ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Seasonal Variation in Heavy-Metal Accumulation in Honey Bees as an Indicator of Environmental Pollution.

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ABSTRACT

Determination of contaminants in biological objects is one of the most important aspects of environmental health control. The objective of this study was to investigate heavy metals accumulation in bees at different seasons in areas with different degrees of technogenic pollution. The concentrations of six microelements in 36 honey bee samples taken from uncontaminated and contaminated environments were analyzed by atom-absorption spectrometry. The contents of heavy metals in bee samples differed significantly by season and location. Cadmium and manganese were found in higher concentrations in summer bees from contaminated apiaries than in autumn ones. On average, levels of cadmium, lead, manganese in contaminated areas exceeded those in control areas by 3.3, 4.5, 2.3 times respectively. There was no discrepancy found in concentration of heavy metals between summer and autumn bees from control apiaries. The index for evaluation of degree of technogenic contamination with used concentrations in different seasonal generations was proposed (ratio of heavy metal concentration of summer and autumn bee generations collected from the same colony). Therefore, honey bees can be used to evaluate contamination in terrestrial ecosystems.

Keywords: *Apis mellifera*, seasonal generations, bioindication, atom-absorption spectrometry

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ISSN: 0975-8585

INTRODUCTION

Studying heavy metal accumulation in living beings is important because of their functions as trace elements in nutrition, but also because they are the main inorganic components of technogenic pollution. Heavy-metal accumulation levels in living organisms, including insects, are the most important biological indicators of environmental health. Analyses of scientific reports and the results of our own studies suggest that heavy-metal pollution indexes in the bodies of western honey bees (*Apis mellifera* L.) have several advantages over other methods of assessing environmental pollution [1-3]. The broad habitat of honey bees and their close relationships with humans and agriculture makes them extremely convenient and economical study subjects.

It is a well-known fact that a number of authors recommend using beehive components other than bees, but such test objects as honey and pollen can be used to evaluate the contamination level of the ecosystem [4-6]. However, the fact that pollen samples represent a mixture gathered from various plants, honey also comes from different floral sources, makes their use as bioindicators quite debatable. The results of Grembecka and Szefer [7] confirm the possibility of classification of honeys from different botanical origins according to their mineral content. The effect of the species property factor on concentration range can be greater than technogenic effect. Raeymaekers [8], after investigating 60 honey samples could not detect expected anomalies in the samples from the industry or from residential areas. Jones [9] studied 50 honey samples and concluded that trustworthy use of honey as a monitoring tool is reduced due to low heavy metals concentrations in honey and their natural variability (differences in botanic origin, foraging range, capture of atmospheric aerosols on the flower, etc.). Heavy metals can penetrate into the honey not only from the environment, but also in contact of honeycombs with the wire [10] and metal surfaces during technological processing. However this is not relating to bioindication of ecosystem contamination issues, but to the quality control of honey as a food product.

The accumulation of heavy metals in bees depends on many factors. Besides local pollution conditions and geochemical peculiarities of a region, the content of microelements in a bee's body depends on her age and the colony's phenology (seasonality). At present, data on differences in heavy-metal accumulation among bees of different seasonal generations is rather limited, making accumulating indexes of these elements difficult to interpret. The aim of this study was to investigate the accumulation of heavy metals in bee bodies summer and autumn generations in areas with different levels of technogenic pollution.

MATERIALS AND METHODS

Honey bee samples were taken from 18 apiaries in Republic of Tatarstan, Russia (in Eastern Europe). Nine apiaries were located in unpolluted (control) regions at least 5 km from motor highways and railways and at least 30 km from large industrial centers. They regions were considered clean, although heavy metal pollution cannot be completely prevented as a result of both global pollution and long-distance (more than 5 km) air pollution from local sources (e.g., industrial works and transport). The 5 km distance was chosen because honey bees generally do not fly beyond a radius of 3 km. The other nine apiaries were near (3 km or less) heavily-used motor highways or large industrial centers.

All total, 36 samples were collected. The course of investigation was carried on the family of foraging bees of grey forest breed living in Dadant-Blatt type of beehive. Summer foraging bees were collected at the end of June, 2012, in the period of intensive foraging. Collecting of foraging bees is accomplished by shaking them off the last forage frame (with honey and beebread) located by the beehive wall in the evening after 8 p.m. It is well-known that adult bees specializing in gathering of nectar and pollen locate in the last frames of the nest. Autumn bees were collected in October from the brood nest frame. By that time there were no summer foraging bees in the hive left. On each of 18 apiaries 100 individual bees were collected from one hive (separate colonies).

