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Assessing Heavy Metal Contamination in District Kasur, Pakistan, Using Honey Bees (*Apis mellifera*) as Bioindicators for Ecological and Food Safety Monitoring

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ABSTRACT

Aims: This study aimed to assess the honey bee (*Apis mellifera*) as a bioindicator of heavy metal contamination in District Kasur, Punjab, Pakistan, for ecological monitoring and food safety.
Materials & Methods: Fresh honey samples were collected from beehives during the summer season in the morning to minimize external metal contamination. Samples were collected from five sites representing different pollution levels, including a forest area located far from tanneries (Changa Manga Jungle, 88 km from the control site and 56 km from Kasur tanneries), a low-pollution area (Village Ganda Singh Border, 75 km from the control site and 16 km from Kasur tanneries), a slightly polluted area (Raiwind Road, 42 km from the control site and 15 km from Kasur tanneries), a moderately polluted area (Village Sehjra, 84 km from the control site and 27 km from Kasur tanneries), and a control site (Lahore Jallo Park, 62 km from Kasur tanneries). We collected 12 individual samples from each site, for a total of 60 experimental samples. We used a Completely Randomized Design (CRD) with five treatments. Heavy metal concentrations were quantified in $mg.kg^{-1}$ using a Flame Atomic Absorption Spectrophotometer (FAAS).
Findings: Results showed that the highest Cu content was recorded in samples collected from Village Sehjra (11.46 $mg.kg^{-1}$); on the other hand, the lowest amount was determined in the forest area (0.09 $mg.kg^{-1}$). Fe expressed the highest contents overall, reaching 15.23 $mg.kg^{-1}$ in the control site (Jallo Park, Lahore), whereas higher amounts were also observed in Village Sehjra (14.12 $mg.kg^{-1}$). Somewhat, high Fe concentration at the control site may reflect background geological characteristics and urban environmental influences rather than direct industrial emissions.
Conclusion: These findings highlight the impact of environmental pollution on heavy-metal bioaccumulation in honey. The results show that honey bees serve as a good bioindicator for assessing environmental quality and ensuring food safety.

Keywords: Bioindicator; Environmental Monitoring; Environmental Pollution; Heavy Metals.

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Introduction

Honey bees belong to the phylum Arthropoda and are strongly related to ants and wasps. They are found globally, but are absent in Antarctica**.** Seven to nine families of honey bees have been identified. About 20, 000 species have been identified. About five species are commonly linked with honey production: *Apis dorsata*, *Apis cerana* (formerly referred to as *Apis indica*), *Apis florea*, *Apis mellifera*, and *Apis andreniformis*. These species belong to *the Apidae* family and are classified in the order *Hymenoptera*. *Apis mellifera* is the most widely administered bee species globally. It is estimated that an individual bee collects nectar from around 1, 000 flowers per day. Still, factors such as floral source, species type, and season can significantly affect honey quality and composition [1].

Honeybees make a natural sweet substance known as honey. The word "honey" means "product" and derives from the Arabic word "han." Its exclusive chemical composition makes it a nutritionally precious food. Honey contains carbohydrates, amino acids such as lysine, arginine, serine, phenylalanine**,** and aspartic acid**.** Beyond its nutritional value, honey and other bee-derived products are bioindicators of environmental conditions [2]. Insects such as honey bees, wasps, butterflies, and ants have received considerable attention because they can indicate slight changes in the environment. They are extensively used for ecological monitoring. *Apis mellifera* (the honeybee) is particularly valuable because it is highly sensitive to changes in environmental quality. Honey bees are therefore considered useful bio-indicators for monitoring environmental contamination across their foraging range. They have a wide foraging range and sensitivity to environmental changes [3]. Heavy metals are environmental pollutants that accumulate in air, soil, sediments, water, and living organisms. These substances are non-biodegradable and can enter the food chain through bioaccumulation, posing risks to both the environment and human health [3, 4]. They come from various natural and anthropogenic sources and may transfer from the environment to beehives and honey bees. Among naturally occurring elements, several heavy metals are toxic to living organisms, including Cu, Pb, As, Hg, Ni, Cr, and Cd. Apiaries situated in industrial areas usually show higher levels of heavy metal contamination than those located far away from industries. Lead (Pb) is considered one of the basic heavy metals emitted from vehicle exhausts. It contaminates flowers and, as a result, accumulates in honey bees during foraging activities [5].

