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Impact of contaminated diet with heavy metals, cadmium and lead on the protein content and longevity of honeybee workers

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Abstract

Heavy metal toxicity is an environmental concern in all areas affected by mining, industrial, and agricultural operations. At high concentrations, heavy metals are lethal to honeybees, but little is known about how different concentrations and doses affect honeybees or whether they will consume contaminated food. We studied the effect of feeding honeybees with a contaminated sucrose solution at two concentrations of 1.5 and 5.00 ppm for both cadmium (Cd) and lead (Pb) on the protein content in the honeybee's body and their survival time. The results indicated that there was a significant difference between the control and the heavy metal contamination under examination; it led to a decrease in the percentage of total protein in the bee's body and a decrease in its survival time, and there was a significant correlation between them.

Introduction

Heavy metals are among the contaminants in the environment. Besides natural activities, almost all human activities also have the potential to contribute to producing heavy metals as side effects. Migration of these contaminants into uncontaminated areas as dust or leachates through the soil and the spreading of heavy metals containing sewage sludge are a few examples of events contributing to contamination of the ecosystems (Gaur and Adholeya, 2004).

Metals released into the environment from different sources are not biodegradable and accumulate, increasing damage in nature by changing their chemical forms and disrupting biological cycles (Perugini *et al.*, 2011). Bees are one of the pollinators that come into contact with large amounts of potentially harmful pollutants during their foraging flights to collect pollen, nectar, or water removed from flowers or reservoirs (Bargańska *et al*., 2016). While many metals can be isolated and transported to honeybees, three of the most common are cadmium (Cd), copper (Cu), and lead (Pb) (Hladun *et al.*, 2015 and Sata *et al*., 2012). In soil, cadmium is actively absorbed by plant roots and transported through plant vessels and reaches nectar and pollen, and subsequently accumulates in pollinators and honeybee products (Bogdanov, 2006 and Selesi, 2013). Lead bio-accumulates in the insect body (Mertz, 1981), and its pollution pollen, honey, and wax in the beehive (Zhou *et al.*, 2018), then bee transport with food to the larvae (Balestra *et al.*, 1992).

Metals can also bioaccumulate in larvae and adult bees, honey, beebread, propolis, and beeswax, negatively affecting all bees stages in the hive (Di *et al*., 2016 and Hladun *et al*., 2016). Elevated concentrations of heavy metals are lethal to honeybees, but little is known about sublethal effects (Di *et al*., 2016). These elements have amphoteric behavior, so their toxicity on bees also depends on their speciation (Emsley, 2003 and Roman, 2010). (Al-Naggar *et al*., 2012) small peaks for Cd, Pb, and Ni were noticed in the X-ray spectra of the wing and hindgut of the forager honeybee as well as of the mid-gut and fat body of the forager bee demonstrated small peaks for Cd, Pb, Fe, and Ni compared to the control.

Some metals are part of vitamins or enzymes, and others such as lead (Pb), do not have known physiological roles and can interfere with biological processes by interacting with macromolecules, replacing or affecting essential metals (Buchwalter *et al*., 2008 and Markert *et al*., 2015). Pb is a non-essential heavy metal, highly toxic, and very persistent in the environment. It holds the second position in the ranking of the 20 most hazardous substances (ATSDR, 2020), being the most harmful metal for the biosphere. Heavy metals are difficult to eliminate once present in soil or water and are reported to cause irreversible damage to the survival, feeding, growth, and behavior of an organism, including honeybees (Nicholson *et al.*, 2003 and Di *et al*., 2016).

The highest hive concentration for some metals including Cd, was found in the royal jelly, which feeds all bees. The independent effect of Cu, cd, is much more susceptible to either metal than larva (Burden *et al*., 2019). The LC_{50} value for foragers fed cu was over 10-fold more than larvae for Cd, and the LC_{50} value for foragers was over 200-fold more than larvae (Burden *et al*., 2019).

This study aimed to evaluate the toxic and sub-lethal effects of lead (Pb) and cadmium (Cd) on the health status of worker bees (*Apis mellifera*) during the sugar feeding of bee colonies at different concentration levels. The correlation between the concentration of lead and cadmium, the survival rate, and the percentage of protein in the worker bee's body was estimated.

Materials and methods

Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. The chemical technologies generate large volumetric sludge and increase the costs (Rakhshaee *et al*., 2009) chemical and thermal methods are both technically difficult and expensive and all of these methods can also degrade the valuable component of soils (Hinchman *et al*., 2000). The study was carried out at the apiary of the Beekeeping Research Section, Dokki, Giza. Five groups were formed: a control group with 3 bee colonies and an experimental group with 12 colonies.

