Original Paper

Diversity of allochtonous substances detected in bee pollen pellets

Marek Kolenčík[1]*, Peter Štrba[1], Gabriela Kratošová[2], Illa Ramakanth[3]
[1]*Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Soil Science and Geology, Slovak Republic*
[2]*VŠB Technical University of Ostrava, Nanotechnology Centre, Ostrava, Czech Republic*
[4]*Rajiv Gandhi University of Knowledge Technologies, Department of Chemistry, AP IIIT, Nuzvid, India*

Article Details: Received: 2016-08-07 | Accepted: 2016-09-13 | Available online: 2017-09-30

http://dx.doi.org/10.15414/afz.2017.20.03.60–65

This paper quantifies the diversity of natural and artificial allochthonous materials in bee pollen pellets and assesses their impact on potential applications. Bee products used in medicine, pharmacology and food products contain honey bee wax, propolis and flower pollens, and bee pollen pellet composition is dependent on the flower's locality and methods used in technological preparation and storage. The quality of commercially available pollen and its positive and negative mode-of-actions are significantly influenced by natural and artificial allochthonous substances. The flower pollen pellets for this study were obtained from the Levice district in the Slovak Republic and analysed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). These visual and chemical analyses confirmed; (i) 4 different botanical pollen species were present in the pellets, (ii) minimal harmful substances were detected; with bee fragments and dead fungal hyphae biomass noted, (iii) different types of soil particles/aggregates were adsorbed; mainly Fe, Si oxides, silicates and alumosilicates and (iv) analysis revealed one artificial Ti-Mn-Fe grain, but this was most likely a residue from technological processes. Determination of all hazardous substances is necessary for bee pollen to be widely commercially available as food nutritional and energy supplements, and this can be achieved by microscopic study and the wide range of current analytical techniques.

Keywords: bee pollen, food sources, pollen pellets, soil particles, artificial contaminants

1 Introduction

Bee pollen comes from different flower types and is primarily collected by the *Apis mellifera* honey bee to feed its larvae in their first stage of growth (Estevinho et al., 2012). Pollen pellets have long been recognised as beneficial in medical therapy and nutrient supplements (Linskens and Jorde, 1997; Villanueva et al., 2002) and medical research has confirmed bee pollen is valuable in the following human health aspects; (i) allergy desensitisation (ii) tumour presence (iii) prostate problems and (iv) arteriosclerosis. It has also proven beneficial in tissue repair, promoting toxic elimination, rapidly decreasing excessive cholesterol and radical scavenging activity (Estevinho et al., 2012; Nogueira et al., 2012; Linskens and Jorde, 1997).

Supplementary bee pollen food products contain carbohydrates, amino acids, proteins, vitamins, lipids, mineral nutrients, trace elements and polyphenol flavonoids (Villanueva et al., 2002; Estevinho et al., 2012; Mărgăoan et al., 2010). Bee pollen, however, also has wide exposure to microbial contamination from bacteria, yeast, fungi and associated aerodispersive inorganic material in air pollution.

Campos et al. (2008) recorded suggestions for quantifying pollen quality, composition and analytical standardisation procedures, and Villanueva et al. (2002) and Mărgăoan et al. (2010) defined and categorised natural and allochthonous substances as follows; (i) additives, (ii) contaminants, (iii) hygiene requirements (iv) packaging, (v) storage and (vi) microbial quality.

Recent publications on pollen pellets lack detailed information on allochthonous inorganic substances. These have relatively small size and specific chemical and physical composition with differing positive and negative effects on human health, and they are often relicts of conventional technological procedures that are very difficult to effectively rectify (Linskens and Jorde, 1997).

This work therefore initially focuses on identifying diverse flower pollen species in pellet-form and determining allochthonous inorganics materials from the Levice

***Corresponding Author:** Marek Kolenčík, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Soil Science and Geology, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic, e-mail: marekkolencik@gmail.com

district in south-western Slovakia, and then discusses their potential impacts in further applications.

