







SUMMER SCHOOL ON ANALYTICAL TOOLS FOR CONTAMINANTS IN HONEY AND OTHER HIVE PRODUCTS

16-18th September, 2024

University of Belgrade - Faculty of Mechanical Engineering, Serbia

Faculty of Mechanical Engoneering Kraljice Marije 16, 11120 Belgrade

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The 1st BeSafeBeeHoney training school will contribute to creation and transfer key knowledge on risk assessment procedures in the honey value chain. The trainees will gain knowledge and practical skills in analytical tools for determination of contaminants in complex matrices, since sample preparation techniques to detection strategies. As output, it is expected to promote a scale-up career development and the idea of interdisciplinary research when it comes to joining honey and by-products production, their nutritional and medicinal properties in correlation with the main threats that honeybee colonies are facing and might be in the near future along with the impact that those situations can create in agro-systems.







AGENDA

16th September 2024

8:30 - 9:00	Participant registration
9:00 - 9:30	Opening ceremony, (Room 211, 2 nd floor)
Training	session - Room 513, 5 th floor
9:30 - 11:00	<u>Nemanja Jovanović</u>
	Digitizing beehives and setting up measuring equipment that monitors the pollutants bees bring into the hive
	Digitizing beehives involves equipping traditional hives with sensors and monitoring devices to track various hive conditions, such as temperature, humidity, and bee activity. This technology helps beekeepers manage hive health more effectively. This training is about digitizing beehives and setting up measuring equipment that monitors the pollutants bees bring into the hive. The first part of the training introduce different types of sensors and their use in beekeeping, beekeeping pollutants, and smart apiculture developments. The second part will be active learning in groups that will try to solve engineering problems in smart apiculture. Participants will be divided into several groups. Groups will quickly need to select a team leader while thinking of new ways to tackle the issue they are facing. After solving the issue, there will be time for presenting the results and discussion. The result of the training should be improved critical thinking, teamwork collaboration, problem assessment, and new approaches to many possible solutions. Personal laptops are necessary for this lecture.
11:00 - 11:30	Coffee break
11:30 - 13:00	<u>Jovetić Milica</u> Directorate for National Reference Laboratories, Ministry of Agriculture, Forestry and Water Management, Serbia
	<u>Nebojša Nedić</u>
	Faculty of Agriculture-University of Belgrade, Serbia
	Contaminants in honey and pollen from urban surroundings
	In recent years, the concept of urban beekeeping, i.e. raising bees in strictly urban areas, has been developed, primarily for the purpose of improving pollination and preserving the flora. Therefore, a detailed examination of the quality and safety of honey produced in this









way is a special challenge, bearing in mind the exposure of urban environments to various pollutions.
The main contaminants of environmental origin are toxic (heavy) metals, pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), bacteria, genetically modified organisms and radioactivity. Harmful substances that can be found in honey and other products due to inadequate beekeeping practices are substances for controlling bee pests and diseases (acaricides, antibiotics, pesticides, etc.) and metals originating from beekeeping equipment and honey storage utensils.
Results in this presentation come from one of the first detailed investigation of honey and pollen from exclusively urban area. Samples were obtained from an experimental stationary apiary of the Belgrade University Faculty of Agriculture, located in the centre of Zemun, a municipality belonging to the Belgrade metropolitan area. The apiary had been monitored by the experts of the same institution to exclude any contamination caused by beekeeping and limit contamination to environmental sources. Twenty three samples of unprocessed polyfloral honey and thirteen samples of bee pollen were collected in years 2015 and 2016, by trained personnel.
The content of 10 metals (Pb, Cd, As, Hg, Cu, Zn, Fe, Mn, Cr and Ni), 15 polycyclic aromatic hydrocarbons (PAHs) and residues of 123 pesticides were analyzed using modern analytical techniques: inductively coupled plasma mass spectrometry (ICP-MS), high performance liquid chromatography with fluorescence detection (HPLC-FLD) and gas chromatography mass spectrometry (GC-MS), respectively.
According to the results for the content of certain metals, it has been concluded that the tested honey met the requirements of Serbian and European regulations of that time. The content of 123 analyzed pesticides in honey was below the limit of the quantification of the applied method. PAH concentrations in honey were below the maximum levels defined for food. Increased concentrations of metals, especially mercury and chromium, as well as PAH, were found in pollen, indicating the air pollution to which the pollen was exposed.
Lunch break
<u>Janis Rusko</u>
Institute of Food Safety, Animal Health and Environment BIOR, Latvia
1. Food safety legislative frameworks and EU institutions









