interference might affect the low recovery of THMs. For example, the determination of As (m/z 75) by ICP-MS can be interfered by ArCl^+ (m/z 75), polyatomic species. Herein, we used dynamic reaction cell (DRC) technology to solve this interference. DRC technology features a scanning quadrupole that removes all interferences created by reactions in the Universal Cell, which enables any reactive gas to be used. By optimizing the cell's quadrupole conditions, only the element of interest is allowed to pass through to the analyzing quadrupole. It is well recognized that ion-molecule reaction chemistry offers the best performance for the reduction of complex polyatomic spectral interference [7]. The recovery rate was

Table 2 Method validation

Validation items	Pb	As	Cd	Hg
Linearity ($n = 3$)				
Concentration range ($\mu\text{g/L}$)	0.2–50.0	0.2–50.0	0.02–5.0	0.02–5.0
Equation	$y = 0.0527x + 0.01181$	$y = 0.0023x + 0.00074$	$y = 0.0112x + 0.00005$	$y = 0.0065 + 0.00014$
Correlation coefficient	0.99976	0.99985	0.99998	0.99995
Recovery ($n = 3$)				
Spike value ($\mu\text{g/mL}$)	4.0	5.0	5.0	0.5
Detection value—Average ($\mu\text{g/g}$) ^a	4.06	6.32	4.48	0.6
Recovery rate—Mean (%) ^b	101.4	105.3	89.7	84.7
Precision ($n = 6$)				
Day 1-Detection value—Average ($\mu\text{g/g}$) ^a	5.48	4.40	0.43	0.50
Day 1-Detection value—RSD (%) ^c	0.81	1.13	0.82	0.57
Day 2-Detection value—Average ($\mu\text{g/g}$) ^a	5.35	4.54	0.44	0.49
Day 2-Detection value—RSD (%) ^c	0.57	1.37	1.07	0.85
Day 3-Detection value—Average ($\mu\text{g/g}$) ^a	5.42	4.47	0.44	0.50
Day 3-Detection value—RSD (%) ^c	0.97	2.80	1.11	1.52
Day 1 to 3-Detection value—Average ($\mu\text{g/g}$) ^a	5.42	4.47	0.44	0.49
Day 1 to 3-Detection value—RSD (%) ^c	1.22	1.53	0.61	0.54
LOQ and MDL				
Concentration range ($\mu\text{g/L}$)	0.01–1.0	0.01–5.0	0.01–2.0	0.01–2.0
Equation	$y = 0.0595x + 0.0002$	$y = 0.0028x + 0.0008$	$y = 0.0104x + 0.0001$	$y = 0.00687x + 0.0001$
Correlation coefficient	0.9999	0.9999	0.9999	0.9997
Slope	0.0595	0.0028	0.0104	0.00687
Ratios of intensities—Average ($n = 7$) ^d	0.0082	0.0011	0.0006	0.0003
Ratios of intensities—SD ($n = 7$) ^d	0.00126	0.00014	0.00004	0.00003
LOQ in test solution ($\mu\text{g/L}$) ^e	0.212	0.500	0.038	0.044
LOQ in solid sample ($\mu\text{g/g}$) ^f	0.106	0.250	0.019	0.022
MDL in test solution ($\mu\text{g/L}$) ^g	0.070	0.165	0.013	0.014
MDL in solid sample ($\mu\text{g/g}$) ^h	0.035	0.0825	0.0065	0.007

^aThe detection value has been subtracted the elemental background from matrix^bThe recovery rate was calculated as detection value ÷ spike value × 100%^cRSD (%) was calculated as SD value ÷ Average × 100%^dRatio of intensities was calculated as the intensities of standard ÷ the intensities of internal standard^eLOQ in test solution was calculated as $10 \times \text{SD} \div \text{slope}$. SD is represented as the standard deviation in ratio of intensities between standard and internal standard^fLOQ in solid sample was calculated as LOQ value in test solution × diluted volume (0.1 L) ÷ sample weight (around 0.2 g)^gMDL in test solution was calculated as $3.3 \times \text{SD} \div \text{slope}$. SD is represented as the standard deviation in ratio of intensities between standard and internal standard^hMDL in solid sample was calculated as MDL value in test solution × diluted volume (0.1 L) ÷ sample weight (around 0.2 g)

