

HONEYBEES – BIOINDICATORS FOR ENVIRONMENTAL QUALITY

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Abstract

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The objective of the study is to use honeybees (*Apis mellifera* L.) as bioindicators for environmental status in the region of Sarnena Sredna gora, county of Stara Zagora. The study comprises six villages (Yagoda, Borilovo, Novo selo, Sulitsa, Ostra mogila, Kanchevo), located on the territory of Sarnena Sredna gora close to the military range of Zmeyovo. In each village, bee samples were taken and 56 samples of whole bees and fecal mass were prepared during the bee season of 2010. The content of copper, zinc, lead, cadmium, cobalt, nickel, manganese and iron was traced in the bee body and in excrements. The analysis was carried out in the scientific laboratory of Trakia University – Stara Zagora through atomic absorption spectrometry according to ISO 11047. The results have been statistically processed (Excel software). Maximum or relatively high values for content of the studied heavy metals and metalloids in the body and fecal mass of bees from the villages included in the study have been found, which makes it possible to comment a possible anthropogenic effect in the area of Sarnena Sredna gora, Zmeyovo military range. Higher content of the studied heavy metals and metalloids in excrements compared to the body of bees has been found, which accounts for the biobarrier function of the bee organism. The elements nickel, cadmium, cobalt and lead are accumulated at the highest degree in the bee fecal mass. The results obtained make it possible to assume that honeybees (*Apis mellifera* L.) respond to changes in their environment and in particular to increased quantities of heavy metals in soil, air, plants. That makes them a reliable indicator and allows their use in biomonitoring of the environment.

Key words: honey bees, bioindicators, environment, heavy metals

Abbreviations used: TPP – thermal power plant; EC – energy complex

Introduction

On the basis of conducted studies it is possible to make a conclusion that the bee organism reacts quickly to various external factors, which makes it possible for honey bees to be used as an accumulation and reactive indicator for biomonitoring related to environmental quality (Crane, 1984; Bilalov et al., 1992; Jeliaskova et al., 2001; Jeliaskova et al., 2002; Porrini et al., 2003; Zhelyazkova et al., 2004; Fakhimzadeh et al., 2005; Stanimirovic et al., 2005; Bianu and Nica, 2006; Gallina et al., 2006; Spodniewska and Romaniuk, 2006). For the purposes of biomonitoring the following can

be traced: development of bee colonies, changes in the behavior of bees and regular reporting of the death rate or apply comparative analysis of by indicator characteristic of bees (content of heavy metals, pesticides, radionucleotids, etc. contaminating agents in the bee organism), reared in areas with varying degree of anthropogenic impact.

In our country the topic about the bioindicator opportunities of bees and bee products is insufficiently developed. Studies have been carried out in areas with varying degree of anthropogenic impact and one-way dependence has been found the high content of some heavy metals and metalloids (copper, manganese, iron)

in the soil of villages located close to TPP of Maritsa East Energy Complex and a stone quarry and the high level of these elements in the bee body and bee products from the same villages – Zhelyazkova I., Personal communication, 2009, 2011; Zhelyazkova et al., 2008; Zhelyazkova et al., 2009a; Zhelyazkova et al., 2009b; Zhelyazkova et al., 2011a; Zhelyazkova et al., 2011b. These results can be commented as indications for anthropogenic impact on the environment.

In the past few years (from 2004) in the town of Stara Zagora and the region cases of atmospheric air and environmental pollution have been observed as a result of which quality, conditions of life and the health status of the population have been disturbed (Berberova et al., 2008; Takuchev, 2011a,b,c,d). The Program for Environmental Protection of Stara Zagora Municipality states that atmospheric air pollution in the region is characterized by specificity relating to the historical development of the economy after 1950, namely: construction of large (TPP), non-typical (military range s) and specific (small enterprises but with varied business) sources of hazardous emissions; presence of urbanized centers; growing and intensive vehicle traffic, etc. (krsz.org/upload/OPOOS_Stara_Zagora_new_2009_2013.doc). To assess atmospheric air pollution in the period 2004-2008 four environmental studies have been conducted by an accredited laboratory at the Environmental Institute “Synlab GmbH” in Stuttgart, Germany, and the initial idea of the monitoring is to establish the possible effect of activities performed at the Central artillery technical testing area of Zmeyovo. Zmeyovo Military range is located in Sarnena Sredna gora to the north-northwest of the town of Stara Zagora (distance from the town six-seven to 30-40 km). It comprises a stretch 40 km long and two-three km wide, with a total area of about 110 km². According to the classification of emission sources the military range is defined as a spatial regular source of emissions from the activities performed at it (testing ammunitions). Since direct emissions from the performed activities cannot be measured practically, the level of atmospheric air pollution from the military range can be assessed by the level of hazardous substances emissions measured on its territory or in close proximity.