2. Basis of food safety risk assessment

This lecture introduces the principles of food safety risk assessment, focusing on chemical hazards, specifically in honey and honey by-products. Key topics include hazard identification, hazard characterization, exposure assessment, risk characterization, and problem formulation. Participants will learn how to define specific risk assessment questions, set objectives, and review context for honey and honey by-products.

3. Types, sources, and characteristics of chemical Hazards

This lecture focuses on the different types of chemical hazards that can pose a food safety risk in honey and honey by-products, including heavy metals like lead and mercury, environmental pollutants such as pesticides and dioxins, veterinary drug residues, and processing-induced contaminants. We will explore the sources of these hazards, their potential health effects, and preventive measures to control them along the food chain.

4. Dietary Exposure Assessment

This lecture explains how dietary exposure to chemical hazards is calculated, focusing on honey and honey by-products. The two main components are occurrence data (concentration of a substance in food) and consumption data (food consumption patterns). We will discuss the sources of occurrence data, including monitoring programs and industry reports, and methods for collecting consumption data, such as national dietary surveys and food diaries. Different approaches for exposure assessment, including deterministic methods using point estimates and probabilistic methods that use distribution of be intakes, will covered with practical examples.

5. Practical: Dietary exposure calculations

In this hands-on workshop, participants will engage in practical exercises to calculate dietary exposure to contaminants in honey and honey by-products using real-world data (for example, EFSA food consumption database). Participants will learn to estimate both chronic and acute dietary exposure and compare these estimates with health-based guidance values to assess risk. The workshop aims to provide practical skills in performing dietary exposure assessments using EFSA's methodologies and databases.

6. Principles of risk communication

This lecture will introduce the fundamental principles of risk communication within the context of food safety, specifically for honey and honey by-products. Topics will include the goals of risk communication, such as informing and educating the public, fostering trust, and promoting transparency. We will explore the key elements of effective risk communication, including clarity and consistency. Additionally, the lecture will cover strategies for addressing uncertainty and managing public perception during a food safety crisis. Examples of successful and unsuccessful risk communication efforts in the EU and globally will be provided to highlight best practices and common pitfalls. Notable cases could include the communication efforts around the neonicotinoid pesticide ban in the EU, which aimed to protect bee populations, and the crisis communication surrounding the contamination of honey with antibiotics, highlighting both effective and ineffective communication strategies.









17:30	Free time (for Belgrade sightseeing)
	 7. Practical: Crisis communication case studies In this interactive workshop, participants will work in small groups to analyze real-world case studies of food safety crises involving honey and honey by-products. Each group will be given a different case study to review, focusing on the communication strategies used by the involved parties. Participants will identify strengths and weaknesses in the communication efforts and propose improvements. 8. Determination of persistent organic pollutants in honey and honey products This lecture will provide an overview of the identification and quantification of POPs in honey and honey products, covering common contaminants like dioxins, PCBs, polycyclic aromatic hydrocarbons (PAHs), endocrine disrupting compounds, and phthalates. It will discuss the relevant EU and international regulations, including maximum residue levels (MRLs). We will explore sample preparation methodologies and analytical techniques such as GC-MS and HRMS, highlighting state-of-the-art methods. Quality assurance practices will be emphasized to ensure accuracy and reliability of results. The lecture will also present case studies, discuss current challenges, and future directions in POPs analysis.

