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# Revealing some Elements and Heavy Metals in Honeybee and Beeswax Samples Collected from Different Environments

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**ABSTRACT**

Honeybees and their products are good bioindicators because they are inextricably linked to the natural environment they inhabit. The objectives of this research were to detect and identify pollution extent by the levels of metals in honeybees (foragers) *(Apis mellifera jemenatica*) and beeswax, including some elements such as K, Ca, Na, Mg, Fe, Mn, Cu, Zn, and heavy metals such as Pb, Ni, Cd, Cr, in different environments in Makkah region of Saudi Arabia. Sampling regions included were as follows: R1, highways; R2, urbanized areas; R3, industrialized areas; and R4, ecologically clean areas. Results of the present study indicating the metal contents in honeybees collected from apiaries in different environmental regions show that the concentrations (mg/g) of metals in bee bodies and wax are found to be of significant value ($P < 0.05$); the highest value among all metals was found to be 0.000. However, it was found that the regions containing the highest concentrations of metals were industrialized areas (R3), urbanized areas (R2), and highways (R1), respectively, whereas the lowest concentration of metals was found in ecologically clean areas (R4). The obtained results showed that the concentrations of metals detected in honeybee samples were higher than those detected in wax samples in all the studied areas. Moreover, it is safe to say that bees and wax are regarded as good indicators of environmental pollution by toxic substances (metals).

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

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## INTRODUCTION

Honey bees and their products are viewed as good indicators of natural contamination in types of big metals, parts/pieces, or different poisons in search of food, etc [1, 2]. Honey bees fly back to their hive, carrying many contaminants deposited on utilitarian plants [3]. The pollution produced by heavy metals (located on the Earth that supports life) has highly increased in at least the last 20 years because of mining and smelting, production and use of farming-based (material that makes plants grow better) and bug-killing chemicals, city-based waste, (things sent out or given off) from traffic, and industrial (chemicals dumped out of a business) and chemicals [4, 5]. Toxicity due to heavy metals is an environmental problem in the affected regions [6]. In addition to affecting plant production and survival, pollinators that rely on these crops are exposed to potentially toxic metal concentrations as a result of environmental contamination with heavy metals [4]. This can lead to reduced species diversity, brood growth, and survival [7].

Honeybee (A. *mellifera* L.) is a species that meets the above criteria and is a good bioindicator; its existence is inextricably linked to the environment it inhabits [8].

Bees are exposed to numerous contaminants during feeding, and their body hair, called corbiculae, can easily adhere to pollutants from the air and during pollen and nectar collection from flowers or through water [9]. Bees are increasingly being used to monitor environmental pollution with metals in rural and urban studies by [10].

Analysis of bee products, such as honey, wax, or pollen, is thought to be helpful indicators of la nd, plant, and air pollution from toxic metals in a region of some square kilometers [11].

Previous studies have shown that honey, propols, and wax contain various toxicants in colonies aro und the world, such as some heavy metals [12]. This is because heavy metals existing in the atmosphere can be deposited and carried back to the hive in the hairy bodies of bees; they can also be taken together with the nectar in flora or honeydew [13]. Heavy metal pollution has spread broadly over the globe, perturbing the environment and posing serious health hazards to humans. Generally, the root causes of this problem are the rapid pace of urbanization, land-use changes, and industrialization, especially in developing countries with an extremely dense population [14]. In a recent study [15], the levels of heavy metal pollution were detected in samples collected from different regions in Saudi Arabia using foraging bees of A. *mellifera jemenatica* and honey samples. The result shows that the levels of heavy metal residues were extremely low and were within the permissible limits. This also denotes that the areas are not polluted by these chemicals. Although trace elements are very important for life, they have potentially harmful effects [16]. Some metals (Fe, Zn, Cu, and Mn) play important roles in biological systems and are therefore referred to as essential elements, whereas others are non-essential elements (Pb, Cd). The non-essential elements can be toxic even in trace amounts [16]. Some elements, such as chromium (Cr) and nickel (Ni), are widely dispersed in the environment because they are released from natural sources and anthropogenic activities and are derived from extensive use in different and specific industries [17]. Lead (Pb) and cadmium (Cd) are deemed to be the main toxic heavy metals, so they are researched most often. Pb occurs in the air, is primarily based on vehicle traffic, and is transferred to crops [4]. Cd, on the other hand, comes from the metal industry and incinerators and is transferred to the soil and then to crops [9, 18].

