ASSESSMENT OF ELEMENTS IN SOIL AND STINGLESS BEE HONEY AND ITS CORRELATION USING NAA AND ICP-MS

Siti Aminah Omar[1], Mohd Noor Hidayat Adenan[2], Salmah Moosa[2], Zalina Laili[1], Md Suhaimi Elias[1], Nazaratul Ashifa Abdullah Salim[1], Azian Hashim[1], Shakirah Abd Shukor[1], Muhd Azfar Azman[1], Munirah Abdul Zali[1], Mohd Razif Mamat[3], Nor Azfa Johari[3], Azira Muhamad[3]

[1]Analytical Chemistry Application Group (ACA), Waste and Environmental Technology Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia

[2]Agroecosystem Management Group, Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia

[3]Malaysia Genome and Vaccine Institute (MGVI), National Institutes of Biotechnology Malaysia, Jalan Bangi, 43000 Kajang, Selangor, Malaysia

sitiaminah@nm.gov.my

ABSTRACT

The objective of this study is to analyse the elemental concentration of two different environmental compartments namely stingless bee honey and its corresponding soil sample collected from the northern and central zone of Peninsular Malaysia that is Baling, Pulau Langkawi, Pulau Pinang, Perlis and Sepang using neutron activation analysis (NAA) and inductively coupled plasma mass spectrometry (ICP-MS). 17 elements were analysed which include Al, As, Ba, Ce, Cr, Cs, Fe, Hf, Fe, La, Mg, Mn, Na, Sb, Th, V and Zn of various concentrations. Soil-honey elemental correlation studies were determined using Pearson's correlation analysis which resulted in mostly weak and moderate correlation of the concentrations of the seventeen elements among the soil and honey samples. Only Cs was found to be highly correlated between stingless bee honey and the soils. Bioaccumulation from soil to honey was found to be relatively low with Na, K, Mg, Mn and Zn showed greater bioaccumulation than all the other elements.

ABSTRAK

Objektif kajian ini adalah untuk menganalisis kepekatan unsur dua petak alam sekitar yang berbeza iaitu madu lebah tanpa sengat dan sampel tanah yang sepadan yang dikumpul dari zon utara dan tengah Semenanjung Malaysia iaitu Baling, Pulau Langkawi, Pulau Pinang, Perlis dan Sepang menggunakan analisis pengaktifan neutron (NAA) dan spektrometri jisim plasma gandingan induktif (ICP-MS). 17 elemen telah dianalisis yang merangkumi Al, As, Ba, Ce, Cr, Cs, Fe, Hf, Fe, La, Mg, Mn, Na, Sb, Th, V dan Zn pelbagai kepekatan. Kajian korelasi unsur tanah-madu ditentukan menggunakan analisis korelasi Pearson yang menghasilkan korelasi kebanyakannya lemah dan sederhana bagi kepekatan tujuh belas unsur antara sampel tanah dan madu. Hanya Cs didapati sangat berkorelasi antara madu lebah tanpa sengat dan tanah. Bioakumulasi dari tanah kepada madu didapati agak rendah dengan Na, K, Mg, Mn dan Zn menunjukkan bioakumulasi yang lebih besar daripada semua unsur lain.

Keywords: stingless bee honey, neutron activation analysis (NAA), Pearson's correlation analysis

INTRODUCTION

Stingless bees (Meliponini) are native to the tropical and subtropical regions of the world including Malaysia. These bees producing *kelulut* honey, which is best described as having a distinct flavour with unique sour taste [1]. Stingless bees are smaller in size than the honeybees (*Apis mellifera*) [2] and also differ in the way the honey is stored in its hives with the former stored in clusters of small resin pots whereas honeybees stored in hexagonal-shaped honeycombs [3]. Stingless bee honey contains carbohydrates, amino acids, phenolic compounds, organic acids, vitamins, minerals, lipids and enzymes [4]. Recently, it has been reported that stingless bee honey contains trehalulose, a bioactive component which is believed to play a major role in its therapeutic properties [5].

