# THE CONTENT OF HEAVY METALS IN HONEY AS INDICATORS OF POLLUTANTS

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**ABSTRACT:**
**Honey and honeydew are natural foods with a very complex composition that contain both, organic and inorganic ingredients. Regardless of the progress of the industry, it can't be replaced by some production process.**
**The quality of honey varies from year to year, and bees can never produce the same honey and honeydew. Weather conditions, grazing, treatment of bees, proximity to industry, roads, etc., greatly affect the quality of the obtained honey. Although minerals and heavy metals are minor constituents of honey, they play a vital role in determining its quality. The goal of the research is to assess the qualitative status of honey based on the content of contaminants, heavy metals from the area of the Tuzla Canton. The research was conducted on 30 (thirty) honey samples. The samples were collected in the period September/October 2022 and constitute the grazing of the specified year.**
**In the samples that were the subject of research, As and Cd did not exceed the limit of quantification (LOQ = 0.009 mg/kg). Current regulations does not define MRL's for these two metals. As for the quantified amount of lead (Pb), it was the same in 12 samples and in 11 samples there was an evident deviation from the MRL. The measured lead (Pb), values range from 0.06 to 5.34 mg/kg. The quality of bee products from the aspect of contamination with heavy metals can serve as bioindicator of environmental pollution, that is, as an indicator of the level of good beekeeping practices.**

**KEYWORDS:** honey; honeydew; heavy metals; Tuzla canton

## INTRODUCTION

Honey is primarily a concentrated sugar solution, composed mainly of glucose and fructose, together with other components such as organic acids, enzymes, vitamins, acetylcholine, flavonoids, minerals and trace elements [1]. The most common ingredients are carbohydrates, mainly fructose and glucose, and water, which together make up more than 99% of honey [2]. The biological activities of honey originate precisely from the compounds present in this natural food. In general, honey consists of approximately 200 different substances [3]. The chemical composition of honey is essentially related to factors such as the geographical region of origin, the flora of the region, the type of soil, the type of bee that produced it, the physiological state of the society, climatic conditions, processing conditions, handling, storage and storage time, the maturity of the honey [4]. The content of minerals and trace elements in honey can be used as an indicator of environmental pollution and an indirect indicator of the geographical origin of honey [5]. The determination of minerals, primarily heavy metals, in honey can be used to assess environmental contamination [6].

The chemical composition of honey from different botanical areas can vary. In this research, the focus is on the healthiness of honey that originates from the area of the Tuzla Canton. The aim of the research is to assess the quality of honey based on the content of contaminants with a focus on heavy metals (As, Cd, Pb) from the area of the Tuzla Canton..

The Tuzla canton is located in the northeastern part of Bosnia and Herzegovina, which in the south is characterized by a distinctly mountainous area (Konjuh, Javornik and Ozren), the valleys of the Spreča and Tinja rivers in the central part, while from the northwest to the southeast of the canton there are the massifs of Skipovac, Trebava and Majevica. [7]. Coal and rock salt are the two most important mineral resources of this region. The salt deposit is the only one of its kind in Bosnia and Herzegovina, while according to coal reserves, this area is the largest energy area of Bosnia and Herzegovina [8]. With over a billion tons of mineral reserves and a 100-year tradition of exploitation, the Tuzla Canton is the most important mining and industrial basin in Bosnia and Herzegovina. With 114,102 hectares of agricultural land, which is 49% of the total territory of the canton, agriculture is also a very important sector in Tuzla Canton [9].

