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HONEY BEE FORAGERS AND SOME BEE PRODUCTS AS POTENTIAL BIOINDICATORS FOR ENVIRONMENTAL CONTAMINATION WITH HEAVY METALS

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ABSTRACT:

Heavy metals are persistent pollutants traveling through the environment-from their industrial source into the atmosphere, soil, and water-and finally into the food chain. They pose the greatest danger to human and animals. Honey bees (Apis mellifera) are good bioindicators of environmental pollution because they accumulate heavy metals in their bodies and products, although showing no immediate effect. Bee-related samples (foragers, honey, and pollen) collected from five sites in Duhok Province in May 2025 were investigated. The assessment confirmed the presence of lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and chromium (Cr) in varying concentrations. Pb levels varied between 0.0003 and 0.0024 mg/kg, while the greatest amount of Pb was detected in pollen. One pollen sample contained Zn levels as high as 20.365 mg/kg. Cu levels fluctuated but didn't show significantly either across locations or sample types. Cr levels were determined to be within WHO-recommended levels, while traces of Cd and Fe were well below the safety limits. Some pollen and honey samples from L2 and L3 showed higher amounts than safety standards, but most were below the limits. The results indicate that pollen and honey are more prone to metal accumulation, confirming contamination at a much lower level.

KEYWORDS: Honeybees, Bioindicators, Heavy Metals, Environmental Contamination, Ecosystem Health.

1. INTRODUCTION

High-density metallic elements that are toxic or hazardous even in trace amounts. They are referred to as "heavy metals" (Ali & Khan, 2018). These include elements such as lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and platinum group metals. In ecosystems, these metals circulate through a chain of contamination involving industries, the air, soil, water, food, and eventually humans (Balali-Mood *et al.,* 2021). Heavy metals are hazardous because they persist in the environment, cannot be degraded biologically, and cause harm to living organisms. Though varied in their chemistry and functions, many of these metals are transition elements on the periodic table (Upadhyay, 2022). Some are essential for physiological processes (e.g., Mo, Mn, Cu, Ni, Fe, Zn) while others, such as Cd, Ni, As, Hg, and Pb, are poisonous at even minimal levels (Kiran *et al*., 2022). Recently, plants, insects, fish, and small animals have been tested as bioindicators for environmental monitoring (Wink, 2025).

Bees are insects of the order Hymenoptera and are composed of about 20,000 species (Engel *et al.,* 2021). They are Keystone pollinators and essential for biodiversity conservation and food production (Patel *et al.,* 2021). Honeybees usually fly 4--5 km from their hives (mostly 2 km because of energy constraints), and so they explore about 12 km² of area (Harrison & Fewell 2002; Jaffe et al. There are two ways that bees can identify pollution: as an increased death rate or by developing toxicants such as heavy metals, herbicides and fungicides in pollen, honey, and larvae (Celli & Maccagnani, 2003). Their activities expose them to air, soil, plants, and water pollutants (Plutino *et al.,* 2022). The long-term bioaccumulation of these chemicals in forager bees renders them as useful sentinels for environmental purposes (Monchanin *et al.,* 2023). In addition to bees, beehive components such as wax, pollen, and propolis are utilized for environmental monitoring (Conti *et al.,* 2022).

The Western honeybee (*Apis mellifera)* is a widely studied species, as it is in continuous contact with the external world between autumn and spring (Di Fiore *et al.,* 2023; Knoll & Cappai, 2024). Honeybees and their corresponding products can be harvested year-round on a laboratory scale to acquire sufficient material for various chemical analyses (Bargańska & Namieśnik, 2010). Their rapid response to environmental changes and the concentration of contaminants in their bodies and products has rendered insects a useful indicator (Salkova & Panayotova-Pencheva, 2016; Ruschioni *et al.,* 2013). The compounds originate from the air, water, soil, and plants near the hive, as well as from the bees themselves, and are absorbed from these sources and converted into honey and other hive products (Margaoan *et al.,* 2024). Nowadays, bees are used to identify metal contamination in both rural and urban settings (Di Fiore *et al.,* 2022). Pollen, a crucial component of honey production, may not necessarily be a direct indicator of pollution levels, but it can represent contamination (Popov Bogdanova *et al.,* 2022). Raw nectar is another way environmental pollutants can enter honey (Bosancic *et al.,* 2020). Heavy metal accumulation in bees can result in physiological problems, even though it may not immediately cause death (Murashova *et al.,* 2020).