In ecological monitoring, selecting a suitable organism as a bio-indicator is an important step. Honey bees are selected because they collect nectar, unintentionally accumulate pollutants, and integrate environmental exposure over time [6]. Honey bees (*Apis mellifera*) are valuable biomonitoring tools because they respond rapidly to minute environmental changes [7]. Several studies have shown that bees and bee products can assess environmental contamination within their foraging area [8, 9].

Various studies have investigated ecological pollution worldwide. However, the use of the honey bee (*Apis mellifera*) as a bio-indicator of heavy metal contamination in District Kasur, Punjab, Pakistan, remains limited. Kasur is well known for its large concentration of tannery industries, which are considered major sources of environmental pollution in the region. Therefore, this study evaluated heavy metal contamination in honey bee (*Apis mellifera*) samples collected from different sites in District Kasur, Pakistan. We analyzed honey samples to assess environmental pollution, particularly heavy metal contamination. These findings will contribute to environmental monitoring, food safety evaluation, and the development of future ecological management strategies for District Kasur, Pakistan.

Materials & Methods

Study Area

The present study was conducted in District Kasur, Punjab, Pakistan. Kasur is widely recognized for its tannery industries. These tanneries are a major source of heavy metal contamination in the surrounding areas. Five sampling sites were selected to characterize different levels of environmental pollution: a forest area situated far from tannery industries (Changa Manga Jungle), a low pollution area (Village Ganda Singh Border), a slightly polluted area (Raiwind Road), a moderately polluted area (Village Sehjra), and a control site (Jallo Park, Lahore).

The control site (Jallo Park, Lahore) was located 62 km away from Kasur Tanneries (Pvt.) Ltd. The distances from the control site to the sampling sites were 88 km (Changa Manga Jungle), 75 km (Village Ganda Singh Border), 42 km (Raiwind Road), and 84 km (Village Sehjra). The consequent distances from Kasur Tanneries (Pvt.) Ltd. to the sampling sites were 56 km (Changa Manga Jungle), 16 km (Village Ganda Singh Border), 15 km (Raiwind Road), and 27 km (Village Sehjra).

These sites show a range of ecological conditions, from comparatively unpolluted forest areas to areas influenced by industrial activities. We collected honey samples directly from local beehives at each sampling site, taking precautions to minimize contamination during collection and handling.

Equipment Used

A magnetic stirrer with a hot plate, a drying oven, filter paper, glassware and sample containers, an analytical balance, a grinder, a Flame Atomic Absorption Spectrophotometer (FAAS) (Infitek; distributed by LABTECH Pakistan), and disposable polyethylene containers were employed in the study:

Sample Collection

Fresh honey samples were collected during the morning hours in the summer season from local beehives. A total of 60 honey samples were collected from five sampling sites, with 12 independent samples collected from 12 separate beehives at each site. We collected each sample individually without compositing, ensuring independent biological replication at each sampling site.

We collected approximately 1 g of fresh honey from each beehive directly into clean, disposable polyethylene containers to minimize contamination. We stored samples at 4°C and transported them to the laboratory for subsequent analysis. Figure 1 shows the sample collection process at Village Ganda Singh Border.

Figure 1) Sample collection process at Village Ganda Singh Border, Pakistan.

Sample Preparation

Approximately 1 g of each honey sample was transferred into a digestion flask, and 12 mL of HNO_3:H_2O_2 (3:1, v/v) was added. The mixtures were heated at 65°C on a hot plate until digestion was complete, then concentrated to 3- 5 mL. After cooling, 10 mL of distilled water was added, and the solutions were filtered and diluted to a final volume of 25 mL with deionized water [9]. The prepared solutions were evaluated for heavy metal amounts using a Flame Atomic Absorption Spectrophotometer (FAAS) (Infitek; distributed by LABTECH Pakistan).

Analysis of Heavy Metals

Heavy metal concentrations of Cu, Pb, Cd, Zn, and Fe in honey samples were determined using a Flame Atomic Absorption Spectrophotometer (FAAS) (Infitek; distributed by LABTECH Pakistan). Calibration standards were prepared at concentrations of 0.00, 1.00, 2.00, and 4.00 $mg.L^{-1}$ for each element. Quality control was ensured by examining reagent blanks and certified reference standard solutions alongside the honey samples.

Data Analyses

Data were assessed using Microsoft Excel. Mean values and standard deviations of all samples were calculated for all heavy metal concentrations. One-way ANOVA was performed to estimate differences in mean heavy metal contents among the five sampling sites. The ANOVA demonstrated statistically significant differences ($p \leq 0.05$). The experiment followed a Completely Randomized Design (CRD) with five treatments and 12 independent replicates per treatment, for a total of 60 experimental samples.