The close source and product connection between plant-honey means that honey inherits different characteristics and shares biological properties with its corresponding botanical origin. [10]. For this reason, honey may contain undesirable compounds or remains of the original plant that was exposed to these substances, including those of anthropic origin. Among the residues that change the natural composition of honey are metals, which, depending on their concentration in food, can pose a risk to human health. An important aspect of honey quality is the presence of metals, which is directly related to the chemical composition of the soil in the areas where the bees feed. Therefore, the content of heavy metals in honey indicates contamination of the nearby soil, caused by volcanic and/or hydrothermal activities and weather conditions, among other factors that pose a risk to human health. [11, 12]. The usual route by which humans ingest and are exposed to metals is through the diet. Some heavy metals are essential elements for normal plant growth such as Co, Fe, Mn, Ni, Zn, and Cu and they play an important role in metabolism, but in higher concentrations the same metals become toxic. These increased levels can cause a decrease in the percentage of biomass in the plant and in many cases lead to the death of the plant. On the contrary, some heavy metals such as Pb, Cd, Cr and Hg are marked with high toxicity to plants [13].

Heavy metals are defined as metals whose density is greater than 5 g/cm$^3$. A total of 53 out of 90 natural elements are heavy metals [14]. In biology, the term "heavy" refers to a number of metals or metalloids that can be toxic to both plants and animals, even when their concentrations are very low. Soil contamination with heavy metals is different from water and air pollution, due to the fact that heavy metals remain longer in the soil than in water or air.[15].

The bee is active in the entire area around the hive although it is opportunistic in the sense that it prefers to gather pollen in nearby flower fields, the bee can move long distances, even up to ten kilometers in exceptional circumstances therefore the hive can maintain areas of seven square kilometers "under its control [16]. Honey can contain high levels of toxic elements, such as Hg, As, Cd and Pb as a result of increased amounts in plant nectar. Due to the large scale of mining and industrial activity, toxic metals are absorbed into the soil, atmosphere and water and consequently into plants. High concentrations of these metals were found in honey from areas with heavy industry, that is, near highways [17].

Cadmium is a non-essential, toxic element and is most often found in the soil in low concentrations, below 3 mg/kg. The availability of cadmium in the soil depends primarily on the type of soil, the form in which Cd is found in the soil, the content of organic matter, the pH of the soil and the cation exchange capacity. Soil contamination with cadmium can be caused by the use of mineral fertilizers, organic fertilizers and fertilizers derived from sewage sludge. Lead is a heavy metal found in exhaust gases from cars and factories. It is the main chemical pollutant of the surrounding environment. It appears in the form of ions $Pb^{2+}$, as lead tetraethyl, lead diethyl and as other alkyl derivatives of lead. The usual content of lead in agricultural soil is 2 to 100 mg/kg [15]. Among the most toxic elements present in nature is As. This element is widely distributed in nature, with the most important arsenic minerals being pyrite, realgar and orpiment. As concentrations found in soil are between 0.1 and 50 μg/g, with a mean value between 5 and 6 μg/g [18]. However, As levels can be much higher in soils polluted by human activity. Arsenic can accumulate in soil as a result of pesticide use, fertilizer application, and fossil fuel burning. Other As sources include industrial deposits and animal waste [19]. A significant part of As was produced by anthropogenic activity as a by-product of the formation of Cu, Pb, Co and Au. Gold minerals contain up to 11% As, while Pb and Cu minerals contain 2-3% As [20]. The toxicity of As varies depending on its chemical composition, whereby inorganic species are more toxic than organic species, and inorganic As is classified as a human carcinogen [21]. The Ordinance on Maximum Permitted Amounts for Certain Contaminants in Food ("Official Gazette of BiH", No. 68/14, 79/16, 84/18) prescribes the maximum permitted amounts of the following contaminants in food: nitrates, mycotoxins, metals, 3-monochloropropanediol (3-MPCD), polycyclic aromatic hydrocarbons, dioxins and dioxin-like polychlorinated biphenyls (PCBs), melamine and its analogues, accumulated radioactivity in the form of Cs134 and Cs137 and other metals: total arsenic (As), copper (Cu), iron (Fe) and nickel (Ni). The maximum permitted amounts for honey are defined as 0.1 mg/kg for lead, 2 mg/kg for copper and 20 mg/kg for iron.

