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BIOMONITORING OF ATMOSPHERIC AEROSOL WITH THE USE OF *Apis mellifera* AND *Pleurozium schreberi*

BIOMONITORING AEROZOLU ATMOSFERYCZNEGO Z WYKORZYSTANIEM *Apis mellifera* I *Pleurozium schreberi*

Abstract: The aim of the carried out research was to assess atmospheric aerosol pollution levels in the area of three apiaries located in the Opole Province and to analyse heavy metals pollution in bee honey and western honey bees. *Pleurozium schreberi* moss was used in analysing atmospheric aerosol pollution with the active biomonitoring method, whereas heavy metals levels were determined with flame atomic absorption spectrometry method (F-AAS). Relative Accumulation Factors (*RAF*) were used in determining increases of analytes concentrations in the moss samples. As a result of the carried out study, the following conclusions have been reached: mosses are good bioindicators of environment pollution thanks to their sorption qualities, similarly to honey bees, which are a bioindicator of environment pollution. According to the Commission Regulation of European Union of 2015 regarding the maximum levels of lead in certain foods (honey), it should not exceed 0.1 mg/kg. On the basis of the carried out study it can be stated that the concentration of this analyte in the analysed honey was below the limit of quantification of the applied analytical method.

Keywords: *Pleurozium schreberi* mosses, honey, bees, heavy metals, biomonitoring

Introduction

Western honey bee (*Apis mellifera*) has been used as a biomonitor in assessment of environment quality in the field and urban studies since 1935. It reacts quickly to various external factors, including changes of atmospheric air pollution (e.g. with heavy metals), which allows to determine the equilibrium between the health condition of humans and their status and life quality [1]. It is an important tool in ecotoxicology for assessing soil, water and air pollution with, among others, heavy metals, radioactive isotopes and pesticides [2]. The pollution accumulated in a bee's organism may indicate the level of environment pollution [3]. As pollinators and collectors of nectar and pollen, they play an important role in maintaining biodiversity and productivity of ecosystems. The form a natural resource by making various products: honey, pollen, wax, propolis and royal jelly. However, due to the ever increasing various environment pollution, the number of bees in Europe has considerably decreased in Europe by 25 % since 1985 [4].

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Pollution of bees and bee products can be divided into two groups: environmental and beekeeping related. Beekeeping practices are an equally dangerous source of pollution of bee products as anthropogenic sources. The most important pollutants are the substances used by beekeepers to fight bee pests. Ecological beekeeping is an alternative method of bee pests fighting, by using minimum quantities of chemical substances. This, in turn, guarantees purity and safety of bee products, free from toxic chemical compounds [5].

If bee feed contains high quantities of heavy metals, their cell defence reactions can be impaired [6]. Study results confirm that various environmental conditions (pollution) influence productivity of colonies: honey yield, foraging behaviours, a colony survival, quality and quantity of products; additionally, these conditions considerably influence metabolic and physiological processes in bees [7]. In consequence of pollution transfer process, occurring between soil, plants, water and air, it also accumulates in bee hives. In consequence, pollution is transferred to royal jelly and, as it is fed to all larvae in the bee family - the presence of heavy metals directly influences all the colony members [8]. Many honey bees, which come into a direct contact with pollution, will not be strong enough to return to their hive and will die during flight. The number of dead bees in front of a hive (in the case of pesticide pollution) is the most important variable and indication of the environment condition. In general, heavy metals themselves do not cause deaths of honey bees, however, they may settle on bees' hairy bodies and be carried into a hive together with pollen [9, 10].

Bee products include a number of substances, such as propolis, royal jelly or pollen, which are known for their medicinal properties and their biological activity was used already in ancient period. Moreover, bee products are regarded as a potential source of natural antioxidants. Studies have been carried out on the potential use of these products in treatment of illnesses in humans [11]. For example, bee pollen has a wide array of therapeutic qualities due to a high content of nutrients and bioactive compounds. Studies on a wider pharmacological use of this product have been under way [12, 13]. The main problems with the use of bee pollen in modern medicine regard its unstable composition as well as the fact that the pollen collected by bees may contain pollutants (heavy metals, pesticides, herbicides, mycotoxins, bacteria or even antibiotics) [14].

