

HONEYBEE PRODUCTS AS POTENTIAL BIOINDICATORS FOR HEAVY METAL CONTAMINATION IN NORTHERN VIETNAM

Tran Thi Ngat^{1,2, ✉}, Truong Xuan Lam^{1,2}, Hoang Gia Minh², Nguyen Thi Phuong Lien^{1,2}

¹Graduate University of Science and Technology, Vietnam Academy of Science and Technology

²Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology

✉To whom correspondence should be addressed. E-mail: tranthingat1012@gmail.com

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SUMMARY

Contamination of heavy metals (As, Cd, Hg, Pb and Sn) in honeybee products (*Apis cerana*) from Northern Vietnam is determined. The study was carried out in two main harvesting seasons of beekeeper farms (April and October), over two years (2018-2019). A total of 72 samples from 24 honeybee hives from 8 provinces and one city were collected. The results showed that the quality of three products in nearly all research sites were met the standards in accordance with the national technical regulation on the limits of heavy metal contamination in food of the Vietnamese Health Ministry, except for the pollen and beeswax from HY2 site. The concentration of Pb was most notably value in this study, which was determined at fairly high levels in pollen (3,767 mg/kg) and beeswax (5,840 mg/kg) from HY2 site. This can be a warning for this metal significant contamination in the habitat. Specially, Hg was not detected in most samples or only recorded without significant. For the environmental types, the mean value of As and Sn in all honeybee's product types in semi-rural area were higher than that in rural area. Thus, the detection of the heavy metals proves that honeybee's products could be good indicators to detect the environmental contaminants and monitor the habitat quality of a particular area.

Keywords: heavy metal, contamination, *Apis cerana*, honey, pollen, beeswax.

INTRODUCTION

The inappropriate use, exploitation and processing of natural resources such as soil, water, forests and the strong development of many industrial sectors are now causing harm to the environment. Specifically, many water sources have been heavily polluted, CO₂ content increased rapidly in the air, residues and debris from factories, transportation are being accumulated in the ground (Bradl, 2002; Fergusson, 1990; He *et al.*, 2005). This seriously affects the growth and development of many flowering plants. In the process of taking water and nutrients from the soil to nourish the tree, a metals residual amount in the soil into the tree

and accumulate in parts such as leaves, plant resin, nectar, and flower pollen. Honeybees take pollen or nectar from these plants as food and the potential for heavy metal residues in the products created by them is very high (Bogdanov, 2006; Bromenshenk *et al.*, 1985; Höffel, 1985; Roman, 2004). With a sufficiently large amount in food can significant impact on the health of consumers (Kabata-Pendias and Mukherjee, 2007). In particular, arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and tin (Sn) are considered to be highly toxic, therein As is a carcinogen, Cd causes bone degeneration, three remain metals cause damage to the central nervous system (Anyanwu *et al.*, 2018). On the other hand, these metallic elements have a strong affinity for sulfur

and in the human body, they often bind to enzymes responsible for controlling the rate of metabolic reactions through Thiol (–SH) groups. Sulfur-metal bonds inhibit the activity of related enzymes, so causing metabolic disorders in the body and adversely affecting human health (Jan *et al.*, 2015).

The honeybee is considered a good biological indicator to assess the environmental condition through the presence of pollutant in their products (Fakhimzadeh *et al.*, 2005; Perugini, 2009). In the world, this issue has been mainly conducted on *Apis mellifera* Linnaeus, 1758 (Costa *et al.*, 2019; Goretti *et al.*, 2020; Naccari *et al.*, 2014; Perna *et al.*, 2012; Perugini *et al.*, 2011; Popescu *et al.*, 2010; Porrini *et al.*, 2014; Ruschiono, 2013; Vincevica-Gaile *et al.*, 2012; Zhelyazkova, 2012) and limited on *Apis cerana* Fabricius, 1793 (Manzoor *et al.*, 2013). *Apis cerana* Fabricius, 1793 is one of the main honeybees species used in beekeeping farms in Vietnam (Pham, 2014; Phung, Vu, 1999). In this study, the contamination of five highly toxic metals (As, Cd, Pb, Sn and Hg) from honey, pollen and beeswax in the Northern provinces and suburb area of Hanoi city, Vietnam was detected.