During the studies conducted on the territory of Stara Zagora region (incl. the site of Zmeyovo military

range, the area of Agrobiochim EAD, some parks and territories in Stara Zagora), the impact of the found pollutants on water, soil and biological materials (plants, animals, products of animal origin, incl. bee honey, humans) has been traced (Eneva and Todorova, 2004; Berberova et al., 2008; Petkov et al., 2010). In relation to that, the existing problem concerning atmospheric air pollution in Stara Zagora region and the proved advantages of honeybees and bee products as biological material in the environmental monitoring determined the objective of this study.

The objective of the study is to use honeybees (*Apis mellifera* L.) as bioindicators for environmental status in the area of Sarnena Sredna gora, Stara Zagora region.

Material and Methods

The study was conducted in the period 2010 – 2011.

Villages from three municipalities in Stara Zagora region located on the territory of Sarnena Sredna gora close to Zmeyovo military range have been included in the sample taking. The villages from which samples have been taken correlate to the stations for intensive monitoring of a Project under a Norwegian programme for cooperation with Bulgaria of Trakia University – Stara Zagora “Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora region”.

The following villages have been included in the study:

I. Maglizh Municipality

- village of Yagoda – located 500 m from the central fire position on the military range;

II. Kazanlak Municipality

- village of Kanchevo – located to the north of station «10th km» on the military range.

III. Stara Zagora Municipality

- village of Ostra mogila and village of Sulitsa – located to the south of station «10th km» on the military range;

- village of Borilovo and village of Novo selo – located to the south of station «3rd km» from the centre for hits of munition fired from the central fire position on the military range ;

In order to fulfill the main goal, bee samples have been taken from each village (twice throughout the active bee season May - July 2010) and 56 samples from

whole bees and 56 samples from fecal mass (large intestine content) have been prepared. Each sample of bees (whole bodies) comprises an average of 150 worker bees. The fecal mass samples have been made after removing the large intestine of the bees (incl. the excrements contained therein), and each sample has been obtained from 200 bees on average.

Indicator characteristics, object of study, were the levels of heavy metals and metalloids in the bee body and in the fecal mass - copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), cobalt (Co), nickel (Ni), manganese (Mn) and iron (Fe). Sample preparation was done at the section of Bee-keeping of the department of Animal Breeding – Non-ruminants and Other Animals. The analysis was done at the scientific laboratory of the Faculty of Agriculture at Trakia University – Stara Zagora through atomic absorption spectrometry according to ISO 11047. The results have been statistically processed on a computer (Excel software).

Results

The analysis of the results obtained concerning content of the studied elements in the bee body shows:

- copper (Cu) – maximum values for quantity of the element have been reported in the body of bees in the villages of Novo selo and Kanchevo and the minimum value is for the bee samples in the village of Borilovo (Table 1).

- zinc (Zn) – the highest level of the element has been determined in bee samples from the village of Ostra mogila. Values close to the maximum ones have been reported in the body of bees from the villages of Borilovo and Kanchevo (Table 2).

- lead (Pb) – the maximum values (above 2 mg.kg⁻¹) have been reported for the bee samples from the villages of Sulitsa and Ostra mogila. For the other villages the lead content in the body of the bees analysed is within 1.6-1.8 mg.kg⁻¹ (Table 1).

- cadmium (Cd) – values from 0.10 to 0.14 mg.kg⁻¹ have been found in the body of bees from the villages of Novo selo, Ostra mogila and Sulitsa. For the bee samples from the other villages, the quantity of cadmium is below 0.1 mg.kg⁻¹ (Table 2).