17th September 2024

Field visit	
9:30 - 12:30	<u>Jelena Ćirić</u>
	Institute of Meat Hygiene and Technology, Serbia
	Visit to the Institute and chemistry laboratory
	https://maps.app.goo.gl/RNxWqefMf24yiZhM6
	Control of food safety and quality is carried out in the Laboratory for biotechnological research and control of food safety and quality. Laboratory tests on samples consist of microbiological, parasitological, immune-enzymatic, molecular biological, physical-chemical, chemical and sensory tests depending on the type of food and include all tests specified by regulations on health and quality regulations with which certain foods must comply. The following food products are tested In the Laboratory for biotechnological research and control of food safety and quality: Meat and meat products, Milk and milk products, Fish and fish products, Fruits and vegetables and their products, Dietary supplements, Eggs and egg products, Protein products, Animal









	feed and water for drinking, Soups and feed additives, Food additives, Honey and other bee products, Cereals and cereal products, oilseeds and other seeds.
12:30 - 14:30	Lunch break
Training see	Ssion - Room 513, 5 th floor
14:30 - 15:30	<u>Andreia Freitas</u>
	National Institute for Agrarian and Veterinary Research (INIAV), Portugal
	Analytical Strategies and EU requirements for veterinary residues monitoring in food of animal origin
	The production of food and intensive animal farming have profound impacts on human, animal, and environmental health, raising significant concerns from a "One Health" perspective. Within animal farms, the ongoing use and release of veterinary medicines can lead to the presence of unwanted residues of these compounds in the food chain and the environment, often through wastewater or the use of manure. The presence of veterinary residues in food of animal origin is of public health concern. To ensure food safety, the European Union has established specific regulations and monitoring requirements for the determination of veterinary medicines in food of animal origin. Honey, as other food products, can contain residues chemicals used in intensive food production due to its direct and indirect exposure during the collection of nectar and pollen. Consumer protection is a primary concern within the European Union, necessitating the mandatory control of veterinary drug residues to ensure that all animal products in the food chain are safe. As a sustainable approach, assessing environmental matrices for potential persistent pharmaceutical pollutants can provide valuable data for establishing risk levels for human, animal, and environmental health. Recent advancements and innovations in analytical strategies offer enhanced insights into the occurrence of persistent pharmaceuticals and contamination levels in food products. In fact, the EU's approach to veterinary medicine residue monitoring, combining regulations, sensitive and specific analytical strategies, and ongoing surveillance, plays a vital role in safeguarding consumer health and ensuring the safety of food of animal origin.
15:30 - 16:00	Coffee break
16:00 - 17:15	<u>Iveta Pugajeva</u>
	Institute of Food Safety, Animal Health and Environment BIOR, Latvia
	Analytical strategies and monitoring of pesticide residues in bee products
	Bee products, such as honey, beeswax, royal jelly, and pollen, are highly valued for their nutritional, medicinal, and therapeutic benefits. However, these products are increasingly subjected to contamination from pesticides used in agricultural practices, which bees are exposed to when foraging. Pesticide residues, including insecticides,









herbicides, and fungicides, can accumulate in bee products, leading to potential health risks for consumers. Additionally, exposure to harmful chemicals poses a significant threat to bee populations, contributing to bee colony decline, which can further disrupt ecosystems and agricultural productivity due to the critical role bees play in pollination. This lecture explains the monitoring programs for pesticide contamination in foodstuffs, especially honey, and analytical strategies employed for detecting and monitoring pesticide residues in bee products. Analytical methods such as multi- and single-residue approaches are discussed, focusing on their sample preparation techniques. Multi-residue methods allow for the simultaneous detection of a wide range of pesticide types in a single analysis, offering efficiency and broadspectrum monitoring. On the other hand, single-residue methods provide higher sensitivity and precision for specific pesticide compounds but are limited in their scope. Finally, current challenges and future perspectives in the field of pesticide residue analysis in bee products are highlighted.

