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RESEARCH ARTICLE

Bees as Bioindicators of Environmental Pollution with Heavy Metals

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ABSTRACT

Honey bee pollen can serve as a biological indicator of environmental pollution. The aim of this study was to examine the presence of heavy metals such as Pb, Zn, Cu, Fe, Mn and Cr in bee pollen collected from nine apiaries in various regions of Northern Iraq. Our findings revealed high levels of heavy metals accumulation in all tested bee pollen samples. In fact, the heavy metal values in the collected bee pollen samples from different locations exceeded the recommended values established by the International Food Standard. Furthermore, significant differences were observed among the pollen samples collected from different locations. The highest value of Pb (53.47 ±3.58 mg/kg) was found in pollen samples from Duhok/Semel/ College of Agriculture, followed by (50.95 ± 1.12 mg/ kg) in Duhok/ Semel /Seje. The highest value of Fe (400.47 ±9.96 mg/ kg) was recorded in pollen samples collected from Duhok/ Semel /Seje. The values of Cu ranged from 30.03 ±0.63 mg/kg in Erbil Center/Qushtapa to (2.67 ± 0.02 mg/ kg) in Duhok/Semel/Seje. The values of Zn varied between the maximum of 94.41 ±3.22 mg/ kg in Duhok/Amedi/Gri village, and the minimum (5.09 ±0.08 mg/ kg) in Duhok/Akre. Additionally, the highest values of Mn (85.55±2.02 mg/kg) and Cr (9.14±0.88 mg/kg) were found in pollen samples collected from Duhok/Semel/College of Agriculture. These investigations highlight that all tested bee pollen samples are highly contaminated with heavy metals, confirming the importance of bee studies in monitoring changes in the natural environment as a result of human activities.

INTRODUCTION

The commonness of metal contamination, even at considerable distances from industrial centers and intensively used economic areas, has become the interest of many researchers (Skorbilowicz et al. 2018). Excessive amounts of toxic elements accumulate in the environment and get into organisms of plants and animals. Harmful effects of metals on living organisms are a major environmental problem (Mustafa et al, 2022). Bees and their products have been suggested as biological indicators of environmental pollution in many studies.

The sources related to human activities are a major cause of pollution and the presence of metals in the environment (Steen et al. 2018; Skorbilowicz et al. 2018). The commonness of metal contamination, even at considerable distances from industrial centers and intensively used economic areas, has become the interest of many researchers. Sources of metals are discharges of municipal and industrial wastewater, road transport, agriculture and dust from urban streets. The main sources of metal contamination in the environment, include both natural and anthropogenic sources (Goretti et al. 2020).

Bees are exposed to numerous contaminants during feeding, their body hair can easily adhere pollutants from the air and during pollen and nectar collection from flowers or through water (Ruschioni et al. 2013). Bee is increasingly being used to monitor the environmental pollution with metals in rural and urban studies (Porrini et al. 2003, Eremia et al. 2010, Steen et al. 2016).

The raw material for honey production may contain pollutants that are characteristic of the environment (Roman et al. 2011), including minerals of natural or anthropogenic origin (Steen et al. 2018; Goretti et al. 2020). The sources of mineral contamination include rock weathering; anthropogenic sources of metals include municipal and industrial wastewaters, road transport, dust from car traffic (Niedzwiecki et al. 2000; Kaniuczak et al. 2003), and agriculture (Klavins et al. 2000).

The pollen powder collected by honey bees from flowers is known as honey bee pollen. Pollen is stored in the chambers of the hives by honeybees. Bee pollen is a ball or pellet of pollen collected in the outdoors by worker honeybees and utilized as the hive's major food supply. Bee pollen is a mixture of flower pollen from different species gathered by forager bees, formed into pellets with nectar and secretions of bees' salivary glands.

The multielement composition of bee products may also reflect environmental pollution, affecting both humans and bees. A continuous decline in the honeybee population has been reported in industrialized countries (Neumann and Carreck 2010). Several factors are supposed to contribute to honeybee declines, such as pests and diseases, global warming, and environmental pollutants associated with industrial and agricultural human activity, including heavy metals (Neumann and Carreck 2010; Feldhaar and Otti 2020).

Due to the many vital roles of honeybees in the environment and agriculture, their health has become a public concern. Thus, it is imperative to assess the pollution to which bees are exposed in their natural habitat. The ease of sampling bees, their ability to accumulate contaminants from air, soil, and water, and the potential for long-term monitoring in contaminated areas were highlighted as advantages of using bees as bioindicators.

The main objective of this work is to evaluate the usefulness of honey bees (*Apis mellifera* L) as bioindicators of the presence of some heavy metals in natural ecosystem and urban areas in Northern Iraq in order to assess the safety of bee products for humans and bees.

