CONTAMINATION OF PROPOLIS USED AS A DIETARY SUPPLEMENT

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ABSTRACT
The aim of the study was to determine the extent of chosen toxic elements (zinc, copper, lead, arsenic and cadmium) bioaccumulation in propolis collected in Opole area. The present study demonstrates that propolis can be used as a dietary supplement. The research material were samples of propolis originated from 3 bee colonies in 30 apiaries (n=3x30=90). Quantitative analysis of studied elements were conducted using Varian ICP-AES plasma spectrometer with mass detection controlled by CETAC-5000 AT ultrasonic nebulizer. The presence of toxic elements was determined in an examined biological materials. The sequence of accumulation level of studied elements in propolis was as follows: Zn>Cu>Pb>As>Cd. An average concentration of zinc, copper, lead, arsenic and cadmium amounted to 56.28, 7.12, 6.91, 0.745, 0.218 mg.kg⁻¹, respectively. Only the copper average content in propolis was within acceptable standards, whereas the mean contents of other elements greatly exceed these standards. All portions of propolis should be subjected to toxicological testing before applying these samples for internal use.

Keywords: propolis, heavy metals, arsenic, cadmium, copper, lead, zinc, accumulation, dietary supplement

INTRODUCTION
The industry and motorization development as well as intensive agriculture based on the chemicalization contributed to a massive increase of environment pollution. Among many pollutants there are elements of toxic properties, which natural content in soil and the atmosphere is negligible (Bayçu and Onal 1993, Celli and Maccagnani 2003, Chlopecka et al. 1996, Jones 1987, Kabata-Pendias and Pendias 1999, Stankovska et al. 2008). They are widely used in the human economy, therefore they are common environmental pollutants. Even at low concentrations they may cause a lot of diseases and abnormalities in the functioning of the human organism.

Honey bee (Apis mellifera L.) is inextricably linked with the external environment, from which it derives not only air and water, but food as well. The products gathered by the bee colony are kind of pooled samples derived from such a large area. The pollutants occurring in a given area can also be accumulated in the raw material collected by bees, and in bees itself (Bogdanov 2006, Iannotti et al. 2000, Roman 2009, Roman 2010).

During the processing of raw materials to propolis by worker bees its purification does not take place. Propolis is an extremely complicated material which has many biologically active compounds valuable to the human organism. Most of these substances belong to the following groups flavonoids, diterpenes, phenolic acids, aromatic esters, alcohol-phenols, keto-phenols, coumarins, lipid and wax, bio-elements, vitamins, proteins and other compounds (Bankova et al. 2000, de Casstro 2001, Marcucci 1995). Propolis contains many minerals and trace elements, which concentration reflects the given region abundance with these chemical elements (Conti and Botrè 2001, Roman 1997). There is a close correlation between the level of heavy metals accumulation in soil and plants and their content in bee products (Kabata-Pendias and Pendias 1999, Roman 1997, Roša 2006, Roman 2009 and 2010). Therefore, only selected portions of propolis collected from bee are suitable for internal use by humans.

The aim of the study was to determine the accumulation degree of chosen toxic elements (arsenic, cadmium, copper, lead and zinc) in propolis collected from bee colonies in Opole area. Can propolis be used as an additive to food supplements?

MATERIAL A METHODOLOGY
The research material for the investigation were samples of propolis originated from stationary apiaries situated in Opole area. Material was collected from May-June to August 2010. Propolis samples originated from 3 bee colonies in 30 apiaries (n=3x30=90). The individual samples were combined into one pooled sample weighting about 30 g, representative for the particular apiary. Propolis was collected directly from the hives, by scraping down with a sharp instrument from their wooden elements (walls and frames) to a clean plastic containers. The received samples were homogenized by freezing, fragmentation and mixing. The 2000 mg of material from each sample was weighted (with precision of 0.1 mg) and diluted with 20 ml of concentrated, spectrally pure, nitric acid solution produced by Merck company. Next, samples were mineralized using the microwave technique at an elevated pressure in the chip-type MD-2000 station manufactured by CEM-USA. Quantitative analysis of studied elements were conducted using Varian ICP-AES plasma spectrometer with mass detection controlled by P-3202 computer cooperating with Philips Scientific analytical combine (PU-7000 model), and CETAC-5000 AT ultrasonic nebulizer. Quantitative analysis were conducted in Analytical Laboratory of Wroclaw University of Environmental and Life Science (Poland). The one-way analysis of variance (ANOVA) was performed to evaluate differences among groups. Mean concentrations of...
RESULTS AND DISCUSSION

Toxicological status of propolis fully reflects the harmful compounds and toxic elements contamination of raw materials and the environment. The concentration of some toxic elements in the propolis samples is high variability. Numerous authors draw an attention to the fact that there is a high correlation between the concentration level of minerals in bee products and their content in an external environment (Barisic et al. 2002, Bayçu and Önal 1993, Conti and Botrè 2001, Leita et al. 1996, Rashed et al. 2009, Roša 2006). Present study demonstrated that propolis was contaminated with toxic elements. This is confirmed by numerous research of other authors, which showed high concentrations of heavy metals in the propolis (Conti and Botrè 2001, Cvek et al. 2008, Kulevanova et al. 1995) also claimed that propolis is much more contaminated with heavy metals than any other bee products. The study results are presented in Table 1. Very high level in this product was observed in the case of zinc, which averaged 55.79 mg·kg⁻¹ and in individual samples ranged from 10.91 to 115.22 mg·kg⁻¹.

