ESVP

Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

2024, Volume 4, Issue 2, Page No: 27-39

Available online at: www.esvpub.com/

Identification of Various Elements and Heavy Metals in Honeybee and Beeswax Samples from Different Environmental Sources

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ABSTRACT

Honeybees and their products act as effective bioindicators due to their close relationship with the environment they inhabit. This study aimed to assess the extent of pollution by measuring the concentration of metals in honeybees (*Apis mellifera jemenatica*) and beeswax, focusing on elements such as K, Ca, Na, Mg, Fe, Mn, Cu, Zn, and heavy metals such as Pb, Ni, Cd, and Cr in different environments in the Makkah region of Saudi Arabia. The sampling areas included: R1 (highways), R2 (urbanized zones), R3 (industrial zones), and R4 (ecologically clean areas). The findings indicate significant differences ($P < 0.05$) in the concentration of metals between bee bodies and wax across these areas. The highest levels were observed in industrial zones (R3), followed by urbanized areas (R2) and highways (R1), with the lowest concentrations found in ecologically clean areas (R4). Notably, the metal concentrations in honeybee bodies were higher than in the wax samples in all study areas. These results suggest that honeybees and beeswax are reliable indicators of environmental pollution, particularly by toxic metals.

Keywords: Honeybee workers, Bee products, Mineral elements, Heavy metals, Environmental pollution

Received: 20 August 2024
Revised: 14 November 2024
Accepted: 18 November 2024

How to Cite This Article: Zafeiraki E, Sabo R, Kasiotis KM, Machera K, Sabová L, Majchrák T. Identification of Various Elements and Heavy Metals in Honeybee and Beeswax Samples from Different Environmental Sources. Int J Vet Res Allied Sci. 2024;4(2):27-39.

Introduction

Honeybees and their by-products are recognized as effective indicators of environmental contamination, particularly from heavy metals, particles, and various other toxins found in their food sources [1, 2]. As honeybees return to their hives, they bring back contaminants that have settled on plants they interact with [3]. The pollution caused by heavy metals has escalated significantly over the past two decades due to activities like mining, smelting, the use of agricultural chemicals (fertilizers and pesticides), urban waste, emissions from traffic, and industrial waste [4, 5]. The toxicity resulting from heavy metals has become a growing environmental issue in affected areas [6]. In addition to impacting plant growth and survival, pollinators that depend on these crops are also exposed to harmful metal concentrations caused by environmental contamination with heavy metals [4]. This exposure can result in reduced species diversity, as well as negative effects on brood development and survival [7].

The honeybee (*Apis mellifera* L.) is a species that fulfills the criteria mentioned above and serves as a valuable bioindicator, with its survival closely linked to the environment it occupies [8]. During their feeding activities, bees encounter various contaminants, and their body hairs, known as corbiculae, easily capture pollutants from the air, as well as from pollen, nectar, and water [9]. Increasingly, bees are being utilized to assess environmental pollution caused by metals, both in urban and rural settings [10]. The analysis of bee-derived products such as wax, honey, and pollen is considered an effective method for detecting land, plant, and air contamination from toxic metals over regions spanning several square kilometers [11].

Previous research has indicated that honey, propolis, and wax in colonies around the world contain various toxic substances, including heavy metals [12]. This occurs because heavy metals present in the atmosphere can be deposited and carried back to the hive by bees' hairy bodies, or they may be absorbed along with nectar from plants or honeydew [13]. The global spread of heavy metal contamination has significantly disrupted ecosystems and poses substantial health risks to humans. The primary causes of this issue are rapid urbanization, land-use changes, and industrialization, especially in densely populated developing nations [14]. A recent study [15] detected heavy metal pollution levels in samples collected from various regions in Saudi Arabia, using foraging bees of *A. mellifera jemenatica* and honey samples. The results revealed that the heavy metal residue levels were very low and within permissible limits, suggesting that these areas are not significantly contaminated by these metals. While trace elements are vital for life, they can be harmful at elevated levels [16]. Essential metals like Fe, Zn, Cu, and Mn play crucial roles in biological systems, while non-essential elements such as Pb and Cd can be toxic even in small quantities [16]. Some metals, such as Cr and Ni, are widely spread in the environment due to both natural sources and human activities, as well as their extensive use in various industries [17]. Lead (Pb) and cadmium (Cd) are particularly toxic and have been the focus of extensive research. Pb is commonly found in the air, primarily from vehicle emissions, and is transferred to crops [4]. Cd, originating from the metal industry and incineration, is transferred to the soil and subsequently to plants [9, 18].