MATERIALS AND METHODS

The methodology involved studying the accumulation of metals in bee pollen as bioindicators of environmental pollution. Bee pollen was sampled from different locations in northern Iraq to assess the levels of metals such as lead (Pb), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and chromium (Cr) on their bodies. The study considered the specifications of each sampling location as a factor, that can influence the accumulation of metals in bee pollen (Sadeghi et al., 2012). Forager bees fly and collect pollen from floral species within a 4 km radius of their hives (Greenleaf et al., 2007). Sampling bees and collecting bee-collected pollen can help reduce the number of samples usually needed for surveys (Roberts et al., 2018; Tremblay et al., 2019).

Pollen samples gathered by honey bees were collected from nine apiaries in different locations in Northern Iraq (Figure 1); all samples were collected from the cites of Duhok, Erbil and their suburbs (rural and urban areas). The sampling process was performed during September and October 2023, when bees collect a large amount of pollen as preparation for winter. Pollen traps were used at the entrance of three hives in each apiary. All samples were preserved in labeled plastic containers and stored in a refrigerator at 5 ºC.

Dried pollen was ground to obtain a homogeneous sample. Approximately 2 g of pollen samples were used and then they were kept in an oven at 70°C until a constant weight was achieved before being used in the analysis (Kacar and İnal 2008).

The heavy metal contents of the samples (total Pb, Cr, Zn, Cu, Mn, and Fe) were determined using Atomic Absorption Spectro-Photometer technique (AAS; Shimadzu GFA7000 France) to detect the previously mentioned heavy metals (Smith et al. 2019; Zaric et al. 2018, 2021).

The chemical analysis was performed at the laboratory of Duhok Environmental Directorate to detect the previously mentioned heavy metals; The elemental amounts of the samples are given in mg/kg.

STATISTICAL ANALYSIS

Heavy metal amounts were determined in pollen samples taken from each of the nine different locations. The data were subjected to one-way analysis of variance, and Tukey´s test was used to determine the differences among groups of different locations. Data are presented as mean and standard error (Bayir and Aygun 2022).

