Research Article

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As, Cr, Cd, and Pb in Bee Products from a Polish Industrialized Region

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Abstract: Bee pollen and bee bread from stationary apiaries in the southwest Polish Legnica-Glogow copper district (LGOM) were analyzed for Cr, Pb, Cd and As by ICP-AES. Their concentrations in both products were As > Cr > Pb > Cd. Concentrations in bee pollen were higher than in bee bread. Average Cr, Pb, As and Cd concentrations in bee products were 0.138, 0.093, 0.325, and 0.019 mg kg⁻¹, respectively. Chromium was the most problematic element in bee pollen because its concentration limit was exceeded in more than 50% of the samples. Differences in Cd level between bee pollen and bread were significant (p < 0.05)

Keywords: bee bread, bee pollen, toxic metals, industrialized region

1 Introduction

Industry, road traffic, and agriculture have increased environmental pollution [1,2]. High Cr, Pb, As, and Cd concentrations – metals widely used in industry - cause many types of disease. In the environment these metals may occur at greater than the maximum allowable concentrations. This is important because they have been detected in pollen, plant nectar, and water – the raw material collected by honeybees. The metals then accumulate in bee pollen and bee bread which are consumed for api-therapeutical purposes.

2 Experimental procedure

2.1 Material

The research material consisted of bee pollen (pollen loads) and bee bread from stationary apiaries in the Legnica-Glogow copper district (LGOM). LGOM is an industrial area in Lower Silesia consisting of five counties with ~0.5 million inhabitants. It is the main center of the copper industry in Poland and one of the largest in the world.

Samples were collected from June to September 2012. The study included 25 apiaries (N=25). Both products were collected from 3 colonies in each apiary (bee pollen – n = 3 × 25 = 75, bee bread – n = 3 × 25 = 75). Bee bread was collected directly from honeycombs using a ceramic knife to avoid contamination. Pollen was collected directly from the hive using bee pollen traps. Individual samples were combined into one pooled bee pollen or bee bread sample per apiary.

2.2 Methods

All samples were dried at 120°C, homogenized in an A-11 laboratory mill (IKA GMBH, Germany) and digested in spectrally pure concentrated nitric acid (Merck, Darmstadt, Germany) using an MD-2000 digestion system (CEM, USA). They were then cooled and diluted to final volume with Milli-Q™ water. Quantitative ICP-AES analyses for Cr, Pb, As and Cd were performed on a PU-7000 spectrometer (Phillips-Unicam, Cambridge, U.K.)
with a 5000uAT ultrasonic nebulizer (CETAC, USA) [11]. The limit of detection (LOD) was 1 ppb.

2.3 Data analysis

Statistical analysis was performed using SAS 9.1.3 software (SAS Institute, 2004). Because the Shapiro-Wilk test showed the distributions were not normal, Kruskal-Wallis and Wilcox tests were applied to verify the significance of differences between metal levels in bee pollen and bee bread. The Spearman rank correlation coefficient was used to verify correlations among element concentrations.

3 Results and Discussion

Bee pollen (pollen loads) contained the highest concentrations of As, Cr, Cd, and Pb (Tables 1, 3). Others have also found elevated toxic metals in bee pollen [12-15], but there have been few publications dealing with bee bread contamination. Our results showed that in both bee products As was present in highest concentration. Although more was found in bee bread (avg. 0.325 mg kg$^{-1}$) than in pollen (0.290 mg kg$^{-1}$), the differences were not significant (Tables 1, 2). Both averages exceed the allowable 0.20 mg kg$^{-1}$ [16,17]. Much lower As content was observed by Roman [18] in pollen loads from two different industrial regions. Similarly, Falco et al. [19] found a mean As content of 0.04 mg kg$^{-1}$ in Polish pollen, with a maximum of 0.19 mg kg$^{-1}$.