2 Material and methods

2.1 Provenance and characterization of pollen grains

Pelletised pollen grains were purchased from a local beekeeper from the Želiezovce (Levice) S-W Slovak district. This region is located in the 'Pannonian flora area' (Futák, 1984) between the Ipeľsko-Rimavská furrow and Danubian lowland phytogeographic districts (Fig. 1).

Flower pollen samples were collected in mid-flowering season and technologically modified by certified procedures (Almeida-Muradian et al., 2005; Schulte et al., 2008). Botanical-palynological identification of pollen grains was settled in the following steps (i) evaluation of general grain morphology; (ii) measurement and calculation of pollen grain uniformity and size by software ImageJ (https://imagej.net/) (iii) detection of specific structures such as surface apertures and (iv) each flower pollen class was finally classified to accommodate "species-specific" features including structure and ornamentation (Almeida-Muradian et al., 2005, Klimko et al., 2000; Lin et al., 2013; Punt et al., 2007; Schulz et al., 2000).

2.2 Physical-chemical determination of flower pollen with natural and artificial allochthonous substances

Scanning electron microscopy (SEM) and chemical composition determined by energy-dispersive X-ray spectroscopy (EDX, of EDS analysis) (QUANTA 450 FEG, FEI Company, USA) were used according to measurements published by Konvičková et al. (2016), and Kořenková et al. (2017). This analysis provided (i) surface flower pollen grain visualisation for identification, characterisation and classification (ii) physical-chemical description of allochthonous substances including their size, morphology, surface and chemical nature and (iii) mineral soil aggregates and residues from flower pollen preparation and modification. A thin layer of gold was dispersed on all sample surfaces to ensure the best conduction.

3 Results and discussion

Botanical-palynological analysis of pollen pellets collected from the Levice district revealed four pollen species; *Trifolium pratense* (Red clover, Fabaceae) (Fig. 2), *Brassica napus* (Oilseed rape, Brassicaceae) (Figs. 4, 6), *Helianthus annuus* (Sunflower, Asteraceae) (Fig. 7) and *Taraxacum officinale* (Dandelion, Asteraceae) (Fig. 8).

