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Review

## Honey bees and their products as indicators of environmental pollution: A review

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**Abstract.** *In the present work a literature review of the experiments that explore the use of honey bees and their products as bioindicator of environmental pollution is presented. The greatest number of studies has been carried out on contaminations with heavy metals, followed by pesticides, radionuclides and other substances. Pb, Cd and Zn have been the most looked for metals. Zn and Cd have been mainly deposited on the surface of the bee body while Ni, Cd, Pb and Co have been released with the excrements most often. In all cases of pesticide implementation certain amounts of them have been always accumulated in the bees and their products. According to the researchers, pollutants accumulate in the bees and their products at different extents. Heavy metals and pesticides have been established in the bodies of honey bees in larger quantities in comparison to honey. Most of the authors reported that bee honey is a suitable tool for monitoring pollution with heavy metals and pesticides but the opposite assertions have been also expressed. A suggestion for the presence of a bio-barrier function of the bee organism against contaminants has been forwarded. It has been established that pollen is the most suitable indicator for radioactive pollution. As a whole, the present review shows that bees and their products are suitable models for bio-monitoring of the environmental pollution of different nature.*

**Keywords:** honey bees, bee products, bioindicators, pollution, heavy metals, pesticides

### Introduction

Environment is the factor together with the genetic background of the human that influences to a great extent his health and quality of life. In recent years as a result of the activity of man (harmful emissions of burnt fuel, deforestation, the deposition of chemical, radioactive and biological refuse, etc.) serious deviations from the normal parameters recording the state of the environment have been observed. The environmental changes on their part have their effect on man directly or indirectly. For example, the contamination of soil and water with heavy metals and chemicals leads to their accumulation in the plants and animals inhabiting the polluted areas. The human as an end consumer accumulates these pollutants, a part of which (for example some heavy metals) are deposited in the bones, teeth and stay there till the end of life having harmed his health in the meantime.

The danger of negative changes in the environment makes scientists look for novel, more efficient methods for monitoring the environment for early detection of pollutants. Alongside with the confirmed and widely used methods attempts are being made recently for testing different biological species (plants, insects, fish and other small animals) as bioindicators of changes (Chovanec et al., 2003; Hijano et al., 2005; Nummelin et al., 2007; Sánchez-Chardi et al., 2007). Bees in this respect, which are in constant contact with the atmosphere, plants, waters and soils are the object of numerous ecological studies aimed at establishing their role in the detection of polluted areas. Great attention is also being paid to honey bee products which also yield information about the state of the surroundings inhabited by bees.

It is well-known that at the time of active pasture bees gather pollen and nectar in the radius of seven square kilometers (Tonelli et al., 1990). Together with the nectar, propolis and pollen they introduce a number of chemical and physical pollutants in the hive, which pass into the honey and affect the production, health and life of the bees.

The pollutants which exert influence on the bees and the bee products can be of different nature. Such are, for example, the contaminations which man willingly or unwillingly produces in his everyday activities (burnt gases, heavy metals and metalloids, radionuclides and other toxic emissions), pesticides used against different harmful agents in agriculture, the veterinary-medical preparations used in the struggle against viral, bacterial and parasitoses in bees, etc. Bogdanov (2006) has divided these pollutants into two main groups – those related to the activity of the bee-keeper and the other caused by the state of the surroundings from which the bees gather nectar and pollen. He has included in the environmental group of pollutants the heavy metals, radioactive isotopes, organic contaminants, pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria and genetically modified organisms. In the group of pollutants driven into the hive by the beekeeper. Bogdanov (2006) mainly relates the acaricidal remedies used in the anti-parasite struggle as well as the chemical remedies for fighting bacterial infections which also leave traces in the honey, pollen, wax, propolis and royal jelly.

The bee acts as a detector of environmental pollution in two ways, as it signals either via high mortality rates the presence of toxic molecules, or via the residues in honey, pollen, and larvae the presence of heavy metals, fungicides and herbicides that are harmless to it. Bee monitoring also contributes to the ecological

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impact statement by culminating in the charting of environmental health maps, which include such data as mortality rates, apicide number, type and risk-level of molecules detected, and so forth (Celli and Maccagnani, 2003).

The aim of the present work is to provide a literature review of the experiments that explore the use of honey bees and their products as bioindicator of environmental pollution.

The results found are presented in a chronological order and are assigned to three main groups of pollutants - heavy metals, pesticides, radioactive and other agents.

