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Mineral and heavy metal variations and contaminations in raw honey of stingless bees, *Heterotrigona itama*, from selected geographical areas of origin in Malaysia

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ABSTRACT

Introduction: Honey contains a complex matrix of different substances consisting of essential minerals, non-nutritive substances, and toxins, often due to environmental sources. The objectives of this study were i) to investigate the variation of mineral and heavy metal content in honey samples of *Heterotrigona itama* from different geographical origins, and ii) to evaluate the nutritional quality and safety of honey from different floral sources for human consumption. **Methods:** A total of 75 samples of raw *H. itama* honey were collected from 11 sites in Sabah, two sites in Sarawak, and two sites in Peninsular Malaysia. The mineral and heavy metal contents of honey were determined in triplicate using inductively coupled plasma mass spectrometry (ICP-MS). **Results:** There were significant differences in the composition of essential minerals and heavy metals in the honey samples according to geographical origins ($p<0.001$). All honey samples tested were below the permitted maximum proportion (ML) for cadmium (Cd) and lead (Pb) specified in the Malaysian Food Act 1983 for honey. However, all honey samples exceeded the ML for Pb set by Codex, with samples from bamboo sites having the highest levels for Pb. **Conclusion:** The composition of minerals and heavy metals in stingless bee honey was influenced by geographical origin. All measured Pb concentrations were above the ML value set by Codex, which raises concerns about possible toxicological risks to human health. Given the toxic nature of Pb in the environment, the measured concentrations emphasise the importance of monitoring Pb in honey from stingless bees.

Keywords: geographical origin, heavy metals, *Heterotrigona itama*, honey, minerals

INTRODUCTION

In Malaysian meliponiculture, *Heterotrigona itama* is a species that is frequently domesticated as it is most abundant in Malaysia and often produces a larger amount of honey compared to other species. Compared to honeybees, stingless bees are harmless to humans and generally easy to rear. They are also more resistant to pests and diseases, and their short flight range makes them easier to keep for crop pollination. The honey produced by stingless bees contains a variety of minerals and heavy metals, the

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composition of which depends on their geographical origin (Ng *et al.,* 2021). The mineral content of honey is usually used to classify it according to its geographical characteristics to assess its originality or authenticity, while heavy metals can serve as biomarkers of environmental pollution in the bees' foraging area, as bees can carry pollutants in plants, air, water, and soil from the environment into their hives. Essential macrominerals, such as potassium (K), sodium (Na), calcium (Ca), and phosphorus (P), are required in large quantities while trace elements, such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), are required in small quantities and are often detected in the honey of stingless bees (Zulhafiz *et al.,* 2019). Heavy metals are chemical elements with a relative density of more than 5 g/cm^3 that occur in high concentrations in waste areas (sewage sludge and mining) or in places of intensive use (industrial by-products and agrochemicals) (Freitas *et al.,* 2009; Amare & Arega, 2018). In honey from stingless bees, the toxic heavy metals cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) are generally present at low levels depending on environmental factors (Salman *et al.,* 2022). A previous study by Salman *et al.* (2022) in Sabah, Malaysia, found that the accumulation of heavy metals in samples of raw stingless bee honey was strongly related to the locations of the hives where the honey was produced, with higher levels of As, Cd, and Pb contamination found in honey from hives produced near urban and industrial areas.

To ensure the food safety of honey, its heavy metal content must comply with the permissible limits of the Codex Commission (FAO/WHO), which are accepted by most international countries. At the national level, the limits set by the Malaysian Food Act 1983 (Act 281) & Food Regulations 1985 (Malaysia & International Law Book Services Legal Research Board, 1994), must be complied with, but so far there are no standards for minerals (i.e., Na, Mg, K, and Ca) and some heavy metals (i.e., Cu, Zn, and Fe), specifically for the honey of stingless bees. However, according to Freitas *et al.* (2009), essential heavy metals, such as Fe, Zn, Cu, and Mg, when present in optimal concentrations play an important role in the biological system, but contribute to toxicity in humans at higher concentrations, while non-essential heavy metals, such as Cd, Pb, As, and Hg, can be toxic even at trace levels. Toxicity results from the inability of heavy metals to be metabolised by the body, leading to an accumulation in human or animal soft tissues without being completely inactivated or destroyed. The accumulation of heavy metals at toxic levels in the environment causes health problems such as headaches, metabolic disorders, respiratory diseases, nausea, vomiting, brain damage, damage to the nervous system, vascular disorders, kidney or bone damage, and irregularities in the reproductive system of humans and animals (Amare & Arega, 2018). Reliable analysis of minerals and heavy metals in honey samples is therefore crucial to prevent consumer poisoning, as there are very few studies on tropical stingless bees.