Findings

The Results show the heavy metal concentrations (i.e., Cu, Pb, Cd, Zn, and Fe) recorded in honey samples collected from five sites exhibiting different pollution levels in District Kasur, Punjab, Pakistan. We observed significant differences among sampling sites for Cu, Pb, Cd, and Zn ($p \leq 0.05$). Fe concentrations did not differ significantly among sites ($F = 2.15$, $p = 0.086$).

Table 1 demonstrates the maximum admissible levels (MALs) of Cu, Pb, Cd, Zn, and Fe in honey bees according to FAO/WHO standards [11]. These standards served as reference thresholds for assessing heavy metal concentrations in honey samples from all five locations.

Table 2 illustrates the calibration standards prepared for each heavy metal, with content values ranging from 0.00 to 4.00 $mg.L^{-1}$, to ensure precise quantification during FAAS analysis.

Control Site (Jallo Park, Lahore)

Table 3 explains the heavy metal concentrations in honey samples collected from the control site (Jallo Park, Lahore). As expected for a control site, Cu and Pb concentrations were generally low across all samples. The maximum concentrations recorded were Cu (0.19 $mg.kg^{-1}$), Pb (0.18 $mg.kg^{-1}$), Cd (0.30 $mg.kg^{-1}$), Zn (0.63 $mg.kg^{-1}$), and Fe (15.23 $mg.kg^{-1}$). The minimum concentrations were Cu (0.09 $mg.kg^{-1}$), Pb (0.05 $mg.kg^{-1}$), Cd (0.14 $mg.kg^{-1}$), Zn (0.07 $mg.kg^{-1}$), and Fe (2.78 $mg.kg^{-1}$).

However, the control site showed the highest Fe concentration. Fe is an abundant element in natural soils and crustal materials. Furthermore, Jallo Park is located within the metropolitan area of Lahore, where vehicle emissions and urban atmospheric deposition may contribute to elevated background Fe levels. Furthermore, ANOVA indicated that Fe concentrations among sites were not statistically significant ($p = 0.086$), suggesting that the observed variation should be interpreted cautiously for the forest site (Changa Manga Jungle)

Table 4 illustrates the heavy metal concentrations in honey samples collected from Changa Manga Jungle, showing the least anthropogenically impacted sampling location. The maximum concentrations recorded were Cu (0.20 $mg.kg^{-1}$), Pb (0.19 $mg.kg^{-1}$), Cd (0.32 $mg.kg^{-1}$), Zn (0.71 $mg.kg^{-1}$), and Fe (19.31 $mg.kg^{-1}$). The minimum concentrations were Cu (0.09 $mg.kg^{-1}$), Pb (0.06 $mg.kg^{-1}$), Cd (0.15 $mg.kg^{-1}$), Zn (0.12 $mg.kg^{-1}$), and Fe (3.76 $mg.kg^{-1}$).

Cu and Pb concentrations at this forest site were among the lowest recorded in the study, supporting its classification as the least anthropogenically influenced location. Fe amounts did not differ significantly from those at the other sites ($F = 2.15$, $p = 0.086$).

Low-Pollution Site (Village Ganda Singh Border)

Table 5 demonstrates the heavy metal concentrations in honey samples collected from Village Ganda Singh Border. The maximum concentrations recorded were Cu (10.21 $mg.kg^{-1}$), Pb (0.90 $mg.kg^{-1}$), Cd (0.35 $mg.kg^{-1}$), Zn (0.75 $mg.kg^{-1}$), and Fe (8.01 $mg.kg^{-1}$). The minimum concentrations were Cu (1.10 $mg.kg^{-1}$), Pb (0.29 $mg.kg^{-1}$), Cd (0.19 $mg.kg^{-1}$), Zn (0.28 $mg.kg^{-1}$), and Fe (3.29 $mg.kg^{-1}$). Pb concentrations exceeded the FAO/WHO maximum admissible limit of 0.30 $mg.kg^{-1}$ in several samples, particularly in samples 8 and 12. Fe concentrations did not differ significantly among sites ($F = 2.15$, $p = 0.086$).

Slightly Polluted Site (Raiwind Road)

Table 6 shows the heavy metal concentrations in honey samples collected from Raiwind Road. The maximum concentrations recorded were Cu (10.46 $mg.kg^{-1}$), Pb (0.92 $mg.kg^{-1}$), Cd (0.41 $mg.kg^{-1}$), Zn (0.77 $mg.kg^{-1}$), and Fe (11.09 $mg.kg^{-1}$). The minimum concentrations were Cu (0.20 $mg.kg^{-1}$), Pb (0.30 $mg.kg^{-1}$), Cd (0.18 $mg.kg^{-1}$), Zn (0.20 $mg.kg^{-1}$), and Fe (2.12 $mg.kg^{-1}$). Pb concentrations exceeded the FAO/WHO maximum admissible limit in multiple samples, and Cd concentration in sample 5 (0.41 $mg.kg^{-1}$) also exceeded the permissible limit of 0.20 $mg.kg^{-1}$. Fe concentrations did not differ significantly among sites ($F = 2.15$, $p = 0.086$).

