Potentially Toxic Metals in Honey from Latvia: Is there Connection with Botanical Origin?

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Abstract: - Distinction based on the botanical origin is widely applied for the recognition of honey types. Although, accurate detection of botanical species can be implemented by pollen analysis, quantification of trace and major elements in honey may be applied as more simplified method for the determination of honey botanical origin. Besides the minerals and essential elements honey may contain the traces of potentially toxic metals, such as Al, As, Cd, Co, Cr, Cu, Ni, Pb and Zn. Current study covers analytical investigation of 80 honey samples collected in Latvia. Quantitative analysis of elements was performed by atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). Botanical origin of honey samples within the statistical analysis was spread into 7 groups. Taking into account mean values of quantified potentially toxic metals possible honey contamination by honey type can be ranged as follows (from most to less contaminated type): commercially manufactured honey mixtures with unknown botanical origin > heather / forest blossom honey > polyfloral honey > meadows blossom honey > linden honey > buckwheat / clover honey > rape / spring blossom honey. Impact of botanical origin on the content of potentially toxic metals in honey might be connected with the specifics of certain growth conditions on the floral specie and possible influence of environmental pollution.

Key-Words: - Honey, botanical origin, heavy metals, ICP-MS, AAS, food analysis, Latvia.

1 Introduction

According to the definition set by the European Union Council Directive 2001/110/EC, “Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances or their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature”. There are several legally agreed parameters that must be applicable for overall characteristics of honey on market, such as detection of sugar content, moisture content, water-insoluble matter content, electrical conductivity, free acid content, diastase activity and hydroxymethylfurfural content [1]. However, these attributes can be applied for the recognition of honey as a food article with the certain quality properties but do not allow the apparent assessment of such important issues as the authenticity of geographical initiation that can be influenced by the site-specific factors, or botanical origin of certain honey sample [2, 3], as well as do not describe possible contamination of honey with potentially toxic elements.

Botanical origin of honey is one of the matters that can characterize differences between colour, taste, flavour and content of physiologically active ingredients [4, 5]. Apart of monofloral honey that is derived mainly from one kind of botanical species and known as more valuable for some floral species [4, 6], polyfloral honey due to its not specified botanical origin is more widespread. There is a wide range of monofloral honey types. Furthermore, the geographical initiation cannot be excluded assessing botanical origin of honey. Widely known monofloral honey from areas of temperate climatic zone is derived from such species as heather, linden, buckwheat, clover [7, 8], while tropical climatic zone allow the obtaining of monofloral honey from orange blossom, acacia, chestnut, rhododendron, eucalyptus, rosemary and other species [3, 5, 6, 9]. Botanical origin of honey can be of wild or natural and agricultural or orchard background, *e.g.* heather honey or rape honey, respectively [10].

Historical method used for the detection of honey botanical origin is pollen analysis or mellisopalynology. However, this method is specific, complicated and time consuming procedure [3, 7, 11]. Mineral and trace element content of honey is widely variable and dependent on geographical, climatic, as well as site specific conditions and may be influenced by botanical origin in great extent [2-4, 11-13].

Quantitative analyses of honey have been carried out in many European countries (Poland, Italy, Greece, Hungary, France *etc.*). The results show quite wide ranges of honey contamination with potentially toxic elements. For example, the highest Pb content has been detected up to 79.1 mg/kg in Slovenian honey [14, 15]. Content of Cr varies from <1.0-37.0 μg/kg in honey from Switzerland [12, 14] up to 0.11-33.8 mg/kg in Slovenian honey samples [14, 15]. Significant influence of botanical origin on the content of elements in honey was detected by Pisani (2008) analyzing polyfloral, sulla, clover and honeydew honey samples collected in Italy, however, content of potentially toxic elements (As, Cd, Sb, Pb) in honey of different types was assessed as very low [16].

The aim of the research is to study the possible pollution levels of potentially toxic elements and evaluation of the accumulation of contaminants in food articles. Latvia, a relatively small country in the East of Europe, was set as the target area for this research due to the absence of large pollution sources and low industrial and agricultural activities within the territory of the country. This study involves analysis of quantitative content of potentially toxic metals (Al, As, Cd, Co, Cr, Cu, Ni, Pb, Zn) in honey samples of different botanical origin collected in Latvia.

2 Materials and Methods

2.1 Sampling

Within the current study 80 honey samples were collected in Latvia in a period of two seasons (2009 and 2010). Honey samples were purchased at the local food markets, supermarkets or straight from the bee-keepers. Taking into account the information provided by honey producer companies on the product trade labels or by the bee-keepers themselves, it was possible to identify botanical origin of 77 samples, while 3 samples were assessed as commercially manufactured honey mixtures, therefore, without certain botanical origin.