The geographical and entomological origins of stingless bee honey can be determined by analysing its mineral and heavy metal contents, as well as other parameters, as shown by the results of Kek *et al.* (2017). In a study on Malaysian raw honey, samples produced by honeybees, *Apis* sp. (Tualang, Gelam, Pineapple, and Borneo) and stingless bees (*H. itama*) were successfully differentiated based on proximate composition (ash, protein, and carbohydrate) and mineral and heavy metal content analysis. The results of Kek *et al.* (2017) also showed that honey of *Apis* sp. and *H.*

itama differed significantly in terms of ash, protein, and carbohydrate, their predominant sugars (fructose, glucose, and sucrose), and main minerals (K and Na). Currently, there are very few studies that focus exclusively on documenting the mineral content and heavy metal contamination of *H. itama* honey from sites of different geographical origins in Malaysia, particularly in the state of Sabah, where the heterogeneous habitats and floral types of agricultural areas, bamboo forests, mixed vegetation, forest parks, and lowland to highland forests occur.

Therefore, the objective of this study was to determine the variations in the content of minerals and heavy metals in different honey from stingless bees sampled from selected geographical areas of origin from sites with different elevations and main floral types. In addition, the present work was also conducted to determine the composition of some heavy metals in stingless bee honey in Malaysia and to assess its health risk for human consumption in accordance with the Malaysian Food Act 1983 and Codex standards.

MATERIALS AND METHODS

Location of study

This study was conducted at selected sites in Sabah, Sarawak, and Peninsular Malaysia. A summary of the geographical origins of the study sites can be found in Table 1. A total of 55 samples of raw *H. itama* honey were collected from 11 sites in Sabah with different geographical origins. A total of 20 samples of raw *H. itama* honey from Sarawak (two sites) and Peninsular Malaysia (two sites) were also collected for comparative studies.

Honey sampling programme in the field

To ensure that all raw honey came from the correct stingless bee species, identification of *H. itama* was done based on Abu Hassan (2016). This bee species has a predominantly large body size of 5-7 mm, is black in colour, and has shiny, translucent wings. The honey of *H. itama* was sampled over a period of six months, from August 2020 to February 2021. Each sampling site included five replicates of honey samples. The *H. itama* honey samples collected in this study were from colonies that had been established for at least five to twelve months. All honey samples of *H. itama* in Sabah, Sarawak, and Peninsular Malaysia were collected directly from the hives in the field. Honey samples from each location were stored directly in polystyrene boxes filled with ice to reduce the effects of the fermentation process on the quality of honey samples. A total of 500 ml of honey was extracted from the hive using a syringe, stored in a glass bottle, and taken to the Faculty of Sustainable Agriculture, University Malaysia Sabah (FSA, UMS) laboratory for further analysis. All honey samples were stored in a glass bottle and kept in a refrigerator at 4°C until further analysis in the laboratory. Honey samples were collected after obtaining consent from the beekeepers.

Determination of mineral and heavy metal contents

Laboratory analyses to determine the content of minerals and heavy metals were conducted from March 2021 to June 2022. Methods for determination of mineral and heavy metal contents in the samples of stingless bee honey were based on Kek *et al.* (2017). A total of 1 ml of stingless bee honey sample was put into a polyurethane cap, then transferred into a polyurethane tube. The polyurethane tube was filled with 6 ml of nitric acid (HNO3) (Fisher Chemical™) and 2 ml of hydrogen peroxide (H2O2) (Fisher Chemical™), then sealed with a stopper. The sample was then microwaved for

Table 1. Description of honey samples from Sabah, Sarawak, and Peninsular Malaysia