## MATERIALS AND METHODS

### MATERIALS

The research was conducted on 30 samples of honey from the area of Tuzla Canton, i.e. from the areas of G. Tuzla, Gračanica, Gradačac, Kalesija, KladANJ, Lukavac, Srebrenik and Tuzla. The samples consisted of monofloral types of honey (linden, acacia), 13 of them, and polyfloral types of honey (floral/meadow), 7 of them, and mixed honey, 10 of them in total.

The samples were collected in the period September/October 2022 and constitute the grazing of the specified year. The condition that the samples had to meet was that they were delivered from stationary pastures in a quantity of at least 500g and in glass packaging. The test was carried out in October 2022, and until the moment of the test, the samples were stored in controlled conditions, without temperature variations, without direct light, in a dry and airy place.

Table 1. Overview of the geographical and botanical origin of the samples

| Sample's mark | Origin | Species |
|---|---|---|
| B1 | Lukavac | Acacia |
| B2 | Tuzla | |
| B3, B4, B5, B6, B7 | Gračanica | |
| L1, L2 | Tuzla | Linden |
| L3 | Gradačac | |
| L4 | Lukavac | |
| L5 | Tuzla | |
| L6 | Srebrenik | |
| P1 | Kladanj | Polyfloral |
| P2, P3, P4, P5 | Tuzla | |
| P6 | Gornja Tuzla | |
| P7 | Gradačac | |
| M1 | Kalesija | Mixed (polyfloral and honeydew) |
| M2 | Tuzla | |
| M3 | Gračanica | |
| M4 | Srebrenik | |
| MD1, MD3 | Tuzla | Honeydew |
| MD2, MD4, MD5, MD6 | Kladanj | |

## METHODS

The technique used to determine the content of heavy metals (Cd, As and Pb) is ICP - OES.

The apparatus on which the samples were measured is manufactured by PerkinElmer, and themodel is Optima 2100 DV. The ICP-OES instrument was invented by Stanley Greenfield (1964) and became an important analytical tool for the determination of about 75-90 elements from different samples.

Inductively coupled plasma is a stream of highly ionized argon that passes through the magnetic field of the coil. A high-frequency magnetic field ionizes argon, which is an inert gas, and plasma is formed. Plasma develops temperatures of 8000K – 10000K, which enables it to determine about 75 elements from the periodic table.

The technique used by ICP to measure samples is optical emission spectrometry (OES), i.e. the apparatus works on the principle of emission. When we introduce the sample into the plasma, which develops a high temperature, electrons are excited, which then go into an excited state. When returning to the basic state, light of a certain wavelength is emitted, which is measured on the detector. In order to transport the sample into the plasma, we must first disperse it, i.e. nebulize it, and we achieve this with nebulizers.

The gases used by ICP are argon, nitrogen and compressed air. Argon is used to form plasmaand clean the system of impurities when starting the device, while nitrogen is used to clean (purge) the optics. The compressed air removes the plasma tail and thus protects the optical parts from destruction.

## RESULTS AND DISCUSSION

The origin of heavy metals in the soil can be anthropogenic or natural and can be related to different soil fractions, which determine the mobility and availability of these metals. Heavy metals such as Pb, Cd and toxic elements such as Cr, As could reflect the presence of pollutants due to environmental contamination or pharmacological (antiparasitic or acaricidal) treatment of honey or incorrect procedures during the processing and conservation stages of honey [22].

The honey bee (Apis mellifera L.) and its products are currently also used as bioindicators of environmental pollution. These insects fly around nectar plants that grow up to 4 km from the hive, but can travel up to 12 km, accumulating pollutants present in the air, soil and water [23]. For this reason, honey can serve as an indicator material to assess the contamination of the environment from which bees collected nectar to make honey.

Honey can also be contaminated during its processing by beekeepers, the equipment and tools used, and the process itself. Materials such as aluminum, stainless steel, and galvanized steel used in honey processing tools and equipment can leach some metals (including Al, Cd, Co, Cr, Cu, Fe, Pb, Ni, and Zn) into honey [24, 25].