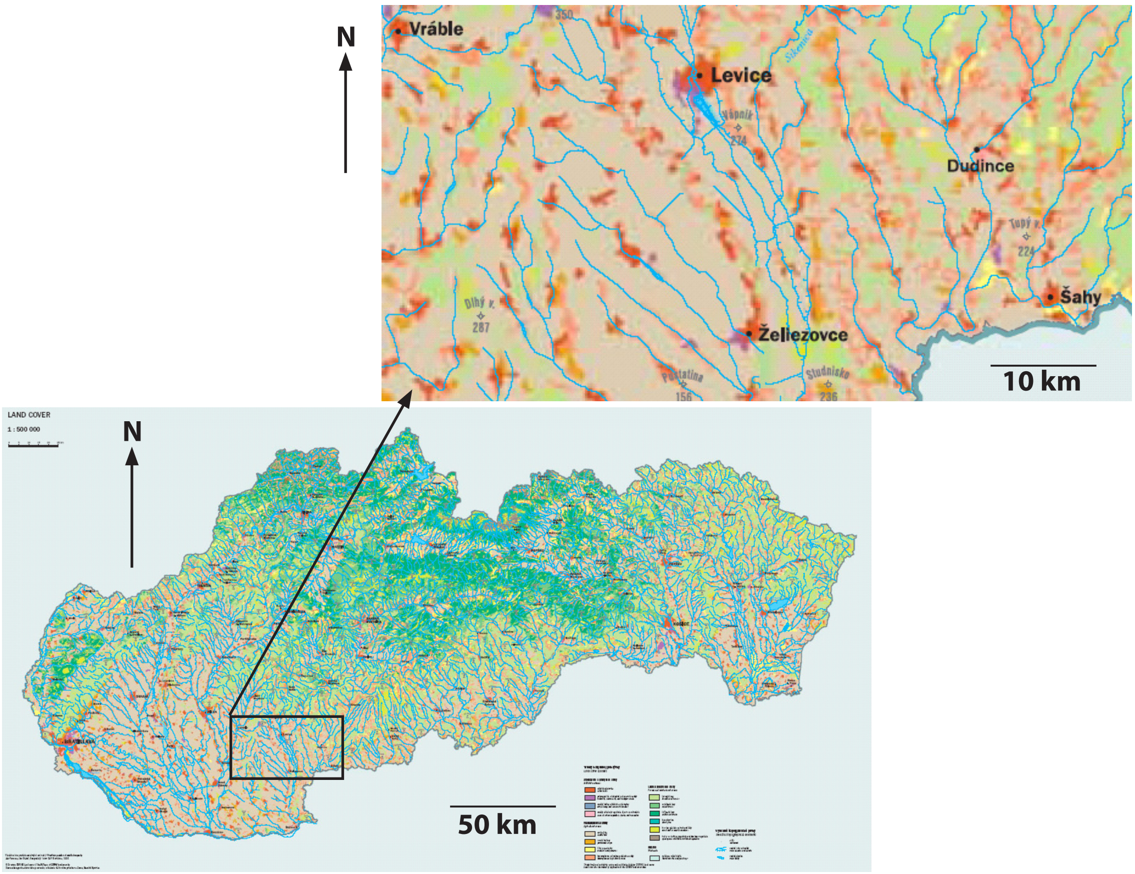

Figure 1 Flower pollen harvested from the Želiezovce, Levice district in SW Slovakia where land cover is dominated by arable land with minor broadleaves forests (Miklós et al., 2002)

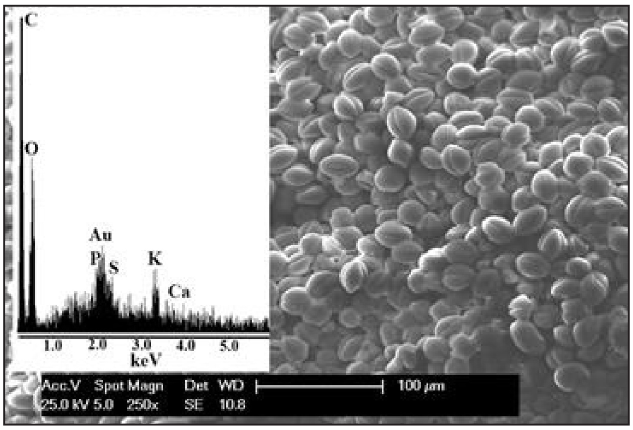

Figure 2 Scanning electron microscopy of *Trifolium pratense* pollen (Red clover, Fabaceae). Inset: energy-dispersive X-ray spectroscopy determined pollen pellet chemical composition, with C, O, P, S, K and Ca the main element components

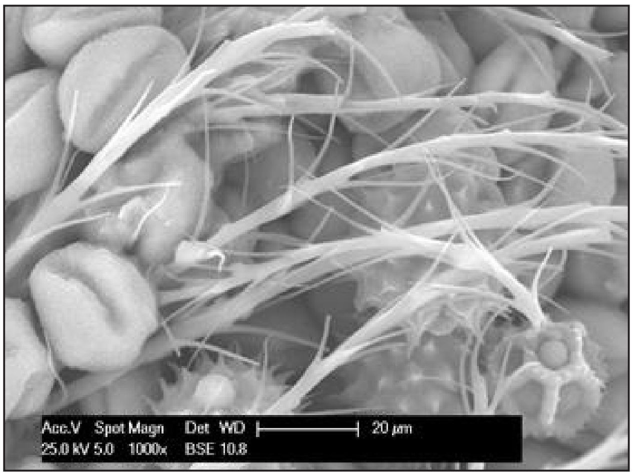

Figure 3 Scanning electron micrograph revealed bee branched body hairs mixed with different pollen grain types