- cobalt (Co) – the highest content of the element has been determined in the bee samples from the vil-

lage of Ostra mogila. Relatively high values (from 1.22 to 1.43 mg.kg⁻¹) have been reported in the body of bees from the villages of Sulitsa, Yagoda and Novo selo (Table 2).

- nickel (Ni) - the highest content of the element has been determined in the bee samples from the village of Sulitsa. Values close to the maximum ones have been reported in the body of bees from the villages of Ostra mogila and Yagoda. Minimum or close to the minimum is the content of nickel in the body of the bees from Borilovo, Kanchevo and Novo selo (Table 2).

- manganese (Mn) – the minimum average value for manganese content in the bee body has been found for the bee samples from the village of Borilovo and the maximum value – for bees in the village of Kanchevo (Table 1).

- iron (Fe) – maximum values for quantity of the element have been reported in the body of bees from the village of Ostra mogila and the minimum value – for the bee samples from the village of Borilovo (Table 1). In the other villages (Novo selo, Sulitsa, Yagoda, Kanchevo) Fe content in the bee body is below 100 mg.kg⁻¹ and it varies within the range of 90 and 98.5 mg.kg⁻¹.

For the bee samples from the village of Ostra mogila (located to the south of station «10th kilometer») maximum or close to these values for content of seven of the studied elements have been determined (Zn, Pb, Cd, Co, Ni, Mn, Fe). For the villages of Sulitsa and Kanchevo (close to station «10th kilometer») and Novo selo (located next to station «3rd kilometer») high values have been reported for four elements: Pb, Cd, Co, Ni for Sulitsa; Cu, Zn, Mn, Fe for Kanchevo; Cu, Cd, Co, Mn for Novo selo, respectively. In bee samples from the village of Borilovo high content of zinc has been found and for the ones in the village of Yagoda (close to station «Central fire site») – of iron.

For the parameter content in the bee body the studied heavy metals and metalloids can be placed in the following descending order: Fe – Zn – Mn – Cu – Pb – Co/Ni – Cd. In the present study an exception are the bee samples from the village of Kanchevo (Mn – Fe – Zn – Cu – Pb – Ni/Co – Cd) and the ones from the villages of Ostra mogila and Borilovo (Zn – Fe – Mn – Cu – Pb – Co/Ni - Cd). A reason for the stated exceptions could be the reported high levels of manganese

for the bee samples in Kanchevo and of zinc for the bee samples from Ostra mogila and Borilovo.

The analysis of the study data (Tables 1 and 2) shows that in excrements the content of the studied heavy metals and metalloids is higher than in bee bodies, i.e. accumulation in the fecal mass is established. An exception is observed concerning Zn quantity where in three of the villages included in the study (Kanchevo, Ostra mogila and Borilovo) a reverse correlation has been reported – zinc content is higher in the bee body (whole body) than in excrements. The calculated correlation

between content of the elements in the body and in the fecal mass (body:fecal mass) for the specific heavy metals and metalloids is as follows: copper (Cu) – from 1:1.3 to 1:1.85; lead (Pb) – from 1:1.94 to 1:3.48; cadmium (Cd) – from 1:1 to 1:5; cobalt (Co) – from 1:1.49 to 1:4.25; nickel (Ni) – from 1:4.44 to 1:7.28; manganese (Mn) – from 1:1.02 to 1:2.02; iron (Fe) – from 1:1.06 to 1:2.17. In relation to that, concerning degree of accumulation of the tested elements in excrements the following existing descending order can be stated: nickel-cadmium-cobalt-lead-iron-manganese-copper.

Table 1
Content of copper (Cu), zink (Zn), manganese (Mn) and iron (Fe) in body and fecal mass of bees from area of Sarnena Sredna gora, Stara Zagora region