Samples were collected into plastic packages using gloves, and were stored at –18°C before analyses.

Immediately before the analyses, the bees were placed in Petri dishes and dried to constant weight in a drying cabinet at 60°C for 16 h. Bees were prepared for analysis using the moist mineralization technique for invertebrates [11]. In this procedure, 1 g of dried bees were placed into a 50 ml flask with 5 ml of nitric acid (HNO_3) and 10 ml of perchloric acid ($HClO_4$), then slowly heated for 30 min at 50°C. After nitrogen oxides

ceased to be discharged, the excess nitric acid was removed by increasing the temperature to 100°C. The solution was carefully evaporated until the perchloric acid began to evaporate. The solution was then cooled, 10 ml of distilled water were added, and the mixture was filtrated through a 1–2.5 µm filter paper and brought to a final volume of 25 ml with distilled water.

Microelements in the samples were quantified by atom-absorption spectrometry with an AAnalyst-400 system (Perkin-Elmer, Waltham, MA, USA) with flame atomization. The following heavy metals were analyzed: cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). All samples were analysed in dublicate.

Standard reference material was used to test the accuracy of the method (Table 1). The absorbance signals on the same mineralized sample of bees were repeatedly (n=20) measured in order to estimate the precision of the analytical method. The relative standard deviation was in the order of 3.5 % for each analysis.

Statistical analyses were performed using STATISTICA 8.0 (Stat Soft, Tulsa, OK, USA). The full range of variation, arithmetic mean, standard deviation (SD), standard error of the mean, median and interquartile range (IQR) were calculated. The distribution of the data was checked for normality with the Shapiro-Wilk W-test. Data with normal and non-normal distributions were analyzed by non-parametric tests. Differences among samples were evaluated using the nonparametric Mann-Whitney *U*-test. Differences were considered statistically significant when $P \leq 0.05$.

Table 1: Certified and obtained values (µg g^{-1} dry weight) for the standard reference material (Grass mixture, SRM 8922-2007, Russian)

Element	Certified value mean ± 95 % C.I.	Obtained value mean ± S.D.; N = 7	Our detection limit
Fe	970±50	960±12	0.0250
Mn	0.509±0.0210	0.51±0.011	0.0057
Zn	0.236±0.0110	0.26±0.005	0.0025
Cu	0.063±0.0060	0.061±0.003	0.0112
Fe	970±50	960±12	0.0250
Mn	0.509±0.0210	0.51±0.011	0.0057

(C.I. = Confidence Interval, S.D. = Standard Deviation, BDL = below detection limit)

RESULTS AND DISCUSSION

The physiological features of honey bees must be considered when interpreting quantitative analyses of microelement content. The summer generation lives for about 6-7 weeks and consists of two main functional-age groups: intrahive workers and foragers. These groups differ in age, division of labor within the hive, food consumption, and degree of contact with atmospheric air. Our method of selecting_samples presupposed the collection of foraging bees which stay in close contact with atmospheric air. Because of their high flight activity, honey bees forage through a large territory around the hive and accumulate environmental pollutants. According to Bromenshenk et al. [12], contaminants enter the hive by different routes such as water, nectar, and pollen, collected by bees. Besides, bees may become contaminated by contact with dusty surfaces or with airborne aerosols. Attachment of the pollutants to the surface hairs or other exterior body parts may occur. Also the contaminants may reach the interior of the bee by ingestion, inhalation, or absorption through the exoskeleton. The results of Leita et al. studies [13] show a high content of zinc and cadmium on the body surface of insects as a consequence of atmospheric fallout (atmospheric fallout), whereas a lead accumulated in the body. Iron-containing granules were found in fat cells and in columnar cells of the midgut of adult worker honey bees [14]. It was shown by energy-dispersive X-ray analyses of air-dried granule preparations, that apart from iron also phosphorus, calcium, potassium, sodium, magnesium, sulfur, magnesium and zinc are always represented. As suggested by the results, function of these granules is storage of surplus ions and toxic metals. This is supported by the fact, that perorally administered lead is accumulated in both the iron rich granules and the spherocrystals [15]. Lead accumulation happens slowly in young bees which feed mainly on pollen and is equally efficient when the contamination begins at foraging bees.