To avoid possible honey sample contamination no metal tools were used for analytical sample preparation. Honey samples were kept in plastic containers or glass vessels with tight plastic covers and until analysis were stored in a cool and dark place.

2.2 Sample preparation

Honey is a substance with naturally formed complicated biological composition that contains high levels of carbohydrates and such constituents as proteins, organic and inorganic acids, amino acids, vitamins, enzymes and hormones, flavonoids and range of minerals and trace elements [5, 15]. Such a complex matrix may influence analysis of single components. Therefore honey sample preparation before operation with quantitative techniques is an important issue. Widely described honey sample pre-treatment may be carried out by honey decomposition in acidic solutions [7, 11, 17].

Experimental mode of several ways of sample preparation such as dissolution of honey in acidified water, cleavage of organic matter by concentrated nitric acid and dry ashing was performed. Wet digestion is widely described method of pre-treatment of biological and environmental samples [*e.g.* 6, 11]. Its principles are based on the matrix decomposition with concentrated acids (mostly nitric acid) with adding hydrogen peroxide as oxidiser and following by sample heating in opened or closed systems or by sample executing under the pressure and microwave influence [*e.g.* 2, 7]. Applying wet digestion for honey decomposition samples were diluted in the mixture of concentrated nitric acid and concentrated hydrogen peroxide (2:1) (only certified ultra pure analytical reagents were used). Then samples were heated at 160 °C until the complete evaporation of nitrogen oxides. Subsequently solutions were filled up with deionised water.

Another method applied for decomposition of honey samples was based on simple honey dissolution in warm deionised water following by acidification with nitric acid [18, 19]. Dry ashing of honey samples also was performed for overall comparison of honey sample pre-treatment methods [5, 11, 20]. However, obtained analytical results from dry ashing were not satisfied in relation with other methods.

Honey dissolution in warm water was selected as the most optimal, enough accurate, analytical reagents saving and less labour-consuming method. Honey sample pre-treatment procedure was performed as stated: precise mass of honey (0.5000 g) was weighted in polypropylene tubes following by dissolution in 50 ml of warmed-up deionised ultra pure water. Then sample solutions were acidified by adding 0.2 ml analytically pure concentrated nitric acid. Sample pre-treatment procedure was carried out for triplicates of every single honey sample [21]. As reference material for the control of analytical performance certified reference sample NCS ZC73017 (GBS-10)-Apple was used.

2.3 Applied analytical methods

Several analytical methods can be used for trace and major element detection in honey. The most recent methods are, for example, inductively coupled plasma mass spectrometry (ICP-MS), inductively plasma atomic emission spectrometry (ICP-AES), varieties of atomic absorption spectrometry (AAS) and methods based on X-ray fluorescence spectrometry such as total reflection X-ray fluorescence spectrometry (TXRF) [*e.g.* 2, 22, 23]. The choice of the appropriate analytical method for honey quantitative analysis should be grounded by the certain accuracy, selectivity and high level of certainty [11, 13, 22].

Quantitative analysis of honey samples within the current study was done by atomic absorption spectrometry on graphite furnace (GFAAS) and atomic absorption spectrometry in flame (FAAS) using "Perkin Elmer AAnalyst 200" spectrometer. By AAS it was possible successfully to quantify such elements as Ca, Cu, Fe, K, Mg, Mn, Na and Zn. However, difficulties arose for quantitative detection of As, Cd, Co, Cr, Ni and Pb due to the low concentrations of these elements in sample solutions. Therefore, for elements that are supposed not to exceed trace levels (*e.g.* Al, As, Ba, Cd, Ce, Co, Cr, Ni, Pb, Rb, Sr, V) in honey samples inductively coupled plasma mass spectrometry (ICP-MS) is more suitable method. It was applied by using "Thermoelectronic" ICP-MS spectrometer. Measurements were performed by background correction. Analyses of blank samples were used for determination of limit of detection and level of quantification.

2.4 Approach of statistical analysis

Current study involves statistical analysis of obtained results only regarding potentially toxic metals (Al, As, Cd, Co, Cr, Cu, Ni, Pb, Zn) detected in honey samples from Latvia.

For statistical data analysis honey samples (total n=80) were divided in 7 groups according to their botanical origin: 1) polyfloral not defined honey (n=33); 2) heather and forest blossom honey (n=16); 3) rape and spring blossom honey (n=5); 4) buckwheat and clover species honey (n=9); 5) linden honey (n=6); 6) meadows blossom honey (n=8) and 7) commercially manufactured honey mixtures with unknown botanical origin (n=3).