The average pollen Cr level was 0.230 mg kg$^{-1}$ (Table 1), above the permissible limit (0.14 mg kg$^{-1}$) [16,17]. More than 50% of bee pollen samples contained excessive Cr. There was less in bee bread (0.138 mg kg$^{-1}$). Values ranged from 0.038 to 0.251 mg kg$^{-1}$; the limit was exceeded in 40% of the samples (Table 3). Differences between Cr concentrations in the bee products were not significant (Table 3). Conti and Bortè [13] found less Cr in all bee pollen samples from areas of varied contamination. Morgano et al. [20] observed varied Cr levels in samples from southeastern Brazil, depending on collection time. They found higher levels of Pb (0.147 mg kg$^{-1}$) and Cd (0.033 mg kg$^{-1}$) in bee loads.

The Pb concentration in bee pollen was higher than in bee bread. The pollen maximum was 0.341 mg kg$^{-1}$ and the average 0.147 mg kg$^{-1}$. The mean in bee bread was 0.093 mg kg$^{-1}$ (Table 3). The highest content in either product did not exceed the allowed maximum of 0.50 mg kg$^{-1}$ [16,17]. Statistical analysis showed no significant differences in lead concentration between pollen and bread (Table 3). Our results for bee bread were lower than those of Madras-Majewska et al. [21], and our results for bee loads were lower than those reported by Szczęsna et al. [5] (0.45–0.98 mg kg$^{-1}$).

Generally, Cd occurred at the lowest concentration. Its mean was higher in bee pollen (0.033 mg kg$^{-1}$) with

### Table 1: Toxic elements in bee pollen loads from Lower Silesia.

| Apiary | Concentration (mg kg$^{-1}$) |
|--------|------------------------------|
|        | Cr  | Pb  | Cd  | As  |
| 1      | 0.087 | 0.025 | 0.015 | 0.142 |
| 2      | 0.127 | 0.101 | 0.020 | 0.097 |
| 3      | 0.115 | 0.025 | 0.020 | 0.225 |
| 4      | 0.079 | 0.284 | 0.090 | 0.275 |
| 5      | 0.107 | 0.250 | 0.001 | 0.475 |
| 6      | 0.407 | 0.327 | 0.066 | 0.758 |
| 7      | 0.095 | 0.025 | 0.001 | 0.245 |
| 8      | 0.110 | 0.043 | 0.003 | 0.312 |
| 9      | 0.945 | 0.25  | 0.047 | 0.135 |
| 10     | 0.227 | 0.142 | 0.031 | 0.299 |
| 11     | 0.613 | 0.321 | 0.009 | 0.523 |
| 12     | 0.089 | 0.032 | 0.024 | 0.121 |
| 13     | 0.265 | 0.103 | 0.056 | 0.228 |
| 14     | 0.078 | 0.219 | 0.072 | 0.107 |
| 15     | 0.131 | 0.022 | 0.095 | 0.782 |
| 16     | 0.439 | 0.128 | 0.012 | 0.126 |
| 17     | 0.099 | 0.113 | 0.092 | 0.252 |
| 18     | 0.176 | 0.038 | 0.023 | 0.199 |
| 19     | 0.312 | 0.341 | 0.011 | 0.338 |
| 20     | 0.145 | 0.181 | 0.007 | 0.089 |
| 21     | 0.201 | 0.218 | 0.003 | 0.225 |
| 22     | 0.236 | 0.066 | 0.029 | 0.111 |
| 23     | 0.184 | 0.169 | 0.044 | 0.406 |
| 24     | 0.155 | 0.098 | 0.021 | 0.377 |
| 25     | 0.334 | 0.159 | 0.030 | 0.392 |
| Median | 0.155 | 0.128 | 0.023 | 0.245 |
| Mean ± SD | 0.230 ± 0.147 ± 0.033 ± 0.290 ± |
|          | 0.200 ± 0.105 ± 0.030 ± 0.188 ± |

SD – standard deviation
$a,b$ – differences between the elements significant at $p < 0.05$
a maximum about 3-fold higher than the average (0.095 mg kg\(^{-1}\)). In bee bread the average was 0.019 mg kg\(^{-1}\) (Table 3). The levels in pollen and bee bread differed significantly (Table 3). Cd in bee pollen from most controlled apiaries has been found to exceed acceptable levels [22-24]. Our results agree; Cd exceeded the standards (0.05 mg kg\(^{-1}\)) by 8% and 20% for bee bread and bee loads.

