CHANGES in the QUANTITY of HEAVY METALS in the HAEMOLYMPH of WORKER BEES FED MICRO-ELEMENT CONTAMINATED SUGAR SOLUTION

Mikro-Element İçeren Şeker Solusyonu ile Beslenen İşçi Arıların Kanında Ağır Metallerin Miktarındaki Değişiklikler

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Abstract: The study was initiated by making 6 colonies (set in 10-frame Dadan Blatt bee-hives) equal by the method of analogues. The bees in the control group (3 colonies) were fed sugar solution without heavy metal additives, while those in the experimental group (3 colonies) were fed sugar solution containing 1,5 ppm Pb, 1,5 ppm Cd, 2,0 ppm Cu, 50,0 ppm Zn, 80,0 ppm Fe, 20,0 ppm Mn, 20,0 ppm Co. At the end of the experimental period (after the end of feeding) haemolymph samples were taken. After feeding the bees with sugar solution contaminated with Pb, Cd, Cu, Zn, Mn, Co and Fe the quantity of all of these elements in the haemolymph of workers increased. The greatest percentage changes occurred in the levels of Mn, Cd and Pb (respectively 21,7 , 17,7 and 8,0 times higher than the control group). However, Cd was among the elements in lowest concentration in the food. On the basis of these results, the authors think that the haemolymph of bees shows promise for biomonitoring heavy metals in the environment (as an accumulative indicator).

Key words: Honey bees, heavy metals, haemolymph, sugar solution, bio-indicator

Özet: Bu çalışma birbirine eşitlenmiş Dadant tipi 6 koloni üzerinde yapılmıştır. Bu kolonilerin 3'ü kontrol olup ağır metaller eklenmeden şeker solusyonu ile, deneme grubunda ise 3 koloniye 1,5 ppm Pb, 1,5 ppm Cd, 2,0 ppm Cu, 50,0 Zn, (0,0 ppm Fe, 20,0 ppm Mn, 20,0 Co içeren şeker solusyonu verilmiştir. Deneme sonucunda (besleme bittikten sonra) arıların kan örnekleri alınmıştır. İçinde Pb, Cd, Cu, Zn, Mn, ve Fe içeren şeker solusyonları ile besleme yapılan işçi arıların kanında bu elementlerin miktarında artış görülmüştür. Yüzde olarak en büyük değişiklik kontrol grubuna göre sırasıyla Mn, Cd, Pb, 21, 7, 17, ve 8,0 kez daha fazla görülmüştür. Cd aslında besinde en az konsantrasyonda olan elementler arasındadır. Bu sonuçlara dayanarak yazar, çevrede ağır metallerin seviyesinin belirlenmesinde ağır metallerin arıların kanında sürekli birikiminin gözlenmesinin bir gösterge olabileceğini önermektedir.

Anahtar Kelimeler: Bal arıları, ağır metaller, hemolimf, şeker solusyonu, biyolojik gösterge

INTRODUCTION

Presently, many countries are considering the use of honeybees to monitor environmental pollution. Those substances that potentially could be monitored in the environment by honeybees include heavy metals, pesticides, and radioactive substances. What makes the honeybee an attractive species for use as a bio-monitor is that: 1) the same species occurs throughout Europe, Russia, the Middle East and Africa, 2) the wide area of activity for the worker bee (up to 2 –3 km away from the apiary), 3) close contact of the bees with the surrounding environment, 4) sensitivity of bees to toxic substances, and 5) the possible use of bee products as indicators for environmental pollution (Bromenshek, 1986; Ravetto *et*

al., 1988; Billalov *et al*., 1992; Makarov *et al*., 1995; Porrini *et al*., 2003; Stark, 2003). This last point is important because bees and bee products can be used as accumulative (determine the chemical substances accumulated in them) and reactive (determine the effect of contaminators on brood, adult individuals, honey productivity) indicators (Billalov *et al*. 1992).

According to data presented by Grigoryan (1970, 1972), honeybee haemolymph, being an internal medium, is an unstable system, which reflects the dynamics of vital processes during the organism's different age periods. In that respect, the blood of bees reacts quickly to various external influences and thus is a suitable biological entity for determining environmental pollution.

The objective of this study was to find out the changes in the quantity of the following elements Pb, Cd, Cu, Zn, Mn, Co, Fe in the haemolymph of worker bees fed with sugar solution contaminated with known amounts of those micro-elements.

MATERIAL AND METHODS

The study was carried out at the Training and Experimental Apiary of the department of Animal Husbandry for non-ruminants and other animals at Trakia University, Stara Zagora during 1999.

Two groups were formed: a control group with 3 bee colonies and an experimental one with 3 colonies. All 6 colonies made equal by the method of analogues – the quantity of honey in bee nests, 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 15 days (4 liters per colony), which was the duration of the experiment. The sugar solution fed to the experimental group contained 1,5 ppm Pb, 1,5 ppm Cd, 2,0 ppm Cu, 50,0 ppm Zn, 80,0 ppm Fe, 20,0 ppm Co, and 20,0 ppm Mn where as that fed to the control group did not have these additional elements. The doses of the micro-elements for this study were determined on the basis of 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.