MATERIAL AND METHODS

In this study, 48 honeybee samples from 16

research locations in 8 Northern provinces were collected in April 2018 and 24 honey bee samples from 8 research locations in districts of Hanoi were collected in April and October 2019 (Table 1). Based on the foraging distance from the honeybee hives, which has given by Crane (1984), the study sites were selected within radius at least 5 km apart (for locations placed in the same district) to ensure the difference among habitats. Moreover, time of sampled collection was chosen when the food sources was adequate and the bee hives were in a period of strong activities.

All collected samples were dried at 120°C for 24 hours and using 0.5 gram of each dried sample for chemical analysis. Object of this study was the concentration of five highly toxic heavy metals (Arsenic, Cadmium, Mercury, Lead and Stannum) in honey, pollen, and beeswax samples. The analysis of the samples have been carried out at the Quality Assurance and Testing Center 1, Directorate for Standards, Metrology and Quality, using ICP- MS mass spectrometer method (EPA, 2007). The data have been processed on IBM SPSS 20 software. The independent sample t-test was used for all variable comparisons. Differences between means at the 95% ($p < 0.05$) confidence level were considered statistical significance. For all samples with concentration below limit of quantification ($< LOQ$), zero was used in the calculation (Voorspoels *et al.*, 2004).

Table 1. Description of sampling sites in Northern provinces and the suburb area of Hanoi city, Vietnam.

Period	Site	Environment	Location information
April 2018	BG1	Rural, non industrial	Cao Xa commune, Tan Yen district, Bac Giang province
	BG2		Cao Thuong commune, Tan Yen district, Bac Giang province
	BK1		Nhu Co commune, Cho Moi district, Bac Kan province
	BK2		Thanh Van commune, Cho Moi district, Bac Kan province
	TQ1		My Binh village, My Bang commune, Yen Son district, Tuyen Quang province

	TQ2		Dau Nui village, My Bang commune, Yen Son district, Tuyen Quang province
	HY1		Hung An commune, Kim Dong district, Hung Yen province
	HY2		Mai Dong commune, Kim Dong district, Hung Yen province
	LC1		30/4 street, Nam Cuong ward, Lao Cai city, Lao Cai province
	LC2		Tong Sanh commune, Bat Xat district, Lao Cai province
	PT1		Dich Qua commune, Thanh Son district, Phu Tho province
	PT2		Yen Luong commune, Thanh Son district, Phu Tho province
	YB1		Dong An commune, Van Yen district, Yen Bai province
	YB2		Que Ha commune, Van Yen district, Yen Bai province
	HB1		Moi Mit village, Yen Mong commune, Hoa Binh city, Hoa Binh province
	HB2		Bun village, Yen Mong commune, Hoa Binh city, Hoa Binh province
April 2019	BV1.M4	Semi-rural	K9 road, Ba Trai commune, Ba Vi district, Hanoi
	TT1.M4		Village 1, Tu nhien commune, Thuong Tin district, Hanoi
	BV2.M4		Muong Phu Vang village, Van Hoa commune, Ba Vi district, Hanoi
	QO.M4		Phuong Cach commune, Quoc Oai district, Hanoi
October 2019	BV2.M10		Muong Phu Vang village, Van Hoa commune, Ba Vi district, Hanoi
	QO.M10		Phuong Cach commune, Quoc Oai district, Hanoi
	TT2.M10		Village 5, Tu nhien commune, Thuong Tin district, Hanoi
	ThTr.M10		Dong My commune, Thanh Tri district, Hanoi

RESULTS AND DISCUSSION

The content of five metals found in the examined samples was reported as shown in Figures 1, 2 and Table 2. The results show that various quantities of five heavy metals were accumulated in honeybee products (honey, pollen and beeswax) and the level for metals depended on the sample site.