| Apiary | Concentration (mg kg\(^{-1}\)) |
|--------|-------------------------------|
|        | Cr   | Pb   | Cd   | As   |
| 1      | 0.061| 0.025| 0.001| 0.062|
| 2      | 0.110| 0.029| 0.009| 0.066|
| 3      | 0.157| 0.103| 0.001| 0.061|
| 4      | 0.251| 0.198| 0.045| 0.062|
| 5      | 0.126| 0.025| 0.010| 0.625|
| 6      | 0.127| 0.137| 0.001| 0.627|
| 7      | 0.142| 0.129| 0.017| 0.892|
| 8      | 0.078| 0.029| 0.012| 0.198|
| 9      | 0.129| 0.101| 0.008| 0.081|
| 10     | 0.216| 0.154| 0.034| 0.242|
| 11     | 0.038| 0.122| 0.003| 0.033|
| 12     | 0.132| 0.104| 0.077| 0.012|
| 13     | 0.098| 0.118| 0.003| 0.554|
| 14     | 0.202| 0.177| 0.021| 0.085|
| 15     | 0.084| 0.099| 0.026| 0.191|
| 16     | 0.221| 0.083| 0.005| 0.332|
| 17     | 0.179| 0.102| 0.012| 0.667|
| 18     | 0.138| 0.091| 0.002| 0.839|
| 19     | 0.125| 0.032| 0.065| 0.062|
| 20     | 0.219| 0.047| 0.001| 0.049|
| 21     | 0.078| 0.028| 0.080| 0.731|
| 22     | 0.133| 0.094| 0.001| 0.338|
| 23     | 0.152| 0.130| 0.009| 0.672|
| 24     | 0.094| 0.065| 0.001| 0.342|
| 25     | 0.148| 0.103| 0.031| 0.299|
| Median | 0.132| 0.101| 0.009| 0.242|
| Mean ± SD | 0.138 ± 0.054 | 0.093 ± 0.024 | 0.325 ± 0.288 |

SD – standard deviation

\(a,b\) – differences between the elements significant at \(p \leq 0.05\)

\(A,B,C\) – differences between the elements significant at \(p \leq 0.01\)

4 Conclusion

Arsenic and chromium are the most problematic toxic elements in bee pollen and bee bread, since these elements’ mean concentrations exceeded the limits. The concentrations were As > Cr > Pb > Cd. Significant interelement correlations were found for Cr and Pb in both bee products (Table 4). Most metal levels were higher in pollen loads than in bee bread. Metal loss may occur during processing pollen loads to bee bread.

| Apiary | Concentration (mg kg\(^{-1}\)) |
|--------|-------------------------------|
|        | Cr   | Pb   | Cd   | As   |
| Pollen loads | Minimum | 0.078 | 0.022 | 0.001 | 0.089 |
|           | Median | 0.155 | 0.128 | 0.023 | 0.245 |
|           | Maximum | 0.945 | 0.341 | 0.095 | 0.782 |
| Bee bread | Minimum | 0.038 | 0.025 | 0.001 | 0.012 |
|           | Median | 0.132 | 0.101 | 0.090 | 0.242 |
|           | Maximum | 0.251 | 0.198 | 0.08  | 0.892 |
| Kruskal-Wallis test | p-value | 0.1277 | 0.1092 | 0.0365 | 0.5802 |
| Wilcoxon test | p-value | 0.1301 | 0.1114 | 0.0374 | 0.5869 |

Table 4: Correlation coefficients (r) between element concentrations.

| Elements | Correlation coefficient (r) |
|----------|-----------------------------|
| pollen loads | bee bread |
| Cr-Pb | 0.402 \(p=0.0464\) | 0.487 \(p=0.0134\) |
| Cr-Cd | -0.004 | 0.089 |
| Cr-As | 0.226 | 0.025 |
| Pb-Cd | 0.043 | 0.163 |
| Pb-As | 0.228 | 0.006 |
| Cd-As | 0.106 | -0.014 |

However, Szczęsna et al. [5] found 0.032 to 0.154 mg kg\(^{-1}\) Cd in pollen; only 9% of their samples exceeded the accepted limit.
As bee products are considered beneficial supplements, consumers may consider their contamination important. Additionally, these products are valuable indicators of environmental pollution.

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