**Table 2.** Results of heavy metal content in honey samples

| Species | Sample mark | Origin | *Concentration As (mg/kg)* | *Concentration Cd (mg/kg)* | *Concentration Pb (mg/kg)* |
|---|---|---|---|---|---|
| ***Acacia*** | *B1* | *Lukavac* | 0,00 | 0,00 | 0,00 |
| | *B2* | *Tuzla* | 0,00 | 0,00 | 1,46 |
| | *B3* | *Gračanica* | 0,00 | 0,00 | 0,06 |
| | *B4* | *Gračanica* | 0,00 | 0,00 | 0,50 |
| | *B5* | *Gračanica* | 0,00 | 0,00 | 0,16 |
| | *B6* | *Gračanica* | 0,00 | 0,00 | 0,00 |
| | *B7* | *Gračanica* | 0,00 | 0,00 | 0,00 |
| | ***N: 7*** | ***Sr.vr.*** | **0,0** | **0,0** | **0,3** |
| | | ***SD*** | **±0,0** | **±0,0** | **±0,5** |
| ***Linden*** | *L1* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *L2* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *L3* | *Gradačac* | 0,00 | 0,00 | 0,00 |
| | *L4* | *Lukavac* | 0,00 | 0,00 | 0,00 |
| | *L5* | *Tuzla* | 0,00 | 0,00 | 4,75 |
| | *L6* | *Srebrenik* | 0,00 | 0,00 | 5,04 |
| | ***N: 6*** | ***Sr.vr.*** | **0,0** | **0,0** | **1,6** |
| | | ***SD*** | **±0,0** | **±0,0** | **±2,5** |
| ***Polyfloral*** | *P1* | *Kladanj* | 0,00 | 0,00 | 0,53 |
| | *P2* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *P3* | *Tuzla* | 0,00 | 0,00 | 0,74 |
| | *P4* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *P5* | *Tuzla* | 0,00 | 0,00 | 4,42 |
| | *P6* | *G. Tuzla* | 0,00 | 0,00 | 0,00 |
| | *P7* | *Gradačac* | 0,00 | 0,00 | 0,00 |
| | ***N: 7*** | ***Sr.vr.*** | **0,0** | **0,0** | **0,8** |
| | | ***SD*** | **±0,0** | **±0,0** | **±1,6** |
| ***Honeydew*** | *MD1* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *MD2* | *Kladanj* | 0,00 | 0,00 | 0,00 |
| | *MD3* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *MD4* | *Kladanj* | 0,00 | 0,00 | 4,88 |
| | *MD5* | *Kladanj* | 0,00 | 0,00 | 0,00 |
| | *MD6* | *Kladanj* | 0,00 | 0,00 | 0,00 |
| | ***N: 6*** | ***Sr.vr.*** | **0,0** | **0,0** | **0,8** |
| | | ***SD*** | **±0,0** | **±0,0** | **±2,0** |
| ***Mixed*** | *M1* | *Kalesija* | 0,00 | 0,00 | 2,43 |
| | *M2* | *Tuzla* | 0,00 | 0,00 | 0,00 |
| | *M3* | *Gračanica* | 0,00 | 0,00 | 0,00 |
| | *M4* | *Srebrenik* | 0,00 | 0,00 | 5,34 |
| | ***N: 4*** | ***Sr.vr.*** | **0,0** | **0,0** | **1,9** |
| | | ***SD*** | **±0,0** | **±0,0** | **±2,5** |
| ***Reference value*** | | | **ND** | **ND** | **max. 0,1** |