The autumn generation of honey bees differs from the summer one in physiology. The amounts of dried substances, nitrogenous compounds, reserved fat, and glycogen increase in the bee bodies, while the

ISSN: 0975-8585

relative amount of water decreases. At the end of the summer, the conditions of the hypopharyngeal glands, fat bodies, and ovaries change. Thus, the autumn generation of bees had fat bodies that were 2-2.5 times more developed than in the summer generation. These physiological changes, and an increased lifetime, in autumn bees are due to better nourishment from collected pollen, contributing to nutritional reserves, as well as few or no brood to be cared for. These autumn bees do not collector convert food. In this region of Russia, physiologically-young autumn bees develop in August and September. We collected the autumn bee samples in October thus excluding the presence of foraging bees in the hive.

Table 2 summarizes the normality tests of the data distributions. Normality tests demonstrated that testing of unified selection of bees (foraging and intrahive) collected from all locations detected normal distribution of iron only. The test of samples from control locations revealed normal distribution of all elements except zinc, samples from contaminated apiaries revealed normal distribution of all elements except cadmium and iron.

Table 2: Distribution of heavy-metal content data in honey bee bodies according to the Shapiro-Wilk *W*-test

		Cd	Pb	Cu	Zn	Mn	Fe
Contaminated areas	*W*	0.7499	**0.9609**	**0.9363**	**0.9633**	**0.9110**	0.8771
	P-value	0.0003	**0.6189**	**0.2503**	**0.6672**	**0.0894**	0.0234
Control areas	*W*	**0.9551**	**0.9169**	**0.9271**	0.8814	**0.9679**	**0.9515**
	P-value	**0.5107**	**0.1140**	**0.1728**	0.0275	**0.7572**	**0.4489**
Summer samples	*W*	0.6893	**0.9290**	0.8798	**0.9086**	0.8866	**0.9500**
	P-value	0.0001	**0.1861**	0.0259	**0.0812**	0.0338	**0.4248**
Autumn samples	*W*	**0.9086**	**0.9144**	**0.9465**	0.8627	0.8426	**0.9069**
	P-value	**0.0811**	**0.1029**	**0.3730**	0.0135	0.0065	**0.0759**
All areas and samples	*W*	0.6119	0.9257	0.9394	0.9263	0.8792	**0.9581**
	P-value	0.0000	0.0186	0.0485	0.0193	0.0010	**0.1879**

Normally-distributed data are indicated in bold type.

Sample histograms of Fe- and Pb-concentration distributions are shown in Figs. 1 and 2. The results of statistical data processing are presented in the Fig. 3. In addition, nonparametric statistical test was used for subsequent analyses to identify differences among samples (among generations and locations). Data analysis indicated that microelement accumulation was dependent on season and apiary location (Tables 3, 4).

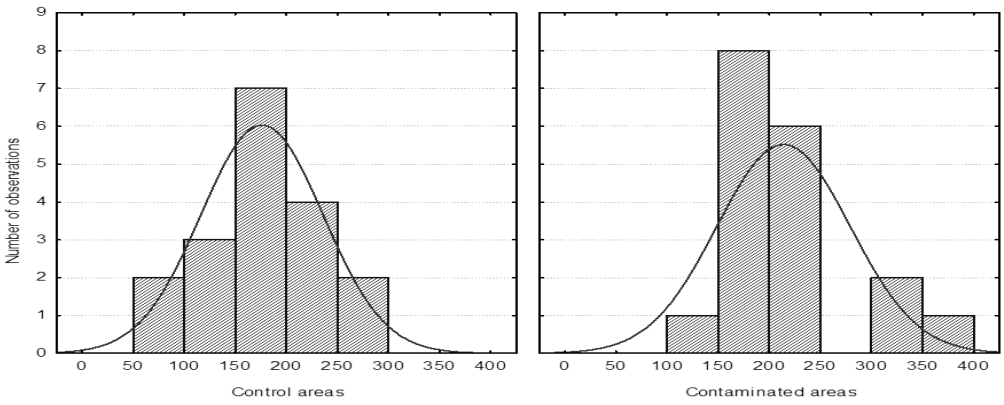

Figure 1: Histogram of iron concentrations in bees from control and contaminated areas

Table 3: Differences in metal content among bee samples of seasonal generations (summer and autumn) according to the Mann-Whitney *U*-test.

		Cd	Pb	Cu	Zn	Mn	Fe
Control areas	*U*	34.0	25.5	33.0	38.0	25.0	22.0
	P-value	0.5660	0.1853	0.5078	0.8253	0.1711	0.1024
Contaminated areas	*U*	**14.0**	18.5	25.0	28.0	**18.0**	29.0
	P-value	**0.0193**	0.0521	0.1711	0.2697	**0.0470**	0.3099
All areas	*U*	115	142	120	125	133	102
	P-value	0.1370	0.5269	0.1839	0.2418	0.3589	0.0577

Significant means are shown in bold type.