No	*Study sites*	*Geographical coordinates*	*Districts/ States*	*Geographical origin*	*Total samples (N)*	*Elevation (m)*
Sabah						
1	Kg. Kipaku	5°43’27.7”N, 116°24’11.9”E	Tambunan	Adjacent to Crocker Range Park	10	650 - 780
	Kg.Kuyungon	5.7083° N, 116.3244° E				
	Kg. Sunsuron	5.7413° N, 116.3799° E				
2	Kg. Magatang	5.3631° N, 116.2194° E	Keningau			287
3	Kg. Bundu Tuhan	5° 58’ 60” N, 116° 31’ 60” E	Ranau	Adjacent to highland forest	5	1206
	Kg. Pasir Putih	5° 56’ 17” N, 116° 39’ 32” E				531.60
4	Kg. Guakon Baru Kg. Nadau	6.1743° N, 116.3187° E 6.0021° N, 116.4329° E	Tamparuli	Adjacent to lowland forest	10	180
	Kiulu	6.0628° N, 116.2825° E	Tuaran			
5	Kg. Chinta Mata	5.0903° N, 115.9476° E	Tenom	Agritourism park and local fruit	5	213
6	Kg. Moyog	5°53’0”N, 116°15’0”E	Penampang	Bamboo	5	260
7	Kuala Abai	6° 22’ 6” N, 116° 25’ 25” E	Kota Belud	Orchard	10	10
	Kg. Bangsal Baru Kg. Palakat	5° 04’ 60” N, 115° 32’ 59.99” E 5° 7’ 2” N, 115° 33’ 26” E	Sipitang			30 - 90
8	Kg. Mandahan	5° 35’ 00” N, 115° 53’ 00” E	Papar	Rubber and Swampy Area	5	32
9	Sikuati	6° 53’ 30” N, 116° 42’ 45” E	Kudat	Secondary forest and orchard	5	16
Peninsular Malaysia						
1	Hulu Langat	3.1131° N, 101.8157° E	Selangor	Orchard and Oil Palm Plantation	10	63
2	Seremban	2.7259° N, 101.9378° E	Negeri Sembilan	Orchard and Oil Palm Plantation		65
Sarawak						
1	Tijirak	1°20’00.5”N 110°23’42.2”E	Kuching	Orchard	10	43
2	Tanjung Manis	2° 8’ 42” N,111° 19’ 54” E	Mukah	Orchard		10

a total of one hour (40 minutes run time, 20 minutes cooling time). The fumes from the microwave sample were removed in the fume chamber (Binder FD 115/E2, Germany). Then, 25 ml of ultrapure water (Elga Option-R 15 PURELAB Water Purification System) was mixed with the sample. An aliquot of 1000 µl was removed from the solution and put into a new container. The extracted solution was mixed with 50 ml of ultrapure water. Of the 50 ml of extracted solution, 10 ml was aliquoted. Triplicate honey samples were prepared for analysis along with blank samples and standard solutions for data quality assurance. The samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS) at the Universiti Malaysia Sarawak (UNIMAS) laboratory. Analyses were performed using ICP-MS (NexION® 2000 ICP Mass Spectrometer), manufactured by Perkin Elmer, United States of America. The carrier gas used was ammonia (NH3), at a flow rate of 0.4 ml/min. The scan mode was set to peak hopping, dwell time per AMU (ms) at 25. The internal standards used were rhodium (Rh) and germanium (Ge), in order to reduce matrix effects and increase the reliability and robustness of the results. The National Institute of Standards and Technology (NIST) library was used as a reference to identify the minerals and heavy metals detected. All samples were analysed at the following wavelengths: Ca, Cu, Fe, Mg, Na, K, Zn, Cd, and Pb were analysed at 317.9 nm, 327.4 nm, 238.2 nm, 285.2 nm, 589.6 nm, 766.5 nm, 206.2 nm, 228.8 nm, and 220.3 nm, respectively.

Health risk and general standards for heavy metals in foodstuffs

To evaluate the composition of heavy metals in *H. itama* honey, the levels of these metals found in this study were compared with the maximum levels specified in food standards, according to the permitted maximum proportion (ML) for Pb in the Codex Alimentarius Standard (FAO/WHO) (Honey) (CXS 193-1995) (FAO & WHO, 2019) and the Malaysian Food Act 1983 (Honey) for Cd and Pb. Other minerals and heavy metals were not included in the analysis as Codex [CXS 12-1981 (CAC, 2019), and CXS 193-1995], Malaysian Food Act 1983 (Act 281) & Food Regulations 1985 (Malaysia & International Law Book Services Legal Research Board, 1994), and Malaysian Standard MS 2683/:2017 (Department of Standards Malaysia, 2017) do not provide a ML value.

