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CONTAMINATION OF *BETULA VERRUCOSA* EHRH. POLLEN BY MICROORGANISMS, MYCOTOXINS AND HEAVY METALS

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ABSTRACT

Pollen samples of silver birch from Ukraine and Slovakia were investigated in order to estimate their contamination with heavy metals (Cd, Pb, Hg, Cr, As, Ni, Se, Co), representatives of the *Enterobacteriaceae* family, mesophilic aerobic bacteria, fungi and mycotoxins. The study is focused on the comparative analysis among pollen samples when complex of biotic, abiotic and anthropogenic factors in different places of growth affect. The obtained results are compared to the existing requirements. It is established excess of limits on the content of cadmium, lead, chromium, the concentrations of enterobacteria and mesophilic aerobic bacteria in some samples of birch pollen. The Tukey test was used to determine the differences between the means at a level of $P < 0.05$. Also in this work, the steps from collection to storage of wind-pollinated pollen were analyzed.

Keywords: Pollen, *Betula verrucosa* Ehrh., habitat, requirements, heavy metals, mycotoxins, microflora

INTRODUCTION

According to **Bogdanov (2004)**, there are sensory, microbiological and physico-chemical quality criteria of pollen. These quality criteria are designed for bee pollen at the national level (**Campos *et al.*, 2008**). International standards there is no exist today (**González *et al.*, 2005; Campos *et al.*, 2008; Brovarskij *et al.*, 2010; Hani *et al.*, 2012**).

Distinguish pollen of entomophilous and anemophilous plants, bee pollen (collected and processed by bees) and natural pollen (obtained by manual collection of flowers) (**Brovarskij *et al.*, 2010**). **Faegri** and **Van der Pijl** gave more precise classification of pollen (**Hani *et al.*, 2012**). They defined two major types of pollen depending on the object of its distribution: biotic pollination, in which the pollen dispersal agent is an animal (an invertebrate or a vertebrate) and abiotic pollination where pollen is dispersed by an inanimate physical agent, such as wind or water. Pollen composition varies greatly depending on the type of its origin (**Campos *et al.*, 2008; Brovarskij *et al.*, 2010**). Bee pollen is used the most commonly as a functional food and medicinal plant product (**Ahmetova *et al.*, 2011; Hani *et al.*, 2012; Bogdanov, 2012**).

According to published studies, microbiological safety is the main pollen quality criterion (**Bogdanov, 2004**). Inside anther pollen is sterile (**Hani *et al.*, 2012**). Microbial contamination of pollen can be attributed to honey bees, weather, plant materials, insects and animals, humans and their agricultural devices, as well as during storage (**González *et al.*, 2005; Brovarskij *et al.*, 2010; Hani *et al.*, 2012**). **Bogdanov (2005)** notes that the risk of contamination of bee products, going on from beekeeping practices than from environmental factors. In the case of anemophilous pollen, which has the allergenic potential, contamination by microorganisms possible by interaction with other bioaerosols, when pollen leaving the pollen sac, during falling of pollen or because contamination of plants by yeast and fungi is widespread (**Śpiewak *et al.*, 1996; Ahapkina, 2007**). Taking into account the nutrient content of pollen, a variety of microorganisms could grow in it (**González *et al.*, 2005**). Microorganisms excrete exoenzymes to environment that destroy the cell wall of pollen and use the necessary substances in their metabolism (**Brovarskij *et al.*, 2010**). It is important to control the microbiological quality, especially the absence of pathogenic germs and fungi following the legislation applied for food production (**Bogdanov, 2004; Hani *et al.*, 2012**).