The chemical composition of honey is strongly influenced by the surrounding natural and anthropogenic factors including types of flora, soil, climatic conditions, beekeeping practice, use of fertilizers and pesticides as well as industrial developments nearby the bee hives [6,7,8,9]. As elements in food reflect its growing conditions, hence it can be used as a marker to determine the origin. Use of multi-elements to classify the geographical and botanical origin of honey has been well established [10,11,12]. Furthermore, there have also been studies that reported the association between chemical composition in honey and other parameters such as soil, plants and water [13,14,15]. The soil composition which is influenced by the geochemical and geological features affects the concentration of elements in nectar, pollen and floral plants which will eventually have an effect on the concentration of elements in honey [16,17]. Indirectly, the elemental contents of honey could also be used as a bioindicator of environmental pollution [18]. As bees foraging for nectars, pollen and waters for quite wide area up to 7km^2**,** thus a large area can be sampled for environmental pollution through some hive flocks [9].

Hence, the aim of this study was to provide an assessment of the concentration level of multi-elements in stingless bee honey and its corresponding soil sample as well as the correlation between the honey and soil. Bioconcentration factors (BAF) were also examined to assess the accumulation of metals from soil to honey.

METHODOLOGY

Study Area

Soil sample and the corresponding stingless bee honey were collected from five different meliponiculture sites in northern and central area of Peninsular Malaysia, namely Baling, Pulau Langkawi, Pulau Pinang, Perlis and Sepang during sampling period in August to September 2020. The geographic coordinate of the sampling points is depicted in Table 1. The soil sample were collected using an auger at 0 – 10cm depth and transferred into a polyethylene bag. At the laboratory, the soil samples were dried in the oven at 60 ^{o}C followed by grounding using agate mortar.

Honey samples were harvested directly from the honey pots of each stingless bee colonies using plastic syringe and stored in airtight plastic container. Approximately 20-50 ml of stingless bee honey were harvested from each plot. Samples were then kept in the freezer at the laboratory at 4 ^{o}C until analysis.

Table 1. Location of the study area

Location	Coordinates
Baling	N 05^{o}35.665’ E 100^{o}46.799’
Pulau Pinang	N 05^{o}19.772’ E 100^{o}14.918’
Pulau Langkawi	N 06°25.189' E 099°46.822'
Perlis	N 06^{o}31.528’ E 100^{o}10.238’
Sepang	N 02^{o}35.827’ E 101^{o}42.577’

Analysis of Soil Sample Using Neutron Activation Analysis (NAA)

Approximately 0.10g of powdered soil sample were weighed into polyethylene vials and heat-sealed. Soil samples, standard reference materials (SRM's) and blank were co-irradiated at the PUSPATI TRIGA Research Reactor facility of the Malaysian Nuclear Agency for two times: 30 seconds and 4 hours for determination of short half-lived and long half-lived radionuclides, respectively. Counting of the γ-ray was done using a gamma spectrometer (EG&ORTEC, USA). Altogether 17 elements in soil samples were analysed using NAA techniques which include Al, As, Ba, Ce, Cr, Cs, Fe, Hf, K, La, Mg, Mn, Na, Sb, Th, V and Zn. The calculation of elements concentration using the NAA technique is based on Equation 1 [19].

$$C_{EL} = \frac{A_{smp}}{A_{std}} \times \frac{W_{std}}{W_{smp}} \times C_{std} \quad (1)$$

where:

C_{EL} = concentration of interested element in the sample (mg/kg)

A_{smp} = net count of the selected peak area of an interested element in a sample

A_{std} = net count of the selected peak area of an interested element in a standard

W_{smp} = Weight of sample used (g)

W_{std} = Weight of standard used (g)

C_{std} = Concentration of interested element in standard (mg/kg)

Analysis of stingless bee honey sample using inductively coupled plasma mass spectrometry (ICP-MS)

Approximately 0.50g of stingless bee honey sample were placed in tetrafluoromethaxil (TFM) vessel and digested with 10 ml concentrated nitric acid (67 % v/v) (trace metal grade) using a microwave digester (MARS 6, CEM, Matthews, USA). Upon completion of the digestion process, the rotor body was let to cool down to room temperature and the digested samples were collected followed by elemental analysis using ICP-MS (NeXion, PerkinElmer, Norwalk, CT, USA). For calibration, multi-element solutions supplied by Perkin Elmer (PerkinElmer, Norwalk, CT, USA) was used. Certified reference materials (Tomato Leaves – 1573a) (National Institute of Standards and Technology (NIST)) was used as quality control material following the same preparation step. All the elements quantified following the NAA technique were analysed.

