

## HONEY BEES AND BEE PRODUCTS AS MONITORS OF THE ENVIRONMENTAL CONTAMINATION

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### Introduction

Honey bees are a good biological indicators because they indicate the chemical impairment of the environment they live in through two signals: the high mortality (in the case of pesticides) and the residues present in their bodies or in beehive products (in the case of pesticides and other contaminants like heavy metals and radionuclides) that may be detected by means of suitable laboratory analyses [1].

Several ethological and morphological characteristics make the honey bee a reliable ecological detector: it is an easy-to-breed, almost ubiquitous organism, with modest food requirements; its body is covered with hairs, which make it particularly suitable to hold the materials and substances it comes into contact with; it is highly sensitive to most plant protection products, revealing when they are improperly spread through the environment (e.g. during flowering, in the presence of wind, etc.); its very high rate of reproduction and relatively short average lifespan, causes the colony to undergo rapid, continuous regeneration; its great mobility and wide flying range allows a vast area to be monitored; it is highly efficient in ground surveys (numerous inspections per day).

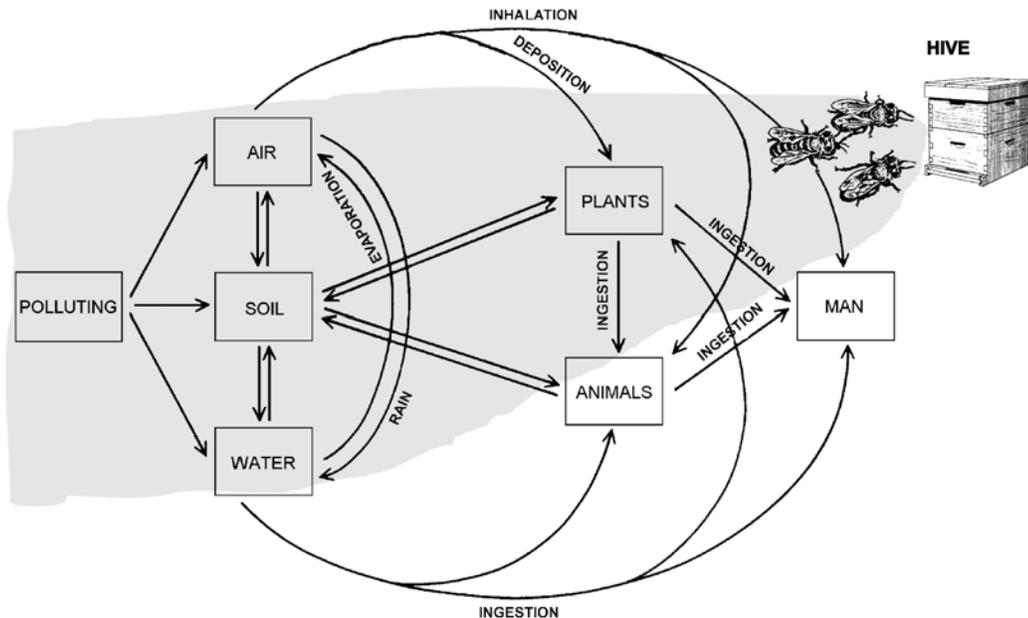


Fig. 1 - Chart of polluting substance diffusion in the environment. The grey area shows the environmental sectors visited by the honey bees.

Furthermore, almost all environmental sectors (soil, vegetation, water, air) are sampled by honey bees (Fig. 1), providing numerous indicators (through foraging) for each season. Finally, a variety of materials are brought into the hive (nectar, pollen, honeydew, propolis and water) and stored according to verifiable criteria.

For about twenty years our research group at the “Guido Grandi” Department of Entomology of the University of Bologna has been studying the use of honey bees as bioindicators of pesticides, heavy metals and radionuclides in many areas of Italy (see Table 1 for monitoring protocols). Recently, we extended our researches even to PAH (Polycyclic Aromatic Hydrocarbons) and to microorganisms (bacteria) [2].