To determine the safety of bee products for both humans and bees, the primary goal of this study is to assess the effectiveness of honey bees (Apis mellifera) as bioindicators of specific heavy metals in urban and natural environments of Northern Iraq. Additionally, to investigate the most effective carrier for contamination.

2. MATERIALS AND METHODS

The experiments were carried out at the University of Duhok in Kurdistan, Iraq, in the Department of Plant Protection and the central laboratory of the College of Agricultural Engineering Sciences. Examining the accumulation of metals in honey bee foragers, honey and pollen as bioindicators of

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environmental contamination was part of the technique (Lambert *et al*., 2012) (Figure 1).

Sampling process:

Sampling sites were categorized into different environmental regions based on land use and pollution risks: L1-Highways, L2-urbanized areas, L3-industrialized areas, L4-ecologically clean areas and L5-semi-urban or transitional areas. The specific locations were Marina in semel (L1), Etet in Duhok (L2), Blan village in Atrush (L3), Dereshki Islam village in Kani Masi (L4), and Sargale village around Amedi (L5) (Aljedani, 2020); (Table 1). Actively foraging individuals were captured at the hive entrances using forceps (Nahar & Ohtani, 2015). 30 bees per apiary from 3 colonies; ten bees from each colony (Klein *et al*., 2019). Bees were stored in sterile containers at -20°C (Skorbiłowicz *et al.,* 2018). A total number of 150 bees was sampled from the five locations.

Honey was taken out from the same three hives where forager bees were collected using sterilized tools and stored in labeled sterilized containers (Correa-Mosquera *et al.,* 2022). At least 300 g of honey was gathered per apiary with ≥100 g from each hive in a package contained 15 tubes. Pollen was collected using pollen traps at the hive entrances, scraping loads from returning forager (Nedić, 2024); The gathered pollen was put in plastic containers with labels and stored at 5°C in a refrigerator (Seaton *et al.,* 2018). Fifteen pollen samples were obtained from five different sites. Over all number of three types of samples collected across five locations were forty-five samples, incorporating replicates at each site to enhance the accuracy and reliability of the dataset.

Figure 1: The locations of the honeybee colonies (sampling sites L1, L2, L3, L4, and L5) are depicted on a map of northern Iraq.

Table 1: Topographical characteristics of the sample collection sites.

Province	Distracts	Sub-distracts	Latitude	Longitude	Altitude
Duhok	Semel	Marina	36°57'02"N	42°45'00"E	531 m
Duhok	Duhok	Etet	36°50'00"N	43°04'32"E	704 m
Duhok	Atrish	Blan	36°49'51"N	43°26'23"E	634 m
Duhok	Kani Masi	Dereshki Islam	37°13'47"N	43°25'52"E	1.271 m
Duhok	Amedi	Sargale	37°04'41"N	43°32'51"E	890 m

Chemical analysis:

Pollen and forager bee samples were oven-dried at 70°C until fully dehydrated (Melo & Almeida-Muradian, 2011). The dried samples were ground into a fine powder using a laboratory grinder to guarantee the homogeneity of the sample. Each sample (approximately 0.5 g per sample) weighed using an analytic balance.