in the range of 101.4–105.4% for Pb, 82.6–89.7% for As, 84.7–87.8% for Cd and 91.7–97.2% for Hg which meet the acceptable quality level of 75–120% (Table 2). The precision is the condition where the results from independent test are acquired with the same method on the same sample in the same or different day by the same or different operator for several replicates using the same analytical instrument [7]. The calculated RSD (%) in inter-day and intra-day precision for the four THMs in spiked samples was all lower than 10% (Table 2). The LOQ in test solution for Pb, As, Cd and Hg was 0.212, 0.500, 0.038, 0.044 µg/L, respectively, and in solid samples was 0.106, 0.250, 0.019, 0.022 µg/g, respectively (Table 2). The MDL in test solution for Pb, As, Cd and Hg was 0.070, 0.165, 0.013, 0.014 µg/L, respectively, and in solid samples was 0.035, 0.0825, 0.0065, 0.007 µg/g, respectively (Table 2). Results obtained from LOQ and MDL indicated that this validated ICP-MS method exhibited excellent sensitivity.

3.2 Monitoring of THMs contamination in selected CMM

CMM has been regarded as a potential source of THMs toxicity, including Pb, As, Cd and Hg, to human and animals [8]. WHO has also recommended that CMM and its products should be determined for the presence of THMs [9]. Herein, an ICP-MS method was used to determine THMs content in a total of 1,887 samples for the 27 selected types of CMM. The maximal, average and median values of all CMM for four THMs are shown in Table 1. Pb was detected in 1,145 samples out of the total samples with the contaminated rate of 61% (Table 1). Among these Pb-contaminated CMM, *Cinnamomi ramulus* (CIR), RS, CUS, KF, *Chuanxiong rhizome* (CHR), *Citri reticulatae pericarpium* (CRP), *Eucommiae cortex* (EC) and *Bupleuri radix* (BR) are most commonly contaminated by Pb with the contaminated rate higher than 80% (Table 1). A total of 7 (6.4%) and 4 (3.5%) samples in CHR and BR, respectively, exceeded the PL of 5.0 µg/g for Pb as established by THP and HKCMMS (Table 4). In addition to CHR and BR, a total of 27 (32%) and 4 (18%) samples in CIR and EC, respectively, exceeded the PL of 5.0 µg/g for Pb as established by HKCMMS (Table 4). However, only 1 (1.2%) and 2 (9.1%) samples in CIR and EC, respectively, exceeded the PL of 10.0 µg/g for Pb as established by WHO and ISO18664:2015 (Table 4).

Moreover, As was detected in 143 samples out of the total samples with the contaminated rate of 7.6% (Table 1). RS, AF and BR are seldom contaminated by As with the contaminated higher than 30% (Table 1). None of the samples exceeded the PL of 3.0 or 5.0 µg/g as set by THP (Table 4). Similarly, none of the samples exceeded the PL of 2.0 or 5.0 µg/g as set by Japanese Pharmacopeia (JP)

(Table 4). Only 3 (2.4%), 2 (1.6%) and 1 (0.8%) samples in *Rehmanniae radix* (RR) exceeded the PL of 2.0 µg/g and 4.0 µg/g, respectively, as set by HKCMMS and ISO18664:2015, respectively (Table 4).

Furthermore, Cd was detected in 1,257 samples out of the total samples with the contaminated rate of 67% (Table 1). Nearly half of all CMM, including HR, CIR, RS, KF, AF, PS, CHR, *Paeoniae alba radix* (PAR), *Ginseng radix* (GR), *Codonopsis radix* (COR), EC, *Armeniacae amarum semen* (AMR) and BR, are most commonly contaminated by Cd with contaminated rate higher than 80% (Table 1). Only 1 (2.6%) sample in HR and 1 (1.1%) sample in PAR exceeded the PL of 0.3 µg/g for Cd as formulated by THP and HKCMMS (Table 4). Among the 27 selected CMM, the ChP only regulated the PL for THMs in AR, *Glycyrrhizae radix et rhizoma* (GRER) and PAR (Table 3). Similarly, only 1 (1.1%) sample in PAR exceeded the PL of 0.3 µg/g for Cd as formulated by ChP (Table 4). A total of 2 (2.7%), 19 (17%), 1 (0.9%) and 2 (1.8%) samples in COR, CHR, AMR and BR, respectively, exceeded the PL of 1.0 µg/g for Cd as formulated by THP and HKCMMS (Table 4). Under the PL of 0.3 µg/g for Cd as formulated by WHO, a total of 74 and 108 samples in CIR and CHR, respectively, with the exceeding rate was 88% and 98%, respectively (Table 4). Also, the exceeding rate in RS, EC, AMR and BR was ranged from 10% to 30% (Table 4). In addition, the exceeding rate in other Cd-contaminated CMM, including *Jujubae fructus* (JF), HR, PS, PAR, GR, COR and *Scutellariae radix* (SR), was lower than 10% (Table 4). Only 1 (0.9%) sample in CHR and 1 (0.9%) sample in BR exceeded the PL of 2.0 µg/g for Cd as formulated by ISO 18664:2015 (Table 4).