All 15 colonies were made equal by the method of strength (Quantity of bees in bee nests), the quantity of capped worker brood, and the age of queens. Additionally, pollen combs were removed from all colonies. Each colony was fed sugar solution (50%) for 21 days (4 liters per colony), which was the duration of the experiment. The sugar solution fed to the experimental group contained 0,5 and 1,5 ppm Pb, 0,5 and 1,5 ppm Cd, whereas that fed to the control group did not have these additional elements. The doses of the microelements of this study were determined based on a prior study conducted under laboratory conditions in small cells (Jeliazkova *et al*., 2001). Those doses showed no negative effect on the vital activity of the worker bees or queen.

1. Longevity of honeybee workers fed on solution sugar contaminated with heavy metals:

Tests were conducted using 100 one day old workers honeybee removed from the emergency boxes and placed in special wooden cages (16 cm x 12 cm x 6 cm) with wire-screened sides and glass fronts. The workers fed with 10 ml sugar syrup (1:1) were placed into each box and the assay was carried out using 3 replicates. The boxes were incubated at 32 ^{o}C and 65% RH. along the experimental period. The feeding solution had been determined; changed daily and dead bees were counted and discarded. At the end of the experiment, bees were sacrificed, and mortality percentages were corrected according to natural mortality (Abbott, 1925 and Fukuda, 1960).

2. Total protein levels % (content) of honey bee workers fed on sugar solution contaminated with different concentrations of cadmium (Cd) or lead (Pb):

Approximately ten newly emerged workers were collected from each colony of different treatments, the workers were frozen at -70 ^{o}C until they were analyzed for protein content. The amount of total protein in newly emerged honeybee workers compared with honeybee workers from control colonies was studied.

3. Determination of crude protein content:

Dry samples of bee workers were ground to a fine powder, then 100 mg. of powder material was used for total nitrogen determination by using the micro-Kjeldahl method. The total protein content was determined in particles of honeybee workers in terms of organic nitrogen content by seems- micro keldahl method according to Loiseleur (1963).

Results and discussion

The effects of a sugary solution containing different concentrations of lead and cadmium on the percentage of protein in the body of honeybee workers. The results are shown in Table (1) and Figure (1). The control group recorded a significant increase in protein (F = 75.49 and P (0.05) = 0.000) compared to the other treatments, where the protein percentage in the bee body reached 32.41±1.31%. However, the lead concentration of 1.5 ppm recorded the lowest percentage in body protein, which was 23.33 ± 0.72%. On the other hand, no significant differences were recorded between the concentrations of lead (0.5 ppm) and cadmium (1.5, 0.5 ppm) in sugary nutritional solutions, as the percentage of protein in bee body was recorded as 26.25 ± 0.00, 25.62 ± 0.00, and 26.63 ± 0.75%, respectively.

Table (1): Impact of heavy metals, cadmium, and lead on the total protein content in all bodies of honeybee workers.

Treatments		Protein± Sd	F	P≤ 0.05	LSD $_{(0.05)}$
Pb Concentration	1.5 ppm	23.33±0.72 c	**75.49**	**0.000**	**1.22**
	0.5 ppm	26.25±0.00 b			
Cd Concentration	1.5 ppm	25.62±0.00 b			
	0.5 ppm	26.63±0.75 b			
Control		32.41±1.31 a			

Means within a column for one metal with the same letter are not significantly different.