Sample digestion was conducted by treating each sample (including honey samples) with 10 ml of concentrated sulphuric acid (H2SO4) followed by 2 ml of perchloric acid (HClO4) (Garba *et al.,* 2024). The digestion process was carried out at 100 °C in a fume hood until the solution became clear (Jones, 2014). Distilled water was used to bring the final volume down to 50 ml then filtered using Whatman filter paper (Hussen, 2022). The concentration of heavy metals: Lead (Pb), Copper (Cu), Zinc (Zn), Chromium (Cr), Cadmium (Cd), and Iron (Fe), were determined using Atomic Absorption Spectrophotometry (AAS). Calibration was performed using certified reference standards (Flamminii *et al.,* 2024).

STATISTICAL ANALYSIS:

Analysis of variations (ANOVA) was applied to compare values of investigated heavy metals in different locations as well as to compare the three carriers (foragers, pollen and honey) of the contamination. Significant differences were found using Duncan's multiple comparison tests (at $p < 0.05$). among groups of samples collected from different locations and transferred by different carriers (Bayir & Aygun, 2022).

3. RESULTS

Heavy metals were detected in three types of samples (foragers, honey and pollen) collected from different environmental regions in May 2025. Results of chemical analysis indicated that all investigated heavy metals were detected in different concentrations. Lead (Pb) concentrations varied among the sample types and locations, ranging from 0.469 mg/kg in honey collected from L3 to 0.889 mg/kg. (WHO permissible range: 0.01-2 mg/kg) Statistical analysis showed that the main effect of location was not significant (P = 0.05), whereas the carrier type exhibited a statistically significant effect (P = 0.01). Zinc (Zn) concentrations were found between 0.133 mg/kg (pollen, L3) and 0.634 mg/kg (foragers, L2).

The carrier type significantly influenced Zn levels (P = 0.01), following the trend: foragers > honey > pollen. Additionally, Zn concentrations in both honey (P = 0.036) and pollen (P = 0.01) were significantly affected by location. Copper (Cu) concentrations, in honey from L3 being 1.599 mg/kg and in pollen from L2 being 7.414 mg/kg, showed no statistically significant difference between locations or carrier types (P > 0.05). Most of the values were under the WHO guideline of 5.0 mg/kg, while some of the pollen samples (L2 and L3) exceed this limit (Table 2).

Table 3 shows that Cadmium (Cd) levels were relatively uniform across all locations and sample types, with values spanning from 0.143 to 0.174 mg/kg. Neither the main effect of location no carrier type was statistically significant (P = 0.423–0.95). All observed concentrations were considerably below the WHO safety limit of (0.005-1.0 mg/kg).

All sites and sample types exhibited Cr concentrations within a narrow range of 0.125-0.169 mg/kg. No statistically significant differences were found concerning the location (P = 0.721) or carrier (P = 0.281). All values were far below the WHO permissible limit (0.01-2.0 mg/kg).

Iron (Fe) concentrations varied from 0.608 mg/kg in foragers (L3) to 1.771 mg/kg in honey (L3). While location had no significant effect, the carrier type was highly significant (P = 0.00), with accumulation following the order; honey > pollen > foragers. However, all Fe value in this study remained within the WHO permissible range (0.5-20 mg/kg).

Table 2: Mean concentration of Pb, Zn, and Cu (mg/kg) in Foragers, Honey, and pollen collected from five locations (L1-L5) in May 2025.