Correlation studies and bioaccumulation factor analysis

Correlation studies were done using SPSS 27.0 package for Windows (SPSS Inc., Chicago, IL, USA). Bioaccumulation factor (BAF) was determined for honey/soil comparisons by dividing the concentration of elements in honey by its concentration in the soil [7].

RESULTS AND DISCUSSION

Multi-elemental Concentrations In Soils

The concentrations of 17 elements in soils from five meliponiculture sites (Baling, Pulau Pinang, Pulau Langkawi, Perlis and Sepang) shown in Figure 1. The descending order of elements concentration in soil was Al>Fe>K>Mg>Na>Ba>Mn>Zn>Ce>V>La>Cr>Th>Cs>As>Hf>Sb ranging from 0.32 to 77905 mg/kg. The average concentration of major elements was ranged from 14552 – 77905 mg/kg (Al), 2793 – 33718 mg/kg (Fe), 18436 – 28079 mg/kg (K), 1574 – 10077 mg/kg (Mg) and 509 – 2658 mg/kg (Na). The concentration of these major elements was within the range reported of surface soil in western Perak [20]. The elemental concentration of Ba, Mn, Zn and V was in the range of 139.3 – 306.3, 22.7 – 413.4, 17.8 – 180.8 and 10.4 – 100.5 mg/kg, respectively. As for rare earth elements, the concentration of Ce, La and Hf was in the range of 17.3 – 132.4, 7.40 – 59.3 and 2.57 – 13.53 mg/kg, respectively. Soil sample from Sepang appeared to have lower concentration of rare earth elements than the other meliponiculture sites. As for heavy metals Cr, As and Sb, their concentration

ranges from 5.76 – 66.1, 1.65 – 17.66 and 0.32 – 20.36 mg/kg, respectively. The Cr and As concentration was much lower than the average concentration of Cr and As in soil from Klang industrial area [21], whereas Sb concentration appeared to be higher which was largely contributed from soils from Perlis.