Foraer honeybee employees were used in researc h because bees (foragers and recipients) are activ ely engaged in the processing of nectar to honey i n their digestive tract and can absorb heavy meta ls from the nectar [19].

Investigating the probability that honeybees will be able to feed on metal-contaminated resources enables to determine the amount of risk to the honeybee population posed by a metal [7]. Foragers are not allowed to discriminate between uncontaminated nectar or pollen and those contaminated with low cadmium, copper, or lead levels. They may even prefer uncontaminated resources to resources that are mildly contaminated with Pb. This could have significant negative repercussions on the health and survival of the colony [7]. This may be especially true because the metals accumulate within the nest over time, leading to toxic effects on the larvae and eventually adult bees, according to one study [7]. The commonness of metal contamination even at considerable distances from industrial centers and intensively used economic areas has become an area of interest for many researchers. This has led the authors of this research to aim to detect and identify the pollution level of metals in honeybee bodies (A. *mellifera jemenatica*) and wax in four different environmental sampling areas in the Makkah region.

## MATERIALS AND METHODS

The following study was conducted to detect and identify the metals according to their different areas of the Makkah region, Saudi Arabia.

A selected sampling of honeybee's foragers (A. *mellifera jemenatica*) (Hymenoptera: Apidae) and beeswax were collected from apiaries located in different environmental areas.

### Sampling Area

The Western region (Makkah region) is one of the most famous areas in beekeeping in the Kingdom of Saudi Arabia. Characterized by geographical and climatic diversity. The sampling regions were divided into four areas: R1- highways (the international coastal highway linking Jeddah and Jazan), R2- urbanized (highly populated area, (Jeddah city)), R3-industrialized area (Jeddah Steel Factory)) and R4- ecologically clean area (the apiary of the research station of Hada Al-Sham, Faculty of Meteorology, Environment and Agriculture of the Dry Zones, King Abdulaziz University) (Figure.1).

### Honeybee Samples Collection and Preparation

Honeybee foragers (A. *mellifera jemenatica*) were collected from each sampling area: The bees (at least 100 individuals) were collected from the entrance of the hive and taking into account the fulfill methodological requirements relating to the conditions for randomization and variability when collecting samples. Bees carefully brushed directly into disposable polyethylene bags. After collection, all samples were frozen at −10 °C in the laboratory freezer. Before analysis, bee bodies were dried in an oven to constant weight at 105 °C and every sample ground in the hand laboratory grinder separately [4].

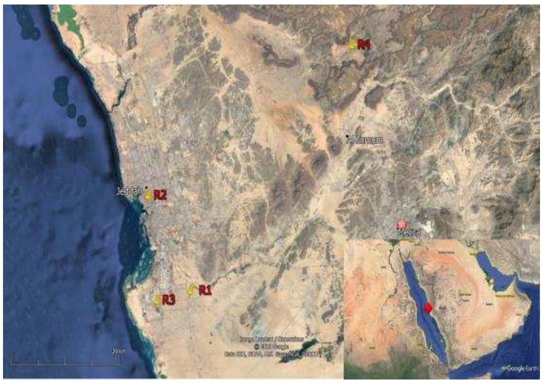


**Figure 1.** Satellite View Showing the Location of Sampling Sites; R1, R2, R3, and R4 (Google Earth Gold Pro).

### Wax Sample Collection and Preparation

Bee's wax was collected from the same hives from which the honeybee's samples were collected in every area, about 5 (gm) were used three repetitions from the samples that were used in this study and were stored at room temperature in tightly closed glass jars [4].

### Determination of Metals

The contents of 12 metals including mineral elements: Potassium (K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), as well as other toxic metals such as Lead (Pb), Nickel (Ni), Cadmium (Cd), Chromium (Cr) in all tested samples were determined using methods of [20].

