SOCIETY OF ECOLOGICAL CHEMISTRY AND ENGINEERING

ECOLOGICAL CHEMISTRY AND ENGINEERING A

CHEMIA I INŻYNIERIA EKOLOGICZNA A

Vol. 17 No. 1

OPOLE 2010

EDITORIAL COMMITTEE

Witold Wacławek (University, Opole) – Editor-in-Chief
Milan Kraitr (Western Bohemian University, Plzen, CZ)
Jerzy Skrzypski (University of Technology, Lodz)
Maria Wacławek (University, Opole)
Grzegorz Formicki (Pedagogical University, Krakow)
Robert Stawarz (Pedagogical University, Krakow)
Tadeusz Majcherczyk (University, Opole) – Secretary

PROGRAMMING BOARD

Witold Wacławek (University, Opole) – Chairman
Jerzy Bartnicki (Meteorological Institute – DNMI, Oslo-Blindern, NO)
Mykhaylo Bratychak (National University of Technology, Lviv, UA)
Bogusław Buszewski (Nicolaus Copernicus University, Torun)
Eugenija Kupcinskiene (University of Agriculture, Kaunas, LT)
Bernd Markert (International Graduate School [IHI], Zittau, DE)
Nelson Marmiroli (University, Parma, IT)
Jacek Namieśnik (University of Technology, Gdansk)
Lucjan Pawłowski (University of Technology, Lublin)
Krzysztof J. Rudziński (Institute of Physical Chemistry PAS, Warszawa)
Manfred Sager (Agency for Health and Food Safety, Vienna, AT)
Mark R.D. Seaward (University of Bradford, UK)
Jiři Ševčik (Charles University, Prague, CZ)
Piotr Tomasik (University of Agriculture, Krakow)
Roman Zarzycki (University of Technology, Lodz)
Tadeusz Majcherczyk (University, Opole) – Secretary

EDITORIAL OFFICE
Opole University
POB 313, ul. Oleska 48, 45–951 OPOLE
phone./fax +48 77 455 91 49
email: waclawek@uni.opole.pl
http://tchie.uni.opole.pl

SECRETARIES
Agnieszka Dołhańczuk-Śródka, phone +48 77 401 60 45, email: agna@.uni.opole.pl
Małgorzata Rajfur, phone +48 77 401 60 42, email: mrajfur@o2.pl

SECRETARIES' OFFICE
phone +48 77 401 60 42
email: mrajfur@o2.pl

Copyright © by
Society of Ecological Chemistry and Engineering, Opole

Ecological Chemistry and Engineering
is partly financed by Ministry of Science and Higher Education, Warszawa
and Provincial Found for Environmental Protection and Water Management, Opole

ISSN 1898–6188

CONTENTS

Editorial 7

Michal BOŠIAK, Radoslav ŽIDEK, Jozef GOLIAN, Branislav ŠIŠKA and Jaroslav ŽIAK – Comparison of Suitability of two Commercially Used Methods ELISA and PCR for Detection of Defatted Soybean Powder 9

Anna CHRZAN, Maria MARKO-WORŁOWSKA and Tomasz ŁACIAK – Influence of the Heavy Metals Polluting the Soil on the Pedofauna of the Selected Parks in Krakow 17

Katarína FATRCOVÁ-ŠRAMKOVÁ, Peter CHLEBO and Eva DUDRIKOVÁ – Risk in Nutrition Habits of Slovak Population 21

Katarína FATRCOVÁ-ŠRAMKOVÁ, Anna KOLESÁROVÁ, Janka NÔŽKOVÁ and Katarína BABINSKÁ – Nutrition Habits and Anthropometric Parameters of Slovak Children 33

Katarína FATRCOVÁ-ŠRAMKOVÁ, Janka NÔŽKOVÁ, Miroslava KAČÁNIOVÁ, Magda MÁRIÁSSYOVÁ and Zlata KROPKOVÁ – Microbial Properties, Nutritional Composition and Antioxidant Activity of *Brassica napus* subsp. *napus* L. Bee Pollen Used in Human Nutrition 45

Krzysztof FRĄCZEK and Jacek GRZYB – Analyses of Bacterial Aerosol Occurring in Health Resorts in Bochnia and Szczawnica 55

Zofia GOC, Katarzyna KILIAN, Grzegorz FORMICKI, Robert STAWARZ, Aldona CIĄGŁO and Anna KUCZKOWSKA-KUŹNIAR – Antioxidant Status and Metal Contents in Human Breast Milk in Relation to Age and Course of Lactation 65

Jacek GRZYB and Krzysztof FRĄCZEK – Occurrence of Fungal Aerosol in Overground and Underground Health Resorts 73

Peter HAŠČÍK, Ivana NOVÁKOVÁ, Miroslava KAČÁNIOVÁ, Martina FIKSELOVÁ and Simona KUWOVÁ – Microbiological Quality of the *Anas platyrhynchos* and the *Fulica atra* Meat 81

Miroslava KAČÁNIOVÁ, Martina FIKSELOVÁ, Peter HAŠČÍK, Vladimíra KŇAZOVICKÁ, Janka NÔŽKOVÁ and Katarína FATRCOVÁ-ŠRÁMKOVÁ – Changes in Microflora of Bee Pollen Treated with UV Light and Freezing during Storage 89

Miroslava KAČÁNIOVÁ, Janka NÔŽKOVÁ, Katarína FATRCOVÁ-ŠRAMKOVÁ, Zlata KROPKOVÁ and Jana KUBINCOVÁ – Antioxidant, Antimicrobial Activity and Heavy Metals Content in Pollen of *Papaver somniferum* L. 97

Adriana KOLESAROVA, Marcela CAPCAROVA, Alexander V. SIROTKIN and Jaroslav KOVACIK – Effect of Lead, Silver and Molybdenum on Steroidogenesis in Porcine Ovarian Granulosa Cells *in Vitro* 107

Eva OKÉNKOVÁ, Jiří KREJČÍ, Jan HRABĚ and Robert VÍCHA – Oxidative Changes of Milk Fat in Dry Milk Stored under Various Conditions 119

Dariusz ROPEK and Krzysztof FRĄCZEK – Effect of the Solid Waste Landfill in Tarnow on the Healthiness of Spring Wheat 129

Dana TANČINOVÁ, Soňa FELŠÖCIOVÁ, Mária DOVIČIČOVÁ, Zuzana MAŠKOVÁ, Roman LABUDA and Soňa JAVOREKOVÁ – Endogenous Contamination of Wheat by Species of Genera *Aspergillus* and *Penicillium* 135

Alena VOLLMANNOVÁ, Ján TOMÁŠ and Tomáš TÓTH – Risk Elements' Input into the Food Chain in Old Loaded Localities 143

REVIEWS

Jerzy SIEPAK – Piotr Konieczka i Jacek Namieśnik, "Quality Assurance and Quality Control in the Analytical Chemical Laboratory. A Practical Approach", Taylor & Francis, Boca Raton, London, New York 2009, 233 ss, ISBN 978-1-4200-8270-8 . . . 157

VARIA

Invitation for ECOpole '10 Conference 161

Zaproszenie na Konferencję ECOpole '10 163

Guide for Authors on Submission of Manuscripts 165

Zalecenia dotyczące przygotowania manuskryptów 167

SPIS TREŚCI

Od Redakcji 7

Michal BOŠIAK, Radoslav ŽIDEK, Jozef GOLIAN, Branislav ŠIŠKA i Jaroslav ŽIAK – Porównanie możliwości zastosowania metody ELISA i PCR do detekcji odtłuszczonej mąki sojowej 9

Anna CHRZAN, Maria MARKO-WORŁOWSKA i Tomasz ŁACIAK – Oddziaływanie metali ciężkich zanieczyszczających glebę na pedofaunę wybranych parków w Krakowie 17

Katarína FATRCOVÁ-ŠRAMKOVÁ, Peter CHLEBO i Eva DUDRIKOVÁ – Zagrożenia w nawykach żywieniowych ludności Słowacji 21

Katarína FATRCOVÁ-ŠRAMKOVÁ, Anna KOLESÁROVÁ, Janka NÔŽKOVÁ i Katarína BABINSKÁ – Nawyki w żywieniu i parametry antropometryczne u słowackich dzieci 33

Katarína FATRCOVÁ-ŠRAMKOVÁ, Janka NÔŽKOVÁ, Miroslava KAČÁNIOVÁ, Magda MÁRIÁSSYOVÁ i Zlata KROPKOVÁ – Skład mikroflory, składniki odżywcze i aktywność antyoksydacyjna w pyłku pszczelim pochodzącym z *Brassica napus* subsp. *napus* L. używanym w żywieniu ludzi 45

Krzysztof FRĄCZEK i Jacek GRZYB – Badania aerozolu bakteryjnego występującego w ośrodkach sanatoryjnych w Bochni i Szczawnicy 55

Zofia GOC, Katarzyna KILIAN, Grzegorz FORMICKI, Robert STAWARZ, Aldona CIĄGŁO i Anna KUCZKOWSKA-KUŹNIAR – Status antyoksydacyjny oraz zawartość metali w mleku ludzkim u kobiet w różnym wieku i okresie laktacji 65

Jacek GRZYB i Krzysztof FRĄCZEK – Badania aerozolu grzybowego w sanatorium nadziemnym i podziemnym 73

Peter HAŠČÍK, Ivana NOVÁKOVÁ, Miroslava KAČÁNIOVÁ, Martina FIKSELOVÁ i Simona KUWOVÁ – Mikrobiologiczna jakość mięsa *Anas platyrhynchos* i *Fulica atra* 81

Miroslava KAČÁNIOVÁ, Martina FIKSELOVÁ, Peter HAŠČÍK, Vladimíra KŇAZOVICKÁ, Janka NÔŽKOVÁ i Katarína FATRCOVÁ-ŠRÁMKOVÁ – Zmiany flory bakteryjnej pyłku pszczelego zamrażanego i eksponowanego na promieniowanie UV w czasie przechowywania 89

Miroslava KAČÁNIOVÁ, Janka NÔŽKOVÁ, Katarína FATRCOVÁ-ŠRAMKOVÁ, Zlata KROPKOVÁ i Jana KUBINCOVÁ – Zawartość metali ciężkich oraz aktywność antyoksydacyjna i antybakteryjna pyłku *Papaver somniferum* L. 97

Adriana KOLESAROVA, Marcela CAPCAROVA, Alexander V. SIROTKIN i Jaroslav KOVACIK – Wpływ ołowiu, srebra i molibdenu na steroidogenezę *in vitro* w komórkach ziarnistych jajników świni 107

Eva OKÉNKOVÁ, Jiří KREJČÍ, Jan HRABĚ i Robert VÍCHA – Zmiany oksydacyjne w tłuszczu mleka proszkowanego przechowywanego w różnych warunkach 119

Dariusz ROPEK i Krzysztof FRĄCZEK – Wpływ składowiska odpadów komunalnych w Tarnowie na zdrowotność pszenicy jarej 129

Dana TANČINOVÁ, Soňa FELŠÖCIOVÁ, Mária DOVIČIČOVÁ, Zuzana MAŠKOVÁ, Roman LABUDA i Soňa JAVOREKOVÁ – Endogenne zanieczyszczenia pszenicy przez gatunki rodzaju *Aspergillus* i *Penicillium* 135

Alena VOLLMANNOVÁ, Ján TOMÁŠ i Tomáš TÓTH – Metale ciężkie w roślinach uprawnych uprawianych w pobliżu dawnych źródeł zanieczyszczeń 143

RECENZJE

Jerzy SIEPAK – Piotr Konieczka i Jacek Namieśnik, "Quality Assurance and Quality Control in the Analytical Chemical Laboratory. A Practical Approach", Taylor & Francis, Boca Raton, London, New York 2009, 233 ss, ISBN 978-1-4200-8270-8 . . . 157

VARIA

Invitation for ECOpole '10 Conference 161

Zaproszenie na Konferencję ECOpole '10 163

Guide for Authors on Submission of Manuscripts 165

Zalecenia dotyczące przygotowania manuskryptów 167

Papers published in the issue have been presented during the 8th Scientific Conference Risk Factors of Food Chain, September 17th, 2008 in Krakow, PL.

Artykuły opublikowane w tym zeszycie były prezentowane w czasie VIII Międzynarodowej Konferencji Naukowej pt.: Risk Factors of Food Chain, 17 września 2008 r. w Krakowie. Organizatorem konferencji był Zakład Zoologii Kręgowców i Biologii Człowieka Uniwersytetu Pedagogicznego w Krakowie kierowany przez Pana Prof. Władysława Zamachowskiego oraz Zakład Fizjologii Zwierząt Uniwersytetu Rolniczego w Nitrze na Słowacji, kierowany przez Pana Prof. Petera Massányego.

Prezentowane artykuły przeszły normalną procedurę recenzyjną i redakcyjną.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Michal BOŠIAK[1], Radoslav ŽIDEK, Jozef GOLIAN,
Branislav ŠIŠKA and Jaroslav ŽIAK

COMPARISON OF SUITABILITY OF TWO COMMERCIALLY USED METHODS ELISA AND PCR FOR DETECTION OF DEFATTED SOYBEAN POWDER

PORÓWNANIE MOŻLIWOŚCI ZASTOSOWANIA METODY ELISA I PCR DO DETEKCJI ODTŁUSZCZONEJ MĄKI SOJOWEJ

Abstract: The aim of this study was to compare the suitability of two methods enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) used for detection of defatted soybean powder. We have analysed 20 artificially contaminated samples prepared by simple dilution with wheat flour. Sample one was prepared as a combination of 1 g of defatted soybean powder with 1 g of wheat flour. Sample has been homogenized by vortexing. Next sample was prepared as combination of one gram of previous sample and 1 g of wheat flour. According to this methodology all twenty samples were prepared. Detection of defatted soybean powder in artificially contaminated samples has been performed using both methods. We detected significant differences between both used methods. We were able to detect presence of defatted soybean powder in artificially contaminated samples with using of both methods. Wheat flour contamination by defatted soybean powder was detected at least in sample 13 (0.012 %/122 mg $\cdot$ kg^{-1}) by PCR method. Defatted soybean powder was not detected in samples 14 up to 20.

Samples from 1 to 10 have not been quantified because absorbance values ranged above detection limit. Samples from 11 to 20 were quantified, but only measured values of sample 16, 17 and 18 were in the guaranteed quantification range provided by ELISA kit manual. We have detected defatted soybean powder contamination in samples 19 and 20 but absorbance values were highly similar to absorbance of the control sample.

Keywords: defatted soybean powder, PCR, ELISA, detection limit, contamination

Allergic reactions to foods are an important medical problem throughout the industrialized world. The occurrence of food allergy appears to be strongly influenced by genetics. In addition, genetic susceptibility alone does not explain the prevalence of food allergy satisfactorily, leaving ample room to consider the importance of environ-

[1] Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak Agricultural University in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, email: m.bosiak@gmail.com

mental influences (external, maternal and gastrointestinal environment) and interactions between the host and the environment [1]. Food allergy is an inadequate immune response to some foods containing a food allergen or allergens. Food allergens are the most common water soluble glycoproteins, mostly with acid pH and molecular weight from 10 to 70 kDa [2]. Allergic reaction involves two actions of the immune system. Immune system produces immunoglobulin E (IgE), a type of protein that works against a specific antigen. This protein is called a food-specific antibody, and it circulates through the blood. The food-specific IgE then attaches to mast cells, the cells are found in all body tissues. They are more often found in areas that are typical sites of allergic reactions. Those sites include nose, throat, lungs, skin and gastrointestinal tract. Clinical allergic people are suffering from a number of the symptoms in the range from allergic coryza, asthma, atopic dermatitis to life threatening anaphylactic shock [3, 4]. An allergic reaction to food can take place within a few minutes to an hour. The process of eating and digesting food affects the timing and the location of manifestation of the symptoms [5]. In adults, the following foods that most often cause allergic reactions include: shellfish, peanuts, tree nuts, fish, hen's egg. The most common foods that cause problems in children are: hen's egg, cow's milk and peanuts. Tree nuts and peanuts are the leading causes of deadly food allergy reactions [6]. Over 90 % of IgE-mediated food allergies in childhood are caused by eight foods: cow's milk, hen's egg, soy, peanuts, tree nuts (and seeds), wheat, fish and shellfish [7]. Food allergy is characterized by acute reactions, such as the oral allergy syndrome, urticaria, angioedema, vomiting, diarrhoea, dyspnoea, allergic asthma, bronchospasm, dermatitis, edema, eczema, rhinitis and even anaphylactic shock [8]. Anaphylaxis is a serious, rapid-onset, allergic reaction that may cause death. Severe anaphylaxis is characterized by life-threatening upper airway obstruction, bronchospasm and/or hypotension [9]. Delayed reactions such as flare up of eczema may occur, but are less frequently reported [10, 11].

Food manufacturers have to declare all food allergens on the labels of their products. Any ingredient used in production of a foodstuff and still present in the finished product, even if in altered form, shall be indicated on the label with a clear reference to the name of this ingredient [12]. Soybean is recognized as one of the major food allergens (14 food allergens), which can be present in food and therefore must be labeled according to the European Directives [13–16]. Moreover, if the origin of the soybean is genetically modified, labeling of this fact is also required according to the European Regulations [17, 18]. In case of allergic reaction to some food that contained specific food allergen and it was not declared on the label is recognized in a consumer's organism, manufacturer will responsible for mayhem.

Many tests for the detection of soy proteins in foods have been described in literature. Efficiency of the detection method depends mostly on food product type and detection tool. Six methods were compared using eight food products by Pedersen et al. [19]. The sandwich ELISA aimed at native soy proteins had the lowest detection limit, but in limited number of products. PCR methods are useful for all products with different detection limit. Advantage of PCR methods is simultaneous detection of GM (genetically modified) products and sensitivity for highly processed food [20]. In spite of DNA resistance to food processing, damage within the DNA fragments is believed to

be caused by the exposure to heat or pressure, enzymatic degradation by nucleases, water activity and pH values [21–23]. Nowadays, enzyme-linked immunosorbent assay (ELISA) is also able to detect presence of soy proteins in denatured and heated soy protein isolate samples [24] or in processed food [25].

Material and methods

Detection of defatted soybean powder frequently used for commercial food production has been performed using two methodologies. PCR method has been used for its DNA resistance to food processing and ELISA method for targeting to soy protein and quantification potential. Both methods were used for analysis of artificially contaminated samples (Table 1) prepared by simple dilution by wheat flour. Sample one was prepared as a combination of 1 g of defatted soybean powder with 1 g of wheat flour. Sample has been homogenized by vortexing. Next sample was prepared as combination of 1 g of previous sample and 1 g of wheat flour. According to this methodology were prepared all twenty samples.

PCR method

Genomic DNA was extracted from each admixed sample by using a NucleoSpin® Food purification kit (Macherey-Nagel, Switzerland). Extracted DNA was quantified at λ = 260 nm by NanoPhotometer™ (Implen GmbH, Germany) and diluted with the appropriate volume of distilled water to a final concentration of 25 ng · cm^3, samples have been stored in –20 °C until needed. Primers have been designed according to Hurst et al [26]. Primers LE1(5′GAAGCAACCAAACATGATCCTC3′)andLE2(5′ATGGAT CTGATAGAATTGACGTTA3′) amplify a 407 bp portion of the lectin gene of Glycine max.

The reaction mixture for PCR was prepared in a PCR reaction tube. The reaction volume of 50 mm^3 of genomic DNA, 0.25 mM dNTP, 1.8 mM $MgCl_2$, 1 μM of the 5′ and 3′ primers, and 1 unit of GoTaq® Hot Start Polymerase (Promega, Medison USA), all topped up with distilled water. The reaction was buffered with Green Go Taq® Flexi Buffer (Promega, Medison USA) and amplified in a thermal cycler (PTC-150 MiniCycler™, MJ Research, Watertown USA). The PCR step-cycle condition was as follows: pre-incubation at 94 °C for 2 min, 40 cycles consisting of denaturation at 94 °C for 45 s, annealing at 61 °C for 45 s, and extension at 72 °C for 1.5 min, followed by a final extension at 72 °C for 10 min. After PCR amplification, agarose gel electrophoresis of the PCR product was carried out using 1 % agarose gel. Fragments were visualized using ethidium bromide and separated by 150 V for 40 min.

ELISA method

The tested samples were prepared according to the accepted sampling techniques (Neogen's® Food Allergen Handbook). The extraction solution was prepared according to the procedural notes. It was preheated to 60 °C by immersing the bottle containing

the extraction solution into the water bath allowing reaching 60 ^{o}C. The samples (1 g) were transferred into the 50 cm^3 plastic tubes. An extraction additive (0.2 g) was added to the samples. The extraction solution was poured (25 cm^3/60 ^{o}C) into the sample plastic tubes. The plastic tubes with samples were capped and the extraction by shaking in water bath at 60 ^{o}C for 15 minutes was performed. After extraction, the plastic tubes were removed from the bath and materials were settled for 5 minutes before the next step. The extracts were filtered by pouring at least 5 cm^3 through a filter and the filtrates as the samples were collected. The clear supernatants were used as the samples. The extracts were cooled to room temperature before beginning analyses.

Twenty red-marked mixing wells for samples and five red-marked wells for controls were removed and placed in the well holder. An equal number of antibody-coated wells were removed and the strip was placed in the well holder. Each control and sample extract was transferred (150 mm^3) to the red-marked mixing wells using a new pipette tip for each. Controls and sample extracts (100 mm^3) were transferred using the 12-channel pipettor to the antibody-coated wells. The wells were mixed in the well holder for 20 seconds. The microwells were incubated for 10 minutes at room temperature (18–30 ^{o}C). The contents of the wells were emptied into a sink. Each antibody well was washed with a wash bottle filled with the wash buffer solution and dumped out. This procedure was repeated 5 times, then the wells were turned upside down and tapped out on the paper towel until the removing washing solution. Conjugate from the blue-labelled bottle (100 mm^3) was using the 12 channel pipettor transferred into all the wells and mixed in the well holder for 20 seconds. The microwells were incubated for 10 minutes at room temperature (18–30 ^{o}C). All the wells were washed with the wash buffer solution as described previously. Conjugate from the green--labelled bottle (100 mm^3) was using the 12 channel pipettor transferred into all the wells and mixed in the well holder for 20 seconds. The microwells were incubated for 10 minutes at room temperature (18–30 ^{o}C). Red Stop solution from the red-labelled bottle (100 mm^3) was using the 12 channel pipettor transferred into all the wells and mixed in the well holder for 20 seconds. The bottoms of the microwells were wiped and read in a microwell reader with λ = 650 nm filter. The results were interpreted using STAT FAX 321/plus microwell reader (Awareness Technology, Palm City, FL).

Results and discussion

Both methods were able to detect soybean contamination. To assess the sensitivity of both methods, we tested the mixed wheat flour samples containing diverse contamination (Table 1) of the defatted soybean powder. 50 ng of the genomic DNA extracted from the mixed samples was amplified in the PCR reaction. Used primers were able to amplify one discrete 407 bp long band. Results shown in Figure 1 demonstrate that the wheat flour contaminated by defatted soybean powder can be detected at least at the level of 0.012 %. Soy DNA was not detected from sample 14 to sample 20, contamination between 61 mg · kg^{-1} and 1 mg · kg^{-1} of defatted soybean powder. Results in Figure 1 show the intensity decreasing of PCR product. Sample 13 (122 mg · kg^{-1} of defatted soybean powder) has the lowest intensity but it is still detectible.

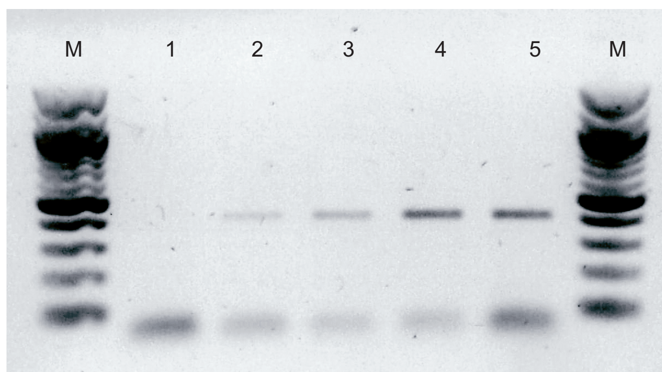
Table 1

Artificial contamination by defatted soybean powder

Soya contamination			PCR	ELISA	
sample	[%]	[mg · kg^{-1}]		[mg · kg^{-1}]	
1	50	—	+	Out of detection range	Out of guaranteed quantification range
2	25	—	+		
3	12.5	—	+		
4	6.25	—	+		
5	3.125	—	+		
6	1.5625	—	+		
7	0.7813	—	+		
8	0.3906	—	+		
9	0.1953	—	+		
10	0.0977	—	+		
11	0.0488	488.3	+	756.9	
12	0.0244	244.1	+	251.3	
13	0.0122	122.1	+	86.3	
14	0.0061	61.0	—	43.1	
15	0.0031	30.5	—	30.1	
16	0.0015	15.3	—	15.3	
17	0.0008	7.6	—	4.9	
18	0.0004	3.8	—	3.2	
19	0.0002	1.9	—	0.9	
20	0.0001	1.0	—	0.6	

Observed detection limit is lower than 1 % described by Meyer et al [27] using similar primers based on soya lectin Le1 gene. Our results are similar to Gryson et al [28] who declare the detection limit for defatted as well as for the full-fat soybean flour samples low as 0.2 % of the soy ingredient on total flour weight basis. Products without processing were tested for detection limit of soybean contamination by Yamakawa et al [29]. Designed primers based on repetitive sequence SIRE1 were able to decrease the detection limit of wheat flour contaminated by soybean flour in a product without processing to level about 0.001 %.

Artificially contaminated samples were tested by ELISA kit. One gram of sample was analysed and calorimetrically quantified. We were not able to detect clear detection limit as by PCR method because of negative control absorbance. According to quantification range provided by ELISA kit manual, we can clearly quantify contamination between 2.5 ppm [mg · kg^{-1}] and 25 ppm [mg · kg^{-1}] of soybean protein.



lane1: sample 14 [61 mg · kg^{-1}]; lane2: sample 13 [122.1 mg · kg^{-1}]; lane3: sample 12 [244.1 mg · kg^{-1}]; lane4: sample 11 [488.3 mg · kg^{-1}]; lane5: sample 10 [977 mg · kg^{-1}]; lane M: 100-bp ladder size standard

Fig. 1. Detection limit of PCR method

Declared quantification range is higher than the limit of quantification 1 ppm [mg · kg^{-1}] defined by Koppelman et al [25]. They have detected soy in following test materials: native soybean meal, soy protein isolate, soy protein concentrate, and textured soy flakes with using sandwich and inhibition ELISA. Competition ELISA format resulted in a sensitive test with a detection limit of 0.02 $\mu g/cm^3$, corresponding to 0.4 μg/g (0.4 ppm) in food samples. Obtained results based on ELISA method are presented in Table 1 in independent column. Samples 1 to 10 contaminated by subsistent percentage of defatted soybean powder have not been quantified because absorbance values ranged above detection limit. Samples from 11 to 20 were quantified, but only measured values of sample 16, 17 and 18 can be considered as accurate. The lowest detection limit of our samples is still higher than detection limit of 0.05 ppm (sandwich ELISA) determined by Pedersen et al [19], and the highest detection limit of our samples is also higher than detection limit of 21 ppm (competitive ELISA) using eight food products with a declared content of soy. We were able to detect soy contamination in samples 19 and 20 but absorbance values were highly similar to absorbance of negative control. Confrontation of ELISA quantification with artificial contamination shows similar contamination data.

Conclusions

We were able to detect presence of defatted soybean powder in artificially contaminated samples with using of both analytical methods. Wheat flour contamination by defatted soybean powder was detected at least in sample 13 (0.012 %/122 mg · kg^{-1}) by PCR method. Defatted soybean powder was not detected in samples 14 up to 20.

Samples from 1 to 10 have not been quantified because absorbance values ranged above detection limit. Samples from 11 to 20 were quantified, but only measured values

of sample 16, 17 and 18 were in the guaranteed quantification range provided by ELISA kit manual. We have detected defatted soybean powder contamination in samples 19 and 20 but absorbance values were highly similar to absorbance of negative control.

According to presented results, we can confirm the high accuracy of selected ELISA kit for detection of soy protein in defatted soybean powder. We are able to quantify detection limit of PCR methods for detection of defatted soybean powder in wheat flour to level above 122 mg · kg^{-1}.

Acknowledgement

This research is supported by a grant from Scientific Grant Agency of Ministry of Education of the Slovak Republic and Slovak Academy of Sciences, no. of project: VEGA 1/3475/06.

References

[1] Dreskin S.C.: *Genetics of food allergy*. Current Allergy Asthma Reports. 2006, **6**, 58–64.
[2] Lehrer S.B., Ayuso R. and Reese G.: *Current understanding of food allergens.* Ann. New York Acad. Sci. 2002, **964**, 69–85.
[3] Wüthrich B.: *Lethal or life-threatening allergic reactions to food.* J. Invest. Allergol. Clinical Immunol. 2000, **10**, 59–65.
[4] Ortolani C., Ispano M., Scibilia J. and Pastorello E.A.: *Introducing chemists to food allergy*. Allergy 2001, **56**, 5–8.
[5] Molkhou P.: *Food Allergies. Present and future problems*. The UCB Institute of Allergy 2000, **49**, 53–56.
[6] Villard-Truc F., Gomez S.A., Deschildre A. and Rance F.: *Oral food challenge in children: who, when, and how? Patient's selection*. Rev. Franc. D. Allergologie D Immunol. Clin. 2006, **46**, 610–624.
[7] Allen K.J., Hill D.J. and Heine R.G.: *Food allergy in childhood*. Med. J. Australia 2006, **185**, 394–400.
[8] Flinterman A.E., Knulst A.C., Meijer Y., Bruijnzeel-Koomen C.A.F.M. and Pasmans S.G.M.A.: *Acute allergic reactions in children with AEDS after prolonged cow's milk elimination diets.* Allergy 2006, **61**, 370–374.
[9] Brown S.G.A., Mullins R.J. and Gold M.S.: *Allergy – Anaphylaxis: diagnosis and management.* Med. J. Australia 2006, **185**, 283–289.
[10] Heine R.G., Elsayed S., Hosking C.S. and Hill D.J.: *Cow's milk allergy in infancy*. Curr. Opin. Allergy Clin Immunol. 2002, **2**, 217–225.
[11] Novembre E. and Vierucci A.: *Milk allergy/intolerance and atopic dermatitis in infancy and childhood.* Allergy 2001, **56**, 105–108.
[12] *Directive 2003/89/EC of European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs*. Off. J. Eur. Communities L308, 15–18.
[13] *Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs*. Off. J. Eur. Communities L109, 29–42.
[14] *Commission Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council*. Off. J. Eur. Communities L75, 33–36.
[15] *Commission Directive 2006/142/EC of 22 December 2006 amending Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council listing the ingredients which must under all circumstances appear on the labelling of foodstuffs*. Off. J. Eur. Communities L368, 110–111.
[16] *Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients*. Off. J. Eur. Communities L310, 11–14.

[17] *Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.* Off. J. Eur. Communities L268, 1–23.

[18] *Regulation (EC) No. 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.* Off. J. Eur. Communities L268, 24–28.

[19] Pedersen H.M., Holzhauser T., Bisson C., Amedeo C., Jensen B.L., Skov P.S., Bindslev-Jensen C., Brinch D.S. and Poulsen L.L.: *Soybean allergen detection methods – A comparison study*. Mol. Nutrit. Food Res. doi:10.1002/mnfr.200700394 Published online: http://www3.interscience.wiley.com/journal/121382715/abstract?CRETRY=1&SRETRY=0.

[20] Zhang M., Gao X., Yu Y., Ao J., Qin J., Yao Y. and Li Q.: *Detection of Roundup Ready soy in highly processed products by triplex nested PCR.* Food Control., 2007, **18**, 1277–1281.

[21] Lindahl T.: *Instability and decay of the primary structure of DNA*. Nature 1993, **362**, 709–715.

[22] Anklam E., Gadani F., Heinze P., Pijnenburg H. and Van Den Eeede G.: *Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant derived food products.* Eur. Food Res. Technol. 2002, **214**, 3–26.

[23] Tilley M.: *PCR amplification of wheat sequences from DNA extracted during milling and baking.* Cereal Chem. 2004, **81**, 44–47.

[24] L'Hocine L., Boye J.I. and Munyana C.: *Detection and quantification of soy allergens in food: study of two commercial enzyme-linked immunosorbent assays*. J. Food Sci. 2007, **72**, 145–153.

[25] Koppelman S.J., Lekemond C.M., Vlooswijk R. and Hefle S.L.: *Detection of soy proteins in processed foods: literature overview and new experimental work.* J AOAC Int. 2004, **87**, 1398–1407.

[26] Hurst C.D., Knight A. and Bruce I.J.: *PCR detection of genetically modified soya and maize in foodstuffs.* Mol. Breed. 1999, **5**, 579–586.

[27] Meyer R., Chardonens F., Hübner P. and Lüthy J.: *Polymerase chain reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products.* Z. Lebensm. Unters. Forsch. 1996, **203**, 339–344.

[28] Gryson N., Messens K. and Dewettinck K.: *PCR detection of soy ingredients in bread.* Eur. Food Res. Technol. 2008, **207**, 345–351.

[29] Yamakawa H., Akiyama H., Endo Y., Miyatake K., Sakata K., Sakai S., Moriyama T., Urisu A. and Maitani T.: *Specific detection of soybean residues in processed foods by the polymerase chain reaction.* Biosci. Biotechnol. Biochem. 2007, **71**, 269–272.

PORÓWNANIE MOŻLIWOŚCI ZASTOSOWANIA METODY ELISA I PCR DO DETEKCJI ODTŁUSZCZONEJ MĄKI SOJOWEJ

Abstrakt: Celem badań było porównanie możliwości zastosowania metod ELISA i PCR do detekcji odtłuszczonej mąki sojowej. Przeanalizowano 20 sztucznie zanieczyszczonych próbek mąki pszennej. Próbka pierwsza została przygotowana poprzez zmieszanie 1 g odtłuszczonej mąki sojowej z 1 g mąki pszennej. Próbka ta została zhomogenizowana przez wirowanie. Następną próbkę przygotowano przez zmieszanie 1 g próbki pierwszej z 1 g mąki pszennej. W ten sposób przygotowano 20 próbek o zmniejszającej się zwartości mąki sojowej. Obydwie metody, tj. ELISA, jak i PCR, umożliwiały stwierdzenie zanieczyszczenia mąką sojową. Odnotowaliśmy statystycznie istotne różnice między wynikami uzyskanymi przy stosowaniu tych dwóch metod badawczych. Metoda PCR pozwalała na wykrycie odtłuszczonej mąki sojowej do próbki 13 (0,012 %/122 mg $\cdot$ kg^{-1}). Używając tej metody, nie można było wykryć mąki sojowej w próbkach od 14 do 20. Zawartość mąki sojowej w próbkach 1 do 10 była zbyt duża i przekraczała zakres oznaczalności metody ELISA. Możliwe było wykonanie oznaczeń w próbkach od 11 do 20, jednak wyłącznie wyniki z próbek 16, 17 i 18 zawierały optymalną ilość mąki sojowej do oznaczeń metodą ELISA. Wykryliśmy również mąkę sojową w próbkach 19 i 20, jednak wartości absorbancji były bardzo zbliżone do absorbancji w próbkach kontrolnych.

Słowa kluczowe: odtłuszczona mąka sojowa, PCR, ELISA, zakres wykrywalności, zanieczyszczenie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Anna CHRZAN[1], Maria MARKO-WORŁOWSKA[1]
and Tomasz ŁACIAK[2]

INFLUENCE OF THE HEAVY METALS POLLUTING THE SOIL ON THE PEDOFAUNA OF THE SELECTED PARKS IN KRAKOW

ODDZIAŁYWANIE METALI CIĘŻKICH ZANIECZYSZCZAJĄCYCH GLEBĘ NA PEDOFAUNĘ WYBRANYCH PARKÓW W KRAKOWIE

Abstract: The high density of the metals in the soil, due to its toxic influence to the microflora, plants as well as to the pedofauna (mesofauna), can negatively affect the functioning of the soil subsystem. In order to evaluate the toxicity of the metals to mesofauna of the soil 3 areas were chosen where the diversity, the number and the content of Cd, Pb, Ni, Cu, Fe and Zn in the soil were detected. The humidity, temperature and pH of the soil were also analyzed. The chosen areas were situated in 3 different city parks in Krakow. The significant differences of the Cd, Pb, Ni, Cu, Fe and Zn content as well as in the density and diversity of the mesofauna were noted. It was detected that cadmium is toxic to the mesofauna. The nickel and the zinc have the similar effects.

Keywords: pedofauna, mesofauna, diversity, abundance, heavy metals

The anthropogenic processes such as: different branches of industry, transport, public utilities, fertilization and the use of pesticides are the main cause of the growth of toxic impact of many metals on environment. The heavy metals deriving from these processes disperse in the environment and pollute air, water, soil and organisms [1, 2].

The high density of the metals in the soil, due to its toxic influence to the microflora, plants as well as to the pedofauna (mesofauna), can negatively affect the functioning of the soil subsystem.

The high content of some heavy metals in the soil can influence negatively the soil subsystem due to its toxic impact on the microflora, plants and pedofauna.

[1] Departament of Ecology, Wildlife Research and Ecoturism, Institute of Biology, University of Education in Krakow, ul. Podbrzezie 3, 31–054 Kraków, Poland, phone 012 662 67 02, email: chrzan@ap.krakow.pl, mmw@ap.krakow.pl

[2] Departament of Zoology, Institute of Biology, University of Education in Krakow, ul. Podbrzezie 3, 31–054 Kraków, Poland, email: tlaciak@ap.krakow.pl

The aim of the research was to evaluate the content of Cd, Pb, Ni, Cu, Fe and Zn and the density and diversity of soil mesofauna in the chosen localities in the parks.

Material and methods

In order to evaluate the toxicity of some metals to soil mesofauna and the density and diversity of the mesofauna 3 localities on which the soil samples were taken in 2007 were chosen. The diversity and density of the mesofauna were detected. The humidity, temperature, soil pH, and Cd, Pb, Ni, Cu, Fe and Zn content were also subjected to the analysis. The methods of taking of the samples, their number, the methods of catching the pedofauna and the way of the evaluation of the content of the metals are presented in the separated article [3]. The selected areas were situated in 3 different city parks in Krakow:

– locality I – Park in Mogila around 300 m distant from the traffic lane,
– locality II – Planty (the green zone) at Gertrudy street near the busy street,
– locality III – Bednarski Park in Podgorze around 200 m distant from the main road.

Results

The soil in the selected localities of the city park had the neutral pH – from 6.75 to 6.9 (Table 1). The small differences in the humidity and the soil temperature were detected (Table 1).

Table 1

Comparison of selected parameters of the soils in the selected localities in Krakow

Selected parameters	Locality I[a]	Locality II	Locality III
Dampness of the soil [%]	12.3	17.18	13
pH value of the soil	6.9	6.75	6.78
Temperature of air in the area [^{o}C]	19.5	26.1	18.6
Temperature of the soil [^{o}C]	12.4	17.5	11.9

[a] Locality I – Park in Mogila around 300 m distant from the traffic lane; Locality II – Planty (the green zone) at Gertrudy street near the busy street; Locality III – Bednarski Park in Podgorze around 200 m distant from the main road.

The significant differences of the Cd, Pb, Ni, Fe and Zn contents as well as in the density and the diversity of the mesofauna were noted. The acceptable content of the metals in the soil containing anthropogenic pollutions according to Kabata-Pendias et al [4] are 70 mg Pb, 150 mg Zn and 1 mg Cd/kg. In the soil of the localities analyzed the instance of exceeding of the acceptable content of the metals was detected only in relation to lead (locality II) and cadmium (Table 2).

Table 2

Contents of heavy metals [mg/kg] in the soils of the selected localities in Krakow

Metal	Locality I[a]	Locality II	Locality III
	[mg/kg]		
Cd	1.896	1.893	2.784
Pb	49.623	100.835	69.207
Ni	10.119	21.155	20.544
Cu	11.01	38.189	17.038
Fe	1074.122	774.836	544.58
Zn	7.173	13.637	9.475

[a] See Table 1.

The content of Cd in the soil in the localities I and II are almost equal (1.896 i 1.893 mg/kg accordingly), whereas in the soil in the III locality the highest (almost 3 times higher than the acceptable content mentioned above) Cd content and at the same time the lowest density and the diversity of the mesofauna were noted (Tables 2, 3).

Table 3

Comparison of mesofauna in the soils of the selected localities in Krakow

Selected parameters	Locality I[a]	Locality II	Locality III
Abundance of pedofauna [sp.no.per m^2]	4280	920	510
Diversity [number of taxonomic groups]	18	15	10
Index of domination in the taxonomic groups [%]	*Acarina* 38.1 *Collembola* 30.6 *Thysanoptera* 9.7	*Collembola* 35.9 *Acarina* 21.8 *Lumbricidae* 11.4	*Acarina* 44.1 *Collembola* 13.7 *Chilopoda* 13.7

[a] See Table 1.

In the soil of Mogilski Grove, as the results concerning the mesofauna reflect, the lowest concentration of Pb, Ni, Cu and Zn was detected. The highest density and the diversity of the mesofauna were detected in above-mentioned locality (Tables 2, 3). In the same locality the biggest number of iron in the soil were noted that is connected with its nearest location from the main source of emission of the Fe-metallurgic complex in Nowa Huta. However, this content of Fe has no significant impact on the mesofauna analyzed. In the soil in the locality II (near the busy road) more than 2 times bigger concentration of Pb, Ni, Zn and 3 times bigger in case of Cu in comparison with the Mogilski Grove were determined. The density of the mesofauna was 4 time smaller in the locality II than in the locality I (Tables 2, 3).

Conclusions

1. As the research data show, cadmium is toxic in relation to the mesofauna that is connected with the fact that the smallest density and the diversity were determined in the soil with the biggest Cd content.

2. The results from the locality situated near the busy road confirm the negative influence of the high concentration of Pb, Ni and Zn in relation to the pedofauna (mesofauna). The biggest density and the diversity of the fauna analyzed were found in the locality in which the smallest content of the Pb and Cd, Ni, Cu and Zn was noted.

3. The research did not show the important influence of the Fe concentration determined for the mesofauna. The statistic of the probability correlation between the content of each metal and the density of the mesofauna indicates the growth, but in case of each metal analyzed $p > 0.05$.

References

[1] Klimowicz Z. and Melke J.: *Zawartość metali ciężkich w glebach w sąsiedztwie szlaków komunikacyjnych na przykładzie wybranych tras*. Roczn. Glebozn. 2000, **51**(3/4), 37–46.
[2] Kalembasa D., Pakuła K. and Becher M.: *Zawartość chromu, niklu, miedzi i manganu w glebach płowych wzdłuż obwodnicy siedleckiej.* J. Elementol. 2005, **10**(2), 315–324.
[3] Chrzan A., Marko-Worłowska M. and Łaciak T: *Influence of selected abiotic factors on pedofauna of different habitats situated near traffic route in Krakow*. Ecol. Chem. Eng. A 2008, **15**(4–5), 319–325.
[4] Kabata-Pendias A. et al: Podstawy oceny chemicznego zanieczyszczenia gleb – metale ciężkie, siarka i WWA. Państwowa Inspekcja Ochrony Środowiska. Bibl. Monit. Środow., Warszawa 1995.