### **Honey bees and their products as indicators of pollutions with heavy metals**

Heavy metals represent some of the most common and dangerous pollutants of the environment. That is probably why a large number of investigators have studied the role of bees and their products as bioindicators for pollution with them.

Nineteen samples of honey taken from grocery shelves, near zinc mines, adjacent to an industrial area, and near a major highway have been subjected to spark source mass spectrometry to determine the concentration of most of 47 elements in the honey (Tong et al., 1975). Certain samples of honey produced by bees in the vicinity of the New York State Thruway have contained elevated levels of certain elements known to be emitted by traffic, such as Al, Ba, Ca, Co, Mg, Ni, Pd, and Si, but the true source of these elements has been uncertain. Honey which has been into contact with metal containers in processing has contained the greatest amounts of Zn and Sn.

The potential use of honey as an indicator in mineral prospecting and environmental contamination studies has been investigated by Jones (1987). Ag, Cd, Cu, and Pb levels content of honey have been investigated in relation to that in the soils collected from within the foraging area. For samples collected over two seasons the following concentrations have been found Ag <0.1 to 6.5 ng. g<sup>-1</sup> (d.w.); Cd <0.3 to 300 ng. g<sup>-1</sup>; Cu 35 to 6510 ng. g<sup>-1</sup>; Pb <2 to 200 ng. g<sup>-1</sup>. Considerable spatial and seasonal fluctuations have been apparent. No correlations have been observed between honey and soil concentrations for either Cu or Pb. It has been concluded that the low concentrations of heavy metals in honey and their inherent variability (due to differences in floral source, foraging range, entrapment of atmospheric particulates on the flower, etc.) detract from the reliable use of honey as a monitoring tool.

Leita et al. (1996) have carried out an experiment using 12 colonies of honey bees bred in hives located near an extrarurban crossroad. They have analyzed the Pb, Cd and Zn deposited on the bee's surfaces and the heavy metal accumulation in the foragers, dead bees, honey products and some environmental markers during nine weeks of the experiment. Results have shown a large amount of Zn and Cd on the bee's surface as a consequence of atmospheric fallout, whereas according to authors Pb seems to be either water-extractable and/or likely accumulated in the body of the insect. Dead bees expelled from the hives have displayed a progressive accumulation of all heavy metals during the experimental period. Royal jelly and honey contained large amounts of heavy metals. The obtained results have suggested that honey bee products may be considered useful parameters to assess the presence of environmental contaminants, whereas the measurements of heavy metals in the dead bees may be considered a suitable tool also to verify a possible dynamics of accumulation of pollutants.

The concentrations of three representative heavy metals (Cd,

Cr and Pb) have been measured by atomic absorption spectroscopy in honeybees, honey, pollen, propolis, and wax (Conti and Botrè, 2001). Samples have been collected from five different sampling points: four from areas surrounding the city of Rome, and the fifth in the city center which receives intensive traffic. All apiaries employed for the study have been specifically constructed without any metal part in order to avoid the risk of contamination of the assayed materials. Experimental data have revealed, in general, statistically significant differences between the background levels of heavy metals recorded from the reference sites and the levels measured in the site located in the center of the city of Rome. According to authors the results have indicated that honeybees and, to a lesser extent, some of their products (pollen, propolis, wax, but not honey), can be considered representative bioindicators of environmental pollution.

Concentrations of <sup>137</sup>Cs in various honey types during the 1990s in Croatia were recorded by Barišić et al. (2002). The research has also documented the levels of <sup>137</sup>Cs, <sup>40</sup>K, Ca, Fe, Rb, Sr, Cu, Zn, Pb, Ni, Mn, and Cr in soils, coniferous tree branches and honey, and has compared the transfer from soil into nectar honey, mixtures of nectar and honeydew honey, and honeydew honey in fir and spruce forests in Croatia. For all of the elemental concentrations investigated, no significant differences have been found between honeydew honey and mixed honey, regardless of the soil type where the honey has been collected from. Elemental transfer factors from soils into nectar honey have been significantly lower than those for honeydew honey.

The research about chemical contamination of honey harvested from beehives situated in Copsa Mica (Sibiu region), famous for the ecological unbalances induced by the non-ferrous metal industry has been carried out by Bratu and Georgescu (2005). The amount of Pb, Cd and Zn contained in the honey samples has been determined using an atomic absorption spectrometer. Amounts of heavy metals above the admitted limit have been discovered.

