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**Original article**

# Honey resistance to air contamination with arsenic from a copper processing plant

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**Summary** — In the region surrounding the Bor smelter plant (East Serbia region), poisoning and mass killing of bee colonies by arsenic exhaust gas occur periodically. Analyzed honey, both from poisoned and healthy honeybee colonies, did not contain increased quantities of arsenic, while pollen and bees at the same time had exceptionally high concentrations. From a toxicological point of view, honey was not contaminated as a food but that cannot be said for pollen.

**honey — pollution — arsenic — *Apis mellifera* — biological indicator**

**Résumé — Résistance du miel à la pollution de l'air par l'arsenic provenant d'une usine de traitement du cuivre.** *Dans la région qui entoure la fonderie de Bor (dans l'est de la Serbie) ont lieu périodiquement des intoxications et des mortalités massives de colonies d'abeilles. On a analysé du miel prélevé dans des colonies intoxiquées et dans des colonies saines. Il ne présentait pas de teneurs en arsenic plus élevées (Tableaux I et II), alors que dans le même temps le pollen et les abeilles avaient des concentrations exceptionnelles (Tableau III). D'un point de vue toxicologique le miel n'est pas un aliment contaminé, mais on ne peut en dire autant du pollen.*

***miel — pollution — arsenic* — Apis mellifica — *indicateur biologique***

**Zusammenfassung — Honigresistenz gegen Luftverschmutzung mit Arsen durch eine kupferverarbeitende Fabrik.** *In der Umgebung einer Borschmelzhütte (in Ost-Serbien) kommt es regelmäßig zu Vergiftungen und Massensterben bei Bienenvölkern aufgrund von arsenhaltigen Abgasen.*
*Analysierter Honig von vergifteten und gesunden Bienenvölkern enthielt jedoch keine erhöhten Mengen an Arsen (Tabelle I und II) während Pollen und Bienen zur gleichen Zeit eine außergewöhnlich hohe Konzentration aufwiesen (Tabelle III).*
*Aufgrund dieser Ergebnisse wird geschlossen, daß Honig als Nahrungsmittel aus toxikologischer Sicht nicht kontaminiert ist, was jedoch von Pollen nicht behauptet werden kann.*

***Honig — Luftverschmutzung — Arsen* — Apis mellifera — *Bioindikator***

## Introduction

In the area surrounding a copper smelting plant (East Serbia region), poisoning and killing of honey bees occur periodically, due to the contamination of air with exhaust smoke. In certain directions and distances from the plant, mass killing and extermination of a large number of honeybee colonies often occur during the period of intensive nectar flow. The cause of this killing is arsenic emitted from Bor smeltery (Terzić and Terzić, 1983; Terzić *et al.*, 1984).

This area, in which intermittent poisoning of bees and bee colonies occurs, is extremely rich with bee pastures. It is probably due to this fact fact, following every massive poisoning of honeybee colonies, apiaries are replenished quickly.

In this report, we present the results of tests of arsenic content in honey and pollen for hives exterminated by arsenic poisoning, hives not poisoned in areas endangered by contamination and hives from uncontaminated areas.

## Materials and Methods

Samples of honey, pollen and worker bees were collected from three areas : 1) from hives in areas contaminated with arsenic in which honeybees had been killed; 2) from hives in apiaries which were situated near the endangered area, but in which poisoning never happened; 3) from apiaries outside the range of potential air contamination from the copper processing plant.

Samples were collected in two basic ways : 1) material (honey, pollen and bees) was collected from 22 apiaries normally located in the copper smelting district, during the period of poisoning and mass killing of bee colonies; 2) material (honey, pollen and bees) was collected from single hives specially placed at various distances (one hive at each distance) downwind from the source of contamination : 11, 16 and 22 kms (area 1), 31 kms (area 2) and 170 kms (area 3).

During the honeybee active flight season, samples were taken approximately once a month. Material was taken by shaking live bees from combs into plastic bags and killing them with ether. Samples were kept at room temperature. Honey samples were taken by cutting out pieces of comb and squeezing the honey. Pollen was collected from cells.

