

Determination of trace-elements and toxic heavy minerals in Thai longan, litchi and Siam weed honeys using ICP-MS

*Wetwitayaklung, P., Wangwattana, B. and Narakornwit, W.

Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University,
Nakhon-Pathom, Thailand. 73000

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Abstract

Eupatorium odoratum (Siam weed), *Litchi chinensis* (litchi) and *Dimocarpus longan* (longan) honeys harvested in Thailand were analyzed for their trace elements (Ag, Al, Ba, Cd, Co, Cs, Cu, Fe, Ga, Li, Mg, Mn, Rb, Se, Sr, and U) and toxic heavy metals (As, Be, Cd, Cr, Ni, Pb, Tl and V) contents using inductively coupled plasma mass spectrometry (ICP-MS). The validation of analytical procedure showed satisfactory results. The maximum and minimum heavy metals in Siam weed, litchi and longan honeys were Mg, (18.71±7.66, 10.19±3.01 and 21.83±5.00 mg/kg) and Be (0.67±0.24, 0.07±0.20 and 0.45±0.18 µg/kg) respectively. The analysis of the 3 honeys revealed sequence of overall toxic metals in the following: Pb > Ni > Cr > Cd > Tl > V > As > Be for Siam weed, Pb > Cr > Cd > Ni > Tl > V > As > Be for Litchi, and Ni > Pb > Cr > Cd > Tl > V > As > Be for Longan honey. The contents of toxic heavy metals in the samples did not exceed the established maximum level in foodstuffs according to Commission Regulation (EC) No.1881/2006. However one litchi honey sample contained Pb (1.07 mg/kg) with the highly deviated content from median of its group and more than the permissible limit regulated by the Ministry of Public Health of Thailand (< 0.5 mg/kg). This may suggested an environmental contaminant of Pb to the sample.

Keywords

Thai honey
Longan
Litchi
Siam weed
Heavy metals

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Introduction

Honey is a natural foodstuff from animal origin that has been known forages as a valuable nutritional and medicinal product. It provides much energy and high nutritional properties that are suitable for all health conditions. In Thailand, popular honeys with different botanical origins for consumers are *Dimocarpus longan* (longan, family Sapindaceae), *Litchi chinensis* (litchi, family Sapindaceae) and *Eupatorium odoratum* (Siam weed, family Asteraceae) honeys. The longan and litchi honeys are derived from orchard trees, but Siam weed honey is derived from wild herb. Thai longan, litchi and Siam weed honeys have been reported to promote antioxidant and antibacterial activities (Montra and Chantawannakul, 2010). The constituents of honey are fructose, glucose, water, vitamins, proteins, enzyme, amino acids, organic acids, ash, phenol compounds and minerals (Ouchemoukh *et al.*, 2007; Alvarez-Suarez, 2010, Mahmoudi *et al.*, 2012). The natural mineral and trace element content of honey is variable, depending on geographical origin, climate, and possibly influenced by the botanical origin of honey (Bogdanov, 2007; Madejczyk and Baralkiewicz, 2008). There are several parameters that are applicable for authenticity and characteristic

properties of honey such as sugar, moisture, water-insoluble, conductivity, free acid, diastase activity and hydroxymethylfurfural contents (Council Directive 2001/110/EC, 2002). These attributes can be qualified and describe honey as a foodstuff but do not apply for analytical minerals in it. However modern analytical techniques, like inductively coupled plasma (ICP) and atomic absorption spectroscopy make it possible to quantify trace minerals and produce many studies in honey authenticity and honey quality control (Fernandez-Torres *et al.*, 2005; Pisani *et al.*, 2008; Chudzinska and Baralkiewicz, 2011; Mahmoudi *et al.*, 2015).

Honey has been proposed over the few past decades as a biological indicator of environmental pollution (Porrini *et al.*, 2002; Podgorski and Kanoniuk, 2004; Onikvar, 2005). One type of hazardous contaminant found in honey is heavy metals, which are toxic to human beings (Mahmoudi *et al.*, 2016) and potentially hazardous to the ecological equilibrium. Metals are deposited on flowers by being absorbed from contaminated soil and water, and then carried to the hive by bees from their journeys gathering nectar and pollen (Porrini *et al.*, 2000). It is well known that elements in living organisms take part in biochemical and physiological functions (Oliveira da Silva, 2005). A

*Corresponding author.
Email: wetwitayaklung_p@su.ac.th

very small amount of several heavy metals in honey is necessary for healthy body functioning, however the consumption of contaminated food with heavy metal can deplete some essential nutrients the in human body, leading to adverse health impacts such as a decrease in immunological defenses, growth retardation, impaired psychosocial faculties, endocrine disruption, disabilities associated with malnutrition, kidney damage and several types of cancer (Oliveira da Silva, 2005; Khan *et al.*, 2008).

The Codex Alimentarius (2001) prescribes about heavy metals in topic of contaminants as "Honey shall be free from heavy metals in amounts which may represent a hazard to human health", however there still has no specific regulations issued for control heavy metals in honeys. The aim of this study was to use honey as bioindicator to determine levels of the trace elements (Ag, Al, Ba, Cd, Co, Cs, Cu, Fe, Ga, Li, Mg, Mn, Rb, Se, Sr, and U) and possible pollution level of toxic heavy metals (As, Be, Cd, Cr, Ni, Pb, Tl and V) in Thai Longan, Litchi and Siam weed honeys using ICP-MS for evaluation the honeys quality, which were insufficient development. These knowledge would give the fundamental understanding of the elements behavior in these honeys.

Materials and Methods

Sample collection

Twenty two honey samples were selected randomly from credible beekeepers in 2014 with declarations of botanical origin. Siam weed honey (EO) was collected in January-February from Northern and North-Eastern forests of Thailand. Litchi honey (LC) was collected in February-March from Northern orchards and Samut-Songkhram province orchards of Thailand. Longan honey (DL) was collected in March-April from Northern orchards of Thailand.

Chemicals

Multi-element calibration standards-2A: one bottle contained 10 mg/L Ag (silver), Al (aluminum), As (arsenic), Ba (barium), Be (beryllium), Ca (calcium), Cd (cadmium), Co (cobalt), Cr (chromium), Cs (cesium), Cu (copper), Fe (Iron), Ga (gallium), K (potassium), Li (lithium), Mg (magnesium), Mn (manganese), Na (sodium), Ni (nickel), Pb (lead), Rb (rubidium), Se (selenium), Sr (strontium), Tl (thallium), U (uranium), V (vanadium), Zn (zinc) in a matrix of 5% HNO₃, 1 mg/L Erbium (internal standard) in a matrix of 0.2% Nitric acid (HNO₃) and 1 µg/L tune solution for ICP-MS 7500cs (Ce, Co, Li, Mg, Tl, Y)

in a matrix of 2% HNO₃ were obtained from Agilent Technologies (California, USA). 65% Nitric acid was purchased from Merck, Darmstadt (Germany). Approx. 30% Hydrogen peroxide pure p.a. was purchased from Poch, Sowinskiego (Poland). ASTM Type I water was used in this experiment.