Due to the increasing environment pollution level, heavy metals (e.g. copper, zinc, cadmium, mercury [15]) can be determined also in honey [16]. Honey contains also iron, aluminium, magnesium, manganese, chlorine, calcium, potassium and sodium [17]. The content of minerals will largely depend on the flowers, from which pollen is collected, therefore geographical location is of key importance [18]. It is recommended to place hives in the areas free from intense traffic communication routes or industrial operations [19] (bees fly intensively within the radius of up to 3 km [5], therefore they cover the area of approximately 50 km^2 [20], making up to 15 foraging flights per day [21]). Taking into consideration that the production of 1 kg of honey requires approximately 100 000 foraging flights, it becomes obvious that the substrates collected by bees for the production may contain various concentrations of analytes [22]. Some European Authorities suggest introduction of maximum residue limit (*MRL*) in honey for cadmium (0.1 mg/kg) and lead (1.0 mg/kg) [23]. Whereas no regulations considering maximum level (*ML*) for polycyclic aromatic hydrocarbons PAH were introduced [4]. Honey may be an important source of vitamins, micro- and macroelements indispensable for human health [24], however, consumption of polluted honey is particularly harmful for infants, children and youth [25].

The objective of the carried out research was to asses heavy metals pollution level of the selected area in the Opole Province, with the use of honey bees and their product - honey. The pollution level of atmospheric aerosol with the selected analytes was additionally assessed with the use of the *moss-bag* active biomonitoring method [26]. The possibility to apply alternative biomonitors, in order to determine the pollution level of the analysed environment with heavy metals, was analysed. Additionally, the possibility to consume honey from the study area was assessed, taking into consideration the pollution levels, which may occur in food products.

Material and methods

Pleurozium schreberi mosses collected in Bory Stobrawskie (Opole Province) were used in the research. The moss collected for the research was taken to the laboratory and cleaned from needles and other impurities.
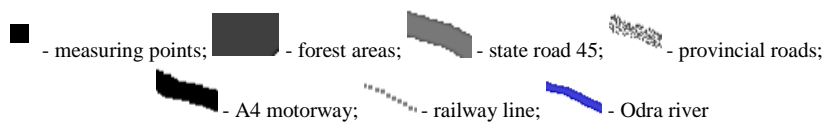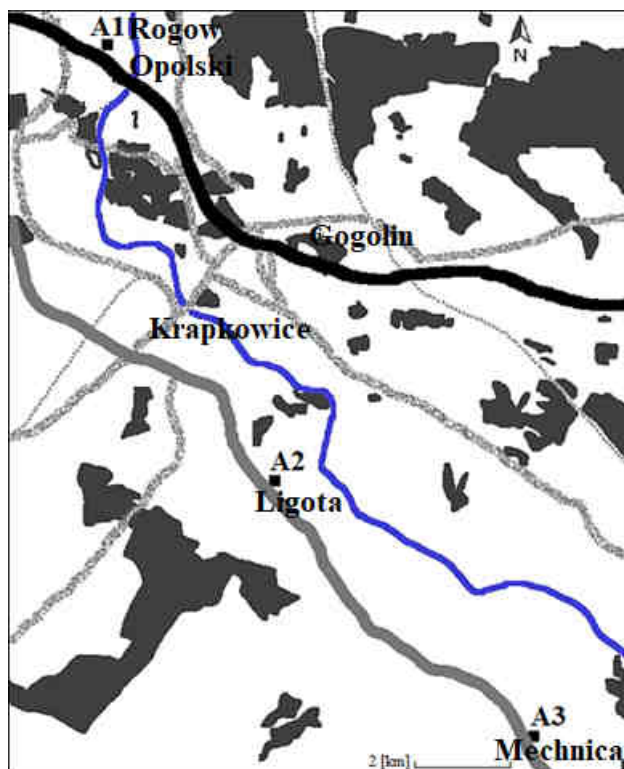
Fig. 1. Measuring points location

The green part of the moss gametophyte was retained, rinsed three times with demineralised water and dried at room temperature for 48 hours until dry mass was obtained. Next, 9 bags were prepared, each with the mass of 2.00 ±0.01 g. Such prepared moss samples were exposed at the height of approximately 1.5 m in three measuring points near every apiary. The moss exposition time was three months: July-September 2018.

The biomonitoring studies with the moss-bag method were carried out in the Opole Province, district Krapkowice. In each aviary located in: Rogow Opolski, Ligota and Mechnica, three measuring points in each were marked (Fig. 1).