Another important issue that needs special care is that of mycotoxins that could theoretically develop in bee pollen after mould spoilage (**Bogdanov, 2005**). Mycotoxins are waste products of microscopic fungi (**González *et al.*, 2005; Antonjak *et al.*, 2009**). Toxic substances from the air, water and soil through the plants get to beekeeping products (**Chekryga, 2006**). Depending on environmental conditions, pollen can also be an optimum medium for growth of molds such as *Fusarium* and *Penicillium* (**Rodríguez-Carrasco *et al.*, 2013**). The main damage is applied to «a storage mould» fungi of the genera *Penicillium, Aspergillus, Mucor* (**Chekryga, 2006**). If collection, storage and marketing practices of pollen are not appropriate, fungi might develop in it as it happens in cereal grains (**González *et al.*, 2005**). In recent years, the problem of mycotoxins becomes more acute (**Gojster *et al.*, 2009**). Studies of mycotoxins of bee pollen in different countries are actual: Spain, Russia, the USA, Slovakia, Algeria, Argentina (**González *et al.*, 2005; Chekryga, 2006; Niu *et al.*, 2010; Kačániová *et al.*, 2011; Hani *et al.*, 2012; Rodríguez-Carrasco *et al.*, 2013**). Mycotoxins have teratogenic, embryotoxic, allergenic, dysbacteriotic and induce genetic damage and carcinogenesis. Currently there are several hundred mycotoxins which are synthesized in the cells of microscopic fungi, and their number increases because of revealing new species of micromycetes (**Antonjak *et al.*, 2009**).

Also it is necessary to determine the levels of heavy metals, radionuclides, pesticides in pollen (**Rachkov, 2008**). Pollen may be polluted by heavy metals and pesticides from the air and soil, despite the fact that pollen grains are the male sex cells and are protected by anthers of flowers (**Brovarskij *et al.*, 2010; Skrebneva *et al.*, 2012**). Content of heavy metals in generative plant organs generally is insignificant, that has the big biological value for conservation of ability to a reproduction. However, researches of some authors indicate that heavy metals are able to move to the reproductive organs of plants. Their content in the generative organs rises with a significant increase of levels of metals in the soils (**Kajgorodov *et al.*, 2010**). Heavy metals are an important ecological factor which, on the one hand, necessary for the plants, and with another hand, (increasing the concentration of these elements in the environment) are the negative factor in their life activity (**Hosue, 1999; Tanashhuk, 2005; Bilanych, 2008; Skrebneva *et al.*, 2012**). Tolerance of plant populations to heavy metals are mainly highly specific and genetically inherited (**Bilanych, 2008**). Stress, caused by heavy metals, a long one and includes all stages of plant development from «seed to seed». It is shown that several zones of protection function in a plant, preventing the penetration and action of heavy metals. The maximum protection is realized at the level of the reproductive organs, especially pollen, for which hypersensitivity to pollutants is determined (**Hosue, 1999**).

Silver birch (*Betula verrucosa* Ehrh., syn. *B. pendula* Roth.) is the representative of wind-pollinated species. Bees collect its pollen only in lack habitual pollen for

them. In Northern Europe silver birch and downy birch are the most commercially important broadleaved tree species (**Siljamo *et al.*, 2008; Hynynen *et al.*, 2010**). In 15 European countries, these two species are included in the list of the 10 most widespread species. In Ukraine, total volume of birch is 75 million m^3, proportion of the total volume is 4%, in Slovakia – <4 million m^3, <1% respectively. Birch produces pollen in abundant quantities (**Sofiev *et al.*, 2005; Hilaire, 2007**). It is very accessible for practical application. It has useful properties (**Danikov, 1993; Kucik *et al.*, 2001; Saarinen *et al.*, 2011**). But on the other hand, the birch pollen is allergenic and has the biocontaminant status (**Mothes *et al.*, 2004; D'Amato *et al.*, 2007; Shamgunova *et al.*, 2010; Pawankar *et al.*, 2011; Vorobec' *et al.*, 2012**; **Rodinkova, 2013**). Individual feature of tree, its age, degree of crop capacity and also the influence of environmental factors and the geographical origin of a tree affect the quality of pollen (**Valetova, 2009**).