Statistical data analysis was performed by using MS Excel 2007. Box-whisker plotting was chosen as the most appropriate descriptive statistical approach within this study for the comparison of different data sets. Such data analysis allows easy determination of the data sets' outliers. The box-whisker plots show range between 25th and 75th quartiles as well as the median values. As outliers were assessed values above and below the 1.5 interquartile box lengths. However, the detected outliers were assessed as not significant and are excluded from graphical images.

3 Results and Discussion

Detection of potentially toxic elements, also known as heavy metals, in biological samples is important issue of concern as well as within the environmental studies as in the researches related to the human health. Drinking water and food articles are the main source of essential elements for animals and humans but in addition they can provide living organisms with unwanted and possibly harmful contaminants [20, 24]. Kabata-Pendias and Mukherjee (2007) differs non essential and highly toxic elements (Be, Bi, Cd, Hg, Pb and Tl) from just non essential elements (Ag, Au, Cs, Hf, In, Ir, Sb, Ta, Te, U, Y, Zr). However, many of elements that are assessed as essential or possibly essential (*e.g.* Al, As, Ba, Co, Cu, Cr, Ni, Sn, Zn) for humans may become potentially toxic if consumed with food and water in elevated concentrations [24]. Therefore monitoring of food articles for the contamination of potentially toxic element is highly significant.

The analysis of honey samples collected in Latvia revealed that in overall potentially toxic metals can be detected in the following sequence: Zn > Al > Cu > Ni > Cr > Pb > Co > Cd > As (based on mean results, n=80). Honey is known as the easily contaminable product if stored or processed by inappropriate, mostly metal alloys, equipment [8], as well as a biological product that can reflect environmental pollution or geochemical specifics of area where bees collect nectar [11, 20]. The overall list of metals detected leads to think of possible contamination at storage and processing as, *e.g.* Al, Cu, Ni and Zn are the ordinary constituents of metallic household and kitchen equipment.

Taking into account the botanical origin of honey collected in Latvia significant differences in the concentrations of potentially toxic metals were detected. The highest variability of zinc concentration was observed for commercially manufactured honey mixtures (0.283-11.599 mg/kg), followed by heather / forest blossom honey and polyfloral honey samples, while rape / spring blossom honey contained the lowest concentration of this element (Fig. 1). Some outliers appeared in the group of polyfloral honey samples, *i.e.* one sample contained 18.89 mg/kg of Zn. Honey analysis in the neighbour country Lithuania revealed obvious honey contamination with Zn up to 41.25 mg/kg in a case when honey sample were kept in a vessel covered with zinc [8]. That approves the importance of equipment materials used for honey storage and processing.

Although the widest spread of aluminium concentration was detected for polyfloral honey (0.088-1.15 mg/kg), the highest mean concentrations refer to the honey mixtures

(0.616 mg/kg) and heather / forest blossom honey (0.485 mg/kg) (Fig. 1). For comparison, nectar honey samples collected in Czech Republic contained Al in higher levels (0.61-5.49 mg/kg) [23]. Other studies showed that rape honey in Poland may contain Al up to 11 mg/kg but buckwheat honey in less levels (up to 2.27 mg/kg) [7].

The highest ranges of variability, as well as the highest mean concentrations of copper were detected for heather / forest honey samples (0.347 mg/kg) and buckwheat / clover honey samples (0.251 mg/kg) (Fig. 1). Regarding the botanical origin in the study by Spanish scientists it has been found out that orange blossom honey may contain much higher values of Cu (42.59-90.90 mg/kg) than other honey types [3], while mean (and maximum) values for rape and buckwheat honey in Poland were 0.174 mg/kg (1.69 mg/kg) and 0.913 mg/kg (1.55 mg/kg), respectively [7].

Range of Ni concentration within the groups of honey samples was not as variable as for other elements. The highest mean content of nickel was detected in heather / forest blossom honey (0.054 mg/kg) (Fig. 2).