Each moss sample after exposition with the mass of 0.400 ±0.001 g d.m. (d.m. - dry mass) were mineralised in the mixture of nitric acid(V) and hydrogen peroxide (HNO_3 65 % : H_2O_2 37 % = 5:3), using a Speedwave Four Berghof, DE microwave oven. Mineralisation process was carried out at temperature 180 °C.

The honey samples from apiaries, in which biomonitoring studies were carried out, was dried in a drier at temperature of 50 °C for approximately 3 weeks and mineralised in the mixture of nitric acid(V) and hydrogen peroxide (HNO_3 65 % : H_2O_2 37 % = 8 : 2) in the same digester as moss.

Bees were collected from apiaries and dried to dry mass at temperature of 80 °C and next mineralised, according to the same procedure as moss samples.

Heavy metals (Mn, Fe, Ni, Cu, Zn, Cd and Pb) were determined with the use of atomic absorption flame spectrometer (F-AAS) type iCE 3500 (series 3000) made by Thermo Scientific, USA.

Quality control

In Table 1, the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer, iCE 3500 are presented. The results were converted into 1 kg of sample. Calibration of the spectrometer was performer with a standard solution from ANALYTIKA Ltd. (CZ). The values of the highest concentrations of the models used for calibration (7.5 mg/dm^3 for Mn, 10 mg/dm^3 for Fe, 5 mg/dm^3 for Ni, Cu, Zn, Pb, 2 mg/dm^3 for Cd) were approved as linear limits for signal dependence on concentration. Concentrations of metals were determined in solution after mineralisation and dilution to were filtered into volumetric flasks of 25 cm^3.

Table 1

The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*)
for the spectrometer iCE 3500 [mg/dm^3] [27]

Metal	*IDL*	*IQL*
Mn	0.0016	0.020
Fe	0.0043	0.050
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

In Table 2, concentrations of heavy metals in certified reference materials BCR-482 lichen, produced at the Institute for Reference Materials and Measurements, Belgium, are shown.

Table 2

Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	BCR-482 lichen		AAS	
	Concentration	Uncertainty	Average	$\pm SD^*$
	[mg/kg d.m.]			
Mn	33.0	0.50	31.70	0.68
Fe	804	160	n.d.	n.d.
Ni	2.47	0.07	2.16	0.32
Cu	7.03	0.19	6.63	0.17
Zn	101	2.20	95.1	2.30
Cd	0.56	0.02	0.53	0.03
Pb	40.9	1.40	38.2	1.00

[*] - standard deviation, [**] - relative difference between the measured (c_z) and certified (c_c) concentration
100 %·$(c_z - c_c)/c_c$, n.d. - not determined

The *RAF* - Relative Accumulation Factors was used to determine increases of concentrations of the analytes in the exposed mosses samples, as defined in [28]:

$$RAF = \frac{C_{i,1} - C_{i,0}}{C_{i,0}}$$

where: $C_{i,1}$ - concentration of an analyte after exposure period [mg/kg d.m.], $C_{i,0}$ - concentration of an analyte before exposure period [mg/kg d.m.].

Results and discussion

Table 3 presents the results of heavy metal concentrations accumulated in moss, honey and bee samples.

Table 3

Heavy metals concentrations determined in moss, honey and bee samples

Sampling sites	Samples	Concentration [mg/kg d.m.]						
		Mn	Fe	Ni	Cu	Zn	Cd	Pb
A1 - Rogow Opolski	M1	309	768	3.97	9.43	37.2	< 0.81	< 4.38
	M2	308	699	3.29	10.1	36.9	< 0.81	< 4.38
	M3	321	766	< 3.13	9.83	43.6	< 0.81	< 4.38
	H	5.22	18.7	< 3.13	1.39	10.0	< 0.81	< 4.38
	HB	149	118	< 3.13	18.4	120	< 0.81	< 4.38
A2 - Ligota	M1	263	750	< 3.13	8.26	37.3	< 0.81	< 4.38
	M2	339	779	< 3.13	9.88	38.2	< 0.81	< 4.38
	M3	-	-	-	-	-	-	-
	H	4.01	20.3	< 3.13	0.86	10.0	< 0.81	< 4.38
	HB	48.9	118	< 3.13	12.0	51.8	< 0.81	< 4.38
A3 - Mechnica	M1	356	841	8.45	10.7	40.8	< 0.81	< 4.38
	M2	335	667	5.60	9.41	36.2	< 0.81	< 4.38
	M3	5.22	727	< 3.13	8.99	40.4	< 0.81	< 4.38
	H	5.12	25.5	< 3.13	1.25	10.0	< 0.81	< 4.38
	HB	62.8	160	< 3.13	14.1	72.4	< 0.81	< 4.38

A1 - apiary; M1 - moss samples; H - honey samples; HB - honeybee samples

Tables 4 and 5 present own research results compared with the data from literature.