At the end of the experimental period, after feeding stopped, about 100 worker bees were taken out of each bee colony (3 samples of bees from the control group and 3 samples of bees from the experimental one) in order to take haemolymph. From the haemolymph of 100 bees from each colony a collective sample was obtained – a total of 6 collective samples, 3 from each group. The haemolymph samples were analyzed for Pb, Cd, Cu, Zn, Fe, Mn, Co content. The analyses was carried out in the laboratory for Physico-chemical analysis of products with animal origin at the National Diagnostic and Scientific Research Institute of Veterinary Medicine, Sofia. The samples were mineralized at 450 ± 30°C in a muffle furnace until they became a greyish-white ash (Bulgarian State Standard 11708/1987). The ash was diluted in 1n HCl and analyzed via an atomic absorption spectrophotometer (Perkin Elmer 3030) in an acetylene – air flame. The obtained results were statistically processed by routine computer methods and the mean values for each group (μg/ml) and the mean error were determined (x ± Sx).

RESULTS AND DISCUSSION

In the haemolymph of bees taken from the control group Fe (30,75 ± 6,15 μg/ml), Zn (9,38 ± 1,88 μg/ml), and Mn (2,41 ± 0,48 μg/ml) were in high concentrations (Table 1). Those results correspond to the findings of Goloskokov & Pimenov (1972). In contrast, the content of Cd (0,06 ± 0,01 μg/ml) and Pb (0,17 ± 0,04 μg/ml) in the blood of bees from the control group was low. This confirms that these elements do not normally comprise an integral part of the haemolymph (Shoven, 1953; Taranov, 1968; Tyshtenko, 1976, 1986).

In the haemolymph of worker bees from the experimental group, Fe (80,46 ± 11,72 μg/ml) occurred in highest concentration and Cd (0,97 ± 0,29 μg/ml) in lowest concentration (Table 1). In addition, a comparatively high quantity of the elements Zn and Mn was recorded (46,79 ± 19,56 μg/ml and 21,29 ± 5,39 μg/ml respectively).

Table 1. Content of micro-elements in the haemolymph of worker bees (μg/ml)

Elements	Groups	
	Control*	Experimental**
	x ± Sx	x ± Sx
Pb	0,17 ± 0,04	1,35 ± 0,21
Cd	0,06 ± 0,01	0,97 ± 0,29
Cu	2,41 ± 0,48	7,26 ± 1,32
Zn	9,38 ± 1,88	46,79 ± 19,56
Mn	0,98 ± 0,19	21,29 ± 5,39
Co	1,86 ± 0,37	8,35 ± 3,37
Fe	30,75 ± 6,15	80,46 ± 11,72

* - fed with pure sugar solution (with no additives)
** - fed with sugar solution contaminated with Pb, Cd, Cu, Zn, Mn, Co, Fe

The comparative analysis of the data about the content of the studied elements in the haemolymph of worker bees from the experimental and control group shows higher values in bees fed with contaminated sugar solution. The relative increase in the studied heavy metals in the experimental group compared to the control group was: Mn – 21,7 times, Cd – 17,7 times, Pb – 8 times, Zn – 4,9 times, Co – 4,5 times, Cu – 3 times and Fe – 2,6 times. Therefore, the elements Mn, Cd and Pb are deposited at the highest relative extent in the blood of bees. This data confirm and supplement the results from another study of ours (Jeliazkova *et al*., 2002). There it was also found that Cd, Mn and Pb are accumulated at the highest degree in the body of bees (whole bees) fed with sugar solution contaminated with heavy metals. However, in the earlier study a greater percentage increase occurred in Cd and a lower percentage increase occurred in Mn (respectively 38,0 and 12,9 times). According to data presented by Grigoryan (1970, 1972) in the haemolymph of bee larvae suffering from European foulbrood that had received with their feed $MnSO_4$ (2 mg/l), the number of haemocytes increased. This is a prerequisite for enhancing the resistance of the honeybee, and it has an effect on the phagocytosis activity of cells.

The low degree of deposition of Fe and Cu in the haemolymph (respectively 2,6 and 3 times more than the control group) that has been established in the present study confirms the results published in earlier work (Goloskokov & Pimenov 1972; Raes *et al*. 1992; Hsu Yuan & Chia Welli 1993; Makarov *et al*. 1995; Jeliazkova *et al*. 2002). According to those reports, these elements are accumulated in the body parts rather than haemolymph (in the chest muscles and in the trophocytes immediately under the hypoderm of the abdomen).

The analysis of the results from the study shows that the haemolymph of worker bees (as an internal medium of the bee organism) is affected by the bee's diet. In this regard we think that the haemolymph can be used for biomonitoring as an accumulative indicator of environmental heavy metal pollution. The most important elements which are to be used as indicators are Mn, Cd, Pb, Zn and Co.

CONCLUSIONS

From the studied of the microelements Pb, Cd, Cu, Zn, Mn, Co, and Fe ingested in the diet of worker bees, the highest absolute content accumulated was Fe in the haemolymph of bees and the lowest Cd and Pb. However, after feeding bees with sugar solution contaminated with Pb, Cd, Cu, Zn, Mn, Co and Fe the quantity of all of these elements in the haemolymph increased. The elements that are deposited at the greatest percentage increase in the blood of bees are Mn, Cd and Pb – from 8 to 21,7 times more compared to the haemolymph of bees that have been given pure sugar solution (with no additives). Thus the use of haemolymph of worker bees looks very promising for bio-monitoring environmental heavy metal pollution (as an accumulative indicator).

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