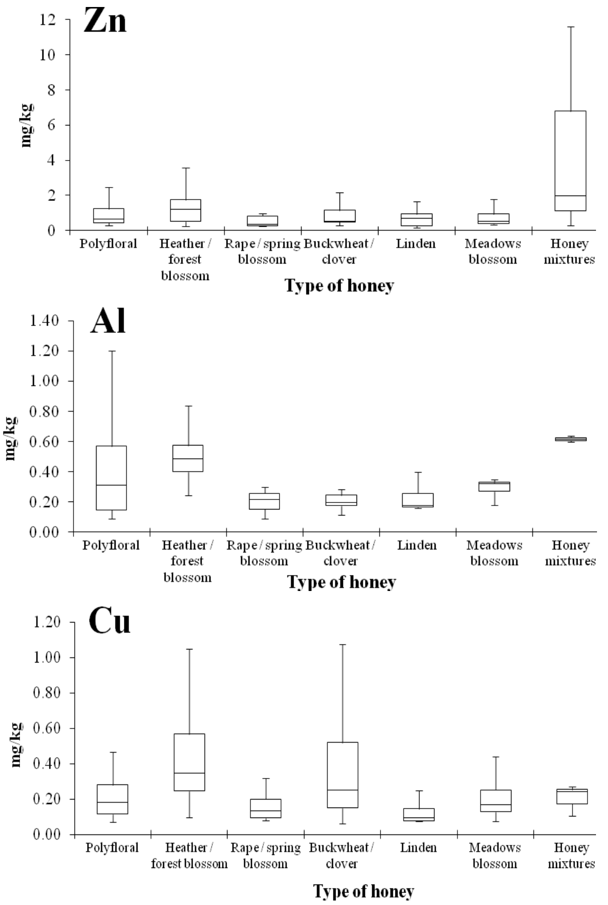

Fig. 1. Box-whisker plots of concentrations of Zn, Al and Cu in honey samples (n=80) of different botanical origin collected in Latvia.

However, detected values of Ni are much lower than detected in Lithuanian honey samples 0.206-0.350 mg/kg [8] and in nectar honey from Czech Republic 0.06-0.40 mg/kg [23].

Different results were gained for lead. The highest levels of Pb were detected in linden honey (mean 0.0143 mg/kg) (Fig. 2), as well as in this group was detected one outlier with Pb concentration of 0.1473 mg/kg. Contamination of linden honey with Pb might be connected with the possible impact of pollution because linden trees widely grow in urban areas or near the roadways. In Hungarian study Pb concentration was detected 0.024-0.163 mg/kg for polyfloral honey and 0.017-0.144 mg/kg for acacia honey [22]. But in Italian study lead was detected 0.0468-0.0525 mg/kg for clover honey and 0.0282-0.3040 mg/kg for polyfloral honey [16]. Lead is an element which in long term can easily accumulate in living organisms and may cause serious adverse effects [24], therefore its presence in food articles should be kept under regular control.

In many samples chromium was detected below the detection limit (<0.02 mg/kg) therefore it was not possible to make a box-whisker plot for this element. However, in ones sample of meadows blossom honey Cr was found 0.31 mg/kg and in one linden honey sample 0.18 mg/kg, that might be resulted from traffic pollution taking into account the geographical origin of these samples. Other studies reveal that Cr might be found up to 0.088 mg/kg in polyfloral honey and up to 0.109 mg/kg in acacia honey in Hungary [22], as well as 0.0051-0.0930 mg/kg in rape honey in Poland [1]. Cr contamination can be connected with pollution created by anthropogenic activities.

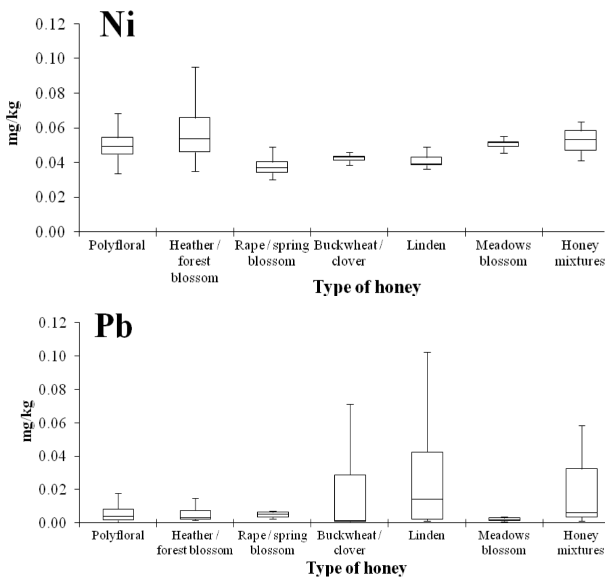

Fig. 2. Box-whisker plots of concentrations of Ni and Pb in honey samples (n=80) of different botanical origin collected in Latvia.