ODDZIAŁYWANIE METALI CIĘŻKICH ZANIECZYSZCZAJĄCYCH GLEBĘ NA PEDOFAUNĘ WYBRANYCH PARKÓW W KRAKOWIE

Instytut Biologii, Zakład Ekologii, Badań Łowieckich i Ekoturystyki, Zakład Zoologii,
Uniwersytet Pedagogiczny w Krakowie

Abstrakt: Zwiększona zawartość metali w glebie może niekorzystnie oddziaływać na funkcjonowanie podsystemu glebowego poprzez toksyczny wpływ na mikroflorę, rośliny, a także pedofaunę i funkcjonowanie podsystemu glebowego. W celu oceny toksyczności metali dla mezofauny glebowej wyznaczono 3 stanowiska badawcze, na których zbadano jej zróżnicowanie, zagęszczenie, a także zawartość Cd, Pb, Ni, Cu, Fe i Zn w glebie. Zbadano również wilgotność, temperaturę oraz pH gleby. Odnotowano różnice w zawartości metali ciężkich oraz w zagęszczeniu i zróżnicowaniu mezofauny. Stwierdzono także toksyczny wpływ kadmu na mezofaunę. Nikiel i cynk ma wpływ na mezofaunę podobny jak kadm.

Słowa kluczowe: pedofauna, mezofauna, zagęszczenie, różnorodność, metale ciężkie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Katarína FATRCOVÁ-ŠRAMKOVÁ[1*], Peter CHLEBO[1]
and Eva DUDRIKOVÁ[2]

RISK IN NUTRITION HABITS OF SLOVAK POPULATION

ZAGROŻENIA W NAWYKACH ŻYWIENIOWYCH LUDNOŚCI SŁOWACJI

Abstract: The aim of the study was to provide a current assessment of the Slovakian adults self-reported dietary, and identify and analyze differences between females and males in anthropometries, food habits, preferences and eating attitude. Data are from a cross-sectional survey of 1 400 adults (700 males and 700 females) from different regions of Slovakia. Participants reported their usual consumption of meat and milk and their products, fruits and vegetables, legumes, sweets, and drinks. The study found that 34.05 % of participants (38.14 % females, and 30.0 % males) had a normal weight, while in 21.86 % of males and 15.72 % of females ($p < 0.01$) prevailed light obesity. Participants reported their usual weekly consumption (number of serves) of foods of vegetable and animal origin. The study found that 62.29 % of females were met the weekly consumption of dairy products three times and more ($p < 0.001$). Males (59.14 %) preferred consumption of pork meat at least once a week and beef (87.14 %). Consumption of poultry was law, with 83.86 % of all participants, having poultry meat at least once a week, 4.21 % eating rabbit meat and 20.43 % having fish (at least once a week). Fresh fruit ($p < 0.01$) as well as vegetable ($p < 0.001$) consumed at least three times a week more females than males (62 % and 42 % of females, respectively; $p < 0.01$). Age and gender difference occurred for more measures, and there were some socio-economic status differences. On the basis of the results of this study, it appears that a significant proportion of the Slovakian adults fall short of current, national dietary and physical activity recommendations for adults. Continual monitoring of these behaviours is essential to help inform research and policy and identify where future efforts should be directed.

Keywords: nutritional habits, food preferences, eating patterns, anthropometries, primary prevention, Slovak inhabitants

The value of the long-term programme of improving public health status in the Slovak Republic "Health for All in the 21st Century" lies in the fact that it represents a model of complex health prevention and health care and its improvement, which was developed according to current needs of medical fields including national health policy. The Report on Public Health Status in the Slovak Republic suggests a positive outcome of implementation of preventive programmes aimed at health promotion, although no

[1] Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[2] University of Veterinary Medicine in Košice, Komenského 73, 041 81 Košice, Slovak Republic.

* Corresponding author: email: katarina.sramkova@gmail.com

significant changes were noted in the structure of mortality stratified according to death causes. Five most common causes of death, like cardiovascular diseases, cancer, external causes (accidents, intoxications, homicides, suicides etc.), respiratory diseases and diseases of digestive system resulted in 95 % of all deaths. Cardiovascular diseases and cancer account quantitatively for the most important causes of death. Trends in mortality changes in the Slovak Republic are similar, to some degree, to those in the European Union.

A trend of mortality due to cardiovascular diseases in the whole Slovak population is sluggish. The incidence of heart attack is decreasing for all age categories, especially in males at productive age. The highest mortality due to cardiovascular diseases in both sexes at the age of 65 years and older was noted in southern and south-eastern regions of the Slovak Republic. This also applies to cerebrovascular diseases, ischemic heart disease, and heart attack. A trend of age-specific mortality due to chronic heart diseases did not almost change in the whole population in the last three decades [1].

The occurrence of non-infectious diseases depends in high range from lifestyle and nutrition belongs also to these risk factors which can be influenced. To decrease the influenced factors in whole population should result in decreasing of morbidity and mortality of these non-infectious diseases [2]. Poor eating habits or exercise regimens and lifestyle choices could lead to chronic disease later in life [3]. Monitoring and evaluation of nutritional habits is very important step to form an opinion on healthy status of population, identification of deficiency in human nutrition and following intervention with the aim of the primary prevention of civilization diseases with the respect of principles in the right human nutrition, mainly in the current national dietary and physical activity recommendations for adults.

The aim of the study was to provide a current assessment of Slovakian adults self-reported dietary, and identify and analyze differences between females and males in anthropometries, food habits, preferences and eating patterns.

Material and methods

Approval to conduct the study was obtained from all appropriate ethics committees as well as participants. Following an established protocols, members of the research team administered the anonymous pencil-and-paper questionnaire to groups of up to 100, 200, 300, and maximum 400 adults on the given the region of Slovakia under their free-time under exam conditions. To ensure that disproportionate sampling did not bias the prevalence estimates, data were weighted by age, gender, and physical activity.

Nutritional habits, food repertoire and anthropometric measurements were assessed in 1400 randomly selected Slovak inhabitants, aged from 25 to 75 years, from different regions of Slovakia. Participants' age was recorded at last birthday. The study recruited 700 females and 700 males (the age of 345 females and 350 males was less than 50 years and 355 females and 350 males were at least 50 years).

With the aim, overweight and obesity prevalence examined by body mass index (BMI) was evaluated in all participants [4].

Waist-to-hip ratio (WHR) was calculated by dividing waist circumference (measured 1 inch ie 2.54 cm above the umbilicus) by hip circumference (measured at the maximum girth over the buttocks). According to waist-to-hip ratio was in the obese participants: the gynoid (in the case of WHR less than 0.85 by females and less than 1 by males) and android (in the case of WHR at least 0.85 by females and at least 1 cm by males) health risk detected [5].

Waist circumference (measured in cm) was used to make a survey of risk of metabolic complications of obesity: adequate risk at the waist circumference less than 80 cm and less than 94 cm by females and by males, respectively; increased risk at the 80 cm and more by females and 94 cm and more by males; very high risk at the minimum 88 cm by females and minimum 102 cm by males [4].

The questionnaire and food frequency questionnaire, which were used to analyze of the nutritional habits of subjects, were composed at the Department of Human Nutrition in Nitra. The data were collected during the year 2007. Evaluation of ingested food was given according to the energy intake of 150–160 kJ (35/38 kcal) per kg a day [6–9].

The statistical evaluation and differences between females and males were analyzed using Statgraphics software. Differences between males and females were evaluated by using t-test and chí-quadratic test.

Results and discussion

A total of 1 400 adults agreed to participate in this study from different regions of Slovakia. Data from all participants aged between 25 and 75 years were analyzed. A summary of the demographic profile of the sample is shown in Tables 1 and 2.

Table 1

Demographic profile of the sample (n = 1400)

Age group [years]	Female		Male		All	
	n	[%]	n	[%]	n	[%]
To 50	345	49.29	350	50.00	695	49.64
50 and more	355	50.71	350	50.00	705	50.36
All	700	100.00	700	100.00	1400	100.00

Table 2

Demographic profile of the sample

Age group [years]	Female		Male		All	
	n	[%]	n	[%]	n	[%]
25–35	143	20.43	142	20.29	285	20.36
36–45	144	20.57	144	20.57	288	20.57
46–55	143	20.43	143	20.43	286	20.43
56–65	138	19.71	134	19.14	272	19.43
66–75	132	18.86	137	19.57	269	19.21
All	700	100.00	700	100.00	1400	100.00

Table 3

A summary of the antropomethric parameters of female

Parameter	Less than 50 years				50 years and more			
	BMI	WHR	Waist	Hip	BMI	WHR	Waist	Hip
Count	345	345	345	345	355	355	355	355
Average	24.1383	0.796319	80.8899	101.551	27.2549	0.845042	90.6845	106.975
Standard deviation	3.9518	0.073797	11.4145	10.304	4.47615	0.0935501	13.7784	12.1205
Coeff. of variation	16.3715 %	9.26727 %	14.1111 %	10.1467 %	16.4233 %	11.0705 %	15.1938 %	11.3302 %
Minimum	16.96	0.62	60.0	74.0	18.29	0.0	60.0	65.0
Maximum	40.63	1.08	130.0	140.0	44.53	1.18	131.0	148.0
Range	23.67	0.46	70.0	66.0	26.24	1.18	71.0	83.0
Stnd. skewness	4.5717	4.17394	6.1358	4.27568	6.03909	–13.743	5.19443	2.21659
Stnd. kurtosis	1.97911	2.86442	3.06241	0.492298	2.82858	70.0023	0.151479	3.43683

Table 4

A summary of the antropometric parameters of males

Parameter	Less than 50 years				50 years and more			
	BMI	WHR	Waist	Hip	BMI	WHR	Waist	Hip
Count	350	350	350	350	350	350	350	350
Average	26.1535	0.914314	92.0429	100.754	28.2186	0.941971	97.9114	104.157
Standard deviation	3.51201	0.104593	10.6468	11.1431	3.91523	0.0894418	12.6128	11.6705
Coeff. of variation	13.4284 %	11.4395 %	11.5672 %	11.0597 %	13.8746 %	9.49517 %	12.8819 %	11.2047 %
Minimum	17.7	0.0	63.0	73.0	19.59	0.57	32.0	56.0
Maximum	53.76	1.22	130.0	195.0	42.27	1.31	140.0	155.0
Range	36.06	1.22	67.0	122.0	22.68	0.74	108.0	99.0
Stnd. skewness	12.9747	–15.591	2.72523	25.9879	4.18217	3.34342	0.581529	2.75388
Stnd. kurtosis	39.1862	66.8618	2.59984	103.582	0.537752	6.61415	8.61759	6.76192

Table 3 and Table 4 show a summary data of the antropomethric parameters of the participants, both males and females.

As shown in Table 3 and Table 4 the normal weight was observed in 34.07 % of respondents (38.14 % of females and 30 % of males). The overweight was higher with males contrary to females (21.86 % of males vs 15.72 % of females), ($p < 0.01$), where in both sexes prevailed light obesity. According to BMI, obesity prevailed in males (21.86 %) rather than with females (53.29 %).

The proportion of obesity was observed rather in the age group of 50 years and more, both males and females. The waist-hip circumference ratio (WHR) has been used to assess distribution of adipose tissue.

The WHR has been reported to be strongly associated with visceral fat, although waist circumference alone may be a better predictor of visceral fat deposition than WHR [2, 10, 11]. The significance of level of visceral (omental and mesenteric) adipose tissue is its reported relationship to higher risk for chronic disease. In response to the evidence supporting waist circumference as a predictor of morbidity and mortality from chronic disease. WHO has published a waist circumference scale to classify overweight and obesity [12]. According to this, in about 52.09 % of participants the gynoid obesity was observed (44 % in the age of less than 50, and 16.94 % in the group of females in the age of 50 and more). In males, the android obesity was higher at the age less under 50 years than over ($p < 0.001$).

As presented in Table 5, males met the recommended weekly requirement of consumed type of meat. Males were more likely than females to consume pork meat ($p < 0.001$) and beef ($p < 0.01$), and poultry, however, there was not significant age differences.

Table 5

Quantity of consumed meat [g], fish [g] and eggs [pieces] a week (average ± SD)

Meat	Consumption	
	Females	Males
Pork	180.88 ± 176.44	234.01 ± 260.07
Beef	70.38 ± 98.75	95.45 ± 176.84
Poultry	223.69 ± 167.68	235.32 ± 265.64
Fish	62.93 ± 81.53	64.42 ± 108.27
Another meat (lamb...)	2.87 ± 28.00	4.10 ± 29.74
Game	6.16 ± 27.00	6.27 ± 21.91
Eggs	2.78 ± 3.42	3.24 ± 3.74

It was reported eating only 62.93 g of fish (females) and 64.42 g (18.29 % males) at least once per week. The proportion of participants consuming the recommended amount of meat decreases with age. Fish never consumed 7.21 % of participants. It was observed that fish consumption rapidly decreased within the last six years, and it is prevailed in females rather than males [2].

The consumed meat amount was evaluated according to the basket of foods [8], which was arranged for one week and supply daily intake of 10 800–11 340 kJ (2400–2700 kcal), or 150–150 kJ (35/38 kcal) per kg body weight a day, respectively. According to this, it was observed higher intake of pork meat and poultry, namely in males, and full insufficient intake of fish, which is associated also with low intake of unsaturated fatty acids.

As shown in Table 6, females reported eating dairy products at least three times in week ($p < 0.001$). Females were more likely than males to consume yogurts, both low-fat and creamy. Males reported consuming low-fat curd and curd classic, in the amount of 3.43 g and 46.52 g per week, respectively. The similar consumption was observed also in other types of fermented liquid milk products, such as kefir, acidophilus milk, etc. Around 15 % of respondents do not consume fermented milk products at all. Milk was relatively popular in all age categories, but consumption of cheese was lowest among individuals aged 65 years and older. It seems, that a high price of the cheese products may play a significant role.

Table 6

Quantity of consumed dairy products [g] a week (average ± sd)

Dairy products	Female	Male
Yogurt low-fat	174.22 ± 268.30	124.22 ± 227.22
Yogurt creamy	164.63 ± 278.13	153.29 ± 322.32
Curd low-fat	30.43 ± 90.25	33.43 ± 112.91
Curd	45.40 ± 102.82	46.52 ± 112.35
Kefir, acidophilous milk	149.50 ± 277.23	151.42 ± 278.83
Cream	82.34 ± 131.38	77.86 ± 167.43
Cheese low-fat	62.47 ± 129.12	50.95 ± 113.84
Cheese	139.19 ± 208.50	137.18 ± 211.18
Processed cheese low-fat	89.75 ± 156.46	63.47 ± 135.80
Processed cheese	159.35 ± 233.28	186.30 ± 265.70
Cottage cheese	28.21 ± 93.71	29.74 ± 95.32
Others	13.62 ± 63.13	13.90 ± 67.88

It is generally known, that milk and milk products are rich in bone minerals, which are normally found in the skeleton and gives an outline of the normal physiology and metabolism of bone. The adult skeleton contains about 1 kg of calcium and is in equilibrium with the plasma calcium at a concentration of about 2.25–2.60 mmol/dm^3. A large number of factors control calcium balance. The mount of calcium within the skeleton changes with age, according to size and composition, increasing during growth and declining with the bone loss of later years [13].

As Table 7 and 8 indicate, any of adults did not meet the recommended daily requirement of four or more serves of vegetable ($p < 0.001$) and fresh fruits ($p < 0.01$). Only 56.28 % of respondents wee more likely to consume at least one (maximum four) serves per week, however, there was no significant age difference. The same percentage

of both females and males reported eating one to four weekly serves of fruit. Females were more likely than males to achieve this. The proportion of adults consuming the recommended amount of fruits and vegetables decreased with age [13].

Table 7

Quantity of consumed fruit [g] a week (average ± SD)

Fruit	Female	Male
Apple	523.09 ± 545.67	487.57 ± 470.57
Peer	49.10 ± 126.90	61.28 ± 183.53
Citrus fruit	273.22 ± 412.02	255.61 ± 359.29
Banana	173.19 ± 238.24	172.99 ± 251.76
Plum, apricot, peach	65.95 ± 292.98	39.79 ± 107.70
Cherry, sour cherry, Cornelian cherry	43.64 ± 126.23	29.73 ± 113.62
Strawberry, raspberry, ribese, gooseberry	58.99 ± 195.72	33.53 ± 81.41
Grape	66.81 ± 290.47	57.99 ± 157.41
Bilberries, cranberries, pineapple	30.06 ± 86.81	31.53 ± 100.46

Table 8

Quantity of consumed vegetables [g] a week (average ± SD)

Vegetables	Female	Male
Potato	374.41 ± 361.44	454.46 ± 409.85
Tomato	252.37 ± 301.67	225.32 ± 286.91
Paprika	169.03 ± 244.74	169.63 ± 234.52
Cucumber	143.79 ± 181.87	141.33 ± 188.57
Cabbage	105.25 ± 146.36	105.86 ± 154.33
Carrot	209.46 ± 249.57	185.23 ± 241.20
Onion, garlic, leek, chive	242.72 ± 272.11	222.20 ± 242.91
Broccoli, cauliflower	60.16 ± 74.74	47.95 ± 117.91
Cole, spinach, lettuce	50.06 ± 67.51	40.57 ± 86.03
Radish, mangold , kohlrabi, parsley	88.87 ± 174.58	73.91 ± 118.35
Others	3.82 ± 29.37	3.18 ± 30.13

Fresh fruit ($p < 0.01$) as well as vegetable ($p < 0.001$) consumed at least three times a week more women than men (62 % and 42 % of females versus 54.71 % and 30.14 % of males respectively; $p < 0.01$).

As presented in Table 9, consumption of legumes is absolutey insufficient in both groups of participants.

Around 32 % of participants reported consuming legumes at least ones a week. When combined, only 20 % of participants usually consumed three serves of legumes per

month. There were not observed any significant differences between males and females. Consumption of legumes was generally low and increases with age. Consumption of potatoes was also lower in all age categories, while that of pastes was too high. The weekly consumption of sweet and sweetened beverages was excessively high.

Table 9

Quantity of consumed legumes [g] a week (average ± SD)

Legumes	Female	Male
Bean	64.88 ± 73.26	62.93 ± 71.86
Pea	55.53 ± 96.71	50.42 ± 57.15
Lentil	60.22 ± 103.35	55.00 ± 73.47
Soya	22.30 ± 80.54	18.83 ± 63.84
Garbanzo and others	3.68 ± 14.68	6.28 ± 44.03

Age and gender difference occurred for more measures, and there were some socio-economic status differences. Insufficient consumption of fruit, vegetable and legumes may lead into depression not only some vitamins but also dietary fiber. Lack of dietary fiber can be associated with other chronic diseases, such as breast cancer, colon cancer, etc it seems that population of Slovakia will need to improve their knowledge about healthy lifestyle [11]. With regard to prevention of obesity and *diabetes mellitus* type 2 is consumption of food with lower glycemic index preferable [16].

In the study were evaluated several eating aspects, which showing different inadequacies in nutrition and possible risks (including low consumption of fish etc.). Rational diet is very necessary to fully support human health and primary to prevent nutrition associated civilization diseases [15]. Lifestyle refers to subject’s behavior resting upon the interaction of environmental conditions, personal characteristics, social factors and economic factors. A healthy lifestyle serves as one of the priorities of the programme of NATIONAL HEALTH PROMOTION PROGRAMME aiming at public education in health issues. By combining health education and effective health promotion strategy, one can achieve improvement in public health. Public health awareness: The most recent survey of public health awareness and behavior in the Slovak Republic suggested that 78 % of men and 72 % women regarded their health status as good, and that women suffered from long-term diseases more than men. The most prevailing conditions in elderly men and women include cardiovascular diseases followed by cancer, while allergy predominates in a younger population. Sixty percent of respondents believe that their life expectancy can be modified by the way they live and care about their health. Ninety percent of respondents from all age categories reported indolence as the main cause of their unhealthy lifestyle.

Conclusion

On the basis of the results of this study, it appears that a significant proportion of the Slovakian adults fall short of current, national dietary and physical activity recom-

mendations for adults. Continual monitoring of these behaviours is essential to help inform research and policy and identify where future efforts should be directed.

Acknowledgement

This work was supported by Cultural and Education Grant Agency under the contract No. 3/5082/07, 301-035SPU-4/2010 and Grant Agency of Slovak University of Agriculture in Nitra under the contract No. 724/02380, ITMS 26220120015.

References

[1] Ministry of Health of the Slovak Republic and Public Health Authority of the Slovak Republic: Objective of national health promotion programme, 2005.
[2] Jurkovičová J.: Vieme zdravo žiť? Univerzita Komenského, Bratislava 2005, 166 pp.
[3] Holben D.H., Hassel J.T. and Holcomb J.P.: *College freshmen do not eat within food pyramid guideliness.* J. Am. Dietetic Assoc. 1998, **98**(9), Suppl., p. A51.
[4] Keller U., Meier R. and Bertoli S.: Klinická výživa, Scientia Medica, Praha 1993, 240 pp.
[5] Kleinwächterová H. and Brázdová Z.: Výživový stav člověka a způsoby jeho zjišťování, IDVPZ, Brno 2001, 102 pp.
[6] Holčíková K., Simonová E., Vojtaššáková A. and et al: Odporúčané dávky potravín. Seminár Výživa a energetický príjem, Výstavníctvo Bratislava, 18. mája 2000.
[7] Kajaba I., Šimončič R., Ginter E. and et al.: *Odporúčané výživové dávky pre obyvateľstvo Slovenska (8. Revízia OVD).* Výživa a zdravie 1999, **44,** 25–29.
[8] Kaláč J.: *Možnosti realizácie správnej výživy na konci tohto storočia*. Recipe 1997, **4**, 48–51.
[9] Vestník MZ SR, 45, 7–8, 28. apríla 1997.
[10] Busetto L., Baggio M.B., Zurlo F. and et al: *Assessment of abdominal fat distribution in obese patients: anthropometry versus computerized tomography*. Int. J. Obes. 1992, **16**(10), 731–736.
[11] Bjorntorp P.: *"PORTAL" adipose tissue as agenerator of risk factors for cardiovascular diseases and diabetes*. Arteriosclerosis 1990, **10**(4), 493–496.
[12] World Health Organization: Obesity: preventing and managing the global epidemic of obesity. Report of WHO Consultation of Obesity, Geneva, June 3–5, 1997.
[13] Heaney R.P.: *Nutrition and the risk of osteoporosis*, [In:] Marcus R., Feldman, D. and Kelsey J. (eds.), Osteoporosis. Academic Press, New York 1996, 483–509.
[14] Kunešová M.: *Obezita – epidemie 3. tisíciletí*. Výživa a potraviny 2000, **55**, 155–156.
[15] Kretter A.: Preferencie spotrebiteľov pre nákup biopotravín. Výživa a potraviny pre tretie tisícročie: Funkčné potraviny, SPU, Nitra 2003, p. 258–262.
[16] Dlouhý P., Hromadová M., Kubíková K. et al: Pyramidy výživy v praxi. Výživa a zdraví 2007. KHS Ústeckého kraja, Ústí nad Labem 2007, 31–32.

ZAGROŻENIA W NAWYKACH ŻYWIENIOWYCH LUDNOŚCI SŁOWACJI

Abstrakt: Celem pracy było zbadanie diety dorosłych Słowaków, określenie różnic w pomiarach antropometrycznych, nawykach i preferencjach żywieniowych występujących między kobietami i mężczyznami. Uzyskane dane pochodzą z badań przekrojowych, którymi objęto 1400 dorosłych osób (700 mężczyzn i 700 kobiet) zamieszkujących w różnych rejonach Słowacji. Uczestnicy badań byli pytani o ilość spożywanego mięsa, mleka i produktów mlecznych, owoców i warzyw, roślin strączkowych oraz słodyczy i napojów. Badania wykazały, że prawidłowa masa ciała występowała u 34,05 % osób (38,14 % kobiet i 30,0 % mężczyzn). U 21,86 % mężczyzn i 15,72 % kobiet ($p < 0,01$) występowała lekka otyłość. Uczestnicy badań podali ilość spożywanych produktów zwierzęcych i roślinnych w ciągu jednego tygodnia (liczba dań). Badania wykazały, że 62,29 % kobiet spożywa produkty nabiałowe trzy lub więcej razy w tygodniu ($p < 0.001$). Mężczyźni (59,14 %) spożywali wieprzowinę i wołowinę (87.14 %) jeden lub więcej razy

w tygodniu. Spośród uczestników badań 83,86 % osób spożywało drób, 4,21 % mięso królików i 20,43 % mięso ryb co najmniej raz w tygodniu. Świeże owoce ($p < 0.01$) i warzywa ($p < 0.001$) były konsumowane co najmniej 3 razy w tygodniu – częściej przez kobiety niż przez mężczyzn (62 % kobiet i 42 % mężczyzn; $p < 0.01$). Różnice związane z płcią i wiekiem wystąpiły również w przypadku innych badanych cech. Różnice te często były związane ze statusem społeczno-ekonomicznym. Przeprowadzone badania wykazały, że znaczna część dorosłych Słowaków zaniedbuje prawidłową dietę i aktywność fizyczną. Ciągły monitoring tych zachowań jest niezbędny dla kształtowania polityki oraz rozpoznania kierunków przyszłych działań.

Słowa kluczowe: nawyki żywieniowe, preferencje żywieniowe, modele żywienia, antropometria, profilaktyka, mieszkańcy Słowacji

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Katarína FATRCOVÁ-ŠRAMKOVÁ[1*], Anna KOLESÁROVÁ[1],
Janka NÔŽKOVÁ[2] and Katarína BABINSKÁ[3]

NUTRITION HABITS AND ANTHROPOMETRIC PARAMETERS OF SLOVAK CHILDREN

NAWYKI W ŻYWIENIU I PARAMETRY ANTROPOMETRYCZNE U SŁOWACKICH DZIECI

Abstract: The aim of the study was to collect and analyse information on dietary patterns, to find out and assess anthropometric measurements and evaluate differences between age groups among with 204 schoolchildren (110 girls and 94 boys), aged 9 to 14 years, from Slovak city Nitra (average age 11.40 ± 1.62 years). Data were compared between two age groups: schoolchildren aged 9 to 11 years (52.45 % of pupils) and 12 to 14 years (47.55 % of pupils). Body fat content was in the younger group 29.28 ± 7.19 % (11.91 ± 5.10 kg) and in the older age group 22.04 ± 6.55 % (11.84 ± 5.15 kg). 18.69 % of younger and 30.93 % of older children do not eat breakfast regularly ($p < 0.05$) and even 14.02 % of younger group and 23.71 % of older group do not eat breakfast at all. The most frequently eaten meat by those children is poultry; just small share of participants (14.95 % and 16.49 %) is consumed fish almost every week. Less than once a week or never drink milk 17.75 % of younger and 14.44 % of older children. Markedly negative founding is fairly deficient (less than once a week) of fruit intake in 5.61 % and 10.31 % of children; as well as vegetable consumption in 14.02 % and 21.65 %; further, legumes consumption (less than twice a month) in 27.10 % and 36.08 % of children.

Keywords: dietary patterns, food preferences, anthropometries, schoolchildren, Slovak Republic

A school-age child begins to make food choices independently, with more peer influence and less parental supervision. It is a period of few apparent feeding problems. Appetite and food intake will naturally increase with the added activities of school and

[1] Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[2] Institute of Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[3] Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava, Sasinkova 2, 813 72 Bratislava, Slovak Republic.

* Correspondence author: email: katarina.sramkova@gmail.com

play. Adolescents who are misinformed about their nutritional needs and who make independent food choice are at risk for developing nutritional deficiencies [1].

Material and methods

The aim of the study was to collect and analyse information on dietary patterns, to find out and assess anthropometric measurements and evaluate differences between age groups among with 204 schoolchildren (110 girls and 94 boys), aged 9 to 14 years (Table 1), from Slovak city Nitra (average age 11.40 ± 1.62 years). The questionnaire used in the study was designed by Babinska et al [2] and adapted at the Department of Human Nutrition in Nitra. It was used to analyze the dietary patterns of subjects. Children's parents completed a questionnaire about food habits. The data were collected in May and in June 2008. Participants' age was recorded at last birthday. An overweight and an obesity prevalence examined by body mass index (BMI) were evaluated in randomly selected group of pupils. Height and weight were measured and body mass index was calculated [kg · m^{-2}].

Table 1

Age composition of analyzed set (n = 360)

Age	n	[%]	n	[%]
9 years	33	16.18		
10 years	38	18.63	107	52.45
11 years	36	17.65		
12 years	23	11.27		
13 years	58	28.43	97	47.55
14 years	16	7.84		
Sum	204	100.00	204	100.00

Based on the data on height and weight we evaluated a Quetelet index – body mass index (BMI), according to following formula: BMI = mass [kg] · height $[m]^{-2}$.

Waist and hip circumference was measured [cm]. Anthropometric parameters and information on dietary patterns were compared between two age groups: 107 schoolchildren aged 9 to 11 years (52.45 % of scholars) and 97 children aged 12 to 14 years (47.55 % of scholars). The younger group compounded of 57 girls (53.27 %) and 50 boys (46.73 %), and older group compounded of 53 girls (54.64 %) and 44 boys (45.36 %). Statistical analysis was performed using Statgraphics Centurion software. The statistical significance of differences was tested by χ^2 test.

Obtained information on nutrition habits of children from Nitra were compared with study on nutrition habits of 1000 children (476 boys and 524 girls) aged 6 to 16 years (average age was 10.8 ± 3.1 years), from whole Slovakia (ten districts – Banska Bystrica, Bardejov, Cadca, Komarno, Levice, Nove Zamky, Nitra, Presov, Stara Lubovna, Ziar nad Hronom) [2, 3].

Results and discussion

Body fat content was in the younger group 29.28 ± 7.19 % (11.91 ± 5.10 kg) and in the older group 22.04 ± 6.55 % (11.84 ± 5.15 kg). Body fat content in girls group was 25.70 ± 6.75 % and in boys group was 25.99 ± 8.86 %, what is above upper limit of recommended range (16 to 22 % for girls and 14 to 20 % for boys, aged 7 to 17 years) in both cases [4]. Most of the children (71.82 % girls and 71.28 % boys) had body fat content above upper limit of mentioned ranges (Table 2). Last years we can see obesity increase also in children, which in consideration of its high prevalence and numerous complications it becomes increasingly serious health problem of childs age in developed countries including Slovakia [5, 6]. Another anthropometric features of children are mentioned in Table 3.

Table 2

Body fat [%]

Girls (n = 110)			Boys (n = 94)			Sum (n = 204)	
Body fat [%]	n	[%]	Body fat [%]	n	[%]	n	[%]
< 16.0	7	6.36	< 14.0	8	8.51	15	7.35
16.0–19.0	12	10.91	14.0–17.0	9	9.57	21	10.29
19.1–22.0	12	10.91	17.1–20.0	10	10.64	22	10.78
> 22.0	79	71.82	> 20.0	67	71.28	146	71.57

Table 3

Characterization of analyzed set ($\bar{x} \pm s$)

Parameter	9–11 years (n = 107)	12–14 years (n = 97)	Sum (n = 204)
Age [years]	10.02 ± 0.80	12.92 ± 0.63	11.40 ± 1.62
Weight [kg]	40.18 ± 11.33	52.31 ± 10.38	45.95 ± 12.45
Height [cm]	146.03 ± 10.53	162.33 ± 8.78	153.78 ± 12.69
Body fat [%]	29.28 ± 7.19	22.04 ± 6.55	25.83 ± 7.77
Body fat [kg]	11.91 ± 5.10	11.84 ± 5.15	11.88 ± 5.11
Waist circumference [cm]	64.87 ± 9.13	70.24 ± 8.52	67.42 ± 9.22
Hip circumference [cm]	80.94 ± 9.06	88.75 ± 8.25	84.66 ± 9.50

Epidemiological research refers to connections between irregular alimentation and increasing risk of obesity. Irregular food intake has also negative influence to a mental activity, which has effect to a prosperity and behavior of child at school [7]. In major part of the examinated children (83.82 %) the lunch has represented the biggest portion of food, but 13.73 % move the major portion of food to suppertime (Table 4). The lunch as the biggest portion of food is more typical for younger than older group ($p < 0.05$). The children have consumed meal on average nearly 5 times a day (4.70 ± 0.80 times girls and 4.66 ± 0.85 times boys per day).

Table 4

Which portion of meal is the biggest?

Meal	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Breakfast	2	1.87	3	3.09	5	2.45
Lunch	95	88.79	76	78.35	171	83.82
Supper	10	9.35	18	18.56	28	13.73

We noticed by evaluating of main meals, that a regular consumption of breakfast was determined in 67.29 % of younger schoolchildren and in 45.36 % of older children ($p < 0.05$) (Table 5). Important detection is, that 18.69 % of younger and 30.93 % of older children did not eat breakfast regularly ($p < 0.05$), and even 14.02 % of younger group and 23.71 % of older group did not eat breakfast at all, eventually they drank only a tea. Breakfast can be an important meal for adults and children alike. The school lunch program was establish to provide a third of the recommended dietary allowance. Home-prepared lunches allow for increased variety [1].

Table 5

Consumption of main meals and its regularity

Consumption	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Breakfast						
Mostly regularly	72	67.29	44	45.36	116	56.86
Mostly irregularly	20	18.69	30	30.93	50	24.51
Do not have breakfast at all or only tea	15	14.02	23	23.71	38	18.63
Lunch						
Regularly complete lunch in canteen	81	75.70	52	53.61	133	65.20
Regularly complete lunch at home	20	18.69	39	40.21	59	28.92
Irregularly at home or in canteen	6	5.61	6	6.19	12	5.88
Regularly fast food from snack bar* instead of lunch	0	0.00	0	0.00	0	0.00
Supper						
Daily hot supper or nearly daily	61	57.01	50	51.55	111	54.41
Mostly cold supper	41	38.32	37	38.14	78	38.24
Irregular supper	5	4.67	10	10.31	15	7.35
Do not have supper at all	0	0.00	0	0.00	0	0.00

* Hot-dog, hamburger, baguette...

In comparison with study of children from whole Slovakia [2, 3] we detected insignificant lower portion of children, which regularly consumed breakfast (56.86 % in

comparison with 61.7 % of children from whole Slovakia, $p \geq 0.05$) and on the contrary slightly more portion of children, which irregularly consumed breakfast (24.51 % versus 21.1 % children from ten districts of Slovakia, $p \geq 0.05$), and did not have breakfast at all (18.63 % versus 17.2 %; $p \geq 0.05$).

The 18.69 % and 40.21 % of children ($p < 0.01$) have consumed regularly complete lunch at home; 75.70 % and 53.61 % of children from individual age groups in school canteen ($p < 0.01$). Regularly lunch have consumed 94.12 % of examined children, ie less than in the study from whole Slovakia (95.2 %; $p \geq 0.05$). In the study from whole Slovakia 0.4 % children had lunch in snack bar, but we did not determine any case.

Regularly supper have consumed 95.33 % and 89.69 % of children of younger and older age group. Mostly cold supper have eaten 38.32 % and 38.14 % of children. More children from Nitra have consumed supper regularly (92.65 %) in comparison with children from whole Slovakia (90.7 %) ($p \geq 0.05$). Irregular consumption of supper was determined mostly in older group from Nitra ($p \geq 0.05$). In the study from ten districts of Slovakia was irregular supper more typical for girls.

We noticed by evaluating of snacks, that the most children (52.34 % younger and 43.30 % older children) have consumed for snack fruit or dairy products sporadically (versus 43 % in study from whole Slovakia), regularly have consumed this foodstuff 40.69 % (versus 45.1 % in study from whole Slovakia; $p \geq 0,05$) (Table 6).

Table 6

Consumption of snacks and its regularity

Consumption	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Morning snack						
Regularly fruit, vegetable or dairy products	46	42.99	37	38.14	83	40.69
Irregularly fruit, vegetable or dairy products	56	52.34	42	43.30	98	48.04
Regularly sweets or fastfood from snack bar*	3	2.80	16	16.49	19	9.31
Do not have snack at all	2	1.87	2	2.06	4	1.96
Afternoon snack						
Regularly fruit, vegetable or dairy products	38	35.51	28	28.87	66	32.35
Irregularly fruit, vegetable or dairy products	54	50.47	46	47.42	100	49.02
Regularly sweets or fastfood from snack bar*	9	8.41	12	12.37	21	10.29
Do not have snack at all	6	5.61	11	11.34	17	8.33
Second supper						
Regularly fruit, vegetable or dairy products	43	40.19	26	26.80	69	33.82
Do not have second supper at all	54	50.47	45	46.39	99	48.53
Regularly bread, cheese, sweets or nuts	10	9.35	26	26.80	36	17.65

* Hot-dog, hamburger, baguette...

Only 1.96 % children did not have snack at all, which is surprising and positive result (similar result 1.8 % was determined in study Babinska et al [2]). For afternoon snack

fruit, vegetable or dairy products have regularly consumed 35.51 % and 28.87 % ($p \geq 0.05$), while sporadically have eaten at afternoon snack time 50.47 % and 47.42 % of children according to age.

Babinska et al [2] quotes, that 91.7 % children usually have afternoon snack, thereof 40.3 % (versus 32.35 % Nitra's schoolchildren) ($p < 0.05$) regularly and 47.6 % (versus 49.02 % pupils from Nitra) irregularly have consumed fruit, vegetable or dairy products.

Second supper has not eaten 50.47 % of younger and 46.39 % of older children. Fruit, vegetable or milk products have regularly eaten 40.19 % pupils from younger and 26.8 % from older group ($p < 0.05$) for second supper. According to Babinska et al [2], mostly younger children have consumed regularly fruit, vegetable or dairy products for second supper, likewise in our experimental set.

The most frequently eaten meat by those children is poultry; just small share of participants (14.95 % and 16.49 %) is consumed fish almost every week.

The meat has not been eaten at all by 3.43 % examined children (Table 7), what are about 1.93 % more children than in whole Slovakia study ($p \geq 0.05$). Preferred type of meat has been poultry, which have consumed up to 88.73 % pupils from Nitra and 92.8 % from whole Slovakia ($p < 0.05$). Then follows a pork, which is usually eaten by 63.73 % of pupils (versus up to 81.6 % pupils from whole Slovakia) ($p < 0.001$). A beef is not eaten by 62.25 % of children from Nitra and 49.4 % from whole Slovakia ($p < 0.001$).

Table 7

Consumption of meat

Meat	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Poultry	97	90.65	84	86.60	181	88.73
Pork	70	65.42	60	61.86	130	63.73
Beef	45	42.06	32	32.99	77	37.75
Do not eat	2	1.87	5	5.15	7	3.43

Weekly average intake of poultry meat has been 2.48 ± 1.02 times in younger and 2.26 ± 0.91 times in older group from Nitra (versus 1.8 times weekly in children from ten districts of Slovakia). Pork was consumed 1.65 ± 0.85 times in younger and 1.74 ± 0.90 times in older children weekly (versus 1.8 times in study from whole Slovakia) and beef was consumed 1.62 ± 0.91 times in younger and 1.34 ± 1.04 times in older group monthly (versus 2 times monthly in whole Slovakia study).

At least one time a week 15.69 % of pupils from Nitra have consumed fish, within the whole Slovakia it was 13.7 % ($p \geq 0.05$) of such pupils (Table 8). Fish meat have included one to three times monthly into menu 41.67 % respondents (in comparison with 54.9 % children from whole Slovakia). Most of the examined children from Nitra have consumed fish even less than once a month (42.65 % from Nitra versus 31.2 % from whole Slovakia; $p < 0,01$).

Table 8

Fish consumption

Frequency	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Every week	16	14.95	16	16.49	32	15.69
1 to 3 times per month	46	42.99	39	40.21	85	41.67
Less than once per month	45	42.06	42	43.30	87	42.65

In a milk consummation monitoring was found, that daily drink milk 60.75 % and 64.95 % of children in quantity 0.31 ± 0.18 and 0.40 ± 0.31 dm^3 in younger and older group. Daily the milk have drunk 62.75 % of all children and 7.35 % have not drunk the milk at all (Table 9). Something more children from older group have drunk it more than younger pupils ($p \geq 0.05$). Less than once a week or never drink the milk 17.75 % of younger and 14.44 % of older children. In the milk consumption at least once a week (83.83 % versus 71.5 %; $p < 0.001$) we have noticed statistically significant difference between children from Nitra and whole Slovakia, but not in daily consumption of the milk (62.75 % versus 62.6 %; $p \geq 0.05$).

Table 9

Consumption of milk, dairy products and cheese

Consumption	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Milk consumption						
Daily	65	60.75	63	64.95	128	62.75
1 to 6 times per week	23	21.50	20	20.62	43	21.08
Less than once per week	12	11.21	6	6.19	18	8.82
Do not drink at all	7	6.54	8	8.25	15	7.35
Dairy products consumption						
Daily	55	51.40	49	50.52	104	50.98
1 to 6 times per week	47	43.93	41	42.27	88	43.14
Less than once per week	5	4.67	7	7.22	12	5.88
Cheese consumption						
Daily	30	28.04	27	27.84	57	27.94
1 to 6 times per week	59	55.14	50	1.55	109	53.43
Less than once per week	14	13.08	15	15.46	29	14.22
Do not eat at all	4	3.74	5	5.15	9	4.41

Dairy products (besides cheese) have consumed daily by around half of the children from both groups (51.40 % and 50.52 %; $p \geq 0.05$); and cheese has consumed more than quarter of children (28.04 % a 27.84 %). Daily dairy products have consumed almost

same part of children from Nitra and whole Slovakia (50.98 % versus 49.2 %; $p \geq 0.05$). Children from Nitra have eaten daily 1.31 ± 0.61 pieces (in younger group) and 1.73 ± 0.81 pieces (in older group) of dairy products. In daily consumption of cheese was not significant differences between younger and older group, but between children from Nitra and whole Slovakia are differences ($p \geq 0.05$). Cheese was daily included into the menu by 27.94 % and 22.7 % of children from these experimental sets (Nitra and whole Slovakia).

Markedly negative founding is fairly deficient (less than once a week) of fruit intake in 5.61 % and 10.31 % of children; as well as vegetable consumption in 14.02 % and 21.65 %; further, legumes consumption (less than twice a month) in 27.10 % and 36.08 % of children (Table 10). At least once a day have consumed fruit and vegetable statistically insignificantly more younger than older pupils, but statistically significantly more children from whole Slovakia than from Nitra in case of fruit, and more children from Nitra in case of vegetable consumption ($p < 0.001$). Daily 65.5 % of children from whole Slovakia have included fruit into diet and vegetable 25.9 %. Alarmingly, insufficient intake of legumes (less than twice a month) was sharply noticed in older pupils and in case of children from Nitra (31.7 % versus 26.0 %), but differences between groups, divided according to gender and region, were not statistically significant ($p \geq 0.05$). Consumed amount of fruit and vegetable was not evaluated.