Instruments

Minerals and trace elements were determined using a 7500ce ICP-MS (Octapole reaction System) (Agilent Technologies, California, USA) with a Mira-Mist nebulizer, Scott type double-pass water cooled spray chamber and nickel sample and skimmer cones. The operating conditions were gas flow rates of standard mode: the plasma, nebulizer, auxiliary and makeup gas flow = 15, 0.83, 0.89 and 0.31 L/min, respectively; ICP RF Power: 1500 W; CeO/Ce = 0.012. Cell gas flow was 4.5 mL/min for He, and 5.5 mL/min for H₂. Three gas modes were used being 1) standard mode for ¹⁰⁷Ag, ²⁷Al, ¹³⁷Ba, ⁹Be, ¹¹¹Cd, ⁵⁹Co, ⁵³Cr, ¹³³Cs, ¹⁶⁶Er, ⁶⁹Ga, ⁷Li, ²⁴Mg, ⁵⁵Mn, ⁶⁰Ni, (^{206±207±208})Pb, ⁸⁵Rb, ⁸⁸Sr, ²⁰⁵Tl, ²³⁸U, ⁵¹V, 2) hydrogen mode for ⁵⁶Fe, ⁸²Se, and 3) helium mode for ⁷⁵As, ⁶³Cu, detection. The Tune was done on masses ⁷Li, ⁸⁹Y, and ²⁰⁵Tl, and ¹⁴⁰Ce was used for oxide and doubly charged interference checks. Mass range was 2-260 a.m.u.

Sample and standard preparation

Approximately 0.3 g sample (weighed to the 4th decimal in each case) were quantitatively transferred into a vessel acid-assisted high performance microwave digestion system (Ethos One (Milestone INC, Milestone, Sorisole, BG, Italy)) by adding 4 mL 65% Nitric acid (HNO₃) and 1 mL 30% H₂O₂. The digestion program was run as follows: 1400W, increased the temperature to 180°C over 15 min and held at 180°C for 15 min then reduced the temperature to 70°C over 30 min (Milestone Srl, 2011). After digestion, samples were added 1 µg/L (final concentration) internal standard in 0.5% HNO₃ and diluted to 25 mL with high purity water. Three replicate digestions of each sample were prepared. The blank was done as a sample with 0.3 g water. Multi-element calibration standards were prepared to 0.05, 0.10, 0.50, 1, 10, 50, 100, 200, 500 µg/L (final concentration) in 0.5% HNO₃ and 1 µg/L (final concentration) internal standard in 0.5% HNO₃ was added to each concentration which had 7 replications.

Method validation

The validations of analytical procedure for determination of minerals and trace elements in the honey samples by ICP-MS were evaluated for

linearity, accuracy, precision, limits of detection (LOD), and limits of quantification (LOQ) according to International Conference on Harmonization (ICH) guidelines (ICH, 2005).

Linearity

Linearity was determined by calibration curves: $y = ax + b$, where y is the signal intensity (counts per second, CPS) and x is the known concentration (0, 0.05, 0.1, 0.5, 0.1, 10, 50, 100, 200 and 500 $\mu\text{g/L}$) of the given metals in the calibration solution. The linearity of the calibration curve was accepted when the correlation factor $r^2 > 0.999$.

Accuracy

Accuracy was analyzed by a recovery test of three levels of added standards 25, 40, 60 $\mu\text{g/L}$ (final concentration of standard in sample) and 1 $\mu\text{g/L}$ internal standard (final concentration of standard in sample) to randomized honey sample (EO3). The standard blank were prepared by added 1 $\mu\text{g/L}$ internal standard (final concentration of standard in sample) to the sample and used to determine the percentage of recovery.

Precision

Precision was evaluated as HorRat (Horwitz ratio) by comparing the experimental relative standard deviations (RSD) of ten replicated samples with predicted RSD_r from the Horwitz equation ($0.66 \times 2^{(1-0.5 \log C)}$), where C is the concentration ratio). The instrumental precision was done by repeatedly injecting 10, 100 and 200 $\mu\text{g/L}$ standard and 1 $\mu\text{g/L}$ internal standard solution (final concentration in solution). Repeatability was determined with the same samples (EO3) and method by the same operator on the same equipment in the same laboratory for 3 consecutive days (intra- and inter-day). The fortified samples, with 10, 100 and 200 $\mu\text{g/L}$ (final concentration of standard in sample) of standard metals and 1 $\mu\text{g/L}$ internal standard (final concentration of standard in sample) were added, were tested for repeatability test.

LOD and LOQ

Limits of detection (LOD) were calculated from the standard deviation (SD) of a blank signal in the following equation $LOD = \bar{x} + 3SD_{bl}$, where \bar{x} is the mean of signal intensities of blanks and SD_{bl} is the standard deviation of signal intensities of blanks. The signal intensities of 0.5% HNO_3 in ultrapure water (18.0 $\text{M}\Omega/\text{cm}$) were repeatedly recorded. The limits of quantification (LOQ) were considered to be approximately three times those of LOD.

Statistical analysis

For statistical analysis, the honey samples were grouped according to their botanical origin as Siam weed, litchi and longan honeys. The statistical data analysis was performed by Microsoft Excel 2013. The Box-whisker plots showing the range between the 25th and 75th quartile and median values were used for comparison of different data sets.