Table 4

Heavy metals concentrations determined in honey samples in comparison to the data from literature

Analytes [mg kg⁻¹]	Current study	[24]	[15]	[20]	[29]	[30] [ppm]	[4]
Mn	4.01-5.22	0.187 ±0.18	0.022439-0.22762	n.a.	n.a.	0.044-6.31	0.079-2.428
Fe	18.7-25.5	2.85 ±0.5	0.950132-3.240654	162.31 ±49.21	0.29-0.92	10.49-80.38	< 0.012-10.054
Ni	< 3.13	0.26 ±0.2	0.011109-0.067599	n.a.	n.a.	0.664; 7.64	< 0.010-0.538
Cu	0.86-1.39	0.15 ±0.1	0.011461-0.096664	1.96 ±0.94	0.09-0.18	10.73-75.5	< 0.015-1.781
Zn	10.0	0.48 ±0.3	0.129515-1.500897	43.88 ±46.58	0.18-1.38	0.336-1.48	< 0.002-4.346
Cd	< 0.81	0.0002 ±0.0	0.002302-0.013221	0.35 ±0.38	< 0.02	n.d	< 0.002-0.009
Pb	< 4.38	0.04 ±0.09	0.00408-0.114437	0.36 ±0.39	< 0.1	n.a.	< 0.003-0.107

n.d. - not detected, n.a. - not analysed

Table 5

Heavy metals concentrations determined in honeybee samples in comparison to the data from literature

Analytes [mg kg⁻¹]	Current study	[3]	[6]	[10]	[31]	[32]	[33]
Mn	48.9-149	n.a	40.5 ±13.08	34-90	20.69-50.80	21-78	41
Fe	118-160	n.a.	177.2 ±54.20	101-421	n.a.	77-227	130
Ni	< 3.13	0.425 ±0.032; 0.358 ±0.037	n.a.	0.25-2.16	0.19-0.47	0.12-1.88	0.48
Cu	12.0-18.4	17.93 ±0.98; 12.82 ±0.92	19.4 ±6.01	11.8-29.2	11.65-19.77	11.8-29.2	21.6
Zn	51.8-120	58.11 ±4.61; 45.92 ±3.64	67.9 ±11.02	65-156	61.14-100.64	59-179	103
Cd	< 0.81	0.024 ±0.002; 0.052 ±0.006	n.a.	0.046-0.33	0.05-0.75	0.03-0.260	0.14
Pb	< 4.38	0.113 ±0.014; 0.127 ±0.017	n.a.	< DL-0.97	0.19-1.67	n.a.	0.65

n.d. - not detected, n.a. - not analysed, DL - Detection Limit

Figure 2 presents values of relative accumulation factor *RAF*, together with statistical parameters determined for the moss samples exposed in apiaries.

Based on the data presented in Table 3 it should be stated that for all three areas it was not possible to determine cadmium and lead - they were below the limit of quantification of the applied analytical F-AAS method. Additionally, one of the bags in apiary A2 was lost during the process, which reduced the number of results obtained for this measuring point. It should be noted that the samples from A1 area (in the vicinity of A4 motorway) contained higher concentrations in the case of bees than those from other locations - this could be observed for zinc or manganese. Copper concentration in bees in this location is the highest, compared to others. This could be influenced by the location of the measuring point - nearby the motorway. Other researchers also confirm that accumulation of heavy metals is related to the environment in which apiaries are located (in this case - urbanised areas). The authors enumerate such sources of analytes as the influence of industry or motor vehicles traffic on pollution of honeybees [6]. The studies carried out in urbanised areas