Thirteen metal elements have been determined in 40 honey samples from Galicia with different environmental origins: rural, urban, and industrial areas (Rodríguez García et al., 2006). The data set of the honey metallic profiles has been studied with a double purpose: first, to make a preliminary evaluation of honey as an environmental indicator in Galicia with the aim of monitoring pollution and, second, to compare the different capabilities of diverse pattern recognition prediction procedures for modeling the environmental surrounding of the hive. A certain level of similarity for urban and industrial samples has been obtained using principal component analysis and cluster analysis, whereas significant differences for urban and industrial honeys have been found in relation to rural honey samples. According to authors the metal profiles of honey seem to provide sufficient information to enable categorization criteria for classifying samples according to their environmental surrounding. Thus, honey could be a potential pollution indicator for the Galician area.

Nine heavy metals, Co, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, have been determined in a total of 192 samples (96 soil, 48 flower, and 48 honey samples), which have been collected from polluted areas (Edfu and Kom Omb cities) and unpolluted areas (Esna and Aneeba cities) at Aswan district, Egypt (Rashed et al., 2009). Metal concentrations have been measured using an atomic absorption spectrophotometer. The results have revealed that honey in polluted areas showed higher concentrations of Cu, Pb, Fe, and Zn than honey from unpolluted areas. This has been related to the pollution input from industrial activities in polluted areas. A study of metal concentrations in flowers has revealed that flowers contained the

highest levels of the studied metals than those in honey. Concentration factors of heavy metal for honey/flower in polluted areas seem to be higher than in that of the unpolluted ones. Element concentrations in honey have been within the safety baseline levels for human consumption. The results have suggested that honey may be useful as an environmental indicator for assessing the presence of environmental pollution with heavy metals.

Zhelyazkova et al. (2010) have carried out a comparative analysis of the content of some heavy metals and metalloids (Cu, Zn, Pb, Cd, Ni, Co, Mn, Fe) in the body of bees, fecal mass, bee products (honey, pollen, wax) and sunflower flowers in areas with different degree of anthropogenic impact. The study has included two settlements in Stara Zagora region (Bulgaria): the town of Gurkovo – an area with low level of anthropogenic impact; the village of Bratya Kunchevi – an area with established anthropogenic impact (working stone quarry on the territory of the village). Accumulation of the studied heavy metals and metalloids in the bee fecal mass has been observed and high level of accumulation in excrements has been exhibited by Pb (1:2,3 - 1:4,0), Cd (1:1,6 - 1:1,9), Ni (1:4,2 - 1:4,3), Co (1:1,6 - 1:2,0). The bio-barrier function of the bee organism has been proved as a result of which heavy and toxic metals such as Pb and Cd have been efficiently excreted by the organism through the fecal mass. It has been established that Fe content has been of higher values in the bee body, pollen and wax in the station village of Bratya Kunchevi (compared to the samples in the town of Gurkovo). According to authors a possible reason for the reported high quantity of iron in the analyzed samples of the village of Bratya Kunchevi could be the working stone quarry on the territory of the village operating a deposition of andesite tuffs (calcium-magnesium-iron silicates). Unsatisfactory indicator characteristics of bee honey for environmental quality have been established in areas with different degree of anthropogenic impact compared to bee organism and pollen.

Sadeghi et al. (2012) have determined the levels of some elements (Hg, Ba, Ca, Fe, Mn, Li, As, Na and K) in the bodies of bees. Honeybee samples have been randomly collected from apiaries located in four counties of the province of Kurdistan. The obtained data have shown that K has had the highest concentration, followed by Na, Ca, Hg, Ba, Fe, Mn, Li and As. Three mineral groups have been differentiated: elements very abundant, in a medium concentration, and trace ones. The first group consisted of K and Na, with a range from 41.857 to 47.871, and 12.653 to 16.183 ppm, respectively. The second mineral group has been composed of Ca, Hg, Ba, and Fe, where Ca was the most abundant element (with a range of 9.077 to 10.058 ppm), followed by Hg (1.12-4.786 ppm), Ba (2.881-3.481 ppm), and Fe (1.050-1.727 ppm). The third mineral group has been composed of Mn, Li and As in range from 0.262 to 0.399, from 0.043 to 0.101, and from 0.017 to 0.068 ppm, respectively.