Results

The linearity of the metals, their calibration equations and limits of detection are shown in Table 1. All metals gave r^2 in the range of 0.999-1, except ^{207}Pb ($r^2 = 0.997$). The limits of detection (LOD) were estimated from blank analysis (9 replicates). The LOD for all metals were less than 1 $\mu\text{g/L}$, except Al, Fe, Mg, and Mn. The limits of quantification (LOQ) calculated as 3LOD were also less than 1 $\mu\text{g/L}$, except Al, Ba, Fe, Mg, and Mn. The percentage recovery limits (8 replicates) of accuracy of all metals were in the range of 80-110%. The instrumental precision (10, 100, 200 $\mu\text{g/L}$), repeatability and intra-day and inter-day precision (10, 100, 200 $\mu\text{g/L}$) with 9 replicates evaluated in terms of HorRats are shown in Table 2. All HorRat values were in range of 0.03-1.03 which did not exceed acceptable limits. The contents of metals in the EO and LC honey samples are shown in Table 3 and the contents of metals in the DL honey samples are shown in Table 4. For metals that report in $\mu\text{g/kg}$ of honey, most EO, LC and DL samples contained less than 200 $\mu\text{g/kg}$, except Ba, Sr and Pb in some of EO; Ba and Pb in some of LC and Ba, Ni and Pb in some of DL samples. The Co and Be were found in the lowest amounts in all types of samples and all LC samples contained no Co. The average content of metals in the EO honeys, ranking from high to low, were Mg, Al, Fe, Rb, Mn, Sr, Cu, Pb, Ba, Ni, Cr, Se, Co, Cd, Tl, Ga, V, Ag, U, Li, Cs, As and Be with the mean and SD of 18.71 \pm 7.66, 4.74 \pm 1.39, 2.65 \pm 0.70, 1.04 \pm 0.53, 0.96 \pm 1.04, 0.31 \pm 0.10, 0.24 \pm 0.14 mg/kg, 211.72 \pm 48.34, 210.23 \pm 26.03, 127.77 \pm 69.45, 119.46 \pm 7.17, 72.86 \pm 28.11, 28.02 \pm 35.01, 25.56 \pm 0.34, 12.34 \pm 0.04, 11.62 \pm 1.67, 9.17 \pm 1.84, 4.60 \pm 0.58, 4.59 \pm 0.34, 3.74 \pm 3.26, 3.53 \pm 1.46, 3.17 \pm 0.77 and 0.67 \pm 0.24 $\mu\text{g/kg}$ respectively. The average content of metals in the LC honeys, ranking from high to low, were Mg, Al, Fe, Cu, Mn, Rb, Pb, Ba, Cr, Sr, Se, Cd, Ga, Ni, Tl, V, Ag, U, Cs, Li, As, and Be with the mean and SD of 10.19 \pm 3.01, 2.68 \pm 0.93, 2.21 \pm 0.70, 1.02 \pm 0.37, 0.39 \pm 0.25, 0.35 \pm 0.10 mg/kg, 271.43 \pm 390.15, 252.92 \pm 187.06, 122.35 \pm 5.91, 35.10 \pm 38.06, 29.98 \pm 14.28, 24.87 \pm 0.27, 13.93 \pm 11.58, 12.61 \pm 0.07, 12.21 \pm 0.40, 8.09 \pm 2.75, 6.31 \pm 2.30,

Table 1. The equations, r^2 , linearity ranges, LOD and LOQ values of the 23 metals.

Metal	equations	r^2	linearity range($\mu\text{g/L}$)	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)
Ag	$y=1.1412x-0.0349$	0.9999	0-50	0.04	0.13
Al	$y=2.1291x+66.467$	0.9997	0-200	3.98	11.95
As	$y=0.0287x+0.0004$	1	0-10	0.005	0.01
Ba	$y=0.3267x-0.0438$	0.9995	0-200	0.54	1.62
Be	$y=0.5026x+0.0067$	1	0-10	0.01	0.03
Cd	$y=0.2346x-0.0673$	0.9998	0-200	0.30	0.90
Co	$y=2.1849x+0.0153$	1	0-50	0.01	0.04
Cr	$y=0.2375x+0.0031$	1	0-100	0.09	0.26
Cs	$y=2.6717x-0.033$	1	0-50	0.02	0.05
Cu	$y=0.3482x+0.0369$	0.9994	0-200	0.14	0.42
Fe	$y=1.051x+7.7406$	0.999	0-500	29.99	89.99
Ga	$y=1.6204x+0.0403$	0.9998	0-100	0.01	0.03
Li	$y=1.9217x+0.1605$	0.9998	0-10	0.08	0.25
Mg	$y=1.5656x+11.736$	1	0-500	16.64	49.92
Mn	$y=2.5064x+0.9965$	1	0-500	2.90	8.70
Ni	$y=0.478x-0.0734$	0.9995	0-200	0.25	0.75
Pb206	$y=0.5285-0.1006$	0.999	0-100	0.02	0.06
Pb207	$y=0.456x-0.0246$	0.997	0-200	0.03	0.08
Pb208	$y=1.1345x+0.0946$	0.9998	0-50	0.03	0.09
Rb	$y=2.0007x+0.5336$	0.9997	0-50	0.04	0.12
Se	$y=0.017x+0.006$	1	0-100	0.05	0.16
Sr	$y=2.6934x+0.1904$	1	0-500	0.13	0.41
Tl	$y=1.5388x-0.2185$	0.9996	0-200	0.15	0.44
U	$y=2.0471x-0.0297$	0.9998	0-100	0.02	0.07
V	$y=2.3082x-0.0962$	1	0-500	0.05	0.15

All equations contained $p < 0.001$, except Li ($p < 0.05$).

3.24±0.30, 3.01±0.53, 2.69±2.46, 1.45±0.62, and 0.07±0.20 $\mu\text{g/kg}$ respectively. No LC honeys contained any Co, and LC5 and LC6 contained no Be. The average content of metals in DL honeys, ranking from high to low, were Mg, Fe, Al, Cu, Rb, Mn, Ni, Ba, Pb, Cr, Sr, Se, Cd, Ga, Co, Tl, Li, V, U, Ag, Cs, As and Be with the mean and SD of 21.83±5.00, 4.75±0.62, 3.52±0.99, 1.54±1.24, 0.89±0.26, 0.67±0.20 mg/kg 511.43±450.62, 397.13±155.79, 165.97±51.57, 115.36±9.25, 102.21±22.52, 38.89±11.94, 25.20±0.78, 22.96±10.03, 12.77±31.68, 12.29±0.20, 8.31±11.25, 7.90±0.92, 4.54±0.95, 4.34±0.49, 3.85±1.00, 2.50±0.71, and 0.45±0.18 $\mu\text{g/kg}$ respectively.

Discussion

Detection of metals in honey is an issue of concern for environmental studies and research related to human health. The analytical method used in this study showed good linearity for all metals ($r^2 > 0.999$), except ^{207}Pb ($r^2 = 0.997$). However ^{207}Pb showed a wider concentration range of linearity (0-200 $\mu\text{g/L}$) than Li (0 – 10 $\mu\text{g/L}$) which had the acceptable $r^2 = 0.9998$. The metals which had a wide linearity range were abundant in nature or the environment, for example, Al, Ba, Cd, Cu, Fe, Mg, Mn, Ni, and Pb. The LOD values for all metals were less than 1 $\mu\text{g/L}$, except Al, Fe, Mg, and Mn. The percentage

recovery limits obtained by the standard addition method of three different calibration concentrations (25, 40, 60 $\mu\text{g/L}$) suggested acceptable accuracy within 80 -110% according to the AOAC 2002. As recommended by AOAC guidelines (AOAC, 2002), the acceptable recovery depends upon the analytical purpose and the concentration and the acceptable percentage recovery for individual assays of residues at 1 ppm and 10 $\mu\text{g/L}$ is 75-120% and 70-125% respectively. The instrumental precision evaluated by repeatedly injecting 10, 100 and 200 $\mu\text{g/L}$ of each metal revealed $\text{HorRat}_{(r)}$ (Horwitz ratio_(repeatability)) values of less than 2. The intra-day and inter-day precision for 10, 100 and 200 $\mu\text{g/L}$ of each metal also gave $\text{HorRat}_{(r)}$ values of less than 2. According to the AOAC International, the European Union, and other European organizations dealing with food analysis, the HorRat value was one of criteria for accepting an analytical method. The AOAC typically accepted $\text{HorRat}_{(r)}$ values in between 0.5 and 2 (AOAC, 2002). The consistently deviation from the ratio with value less than 0.5 indicated excellent training and experience. On the other hand, consistent deviations with values more than 2 may indicate inhomogeneity of the test samples, a need for further method optimization or training, operating below the limit of determination, or an unsatisfactory method (Horwitz and Albert, 2006). Therefore, the instrumental precision evaluated by repeated injection showed a