Heavy Metals mg/kg	Locations	Foragers	Honey	Pollen	Main effect of Locations
Pb WHO permissible range 0.01 – 2.0	L1	0.646 b-e	0.547 de	0.664 a-e	0.619 A
	L2	0.837 ab	0.538 de	0.820 ab	0.732 A
	L3	0.837 ab	0.469 e	0.616 b-e	0.641 A
	L4	0.707 a-d	0.807 a-c	0.473 e	0.662 A
	L5	0.820 ab	0.586 c-e	0.889 a	0.765 A
	Main effect of Carriers	0.769 A	0.589 B	0.692 AB	P=0.155
	P-value		0.05		
				0.01	
Heavy metals mg/kg	**Locations**	**Foragers**	**Honey**	**Pollen**	**Main effect of Locations**
Zn WHO permissible range 0.1 – 25.0	L1	0.394 b-e	0.313 d-f	0.221 ef	0.309 A
	L2	0.634 a	0.626 ab	0.143 f	0.468 A
	L3	0.486 a-d	0.571 a-c	0.133 f	0.397 A
	L4	0.383 c-e	0.122 f	0.306 d-f	0.27 A
	L5	0.39 c-e	0.133 f	0.395 b-e	0.306 A
	Main effect of Carriers	0.457 A	0.353 A	0.24 B	P=0.036
	P-value		0.01		
				0.01	
Heavy metals mg/kg	**Locations**	**Foragers**	**Honey**	**Pollen**	**Main effect of Locations**
Cu WHO permissible range 0.05 – 5.0	L1	4.651 a	1.753 a	4.127 a	3.51 A
	L2	2.17 a	5.114 a	7.414 a	4.899 A
	L3	5.5 a	1.599 a	5.994 a	4.364 A
	L4	1.907 a	4.42 a	1.537 a	2.621 A
	L5	2.676 a	4.898 a	2.003 a	3.192 A
	Main effect of Carriers	3.381 A	3.557 A	4.215 A	P=0.501
	P-value		0.721		
				0.162	

4. DISCUSSION

Pollution from heavy metals around Duhok city is an increasing environmental and public health concern, primarily due to some petroleum industrial activities, and inadequate waste management. The most expected sources of pollution with heavy metals represents in the lack of modern waste treatment facilities results in the leaching of heavy metals from landfills waste, particularly around urban centers. The mountainous region of northern Iraq is rich in minerals, and some small-scale or unregulated mining operations may contribute to the release of chromium and zinc into the environment. Rivers may also be contaminated by runoff from polluted sites, negatively affecting water quality. Several studies conducted mainly after 2010 have shown elevated levels of heavy metals in drinking water sources and some crops, especially near oilfields and former conflict zones. This study corroborates the findings of Abu-Almaaly (2021) who reported possible contamination that he attributed to the amounts of Pb in Iraqi honey, which he postulated might exceed 4.657 mg/kg from different urban areas. In contrast, Pb concentrations in honey samples analyzed by bayir and Ayrgun (2022) from Turkey were between 0.192 and 0.358 mg/kg, showing significant differences between rural and urban areas ($P < 0.05$). Further evidence is provided by Erdoğan *et al.,* (2023), where the range of Pb in Turkish bee pollen was < 0.005-0.622 mg/ kg according to the exposure conditions of the environment.

Table 3: Mean concentration of Cd, Cr, and Fe (mg/kg) in Foragers, Honey, and pollen collected from five locations (L1-L5) in May 2025.

Heavy metals mg/kg	**Locations**	**Foragers**	**Honey**	**Pollen**	**Main effect of Locations**
	L1	0.166 a	0.166 a	0.174 a	0.169 A
	L2	0.166 a	0.148 a	0.143 a	0.152 A
Cd WHO	L3	0.178 a	0.161 a	0.157 a	0.165 A
permissible range	L4	0.145 a	0.176 a	0.174 a	0.165 A
	L5	0.152 a	0.166 a	0.162 a	0.16 A
0.005 – 1.0	**Mian effect of Carriers**	0.161 A	0.164 A	0.162 A	P=0.534
	P-value		0.95		
				0.423	

Heavy metals mg/kg	**Locations**	**Foragers**	**Honey**	**Pollen**	**Main effect of Locations**
	L1	0.133 a	0.155 a	0.167 a	0.152 A
	L2	0.156 a	0.139 a	0.125 a	0.14 A
Cr WHO	L3	0.169 a	0.152 a	0.159 a	0.16 A
permissible range	L4	0.163 a	0.166 a	0.149 a	0.159 A
	L5	0.152 a	0.147 a	0.144 a	0.148 A
0.01 – 2.0	**Mian effect of Carriers**	0.155 A	0.152 A	0.149 A	P=0.188
	P-value		0.721		
				0.281	