Earlier own researches have shown that pollen of *Betula verrucosa* Ehrh., collected in Ukraine and Slovakia, shows high antioxidant activity *in vitro*, that as it is supposed, is caused by protective properties of the protein-lipid complex of pollen (**Shevcova *et al.*, 2013**). Also results on the microflora of Ukrainian pollen samples by representatives of *Enterobacteriaceae* family, anaerobic bacteria and fungi have been published (**Shevtsova *et al.*, 2013**).

In order to determine the degree of contamination of *Betula verrucosa* Ehrh. pollen by heavy metals, mycotoxins during conservation, and whether it affects its biological activity the following results have been received.

MATERIAL AND METHODS

Collection of samples

Pollen of *Betula verrucosa* Ehrh. (BV) has been prepared prior to the beginning of anthesis in Ukraine from 18 to 24 April 2011 and in Slovakia from 15 to 21 April 2013. The choice of collecting places of silver birch pollen based on the selection of the various conditions of trees growth, the degree of anthropogenic influence, belonging to different Chernobyl zones in Ukraine, and also to rather close locating from each other for possibility to collect pollen samples during flowering period. In total, 7 Ukrainian samples of birch pollen and 3 Slovak have been prepared. Heavy metals and mycotoxins were defined only for several samples. Topographic indicators (Latitude, Longitude and Altitude) are shown in Table 1. These samples are: BV1 – from Kyiv park zone near the road; BV3 – from Hotsky Kyiv region forest; BV4 – from the highways and housing estate of Ivankiv, Kyiv region in III Chernobyl zone; BV6 – from the territory of Borodyanka airdrome, Kyiv region, IV Chernobyl zone. All Slovak birch pollen samples has been prepared in the city of Nitra: near the highway (BV7), the Botanical Garden of the Slovak Agricultural University (BV8), from the housing estate near the highways (BV9). Based on the results of microbiological analysis, mycotoxins and heavy metals were determined for only one sample – BV9.

Table 1 Topographic data of sampling sites of *Betula verrucosa* Ehrh. pollen

Sampling site	Pollen sample	Latitude	Longitude	Altitude, m
Kyiv, Ukraine	BV1	50.27	30.31	136
Hotsky, Ukraine	BV3	49.53	31.37	126
Ivankiv, Ukraine	BV4	50.55	29.53	92
Borodyanka, Ukraine	BV6	50.38	29.58	118
Nitra, Slovakia	BV7- BV9	48.18	18.05	149

The samples were collected aseptically, placed in sterile plastic bags and brought to the laboratory. Pollen was dried at room temperature in the shade and kept in a freezer at a temperature of –7ºC.

Determination of heavy metals

Pollen samples were sent to the Environment laboratory EL spol. s r.o., Spišská Nová Ves, Slovakia to investigate the content of heavy metals. Their content was determined by electrothermal atomic absorption spectrometry (ETAAS), hydride generation atomic absorption spectrometry (HG-AAS) and atomic absorption spectrometry (AAS-AMA). Results are presented as mean ± standard deviation.

Determination of microflora

The concentration of bacteria in the Slovak pollen samples was determined by dilution plating. One gram of each sample was suspended in 99 ml of distilled water. After vigorous shaking, 10-fold serial dilutions were made up to 10^{-3}. The 1 ml aliquots of each dilution were spread on duplicate sets of media appropriate for determination of enterobacteria, mesophilic aerobic bacteria and fungi. The serial dilutions were inoculated on nutrient media. The plates with enterobacteria were incubated on Endo agar for 24 hours at 37°C, mesophilic aerobic bacteria were incubated on GTK medium for 48 hours at 30°C and fungi on Sabouraud's medium for 54 hours at 25°C respectively. The data are reported as colony forming units (cfu) per 1 g of pollen and log cfu/g. Fungi were isolated and determined using the «Dictionary of the Fungi» **(Kirk *et. al.*, 2001)**.