Statistical analysis

The assays were performed in triplicate and the results were expressed as average values. All data were checked for normality using the Shapiro-Wilk test before statistical analyses were performed. Data were tested for significant differences using the one-way analysis of variance test (ANOVA; $p<0.05$). If ANOVA showed a significant difference, a post-hoc test was performed ($p<0.05$). Statistical tests and data manipulations were performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, U.S.A.) and IBM SPSS Statistics version 26.0 (IBM Corporation, New York, U.S.A.).

RESULTS

Determination of the variation of minerals in honey samples of stingless bee, *H. itama*, from sites differing in geographical origins

This study found that the mineral content of stingless bee honey varied according to the geographical origins of the samples in Sabah, Sarawak, and Peninsular Malaysia for Na ($F_{9,65} = 7.53$, $p<0.001$), Mg ($F_{9,65} = 4.37$, $p<0.001$), K ($F_{9,65} = 52.17$, $p<0.001$), and Ca ($F_{9,65} = 8.19$, $p<0.001$) (Table 2). Overall, the most abundant mineral detected in honey from stingless bees was K (Peninsular Malaysia, mean = 1753.07 mg/kg; Sarawak, mean = 1572.59 mg/kg; Sabah, mean = 1525.34 mg/kg), followed by Ca (Peninsular Malaysia, mean = 157.57 mg/kg; Sarawak, mean = 131.55 mg/kg; Sabah, mean = 107.09 mg/kg), and Mg (Sarawak, mean = 86.61 mg/kg; Peninsular Malaysia, mean = 72.53 mg/kg; Sabah, mean = 178.34 mg/kg). In Sabah, the results for mineral content showed that honey from orchards had the highest mean value for Na (77.81 mg/kg), while honey from the sites adjacent to lowland forest had the lowest mean value for Na (2.20 mg/kg). The highest mean value for Mg (136.42 mg/kg) was found in honey samples from bamboo sites, while the lowest mean value for Mg (30.09 mg/kg) was found in samples from rubber and swamp sites. The highest mean value for K (3797.47 mg/kg) was also found in samples from bamboo sites; the lowest mean value for K (817.01 mg/kg) was found in samples from the agritourism park and orchards (Tenom). The highest mean Ca value (169.01 mg/kg) was found in samples from sites adjacent to highland forest; the lowest mean value for Ca (65.94 mg/kg) was found in samples from sites adjacent to Crocker Range Park.

Table 2. Mineral content of *H. itama* honey in Sabah based on different geographical areas

Geographical Areas	*Total sites*	*Total Samples*	*Na*** (mg/kg)*		*Mg** (mg/kg)*		*K*** (mg/kg)*		*Ca*** (mg/kg)*	
			Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Adjacent to Crocker Range Park	4	10	10.54^{b}	3.48	44.47^{a}	4.23	1279.24^{bcd}	45.39	65.94^{a}	11.06
Adjacent to highland forest	2	5	53.91^{cd}	1.42	91.12^{ab}	10.21	1661.72^{de}	41.76	169.01^{cd}	43.40
Adjacent to lowland forest	3	10	2.20^{a}	1.12	83.94^{ab}	5.81	1206.04^{bc}	66.99	74.74^{ab}	10.68
Agritourism park and local fruit	1	5	19.91^{b}	0.00	89.59^{ab}	0.00	817.01^{a}	0.00	139.68^{cd}	0.00
Bamboo	1	5	37.49^{bcd}	0.00	136.42^{b}	0.00	3797.47^{f}	0.00	86.31^{b}	0.00
Orchard	3	10	77.81^{d}	17.80	81.80^{ab}	18.68	1363.27^{bcde}	189.96	122.61^{cd}	10.71
Rubber and Swampy Area	1	5	68.64^{d}	0.00	30.09^{a}	0.00	933.48^{b}	0.00	88.23^{bc}	0.00
Secondary forest and orchard	1	5	68.94^{d}	0.00	69.28^{ab}	0.00	1144.46^{b}	0.00	110.22^{bc}	0.00
Sabah (Mean Total)	16	55	42.43	2.98	78.34	4.87	1525.34	43.01	107.09	9.48
Peninsular Malaysia	2	10	21.55^{bc}	0.06	72.53^{ab}	14.57	1753.07^{e}	43.65	157.57^{d}	4.72
Sarawak	2	10	44.52^{bcd}	14.84	86.61^{ab}	12.54	1572.59^{cde}	79.42	131.55^{cd}	7.75

SE: Standard error
**significance at $p<0.01$; ***significance at $p<0.001$
Different superscript letters indicate significant difference ($p<0.05$) between the samples and standards as indicated by Dunn's test for multiple comparisons. Values with at least one similar alphabet in the same row are not significantly different ($p>0.05$).