To analyze some elements, Bee, and wax, samples were prepared. Five gm of bee and wax was burned until turned into ashes and the rest was calcined 13 h. in a furnace at 450$^{o}$C. Residual ash was dissolved in 10 ml 0.5M $HNO_3$ and filtered through quantitative filter paper, and according to the [20]. During the analysis of all samples to create calibration standards, the basic standards of concentration 1000 ppm, Merck were used. All the analyses were made in three independent replications for each sample. The method was validated using certified reference material (NIST –1515). The results were expressed as milligram per gram (mg/g) for bees and wax.

### Statistical Analysis

Statistical analysis for obtained results was carried out with the aid of the (SPSS) [21] computer software program for detection and identification of some metals (mean ± SD) in bee and wax according to their origin from different environments of the region. All data were analyzed using analysis of variance one-way and two-way ANOVA significant differences among the means ($P < 0.05$).

## RESULTS

The results of the current study showed the importance of the selection of the apiary site and the impact on honeybees and the extent of contamination of its products with different metals. Four different environmental regions were identified in the Makkah of Saudi Arabia, which are (R1- highways, R2- urbanized, R3-industrialized, and R4- ecologically clean). That the samples conducted to detect were collected from foragers honeybee workers (*A. mellifera jemenatica*) (Hymenoptera: Apidae) and the bee products (wax) samples. To detect pollution by some metals; (Potassium (K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Lead (Pb), Nickel (Ni), Cadmium (Cd), Chromium (Cr)). The current results indicate:

### Determination and Detection of some Metals in Honeybee Samples from Different Environmental Regions:

The results of the present study showing in the honeybee of various environmentally diverse regions it was found that in the region highways (R1) were the highest concentrations of following metals ((Ca), (Na), (K), (Mg), (Fe), (Zn), (Cu), (Mn) and (Cr)), respectively, they include most minerals, where the concentration of metals amounted to (45.464, 37.106, 36.846, 22.582, 10.028, 3.975, 3.225, 1.668, and 1.003) (mg/g), respectively. While the lowest concentrations were in the following metals (Cd), (Ni), and (Pb), respectively, where the concentration of metals amounted to (0.075, 0.265, and 0.236) (mg/g), respectively. On the other hand, in the region urbanized (R2) were the highest concentrations of the following metals ((Ca),(Na),(K),(Mg),(Fe),(Zn),(Cr),(Mn),(Cu) and (Ni)) respectively, they include most minerals, where the concentration of metals amounted to (63.604, 61.730, 45.079, 31.115, 27.277, 10.602, 8.266, 3.782, 3.559 and 2.617)(mg/g), respectively. While the lowest concentrations were in the following metals (Pb) and (Cd), respectively, where the concentration of metals amounted to (0.307 and 0.079) (mg/g), respectively. In contrast, it was found in the region industrialized (R3) were the highest concentrations of following metals ((Ca),(Na),(K),(Mg),(Fe),(Zn),(Mn),(Cu) and (Cr)) respectively, they include most minerals, where the concentration of metals amounted to (69.005, 55.722, 51.825, 32.179, 27.857, 7.052, 5.036, 3.885, 1.190) (mg/g), respectively. While lowest concentrations were in the following metals (Pb), (Cd), and (Ni), respectively, where the concentration of metals amounted to (0.562, 0.079, and 0.290) (mg/g), respectively. And finally, it was found in the region ecologically clean (R4) were the highest concentrations of following metals ((K),(Mg),(Ca),(Na),(Fe),(Zn) and (Mn)) respectively, they include most minerals, where the concentration of metals amounted to (65.254, 10.050, 8.850, 7.882, 4.386, 1.122 and 1.059)(mg/g) respectively. While lowest concentrations were in metal (Cu) where the concentration of metal amounted to (0.211) (mg/g). While the following minerals were (Ni) (Cd), (Cr), (Pb), and (Cu) completely free of any metal contamination (0.000) (mg/g) the Figure (2).