### Monitoring of pesticides

As stated previously, honey bees are extremely sensitive to pesticides. The number of dead bees in front of the hive is therefore the most important variable to be considered for these contaminants [3] and varies according to a number of factors: the toxicity (for bees) of the active ingredient used ( $LD_{50}$ ) [4], the presence and extension of bloom among cultivated or spontaneous plants, the presence of honey bees on the site and at the time of the chemical treatment, the means used to distribute the pesticide, the presence of wind, etc.

Many bees directly struck by an insecticide will not have enough strength to return to their hive and will die in the field or during their return flight. Other bees only marginally hit,

while visiting the flowers of the treated species (whenever the active ingredients have no repellent effect) or gathering nectar and pollen from spontaneous species contaminated by “drift”, will eventually die in the hive. In this case the honey bee acts as a direct indicator.

In the case of compounds that are not particularly dangerous, the insect acts as an indirect indicator, i.e. not sensitive but exposed, and will provide us with information in form of residues.

With our monitoring scheme several results may be obtained: weekly mortality, active ingredients responsible of the bee killing, periods and areas at highest risk (Fig. 3), treated crops, errors of the growers in plant protection management. It is also possible to assess the degree of environmental hazard processing the data through specific indexes [5].



Fig. 2 - Monitoring station, constituted of two hives equipped with dead bee collecting traps (“underbasket”)

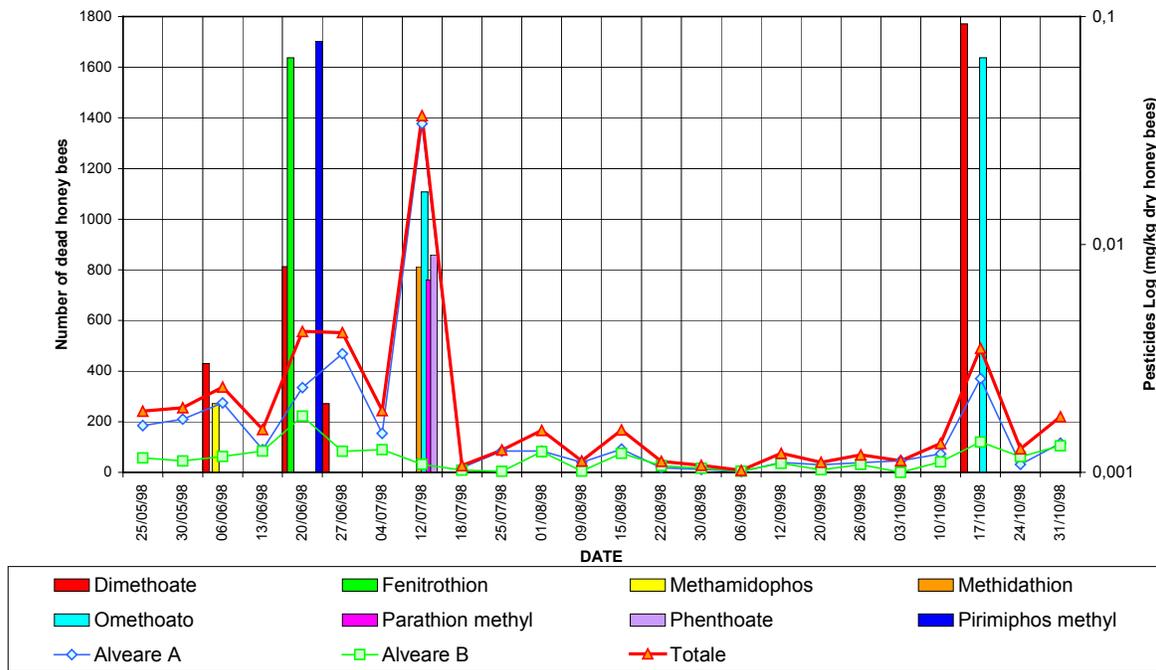


Fig. 3 - Weekly number of dead bees and pesticides residues in a monitoring station located in Bologna province.