Heavy metals mg/kg	**Locations**	**Foragers**	**Honey**	**Pollen**	**Main effect of Locations**
	L1	0.936 de	1.332 c	1.588 ab	1.285 A
	L2	0.699 fg	1.659 a	1.398 bc	1.252 A
Fe WHO	L3	0.608 g	1.771 a	1.378 bc	1.252 A
permissible range	L4	0.764 e-g	1.023 d	1.318 c	1.035 A
	L5	0.771 e-g	1.33 c	0.901 d-f	1:00 AM
0.5 – 20.0	**Mian effect of Carriers**	0.755 C	1.423 A	1.317 B	P=0
	P-value		0		
				0	

** At $P < 0.05$, the mean with distinct letters is statistically different.

Results concerning Zinc (Zn) concentrations; the values remained well within the limits laid down by the World Health Organization (0.1–25.0 mg/kg). In contrast, Bayır and Aygün (2022) reported that the content of Zn in Turkish bee pollen varied from 10.25 mg/kg in rural areas to 20.27 mg/kg in the urban setting. Even higher levels were noted by Erdoğan *et al.* (2023) with values recorded between 13.274 and 57.844 mg/kg, which can be ascribed to variation in soil mineral content and plant diversity.

Most of the values of copper concentrations were under the WHO guideline of 5.0 mg/kg, while some of the pollen samples (L2 and L3) exceed this limit. Earlier studies in Iraq reported a lower level of Cu in honey, with an average of about 0.1 mg/ kg (Dhahir & Hemed, 2015), whereas Bayır and Aygün (2022) reported levels much higher in urban areas of Turkey, as high as 17.18 mg/kg, thus illustrating the effect of anthropogenic sources.

Table 3 shows that Cadmium (Cd) levels were relatively uniform across all locations and sample types, neither the main effect of location nor carrier type was statistically significant. All observed concentrations were considerably below the WHO safety limit of (0.005-1.0 mg/kg). In comparison, Dhahir and Hemed (2015) recorded wider variability in Cd levels in Iraqi honey (0.108–0.820 mg/kg), while Bayır and Aygün (2022) noted significantly lower concentrations in Turkish bee samples, ranging from 9.52 to 20.78 µg/ kg ($P < 0.05$).

All sites and sample types showed Cr concentrations in a small range, no significant differences were discovered concerning the location or carrier. All values were far below the WHO permissible limit (0.01-2.0 mg/kg). Turkish samples analyzed by Bayır and Aygün (2022) reported Cr values between 58.24 and 99.24 µg/ kg, with greater concentrations in urban zones answering environmental influence on Cr distribution.

Iron (Fe) concentrations varied between foragers and honey samples (L3). While location had no significant effect, the carrier type was highly significant with accumulation following the order; honey > pollen > foragers. However, all Fe value in this study remained within the WHO permissible range (0.5-20 mg/kg). The levels observed were found to be much higher than Fe concentrations in the report of Dhahir and Hemed (2015), which ranged between 0.002–0.034 mg/kg in Iraqi honey. On the contrary, extremely high Fe concentrations have been documented in Turkish bee pollen, which ranged from 28.60 to 725.36 mg/kg (Altunatmaz *et al.,* 2017) and reached levels of even 811.043 mg/kg in the East Black Sea Region (Erdoğan *et al.,* 2023) attributable to huge differences relating to floral and soil properties.

CONCLUSION

It can be concluded that the studied locations were slightly contaminated with the heavy metals investigated, most of them within the limits of the World Health Organization (WHO). Pollen and honey were more effective than forager bees for transferring this contamination.

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Ethical statement:

This work has not been applied to animals or humans.

Author Contributions:

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.
Concept, design, and supervision: Z. N. A.,
Acquisition, analysis, or interpretation of data: N. M. S.,
Drafting of the manuscript: Z. N. A. and N. M. S.

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