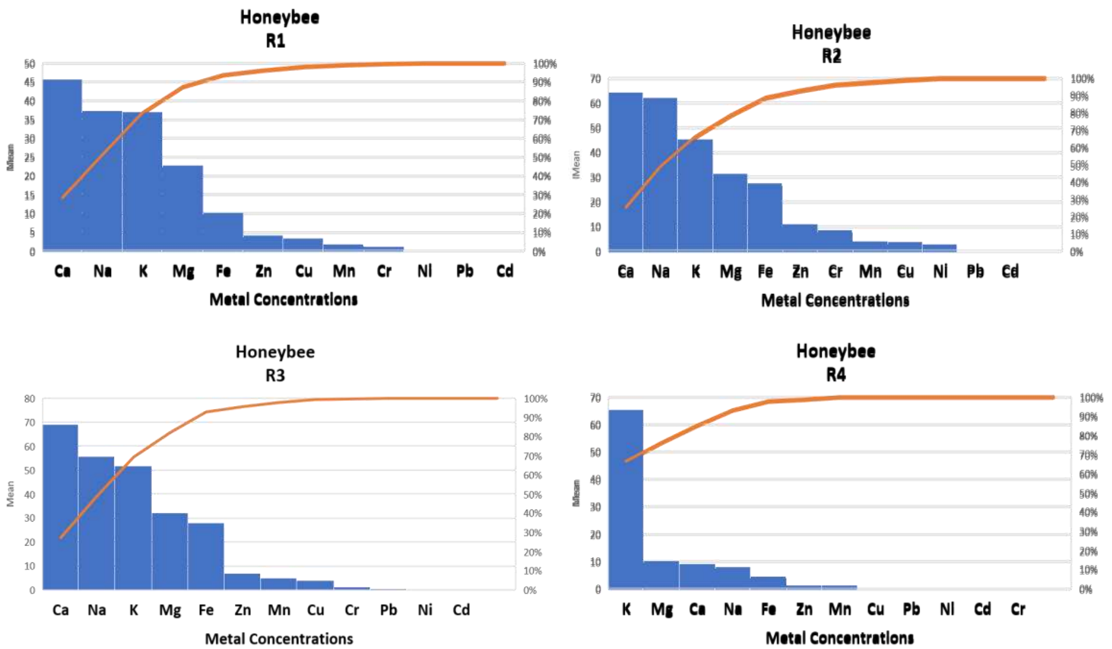


**Figure 2.** The Concentration of Metals (mg/g) in Honeybee Was Collected from Different Environmental Regions.

**Determination and Detection of some Metals in Wax Samples from Different Environmental Regions:**

As shown from the results in Figure 3. obtained from the analysis are found metals concentrations in wax from the various environmentally diverse regions it was found that in the region highways (R1) were the highest concentrations of the following metals ((Ca), (Zn), (Na), (Mg), (Fe), (K), (Cr) and (Cu)) respectively, they include most metals, where the concentration of metals amounted to (19.699,19.699,10.500,6.802,5.972,3.924,1.768 and 1.034) (mg/g), respectively, while the lowest concentrations were in following metals ((Ni), (Mn), (Pb) and (Cd)), respectively, where the concentration of metals amounted to (0.474,0.365,0.114 and 0.059) (mg/g) respectively. Further analysis showed that in the region urbanized (R2) were the highest concentrations in wax samples of following metals ((Ca), (Na), (Mg), (Fe), (K), (Cr), (Zn), and (Cu)) respectively, they include most minerals, where the concentration of metals amounted to (28.041, 25.012, 10.480,9.698,5.959,2.016,1.542 and 1.139) (mg/g) respectively. While the lowest concentrations were in the following metals ((Ni), (Mn), (Pb), and (Cd), respectively, where the concentration of metals amounted to (0.598, 0.501, 0.137, and 0.060) (mg/g) respectively. The results, as shown in Figure 3, indicate that the region industrialized (R3) were the highest concentrations of following metals ((Ca), (Na), (Mg), (K), (Fe), (Zn), (Cr), (Cu) and (Mn)), respectively, they include most minerals, where the concentration of metals amounted to (64.961,52.439,34.369,27.024,18.516,6.272,2.307,1.913,1.311) (mg/g) respectively. While lowest concentrations were in the following metals ((Ni), (Pb) and (Cd)) respectively, where the concentration of metals amounted to (0.678,0.215 and 0.075) (mg/g) respectively. From the data in Figure 3, it is apparent they were highest concentrations in the region ecologically clean (R4) of following metals; ((K), (Mg), (Na), (Fe) and (Ca)) respectively, amounted at (84.679, 19.443,13.010,11.195 and 9.504) (mg/g) respectively. While lowest concentrations were in metal; ((Zn) (Mn), (Cu) and (Cr)) where the concentration of metal amounted to (0.776,0.414,0.095 and 0.003) (mg/g), whereas the following metals were (Ni) (Cd) and (Pb) completely free of any metal contamination (0.000) (mg/g), the Figure (3).