Forager honeybees were selected for this study because they are actively involved in nectar-to-honey processing within their digestive system, allowing them to absorb heavy metals from the nectar they collect [19]. Investigating the likelihood that honeybees feed on metal-contaminated resources is essential to assess the level of risk these metals pose to honeybee populations [7]. Foragers cannot distinguish between clean nectar or pollen and those contaminated with trace amounts of cadmium, copper, or lead. They may even show a preference for uncontaminated resources over mildly contaminated ones, which could have serious consequences for the health and survival of the colony [7]. This is particularly concerning because metals accumulate in the hive over time, potentially causing toxic effects on both the larvae and adult bees, as highlighted by one research [7]. The widespread occurrence of metal contamination, even at significant distances from industrial zones and highly utilized agricultural areas, has attracted the attention of numerous researchers. As a result, the objective of this study is to measure and identify the pollution levels of metals in honeybee bodies (*A. mellifera jemenatica*) and beeswax from four distinct environmental locations in the Makkah region.

Materials and Methods

This study aimed to identify and analyze the metal content in various regions of the Makkah area, Saudi Arabia. Forager honeybees (*A. mellifera jemenatica*) (Hymenoptera: Apidae) and beeswax samples were gathered from apiaries situated across different environmental zones.

Sampling area

The Makkah region, located in the western part of Saudi Arabia, is renowned for its beekeeping activities, owing to its diverse geography and climate. The sampling locations were categorized into four distinct zones: R1 - highways (the coastal road connecting Jeddah to Jazan), R2 - urbanized (Jeddah city, a densely populated area), R3 - industrial area (Jeddah Steel Factory), and R4 - ecologically clean zone (the research station apiary at Hada Al-Sham, Faculty of Meteorology, Environment, and Agriculture of Dry Zones, King Abdulaziz University) **(Figure 1)**.

Honeybee samples collection and preparation

Honeybee foragers (*A. mellifera jemenatica*) were gathered from each designated sampling location. A minimum of 100 bees were collected from the hive entrance, ensuring adherence to methodological guidelines for randomization and variability in the sampling process. The bees were gently brushed into disposable polyethylene bags. Following collection, the samples were immediately frozen at −10 °C in a laboratory freezer. Before analysis, the bee bodies were oven-dried at 105 °C until a constant weight was achieved, and each sample was ground separately using a hand-held laboratory grinder [4].

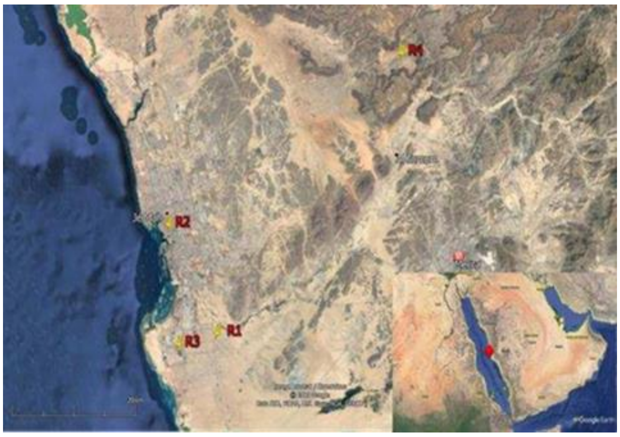

Figure 1. Satellite image depicting the sampling site locations: R1, R2, R3, and R4 (Google Earth Gold Pro).

Wax sample collection and preparation

Beeswax was harvested from the same hives as the honeybee samples in each area. Approximately 5 grams of beeswax were taken in triplicate from the samples used in this research and stored at room temperature in sealed glass containers.

Determination of metals

The concentrations of twelve metals, including essential elements like Potassium (K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), and toxic metals such as Lead (Pb), Nickel (Ni), Cadmium (Cd), and Chromium (Cr), in all samples were measured using the methods described by [20].

For the analysis of these elements in bee and wax samples, 5 grams of each was first incinerated until it became ash, followed by calcination for 13 hours at 450 °C. The residual ash was then dissolved in ten mL of 0.5M HNO3 and filtered through quantitative filter paper, as per [20]. Calibration standards for the analysis were created using a 1000 ppm concentration from Merck. Each sample was analyzed in three independent replications. To ensure accuracy, the method was validated with certified reference material (NIST–1515). The results were expressed in milligrams per gram (mg/g) for both bees and wax.

Statistical analysis

Statistical analysis of the results was performed using the SPSS software [21] to assess and identify the concentrations of metals (mean ± SD) in both bee and wax samples from various environmental sources within the region. The data were analyzed using one-way and two-way analysis of variance (ANOVA) to determine significant differences between the means, with a significance level set at $P < 0.05$.