Table 10

Fruit, vegetable and legumes consumption

Consumption	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Fruit consumption						
Minimally once per day	61	57.01	54	55.67	115	56.37
Minimally once per week	40	37.38	33	34.02	73	35.78
Less than once per week	6	5.61	10	10.31	16	7.84
Vegetable consumption						
Minimally once per day	50	46.73	42	43.30	92	45.10
Minimally once per week	42	39.25	34	35.05	76	37.25
Less than once per week	15	14.02	21	21.65	36	17.65
Legumes consumption						
Minimally 2 times per week	23	21.50	23	23.71	46	22.55
Almost every week	55	51.40	39	40.21	94	46.08
Less than 2 times per month	29	27.10	35	36.08	64	31.37

Sweets eat daily more than half of children from both groups (58.88 % and 62.89 %) (Table 11). Insufficient daily intake of sweets in our and compared experimental sets concerned 60.78 % of children from Nitra and 55.2 % children from whole Slovakia. Significant differences between Nitra and whole Slovakia sets, as well as between younger and older pupils within Nitra set was not determined ($p \geq 0.05$).

Table 11

Sweets and drinks consumption

Consumption	9–11 years (n = 107)		12–14 years (n = 97)		Sum (n = 204)	
	n	[%]	n	[%]	n	[%]
Sweets consumption						
Almost never	1	0.93	0	0.00	1	0.49
3 times per month and less	4	3.74	2	2.06	6	2.94
Minimally once per week	39	36.45	34	35.05	73	35.78
Daily	63	58.88	61	62.89	124	60.78
Drinks consumption						
Perhaps 5 glasses	89	83.18	89	91.75	178	87.25
Less than 3 glasses	10	9.35	3	3.09	13	6.37
During a day forget to drink; at an evening fill up	8	7.48	5	5.15	13	6.37

Incorrect fresh schedule (daily water intake less than 3 glasses or unequal splitting of drinks during the day) has got 16.83 % of younger and 8.24 % of older children. Similarly, in both groups we have not noticed significant differences in incorrect fresh schedule defined by daily intake less than 3 glasses or unequal splitting of drinks during the day and its evening refilling. This way is characterized fresh schedule in 12.74 % children from Nitra and in 13.1 % children from whole Slovakia.

In the study is analyzed also intake of particular foodstuffs as for example honey with children or biofoodstuffs in family. Honey is consumed often by 15.20 % of pupils and never by 19.61 % children. Allergic to honey is 2.45 % children. Biofoodstuff is never consumed in 49.02 % children's families; thereof 21.57 % do not know them at all, and occasionally are biofoodstuff consumed in 11.27 % children's families. According to Kretter [8] biofoodstuffs represent a new quality level in the supply of foodstuffs. Their consumption in Slovakia is however minimal. The causes are price, information and habit barrier.

According to observation and evaluation of nutrition habits of children from Nitra we positively evaluate the poultry preference, which was observed in our experimental set, as well as in the set from whole Slovakia. Selection of meat with lower content of fat is important step to reduction of saturated fatty acids and also fats in total [3].

In the experimental set is very low intake of fishes and legumes, even more children than in set from ten districts. According to actual recommendations for children with this age, the optimal intake of fishes is twice a week. We positively evaluate daily intake of fruit and vegetable in more children from Nitra's district in comparison with set of children from whole Slovakia.

From monitoring of frequency and preference of poultry meat and the fishes consumption in 298 high scholars [9] we figured out, that poultry meat consumed the most children, 2 to 3 times a week (46.98 % pupils) and once a week 33.89 %. Between girls and boys were significant differences in preference of poultry meat (more girls

have preferred it – 81.18 % girls and 68.75 % boys). Most of the older pupils included the fishes into their diet only several times a year (34.40 % boys and 28.57 % girls).

Comparison of our results and results from Babinska et al [2, 3] with previous epidemiologic studies of children nutrition manners in Slovakia at the end of nineties years [10, 11] referred to ongoing irregular breakfast intake.

Bederova [12] declares, about evaluation of nutrition schedule of children and adolescents (from elementary and high schools), that in meat intake dominated the pork and permanent insufficient was the fish consumption.

In general, regular nutrition schedule and regular distribution of meals during a day are considered as optimal for mental performance of a person [7, 13].

Conclusion

Results from our study provide information about actual problems in children nutrition in Nitra, as well as comparison of nutrition habits of children from Nitra with children from different districts of Slovakia. Overall, we can positively evaluate preference of poultry meat in the experimental set of scholars from Nitra, and also daily consumption of fruit and vegetable in major part of set. The most important observed deficiencies are: the breakfast skipping and insufficient intake of fishes and legumes. These factors can negatively influence nutritive value of food and consequently health and nutritive conditions of child body and its mental performance.

An aim of dietary counseling is oriented to prevention of nutrition-related diseases, which should be provided by pediatricians, and it should also include recommendations for regular dietary patterns.

Acknowledgement

This work was supported by Cultural and Education Grant Agency under the contract No. 3/5082/07, No. 301-035SPU-4/2010, 6A 3/2010 Grant Agency of Slovak University of Agriculture in Nitra under the contract No. 724/02380 and by project "Healthy city Nitra".

References

[1] Herbert V. and Subak-Sharpe G.J.: Total nutrition. St. Martin's Press, New York 1995, 811 pp.

[2] Babinská K., Vitáriušová E., Rosinský J., Babinská K. ml., Košťálová Ľ., Hlavatá A., Pribilincová Z. and Kovács L.: *Stravovací režim školákov na Slovensku*. Pediatr. Prax. 2007, **4**, 217–220.

[3] Babinská K., Vitáriušová E., Hlavatá A., Rosinský J., Babinská K.M., Košťálová Ľ., Pribilincová Z. and Kovács L.: Stravovacie návyky žiakov základných škôl na Slovensku. Nové trendy vo výžive detí. Lekárska fakulta Univerzity Komenského v Bratislave, Bratislava 2008, 17–28.

[4] URL: www.lymfo.sk/vaha.html

[5] Fussenegger D., Pietrobelli A. and Widhalm K.: *Childhood obesity: political developments in Europe and related perspectives for future action on prevention.* Obes. Rev. 2008, **9**(1), 76–82.

[6] Hlavatá A.: *Obézne dieťa v ambulancii lekára pre deti a dorast. Klinické odporúčania I.* Pediatria pre prax. 2007, **8**, 12–16.

[7] Bellisle F.: *Effects of diet on behaviour and cognition in children*. British J. Nutrit. 2004, **92**, 27–32.

[8] Kretter A.: Preferencie spotrebiteľov pre nákup biopotravín. Výživa a potraviny pre tretie tisícročie: Funkčné potraviny. SPU, Nitra 2003, 258–262.

[9] Šramková K. and Kopčeková J.: *Aktuálne poznatky o frekvencii a preferencii konzumácie hydinového mäsa a rýb u stredoškolských študentov.* 1. medzinárodné vedecké hydinárske dni. Nitra, 12.–14. septembra 2005. Zborník referátov. SPU, Nitra 2005, 24–29.
[10] Béderová A., Babinská K. and Béder I.: *Aktuálny spôsob výživy detí a mladistvých v regiónoch Banská Bystrica a Prešov v r. 1999*. Det lekár, 2003, **10**, 25–30.
[11] Béderová A., Babinská K. and Krajčovičová-Kudláčková M.: *Spôsob výživy detí a mládeže, aktuálny príjem makro a mikronutrientov v dvoch regiónoch SR v roku 1998*. Hygiena 2000, **45**, 204–213.
[12] Béderová K.: *Prednosti školského stravovania.* Výživa a zdravie 2004, **49**, 10–11.
[13] Muthayya S., Thomas T. and Srinivasan K. et al: *Consumption of a mid-morning snack improves memory but not attention in school children.* Physiol. Behav. 2007, **90**, 142–150.

NAWYKI W ŻYWIENIU I PARAMETRY ANTROPOMETRYCZNE U SŁOWACKICH DZIECI

Abstrakt: Celem pracy było zebranie oraz analiza danych dotyczących nawyków żywieniowych, wybór pomiarów antropometrycznych oraz ocena różnic występujących miedzy dziećmi z różnych grup wiekowych. W badaniach wzięło udział 204 osoby (110 dziewcząt i 94 chłopców) w wieku szkolnym (średnia wieku 11,40 ± 1,62 lat) mieszkających w słowackim mieście Nitra. Dzieci zostały przyporządkowane do 2 grup wiekowych, mianowicie 9–11 lat (52,45 %) i 12–14 lat (47,55 %). Zawartość tkanki tłuszczowej w grupie młodszych dzieci wynosiła 29,28 ± 7,19 % (11,91 ± 5,10 kg), a w grupie dzieci starszych 22,04 ± 6,55 % (11,84 ± 5,15 kg). Spośród badanych dzieci 18,69 % młodszych i 30,93 % starszych osób nie spożywa regularnie śniadań ($p < 0,05$). Wśród dzieci młodszych 14,02 %, a wśród dzieci starszych 23,71 % osób w ogóle nie spożywa śniadań. Głównym rodzajem mięsa spożywanego przez badaną grupę dzieci jest mięso drobiowe. Tylko 14,95 do 16,49 % dzieci jada ryby raz w tygodniu. Spośród młodszych dzieci 17,75 %, a spośród starszych dzieci 14,44 % pije rzadziej niż raz w tygodniu lub w ogóle nie pije mleka. Owoce jedzone są rzadziej niż raz w tygodniu przez 5,61 do 10,31 % dzieci. Warzywa jedzone są rzadziej niż raz w tygodniu przez 14,02 % dzieci młodszych i 21,65 % dzieci starszych. Rośliny strączkowe są spożywane rzadziej niż 2 razy w miesiącu przez 27,10 do 36,08 % dzieci.

Słowa kluczowe: zwyczaje żywieniowe, preferencje żywieniowe, antropometria, dzieci szkolne, Republika Słowacka

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Katarína FATRCOVÁ-ŠRAMKOVÁ[1*], Janka NÔŽKOVÁ[2], Miroslava KAČÁNIOVÁ[3], Magda MÁRIÁSSYOVÁ[4] and Zlata KROPKOVÁ[5]

MICROBIAL PROPERTIES, NUTRITIONAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Brassica napus* subsp. *napus* L. BEE POLLEN USED IN HUMAN NUTRITION

SKŁAD MIKROFLORY, SKŁADNIKI ODŻYWCZE I AKTYWNOŚĆ ANTYOKSYDACYJNA W PYŁKU PSZCZELIM POCHODZĄCYM Z *Brassica napus* subsp. *napus* L. UŻYWANYM W ŻYWIENIU LUDZI

Abstract: An aim of this work was to characterize microbial properties, a nutritional composition and an antioxidant activity of *Brassica napus* subsp. *napus* L. bee pollen sample, which can be possibly used in human nutrition. A plate diluting method was applied for quantitative cfu (*colony forming units*) counts determination. The mean number of mesophilic aerobic sporulating microorganisms ranged 3.78–4.56 log cfu $\cdot$ g^{-1}, the number of mesophilc anaerobes sporulating microorganisms ranged 2.54–4.63 log cfu $\cdot$ g^{-1}, the number of coliforms bacteria 0–3.74 log cfu $\cdot$ $g^{-1}$and the cells number of *Escherichia coli* 0–3.71 log cfu $\cdot$ g^{-1}. The mean number of microscopic fungi ranged from 2.48 to 4.20 log cfu $\cdot$ g^{-1}. The antioxidant activity of bee pollen ranged from 1.25 to 1.93 I/I_0 (in case of the freeze-dried and frozen bee pollen, respectively). The highest total flavonoids content (128.33 mg $\cdot$ kg^{-1}) was occurred in the frozen pollen. The highest value of the flavonoid kaempferol achieved in the dried bee pollen, whereas the freeze-dried form contains the most of other three flavonoids (quercetin, luteolin, apigenin). The sum of proteins (average 251.13 ± 33.06 g $\cdot$ kg^{-1}) decreased in the order: freeze-dried > dried > frozen bee pollen. The freeze-dried form of pollen was characterized with the highest value of the calcium concentration, and the frozen treatment

[1] Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[2] Institute of Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[3] Department of Microbiology, Faculty of Biotechnology and Food Industry, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[4] Food Research Institute, Centre in Modra, Slovak Republic.

[5] Department of Statistics and Operation Research, Faculty of Economics and Management, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

* Corresponding author email: katarina.sramkova@post.sk

with the lowest content (2040 mg · kg^{-1} versus 1800 mg · kg^{-1}). The zinc was presented in amount 36.97 ± 4.15 mg · kg^{-1}. The most of the zinc was contained in the freeze-dried pollen.

Keywords: *Brassica napus* subsp. *napus* L. bee pollen, microbiological quality, bacteria, microscopic fungi, antioxidative properties, nutritional composition

Honeybee-collected pollen is recognized as a well balanced food [1]. Bee pollen, ie a floral pollen collected by a honey bee for its protein content, has been used as a nutrient rich health food for many centuries [2], and its benefits have been widely lauded [3–6]. The German Federal Board of Health has recently officially recognized pollen as a medicine [4]. More specifically, the ingestion of bee pollen by rats has been shown to decrease the level of the lipid oxidation products, malondialdehyde and conjugated dienes, in the erythrocytes [7], thus suggesting the antioxidant role for bee pollen. The same workers also demonstrated the immunostimulation activity on primary and secondary levels of IgM and IgE in rabbits fed on bee pollen-containing diet for 1 month [8]. But there are no official international pollen standards, yet. The pollen is collected by special pollen traps. Fresh, bee collected pollen contains about 20–30 g water per 100 g. This high humidity is an ideal culture medium for microorganisms like bacteria and yeast. For prevention of spoilage and for preservation of a maximum quality the pollen has to be harvested daily and immediately placed in a freezer [9].

The aim of this work was to characterize the microbial properties, the nutritional composition and the antioxidant activity of *Brassica napus* subsp. *napus* L. bee pollen sample, which can be possibly used in human nutrition.

Material and methods

A. Bee pollen samples preparation

Samples of bee-collected pollen (*Brassica napus* subsp. *napus* L.) were obtained from beekeepers, which respected qualitative criteria for gathering, drying and storing as proposed by Bogdanov [9]. The samples were collected during the spring season 2007 from different regions of western Slovakia. The fresh bee pollen was stored at –18 °C, 20 % moisture, approximately six months until analysed. The pollen samples were dried (9–11 % moisture) approximately 8 hours at maximum temperature 35 °C. The moisture was tested by thermogravimetric analyzer WPS 50SX/1 by RADWAG. The lyophilized samples of bee pollen were dried in the table laboratory lyophilizator LYOVAC GT 2 by Amsco/Finn-Aqua, 80 hours without heating, until 2 % moisture. The drying process was realized without heating so the nutritive compounds of the pollen were not changed.

B. Microbiological analysis

Determination of colony forming units (cfu) counts in pollen samples. The plate diluting method was applied for quantitative cfu counts determination of respective groups of microorganisms in 1 g of *Brassica napus* subsp. *napus* L. bee pollen sample.

The gelatinous nutritive substrate in Petri dishes was inoculated with 1 cm^3 of the pollen samples by flushing on a surface, in three replications. The basic dilution (10^{-1}) was prepared as follows: 5 g of the pollen content was added to the test tube containing 45 cm^3 of distilled water.

Media and culture conditions. The composition of nutritive substrates, for the total mesophilic sporulating anaerobes and the aerobes bacteria, the coliforms bacteria, and *Escherichia coli*, was according to the directions for use declared by the producer (Biomark laboratories). The total mesophilic sporulating anaerobes bacteria were grown on Meat Peptone agar (anaerobiosis), at 37 ^oC during 72 hours. The total mesophilic sporulating aerobes bacteria were grown in Meat Peptone agar (aerobiosis), at 37 ^oC during 72 hours. The coliforms bacteria were grown on Mac Conkey agar (aerobiosis), at 37 ^oC during 24 hours. *Escherichia coli* were grown on Violet red bile agar (aerobiosis), at 37 ^oC during 24 hours. The composition of these nutritive substrates was according to the directions for use declared by the producer (Biomark laboratories). The bacteria were determined according to Holt et al [10].

Isolation and morphological characterization of fungi. For determination of the fungi colony-forming units (cfu) 5 g of the sample was soaked in 45 cm^3 sterile tap-water, containing 0.02 % Tween 80 and then 30 min shaken. The dilutions (from 10^{-1} to 10^{-5}) in sterile tap-water with 0.02 % Tween 80 were prepared and 1 cm^3 aliquots were inoculated on each of three plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25 ^oC. The total fungi cfu $\cdot$ g^{-1} counts in the samples were determined after 5 days of incubation.

Malt agar and Czapek-Dox agar were used to isolate and identify individual genera and species. After isolation, or in some cases monosporic isolation, individual species were identified on the basis of their macro- and micromorphology in accordance with other scientific reports [11–13].

C. Antioxidant activity

The antioxidative properties were evaluated using the voltammetric procedure based on the protective effect of antioxidants against the oxidative DNA damage. The method was employed using a disposable DNA biosensor fabricated as a screen-printed electrode chemically modified by calf thymus double stranded (ds) DNA.

Preparation of the DNA biosensor: the working carbon electrode (SPE with 25 mm^2 geometric surface area) of the three-electrode screen-printed assembly (including also silver/silver chloride reference electrode and carbon counter-electrode, FACH, Prešov, Slovakia) was chemically modified in the laboratory by covering it with 5 mm^3 of the DNA stock solution and leaving it to dry overnight.

Use of the DNA-Based Biosensor: the procedure reported previously [14] was exploited. Briefly, the new DNA sensor (DNA/SPE) was pretreated by immersion into 10 $mmol/dm^3$ phosphate buffer, pH 7.0, for 15 min and rinsed with water. Then, the $[Co(phen)_3]^{3+}$ marker was accumulated from 5 cm^3 of its 5×10^{-7} mol/dm^3 solution in 0.010 mol/dm^3 phosphate buffer under stirring for 120 s at an open circuit. The

differential pulse voltammogram (DPV) was recorded immediately from +0.4 to –0.5 V at the pulse amplitude of 100 mV and the scan rate of 25 mV/s using a computerised voltammetric analyser ECA pol, model 110 (Istran, Bratislava, Slovakia), fitted with the DNA/SPE assembly. With the software used, the current was measured with 2 mV scan step at this scan rate. The marker DPV peak current (I_0) at –0.130 V was evaluated against the base-line using the standard software and was corrected by the subtraction of the mean marker DPV peak current measured at the unmodified SPE (n = 10) under identical conditions. Subsequently, the DNA/SPE was regenerated by the removal of the accumulated $[Co(phen)_3]^{3+}$ ions from the DNA layer treating the sensor in the solution of a high ionic strength (0.100 mol/dm^3 phosphate buffer pH 7.0) under stirring during 120 s. The negligible marker peak current was checked by the DPV record in blank. The peak current I_0 was obtained in triplicates.

The DNA damage and the antioxidative effects of the plant extracts were detected after 5 min incubation of the sensor in the cleavage mixture (2 × 10^{-4} mol/dm^3 $FeSO_4$, 4 × 10^{-4} mol/dm^3 EDTA, 9 × 10^{-3} mol/dm^3 H_2O_2, in 0.010 mol/dm^3 phosphate buffer solution with 10 % methanol without or with the addition of the plant extract under the application of the electrode potential of –0.5 V in aerobic conditions at room temperature). The marker peak current I was obtained in triplicates using the marker accumulation/measurement/sensor regeneration scheme as described above and employing the same DNA/SPE for the given composition of the cleavage mixture. The average signals I_0 and I were calculated from the second and the third measurements. To compensate for the differences in the properties of the individual strips of the DNA-biosensor, the normalised (relative) signal value I/I_0 was obtained which represents the survived portion of the original DNA.

D. Total flavonoids and selected flavonoids analysis

HPLC determination of flavonoids. The chromatographic separation were performed on a Purospher Star RP-18e (Merck) column (250 × 4 mm I.D., 5 μm), protected by a Merck Purospher Star (4 × 4 mm, 5 μm) guard column. The HPLC system consisted of Shimadzu LC 10ADvp series pumping system, SPD 10AV/VP UV/VIS detector set at 360 nmand C-R6A chromatography data station software. Two solvents were used with constant flow rate 1 cm^3/min.

The injection volume was 20 mm^3. Solvent A consisted 0.05 % of TFA/methanol (95:5, V/V), solvent B included methanol/0.05 % TFA (95:5, V/V). For the elution program, the following proportions of solvents B were used: 0–15 min, 40 % B; 15–30 min, 40–55 % B; 30–35 min, 55–70 % B. The ethanolic extracts were injected under this conditions as well as the mixture of authentic samples of quercetin, luteolin, kaempferol and apigenin.

The correlation between antioxidant activity and content of flavonoids was analysed using SAS 9.1.3 software.

E. Minerals and proteins analysis

Minerals – calcium, zinc and total proteins were determined by standard methods in the accredited analytic laboratories – BEL/NOVAMANN International Ltd. Nove Zamky and EL Ltd. Spisska Nova Ves. The samples of pollen were homogenized, and then they were further processed according to determined chemical compounds.

Zinc concentration was determined using electrothermal atomizer atomic absorption spectrometry (ETA-AAS). Determination of calcium in tested pollen samples was realized by the inductively coupled plasma-atomic emission spectrophotometry (ICP-AES) method.

For quantitative determination of proteins content in analysed pollen samples was used Kjeldahl method.

Results and discussion

Microbiological analysis

The mean number of mesophilic aerobic sporulating microorganisms ranged 3.78––4.56 log cfu · g^{-1}, the number of mesophilc anaerobes sporulating microorganisms ranged 2.54–4.63 log cfu · g^{-1}, the number of coliforms bacteria 0–3.74 log cfu · g^{-1} and the cells number of *Escherichia coli* 0–3.71 log cfu · g^{-1}. The mean number of microscopic fungi ranged from 2.48 to 4.20 log cfu · g^{-1} (Fig. 1). Five species of microscopic fungi were isolated from *Brassica napus* subsp. *napus* L. bee pollen. The most frequently microscopic fungi species were *Alternaria alternata* and *Cladosporium cladosporoides* (Fig. 2).

From the ecological point of view should be mentioned, that microscopical fungi of genus *Cladosporium* and *Alternaria*, which were mostly isolated from examined

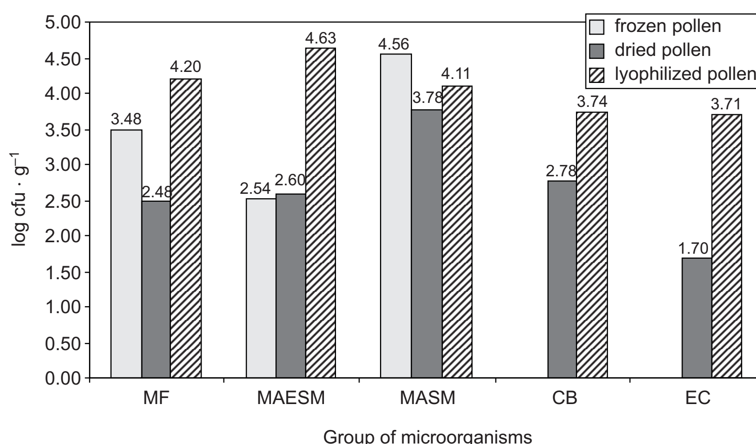


Fig. 1. The number of microorganisms in *Brassica napus* subsp. *napus* L. bee pollen [log cfu · g^{-1}]: MF – microscopic fungi, MAESM – mesophilc anaerobes sporulating microorganisms, MASM – mesophilc aerobes sporulating microorganisms, CB – coliforms bacteria, EC – *Escherichia coli*

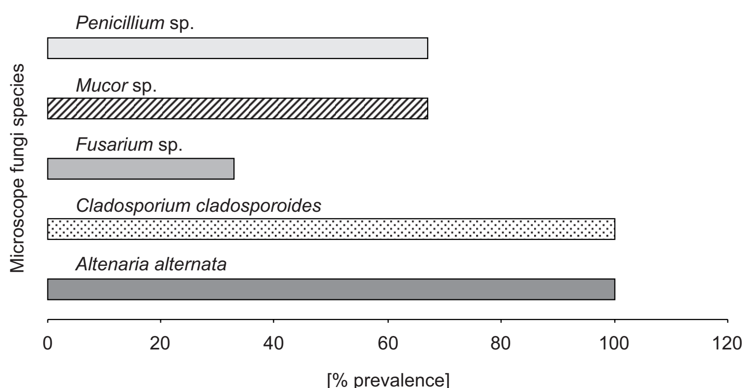


Fig. 2. The prevalence of microscopic fungi in *B. napus* subsp. *napus* L. bee pollen samples [%]

samples, very often saprophyte on sweet products and honeydew. When these microscopics fungi have suitable surroundings, they can multiply on these substrates as much as possible, and then they cover them with sensorial visible black sediment coatings [15].

The freshly collected bee pollen contains approximately 20 % of water, and this is the reason why it can get mouldy. How big attention beekeeper gave to storage of bee pollen, it is possible to find out from laboratory analysis. We expect that microscopic fungi of genus *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus* and other can participate on mouldy bee pollen. Therefore, amount of these microscopic fungi in degraded bee pollen will be substantial higher. Dry bee pollen contains just a little water what averts expansion of unwelcomed microscopic fungi.

Antioxidant activity, total flavonoids and selected flavonoids analysis

The antioxidant activity of the bee pollen samples ranged from 1.25 to 1.93 I/I_0 (in case of freeze-dried and frozen bee pollen, respectively) (Table 1).

Table 1

The antioxidant activity of bee pollen compounds (I/I_0)

Pollen	Antioxidant activity (I/I_0)
Frozen	1.93 ± 0.02
Dried	1.83 ± 0.02
Freeze-dried	1.25 ± 0.02

The dietary antioxidants, flavonoids and other components have been investigated extensively [16–18], and it has been demonstrated that each constituent of floral pollen possesses its own distinct flavonoid/phenolic HPLC profile [16, 18]. On the basis of the findings by Campos et al [19], it is concluded that the free radical scavenging effectiveness of a bee pollen is determined by its constituent pollens, and that the free radical scavenging effectiveness of a bee pollen values for the constituent pollens are

consistent (always the same) for each pollen species. Differences in the nature and levels of the flavonoids and other phenolics would suggest that the effectiveness of various floral pollens (and therefore of the bee pollen mixes) as antioxidants/free radical scavengers may vary widely [19]. The free radical scavenging effectiveness values are in large part determined by the free radical scavenging activity of the flavonoid/phenolic constituents, although other constituents, perhaps proteins can contribute up to half the activity. This activity can decrease significantly on storage (ageing), and it is therefore proposed that the freshness of bee pollen may be determined from its free radical scavenging capacity relative to that of fresh bee pollen with the same floral pollen mix.

The highest total flavonoids content (128.33 $mg \cdot kg^{-1}$) was occurred in the frozen pollen. The highest value of the flavonoid kaempferol achieved in the dried bee pollen, whereas the freeze-dried form contains the most of other three flavonoids (quercetin, luteolin, apigenin) (Table 2).

Table 2

The content of flavonoids in pollen [$mg \cdot kg^{-1}$]

Flavonoids [$mg \cdot kg^{-1}$]	Pollen		
	Frozen	Dried	Freeze-dried
Quercetin	7.67 ± 0.03	11.52 ± 0.04	16.89 ± 0.02
Luteolin	33.60 ± 0.52	37.59 ± 0.09	40.15 ± 0.14
Kaempferol	57.13 ± 0.82	61.16 ± 0.39	57.08 ± 0.61
Apigenin	29.76 ± 1.33	17.70 ± 0.47	32.09 ± 0.69
Flavonoids	128.33 ± 2.05	115.33 ± 3.86	121.33 ± 3.30

There were observed the statistically significant strong dependence between antioxidant activity and individual flavonoids, namely quercetin, ($p < 0.001$), luteolin ($p < 0.01$), but not between kaempferol, apigenin, and total flavonoids (Table 3).

Table 3

Correlation matrix for antioxidant activity and flavonoids content

	AA (DNA biosenzor)	Flavonoids	Quercetin	Luteolin	Kaempferol	Apigenin
AA (DNA biosenzor)	1	$p \geq 0.05$ NS	$p < 0.001$ $r = -0.95499$	$p < 0.01$ $r = -0.86507$	$p \geq 0.05$ NS	$p \geq 0.05$ NS
Flavonoids		1	$p \geq 0.05$ NS	$p \geq 0.05$ NS	$p \geq 0.05$ NS	$p \geq 0.05$ NS
Quercetin			1	$p < 0.001$ $r = 0.96886$	$p \geq 0.05$ NS	$p \geq 0.05$ NS
Luteolin				1	$p \geq 0.05$ $r = 0.95397$	$p \geq 0.05$ NS
Kaempferol					1	$p < 0.001$ $r = -0.97444$
Apigenin						1

AA – antioxidant activity.

Minerals and proteins analysis

The sum of proteins (average 251.13 ± 33.06 g · kg^{-1}) decreased in the order: freeze-dried > dried > frozen bee pollen. Somerville and Nicol [20] investigated the crude protein levels in pollen pellets. Pollens collected from species of the same genus demonstrated similar protein profiles.

The freeze-dried form of pollen was characterized with the highest value of calcium concentration, and the frozen treatment with the lowest content (2040 mg · kg^{-1} versus 1800 mg · kg^{-1}). The zinc was presented in amount 36.97 ± 4.15 mg · kg^{-1}. The most of zinc was contained in the freeze-dried pollen.

In the study by Szczesna [21] calcium content of *Brassicaceae* honey bee-collected pollen ranged from 542 to 1080 mg · kg^{-1} dry matter (d.m.) (average 782 ± 23 mg · kg^{-1} d.m.), content of *Artemisia* ranged from 798 to 827 mg · kg^{-1} d.m. (812 ± 21 mg · kg^{-1} d.m.), content of multifloral pollen ranged from 648 to 796 mg · kg^{-1} d.m. (718 ± 63 mg · kg^{-1} d.m.). The zinc content of *Brassicaceae* honey bee-collected pollen ranged from 31.9 to 39.9 mg · kg^{-1} d.m. (average 35.7 ± 3.6 mg · kg^{-1} d.m.), the content of *Artemisia* ranged from 25.6 to 31.2 mg · kg^{-1} d.m. (28.4 ± 4.0 mg · kg^{-1} d.m.), the content of multifloral pollen ranged from 34.1 to 53.6 mg · kg^{-1} d.m. (41.5 ± 7.6 mg · kg^{-1} d.m.). The differences in the contents of individual elements as reported by different authors can be explained by differences in geographic and botanical origin of the pollen samples tested. The high concentration of the tested elements, especially in the pollen of *Brassicaceae* family, makes that variety of pollen an important potential source of macro- and micronutrients. In comparison with recommended dietary intakes of elements, the obtained contents of bee pollen point to a high nutritive value of the product, which can be recommended as a natural source of macro- and micronutrients [22]. The product can be successfully used as different dietary formulas and supplements in order to enrich our food rations with valuable nutrients performing important functions in the human body.

Conclusions

According to microbiological analyses the bee pollen is the ideal cultural medium for microorganisms like bacteria and yeast. The drying (moisture 10–11 %), freezing (–18 to –20 ^{o}C) and lyophilization were not enough efficient ways to preserve hygienic quality of bee pollen. It is important to look for the treatment which will protect bee pollen from another microorganisms spreading, and it allows decreasing of number microorganisms as much as possible.

There have to be other studies to determine the quality parameters of bee pollen with different botanical origin. Microbial properties, nutritional composition and antioxidant activity of bee pollen were largely investigated, but enormous botanical diversity in pollen pellets causes that there are still many gaps to be solve to standardize quality of bee pollen as food supplement or medicine.

Acknowledgement

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704, VEGA 1/0887/10 and KEGA 301-035SPU-4/2010 and ITMS 26220120015. This work has been supported by the Excellence Center for Agrobiodiversity Conservation and Benefit project implemented under the Operational Programme Research and Development financed by European Fund for Regional Development.

References

[1] Gonzáles-Güerca M.C., Almaraz-Abarca N., Ávila-Reyes J.A., Herrera-Corral J. and Naranjo-Jiménez N.: Apitec 2001, **28**, 19–23.
[2] Campos M.G., Mitchel K., Cunha A. and Markham K.R.: Phytochem. Anal. 1997, **8**, 181–185.
[3] Hanssen M.: The healing power of pollen and other products from the beehive – propolis, royal jelly, honey. Thorsons Publ. Ltd., Northampshire (UK) 1979.
[4] Linskens H.F. and Jorde W.: Econom. Bot. 1997, **51**, 77–78.
[5] Lyngheim L. and Scangetti J.: Bee pollen – nature's miracle health food. Wilshire Book Co., Hollywood (CA) 1979.
[6] Flynn A., Moreiras O., Stehle P., Fletcher R.J., Muller D.J.G. and Rolland V.: Eur. J. Nutrit. 2003, **42**, 118–130.
[7] Dudov I.A. and Starodub N.F.: Ukr. Biokhim. Zh. 1994, **66**(6), 94–96.
[8] Dudov I.A., Morenets A.A., Artinkh V.P. and Starodub N.F.: Ukr. Biokhim. Zh. 1994, **66**(6), 91–93.
[9] Bogdanov S.: Apiacta 2004, **38**, 334–341.
[10] Holt G.J., Krieg N.R., Sneath P.H.A., Staley J.T. and Williams S.T.: Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore 1994.
[11] Hoog G.S., Guarro J., Gene J. and Figueras M.J.: Atlas of clinical fungi. Centralbureau voor Schimmelcultures, Rovira 2000.
[12] Klich M.A.: Identification of common *Aspergillus* species. Ponsen & Looijen, Wageningen 2002.
[13] Samson R.A., Van Reenen-Hoekstra E.S., Frisvad J.C. and Filtenborg O.: Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Utrecht 2002.
[14] Heilerová Ľ., Bučková M., Tarapčík P., Šilhár S. and Labuda J.: Czech J. Food Sci. 2003, **21**, 78–84.
[15] Miller J.D.: Atmos. Environ., 1992, **32**, 2163–2172.
[16] Campos M.G., Markham K. and Proença da Cunha A.: Bull. Groupe Polyphen. 1996, **18**, 54–55.
[17] Ferreres F., Tomas-Barberan F.A., Tomas-Lorente F., Nieto J.L., Rumbero A. and Olias J.M.: Phytochemistry 1989, **28**, 1901–1903.
[18] Campos M.G., Webby R.F., Markham K.R., Mitchell K.A. and Cunha A.P. Da: J. Agric. Food Chem. 2003, **51**, 742–745.
[19] Somerville D.C. and Nicol H.I.: Australian J. Exp. Agricult. 2006, **46**, 141–149.
[20] Szczęsna T.: J. Apicult. Sci. 2007, 51, 5–13.
[21] Wade C.: Health from the hive. Keats Publishing, New Cannaa (CT) 1992.

SKŁAD MIKROFLORY, SKŁADNIKI ODŻYWCZE I AKTYWNOŚĆ ANTYOKSYDACYJNA W PYŁKU PSZCZELIM POCHODZĄCYM Z *Brassica napus* subsp. *napus* L. UŻYWANYM W ŻYWIENIU LUDZI

Abstrakt: Celem pracy była charakterystyka mikroflory, składników odżywczych i aktywności antyoksydacyjnej w pyłku pszczelim wyprodukowanym z *Brassica napus* subsp. *napus* L. Oznaczono wskaźnik cfu (jednostki tworzenia kolonii). Średnia liczebność mezofilowych aerobowych sporulujących mikroorganizmów wynosiła 3,78–4,56 log cfu $\cdot$ g^{-1}. Liczebność mezofilowych anaerobowych sporulujących mikroorganizmów wynosiła 2.54–4.63 log cfu $\cdot$ g^{-1}. Liczebność bakterii coli wynosiła 0–3,74 log cfu $\cdot$ g^{-1}. Średnia liczebność grzybów wynosiła 2.48 do 4.20 log cfu $\cdot$ g^{-1}. Aktywność antyoksydacyjna w pyłku pszczelim wynosiła 1,25–1,93 I/I_0 (odpowiednio dla pyłku liofilizowanego oraz pyłku zamrażanego). Największa całkowita zawartość flawonoidów (128,33 mg $\cdot$ kg^{-1}) występowała w pyłku zamrażanym. Największa zawartość

flawonoidu kemferolu występowała w suszonym pyłku pszczelim, natomiast pyłek liofilizowany zawierał największe ilości pozostałych flawonoidów (kwercetyny, luteoliny, apigeniny). Sumaryczna zawartość białka (średnia 251,13 ± 33,06 g · kg^{-1}) zmniejszała się w kolejności: pyłek liofilizowany > suszony > zamrażany. Liofilizowana postać pyłku charakteryzowała się największą zawartością wapnia (2040 mg · kg^{-1}), natomiast najmniejszą zawartość wapnia stwierdzono w pyłku zamrażanym (1800 mg · kg^{-1}). Zawartość cynku w badanym pyłku wynosiła 36,97 ± 4,15 mg · kg^{-1}. Najwięcej cynku znajdowało się pyłku liofilizowanym.

Słowa kluczowe: *Brassica napus* subsp. *napus* L., pyłek pszczeli, mikrobiologiczna jakość, bakterie, mikroflora grzybowa, właściwości antyoksydacyjne, składniki odżywcze

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Krzysztof FRĄCZEK[1] and Jacek GRZYB[1]

ANALYSES OF BACTERIAL AEROSOL OCCURRING IN HEALTH RESORTS IN BOCHNIA AND SZCZAWNICA

BADANIA AEROZOLU BAKTERYJNEGO WYSTĘPUJĄCEGO W OŚRODKACH SANATORYJNYCH W BOCHNI I SZCZAWNICY

Abstract: The main aim of the research was evaluation of the bacterial aerosol occurring in sanatorium chambers in the Health Resort of Salt Mine in Bochnia and in the sanatorium rooms of Przedsiębiorstwo Uzdrowisko Szczawnica S.A., with the definition of its particle fractions, depending on the sizes of aerodynamical diameters into: over 7.0 μm, 7.0–4.7 μm, 4.7–3.3 μm, 3.3–2.1 μm, 2.1–1.1 μm, and 1.1–0.65 μm. Microbiological research of the air was carried out during spring 2008 in healing chambers of the Health Resort of Salt Mine in Bochnia and in the sanatorium rooms of the "Health Resort Enterprise Szczawnica". The measurements were performed using 6-step Graseby-Anderson impactor from the respiratory zone (oral and nasal cavity location) of people in the state of so-called "original sterility", which is before introduction of the bathers and personnel into the sanatorium chambers and rooms but also during the presence of patients and during treatment operations. As a result of the analyses, significant differences were found in the amount of aerosol in various examination points. On the basis of the gained results it may be concluded, that the highest concentration of bacterial aerosol in the Health Resort of Salt Mine in Bochnia ocurred in Wazyn Chamber (in the part of the gym and the bedroom), whereas in the "Health Resort Enterprise Szczawnica" – inside the mineral baths room and in the corridor leading to the bathers' rooms in the Inhalatorium building. Significantly lower level of the bacterial aerosol was observed during the patients' stay in relation to the period between the stays (the lack of patients).

Keywords: bacterial aerosol, sanatorium, air

The degree of air pollution plays an important role in health conditions of an environment. This becomes more and more live issue, because constantly progressing environment degradation is among others the factor which causes permanent growth of bacterial and mycological contamination of atmospheric air [1, 2].

[1] Department of Microbiology, University of Agriculture in Krakow, al. A. Mickiewicza 24/28, 30–058 Kraków, Poland, phone 12 662 41 81, email: rrfracze@cyf-kr.edu.pl, rrgrzyb@cyf-kr.edu.pl

According to the reports, aeromicroflora of closed rooms is responsible for the respiratory system problems as well as for other health issues among children, and is considered as a direct infectious agent in hospitals [2, 3].

In relation to this, specific healing environmental conditions, which occur in underground subterraneotherapy chambers and in overground health resorts, presently become very significant. This is why in recent years a new tendency is observed for applying in therapeutics subterraneotherapy, which has become an efficient method for dealing with harmful influence of pollutants on the Earth surface on the human organism [1, 4].

According to the above-mentioned facts, the present research, aimes at proving if important differences occur in the size of aerosol particles between the environment of the subterraneotherapy chambers in Salt Mine in Bochnia and in chambers of overground health resort of "Health Resort Enterprise Szczawnica".

Material and methods

The analyses concerning the occurrence of different particles of biological aerosol were carried out in spring 2008 in the air temperature above 14 ^{o}C. As an overground sanatorium, "Health Resort Enterprise Szczawnica" was appointed. It is located in one of the oldest and the most beautiful Polish health resorts – Szczawnica. The town – completely without industry, and located nearby three National Parks – has an exceptional healing microclimate and clean, allergen-free air. Presently, as a part of "Health Resort Enterprise Szczawnica" Sanatorium "Papiernik" and "Inhalatorium", Nature Treatment Institute, Spa Clinic and Pump Room operate. This resort is specialized in treatment of respiratory ways diseases, including allergic diseases of respiratory system, asthma and activity system issues.

The research concerning the occurrence of bioaerosol was carried out also in underground healing chambers in Salt Mine in Bochnia. This resort specializes itself also in respiratory ways diseases, including lingering nose and throat inflammations, voice apparatus diseases, allergies of respiratory system and asthma. In this case specific microclimate and saline aerosol floating in the air of healing chambers are the healing factors.

The measurements of bacterial aerosol were performed in two measuring sessions. In each tested resort, 6 examination points were chosen, in which samples were taken. The bioaerosol was analyzed inside the sanatorium rooms and healing chambers, during the presence of the personnel and bathers, and also in the state of so-called "original microbiological cleanliness", which was during the days off-work, when no healing operations were performed. Moreover, bioaerosol samples were taken in the external environment in the surroundings of the resort buildings.

The air samples were taken by means of 6-step Anderson impactor (model 10–710, Anderson Instruments, Atlanta, Ga, USA), which during the analyses was placed 1–1.5 m over the floor, in order to take the bioaerosol from the human respiratory zone. During the measuring sessions, the measurements of the air temperature and relative humidity were performed at the same time. Microbiological analyses included evalua-

tion of the general number of bacteria on the Trypticase Soy Agar Medium (Trypticase Soy Agar, TSA, Emapol, Gdansk, Poland) with 5 % addition of defibrinated sheep blood. Conditions of air samples incubation were as following: 1 day in 37 ^{o}C, 3 days in 22 ^{o}C, and then 3 days in 4 ^{o}C. Concentration of the tested aerosol was expressed as an amount of colony forming units on the microbiological medium, present in 1 m^3 of taken air [cfu/m^3].