Roman and Popiela-Pleban (2012) have determined the extent of bioaccumulation of toxic elements Zn, Cu, Pb, As and Cd in propolis collected in Opole area. The sequence of accumulation level of the studied elements in propolis has been as follows: Zn>Cu>Pb>As>Cd. An average concentration of Zn, Cu, Pb, As and Cd has amounted to 56.28, 7.12, 6.91, 0.745, 0.218 mg.kg<sup>-1</sup>, respectively. Only the Cu average content in propolis has been within acceptable standards, whereas the mean contents of the other elements have greatly exceeded these standards.

Zhelyazkova (2012) has tested honeybees as bioindicators for environmental status in the region of Sarnena Sredna gora, county of Stara Zagora, Bulgaria. The study comprised six villages, located close to the military range of Zmeyovo. In each village bee samples

were taken and samples of whole bees and fecal mass were prepared. The content of Cu, Zn, Pb, Cd, Co, Ni, Mn and Fe were traced in the bee body and in excrements. The analysis was carried out through atomic absorption spectrometry according to ISO 11047. Maximum or relatively high values for content of the studied heavy metals and metalloids in the body and fecal mass of bees were found, which has made it possible to comment a possible anthropogenic effect in the area of Sarnena Sredna gora, Zmeyovo military range. Higher content of the studied heavy metals and metalloids in excrements compared to the body of bees was found, which has accounted for the bio-barrier function of the bee organism. The elements Ni, Cd, Co and Pb have been accumulated at the highest degree in the bee fecal mass. The author assumes that honeybees respond to changes in their environment and in particular to increased quantities of heavy metals in soil, air, plants and that makes them a reliable indicator and allows their use in bio-monitoring of the environment.

Honey samples have been collected in different places of Slovakia aiming to determine the concentration of heavy metals in honey (Lazor et al., 2012). The results obtained in site Prievidza during the 2006 – 2008 period were as follows: 0.0599 mg.kg<sup>-1</sup> As, 0.0948 mg.kg<sup>-1</sup> Cd, 0.0747 mg.kg<sup>-1</sup> Cr, 0.0394 mg.kg<sup>-1</sup> Hg and 0.1252 mg.kg<sup>-1</sup> Pb and in site Šafa: 0.0862 mg.kg<sup>-1</sup> As, 0.0942 mg.kg<sup>-1</sup> Cd, 0.0736 mg.kg<sup>-1</sup> Cr, 0.0341 mg.kg<sup>-1</sup> Hg and 0.1626 mg.kg<sup>-1</sup> Pb. The authors suggested that honey could be used to detect contaminating agents in the environment.

Dima et al. (2012) evaluated the use of pollen as bioindicator of environmental pollution. The content of Fe, Mn, and Zn from pollen samples uniformly distributed in Dambovita County has been determined using the Energy Dispersive X-Ray Fluorescence Spectrometry. The samples have been collected from private farms, kipped in industrial-urban and non industrial rural areas. The authors have found differences between the two kinds of samples and have concluded that pollen can be used for bio-monitoring the environment for Fe, Mn, and Zn.

Total arsenic (t-As) and inorganic As (i-As) concentrations were determined by Bastías et al. (2013) in 227 samples of honey harvested during the years 2007, 2008, and 2009 in the areas of San Pedro de Atacama, Atacama, Chiloé, and Futaleufú, with the last town located 156 km from the Chaitén Volcano (latest eruption in 2008). These analyses have been conducted using an atomic absorption spectrophotometer coupled with a hydride generator. In the honey samples the concentrations of t-As ranged from 2.2 to 171.9 µg.kg<sup>-1</sup>, and the i-As concentrations - from none detected (ND) to 24.6 µg.kg<sup>-1</sup>, with the area of San Pedro de Atacama having the highest As concentrations. The samples of honey from Futaleufú showed higher As concentrations after the eruption of the Chaitén Volcano in 2008. According to authors the study demonstrates that As pollution in honey may originate from both natural and anthropogenic sources. The results have indicated that it is appropriate to use honey as a bioindicator of environmental pollution.

Ruschioni et al. (2013) have carried out bio-monitoring with honeybees aiming to assess the presence of heavy metals (Cd, Cr, Ni, Pb) in all of the ten nature reserves of the Marche Region (central-eastern Italy). The study was performed during the spring and summer seasons when honeybees were active, over 3 years (2008–2010). Twenty-two colonies of honeybees bred in hives were used. Samples of live and dead honeybees and of honey were collected from 11 sampling stations from May to October in each year. No pesticide pollution was found. Significant differences in

heavy metal concentrations were found among years, months and sites, and in particular situations. The analysis has revealed that high heavy-metal concentrations have occurred exclusively in live honeybees. The most detected heavy metal was Cr, which exceeded the threshold more often than for the other elements, followed by Cd and Pb; Ni has never exceeded the threshold.

