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Determination of lead and cadmium contents in some bee products collected from north Upper Egypt

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ABSTRACT

Bee products have been a good bioindicator for environmental monitoring to measure pollution with heavy metals as the accumulation of pollutants in the different products of honey bee colonies. The main objective of this research is to evaluate pollution levels of heavy metals such as lead (Pb) and cadmium (Cd) in bee bread and old, new bee wax in apiaries spread across different regions of Upper Egypt. The finding showed that samples collected from apiaries in industrial, electric power, and quick road regions contained a higher concentration of Pb and Cd than samples collected from rural villages. It was found that high lead levels in bee bread, old bee wax, and new bee wax were 1.17±0.4, 2.25±0.35, and 1.38±0.35 mg/kg, respectively, in industrial regions, while the lowest concentrations in rural regions were 0.29±0.05, 0.87±0.20, and 0.25±0.12 mg/kg of bee bread, old bee wax, and new bee wax, respectively. High concentrations of Cd were obtained in samples collected from industrial areas near electricity and road traffic, respectively. While the rural regions with the lowest concentration of Cd were 0.06±0.05, 0.04±0.03, and 0.03±0.02 mg/kg for bee bread and bee wax, old and new, respectively. These results are a very important indicator for knowing the regions least exposed to polluting with Pb and Cd to obtain pure bee products and bee colonies healthy fitness.

Keywords: Heavy metals, Lead, Cadmium, Bee bread, Bee wax.

INTRODUCTION

Metals are released into surrounding regions from different sources and are non-degradable and accumulate, which increases harm to nature as a result of changing their chemical forms and leads to disturbance of biological cycles (Perugini *et al.*, 2011). Human activities have changed the quantity and distribution of heavy metals in the environment and these pollutants have been concerns raised about its dangers to plants, animals and humans (Gbaruku and Friday, 2007). Heavy metals are released into the environment and pollute the raw materials of bee products (nectar, honeydew, pollen) through the air, water, plants and soil and are then transported to the beehive by the bees (Bogdanov, 2006). Bees one of insect pollinators that come into contact with a large quantity of potentially harmful pollutants during their foraging flight in search of pollen, nectar, or water extracted from flowers or reservoirs (Bargańska *et al.*, 2016). Honey bees (*Apis mellifera* L.) and its products are in between the most important living organisms that are affected by environmental conditions and contamination have a great ability to sense environmental changes; they are considered a bio indicator of many toxic environmental factors and heavy metals that exist in nature (Al Naggar, 2013). Therefore, bees and their products (honey, royal jelly, bee bread, propolis) are used as a vital indicator in determining environmental pollution and detecting heavy metals (Salkova and

Panayotova-Pencheva, 2016). Apiaries in urban and industrial areas are considered more polluted than apiaries in cultivated areas and natural forests; and specific lead concentrations in the samples vary depending on the dry seasons (Roman, 2010). Many minerals can be found in honey bee colonies and isolated; especially cadmium, copper and lead are the most common (Hladun *et al.,* 2015 and Sata *et al.,* 2012). Lead the main toxic heavy metal found in the air and arises mainly from diesel engines, which in turn directly pollute the air and thus the nectar and pollen visited by bees (Tomczyk *et al.,* 2023). Worker bees collect pollen from different flowering plants and store it in the bee colonies in the form of bee bread, which is one of the natural products of bees (Romeh, 2021). Bee bread consists of pollen and nectar enzymatic secretions of bees secreted orally, and bees use it as an essential protein source in their diet (El Ghouizi *et al.,* 2023). Lead bio accumulates in the insect's body (Mertz, 1981). And pollute pollen, honey and bee wax in honey bee colonies (Zhou *et al.*, 2018). Bees transport food to the larvae (Balestra *et al.,* 1992). Heavy metals also bio accumulate in bee larvae, adult bees and all bee products; which negatively affects all stages of bee growth in the colony (Di *et al.,* 2016). Little is known about sub-lethal effects, but high concentrations of heavy metals are lethal to honey bees (Hladun *et al.,* 2016). Moreover, Pb toxicity causes great harm to humans, such as anemia, as well as kidney brain damage (Wani *et al.,* 2015)**.** Heavy metals vary depending on their types in toxicity to bees, because they have an oscillatory behavior (Emsley, 2011 and Roman, 2010). Concentration of heavy metals in the environment which below sub lethal risks to human health, its directly affects the activity and survival of insect pollinators. This paper aims to measure the extent of environmental pollution with heavy metals, especially lead and cadmium, by using some products of bee colonies, namely bee wax and bee bread, as an indicator to determine the extent of pollution in different areas of northern Upper Egypt.

MATERIALS AND METHODS

This study was conducted during the year 2023 to determine the extent of environmental pollution with heavy metals in North Upper Egypt by measuring the levels of lead and cadmium in bee colony products (bee bread and bee wax old & new).