Determination of mycotoxins

Mycotoxins in pollen samples were also determined in the Environment laboratory EL spol. s r.o., Spišská Nová Ves, Slovakia. HPLC method with fluorescence detection was used (HPLC/FD). Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The heavy metal content in the pollen has been analyzed to assess the status of environmental pollution by these metals. It is necessary to investigate the content of toxicants and functional status of individual organs and tissues of the plant for an assessment of the negative influence of environmental factors on the physiological reactions of plants (**Koljasnikova *et al.*, 2011**). Based on the expression of a significant part of genes of a genome of an adult plant in pollen, it is possible to judge the stability of any species of a plant to this or that environmental factor by the reaction of pollen to this environmental influence (**Ljah *et al.*, 2004**). Accumulations of pollutants in plants are complex enough and depend on many factors: the nature of pollutant, its concentration, morphological and physiological characteristics of plants, their age, environmental conditions (**Kajgorodov *et al.*, 2010**).

According to the study, pollen of silver birch from different habitats differs by the heavy metal content between samples, in some cases exceeds the requirements (Tab 2 and 3).

Table 2 The heavy metal content in *Betula verrucosa* Ehrh. pollen, mg/kg

Heavy metal	BV1		BV3		BV6		BV9		Mean
	$x \pm S_x$	V%	$x \pm S_x$	V%	$x \pm S_x$	V%	$x \pm S_x$	V%	x
Cd	0.103 ± 0.005	18	0.560 ± 0.005	9	0.210 ± 0.005	18	0.046 ± 0.005	18	0.230
Pb	0.33 ± 0.05	18	0.31 ± 0.05	18	0.52 ± 0.05	18	0.24 ± 0.05	18	0.35
Hg	0.003 ± 0.002	13	0.004 ± 0.002	13	0.005 ± 0.002	13	0.005 ± 0.002	13	0.004
Cr	0.50 ± 0.3	17	0.51 ± 0.3	17	0.87 ± 0.3	17	0.84 ± 0.3	17	0.68
As	<0.3 ± 0.3	–	<0.3 ± 0.3	–	<0.3 ± 0.3	–	<0.3 ± 0.3	–	<0.3
Ni	1.56 ± 0.2	19	2.41 ± 0.2	19	2.10 ± 0.2	19	0.67 ± 0.2	19	1.69
Se	0.24 ± 0.10	19	0.67 ± 0.10	19	0.12 ± 0.10	19	0.19 ± 0.10	19	0.56
Co	<0.1 ± 0.1	–	0.15 ± 0.1	14	0.11 ± 0.1	14	ND	–	0.13

Legend: x ± Sx – mean ± standard deviation, V – coefficient of variation in %, mean – arithmetic mean, ND – not determined

Table 3 Requirements of heavy metals content in bee pollen, mg/kg

Source	Cd	Hg	Cr	As	Pb
Chlebo *et al*., 2001	0.100	0.050	0.500	1.000	–
Polish standard, 2007	0.05	0.02	–	0.20	0.50
Campos *et al*., 2008	0.03	0.01	–	–	0.5

Lead and cadmium are considered as major toxic heavy metals and, thus, the most frequent subject of researches (**Brovarskij *et al.*, 2010; Koljasnikova *et al.*, 2011**). Cadmium in soil doesn't decompose, remains in it, which results in contamination of a plant, mainly because of diffusion into the root system. Industrial gases and transport are the typical sources of lead and cadmium (**Solnceva *et al.*, 2010**). Different studies show that pollen is very suitable for the cadmium indication, because it contains a lot of protein substances with groups that cadmium joins in with (with sulfhydryl groups -SH). Content of cadmium is in 1.03-5.6 times greater than the requirements in all pollen samples, except Slovak (BV9). Content of lead is in normal in all pollen samples, except its insignificant excess (1.04 time) in pollen sample from the territory of airdrome (BV6). Also in these samples, BV6 and BV9, the chromium content is exceeded the requirements in 1.68-1.74 times.