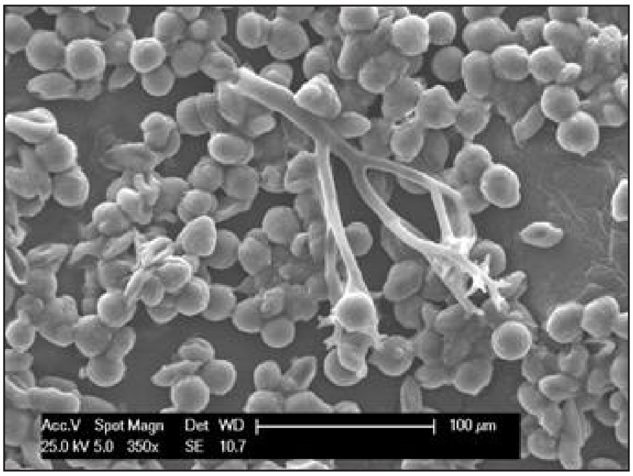

Figure 4 Microscopically observable dead biomass of imperfect fungi (phylum Deuteromycota) with pollen grains of *Brassica napus* (Oilseed rape, Brassicaceae) (Scanning electron microscopy)

The identified pollen samples corresponded with Levice arable land vegetation (Fig. 1, Miklós et al., 2002) with rape, maize, sunflower and cereal crops typical for this region. Fodder plants were alfalfa and red clover and the many weed plant species included *Taraxacum officinale* dandelion. This supported Štrba and Kosár's (2012) observation of the occurrence and uniformity of synanthropic, invasive and expansive species in vascular plant flora diversity in similar agricultural ecosystems.

EDS analysis in Figure 2 inset reveals that *Trifolium pratense* (Red clover, Fabaceae) pollen chemical composition comprises C, O, P, S, K and Ca main elements. It also highlights that nutritive element content agrees with Mărgăoan et al. (2010). Similar chemical composition was also confirmed in other pollen-pellet species including *Brassica napus* (Oilseed rape, Brassicaceae), *Helianthus annuus* (Sunflower, Asteraceae) and *Taraxacum officinale* (Dandelion, Asteraceae). Moreover, Campos et al. (2008) report that pollen is one of the most significant human diet products because it has high concentrations of reducing sugars, amino acids, un/saturated fatty acids, heavy metals (Fe, Cu, a Zn) and high K/ Na ratio. Moreover, our results identified that pollen pellets contained C, P, S and K macronutrients as platform components for the above-mentioned organic substances (Mărgăoan et al., 2010).

Standardised pollen quality revealed the characteristic spectrum; including water content not higher than 6%, monofloral and multifloral pollen for specific nutritional and therapeutic purposes, packaging precluding atmospheric humidity and moisture because pollen can absorb water from the air and the absence of additives and contaminants. These factors enhance pollen's use in standardised technical and hygenic regulation of food industry products (Campos et al., 2008; Villanueva et al., 2002; Estevinho et al., 2012; Mărgăoan et al., 2010).

Microscopic observation identified the presence of fragments of bees, plants and harvesting process materials in the pollen pellets (Campos et al., 2008). Results confirmed pollen species, branched hairs from the bee body surfaces (Fig. 3) and microscopic dead fungal biomass; after some difficulty identified as imperfect fungi of phylum Deuteromycota (Fig. 4). However, fungal hyphae occurrence was rare compared to bulk pollen materials. The hyphae presence could be due to (i) accidental bee transfer to the pollen or (ii) microbial contamination hazardous to human health which appeared at high moisture levels and was often accompanied by other pathogenic microorganisms such as gram positive and negative bacterial spores, yeasts and acarid mites (Campos et al., 2008; Nogueira et al., 2012) – although evidence of these pathogens was not confirmed in our samples. Negative synergic effects of microbial contamination could decrease pollen quality or introduce harmful human health reactions such as allergies (Kačániová et al., 2004; Kačániová et al., 2011) and therefore mycogenic analysis is beneficial in evaluating presence and activity of microscopic fungi, and preclude any of the abundant mycotoxins (Linskens and Jorde, 1997; Kačániová et al., 2011).