Likewise, Hg was detected in 17 (0.9%) samples out of the total samples (Table 1). The contaminated rate for Hg in all selected CMM was not higher than 10% (Table 1). Only 1 (0.7%) sample in GRER exceeded the PL of 0.2 µg/g for Hg as regulated by THP, ChP and HKCMMS (Table 4). Similarly, only 1 (2.6%) sample in HR exceeded the PL of 0.2 µg/g for Hg as regulated by THP and HKCMMS (Table 4).

3.3 Comparison of PL among different countries/organizations

As shown in Table 3, we further summarized the PL set by different countries/organizations for the four THMs of the all selected CMM. The Taiwan authority has renewed the PL for the four THMs in 355 types of CMM in 2016 and brought it into the THP [10]. The general rules of the PL in THP were established as 5.0, 3.0, 1.0, and 0.2 µg/g for Pb, As, Cd, and Hg, respectively, in most CMM [10]. For specific CMM, the PL was 10.0 µg/g in CUS or 15.0 µg/g in CIR and EC for Pb, 5.0 µg/g in ASR, RR, *Poria* (P), CHR, AMR, BR and SR for As, 0.3 µg/g in AR, HR, GRER and PAR or 1.5 µg/g for Cd and 0.5 µg/g for Hg (Table 3) [10]. The ChP has drawn

Table 3 The permissible limit of Pb, As, Cd, and Hg for 27 selected types of Chinese materia medica in different countries/organizations

Countries/organizations	Permissible limit (µg/g)				CMM
	Pb	As	Cd	Hg	
Taiwan ^a	5.0	3.0	1.0	0.2	JF, CC, RS, KF, AF, CAS, PS, BS, GR, COR, TECP, CRP, AAS
	5.0	3.0	0.3	0.2	AR, HR, GRER
	15.0	3.0	1.0	0.2	CIR, EC
	5.0	5.0	1.0	0.2	ASR, RR, P, CHR, AMR, BR, SR
	10.0	3.0	1.0	0.2	CUS
	5.0	5.0	0.3	0.2	PAR
Japan ^b	N/A	5.0	N/A	N/A	AR, GRER, ASR, RR, P, BR, SR
	N/A	2.0	N/A	N/A	GR
China ^c	5.0	2.0	0.3	0.2	AR, GRER, PAR
Hong Kong ^d	5.0	2.0	1.0	0.2	AR, HR, GRER, CIR, ASR, RR, CUS, KF, AF, CAS, PS, CHR, PAR, GR, COR, CRP, EC, AMR, BR, SR
WHO ^e	10.0	N/A	0.3	N/A	25 selected types of CMM (CC and TCEP excluded)
ISO ^f	10.0	4.0	2.0	3.0	27 selected types of CMM

^aTaiwan herbal pharmacopeia^bJapanese pharmacopeia^cChinese pharmacopoeia^dHong Kong Chinese materia medica standards^eWorld Health Organization^fISO18664:2015**Table 4** The percentage of Chinese materia medica exceeding permissible limits in different countries/organizations

CMM	Percentage of samples exceeding permissible limits (%)													
	Taiwan ^a			China ^b		Hong Kong ^c				WHO ^d		ISO ^e		
	Pb	Cd	Hg	Cd	Hg	Pb	As	Cd	Hg	Pb	Cd	Pb	As	Cd
JF	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–
HR	–	2.6	2.6	–	–	–	–	2.6	2.6	–	2.6	–	–	–
GRER	–	–	0.7	–	0.7	–	–	–	0.7	–	–	–	–	–
CIR	–	–	–	–	–	32	–	–	–	1.2	88	1.2	–	–
RR	–	–	–	–	–	–	2.4	–	–	–	–	–	0.8	–
RS	–	–	–	–	–	–	–	–	–	–	23	–	–	–
PS	–	–	–	–	–	–	–	–	–	–	7.1	–	–	–
CHR	6.4	17	–	–	–	6.4	–	17	–	–	98	–	–	0.9
PAR	–	1.1	–	1.1	–	–	–	–	–	–	1.1	–	–	–
GR	–	–	–	–	–	–	–	–	–	–	8.4	–	–	–
COR	–	2.7	–	–	–	–	–	2.7	–	–	9.6	–	–	–
EC	–	–	–	–	–	18	–	–	–	9.1	23	9.1	–	–
AMR	–	0.9	–	–	–	–	–	0.9	–	–	33	–	–	–
BR	3.5	1.8	–	–	–	3.5	–	1.8	–	–	13	–	–	0.9
SR	–	–	–	–	–	–	–	–	–	–	2.8	–	–	–