Table 2. The %recovery and precision of the 23 metals.

Metal	accuracy range (%recovery limit)			precision (Horrat)															
				instrumental			10 μg/L				100 μg/L				200 μg/L				
	25 μg/L	40 μg/L	60 μg/L	10	100	200	day1	day2	day3	interday	day1	day2	day3	interday	day1	day2	day3	interday	
Ag	96.32-100.29±1.69	84.13-86.52±0.88	94.89-98.02±1.17	0.04	0.22	0.10	0.24	0.12	0.16	0.31	0.13	0.17	0.18	0.24	0.14	0.11	0.17	0.17	
Al	101.35-109.18±3.01	95.35-101.57±2.55	95.33-100.30±2.23	0.66	0.27	0.11	0.84	0.63	0.30	0.65	0.14	0.10	0.10	0.34	0.14	0.08	0.16	0.29	
As	99.67-102.11±0.94	98.01-105.10±2.42	86.47-92.08±1.72	0.08	0.16	0.12	0.46	0.09	0.09	0.29	0.09	0.08	0.20	0.22	0.14	0.09	0.12	0.17	
Ba	97.52-100.29±1.03	96.95-98.83±0.85	96.90-99.75±1.08	0.03	0.10	0.05	0.16	0.07	0.04	0.15	0.12	0.07	0.09	0.14	0.07	0.07	0.09	0.10	
Be	97.30-103.55±1.83	99.78-104.33±1.47	95.24-103.71±3.00	0.09	0.27	0.15	0.48	0.10	0.09	0.34	0.09	0.14	0.11	0.19	0.18	0.10	0.20	0.23	
Cd	81.64-84.49±0.94	81.75-82.99±0.50	80.58-82.79±0.76	0.05	0.15	0.09	0.38	0.07	0.04	0.34	0.10	0.07	0.12	0.28	0.09	0.14	0.11	0.15	
Co	97.71-101.89±1.70	99.03-102.38±1.02	97.64-100.72±1.16	0.06	0.20	0.11	0.41	0.11	0.07	0.38	0.17	0.14	0.14	0.23	0.12	0.11	0.17	0.15	
Cr	96.92-100.28±1.22	96.53-99.97±1.10	98.38-95.41±1.31	0.06	0.18	0.11	0.35	0.10	0.05	0.32	0.12	0.09	0.11	0.21	0.10	0.08	0.13	0.11	
Cs	98.72-102.95±1.47	99.56-101.63±0.71	98.37-100.75±0.84	0.05	0.12	0.07	0.18	0.08	0.05	0.18	0.12	0.08	0.10	0.15	0.05	0.08	0.09	0.10	
Cu	95.76-92.66±1.20	95.98-90.86±2.08	92.57-105.36±4.26	0.09	0.17	0.11	0.36	0.11	0.07	0.22	0.10	0.08	0.14	0.45	0.17	0.09	0.14	0.39	
Fe	93.54-109.23±4.78	90.47-106.13±5.11	93.14-109.37±6.09	0.36	0.21	0.06	0.35	0.12	0.16	0.36	0.11	0.09	0.12	0.32	0.19	0.10	0.17	0.20	
Ga	101.68-99.13±1.19	98.35-101.53±1.12	98.78-101.11±0.86	0.06	0.16	0.09	0.31	0.08	0.06	0.29	0.11	0.08	0.11	0.17	0.10	0.08	0.13	0.11	
Li	99.51-103.80±1.82	99.69-101.73±0.80	99.43-106.34±2.60	0.09	0.25	0.17	0.25	0.16	0.12	0.27	0.12	0.15	0.20	0.34	0.17	0.12	0.21	0.33	
Mg	80.97-95.96±5.82	91.63-103.84±4.33	95.53-104.66±3.26	0.34	0.22	0.10	0.49	0.11	0.07	0.32	0.11	0.08	0.07	0.26	0.12	0.09	0.17	0.26	
Mn	103.40-98.40±1.66	100.36-103.20±0.91	98.38-104.08±1.52	0.06	0.21	0.09	0.37	0.09	0.06	0.28	0.15	0.10	0.10	0.20	0.09	0.08	0.14	0.13	
Ni	84.34-87.97±1.34	94.71-98.17±1.04	83.55-86.48±1.04	0.07	0.21	0.10	0.44	0.10	0.05	0.41	0.13	0.12	0.11	0.19	0.12	0.07	0.13	0.12	
Pb206	97.28-100.01±0.99	96.24-98.19±0.63	88.67-90.90±0.73	0.03	0.08	0.10	0.07	0.08	0.07	0.05	0.16	0.13	0.13	0.16	0.18	0.13	0.14	0.19	
Pb207	93.08-95.36±0.89	95.26-97.19±0.62	93.11-95.74±0.91	0.04	0.09	0.08	0.06	0.08	0.07	0.05	0.15	0.12	0.11	0.16	0.21	0.52	0.14	0.25	
Pb208	88.30-94.34±2.19	87.59-89.04±0.60	94.34-97.94±1.27	0.05	0.10	0.09	0.04	0.06	0.05	0.05	0.08	0.05	0.06	0.16	0.08	0.07	0.06	0.20	
Rb	98.60-101.24±1.07	101.84-104.10±0.76	98.82-101.85±1.07	0.07	0.16	0.11	0.33	0.08	0.07	0.26	0.10	0.10	0.11	0.17	0.11	0.09	0.12	0.13	
Se	97.15-101.09±1.26	91.97-98.11±1.90	93.50-100.16±1.93	0.08	0.27	0.09	0.28	0.13	0.05	0.18	0.08	0.07	0.17	0.19	0.15	0.07	0.13	0.16	
Sr	100.49-104.28±1.25	101.32-104.77±1.33	99.65-103.04±1.17	0.06	0.15	0.09	0.30	0.09	0.06	0.25	0.11	0.09	0.11	0.16	0.09	0.08	0.12	0.12	
Tl	97.37-101.19±1.39	96.14-97.96±0.63	96.62-100.00±1.22	0.08	0.08	0.09	0.04	1.03	0.04	0.57	0.07	0.07	0.06	0.16	0.09	0.05	0.06	0.20	
U	104.09±0.86	99.98-103.47±1.23	99.70-103.34±1.46	0.05	0.11	0.08	0.05	0.05	0.05	0.05	0.08	0.06	0.07	0.15	0.12	0.08	0.08	0.22	
V	98.79-103.89±1.81	100.01-102.54±0.80	98.49-101.56±1.27	0.07	0.18	0.10	0.35	0.09	0.07	0.31	0.11	0.10	0.11	0.18	0.10	0.10	0.13	0.11	

accuracy n = 8; precision n = 9

Table 3. The concentration mean ± standard deviation of minerals and trace elements of EO and LC honey samples by ICP-MS