Results and Discussion

The findings of this study emphasized the significance of choosing appropriate apiary locations and the influence of these sites on honeybees, as well as the level of contamination in their products due to various metals. Four distinct environmental zones in the Makkah region of Saudi Arabia were identified: R1 (highways), R2 (urbanized), R3 (industrialized), and R4 (ecologically clean). Samples were collected from foraging honeybees (*A. mellifera jemenatica*) (Hymenoptera: Apidae) and their wax products to assess contamination with metals including Potassium (K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Lead (Pb), Nickel (Ni), Cadmium (Cd), and Chromium (Cr). The results from this analysis revealed:

Determination and detection of some metals in honeybee samples from different environmental regions

The findings of this study revealed variations in the concentrations of metals across different environmental regions in honeybees. In the highway region (R1), the highest levels of metals were observed for Calcium (Ca), Sodium (Na), Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), and Chromium (Cr), with concentrations of 45.464, 37.106, 36.846, 22.582, 10.028, 3.975, 3.225, 1.668, and 1.003 mg/g, respectively. On the other hand, the lowest concentrations were found for Cadmium (Cd), Nickel (Ni), and Lead (Pb), with values of 0.075, 0.265, and 0.236 mg/g, respectively. In the urbanized region (R2), the highest concentrations were observed for Calcium (Ca), Sodium (Na), Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn), Chromium (Cr), Manganese (Mn), Copper (Cu), and Nickel (Ni), with values of 63.604, 61.730, 45.079, 31.115, 27.277, 10.602, 8.266, 3.782, 3.559, and 2.617 mg/g, respectively. The lowest concentrations were seen for Lead (Pb) and Cadmium (Cd), with levels of 0.307 and 0.079 mg/g, respectively. In the industrialized region (R3), the highest concentrations were found for Calcium (Ca), Sodium (Na), Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), and Chromium (Cr), with concentrations of 69.005, 55.722, 51.825, 32.179, 27.857, 7.052, 5.036, 3.885, and 1.190 mg/g, respectively. The lowest concentrations were recorded for Lead (Pb), Cadmium (Cd), and Nickel (Ni), with concentrations of 0.562, 0.079, and 0.290 mg/g, respectively. Lastly, in the ecologically clean region (R4), the highest concentrations were found for Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na), Iron (Fe), Zinc (Zn), and Manganese (Mn), with concentrations of 65.254, 10.050, 8.850, 7.882, 4.386, 1.122, and 1.059 mg/g, respectively. The lowest concentration was observed for Copper (Cu), at 0.211 mg/g. Moreover, Nickel (Ni), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Copper (Cu) were completely free from metal contamination in this region, with values of 0.000 mg/g, as shown in **Figure 2**.

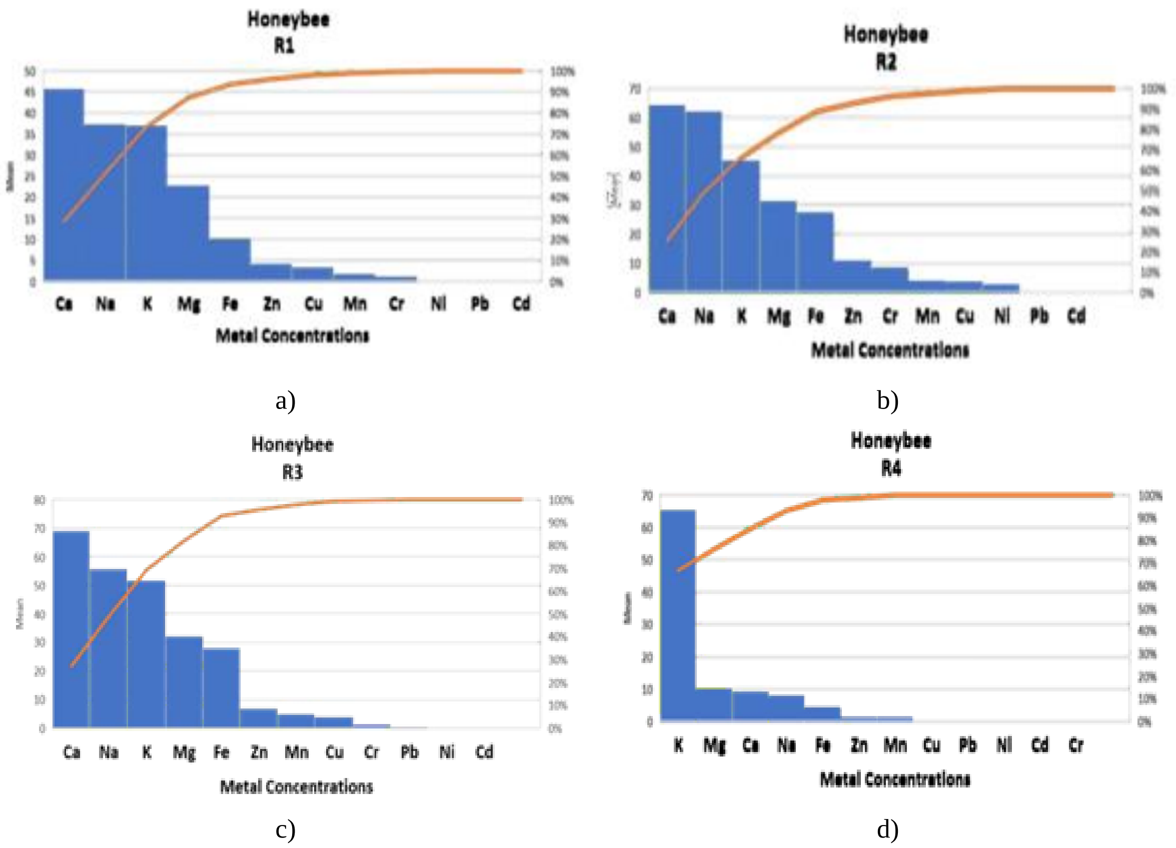

Figure 2. The concentration of metals (mg/g) in honeybees was collected from different environmental regions.