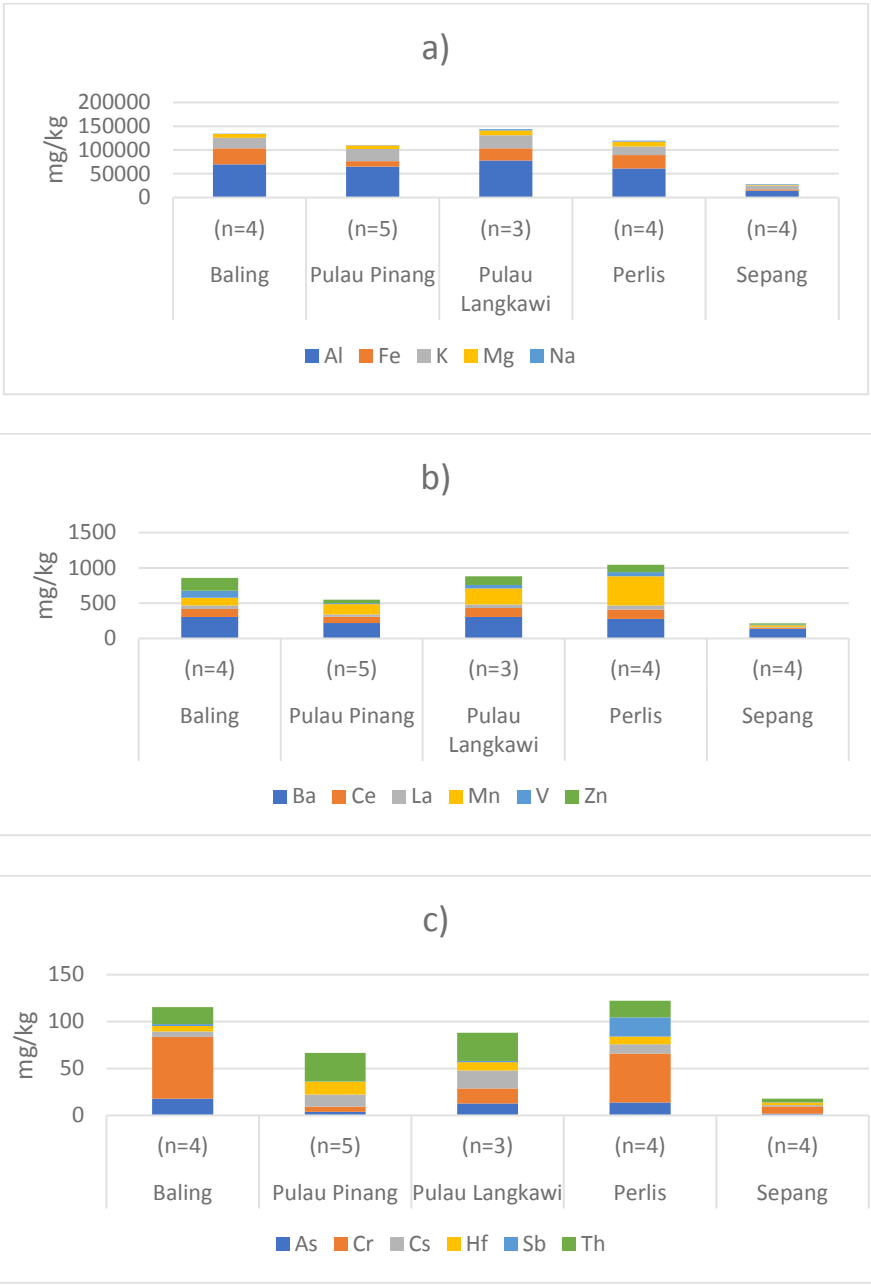

Figure 1: Mean of the elemental concentrations found in soil **a)** Al, Fe, K, Mg, Na **b)** Ba, Ce, La, Mn, V, Zn **c)** As, Cr, Cs, Hf, Sb, Th

Multi-elemental concentrations in stingless bee honey

The concentrations of 17 elements in stingless bee honey is shown in Figure 2. The most abundant element in stingless bee honey is K (983 – 3503 mg/kg). High K concentration in Malaysia's stingless bee honey were previously reported [1,3,22]. This is followed by Na and Mg (36.28 – 1168 mg/kg), although variations could be seen across all five meliponiculture sites in this study. Honey sample from Sepang showed the highest Na concentration, probably due to its hives located nearby to the seashore. Minor elements found in stingless bee honey include Mn, Al, Fe, Zn and Ba in the range 1.0 - 7.6 mg/kg. The concentration of Mn, Al and Ba were

higher than previously reported of Malaysian's honey [23], whereas Fe and Zn concentration were found to be lower. Other elements can be classified as trace elements in stingless bee honey honey (< 1 mg/kg) which include Cr, Cs, V, As, La, Hf, Ce, Sb and Th.