Results and discussion

The results of measurements of the bacterial aerosol occurring in the air outside and in the rooms and chambers of the two health resorts are shown in Figures 1, 2. While comparing the gained results, it may be concluded, that in the chosen examination points in the area of the underground sanatorium Health Resort of Salt Mine in Bochnia, the measured amounts of bacteria concentration, during the presence of bathers in the healing chambers, are within the ranges from 189 cfu/m^3 to 11688 cfu/m^3. In the state of so-called "original microbiological cleanliness", which is the time when people are absent – from 63 cfu/m^3 to 2997 cfu/m^3. In relation to the measurements of the bacterial aerosol in the examination points, located in the underground sanatorium chambers, it was shown, that mean value of bacteria concentration in the air in Wazyn Chamber in the part designed for the gym, was much higher than the mean concentrations found in the other places in the mine. Basing on the results analysis, it is clearly seen, that generally the highest concentration of bacteria in the tested air was in Wazyn Chamber (in the gym and bedroom) and on the ramp between levels IV and VI (points B3, B4 and B2, respectively). However, the lowest concentration of this bioaerosol was found in the air of the gangway leading the air from the shaft Trynitatis, which constitutes the "inside background" and was significantly lower than the results gained from the measurements for the "outside background" (points B6 and B7, respectively). More-

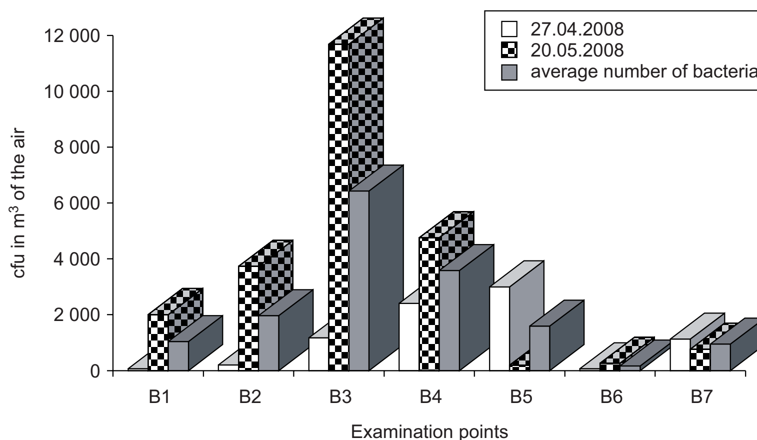


Fig. 1. Bacterial aerosol in the external environment and in the area of the underground sanatorium – "Health Resort of Salt Mine in Bochnia"

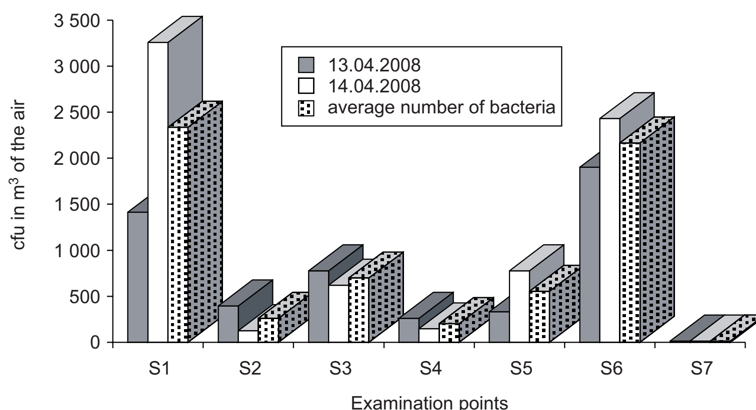


Fig. 2. Bacterial aerosol in the external environment and inside the sanatorium rooms in the overground health resort – "Health Resort Enterprise Szczawnica"

over, the concentration values of bioaerosol in healing chambers were found to be higher than the values measured for the "inside background".

The observed trend is coherent with the present state of knowledge of the origin of the tested bacterial aerosol, in which people are considered as the biggest and constantly active source of its emission in the interiors. The above specified concentrations of bacteria occurring in the healing chambers in Salt Mine in Bochnia are also the result of constant air circulation, which occurs between the earth surface and each levels in the Mine. The appearance of microflora in the chambers is also influenced by the horizontal air flows, temperature, and the lack of sun rays and the dampness from brines [5, 6].

In relation to the total concentration of bacterial aerosol in sanatorium chambers of the overground health resort "Health Resort Enterprise Szczawnica", the tested bioaerosol values during the presence of patients and therapeutic procedures were found to be within the range between 127 cfu/m^3 and 3258 cfu/m^3. It is also noticeable, that the concentration of bacteria in the air inside the tested rooms in the day off-work was lower and was from 261 cfu/m^3 to 1901 cfu/m^3. The highest concentration of the tested bacterial aerosol was in the room in the Nature Treatment Institute, in which mineral baths are performed and in the corridor leading to the rooms of bathers in the Inhalatorium building – "inside background" (points S1 and S6, respectively). However, the lowest bioaerosol value was found in the chamber inhalations room and in the room, in which whirl massage of limbs was performed (points S4 and S2, respectively). The results, shown in the graph 2 indicate, that the mean values of bacteria concentration in the air inside the mineral baths room and in the corridor of the Inhalatorium building, were significantly higher than in the other measuring points. The comparison of the measurement results for the "inside background" and "outside background" shows, that the concentration of bacterial aerosol in the outside environment was significantly lower than the values of "the background" measured inside the tested sanatorium chambers. On the other hand, the concentration values of the tested bioaerosol measured in chosen rooms were lower than the values measured for the "outside background".

It is known from the literature, that in normal conditions in rooms, in which people stay, bacterial concentrations are higher than in the outside environment, and generally they do not transgress the range of 10000 cfu/m^3. The degree of air pollution in closed rooms depends on the size of rooms, furnishings, amount of people who use the room, location and outdoor air properties. The range of impact of the external meteorological factors and the penetration speed of these influences inside the room depends on the way of air exchange, character of rooms and on their isolation from the surroundings [7–9].

On the basis of the results of the measurements of the bacterial aerosol concentration, it may be noticed that generally higher concentrations of bacteria occurred in the air during the presence of bathers in the underground sanatorium chambers than in rooms of the overground health resort. It should be noticed, that in this period the maximum bacterial aerosol concentration in Salt Mine in Bochnia, which was 11688 cfu/m^3 (Fig. 1), was over three times higher than the maximum concentration of the tested bioaerosol – 3258 cfu/m^3, found in the sanatorium rooms of "Health Resort Enterprise Szczawnica" However, concerning the measured values of bacterial aerosol concentrations for the "inside background", in both health resorts, the mean amount of bacteria in the tested "background" air inside the rooms in the overground sanatorium was found to be significantly higher than the mean amount of bacteria found in the "background" air of the underground sanatorium chambers. The analysis of the gained results reveals clearly the low level of bacteriological contamination in the state of so-called "original microbiological cleanliness", which is before the introduction of the bathers into the sanatorium rooms or chambers. Such result may be explained by better environmental conditions. The above observations emphasize the value of underground treatment, in which avoiding the superinfection of the patients is essential. The presence of small biological environmental contamination is considered to be one of the most important factors of pulmonological subterraneotherapy, because it allows the temporary isolation of the patient from the harmful influence of the external environment [1].

The usage of 6-step Anderson impactor in the research has allowed to collect data about fractional (grain size) distribution of the bacterial aerosol in the tested rooms. Tables 1 and 2 show grain size distribution of the air microflora found in the external environment, in the air of the sanatorium rooms and in the underground healing chambers. The analysis of fractional distribution occurring in the rooms of the overground sanatorium as well as in the underground healing chambers indicates the presence of bacteria mainly in the range of diameters from 1.1 to 4.7 μm. The gained result shows, that bacterial microorganisms were present there mainly as single cells (bacteria 1.1–3.3 μm) and small bacterial or dust-bacteria aggregates (2.1–4.7 μm). The share of large microorganisms' aggregates in the bioaerosol composition (>3.3 μm) was lower than their smaller forms. Such distribution of the particles' aerodynamic diameters indicates the additional emission from the reservoir, which is human organism (the increased emission during breathing and exfoliation of the epidermis during the bathers and personnel presence in the sanatorium rooms or chambers) [6].

Table 1

The concentration of bacterial aerosol in the external environment and inside the rooms of the overground sanatorium – "Health Resort Enterprise Szczawnica" on 13th April 2008 (measure without the bathers) and on 14th April 2008 (measure during the presence of the bathers)

Examination point	Concentration [cfu/m^3] of each fraction of bacterial aerosol (the range of particle diameters [µm])											
	≥ 7		7.0–4.7		4.7–3.3		3.3–2.1		2.1–1.1		1.1–0.65	
	13.04. 2008	14.04. 2008	13.04. 2008	14.04. 2008	13.04. 2008	14.04. 2008	13.04. 2008	14.04. 2008	13.04. 2008	14.04. 2008	13.04. 2008	14.04. 2008
S1 Nature Treatment Institute building (room – mineral bath)	212	813	113	445	261	749	594	820	226	417	7	14
S2 Nature Treatment Institute building (room – whirl massage of the limbs)	35	57	7	7	49	7	78	14	163	35	64	7
S3 room in the building of the Pump Room	120	85	71	35	254	155	205	212	127	134	0	0
S4 Inhalatorium building (room – chamber inhalations)	49	42	14	7	57	14	99	35	42	42	0	7
S5 Inhalatorium building (room – individual inhalations)	42	85	14	92	57	226	113	240	92	127	14	7
S6 Inhalatorium building – the corridor (inside background)	339	269	290	445	389	622	622	657	261	410	0	28
S7 nearby the sanatorium building (outside background)	14	7	0	7	0	0	0	0	0	0	0	0

Table 2

Bacterial aerosol concentration in the external environment and inside the healing chambers in the area of the underground sanatorium – "Health Resort of Salt Mine in Bochnia" on 20th May 2008 (measure during the stay of the bathers) and on 27th April 2008 (measure without the bathers)

Examination point	Concentration [cfu/m^3] of each fraction of bacterial aerosol (the range of particle diameters [μm])											
	≥ 7		7.0–4.7		4.7–3.3		3.3–2.1		2.1–1.1		1.1–0.65	
	27.04. 2008	20.05. 2008	27.04. 2008	20.05. 2008	27.04. 2008	20.05. 2008	27.04. 2008	20.05. 2008	27.04. 2008	20.05. 2008	27.04. 2008	20.05. 2008
B1 The Koldras Chamber – the middle	7	289	14	431	7	184	21	558	21	544	0	0
B2 ramp between levels IV and VI	64	558	49	686	28	749	49	947	7	700	7	92
B3 The Wazyn Chamber – the gym	403	926	283	1194	184	2226	191	4346	113	2869	0	127
B4 The Wazyn Chamber – the bedroom	417	318	318	283	445	1011	537	1767	678	1364	14	14
B5 The gangway behind the Wazyn Chamber in the direction of Sutoris shaft	862	35	297	42	403	21	841	14	509	49	85	28
B6 Inside background – the gangway leading the air from the Trynitatis shaft	0	85	7	57	21	21	7	64	14	35	0	0
B7 Outside background in the open area, nearby the Trynitatis shaft	120	375	163	127	219	67	297	85	113	64	219	49

Analyzing the grain size distribution for the "outside background", chosen for the underground as well as for the overground health resort, it may be stated, that in the external environment bacteria were most often present as dust-bacteria aggregates. Other researchers gained similar results, for example Lis et al [9], who found, that the most particles of the bacterial aerosol in the external environment have the diameter larger than 5 μm. This is probably due to the dust pollutants, emitted in cities to the atmosphere from the low emission sources [9, 10].

Conclusions

1. The research has shown, that concerning microbiological cleanliness, the state of the air in the tested overground sanatorium rooms and in the underground subterraneotherapy chambers is good.
2. It was found, that the environment in the underground healing chambers, as well as in the rooms of the overground sanatorium is bacteriologically less contaminated in the state of so-called "initial microbiological cleanliness", which is before entering the bathers.
3. The research shows the purposefulness of using the environment of Wazyn Chamber in Salt Mine in Bochnia in the subterraneotherapy treatment.
4. Data gained in the research shows that the tested health resort in Szczawnica has profitable biological factors which determine its role in allergic diseases treatment.

Acknowledgement

The research financed by the science resources in 2007–2008 as the research project N N305 2552 33.

References

[1] Doleżal M., Doleżal M. and Żuława G.: *Mikroflora komór podziemnych stacji badawczej subterraneoterapii w Wieliczce*. Biozanieczyszczenia w Budynkach a Zdrowie. Zesz. Nauk. AWF w Krakowie 1983, **35**, 55–65.
[2] Di Georgio C., Krempff A., Guiraud H., Binder C. and Tiretf G.D.: *Atmospheric pollution by airborne microorganisms in the city of Marseilles*. Atmos. Environ. 1996, **30**(1), 155–160.
[3] Górny R.L.: *Biologiczne czynniki szkodliwe: normy, zalecenia i propozycje wartości dopuszczalnych*. Podstawy i Metody Oceny Środowiska Pracy 2004, **3**(41), 17–39.
[4] Rozporządzenie Ministra Zdrowia z dnia 13 kwietnia 2006 r. w sprawie zakresu badań niezbędnych do ustalenia właściwości leczniczych naturalnych surowców leczniczych, właściwości leczniczych klimatu, kryterium ich oceny oraz wzoru świadectwa potwierdzającego te właściwości.
[5] Skrzyńska J.: *Mikrobiologiczne badanie powietrza w podziemiach kopalni Wieliczki*. Przegl. Lekarski 1948, **4**(2), 1–11.
[6] Wlazło A., Górny R.L., Złotowska R., Ławniczek A., Łudzień-Izbińska B., Harkawy A.S. and Janczyk E.: *Narażenie pracowników na wybrane szkodliwe czynniki biologiczne w bibliotekach województwa śląskiego*. Med. Pracy 2008, **59**(2), 159–170.
[7] Cianciała M., Smyk B., Bukowski Z. and Kukliński P.: *Kształtowanie się zespołów mikroflory w powietrzu ośrodków alergologicznych w Szczawnicy i w Krakowie*. Balneol. Polska 1968, **13**(2–4), 265–273.
[8] Kotowski J. and Gajewski M.: Raport z badań mikrobiologicznych powietrza w Komorze Ważyn Kopalnia Soli "Bochnia". Instytut Medycyny Uzdrowiskowej, Warszawa 1988.

[9] Lis D., Pastuszka J.S. and Uflig K.: *Mikrobiologiczna jakość powietrza w pomieszczeniach administracyjno-biurowych wysypiska odpadów komunalnych na przykładzie Miejskiego Zakładu Składowania Odpadów w Sosnowcu*. Ochr. Powiet. Probl. Odpad. 1999, **33**(5), 199–202.
[10] Górny R.L. and Dutkiewicz J.: *Bacterial and fungal aerosols In indor environment In Central and Eastern European countries*. Ann. Agric. Environ. Med. 2002, **9**(1), 17–23.

BADANIA AEROZOLU BAKTERYJNEGO WYSTĘPUJĄCEGO W OŚRODKACH SANATORYJNYCH W BOCHNI I SZCZAWNICY

Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Zasadniczym celem pracy było określenie charakterystyki aerozolu bakteryjnego występującego w komorach sanatoryjnych Uzdrowiska Kopalnia Soli Bochnia oraz w pomieszczeniach sanatoryjnych Przedsiębiorstwa Uzdrowisko Szczawnica, z jego rozkładem na poszczególne frakcje cząstek w zależności od średnic aerodynamicznych: powyżej 7,0 μm, 7,0–4,7 μm, 4,7–3,3 μm, 3,3–2,1 μm, 2,1–1,1 μm i 1,1–0,65 μm. Badania mikrobiologiczne powietrza przeprowadzone zostały w okresie wiosny 2008 r. w komorach leczniczych Uzdrowiska Kopalni Soli w Bochni oraz w pomieszczeniach sanatoryjnych Przedsiębiorstwa „Uzdrowisko Szczawnica" S.A. Pomiary zostały przeprowadzone za pomocą sześciostopniowego impaktora Graseby-Andersena ze strefy oddechowej (położenie jamy ustnej i nosowej) człowieka w stanie tzw. pierwotnej jałowości, tj. przed wprowadzeniem kuracjuszy i personelu do komór oraz pomieszczeń sanatoryjnych, a także w czasie trwania turnusu i wykonywania zabiegów leczniczych. W wyniku przeprowadzonych analiz wykazano znaczne różnice w ilości występującego aerozolu bakteryjnego na różnych stanowiskach pomiarowych. Na podstawie otrzymanych wyników można stwierdzić, że największe stężenie aerozolu bakteryjnego w Uzdrowisku Kopalni Soli w Bochni występowało w Komorze Ważyn (w części hali sportowej oraz sypialnej), natomiast w Przedsiębiorstwie „Uzdrowisko Szczawnica" wewnątrz pomieszczenia, w którym są przeprowadzane zabiegi kąpieli mineralnej i na korytarzu prowadzącym do pokoi kuracjuszy w budynku Inhalatorium. Zaobserwowano wyraźnie wyższy poziom występującego aerozolu bakteryjnego w trakcie trwania turnusów leczniczych i wykonywania zabiegów w porównaniu z okresem międzyturnusowym (brak kuracjuszy).

Słowa kluczowe: aerozol bakteryjny, sanatorium, powietrze

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Zofia GOC[1], Katarzyna KILIAN, Grzegorz FORMICKI,
Robert STAWARZ, Aldona CIĄGŁO
and Anna KUCZKOWSKA-KUŹNIAR

ANTIOXIDANT STATUS AND METAL CONTENTS IN HUMAN BREAST MILK IN RELATION TO AGE AND COURSE OF LACTATION

STATUS ANTYOKSYDACYJNY ORAZ ZAWARTOŚĆ METALI W MLEKU LUDZKIM U KOBIET W RÓŻNYM WIEKU I OKRESIE LAKTACJI

Abstract: The aim of the study was to investigate the concentration of chosen trace elements, activity of catalase (CAT) and glutathione content (GSH) in human milk. The study subjects were recruited from among the lactating women from Malopolska province in Poland. Milk was taken from 23 mothers classified into three groups of age (20–25, 26–31 and 32–37 years) and two groups of lactation period (colostrums; 1–3 days; and transitional milk, over 4th day). CAT and GSH values were determined by the spectrophotometric method. Cu, Zn, Mg, Fe and Cd concentrations were determined by atomic absorption spectrophotometry (AAS). The results indicate no significant differences between activity of catalase taking into account the age of mothers and the day of lactation. Similarly glutathione level showed insignificant differences between studied groups. Our results indicate that human milk of women from Malopolska province, Poland, may contain significant amount of cadmium. We found statistically significant correlation between some metals in milk from women in different age and period of lactation. It concerned: Fe vs Cd and Cd vs Cu correlation**.** The result of our study indicate that the activity of antioxidant enzymes did not change during the course of lactation and in relation to mother's age.

Keywords: catalase, glutathione, breast milk, trace elements, heavy metals

Human milk is considered as optimal food for newborn infants during the first months of life in relation with its nutritional and protective characteristics. Properties of breast milk change during the course of lactation. Breast milk has three different stages: colostrum, transitional milk, and mature milk. Colostrum is the first stage of breast milk which lasts for several days after the birth. It is rich in proteins, fat-soluble vitamins, minerals, and immunoglobulin. The protein concentration is much higher in colostrum

[1] Institute of Biology, Pedagogical University of Krakow, ul. Podbrzezie 3, 31–054 Kraków, Poland, email: zosiak0@vp.pl

than in mature milk but much of it has the form of secretory immunoglobulin A (IgA). The immunoglobulin is probably not absorbed by the gut and therefore it is not nutritionally available. Although, it is thought to have a protective function against infections [1]. Transitional milk occurs after colostrum, lasts for approximately two weeks and has high level of fat, lactose, water-soluble vitamins, and contains more calories than colostrum. Mature milk is the final milk that is produced. It contains about 90 % of water, which is necessary to maintain hydration. The other 10 % is comprised of carbohydrates, proteins, and fats which are necessary for both growth and energy.

Breast milk is also a source of antioxidants. They have specific properties to neutralize free radicals which can damage cellular structures like DNA, proteins, carbohydrate and cause lipid peroxidation. Vitamins, proteins and enzymes are involved in the antioxidant protection [2]. One of the most active antioxidant enzyme is catalase (CAT, E.C.1.11.1.6). Catalase is a hemoprotein composed of four identical subunits which contains haem-bound iron in its activate site. The heme group is responsible for enzymatic activity of the enzyme. The presence of CAT is documented in human and cow milk [3, 4]. It plays very important role in the decomposition of hydrogen peroxide into molecular oxygen and water to prevent accumulation of toxic peroxides in the cell. Milk from mothers of preterm and full term infants has equal resistance to oxidative stress. Catalase activity in human milk increases with time [5].

Protective mechanisms against oxidation involves also tripeptyde glutathione. Glutathione (γ-glutamylocysteinyloglicyn) is a non-enzymatic antioxidant which exhibits reductive properties conditioned by the thiol group. Breast milk becomes an important source of dietary (GSH) since infant's GSH synthetic capacity may not be well developed [6]. Glutathione and its precursors are present in the colostrum. Additionally, GSH takes part in detoxification processes of metals like Cu, Ag, Cr in organism and form stable complexes with them [2].

Breast milk includes nutritional and trace elements, on the other hand there is documented that xenobiotics like cadmium and lead are also present [7]. A plentiful supply of breast milk from a mother eating an adequate diet should provide all the neonate's requirements of minerals and trace elements because they have a high level of bioavailability so even low concentration can be well utilized [1]. We investigated concentration of some trace elements (Fe, Cu), nutritional elements (Mg, Zn) and pollutant Cd. Zinc and copper are present in several enzymes and proteins and interact in various tissues. Metallothionein is considered to regulate the transportation of zinc and copper [7]. Zinc and copper have been found to be bound partially to the same proteins, eg lactalbumin in colostrum and transitional milk [8]. Zinc is present mainly in association with the low-molecular-weight components and proteins may contribute to its high bioavailability [9]. The bioavailability of some elements can have an influence on other elements level, for example high zinc/copper ratio can cause a decrease in the absorption of copper from diet and vice versa [10]. Moreover, the stage of lactation is a very significant factor which can cause changes in trace elements' level what is well documented for copper, iron and zinc. Iron concentration in milk is not dependent on maternal iron status, similarly zinc level [10–13]. Milk can also contain heavy metals like cadmium.

During lactation, Cd is transported from maternal plasma to mammary gland and secreted into breast milk [7].

The aim of the study was to investigate the concentration of nutritional and trace elements, and also activity of catalase (CAT) and glutathione content (GSH) in human milk in relation with mother's age and period of lactation.

Materials and methods

The study subjects were recruited from among the lactating women from Malopolska province in Poland who had given birth to a mature baby at the Czerwiakowski Hospital in Krakow. Women had been informed about the aim of the study and gave their permission for collecting milk. All women were in good health condition. We classified mothers taking into account two factors: age and stage of lactation. According to the various age of the women they were segregated into three groups: 20–25, 26–31 and 32–37 years. In addition women were segregated into two groups of different stage of lactation. First group consisted of women in early stage of lactation (1 to 3 days – colostrum). The women from the second group had lactation for more than 4 days (transitional milk).

Milk samples were collected into 5 cm^3 sterile polypropylene containers by manual expression, always in the morning hours between 9 am and 11 am.

Enzymatic analyses were performed immediately after the samples collection. Catalase activity was measured using method described by Bartosz (1995). Fresh milk was centrifuged at 12.000 g (10 min) and then was skimmed by vacuum suction. Briefly, 0.1 cm^3 of the supernatant was mixed with 0.9 cm^3 potassium – phosphate buffer (pH = 7.0) and 0.5 cm^3 solution of 54 mmol H_2O_2 in this buffer. Then we recorded a decrease in the absorbance at λ = 240 nm during 1 minute. We assumed that one unit of catalase decomposed 1 μmol H_2O_2 during one minute. We also measured total protein level using Lowry method.

Glutathione was measured by Ellman's method (1959). The volume of 500 mm^3 of each sample was mixed with 500 mm^3 TCA and 500 mm^3 EDTA to get rid of proteins. Then the samples were incubated in a fridge temperature for 10 minutes. Next samples were centrifuged at 6300 g for 5 minutes. The volume of 200 mm^3 of supernatant was mixed with 2.3 cm^3 H_2O, 100 mm^3 EDTA, 300 mm^3 TRIS and 100 mm^3 DTND. Blank assay was prepared by mixing 2.3 cm^3 H_2O, 200 mm^3 EDTA, 300 mm^3 TRIS, 100 mm^3 DTND and 100 mm^3 TCA. The absorbance was measured spectrophotometrically towards blank assay at λ = 412 nm. Glutathione content was expressed in milimol per liter of human milk.

For metals determination aliquots of milk (2 cm^3) were placed in a separate mineralization tubes and mixed with 2 cm^3 of concentrated HNO_3 and heated at 120 ^oC for 240 minutes in a thermostat-controlled digestion block. After cooling the samples were filled to the volume of 5 cm^3 with demineralized water. Trace elements: iron, copper; nutritional elements: magnesium, zinc and xenobiotics: cadmium were measured by atomic absorption spectrofotometry (AAS). The concentration of the elements were expressed in miligrams per 1 dm^3 of milk.

Statistical analysis of catalase activity and glutathione content was performed using one-way ANOVA. Correlation coefficients, between six elements (Cd, Fe, Zn, Cu, Mg), catalase and glutathione were calculated by Spearman's correlation test.

Results and discussion

Milk samples were classified into three groups of age (20–25, 26–31, 32–37) and two groups of stage of lactation (1–3 days colostrum, over 4 days transitional milk). Average concentration, mean and standard error of nutritional and trace elements in relation with age are listed in Table 1. The same characteristics in relation with stage of lactation are listed in Table 2.

Table 1

Elements concentration in breast milk in relation with maternal age. Values expressed in mg/dm^3, Cd in μg/dm^3

	20–25 age			26–31 age			32–37 age		
	mean	min.–max	S.E.	mean	min.–max	S.E.	mean	min.–max	S.E.
Mg	26.00	16.31–37.92	3.67	41.28	33.79–53.32	2.78	44.10	30.58–56.02	6.71
Zn	4.60	3.04–7.77	0.74	3.83	1.43–7.35	0.99	5.62	1.40–8.30	1.56
Cu	0.31	0.23–0.49	0.04	0.41	0.17–0.60	0.07	0.44	0.17–0.97	0.14
Fe	3.15	1.01–8.93	1.33	1.16	0.66–2.93	0.44	12.37	1.57–26.94	5.58
Cd	0.06	0.04–0.07	0.01	0.06	0.05–0.07	0	0.06	0.05–0.06	0

Table 2

Comparison of elements concentration between colostrum and transitional breast milk. Values expressed in mg/dm^3, Cd in μg/dm^3

	Colostrum			Transitional milk		
	mean	min.–max	S.E.	mean	min.–max	S.E.
Mg	33.68	21.08–55.23	5.33	45.96	29.02–66.67	6.06
Zn	5.59	3.04–8.30	0.80	3.99	1.43–7.77	1.19
Cu	0.33	0.17–0.49	0.04	0.39	0.17–0.60	0.07
Fe	1.42	0.37–2.93	0.43	0.94	0.66–1.57	0.17
Cd	0.06	0.05–0.07	0.00	0.06	0.04–0.07	0.00

The concentrations of all tested elements were not statistically significant in relation with mothers age, however our results show differences in mean concentrations of the metals. We have found higher mean concentrations of Zn and Cu in older women (32–37) than in younger ones (20–25). This data is in accordance with those presented in earlier studies [14, 15]. In other studies Fe level of 12.37 mg/dm^3 was higher in older women (age of 32–37) than in younger ones. The obtained results for iron, copper and zinc in colostrum are similar to those published by Costa et al [16]. Well known

decrease in milk zinc level during lactation [10, 12, 17, 18], is confirmed by our study. The decline of that element can be explained by the fact that trace elements are bound by proteins in milk and play role as cofactors of enzymes. Thus the observed changes may result from the changes in women metabolism, nutrition and decreasing zinc requirements of the growing infants. Regarding the changes of elemental concentrations during breast feeding, also Fe and Cu showed decreasing tendency. Copper level found in our study is somewhat higher than copper level reported by other authors, but the compared values are in similar range. Almeida et al reported [17] that there is dependence between Zn and Cu concentration in serum and in colostrum. While copper level is high in the mother's blood and low in colostrum, contrary Zn level is the highest in colostrum because of its essential function for development of the infant [19]. The presence of iron and copper is important for the bacteriostatic properties of human milk. Newborn infants require large amounts of these minerals, but they intake low volume of milk, so colostrum contains high concentration of copper and iron [11, 12]. Mean magnesium level tend to increase with age (26.00–44.10 mg/dm^3). Such a tendency may be confirmed by Lipsman et al [20]. This fact can be caused by differences in bone mineralization between young and adolescent pregnant women [21]. Magnesium concentration in milk increased during the course of lactation, the same tendency is shown by Karra et al [22]. However, Dorea [23] indicates that factors like stage of lactation, maternal metabolic condition, dietary habits and magnesium intake do not have influence on Mg concentration in milk. We have also found that toxic element Cd was present in tested milk, the presence of cadmium in breast milk is well documented by other research. The cadmium concentrations estimated in our studies were lower than concentrations of range 0.07–1.23 μg/dm^3, indicated by Honda et al [7, 10]. Low cadmium concentrations suggest that there might be some maternal homeostatic processes preventing the transfer of cadmium into milk [17]. All the more that we have not found increase in the concentration of cadmium in relation with mother's age. Moreover we have not observed any changes in cadmium content in milk during the course of lactation. Similar observation were also made by Rossipal et al [10].

No significant correlations were found between the studied elements except for Cd vs Cu and Cd vs Fe (Table 3).

Table 3

Spearman correlation coefficients between nutritional elements and cadmium

	Trace elements	
	Cu	Fe
Cd	0.880*	–0.509*

* $p < 0.05$

The interactions between Cd and Cu in kidneys and liver are well known [23]. On the other hand, Honda et al [7] did not show such relations in milk. Our research point to the strong positive correlation Cd vs Cu (r = 0.880). Such a trend is probably a result of protective function of Cu.

Human breast milk is an essential source of Fe for newborn infants. It is important constituent of hemoglobin and negative correlation between Cd vs Fe ($r = -0.509$), suggest that cadmium has toxic influence on iron uptake and transportation. Antagonism between cadmium and iron is generally attributed to the divalent metal transporter (DMT1) in digestive tract. It is established that the presence of cadmium decreases iron uptake by DMT-1 transporter [25]. We did not find any significant differences in activity of catalase between women in different age and stage of lactation ($p = 0.05$). Similarly glutathione content did not show significant fluctuations. Moreover there was no correlation between enzymes ($p = 0.05$).

The highest mean activity of CAT occurred in milk from mothers in the age of 26–31. We have also observed higher activity of CAT in transitional milk in comparison with colostrum. This results do not confirm clearly generally accepted increase of catalase activity in relation to time progress [5].

Table 4

Catalase activity and glutathione concentration in different groups of age, CAT [U/mg of protein], GSH [mmol/dm^3]

	CAT			GSH		
	mean	min.–max	S.E.	mean	min.–max	S.E.
I group	0.12	0.07–0.14	0.01	0.433	0.31–0.65	0.063
II group	0.23	0.05–0.70	0.06	0.297	0.11–0.51	0.054
III group	0.14	0.05–0.37	0.06	0.209	0.06–0.51	0.062

Table 5

Catalase activity and glutathione concentration in colostrums and transitional milk, CAT [U/mg of protein], GSH [mmol/dm^3]

	GSH			CAT		
	mean	min.–max	S.E.	mean	min.–max	S.E.
Colostrum	0.30	0.06–0.65	0.05	0.14	0.05–0.37	0.03
Transitional milk	0.29	0.11–0.47	0.06	0.20	0.05–0.70	0.06

We observed the highest mean content of GSH in the youngest women (20–25) and the lowest mean content was detected in the oldest women (31–37). Our values are in the range of data showed by other authors. Some data suggest that the content of GSH decreases over 1 month of lactation. Our study indicate only a slightly decreasing trend between colostrum and transitional milk [6]. GSH plays an important role in detoxification of hydrogen peroxide, other peroxides and free radicals take part in the detoxification of a variety of xenobiotics [26]. Thus it seems that the presence of GHS in human milk reflects the antioxidative status of the mothers' organisms and plays role in the protection of infants against reactive oxygen species.

Conclusion

Breast milk composition is dependant on various factors including the age of lactating woman and the stage of lactation. Catalase and glutathione in milk play very important role in antioxidant defence of the infants in early stage of their life. We can observe some tendencies in changing levels of elements, decreasing tendency with course of lactation for Zn, Fe and increasing for Mg and Cu. Additionally, there are significant positive correlations between Cd vs Cu and negative Cd vs Fe. This suggest that Cd has strong influence on other elements. Cadmium concentration did not change during lactation, what suggest that there are some homeostatic mechanisms in mother's organism which prevent newborn against toxicity of Cd.

References

[1] Emmett P.M. and Rogers I.S.: Early Human Development 1997, **49**, 7–28.
[2] Kulikowska-Karpińska E. and Moniuszko-Jakoniuk J.: Polish J. Environ. Stud. 2004, **13**, 5–13.
[3] Lindmark-Månsson H. and Åkesson B.: British J. Nutrit. 2000, **84**, 103–110.
[4] Saugstad O.D.: Acta Paediat. Scandin. 1990, 881–892.
[5] Friel J.K., Martin S.M., Langdon M., Herzberg G.R. and Buettner G.R.: Pediatr. Reas. 2002, **51**, 612–618.
[6] Ankrah N.-A., Appiah-Opong R. and Dzokota C.: J. Tropical Pediatr. 2000, **46**, 111–113.
[7] Honda R., Tawara K., Nishijo M., Nakagawa H., Tanebe K. and Saito S.: Toxicology 2003, **186**, 255–259.
[8] Kantola M. and Vartiainen T.: J. Trace Elem. Med. Biol. 2001, **15**, 11–17.
[9] Góes H.C.A., Torres A.G., Donangelo C.M. and Trugo N.M.F.: Nutrition 2002, **18**, 590–594.
[10] Rossipal E. and Krachler M.: Nutrition Res. 1998, **18**, 11–24.
[11] Dorea J.G.: Nutrition 2000, **16**, 209–220.
[12] Arnaud J. and Favier A.: Sci. Environ. 1995, **159**, 9–15.
[13] Dorea J.G.: Nutrition Res. 2000, **20**, 1647–1687.
[14] Picciano M.F. and Guthrie H.A.: Amer. J. Clinical Nutrit. 1976, **29**, 242–254.
[15] Lin H.E., Jong Y.J., Chiang C.H. and Yang M.H.: Biol. Trace Element Res. 1998, **62**, 31–41.
[16] Da Costa R.S.S., Tavares do Carmo M.G., Saunders C., Lopes R.T, Jesus E.F.O. and Simabuco S.M.: J. Food Composit. Anal. 2002, **15**, 27–33.
[17] Almeida A.A., Lopes C.M., Silva A.M. and Barrado E.: J. Trace Elements Med. Biol. 2008, **22**, 196–205.
[18] Dhonukshe-Rutten R.A.M., Vossenaar M., West C.E., Schümann K., Bulux J. and Solomons N.W.: J. Pediat. Gastroenterol. Nutrit. 2005, **40**, 128–134.
[19] Domellof M., Lonnerdal B., Dewey K.G., Cohen R.J. and Hernell O.: Amer. J. Clin. Nutrit. 2004, **79**, 111–115.
[20] Lipsman S., Dewey K.G. and Lönnerdal B.: J. Pediatr. Gastroenterol. Nutrit. 1985, **4**, 426–34.
[21] Chan G.M., Ronald N., Slater P., Hollis J. and Thomas M.R.: J. Pediatr. 1982, **101**, 767–770.
[22] Karra M.V. and Kirksey A.: J. Pedriatr. Gastroenterol. Nutrit. 1988, **7**, 100–106.
[23] Dórea J.G.: J. Amer. Coll. Nutrit. 2000, **19**, 210–219.
[24] Honda R. and Nogawa K.: Arch. Toxicol. 1987, **59**, 437–442.
[25] Martelli A., Rousselet E., Dycke C., Bouron A. and Molis J.M.: Biochimie 2006, **88**, 1807–1814.
[26] Sen Ch.K.: Nutrit. Biochem. 1997, **8**, 660–672.

STATUS ANTYOKSYDACYJNY ORAZ ZAWARTOŚĆ METALI W MLEKU LUDZKIM U KOBIET W RÓŻNYM WIEKU I OKRESIE LAKTACJI

Zakład Zoologii Kręgowców i Biologii Człowieka Instytutu Biologii
Uniwersytet Pedagogiczny im. Komisji Edukacji Narodowej w Krakowie

Abstrakt: Celem badań było określenie koncentracji metali ciężkich, a także zawartości enzymów antyoksydacyjnych: katalazy (CAT) i zredukowanego glutationu (GSH) w mleku ludzkim. Kobiety biorące udział w eksperymencie były rekrutowane spośród matek w różnych okresach laktacji, pochodzących z terenu województwa małopolskiego. Próbki mleka zostały pobrane od 23 zdrowych kobiet z klas wiekowych: 20–25, 26–31, 32–37 lat. Dodatkowo został dokonany podział na grupy ze względu na okres laktacji, pierwsza: 1–3 dzień laktacji – siara, druga: powyżej 4 dnia – mleko przejściowe. Aktywność katalazy była mierzona spektrofotometrycznie według metody Bartosza (1995), natomiast poziom glutationu oznaczano spektrofotometrycznie przy użyciu metody Ellmana (1965). Koncentrację pierwiastków, takich jak: Zn, Mg, Cu, Fe oraz Cd oznaczano za pomocą spektrofotometrii absorpcyjnej (AAS). Aktywność katalazy, a także poziom glutationu zredukowanego nie wykazały statystycznie istotnych różnic pomiędzy grupami wiekowymi oraz okresem laktacji. Wyniki naszych badań pokazują, że mleko kobiet z regionu Małopolski zawiera pewne ilości kadmu. Dodatkowo zostały wykazane statystycznie istotne korelacje pomiędzy zawartością metali w próbkach mleka od kobiet w różnym wieku i okresie karmienia, dotyczyło to związku pomiędzy: Fe i Cd oraz Cd i Cu. Pomiędzy pozostałymi badanymi pierwiastki nie zaobserwowano statystycznie istotnych korelacji.

Słowa kluczowe: katalaza, glutation; mleko ludzkie, mikroelementy, metale ciężkie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Jacek GRZYB[1] and Krzysztof FRĄCZEK[1]

OCCURRENCE OF FUNGAL AEROSOL IN OVERGROUND AND UNDERGROUND HEALTH RESORTS

BADANIA AEROZOLU GRZYBOWEGO W SANATORIUM NADZIEMNYM I PODZIEMNYM

Abstract: For many years sanatorium therapeutics has been very popular in Poland as well as abroad. Detailed pulmonological research showed the appropriateness of such treatment in respiratory diseases. According to this, specific environmental conditions, which occur in the underground subterraneotherapy chambers and rooms of overground sanatoriums become more and more important. The main aim of the research was to define and divide the particles of biological aerosol into fractions (depending on their aerodynamical diameters) into: over 7.0 μm, 7.0–4.7 μm, 4.7–3.3 μm, 3.3–2.1 μm, 2.1–1.1 μm and 1.1–0.65 μm which occur in the underground subterraneotherapy chambers in Salt Mine in Bochnia and in overground sanatorium chambers in "Health Resort Szczawnica". The microbiological analyses of the air were carried out during the winter and spring of 2008, in 2 underground sanatorium chambers in Bochnia and in sanatorium chambers in Szczawnica. The measurements were carried out by means of the six-step Graseby-Anderson impactor from the human's respiratory zone (oral and nasal cavity position) in the state of so called "original sterility" which is before introducing sick people and the personnel into the chambers and during the period of visitors stay. As a result of the analyses, significant differences between the amount of fungal aerosol in different places of chambers were showed. It has been ascertained that the biological aerosol occurred in subterraneotherapy chambers in definitely higher concentrations during the treatment activity than during the period of the break between the turns. The highest concentrations of fungi in Salt Mine in Bochnia ascertain in Koldras Chamber and gymnasium in Wazyn Chamber; in Szczawnica the highest concentration was in the pump room.

Keywords: health resorts, fungi, air, salt mine

The degree of air pollution has great influence on human organism. Dust and gas pollutants play the main role in atmospheric air contamination, but the recent research has shown, that microbiological contamination is equally, and sometimes even more important [1–7].

[1] Department of Microbiology, University of Agriculture in Krakow, al. A. Mickiewicza 24/28, 30–059 Kraków, Poland, email: rrgrzyb@cyf-kr.edu.pl, rrfracze@cyf-kr.edu.pl

The occurring air contamination is the reason why immunological barrier in humans weakens, which results in potentially pathogenic and sometimes even saprophytic microorganisms becoming the cause of diseases. The degree of microbiological contamination of air in closed rooms, especially these, in which people of weak immunological barrier and ill people dwell becomes particularly important [8–10].

The above-mentioned phenomena influence the popularity of health resorts as well in Poland as abroad. The detailed pulmonological research shows the purposefulness of undertaking such cures in treatment of different types of respiratory ways diseases. In order to enable the effective therapy, specific environmental conditions, which occur in the underground subterraneotherapy chambers and in overground health resorts, become essential [5, 10–13].

The main aim of the research was to characterize the fungal aerosol occurring in sanatorium chambers in Salt Mine in Bochnia and in rooms of overground health resort in Szczawnica, regarding the part of overground sanatorium in Szczawnica, taking the share of respirable fraction into consideration.

Materials and methods

The research concerning the occurrence of fungal bioaerosol was carried out in the underground subterraneotherapy chambers in Salt Mine in Bochnia and in sanatorium rooms in Health Resort Szczawnica. The first one, as well as the second sanatorium specializes itself in treatment of respiratory ways diseases, including lingering nose and throat inflammations, voice apparatus diseases, allergic diseases of respiratory ways and asthma. The healing factors are: the specific microclimate in both of the health resorts and saline aerosol floating in all underground chambers in Salt Mine in Bochnia [5, 11].

The measurements were carried out during the periods of presence of bathers, but the part of the measurements was performed in so called state of "primary cleanliness", which is before entering of bathers and personnel into the sanatorium chambers (marked in the Tables as BK) and during the presence of bathers and performing therapeutic operations (marked as ZK).

Table 1 compares measuring points, in which measurements were carried out in underground and in overground sanatorium.

The air samples were taken using six-step Graseby-Anderson impactor, which was placed in the tested room on the height of 1.0–1.5 m over the floor to take the sample of bioaerosol from the respiratory zone (oral and nasal cavity position) of people. The microbiological analyses included evaluation of the general amount of fungi on the MEA medium (*Malt Extract Agar*, OXOID, Basingstoke, Great Britain).

The samples were incubated in thermostats in 30 ^{o}C for 4 days. The method was compatible with the Polish Standard PN-89/Z-04111/03 [14].

Table 1

The description of measuring points

No.	Measuring points	Point description
Salt Mine in Bochnia		
1	B1	The Koldras Chamber
2	B2	The ramp between levels IV and VI
3	B3	The Wazyn Chamber – the gym
4	B4	The Wazyn Chamber – the bedroom
5	B5	The gangway behind the Wazyn Chamber (in the Sutoris shaft direction)
6	B6	Inside control (background – IC) – the gangway leading the air to the levels IV and VI of mine from the Trinitatis shaft
7	B7	Outside control (background – OC) – in the open air, next to the Trinitatis shaft
Sanatorium chambers in Szczawnica Health Resort		
8	S1	Nature Treatment Institute building – inside the room of mineral baths
9	S2	Nature Treatment Institute building – inside the room of the whirl massage of limbs
10	S3	Pump Room building – inside
11	S4	Inhalatorium building – inside the room of chamber inhalations
12	S5	Inhalatorium building – inside the room of individual inhalations
13	S6	Inside control (background – IC) Inhalatorium building – in the corridor leading to the rooms of the sanatorium bathers
14	S7	Outside control (background – OC) – in the open air, nearby the building of the Management of Przedsiębiorstwo "Uzdrowisko Szczawnica" SA

Legend: IC – inside control; OC – outside control.

The concentration of the tested aerosol was expressed as an amount of spores or mycelium hyphae fragments able to develop in the form of the colony – as colony forming units (cfu) present in one cubic meter [m^3] of the taken air.