Villages	Parameters	n	Quantity of heavy metals and metalloids,			
			(mg.kg ⁻¹) - $\bar{x} \pm S_x$			
			Cu	Zn	Mn	Fe
Ostra mogila	bee body	8	16.19±1.57	111.09±10.04	64.02±5.54	100.90±7.49
	fecal mass	8	21.16±0.48	68.44±10.26	60.00±4.69	119.11±4.14
Borilovo	bee body	9	15.75±0.94	97.87±5.43	30.69±1.43	75.74±3.56
	fecal mass	9	26.79±0.26	59.44±4.20	53.98±4.14	122.91±8.46
Novo selo	bee body	9	21.19±0.40	75.04±1.49	69.35±2.68	90.14±2.96
	fecal mass	9	39.20±3.76	139.77±7.24	104.38±3.3	195.88±19.3
Sulitsha	bee body	10	17.29±0.86	58.23±5.96	53.29±7.58	92.19±7.68
	fecal mass	10	27.17±3.18	86.50±13.02	95.39±11.9	124.57±10.3
Yagoda	bee body	10	18.91±1.03	71.08±5.10	43.43±3.02	94.58±2.71
	fecal mass	10	25.99±1.36	88.49±4.29	87.96±6.63	140.76±6.52
Kunchevo	bee body	10	20.92±1.51	97.58±7.46	113.94±7.9	98.49±4.25
	fecal mass	10	34.67±1.05	43.09±2.43	116.44±11.3	104.39±4.22

Table 2
Content of lead (Pb), cadmium (Cd), cobalt (Co) and nichel (Ni) in body and fecal mass of bees from area of Sarnena Sredna gora, Stara Zagora region

Villages	Parameters	n	Quantity of heavy metals and metalloids,			
			(mg.kg ⁻¹) - $\bar{x} \pm S_x$			
			Pb	Cd	Co	Ni
Ostra mogila	bee body	8	2.47±0.31	0.11±0.005	1.52±0.15	1.33±0.08
	fecal mass	8	4.79±0.50	0.11±0.01	2.26±0.20	6.10±0.33
Borilovo	bee body	9	1.59±0.09	0.06±0.01	0.92±0.07	0.98±0.03
	fecal mass	9	3.91±0.31	0.18±0.005	3.91±0.30	4.37±0.21
Novo selo	bee body	9	1.62±0.08	0.14±0.03	1.35±0.05	1.11±0.06
	fecal mass	9	5.02±0.39	0.32±0.02	3.60±0.26	6.72±0.26
Sulitsha	bee body	10	2.51±0.45	0.10±0.02	1.43±0.19	1.42±0.22
	fecal mass	10	8.74±2.36	0.21±0.02	5.03±0.59	6.33±0.93
Yagoda	bee body	10	1.65±0.06	0.05±0.01	1.22±0.08	1.28±0.07
	fecal mass	10	4.11±0.20	0.25±0.02	2.70±0.08	5.68±0.32
Kunchevo	bee body	10	1.78±0.05	0.07±0.003	0.88±0.05	1.08±0.04
	fecal mass	10	3.68±0.22	0.15±0.003	1.63±0.15	7.86±1.16

Discussion

The content of macro and micro elements in the body of bees varies in wide ranges and depends on a number of factors: area of bee keeping (including types of soils and nectariferous plants) and its ecological status, method of rearing bee colonies (incl. supplemental feeding), age of worker bees, physiological and health status of bee specimens and bee colonies, etc. (Table 3).

Table 3 shows the results from the studies of some authors concerning limits of variation for content of heavy metals and metalloids in the bodies of bees (Goloskokov and Pimenov, 1972; Ursu and Eremiya (1984); Fakhimzadeh and Lodenius (2000); Conti and Botre (2001); Fakhimzadeh et al. (2005); Stanimirovic et al. (2005); Bianu and Nica (2006); Spodniewska and Romaniuk (2006); Wallner K. (2010). For the region of Stara Zagora data have been included from studies if bee samples from villages in the municipalities of Pavel banya and the town of Gurkovo as mountainous (background) areas and from villages located in proximity to TPP of Maritsa East Energy Complex and to a stone quarry as areas with proven anthropogenic impact (Zhelyazkova I., Personal communication, 1999, 2009; Zhelyazkova et al. (2008); Zhelyazkova et al. (2009a, b); Zhelyazkova et al. (2011a,b). On the basis of the comparative analysis between the results of this study and the specified reference data (Tables 1, 2 and 3) it can be assumed that the values obtained in the study concerning content of heavy metals and metalloids in the body of bees (except for cadmium) refer to urban and industrial areas in Stara Zagora region. In regard to that a possible anthropogenic impact in the studied area (Sarnena Sredna gora, Zmeyovo military range) can be commented.