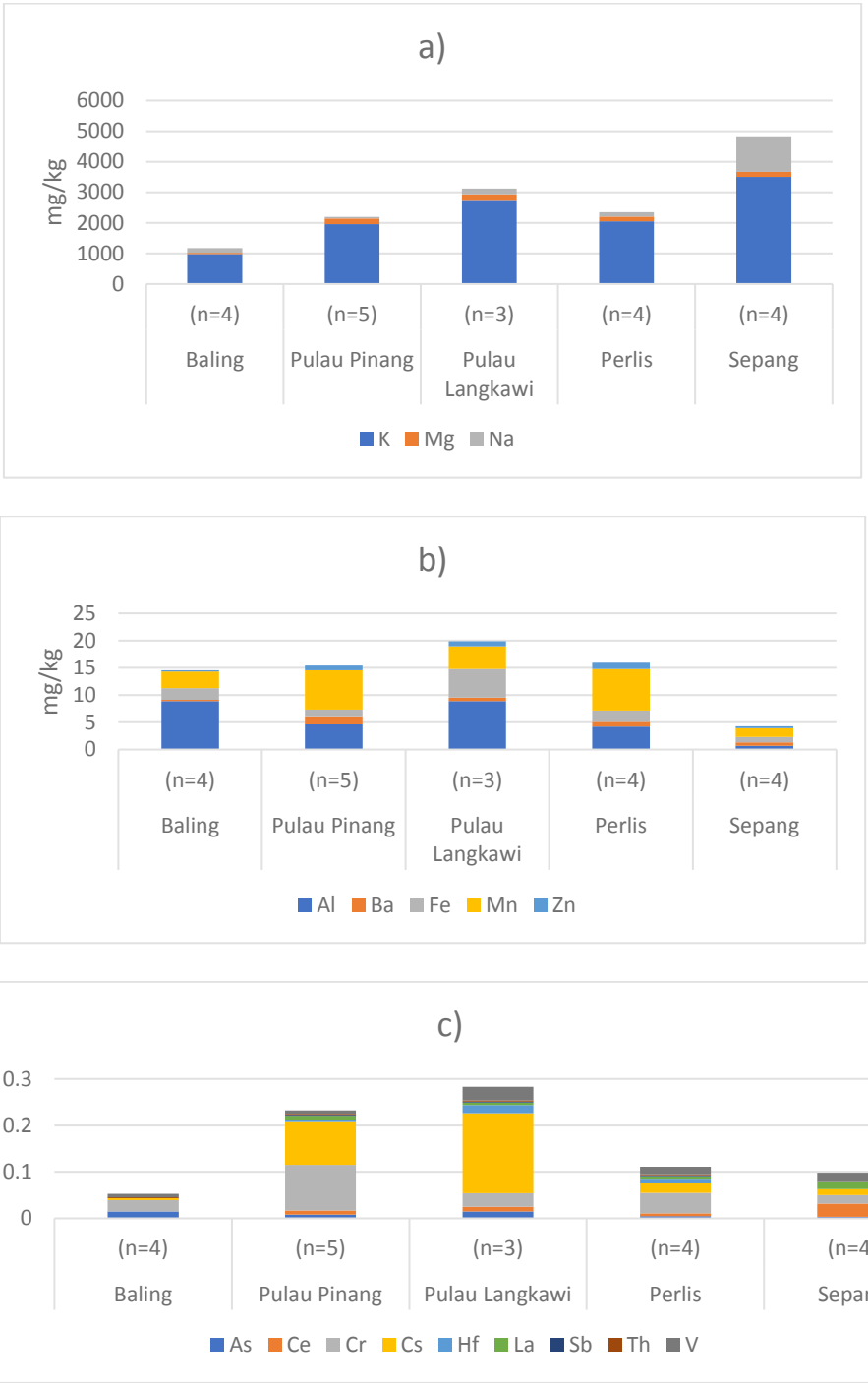

Figure 2: Mean of the elemental concentrations found in honey **a)** K, Mg, Na **b)** Al, Ba, Fe, Mn, Zn **c)** As, Ce, Cr, Cs, Hf, La, Sb, Th, V

Relationship Between Elemental Concentrations In Stingless Bee Honey And In Soils

Pearson correlation coefficient of elements in stingless bee honey and those in soils are listed in Table 2. The descending order of the significant correlation based on the *P* value were Cs>K>Al>Mn>Zn>Ba>Cr>Mg>Na>V>Fe>As. Notably, only Cs showed strong correlation between the pair, while the other honey-soil correlation can be described as moderate (3) and weak (8). It is postulated that the

weak and negative correlation between soil and honey could derived from the complexities of honey collection by the bees [9]. Other studies have shown that soil mineral availability affect the flower plants and nectar compositions [7,14].

Table 2. Correlation coefficients of elemental composition between stingless bee honey and soils.

Elements	Correlation coefficients	*P* value
Al	0.443	0.051
As	-0.002	0.992
Ba	-0.255	0.278
Ce	-	-
Cr	-0.233	0.323
Cs	0.844	<0.001
Fe	0.014	0.953
Hf	-	-
K	-0.534	0.015
La	-	-
Mg	-0.194	0.411
Mn	0.368	0.111
Na	-0.163	0.493
Sb	-	-
Th	-	-
V	-0.153	0.520
Zn	0.279	0.234

Metals Accumulation From Soil To Honey

Table 3 summarized the bioaccumulation factor (BAF) of elements from the five meliponiculture sites. The BAF is a quantitative approach that characterize the accumulation of heavy metals or any other substances within the tissue of an organism in relation to the surrounding environment such as soil or water [24]. Only Na from Sepang samples had BAF value greater than 1, while other elements had significantly low value. Low BAF value for honey/soil from Hungary was also reported previously [7]. The BAF value was greater for Na, K, Mg, Mn and Zn than all the other elements. The observation on the BAF value showed that high elemental concentrations in soil resulted in high concentration in honey for certain elements. For toxic metals such as Al, As, Fe, Cr, Sb and Zn, the translocation was found to be very low, signalling that there was very little movement of these elements from the soil to honey.