Results and discussion

Fungal aerosol gets into the rooms mainly from the outside environment (soil, water, plants etc.), during the whole year its migration intensity varies, but in winter it is the lowest. In winter the soil is frozen and covered with snow – such conditions do not promote the natural emission of these microorganisms from soil. As a result, the concentration of fungal aerosol in the outer environment is usually lower than in flats. Penetration of fungal aerosols into interiors is the main reason for biological contamination of the interior environments [2, 4, 12].

The results, gained from the measurements of the fungal aerosol performed in two different types of sanatoriums – on as well as under the Earth surface, are presented in the Tables 2 and 3. In the tested sanatorium chambers in Salt Mine in Bochnia, the fungal aerosol occurred in values from 7 to 614 cfu in 1 m^3, whereas in the sanatorium rooms in Szczawnica – from 0 to 706 cfu in 1 m^3 (Tables 2 and 3). The fungal aerosol

concentration in the external environment (outside background level) was from 282 to 3010 cfu in 1 m^3 and from 14 to 290 cfu in 1 m^3, respectively.

Table 2

The number of airborne fungi in the underground chambers in Salt Mine in Bochnia in winter and spring 2008

Examination point	Winter 2008		Spring 2008	
	[number of colony forming units (cfu) in 1 m^3 of the air]			
	Patients absent – PA (17.02.08)	Patients present – PP (16.01.08)	Patients absent – PA (27.04.08)	Patients present – PP (20.05.08)
B1	7	56	77	565
B2	70	197	91	134
B3	99	133	105	566
B4	70	155	49	7
B5	35	126	56	614
B6 – IC	21	148	63	120
B7 – OC	282	346	311	3010

Legend: the same as for the Table 1.

The fungal aerosol concentration in sanatorium chambers in Bochnia was definitely lower (even 400 times lower) than in the external environment, and it was irrespective of the presence or absence of the bathers (Table 2). The situation was more complex in Szczawnica – during the two winter measurement sessions the amount of fungi in the sanatorium rooms was higher than in the examination point which was the outside background. Similar relationship was found during the spring measurements with present bathers. Different results were found during the spring measurements without the bathers, when the amounts in the sanatorium were a few times higher than outside (Table 3).

Table 3

The number of airborne fungi in rooms of overground sanatorium health resort Szczawnica in winter and spring 2008

Examination point	Winter 2008		Spring 2008	
	[number of colony forming units (cfu) in 1 m^3 of the air]			
	Patients absent – PA (23.01.08)	Patients present – PP (03.02.08)	Patients absent – PA (13.04.08)	Patients present – PP (14.04.08)
S1	154	233	120	63
S2	368	367	91	28
S3	310	165	71	706
S4	0	240	28	21
S5	14	452	28	21
S6 – IC	63	1575	35	226
S7 – OC	56	92	290	14

Legend: the same as for the Table 1.

In the sanatorium chambers in Salt Mine in Bochnia, there was found visibly inhibiting influence of the specific environment saturated with sodium chloride on the fungal spores. The air is pumped into the mine by the Trinitatis shaft, next to which the examination point – outside background (B7) is located. The air then flows to the B6 point, covering the distance of about 1000 m. In this part the fungal spores' concentration decreases even 25 times. Then, the amount of fungi spores increases along the way of the flowing air. This is undoubtedly caused by the dust collected on the floor of the chambers and corridors, which then is floating in the air. The influence of the bathers' presence on the amount of fungal spores in the air is ambiguous – in winter no changes in the abundance were observed, whereas in spring the amount of fungi in the chambers was a few times higher than in the inside control point.

On the basis of the results concerning the amount of fungal aerosol, it may be also concluded, that its highest values occurred in the underground sanatorium in Bochnia in Wazyn Chamber – in the gym (from 99 to 566 cfu in 1 m^3) and in the gangway which leads the air from the Wazyn Chamber (from 35 to 614 cfu in 1 m^3). The smallest amounts of the bioaerosol (7 cfu in 1 m^3) were found in the state of so called "original sterility", which is before entering the bathers into the sanatorium chambers in Koldras Chamber and in the bedroom part of the Wazyn Chamber – during the bathers' presence (also 7 cfu in 1 m^3).

In the sanatorium rooms in Szczawnica, the highest amounts of fungi were found in the outside control point (Inhalatorium building – the corridor – from 35 to 1575 cfu in 1 m^3) and inside the Pump Room building – from 71 to 706 cfu in 1 m^3. The smallest amounts of the tested bioaerosol (7 cfu in 1 m^3) were found in the Inhalatorium building, in the room of chamber inhalations (0 cfu in 1 m^3 – without the bathers and 21 cfu in 1 m^3 – with the bathers).

The comparison of the gained results with literature data is difficult because of the minute amount of the available publications as well as the differences in methods.

In normal conditions, the fungal aerosol concentration in rooms, in which people are present, is within the range of 10 to 1.000 cfu in 1 m^3 [15, 16], but according to the other microbiological analyses, the proposed highest admissible fungal aerosol concentration in flats and offices is defined as 500 cfu in 1 m^3 [17] or only in offices as 200 cfu in 1 m^3 [18].

It should be emphasized, that although many bathers were present in the sanatorium rooms in both of the health resorts, who moreover were intensively moving, the concentrations of fungal aerosol are generally lower than in the habitable rooms.

The analysis of the grain size distribution shows, that the share of the respirable fraction of the fungal aerosol in the saantorium chambers in the salt Mine in Bochnia in the period between turns is significantly higher and is from 62.5 to 100 % (average 88.5 %) than during the treatment turn, when it ranges between 0 and 96.6 % (average 73.7 %) (Table 3). This means, that the concentration of the respirable fraction of the fungal aerosol does not depend on the amount of the patients who are present in the healing rooms. Average concentration of the fungal bioaerosol in sanatorium chambers in Bochnia amounts 81 % and is very close to the amount given by Pastuszka [4]. In the sanatorium rooms in Szczawnica, the share of the respirable fraction of the fungal

aerosol is related to the presence of the patients. When they are present, this fraction is on average 92.9 %, and when they are absent, it decreases to 79.5 % (Table 4).

Table 4

The proportion of respirable fraction of general number of fungi in the chambers in Salt Mine in Bochnia in winter and in spring 2008

Examination point	Winter 2008		Spring 2008	
	[%]			
	Patients absent – PA (17.02.08)	Patients present – PP (16.01.08)	Patients absent – PA (27.04.08)	Patients present – PP (20.05.08)
B1	100.0	87.5	100.0	82.7
B2	80.0	85.8	100.0	89.6
B3	100.0	73.7	66.7	52.5
B4	90.0	91.0	85.7	0.0
B5	100.0	77.8	62.5	96.6
B6 – IC	66.7	100.0	88.9	82.5
B7 – OC	70.2	91.9	54.3	67.6

Legend: the same as for the Table 1.

Table 5

The proportion of respirable fraction of general number of airborne fungi in the chambers in Szczawnica Health Resort Mine in winter and in spring 2008

Examination point	Winter 2008		Spring 2008	
	[%]			
	Patients absent – PA (17.02.08)	Patients present – PP (16.01.08)	Patients absent – PA (27.04.08)	Patients present – PP (20.05.08)
S1	72.7	97.0	94.2	88.9
S2	76.9	88.6	100.0	100.0
S3	86.5	95.8	90.1	98.0
S4	0.0	94.2	75.0	100.0
S5	100.0	100.0	100.0	66.7
S6 – IC	88.9	97.8	100.0	90.7
S7 – OC	100.0	92.4	100.0	50.0

Legend: the same as for the Table 1.

According to the same author, for the external environment, the share of the respirable fraction is usually 60 % in winter. In the research to the present paper, for the external environment in winter, higher share of the respirable fraction was found, and it was 71 % for Bochnia, and 85.6 % for Szczawnica, respectively.

Information about the concentration and distribution of the microflora particle sizes in the air in the chosen examination points allows to define their abilities to potentially influence the human organism [5, 16].

Conclusions

1. The fungal aerosol concentrations in winter and in spring in sanatorium chambers in Bochnia were definitely lower than in the outside environment, and it was with the presence of the bathers as well as without them. In Szczawnica health resort, similar results were gained only during the absence of the patients.

2. Specific conditions in the sanatorium chambers in Salt Mine in Bochnia cause the decrease of the amount of fungal bioaerosol.

3. The proportional share of the respirable fraction of the fungal bioaerosol in the underground sanatorium was independent of the tourists' presence, but the opposite relation was found in the overground health resort.

4. The ascertained amounts of the fungal bioaerosol in each case fulfill the criteria of the Polish Standard, however they transgress the values suggested by the other authors several times.

Acknowledgement

The research financed by the science resources in 2007–2008 as the research project N N305 2552 33.

References

[1] Nevalainen A.: *Bacterial aerosols in indoor air*, Ph.D. thesis, University of Kuopio, Kuopio, Finland, 1989.

[2] Lis D., Pastuszka J.S. and Górny R.L.: *Występowanie aerozolu bakteryjnego i grzybowego w mieszkaniach, biurach i środowisku zewnętrznym Górnego Śląska*, Roczn. PZH 1997, **48**(1), 59–68.

[3] Lis D., Pastuszka J.S. and Uflig K.: *Mikrobiologiczna jakość powietrza w pomieszczeniach administracyjno-biurowych wysypiska odpadów komunalnych na przykładzie Miejskiego Zakładu Składowania Odpadów w Sosnowcu*, Ochr. Powiet. i Probl. Odp. 1999, **33**(5), 199–202.

[4] Pastuszka J.S.: *Narażenie na aerozole ziarniste, włókniste i biologiczne (bakterie i grzyby mikroskopijne) populacji generalnej Górnośląskiego Okręgu Przemysłowego*, Pr. Nauk. Inst. Inż. Ochr. Środow. Polit. Wrocław., OWPW, 2001, **73,** Monografie 40, 7–131.

[5] Wlazło A., Pastuszka J.S. and Łudzeń-Izbińska B.: *Ocena narażenia na aerozol bakteryjny pracowników niedużej oczyszczalni ścieków*, Med. Pracy 2002, **53**(2), 109–114.

[6] Barabasz W., Albińska D., Frączek K., Grzyb J., Barabasz J. and Kosińska B.: *Mikrobiologiczne i zdrowotne zagrożenia ludzi wokół składowisk odpadów i oczyszczalni ścieków*, Gosp. Odpad. Komunal. 2003, **IX**, 155–168.

[7] Marcinowska K., Frączek K., Barabasz W. and Grzyb J.: *Wpływ składowiska odpadów komunalnych w Ujkowie Starym – gmina Bolesław na jakość mikrobiologiczną powietrza*, Acta Agr. et Silv., Ser. Agr. 2004, **XLII**, 289–295.

[8] Torochtin M.: *Wykorzystanie mikroklimatu kopalni soli w leczeniu i profilaktyce przewlekłych niespecyficznych chorób układu oddechowego*, Probl. Uzdrow. 1981, **1**(4), 7–12.

[9] Doleżal M., Doleżal M. and Żuława G.: *Mikroflora komór podziemnych stacji badawczej subterraneoterapii w Wieliczce. Biozanieczyszczenia w budynkach a zdrowie*, Zesz. Nauk. AWF w Krakowie 1983, **35**, 55–65.

[10] Latour T., Jastrzębska B. and Nowacka A.: *Mikroklimat i warunki środowiskowe w podziemnych komorach leczniczych na terenie Kopalni Soli w Wieliczce*, Inst. Med. Uzdrow., Poznań 1989.

[11] Olechnowicz-Bobrowska B., Wojkowski J., Frączek K. and Grzyb J.: *Charakterystyka mikroklimatyczna i mikrobiologiczna komór leczniczych w kopalni soli w Bochni i Wieliczce,* [in:] VI Krajowy Kongres Ekologiczny EKO-MED Tarnów, "Środowiskowe zagrożenia dla społeczeństwa u progu XXI wieku", Tarnów 2000, 151–156.

[12] Bis H., Grzyb J., Barabasz W. and Frączek K.: *Kształtowanie się liczebności grzybów – Micromycetes w komorach sanatoryjnych Kopalni Soli w Bochni i Wieliczce*, Acta Agr. et Silv., Ser. Agr. 2004, **XLII**, 29–39.
[13] Grzyb J., Bis H., Barabasz W., Frączek K. and Chmiel M.: *Badania nad występowaniem bakterii w powietrzu komór sanatoryjnych w kopalniach soli w Bochni i Wieliczce*, Acta Agr. et Silv., Ser. Agr. 2004, **XLII**, 163–175.
[14] Polska Norma, PN-89/Z-04111/03, *Ochrona czystości powietrza; Badania mikrobiologiczne; Oznaczanie liczby grzybów mikroskopowych w powietrzu atmosferycznym (imisja) przy pobieraniu prób metodą aspiracyjną i sedymentacyjną.*
[15] De Koster J.A. and Thorne P.S.: *Bioaerosol concentrations in noncomplaint, complaint and intervention homes in the Midwest*, Amer. Ind. Hyg. Assoc. J. 1995, **56**, 576–580.
[16] Górny R.L. and Dutkiewicz J.: *Bacterial and fungal aerosols in indoor environment in Central and Eastern european countries*, Ann. Agric. Environ. Med. 2002, **9**, 17–23.
[17] Reynolds S.J., Streifel A.J. and McJilton C.E.: *Elevated air borne concentrations of fungi in residential and Office environment*, Amer. Ind. Assoc. J. 1990, **51**, 601–604.
[18] Yang C.S., Hung L.-L., Lewis F. and Zampiello A.: *Airborne fungal populations in non-residential buildings in the United States*, Proc. Indust. Air 1993, **4**, 219–224.

BADANIA AEROZOLU GRZYBOWEGO W SANATORIUM NADZIEMNYM I PODZIEMNYM

Katedra Mikrobiologii
Uniwersytet Rolniczy w Krakowie

Abstrakt: Lecznictwo sanatoryjne zarówno w Polsce, jak i za granicą jest bardzo rozpowszechnione. Jak wynika z wielu badań szczególnie w schorzeniach dróg oddechowych takie leczenie przynosi bardzo dobre efekty. Na te efekty w znaczący sposób wpływa specyficzny mikroklimat panujący zarówno w podziemnych komorach subterraneoterapii, jak i w pomieszczeniach sanatoriów naziemnych. Zasadniczym celem wykonywanych badań było oznaczenie i rozdział cząstek aerozolu biologicznego na frakcje (w zależności od ich średnic aerodynamicznych: powyżej 7,0 μm; 7,0–4,7 μm; 4,7–3,3 μm, 3,3–2,1 μm; 2,1–1,1 μm i 1,1–0,65 μm) występującego w komorach leczniczych w Kopalni Soli w Bochni oraz w pomieszczeniach sanatoryjnych Przedsiębiorstwa „Uzdrowisko Szczawnica". Badania mikrobiologiczne powietrza zostały przeprowadzone w okresie zimy i wiosny 2008 r. w dwóch podziemnych komorach sanatoryjnych w Bochni oraz w pomieszczeniach sanatoryjnych w Szczawnicy. Pomiary zostały wykonane za pomocą sześciostopniowego aeroskopu Graseby-Andersena ze strefy oddechowej (tj. na wysokości jamy ustnej i nosowej) człowieka w stanie tzw. „pierwotnej jałowości", tj. przed wprowadzeniem chorych i personelu do pomieszczeń sanatoryjnych oraz w czasie przebywania tam kuracjuszy. W wyniku przeprowadzonych analiz ilościowych stwierdzono znaczne zróżnicowanie aerozolu grzybowego na różnych stanowiskach pomiarowych. W czasie turnusów sanatoryjnych liczebności grzybów były znacząco większe w stosunku do tych, które występowały podczas nieobecności kuracjuszy – zarówno w sanatorium nadziemnym, jak i podziemnym. Największe stężenia grzybów w Kopalni Soli w Bochni zanotowano w Komorze Kołdrasa oraz na boisku sportowym w Komorze Ważyn; z kolei w sanatorium w Szczawnicy największe stężenia bioaerozolu grzybowego stwierdzono w pijalni wód mineralnych.

Słowa kluczowe: uzdrowiska, grzyby, powietrze, kopalnia soli

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Peter HAŠČÍK[1], Ivana NOVÁKOVÁ[2],
Miroslava KAČÁNIOVÁ[2*], Martina FIKSELOVÁ[3]
and Simona KUWOVÁ[4]

MICROBIOLOGICAL QUALITY OF THE *Anas platyrhynchos* AND THE *Fulica atra* MEAT

MIKROBIOLOGICZNA JAKOŚĆ MIĘSA *Anas platyrhynchos* I *Fulica atra*

Abstract: The aim of our experiment was monitoring of the microbiological quality of the *Anas platyrhynchos* and the *Fulica atra* meat after the slaughter and seven days of maturing process. We followed total count of microorganisms, number of coliforms bacteria and number of mesophilic anaerobic sporulating microorganisms. The evaluation of microorganisms was done by Codex Alimentarius SR. We noticed that the count of coliform bacteria was negative after slaughter in both experimental groups. The count of mesophilic anaerobic sporulating bacteria in the meat of the wild ducks ranged from 1.78–2.12 log cfu $\cdot$ g^{-1} and in the meat of the fulicas was found from 4.98 to 5.95 log cfu $\cdot$ g^{-1}. From the statistical point of view it was a high significant difference ($p \le 0.001$). The total count of microorganisms in the meat of the wild ducks was zero. In the meat of the fulicas ranged from 5.18 to 6.25 log cfu $\cdot$ g^{-1}. The statistical differences between the meat samples from the wild ducks and the fulicas were significant ($p \le 0.001$). The count of coliforms in the mature meat of wild ducks varied from 1.12–1.73 log cfu $\cdot$ g^{-1}. The statistical differences between the meat samples from the wild ducks and the fulicas were not significant ($p \ge 0.05$). The count of mesophilic anaerobic sporulating microorganisms in the wild duck mature meat samples varied from 1.95–2.24 log cfu $\cdot$ g^{-1} and in the mature meat of the fulicas ranged from 5.00 to 6.00 log cfu $\cdot$ g^{-1} The significant differences between the meat samples of the wild ducks and the fulicas were determined ($p \le 0.001$). The total count of microorganisms in the mature meat samples of the wild ducks ranged from 1.18–2.24 log cfu $\cdot$ g^{-1}, ie on average 1.99 log cfu $\cdot$ g^{-1}. Higher values were detected in the mature meat samples of the fulicas. The values varied from 5.24–6.30 log cfu $\cdot$ g^{-1}, ie on average 5.69 log cfu $\cdot$ g^{-1}. The comparison of meat samples of both experimental animals showed high significant differences ($p \le 0.001$).

Keywords: microorganisms, *Fulica atra*, *Anas platyrhynchos*, meat, maturing

[1] Department of Animal Products Evaluation and Processing, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[2] Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[3] Department of Plant Processing and Storage, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[4] Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

* Corresponding author: email: Miroslava.Kacaniova@uniag.sk

Hoofed game is nowadays a basic part in production and consumption of game. The high percentage creates feather game, too [1].

Game is an economically significant product. It plays an eminent role like a complement of menu. Feather game, with production of 257 Mg, creates 10% from market production of game [2, 3].

Although the meat production is one of the main indicator, according to Hascík et al [3, 4] it is necessary to pursue the nutrition quality of the meat, adherence to the sanitary code and the microbiological requirements. They are coupled with eventual contamination and consecutive devaluation of the meat.

The maturing processes are running in the muscles up to point of time after death of game until the supplies of glycogen and energically valued phosphates are available. The production of enzymes and products of proteins metabolism induce specifically taste of game meat. Game meat „the red meat" must be matured few days (5 or 7) in cold conditions, in order to get required tenderness and rich taste [5]. It is necessary to avoid microbial contamination.

The meat is an ideal nutrient medium for microorganisms. It has a high content of water, nitrogenous and mineral compounds, growing factors and pH which is ideal for microorganisms. The meat as a food of animal origin, is exposed to negative surrounding factors. These factors are responsible for surviving and propagation of microorganisms [6, 7].

Contamination of the meat is subjected to these factors: the illnesses of the animals (microorganisms penetrate into the muscles and apparatus), the delay of the evisceration, inexpert examination of the carcass and breach of sanitation [8].

Steinhauserova et al [9] mention, that the spectrum of microorganisms on the surface of the meat is very extensive. The most frequently Gram-negative bacteria are: *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Enterobacter*, *Citrobacter*, *Moraxella*, *Pseudomonas*, *Escherichia*, *Serratia*, *Psychrobacter*, *Shewanella* and *Yersinia.* From the Gram-positive bacteria are present: *Bacillus*, *Brochotrix*, *Carnobacterium*, *Micrococcus*, *Microbacterium* and *Lactobacillus.*

Material and methods

As a biological material were used *Fulica atra* (fulica) and *Anas platyrhynchos* (wild duck) of the both sexes. In the autumn was made the catching of feather game by the nets. Among 30 pieces of fulicas and 30 pieces of wild ducks were chosen randomly 15 pieces of female and 15 pieces of male subjects. They were transported immediately on the Department of Animal Products Evaluation and Processing SPU Nitra, where they were slaughtered. For the quantitative microbial analysis (total count of microorganisms, number of coliform bacteria and count of mesophilic anaerobic sporulating microorganisms) of the meat was taken the thigh and breast muscle, 45 minutes after slaughter, ie before chilling of the meat and after 7 days of maturing process. It was cut (with sterile scalpel) 17 samples of particular muscle. The sections was 5 cm^2 in depth of 5 mm. The samples were preserved at 4 ^{o}C. The various pieces from each animal were homogenised on meat-mincer (size of slots was 3 mm). The homogenisate in

amount of 10 g was added into the sterile flask with 90 cm^3 of saline solution. The flasks were shaked for 30 min on the beater. The particular steps of isolation are presented in Table 1.

Table 1

Isolated species of microorganisms of the *Fulica atra* and the *Anas platyrhynchos* meat and their fundamental identification signs

Cultivated microorganisms	Nutritive substrate	Temperature of cultivation [°C]	Time of cultivation [h]	Colour of colony
Coliform bacteria	VRB agar (8)	37	24–48	Reddish purple
MASM	Nutrient agar	25	38–72	Light amber
Total microorganisms count	GTY agar (9)	30	48–72	Yellow

VRB – Violet red bile agar; MASM – mesophilic anaerobic sporulating microorganisms; GTY – Glucose tryptone yeast agar.

The basic statistical characteristic (arithmetical average, standard deviation, min., max and variation index) was evaluated by statistical program SAS. Differences among various groups in the experiment were tested by the t-test and the Schefe test.

Results and discussion

At the evisceration and the carcase splitting it is not possible to avoid microbial contamination. A mixture of bacteria, fungi and yeast, which are in contact with game, has various effects on quality of the meat as foodstuff. They can activate positive also negative processes in the meat by Winkelmayer et al [5].

In the meat microorganisms are attendant which are responsible for the alimentary diseases. Microorganisms affect by their metabolism quality of the meat especially sensorial characteristic. Microorganisms participate in metabolism of proteins and lipids and products of metabolism result in unpleasant smell and taste of the meat. Other negative marks are production of visual colonies and mould films on the surface of the meat or changes of the meat pigmentation. The consummation of the bad meat can cause enteric diseases [10].

In our experiment had been monitoring the same groups of microorganisms in the meat of the fulicas and the wild ducks after the slaughter (Table 2) and after the 7 days of maturing process (Table 3).

The count of coliform bacteria was negative after slaughter in the both experimental groups. Kacaniova et al [11, 12] confirmed the suitable quality of the fulica and the wild duck meat on the count of coliforms, that is in accordance with the Codex Alimentarius SR [13]. The standard for the count of coliform bacteria is 5 log cfu $\cdot$ g^{-1}.

The count of mesophilic anaerobic sporulating bacteria in the meat of the wild ducks ranged from 1.78–2.12 log cfu $\cdot$ g^{-1} and in the meat of the fulicas it was from 4.98 to 5.95 log cfu $\cdot$ g^{-1}. From the statistical point of view there was a high significant difference ($p \le 0.001$). The attained results correspond with assignments of Kacaniova et al

[12]. They observed the increased count of mesophilic anaerobic sporulating bacteria in the meat of the wild ducks, as well. According to the Codex Alimentarius SR [13] we can conclude that the microbial quality of the wild duck meat accommodated to the norm and the microbial quality of the fulica meat was inconvenient. The norm for mesophilic anaerobic sporulating microorganisms is 2 log cfu $\cdot$ g^{-1} according to the Codex Alimentarius SR [13].

Table 2

Representation of individual microorganisms groups of *Anas platyrchynos* and *Fulica atra* meat in log cfu $\cdot$ g^{-1}

Groups of microorganisms	Basic of statistical characteristics	Animal brand	
		Anas platyrhynchos	*Fulica atra*
Coliform bacteria	$\bar{x}$	0.00	0.00
	min.	0.00	0.00
	max	0.00	0.00
	s_x	0.00	0.00
	v %	0.00	0.00
Mesophilic anaerobic sporulating microorganisms	$\bar{x}$	1.99	5.39
	min.	1.78	4.98
	max	2.14	5.95
	s_x	0.12	0.32
	v %	5.97	5.84
Total count of microorganisms	$\bar{x}$	0.00	5.62
	min.	0.00	5.18
	max	0.00	6.25
	s_x	0.00	0.42
	v %	0.00	7.53

$\bar{x}$ – average; s_x – standard deviation; v % – coefficient of variation.

The total count of microorganisms in the meat of the wild ducks was zero. In the meat of fulicas ranged from 5.18 to 6.25 log cfu $\cdot$ g^{-1}. On the base of average results we can conclude that the total count of microorganisms is conformable with the Codex Alimentarius SR [13]. The norm for the total count of microorganisms is 5.69 log cfu $\cdot$ g^{-1}. But we must stress that 30 % samples of the fulica meat had excess values.

The increasing count of microorganisms noticed Kacaniova et al [11] at the samples of the wild duck meat (0.00–6.37 log cfu $\cdot$ g^{-1}), but in other experiments [12] are the total counts of microorganisms the same as in our experiment, ie zero. The statistical differences between the meat samples from the wild ducks and the fulicas were significant ($p \leq 0.001$).

In the past storage of game meat was usually at 0 to 4 °C. The base of the 7 days maturing process is to supply the mature meat for consumer. Profoundness autolysis occurs right after the 7 days maturing process [14]. Then we had observed microbial contamination of the wild duck and the fulica meat at the end of maturation.

Table 3

Representation of individual microorganisms groups of *Anas platyrchynos* and *Fulica atra* meat in log cfu · g^{-1} after 7 days of maturing

Groups of microorganisms	Basic of statistical characteristics	Animal brand	
		Anas platyrhynchos	*Fulica atra*
Coliform bacteria	$\bar{x}$	1.27	2.03
	min.	1.12	0.00
	max	1.73	3.43
	s_x	0.17	1.45
	v %	13.63	71.38
Mesophilic anaerobic microorganisms	$\bar{x}$	2.08	5.43
	min.	1.95	5.00
	max	2.24	6.00
	s_x	0.11	0.31
	v %	5.43	5.62
Total count of microorganisms	$\bar{x}$	1.99	5.74
	min.	1.18	5.24
	max	2.24	6.30
	s_x	0.30	0.39
	v %	15.19	6.86

$\bar{x}$ – average; s_x – standard deviation; v % – coefficient of variation.

All samples of the wild duck and the fulica meat were contaminated by the coliform bacteria, it comes to this, that in the term of faecal contamination the muscles of game were not clean. The count of coliforms in the mature meat of the wild ducks was in the rate of 1.12–1.73 log cfu · g^{-1}. In the mature meat of the fulicas was from 0.00 to 3.43 log cfu · g^{-1}. The other attributes were measured by Kacaniova et al [11, 14]. The count of coliforms was zero in the meat of wild ducks as well as in the meat of fulicas. These results had confirmed the suitable microbial quality of feather game according to the Codex Alimentarius SR. The Codex Alimentarius [13] determinated 5 log cfu · g^{-1} as the maximum of the count of coliforms. Our results correspond with the norm listed in the Codex Alimentarius SR. The statistical differences between the meat samples from the wild ducks and the fulicas were not significant ($p \geq 0.05$).

The count of mesophilic anaerobic sporulating microorganisms in the wild duck mature meat samples was within range of 1.95–2.24 log cfu · g^{-1} and in the mature meat of fulicas it was from 5.00 to 6.00 log cfu · g^{-1}. The average values were inconvenient according to the Codex Alimentarius SR [13]. The norm for mesophilic anaerobic sporulating bacteria is 2 log cfu · g^{-1}. The significant differences between the meat samples from the wild ducks and the fulicas were significant ($p \leq 0.001$).

The total count of microorganisms in the mature meat samples of the wild ducks was in the rate of 1.18–2.24 log cfu · g^{-1}, ie on the average 1.99 log cfu · g^{-1}. These values are suitable with the norm in the Codex Alimentarius SR [13]. The higher values were detected in the mature meat samples of the fulicas. The values were within rate of

5.24–6.30 log cfu · g^{-1}, on average 5.69 log cfu · g^{-1}. The comparison of the meat samples of both experimental animals showed high significant differences ($p \leq 0.001$).

The statistical significant differences ($p \leq 0.001$) were found among the count of coliforms and the total count of microorganisms in the meat of the wild ducks immediately after the slaughter and after the 7 days maturing process. In the counts of mesophilic anaerobic sporulating microorganisms after the slaughter and after the 7 days maturing process were not found statistical significant differences ($p \geq 0.05$).

The similar tendency of the statistical significant differences ($p \leq 0.001$) showed values between the counts of coliforms in samples of the fulica meat after the slaughter and maturation. In the counts of mesophilic anaerobic sporulating microorganisms and in the total counts of microorganisms after the slaughter and the maturing process were not found statistical significant differences ($p \geq 0.05$).

Conclusion

The zero values of the counts of coliform bacteria in the meat of the wild ducks and the fulicas after the slaughter as well as values 1.27 log cfu · g^{-1} (the wild duck mature meat) and 2.30 log cfu · g^{-1} (the fulica mature meat) give the good premise to consummation of this meat. The count of mesophilic anaerobic sporulating microorganisms in the meat of the wild ducks had allowed values according to the Codex Alimentarius SR. After the 7 days of maturing process the value of mesophilic anaerobic sporulating microorganisms count increased about 0.08 log cfu · g^{-1} above the norm. In the fresh and the mature meat of the fulicas were observed the high values of the mesophilic anaerobic sporulating microorganisms counts, where the value of the count of mesophilic anaerobic sporulating microorganisms were about 2.69 higher than it is allowed. After maturation the value of the count of mesophilic anaerobic sporulating microorganisms was about 2.69 higher than is allowed by the Codex Alimentarius SR. The total count of microorganisms in the meat samples of the wild ducks after the slaughter was zero and the values increased after maturation on 1.99 log cfu · g^{-1}, which is in accordance with the Codex Alimentarius SR. In the meat samples of fulicas after the slaughter was 5.62 log cfu · g^{-1}, ie close to maximum of the allowed norm. The total count of microorganisms in the mature meat samples of the fulicas was about 2.13 % higher than in the fresh meat. This value falls outside the Codex Alimentarius norms.

On the base of our experiments we can conclude that the maturing process of game meat is not relevant problem in term of negative influence of the meat quality, but in many cases the microbial quality is better than in the fresh meat.

References

[1] Slamečka J., Mertin D., Hell P., Mojto J. and Jurčík R.: *Slaughter yield and duality of the meat of free Libiny pheasants and pheasants from farm breeding.* Folia Venatoria 2003, **33**, 135–143.

[2] Hell P., Slamečka J., Gašparík J. and Vodňanský M.: *Valuation of the production of venison in Slovakia in the years 1969–2004*. Folia Venatoria 2005, **35**, 115–124.

[3] Haščík P., Gašparík J., Čuboň J., Kulíšek V., Kačániová M. and Massanyi P.: *Comparison study of wild duck Anas Platyrhanchos meat efficiency from different hunting grounds.* Folia Veanatoria 2005, **35**, 139–146.
[4] Haščík P., Gašparík J., Čuboň J., Kulíšek V. and Krivánek L.: *Nutritional composition of Muscle of the Most Valuable Part of Pheasant Carcasses from phesantries.* Folia Venatoria 2004, **34**, 161–166.
[5] Winkelmayer R., Lebesorger P. and Zenka H.F.: *Hygieny zveřiny*. Středoeurópský institút ekologie zveře, Wien–Brno–Nitra 2005, pp. 168.
[6] Görner F. and Valík Ľ.: *Aplikovaná mikrobiológia požívatín*. Bratislava – Malé Centrum 2004, pp. 528.
[7] Kačániová M., Čuboň J., Haščík P. and Pavličová S.: *Effect of Enterococcus faecium on some characteristic in ceca of chickens and its typisation by PCR.* Acta Fytotechn. Zootechn., 2005, **8**, 17–20.
[8] Winkelmayer R.: Wildfleischhygiene. Zentralstelle Őster. Landesjagdverbände, Wien 2000.
[9] Steinhauserová I.: *Specifická mikroflóra baleného masa*. Czech J. Food Sci. 2000, **4**, 159–163.
[10] Grieger C.: Mikrobiológia poťivatín ťivočíšneho pôvodu. Príroda, Bratislava 1991, pp. 272.
[11] Kačániová M., Mindek S., Haščík P., Košťálová D. and Petrová J.: *Microbiological quality of will duck meat*. Rizikové faktory potravového reťazca, Nitra 2004, 98–100.
[12] Kačániová M., Haščík P., Čuboň J., Petrová J. and Mindek. S.: *Mikrobiologická kvality mäsa divých kačíc*. I medzinárodné vedecké hydinárske dni (Zborník na CD), SPU, Nitra 2005, 20–22.
[13] Potravinový Kódex V Úplnom Znení – I., II. A III. Časť. Aktualizované znenie. Epos, Bratislava 2006, pp. 736.
[14] Kačániová M., Haščík P., Gašparík J., Čuboň J., Pavlačová S. and Petrová J.: *Microbial colonisation of Fulica atra gastrointestinal tract and its effect on microbiological duality of meat.* Folia Venatoria 2007, **36–37**, 135–144.

MIKROBIOLOGICZNA JAKOŚĆ MIĘSA *Anas platyrhynchos* I *Fulica atra*

Abstrakt: Celem badań był monitoring mikrobiologicznej jakości mięsa z *Anas platyrhynchos* i *Fulica atra* bezpośrednio po uboju oraz po 7 dniach dojrzewania. Oznaczono całkowitą liczebność mikroorganizmów, liczebność bakterii Coli oraz liczebność mezofilowych anaerobowych mikroorganizmów sporulujących. Nie stwierdzono obecności bakterii Coli bezpośrednio po uboju. Liczebność mezofilowych anaerobowych mikroorganizmów sporulujących w mięsie *Anas platyrhynchos* wynosiła 1,78–2,12 log cfu $\cdot$ g^{-1}. Liczebność tych organizmów w mięsie *Fulica atra* wynosiła 4,98–5,95 log cfu $\cdot$ g^{-1}. Różnice między badanym mięsem były statystycznie istotne ($p < 0{,}001$). Całkowita liczebność mikroorganizmów w mięsie *Fulica atra* wynosiła 5,18 do 6,25 log cfu $\cdot$ g^{-1}. Parametr ten wykazał wartość zerową w mięsie *Anas platyrhynchos*. Różnice między badanym mięsem pod względem całkowitej zawartości mikroorganizmów były statystycznie istotne ($p < 0{,}001$). Liczebność bakterii Coli w dojrzałym mięsie *Anas platyrhynchos* wynosiła 1,12–1,73 log cfu $\cdot$ g^{-1}. Różnice między mięsem *Anas platyrhynchos* i *Fulica atra* pod względem tego parametru nie były istotne statystycznie ($p < 0{,}05$). Liczebność mezofilowych anaerobowych mikroorganizmów sporulujących w dojrzałym mięsie *Anas platyrhynchos* wynosiła 1,95–2,24 log cfu $\cdot$ g^{-1}, a w dojrzałym mięsie *Fulica atra* 5,00–6,00 log cfu $\cdot$ g^{-1}. Różnice te były statystycznie istotne ($p < 0{,}001$). Całkowita liczebność mikroorganizmów w próbkach dojrzałego mięsa *Anas platyrhynchos* wynosiła 1,18–2,24 log cfu $\cdot$ g^{-1} (średnio 1,99 log cfu $\cdot$ g^{-1}). Większą liczebność mikroorganizmów stwierdzono w dojrzałym mięsie *Fulica atra*, gdzie mieściła się ona w granicach 5,24–6,30 log cfu $\cdot$ g^{-1} (średnio 5,69 log cfu $\cdot$ g^{-1}). Porównanie dojrzałego mięsa obu gatunków pod względem tego parametru wykazało statystycznie istotne różnice ($p < 0{,}001$).

Słowa kluczowe: mikroorganizmy, *Fulica atra, Anas platyrhynchos,* mięso, dojrzewanie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Miroslava KAČÁNIOVÁ[1], Martina FIKSELOVÁ[2],
Peter HAŠČÍK[3], Vladimíra KŇAZOVICKÁ[1],
Janka NÔŽKOVÁ[4] and Katarína FATRCOVÁ-ŠRÁMKOVÁ[5]

CHANGES IN MICROFLORA OF BEE POLLEN TREATED WITH UV LIGHT AND FREEZING DURING STORAGE

ZMIANY FLORY BAKTERYJNEJ PYŁKU PSZCZELEGO ZAMRAŻANEGO I EKSPONOWANEGO NA PROMIENIOWANIE UV W CZASIE PRZECHOWYWANIA

Abstract: The aim of this work was observation of microbial community of bee pollen of poppy (*Papaver somniferum*), rape (*Brassica napus*) and sunflower (*Helianthus annus*) which were treated under freezing, UV light and then stored for 6 weeks. From among microbiological parameters were tested counts and representation of microscopic fungi, total counts of microorganisms, counts of mesophilic aerobic and anaerobic sporulating microorganisms, count of coliforms bacteria and count of cells of *Escherichia coli.* Counts of microscopic fungi in the pollen treated with UV light ranged from 1.86 log cfu $\cdot$ g^{-1} in the rape pollen after the 5th week of storage to 3.94 log cfu $\cdot$ g^{-1} in the sunflower pollen after the 1st week of storage. Counts of mesophilic anaerobic sporulating microorganisms ranged from 2.54 log cfu $\cdot$ g^{-1} after the 6th week of storage in the poppy pollen to 4.27 log cfu $\cdot$ g^{-1} in the sunflower pollen after the first week. Mesophilic aerobic sporulating microorganisms varied from 2.43 log cfu $\cdot$ g^{-1} in poppy pollen after the 6th week of UV light treatment to 3.60 log cfu $\cdot$ g^{-1} in rape pollen after the first week. Counts of coliforms bacteria ranged from 0 log cfu $\cdot$ g^{-1} since the 4th week in sunflower pollen and since 4th week in rape pollen to 3.33 log cfu $\cdot$ g^{-1} in sunflower pollen after the 1st week of UV light treatment. Counts of microscopic fungi in the pollen treated by freezing ranged from 2.13 log cfu $\cdot$ g^{-1} after the 5th week of storage in the pollen of rape to 4.05 log cfu $\cdot$ g^{-1} in

[1] Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[2] Department of Plant Processing and Storage, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[3] Department of Animal Products Evaluation and Processing, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

[4] Institute of Biodiversity Conservation and Biosafety, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic.

[5] Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic

* Corresponding author: email: Miroslava.Kacaniova@uniag.sk

the pollen of sunflower after the first week. Counts of mesophilic anaerobic sporulating microorganisms varied from 2.13 log cfu · g^{-1} after the 5th week in the pollen of rape to 4.65 log cfu · g^{-1} after the first week in the pollen of sunflower. Mesophilic aerobic sporulating microorganisms were found from 2.60 log cfu · g^{-1} after the 6th week of freezing of poppy pollen to 4.02 log cfu · g^{-1} after the first week of freezing in the pollen of sunflower. Counts of coliforms bacteria ranged from 0 log cfu · g^{-1} after the 3rd week in the rape pollen to 3.21 log cfu · g^{-1} after first week of freezing in the poppy pollen. *Alternaria* sp., *Cladosporium* sp. and *Penicillium* sp. were the most frequent species among microbiological fungi during whole time of observation. We can conclude that counts of microorganisms during 6 weeks storage treated with UV light and freezing decreased in all kinds of pollen.

Keywords: bee pollen, poppy, rape, sunflower, microorganisms, storage

The quantity and quality of pollen collected by honeybees affect reproduction, brood rearing and longevity, thus ultimately the productivity of the colony [1]. Apart from small quantities in nectar, honeybees obtain all the proteins, lipids, minerals and vitamins they need for brood rearing and adult growth and development from pollen [2]. The proportions of these nutrients can vary widely among pollens of different plant species [3], but few complete analyses are available for the chemical composition of pollens. Pollen analyses are generally carried out on bee-collected pollens because of the ease of collection. Bees do not consume fresh pollen. During collection and storage the pollen composition is changed through the addition of mainly nectar, but also glandular secretions [4]. Together with a specific bacterial flora associated with stored pollen, this increases the digestibility and nutritive value of pollen for honeybees.

Honeybee collected pollen, a traditional product of beekeeping, is used as an ingredient in diet cooking and is thought to be a source of physiologically active elements. Commercial production of biologically active food supplements based on honeybee pollen has recently been growing. With physiological value of pollen having been studied well enough, scientists have limited access to publications on hygienic aspects of pollen which would involve microbiological analysis. This article describes microbiological quality of pollen as a food substance in respect of the set sanitary standards.

Materials and methods

Bee-collected pollen of common poppy (*Papaver somniferum* L.), rape (*Brassica napus*) and sunflower (*Helianthus annuus*) was studied. Eighteen samples of bee-collected pollen were gathered from beekeepers, during a last spring season (year 2007). There were respected qualitative criteria for gathering, drying and storage of bee pollen according to criteria proposed by Bogdanov [5]:

a) Bee pollen was obtained from selected beekeepers. Health and hygiene conditions of bee families were controlled before a season starts.

b) The pollen was collected by special pollen traps.

c) Pollen was harvested daily and in the shortest time placed to a freezer (–18 to –20 ^{o}C) for prevention of spoilage and for preservation of a maximum quality.

d) Purification of frozen bee pollen pellets from different impurities was done most efficiently by air with special constructed purifier.

e) The frozen and purified bee pollen was dried as the gentlest way as possible to keep high nutritional value of pollen. Firstly, pollen was defrosted 2–3 hours in room conditions. Time of drying in a drying-oven was 6–8 hours. The maximum temperature was 35–40 ^{o}C. The pollen was dried until humidity was 10–11 %.

f) Dried pollen was stored under cool conditions (around 8 ^{o}C), in sterilized containers.

g) During all stages of manipulation with bee pollen were kept as sterilized conditions as possible to avoid contamination.

Determination of colony forming units (cfu) counts in pollen samples

Plate diluting method was applied for quantitative cfu counts determination of respective groups of microorganisms in 1 g of pollen sample. Gelatinous nutritive substrate in Petri dishes was inoculated with 1 cm^3 of pollen samples by flushing on surface, in three replications. Basic dilution (10^{-1}) was prepared as follows: 5 g of pollen content was added to the test tube containing 45 cm^3 of distilled water.