The obtained result from samples of honeybee products collected in 8 Northern provinces was illustrated in Figure 1. The widest range of arsenic

(As) concentration was detected in beeswax samples (0-0.093 mg/kg) while the highest median value refers to pollen samples (0.013 mg/kg). The figures showed that this element was not detected in all honey samples.

The highest variable range of cadmium (Cd) was gained for honey samples (0-0.059 mg/kg), followed by beeswax samples (0-0.050 mg/kg) and lowest in pollen samples (0,036-0.085 mg/kg). However, the highest median value was observed for pollen samples (0.047 mg/kg). Some exceptions were found in groups of pollen

and beeswax samples, i.e. one honey sample contained 0.120 mg/kg and one beeswax sample showed 0.355 mg/kg for Cd concentration.

However, this content was within the allowed range in Ministry of Health for supplement products.

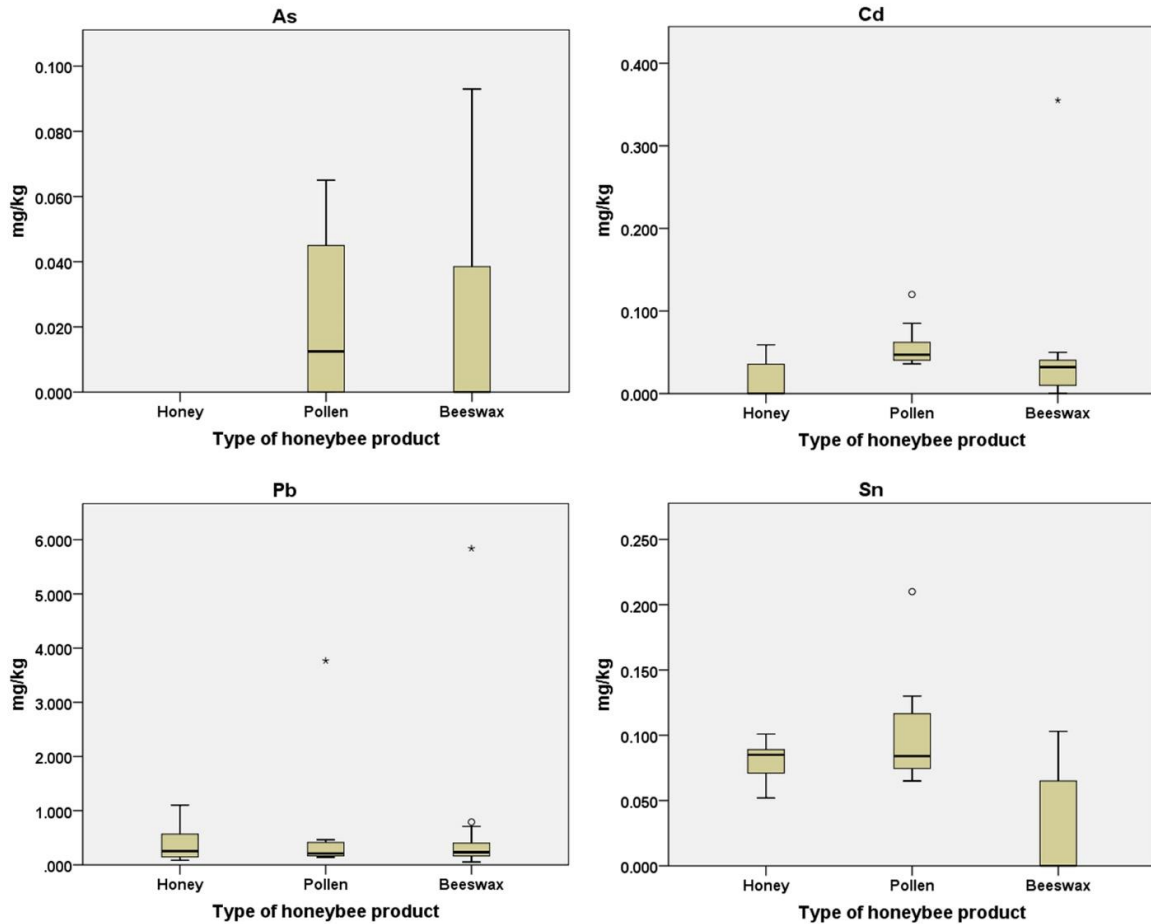


Figure 1. Box-whisker plots of heavy metals concentration in honeybee products of Northern provinces, Vietnam (n=16).