The data from the study reveal that maximum and relatively high values for all studied heavy metals and metalloids in bee samples have been reported for the villages located to the north and south of *station «10th kilometer»* of Zmeyovo military range – the villages of Kanchevo, Ostra mogila and Sulitsa. The results obtained in this study correspond to the results from studies concerning content of the elements Cu, Zn, Pb, Cd, Co, Ni, Mn and Fe in plants collected in that area (Berberova et al., 2008; [http:// www.uni-sz.bg/sites/userfiles/5/file/](http://www.uni-sz.bg/sites/userfiles/5/file/)

ResultsWaterSoilsPlantPoligonStZVer1.doc). According to the above studies, in plant samples from the villages close to station „10th kilometer” the content of iron, copper, zinc and manganese has minimum or relatively high values compared to other villages in proximity to the military range. High positive correlation ($r = +0.8$) concerning content of Mn in plants and soils is reported.

The established high levels of lead in the body of bees from the villages of Sulitsa and Ostra mogila (direction south of station „10th kilometer”) can be related to the quality of atmospheric air, possible transfer of metal aerosols from other regions and/or retention of pollutants in the air above both villages located in a

Table 3
Content of heavy metals and metalloids in bee bodies (references)

Heavy metals and metalloids/ Kind of regions	Quantity of heavy metals and metalloids, mg.kg ⁻¹	
	Variation limits	Including for region
Cu		
bees from clean regions	9 – 21	9 – 15.2
bees from industrial regions	13 – 27	13 – 22.5
Zn		
bees from clean regions	3 – 122.5	12 – 84
bees from industrial regions	55 – 229	22 – 113.4
Pb		
bees from clean regions	0.2 – 2.4	1.7 – 2.0
bees from industrial regions	0.3 – 15.5	1.2 – 2.1
Cd		
bees from clean regions	0.01 – 0.3	0.1 – 0.19
bees from industrial regions	0.03 – 6.2	0.06 – 0.2
Co		
bees from clean regions	0.07 – 3	1.3 – 1.5
bees from industrial regions	1.9 – 2.1	1.9 – 2.0
Ni		
bees from clean regions	0.6 - 4	0.6 – 1
bees from industrial regions	0.4 – 1.2	0.4 – 1.2
Mn		
bees from clean regions	8 – 75	17 – 45.5
bees from industrial regions	27 – 52.6	27 – 52.6
Fe		
bees from clean regions	39 – 134	39 – 96
bees from industrial regions	79 – 172.5	79 - 117

hollow. In confirmation to this is the fact that the element lead is very slowly transported from the soil through plants (Bogdanov, 2006) and this element as a pollutant is mainly in the atmospheric air. In relation to that one can assume that the defined maximum values (above 2 mg.kg^{-1}) for Pb quantity in bee samples from the villages of Sulitsa and Ostra mogila are due to surface pollution, i.e. sources of lead aerosols are inhaled by bees and stick to the hairs of their bodies. Some elements from the study of atmospheric air condition in Stara Zagora region (quantity of fine dust and content of heavy metals in it) confirm our assumptions in that direction (<http://www.uni-sz.bg/sites/userfiles/5/file/MonitoringAir2011.doc>; www.stz.riew.e-gov.bg/details.php).

The analysis of the results obtained (Tables 1 and 2) for bee samples from the villages close (in southern direction) to *station „3rd kilometer”* of Zmeyovo military range shows the following: in the body of bees from the village of Novo selo maximum values have been found for content of the elements copper and cadmium and relatively high values for cobalt and manganese; in bee samples from the village of Borilovo minimum values have been determined for the elements copper, manganese and iron and relatively low values for lead, cadmium, cobalt and nickel. The results show that in the village located closer to the station *”3rd kilometer”* (the village of Borilovo) no environmental pollution is recorded through honey bees unlike the village of Novo selo located at a greater distance from the specified station. Possible reasons about that could be the location of apiaries, the type and location of the main seasonal nectariferous plants, the direction and strength of winds, etc. The obtained high values for content of cobalt and manganese in the body of bees from the village of Novo selo correspond to the studies of soils where higher levels have been defined for these elements compared to the other controlled villages (<http://www.uni-sz.bg/sites/userfiles/5/file/ResultsWaterSoilsPlantPoligonStZVer1.doc>).