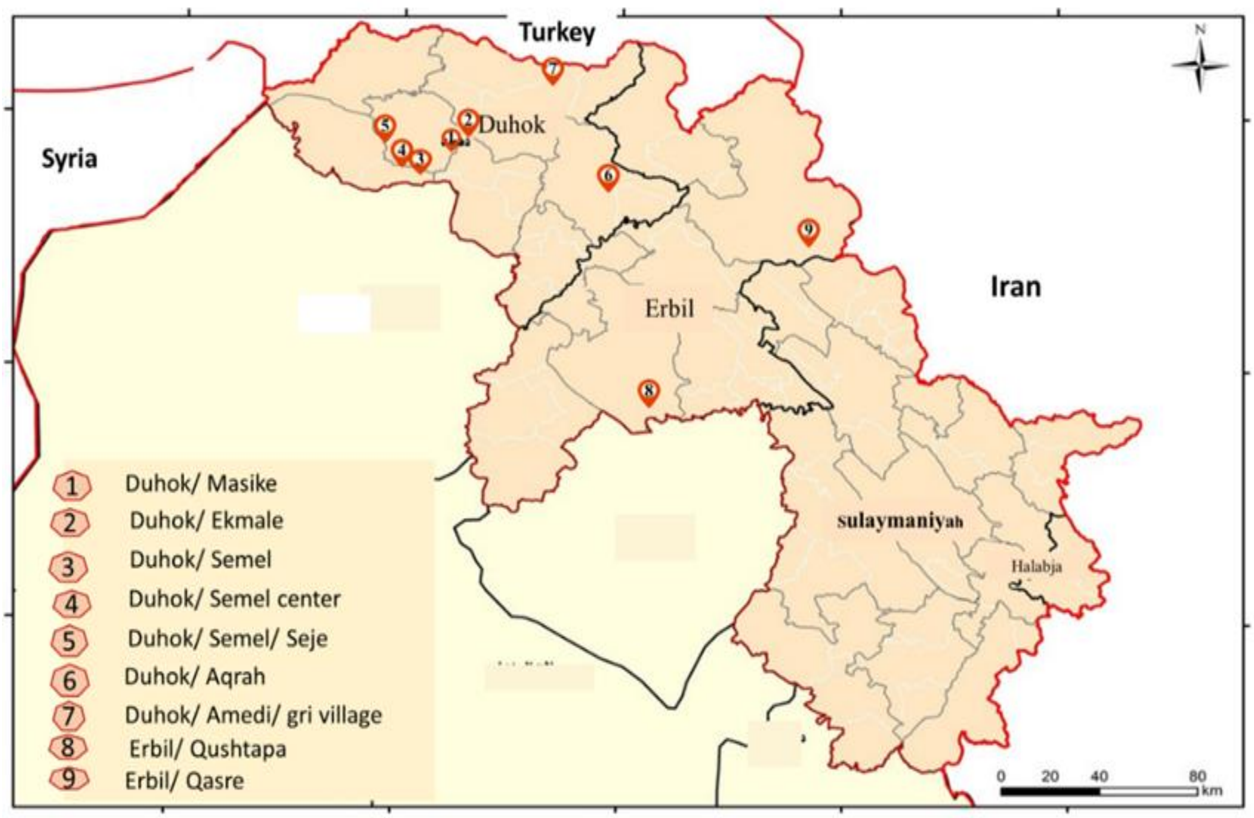

Figure 1. Map of Northern Iraq shows the locations of the honey bee colonies (sampling sites

Table 1. Topographical features of the locations of collected samples.

Province	Districts	Sub- Districts	Latitude	Longitude	Altitude
Duhok	Center	Masike	36°52'25"N	42°56'32"E	537 m
Duhok	Center	Ekmale	36°55'03"N	43°02'59"E	848 m
Duhok	Semel	College of Agriculture	36°51'28"N	42°52'04"E	472 m
Duhok	Semel	Center	36°51'45"N	42°52'26"E	481 m
Duhok	Semel	Seje	36°55'01"N	42°55'20"E	794 m
Duhok	Akre	Center	36°44'26"N	43°52'21"E	670 m
Duhok	Amedi	Gri village	34°09'21"N	45°22'14"E	238 m
Erbil	Qushtapa	Center	35°59'47"N	44°02'12"E	402 m
Erbil	Qasre	Center	36°33'31"N	44°49'30"E	1.087 m

RESULTS AND DISCUSSION

Table 2. Heavy metal values in bee pollen samples.

Location	Heavy metals					
	Pb	Zn	Cu	Fe	Mn	Cr
Duhok Center/ Masike	36.59±1.24 b	75.48±2.23 ab	24.33±0.21 a	252.89±0.22 b	47.01±1.42 bc	5.95±0.12 b
Duhok/ Ekmale	20.51±0.22 c	53.71±0.95 b	12.64±0.07 b	189.36±7.62 bc	34.95±1.16 c	5 ±0.15 b
Duhok/ Semel / College of Agriculture	53.47±3.58 a	53.92±1.02 b	8.98±0.02 c	392.18±9.92 a	85.55±2.02 a	9.14±0.88 a
Duhok/ Semel	20.82±0.15 c	60.93±1.62 b	17.1 ±0.15 b	179.11±6.75 c	54.44±1.12 bc	2.69±0.02 cd
Duhok/ Semel /Seje	50.95±1.12 a	54.61±1.01 b	2.67 ±0.02 d	400.47±9.96 a	69.65±1.24 b	3.68 ±0.05 c
Duhok/ Akre	35.11±0.26 b	5.09 ±0.08 c	26.3 ±0.35 a	98.58 ±4.56 d	35.53±0.98 c	3.25±0.02 c
Duhok/ Amedi/ Gri village	20.17±0.66 c	94.41±3.22 a	25.86±0.55 a	202.24±7.32 bc	62.92±1.32 b	4.43 ±0.12 bc
Erbil Center/ Qushtapa	17.58±0.16 d	54.13±1.25 b	30.03±0.63 a	117.166±5.85 d	53.74±1.03 bc	1.93 ±0.02 d
Erbil / Qasre	21.20±0.85 c	68.48±2.02 ab	26.52±0.27 a	253.24±8.96 b	19.43±0.47 d	4.72 ±0.06 b

(a-d) Different letters in the same column indicate significant differences ($P < 0.05$).

The mean values and standard errors of Pb, Zn, Cu, Fe, Mn and Cr in pollen samples are given in Table 2. In pollen samples, the differences among the locations were statistically significant (P< 0.05). The highest value of Pb (53.47 ± 3.58 mg/kg) was detected in pollen samples from Duhok/ Semel / College of Agriculture, followed by (50.95 ± 1.12 mg/ kg) in Duhok/ Semel /Seje; Both pollen samples represented by maximum values were collected from locations characterized by the presence of petroleum refinery stations.