The mercury (Hg) element was not found for all honey samples and most of the remain samples of other two products. The data obtained show that three pollen samples and one beeswax sample were recorded with the number below the quantitation limit (<0.017 mg/kg), only one pollen sample was determined with the number at very low levels (0.022 mg/kg).

The range of lead (Pb) concentration within the samples of honeybee products was not too wide. The figure illustrate that the wide variability of this element was defined for honey

samples (0.085-1.010 mg/kg) and the narrowest range refers to pollen samples (0.139-0.463 mg/kg). The median concentrations of Pb were not much difference between honeybee product types and can be arranged in the following sequence: honey (0.254 mg/kg) > beeswax (0.234 mg/kg) > pollen (0.210 mg/kg). Determination some outliers in pollen and beeswax samples, which much exceed the variable range in two these product types, one pollen samples contained 3.767 mg/kg and one beeswax samples showed up to 5.840 mg/kg for

Pb concentration. Compared with the Vietnamese Health Ministry standards (Pb=3 mg/kg), this content exceeds the permitted level in supplements, which may harm consumer's health if used for a long time. Specially, both these samples were collected in HY2 site. This result reflects the habitat condition at HY2 site. Contamination of Pb in some pollen and beeswax samples may related to the impact of the pollution in the environment where those samples were collected. Some reasons may be given to explain for the issue as follows: HY2 site is one of the largest logan growing areas in the Northern Vietnam. The overused of fertilizers and pesticides for agriculture purposes may lead to accumulate Pb element and may be caused contamination for soil and water sources; on the other hand, this site is located near to the provincial road with a lot of

traffic activities, and it could also be a reason to make Pb concentration increased in the environment. Therefore, the honeybee hives should be moved to another location. At the same time, the local authorities and farmers should consider and give specific effort to protect the living environment in this area.

The widest range of stannum (Sn) concentration was revealed clearly for beeswax samples (0-0.103 mg/kg) while the highest median concentrations of this element were observed for honey samples (0.085 mg/kg) and pollen samples (0.084 mg/kg). In the group of pollen samples, one exception was detected with Sn concentration of 0.210 mg/kg in TQ1 site. However, this content was still within the permitted limits and could not be able affected human health.