ISSN: 0975-8585

Statistical analysis of samples of different generations of bees from control apiaries did not reveal discrepancies of heavy metals in summer and autumn bees. Meanwhile, samples from contaminated apiaries displayed distinct differences in the content of two metals: cadmium (P=0.02) and manganese (P=0.05) (Table 4). Values of mean, SD, median and IQR for cadmium is higher in summer bees than autumn ones by 2.6, 3.5, 1.5, and 5 times respectively; values for manganese are 1.8, 0.9, 2.3, and 1.7 times higher. In summer bee samples from control and contaminated apiaries, statistically significant differences were detected for three metals: cadmium (P=0.0004), lead (P=0.0004) and manganese (P=0.0054) (Table 4).

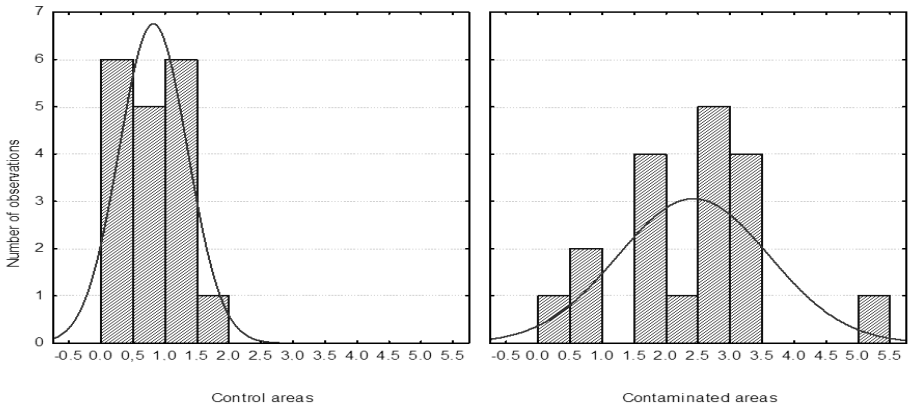

Figure 2: Histogram of lead concentrations in bees from control and contaminated areas

Values of mean, SD, median and IQR for cadmium is 3.3, 9.3,1.7, 6.3 times higher for summer bees samples from contaminated locations comparing to samples from control apiaries; those values are 4.5, 1.7, 7, 0.9 times higher for lead; and 2.3, 2.1, 3.0, 2.9 times higher for manganese. Differences were not found in autumn bees samples from different locations. Even though there were no statistically significant differences found for other elements, it should be noted that the highest values of full range of variation and likely range of variation (characterized by IQR) for all metals with the exception of iron, can be found for samples from contaminated locations (Fig. 3). Apart from technogenic factor, it may also be a consequence of geochemical anormalities but research of the causes and identification of heavy metal sources for bee samples were not the objectives of this study.

Table 4: Differences in metal content among bee samples from contaminated and control areas according to the Mann-Whitney *U*-test.

		Cd	Pb	Cu	Zn	Mn	Fe
Summer samples	*U*	**0.0**	**0.0**	26.0	21.0	**9.0**	34.0
	P-value	**0.0004**	**0.0004**	0.2004	0.0851	**0.0054**	0.5660
Autumn samples	*U*	34.0	20.0	39.0	38.0	33.0	23.0
	P-value	0.5660	0.0703	0.8946	0.8253	0.5078	0.1223
All samples	*U*	**69.0**	**33.0**	130.0	115.0	107.0	115.0
	P-value	**0.0033**	**0.0001**	0.3113	0.1370	0.0818	0.1370

Significant means are shown in bold type.

Published data on microelement content in bees of different season generations is extremely limited. Höffel and Müller [16,17] compared the contents of Pb and Cd in winter and summer bees and found no significant differences between seasons. Veleminsky et al. [18] reported that Zn, Cu, Pb, and Cd contents in winter bees were lower than in summer and autumn ones, which makes them less appropriate for the objectives of the monitoring. Van der Steen et al. [19] studied the concentration of trace metals in adult honey bee samples from three locations in the period of July–September at 2-week intervals. Temporal differences were markedly more significant than the spatial differences. The authors came to a conclusion, that the temporal fluctuations over a 3-month period are probably greater than the differences between locations.