Moderately Polluted Site (Village Sehjra)

Table 7 shows heavy metal concentrations in honey samples collected from Village Sehjra, which had the highest overall heavy metal burden among the sampling sites. The maximum concentrations recorded were Cu (11.46 $mg.kg^{-1}$), Pb (0.96 $mg.kg^{-1}$), Cd (0.44 $mg.kg^{-1}$), Zn (0.93 $mg.kg^{-1}$), and Fe (14.12 $mg.kg^{-1}$). The minimum concentrations were Cu (1.05 $mg.kg^{-1}$), Pb (0.27 $mg.kg^{-1}$), Cd (0.21 $mg.kg^{-1}$), Zn (0.29 $mg.kg^{-1}$), and Fe (3.25 $mg.kg^{-1}$). Lead and Cadmium contents exceeded the FAO/WHO maximum admissible limits in various samples, showing considerable heavy metal contamination at this site. Fe concentrations ranged from 3.25 to 14.12 $mg.kg^{-1}$ and were not significantly dissimilar from those recorded at the other sites ($F = 2.15$, $p = 0.086$).

Table 1) Maximum Admissible Levels (MAL) of heavy metals in honey bee according to FAO/WHO standards.

Heavy Metals	FAO/WHO Maximum Permissible Values ($mg.kg^{-1}$)
Cd	0.2
Pb	0.3
Fe	425.5
Cu	73.3

Table 2) Calibration standards and their concentrations used for heavy metal analysis.

Standard No.	Concentration ($mg.kg^{-1}$)
Standard I	0.00
Standard II	1.00
Standard III	2.00
Standard IV	4.00

Table 3) Heavy metal concentrations ($mg.kg^{-1}$) in honey samples collected from the control site (Lahore Jallo Park, 62 km from Kasur Tanneries, Pvt., Ltd.).

Sample No.	Cu	Pb	Cd	Zn	Fe
1	0.09	0.12	0.27	0.17	7.20
2	0.15	0.05	0.30	0.07	8.11
3	0.17	0.10	0.14	0.15	4.69
4	0.12	0.18	0.22	0.63	15.23
5	0.14	0.16	0.23	0.27	2.78
6	0.16	0.14	0.29	0.25	12.71
7	0.11	0.18	0.26	0.48	8.71
8	0.12	0.11	0.17	0.25	9.39
9	0.14	0.07	0.21	0.10	4.72
10	0.11	0.12	0.25	0.27	6.31
11	0.18	0.13	0.22	0.31	10.68
12	0.19	0.16	0.19	0.63	8.51
Mean	0.14	0.13	0.23	0.30	8.25

Note: Values represent heavy metal concentrations ($mg.kg^{-1}$) measured in 12 independent honey samples collected from separate beehives at the control site (Jallo Park, Lahore). Mean values represent the arithmetic mean of the 12 independent samples.

Table 4) Heavy metal concentrations ($mg.kg^{-1}$) in honey samples collected from the forest area far from tanneries (Changa Manga Jungle, 88 km from control site, 56 km from Kasur Tanneries, Pvt., Ltd.).

Sample No.	Cu	Pb	Cd	Zn	Fe
1	0.09	0.11	0.27	0.12	9.23
2	0.10	0.08	0.29	0.44	3.76
3	0.13	0.19	0.32	0.70	5.33
4	0.09	0.10	0.23	0.50	8.17
5	0.16	0.16	0.15	0.17	6.27
6	0.14	0.08	0.24	0.45	19.31
7	0.12	0.13	0.27	0.69	6.69
8	0.20	0.14	0.18	0.65	7.95
9	0.10	0.06	0.19	0.47	4.75
10	0.12	0.12	0.22	0.71	11.03
11	0.11	0.13	0.24	0.25	5.78
12	0.13	0.09	0.30	0.54	8.29
Mean	0.12	1.12	0.24	0.47	8.05

Note: Data are expressed as concentrations ($mg.kg^{-1}$) determined in 12 independent honey samples collected from separate beehives. Mean values represent the arithmetic mean of the independent samples.