Chemical determination of arsenic in honey, pollen and bees was performed using a spectrophotometric determination method for micro quantities of arsenic in biological materials (Terzić *et al.*, 1982). Sensitivity of the method is 0.1 µg. Sample size was 10 g of raw material. The samples were mineralized by wet digestion using strong mineral acids ($H_2SO_4$ (4 ml); $HNO_3$ (40 ml); $HClO_4$ (5 ml)). Arsenic in the digest was determined by the principle of arsine liberation by adding reagent KJ, $SnCl_2$ and granulated zinc, and by introducing released arsine into absorbtion dilution (silver diethyldithiocarbamate and L-ephedrine in chloroform). The resulting red—violet colored complex (arsenic diethyldithiocarbamate) can be read on a spectrophotometer (at 520 nm). Arsenic content was given in mg/kg to raw (wet) weight.

## Results

The arsenic content in samples of honey from 22 apiaries in the area affected by poisoning and excess killing in 1978 ranged from 0.028 to 0.067 mg/kg, and the average value was 0.037 mg/kg (Table I).

Honeybee colonies placed in the same area of contamination and excess killing contained 0.020—0.053 mg/kg of arsenic, with the average value for honey of 0.032 mg/kg. All colonies from which samples were taken in this area were destroyed in later mass losses due to the arsenic poisoning from exhaust smoke.

Honey samples taken from localities which were situated at the edge of the

endangered zone contained 0.027—0.036 mg/kg of arsenic, with the average value of 0.030 mg/kg. Mass poisoning of bees never occurred in this zone.

In the honey samples taken from hives in apiaries 170 kms away (Stražilovo, Fruška Gora), the arsenic content ranged from 0.014 to 0.018 mg/kg, with the average value 0.015 mg/kg (Table II).

The arsenic content in pollen, poisoned bees and bees killed by poisoning from exhaust smokes in the closer and more distant areas surrounding the copper smeltery ranged from 1.090 to 9.640 mg/kg (Terzic *et al.*, 1984), while the arsenic content in pollen and bees from the control group from Strazilovo, Fruska Gora, ranged from 0.020 to 0.083 mg/kg.

## Discussion

The arsenic content in honey is considerably lower than that in pollen and bees from all apiaries in the endangered zone, which agrees with results obtained in the United States (Bromenshenk, 1985).

By comparing the average arsenic content in honey from the control group (Stražilovo, Fruška Gora) with that from apiaries in other regions (0.015—0.037; 0.030—0.032 mg/kg), it was noticed that, from the toxicological point of view, these quantities are negligible. Considering that the maximum allowed quantities of arsenic in food range up to 1.40 mg/kg (Dreisbach, 1977), then the honey from

**Table I.** Arsenic in honey from 22 different apiaries after mass lethal bee poisoning.

| *Locality and distances from the source of contamination* | *Arsenic content in mg/kg (wet wt)* |
|---|---|
| Bor (1 km) | 0.028 |
| Brestovac (5 km) | 0.037 |
| Krivelj (5.5 km) | 0.051 |
| Krivelj (5.5 km) | 0.031 |
| Krivelj (5.5 km) | 0.042 |
| Bučje (7.5 km) | 0.038 |
| Donja Bela Reka (9 km) | 0.041 |
| Rgotina (14 km) | 0.036 |
| Metovnica (15 km) | 0.030 |
| Koprivnica (17.5 km) | 0.031 |
| Koprivnica (17.5 km) | 0.067 |
| Vlaole (21 km) | 0.043 |
| Vlaole (21 km) | 0.038 |
| Vražogrnac (22 km) | 0.033 |
| Zaječar (26 km) | 0.029 |
| Zaječar (26 km) | 0.032 |
| Zaječar (26 km) | 0.030 |
| Zaječar (26 km) | 0.034 |
| Žagubica (27 km) | 0.032 |
| Žagubica (27 km) | 0.032 |
| Veliki Izvor (27 km) | 0.041 |
| Šipikovo (30 km) | 0.031 |