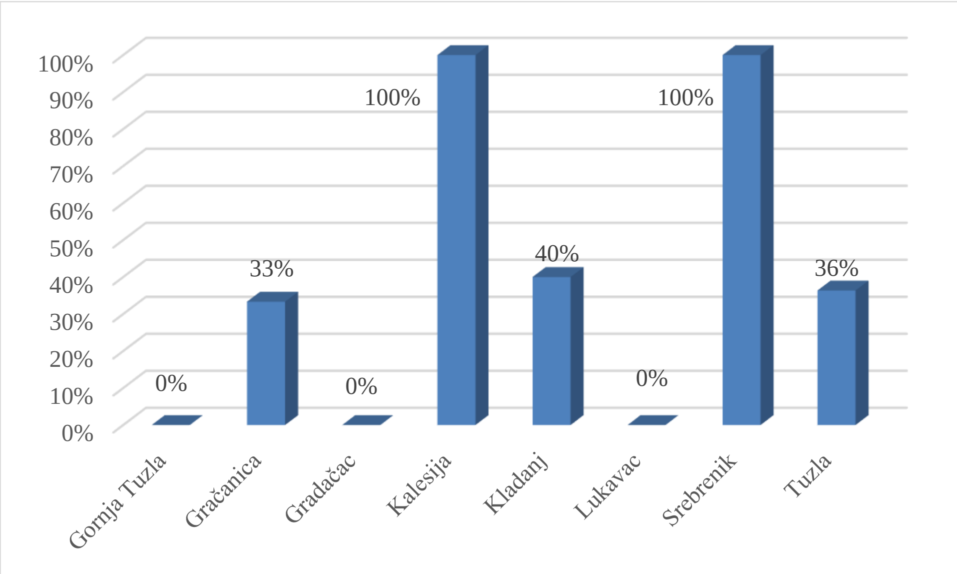
ND – not defined

In the samples that were the subject of research, As and Cd did not exceed the limit of quantification (LOQ), otherwise, for the method used, the limit of detection (LOD) was 0,003 mg/kg, and the limit of quantification was 0,009 mg/kg. Current regulations do not define MRLs for these two metals.

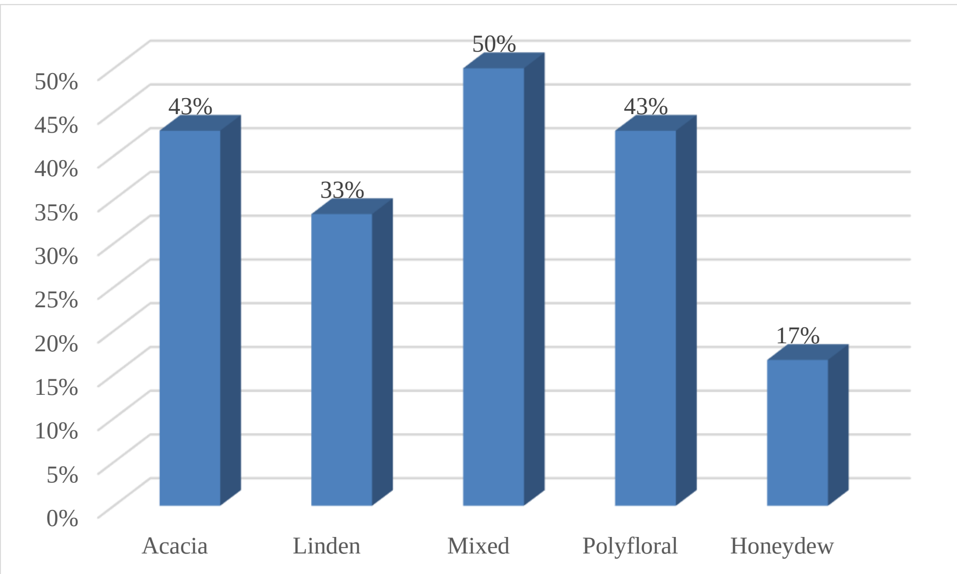
As for the quantified amount of lead, it was the same in 12 samples and in 11 samples there was an evident deviation from the MRL. It was quantified in all types of honey and from all areas except Gradačac and Lukavac and Gornja Tuzla. The measured lead values range from 0,06 to 5,34 mg/kg.