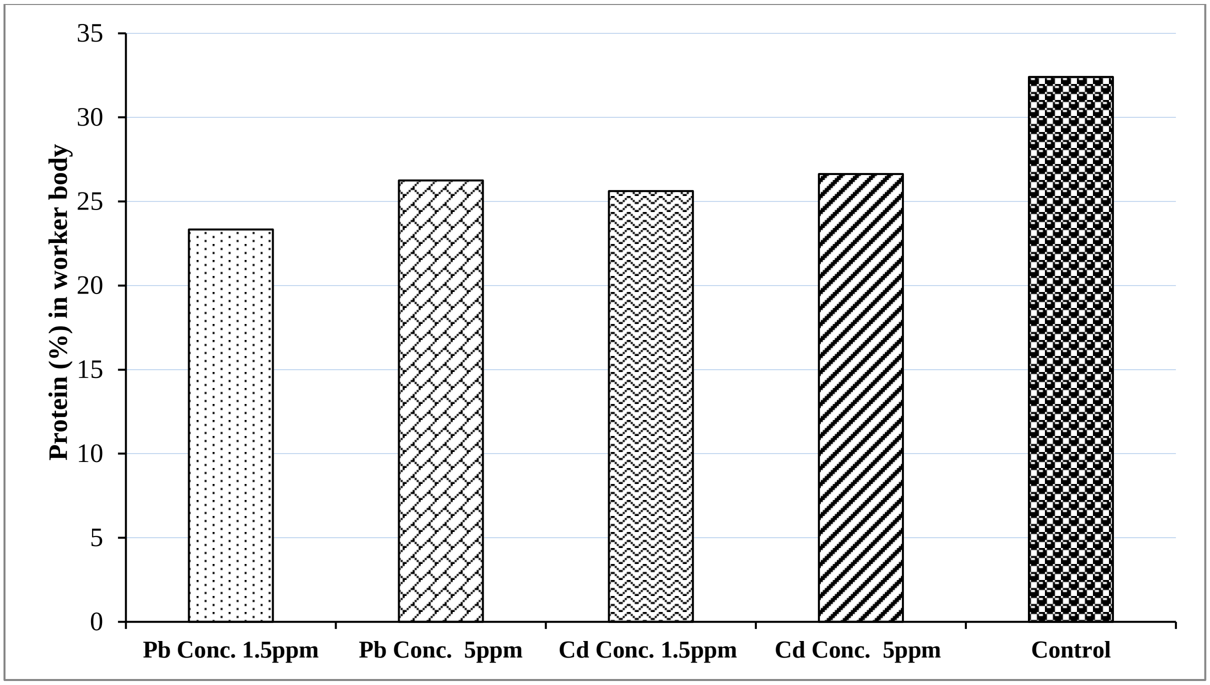

Figure (1): Illustrate sugary feeding contaminated with different concentrations of cadmium (Cd) or lead (Pb) on protein (%) in the body of honeybee workers.

Table (2) and Figure (2) observed Significant differences between the control and all concentrations of Cd and Pb treatments, F = 21.76; *P* = 0.0001, where the longevity of honeybee workers reached 18.77 ± 0.45 days; However, Cd concentration significant at 0.5 ppm and other treatments longevity was 15.83 ± 0.11 days. On the other hand, there is no significance between Pb concentration 0.5 ppm and Cd concentration 1.5 ppm recorded were 14.56±0.38 and 14.37±1.13 days respectively; while the minimum life span of the workers was 13.06 ± 0.24 days recorded with Pb concentration of 1.5 ppm.

Table (2): Impact of heavy metals cadmium and lead on the longevity of honeybee workers at different concentrations.

Treatments		Age(day)± Sd	F	P≤ 0.05	LSD (0.05)
Pb Concentration	1.5 ppm	13.06±0.24 d	21.76	0.0001***	1.27
	0.5 ppm	14.56±0.38 c			
Cd Concentration	1.5 ppm	14.37±1.13 c			
	0.5 ppm	15.83±0.11 b			
Control		18.77±0.45 a			

Means within a column for one metal with the same letter are not significantly different.

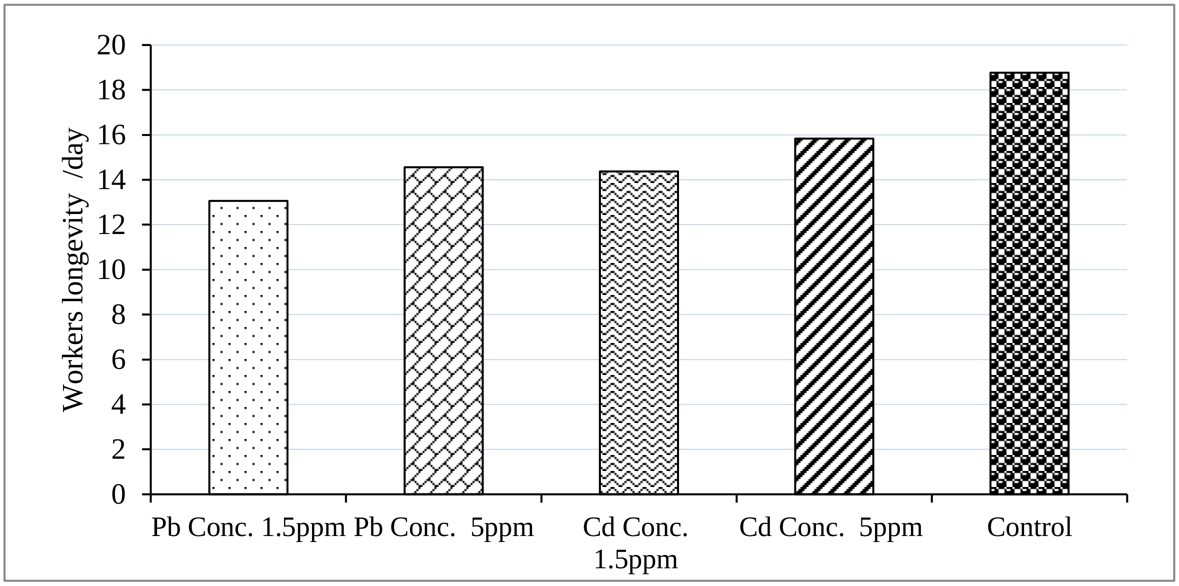

Figure (2): Illustrate of heavy metals, cadmium, and lead on the longevity of honeybee workers at different concentrations.