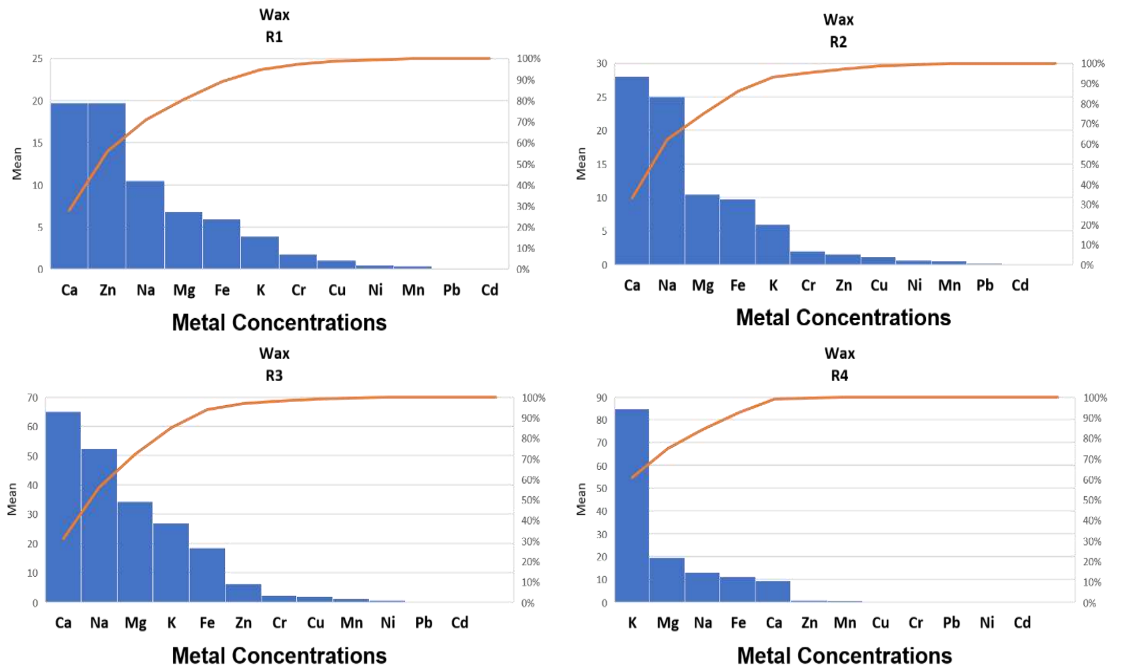


**Figure 3.** The Concentration of Metals (mg/g) in Wax was Collected from Different Environmental Regions

**Trace of Metals in Honeybee Body and Wax Samples:**

Interestingly, this study found that there is a comparison between different regions in terms of their containing concentrations (mg/g) of the metals, region Industrialized (R3), Urbanized (R2), and Highways (R1) respectively have high concentrations, while the lowest concentration was Ecologically clean (R4), Figure (4). A comparison between the bee and wax results reveals, comparing the two results, concentrations of metals detected in honeybees are higher than wax in all regions studied, Figure (5).