18th September 2024

Training session - Room 513, 5 th floor	
9:30 - 10:30	<u>Ivana Varenina</u>
	Croatian Veterinary Institute, Croatia
	Determination and quantification of xenobiotics in bees and bee products
	The honey bee (Apis mellifera) is one of the most important insects in agricultural production and contributes significantly to the conservation of ecosystems and biodiversity through its pollination of cultivated and wild plant species in nature. Among the challenges that affect the growth, reproduction and sustainability of bee colonies, diseases, climate change and the presence of various pollutants (heavy metals, pesticides, etc.) pose the greatest challenge. Although each of these stressors in its explicit form can seriously disrupt the balance in the bee community, the most likely explanation for the decline of bee populations in the world is probably their synergistic effect. Laboratories have developed selective, precise and sensitive methods to determine the contaminants present , thus ensuring food safety . By analysing pollen, wax and bees as bioindicators of environmental pollution, it is possible to monitor the exposure of bees to pollutants and the sustainability of bee colonies. Analytical methods for the detection and quantification of target residues consist of two parts: Sample clean-up and instrumental analysis. Analysing food of animal origin is a major challenge due to the complex nature of the samples. For this reason, the methods for the determination of antibiotics and pesticides use the highly selective QuEChERS technique for sample extraction, which can easily remove unwanted components of the matrix. The QuEChERS extraction procedure enables rapid and specific extraction of selected analytes, which ensures









	high sensitivity of the method and the possibility of determining residues in the ppb concentration range. Taking into account the physico-chemical properties of the analytes to be determined, the technique of high-performance liquid chromatography UHPLC- MS/MS coupled with mass spectrometry is the most suitable technique for the separation and detection of residues of these chemical substances in the methods for the determination of antibiotics or pesticides. The multi-residue method for the determination of antibiotics covers different classes of antibiotics: quinolones, macrolides, lincosamides, sulfonamides and tetracyclines. The method for the determination of pesticides covers numerous groups of compounds such as carbamates, organophosphates, neonicotinoids and/or pyrethroids, which are analysed, as well as glyphosate, the world's best-selling herbicide and the most abundant pollutant in the environment. The analytical methods used to quantify the metals consist of microwave digestion of the samples, followed by ICP-MS analysis(inductively coupled plasma mass spectrometry). The removal of the organic fraction from the samples prior to instrumental analysis is extremely important in order to reduce the influence of the matrix itself, which can have a direct impact on the result obtained.
10:30 - 11:00	Coffee break
11:00- 11:30	<u>Krišs Dāvids Labsvārds</u>
	Institute of Food Safety, Animal Health and Environment BIOR, Latvia
	Application of chemometrics and various modern methods of analytical chemistry for honey samples
	Apiculture has an important role in nature and the economy. Honey manufacturers provide honey bee hive colonies that can play an important role in local plant pollination while producing outcome - the honey, is demanded sweetener. The combination of several modern instrumental methods has been proposed as an appropriate tool not only for adulteration problems but also for mislabelling of honey floral origins.
	The studies include physicochemical parameter evaluation of natural monofloral and polyfloral honey of Latvian origins. The 6 most common (buckwheat (Fagopyrum esculentum), clover (Trifolium repens), heather (Calluna vulgaris), linden (Tilia cordata), rapeseed (Brassica napus), willow (Salix cinerea)) and few less common (facelia (Phacelia tanacetifolia), umbellifers (Apiaceae), Raspberry (Rubus) and horse chesnut (Aesculus hippocastanum)) types of monoflorals were investigated by several instrumental methods.
	The physicochemical parameters and instrumentation were the following: The isotope ratio signatures of δ_{13} C and δ_{15} N in honey and honey proteins were obtained with isotope ratio mass spectrometry (IRMS); the polyphenolic compound profile was obtained with ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS); aliphatic and aromatic compound chemical patterns were investigated by nuclear magnetic resonance (NMR); volatile organic compound profile was obtained with gas chromatography-mass spectrometry (GC-MS); the chemical bond patterns were analyzed with Fourier transformation infrared