Determination and detection of some metals in wax samples from different environmental regions

As depicted in **Figure 3**, the analysis of metal concentrations in wax samples from different environmental regions revealed varying levels of metals. In the highway region (R1), the highest concentrations were observed for Calcium (Ca), Zinc (Zn), Sodium (Na), Magnesium (Mg), Iron (Fe), Potassium (K), Chromium (Cr), and Copper (Cu), with values of 19.699, 19.699, 10.500, 6.802, 5.972, 3.924, 1.768, and 1.034 mg/g, respectively. The lowest concentrations were found for Nickel (Ni), Manganese (Mn), Lead (Pb), and Cadmium (Cd), with levels of 0.474, 0.365, 0.114, and 0.059 mg/g, respectively. In the urbanized region (R2), the highest concentrations were noted for Calcium (Ca), Sodium (Na), Magnesium (Mg), Iron (Fe), Potassium (K), Chromium (Cr), Zinc (Zn), and Copper (Cu), with concentrations of 28.041, 25.012, 10.480, 9.698, 5.959, 2.016, 1.542, and 1.139 mg/g, respectively. The lowest concentrations were observed for Nickel (Ni), Manganese (Mn), Lead (Pb), and Cadmium (Cd), with values of 0.598, 0.501, 0.137, and 0.060 mg/g, respectively. In the industrialized region (R3), the highest concentrations of metals were recorded for Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Iron (Fe), Zinc (Zn), Chromium (Cr), Copper (Cu), and Manganese (Mn), with levels of 64.961, 52.439, 34.369, 27.024, 18.516, 6.272, 2.307, 1.913, and 1.311 mg/g, respectively. The lowest concentrations were found for Nickel (Ni), Lead (Pb), and Cadmium (Cd), with concentrations of 0.678, 0.215, and 0.075 mg/g, respectively. Finally, in the ecologically clean region (R4), the highest concentrations were observed for Potassium (K), Magnesium (Mg), Sodium (Na), Iron (Fe), and Calcium (Ca), with values of 84.679, 19.443, 13.010, 11.195, and 9.504 mg/g, respectively. The lowest concentrations were noted for Zinc (Zn), Manganese (Mn), Copper (Cu), and Chromium (Cr), with concentrations of 0.776, 0.414, 0.095, and 0.003 mg/g, respectively. Additionally, Nickel (Ni), Cadmium (Cd), and Lead (Pb) showed no contamination, with concentrations of 0.000 mg/g, as indicated in **Figure 3**.