provide data on pollution levels but also allow for assessing the possibility of using bees in long-term monitoring of anthropogenic changes and their use as the source of information about the changing environment conditions [21].
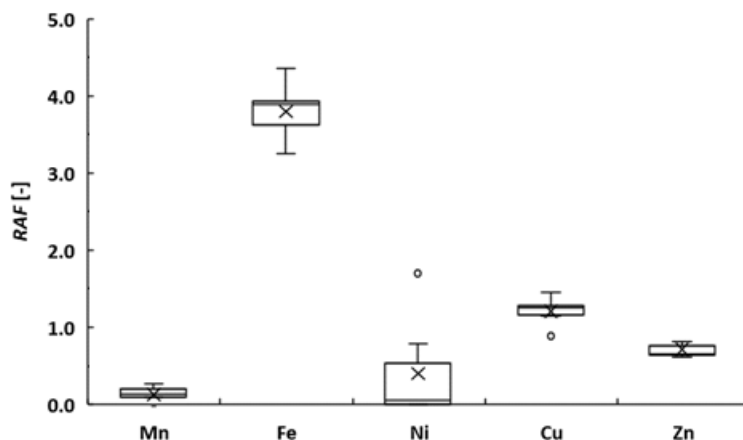

Fig. 2. *RAF* values determined for the moss samples; (x) average, (–) median, (°) outlier

The analysis of the results presented in Table 3 shows that there are no statistically relevant differences between heavy metals concentrations determined in the mosses exposed in three apiaries. The content of analytes in mosses was on a similar level for the three areas. According to the information obtained thanks to this biomonitor, the environment pollution level is similar for the studied areas. These results are confirmed also by Figure 2, which shows small increases of analytes concentrations. The mosses used in exposition were collected from the areas with high levels of heavy metals pollution [34].

The results of heavy metals content in honey were compared with the data from literature (Table 4). Concentrations of heavy metals accumulated in the honey used in the research were lower than the results presented in the quoted literature references. The limit of quantification of the used equipment is relevant in the case of such elements as cadmium or lead. The use of equipment with the appropriate low limit of quantification guarantees the possibility to determine even trace quantities of a given element [19]. In most cases, the maximum limit for lead was not exceeded (0.1 mg/kg) [35]. Some studies indicate that heavy metals pollution of honey in urban environment is unlikely, if all beekeeping good practices are followed [4]. Others demonstrate that in spite of certain heavy metals content (micropollution), honey is fit for human consumption [30].

Literature sources frequently describe studies, which refer to the results of heavy metals content in bees and honey. The research carried out in Saudi Arabia did not determine concentrations of Cd, Cr, Ni and Pb (the results were below the limit of quantification of the applied analytical method) in bee samples, interpreting it as the lack of pollution in the study area. It was emphasised that, in order to maintain this situation, standardisation and implementation of the appropriate beekeeping techniques are required [18]. It was indicated that the simultaneous use of honey and bees is more effective in environment monitoring than the use of honey only. In the research carried out in the vicinity of the power plant, concentration of cadmium was below limit of quantification, as

in our samples [17]. Other studies indicate the possibility of the use of bees and honey as effective bioindicators in assessment of environment pollution. Nevertheless, the content of heavy metals below limit of quantification in honey may be caused by the fact that bees, by accumulating toxic metals, form a natural, biological barrier, which protects honey from pollution by analytes [36].

Conclusion

Biomonitoring studies carried out in the Opole Province in three apiaries allowed to assess heavy metals pollution level of the atmospheric aerosol in which the bee hives, bees in these hives and honey produced by the bees were located.

Out of the three biosorbents, the lowest heavy metals concentrations were accumulated in honey. Monitoring with the exclusive use of honey only may be an ineffective tool in assessing environment pollution level. The honey is fir for human consumption due to low or below limit of quantification concentrations of toxic metals.

Mosses showed similar heavy metals concentrations in all three areas. The increases versus the samples prior to exposition are low, which indicates relatively low pollution of the studied area with analytes.

Bees proved to be the most sensitive biomonitor. The accumulated most pollution and some of them correlated with the apiaries location area (apiary 1 nearby the motorway). The possibility of including honey bees in the air monitoring systems should be considered. This is supported not only by their characteristics of accumulating analytes but also economically justified cost of the material availability [2]. Own studies and literature indicate that bees are a good bioindicator of environment heavy metals pollution level [37]. They offer the possibility to analyse and describe changes in toxic metals concentrations in time or define sources of such pollution [33], as well as mosses [38].

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