The lowest concentrations of cobalt were detected in linden honey but the highest variability for the heather / forest blossom (0.0047-0.0133 mg/kg) honey and meadows blossom honey (0.0064-0.0134 mg/kg) while the highest mean was detected for manufactured honey mixtures (Fig. 3).

The widest range of cadmium concentration was observed for polyfloral honey while the highest mean value refers to commercially manufactured honey mixtures (0.0056 mg/kg) (Fig. 3). In the group of polyfloral honey some outlier values were detected with concentration of Cd 0.027 mg/kg and 0.012 mg/kg. Other studies show that concentrations of Cd in honey can be detected in higher levels, *e.g.* for rape honey up to 0.082 mg/kg and for buckwheat honey 0.0025-0.030 mg/kg in Poland [7].

Wide variability of arsenic concentration was detected for heather / forest blossom honey, following by linden and polyfloral honey groups. The highest mean concentrations refer to linden honey (0.0031 mg/kg) and heather / forest blossom honey (0.0029 mg/kg) (Fig. 3). Arsenic has been quantified in acacia honey up to 0.013 mg/kg and up to 0.006 mg/kg in polyfloral honey in Hungary [22] and 0.0046-0.0055 mg/kg in clover honey and 0.0028-0.0111 mg/kg in polyfloral honey in Italy [16].

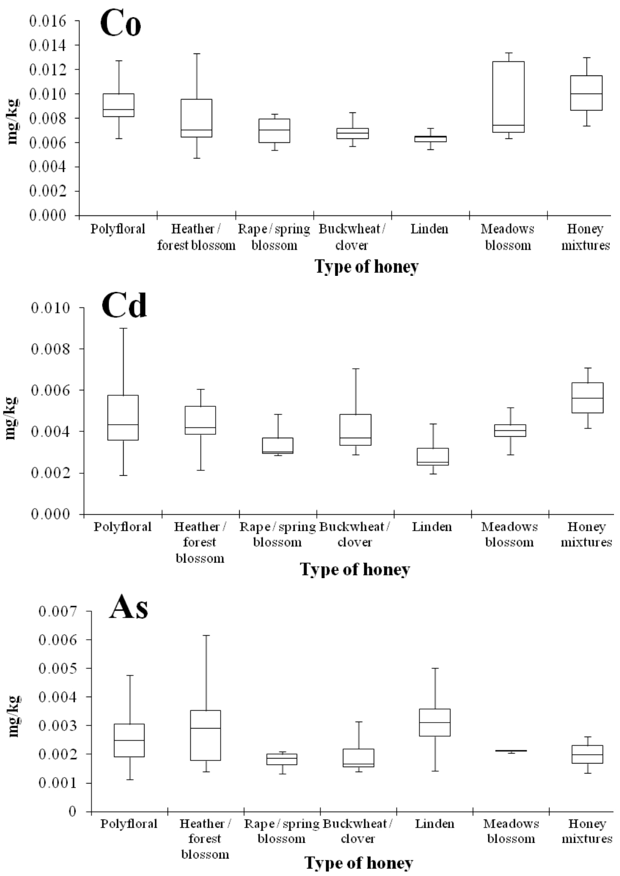

Fig. 3. Box-whisker plots of concentrations of Co, Cd and As in honey samples (n=80) of different botanical origin collected in Latvia.

4 Conclusions

1) Impact of botanical origin on the content of potentially toxic metals in honey is obvious. Overall average concentrations were detected lower than in similar studies worldwide, however, due to appearance of outliers significant contamination risks remain in some cases. Taking into account mean values of quantified potentially toxic metals possible honey contamination by honey type can be ranged as follows (from most to less contaminated type): commercially manufactured honey mixtures with unknown botanical origin > heather / forest blossom honey > polyfloral honey > meadows blossom honey > linden honey > buckwheat / clover honey > rape / spring blossom honey.

2) According to detected variability between the groups of honey samples and quantified elements, impact of botanical origin on element content in honey can be connected with the growing conditions of the floral plant species. For example, some elements were detected in higher levels in linden honey (Pb and As) and it is known that linden trees are widely grown in urban areas or near the traffic lines that can be a source of element contamination. Agricultural activities and use of agrochemicals and fertilizers also can become a source of contaminants for some types of honey such as rape honey or buckwheat honey.

3) Honey storage and processing equipment can be assessed as one of the honey contamination ways, especially for such elements as Al, Cu and Zn. This fact can be attributed to commercially manufactured honey mixtures that might be processes with inappropriate equipment.

4) For more precise and detailed assessment of honey contamination multi-factor analysis should be applied taking into account not only botanical origin but also of geographical origin and site specific peculiarities.

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