Some of the new insecticides do not cause high bee mortality, but they can provoke severe behavioural effects, even at low doses. These active ingredients are also difficult to detect with chemical analysis and therefore our tools (mortality and residues) are not able to reveal them. In a near future it will become necessary to change the monitoring strategies, in relation to these new effects.

Table 1 – Monitoring protocols adopted for different contaminants

|                                      | <b>Pesticides</b>          | <b>Heavy metals</b>                                    | <b>Radionuclides</b>            |
|--------------------------------------|----------------------------|--|---------------------------------|
| <b>Hives per station</b>             | two                        | two  | two                             |
| <b>Used matrixes</b>                 | dead bees                  | foraging bees<br>"fresh" honey                         | dead bees                       |
| <b>Dead bee collection traps</b>     | "underbasket"              | -  | "underbasket"                   |
| <b>Frequency of sample gathering</b> | weekly                     | foraging bees: every 15 days<br>"fresh" honey: monthly | weekly                          |
| <b>Critical mortality threshold</b>  | 250 dead bees/week/station | -  | -                               |
| <b>Analysis</b>                      | chemical and palynological | chemical and palynological                             | radiochemical and palynological |
| <b>Other tools</b>                   | crop-growing maps          | Crop-growing maps                                      | crop-growing maps               |

### Monitoring of heavy metals

The fundamental aspect that differentiates heavy metals from other pollutants, like pesticides, is their introduction into the territory and their environmental fate. Pesticides are scattered both in time and space and, depending on the type of chemical compound, they are degraded by various environmental factors over a longer or shorter period of time. Heavy metals, on the other hand, are emitted in a continuous manner by various natural and anthropical sources and, since they are not degraded, they are continuously kept "in play", thus entering the physical and biological cycles.

Heavy metals present in the atmosphere can deposit on the hairy bodies of bees and be brought back to the hive with pollen, or they may be absorbed together with the nectar of the flowers, or through the water or the honeydew. A number of variables have to be considered when using bees, or beehive products such as honey, to monitor heavy metals in the environment: the weather (rain and wind can clean the atmosphere or transfer heavy metals to other environmental sectors), the season (the nectar flow, which is usually greater in spring than in summer and autumn, could dilute the pollutant), the botanical origin of the honey (the nectar of flowers with an open morphology and the honeydew are much more exposed to pollutants).

To compare the two matrixes, 43 samples of honey bees from 16 hives and 74 samples of honey from 29 hives were examined in the same conditions and using the same procedures. The statistical analyses showed a slightly higher degree of reliability for honey, statistically significant only for chromium (U test  $p < 0.05$ ).

To better investigate the matrix honey bees, we analysed 178 samples of foragers, caught on their return to their hives in three different areas: urban, industrial and natural (Fig. 4). The amount of metal accumulated inside the bee and deposited on the bee surface was analysed.

The lead in the urban and industrial areas was found in higher quantities inside the bee than on the bee surface, to a highly significant degree ( $p < 0.0001$ ) while the ratio was inverted in the natural areas ( $p < 0.0005$ ). With regard to nickel, a significant difference was found only in the natural area ( $p < 0.05$ ), where the amount was again higher on the bee surface. For chromium a significantly higher amount was found on the bee surface in all three environments (urban:  $p < 0.05$ ; industrial:  $p < 0.005$ ; natural:  $p < 0.005$ ).

The results for lead could indicate that persistent contamination induces higher absorption of pollutants, by inhalation or ingestion, into bee bodies during foraging. On the other hand, the higher levels of all three metals on the bee surface in natural areas could suggest that the pollutants are scattered throughout the atmosphere and they do not impregnate or deposit on the environmental components visited by bees. Nickel and chromium differ from lead in the two most highly contaminated areas. This discrepancy is probably due to their different environmental fate. However, it also reflects the high number of cases in which the values recorded inside and on the bee were equal, because they were below the limit detectable by the instrument [6].