Ljah *et al.* (2004) studied the sensitivity of the male gametophyte of *Betula pendula* Roth. to heavy metals by the ability of pollen to germinate on artificial nutrient medium with salts of heavy metals (Pb, Cr, Cu, Zn) in concentration of 0.1, 1.0 and 10 mg/l. It has appeared pollen of *Betula pendula* Roth. was the most sensitive to Cu and Zn. These metals inhibited the growth of the pollen tubes. It was found that pollen of *Betula pendula* Roth. is applicable for bioindication of pollution of the environment by heavy metals (**Hmelevskaja, 2008; Solnceva *et al.*, 2010**). In Slovakia **Supuka (1993)** showed direct dependence the dynamics of accumulation of pollutants, including heavy metals, in the leaves of *Betula pendula* Roth. in urban environments, near highways and recreational areas from the level of soil contamination (**Solnceva *et al.*, 2010**).

The results of research of microflora of Slovak birch pollen samples are presented in Table 4 and Figure 1. Results of the microflora of Ukrainian birch pollen samples have been presented earlier (**Shevtsova *et al.*, 2013**). According to Tukey's test, there are no significant differences on the number of enterobacteria between pollen samples collected in different places in Nitra, Slovakia. Furthermore, according to **Campos *et al.* (2008)** number of enterobacteria in the pollen could not exceed 100 cfu/g. Comparing this recommendation with our result reveals that *Betula verrucosa* Ehrh. pollen from Slovakia has a poor microbial quality.

There are differences in the total number of aerobic bacteria (TAB). According to **Campos *et al.* (2008)** requirements, TAB could not exceed 10^5 cfu/g. Pollen sample BV7, collected near the highway, exceeds this requirement and is significantly different from the other two samples (BV8 and BV9), which have acceptable counts of TAB. Moulds and yeasts could not exceed $5x10^4$ cfu/g.

Table 4 Microflora of three pollen samples of *Betula verrucosa* Ehrh. from Slovakia (Results are presented as cfu/g and log cfu/g)

Pollen sample	Representatives of the *Enterobacteriaceae* family		Mesophilic aerobic bacteria	
	10^2 cfu/g	log cfu/g	10^2 cfu/g	log cfu/g
BV7	1180	5.07[a]	2638	5.42[a]
BV8	1212	5.08[a]	762	4.88[b]
BV9	1380	5.14[a]	932	4.97[b]

Legend: [ab]Means within a column with the same letters are not significantly different according to Tukey's multiple range test ($P \leq 0.05$)

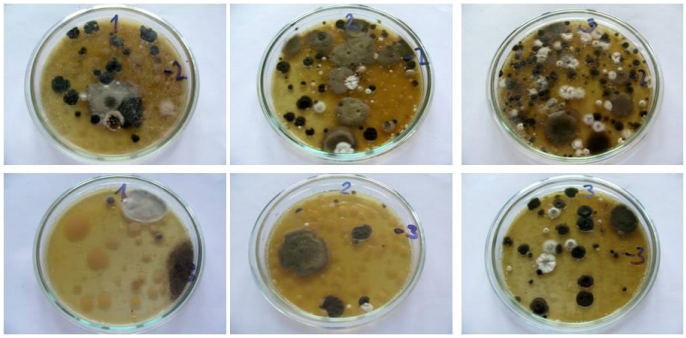

Figure 1 Present fungi on *Betula verrucosa* Ehrh. pollen from Slovakia

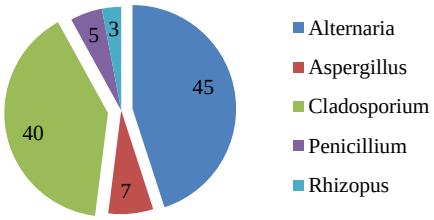

Figure 2 Frequency of microscopic filamentous fungi on *Betula verrucosa* Ehrh. pollen from Slovakia

The fungi colonies were isolated and identified at genus level. Birch pollen samples is contaminated by the genera *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus*, yeasts (Figure 1), and *Rhizopus* were identified only on the pollen sample BV8. The most frequent genera of microscopic fungi (Figure 2) in the Slovak birch pollen was *Alternaria* and *Cladosporium*. The similar results with microscopic fungi isolation in pollen samples found **Kačániová and Fikselová, 2007**.