In addition, inorganic materials in aerodispersive environments can influence pollen pellet quality parameters. They include agricultural fertilizers, pesticides, veterinary antibiotic residues and organic molecules from other sources (Chauzat et al., 2006; de

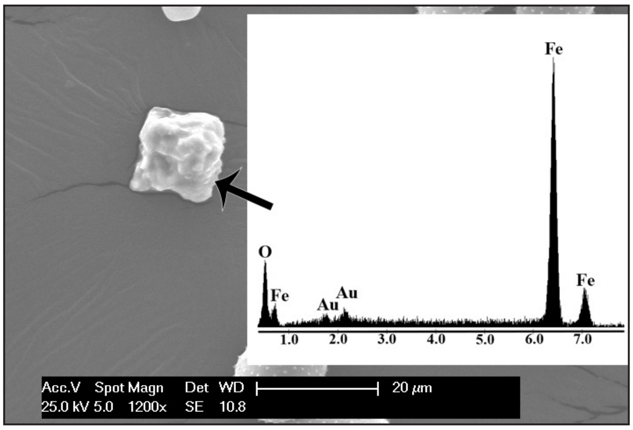

Figure 5 Scanning electron micrograph depicts 10 to 12 µm sized particle with square-shape background and no other symmetric-facet morphology; Inset: Energy-dispersive X-ray spectrum analysis reveal O and Fe chemical elements corresponding to mineral Fe oxide

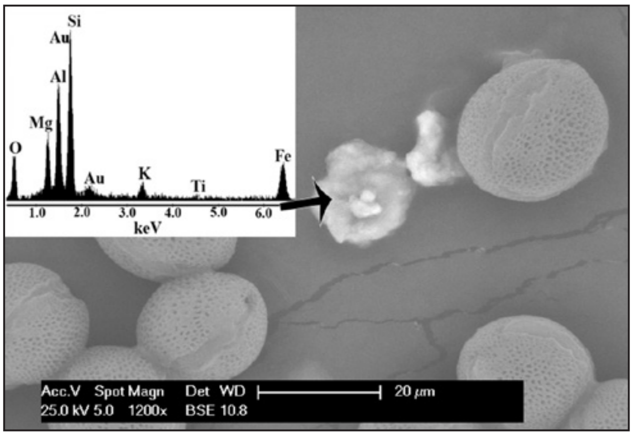

Figure 6 Scanning electron microscopy (SEM) of heterogeneous 15µm soil aggregate lacking defined morphology; but with mineral different mineral types, mainly alumo-silicates, associated with pollen from *Brassica napus* (Oilseed rape, Brassicaceae). Inset: Energy-dispersive X-ray spectrum showing Si, Al, O, Mg, K, Fe and Ti elements confirming non-homogenous soil aggregate

Oliveira, 2016). Furthermore, these additives can be incorporated in pellets and produce negative effects in biological, pharmacological and human organoleptic sensory pathways (Campos et al., 2008).

Our results confirmed that not all natural and artificial allochtonous particles can have their effects nullified or be completely removed from pellets, particularly those produced in technological processes. Others are small with non-respirable size (Fig. 5–8) and have been incorporated in situ during pellet formation; especially in rain and wind-driven meteorological conditions (Knox et al., 1997; Ormstad et al., 1998; Campos et al., 2008). Pollen species also depend on individual levels of adherence to inorganic mineral surfaces. This depends on pollenkit content; the viscous organic substances on the pollen surface which generate strong energy forces between close molecules; such as Van der Wall attraction (Lin et al., 2013). Although the pollen surface is responsible for initial inorganic particle-surface attachment, it is also able to resist attachment during technologically allochtonous material purification and elimination processes with the aid of specially constructed purifiers based on bulk density separation. These inorganic materials have absolute resistant properties in freezing, drying at 40 °C and lyophilisation, even when they are slightly modified (Campos et al., 2008).