Heavy metals determination in bee products:

1. **Areas of study:**

In North Upper Egypt (Beni Suef Governorate), four areas were identified according to different human activities, which are: industrial areas, areas close to electricity, areas close to roads (traffic), and agricultural rural areas. Five apiaries randomly were selected from each of these areas to samples collection and examination.

2. **Sample collection and preparation:**

Bee bread, bee wax old and bee wax new were choosing for determined the contamination with some heavy metals Pb and Cd in this experiment. Each region was represented by three apiaries, and samples were taken from three different colonies from each apiary. Bee bread samples additionally bee wax new and old (samples of bee wax new from newly built combs in the same season, and samples of bee wax old from combs built more than a year ago) each sample (10 × 10 cm) taken from each colony. When the samples were returned to the laboratory, each sample of the same type for each apiary was collected to represent one sample of bee bread or bee wax old and new each of separately and kept in frozen at -20°C. Twenty samples were collected for each type.

3. **Heavy metals determination:**

The residues of heavy metals (Pb, Cd) were determined by an Atomic Absorption Spectrophotometer 200 Series AA Systems. According to Feldsine *et al.* (2002) in laboratories of Faculty of post graduate studies for advanced sciences the metals concentrations in bee (bee wax and bee bread) samples were determined against standard solutions of known contents

which were analyzed concurrently. The concentrations of heavy metals were determined in mg/kg.

4. The international standards of heavy metals in bee products:

Pursuant to the European legislation specification of heavy metal in bee wax and bee bread as follow:

For Bee wax were set to 5 mg kg^{-1} for Pb and 3 mg kg^{-1} for Arsenic and 1 mg kg^{-1} for Hg (Codex Alimentarius Commission, 2011). According to the FAO/Who Expect committee the recommended limits on wax were of 2 mg kg^{-1} for Pb in wax and proposed a maximum residue limit (MRL) for the EU of 1 mg kg^{-1} (European commission, (EC) 2001). The committee of European food safety authority (EFSA); Formicki *et al.* (2013) recommended that the levels for Lead should be set as low as possible due to its dangerous effects on human being.

5. Statistical analysis:

The records in this work are the average group treated± standard deviation (Sd). The differences between the averages were analyzed through the analysis of the one-way variation (ANOVA), followed by the Duncan test. For the analysis of statistical data, the program me package was separated while the average was separated using the less significant differential method, when "F" test significant was obtained according to Steel and Torrie (1980). (SPSS)

RESULTS AND DISCUSSION

Heavy metals residues in honey bee products:

1. Bee bread

The data presented in Table (1) showed the different concentrations of Pb in all bee bread samples collected from all apiaries in the different areas tested. High levels of Pb concentration (1.17 ± 0.43 mg/kg) were determined in bee bread samples collected from apiaries in industrial areas, while the lowest average of Pb concentration was in bee bread samples from rural areas (0.29 ± 0.05 mg/kg). While the average concentration of Pb was sampled of bee bread in the electric power areas and traffic roads, reaching 0.80±0.10 and 0.62±0.09 mg/kg, respectively. In the same Table (1) the maximum, minimum and average values ± Sd of Cd mg/kg are shown in all tested bee bread samples that were collected from the study areas. The highest average of Cd contents (0.24 ± 0.04 mg/kg) were estimated in bee bread samples from industrial areas; while the areas of Electric power and Roads (traffic) samples were 0.20±0.02 and 0.17±0.02 mg/kg, respectively. The lowest average of Cd was found in bee bread sample from rural area (0.06±0.05 mg/kg). Honey products (bee bread) have become one of the important tools for eco-pollution index due to their properties of bioaccumulation of trace elements and toxic metals in the environment.

Table 1. Concentrations of lead and cadmium (mg/kg) in bee bread in the different areas.

The metals / Sample areas	Pb concentration			Cd concentration		
	Max.	Min.	Average± Sd	Max.	Min.	Average ±Sd
Industrial areas	1.90	0.87	1.17±0.43a	0.28	0.19	0.24±0.04a
Electric power	0.86	0.65	0.80±0.10b	0.23	0.17	0.20±0.02ab
Roads (traffic)	0.76	0.50	0.62±0.09b	0.19	0.13	0.17±0.02b
Rural areas	0.33	0.22	0.29±0.05c	0.12	0.01	0.06±0.05c
F	12.898			26.84		
P≤0.05	0.003			0.00		
$LSD_{(0.050)}$	0.304			0.048		

Table 2. Concentration of Lead in old and new bees wax (mg/kg) in the different areas.