The previously mentioned first location is close to the highway; pollen samples from this location also showed high values of Fe (392.18 ± 9.92 mg/ kg) and Mn (85.55±2.02 mg/ kg). Minimum value of Pb was observed in Erbil/ Qushtapa (17.58 ± 0.16 mg/ kg). Values of Zn were restricted between maximum (94.41±3.22 mg/ kg) in Duhok/ Amedi/ Gri village, and minimum (5.09 ±0.08 mg/ kg) in Duhok/ Akre. This little amount of Zn in Akre can be explained that the pollen samples were collected by bees reared in apiary located far from the human activities. Values of Cu were ranged from 30.03 ±0.63 mg/ kg in Erbil Center/ Qushtapa to 2.67 ±0.02 mg/ kg 8.98 ±0.02 in Duhok/ Semel /Seje. According to the regulation of WHO (World Health Organization), the optimum limit of Cu should be 20.0 mg/kg, the above obtained results showed a very low concentration of Cu in Duhok/ Semel /Seje. The highest value of Fe (400.47±9.96 mg/ kg) was recorded in bee samples collected from Duhok/ Semel /Seje; this location was characterized by the presence of petroleum refinery and industrial stations, while the minimum value of Fe (98.58 ±4.56 mg/ kg) was found in pollen samples collected from Duhok/ Akre.

Values of Mn were ranged from 85.55±2.02 mg/ kg in bee samples collected from Duhok/ Semel / College of Agriculture; to 19.43± 0.47 mg/ kg in Erbil / Qasre. Values of Fe were ranged from 98.58± 4.56 mg/ kg in Duhok/ Akre to 392.18± 9.92 mg/ kg in Duhok/ Semel / College of Agriculture. The highest value of Cr (9.14± 0.88 mg/ kg) was found in bee samples collected from Duhok/ Semel / College of Agriculture; while the minimum value (1.93± 0.02 mg/ kg) of Cr was found in bee samples from Erbil / Qushtapa.

Overall results showed that the Pb, Zn, Cu, Fe, Mn and Cr values of pollen samples were highest at sites around the cities and lowest in the rural sites. The values of All studied elements in pollen samples were higher than the International Food Standard values (Codex Alimentarius 2015). There are very wide range observed between our results and those found by Bayir and Aygun (2022) in Turkey, particularly in the values of Fe. Values of Pb and Zn of all tested pollen samples in the current study were higher than those found by Bayir and Aygun (2022) in Turkey, and also higher than those determined by Formicki et al. (2013) from Poland.

Several key points were addressed as a result of these investigations of metal contamination in bees as bioindicators of environmental pollution; the study demonstrated that bees, particularly honey bees (*Apis mellifera* L), are effective bioindicators of metal contamination in urban areas. Their close interaction with the environment through foraging activities makes them sensitive to environmental pollutants, making them valuable indicators of environmental quality (Sadeghi et al. 2012, Steen et al. 2012). The main sources of metals in bee samples were identified as motorization, industry, and municipal activities within urban areas. These anthropogenic sources contribute to the presence of metals such as copper, chromium, zinc, manganese, and iron in the environment, which are subsequently accumulated by bees (Steen et al. 2016; Eremia et al. 2010).

Bees offer a practical and cost-effective means of monitoring environmental pollution over extended periods, providing valuable data on toxic metal emissions from various sources. Conti and Botre (2001) found higher Cr contents in pollen samples obtained from inner-city locations compared to urban locations (P < 0.01). Taha et al. (2017) found that the Cu content of pollen samples taken from colonies placed at different distances from a cement factory was higher in samples close to the factory (P < 0.05). Taha (2015) found significant statistical differences among pollen obtained from different plants. Arslan and Arıkan (2013) did not find a significant statistical difference among the Fe values of pollen samples obtained from colonies placed at different distances from the highway. Overall, the investigations emphasized the importance of bees as bioindicators for assessing metal contamination in urban environments and their potential role in environmental monitoring and management. As well as confirmed the significance of bee studies for monitoring changes in the natural environment due to human activities. Establishing a network of apiaries in contaminated areas can facilitate ongoing monitoring of metal pollution, offering insights into long-term trends and the impact of anthropogenic changes on environmental quality.

CONCLUSION

Metal contamination in bees as bioindicators of environmental pollution revealed significant findings. The presence of metals such as lead (Pb), chromium (Cr), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) were detected in all pollen samples, indicating environmental contamination. The main sources of these metals in these samples were attributed to activities related to motorization, industry, and municipal economy within urban areas. Statistical analysis showed a common origin for the majority of the studied metals, emphasizing the impact of anthropogenic activities on environmental metal pollution.

The study highlighted the geographical variability of metal concentrations in bee pollen samples, with higher contents observed in industrial areas due to accumulation of contaminants. Bees were recognized as effective bioindicators of changing natural environments resulting from human activities, making them valuable for bio-monitoring purposes. Overall, the research underscored the importance of studying bees as bioindicators for metal contamination, providing valuable insights into the environmental impact of anthropogenic activities. The findings contribute to the understanding of metal pollution dynamics and the role of bees in monitoring environmental changes in industrial, urban, and rural areas in northern Iraq.

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