Our results in the accompanying figures reveal a wide range of inorganic materials associated with different pollen species:

A/ Figure 5 depicts the Fe oxide solid-particle soil component which acts as geochemical barrier to absorb the hazardous heavy metal elements such as Pb, Cd, As (Čurlík, 2011).

B/ Figure 6 shows the occurrence of non-homogenous soil aggregates containing Si, Al, O, Mg, K, Fe and Ti elements. Si, Al, K, Mg in addition, Ti and Fe are in oxide form and silicates and alumosilicates. The oxides, silicates and alumosilicates are natural polen pellet contaminants distributed by air from soil systems (Knox et al., 1997; Ormstad et al., 1998).

C/ Figure 7 highlights that quartz (SiO_2) is the most resistant and abundant soil and sediment material in the sample area. Its multiple re-deposition and transport is deduced from fragment morphology and size (Čurlík, 2011).

D/ Figure 8 reveals the sole particle with Ti – Mn – Fe chemical composition, no natural facet morphology and lacking correspondence to naturally occurring mineralogical groups. Chemical analytic determination of its origin in Figure 8 (inset) proved difficult because the absence of oxygen precludes classified as oxides or sulphates. The most logical deduction is that this particle originates from artificial technological processes because similar material types have been identified as light alloys or multi-layer metal products with excellent thermal, electrical, and mechanical properties (Kim et al., 2013). These and similar high-content artificial materials cause problems when combined with pollen because they accelerate harmful human health effects (Linskens and Jorde, 1995; Ruby et al., 1999).

The 0.05 to 0.01 mm size-dependency of all evaluated particles enables the general classification of 'coarse dust'. This is contrasted with the less than 10 µm particle size of dangerous human-inhalable particles, which normally have particular morphology and surface-to-volume ratio. Moreover, Ormstad et al. (1998) assert that it is inhalable fractions smaller than 2.5 µm which create the most harmful human effects.

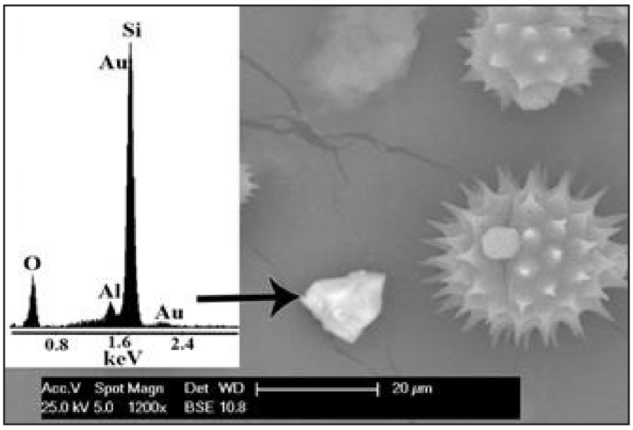

Figure 7 SEM of 9–12µm angular quartz grain fragments associated with pollen from *Helianthus annuus* (Sunflower, Asteraceae) Inset: Energy-dispersive X-ray spectrum with Si and O elements confirming quartz mineral

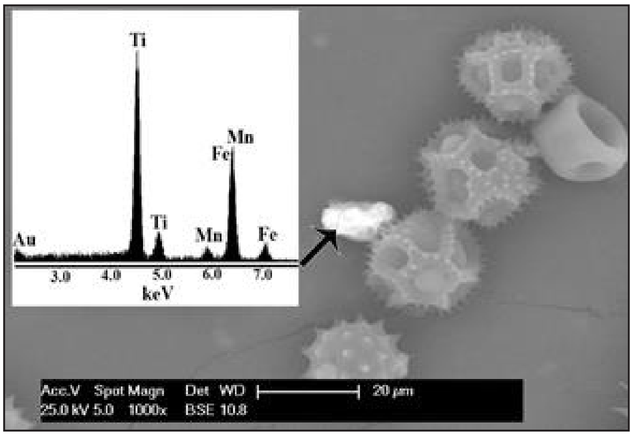

Figure 8 SEM of prolonged 5 to 10µm spherical grain accompanying pollen from *Taraxacum officinale* (Dandelion, Asteraceae) Inset: Energy-dispersive X-ray analysis of the grain with Ti-Mn-Fe chemical nature corresponding to allochtonous substance artificial origin