High concentrations of heavy metals are lethal to honeybees, but little is known about sublethal effects (Di *et al*., 2016). These elements have amphoteric behavior, so their toxicity to bees also depends on their type (Emsley, 2003 and Roman, 2010). (Zhelyazkova *et al*., 2004) feeding the bees with a sugar solution contaminated with lead, cadmium, copper, zinc, manganese, copal, and iron increased the amount of all these elements in the hemolymph.

The elements in the greatest quantity in the blood of bees are manganese, cadmium, and lead - 8 to 21.7 times more than in the hemolymph of bees fed only with sugar solutions (No additives). Gauthier *et al*. (2016) after 10-day lead exposure, the level of lipid peroxidation in honeybees was also not elevated, indicating that non-enzymatic antioxidant defense could act as protection after lead intoxication. Chronic and acute toxicity tests were conducted on larvae and foragers using solutions of 50% sucrose, which contained cadmium (Cd), copper (Cu), and lead (Pb) found that mortality increased in both larvae and foragers in a dose-dependent manner.

Control larvae had higher relative growth indices from 16 days 6 to day 10 compared to all metal treatments, demonstrating the substantial negative effects of metals on development. (Schmarsow *et al*., 2023) a single sub-lethal dose of Pb negatively affected the body proteins of bees despite the nutritional condition but did not disturb the FAs profile of the workers.

Nutrition plays an important role in preventing Pb-induced toxicity in honeybees. Engels *et al*. (1990) portend that when total protein content decreased, the manlier of deformed bees increased, in general, the relationship between worker size, protein content, and longevity, suggested large and blogger life probity, as a response, the activity of natural defense factors of the bee organism is a triggered chance in the protein composition why protect the natural protective function of the hemolymph with lysozymes, protein, hemocytes and antibiotic contained in it play an important role.

Soil can be contaminated with Pb from several other sources such as industrial sites, leaded fuels, old lead plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Contamination is long-term. Without remedial action, high soil lead levels will never return to normal (Traunfeld and Clement, 2001). In the environment, lead is known to be toxic to plants, animals, and microorganisms. Effects are generally limited to especially contaminated areas. Pb contamination in the environment exists as an insoluble form, and the toxic metals pose serious human health problems, namely, brain damage and retardation (Cho-Ruk *et al.,* 2006).

Nevertheless, a few numbers of studies have addressed the effects of lead on honeybee physiology and survival (Filipiak *et al*., 2017 and Ptaszyńska *et al*., 2018). Relationships between honeybee mortality and concentration of Cd and Cu in po4-Cd accumulated in bees to 2.87-4.23 mg/kg (Conti and Botre, 2001). In metal-polluted areas, foragers accumulated 0.05-0.06mg/kg cd (Perugini *et al.*, 2011). When hives were located in industrial areas that were rich in arsenic and Cd, honeybee hive fecundity declined (Bromenshenk *et al.,* 1991). Egyptian agriculture areas contain roared Cu and Cd concentrations of 11 and 0.41 respectively (Rashed *et al.,* 2009). Esmael *et al.* (2016) found that the degree of development of the hypopharyngeal glands of newly emerged workers fed on the experimental diets at different concentrations of cadmium showed the highest degree of development of hypopharyngeal gland was recorded in workers fed on the control diets (without heavy metals) with mean (2.86±0.06), followed by 1ppm (2.73±0.09), 3 ppm (2.73±0.28), 5 ppm (2.72±0.29) and 0.5 ppm (2.62±0.11).

The use of a high concentration of 10 ppm of cadmium decreases the degree of hypopharyngeal gland development. Determination of the longevity of worker bees: The highest longevity (19.00±0.92) was recorded in workers fed on (5 ppm cadmium) diet followed by (18.00±0.57) in 0.5 ppm, 3 ppm (17.50±0.50), control (17.00±1.08), 10 ppm (17.00±0.41) and (16.75±1.11) was recorded in bees fed on 1 ppm diets. The degree of development of the hypopharyngeal glands of newly emerged workers fed experimental diets at different concentrations of chromium. The highest mean (2.95±0.09) was recorded in workers fed on the control diet, followed by (2.75±0.06) on 1 ppm, 3 ppm (2.60±0.18),0.5 ppm (2.50±0.10), 10 ppm (2.05±0.11) and 5 ppm (1.97±0.01). The highest longevity (20.00±0.91) was recorded in workers fed on 10 ppm diet, followed by (18.50±1.04) in bees fed on the control diets, 0.5 ppm (17.50±0.29), 5 ppm (17.50±0.50), 1 ppm (17.00±0.71) and 3 ppm (16.75±1.43).

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