Table 3. Bioaccumulation factor of elements from different meliponiculture sites.

Element	Baling	Pulau Pinang	Pulau Langkawi	Perlis	Sepang
Al	0.00003	0.00006	0.00015	0.00007	0.00004
As	0.0001	0.0025	0.0010	0.0004	0.0017
Ba	0.0007	0.0068	0.0017	0.0029	0.0047
Ce	-	0.00010	0.00007	0.00004	0.00167
Cr	0.0004	0.0170	0.0020	0.0008	0.0028
Cs	0.0007	0.0073	0.0083	0.0020	0.0076
Fe	0.00002	0.00010	0.00021	0.00007	0.00042
Hf	-	0.00003	0.00063	0.00013	-
K	0.044	0.070	0.094	0.111	0.431

La	-	0.00022	0.00012	0.00011	0.0021
Mg	0.003	0.030	0.017	0.015	0.103
Mn	0.028	0.041	0.017	0.018	0.072
Na	0.311	0.034	0.078	0.080	1.55
Sb	-	0.0026	0.0018	0.0001	-
Th	0.00006	0.00003	0.00007	0.00013	-
V	0.00008	0.00050	0.00062	0.00030	0.00187
Zn	0.0037	0.0165	0.0088	0.0130	0.0222

CONCLUSION

Elemental concentration in stingless bee honey and the corresponding soil samples from several meliponiculture sites in Peninsular Malaysia were examined. Analysis results showed that stingless bee honey from Malaysia contains essential elements such as K, Na, Mg and Mn with various concentrations across all meliponiculture sites in this study. Correlation studies showed that Cs is highly correlated between the soil-honey pair. BAF value indicated that there was very little bioaccumulation especially for toxic metals.

ACKNOWLEDGMENTS

The authors wished to thank the staff from the Analytical Chemistry Application group (ACA) for their help during the sampling process and irradiation of the soil samples using NAA technique.

REFERENCES

[1] Ramly N S, Sujanto I S R, Tang J Y H, Ghani A A, Alias N, Mohamed S, Zakaria A J and Ngah N 2021 Physicochemical properties and mineral elements in honey from various species of malaysian stingless bee *Biosci, Res.* **18**(SI-2) 34-44.

[2] Sujanto I S R, Ramly N S, Tang J Y H, Ghani A A, Alias N, Mohamed S and Ngah N 2022 Influence of spatial variation on the physicochemical properties and mineral content of stingless bee honey (Heterotrigona itama) in Terengganu, Malaysia. Asian J. Agric & Biol. 4 202104176.

[3] Kek S P Chin N L , Yusof Y A, Tan S W , Lee S C 2014 Total phenolic contents and colour intensity of Malaysian honeys from the Apis spp. and Trigona spp. bees Agricul.and Agricultural Sci, Procedia 2150-155.

[4] Fatima I J, Mohd Hilmi A B, Salwani I and Lavaniya M 2018 Physicochemical characteristics ofof malaysian stingless bee honey from Trigona Species IIUM Med. J. Malaysia=17(1) 187-191.

[5] Fletcher M T, Hungerford N L, Webber D, de Jesus M D, Zhang J, Stone I S J, Blanchfield J T, and Zawawi N 2020 Stingless bee honey, a novel source of trehalulose: a biologically active disaccharide with health benefits Sci. Rep. 1012128

[6] Moniruzzaman M, Chowdhury M A, Rahman M A, Sulaiman S A, Gan S H 2014 Determination of mineral, trace element and pesticide levels in honey samples originating from different regions of Malaysia compared to manuka honey. Biomed. Res. Int. 359890

[7] Czipa N, Diósi G, Phillips C and Kovács B 2017 Examination of honeys and flowers as soil element indicators Environ. Monit. Assess. 189 412

[8] Skorbiłowicz M, Skorbiłowicz E, Cieśluk I 2018 Bees as bioindicators of environmental pollution with metals in an urban area. J. of Ecol.Eng. 19(3) 229-23.

[9] Kastrati G, Paçarizi M, Sopaj F, Tašev K, Stafilov T and Mustafa MK 2021 Investigation of concentration and distribution of elements in three environmental compartments in the region of Mitrovica, Kosovo: Soil, honey and bee pollen Int. J. Environ. Res. Public Health 18 2269.