Table 5) Heavy metal concentrations ($mg.kg^{-1}$) in honey samples collected from the low-polluted area (Village Ganda Singh Border, 75 km from control site, 16 km from Kasur Tanneries, Pvt., Ltd.).

Sample No.	Cu	Pb	Cd	Zn	Fe
1	2.09	0.71	0.19	0.28	4.30
2	1.10	0.39	0.30	0.31	6.81
3	1.11	0.79	0.31	0.49	5.40
4	1.20	0.60	0.27	0.30	4.19
5	2.14	0.29	0.23	0.35	3.29
6	7.17	0.57	0.31	0.40	4.38
7	9.09	0.29	0.19	0.33	3.69
8	1.20	0.90	0.31	0.60	6.10
9	8.09	0.65	0.35	0.75	6.81
10	1.16	0.79	0.21	0.51	8.01
11	8.09	0.49	0.24	0.34	6.90
12	10.21	0.82	0.29	0.41	7.29
Mean	4.39	0.61	0.27	0.42	5.60

Note: Values represent heavy metal concentrations ($mg.kg^{-1}$) measured in 12 independent honey samples collected from separate beehives at the sampling site. Mean values represent the arithmetic mean of the 12 independent samples.

Table 6) Heavy metal concentrations ($mg.kg^{-1}$)in honey samples collected from the slightly polluted area (Raiwind Road, 42 km from control site, 15 km from Kasur Tanneries, Pvt., Ltd.).

Sample No.	Cu	Pb	Cd	Zn	Fe
1	10.46	0.91	0.19	0.51	6.51
2	1.23	0.78	0.42	0.48	6.11
3	1.79	0.64	0.34	0.45	6.81
4	1.17	0.75	0.27	0.33	5.39
5	1.70	0.51	0.41	0.25	5.41
6	0.20	0.49	0.22	0.41	3.49
7	7.49	0.30	0.26	0.38	4.17
8	1.20	0.69	0.33	0.20	3.01
9	9.19	0.35	0.18	0.77	4.69
10	9.10	0.92	0.27	0.52	2.12
11	1.05	0.79	0.25	0.40	11.09
12	1.12	0.83	0.29	0.52	4.29
Mean	3.81	0.66	0.29	0.44	5.26

Note: Data are expressed as concentrations ($mg.kg^{-1}$) determined in 12 independent honey samples collected from separate beehives. Mean values represent the arithmetic mean of the independent samples.

Table 7) Heavy metal concentrations (mg.kg^{-1}) in honey samples collected from the moderately polluted rural area (Village Sehjra, 84 km from control site, 27 km from Kasur Tanneries, Pvt., Ltd.).

Sample No.	Cu	Pb	Cd	Zn	Fe
1	11.46	0.95	0.23	0.48	6.51
2	2.23	0.89	0.33	0.55	8.11
3	1.97	0.65	0.41	0.36	4.72
4	1.17	0.83	0.29	0.31	5.39
5	1.70	0.57	0.38	0.47	7.23
6	1.20	0.51	0.30	0.55	4.33
7	7.49	0.37	0.21	0.29	14.12
8	1.20	0.76	0.33	0.61	13.71
9	9.19	0.27	0.27	0.93	4.72
10	10.10	0.96	0.37	0.47	4.14
11	1.05	0.69	0.34	0.89	11.21
12	1.12	0.91	0.44	0.37	3.25
Mean	4.16	0.70	0.33	0.52	7.29

Note: Values represent heavy metal concentrations (mg.kg^{-1}) measured in 12 independent honey samples collected from separate beehives at the sampling site. Mean values represent the arithmetic mean of the 12 independent samples.

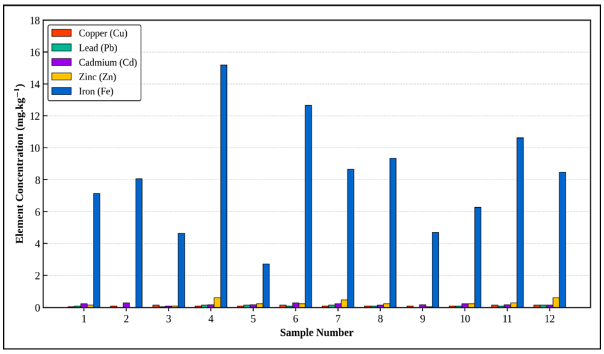

Figure 2) Concentrations of heavy metals (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from the control site (Lahore Jallo Park, 62 km from Kasur Tanneries, Pvt., Ltd.).