Metal	Conc in honey	EO1	EO2	EO3	EO4	EO5	EO6	EO7	LC1	LC2	LC3	LC4	LC5	LC6
Al		4.23±0.23	3.85±1.35	4.57±0.76	6.24±3.92	3.40±0.97	3.81±1.37	7.10±0.62	2.83±0.55	3.52±0.64	3.63±1.99	3.00±0.60	1.46±0.49	1.61±0.35
Cu		0.53±0.02	0.27±0.04	0.17±0.00	0.27±0.01	0.15±0.01	0.16±0.01	0.14±0.01	1.23±0.06	1.20±0.06	1.17±0.02	1.41±0.15	0.52±0.03	0.58±0.03
Fe		2.77±0.60	2.54±0.47	1.89±0.63	2.35±0.19	1.93±0.26	3.17±0.61	3.86±0.25	1.41±0.10	1.52±0.06	1.86±0.15	2.99±0.13	2.95±0.47	2.94±0.64
Mg	mg/kg	18.82±0.52	17.02±0.60	12.03±0.51	9.02±0.13	20.66±1.47	20.42±2.09	32.99±0.57	9.94±0.40	7.90±0.12	8.31±0.49	9.22±0.50	9.64±0.34	16.12±0.22
Mn		2.68±0.06	0.33±0.02	0.33±0.01	0.29±0.01	0.43±0.02	0.37±0.02	2.26±0.07	0.15±0.01	0.24±0.01	0.32±0.08	0.32±0.12	0.46±0.04	0.85±0.03
Rb		1.02±0.06	1.94±0.02	1.01±0.03	0.44±0.01	0.61±0.01	0.76±0.04	1.52±0.02	0.37±0.01	0.38±0.02	0.36±0.01	0.20±0.01	0.32±0.00	0.51±0.01
Ag		4.99±0.10	5.55±1.18	4.42±0.38	4.62±1.23	4.77±0.54	4.03±0.27	3.82±0.20	4.47±0.42	7.03±5.61	3.95±0.41	6.92±3.57	5.23±1.13	10.25±9.66
As		3.70±1.49	3.06±0.86	2.18±0.64	2.14±0.47	4.19±0.58	3.56±0.78	3.37±0.64	3.30±0.38	1.19±0.28	1.71±0.48	2.17±0.71	0.54±0.25	1.08±0.75
Ba		230.46±18.68	213.59±30.04	153.67±1.34	220.89±2.59	210.91±28.03	227.97±7.04	214.16±2.97	147.89±37.29	198.19±4.40	199.24±16.44	149.47±7.66	1991±47.38	631.74±44.68
Be		0.46±0.07	0.38±0.33	0.78±0.34	0.49±0.02	0.68±0.28	0.88±0.45	1.04±0.29	0.13±0.04	0.25±0.14	0.19±0.02	0.21±0.10	0.00	0.00
Cd		25.97±0.44	25.20±0.17	25.24±0.14	25.25±0.08	25.59±0.39	26.03±0.56	25.61±0.33	25.37±0.80	24.64±0.42	24.77±0.04	24.91±0.01	24.56±0.09	24.88±0.26
Co		0.00	19.65±1.28	63.66±48.26	15.37±2.98	90.12±83.56	7.33±7.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr		114.96±5.88	117.40±13.56	118.42±0.98	110.93±2.07	116.75±8.56	132.42±2.35	125.35±7.53	123.48±3.35	123.30±3.02	122.36±1.45	131.43±9.15	120.48±4.09	113.03±2.54
Cs		4.58±0.15	3.63±0.26	2.97±0.22	2.13±0.15	2.42±0.30	2.69±0.23	6.25±0.08	2.46±0.16	3.69±0.22	3.58±0.09	2.53±0.12	2.71±0.10	3.06±0.04
Ga	μg/kg	12.79±1.37	11.70±1.99	7.95±0.22	12.56±0.20	11.63±1.88	12.37±1.26	12.32±0.25	7.40±2.24	10.73±0.31	10.68±0.96	7.44±0.47	9.98±2.85	37.37±2.61
Li		0.18±0.26	5.52±0.79	9.58±0.6	0.66±0.61	1.81±0.21	3.97±0.99	4.46±0.29	2.20±0.72	5.71±0.08	5.76±0.07	0.00	1.07±0.12	1.41±0.48
Ni		56.61±4.52	219.08±159.46	166.90±89.72	108.75±7.43	206.57±140.74	85.50±4.93	52.00±8.62	12.54±0.04	12.55±0.18	12.68±0.05	12.57±0.09	12.66±0.06	12.68±0.07
Pb		297.34±50.66	226.27±26.63	140.09±19.94	180.77±16.72	197.11±64.43	216.27±15.04	224.19±3.27	1066.17±89.61	118.77±30.68	124.89±16.02	140.73±8.87	87.32±29.28	90.14±9.09
Se		26.47±2.20	92.86±81.28	79.36±61.74	79.49±12.77	98.75±81.86	92.54±55.42	40.53±7.85	12.33±2.15	25.05±13.99	21.86±6.00	26.67±1.94	43.18±17.80	50.77±14.49
Sr		206.95±3.50	324.68±5.15	476.75±3.12	162.42±1.31	354.07±12.41	356.95±9.93	295.82±5.59	1.17±0.98	24.19±1.03	26.63±4.29	14.74±2.89	34.66±2.99	109.18±1.47
Tl		12.28±0.07	12.37±0.21	12.33±0.37	12.33±0.08	12.32±0.13	12.34±0.17	12.39±0.38	13.02±0.09	11.94±0.20	12.10±0.11	12.02±0.06	12.03±0.05	12.14±0.11
U		4.48±0.64	4.37±1.17	4.01±0.25	4.59±0.98	4.79±0.72	5.02±0.23	4.85±0.45	3.38±0.80	2.72±0.28	3.44±0.25	3.51±0.52	3.02±0.14	3.38±0.42
V		8.21±0.28	7.20±0.35	7.67±0.35	9.10±2.38	9.92±0.29	12.71±2.62	9.38±1.86	6.31±0.51	5.86±0.07	6.20±0.42	6.96±1.11	11.30±1.48	11.88±1.52

All metals were measured in triplicate. (n = 3)

good instrumental function, and intra-day and inter-day or repeatability evaluation suggested accepted variation in sample preparation (same samples and conditions) between the 3 days, operating at the limit of determination and a satisfactory method. Overall, the validation parameters in our analysis, including linearity, accuracy, precision and limits of detection and quantitation, were found to be satisfactory.