Determination of the variation of heavy metals in honey samples of stingless bee, *H. itama*, from sites differing in geographical origins

The heavy metal content of stingless bee honey varied according to the geographical origins of the samples in Sabah, Sarawak, and Peninsular Malaysia for Cu ($F_{9, 65}$ = 131.34, p<0.001), Zn ($F_{9, 65}$ = 45.74, p<0.001), Fe ($F_{9, 65}$ = 4.93, p<0.001), Cd ($F_{9, 65}$ = 11.82, p<0.001), and Pb ($F_{9, 65}$ = 166.95, p<0.001) (Table 3). Table 3 showed that honey from the bamboo sites of Sabah had the highest mean value for Cu (2.84 mg/kg), while honey from the rubber and swamp sites had the lowest mean value for Cu (0.17 mg/kg). The highest mean value for Zn (2.05 mg/kg) was found in honey from Sabah sourced from bamboo sites, while the lowest mean value for Zn (0.34 mg/kg) was found in samples from a rubber and swamp area. The highest mean value for Fe (3.31 mg/kg) was found in honey from Sabah sourced from sites adjacent to the Crocker Range Park; the lowest mean value for Fe (0.87 mg/kg) was found in honey from rubber and swamp sites (Table 3). The mean value for Cd (0.03 mg/kg) was the same for all honey samples from stingless bees from all sites in this study (Table 3). The highest mean value for Pb (0.65 mg/kg) was found in honey samples from bamboo sites; the lowest mean value for Pb (0.21 mg/kg) was found in honey from secondary forests and orchards. The most common heavy metals detected in honey from Sabah were Fe (1.73 mg/kg), followed by Zn (0.85 mg/kg), Cu (0.68 mg/kg), Pb (0.30 mg/kg), and Cd (0.03 mg/kg). All samples of honey from stingless bees from sites in Sabah, Peninsular Malaysia, and Sarawak with different geographical areas had Cd and Pb levels below the permitted ML set by the Malaysian Food Act 1983, but exceeded the ML for Pb set by Codex, with samples from bamboo sites having the highest levels of Pb.

DISCUSSION

This study showed that the variation in the mineral content of honey from stingless bees was strongly dependent on the geographical origin of the honey (Table 2; p<0.001), which is also consistent with the results of previous studies on the mineral content of honey. A study by Zulhafiz *et al.* (2019) found that honey samples from the forest area had a higher total mineral content than samples from the suburban area. According to Bogdanov (2011), the mineral content of honey depends mainly on the botanical origin of the honey, with light-coloured blossom honey having a lower mineral composition than dark-coloured honey, e.g., honeydew, chestnut, and heather. In addition, the different mineral content depends on the plant uptake by different soil types, floral types, and the environmental conditions under which the bees collect their nectar for honey production and the type of bee species (Adalina, Kusmiati & Pudjiani, 2020). Differences in the mineral content of honey in this study could also be due to the materials used by bees for nest or hive construction, such as resin and wax, which vary depending on the availability of natural sources in the environment, which was also confirmed by the results of Zulhafiz *et al.* (2019). Other factors not considered in this study that could influence the mineral composition of each honey analysed include soil type, seasonal variation, and time of flowering.

In this study, all minerals were present in detectable amounts in honey from all geographical origins, with K being the most abundant element, followed by Ca, Na, and Mg (Table 2). The study on Brazilian stingless bees by Biluca *et al.* (2016) was also consistent with the results of this study, which found that K was the most abundant mineral in the honey of stingless bees (*Melipona bicolor, Scaptotrigona bicunctata, Melipona quadriasciata, Melipona marginata, Tetragonisca angustula, Melipona mondury, Melipona rufivestris mondory, Tetragona clavipes, Melipona scutellaris,* and *Trigona fuscipennis*), followed by Ca, Mg, and Na. In honeybee honey, Tualang (*Apis dorsata*) and Acacia (*Apis* spp.) honey also showed similar results in terms of higher K elements, followed by Ca and Na (Muhammad & Sarbon, 2021). Minerals found in honey samples, such as Na, Mg, K, and Ca, are of nutritional and health importance when consumed in small amounts. According to Kar, Choudhary & Bandyopadhyay (1999), honey contains some elements such as Zn, Cu, Ca, Mg, Cr, and Mg, which have been reported to play an important role in maintaining normal glucose tolerance and insulin secretion from the pancreatic β-cells. The minerals in honey also protect tissues from free radical damage (Kek *et al.,* 2017).