Penicillium genus belongs to the widespread saprophytic-parasitic microscopic fungi in soil, water and various organic substrates. Their negative influence is in production of mycotoxins (ochratoxins) in moulding processes of plants and can cause allergic reactions in humans and animals. Representatives of the genus *Aspergillus* are widespread in soil, on plant and animal residues as saprophytes. Some *Aspergillus* species, e.g., *Aspergillus flavus*, *Aspergillus parasiticus*, produce toxins in the plant substrate (aflatoxins). Species of the genus *Rhizopus* are present in fruits. They contribute to their decay and fermentation. Some species are pathogenic for animals and humans (**Brovarskij *et al.*, 2010**). Fungi belonging to the genera *Penicillium*, *Alternaria*, *Aspergillus* and *Cladosporium* possess strong allergenic properties (**Dziadzio *et. al.*, 2001; Ryzhkin *et. al.*, 2002; Kirk *et. al.*, 2001; Gannibal, 2003**). Fungal allergenic activity has been most associated with spores, though other parts of the fungus, such as mycelial fragments, may also contribute to allergenicity. Fungal growth is enhanced by damp conditions, and fungi often grow outdoors on leavers, in soil, and plant litter (**Dziadzio *et. al.*, 2001**). The number of fungal spores in the air, as a rule, much higher than concentration of pollen (**Ryzhkin *et. al.*, 2002**).

According to the content of mycotoxins samples of *Betula verrucosa* Ehrh. pollen are not differ (Tab 5). Among the mycotoxins, aflatoxins and ochratoxin A occupy especial places because of its high occurrence and toxicity. The European Commission has established maximum allowable limits for these toxins in some food products (**González *et al.*, 2005**). According to recommendations of **Campos *et al.* (2008)** sum of aflatoxins B1+B2+G1+G2 could not exceed 4 mg/kg, in our case the value is not more than 4 µg/kg.

Table 5 Mycotoxin content in pollen of *Betula verrucosa* Ehrh., µg/kg

Mycotoxins	BV1	BV3	BV4	BV9
	$x \pm S_x$	$x \pm S_x$	$x \pm S_x$	$x \pm S_x$
Sum of aflatoxins B1,B2,G1,G2	<4 ± 4.0	<4 ± 4.0	<4 ± 4.0	<4 ± 4.0
Fumonisin B1	<100 ± 100	<100 ± 100	<100 ± 100	<100 ± 100
Fumonisin B2	<100 ± 100	<100 ± 100	<100 ± 100	<100 ± 100
Deoxynivalenol, mg/kg	<0.1 ± 0.10	<0.1 ± 0.10	<0.1 ± 0.10	<0.1 ± 0.10
Zearalenone	<10 ± 10	<10 ± 10	<10 ± 10	<10 ± 10
T-2 toxin	<10 ± 10.0	<10 ± 10.0	<10 ± 10.0	<10 ± 10.0
Ochratoxin A	<0.5 ± 0.5	<0.5 ± 0.5	<0.5 ± 0.5	<0.5 ± 0.5

Legend: x ± Sx – mean ± standard deviation

Requirements for the content of deoxynivalenol (DON) in cereals and processed product could not exceed 8 and 12 mg/kg respectively. DON is secondary metabolite of *Fusarium* fungi, in particular *Fusarium graminearum* and *Fusarium culmorum*, affecting standing plants. DON is less toxic than other trichothecenes, for example, T-2 toxin (**Fisinin *et al.*, 2012**). T-2 toxin affects hemopoietic and immunocompetent organs, functions of the gastrointestinal tract (**Metodicheskie, 1984**). Fumonisin produced by fungi *Fusarium moniliforme* and *F. proliferatum*. *F. graminearum* is the main producer of zearalenone. Spores live in the soil, from where they get on vegetating plants and, under favorable conditions (high humidity), germinate, affecting the spica or spadix and forming waste products (**Mhitarov, 2001**).