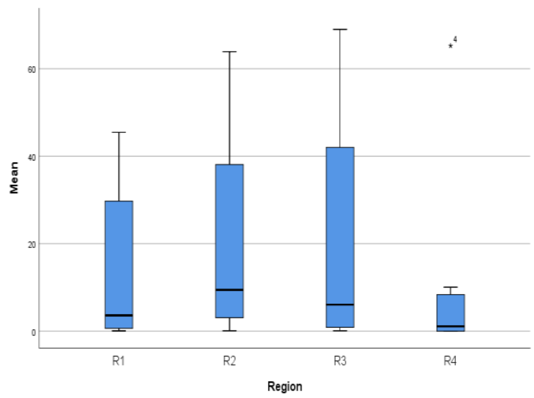


**Figure 4.** A Comparison between Different Regions in terms of Containing Concentrations (mg/g) of the metals.

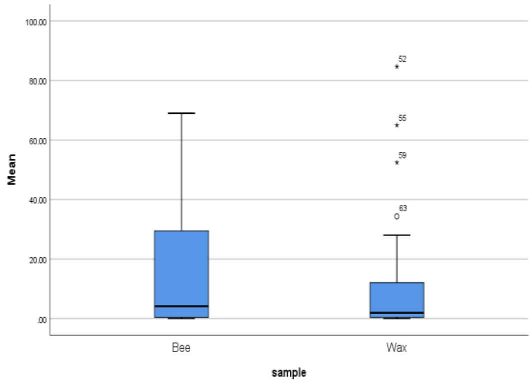


**Figure 5.** The contamination of bee and wax by metals according to their origin from different regions.

This study did detect evidence for finding some of the metals (mg/g) in bee bodies and wax samples, from statistical tests we showed that revealed levels of concentration of metals in bee bodies were highest than wax samples compared to tested metals. Furthermore, the results of this study show a sensitivity difference between bees and wax in different regions. Firstly, in the region (R1): there is no sensitivity difference between bee and wax for Cd, Cr, and Ni but in other metals, there were sensitivity differences. Secondly, in the region (R2): it can be noted there is a sensitivity difference between bee and wax for all study parameters except Ni there is no significant difference between bee and wax. Besides, in the region (R3): It can be noted there is no significant difference between bee and wax for Cd and Na, but there is a significant difference for other parameters. Finally, in the region (R4): It can be noted there is a significant difference between bee and wax for all study parameters, Table 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 * indicate 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 *indicate P<0.05, Significant.

## DISCUSSION

It is difficult to assess the extent of pollution caused by human exposure to hazardous toxic metals in the environment. The most direct approach to revealing the heavy metal status in the environment is the chemical analysis of the environment matrix [4]. However, indirect methods for assessing environmental cleanliness based on the use of living organisms as bioindicators for determining the quality of the environment were widely used [22]. Pollinator guides offer new ways to evaluate ecosystem health because of the relationship between species diversity and abundance changes from the log-normal standard expected from ecological principles [23]. Honeybees are considered to be the most important environmental pollution indicators [24]. The results of this study confirmed detected the varying levels of most of the tested metals in bee bodies of foragers honeybee workers (*A. mellifera jemenatica*) compared to the bee products (wax) samples, was samples inhabiting in different regions and environments in the Makkah region of Saudi Arabia. This is what they have explained [4]. That the content of macro-and micro-elements in the body of bees varied across a wide range of sand depended on several factors, including soil types and nectariferous plants, beekeeping methods, and bee workers' physiological and health status and the periods of the year [25]. About toxic metals, the beekeeping area's ecological status is crucial [25, 26].

The results of the present study confirmed that most regions containing concentrations of the metals were Industrialized (R3), while the lowest in the Ecologically clear (R4), this can be explained that by what he said by [27] contaminated heavy metal discovered in and around urbanized and industrialized areas, mining sites, and heavily-used agricultural regions, in many regions around the globe. Previous studies have shown that many heavy metals are used by crops growing in contaminated soil and demonstrate high concentrations of plant tissue relative to crops cultivated in control soils [7, 27, 28].

Furthermore, the accumulation of heavy metals such as copper, cadmium, lead, zinc, and nickel showed in their leaves and flowers [7, 28, 29]. In addition to influencing the productivity and survival of crops, pollinators that rely on these crops are exposed to possibly toxic metals. They [30] may lead to a decrease in the diversity of species, brood development, and survival of wild and managed pollinator species, as demonstrated in regions known to have high concentrations of metal contamination.