Although mineral content is not yet considered as a quality parameter for stingless bee honey by the Malaysian Food Act 1983, and Codex, the determination of K, Na, Mg, and Ca contents of *H. itama* honey in Sabah, Sarawak, and Peninsular Malaysia is useful to determine its nutritional value and geographical origin. Moreover, macromineral elements, such as K, Ca, and Na, as well as trace minerals, such as Fe, Cu, Zn, and Mg, play a critical role in the biological system. Data on the mineral content of *H. itama* honey in this study also helps to establish food safety guidelines for certain consumers, as people with hypertension and chronic kidney problems should consume foods high in Na, Mg, and K only in low or moderate amounts. The K content of *H. itama* honey from Sabah differed significantly between the eight geographical areas (Table 2, $p<0.001$) and ranged from 817.01 mg/kg to 3797.47 mg/kg. Consumption of K in the diet is often associated with better blood pressure control, supports muscle and nerve functions, and contributes to good heart and kidney functions (Chan *et al.,* 2023). Zulhafiz *et al.* (2019) found a much lower K content in a similar species of *H. itama* in suburban and degraded forest honey in Peninsular Malaysia, ranging from 484.11 mg/kg to 761.22 mg/kg. Similarly, Muhammad & Sarbon (2021) also found a much lower K content of 370 mg/kg in stingless bee honey from Marang, Terengganu. However, in this study, a much higher K content was found, with the highest content measured in the sites originating from bamboo forests (3797.47 mg/kg), followed by natural highland forests (1661.72 mg/kg) and orchards (1363.27 mg/kg). This suggests that honey reflects the mineral composition of the floral from which the stingless bees forage their food and nesting materials. Wang *et al.* (2020) identified different minerals in edible bamboo shoots and K was the richest macroelement in various bamboo shoots, followed by P and Mg. This therefore explains why the K content was highest in honey from bamboo sites. Previous research has also shown that the contents of K and other minerals in honey were generally much higher in natural forests (Riswahyuli *et al.,* 2020) and orchards (Pohl, 2009). The total Ca content in highland forest sites (169.01 mg/kg) was significantly higher than in the agritourism park and local fruit sites (139.68 mg/kg), orchard sites (122.61 mg/kg), and other sites in Sabah (Table 2, $p<0.001$). In contrast, the lowest Ca content was found in sites adjacent to Crocker Range Park (65.94 mg/kg), lowland forest (74.74 mg/kg), and bamboo sites (86.31 mg/kg). Ca is the most abundant mineral element in our body, accounting for about 2% of body weight and is responsible for skeletal and regulatory functions (Martinez de Victoria, 2016). Total Mg content was significantly higher in bamboo sites than in other geographical areas of origin, with 136.42 mg/kg compared to 91.12 mg/kg in highland forest sites, 89.59 mg/kg in agritourism park and local fruit sites, 83.94 mg/kg in lowland forests, and 81.80 mg/kg in orchards (Table 2; $p<0.01$). Honey from the orchards in Sabah had the highest average value for Na (77.81 mg/kg), while honey from the sites adjacent to the lowland forests had the lowest average value for Na (2.20 mg/kg) (Table 2).

Just like other foods, honey can be contaminated by the environment, but in general the levels of contamination found do not pose a health risk (Bogdanov, 2011). However, the high concentration of heavy metals in foods such as honey can be a source of disease for humans (Liao *et al.*, 2021). The heavy metals in honey in this study consisted of essential heavy metals (Cu, Zn, Fe), which are only needed in small amounts, while toxic heavy metals (Cd, As, Pb) can cause health problems as toxicity progresses. Pb, As, and Cr are not essential toxic metals, but naturally occurring elements in the environment that can enter the human health system through food, water, and air (ATSDR 2023). Table 3 showed that all honey of *H. itama* from Sabah, Sarawak, and Peninsular Malaysia had Cd and Pb levels that were below the permitted ML specified in the Malaysian Food Act 1983. However, the honey exceeded the ML for Pb set by Codex, raising concerns about possible toxicological risks to human health. The Codex Alimentarius Commission has set a ML for Pb in honey from honeybees (0.1 mg/kg) and the Global Monitoring System (GEMS) analysis was based on data from only 18 countries