Based on the received results, growth conditions (complex of conditions of abiotic and biotic environment), weather, the conditions of collection, drying, storage and time factor affect on the contamination of wind-pollinated species pollen. The presence of heavy metals in pollen indicates the contamination of soil or air, from where they come in the above-ground organs of plants with dust and aerosol particles, passing the physiological barriers at root level (**Kajgorodov *et al.*, 2008**). As it has appeared, all investigated pollen samples exceeded the existing requirements at least on one heavy metal. Park, housing estate, airdrome, suburban forest, where birch grows, are influenced by mobile sources of pollution, namely motor transport. **Campos *et al.* (2008)** recommends to collect pollen in areas which are at least 3 km distant from sources of contamination such as heavy traffic, industrial centres or pesticide treated agricultural areas.

Sterile conditions of collecting and transporting pollen inside the sterile room for drying does not guarantee the absence of microorganisms on pollen. Even when **Śpiewak *et al.* (1996)** placed the branches of *Betula verrucosa* Ehrh. before flowering into the previously sterilised chamber, where there were no considerable air movement, as a result pollen was contaminated by Gram-positive and Gram-negative bacteria, thermophilic actinomycetes, fungi also including from the relatively ecologically clean region. Besides, the epiphytic microflora of plants is normal. Microbiological quality of pollen has the importance. Whether these indicators exceed the aspects. In our case, the analyzed pollen samples contain enterobacteria that exceed recommended values, content of aerobic bacteria have distinctions between collection sites. Enumeration of fungal colonies was not performed.

Pollen was dried at room temperature, that, most likely, has affected its contamination by mycotoxins. **Serra** and **Alegret** recommend the avoidance of natural pollen drying, because at low temperatures, fungal growth and mycotoxin production might occur (**Hani *et al.*, 2012**). Drying must be rapid (**González *et al.*, 2005**). If raw pollen was stored without processing, fungi can flourish under appropriate conditions (humidity and temperature) as pollen is a suitable plant product either for fungal growth or mycotoxin production. Aflatoxins and ochratoxin A are thermostable and carcinogenic molecules, thus, pollen dried

naturally or not may present a threat for human and animal health (**Hani *et al.*, 2012**). The norm on mycotoxins has not been exceeded (Tab 5).

Storage conditions were appropriate: in hermetically occluded plastic bags and glass jars in a freezer at the temperature of –7 ºC. As for the time factor, **Bogdanov (2012)** confirm that pollen keeps its sensory and microbiological quality for a storage period of 2 years, if stored in a cool, dry and dark place. According to the content of mycotoxins samples of birch pollen do not differ, but samples of the Ukrainian pollen were stored 2.5 years prior to analysis, the Slovak pollen samples – six months. This result indicates an error in drying conditions of pollen.

Thus, this study allows to analyze the influence of growth conditions and stages of preparation without participation of bees on pollen quality of anemophilous species. The human factor includes four steps before pollen use: collection, drying, packaging and storage. It is important to follow the recommendations on each of them. But even at some unconformities to requirements, pollen of *Betula verrucosa* Ehrh. has shown strong antioxidant activity *in vitro* from different habitats (**Shevcova *et al.*, 2013**). This result can be useful for further study of useful properties of birch pollen, in developing international microbiological quality parameters and standard processing protocols for pollen.

CONCLUSION

In this paper pollution of *Betula verrucosa* Ehrh. pollen by heavy metals, microorganisms and mycotoxins were examined. Pollen of silver birch from different habitats differs by the heavy metal content between samples, in some cases exceeds the requirements. Pollen samples from Slovakia have a poor microbiological quality. Mycotoxins content is the same for all Ukrainian and Slovak pollen samples. Place of growth affects the quality of the pollen, as well as the conditions from collection to its consumption.

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