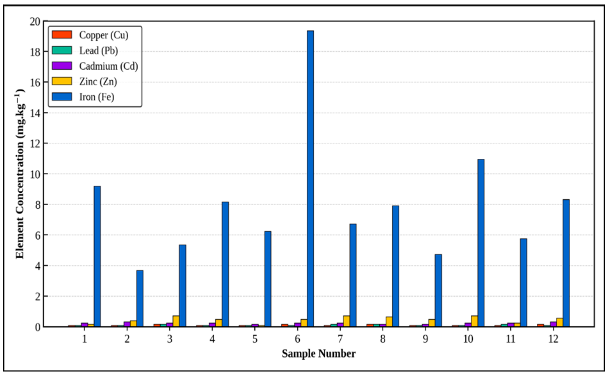

Figure 3) Concentrations of heavy metals (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from the forest area (Changa Manga Jungle, 88 km from the control site, 56 km from Kasur Tanneries, Pvt., Ltd.)

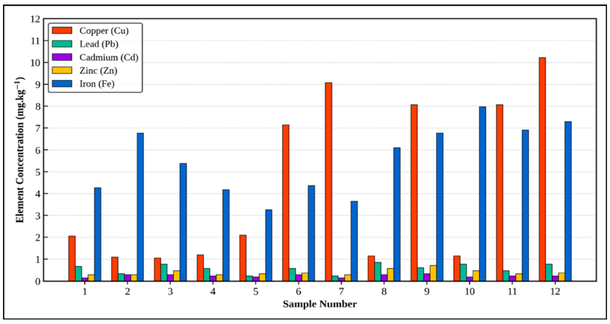

Figure 4) Concentrations of heavy metals (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from the low polluted area (Village Ganda Singh Border, 75 km from the control site, 16 km from Kasur Tanneries, Pvt., Ltd.).

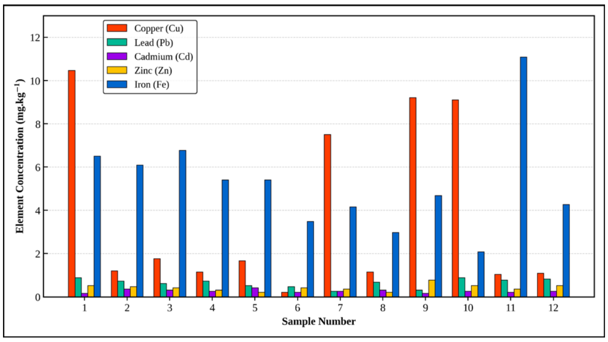

Figure 5) Concentrations of heavy metals (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from the slightly polluted area (Raiwind Road, 42 km from the control site, 15 km from Kasur Tanneries, Pvt., Ltd.).

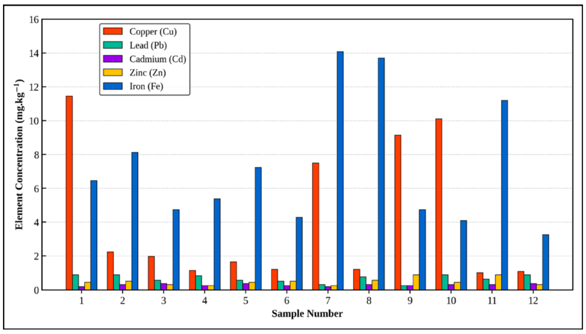

Figure 6) Concentrations of heavy metals (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from the moderately polluted rural area (Village Sehjra, 84 km from the control site, 27 km from Kasur Tanneries, Pvt., Ltd.)

Discussion

The current study assessed heavy metal concentrations (Cu, Pb, Cd, Zn, and Fe) in honey samples collected from five sites in District Kasur, Punjab, Pakistan, illustrating different levels of environmental contamination: the control site (Jallo Park, Lahore), a forest area (Changa Manga Jungle), a low-pollution area (Village Ganda Singh Border), a slightly polluted area (Raiwind Road), and a moderately polluted rural area (Village Sehjra). The findings confirmed that the honey bee is a reliable bio-indicator of environmental heavy metal pollution. Cu, Pb, Cd, and Zn levels generally increased at sites influenced by anthropogenic activities, mainly those near the Kasur tannery industries. In contrast, Fe exhibited a different spatial distribution, with the highest amount recorded at the control site, indicating that factors other than tannery activities may also contribute to Fe accumulation. Heavy metals may accumulate in honeybee products due to pollution from vehicles, factories, agricultural activities, and chemical waste discharged by industrial operations. During flight, feeding, and water consumption, forager bees can readily pick up pollutants from industrial emissions, vehicle exhaust emissions, and chemical waste dispersed throughout the environment. The environment is continually changing because intensive agricultural operations are required to meet the demands of an expanding human population. Among many suggested strategies, one of the most widely adopted techniques for detecting ecological changes, both early and long-term, is biomonitoring [10].