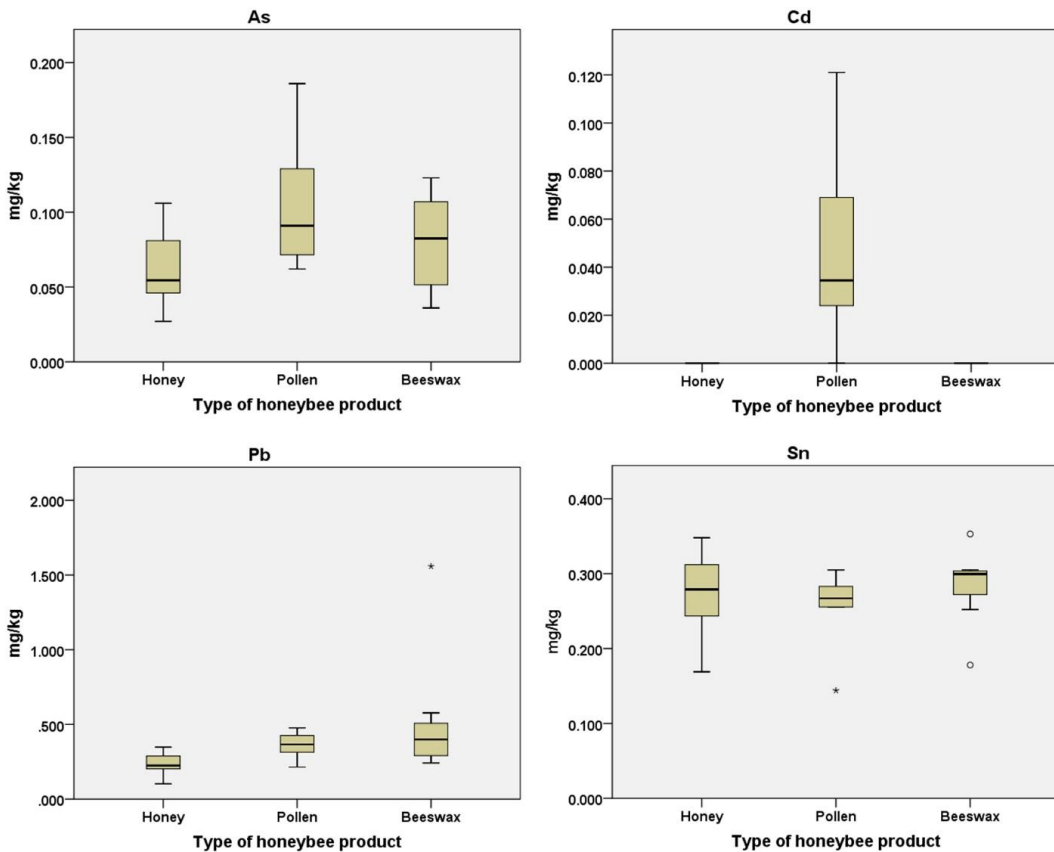


Figure 2. Box-whisker plots of heavy metals concentration in honeybee products of the Hanoi city's suburb area (n=8).

The analysis result from samples of honeybee products collected in the suburb area of Hanoi city showed in Figure 2. The variable range and median value of As element were detected as the following sequence: pollen (0.062-0.186 mg/kg, 0.091 mg/kg) > beeswax (0.036-0.123 mg/kg, 0.083 mg/kg) > honey (0.027-0.106 mg/kg, 0.055 mg/kg).

As showed in the Fig. 2, Cd element was not detected in most honey and beeswax samples, its content was below the quantitation limit (<0.025 mg/kg) in some other samples, therefore it is impossible to make box- whisker plots for this element in two these sample types. However, the range of Cd concentration was relatively wide (0-0,121 mg/kg), and 0.035 mg/kg was median value for pollen samples.

Among a total 24 samples belonging to 3 product types, Hg element was not detected from 7 honey samples, 3 pollen samples and one beeswax samples. Determination of this metal in one pollen samples was at very low level (0.018 mg/kg) and it was found under the quantitation limit (<0.017 mg/kg) in other remain samples.

The highest range of variability and highest median concentration of Pb were defined for beeswax samples (0.242-0.577 mg/kg, 0.399 mg/kg). To opposite, the lowest range and median value were found for honey samples (0.102-0.348 mg/kg, 0.225 mg/kg). This element was detected outside the variable range of Pb element (1.558 mg/kg) from beeswax sample (QO.M4). However, this value was not cause concern for the health of consumers.

In contrast to all other metals, the wide variability of Sn concentration was determined for honey samples (0.169-0.348 mg/kg) while the highest median value refers to beeswax samples (0.330 mg/kg). Some exceptions were found for pollen and beeswax samples, in which one beeswax samples contained 0.353 mg/kg of this element and gained above the variable range.