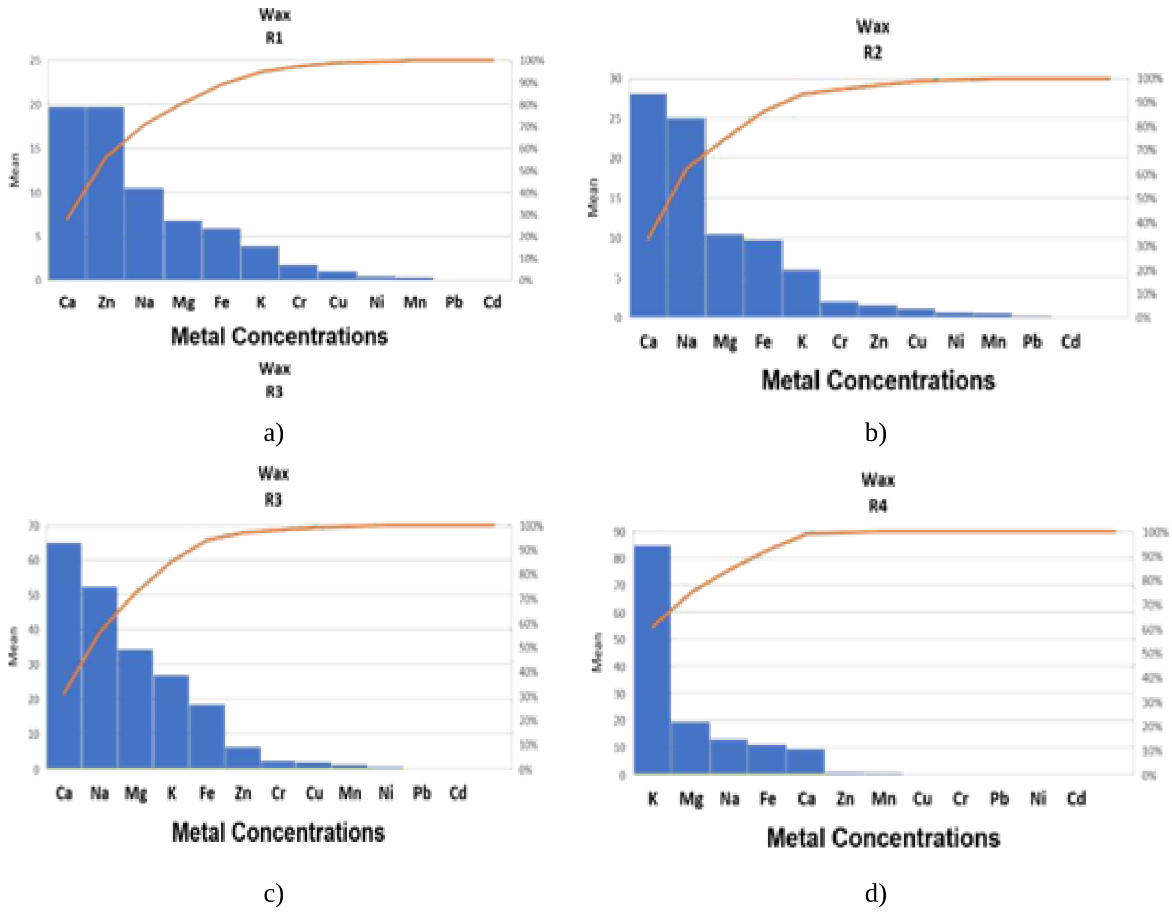

Figure 3. The concentration of metals (mg/g) in wax was collected from different environmental regions.

Trace of metals in honeybee body and wax samples

This study highlights a notable difference in metal concentrations (mg/g) across various regions. The industrialized (R3), urbanized (R2), and highways (R1) regions exhibit the highest concentrations, whereas the ecologically clean (R4) region shows the lowest, as illustrated in **Figure 4**. When comparing the metal concentrations found in honeybees and wax, the results indicate that honeybees consistently show higher levels of metals than wax across all regions examined, as shown in **Figure 5**.

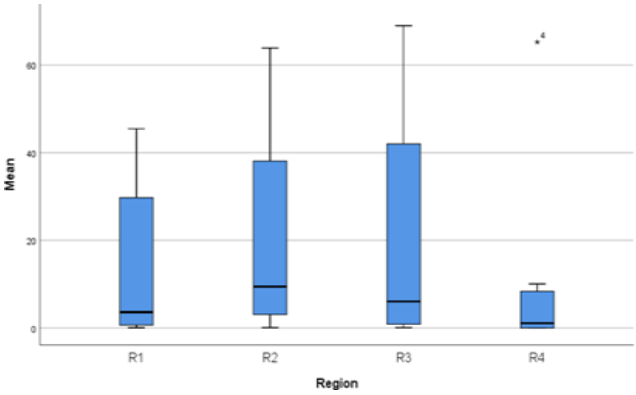

Figure 4. A comparison between different regions in terms of containing concentrations (mg/g) of the metals.

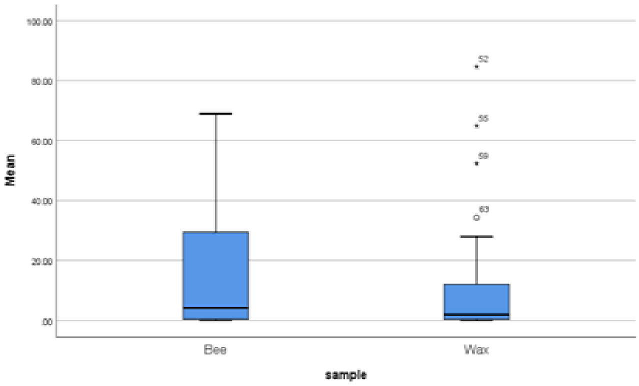

Figure 5. The contamination of bees and wax by metals according to their origin from different regions.

The study identified the presence of various metals (mg/g) in both bee bodies and wax samples. Statistical analysis demonstrated that the metal concentrations in bee bodies were generally higher than those in wax samples across the metals tested. Additionally, the findings reveal varying sensitivity levels between bees and wax across different regions. In region (R1), no sensitivity differences were observed for Cd, Cr, and Ni between bees and wax, but differences were found for other metals. In region (R2), sensitivity differences were seen for all parameters, except for Ni, where no significant variation between bees and wax was detected. In region (R3), there was no significant difference for Cd and Na, but notable differences were observed for other metals. Lastly, in the region (R4), significant differences between bees and wax were found for all studied parameters, as shown in **Tables 1 and 2**.

Table 1. The detection and identification of some metals (mean ± SD) in bee and wax according to their origin from different environments of the region (R1-R2).