^aTaiwan Herbal Pharmacopeia^bChinese Pharmacopoeia^cHong Kong Chinese Materia Medica Standards^dWorld Health organization^eISO18664:2015

up the PL of 5.0, 2.0, 0.3 and 0.2 µg/g for Pb, As, Cd and Hg, respectively, in eleven types of CMM, whereas, the PLs were varied in some specific CMM [11]. Herein, only AR, GRER and PAR were included (Table 3). The JP only proposed the PL of 2.0 or 5.0 µg/g for As in certain CMM [12]. The PL for As was 2.0 µg/g in GR and 5.0 µg/g in AR, GRER, ASR, RR, P, BR and SR (Table 3). The nine volumes of HKCMMS covering standards for a total of 299 CMM have been published. The PL of 5.0, 2.0, 1.0 and 0.2 µg/g for Pb, As, Cd and Hg, respectively, as a general rule in most CMM [13]. The WHO only recommended the PL of 10.0 µg/g for Pb and 0.3 µg/g for Cd in dried plant materials [14]. The International Organization for Standardization (ISO) also released ISO 18,664:2015 and published the PL of 10.0, 4.0, 2.0, and 3.0 µg/g for Pb, As, Cd, and Hg, respectively, in all CMM except minerals [15].

Taking CIR for example, the PL of 15.0, 5.0 and 10 µg/g for Pb has been established by THP, HKCMMS and WHO, respectively, with the exceeding rate of 0%, 32% and 1.2%, respectively (Table 4). The PL of 1.0 and 0.3 µg/g for Cd in CIR has been set by THP and WHO, respectively, with the exceeding rate of 0% and 88% (Table 4). Another example is CHR. Similarly, the PL of 1.0 and 0.3 µg/g for Cd has been drawn up by THP and WHO, respectively, with the exceeding rate of 27% and 98%, respectively (Table 4). The diverse PL for the four THMs set by various countries/organizations is the major reason for the difference in the exceeding rate in CMM. As compared to other countries/organizations, the THP did set a more practical and reasonable PL. The THP established the PL for the four THMs based on the inspection data collected from several certificated Chinese herbal medicine pharmaceutical factories in Taiwan and on the 80th percentile values of the Taiwan Food and Drug Administration (TFDA) surveys from 2006 to 2013 [16–23] as well as referred to the PL established by other countries/organizations. There is an increasing number of people worldwide using CMM as remedy, health food and dietary supplements, therefore, it is necessary to establish the unifying PL for THMs in CMM. One limitation existed that no clear correlation was found between the levels of THMs in soil and collection site in CMM because our CMM samples were collected from herbal markets or imported from trading agent in different provinces of China. It should also be noted that the present results did not represent the exact quality of the CMM sold in Taiwan and our company only imported the qualified CMM.

4 Conclusions

This study provides insights into THMs, including Pb, As, Cd and Hg, contamination in the imported 27 types of selected CMM. Overall, Pb and Cd are the most abundant

contaminant in selected CMM. In diverse permissible limits (PL) established by different countries/organizations, CIR and EC usually exceeded the PL of 0.3 µg/g for Cd and occasionally exceeded the PL of 5.0 µg/g for Pb. CHR seldom exceeded the PL of 1.0 µg/g for Cd. EC, RS, AMR, and BR occasionally exceeded the PL of 0.3 µg/g for Cd. We hope to raise awareness of THMs contamination in CMM that affects safe medication use among farmers, manufacturer and consumers.

Author contributions Dr. Mei-Yin Chien and Dr. Chao-Hsiang Chen were involved in conception and design of study. Dr. Chih-Min Yang and Dr. Chih-Min Huang were involved in acquisition of data. Dr. Mei-Yin Chien, Dr. Chih-Min Yang and Dr. Chih-Min Huang were involved in analysis and interpretation of data. Dr. Mei-Yin Chien was involved in drafting the manuscript. Dr. Chao-Hsiang Chen was involved in revising the manuscript critically for important intellectual content. Dr. Mei-Yin Chien, Dr. Chih-Min Yang, Dr. Chih-Min Huang and Dr. Chao-Hsiang Chen were involved in approval of the version of the manuscript to be published.

Compliance with ethical standards

Conflicts of interest All authors listed in this study from our company and the results obtained from the present study were part of the quality control process in our company. Therefore, we declare that there is no conflict of interests.

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