In our study, we did detect evidence for finding some of the metals (mg/g) in bee bodies and wax samples, from statistical tests we showed that revealed levels of concentration of metals in bee bodies were highest than wax samples compared to tested metals. We found a significant value (P<0.05) at (0.000). Furthermore, [4] the bee body was found to be the most effective barrier for transferring Cd to honey, the highest sensitivity to heavy metal pollution was observed in honeydew honey compared to nectar honey (P<0.05), bees were first demonstrated to be used as biofilters for toxic metals and to prevent contamination of honey. Numerous studies use bees and wax in various countries to biomonitor heavy metals in environments. The obtained results are in agreement with other findings. The results in this study demonstrate that the highest concentration of the element Cadmium (Cd) was found in the bee and wax samples in the region of Industrialized (R3) amounted to (0.081 in bee and 0.075 in wax) (mg/g). While the ecologically clean (R4) region was completely free of any contamination, we agree with what the researcher has reached that metals and metalloids – like cadmium – that are not detected pre-ingestion at sublethal, yet toxic concentrations may be readily consumed and pose a significant threat to the health and survival of the colony [31, 32]. Furthermore, this is what he reached [33] the concentration of heavy metals in urban and agricultural woodland bee bodies located in south-western Poland was tested. Cd levels (0.6 and 0.7 mg $kg^{-1}$ d.m.) and Pb levels (1.98 and 1.91 mg $kg^{-1}$ d.m.) were found. [24] it was noted that the levels of Pb and Cd in the Moldavian forest area in honeybee organs, were significantly smaller than in industrial zone specimens. [34] Concentrations of Cd in Italian bee bodies were considerably greater than in the hon-ey sample. Moreover, according to [35], the concentration of Cd and Pb in bee bodies depended on the season and could lead to environmental activists, not from anthropogenic activity.

The absence of cadmium contaminated food being reject-ed by the bees is particularly interesting since[36] showed that cadmium is highly toxic to the honeybee, even at the concentrations they tested. In foragers, concentrations similar to those they used significantly increased adult mortality [36]. In previous studies, scientists have proven that the working honeybees flying, due to their active contact with the atmosphere and environment components, reflects the level of pollution area. The heavy metals present in the atmosphere can be stored on the bees' body brushes, in pollen, or can be absorbed with nectar, mildew, or water. The norms regarding the maximum permissible concentrations of heavy metals in the bees' bodies are not established. However, it is clear, that excessive levels are important reasons for regression and even the disappearance of species *A. mellifera* [24].

Honeybee Products (wax) was regarded as a potential indicator of environmental pollution as a consequence of a bio-accumulative method in the periphery of urban and industrial regions as well as in extra-urban crossroads where traces of certain mineral compounds and/or heavy metals have been discovered [37]. They also discovered small and variable levels of heavy metals in honey, attributing variability among others to variables like the floral source, season, time of year, and rainfall. For this reason, he found that as a reliable and delicate indicator, honey could not be used [38]. The honeybee itself was a better bioindicator of industrial and urban heavy metal pollution., this agrees with [39] Honey and honeybees were evaluated to identify potential thermoelectric power plant contamination in Mugla (Turkey). The analyzed honey samples did not find toxic concentrations of heavy metals, but they discovered greater concentrations of Pb and Cd in honeybees, verifying better use of honeybees as heavy metal pollution bioindicators than honey. The findings of this research indicate that in all areas studied, the levels of metals found in honeybees are greater than wax., this is consistent with [34] When statistically significant differences were observed between heavy metal levels in honeybees and, to a lesser extent, pollen, propolis and wax (but not honey) in areas around the city of Rome and levels measured in the center of the city. Other scientists, however, indicated greater levels of heavy metals in urban and industrial polluted honey than honey from unpolluted rural regions [40-42].

This finding further emphasizes the elevated level of environmental pollution with these metals at such locations and the possibility of using honeybee workers as a bioindicator for environmental pollution with heavy metals, maybe during active movement, the metals enter the bees' body through the air spray and are absorbed by both through the surface of the porous body and breathing. Our study did detect evidence for finding some of the metals (mg/g) in bee bodies and wax in different regions. Firstly, in the region (R1): there is no sensitivity difference between bee and wax for Cd, Cr, and Ni but otherwise metals were a sensitivity difference. Secondly, the present study indicated that in the region (R2) it can be noted that there is a sensitivity difference between bee and wax for all study parameters except Ni there is no significant difference between bee and wax. Also, in the region (R3) it can be noted that there is no significant difference between bee and wax for Cd and Na, but there is a significant difference for other parameters. Finally, in the region (R4) it can be noted that there is a significant difference between bee and wax for all study parameters.