In addition, in order to compare and re-verify heavy metal content in honeybee products to see

whether they were really high as given by Nguyen *et al.* (2015), honey and beeswax samples were collected again at beekeeping farm (TT1.M4). Four heavy metals (As, Cd, Hg, and Pb) were detected from honey and beeswax samples in the previous research (Nguyen *et al.*, 2015), and the level of most heavy metals in this study were detected with much lower than that. Specially, Hg element with high toxicity was not detected from honey sample and determined below the quantitation limit (<0.017 mg/kg) for beeswax in this analysis while this element was found with much higher levels in the previous study, i.e. 15.155 mg/kg for honey sample and up to 29.034 mg/kg for beeswax sample. Similarly, the recorded of As and Pb concentrations at 0.090 mg/kg and 0.204 mg/kg for honey sample (while 9.728 mg/kg and 3.072 mg/kg in previous study), and at 0,114 mg/kg and 0.577 mg/kg for beeswax sample (while 10.385 mg/kg and 11.279 mg/kg in previous study). All figures showed that As, Hg and Pb concentrations in that study exceeded too much the permitted levels. Using products contained a toxic metal with high level, it was likely to threaten human health (Ru *et al.*, 2013). However, the phenomenon of poisoning honey bee products has not been determined in Vietnam. So, previous analytical data may be inaccurate and probably due to some reasons such as the pollution from product containers and so on.

The data from Table 2 showed that in honey samples, concentrations of nearly all metals (As, Cd, Sn) except Pb between rural and semi-rural area differed significantly ($p < 0.05$). As results, mean value of As was 0.0620 mg/kg in semi-rural area and this value of Cd was 0.0153 mg/kg in rural area. For Sn, mean value of this metal in semi-rural area (0.2733 mg/kg) was higher than that in rural area (0.0813 mg/kg).

In pollen and beeswax samples, concentrations of As and Sn differed significantly between rural and semi-rural area ($p < 0.05$) but there was no significant different for the remained metals (Hg, Cd, Pb). Mean concentration of both As and Sn in semi-rural area were higher than that in rural area. In particular, the mean value proportion of the two

metals between semi-rural area and rural area were more than four times (4,64:1) for As in both sample types, more than two times

(2,58:1) for Sn in pollen samples and more than ten times (10,52: 1) for Sn in beeswax samples.

Table 2. Mean (mg/kg) and standard deviation (SD) of honeybee products in the environmental types (rural: n=16, Semi-rural: n=8).

Sample type	Environment	As	Cd	Hg	Pb	Sn
Honey	Rural area	0	0.0153±0.022	0	0.3713±0.306	0.0813±0.014
	Semi-rural area	0.0620±0.026	0	0	0.2351±0.080	0.2733±0.058
Pollen	Rural area	0.0224±0.025	0.0547±0.022	0.0055±0.11	0.4794±0.884	0.0997±0.036
	Semi-rural area	0.1039±0.043	0.0470±0.041	0.0036±0.008	0.3626±0.085	0.2575±0.049
Beeswax	Rural area	0.0189±0.029	0.0460±0.084	0	0.6439±1.401	0.0271±0.041
	Semi-rural area	0.0801±0.033	0	0	0.5245±0.431	0.2851±0.051

CONCLUSION

For the honeybee's product type, the most remarkable thing is the maximum values and even the exceptional concentration of heavy metals was determined for pollen and beeswax samples, which may be a suggestion about the probability of their contamination in two these types was higher than in honey samples. Moreover, all recorded data in this study showed that the quality of honey and remained products in all of the research sites met the standards in accordance with the national technical regulation on the limits of heavy metal contamination in food of the Vietnamese Health Ministry, except the pollen and beeswax samples in HY2 site. This can be a warning for Pb element with significant contamination in the area's habitats. Using agrochemicals, fertilizers or traffic activities also can become contamination sources for pollen and beeswax in this sampling site.

For the environmental type, the average concentration of As and Sn in all honeybee's

product types in semi-rural area higher than in rural area.

The analysis of the results obtained from the study about the presence of the heavy metals in three products of honeybee (*Apis cerana*) reflect their quality and environmental status. Therefore, honeybee could be used as a bio-indicator for habitat assessment.