Foods with adsorbed soil particles introduce dangers in oral ingestion and subsequent gastrointestinal problems. This was identified in Hooda et al's. (2004) 'human health risk assessment' research. The bioavailability of soil particle macro-, micro and trace element mineral nutrients for human and animals is most closely related to increased mobility and potential toxicity and directly dependent on combinations of individual characteristics. The major criteria here include soil material type, dose, time-exposure dependency, particle size and morphology. The chemical and physical attributes include crystallinity, leaching and release ability, reactivity and inertia (Ruby et al., 1999; Hooda et al., 2004). Our results detected inorganic substances and minerals in pollen pellets which maintain significant chemical and physical resistance in a wide range of environments, and Ruby et al. (1999) and Hooda et al. (2004) add that human absorption is independent of metal biological availability.

4 Conclusions

Pollen pellets obtained from the Levice district in the Slovak Republic contained the following four pollen species; *Trifolium pratense* (Red clover, Fabaceae), *Brassica napus* (Rape Oilseed, Brassicaceae), *Helianthus annuus* (Sunflower, Asteraceae) and *Taraxacum officinale* (Dandelion, Asteraceae). Interestingly, microscopic inspection of the resultant pollen pellets revealed one Ti-Mn-Fe grain, but this is considered a residue from technological processes or harvesting treatments The pellets also contained attached branched hairs from bee body surfaces, dead fungal hyphae biomass, fine soil mineral particles and aggregates including quartz (SiO2), Fe oxide and alumosilicate. However, these foreign substances occur rarely compared to bulk pollen materials and do not lower food quality or pose particularly harmful effects to human health. Finally, pollen pellets are widely recognised as one of the most excellent nutritional food sources, and this has inspired our new research into the pollen content of protein, lipid, ash and other organic substances; with publication of results in the near future.

Acknowledgments

We would like to thank Dr. Raymond Joseph Marshall for a thorough correction of the English language. This work was financial support by European Union foundation (Erasmus plus program -academic stuff mobility), Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and the Slovak Academy of Sciences via grants VEGA 1/0164/17 and KEGA 014SPU-4/2016, and we also appreciate the Ministry of Education of the Czech Republic grant scheme SP2016/57.

References

ALMEIDA-MURADIAN, L.B. et al. (2005) Chemical composition and botanical evaluation of dried bee pollen pellets. In *Journal of Food Composition and Analysis*, vol. 18, pp. 105–111.

CAMPOS, M.G.R. et al. (2008) Pollen composition and standardisation of analytical methods. In *Journal of Apicultural Research*, vol. 47, no. 2, pp. 154–161.

CHAUZAT, M.P. et al. (2006) A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees in France. In *Journal of Economic Entomology*, vol. 99, no. 2, pp. 253–262.

ČURLÍK, J. (2011) Potentially toxic microelements and their distribution in soils of Slovakia. Bratislava: Suma print (in Slovak).

DE Oliveira, R. C. et al. (2016) Bee pollen as a bioindicator of environmental pesticide contamination. In *Chemosphere*, vol. 163, pp. 525–534.

ESTEVINHO, L.M. et al. (2012) Portuguese bee pollen: Palynological study, nutritional and microbiological evaluation. In *International Journal of Food Science and Technology*, vol. 47, pp. 429–435.

FUTÁK, J. (1984) Phytogeographical division of Slovakia. In *Flóra Slovenska IV/1*. Bratislava: Veda, pp. 418–419 (in Slovak).

HOODA, P.S. et al. (2004) The potential impact of soil ingestion on human mineral nutrition. In *Science of The Total Environment*, vol. 333, pp. 75–87.