The content of this element observed by Dogan et al. (2006) in propolis samples from different regions of Turkey was 176-676 mg·kg⁻¹. Cvek et al. (2008) demonstrated even the value of 9326 mg·kg⁻¹. In turn, the maximum copper content was determined as 18.32 mg·kg⁻¹, and the average was 8.94 mg·kg⁻¹. Roman (1997) observed several times higher concentration of that metal in the propolis from the region of Głogów which ranged from 23.51 to 34.15 mg·kg⁻¹, while Dogan et al. (2006) received higher value in the propolis from different regions of Turkey - an average of i.e. 45-96 mg Cu·kg⁻¹. Lead is one of the most burdensome heavy metals in the environment. Its concentration was also high in propolis and reached an average level of 6.54 mg·kg⁻¹ (maximum 18.29 mg·kg⁻¹). In other studies, Roman (2000) showed the mean level of lead concentration amounted to 18.39 mg Pb·kg⁻¹ in the propolis from Głogów and from Rudna area from 6.73-17.83 mg·kg⁻¹ on average (Roman 2000). Similar results were obtained when examining the mean concentration of this element in propolis from the region of Opole (6.62-13.63 mg·kg⁻¹). It can be thus concluded that lead concentration in propolis was high and relatively stable. The present study demonstrated that arsenic content in propolis amounted to 0.698 mg·kg⁻¹ on average, and in individual samples ranged from 0.007 to 1.806 mg·kg⁻¹. The average concentration of arsenic should be considered as a very high in the propolis. Very similar results were shown by Roman (1997) in propolis originating from the Opole and LGOM area, i.e. 0.561 and 0.670 mg·kg⁻¹, respectively. This is most likely the result of a significant contamination of soils in the areas of metallurgical and chemical industries and extensive urban areas, where the accumulation of arsenic in the soil reaches up to 2500 mg·kg⁻¹ (Kulevanova et al. 1995). Generally, the cadmium concentration in the examined bee product was the smallest and was an average of 0.203 mg·kg⁻¹. However, the dispersion of results was very wide from 0.006 to 0.811 mg·kg⁻¹. In earlier studies conducted by Roman (1997), lower levels of cadmium, ranged from 0.043 to 0.116 mg·kg⁻¹, were observed in propolis originating from the region of the copper industry, but significantly higher in the product coming from the region of the cement industry - an average of 0.513 to 0.795 mg·kg⁻¹. Moreover, Roman (2000) noted higher cadmium content in the propolis from the Walbrzych region (0.260 mg·kg⁻¹).

The results of the present study and review of other authors results allow to conclude unequivocally that the level of toxic trace elements concentration in propolis depends on the state of environmental pollution in the area of the material sampling.

Only the copper average content in propolis did not exceed the maximum allowable standards (Polish Standard) (PN 1998), which is set at 10 mg·kg⁻¹. In case of other elements mean values were significantly exceeded. It demonstrates that bee products show significant contamination level. Therefore, only selected portions of propolis can be used to produce natural medicines and dietary supplements for humans. Each lot of propolis intended for that purpose must be subjected to toxicological analysis.

CONCLUSION

In present study propolis has shown high contamination level by toxic elements. Zinc, lead and arsenic showed the highest level of concentration of studied elements in relation to current standards. The sequence of accumulation level of studied elements in propolis was as follows: Zn >> Cu > Pb > As > Cd.

All portions of propolis should be subjected to toxicological testing before applying these samples for internal use as a dietary supplement.

Table 1 Concentration of chosen elements in bee propolis (N=90)

| No. of sample | Chemical elements (in mg·kg⁻¹) |
|---------------|-------------------------------|
|               | As   | Cd   | Cu  | Pb   | Zn   |
| Minimum       | 0.007| 0.006| 1.09| 0.39 | 10.91|
| Maximum       | 1.806| 0.811| 18.32| 18.29| 115.22|
| Average       | 0.698 A | 0.203 B | 8.94 C | 6.54 D | 55.79 E |
| SD            | 0.577| 0.184| 4.89| 4.51 | 33.21 |
| Variation coefficient in % | 81.7 | 90.6 | 54.7 | 69.0 | 59.5 |
| LOQ           | ≤0.001| ≤0.01| ≤0.01| ≤0.100| ≤0.001 |

A, B, C, D, E - differences between the elements assessed highly significant on a level of p<0.01
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