The sequence of estimate total metal concentration of the 3 unifloral honeys by sum of the average value of each metal was as follow: DL (34.64 mg/kg) > EO (29.50 mg/kg) > LC (17.68 mg/kg). Comparisons of metals in these different unifloral honeys revealed

that Sr was significantly higher in EO honeys, Ba and Ni were significantly higher in DL honeys and Co and Ni were significantly lower in LC honeys. This finding suggested that Sr, Co and Ni might be used for classification of these honeys. This agreed with previous study of Oroian *et al.* (2015) that trace elements can be used for discrimination of unifloral honeys.

These Non-essential and highly toxic elements such as Be, Cd, Hg, Pb and Tl can be transferred from soil to the human food chain and produce toxicity (Kabata-Pendias and Mukherjee, 2007). Toxic metals, especially heavy metals, could imitate

Table 4. The concentration mean \pm standard deviation of minerals and trace elements of DL honey samples by ICP-MS

Metal	Conc in honey									
	DL1	DL2	DL3	DL4	DL5	DL6	DL7	DL8	DL9	
Al	5.97 \pm 1.64	3.34 \pm 0.35	2.99 \pm 0.66	3.57 \pm 1.36	3.32 \pm 0.84	3.18 \pm 0.79	3.62 \pm 0.97	3.34 \pm 0.19	2.38 \pm 0.38	
Cu	4.77 \pm 3.47	1.36 \pm 0.02	1.71 \pm 0.03	1.22 \pm 0.05	0.81 \pm 0.02	0.83 \pm 0.09	1.17 \pm 0.02	1.05 \pm 0.04	0.94 \pm 0.01	
Fe	4.59 \pm 0.66	4.88 \pm 0.33	4.75 \pm 0.34	5.09 \pm 0.40	3.92 \pm 0.18	3.93 \pm 0.64	5.24 \pm 0.54	5.86 \pm 0.31	4.50 \pm 0.19	
Mg	28.16 \pm 0.34	16.95 \pm 0.77	17.04 \pm 0.45	18.76 \pm 0.58	24.98 \pm 0.48	24.51 \pm 1.35	17.62 \pm 0.61	18.87 \pm 0.43	29.60 \pm 0.09	
Mn	0.67 \pm 0.09	0.59 \pm 0.02	0.77 \pm 0.07	0.50 \pm 0.01	0.48 \pm 0.02	0.49 \pm 0.04	0.56 \pm 0.03	0.84 \pm 0.06	1.10 \pm 0.03	
Rb	0.57 \pm 0.01	1.13 \pm 0.02	1.13 \pm 0.02	0.52 \pm 0.00	0.83 \pm 0.05	0.90 \pm 0.04	1.19 \pm 0.02	0.62 \pm 0.00	1.09 \pm 0.05	
Ag	4.93 \pm 0.35	4.47 \pm 0.89	3.84 \pm 0.25	4.13 \pm 0.33	4.07 \pm 0.27	5.30 \pm 2.35	4.37 \pm 0.74	4.12 \pm 0.49	3.84 \pm 0.21	
As	3.37 \pm 0.21	2.03 \pm 0.30	2.45 \pm 0.92	2.93 \pm 0.34	1.80 \pm 0.30	1.89 \pm 0.49	2.24 \pm 0.74	3.78 \pm 0.99	1.98 \pm 0.48	
Ba	638.15 \pm 56.86	313.34 \pm 8.03	277.96 \pm 23.19	262.29 \pm 5.79	366.77 \pm 51.36	383.67 \pm 16.42	273.07 \pm 11.23	679.73 \pm 472.00	379.20 \pm 129.60	
Be	0.64 \pm 0.21	0.45 \pm 0.07	0.48 \pm 0.11	0.59 \pm 0.13	0.53 \pm 0.03	0.56 \pm 0.22	0.42 \pm 0.08	0.28 \pm 0.11	0.06 \pm 0.01	
Cd	24.96 \pm 0.26	25.22 \pm 0.06	25.02 \pm 0.06	24.76 \pm 0.09	24.98 \pm 0.09	24.86 \pm 0.46	27.23 \pm 2.30	24.71 \pm 0.19	25.02 \pm 0.26	
Co	96.47 \pm 91.02	12.46 \pm 30.54	0.00	0.00	0.00	5.96 \pm 0.68	0.00	0.00	0.00	
Cr	128.60 \pm 20.54	110.72 \pm 7.58	106.58 \pm 4.89	102.45 \pm 4.37	105.92 \pm 2.19	122.24 \pm 23.26	116.68 \pm 18.46	123.65 \pm 3.11	121.40 \pm 3.60	
Cs	4.34 \pm 0.14	4.45 \pm 0.29	4.01 \pm 0.04	2.31 \pm 0.05	3.29 \pm 0.05	4.71 \pm 0.16	3.02 \pm 0.15	3.05 \pm 0.05	5.50 \pm 0.06	
Ga	38.47 \pm 3.89	17.68 \pm 0.39	15.39 \pm 1.01	14.35 \pm 0.41	20.76 \pm 3.02	21.80 \pm 0.82	15.03 \pm 0.75	41.27 \pm 30.45	21.93 \pm 8.73	
Li	0.00	3.77 \pm 0.24	0.81 \pm 0.20	2.42 \pm 0.77	2.31 \pm 0.31	12.47 \pm 1.13	3.61 \pm 0.42	14.20 \pm 0.91	35.19 \pm 1.33	
Ni	356.24 \pm 10.67	822.42 \pm 70.42	1455.09 \pm 45.29	599.70 \pm 14.77	393.09 \pm 9.43	261.95 \pm 52.77	689.12 \pm 6.71	12.60 \pm 0.07	12.68 \pm 0.07	
Pb	219.93 \pm 68.34	188.53 \pm 10.42	230.33 \pm 31.14	154.88 \pm 12.18	157.10 \pm 33.01	144.51 \pm 37.93	212.53 \pm 19.47	103.38 \pm 16.61	82.52 \pm 1.11	
Se	44.10 \pm 25.77	47.36 \pm 14.67	35.43 \pm 11.74	58.09 \pm 47.69	52.60 \pm 35.48	29.74 \pm 10.84	25.76 \pm 3.94	28.09 \pm 17.86	28.85 \pm 15.80	
Sr	98.76 \pm 8.66	103.06 \pm 0.28	74.97 \pm 2.78	131.16 \pm 3.69	77.85 \pm 3.66	109.97 \pm 9.21	84.97 \pm 6.43	98.28 \pm 14.79	140.90 \pm 1.59	
Tl	12.20 \pm 0.10	12.79 \pm 0.79	12.17 \pm 0.09	12.25 \pm 0.14	12.26 \pm 0.13	12.31 \pm 0.14	12.18 \pm 0.27	12.11 \pm 0.12	12.30 \pm 0.05	
U	4.71 \pm 0.98	3.82 \pm 0.31	3.71 \pm 0.14	5.66 \pm 1.16	3.82 \pm 0.72	3.93 \pm 0.36	4.79 \pm 0.94	6.42 \pm 3.23	4.03 \pm 0.44	
V	9.93 \pm 2.96	7.96 \pm 1.42	7.30 \pm 0.50	8.04 \pm 0.87	7.40 \pm 0.65	8.16 \pm 0.04	7.12 \pm 0.62	6.84 \pm 1.16	8.34 \pm 3.75	