The concentration of trace elements in bee products varies considerably, influenced by several factors, including ecological quality, apiary location, the physiological and health conditions of bees and bee colonies, colony-rearing methods, and the age of worker bees [11]. During foraging activities, bees are exposed to numerous pollutants: **(i)** the body of honeybees is covered with hairs that trap contaminants from the environment, and **(ii)** these contaminants are introduced into hive products through the collection of nectar and pollen from different plants, as well as through drinking water [3].

In the present study, Cu concentrations in honey samples collected from the moderately polluted rural area (Village Sehjra) ranged from 1.05 to 11.46 $mg.kg^{-1}$, Pb from 0.27 to 0.96 $mg.kg^{-1}$, Cd from 0.21 to 0.44 $mg.kg^{-1}$, Zn from 0.29 to 0.93 $mg.kg^{-1}$, and Fe from 3.25 to 14.12 $mg.kg^{-1}$. Compared with the control site and the forest area, Village Sehjra usually showed higher amounts of Cu, Pb, Cd, and Zn, signifying a greater impact of anthropogenic activities. However, the highest individual Fe amount (15.23 $mg.kg^{-1}$) was noted at the control site (Jallo Park, Lahore). This observation illustrates that Fe accumulation may also be influenced by natural geogenic sources and urban atmospheric deposition rather than solely by industrial emissions. Likewise, the amounts of Cu (1.11–9.15 $mg.kg^{-1}$), Pb (0.30–0.91 $mg.kg^{-1}$), Cd (0.20–0.30 $mg.kg^{-1}$), and Fe (3.06–7.33 $mg.kg^{-1}$) reported in previous studies were generally lower, whereas Zn reached 24.2 $mg.kg^{-1}$ in some regions [12, 13]. These results were generally similar to those reported by [14].

Comparison with the FAO/WHO Maximum Admissible Levels (MAL) indicated that Cu concentrations remained below the permissible limit of 73.3 $mg.kg^{-1}$ at all sampling sites. In contrast, several individual honey samples collected from the low-, slightly-, and moderately polluted sites exceeded the MAL for Pb (0.30 $mg.kg^{-1}$), indicating a potential food safety concern. Likewise, several individual honey samples contained Cd concentrations above the recommended limit of 0.20 $mg.kg^{-1}$, particularly at the slightly and moderately