KAČÁNIOVÁ, M. et al. (2004) Microflora of the honeybee gastrointestinal tract. In *Folia Microbiologica*, vol. 49, no. 2, pp. 169–171.

KAČÁNIOVÁ, M. et al. (2011) Mycobiota and mycotoxins in bee pollen collected from different areas of Slovakia. In *Journal of Environmental Science and Health – Part B Pesticides, Food Contaminants and Agricultural Wastes*, vol. 46, pp. 623–629.

KIM, H., Suh, D.W. and Kim, N.J. (2013) Fe-Al-Mn-C lightweight structural alloys: A review on the microstructures and mechanical properties. In *Science and Technology of Advanced Materials*, vol. 14, pp. 1–12.

KLIMKO, M., Kluza, M. and Kreft, A. (2000) Morphology of pollen grains in three varieties of *Helianthus annuus* L. In *Roczniki Akademii Rolniczej w Poznaniu CCCXXII Botanika*, vol. 3, pp. 135–142.

KNOX, R.B. et al. (1997) Major grass pollen allergen Lol p 1 binds to diesel axhaust particles: Implications for asthma and air pollution. In *Clinical and Experimental Allergy*, vol. 27, pp. 246–251.

KONVIČKOVÁ, Z. et al. (2016) Antimicrobial bionanocomposite–from precursors to the functional material in one simple step. In *Journal of nanoparticle research*, vol. 18, pp. 368.

KOŘENKOVÁ, L. et al. (2017) Physiological response of culture media-grown barley (*Hordeum vulgare* L.) to titanium oxide nanoparticles. In *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, vol. 67, pp. 285–291.

LIN, H., Gomez, I. and Meredith, J.C. (2013) Pollenkitt wetting mechanism enables species-specific tunable pollen adhesion. In *Langmuir*, vol. 29, pp. 3012–3023.

LINSKENS, H.F. and Jorde, W. (1997) Pollen as food and medicine – A review. In *Economic Botany*, vol. 51, no. 1, pp. 78–86.

MĂRGĂOAN, R. et al. (2010) Bee collected pollen–General aspects and chemical composition. In *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies*, vol. 67, no. 1–2, pp. 254 –259.

MIKLÓS, L. et al. (2002) *Landscape atlas of the Slovak Republic*. Bratislava: Ministerstvo životného prostredia SR.

NOGUEIRA, C. et al. (2012) Commercial bee pollen with different geographical origins: A comprehensive approach. In *International Journal of Molecular Sciences*, vol. 13, pp. 11173–11187.

ORMSTAD, H., Johansen, B.V. and Gaarder, P.I. (1998) Airborne house dust particles and diesel exhaust particles as allergen carriers. In *Clinical and Experimental Allergy*, vol. 28, pp. 702–708.

PUNT, W. et al. (2007) Glossary of pollen and spore terminology. In *Review of Palaeobotany and Palynology*, vol. 143, pp. 1–81.

RUBY, M.V. et al. (1999) Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. In *Environmental Science and Technology*, vol. 33, pp. 3697–3705.

SCHULTE, F. et al. (2008) Chemical characterization and classification of pollen. In *Analytical Chemistry,* vol. 80, pp. 9551–9556.

SCHULZ, S. et al. (2000) Composition of lipids from sunflower pollen (*Helianthus annuus*). In *Phytochemistry*, vol. 54, pp. 325–336.

ŠTRBA, P. and KOSÁR, G. (2012) Diversity of vascular plants in agricultural landscape of central part of Žitný ostrov region. In *Biodiversity in agricultural landscape and ecosystem. International conference of the project REVERSE-INTERREG IVC. Piešťany: 13th of June 2012*. Piešťany: Centre of Plant Production Piešťany, pp. 13–16 (in Slovak).

VILLANUEVA, M.T.O. et al. (2002) The importance of bee-collected pollen in the diet: A study of its composition. In *International Journal of Food Sciences and Nutrition*, vol. 53, pp. 217–224.