	spectroscopy (FT IR); the low molecular weight organic acid profile was obtained with
	spectroscopy (FTR), the low motecular weight organic acid profile was obtained with ion exchange chromatography (IEC) the elemental profile was obtained with the inductively coupled plasma-mass spectrometry (ICP-MS) methods. Thus, provides with extensive chemical profile, which was compared with honey's antibacterial and antifungal properties as well. The nitrogen isotope ratio was a selective criterion for heather (Calluna Vulgaris) monofloral honey discrimination from other sources. The polyphenol profile provided by UPLC-HRMS can be used for buckwheat (Fagopyrum esculentum) honey evaluation. The NMR aliphatic and aromatic compound profile can be the most comprehensive tool for evaluating honey floral origins. The VOC profile by GC-MS was a useful method to determine heather and buckwheat honey. With the ICP-MS method successfully 30 different element concentrations were quantified and compared to floral origins. Studies suggest typical element patterns even for clover (Trifolium repens), willow (Salix cinerea), and rapeseed (Brassica napus), while with other considered methods it was not possible to distinguish. FT IR spectrometry did not show promising results with floral origins evaluation but after experiments with different adulteration methods, it showed that it can be used to distinguish counterfeit honey from natural. Macro- and trace element profiles show characteristic concentrations of elements in the honey of Latvian origins and can be successfully used for floral origins evaluation because of different plant bioremediation abilities. Although the classic chemometric approaches were used (PCA, ANOVA), the need for low-level or mid-level data fusion is necessary since data includes various instrumental approaches. Achieved results related to physicochemical and antibacterial properties will be implemented further for "medical grade" honey preposition to wound healing bandages. The instrumental method capabilities will be summarized to make a simplified decision tree scheme for beekeepers.
11:30 - 12:00	<u>Mohammad Nausad</u>
11:30 - 12:00	<u>Mohammad Nausad</u> Jožef Stefan Institute, Slovenia
11:30 - 12:00	









	particular focus on the targeted analysis of known, usually performed by gas or liquid chromatography coupled with tandem mass spectrometry (GC-MS/MS and LC- MS/MS). The increasingly prominent high-resolution mass spectrometry (HRMS) has opened new avenues for non-targeted and suspect screening approaches, facilitating the discovery of unknown and known unknown, respectively. Data preprocessing and mass spectral library-supported annotation are crucial segments in particular in non- targeted and suspect screening workflows that have enabled the identification of a diverse array of contaminants, including pesticides, acaricides, herbicides, and environmental pollutants, at trace levels Complementing these analytical approaches metabolic modeling with KBase enhances the understanding of the metabolism of bees. It supports targeted conservation and health strategies by uncovering the effects of contaminants on the metabolic pathways and also for the identification of biomarkers. It can be concluded, establish honeybees as biomonitoring tools and sentinel species for environmental pollution, ultimately developing an early warning system for environmental hazards.
12:00 - 12:30	<u>Romans Pavļenko</u>
	Institute of Food Safety, Animal Health and Environment BIOR, Latvia
	Determination of plant toxins in honey by liquid chromatography- mass spectrometry
	Plant toxins are natural chemicals plants produce to defend against threats like bacteria, fungi, insects, and predators. They can be found in foods and may harm the liver if consumed. Grayanotoxins are a group of closely related neurotoxins produced by Rhododendron species and other plants in the Ericaceae family. Honey containing pollen from these plants, known as mad honey, also contains grayanotoxins. Consuming the plant or its products, including mad honey, can lead to rare poisoning called grayanotoxin poisoning. Pyrrolizidine alkaloids are one of the groups of plant-synthesized alkaloids that a plant accumulates in its body to prevent harm from insects, pests and animals. More than 6000 plants are known, several of which are banned for human consumption. The toxicological properties of these compounds can vary considerably, and many pyrrolizidine alkaloids are cytotoxic, hepatoxic, genotoxic and carcinogenic.
	This lecture provides information about occurrence data of plant toxins in honey, European Union regulations, determination methods, as well as the challenges that can be encountered during method development.
12:30 - 13:00	<u>Anna Skrastiņa</u>
	Institute of Food Safety, Animal Health and Environment BIOR, Latvia
	Determination of PFAS and PAH in honey
	Persistent organic pollutants (POPs), such as per- and polyfluoroalkyl substances (PFAS) and polycyclic aromatic hydrocarbons (PAHs), are commonly found in surface water, groundwater, wastewater, sediments, soil, as well as in animals and humans.