Table 3. Heavy metals content of *H. itama* honey sampled from sites with different geographical areas

Geographical Areas	*Total Sites*	*Total Samples*	*Cu*** (mg/kg)*		*Zn*** (mg/kg)*		*Fe** (mg/kg)*		*Cd*** (mg/kg)*		*Pb*** (mg/kg)*	
			Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Adjacent to Crocker Range Park	4	10	0.57^{c}	0.09	0.74^{b}	0.08	3.31^{de}	0.66	0.03^{cd}	0.00	0.29^{c}	0.01
Adjacent to highland forest	2	5	0.47^{bc}	0.10	0.88^{b}	0.14	1.87^{e}	0.02	0.03^{bcd}	0.00	0.24^{ab}	0.01
Adjacent to lowland forest	3	10	0.21^{ab}	0.02	0.58^{b}	0.01	1.17^{bc}	0.04	0.03^{de}	0.00	0.29^{bc}	0.01
Agritourism park and local fruit	1	5	0.43^{c}	0.00	0.73^{b}	0.00	1.25^{c}	0.00	0.03^{ab}	0.00	0.23^{a}	0.00
Bamboo	1	5	2.84^{d}	0.00	2.05^{c}	0.00	1.69^{e}	0.00	0.03^{e}	0.00	0.65^{d}	0.00
Orchard	3	10	0.47^{c}	0.04	0.87^{b}	0.08	2.34^{de}	0.34	0.03^{ab}	0.00	0.25^{abc}	0.01
Rubber and Swampy Area	1	5	0.17^{a}	0.00	0.34^{a}	0.00	0.87^{a}	0.00	0.03^{a}	0.00	0.23^{a}	0.00
Secondary forest and orchard	1	5	0.27^{bc}	0.00	0.59^{b}	0.00	1.37^{cde}	0.00	0.03^{a}	0.00	0.21^{a}	0.00
Sabah (Mean Total)	16	55	0.68	0.03	0.85	0.04	1.73	0.13	0.03	0.00	0.30	0.00
Peninsular Malaysia	2	10	0.40^{c}	0.02	0.38^{a}	0.02	1.03^{b}	0.03	0.03^{cd}	0.00	0.24^{ab}	0.01
Sarawak	2	10	0.45^{bc}	0.08	0.69^{b}	0.06	1.25^{cd}	0.04	0.03^{abc}	0.00	0.22^{a}	0.00

SE: Standard error
**significance at $p<0.01$; ***significance at $p<0.001$. Different superscript letters indicate significant difference ($p<0.05$) between the samples and standards as indicated by Dunn's test for multiple comparisons. Values with at least one similar alphabet in the same row are not significantly different ($p>0.05$).

worldwide (CAC, 2022). Previous studies have shown that ML of Pb in honey vary from region to region due to different environmental conditions, floral sources, and national practices. For example, Pb concentrations in honey from Croatia (Bilandžić *et al.,* 2011), the Middle East, North Africa, the Arab States (Théolier, Dominguez & Godefroy, 2024), and Romania (Bartha *et al.,* 2020) ranged from low to several times higher than the ML recommended by Codex. In this study, the mean concentrations of Pb in honey from stingless bees ranged from 0.21 mg/kg in secondary forest and orchard honey to 0.65 mg/kg in bamboo honey (Table 3), indicating that the levels of this toxic metal are of concern. Higher ML levels were also found in honey from all geographical areas where the honey samples were collected from hives far away from sources of Pb contamination such as urban areas and motorways. This indicated that there was no evidence of contamination from vehicles with internal combustion engines. One possible explanation for this is the high Pb concentration in the soils of the study areas. This result is also confirmed by a study in Croatia by Tlak Gajger *et al.* (2024), who found that the Pb content in all analysed honey exceeded the Codex ML value of 0.1 mg/kg, regardless of where the samples were taken. However, honey from stingless bees, which mostly originates from tropical countries, is not currently considered a food of concern for Pb under the Codex. Thus, there is also the possibility that the Codex ML value for environmental contaminants in honey from honeybees (*Apis* spp.) differs from the Pb contaminants in honey from stingless bees, which therefore requires further investigation.