The results from the study reveal that in bee samples from the village of Yagoda located 500 m from *station „Central fire position”* of Zmeyovo military range, relatively high values have been determined only for content of the elements cobalt, nickel and iron (Tables 1 and 2). The data obtained correspond to the results

from studies of soils with the area of the above station (the village of Yagoda), according to which relatively high is the quantity of iron, chrome, copper, nickel and zinc for both soil depths ($0\div 20$ and $20\div 40$ cm). That allows it to comment a relatively high load of the soil ecosystem with the studied elements for the area in proximity to the central fire site at the military range (<http://www.uni-sz.bg/sites/userfiles/5/file/ResultsWaterSoilsPlantPoligonStZVer1.doc>). In relation to that, as a possible reason for the relatively high values obtained in the present study for content of cobalt, nickel and iron in the body of bees from the village of Yagoda could be assumed the transfer of the above elements and their compounds from the soil through the root and stems of plants to the flowers where stamens and stigma are, providing nectar and pollen for the bees.

From the data (Tables 1 and 2), it becomes clear that in the excrements of bee the content of the studied heavy metals and metalloids is higher compared to the body. The elements nickel, cadmium, cobalt and lead are accumulated to the highest extent in the fecal mass of bees. The specified results could account for the bio-barrier function of the bee organism found in a number of studies because of which toxic metals such as lead and cadmium are eliminated through the excrements and are not found in the bee honey – a basic bee product used for human consumption. The high cumulative capacity of lead in the fecal mass of bees determined in this study correlates to the findings of Goloskov and Pimenov (1972) for studies of bees (*Hymenoptera, Apidae*) and of Urbini et al. (2006) in experiments with wasps *Polistes dominulus* (*Hymenoptera, Vespidae*). Low level of accumulation in the fecal mass has been reported for copper, zinc and iron, which shows that the specified elements are deposited in other parts of the bee body and are not effectively excreted by the bee organism. Similar results have been found by Raes et al. (1992), Hsu Yuan and Chia Welli (1993), according to which Fe is deposited in special cells (trophocytes) immediately under the hypoderm of the abdomen, and Cu and Zn are accumulated in the chest muscles and the haemolymph (the bee blood).

In conclusion, the results obtained from the study reveal that honeybees (*Apis mellifera L.*) react to the changes in the environment they dwell in and can be used as bioindicators.

Conclusion

Based on the results from the study, a descending order for content of the studied heavy metals and metalloids is determined in the bee body: Fe – Zn – Mn – Cu – Pb – Co/Ni – Cd. The high levels of manganese for the body of bees in the village of Kanchevo and of zinc for bee samples from Ostra mogila and Borilovo lead to a change in the specified descending order, respectively: for bee samples from the village of Kanchevo (Mn – Fe – Zn – Cu – Pb – Ni/Co – Cd); for samples from the villages of Ostra mogila and Borilovo (Zn – Fe – Mn – Cu – Pb – Co/Ni - Cd).

The established maximum or relatively high levels for content of the studied heavy metals and metalloids (Cu, Zn, Pb, Cd, Co, Ni, Mn, Fe) in the body and the fecal mass of bees from the villages included in the study allows us to comment a possible anthropogenic impact in the area of Sarnena Sredna gora, Zmeyovo military range. A number of the specified results are confirmed by the studies of soil, air and plants within the same area.

The content of the studied heavy metals and metalloids is higher in excrements compared to the bee body, which accounts for the biobarrier function of the bee organism. The elements nickel, cadmium, cobalt and lead are accumulated to the highest extent in the bee fecal mass.

The analysis of the results obtained from the study concerning the content of heavy metals and metalloids in the body of bees from the area of Sarnena Sredna gora (Stara Zagora region) makes it possible to conclude that honey bees (*Apis mellifera L.*) react to changes in the environment they dwell in, particularly to increased quantities of heavy metals in soil, air, plants. That makes them a reliable indicator and allows us to use them in environmental biomonitoring.

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