polluted sites. Fe concentrations remained well below the FAO/WHO MAL of 425.5 $mg.kg^{-1}$ at all sampling sites.
In spring, previously reported cadmium and copper concentrations in honey samples were 0.31 $mg.kg^{-1}$ and 9.57 $mg.kg^{-1}$, respectively. Copper concentrations remained within the internationally accepted permissible limit, whereas cadmium concentrations exceeded the recommended guideline value. The lead concentration (0.91 $mg.kg^{-1}$) was below the Maximum Allowable Limit (1.5 $mg.kg^{-1}$) reported in that study. Similarly, [14] reported that Fe had the highest concentration, followed by Pb, Zn, and Cu. However, the concentrations differed from those observed in the present study and from those reported for honey samples collected in Saudi Arabia. Honey samples collected in spring had mean Zn, Pb, Fe, and Cd concentrations of 0.49, 0.13, 8.48, and 0.25 $mg.kg^{-1}$, respectively. Reference [15] recorded the highest mean Cd concentration (0.14 $mg.kg^{-1}$) in honey collected from Al-Fayoum during spring. Similarly, the highest mean concentrations of other metals were Zn (0.28 $mg.kg^{-1}$) from Kafr El-Zayat, Pb (0.35 $mg.kg^{-1}$) from El-Mehala El-Kobra, Cu (3.23 $mg.kg^{-1}$) from Kafr El-Sheikh, and Fe (11.20 $mg.kg^{-1}$) from Al-Fayoum. Another study reported maximum mean concentrations of Cu (4.31 $mg.kg^{-1}$), Pb (0.69 $mg.kg^{-1}$), Cd (0.27 $mg.kg^{-1}$), Zn (0.40 $mg.kg^{-1}$), and Fe (5.59 $mg.kg^{-1}$) in honey samples harvested from two different localities. Rajpoot and Agrawal (2016) recorded maximum mean concentrations of Cu (0.58 $mg.kg^{-1}$) from the Gwalior region, Pb (0.39 $mg.kg^{-1}$) from the Morena region, Cd (0.28 $mg.kg^{-1}$) from the Guna region, Zn (1.69 $mg.kg^{-1}$) from the Gwalior region, and Fe (4.25 $mg.kg^{-1}$) from the Morena region. A related study found that honeybee samples collected from an agroforestry zone had a mean Cr concentration of 0.19 $mg.kg^{-1}$. In contrast, samples collected near an airport in the Brest district of Belarus contained a mean Cr concentration of 0.24 $mg.kg^{-1}$ [16]. The same study reported Cu concentrations in honey ranging from 0.621 to 2.931 $mg.kg^{-1}$, whereas other studies reported concentrations ranging from 0.395 to 0.950 $mg.kg^{-1}$ [17], 0.45 to 2.15 $mg.kg^{-1}$ [18], and 0.15 to 0.71 $mg.kg^{-1}$**.** Reference [17] also reported concentrations ranging from 1.258 to 4.566 $mg.kg^{-1}$ for another investigated metal, whereas another study reported concentrations between 1.039 and 1.635 $mg.kg^{-1}$. Fe concentrations in honey have been reported to range from 1.15 to 13.18 $mg.kg^{-1}$**.** Sholars [19] reported Fe concentrations between 10.90 and 61.15 $\mu g\ kg^{-1}$, whereas other researchers reported concentrations ranging from 7.40 to 92.38 $mg\ kg^{-1}$, and another study reported values ranging from 6, 266 to 14, 500 $\mu g\ kg^{-1}$.
The high Fe concentrations noted in Village Sehjra, Changa Manga Jungle, and the control site (Jallo Park) may be attributed to natural geogenic sources, including atmospheric dust deposition, and agricultural practices. Although the highest Fe concentration was noted at the control site (Jallo Park), this site is situated within the metropolitan area of Lahore, where traffic, road dust suspension, and urban atmospheric deposition contribute considerably to Fe accumulation. In addition, Fe amounts did not vary significantly among sampling sites, indicating that the noted differences should be interpreted carefully. These findings align with previous studies reporting elevated Fe contents in honey collected from agricultural and forest areas, as well as urban environments [19].
The unpredictably high Zn amount observed

at the control site (maximum 0.63 $mg.kg^{-1}$) needs careful consideration. Jallo Park was selected as the reference site because it lies outside the direct influence of the Kasur tannery industries. It is situated within the greater Lahore metropolitan area, which is exposed to substantial traffic and urban road runoff, both recognized sources of Zn pollution. These findings are consistent with studies reporting elevated Zn contents in urban honey samples collected from cities with high traffic volume [16, 17].

This research confirms that heavy metals may pose risks to ecological and human health when their contents exceed globally accepted permissible limits. Even so, the tolerable limit of Cu is 73.3 $mg.kg^{-1}$, and extreme amounts may become toxic [20]. WHO guideline values for Pb and Cd are 0.01 and 0.025 $mg.kg^{-1}$, respectively. High Pb concentrations may cause neurotoxicity and kidney damage. Cd is recognized as a carcinogenic metal when found at extreme concentrations [21]. Iron is an important element and a major component of hemoglobin, but excessive Fe ingestion may produce severe health impacts [22]. The detection of elevated Pb and Cd levels in several honey samples from contaminated sites highlights the need for continuous monitoring of honey produced in District Kasur, particularly near tannery industries, to protect human health and environmental quality.

These results emphasize the importance of regular monitoring of honey bees as a bio-indicator of environmental pollution to ensure food safety and protect ecological integrity and public health in industrially polluted areas such as District Kasur, Punjab, Pakistan.

Conclusion

The current study revealed that Honey bees (*Apis mellifera*) proved to be an effective bio-indicator of environmental heavy metal contamination in District Kasur, Punjab, Pakistan. Cu, Pb, Cd, Zn, and Fe contents differed among sampling sites, reflecting differences in environmental conditions. The highest Cu amount (11.46 $mg.kg^{-1}$) was observed in the fairly polluted rural area (Village Sehjra). On the other hand, the highest individual Fe content (15.23 $mg.kg^{-1}$) was recorded at the control site (Jallo Park, Lahore), suggesting that natural sources and urban atmospheric deposition may also impact Fe accumulation. Multiple individual honey samples collected from polluted sites possessed Pb and Cd content above the recommended admissible values, indicating potential food safety concerns. Overall, these findings show that honey bees are a practical, non-destructive tool for ecological and food safety monitoring and support their use in environmental pollution assessment, particularly in industrially affected areas such as District Kasur, Pakistan.

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