Region	Metals	Independent t-test			P-value
		Samples	Mean	SD	
R1	**K**	Bee	36.846	2.234	*0.000**
		Wax	3.924	0.085	
	Ca	Bee	45.464	8.694	*0.030**
		Wax	19.699	2.037	
	Na	Bee	37.106	3.387	*0.005**
		Wax	10.500	0.414	
	Mg	Bee	22.582	1.476	*0.003**
		Wax	6.802	0.102	
	Fe	Bee	10.028	1.228	*0.015**
		Wax	5.972	1.224	
	Mn	Bee	1.668	0.062	*0.000**

		Wax	0.365	0.043	
	Cu	Bee	3.225	0.189	*0.000**
		Wax	1.034	0.068	
	Zn	Bee	3.975	0.129	*0.003**
		Wax	1.283	0.381	
	Pb	Bee	0.236	0.046	*0.023**
		Wax	0.114	0.037	
	Ni	Bee	0.265	0.018	*0.214*
		Wax	0.474	0.202	
	Cd	Bee	0.075	0.007	*0.059*
		Wax	0.059	0.001	
	Cr	Bee	1.003	0.018	*0.190*
		Wax	1.768	0.679	
R2	**K**	Bee	45.079	0.536	*0.000**
		Wax	5.959	0.096	
	Ca	Bee	63.904	0.716	*0.000**
		Wax	28.041	0.729	
	Na	Bee	61.730	1.247	*0.000**
		Wax	25.012	0.903	
	Mg	Bee	31.115	0.236	*0.000**
		Wax	10.480	0.336	
	Fe	Bee	27.277	0.670	*0.000**
		Wax	9.698	0.633	
	Mn	Bee	3.782	0.236	*0.000**
		Wax	0.501	0.051	
	Cu	Bee	3.559	0.084	*0.000**
		Wax	1.139	0.035	
	Zn	Bee	10.602	2.476	*0.024**
		Wax	1.542	0.012	
	Pb	Bee	0.307	0.015	*0.000**
		Wax	0.137	0.004	
	Ni	Bee	2.617	2.007	*0.223*
		Wax	0.598	0.011	
	Cd	Bee	0.079	0.006	*0.026**
		Wax	0.060	0.001	
	Cr	Bee	8.266	0.405	*0.000**
		Wax	2.016	0.312	

(R1- highways, R2- urbanized, R3-industrialized and R4- ecologically clean) SD = Std. Deviation * indicates P < 0.05, Significant.

Table 2. The detection and identification of some metals (mean ± SD) in bee and wax according to their origin from different environments of the region (R3-R4).

Region	Metals	Independent t-test			P-value
		Samples	Mean	SD	
R3	**K**	Bee	51.825	1.022	*0.000**
		Wax	27.024	0.185	
	Ca	Bee	69.005	0.423	*0.000**
		Wax	64.961	0.339	
	Na	Bee	55.722	1.225	*0.060*

		Wax	52.439	1.817	
	Mg	Bee	32.179	1.073	*0.031**
		Wax	34.369	0.449	
	Fe	Bee	27.857	2.460	*0.017**
		Wax	18.516	0.620	
	Mn	Bee	5.036	0.076	*0.000**
		Wax	1.311	0.030	
	Cu	Bee	3.885	0.183	*0.002**
		Wax	1.913	0.036	
	Zn	Bee	7.052	0.081	*0.009**
		Wax	6.272	0.276	
	Pb	Bee	0.562	0.019	*0.000**
		Wax	0.215	0.035	
	Ni	Bee	0.290	0.006	*0.002**
		Wax	0.678	0.032	
	Cd	Bee	0.081	0.005	*0.149*
		Wax	0.075	0.003	
	Cr	Bee	1.190	0.031	*0.000**
		Wax	2.307	0.132	
R4	**K**	Bee	65.254	0.456	*0.000**
		Wax	84.679	0.167	
	Ca	Bee	8.850	0.135	*0.002**
		Wax	9.504	0.096	
	Na	Bee	7.882	0.055	*0.000**
		Wax	13.010	0.119	
	Mg	Bee	10.050	0.082	*0.000**
		Wax	19.443	0.121	
	Fe	Bee	4.386	0.022	*0.000**
		Wax	11.195	0.061	
	Mn	Bee	1.059	0.004	*0.000**
		Wax	0.414	0.005	
	Cu	Bee	0.211	0.003	*0.000**
		Wax	0.095	0.003	
	Zn	Bee	1.122	0.005	*0.000**
		Wax	0.776	0.008	
	Pb	Bee	0	0	-
		Wax	0	0	
	Ni	Bee	0	0	-
		Wax	0	0	
	Cd	Bee	0	0	-
		Wax	0	0	
	Cr	Bee	0	0	-
		Wax	0.003	0	

(R1- highways, R2- urbanized, R3-industrialized and R4- ecologically clean) SD = Std. Deviation *indicates P < 0.05, Significant.

Evaluating the extent of pollution from human exposure to hazardous toxic metals in the environment is challenging. A direct method for assessing the presence of heavy metals in the environment is through chemical analysis of environmental matrices [4]. However, indirect methods, such as using living organisms as bioindicators, have been widely utilized to gauge environmental quality [22]. Pollinators, such as honeybees, provide valuable insights into ecosystem health, given the connection between changes in species diversity and abundance and ecological principles [23]. Honeybees are widely recognized as key indicators of environmental pollution [24]. This study's findings align with these observations, showing varying concentrations of many tested metals in the bodies of foraging honeybee workers (*A. mellifera jemenatica*) compared to wax samples collected from different regions and environments within Makkah, Saudi Arabia. As noted [4], the presence of macro- and micro-elements in bee bodies fluctuates significantly, influenced by factors such as soil types, nectar sources, beekeeping practices, as well as the physiological and health conditions of the bee workers throughout different times of the year [25]. In terms of toxic metals, the ecological condition of the beekeeping area plays a critical role [25, 26].