[10] Shadan A F, Mahat N A, Wan Ibrahim W A, Ariffin Z and Ismail D 2018 Provenance establishment of stingless bee honey using multi-element analysis in combination with chemometrics techniques J. Forens.Sci. 63(1) 80-85.

[11] Kek S P, Chin N L, Tan S W, Yusof Y A and Chua L S 2017 Classification of honey from its bee origin via chemical profiles and mineral content Food Anal. Meth. 10 19–30.

[12] Jovetić M, Trifković J, Stanković D, Manojlović D, Milojković-Opsenica D 2017 Mineral Content as a Tool for the Assessment of Honey Authenticity Journal of AOAC INTERNATIONAL, 100(4):862–870.

[13] Schmidlová S, Javurková Z, Tremlová B, Hernik J, Prus B, Marcincák S, Marcincáková D, Štarha P, C˘ížková H, Kružík V, Bodor Z, Benedek C, Titera D, Borzikova J and Pospiech M 2024 Exploring the influence of soil types on the mineral profile of honey: implications for geographical origin prediction Foods 13 2006.

[14] Atanasov A, Hristakov I, Kuncheva G, Koszel M and Dochev V 2023 Assessment of heavy metals in soil, oilseed rape (Brassica napus L.) and honey Plant Soil Environ. 69(8) 400-407.

[15] Mititelu M, Udeanu DI, Nedelescu M, Neacsu S M, Nicoara A C, Oprea E, Ghica M 2022 Quality control of different types of honey and propolis collected from romanian accredited beekeepers and consumer's risk assessment Crystals 12(1) 87.

[16] Baroni M V, Podio N S, Badini R G, Inga M, Ostera H A, Cagnoni M, Gautier E A, García P P, Hoogewerff J and Wunderlin D A 2015 Linking Soil, water and honey composition to assess the geographical origin of argentinean honey by multielemental and isotopic analyses J. Agric. Food Chem. 63(18) 4638–464.

[17] Uršulin-Trstenjak N, Puntarić D, Levanić D, Gvozdić V, Pavlek Ž, Puntarić A, Puntarić E, Puntarić I, Vidosavljević D, Lasić D and Vidosavljević M 2017 Pollen, physicochemical, and mineral analysis of Croatian acacia honey samples: Applicability for identification of botanical and geographical origin J.of Food Qual. 8538693.

[18] Nascimento A S, Chambó E D, Oliveira D J, Andrade B R, Bonsucesso J S and Lopes de Carvalho C A 2018 Honey from stingless bee as indicator of contamination with metalsSociobiol. 65(4) 727–736.

[19] Elias M S, Ibrahim S, Samuding K, Rahman S A and Wo Y M 2018 Assessment of toxic elements in sediments of Linggi River using NAA and ICP-MS techniques Meth. X 5 454–465.

[20] Azman M A, Hamzah S, Rahman S A, Elias M S, Salim N A A, Hashim A, Shukor S A and Kamaruddin A H C 2015 Trace element analysis of soil type collected from the Manjung and central Perak AIP Conf. Proc. 1659 050003

[21] Elias M S, Azman M A, Daung J A D, Hashim A, Omar S A, Salim N A A, Shukor S A and Laili Z 2024 Evaluation of elemental pollution in soil samples from Klang industrial area IOP Conf. Ser.: Mater. Sci. Eng. 1308 012017

[22] Cheng M, Ismail M, Chan K W, Ooi D J, Ismail N, Zawawi N, Lila M A and Esa N M 2019 Comparison of sugar content, mineral elements and antioxidant properties of Heterotrigona itama honey from suburban and forest in Malaysia Mal. J. Med. Health Sci. 15 104-112.

[23] Chua L S, Abdul-Rahaman N, Sarmidi M R, Aziz R 2012 Multi-elemental composition and physical properties of honey samples from Malaysia Food Chem.135(3) 880-887.

[24] Bora F D, Babeș A C, Călugăr A, Jitea M I, Hoble A, Filimon R V, Bunea A, Nicolescu A, Bunea CI 2023 Unravelling heavy metal dynamics in soil and honey: a case study from Maramureș region, Romania Foods 12(19)3577.