Formicki et al. (2013) have studied concentrations of Cd, Ni, Pb, Fe, Mg, and Zn in multi-floral honey, propolis, bee pollen, and wax coming from apiaries situated in different locations in Malopolska Voivodeship in Poland. The authors have established that honey contained the lowest concentrations of all the tested metals, with Cd and Pb concentrations well below allowable levels. Other products have showed much higher concentration of Cd and Pb. Propolis and pollen from certain areas were significantly contaminated with Pb. High metal contents occurred in beeswax. Positive correlations between metals occurred in wax: Cd vs. Ni, Cd vs. Fe, Ni vs. Fe, Ni vs. Mg, Pb vs. Fe, and Fe vs. Mg. Metal contents in honey did not correlate with metal contents in other tested products. Correlations between Fe concentrations in honey and wax and between Mg in bee pollen and propolis were exceptional. The authors consider that honeybee products may be useful in monitoring environmental contamination by metals.

Steen et al. (2015) have explored whether honeybees could be used as a reliable alternative to the standard mechanical devices for monitoring air quality, in particular with respect to the concentration of the heavy metals Cd, Pb and V. They have tested whether the concentrations of these metals in adult honeybees and in ambient air have been positively correlated, and whether differences in concentration between locations have been similar for bees and air. Measurements have been conducted over two-month period at three distinct locations in the Netherlands with each three replicate honeybee colonies placed next to mechanical monitoring devices and they have shown that a significant positive relationship between the concentrations in bees and in air could only be established for V. Also, only in the case of V, the differences between the three locations in mean concentration have been similar for bees and air. Both outcomes have been probably due to the relatively large range over which the concentrations of V varied, both in bees and in air, as compared to Cd and Pb. However, for V, as well as for Cd and Pb, the concentrations in ambient air have been about two orders of magnitude below the established air quality standards. Therefore, the authors have concluded that in the Netherlands, both variation and levels of the atmospheric concentrations of these metals are too low to establish a relationship between the concentration in bees and in air that is useful to present honeybees as an alternative to mechanical devices in monitoring air pollution. However, they consider that in countries with larger variation and higher levels of the atmospheric concentrations of these metals, further exploration of the potential of honeybees in bio-monitoring air pollution may be worthwhile.

A study by Aghamirlou et al. (2015) has indicated that all types of honey contain metals and the metal concentrations vary among different regions because of some variables. Findings have showed that Zn and As have had the maximum and minimum concentrations, respectively. The authors consider that some geological and geochemical parameters may affect the honey chemistry. They also have pointed that the proximity to the industries, having different types of soil, using various fertilizers, and the diversity in the practice of growing plants probably led to some differences between regions. In their study, it has been difficult to compare the obtained results with related standards, because some standards were based on daily or weekly intake of metals, while there was no data about daily

consumption of honey by people. Finally, according to literature, authors have mentioned some recommendations, namely: limiting the consumption of hazardous fertilizers, monitoring the soil in agricultural regions, considering the distance of agricultural lands and flower gardens with industries, controlling the quality of food products, providing some accurate standard limits for hazardous compounds in the foods, and monitoring the water that is used for agriculture and floriculture.

### **Honey bees and their products as indicators of pollution with pesticides**

According to some authors the utilization of different types of pesticides in agriculture can be established by detecting traces of them in bees and bee products.

According to Porrini et al. (2003) in many cases, pollution caused by abuse or by erroneous application of pesticides could not be proven without the help of honey bees. This group of authors has carried out a monitoring of pesticides with honey bees. This work has been applied in some areas surrounding Bologna. Each monitoring station consisted of two beehives equipped with collection cages for dead bees. Once a week, families were checked and the number of dead bees was recorded. When the mortality rate exceeded the critical threshold (250 bees/week/station), laboratory analysis was carried out. The authors indicated periods of major bee poisoning risk, and identified the most frequently used pesticides (also those that are prohibited) and the crops treated. These studies with honey bees have revealed the type of plant protection management applied to the area under investigation and allowed to prove the application of molecules not permitted under certain circumstances or even forbidden.