All metals were measured in triplicate. (n = 3)

the action of essential elements and interrupt the metabolic process in the body which may result in serious health problems. Undoubtedly, metal toxicity is problematic when consumers receive high doses of contaminated products. In addition to acute toxicity, long term exposure to small amounts of metals may cause chronic toxicity due to their cumulative toxic effects over time. For these reasons, the United States Pharmacopoeia (USP) always recommends maximum acceptable limits of toxic heavy metals such as As, Cd, Hg and Pb in nutritional supplements (United States Pharmacopoeia, 2013). The European Medicines Agency (EMA) also established a guideline on the specification limits for residues of metal catalysts, and classified metals into three classes based on their potential risk to human health. Class 1 metals are metals of significant safety concern (Class 1A: Pt, Pd; Class 1B: Ir, Rh, Ru, Os; Class 1C: Mo, Ni, Cr, V). Class 2 metals are metals with low safety concern (Cu, Mn) and class 3 metals are metals with minimal safety concern (Fe, Zn) (European Medicines Agency, 2007). According to Kabata-Pendias and Mukherjee, USP and EMA, the present study classified As, Be, Cd, Cr, Ni, Pb, Tl and V as toxic heavy metals and classified Ag, Al, Ba, Cd, Co, Cs, Cu, Fe, Ga, Li, Mg, Mn, Rb, Se, Sr, and U as minerals and trace elements. The top 3 metals with the highest concentrations found in all the honey samples were minerals including Mg, Al and Fe, while the top 2 metals with the lowest concentrations found in all the honey samples were toxic metals including As and Be. The highest Mg concentration was found in DL9 (29.60 \pm 0.09 mg/kg). The lowest Be concentration was found in DL9 (0.06 \pm 0.01 μ g/

kg). LC5 and LC6 contained no Be. The sequence of sum of average concentration of classified potentially toxic heavy metals of EO, LC and DL honeys was as follows: Pb > Ni > Cr > Cd > Tl > V > As > Be for EO, Pb > Cr > Cd > Ni > Tl > V > As > Be for LC, and Ni > Pb > Cr > Cd > Tl > V > As > Be for DL. These results revealed that the botanical origin of honeys harvested in Thailand were differences in the amount of toxic heavy metals.

Honey is known as a biological product that can reflect environmental pollution (Porrini *et al.*, 2000; Formicki *et al.*, 2013). The standard deviation (SD) of the amount of metals in EO, LC and DL honeys revealed high variations of metal concentrations in the samples. The metals with high variation of concentrations in each honey sample were as follows: Co and Mn in the EO samples; Ga and Pb in the LC samples and Co, Cu, Li and Ni in the DL samples. These presented that the contents of Co, Cu, Ga, Li, Mn, Ni, and Pb were highly scattered in the honey samples. These variability of metals content between types of honey were impact of biological origin and can be linked to growing condition of honey origins flora (Vincevica-Gaile, 2012). The result suggested that the samples might have been contaminated from geochemical specifics of the area where the bees collected nectar or a polluted environment. This could be seen in the cases of Co, Pb and Ni. A majority of the honey samples (2 of 7 EO samples, 6 of 6 LC samples and 6 of 9 DL samples) did not contain Co. For Pb and Ni, the Pb content in LC1 and Ni content in DL3 appeared to shoot out of range of other samples in their groups. The Box-whisker plots of concentrations of toxic metals As, Be, Cd,

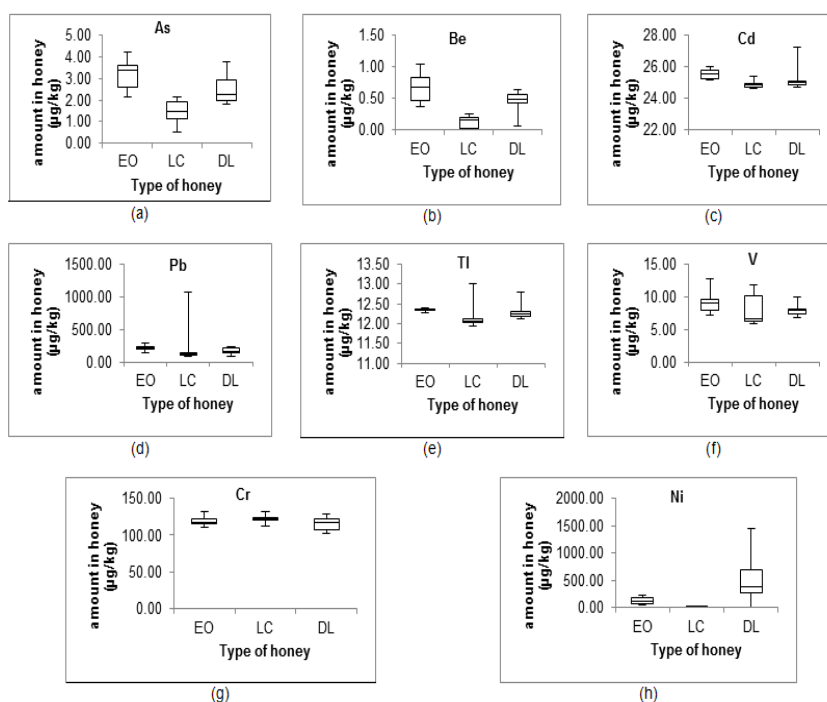


Figure 1. The Box-whisker plots of concentrations of toxic metals: (a) As, (b) Be, (c) Cd, (d) Pb, (e) Tl, (f) V, (g) Cr and (h) Ni.