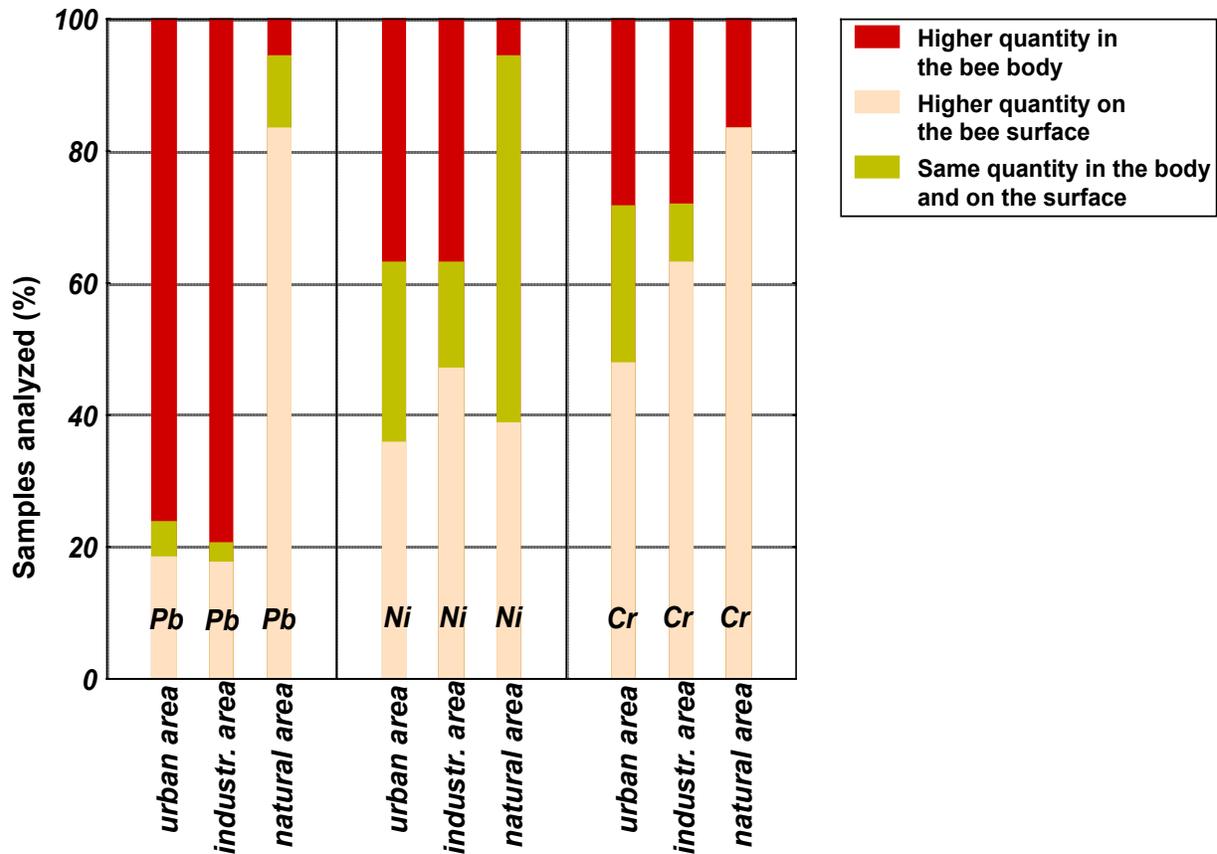


Fig. 4 - Quantity of heavy metals detected internally and externally in honey bees in relation to three different environments.

### Monitoring of radionuclides

The use of honey bees and hive products to monitor radioactivity dates back to the end of the 1950s, but it was not until the Chernobyl state of emergency (April - May 1986) that the excellent efficacy of bees in detecting radioisotopes was unequivocally demonstrated. In a research project carried out by our team, again in the context of Chernobyl [7], numerous samples of honey bees, wax and pollen were analysed. The findings demonstrated that pollen was the most efficient indicator of atmospheric radionuclide contamination. Honey bees can also be used profitably for monitoring, while there are numerous obstacles to the use of honey.