Samples of honeybees from 14 beehive monitoring stations located in 3 townships in the province of Bologna were aiming to evaluate the concentration of 32 organophosphorus pesticides and 5 carbamates (Ghini et al., 2004). The most contaminated samples were from Granarolo Emilia where cereals (wheat, sorghum, and corn), sugar beets, and potatoes were the main agriculture products. Thirty-five pesticides were detected, with organophosphorus being the most abundant one. Malathion was detected in 58% of the samples (mean level 0.360 mg/kg) followed by fenitrothion in 53% of the samples (mean level 0.544 mg/kg) and pirimiphos methyl in 48% of the samples (mean level 0.006 mg/kg). Temporal trends have showed that the maximum detection frequency occurred in late spring and was associated with the use of treatment products and less rainfall. The results have demonstrated the feasibility of using honeybees for assessing pesticide exposure in agriculture settings.

A field survey has been performed on French apiaries to monitor weakness of honey bee colonies (Chauzat et al., 2006). Five colonies have been randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 years colonies were visited four times per year: after winter, before summer, during summer, and before winter. Pollen loads from traps were collected at each visit. Multiresidue analyses have been performed in pollen to search residues of 36 different molecules. Specific analyses have been conducted to search fipronil and metabolites and also imidacloprid and metabolites. Residues of 19 searched compounds have been found in samples. Contamination by pesticides ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues have been the most concentrated of all residues (mean concentrations - 925.0 and 487.2 µg/kg, respectively). Fipronil and metabolite contents have been superior to the limit of detection in 16 samples. Residues of fipronil have been found in 10 samples. Nine samples contained

the sulfone compound, and three samples contained the desulfinyl compound. Residues of imidacloprid and 6-chloronicotinic acid have been found in 69% of samples. Imidacloprid contents has been quantified in 11 samples with values ranging from 1.1 to 5.7 µg/kg. 6-Chloronicotinic acid content has been superior to the limit of quantification in 28 samples with values ranging from 0.6 to 9.3 µg/kg.

The pollution of six agricultural areas of Greece by insecticides used in crop protection has been investigated utilizing bee honey produced in those areas as bioindicator (Balayiannis and Balayiannis, 2008). Honey samples collected randomly from apiaries located in those areas have been analyzed for pesticide residues with a multi-analytical method, able to determine simultaneously up to 10 organophosphorous insecticides from the same honey extract. Findings concerning the acaricide coumaphos have also been included, even though it is not used in crop protection. The above areas are planted to a large extent with citrus trees or cotton or sunflower crops which are good forages for honeybees. The main pests of those crops are insects; hence, insecticides are used on a large scale for crop protection. The most contaminated samples originated from citrus groves; 16 out of 19 had pesticide residues: 4 samples had chlorfenvinphos (21.05%), 10 had chlorpyrifos (52.63%) and 2 - phorate (10.53%). Out of 17 samples from cotton fields, residues have been found in 8, phorate in 6 (35.29%), chlorfenvinphos in 1 (5.88%), and chlorpyrifos in 1 (5.88%). Out of nine samples from fields of sunflower, four had phorate residues (44.44%). In brief, from the 50 analyzed samples,

residues of chlorfenvinphos have been detected in 5 samples (10%), residues of chlorpyrifos in 11 samples (22%), and residues of phorate in 12 samples (24%). Their levels ranged between 0.70 and 0.89 µg/kg. Coumaphos residues ranged from 0.10 up to 4.80 µg/kg and have been derived exclusively from beehives treated with Perizin (the commercial formulation of coumaphos) for *Varroa* control. This study has indicated that in agricultural areas with developed apiculture useful information about the occurrence and the distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples used as bioindicators. It has also shown that, very often, the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey.

### Honey bees and their products as indicators of pollutions with radioactive and other agents

Samples of honey, pollen and honey bees have been collected in some regions of Italy after the accident in Chernobyl Nuclear Power Plant (1986) and subjected to gamma spectrometry in order to assess their possible use as markers of the radioactive environmental contamination (Tonelli et al., 1990). Pollen has resulted in the best indicator, since it reflects exactly the air contamination and according to authors it is suitable for obtaining a map of fallout. The authors have determined that bees can be used for the purpose too, even if their collection is more difficult, whereas honey has given only an indication.