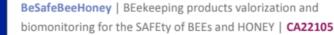
13:00 - 14:30	Lunch break
	This lecture focuses on methods for determining POPs in honey and data on their occurrence.
	POPs can enter the raw materials of honey (pollen, nectar) via air, water, and soil. They are then transported to the beehive and ultimately end up in honey, posing a risk to bees and honey consumers.
	processes such as vehicle exhaust, gas- or oil-fired heating, incinerators, and other industrial emissions.
	rings and occur naturally in fossil fuels or as anthropogenic pyrolysis products. They are produced through the incomplete combustion of organic matter and industrial
	PAHs are a group of organic compounds consisting of two or more fused aromatic
	surface protectants in various industrial, commercial, and consumer products because of their exceptional water and oil repellency, surface activity, and thermal stability.
	atom. Since the mid-20th century, they have been widely used as surfactants and
	PFAS are a class of synthetic chemicals containing at least one fully fluorinated carbon
	Increasing concern has arisen over their presence in the environment due to their toxicity, bioaccumulative nature, and persistence.

Field visit

16:00 - 18:00	<u>Nebojša Nedić and Denis Vojt</u>
	Visit to the urban apiary of the Faculty of Agriculture University of Belgrade in cooperation with the Faculty of Mechanical Engineering of the University of Belgrade
	https://maps.app.goo.gl/TncD6PCeQJpkS8C69
	The apiary was founded in the sixties of the twentieth century and since then has been a demonstration apiary for students of the Faculty of Agriculture University of Belgrade. It is located in the courtyard of the Faculty and the busy part of the urban zone of the municipality of Zemun. The apiary is surrounded by various trees from the Zemun Park, and dominantly the sofora, the Danube River is nearby, and with the students of the Faculty and the citizens of Belgrade, the apiary has been living its seventh decade in an urban environment. The visit will include the inspection and preparation of bee colonies for wintering: Assessment of the strength of colony The status of the queen bee The quality and arrangement of the combs in the nest Checking the supply and distribution of food in the hive Prevention from robbery bees Protect hives from intruders Varroa infestation check and signs of other bee diseases









19th September 2024

WORKSHOP – SYSTEMATIC REVIEW

Room 513, 5th floor

<u>Marta Leite</u>

National Institute for Agrarian and Veterinary Research (INIAV), Portugal

Description:

A systematic review can be defined as a detailed and comprehensive plan and search strategy that uses reproducible and rigorous methods to systematically search and synthetize a particular research question. In this workshop, the process on how to conduct a systematic review will include a theoretical part concerning the formulation of an answerable research question, definition of inclusion and exclusion criteria, and search tools for the evidence; and a practical part where the trainees will be in contact with systematic review platforms for article selection on a specific research question previously defined.

Outcomes:

Data collection is an extensive systematized procedure that allows to gather information towards the response to primary questions, thus permitting to analyse current information about a specific topic, and consequently formulating strategies to overcome practices' gaps, to improve existing methods or procedures, and to implement new approaches. This workshop will therefore guarantee that the trainees will be capable of delineating a data collection plan for application of a specific systematized procedure, ultimately aiming at a complete assessment of emerging contaminants in honey. Specifically, trainees will be able to:

- Recognize the research design of a systematic review
- Recognize the importance of using precise and accurate methods to conduct literature review
- Identify review questions and the elements of a well-defined review question
- Understand each step of the systematic review

09:00 - 09:30	Participant registration
09:30 - 11:00	The process of Systematic Review (theory)
11:00 - 11:30	Coffee break
11:30 - 13:00	Practical session
13:00 - 14:30	Lunch break
14:30 - 17:00	Practical session