In our research projects, the monitoring of pesticides is often coupled with that of radionuclides, that can be released into the environment from nuclear power plants, factories or health establishments.

Towards the end of April 1998, an incident occurred at the Algeciras steel works in southern Spain with emissions of cesium 137, coming from a radioactive source no longer in use. In May 1998, our radiochemical laboratory detected an anomalous presence of <sup>137</sup>Cs in honey bee samples taken from monitoring stations in the Bologna province (Fig. 5).

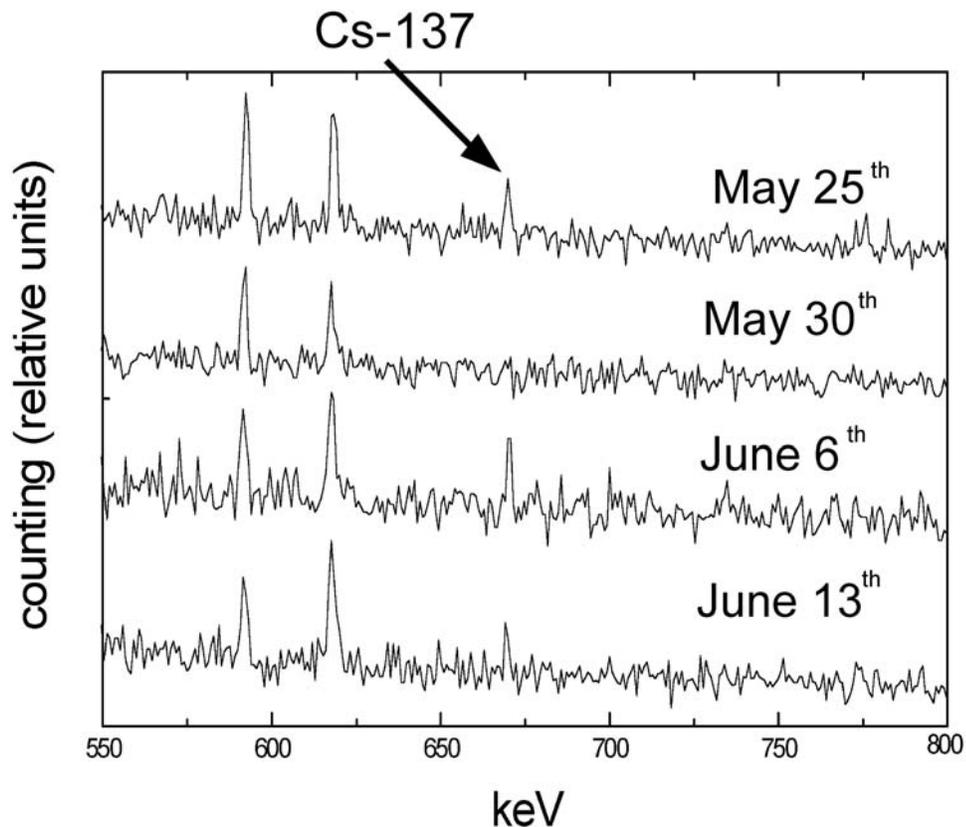


Fig. 5 - Comparative gamma spectrum of honey bees samples collected in Bologna province (1998).

The hypothesis that the anomalous radioactivity derived from active nuclear plants can be ruled out, as the  $^{137}\text{Cs}$  was not accompanied by the other radionuclides produced during fission. The fact that the presence of  $^{137}\text{Cs}$  was interrupted for a week and then resumed is not unusual as the transport and soil deposition of air-dispersed pollutants is strictly linked to wind and precipitation. The levels of radioactivity were negligible and many times below every alarm threshold, but the bee matrix promptly revealed the presence, albeit minimal, of  $^{137}\text{Cs}$  in the atmosphere with an efficiency above that of traditional monitoring techniques.

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