Media and culture conditions

The composition of nutritive substrates, for total mesophilic sporulating anaerobic and aerobic microorganisms, coliform bacteria, and *Escherichia coli*, was according to the directions for use declared by the producer (Biomark laboratories). Total mesophilic sporulating anaerobic microorganisms were grown in Meat Peptone agar (anaerobiosis), at 37 ^{o}C during 72 hours. Total mesophilic sporulating aerobic microorganisms were grown in Meat Peptone agar (aerobiosis), at 37 ^{o}C during 72 hours. Coliform bacteria were grown in Mac Conkey agar (aerobiosis), at 37 ^{o}C during 24 hours. *Escherichia coli* were grown in Violet red bile agar (aerobiosis), at 37 ^{o}C during 24 hours. The composition of these nutritive substrates was according to the directions for use declared by the producer (Biomark laboratories). Bacteria were determined according to Holt et al [6].

Isolation and morphological characterization of fungi

For determination of fungi colony-forming units (cfu) 5 g of sample was soaked in 45 cm^3 sterile tap-water containing 0.02 % Tween 80 and then 30 min shaken. Dilutions (from 10^{-1} to 10^{-5}) in sterile tap-water with 0.02 % Tween 80 were prepared and 1 cm^3 aliquots were inoculated on each of three plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25 ^{o}C. Total fungi cfu $\cdot$ g^{-1} counts in samples were determined after 5 days of incubation.

Malt agar and Czapek-Dox agar were used to isolate and identify individual genera and species. After isolation, or in some cases monosporic isolation, individual species were identified on the basis of their macro- and micromorphology in accordance with other scientific reports [7–9].

Results and discussion

Changes in microbiological properties of bee pollen by application of gamma irradiation and ozone treatment were tested by York et al [10]. Gamma irradiation at 7.5 kGy reduced the total microbial loads below detection levels (>10^2 cfu · g^{-1}), but after ozone treatment of up to 18 ppm for 8 h the total aerobic bacteria were found in concentrations of more than 10^3 cfu · g^{-1}.

Our results in Table 1 show microbial community of bee pollen of poppy (*Papaver somniferum*), rape (*Brassica napus*) and sunflower (*Helianthus annus)* treated under freezing, UV light and submitted to storage for 6 weeks. The results of microbiological quality of bee pollen during storage after six weeks and UV light treatment show, growing number of all microbial groups in the second week. This numbers after next weeks are gradually reduced. The same results of microbiological quality of bee pollen after six weeks with freezing treatment were achieved.

Table 1

The number of microorganisms in bee pollen [log cfu · g^{-1}] during its storage

Sample	UV light				Freezing			
	MF	MAESM	MASM	CB	MF	MAESM	MASM	CB
1st week								
Poppy	3.56	3.32	3.43	3.28	3.67	3.93	3.56	3.23
Rape	2.76	2.87	3.60	3.27	2.69	3.01	3.37	0.57
Sunflower	3.94	4.27	3.58	3.33	4.65	4.56	4.62	3.21
2nd week								
Poppy	3.38	3.83	3.74	2.10	3.61	3.47	3.67	0.90
Rape	3.10	2.93	3.25	0.57	2.53	2.82	3.18	0.67
Sunflower	3.44	3.90	0.56	1.23	3.41	4.13	3.40	2.61
3rd week								
Poppy	2.90	3.44	3.52	2.77	3.48	3.09	3.49	0.77
Rape	2.41	2.58	3.30	0.57	2.39	2.68	3.12	0.00
Sunflower	3.23	3.81	3.48	0.49	3.28	4.01	3.35	1.61
4th week								
Poppy	3.07	3.21	3.24	2.57	3.35	3.25	3.13	0.67
Rape	2.12	2.37	3.16	0.00	2.16	2.47	3.01	0.00
Sunflower	3.09	3.61	3.39	0.00	3.16	3.996	3.28	1.39
5th week								
Poppy	2.90	3.10	2.54	1.79	3.29	3.03	3.28	0.57
Rape	1.86	2.33	2.99	0.00	2.13	2.13	2.86	0.00
Sunflower	2.90	3.54	3.25	0.00	3.05	3.63	3.33	1.33
6th week								
Poppy	2.25	2.54	2.43	1.97	2.52	2.41	2.60	1.00
Rape	2.82	2.60	3.16	0.00	2.45	2.63	3.01	0.00
Sunflower	3.06	3.63	3.29	0.00	3.10	3.68	3.28	1.39

MF – microscopic fungi, MAESM – mesophilic anaerobic sporulating microorganisms, MASM – mesophilic aerobic sporulating microorganisms, CB – coliform bacteria.

Kačániová et al [11] published similar results in pollen microbiology. It is very important to continue similar research of pollen, because standards in this area are deficient.

These findings match the data obtained by the researches in China in the number of aerobic bacteria identified in pollen, which were 10^3–10^7 cfu · g^{-1} [12]. The level of pollen contamination with moulds generally exceeded the values determined by researchers in fresh pollen in Spain [13] and in French pollen [14].

Nowadays huge attention is focused on microscopic fungi, because it was discovered that some of them are able to produce toxic metabolites, called mycotoxins, which can threat health of consumer of contaminated food [15]. In our research microscopic fungi found in bee pollen samples correspond to the genus *Alternaria* sp., *Cladosporium* sp. and *Penicillium* sp. as the most abundant (100 %, 1000 % and 78 %, respectively). The rest was represented by *Fusarium* sp. (56 %), *Mucor* sp. (56 %) and *Trichoderma* sp. (33 %).

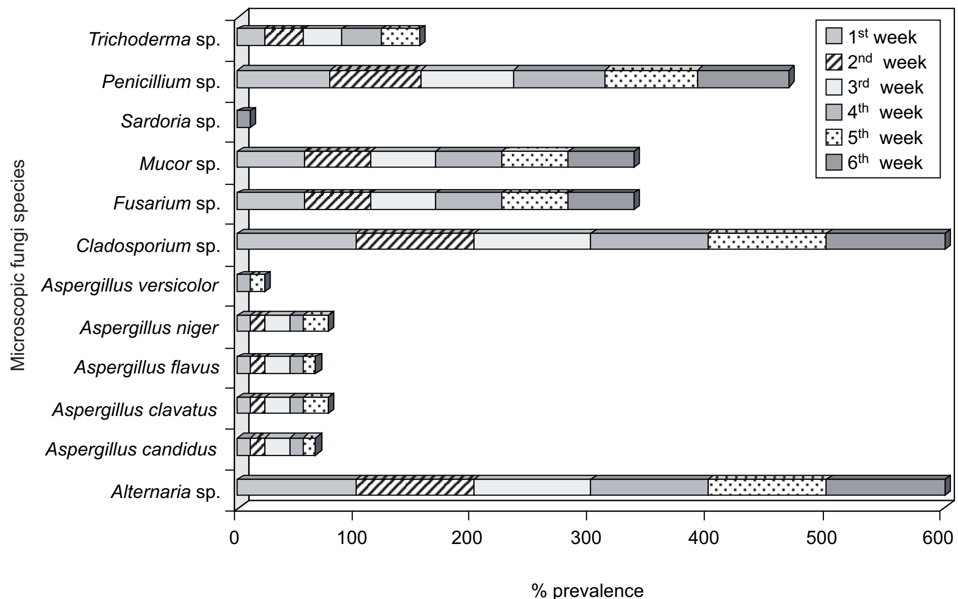


Fig. 1. Prevalence of microscopic fungi in bee pollen samples during its storage (%)

If microorganisms are responsible for fermentation and the accompanying chemical changes of pollen stored in comb cells by honey bees, the moulds may be a component of the required microbial complement. They could contribute antibiotics, organic acids and enzymes, products for which they are utilized industrially. These compounds may limit the growth of deleterious microorganisms and provide enzymes for utilization of nutrients [16].

The high water content in bee pollen is an ideal cultural medium for microorganisms as bacteria and yeast [5]. The results confirmed that the bee pollen samples contain

higher diversity of different genus of microscopic fungi than the samples of flower pollen.

Conclusion

Based on our findings, presence of moulds of up to 10^4 cfu · g^{-1} seems to be normal, which is a permissible level in Europe. In this connection studies of microflora content in relatively good quality pollen are required for the estimation of its risks for human health. From the hygienic point of view the microbiological safety is the main quality criterium. It is important to control the microbiological quality of pollen, especially the absence of pathogenic germs and yeasts. In our studies of microbiological quality we documented positive influence of UV radiation and freezing to bee pollen during its storage.

Acknowledgement

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704.

References

[1] Human H. and Nicolson S. W.: *Nutritional content of fresh, bee-collected and stored pollen of Aloe greatheadii var. davyana (Asphodelaceae)*. Phytochemistry 2006, **67**(14), 1486–1492.
[2] Loidl A. and Crailsheim K.: *Free fatty acids digested from pollen and triolein in the honeybee (Apis mellifera carnica Pollmann) midgut*. J. Comp. Physiol. B 2001, **171**, 313–319.
[3] Roulston T.H. and Cane J.H.: *Pollen nutritional content and digestibility for animals.* J. Plant Syst. Evol. 2000, **222**, 187–209.
[4] Roulston T.H.: *Pollen as a reward*. [in:] A. Dafni, P.G. Kevan and B.C. Husband (eds.), Practical Pollination Biology, Enviroquest, Cambridge 2005, 234–260.
[5] Bogdanov S.: *Quality and standards of pollen and beeswax*, Apiacta 2004, **38**, 334–341.
[6] Holt G.J., Krieg N.R., Sneath P.H.A., Staley J.T. and Williams S.T.: Bergey's Manual of Determinative Bacteriology. Williams and Wilkins. Baltimore 1994, 787.
[7] Hoog G.S., Guarro J., Gene J. and Figueras M.J.: Atlas of clinical fungi. Centralbureau voor Schimmelcultures, Rovira 2000.
[8] Samson R.A., Van Reenen-Hoekstra E.S., Frisvad J.C. and Filtenborg O.: Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Utrecht 2002.
[9] Klich M.A.: *Identification of common Aspergillus species*. Ponsen&Looijen, Wageningen 2002, 116 p.
[10] York H.S., Lim S.I. and Byun M.W.: *Changes in microbiological and physicochemical properties of bee pollen by application of gamma irradiation and ozone treatment*. Food Protect. 1989, **61**(2), 217–220.
[11] Kačániová M., Fikselová M., Nôžková J., Šrámková K., Sudzina M., Kňazovická V. and Tonková M.: *Microbiological quality of bee pollen as foodstuff*, [in:] Bezpečnost a kvalita surovín a potravín, III. vedecká konferencia s medzinárodnou účasťou, Nitra 31.1.–1.2.2008, 264–267.
[12] Yu Shao Y. et al: *Research of bacteria effect on the quality of honeybee collected pollen.* J. Hyg. Res. 1989, **18**(2), 30–33.
[13] Serra Bonvehi J. and Escolá Jordá R.N.: *Nutrient composition and microbiological quality of honeybee collected pollen in Spain.* J. Agricult. Food Chem. 1997, **45**(3), 725–732.
[14] Fleche C., Clément M.C., Zeggane S. and Faucon J.P.: *Contamination of bee products and risks for human health: the situation in France*. Rev. Sci. Techn. de l'Office International des Epizooties 1997, **16**(2), 609–619.

[15] Greenberger P.A. and Flais M.J.: *Bee pollen-induced anaphylactic reaction in an unknowingly sensitized subject.* Ann. Allergy Asthma Immunol. 2001, **86**, 239–242.
[16] Gilliam M., Prest D.B. and Lorenz B.J.: *Microbiology of pollen and bee bread: taxonomy and enzymology of molds*. Apidology 1989, **20**, 53–68.

ZMIANY FLORY BAKTERYJNEJ PYŁKU PSZCZELEGO ZAMRAŻANEGO I EKSPONOWANEGO NA PROMIENIOWANIE UV W CZASIE PRZECHOWYWANIA

Abstrakt: Celem pracy była obserwacja mikroorganizmów pyłku pszczelego pochodzącego z *Papaver somniferum*, *Brassica napus* i *Helianthus annus*, który naświetlono UV, zamrożono i przechowywano przez 6 tygodni. Spośród parametrów mikrobiologicznych oznaczono liczebność i skład gatunkowy grzybów, całkowitą liczebność mikroorganizmów, liczebność mezofilnych aerobowych i anaerobowych sporulujących mikroorganizmów, liczebność bakterii *coli* oraz liczebność *Escherichia coli*. Liczebność grzybów w pyłku *Brassica napus* naświetlanym UV wynosiła od 1.86 log cfu · g^{-1} po 5 tygodniach przechowywania do 3.94 log cfu · g^{-1} w pyłku *Helianthus annus* po 1 tygodniu przechowywania. Liczebność mezofilowych anaerobowych sporulujących mikroorganizmów wynosiła od 2.54 log cfu · g^{-1} w pyłku z *Papaver somniferum* po 6 tygodniach przechowywania do 4.27 log cfu · g^{-1} w pyłku *Helianthus annus* przechowywanym przez tydzień. Liczebność aerobowych sporulujących mikroorganizmów wynosiła od 2.43 log cfu · g^{-1} w pyłku *Papaver somniferum* przechowywanym przez 6 tygodni w obecności UV do 3.60 log cfu · g^{-1} w pyłku *Brassica napus* po pierwszym tygodniu przechowywania. Liczebność bakterii coli w pyłku *Helianthus annus* i *Brassica napus* wynosiła od 0 w 4 tygodniu przechowywania do 3.33 log cfu · g^{-1} w pyłku *Helianthus annus* po 1 tygodniu naświetlania UV. Liczebność grzybów wynosiła od 2.13 log cfu · g^{-1} w zamrażanym przez 5 tygodni pyłku *Brassica napus* do 4.05 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność mezofilnych anaerobowych sporulujących mikroorganizmów wynosiła od 2.13 log cfu · g^{-1} w zamrażanym przez 5 tygodni pyłku *Brassica napus* do 4.65 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność mezofilowych aerobowych sporulujących mikroorganizmów wynosiła od 2.60 log cfu · g^{-1} w pyłku *Papaver somniferum* zamrożonym przez 6 tygodni do 4.02 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność bakterii coli wynosiła od 0 w pyłku *Brassica napus* zamrożonym przez 3 tygodnie do 3.21 log cfu · g^{-1} w pyłku *Papaver somniferum* zamrożonym przez tydzień. Spośród grzybów najczęściej występowały *Alternaria* sp., *Cladosporium* sp. i *Penicillium* sp. Zamrażanie połączone z ekspozycją na promieniowanie UV powodowało spadek liczebności mikroorganizmów we wszystkich rodzajach pyłku po 6 tygodniach przechowywania.

Słowa kluczowe: pyłek pszczeli, mak polny, rzepak, słonecznik, mikroorganizmy, przechowywanie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Miroslava KAČÁNIOVÁ[1*], Janka NÔŽKOVÁ[2],
Katarína FATRCOVÁ-ŠRAMKOVÁ[3], Zlata KROPKOVÁ[4]
and Jana KUBINCOVÁ[5]

ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND HEAVY METALS CONTENT IN POLLEN OF *Papaver somniferum* L.

ZAWARTOŚĆ METALI CIĘŻKICH ORAZ AKTYWNOŚĆ ANTYOKSYDACYJNA I ANTYBAKTERYJNA PYŁKU *Papaver somniferum* L.

Abstract: An aim of the study was to measure a total flavonoids content, as well as, individually the content of quercetin, luteolin, kaempferol, apigenin in *Papaver somniferum* L. bee pollen, then to analyse a reduction power of bee pollen (dried, frozen, freeze-dried), and also to determine an antibacterial activity of bee pollen extracts, obtained with different concentrations of ethanol. Heavy metals concentrations were analysed in bee gathered (in case of all three treatment) and flower pollen samples. The reduction power of bee pollen compounds was 3592.56 ± 105.29 $\mu g \cdot cm^{-3}$. The highest value achieved the freeze-dried pollen. Comparison of the flavonoids content (averaged 262.33 ± 4.42 $mg \cdot kg^{-1}$) refers on higher values in the frozen bee pollen than in the dried and freeze-dried forms. In the freeze-dried pollen was the highest content of two flavonoids (quercetin and apigenin), but in the case of the other two analysed flavonoids (luteolin and kaempferol) their content was the highest in the dried bee pollen. The obtained results characterize *Papaver somniferum* L. bee pollen ethanolic extract samples as the product with the broad antimicrobial effect. From the heavy metals, in flower and bee pollen, the lead level was 0.64 $mg \cdot kg^{-1}$ and less than 0.1 $mg \cdot kg^{-1}$, respectively. Consecutively, the contents of mercury were 0.019 $mg \cdot kg^{-1}$ in the flower pollen and ranging from 0.004 to 0.005 $mg \cdot kg^{-1}$ in the frozen, freeze-dried and dried bee pollen. The cadmium concentration in the flower pollen was 0.12 $mg \cdot kg^{-1}$, and in the bee pollen ranged from 0.22 to 0.26 $mg \cdot kg^{-1}$.

Keywords: *Papaver somniferum* L. bee pollen, flower pollen, flavonoids, reduction power, antibacterial activity, antifungal activity, heavy metals, contamination

[1] Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[2] Institute of Biodiversity Conservation and Biosafety, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[3] Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[4] Department of Statistics and Operation Research, Faculty of Economics and Management, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[5] Food Research Institute, Biocentre in Modra, Kostolná 7, 900 01 Modra, Slovak Republic.

* Correspondence: email: miroslava.kacaniova@uniag.sk

Introduction

A bee gathered pollen is regarded as valuable special food and is used also in an apitherapy [1, 2]. Bee-collected pollen ("bee pollen") is promoted as a health food with a wide range of nutritional and therapeutic properties [3]. This beehive product also has several useful pharmacological properties, such as antibiotic, antineoplastic, antidiarrhoeatic and as an antioxidant agent [4]. Also bee-pollen, as well as other apicultural products, has gained increased attention for its therapeutic properties, such as antibacterial [5, 6], antifungal [5], anti-caryogenic [7] and immunomodulatory [8] effects.

The antioxidant activity of honeybee-collected pollen has been recognized as a free radical scavenger and as a lipid peroxidation inhibitor [4, 9]. This activity has been associated with the phenolic pollen content [4]. Usually, honeybee-collected pollen is a mixture of pollen pellets from different botanical origins. Each one is an important source of flavonol glycosides [10], and in some species, of hydroxycinnamic acids [4]. These compounds are found in a species-specific profile [4], which suggests that honeybee-collected pollen from different areas or seasons could have different antioxidant activities. In spite of the relevance of honey-bee collected pollen as an antioxidant substance, there is not enough systematic information about the antioxidant activity levels associated to the flavonol content and profile of honeybee-collected pollen from different botanical origins [11]. Bee-collected pollen is an apicultural product, which is composed of nutritionally valuable substances and contains considerable amounts of polyphenol substrates, which may act as potent antioxidants. It was concluded, that pollen and propolis extracts inhibit respiratory burst within cancer cell lines, probably by their antioxidant potentials [12]. The bioactive properties of apicultural pollen extracts can be increased using a solvent suitable for its extraction, improving the activity of free radicals sequestration (antirust activity) [13]. Appropriate extracts of pollen can be used as functional food or alimentary supplement. They had the amount of phenolic composites and their capacity of sequestered free radicals which are responsible for carcinogenesis [14]. Pollen grains have specific characteristics according to the floral species or cultivation methods, but the quality depends on the collection process, cleanness, drying and storage applied by beekeepers with the objective to increase the product shelf-life.

The aim of our study was to measure the total flavonoids content, as well as, individually the content of quercetin, luteolin, kaempferol, apigenin in *Papaver somniferum* L. bee pollen. Then, to analyse the reduction power of bee pollen (dried, frozen, freeze-dried), and also to determine the antibacterial activity of bee pollen extracts, obtained with different concentrations of ethanol. The heavy metals concentrations were analysed in bee gathered (in case of all three treatments) and flower pollen samples of *Papaver somniferum* L.

Material and methods

Samples preparation

Samples of bee-collected pollen were obtained from beekeepers, which respected qualitative criteria for gathering, drying and storing as proposed by Bogdanov [2]. The

samples were collected during the spring season 2007 from different regions of western Slovakia. The flower pollen samples were obtained in the year 2007. A poppy is a self-pollinated plant species and anthers are opened inside of a flower bud before it opens. For this reason the pollen was collected from plants of different genotypes closely before flowering. The samples of flower pollen were stored in dry place at room temperature until analysed. The fresh bee pollen was stored at –18 ^{o}C, 20 % moisture, approximately six months until analysed. The dried pollen samples were dried (9–11 % humidity) approximately 8 hours at maximum temperature 35 ^{o}C. The moisture was tested by thermogravimetric analyzer WPS 50SX/1 by RADWAG. The lyophilized samples of bee pollen were dried in the table laboratory lyophilizator LYOVAC GT 2 by Amsco/Finn-Aqua, 80 hours without heating, until 2 % moisture. The drying process was realized without heating so the nutritive compounds of the pollen were not changed.

Total flavonoids and selected flavonoids analysis

HPLC determination of flavonoids. Chromatographic separation were performed on a Purospher Star RP-18e (Merck) column (250 × 4 mm I.D., 5 μm), protected by a Merck Purospher Star (4 × 4 mm, 5 μm) guard column. The HPLC system consisted of Shimadzu LC 10ADvp series pumping system, SPD 10AV/VP UV/VIS detector set at 360 nmand C-R6A chromatography data station software. Two solvents were used with constant flow rate 1 cm^3/min.

The injection volume was 20 mm^3. Solvent A consisted 0.05 % of TFA/methanol (95:5, V/V), solvent B included methanol/0.05 % TFA (95:5, V/V). For the elution program, the following proportions of solvents B were used: 0–15 min, 40 % B; 15–30 min, 40–55 % B; 30–35 min 55–70 % B. The ethanolic extracts were injected under this conditions as well as a mixture of authentic samples of quercetin, luteolin, kaempferol and apigenin.

The correlation between content of flavonoids and antioxidant activity (expressed as RP_{AA}) was analysed using SAS 9.1.3 software.

Reduction power. The reduction power of pollen compounds was evaluated spectrophotometrically by the modified method Prieto [15]. This method is established on reduction of Mo(VI) to Mo(V) with an effect of reduction parts in the presence of phosphorus under formation of green phosphomolybdenum complex. Solution absorbance of reducing sample was measured at λ = 705 nm (UV-1601, Shimadzu, Tokyo Japan) toward blank experiment (distilled water). The reduction power of compounds (RP_{AA}) expressed as quantity of ascorbic acid necessary to achieve the same effect in $\mu g \cdot cm^{-3}$ was calculated using the equation: $RP_{AA} = (A_{705\ nm} - 0.0011) / 0.00236$.

Microbiological analysis

Preparation of bee pollen ethanolic extracts (BPEE). The bee pollen samples (2 g) were milled, homogenized and extracted individually using 15 cm^3 of the ethanol solution as the extraction solvent in different concentrations (BPEE 50, BPEE 70, and

BPEE 90 %) at temperature of 70 °C for 30 min with constant agitation. The supernatant was separated and the solid residue was re-extracted. Then, the ethanol extracts of pollen were combined and stored at 5 °C for further analysis. All samples were extracted in duplicate [16].

Antimicrobial activity of bee pollen ethanolic extracts (BPEE). The disk diffusion assay described in detail by Bauer et al [17] was used in this study, with some modifications. The stock of bacterial cultures (*Staphylococcus* sp., family *Enterobacteriaceae*) and fungal (*Penicillium citrininum*, *P. crustosum*, *P. expansum*, *P. brevicompactum* and *P. chrysogenium*) were grown in the nutrient agar at 26–27 °C for 24 h and the Malt agar at 25 °C for 48 h in a shaker. The aliquots of 40 mm^3 of bee pollen ethanolic extract were applied in a paper disk and placed in plates containing the nutrient agar that was previously inoculated with active cultures of these microorganisms with sterile swabs. Antimicrobial activity was assessed by measuring the diameter of the inhibition zone around each disk after 24 hours of inoculation at 37 °C. The control (40 mm^3 of 96 % ethanol) used in all the plates and extracts analysed in duplicate. Chloramphenicol (40 mm^3) was used as the positive control for bacteria.

Heavy metals analysis

Heavy metals in flower and bee pollen were analysed by standard methods in the accredited analytic laboratory BEL/NOVAMANN International Ltd. Nové Zámky. The samples of pollen were homogenized, and then they were further processed according to determined chemical compounds.

An Advanced Mercury Analyzer (AMA 254) was assessed for determination of mercury in analysed samples of pollen. The method is based on sample catalytic combustion, preconcentration by gold amalgamation, thermal desorption, and atomic absorption spectrometry.

In the case of cadmium and lead determination was used electrothermal atomic absorption spectrometry (ETA-AAS).

Results and discussion

Total flavonoids and selected flavonoids analysis

The reduction power of bee pollen compounds was $3592.56 \pm 105.29\ \mu g \cdot cm^{-3}$. In the present submission we have compared analysed results of dried, frozen and freeze-dried bee pollen. The highest value achieved the freeze-dried pollen (Table 1).

An antioxidant ability of pollen seems to be due to phenolic compounds [18]. High levels of phenolic constituents are often accompanied by a high antioxidative capacity of pollen; however, according to the reports of Campos et al [3] and Campos et al [19], no direct correlation between flavonoids and a radical-scavenging activity was found. A gradual decrease of RSA in pollen stored for 4 years was not accompanied by a parallel reduction of flavonoids [3] and some pollen with high levels of phenolics did not present significant antiradical activity [19]. Pollen, containing more than 6 % of

water will ferment upon storage. Storage for one year or longer will reduce the free radical scavenging capacity of pollen [3].

Table 1

The reduction power (RP_{AA}) of bee pollen compounds [$\mu g \cdot cm^{-3}$]

Pollen	RP_{AA} [$\mu g \cdot cm^{-3}$]
Frozen	3452.67 ± 4.64
Dried	3653.67 ± 4.64
Freeze-dried	3671.33 ± 3.40

Comparison of flavonoids content (averaged 262.33 ± 4.42 $mg \cdot kg^{-1}$) refers on higher values in the frozen bee pollen than in the dried and freeze-dried forms (Table 2). In the freeze-dried pollen was the highest content of two flavonoids (quercetin and apigenin), but in the case of the other two analysed flavonoids (luteolin and kaempferol) their content was the highest in the dried bee pollen. For any from four investigated flavonoids the highest content was not determined in the frozen bee pollen. Sum of particular four selected flavonoids decreased in the order: dried > freeze-dried > frozen bee pollen.

Table 2

The content of flavonoids in bee pollen [$mg \cdot kg^{-1}$]

Flavonoids [$mg \cdot kg^{-1}$]	Pollen		
	frozen	dried	freeze-dried
Quercetin	2.05 ± 0.14	3.99 ± 0.06	5.19 ± 0.10
Luteolin	1105.80 ± 0.57	1390.67 ± 0.35	1340.58 ± 0.59
Kaempferol	12.96 ± 0.75	22.40 ± 0.77	23.61 ± 0.45
Apigenin	4.60 ± 0.46	6.56 ± 0.29	15.65 ± 0.67
Total flavonoids	266.00 ± 3.74	258.67 ± 1.70	262.33 ± 2.87

In investigations by Leja et al [20] great variability regarding content of total phenols, phenylpropanoids, flavonols and antioxidant capacity in 12 examined pollens was found. In some of them (*P. tanacetifolia* and *S. alba*) a very high antioxidant activity, expressed as a radical-scavenging activity, inhibition of a lipid peroxidation and a hydroxyl radical scavenging activity, corresponded to high levels of total phenols, phenylpropanoids and flavonols. A variability of total antioxidant activity in investigated species seems to correspond to their phenylpropanoid contents, being manifested by the significant positive correlation coefficient [20].

There were observed the statistically significant strong dependence between antioxidant activity and individual flavonoids, namely quercetin, luteolin, kaempferol ($p < 0.001$), and apigenin ($p < 0.05$), but not between total flavonoids (Table 3).

Table 3

Correlation matrix for antioxidant activity and content of flavonoids

	AA (RP_{AA})	Flavonoids	Quercetin	Luteolin	Kaempferol	Apigenin
AA (RP_{AA})	1	$P \geq 0.05$ NS	$P < 0.001$ $r = 0.94759$	$P < 0.001$ $r = 0.97081$	$P < 0.001$ $r = 0.99064$	$P < 0.05$ $r = 0.68926$
Flavonoids		1	$P \geq 0.05$ NS	$P < 0.05$ $r = -0.67444$	$P \geq 0.05$ NS	$P \geq 0.05$ NS
Quercetin			1	$P < 0.01$ $r = 0.84820$	$P < 0.001$ $r = 0.94193$	$P < 0.01$ $r = 0.87972$
Luteolin				1	$P < 0.001$ $r = 0.95397$	$P \geq 0.05$ NS
Kaempferol					1	$P < 0.05$ $r = 0.70517$
Apigenin						1

AA – antioxidant activity.

Microbiological analysis

The antimicrobial activity of *P. somniferum* L. bee pollen extracts was analysed according to the disk diffusion assay, and the results are shown in Table 4. The obtained results characterize *P. somniferum* L. bee pollen ethanolic extract samples as the product with the broad antimicrobial effect. The strongest antibacterial effect was shown by *P. somniferum* L. bee pollen ethanolic extract of 90 % concentration against *Staphylococcus* sp. and family *Enterobacteriaceae.* The strongest antifungal effect was shown by *P. somniferum* L. bee pollen ethanolic extract samples of 90 % concentration against *Penicillium citrininum* and *Penicillium crustosum* strains. The least antifungal effect was shown by *P. somniferum* L. bee pollen ethanolic extract samples of 50 % concentration against to *Penicillium chyrysogenium* strains. Chloramphenicol the antibiotic was used for comparison, showing different ways of action to each microorganism. The control proved that the ethanol used in the extractions did not have inhibiting action.

Table 4

Antimicrobial activity [mm] of *Papaver somniferum* L. bee pollen ethanolic extracts (BPEE)

BPEE	*Enterobacteriaceae*	*Staphylococcus* sp.	*Penicillum citrininum*	*Penicillium crustosum*	*Penicillium expansum*	*Penicillium brevicompactum*	*Penicillium chrysogenium*
50 %	6.0	5.0	4.0	4.5	4.0	2.0	0.0
70 %	6.5	5.5	4.0	6.0	3.5	3.0	0.0
90 %	7.0	6.0	5.0	9.0	1.5	1.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A	9.0	11.0	6.0	4.0	5.0	0.0	5.0

Pollen ethanolic extracts in Carpes et al. [16] study of all pollen at 60 % showed the same inhibition degree against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Bacillus cereus* and *Staphylococcus aureus* bacteria. In Carpes et al [16] study *Bacillus subtilis* bacteria were inhibited for Parana pollen in a pollen ethanolic extracts at 40 %, 50 %, 60 % and 70 % and for Alagoas pollen in PEE at 50 %, 70 % and 90 %. In this study, the pollen ethanolic extracts of Parana pollen at 90 % showed the biggest clear zones around each disk (7.0 mm) against *Klebsiella* sp. Nevertheless, *Klebsiella* was also inhibited by the pollen from Alagoas in extracts of 60 and 70 %. The Alagoas extract pollen at 70 %, contained the highest antibacterial activity. *Pseudomonas aeruginosa* bacteria were inhibited by 80 % and 90 %, and *Staphylococcus aureus* bacteria were inhibited by extracts of pollen at 50 %, 60 %, 70 % and 80 % of ethanol solution.

Heavy metals analysis

From the heavy metals in flower and bee pollen the lead level was 0.64 $mg \cdot kg^{-1}$ and less than 0.1 $mg \cdot kg^{-1}$, respectively (Table 5). Consecutively, the contents of mercury were 0.019 $mg \cdot kg^{-1}$ for the flower pollen and ranging from 0.004 to 0.005 $mg \cdot kg^{-1}$ for frozen, freeze-dried and dried bee pollen. The cadmium concentration in the flower pollen was 0.12 $mg \cdot kg^{-1}$ and in the bee pollen ranged from 0.22 to 0.26 $mg \cdot kg^{-1}$. The content of cadmium was lower in the flower pollen than in the bee pollen, but other heavy metals like the lead and mercury had higher levels in the flower pollen.

Table 5

The content of heavy metals in flower and bee pollen [$mg \cdot kg^{-1}$]

Heavy metals [$mg \cdot kg^{-1}$]	Flower pollen (year 2007)	Bee pollen (year 2007)	
Lead (Pb)	0.64	Dried	< 0.1
		Frozen	< 0.1
		Freeze-dried	< 0.1
Mercury (Hg)	0.019	Dried	0.005
		Frozen	0.004
		Freeze-dried	0.004
Cadmium (Cd)	0.12	Dried	0.25
		Frozen	0.22
		Freeze-dried	0.26

Chlebo and Čermáková [20] investigated; that a bee pollen contamination can comes from two sources – by industrial emissions and agricultural pesticides. They determined following content of risk chemical elements in 16 samples of bee pollen, from 4 high industrialized regions of Slovakia (Stredný Spiš, Ružomberok, Horná Nitra, and Bratislava): a lead (Pb) 1.70 $mg \cdot kg^{-1}$ (year 1990) and 0.38 $mg \cdot kg^{-1}$ (year 1999), a mercury (Hg) 0.018 $mg \cdot kg^{-1}$ (year 1990) and 0.003 $mg \cdot kg^{-1}$ (year 1999), a cadmium (Cd) 0.180 $mg \cdot kg^{-1}$ (year 1990) and 0.043 $mg \cdot kg^{-1}$ (year 1999). In

comparison with our results the level of cadmium was higher in bee pollen samples, and the level of mercury was slightly higher in flower and bee pollen samples (Table 5).

Conclusions

Further studies of the antimicrobial and the antioxidant properties and the antioxidant components of bee pollen from different botanical origins are required. There is also important problem with the heavy metals content in bee pollen, which has to be closely investigated; in consequence that bee pollen is regarded as valuable food supplement.

Acknowledgement

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704.

References

[1] Isla M.I., Moreno M.I.N., Sampietro A.R. and Vattuone M.A.: J. Ethnopharmacol. 2001, **76**(2), 165–170.
[2] Bogdanov S.: Apiacta 2004, **38**, 334–341.
[3] Campos M.G., Webby R.F., Markham K.R., Mitchell K.A. and Cunha A.P. Da: J. Agric. Food Chem. 2003, **51**, 742–745.
[4] Campos M.G., Mitchel K., Cunha A. and Markham K.R.: Phytochem. Anal. 1997, **8**, 181–185.
[5] García M., Pérez-Arquillue C., Juan T., Juan M.I. and Herrera A.: Food Sci. Tech. Inter. 2001, **7**(2), 155–158.
[6] Proestos C., Chorianopoulos N., Nichas G.J.E. and Komaitis M.: J. Agri. Food Chem. 2005, **53**(4), 1190–1195.
[7] Almas K., Mahmoud A., Dahlan A.: Almas K.., Mahmoud A. and Dahlan A.: Indian J. Dentist Resour. 2001, **12**, 21–70.
[8] Gebara E.C.E., Lima L.A. and Mayer M.P.A.: Braz. J. Microbiol. 2002, **33**(4), 365–369.
[9] Campos M.G., Cunha P.A., Navarro M.C. and Utrilla M.P.: Polyphenols 1994, **94**, 415–426.
[10] Wiermann R. and Vieth K.: Protoplasma 1983, **118**, 230–233.
[11] Almaraz-Abarca N., Campos M. Da G., Ávila-Reyes J.A., Naranjo-Jiménez N., Herrera-Corral J. and González-Valdez L.S.: Interciencia 2004, **29**, 574–578.
[12] Aliyazicioglu Y., Deger O., Ovali E., Barlak Y., Hosver I., Tekelioglu Y. and Karahan S.C.: Int. Immunopharm. 2005, **5**, 1652–1657.
[13] Di Paola-Naranjo R.D., Sánchez J.S., Paramás A.M.G. and Gonzalo J.C.R.: J. Chromatography A, **2004**, 1054(1/2), 205–210.
[14] Tang B., Zhang L. and Geng Y.: Talanta, 2005, **65**(3), 769–775.
[15] Prieto P., Pineda M. and Aquilar M.: Analytical Biochemistry 1999, **269**, 337–341.
[16] Carpes S.T., Begnini, R., De Alencar S.M. and Masson M.L.:.Ciênc. agrotec., 2007, **31**(6), 1818–1825.
[17] Bauer A.W., Kirby W.M., Sherris J.C. and Turck M.: Am. J. Clin. Pathol., 1966, **45**, 493–496.
[18] Markham K.R. and Campos M.G.: Phytochemistry 1996, **43**, 763–767.
[19] Campos M.G., Webby R.F., Mitchell K.A., Coleta M., Markham K.R. and Cunha A.P.: Polyphenols Communications 2000, September 11–15. Freising-Weihenstephan Germany.
[20] Leja M., Mareczek A., Wyżgolik G., Klepacz-Baniak J. and Czekońska K.: Food Chem. 2007, **100**, 237–240.
[21] Chlebo R. and Čermáková T.: Nutrition and Food in 3rd Millennium – proceeding. SPU, Nitra 2001, 171–173 [in Slovak]

ZAWARTOŚĆ METALI CIĘŻKICH ORAZ AKTYWNOŚĆ ANTYOKSYDACYJNA I ANTYBAKTERYJNA PYŁKU *Papaver somniferum* L.

Abstrakt: Celem pracy było zmierzenie całkowitej zawartości flawonoidów oraz kwercetyny, luteoliny, kemferolu i apigeniny w pyłku pszczelim pochodzącym z *Papaver somniferum*. Następnie zbadano siłę redukcyjną pyłku pszczelego (suszonego, mrożonego, suszonego i mrożonego) oraz aktywność antybakteryjną ekstraktów pyłku pszczelego uzyskanych przy użyciu etanolu o różnych stężeniach. Zawartość metali ciężkich zbadano w pyłku zebranym przez pszczoły oraz w próbkach pyłku pobranych z kwiatów. Siła redukcyjna pyłku pszczelego wynosiła 3592,56 ±105,29 $\mu g \cdot cm^{-3}$. Największą siłę redukcyjną miał pyłek suszony i zamrożony. Największą zawartość flawonoidów stwierdzono (średnio 262 ± 4.42 $mg \cdot kg^{-1}$) w zamrożonym pyłku pszczelim. W pyłku, który był jednocześnie wysuszony i zamrożony, występowała największa ilość kwercetyny i apigeniny. Pozostałe flawonoidy (luteolina i kemferol) występowały w największej ilości w suszonym pyłku pszczelim. Uzyskane rezultaty wskazują na bardzo szerokie działanie antybakteryjne etanolowych ekstraktów pyłku pszczelego z *Papaver somniferum*. Zawartość ołowiu w pyłku pszczelim oraz próbkach zebranych bezpośrednio z kwiatów wynosiła odpowiednio 0,64 $mg \cdot kg^{-1}$ i mniej niż 0,1 $mg \cdot kg^{-1}$. Zawartość rtęci wynosiła 0,019 $mg \cdot kg^{-1}$ w pyłku zebranym bezpośrednio z kwiatów. Natomiast w pyłku pszczelim mrożonym, suszonym oraz suszonym i mrożonym zawartość rtęci wynosiła od 0,004 do 0,005 $mg \cdot kg^{-1}$. Zawartość kadmu w pyłku zebranym z kwiatów wynosiła 0,12 $mg \cdot kg^{-1}$, a w pyłku pszczelim od 0,22 do 0,26 $mg \cdot kg^{-1}$.

Słowa kluczowe: pyłek pszczeli z *Papaver somniferum* L., pyłek kwiatowy, flawonoidy, siły redukcyjne, aktywność antybakteryjna, aktywność antygrzybiczna, metale ciężkie, zanieczyszczenia

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Adriana KOLESAROVA[1*], Marcela CAPCAROVA[1],
Alexander V. SIROTKIN[2] and Jaroslav KOVACIK[1]

EFFECT OF LEAD, SILVER AND MOLYBDENUM ON STEROIDOGENESIS IN PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

WPŁYW OŁOWIU, SREBRA I MOLIBDENU NA STEROIDOGENEZĘ *IN VITRO* W KOMÓRKACH ZIARNISTYCH JAJNIKÓW ŚWINI

Abstract: The present study was carried out to investigate possible effects of lead (Pb), silver (Ag) and molybdenum (Mo) administrations on porcine ovarian granulosa cells in relation to progesterone (P_4) release. Ovarian granulosa cells were incubated with/without lead acetate, silver nitrate and ammonium molybdate for 18 hours: 1.0 mg/cm^3; 0.5 mg/cm^3; 0.33 mg/cm^3; 0.17 mg/cm^3; 0.09 mg/cm^3 and the control group without metal addition. The release of progesterone by granulosa cells was assessed by RIA. The release of steroid hormone P_4 was significantly ($p < 0.05$) inhibited after Pb administration at the dose 1.0 mg/cm^3. Secretion of P_4 by granulosa cells was decreased by Ag addition at the doses 0.5 mg/cm^3; 0.33 mg/cm^3; 0.17 mg/cm^3 and 0.09 mg/cm^3. Significant ($p < 0.05$) increase of P_4 release after Mo addition was found. Data obtained from these *in vitro* studies indicate new knowledge that release of steroid hormone progesterone by porcine granulosa cells is associated with doses and variety of chemical treatments (Pb, Ag, Mo). Obtained data indicate the interference of these endocrine disruptors in the pathways of steroidogenesis of porcine ovarian granulosa cells.

Keywords: lead, silver, molybdenum, progesterone, steroidogenesis, granulosa cells

Environmental pollution is one of the major issues of today's world [1]. Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues [2].

Lead is a ubiquitous environmental and industrial pollutant [3–6] found in air, water, brass plumbing fixtures, soil [7, 8], and some foods, vegetables and rice [7]. An

[1] Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, 949 76 Nitra, Slovak Republic,

[2] Institute for Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra, 951 41 Luzianky, Slovak Republic,

* Corresponding author: phone +42 13 741 41 19, email: adrianakolesarova@yahoo.com

accumulation of Pb in granulosa cells of the rat ovaries [9], sheep ovaries [10], the liver and kidney of brown hares was reported [11]. Lead can induce ovarian changes in sheep [10] and ovarian granulosa cell toxicities [9].

The primary use of **silver** (Ag) is in industrial applications, including use as conductors, switches, and contacts; plating applications; silver brazing or soldering; and miscellaneous uses such as mirrors, batteries, and catalysts. The second most important use is the production of coins, jewellery, and tableware [12]. Silver salts are also used as disinfectants. Recently, production of silver nanoparticles has been used in various nanotechnologies [13] and suggested as antibacterial agent [14]. The highest concentrations of Ag are usually found in the liver and spleen, and to some extent, in muscles, skin, and brain after ingestion. Water-soluble Ag compounds such as silver nitrate have a local corrosive effect. Repeated exposure in animals causes growth retardation and degenerative changes in the liver. The metal Ag preferentially accumulated in the kidney of scallop, with much lower concentrations in the other organs [15].

Molybdenum (Mo) is an essential trace element [16]. It is of considerable industrial importance, especially in the production of strong, high quality steels for making heavy machines and industrial hardware [17]. Mo is found in all foods and beverages [18], usually at low levels of less than 1 mg/kg. Animal offal and nuts appear to be the only foodstuffs that contain its relatively high levels. In certain conditions, such as when soil is either naturally rich in Mo or has been contaminated by industrial activity, certain food crops and other plants may accumulate unusually high levels of the metal. High levels of Mo have also been detected in plants grown on soil which has been treated with sewage sludge and certain fertilisers [17]. Mo is an essential component of several enzymes [19, 20], including xanthine oxidase and xanthine dehydrogenase [20]. It fulfils important cell functions [21]. Polyoxomolybdates as discrete molybdenum--oxide cluster anions have been investigated in the course of study of their medical applications [22]. Potential anticancer cytostatic and cytotoxic effects of piroxicam complexes with MoO_2^{2+} on human promyelocytic leukemia HL-60 cells have been investigated [23]. Polyoxomolybdates provide promising, novel anti-tumor agents, especially for cancers that are difficult to treat [24]. The effects of tetrathiomolybdate analogue (ATN-224) on endothelial and tumor cell growth were evaluated in cell culture experiments *in vitro*. ATN-224 inhibits superoxide dismutase 1 (SOD1) in tumor and endothelial cells [25].