Cr, Tl, Pb, Ni and V are shown in Figure 1. It can be seen that As, Be, Cd, Pb, Tl and V (Figure 1(a-f)) were found most in the EO samples, followed by the DL and LC samples respectively. The distribution of the concentrations of the metals in the studied samples was also different. The distribution of As concentrations was quite in symmetry in the LC samples but skewed to the left (a majority of the samples had high concentrations of As) in the EO samples and skewed to the right (a majority of the samples had low concentrations of As) in the DL samples. The distribution of Be concentrations was quite in symmetry in the DL samples but skewed to the left in the EO and LC samples. The distribution of Cd concentrations in all honey samples was skewed to the left. The distribution of Pb concentrations in the DL was skewed to the right, but skewed to the left in the EO and LC samples. The distribution of Tl concentrations in the DL samples was skewed to the left, but skewed to the right in the EO and LC samples. The distribution of V concentrations in the LC samples was skewed to the right, but skewed to the left in the others. The Box-whisker plots of concentrations of Cr and Ni are shown in Figure 1(g-h). The distribution of Cr concentrations in the LC and DL samples was skewed to the left but skewed to the right in the EO samples. The distribution of Ni concentrations in the LC samples was in symmetry, but skewed to the right in the others. Although no specific legislation exists on maximum residual limits (MRLs) of heavy metals in honey (EUR-lex, 2006),

the Commission Regulation ((EC) No 1881/2006 of 19 December 2006) set maximum levels for contamination of Pb in food supplements at 3 mg/kg wet weight, Tin (Sn) in canned food at 200 mg/kg wet weight, Cd in food supplements (dried seaweed, dried bivalve molluscs) at 3 mg/kg wet weight and Hg in the muscle meat of some fish at 1 mg/kg wet weight. In addition, the Ministry of Public Health of the Kingdom of Thailand established the maximum metal content in honey as As not more than 0.2 mg/kg and Pb not more than 0.5 mg/kg. According to the Commission Regulation ((EC) No 1881/2006) and the Ministry of Public Health of Thailand, the presence of Pb and Cd in the EO, LC and DL honey samples was considered safe, except the Pb content in LC1 that did not pass the criteria established by the Ministry of Public Health of Thailand. Bogdanov (2006) reported that contamination of Pb in honey in polluted and non-polluted area was not significantly different, but honey from polluted areas often contained high Pb content. Pb was not transported by plants, but had origin from traffic that contaminated in air and polluted to nectar and honey directly. The LC1 showed one outlier of Pb content from the median of Pb concentration of the LC samples. These suggested that the high variation of Pb content in LC1 might come from contamination or pollution.

The amount of Cu, Mg, As, Cd, Co and Pb in Thai longan honey were lower than Malaysian longan honey which contained 2.42, 35.47, 0.057, 0.050, 0.065 and 0.723 mg/kg of Cu, Mg, As, Cd,

Co and Pb respectively (Moniruzzaman *et al.*, 2014). The level of Mg in longan honey of this study 16.95-29.60 mg/kg was close to that previously reported in Thai longan honey (13.10-27.10 mg/kg) (Tantidanai-Sungayuth *et al.*, 2012). The mean concentration of Ba, Cu, Fe, Mg, Mn, Rb, and Sr in Thai EO, LC and DL honeys compared to that reported for linden, vitex, rape and acacia honeys from China by Chen *et al.* (2014) as followed. The mean concentration of Ba in EO, LC and DL honeys were higher than vitex (0.03 mg/kg), rape (0.03 mg/kg) and acacia (0.05 mg/kg) honeys, but lower than linden (0.45 mg/kg) honey. The mean concentration of Cu in EO, LC and DL honeys were higher than linden (0.08 mg/kg), vitex (0.06 mg/kg), rape (0.11 mg/kg) and acacia (0.10 mg/kg) honeys. The mean concentration of Mg in EO and DL honeys were higher than linden (17.69 mg/kg), vitex (7.37 mg/kg), rape (17.04 mg/kg) and acacia (13.00 mg/kg) honeys, and LC higher than vitex honey. The mean concentration of Mn in EO, and DL honeys were higher than vitex (0.09 mg/kg), rape (0.44 mg/kg) and acacia (0.24 mg/kg) honeys, but lower than linden (1.13 mg/kg) honey. The mean concentration of Mn in LC honeys was higher than vitex, and acacia honeys, but lower than linden, rape honeys. The mean concentration of Fe in EO, and DL honeys were higher than linden (0.64 mg/kg), vitex (2.23 mg/kg), rape (1.24 mg/kg) and acacia (2.20 mg/kg) honeys, and LC honey was close to acacia honey. (Chen *et al.*, 2014). The mean concentration of Rb in EO, and DL honeys were higher than vitex (0.41 mg/kg), rape (0.43 mg/kg) and acacia (0.42 mg/kg) honeys, but lower than linden (1.68 mg/kg) honey. The mean concentration of Rb in LC honeys were lower than linden, vitex, rape and acacia honeys. The mean concentration of Sr in EO honeys were higher than vitex (0.09 mg/kg), rape (0.07 mg/kg) and acacia (0.12 mg/kg) honeys, but lower than linden (0.56 mg/kg) honey. The mean concentration of Sr in DL honeys were higher than vitex and rape honeys, but lower than linden and acacia honeys. The mean concentration of Sr in LC honeys were lower than linden, vitex, rape and acacia honeys.

Conclusion

On account of this research, Thai Longan, Litchi and Siam weed honeys contained differences in amount of metals. These could come from difference in botanical origins of nectar and geochemical specifics of the area. The Mg and Be were maximum and minimum of the studied metals in these honey samples respectively. The toxic heavy metals in Longan, Litchi and Siam weed honey samples met

the regulation requirement of the Commission Regulation ((EC) No 1881/2006) and the Ministry of Public Health of Thailand, except one sample was outlier due to Pb contamination. By sum values of toxic heavy metals (DL = 841.08 µg/kg, EO = 509.86 µg/kg and LC = 453.05 µg/kg), the DL honey was possible the most contaminated. Figure 1 illustrated that EO honey harvested from the forest had the highest median of toxic heavy metal concentrations of As, Be, Cd, Pb, Tl and V when compared to LC and DL honeys. However the mean value of Cd, Cr, Pb, Tl and V contents of the 3 uniflora honeys were not statistically significant difference based on one-way ANOVA ($p < 0.05$). Although some elements are essential for human life, they can become toxic after long-term low dose, or short-term high dose exposure. Therefore, it is crucially important to monitor the amounts of minerals and contaminated toxic heavy metals in foodstuffs such as honey and this helps to control its quality.

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