Headache, bad concentration and attention, irritability, memory loss can represent early symptoms of adverse effects exposure to Pb due to negative effect to the central nervous system [27]. Experimental studies have shown that Pb is potentially carcinogenic and is classified according to IARC as a probable carcinogen [21].

Based on the obtained results, two samples of acacia honey (B4 and B5) from the area of Gračanica contain an amount of lead above the permitted amount. Only one sample of acacia honey was analyzed from the Tuzla area, and the amount of lead above the prescribed value was detected in it. Two samples of linden honey (L5 and L6) contain an amount of lead above the permitted amount. Out of a total of six analyzed samples of medljikovac honey, one sample (MD4) from Kladnje contains an amount of lead above the prescribed value. Three (P1, P3 and P5) out of a total of seven samples of mixed honey contain more lead than the prescribed value. Samples P3 and P4 are from the area of Tuzla, while the third defective sample is from the area of Kladanj and is the only one from that region. In two (M1 and M4) out of a total of four samples of mixed honey, the lead content was quantified higher than the prescribed value, and these samples are from the area of Kalesija and Srebrenik.




**Graph 1**. Overview of % defective samples from the aspect of lead content by municipalities of Tuzla Canton




**Graph 2**. Overview of % defective samples from the aspect of lead content by botanical types from Tuzla Canton

Graph 1. shows that with regard to the municipalities of Gornja Tuzla, Gradačac and Lukavac, there are no samples with an increased concentration of lead in the tested samples. While all the samples that participated in the research from the areas of Srebrenik and Kalesija have an increased concentration of lead.

From graph 2., it is evident that the most samples of mixed honey have an increased concentration of lead, while the increased concentration of lead is the least in the honeydew samples.

Table 3. shows the results of other studies, which refer to the measured lead content in honey samples of different botanical species.

Table 3. Levels of heavy metals reported in honey samples from different countries [27]

| Origin | France | Croatia | Italy | Israel | Malaysia | Romania | USA |
|---|---|---|---|---|---|---|---|
| Pb (µg/kg) | 280.00 - 1080.00 | 4130.0 - 21590.0 | 100.00 - 1533.00 | 150.00 - 8220.00 | nd - 1017.00 | 20.00 - 6000.00 | <983.30 - 1534.80 |

nd – not defined

## CONCLUSION

Lead (Pb), contained in the air and originating mainly from motor traffic, can contaminate the air, and then directly nectar and honeydew. In general, Pb is not translocated by plants. Proximity to industry, beekeeping and agricultural practices as well as other anthropogenic influences can be the source and consequence of elevated lead concentrations in food. The general conclusion is that the contamination according to the types of samples and according to the regions is not constant or consistent, but it certainly leads to the conclusion that a more detailed investigation is necessary. According to the variability of the results, it can be concluded that this contamination can be caused by the use of galvanized or welded metal fittings. Honey is an acidic product that can react with surfaces containing lead and cause migration of lead into the honey. Namely, the wire for fixing the clock bases can be galvanized or made of stainless steel (so-called chrome or stainless steel wire). Stainless steel wire can be used multiple times when replacing honeycombs and installing new clock bases, while galvanized wire must be changed every time when replacing honeycombs, i.e. installing new clock bases. The wire in the frames must not be rusted (corroded), so as not to cause chemical contamination of the bee products. The beekeeper's knife, equipment for inserting clock bases and queen grids should be made of stainless material (galvanized, plasticized, plastic or chrome-plated queen grid, etc.) or that they are not rusted, and that they are made of a material that allows them to be easily cleaned, washed and disinfected. All equipment for brewing, filtering, storage and packaging should also meet all minimum sanitary, technical and hygienic standards.

During the collection of samples, auditing of the implementation of good beekeeping practices was not carried out, since this was not an aspect of the research, but certainly the results of this research point to the need to record and investigate the impact of inadequate practices on the healthiness of the product.

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