**Table II.** Arsenic content in honey, expressed in mg/kg, during the bee's active flying season with various exposures to contamination.

| *Sampling period* | *Area outside the contamination* | | *Area exposed to lethal contamination* | | |
|---|---|---|---|---|---|
| | *Strazilovo* [a] *(170 km)* | *Lubnicka reka* [a] *(31 km)* | *Rgotina* [a] *(14 km)* | *Koprivnica* [a] *(17.5 km)* | *Vrazogrnac* [a] *(22 km)* |
| April | 0.014 | 0.027 | 0.028 | 0.027 | 0.022 |
| May | 0.015 | 0.029 | 0.039 | 0.034 | 0.030 |
| June | 0.016 | 0.036 | 0.037 | 0.032 | 0.021 |
| July | 0.014 | 0.035 | 0.042 | 0.038 | 0.033 |
| August | 0.027 | 0.027 | 0.033 | 0.053 | 0.020 |

[a] Apiary locality and distance from the source of contamination.

hives placed in the area surrounding the Bor smeltery can be considered as being uncontaminated with arsenic. Unfortunately, this was not the case with pollen and with worker bees contaminated and killed by poison. Interestingly, the content of arsenic in honey from the apiaries which are place adjacent to the zone endangered by contamination is almost identical to the quantity found in honey from the contaminated apiaries (0.036—0.032 mg/kg).

**Table III.** Content of arsenic in bees and pollen.

| *Sampling period* | *Area outside lethal contamination zone* | | | | *Area periodically endangered by lethal contamination* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Strazilovo (170 km)* | | *Lubnicka reka (31 km)* | | *Rgotina (11 km)* | | *Koprivnica (16 km)* | | *Vrazogrnac (22 km)* | |
| | *bees* | *pollen* | *bees* | *pollen* | *bees* | *pollen* | *bees* | *pollen* | *bees* | *pollen* |
| April | 0.120 | 0.171 | 0.315 | 0.110 | 0.896 | 1.387 | 0.719 | 0.350 | 0.635 | 5.573 |
| May | 0.115 | — | 1.196 | 1.528 | 0.752 | 1.528 | 0.438 | 0.550 | 0.697 | 0.775 |
| June | 0.105 | 0.104 | 1.190 | 1.143 | 1.850 | 2.768 | 1.510 | 2.460 | 1.276 | 3.595 |
| July | 0.131 | 0.105 | 1.090 | 0.880 | 3.200 | 3.200 | 1.520 | 3.120 | 2.350 | 2.260 |
| August | — | — | 0.605 | 0.380 | 2.150 | 4.970 | 1.710 | 5.660 | 2.070 | 1.610 |

In our opinion there are 3 explanations for the absence of an increased concentration of arsenic in honey from hives affected by excess poisoning : 1) nectar is secreted in flowers during a relatively short time period and worker bees gather it before it becomes contaminated with arsenic from the air, whereas pollen is collected from flowers which normally have a longer period of flowering; 2) it is possible that during the process of making honey from nectar bees retain particle pollutants in their intestinal tract, thus reducing the concentration of arsenic in the honey; 3) plant incorporation into nectar is different from that for pollen.

Somewhat higher quantities of arsenic in honey from the whole region of East Serbia (around 0.032 mg/kg) as compared to the control group from Stražilovo, Fruška Gora (around 0.015 mg/kg) could be explained by differences in the normal quantity of arsenic in various regions.

Our results point to the possibility of using honeybees to follow the contamination of areas, on a larger scale, with arsenic and with other elements. It is also important to map chemical contamination for regions in which large contaminators, such as the Bor smeltery, are located. Similar investigations, carried out in the Puget Sound copper smelter area in the U.S.A., confirm the reliability and practicality of this method (Bromenshenk *et al.*, 1985; Bromenshenk, 1986; 1987).

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