Progesterone (P_4) is an ovarian steroid [26, 27] produced by porcine ovarian granulosa cells [28, 29] and *corpus luteum* of pigs [30]. It is essential for normal ovarian cycle [26, 27], sexual maturation [31], breast development and embryo development [26, 27]. It is among the intraovarian signals that contribute to regulation of ovarian follicular development and remodeling [30].

The general objective of this *in vitro* study was to examine the secretory activity of porcine ovarian granulosa cells after lead, silver and molybdenum administrations. The study also aimed at examining release of progesterone by porcine ovarian granulosa cells after metal additions.

Material and methods

Preparation, culture and processing of granulosa cells from ovaries

Slovakian White gilts at the ages of 100–120 days were kept under standard conditions at the Experimental Station of the Animal Production Research Centre Nitra. Conditions of their care, manipulations and use corresponded to the instruction of EC no. 178/2002 and related EC documents, and they were approved by local ethics commission. Porcine ovaries at the early and mid-follicular phase of the estrous cycle were obtained from healthy gilts without visible reproductive abnormalities. Ovaries were transported to the laboratory at 4 ^{o}C and washed in sterile physiological solution. Follicular fluid was aspirated from 3–5 mm follicles. Granulosa cells were isolated by centrifugation for 10 min at 200xg followed by washing in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and resuspended in the same medium supplemented with 10 % fetal calf serum (BioWhittaker™) and 1 % antibiotic--antimycotic solution (Sigma, St. Louis, Mo, USA) at a final concentration of 10^6 cells/cm^3 (determined by haemocytometer). Portions of the cell suspension were dispensed to 24-welled culture plates (Nunc™, Roskilde, Denmark, 1 cm^3 per well) for radioimmunoanalysis (RIA). The plate wells were incubated at 37.5 ^{o}C and 5 % CO_2 in humidified air until a 75 % confluent monolayer was formed (5–7 days). At this point, the medium (1 cm^3 per well) was renewed and ovarian granulosa cells were incubated with the same supplements (10 % fetal calf serum, 1 % antibiotic-antimycotic solution) and with or without chemical substances: lead acetate $Pb(CH_3COO)_2 \cdot 3H_2O$, silver nitrate $AgNO_3$ and ammonium molybdate $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$. The concentrations were diluted as described in Table 1. Further culture was performed for 18 h, and then the culture media from plate wells were aspirated and kept at –20 ^{o}C for further assay.

Table 1

Lead, silver and molybdenum concentrations used in the study

Group	$Pb(CH_3COO)_2 \cdot 3H_2O$, $AgNO_3$, $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$ [mg/cm^3]	Medium [cm^3]	Dilutio rate	Concentrations of $Pb(CH_3COO)_2 \cdot 3H_2O$, $AgNO_3$, $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$ [mg/cm^3]
Control	0	1	0:1	0
Max	1	0	1:0	1.0
A	0.5	0.5	1:1	0.5
B	0.33	0.67	1:2	0.33
C	0.17	0.83	1:5	0.17
D	0.09	0.91	1:10	0.09

Maximum used dose: 1.0 mg $Pb(CH_3COO)_2 \cdot 3H_2O$/cm^3 = 0.546 mg Pb/cm^3; Maximum used dose: 1.0 mg $AgNO_3$/cm^3 = 0.6349 mg Ag/cm^3; Maximum used dose: 1.0 mg $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$/cm^3 = 0.0776 mg Mo/cm^3.

Immunoassay

Concentrations of P_4 were determined in 25–100 mm^3 incubation medium by RIA. This substance was assayed using RIA kits (Immunotech SAS, Marseille Cedex, France) according to the manufacturer's instructions [3, 31, 32]. All RIA were validated for use in samples of culture medium. RIA assay sensitivity for P_4 was 0.05 ng/cm^3. Inter- and intra-assay coefficients of variation did not exceed 9.0 % and 5.8 %, respectively.

Statistical analysis

Each experimental group was represented by four culture wells of cultured granulosa cells. Assay of substance in incubation medium was performed in duplicate. The data presented are means of values obtained in three separate experiments performed on separate days using separate pools of ovaries from 10–12 animals. Significant differences between the control and experimental groups were evaluated by paired t-test using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at the level $p < 0.05$.

Results

Release of progesterone by porcine ovarian granulosa cells

The reduction of the monolayer of granulosa cells after Pb addition was found by light microscopy (Fig. 1). The release of P_4 by porcine ovarian granulosa cells was 23.37 ± 1.28 ng/cm^3 in the control group. Release of steroid hormone P_4 by granulosa

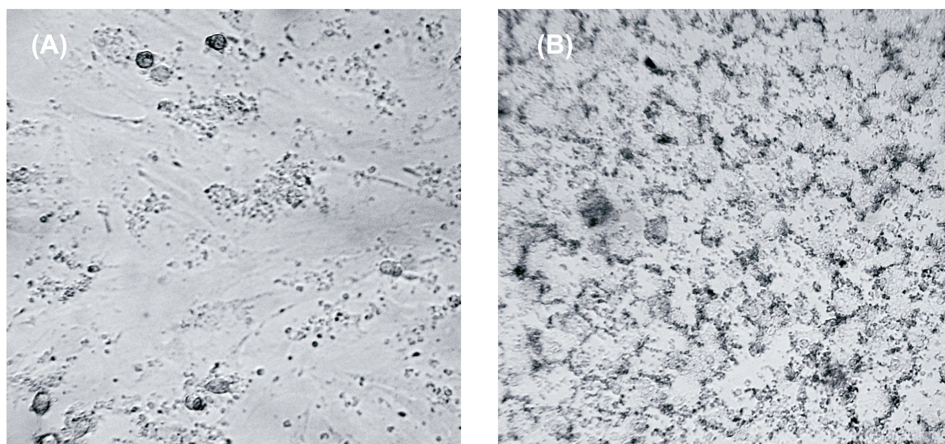


Fig. 1. Effect of lead on monolayer of ovarian granulosa cells. A – Control represents culture medium without lead addition. B – Group Max received lead acetate at 1.0 mg/cm^3. Light microscopy (Magnification 45x)

cells in the group Max (6.95 ± 0.84 ng/cm^3) was significantly ($p < 0.05$) inhibited after Pb administration. Groups A (21.07 ± 3.23 ng/cm^3), B (27.72 ± 3.33 ng/cm^3), C (28.03 ± 3.02 ng/cm^3) and D (25.98 ± 0.94 ng/cm^3) showed no significant ($p > 0.05$) differences in comparison with control group (Fig. 2). The lowest amount of P_4 was released by ovarian cells in the experimental group Max with the highest Pb administration used in this study.

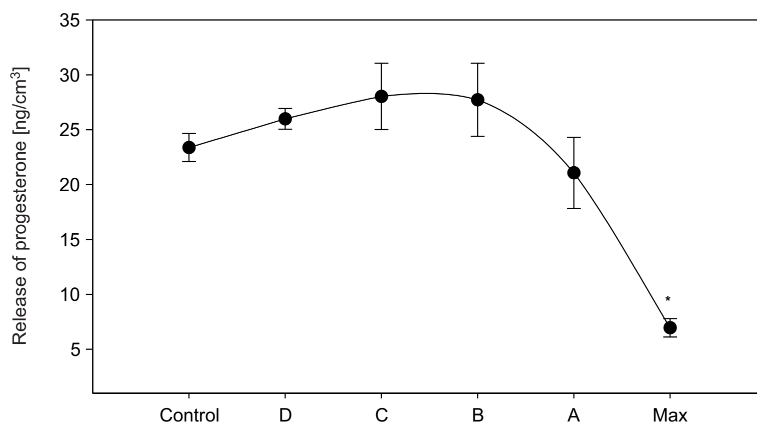


Fig. 2. Effect of lead on progesterone release by porcine ovarian granulosa cells. Control represents culture medium without lead addition. Group Max received lead acetate at 1.0 mg/cm^3; group A 0.5 mg/cm^3; group B 0.33 mg/cm^3; group C 0.17 mg/cm^3; and group D 0.09 mg/cm^3. Values are means ± SEM. * Significant differences in comparison with control $p < 0.05$ were evaluated by paired t-test. RIA

The reduction of the monolayer of granulosa cells after Ag addition by light microscopy (Fig. 3) was found, too. The release of P_4 by ovarian granulosa cells was 24.72 ± 1.56 ng/cm^3 in the control group (Fig. 4). Release of P_4 by granulosa cells of

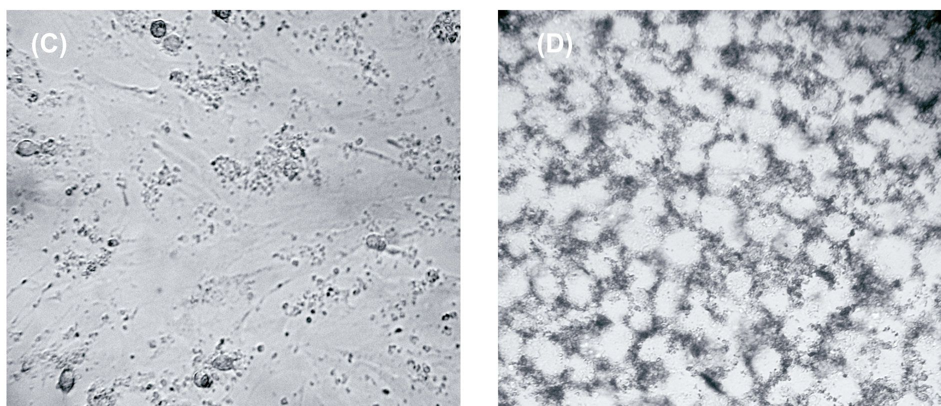


Fig. 3. Effect of silver on monolayer of ovarian granulosa cells. C – Control represents culture medium without silver addition. D – Group Max received silver nitrate at 1.0 mg/cm^3. Light microscopy (Magnification 45x)

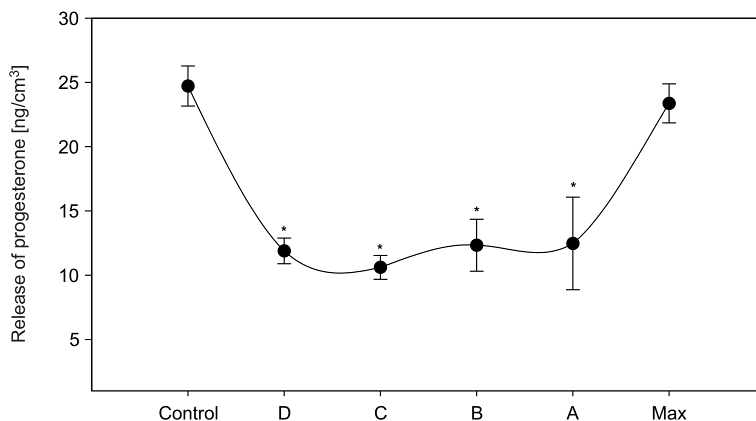


Fig. 4. Effect of silver on progesterone release by porcine ovarian granulosa cells. Control represents culture medium without silver addition. Group Max received silver nitrate at 1.0 mg/cm^3; group A 0.5 mg/cm^3; group B 0.33 mg/cm^3; group C 0.17 mg/cm^3; and group D 0.09 mg/cm^3. Values are means ± SEM. * Significant differences in comparison to control $p < 0.05$ were evaluated by paired t-test. RIA

experimental groups A (12.47 ± 3.60 ng/cm^3), B (12.33 ± 2.02 ng/cm^3), C (10.61 ± 0.93 ng/cm^3) and D (11.89 ± 1.00 ng/cm^3) showed significant ($p < 0.05$) inhibition compared with the control group (Fig. 4). Similar release of P_4 was found in control and Max groups (23.37 ± 1.52 ng/cm^3).

The reduction of the monolayer of granulosa cells after Mo addition was found with light microscopy (Fig. 5). The release of P_4 by porcine ovarian granulosa cells was 17.01 ± 2.53 ng/cm^3 in the control group. P_4 release by granulosa cells of experimental groups A (21.59 ± 1.38 ng/cm^3), B (22.29 ± 2.85 ng/cm^3), C (20.93 ± 0.99 ng/cm^3) and D (16.61 ± 1.60 ng/cm^3) showed no significant ($p > 0.05$) differences in comparison

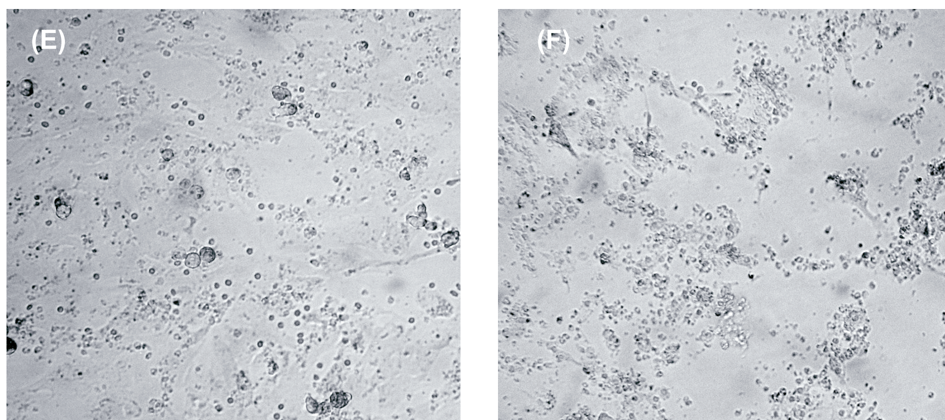


Fig. 5. Effect of molybdenum on monolayer of ovarian granulosa cells. E – Control represents culture medium without molybdenum. F – Group Max received ammonium molybdate at 1.0 mg/cm^3. Light microscopy (Magnification 45x)

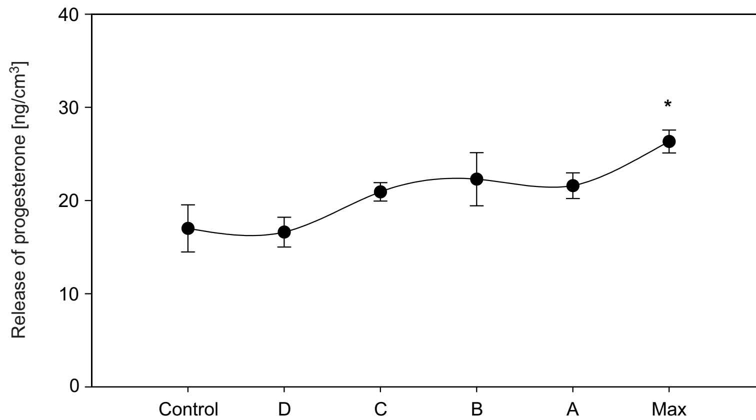


Fig. 6. Effect of molybdenum addition on progesterone release by porcine ovarian granulosa cells. Control represents culture medium without molybdenum addition. Group Max received ammonium molybdate at 1.0 mg/cm^3, group A 0.5 mg/cm^3, group B 0.33 mg/cm^3, group C 0.17 mg/cm^3, group D 0.09 mg/cm^3. Values are means ± SEM. *Significant differences in comparison with control $p < 0.05$ were evaluated by paired t-test. RIA

with control group (Fig 6). Significant ($p < 0.05$) increase comparing with the control group was found in group Max (26.33 ± 1.23 ng/cm^3).

Discussion

Our reports confirm previous data about influence of heavy metals on cellular processes [3, 4, 11, 33]. Neurodevelopmental toxins, such as heavy metals, interrupt growth factor signalling [34]. The effect of metals on organisms can range from acute mortality to chronic effects such as reductions in growth and reproductive output [35]. The levels of elements in follicular fluid (FF) of patients and evaluate the relationship between the concentration of elements in FF, follicular volume, and blood was determined by Silberstein [36].

Lead is the most extensively studied reproductive and developmental toxicant [3, 4, 11]. An accumulation of Pb in granulosa cells of the rat ovaries [9], sheep ovaries [10], chicken granulosa cells [4], human ovarian granulosa cells [37] and porcine granulosa cells [3, 5] was reported. Our observations represent the demonstration of Pb influence on secretory activity of porcine ovarian granulosa. In our study isolated ovarian granulosa cells were able to survive in culture and the release hormones P_4. In our experiments steroid hormone progesterone was released by porcine ovarian granulosa cells. Our observations confirm previous reports on the production of progesterone by porcine ovarian granulosa cells [4, 28, 31]. Pb can cause a reduction in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) binding, which significantly alters steroid production *in vitro* and exerts a direct influence on granulosa cell function [38]. In our study P_4 release by granulosa cells was inhibited after Pb addition at the highest dose 1.00 mg/cm^3. The Pb concentrations (0.5–0.046 mg/cm^3) used in the

present study did not induce the release of P_4 by porcine ovarian granulosa cells [3]. On the contrary, the P_4 release by ovarian granulosa cells of pregnant gilts was significantly stimulated by Pb addition at doses of 0.25 mg/cm^3 and 0.063 mg/cm^3 [3]. Similarly, the P_4 release by granulosa cells of pregnant gilts was significantly stimulated by Hg addition at the doses of 0.25 mg/cm^3 and 0.083 mg/cm^3. P_4 release by ovarian cells of pregnant gilts was not influenced by FSH (1.0 ng/cm^3) + Pb (0.083 mg/cm^3) + Hg (0.083 mg/cm^3) but it was inhibited by the FSH (10 ng/cm^3) + Pb (0.25 mg/cm^3) + Hg (0.25 mg/cm^3) administrations. Further observations suggest possible involvement of the heavy metals Pb and Hg, and pituitary hormone FSH, in the regulation of P_4 release by porcine ovarian granulosa cells of pregnant gilts. Progesterone release by chicken granulosa cells was stimulated after 0.33 mg/cm^3 lead addition [4]. In another report, lead seemed not to exert a specific effect on the steroidogenesis in cultured human granulosa cells, but lead application *in vitro* at 1.600 μM (331.5 mg/dm^3) resulted in a significant decrease in progesterone production. The lead levels measured in the ovarian follicular fluid seemed not to pose a hazard with respect to progesterone secretion by the ovary [37]. The highest production of P_4 by porcine ovarian granulosa cells, in the case cadmium treatment, was found in the group with addition of 10 ng/cm^3 cadmium chloride ($CdCl_2$), and when the dose of cadmium was increased to 20 ng/cm^3 $CdCl_2$ its production decreased [29]. Cadmium-induced alterations in the production of progesterone by the human granulosa cells were determined after exposure to concentrations of 8, 16, 32 and 64 μM $CdCl_2$ for 2, 4, 8, 24 and 48 h [37]. Data obtained from *in vitro* study indicate that the hormonal release by porcine ovarian granulosa cells is associated with the dose of the metals administration, animal species, and also depends on pregnancy of animals.

The increased use of nano-sized metallic materials is likely to result in the release of these particles into the environment [39]. Silver is not toxic to humans and is not known to cause cancer, reproductive or neurological damage or other chronic adverse effects. Microorganisms could develop resistance to antibacterial silver because an organism would have to undergo simultaneous mutations in every one of its critical functions within a single generation to avoid the effects of silver [40]. This study was conducted to test the effect of Ag on secretory activity of porcine ovarian granulosa cells. In our experiments steroid hormone progesterone was released by porcine ovarian granulosa cells after experimental Ag administration. The release of steroid hormone P_4 by granulosa cells was inhibited by Ag addition at the doses 0.5 mg/cm^3, 0.33 mg/cm^3, 0.17 mg/cm^3 and 0.09 mg/cm^3. Similar release of P_4 was found in control and group with the highest experimental Ag (1.00 mg/cm^3) administration. In other report the progesterone-AAG (α_1-acid glycoprotein, orosomucoid) interaction was inhibited by $Hg^{2+} > Ag^+ > Cu^+ > Fe^{2+}$ [41]. The *in vitro* steroid binding process was found to be sensitive to the presence of certain metal ions. The cations seemed to interfere directly with SH groups at the progesterone binding site. The most potent inhibitor of the binding was Hg(II) (50 % decrease in binding in the presence of 8 μmol · dm^{-3}) followed by Cu(II) (10 μmol · dm^{-3}), Ag(I) (13 μmol · dm^{-3}), Zn(II) (17 μmol · dm^{-3}) and Fe(II) (2.5 mmol · dm^{-3}) [42]. The role of Ag in control of porcine ovarian granulosa cells functions related to P_4 is not known yet. The results of our investigation

show that Ag, when given at the highest dose, did not affect P_4 output by porcine ovarian granulosa cells, but Ag at lower doses decreased release of P_4 by granulosa cells. This chemical element can be suppressor of ovarian steroidogenesis and potential risk factor for reproductive functions regulated by steroid hormones.

Exposure to a number of metals can affect neuroendocrine and thyroid signalling, which can result in adverse effects on development, behaviour, metabolism, reproduction, and other functions [43]. The testes are more sensitive to Mo exposure to the female reproductive organs [44]. Our studies are first contribution about the effect of Mo on the ovarian chicken granulosa cells [4] and porcine ovarian granulosa cells. Progesterone release by porcine ovarian granulosa cells was stimulated by the addition of Mo at the dose 1.0 mg/cm^3. Our previous study [4] shows that progesterone release by chicken granulosa cells was stimulated by Mo doses 0.17 mg/cm^3 and 0.33 mg/cm^3. The effect of an induced Cu deficiency on the fertility of South Africa Mutton Merino ewes (*Ovis aries*) was investigated. The incidence of estrus of adult ewes suffering from an induced Cu deficiency by supplementing molybdenum (Mo – 38 mg Mo/kg feed) and sulphur (S – 0.34 %) to their diet was compared with that of a control group (Mo – 1.3 mg/kg; S – 0.22 %). No significant differences in plasma progesterone concentrations were recorded during the estrus cycles. It is suggested that Mo and S induced Cu deficiency inhibits gonadothropin releasing hormone (GnRH) release or the production of FSH and/or LH to such an extent that cyclicity in the ewe is suppressed [45]. Meador et al [46] show that Mo, membrane-permeable form of the Ca^{2+} – chelating agent EGTA, or protease inhibitors substantially increases detectable rat uterine progesterone receptors. Thiomolybdate depressed estradiol production in a dose-dependent manner at doses >1 $\mu g/cm^3$ and prevented the characteristic clumped appearance of granulosa cells in this serum-free system. Although the supplementation of copper alone had no effect at physiological doses, the use of the equimolar copper and thiomolybdate media ameliorated the effect of tetrathiomolybdates on both estradiol production and cellular morphology [47]. In our present study the effect of Mo addition on the progesterone release by granulosa cells was examined. The report shows a direct influence of Mo on granulosa cells functions (steroidogenesis).

Data obtained from these *in vitro* studies bring new knowledge that release of steroid hormone progesterone by porcine granulosa cells is associated with doses and variety of chemical treatments (Pb, Ag, Mo). Obtained data indicate the interference of these endocrine disruptors in the pathways of steroidogenesis of porcine ovarian granulosa cells.

Acknowledgement

The authors are thankful to Katarina Tothova and Ing. Zofia Kuklova for skillful technical assistance. This work was financially supported by APVV SK-PL-0007-09, APVV project 0299-06 and VEGA scientific grant 1/0696/08.

References

[1] Ishaq M., Khan M.A., Jan F.A. and Ahmad I.: Environ. Monit. Assess. 2009 Jul 10 in press.
[2] Järup L.: Brit. Med. Bull. 2003, **68**, 167–182.

[3] Kolesarova A., Roychoudhury S., Slivkova J., Sirotkin A., Capcarova M. and Massanyi P.: *Toxic hazardous substances and environmental engineering*, J. Environ. Sci. Health, Part A. 2010, **45**, in press.
[4] Kolesarova A., Capcarova M., Sirotkin A. and Massanyi P.: Int. J. Poult. Sci. 2009, **8**, 890–895.
[5] Kolesarova A., Slivkova J., Sirotkin A., Massanyi P. and Capcarova M.: Slovak J. Animal Sci. 2009, **42**, 35–41
[6] Shan G., Tang T. and Zhang X.: J. Huazhong Univer. Sci. Technol. Med. Sci. 2009, **29**, 68–72.
[7] Zhuang P., Zou B., Li N.Y. and Li Z.A.: Environ. Geochem. Health 2009, **29** [Epub ahead of print].
[8] Chrastný V., Komárek M. and Hájek T.: Environ. Monit. Assess., 2009, **42**(3), 320–331.
[9] Nampoothiri L.P. and Gupta S.: Reproduct. Toxicol. 2006, **21**, 179–185.
[10] Bires J., Maracek I., Bartko P., Biresova M. and Weissova T.: Veter. Human Toxicol. 1995, **37**, 349–356.
[11] Kolesarova A., Slamecka J., Jurcik R., Tataruch F., Lukac N., Kovacik J., Capcarova M., Valent M. and Massanyi P.: *Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters*, J. Environ. Sci. Health, Part A Toxic/Hazard. Substan. Environ. Eng. 2008, **43**, 646–650.
[12] The Silver Institute, 2005, http://www.silverinstitute.org/silver
[13] Miao A.J., Schwehr K.A., Xu C., Zhang S.J., Luo Z., Quigg A. and Santschi P.H.: Environ. Pollut. 2009, **157**, 3034–3041.
[14] Fabrega J., Fawcett S.R., Renshaw J.C. and Lead J.R.: Environ. Sci. Technol. 2009, **43**, 7285–7290.
[15] Saaverda Y., González, A. and Blanco J.: Food Addit. Contamin., Part A Chem., Anal., Control, Expos. Risk Assess. 2008, **25**, 1339–1344.
[16] Sun Hu C., Tan Q., Liu J. and Liu H.: Botany (London), 2009, **104**, 345–356.
[17] Reilly C.: Blackwell Publishing Ltd. 2004, 238 p.
[18] Anke M., Groppel B., Krause U., Arnhold W. and Langer M.: J. Trace Elements and Electrolytes in Health and Disease 1991, **5**, 69–74.
[19] Zahalak M., Pratte B., Werth K.J. and Thiel T.: Molec. Microbiol. 2004, **51**, 539–549.
[20] Mendel R.R. and Haunsch R.: J. Exp. Bot. 2002, **53**, 1689–1698.
[21] Tejada-Jiménez M., Galván A., Fernández E. and Llamas A.: Curr. Opinion Plant Biol. 2009, **12**, 358–363.
[22] Ogata A., Yanagie H., Ishikawa E., Morishita Y., Mitsui S., Yamashita A. and Hasumi K. et al: Brit. J. Cancer 2008, **98**, 399–409.
[23] Christofis P., Katsarou M., Papakyriakou A., Sanakis Y., Katsaros N. and Psomas G.: J. Inorg. Biochem. 2005, **99**, 2197–2210.
[24] Ogata A., Mitsui S., Yanagie H., Kasano H., Hisa T., Yamase T. and Eriguchi M.: Biomed. Pharmacother. 2005, **59**, 240–244.
[25] Juarez J.C., Betancourt O. Jr., Pirie-Shepherd S.R., Guan X., Price M.L., Shaw D.E., Mazar A.P. and Don~ate F.: Clin. Cancer Res. 2006, **12**, 4974–4982.
[26] Hagan C.R., Faivre E.J. and Lange C.A.: Steroids 2008, **74**, 568–572.
[27] Arnhold I.J., Lofrano-Porto A. and Latronico A.C.: Hormone Res. 2009, **71**, 75–82.
[28] Meszarosova M., Sirotkin A.V., Grossmann R., Darlak K. and Valenzuela F.: Animal Reproduct. Sci. 2008, **108**, 196–207.
[29] Massanyi P., Uhrin V., Sirotkin A., Paksy K., Forgacs Zs., Toman R. and Kovacik J.: Acta Veter., Brno 2000, **69**, 101–106.
[30] Mahajan D.K.: Sourcebook of Models for Biomedical Research: Humana Press, 2008, 425–436.
[31] Kolesárová A., Sirotkin A. and Kováčik J.: Endokrinné a vnútrobunkové mechanizmy dospievania prasničiek. SPU, Nitra 2008, 131 pp.
[32] Makarevich A.V. and Sirotkin A.V.: Veter. Med. 1999, **44**, 71–78.
[33] Silberstein T., Saphier O., Paz-Tal O., Trimarchi J.R., Gonzalez L. and Keefe D.L.: J. Elements in Med. Biol. 2006, **20**, 205–207.
[34] Waly M., Olteanu H., Banerjee R., Choi S.W., Mason J.B., Parker B.S., Sukumar S., Shim S., Sharma A. and Benzecry J.M.: Molec. Psychiat. 2004, **9**, 358–370.
[35] Jarvinen A.W. and Ankley G.T.: SETAC Press. Pensacola 1999, 1–358.
[36] Silberstein T., Saphier O., Paz-Tal O., Gonzalez L., Keefe D.L. and Trimarchi J.R.: Retility and Sterility 2009, **91**, 1771–1774.
[37] Paksy K., Rajczy K., Forgacs Z., Lazar P., Bernard A., Gati I. and Kaali G.S.: J. Appl. Toxicol. 1997, **17**, 321–327.

[38] Priya P.N., Pillai A. and Gupta, S.: Indian J. Exp. Biol. 2004, **42**, 143–148.
[39] Chae Y.J., Pham C.H., Lee J., Bae E., Yi J. and Gu M.B.: Aquat. Toxicol. 2009, **94**, 320–327.
[40] Platt N.: Polym. Paint Colour J. 2006, **196**, 42–43.
[41] Kerkay J. and Westphal U.: Arch. Biochem. Biophys. 1969, **129**, 480–489.
[42] Kontula K., Janne O., Lukkainen T. and Vihko R.: J. Clinic Endocrinol. Metabol. 1974, **38**, 500–503.
[43] Meeker J.D., Rossano M.G., Potas B., Diamond M.P., Puscheck E., Daly D., Paneth N. and Wirth J.J.: Environ. Res. 2009, **109**, 869–873.
[44] Bersényi A., Berta E., Kádár I., Glávits R., Szilágyi M. and Fekete S.G.: Acta Veter. Hungar. 2008, **56**, 41–55.
[45] Du Plessis S.S., Van Niekerk F.E. and Coetzer W.A.: Small Ruminant Res. 1999, **33**, 71–76.
[46] Meador J., Ilenchuk T.T. and Walters M.R.: J. Steroid Biochem. 1988, **30**, 245–251.
[47] Kendal N.R., Marsters P., Scaramuzzi R.J. and Campbell B.K.: Reproduction 2003, **125**, 657–665.

WPŁYW OŁOWIU, SREBRA I MOLIBDENU NA STEROIDOGENEZĘ *IN VITRO* W KOMÓRKACH ZIARNISTYCH JAJNIKÓW ŚWINI

Abstrakt: Zbadano wpływ ołowiu (Pb), srebra (Ag) i molibdenu na wydzielanie progesteronu (P_4) przez komórki ziarniste jajników świni. Komórki ziarniste inkubowano w obecności octanu ołowiu, azotanu srebra i molibdenianu amonu przez 18 godzin: 1.0 mg/cm^3; 0.5 mg/cm^3; 0.33 mg/cm^3; 0.17 mg/cm^3; 0.09 mg/cm^3. Założono również grupę kontrolną komórek ziarnistych, które nie były eksponowane na jony metali. Wydzielanie progesteronu przez komórki ziarniste zostało zbadane metodą RIA. Wydzielanie sterydu P_4 przez komórki ziarniste zostało zahamowane w sposób statystycznie istotny ($p < 0.05$) przez ołów podany w dawce 1 mg/cm^3. Wydzielenie P_4 zmniejszyło się w obecności Ag w stężeniach 0.5 mg/cm^3; 0.33 mg/cm^3; 0.17 mg/cm^3 i 0.09 mg/cm^3. Stwierdzono statystycznie istotny wzrost wydzielania P_4 po podaniu Mo ($p < 0.05$). Wyniki uzyskane w przedstawionym eksperymencie prowadzonym *in vitro* wykazują, że wydzielanie progesteronu przez komórki ziarniste jajników świni może być modyfikowane przez różne czynniki chemiczne (Pb, Ag, Mo) stosowane w zróżnicowanych dawkach. Odnotowane zjawisko jest prawdopodobnie związane z zaburzeniem niektórych szlaków steroidogenezy zachodzącej w komórkach ziarnistych jajników świni.

Słowa kluczowe: ołów, srebro, molibden, progesteron, steroidogeneza, komórki ziarniste

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Eva OKÉNKOVÁ[1], Jiří KREJČÍ[1],
Jan HRABĚ[1] and Robert VÍCHA[2]

OXIDATIVE CHANGES OF MILK FAT IN DRY MILK STORED UNDER VARIOUS CONDITIONS

ZMIANY OKSYDACYJNE W TŁUSZCZU MLEKA PROSZKOWANEGO PRZECHOWYWANEGO W RÓŻNYCH WARUNKACH

Abstract: Oxidative changes of milk fat in whole dry milk during 50 days of storage under various conditions were examined. Whole dry milk with 26.47 % of fat and insolubility index 1.27 was taken as a sample. Whole dry milk was manufactured by roller drying in YOG s. r. o. Bojkvice. Whole dry milk was stored in desiccators at temperature 37 °C in thermoregulator under various water activities (0.23 and 0.82). Water activity was made by 100 cm^3 of saturated salt solution. Water activity 0.23 was made by saturated solution of potassium acetate and water activity 0.82 was made by saturated solution of potassium bromide. The milk powder was stored for 50 days. The sample with water activity 0.82 became dark brown during storage thanks to products of Maillard reaction. The oxidative changes were examined as a content of hydroperoxides, TBARS (*thiobarbituric reactive substances*), peroxide value, neutralization number, content of conjugated dienes and fatty acids composition. The content of hydroperoxides, TBARS and fatty acids, especially unsaturated fatty acids (oleic acid and linoleic acid) decreased during storage. Neutralization number and peroxide value increased during storage. All chemical parametrer were significantly changed during 50 days of storage under various water activities.

Keywords: oxidative changes, milk fat, storage conditions, water activity, TBARS, hydroperoxides

Dairy products are an important group in human nutrition. They are consumed as such or are used in preparation of many food items to provide specific functional properties [1]. Proteins, carbohydrates and lipids in foods or food ingredients undergo inevitable chemical changes during storage, due to interactions amongst themselves [2]. The Maillard reaction between amino acids residues and carbohydrates, and oxidative changes of amino acid residues by lipid peroxides are typical examples concerning proteins changes [3]. Water activity (A_w) is considered to be a principal factor governing these reactions [4]. Milk lipids may undergo chemical and physical changes

[1] Department of Food Engineering, Thomas Bata University in Zlín, nám. T.G. Masaryka 275, Zlín 762 72, Czech Republic, email: okenkova.e@seznam.cz

[2] Department of Chemistry, Thomas Bata University in Zlín, nám. T.G. Masaryka 275, Zlín 762 72, Czech Republic.

during technological processing such as autooxidation, oxidation and formation of trans fatty acids [5]. Fat oxidation leads to production of low molecular weight substances such as aldehydes, ketones and lactones which can influence the properties of dairy products in inadvisable way for example odour, flavor and color [6]. High content of fat in whole dried milk is one of the major factors which participate in oxidized flavor, other factors are technology of drying, storage conditions above all storage temperature and water activity.

In the present paper, the effect of A_w on the chemical changes in milk fat of whole roller dried milk stored at temperature 37 °C was desribed. The oxidative changes were examined as a content of hydroperoxides, TBARS (thiobarbituric reactive substances), peroxide value, neutralization number, content of conjugated dienes and fatty acids composition in dried milk stored under two A_w (0.23 and 0.82).

Materials and methods

Whole roller dried milk was manufactured from pasteurized cow milk with fat content around 3.5 %. Whole dried milk with 26.47 % of fat, insolubility index 1.85, WPNI 3.66 and raw protein 27.31 % was used as a sample. Fat content was made after cold extraction according to Davídek [7], Whey protein nitrogen index (WPNI) according to Niro method No. A 21a and insolubility index according to ČSN ISO 57 0105 [8]. Saturated solutions of following salts were placed in desiccators to adjust the A_w parameter [9, 10]: potassium acetate for $A_w = 0.23$ and potassium bromide for $A_w = 0.82$. Twenty grams of whole milk powder were put in Petri dish (Φ 15 cm) and the dish was placed in the desiccators and then stored in incubators at a temperature 37 °C. Twenty grams of whole milk powder were packed in polyethylene sachet and then stored as a control sample in refrigerator at 6 ± 2 °C. All samples were analyzed under same conditions and were measured three times and compared with control.

Thiobarbiruc acid reactive substances (TBARS)

TBARS were measured by method of King [10] and results are expressed as an absorbance at $\lambda = 450$ nm, as measured on a Libra S6 spectrophotometer (Biochrom, Cambridge, England). As a first step 2 grams of whole milk powder were reconstituted in 20 cm^3 of distilled water at 30 °C and then the method of King [10] was used. Then 1 cm^3 of trichloracetic acid with a concentration 1 $g \cdot cm^{-3}$ (Lachema, Brno) and 2 cm^3 of ethanol were added to 20 cm^3 reconstituted milk. After 5 minutes the mixture was filtrated through Filtrak 389. Next, 1 cm^3 of TBA solution (Sigma Aldrich, Inc., St Louis, MO, USA), with a concentration of 1.4 g of 2-thiobarbituric acid (TBA) in ethanol to 100 cm^3, was added to 4 cm^3 of clear filtrate. Filtrate with TBA solution was placed in 60 °C water bath for 60 minutes and after cooling the absorbance at $\lambda = 450$ nm was measured. The water modification was made with 2 cm^3 of TBA, with a concentration of 0.05 M were added to 4 cm^3 of clear filtrate which was prepared same as for ethanol modification. Filtrate with TBA solution was placed in 100 °C water

bath for 15 minutes and after cooling the absorbance at λ = 450 nm was measured. Distilled water was used as a blank sample.

Hydroperoxides

Hydroperoxides were measured by method of Ostdal [9] and expressed as an absorbance at λ = 500 nm, as measured on a Libra S6 spectrophotometer (Biochrom, Cambridge, England). 1 gram of whole milk powder was reconstituted in 10 cm^3 of distilled water and after reconstitution the method of Ostdal [9] was applied. 2 cm^3 of reconstituted milk were mixed with 2 cm^3 of methanol and 4 cm^3 of chloroform. Mixture was shaken 30 second and then centrifuged at 1500 xg for 10 minutes using HERMLE Z 300 K (Labortechnik, Wehinaen, Germany). 1 cm^3 of lower chloroform phase was taken and mixed with 1 cm^3 iron(II) thiocyanate solution. Iron(II) thiocyanate solution was prepared by mixing 250 mm^3 of solution I with 250 mm^3 of solution II and adding approximately 25 cm^3 of solution III to yield 25 cm^3 [solution I was prepared by mixing 0.8 % barium chloride dihydrate (Lachema, Brno) with 1 % $FeSO_4 \cdot 7H_2O$ (Lachema, Brno); the solution was filtered and the filtrate was used for the final solution; solution II was 30 % ammonium thiocyanate (Lachema, Brno); solution III was mixture of chloroform and methanol 1:1]. The reaction between chloroform and iron(II) thiocyanate solution run 5 minutes at room temperature and then the absorbance at λ = 500 nm was measured. Distilled water was used as a blank sample.

Extraction of fat by solution of chloroform and methanol

Extraction of fat from the sample was made by the mixture of chloroform and methanol (2:1). The sample was first homogenized with seventeenfold volume of chloroform and methanol for 3 minutes. Suspension was filtered through glass frit S_1 after homogenization. Filtrate was washed by twenty percent of distilled water and water phase was separated. Chloroform phase was washed by mixture of chloroform, methanol and water (3:48:47). Lower chloroform phase was taken to the flask and the phase was evaporated at temperature 37 ^oC.

Neutralization number

10.0000 grams of fat were dissolved in 50 cm^3 of a mixture of ethanol and ether (1:1). Then the mixture of ethanol, ether and oil was titrated by KOH (0.1 mol/dm^3) to turns colour to red [15].

$$k = \frac{a \cdot 5.611}{q}$$

a ... 0.1 mol/dm^3 KOH [cm^3],
q ... weight of fat [g].

Peroxide value

5.0000 grams of fat were dissolved in 50 cm^3 of mixture of acetic acid and chloroform (3:2). After dissolving 2 mm^3 of KI were added. This mixture reacted for 60 seconds and then 100 cm^3 of distilled water and 2 mm^3 of amyloid solution were added. This system was titrated by $Na_2S_2O_3$ (0.01 mol/dm^3) till discolouration. The blank sample, which did not contained fat, was made under the same conditions [15].

$$p = \frac{10 \cdot (b - a)}{q}$$

b ... 0.01 mol/dm^3 $Na_2S_2O_3$ [cm^3],
a ... 0.01 mol/dm^3 $Na_2S_2O_3$ for blank sample [cm^3],
q ... weight of fat [g].

Preparation of fatty acid methyl esters for gas chromatography analysis (GC-MS)

Methyl esters were prepared as described by Davídek [7]. Fat sample extracting from the sample was boiled with 0.5 N methanol solution of NaOH according to Table 1 for 10 minutes in the atmosphere of nitrogen. Then aliquot amount of 12–15 % methanol solution of BF_3 (amount according Table 1) was added and the solution was boiled for 2 minutes. Next, 5 cm^3 of heptane was added and the boiling was other 1 minute. 2 cm^3 of saturated solution of NaCl was added after boiling. Solution was decanted to separator funnel and 15 cm^3 of heptane and 40 cm^3 of saturated solution of NaCl were added. Lower heptane layer was removed and water solution of saturated NaCl was washed by other 15 cm^3 of heptane. Both heptane phases were united and dried by anhydrous Na_2SO_4. Fatty acid methyl esters were determined by gas chromatorgaphy with mass spectroscopy.

Table 1

Conditions for fatty acid methyl esters preparation

Weight of fat [mg]	Amount of NaOH [cm^3]	Amount of BF_3 [cm^3]
100–250	4	5
250–500	6	7
500–750	8	9
750–1000	10	12

Conjugated dienes

0.0250 grams of fat were dissolved in ethanol for UV in 25 cm^3 volumetric flask. Then the absorbance of UV light was measured with UV spectrometr as UV spectrum of dissolved fat.

Results and discussion

Oxidative changes of milk fat during storage under various conditions were examined. Whole roller dried milk was used as a sample. Storage was at temperature 37 ^{o}C and two valued of water activity (0.23 and 0.82). The sample turned dark brown during storage under water activity 0.82 thanks to products of Maillard reaction. The oxidative changes were examined as a content of hydroperoxides, TBARS, peroxide value, neutralization number, content of conjugated dienes and fatty acids composition. Gained results were compared with other similar studies. Initial chemical parameters can be seen from Table 3 and was measured immediately after manufacturing of sample.