The current research reinforces the concept that due to local environmental exposure; an elevate d concentration of heavy metals is reflected in honeybee workers. Sever-al researchers commend the option of using bees to monitor environmental purity[43,44]. A survey was conducted to determine levels of heavy metals in honeybee employees. Heavy metal concentrations ranged from 3.53- 6.26, 27.65- 30.80, 0.05- 0.19, 375.4- 446.5 and 3406.35- 5161.25 ppm respectively for Cu, Zn, Cd, Pb and Fe. Similarly,[34]. In honeybee employees, levels of Cd and Pb ranged from 2.87 to 4.23 ppm for Cd and 0.61 to 1.25 ppm for Pb. The levels published in Cu, Zn, and Cd were lower than those published in this report [44] and Pb was lowest than those reported by [34]. These differences may be attributed to the varying degrees of heavy metal contaminations at each location. Honeybees (*A. mellifera* L.) have great potential for detecting and monitoring environmental pollution, given their wide-ranging foraging behavior [8]. Honeybees collect particles deposited in the flowers and other places where bees collect resins (propolis) and water along with nectar, pollen, water, and propolis. The branched hairs on the bee's body, about pollen, gathered from the anthers, readily retain non-floral particles from atmospheric deposition. Each honeybee can thus function as a micro-sampler of the setting and a colony of honeybees as a sampler unit. About a quarter of the colony's population is a forager honeybee worker during the active foraging period of the honeybee colony [10].

Meanwhile, K has been the most efficient mineral element content environment variable than Ca, Na, Mg, Fe, Zn, and Cr. On the other hand, Mn was the least environmental variable of levels of bee and wax heavy metals. While Cr was the most efficient heavy metal content environment variable than Pb and Ni. In contrast, Cd was the least environmental variable of the bee and wax heavy metals concentrations. On the other hand, as for the comparison in terms of regions, it was found that the most regions containing concentrations of the metals were Industrialized (R3), Urbanized (R2), and Highways (R1) respectively have high concentrations, while the lowest concentration was Ecologically clean (R4). This is similar to the study results Carried out by [15] who found that the highest contamination level among the heavy metals in question was iron (Fe). In the honeybee samples, the highest Fe concentrations were in the Makkah region (8.794) Asir (6.222) Jazan (6.205), and Al-Baha (2.088). In the honey samples, the highest Fe concentrations were found in bees of Asir (1.904) Jazan (1.843) Al-Baha (1.340), and Makkah (0.907). In addition, there found that the most concentrated mineral element is potassium (K) in four agricultural areas, this is consistent with the results of the present study. This is consistent with the results of the study [4] Potassium is the most abundant component, Mg and Ca levels in bee organs were significantly lower And these components' concentrations in honey were about seven times lower. Mn, Fe, Zn, and Cu with that discovered detected in bee bodies.

The content of heavy metals in the bee's body depending on a large number of factors: the extent and the location of the apiary, type of soil and nectar plant from the area, the ecological status of the area, the methodology of increasing bee families (including food stimulation supplements), working bees age, physiological status and health of bee colonies, etc. [25]. Generalizing present research results, we can conclude that concentrations of metals in the bee's body in all research areas are far away and much lower, compared with the noxious dose for bees established by [24].

## CONCLUSION

One of the more significant findings to emerge from this study is: the effectiveness and the usefulness of honeybees and wax in the assessment of environmental clearness and evaluate the effectiveness as a bioindicator. The following conclusions can be drawn from the present study when were that the analysis of honeybee workers (*A. mellifera jemenatica*) and their products (wax). For the determination of the environmental pollution with metals by comparing data obtained by different sampling sites in Saudi Arabia. It was found that most regions containing concentrations of the metals were Industrialized (R3), Urbanized (R2), and Highways (R1) respectively, while the lowest concentration was recorded in Ecologically clean (R4). So, beekeepers should be interested in identifying bee-sensitive and beekeeping sites to obtain good products and free from any environmental contaminants. However, when compared to the mineral levels in this study with international standards they are still within the permissible limits. So, further data collection is required to determine exactly. Because of the recommendation that the quality and safety of honeybees and their products are related to the health and safety of human beings, so it is necessary to be paid attention to.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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