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TIỀM NĂNG SỬ DỤNG CÁC SẢN PHẨM ONG MẬT LÀM CHỈ THỊ SINH HỌC CHO Ô NHIỄM KIM LOẠI NẶNG Ở MIỀN BẮC VIỆT NAM

Trần Thị Ngát^{1,2, ✉}, Trương Xuân Lam^{1,2}, Hoàng Gia Minh², Nguyễn Thị Phương Liên^{1,2}

¹Học viện Khoa học và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

²Viện Sinh thái và Tài nguyên sinh vật, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

TÓM TẮT

Hàm lượng các kim loại nặng (As, Cd, Hg, Pb and Sn) trong các sản phẩm ong mật (*Apis cerana*) ở miền Bắc Việt Nam được xác định. Nghiên cứu tiến hành vào 2 mùa thu hoạch chính của các trại nuôi ong (Tháng 4 và tháng 10) trong vòng 2 năm (2018-2019). Tổng cộng 72 mẫu từ 24 tổ ong của 8 tỉnh và 1 thành phố được thu thập. Kết quả cho thấy, chất lượng của 3 loại sản phẩm ong mật tại hầu hết các điểm nghiên cứu đều đạt tiêu chuẩn theo quy chuẩn kỹ thuật quốc gia về giới hạn ô nhiễm kim loại nặng trong thực phẩm của Bộ Y tế Việt Nam, trừ phấn hoa và sáp ong từ điểm HY2. Đáng chú ý nhất trong nghiên cứu này là hàm lượng Pb, được xác định ở mức khá cao trong phấn hoa (3.767 mg/kg) và sáp ong (5.840 mg/kg) từ điểm HY2. Đây có thể là một cảnh báo về sự ô nhiễm kim loại này trong môi trường sống. Đặc biệt, Hg không được phát hiện trong hầu hết các mẫu hoặc chỉ được ghi nhận với hàm lượng không đáng kể. Đối với các loại môi trường, giá trị trung bình của As và Sn trong tất cả các loại sản phẩm của ong mật ở khu vực bán nông thôn cao hơn ở khu vực nông thôn. Như vậy, các sản phẩm của ong mật có thể sử dụng như chỉ thị sinh học để phát hiện các chất gây ô nhiễm môi trường và giám sát chất lượng môi trường sống của một khu vực cụ thể.

Từ khóa: Kim loại nặng, ô nhiễm, *Apis cerana*, mật ong, phấn hoa, sáp ong.

Appendix**Table A.1.** Heavy metals concentration in honey sample from research sites (mg/kg).

Site	As	Cd	Hg	Pb	Sn
BG1	ND	0.059	ND	0.891	0.101
BG2	ND	<LOQ	ND	0.145	0.052
BK1	ND	<LOQ	ND	0.165	0.096
BK2	ND	0.035	ND	0.650	0.087
HB1	ND	<LOQ	ND	0.085	0.062
HB2	ND	<LOQ	ND	0.379	0.064
HY1	ND	<LOQ	ND	0.288	0.083
HY2	ND	0.053	ND	1.101	0.088
LC1	ND	0.039	ND	0.579	0.083
LC2	ND	0.023	ND	0.369	0.087
PT1	ND	<LOQ	ND	0.132	0.071
PT2	ND	<LOQ	ND	0.147	0.097
TQ1	ND	<LOQ	ND	0.220	0.081
TQ2	ND	0.036	ND	0.557	0.090
YB1	ND	<LOQ	ND	0.147	0.087
YB2	ND	<LOQ	ND	0.086	0.071
BV1.M4	0.052	ND	ND	0.232	0.211
BV2.M4	0.072	ND	<LOQ	0.348	0.278
BV2.M10	0.042	<LOQ	ND	0.342	0.348
QO.M4	0.057	ND	ND	0.201	0.298
QO.M10	0.050	ND	ND	0.217	0.276
ThTr.M10	0.106	<LOQ	ND	0.235	0.326
TT1.M4	0.090	ND	ND	0.204	0.280
TT2.M10	0.027	ND	ND	0.102	0.169
LOD	0.0075	0.006	0.005	0.0075	0.015
LOQ	0.025	0.020	0.017	0.025	0.050

ND: not detected, LOD: Limit of detection, LOQ: Limit of quantitation

Table A.2. Heavy metals concentration in pollen sample from research sites (mg/kg).