Sample areas	Bee wax	Max.	Min.	Average± Sd
Industrial areas	Old	2.8	1.97	2.25±0.35 a
	New	1.86	1.08	1.38±0.35 c
Electric power	Old	2.13	1.53	1.86±0.26 b
	New	0.96	0.18	0.71±0.33 d
Roads (traffic)	Old	1.57	1.03	1.33±0.20 c
	New	0.99	0.69	0.80±0.11 d
Rural areas	Old	1.16	0.68	0.87±0.20 d
	New	0.4	0.11	0.25±0.12 e
F	32.28			
P≤0.05	0.00			
LSD $_{(0.050)}$	0.33			

Data in Table (2) revealed high, low and average ±Sd of Pb concentrations in bees wax old and new (mg/kg) in the different areas. In general, different concentrations of lead were found in old and new bees wax samples collected from all areas. The highest average lead concentrations were determined in old and new bees wax samples collected from industrial areas, recorded 2.25±0.35 and 1.38±0.35 mg/kg, respectively, compared to samples from the other areas. While the rural areas recorded the lowest concentration of Pb were 0.87±0.20 and 0.25±0.12 mg/kg in old and new bee wax, respectively. While the values of lead concentrations in samples of electric power and roads (traffic) areas in bee wax old and new were relatively close to each other and were 1.86±0.26, 0.71±0.33, and 1.33±0.20, 0.80±0.11 mg/kg, respectively.

On the other hand, Cd was found in all the tested old and new bee wax samples collected from different areas, as shown in Table 3. High levels of Cd content (0.31±0.03 mg/kg) were determined in old bee wax samples collected from industrial areas compared to other samples, and new bee wax samples collected from rural areas were 0.04±0.03 and 0.03±0.02 mg/kg, respectively.

Table 3. Concentration of cadmium in old and new bees wax (mg/kg) in the different areas.

Sample areas	Bee wax	Max.	Min.	Average± Sd
Industrial areas	Old	0.34	0.28	0.31±0.03 a
	New	0.2	0.11	0.17±0.04 c
Electric power	Old	0.27	0.18	0.22±0.04 b
	New	0.2	0.14	0.17±0.02 c
Roads (traffic)	Old	0.19	0.1	0.14±0.04 c
	New	0.13	0.05	0.09±0.03 d
Rural areas	Old	0.08	0.01	0.04±0.03 e
	New	0.06	0.01	0.03±0.02 e
F (value)	44.11			
P≤0.05	0.00			
LSD $_{(0.050)}$	0.04			

In these experiments, different levels of lead and cadmium concentrations were determined in some products of honey bee colonies, namely bee bread and old & new bee wax. This has used several research teams to use of honey bee colonies products as a biological marker of the environmental pollution e.g. (Munoz and Palmero 2006, Naccari *et al.*, 2014, Sitarz-Palczak *et al.*, 2015). These experiments were conducted in four different regions according to human activity to estimate the extent of environmental pollution: industrial areas, electric power, roads (traffic), and rural areas. In general, industrial areas recorded the highest pollution levels for lead and cadmium concentrations in all samples tested for some honey bee colonies products (bee bread and bee wax old & new), followed by electrical power and road (traffic) areas. While rural areas were lowest polluted with lead and cadmium, these results finding are came consisted with many authors. Lead is highly toxic, and it's traces stay for a long time in the environment. It represents the second level in the ranking of the 20 most hazardous substance as it is the most harmful metal for the biosphere (ATSDR, 2020). Al-Naggar *et al.* (2013) reported that small peaks for Cd, Pb and Ni were noticed in the X-ray spectra of the wing and hind gut of the forager honey bee as well as of mid-gut and fat body of forager bee demonstrated small peaks for Cd, Pb, Fe and Ni compared to the control. The air and soil contain heavy metals, mainly from industry and traffic, which can also contaminate the bee colony and its products. Bees hairy bodies can carry heavy metals from the atmosphere and transport them into the hive along with pollen (Pehlivan and Gl, 2015). Many reports indicated that honey samples Clustered from industrial areas have a higher percentage of heavy metals (cadmium, lead, mercury, zinc, copper, nickel and chromium) more than those found in natural regions (Tuzen and Soylak, 2005). Honey is contaminated with heavy metals in two ways: the environment in which the honey bees colonies and the method of raising the bees. The bee foraging area is approximately 7 km^2 and includes and heavy metals in honey may be largely dependent on the soil composition, as well as various types of floral plants and foods (Sereviciene *et al.*, 2022). This significant variation could be due to bee colonies areas, beekeeping methods, environmental contamination (Iwegbue *et al.*, 2015). Addition to assessing heavy metal pollution, heavy metal levels are relatively higher in urban areas than in rural areas.

Conclusion

Previous results revealed the possibility of using honey bee colony products (bee bread and bee wax) as an indicator to measure the extent of environmental pollution in the vicinity of colony areas, and that rural areas are the least polluted with heavy metals, especially cadmium and lead, under study, and are safe from the health risks of heavy metals from long-range air pollution and less than the normal limit allowed. The pollution levels of heavy metals such as lead (Pb) and cadmium (Cd) in bee bread and old, new bee wax in apiaries spread across different regions in Upper Egypt. The finding showed that samples collected from apiaries in industrial, electric power, and quick road regions contained a higher concentration of Pb and Cd than samples collected from rural villages. So, the research recommends that it is necessary to select the regions least exposed to polluting with Pb and Cd to obtain pure bee products and bee colonies healthy fitness, especially the rural villages.

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