ISSN: 0975-8585

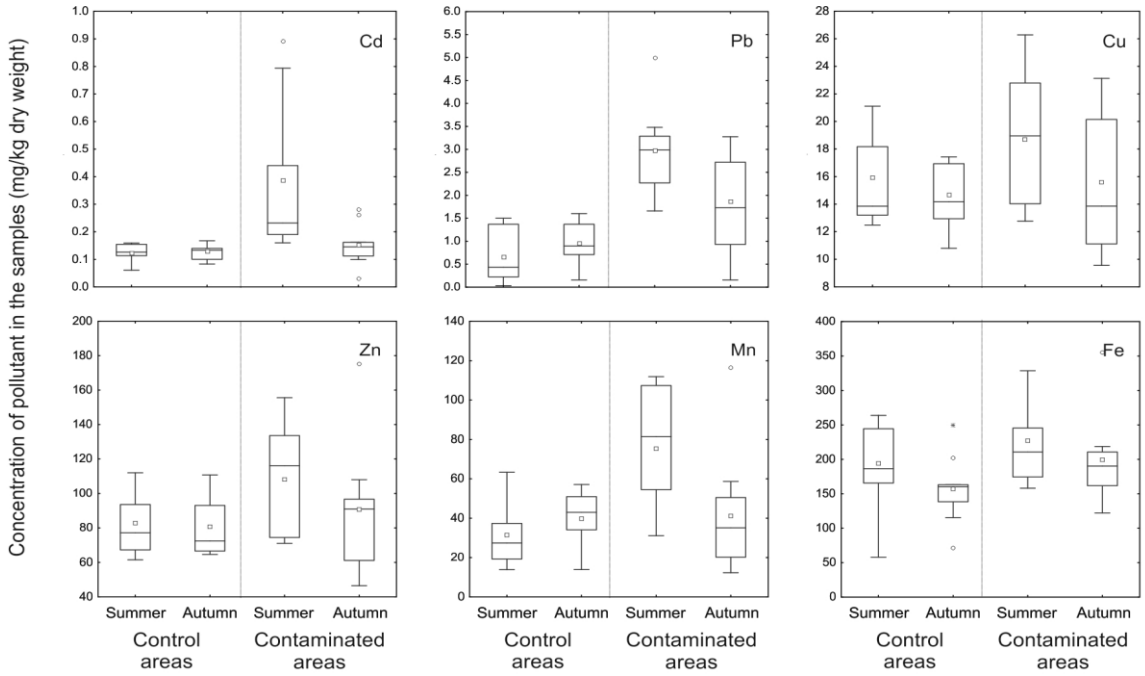

Figure 3: Box plots of concentration data for six heavy metals in the bodies of bees in contaminated and control environments in two seasons. Full range of variation (between minimum and maximum non-outliers whiskers), the likely range of variation (the IQR; box), outliers (open circles), extremes (asterisks), median (horizontal line), and mean (open square within box)

CONCLUSIONS

Since heavy metals accumulate primarily in the body's fat according to research findings, it could be supposed that their content is higher in autumn than summer bees. However this hypothesis was not confirmed. Differences in heavy-metal content in samples of different seasonal generations from control apiaries were not found. Meanwhile, for samples from contaminated areas there were found distinct differences between the content of two metals (cadmium and manganese) in summer and autumn bees. Our investigation revealed distinct excess in microelement contents in summer bees, collected from contaminated areas compared to summer ones, collected from control locations. Three of six analyzed elements (Mn, Pb, Cd) were found in higher concentrations. These differences were probably due to the intensive contact of summer-foraging bees with their environments. The findings let us make a conclusion, that the spatial differences are greater than the seasonal differences (P-values have greater significance in the first case than in the second).

Ratio of heavy metal concentration of summer and autumn bee generations collected from the same hive (colony) can be used for evaluation of ecosystem contamination levels. This index is more convenient in use than simple comparison of concentrations among the bees of the same seasonal generation collected from areas with different contamination levels since it does not depend on breed differences and geochemical peculiarities of the region. Breed factor is difficult to keep under control, which gives the proposed index the special relevance in network monitoring on large territories.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of all who contributed to this paper and, particular: Prof. Venera Z. Latypova from Kazan Federal University, who were involved in the sample preparation and the data analysis. This study was performed in Kazan Federal University and funded by the Ministry of Education and Science of Russian Federation.

Ethical Standards

The experiments conducted in this study complied with the current laws of Tatarstan, Russia, where they were conducted.

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