Site	As	Cd	Hg	Pb	Sn
BG1	<LOQ	0.036	ND	0.208	0.065
BG2	<LOQ	0.039	ND	0.139	0.084
BK1	0.042	0.085	ND	0.431	0.113
BK2	<LOQ	0.06	ND	0.307	0.105
HB1	0.062	0.045	<LOQ	0.298	0.079
HB2	0.050	0.040	0.022	0.160	0.073
HY1	0.065	0.074	<LOQ	0.463	0.127
HY2	0.048	0.064	<LOQ	3.767	0.106
LC1	<LOQ	0.05	ND	0.199	0.120
LC2	<LOQ	0.041	ND	0.142	0.072
PT1	<LOQ	0.049	ND	0.142	0.068
PT2	0.031	0.120	ND	0.211	0.083
TQ1	0.035	0.037	ND	0.453	0.210
TQ2	0.025	0.041	ND	0.397	0.130
YB1	<LOQ	0.043	ND	0.180	0.084
YB2	<LOQ	0.051	ND	0.173	0.076
BV1.M4	0.090	0.040	<LOQ	0.374	0.287
BV2.M4	0.107	0.098	0.018	0.476	0.279
BV2.M10	0.066	0.121	<LOQ	0.455	0.255
QO.M4	0.062	<LOQ	<LOQ	0.215	0.276
QO.M10	0.092	0.038	ND	0.396	0.258
ThTr.M10	0.151	0.031	ND	0.357	0.256
TT1.M4	0.186	0.021	<LOQ	0.283	0.305
TT2.M10	0.077	0.027	ND	0.345	0.144
LOD	0.008	0.006	0.005	0.008	0.015
LOQ	0.025	0.020	0.017	0.025	0.050

ND: not detected, LOD: Limit of detection, LOQ: Limit of quantitation

Table A.3. Heavy metals concentration in beeswax sample from research sites (mg/kg).

Site	As	Cd	Hg	Pb	Sn
BG1	<LOQ	<LOQ	ND	0.186	0.072
BG2	<LOQ	<LOQ	ND	0.169	0.058
BK1	<LOQ	0.033	ND	0.351	ND
BK2	<LOQ	0.031	ND	0.711	0.098
HB1	<LOQ	0.042	ND	0.245	<LOQ
HB2	0.028	0.049	<LOQ	0.427	<LOQ
HY1	0.032	0.039	ND	0.373	<LOQ
HY2	<LOQ	0.020	ND	5.840	<LOQ
LC1	<LOQ	0.034	ND	0.223	<LOQ
LC2	<LOQ	0.050	ND	0.132	<LOQ
PT1	0.045	0.023	ND	0.107	<LOQ
PT2	0.093	0.355	ND	0.790	<LOQ
TQ1	<LOQ	0.035	ND	0.327	0.103
TQ2	<LOQ	<LOQ	ND	0.212	0.075
YB1	0.051	<LOQ	ND	0.052	<LOQ
YB2	0.054	0.025	ND	0.157	<LOQ
BV1.M4	0.099	<LOQ	<LOQ	0.324	0.292
BV2.M4	0.098	<LOQ	<LOQ	0.378	0.305
BV2.M10	0.049	<LOQ	<LOQ	0.258	0.252
QO.M4	0.067	<LOQ	ND	1.558	0.353
QO.M10	0.054	<LOQ	<LOQ	0.421	0.300
ThTr.M10	0.123	<LOQ	<LOQ	0.438	0.302
TT1.M4	0.115	<LOQ	<LOQ	0.577	0.299
TT2.M10	0.036	ND	<LOQ	0.242	0.178
LOD	0.008	0.006	0.005	0.008	0.015
LOQ	0.025	0.020	0.017	0.025	0.050

ND: not detected, LOD: Limit of detection, LOQ: Limit of quantitation.