The findings of this study indicate that the highest concentrations of metals were found in the Industrialized (R3) region, while the lowest were in the Ecologically clean (R4) region. This can be explained by previous observations [27] that contaminated heavy metals are commonly found in and around urbanized, industrialized areas, mining sites, and regions with intensive agricultural activity, across various parts of the world. Prior research has shown that heavy metals are often absorbed by crops grown in contaminated soils, leading to elevated metal concentrations in plant tissues compared to crops grown in uncontaminated soils [7, 27, 28].

The accumulation of heavy metals such as copper, cadmium, lead, zinc, and nickel has been observed in the leaves and flowers of plants [7, 28, 29]. This contamination not only affects the productivity and survival of crops but also exposes pollinators that depend on these crops to potentially toxic metals. These pollutants can reduce species diversity, brood development, and survival rates in both wild and managed pollinator populations, particularly in areas with high metal contamination, as noted by [30].

In our research, we identified the presence of various metals (mg/g) in both bee bodies and wax samples. Statistical analysis revealed that the concentration of metals was higher in bee bodies compared to wax samples across the tested metals (P-value < 0.05). Additionally, it was found that the bee body serves as the most efficient barrier to prevent the transfer of cadmium (Cd) into honey, with honeydew honey showing higher sensitivity to heavy metal pollution compared to nectar honey ($P < 0.05$). Bees were first identified as biofilters for toxic metals, helping to prevent honey contamination. Many studies have utilized bees and wax in different countries as bioindicators to monitor heavy metals in the environment, and our results align with previous research.

This study shows that the highest concentration of cadmium (Cd) was found in both bee bodies and wax samples from the Industrialized (R3) region, with values of (0.081 mg/g in bees and 0.075 mg/g in wax). In contrast, the Ecologically clean (R4) region showed no contamination. These findings support previous research that indicates metals like cadmium, which may not be detected in sublethal yet toxic concentrations before ingestion, can still pose significant risks to colony health and survival [31, 32]. Furthermore, similar studies conducted in southwestern Poland tested heavy metal concentrations in urban and agricultural woodland bee bodies, finding Cd levels (0.6 and 0.7 mg/kg) and Pb levels (1.98 and 1.91 mg/kg) [33]. In the Moldavian forest area, levels of Pb and Cd in honeybee organs were notably lower than those found in industrial zone specimens [34]. Additionally, in Italy, Cd concentrations in bee bodies were found to be significantly higher than in honey samples, and research by Al Naggar *et al*. [35] indicated that the concentration of Cd and Pb in bee bodies varied seasonally, potentially due to environmental factors rather than anthropogenic activity.

The lack of rejection of cadmium-contaminated food by the bees is noteworthy, especially since [36] demonstrated that cadmium is highly toxic to honeybees, even at the concentrations tested. In foraging bees, the concentrations they used significantly increased adult mortality rates [36]. Previous studies have shown that working honeybees, due to their constant interaction with the atmosphere and environmental elements, serve as indicators of the pollution level in a given area. Heavy metals present in the air can accumulate on the bees' body brushes, in pollen, or be absorbed through mildew, nectar, or water. While there are no established norms for the maximum allowable concentrations of heavy metals in bee bodies, it is evident that excessive levels contribute to regression and, in some cases, the disappearance of species such as *A. mellifera* [24].

Honeybee products, particularly wax, have been identified as potential indicators of environmental pollution due to their bioaccumulative nature in industrial and urban areas, as well as in peripheral areas near highways where traces of certain mineral compounds and heavy metals have been detected [37]. In addition, small and varying levels of heavy metals have been found in honey, with variability attributed to factors such as the floral source, time of year, season, and rainfall. As a result, honey has been deemed an unreliable and less sensitive indicator for heavy metal contamination [38]. In contrast, honeybees themselves have proven to be more effective bioindicators of heavy metal pollution in industrial and urban environments, a conclusion supported by Silici *et al*. [39]. In a study in Mugla, Turkey, honey and honeybees were assessed for potential contamination from a thermoelectric power plant. While no toxic concentrations of heavy metals were found in the honey samples, higher levels of Pb and Cd were detected in the honeybees, confirming the greater reliability of honeybees as bioindicators of heavy metal pollution compared to honey. The results from our research show that in all areas studied, metal concentrations in honeybees were higher than in wax. This aligns with findings from [34], where significant differences in heavy metal levels were found between honeybees and, to a lesser extent, pollen, propolis, and wax (but not honey) in areas surrounding the city of Rome, compared to the city center. Other studies, however, have found higher heavy metal concentrations in industrial and urban honey than in honey from unpolluted rural regions [39-41].