**Table 1.** Comparative data about the use of honey bees and their products as indicator of environmental pollution with heavy metals

Investigated products and origin	Pollutants	Results / Conclusions	Authors
Honey taken from grocery shelves, near zinc mines, adjacent to an industrial area, and near a major highway.	47 different elements	The samples have contained elevated levels of certain elements known to be emitted by traffic, such as Al, Ba, Ca, Cu, Mg, Ni, Pd, and Si. Honey which has been in contact with metal containers in processing contained the greatest amounts of Zn and Sn.	Tong et al. (1975)
Honey has been investigated in relation to that in the soils collected from within the foraging area of the bees.	Ag, Cd, Cu, Pb	Considerable spatial and seasonal fluctuations in the results have been apparent. No correlations have been observed between honey and soil concentrations for either Cu or Pb.	Jones et al. (1987)
12 colonies of honey bees bred in hives located near an extraurban crossroad: the bees' surfaces, foragers, dead bees, honey products.	Pb, Cd, Zn	Dead bees expelled from the hives have displayed a progressive accumulation of all heavy metals. Royal jelly and honey contained large amounts of heavy metals. Zn and Cd accumulate mainly on the bee's surface and Pb - in the body.	Leita et al. (1996)
Honey bees, honey, pollen, propolis, and wax have been collected from areas surrounding the city of Rome, and the city center which receives intensive traffic.	Cd, Cr, Pb	Honey bees and, to a lesser extent, some of their products (pollen, propolis, wax, but not honey), can be considered representative bio-indicators of environmental pollution.	Conti and Botrè (2001)
Nectar-honey, mixed honey, honeydew honey, soils, coniferous tree branches.	<sup>137</sup> Cs, <sup>40</sup> K, Ca, Fe, Rb, Sr, Cu, Zn, Pb, Ni, Mn, Cr	Elemental transfer factors from soils into nectar honey have been significantly lower than those for honeydew honey. For all of the elemental concentrations no significant differences have been found between honeydew honey and mixed honey, regardless of the soil.	Barišić' et al. (2002)

Honey from beehives situated in region ecologically unbalanced by non-ferrous metal industry.	Pb, Cd, Zn	Amounts of heavy metals above the admitted limit have been discovered.	Bratu and Georgescu (2005)
Honey from different environmental origins: rural, urban, and industrial areas.	13 metal elements	Honey could be a potential pollution indicator.	Rodríguez García et al. (2006)
Samples of soil, flower, and honey from polluted areas and unpolluted areas.	Co, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Concentration factors of heavy metal for honey/flower in polluted areas seem to be higher than in that of the unpolluted ones.	Rashed et al. (2009)
Bodies of bees, fecal mass, bee products (honey, pollen, wax) and sunflower flowers from areas with different degree of anthropogenic impact.	Cu, Zn, Pb, Cd, Ni, Co, Mn, Fe	The biobarrier function of the bee organism has been proved as a result of which heavy and toxic metals such as Pb and Cd have been efficiently excreted by the organism through the fecal mass.	Zhelyazkova et al. (2010)
Bodies of bees from different regions.	Hg, Ba, Ca, Fe, Mn, Li, As, Na, K	Three minerals groups have been differentiated: elements very abundant, in a medium concentration, and trace ones. Bee honey is a suitable bioindicator.	Sadeghi et al. (2012)
Propolis.	Zn, Cu, Pb, As, Cd	Only the copper average content in propolis has been within acceptable standards, whereas the mean contents of other elements have greatly exceeded these standards.	Roman and Popiela-Pleban (2012)
Bee bodies and bee excrements from villages located close to a military range.	Cu, Zn, Pb, Cd, Co, Ni, Mn, Fe	Higher content of the studied heavy metals and metalloids in excrements compared to the body of bees has been found, which has accounted for the bio-barrier function of the bee organism.	Zhelyazova (2012)
Honey from different regions.	Heavy metals	The authors suggested that honey could be used to detect contaminating agents in the environment.	Lazor et al. (2012)
Pollen samples collected from private farms, kipped in industrial-urban and non industrial rural areas.	Fe, Mn, Zn	Pollen can be used for bio-monitoring of environment.	Dima et al. (2012)
Honey from different regions, including ones situated near the Chaitén Volcano, latest erupted in 2008.	Total As, inorganic As	Pollution with As in honey may originate from both natural and anthropogenic sources. It is appropriate to use honey as a bioindicator of environmental pollution.	Bastias et al. (2013)
Samples of live and dead honey bees and honey during the spring and summer seasons over 3 years' period.	Cd, Cr, Ni, Pb	Significant differences in heavy metal concentrations have been found among years, months and sites, and in particular situations. The analysis has revealed that high heavy-metal concentrations have occurred exclusively in live honey bees.	Ruschioni et al. (2013)
Honey, propolis, bee pollen, and wax from different regions.	Cd, Ni, Pb, Fe, Mg, Zn	Metal contents in honey have not been correlated with metal contents in other tested products. High metal contents have occurred in beeswax. Honey contained the lowest concentrations of all tested metals.	Formicki et al. (2013)
Concentrations of some metals have been tested in honey bees and air.	Cd, Pb, V	In countries with larger variation and higher levels of the atmospheric concentrations of these metals, further exploration of the potential of honey bees in bio-monitoring of air pollution may be worthwhile.	Steen et al. (2015)
Honey from different types and regions.	Zn, As	All types of honey contain metals and the metal concentrations vary among different regions because of some geological, geochemical and anthropological factors.	Aghamirlou et al. (2015)