Pb accumulation was highest at bamboo sites compared to the other sites in this study, which is probably due to the high Pb contamination in soils. Pb, Cd, and Zn are known as potential air or soil contaminants of anthropogenic origin, but also occur as natural components of soil minerals (Bogdanov, 2011). Pb, one of the most abundant heavy metal elements in soil, cannot be degraded and is bioaccumulative (ATSDR, 2023). In this study, Pb was most likely absorbed by the bamboo and this in turn led to the accumulation of these metals by bees in their beehives and honey pots, which were then detected in the honey. Bamboo has a large rhizome root system and is generally distributed in shallow soil layers (0–30 cm), but these plants have a high Pb absorption capacity (Liao *et al.*, 2021). In addition, heavy metals in honey are stable for a long time because they originate from the soil, are transported through the root system to the nectar-producing plants, passed into the nectar, and then enter the honey produced by the bees (Kek *et al.*, 2017). As the area is covered with bamboo, it is possible that stingless bees utilised parts of the bamboo as a food source, especially the soft, chewable parts of the bamboo shoots (Table 3). Results from Bian *et al.* (2020) have shown that some bamboo species have a high adaptability to metal-containing environments and a high capacity to absorb heavy metals. The bamboo tissues in the rhizome and culm can accumulate a large amount of heavy metals, which mainly accumulate in the cell wall, vacuole, and cytoplasm.

Honey samples from bamboo sites in Sabah had the highest average values for Mg, K, Zn, Cu, and Pb than other forest sites (Crocker Range Park, highland, and lowland forests), mixed vegetation (agritourism and local fruits, secondary forest, and orchard), and agricultural sites (orchard, rubber and swampy area) (Table 2 and Table 3). A previous study result showed that Pb content in honey from bamboo sites was very high and exceeded international food standards, which was probably due to the high heavy metal load in the polluted environment that bamboo absorbed from soils (Bian *et al.*, 2020). The higher content of essential minerals (Mg, K) and essential heavy metals (Zn and Cu) in honey from bamboo sites could therefore be due to the natural content of these elements in bamboo, which were analysed together with Pb in honey in this study. According to Chongtham *et al.* (2021), bamboo shoots are naturally rich in macro- and microminerals, and are a good source of K, Mg, Fe, Ca, Zn, and P, which are also recommended as a healthy food to promote bioactive compounds. Honey samples from agricultural sites (orchard, rubber and swampy area) and mixed vegetation (secondary forest and orchard) had the highest mean value of Na (Table 2). The higher average value of Na in honey from agricultural and mixed vegetation areas compared to other sites in this study could be related to the use of fertilisers that release Na. Na is required as an essential element for many agricultural crops such as fruit trees. Na-based fertilisers include Na2SO4, NaNO3, and NaCl, suggesting that Na levels were highest in the agricultural and mixed vegetation sites in this study. The remaining honey samples from sites in other geographical areas had low to moderate average values for mineral and heavy metal contents (Table 2 and Table 3). In general, these results suggested that honey reflects the mineral components of its geographical origin from which the stingless bees collect their food. The content of heavy metals in honey thus depends on the type of soil in which the plants and nectar are found, revealing the botanical origin of each honey sample.

CONCLUSION

This study concluded that the composition of minerals and heavy metals in honey varied according to geographical locations in Sabah, Sarawak, and Peninsular Malaysia. In terms of national food standards, all samples of stingless bee honey did not exceed the maximum permissible levels for Cd and Pb as stipulated by the Malaysian Food Act 1983. As for the international food standards as stipulated by Codex, the honey samples were found to exceed the ML levels for Pb, raising concerns about possible risks to human health. Considering the toxic nature of Pb, which was present in all geographical areas of this study, this emphasised the importance of continuous monitoring of Pb levels in honey from stingless bees.

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Authors' contributions

Benedick S, lead researcher, conceptualised and designed the study, advised on data analysis and interpretation, and reviewed the manuscript; Binjamin B and Johny @ Hasbullah MIJ, conducted the data collection in Sabah, Sarawak and Peninsular Malaysia, prepared the draft manuscript and conducted the data analysis and interpretation; Gobilik J, Chin CFS, Lum MS, Ador K, and Mohd Yaakub N, assisted in drafting the manuscript and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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