This finding further highlights the increased levels of environmental pollution with these metals at specific locations, suggesting that honeybee workers could serve as effective bioindicators for environmental heavy metal contamination. The metals likely enter the bees' bodies during their active foraging activities, through airborne particles that are absorbed via both the porous surface of their bodies and through respiration. Our study found detectable concentrations of several metals (mg/g) in both bee bodies and wax samples from various regions. In the region (R1), no significant sensitivity difference was observed between bee bodies and wax for Cd, Cr, and Ni, while other metals showed a sensitivity difference. In region (R2), a sensitivity difference was observed between bee bodies and wax for all parameters except Ni, where no significant difference was found. In region (R3), no significant difference was observed between bee bodies and wax for Cd and Na, but differences were significant for the other metals. Finally, in the region (R4), a significant difference was found between bee bodies and wax for all parameters.

This study supports the idea that honeybee workers reflect elevated concentrations of heavy metals because of local environmental exposure. Numerous studies advocate for using bees as bioindicators to assess environmental purity [42, 43]. A survey was carried out to evaluate heavy metal concentrations in honeybee workers, with levels ranging from 3.53 to 6.26 ppm for Cu, 27.65 to 30.80 ppm for Zn, 0.05 to 0.19 ppm for Cd, 375.4 to 446.5 ppm for Pb, and 3406.35 to 5161.25 ppm for Fe. Similarly, in honeybee workers, Cd and Pb concentrations were found to be between 2.87 and 4.23 ppm for Cd and 0.61 to 1.25 ppm for Pb. The concentrations of Cu, Zn, and Cd in this study were lower than those reported previously [43], and Pb levels were also lower than those reported by Conti and Botrè [34]. These variations can likely be explained by the differing levels of heavy metal contamination present at each sampling site. Honeybees (*A. mellifera*) are highly effective in detecting and tracking environmental pollutants due to their extensive foraging behavior [8]. While foraging, honeybees gather particles from a variety of sources such as flowers, resins (propolis), water, nectar, and pollen. The bee's body, covered with branched hairs, effectively traps non-floral particles from the air, particularly those collected from the anthers of flowers. In this way, each bee acts as a small-scale sampler of its environment, and the entire colony serves as a collective monitoring unit. During the active foraging period, around a quarter of the colony consists of foraging honeybee workers [10].

In this study, potassium (K) emerged as the most significant environmental variable compared to other minerals such as calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), and chromium (Cr). Manganese (Mn), on the other hand, was the least variable among the heavy metals in bee bodies and wax. Chromium (Cr) was the most efficient environmental variable in terms of heavy metal content, surpassing lead (Pb) and nickel (Ni), while cadmium (Cd) had the least influence on metal concentrations in bee bodies and wax. When comparing different regions, it was observed that the highest metal concentrations were found in the industrialized (R3), urbanized (R2), and highway (R1) areas, with the lowest concentrations detected in the ecologically clean (R4) region. This finding aligns with the research by Aljedani [15], which identified iron (Fe) as the most prevalent heavy metal in honeybee samples. The highest concentrations of iron were recorded in the Makkah (8.794), Asir (6.222), Jazan (6.205), and Al-Baha (2.088) regions. Similarly, iron concentrations in honey were highest in Asir (1.904), Jazan (1.843), Al-Baha (1.340), and Makkah (0.907). Additionally, potassium (K) was the most concentrated mineral in four agricultural areas, which is consistent with the findings of the present research. These results are also in agreement with the study by Dżugan *et al*. [4], which found that potassium was the most abundant element in bee bodies, with magnesium (Mg) and calcium (Ca) levels being significantly lower. Furthermore, concentrations of manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu) in bee bodies were higher than those found in honey.

The concentration of heavy metals in a bee's body is influenced by various factors, including the apiary's location, soil type, nectar plant species in the area, the ecological condition of the environment, beekeeping practices (such as the use of food supplements), the age and health of the working bees, and the overall physiological state of the colonies [25]. Based on the findings of this study, it can be concluded that the metal concentrations found in the bees' bodies across all research locations are significantly lower than the harmful thresholds for bees as defined by [24].

Conclusion

A key takeaway from this research is the demonstrated effectiveness of honeybees and wax in evaluating environmental cleanliness and their potential as bioindicators. Based on the analysis of honeybee workers (*A. mellifera jemenatica*) and their products (wax) to assess metal pollution, data from various sampling locations in Saudi Arabia indicated that the highest concentrations of metals were found in the Industrialized (R3), Urbanized (R2), and Highways (R1) areas, with the lowest levels observed in Ecologically Clean (R4) regions. Beekeepers should focus on identifying sensitive beekeeping locations to ensure the production of high-quality, uncontaminated products. When compared to international standards, the mineral concentrations found in this study remain within permissible limits. However, further research is necessary for a more precise understanding. Given the importance of honeybees and their products for human health and safety, it is crucial to prioritize their quality and safety.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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