**Table 2.** Comparative data about the use of honey bees and their products as indicator of environmental pollution with pesticides and other agents.

Investigated products and origin	Pollutants	Results / Conclusions	Authors
Dead honey bees	Pesticides	The honey bees can be used for detection of pollution with pesticides.	Porrini et al. (2003)
Honey bees	Organophosphorus pesticides, carbamates	The results have demonstrated the feasibility of using honey bees for assessing pesticide exposure in agriculture settings.	Ghini et al. (2004)
Pollen	36 different molecules of pesticides	Contamination by pesticides ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues have been the most concentrated of all residues. Bee pollen is suitable for detection of residues of pesticides.	Chauzat et al. (2006)
Honey	Insecticides	In agricultural areas with developed apiculture, useful information about the occurrence and the distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples, used as bioindicators. Very often, the chemicals used by apiculturists inside the hives in order to control diseases are the main pollutants of the produced honey.	Balayiannis and Balayiannis (2008)
Honey, pollen and honey bees have been collected in Italy after the Chernobyl accident.	Radioactive contamination	Pollen is the best indicator for radioactive contamination.	Tonelli et al. (1990)
Honey	SO <sub>2</sub>	The amount of sulphate determined in honey correlated with total yearly emissions of SO <sub>2</sub> observed by an electronic monitoring.	Ponikvar et al. (2005)

A bee monitoring net consisting of 32 apiaries and covering an area of about 700 km<sup>2</sup> has been configured surrounding the Šoštanj coal-fired power plant (Ponikvar et al., 2005). The results of investigations have revealed that the amount of sulphate determined in honey from this area correlated with total yearly emissions of SO<sub>2</sub> observed by an electronic monitoring. When exhaust gases at the power plant were purified, a corresponding decrease in the amounts of sulphate in honey has been observed. According to authors, the proposed bee monitoring net is an example of using bio-monitoring for tracing environmental pollution, which could be also applied to estimate the impact of NO<sub>x</sub> gases or to trace other pollutants.

The extraction from the above mentioned studies is shown in Table 1 and Table 2.

## Conclusion

The summing-up and analysis of the presented literature data give us grounds for drawing the following conclusions:

The greatest number of the studies on bees and their products as bioindicators of the environmental pollutions have been carried out on contaminations with heavy metals followed by that with pesticides, radionuclides and other substances.

Of the metals, Pb, Cd and Zn are the ones most looked for and Fe, Mn, Ni, Cr, Cu, As and others more rarely. It has been established that Zn and Cd are mainly deposited on the surface of the bee body while Ni, Cd, Pb and Co are released with the excrements most often.

It has become clear from the literature references made that in all cases of pesticide implementation certain amounts of them are always accumulated in bees and their products.

Honey is most often tested for pollutants followed by the bee body, pollen, wax, propolis and faecal masses of bees. Studies on royal jelly, nectar honey and honeydew are in single numbers.

As a whole, the authors quoted in the present study think that bees and their products are suitable bioindicators of environmental pollutions. According to the studies of those of them who have compared the role of different bee products as bioindicators we have arrived to the conclusion that pollutants accumulate in bees and their products at different extent. Higher content of heavy metals and metalloids was found in excrements of bees compared to the body, which accounts for the bio-barrier function of the bee organism. Honeybees and, to a lesser extent, some of their products (pollen, propolis, wax), can be considered representative bioindicators of environmental pollution. Other authors, however, have assumed that bee honey is not among the appropriate bioindicators as compared to pollen and bees have only yielded tips for their possible use as bioindicator.

Generally it can be stated that the present literature review undisputedly shows that bees and their products are a suitable model for bio-monitoring of environmental pollution of various kind which renders them a promising subject for future studies in this field.

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### Thesis:

**Hristova D**, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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