Table 2

Initial content of fatty acids in sample without storage

Fatty acid	Content [%]
Caproic acid (C6:0)	1.170 ± 0.125
Caprylic acid (C8:0)	0.900 ± 0.206
Capric acid (C10:0)	2.380 ± 0.266
Lautic acid (C12:0)	3.310 ± 0.308
Myristic acid (C14:0)	12.570 ± 0.885
Palmitic acid (C16:0)	40.330 ± 1.113
Stearic acid (C18:0)	12.390 ± 0.494
Oleic acid (C18:1)	25.540 ± 0.771
Linoleic acid (C18:2)	1.950 ± 0.142

Table 3

Initial values of monitored parameters in sample without storage

Chemical parameters	Value
Hydroperoxides	0.131 ± 0.155
TBARS (ethanol solution)	0.398 ± 0.012
TBARS (water solution)	0.343 ± 0.006
Peroxide value [$\mu gO_2/g$]	1.580 ± 0.004
Neutralization number [mg KOH/g]	0.390 ± 0.0067
Conjugated dienes (absorbance)	0.681 ± 0.173

Hydroperoxides and peroxide value

The influence of water activity on stability of dried milk was main point of many studies of Ostadal [9], Celestino [14] and Hedegaard [13] and but the higher focus was devoted to protein composition of milk. Oxidative stability of dried milk under two different water activities is written in this paper.

As can be seen from Table 4 and 5, the content of hydroperoxides was rapidly increased in desiccators with A_w = 0.23. In sample stored in desicator with A_w = 0.82 has there were not detected any hydroperoxide probably because of the disintegration of present hydroperoxide to another oxidative products. Presented results show that under A_w = 0.82 the creation and disintegration of hydroperoxides is rapidly quick and, therefore, the content of hydroperoxides is immeasurable after 16 days of storage.

Table 4

Chemical parameters of sample after 16 days of storage*

Chemical parameters	Control	A_w 0.23	A_w 0.82
Hydroperoxides	0.101 ± 0.035[a]	0.02 ± 0.007[b]	no reaction[c]
TBARS (ethanol solution)	0.576 ± 0.004[a]	0.244 ± 0.005[b]	0.282 ± 0.004[c]
TBARS (water solution)	0.349 ± 0.002[a]	0.355 ± 0.002[b]	0.296 ± 0.005[c]
Peroxide value [μgO_2/g]	3.014 ± 0.004[a]	20.950 ± 0.004[b]	19.160 ± 0.005[c]
Neutralization number [mg KOH/g]	1.134 ± 0.001[a]	3.180 ± 0.035[b]	3.280 ± 0.007[c]
Conjugated dienes (absorbance)	0.723 ± 0.007[a]	0.924 ± 0.001[b]	0.728 ± 0.018[c]

* Chemical parameters are presented by mean ± standard deviation. Mean values having the same superscript letter in each line are not significantly different ($p \geq 0.05$).

Table 5

Chemical parameters of sample after 50 days of storage*

Chemical parameters	Control	A_w 0.23	A_w 0.82
Hydroperoxides	0.055 ± 0.017[a]	0.135 ± 0.001[b]	no reaction [c]
TBARS (ethanol solution)	0.613 ± 0.008[a]	0.208 ± 0.001[b]	0.247 ± 0.008[c]
TBARS (water solution)	0.437 ± 0.007[a]	0.320 ± 0.015[b]	0.359 ± 0.002[c]
Peroxide value [μgO_2/g]	6.650 ± 0.012[a]	49.550 ± 0.016[b]	34.360 ± 0.005[c]
Neutralization number [mg KOH/g]	3.765 ± 0.002[a]	6.620 ± 0.008[b]	6.220 ± 0.001[c]
Conjugated dienes (absorbance)	1.001 ± 0.016[a]	0.669 ± 0.003[b]	0.567 ± 0.009[c]

* Chemical parameters are presented by mean ± standard deviation. Mean values having the same superscript letter in each line are not significantly different ($p \geq 0.05$).

Content of hydroperoxides and peroxide value are in good correlation in spite of the fact that the method of detection of hydroperoxides cannot detect hydroperoxides under A_w = 0.82. This fact can be due to restricted possibilities of used method for detection of hydroperoxides according to Ostdal [9]. Determination of peroxide value is straight reaction between fat and reactionary chemicals and this can caused better detection of lower concentration of hydropexides than reaction according to Ostdal [9].

The results obtained for hydroperoxides content in this study correlate with available literature, eg with Celestino [14] and Hedegaard [13]. Celestino [14] and Hedegaard [13] examined only the influence of storage time but our paper includes the influence of water activity too. Celestino [14] used spray-dried whole milk as a sample. They found the rapid increasing tendency of hydroperoxides content during storage. While the creation of hydroperoxides has close contexture with ways of drying, subsequent fat

changes are influenced by storage time and storage conditions. Hadegaard [13] founded the same development of hydroperoxides content as is reported in this paper. Their paper indicated as a major factor influencing hydroperoxide content storage time. In general, storage time can be considered as a major factor influencing the formation of primary oxidative products. In studies of Celestino [14] and Hedegaard [13] was used spray dried whole milk as a sample while in this paper was used roller dried whole milk.

Changes of hydroperoxides content under water activity are clearer and quicker than those that proceed during storage without the influence of higher water activity. Content of hydroperoxides established as peroxide value and expressed as absorbance was used for comparison with hydroperoxide value. Peroxide value is shown as a better method for the detection of lower hydroperoxides concentration as can be seen from presented data. These data showed significant ($p \geq 0.05$) changes of fat under various A_w.

TBARS

Thiobarbituric acid reactive substances (TBARS) were measured according to King [10] and were expressed as absorbance at $\lambda = 450$ nm. Water and ethanol modifications were used for comparing the better detection of TBARS. Measuring of absorbance at $\lambda = 450$ nm was used because Patton and Kurts [11] and Jenings [12] found the more intensively absorbing yellow pigment at $\lambda = 450$ nm for dairy products.

The content of TBARS in milk powders stored under different A_w was significantly lower than control as can be seen from Table 4 and 5. This difference may be caused by fact that A_w has the influence only on the rise of primary oxidative product. The difference in TBARS content between A_w 0.23 and 0.82 are significant ($p \geq 0.05$) in spite of not very different values of absorbance. Though, TBARS detected under $A_w =$ $= 0.82$ has higher value of absorbance than those under $A_w = 0.23$. This fact confirmed increasing oxidative changes of lipid in milk powder stored under higher water activity. The same results as for ethanol solution were found for water solution too.

Absorbance value of water solution is higher than ethanol because of different reaction conditions which are used for creation of yellow pigment. Reaction temperature 100 ^{o}C used for water modification can lead to higher absorbance value close to same content of TBARS.

Fatty acids composition and neutralization number

Fatty acids composition is in closed accordance with neutralization number. Neutralization number is an indicator of hydrolysis of triacylglycerols of milk powder fat. Increasing value of neutralization number is an indicator of triacylglycerols decline. Triacylglycerols hydrolysis is influenced by water activity as can be seen from Table 4 and 5. Moreover, there was found difference between two-tested A_w. The neutralization number of milk powder stored under both A_w was twice higher as control.

Content of fatty acids is changed with water activity as can be seen from Table 6. Content of oleic acid (C18:1) and linolenic acid (C18:2) dropped during storage and this changes are closed with increasing values of TBARS, hydroperoxides and decrease of

linolenic acid is oscillated with formation of conjugated dienes. Content of other fatty acids is increased because of triacylglycerols hydrolysis. Slight significant changes of fatty acids were found in comparison with control and milk powder stored under various A_w as can be seen from Table 6.

Table 6

Content of fatty acids after 50 days of storage [%]*

Fatty acid	Control	A_w 0.23	A_w 0.82
Caproic acid (C6:0)	1.591 ± 0.114^a	1.628 ± 0.142^a	1.370 ± 0.120^b
Caprylic acid (C8:0)	1.246 ± 0.144^a	1.279 ± 0.245^a	1.109 ± 0.227^a
Capric acid (C10:0)	3.277 ± 0.276^a	3.125 ± 0.324^a	2.817 ± 0.229^b
Lauric acid (C12:0)	4.606 ± 0.323^a	3.758 ± 0.417^b	4.232 ± 0.185^c
Myristic acid (C14:0)	15.198 ± 0.741^a	14.837 ± 1.246^a	15.338 ± 0.668^a
Palmitic acid (C16:0)	46.967 ± 0.590^a	51.077 ± 2.048^b	47.300 ± 0.700^a
Stearic acid (C18:0)	15.639 ± 0.479^a	13.039 ± 0.542^b	9.847 ± 0.460^c
Oleic acid (C18:1)	16.922 ± 0.834^a	15.010 ± 0.854^b	17.991 ± 0.623^c
Linoleic acid (C18:2)	0 ± 0^a	0 ± 0^a	0 ± 0^a

* Fatty acids are presented by mean ± standard deviation. Mean values having the same superscript letter in each line are not significantly different ($p \geq 0.05$).

Conjugated dienes

Conjugated dienes are formed immediately after peroxides. Conjugated dienes are formed from unsaturated fatty acids especially from linolenic acid (C18:2). Conjugated dienes absorb ultraviolet radiation strongly at $\lambda = 233$ nm. Thus oxidation is following by dissolving the lipid in a suitable organic solvent (ethanol for UV spectroscopy in our case) and measuring the change in its absorbance with UV-visible spectrophotometer.

Milk powder stored under higher Aw has higher content of conjugated dienes than control after 16 days of storage while conjugated dienes detect after 50 days of storage are lower for milk powder stored under higher A_w. These differences are due to the fact that conjugated dienes are later broken down into secondary products, which do not strongly absorb UV-visible light and this leads to a decrease in absorbance. Higher water activity accelerated the oxidative processes and this fact leads to differences between control and samples stored under A_w. Oxidative changes of milk powder stored under standard condition in refrigerator are slower and conjugated dienes in control sample are increased during time of our experiment.

Conclusions

Our research indicates that water activity has influence on oxidative changes of whole milk powder. Whole dried milk is ideal substrate for oxidative changes because of its high fat content. Changes of milk fat stability during storage under various water activities were measured. The oxidative changes were examined as a content of hydroperoxides, TBARS, peroxide value, neutralization number, content of conjugated dienes and fatty acids composition. The content of hydroperoxides, TBARS and fatty

acids especially unsaturated fatty acids (oleic acid and linoleic acid) had decreased during storage. Neutralization number and peroxide value had increased during storage. Significant changes of all monitored chemical parameters were found during 50 days of storage under various water activities.

Research about oxidative changes of milk powder stored under various temperature and water activities is in progress.

References

[1] Meshref A. and Rwaily Al.: Pakistan J. Nutrit. 2008, **7**, 118–125.

[2] Kato H.: Interactions of food constituents, Eds. Namiki M. and Matsushita S., Kodansha, Tokyo 1980, p. 5.

[3] Hurrell R.F. and Carpenter K.J.: Protein Crosslinking, Ed. Fiedman M., Plenum Press 1977, vol. 86 B, p. 255.

[4] Labuza T.P., Tannenbaum S.R. and Karel M.: Food Technol. 1970, **24**, 543.

[5] Semma M.: *Trans fatty acids: properties, benefits and risks*. J. Health Sci. 2002, **48**, 7–13.

[6] Frankel E.N.: J. Amer. Oil Chem. Soc. 1984, **61**, 1908–1917.

[7] Davídek J. et al: Laboratorní příručka analýzy potravin, SNTL, Praha 1977.

[8] ČSN ISO 57 0105, Metody zkoušní mléčných výrobků sušených a zahuštěných. Praha: Úřad pro normalizaci a měření, Praha 1965.

[9] Ostdal H., Andersen H.J. and Nielsen J.H.: *Antioxidative activity of urate in bovine milk*. J. Agric. Food Chem. 2002, **48**, 5588–5592.

[10] King R.L.: *Oxidation of milk fat globule membrane material. I. thiobarbituric acid reaction as a measure of oxidized flavour in milk and models* systems. J. Dairy Sci. 1962, **45**, 1165–1171.

[11] Patton S. and Kurtz G. W.: *A note on the thiobarbituric acid test for milk oxidation*. J. Dairy Sci. 1955, **38**, 901.

[12] Jennings W.G., Dunkley W.L. and Reiber H.G.: *Studies of certain red pigments formed from 2-thiobarbituric acid.* Food Res. 1955, **20**, 13–22.

[13] Hedegaard R.V., Kristen D., Nielsen J.H., Frost M.B., Ostdal H., Hemansen J.E., Kroger-Ohlsen M. and Skibsted L.H.: *Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk.* J. Dairy Sci. 2006, **89**, 495–504.

[14] Celestino E.L., Iyer M. and Roginski H.: *The effects of refrigerated storage of raw milk on the quality of whole milk powder stored for different periods*. Int. Dairy J. 1996, **7**, 119–127.

[15] Lékopis, vydal Avicenum – Zdravotnické nakladatelství Praha, Praha 1987.

ZMIANY OKSYDACYJNE W TŁUSZCZU MLEKA PROSZKOWANEGO PRZECHOWYWANEGO W RÓŻNYCH WARUNKACH

Abstrakt: Badano zmiany oksydacyjne powstające w tłuszczu mleka suszonego przechowywanego przez 50 dni w różnych warunkach. Próbki przygotowano z mleka o wskaźniku nierozpuszczalności 1.27 i zawartości tłuszczu 26,47 %. Badane mleko zostało wyprodukowane w zakładach YOG Bojkovice. Mleko przechowywano w eksykatorze w temperaturze 37 ^{o}C. Aktywność wody na poziomie 0,23 uzyskano dzięki zastosowaniu nasyconego roztworu octanu potasu, natomiast aktywność wody na poziomie 0,82 uzyskano, stosując nasycony roztwór bromku potasu. Proszek mleczny był przechowywany przez 50 dni. Próbki mleka przechowywane przy aktywności wody 0,82 przybrały kolor ciemnobrązowy w wyniku reakcji Maillarda. Zmiany oksydacyjne zostały zmierzone jako zawartość wodoronadtlenków, TBARS, zawartość nadtlenków, liczba zobojętnienia, zawartość dienów sprzężonych oraz skład kwasów tłuszczowych. Zawartość wodoronadtlenków, TBARS, kwasów tłuszczowych, a w szczególności nienasyconych kwasów tłuszczowych (kwas oleinowy, kwas linolowy) uległy zmniejszeniu w czasie przechowywania. Wartości liczby zobojętnienia i zawartości nadtlenków wzrosły w czasie przechowywania. Wszystkie parametry chemiczne uległy znacznym zmianom w czasie 50 dni przechowywania przy zróżnicowanej aktywności wody.

Słowa kluczowe: zmiany oksydacyjne, tłuszcz mleka, warunki przechowywania, aktywność wody, TBARS, wodoronadtlenki

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Dariusz ROPEK[1] and Krzysztof FRĄCZEK[2]

EFFECT OF THE SOLID WASTE LANDFILL IN TARNOW ON THE HEALTHINESS OF SPRING WHEAT

WPŁYW SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE NA ZDROWOTNOŚĆ PSZENICY JAREJ

Abstract: Present work aimed at investigating the degree of spring wheat infection by fungal pathogens in the immediate vicinity of the landfill. The field experiment was conducted in 2006 and 2007 in Tarnow. During vegetation period leaf and ear infection with phytopathogenic fungi was assessed on a 9-degree scale. Both in 2006 and 2007 spring wheat was most strongly attacked by *Erysiphe graminis* on plots located in zone I on the southern, eastern and northern side of the landfill. Leaves and ears infestation by *Septoria nodorum* was lowest on plots located in zone II on the western side of the landfill. Index of *Puccinia recondita* infection was low. Cultivation of spring wheat close to active sector favours stronger plant infection by *Erysiphe graminis* and *Septoria nodorum*.

Keywords: municipal landfill site, fungal diseases, spring wheat

As municipal landfill sites affect their natural environment [1] they pose an important economic and social problem. Moreover, the areas adjoining landfills are exposed to microbiological or chemical pollution, which leads to degradation of conditions of agricultural production. Dispersal of gaseous, dust and microbial pollutants in the atmosphere may pose a hazard not only to plants and animals but also to human life and health [2, 3].

Agricultural activity is often conducted in the vicinity of municipal landfill sites. Landfill sites may disturb the balance in the environment eg through change of habitat conditions, which is visible as a sudden increase in population of pests. Fungal disease development depends on many abiotic factors such as climatic conditions and agrotechnical factors [4, 5]. Also other factors, such as industrial or traffic pollution

[1] Department of Agricultural Environmet Protection, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone 12 662 44 02, email: rrropek@cyf-kr.edu.pl

[2] Department of Microbiology, University of Agriculture, al. A. Mickiewicza 24/28, 30–058 Kraków, Poland, email: rrfracze@cyf-kr.edu.pl

may influence plants infection by phytopathogenic fungi [6, 7]. Municipal landfill sites also emit numerous pollutants which may directly affect plant pathogenic organisms but this effect has not been fully investigated yet. Phytopathogenic fungi developing on wheat inhibit its growth and as a result decrease the yield and worsen seed quality [4].

The present research aimed to investigate the occurrence of spring wheat infection by fungal pathogens in the area immediately adjoining a municipal landfill site.

Material and methods

The field experiment was conducted in 2006 and 2007 in Tarnow. The solid waste landfill site in Tarnow, around which the studies were carried out, is located in the northern city quarter. Observations were conducted on experimental plots located in the immediate vicinity of the landfill. The experimental points were set up on each side of the landfill in two zones: below 250 m and 250–500 m from its boundaries. Labelling of experimental plots is presented in Table 1. Spring wheat was cultivated on 20 m^2 plot. Spring wheat, Zura c.v. was seeded in 2006 in the second decade of April and in 2007 in the third decade of March. Tillage was carried out according to the rules of agrotechnics on all plots.

Table 1

Soil sampling sites in the vicinity of the municipal landfill site in Tarnow

Point	Localization of points with respect to landfill site	
	Direction	Zone [m]
W I	West	below 250
W II	West	250–500
N I	North	below 250
N II	North	250–500
E I	East	below 250
E II	East	250–500
S I	South	below 250
S II	South	250–500

Wind distribution in the area of Tarnow city is as follows: north winds – 6 %, north-east winds – 7.1 %, east winds – 16.7 %, south-east winds – 4.8 %, south winds – 14.8 %, south-west winds – 7.4 %, west winds – 22.6 %, north-west winds – 8.8 % and calm air – 11.8 %. Measurements of emission and composition of biogas were conducted in places where plants where cultivated. The measurements were carried out using al device for measuring landfill gas composition – Polytector II G 750(Germany). The contents of biogas components in the air surrounding the landfill are presented in Table 2. The lowest methane concentrations occurred in zone II on the eastern, northern and western side of the landfill.

Table 2

Mean value of biogas components in the air surrounding the municipal landfill site in Tarnow (from April 2006 to October 2007)

Indicator	Unit	Measuring point							
		S I	S II	E I	E II	N I	N II	W I	W II
Methane (CH_4)	ppm	0.9	0.4	0.7	0.5	0.5	0.0	0.0	0.0
Hydrogen sulfide H_2S	ppm	Not registered							
Carbon dioxide (CO_2)	%	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.04
Oxygen (O_2)	%	20.9	20.9	20.9	20.9	20.9	20.9	20.9	20.9

Leaf and ear infection with *Erysiphe graminis, Puccinia recondita* f. sp. *Tritici* and *Septoria nodorum* were assessed on a 9-degree scale: 9 – 0–5 %, 8 – 6–15 %, 7 – 16–25 %, 6 – 26–40 %, 5 – 41–60 %, 4 – 61–75 %, 3 – 76–85 %, 2 – 86–95 %, 1 – 96–100 % damages [8]. The results were verified statistically using Statistica programme. Three factor ANOVA was conducted, Newman-Keuls critical intervals were computed and the value of the final step was used for means differentiation at significance level $p < 0.05$.

Results and discussion

The obtained results revealed that at earing maturity spring wheat leaves were more strongly attacked by *Erysiphe graminis* in zone I than in zone II (Table 3). The decreased plant infection by *Erysiphe graminis* was found on plots located on the western side of the landfill. At grain milk maturity plants infestation by *Erysiphe graminis* was the highest on plots located in the I zone on the eastern and southern site of landfill.

Table 3

Mean index of spring wheat infection by *Erysiphe graminis*

Direction (a)	Index of leaves infection [9 ^{o}] at earring maturity						
	Year (c)						
	2006		2007		Mean 2006–2007		
	Zone (b)						Mean
	I	II	I	II	I	II	
South	8.48	8.70	8.14	8.41	8.31	8.56	8.43
East	8.44	8.69	7.94	8.38	8.19	8.54	8.36
North	8.43	8.56	8.02	8.50	8.23	8.53	8.38
West	8.60	8.64	8.36	8.55	8.48	8.60	8.54
Mean	8.57		8.29		8.30	8.55	
$LSD_{p < 0.05}$; a – 0.094, b – 0.050, c – 0.050, a × b × c – 0.257							

Table 3 contd.

Direction (a)	Index of leaves infection [9 °] at grain milk maturity						
	Year (c)						
	2006		2007		Mean 2006–2007		
	Zone (b)						Mean
	I	II	I	II	I	II	
South	8.65	8.84	8.42	8.74	8.54	8.79	8.66
East	8.62	8.82	8.49	8.80	8.56	8.81	8.68
North	8.56	8.84	8.56	8.78	8.56	8.81	8.69
West	8.88	8.85	8.74	8.71	8.81	8.78	8.80
Mean	8.76		8.66		8.62		8.80
$LSD_{p<0.05}$; a – 0.061, b – 0.032, c – 0.032, a × b × c – 0.167							

Plants infestation by *Puccinia recondite* was similar on all plots, irrespective of their location with respect to the landfill (Table 4).

Table 4

Mean index of spring wheat infection by *Puccinia recondita* f. sp. *tritici*

Direction (a)	Index of leaves infection [9 °]						
	Year (c)						
	2006		2007		Mean 2006–2007		
	Zone (b)						Mean
	I	II	I	II	I	II	
South	8.77	8.92	8.46	8.93	8.62	8.93	8.77
East	8.49	8.66	8.43	8.72	8.46	8.69	8.58
North	8.54	8.73	8.49	8.51	8.52	8.62	8.57
West	8.93	8.92	8.9	8.73	8.92	8.83	8.87
Mean	8.75		8.65		8.63	8.77	
$LSD_{p<0.05}$; no significant difference							

Spring wheat leaves were more strongly attacked by *Septoria nodorum* than ears (Table 5). Among compared plots the highest plant leaves infestation was observed in zone I on eastern and northern site of landfill. The plots on these sites were placed closest to the active sector. Obviously lower plants infestation by phyto-pathogenic fungi was observed on plots situated at a long distance from the active landfill sector.

Table 5

Mean index of leaves and ears of spring wheat infection by *Septoria nodorum*

Direction (a)	Index of leaves infection [9°]						
	Year (c)						
	2006		2007		Mean 2006–2007		
	Zone (b)						Mean
	I	II	I	II	I	II	
South	8.14	8.46	8.10	8.33	8.12	8.40	8.26
East	8.05	8.04	7.75	7.89	7.90	7.97	7.93
North	8.36	8.41	7.72	8.40	8.04	8.41	8.22
West	8.43	8.44	8.27	8.36	8.35	8.40	8.38
Mean	8.29		8.10		8.10	8.29	
$LSD_{p < 0.05}$; a – 0.085, b – 0.045, c – 0.045, a × b × c – 0.234							

Direction (a)	Index of ears infection [9°]						
	Year (c)						
	2006		2007		Mean 2006–2007		
	Zone (b)						Mean
	I	II	I	II	I	II	
South	8.25	8.57	8.29	8.45	8.27	8.51	8.39
East	8.28	8.31	8.17	8.30	8.23	8.31	8.27
North	8.18	8.17	8.12	8.24	8.15	8.21	8.18
West	8.54	8.60	8.23	8.55	8.39	8.58	8.48
Mean	8.36		8.29		8.26	8.40	
$LSD_{p < 0.05}$; a – 0.077, b – 0.041, c – 0.041, a × b × c – 0.211							

The gaseous (methane) and microbiological pollutants originates on municipal waste landfill sites and moves to the adjoining terrains with air currents. The plots situated on the eastern and northern side of the landfill in zone I are most exposed to the emission from the landfill. The highest methane concentrations were registered on these plots. It is connected with east wind prevailing in this area. Other authors also found that pollution may negatively affect plant healthiness [6, 7]. According to Stompor-Chrzan [6] ears of winter wheat were attacked more intensively by *Septoria nodorum* when cultivars were grown near the Nitrogen Plant. On the other hand, *Erysiphe graminis* infection was more intensive on control plantation. The obtained results evidence that pollutants originating from municipal landfill site may have stimulating effect on the course of some fungal disease development on spring wheat.

Conclusions

Spring wheat leaves and ears infections by phytopathogenic fungi depended on the localization of plots with respect to the landfill. Plants growing in the immediate vicinity of the active landfill sector were most strongly attacked by *Erysiphe graminis* and *Septoria nodorum*.

Acknowledgement

The research project has been supported by a grant No 2P06R 018 30 from KBN – The Polish State Committee for Scientific Research.

References

[1] Frączek K.: Acta. Agr. Silv. Ser. Agr. 2004, **42**, 87–96.
[2] Chan Y.S.G., Chu L.M. and Wong M.H.: Environ. Pollut. 1997, **97**(1–2), 39–44.
[3] Dobrzyńska D., Kulig A. and Kutla G.: Pr. Nauk. Polit. Warszaw. 1998, **26**, 153–172.
[4] Kuś J. and Mróz A.: Prog. Plant Protect./Post. Ochr. Roślin 2001, **41**(2), 740–743.
[5] Korbas M.: Prog. Plant Protect./Post. Ochr. Roślin 2004, **44**(1), 147–154.
[6] Stompor-Chrzan E.: Prog. Plant Protect./Post. Ochr. Roślin 1998, **5**(8–9), 751–755.
[7] Nadolnik M.: Prog. Plant Protect./Post. Ochr. Roślin, 1998, **5**(8–9), 743–749.
[8] Metodyka badania wartości gospodarczej odmian (WGO) roślin uprawnych. 1. Rośliny rolnicze. 1.1. Zbożowe. Jęczmień, Owies, Pszenica zwyczajna, Pszenżyto, Żyto. Centralny Ośrodek Badania Odmian Roślin Uprawnych, Słupia Wielka 1998.

WPŁYW SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE NA ZDROWOTNOŚĆ PSZENICY JAREJ

[1] Katedra Ochrony Środowiska Rolniczego, [2] Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem pracy było zbadanie stopnia porażenia pszenicy jarej przez patogeny grzybowe w strefie bezpośrednio przylegającej do składowiska odpadów komunalnych. Badania przeprowadzono w 2006 i 2007 roku w Tarnowie. W okresie wegetacji prowadzono obserwacje występowania objawów chorobowych powodowanych przez patogeny grzybowe. Stopień porażenia roślin oceniano w skali 9°. Zarówno w 2006, jak i 2007 r. najsilniej porażone przez mączniaka rośliny pszenicy jarej obserwowano na poletkach znajdujących się w I strefie po południowej, wschodniej i północnej stronie składowiska. Objawy porażenia liści i plew roślin przez septoriozę obserwowano w najmniejszym nasileniu na poletkach po zachodniej stronie w II strefie. Stopień porażenia liści przez rdzę brunatną był niewielki. Uprawa pszenicy jarej w pobliżu czynnego sektora skaldowska sprzyjała silniejszemu porażeniu roślin przez mączniaka i septoriozę.

Słowa kluczowe: składowisko odpadów komunalnych, choroby grzybowe, pszenica jara

Vol. 17, No. 1 2010

Dana TANČINOVÁ[1], Soňa FELŠÖCIOVÁ,
Mária DOVIČIČOVÁ, Zuzana MAŠKOVÁ,
Roman LABUDA[2] and Soňa JAVOREKOVÁ

ENDOGENOUS CONTAMINATION OF WHEAT BY SPECIES OF GENERA *Aspergillus* AND *Penicillium*

ENDOGENNE ZANIECZYSZCZENIA PSZENICY PRZEZ GATUNKI RODZAJU *Aspergillus* I *Penicillium*

Abstract: The aim of this study was to analyse the endogenous mycobiota of superficially sterilised wheat grains with the focus on *Aspergillus* (including two teleomorphs) and *Penicillium* genera. The Slovak wheat samples (*Triticum aestivum* L.) were harvested in the season 2006. The total of 6 wheat samples grown under conditions of the conventional and 12 of the ecological farming system were investigated for the presence of microscopical fungi. A total of 17 genera were recovered as members of the endogenous mycobiota on Dichloran Rose Bengal Chloramphenicol agar (DRBC) and Dichloran Yeast Extract 18 % Glycerol agar (DYSG). On DRBC were detected *Aspergillus* and *Penicillium* species only from the ecological agriculture, namely *A. candidus*, *A. flavus, A. niger*, *Emericella nidulans*, *Eurotium amstelodami*, *E. chevalieri*, *Eurotium* sp., *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. corylophilum, P. crustosum, P. griseofulvum, P. viridicatum* and *Penicillium* sp. On DYSG were detected *Eurotium* species (*E. amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*) and *Penicillium* species (*P. griseofulvum*, *P. hordei*) both from ecological and conventional agriculture. From the ecological wheat was isolated a wider spectrum of fungi on DYSG in comparison with the conventional agriculture, namely *A. flavus*, *A. ochraceus*, *A. sydowii*, *Emericella nidulans, E. amstelodami, E. chevalieri, E. repens, E. rubrum, Eurotium* sp. *P. aurantiogriseum*, *P. crustosum*, *P. solitum* and *Penicillium* sp. The isolates of potentially toxigenic species of *Aspergillus*, *Emericella* and *Penicillium* were tested for their ability to produce particular toxic metabolites, ie mycotoxins *in vitro* by means of a thin layer chromatography (TLC). All the tested isolates were obtained from the samples of ecological agriculture. Out of 18 screened isolates 11 produced at least one mycotoxin and a production was vague in 2 isolates. One isolate (out of one) produced sterigmatocystin, 6 (out of 11) cyclopiazonic acid (production was vague in 2 isolates), and patulin 3 (out of 3). Conversely, none of potentially aflatoxinogenic isolates (*Aspergillus flavus*) tested in this study produced aflatoxins. Two isolates were tested for the production of ochratoxin A, *Aspergillus niger* did not produce ochratoxin A and in *A. ochraceus* production was unclear.

Keywords: fungi, *Aspergillus*, *Penicillium*, *Triticum aestivum* L., wheat

[1] Department of Microbiology, Faulty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2 949 76 Nitra, Slovak Republic, email: dana.tancinova@uniag.sk

[2] Romer Labs Division Holding GmbH, Technopark 1, A-3430 Tulln, Austria.

Mould growth in grain normally occurs both in the field and in the storage. Mould growth can spoil the nutritional aspects of the grain and can also result in the production of secondary metabolites that are highly toxic to animals and humans. *Aspergillus* and *Penicillium* species belong to the group of the storage fungi [1]. The members of the genus *Aspergillus* are common contaminants of diverse substrates [2]. The most important possible consequence of their presence in foods and feeds is mycotoxin contamination [3]. Several potentially toxigenic species have been reported to be dominant on cereal grains at low water activities: *A. candidus*, *A. flavus*, *A. niger*, *A. versicolor*, *A. penicillioides* and *Eurotium* spp. (Lacey et al. 1991; Sauer et al. 1992, [4]). The most important mycotoxins, potentially produced by food-borne aspergilli, are aflatoxins, ochratoxin A, sterigmatocystin, cyclopiazonic acid and patulin [5, 6]. Out of genus *Penicillium*, dominant fungi in wheat kernels are: *P. aurantiogriseum*, *P. hordei*, *P. verrucosum*, *P. cyclopium*, *P. polonicum* [7]. According to Pitt and Leistner (1991, [1]) *Penicillium* species can produce 27 different mycotoxins, with three being the most important: ochratoxin, patulin, and citrinin. Ochratoxin A is a potent nephrotoxin, teratogen, and carcinogen. The main, if not the only producer of OTA in European cereals is *P. verrucosum* [8]. Patulin produces adverse neurological and gastrointestinal effects and is produced eg by *P. expansum*, *P. griseofulvum* (Damoglou, Campbell, 1986, [1]). Finaly, citrinin is a nephrotoxin and is produced mainly by *P. citrinum*, *P. expansum*, and *P. verrucosum* [9].

The aim of this study was to investigate endogenous microscopic filamentous fungal contamination of wheat grains grown under conditions of the so-called conventional and ecological agriculture in Slovakia in year 2006 with the focus on genera *Aspergillus* and *Penicillium*. The ability of isolates of potentially toxigenic species to produce the most important mycotoxins was determined by the means of thin layer chromatography.

Materials and methods

The total of 18 samples of wheat grains (*Triticum aestivum* L.) grown under conditions of the so-called conventional (6 samples) and the so-called ecological (12 samples) agriculture and harvested in year 2006 in Slovakia was mycologicaly investigated for the endogenous presence of *Aspergillus* and *Penicillium* species. For this purpose, direct plating method was used. Out of each sample, the amount of 200 morphologically indefectible grains was superficially sterilised and plated on DRBC (*Dichloran Rose Bengal Chloramphenicol* agar; Merck, Germany; 100 grains) and DYSG (*Dichloran Yeast Extract 18 % Glycerol* agar [8]; 100 grains). The superficial sterilisation was achieved by poring grains into 0.4 % solution of chloramine for 2 minutes; grains were consequently 3 times rinsed in sterile distilled water and dried on sterile filter paper. The members of genera *Aspergillus* and *Penicillium* including their perfects were consequently isolated on diagnostic media of CYA (*Czapek Yeast Extract* agar [10]), MEA (*Malt Extract* agar [10]), CY20S (*Czapek Yeast Extract* agar with 20 % Sucrose [10]) and CYA, MEA, CREA (*Creatine-Sucrose* agar [11]), YES (*Yeast Extract* agar [2]; 1000 cm^3 of distilled water), respectively. In all cases, cultivation proceeded for 5–7 days in the dark at 25 ± 1 ^{o}C. To determine particular species,

diagnostic literature was used as follows: Pitt [12], Klich [10], Samson et al [2] and Kubatova [13] for aspergilli and Ramirez [14], Pitt et Hocking [9], Samson et al [2], Samson et Frisvad [15] for penicillia. The ability of selected isolates of potentially toxigenic species to produce relevant mycotoxins *in vitro* conditions was screened by the means of thin layer chromatography (TLC) according to Samson et al [16] modified by Labuda et Tancinova [17]. The cultivation for screening of extracellular metabolites (griseofulvin, patulin, aflatoxin B_1, ochratoxin A) was carried out on YES and for intracellular (sterigmatocystin, cyclopiazonic acid, penitrem A, roquefortin C) on CYA; the conditions of cultivation as described above. In each tested isolate, 3 pieces of mycelium together with the cultivation medium of area of approximately 5 × 5 mm were cut from colonies and extracted in 1000 cm^3 of chloroform-methanol (2:1, v/v) on vortex for 2 minutes. 20 mm^3 of liquid phase of extracts along with standards (Sigma, Germany) were applied on TLC plate (Marchey-Nagel, Germany) and consequently developed in solvent system toluene:ethylacetate:formic acid (5:4:1, v/v/v). The visualisation of extrolites was carried out as follows: cyclopiazonic acid directly in daylight after spraying with the Ehrlich reagent (violet-tailed spot); patulin by spraying with 0.5 % methylbenzothiazolone hydrochloride (MBTH, Merck, Germany) in methanol, heated at 130 ^oC for 8 min and then detectable as a yellow-orange spot; penitrem A after spraying with 20 % $AlCl_3$ in 60 % ethanol, heated at 130 ^oC for 8 min and then detectable as a dark green to black spot on daylight; roquefortin C after spraying with $Ce(SO_4)_2 \cdot 4\,H_2O$ visible as orange spot. Directly under UV light (λ = 365 nm) were visualised following mycotoxins: sterigmatocystin (reddish spot), ochratoxin A (bluish-green), griseofulvin (blue).

Results and discussion

Table 1 shows the results from investigation of the endogenous contamination of wheat grains. From 6 samples of wheat grains grown in the so-called conventional agriculture, no member of genus *Aspergillus* and its perfects was isolated on DRBC and on DYSG were recovered 23 isolates of 4 species of genus *Eurotium*, what represented 3.83 % infestation from the total of 600 investigated kernels. *E. amstelodami* with total number of 17 isolates appeared to be the most encountered species. However, all the isolates were recovered from a single sample of wheat. From 12 samples obtained from the so-called ecological farming system were isolated 19 isolates of 8 species of 3 genera *Aspergillus*, *Eurotium* and *Emericella* on DRBC. That represented 0.42 %, 0.08 % and 1.1 % infested kernels, respectively out of 1185 investigated. On DYSG were detected 46 isolates of 9 species of the same three genera, what represented 0.58 %, 0.17 % and 3.08 % infested grains, respectively out of 1200 tested. In the case of samples from ecological agriculture, the most encountered species on DRBC was *Eurotium chevalieri* and *E. amstelodami* on DYSG. These species are xerophilic and they can be considered as members of typical mycobiota of wheat grains [2, 5, 7]. Genera *Eurotium* and *Emericella* are perfect microscopic filamentous fungi, which have their imperfects in the genus *Aspergillus* [2]. From the list of isolated, all the species except one (*A. sydowii*) are known to produce at least one toxic product in their

Table 1

The endogenous contamination of wheat grains by species of genera *Aspergillus* (and relevant teleomorphs) and *Penicillium* on DRBC and DYSG

Species and genera	DRBC[1]		DYSG[2]	
	Conventional farming system	Ecological farming system	Conventional farming system	Ecological farming system
	Number of isolates	Number of isolates	Number of isolates	Number of isolates
Aspergillus candidus		1		
Aspergillus flavus		2		5
Aspergillus niger		2		
Aspergillus ochraceus				**1**
Aspergilus sydowii				**1**
Aspergillus	**0**	**5**	**0**	**7**
Emericella nidulans		1		2
Emericella	**0**	**1**	0	**2**
Eurotium amstelodami		5	17[3]	20
Eurotium chevalieri		6	1	8
Eurotium repens		1	1	3
Eurotium rubrum			4	2
Eurotium sp.		1		4
Eurotium	**0**	**13**	**23**	**37**
Penicillium aurantiogriseum		12		11
Penicillium chrysogenum		2		
Penicillium corylophilum		2		
Penicillium crustosum		1		3
Penicillium griseofulvum		3	1	
Penicillium hordei			2	
Penicillium viridicatum		4		
Penicillium solitum				1
Penicillium sp.		5		5
Penicillium	**0**	**29**	**3**	**19**
Number of tested wheat grains	**600**	**1185**	**600**	**1200**

DRBC[1] – Dichloran Rose Bengal Chloramphenicol agar; DYSG[2] – Dichloran Yeast Extract 18 % Glycerol agar; [3] only from one sample of wheat.

secondary metabolism. All of the identified species except one (*Emericella nidulands*) are common on grains of cereals including wheat [2, 18]. According to several studies the species of the genus *Penicillium* belong to the dominant cereals microbiota [19–21]. *Penicillium* spp. isolates were not detected in any of the 6 wheat samples from

conventional agriculture on DRBC and on DYSG were found only two species: *P. griseofulvum* and *P. hordei*, what presented 0.5 % ratio from 600 kernels. They are terverticillate species from subgenus *Penicillium* and their primary occurrence is in cereals. According to Pitt et Hocking [9] many species in Section *Penicillium* appear to have their primary natural habit on cereal grains. Taxa in *Penicillium* subg. *Penicillium* are very important in foods and feedstuffs, too because of their widespread occurrence and their ability to produce several potent mycotoxins (Frisvad, 1986, [22]; Mantle, 1987, [22]). From 12 ecological wheat samples 7 *Penicillium* species, namely *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. corylophilum*, *P. crustosum*, *P. griseofulvum*, *P. viridicatum* and *Penicillium* sp. were isolated on DRBC agar. The highest number of these isolates was diagnosed to the species *P. aurantiogriseum* (12), likewise on DYSG (11), where were recovered 4 *Penicillium* species: *P. aurantiogriseum, P. crustosum, P. solitum* and *Penicillium* sp. Out of 1185 tested wheat kernels *Penicillium* spp. isolates presented 2.4 % infestation found on DRBC and 1.6 % on DYSG agar.

Toxin-producing micromycetes are widespread in nature, and when occurring in grains they often reduce both the yield and the quality of grains [23]. Table 2 shows results from screening of selected isolates for *in vitro* production of mycotoxins by means of TLC. The most important mycotoxins in general are aflatoxins [5]. The main causal agent of their presence in cereals is *Aspergillus flavus* (Cotty et al 1994, Cotty 1997, [3]). Beside B aflatoxins, *A. flavus* is also a potential producer of another mycotoxin,

Table 2

In vitro production of mycotoxins by endogenous aspergilli and penicillia isolated from wheat grains tested by means of thin layer chromatography

Species	Number of tested isolates	Detected toxin	Evaluation		
			+	±	–
Aspergillus flavus	7	Af B_1			7
	7	CPA	2	2	3
Aspergillus niger	2	OA			2
Aspergillus ochraceus	1	OA		1	
Emericella nidulans	1	SC	1		
Penicillium crustosum	4	PA	4		
	4	ROC	4		
Penicillium griseofulvum	3	CPA	3		
	3	GRI	3		
	3	P	3		
	3	ROC	3		

Af B_1 – aflatoxin B_1; CPA – cyclopiazonic acid; GRI – griseofulvin; OA – ochratoxin A; P – patulin; PA – penitrem A; ROC – roquefortin C; SC – sterigamtocystin; + production of mycotoxin confirmed; ± production of mycotoxin is not clear; – no production of mycotoxin.

cyclopiazonic acid [2, 18, etc.]. In this study, all of the 7 isolates of this species were tested for the production of both of these mycotoxins by means of TLC. None of isolates showed potential to produce aflatoxin B_1. That is in an agreement with previous results of authors, who were dealing with commodities of Slovak origin, where none of the screened isolates of *A. flavus* was a producer of aflatoxins [21, 24–26]. All these results are in understanding with the authoritative publication of Frisvad et al [18], according to which aflatoxin producing isolates of *A. flavus* are typical for tropics and subtropics. The production of cyclopiazonic acid was found in 2 isolates, 3 isolates did not show the production of CPA and the production by 2 isolates was unclear. Production of another mycotoxin, which is important in cereals, nephrotoxic and carcinogenic ochratoxin A (JECFA, 2001, [18]), was tested in 2 isolates of *A. niger* with negative results and in single isolate of *A. ochraceus* with vague result. Single isolate of *Emericella nidulans* showed itself to be a producer of sterigmatocystin (Table 2). Terverticillate penicillia are very efficient mycotoxin producers [22], what was confirmed also with our results. Four isolates of *P. crustosum* produced penitrem A and neurotoxin roquefortin C and 3 tested isolates of *P. griseofulvum* produced cyclopiazonic acid, griseofulvin, patulin and roquefortin C.

Conclusion

During the investigation of endogenous presence of members of genera *Aspergillus* and *Penicillium* in wheat grains of Slovak origin grown in the so-called conventional and ecological farming system in year 2006 in general higher infestation was found in samples from ecological agriculture. In samples from conventional agriculture following species were detected: *Eurotium amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*, *Penicillium griseofulvum*, *P. hordei*. Higher diversity of both *Aspergillus* and *Penicillium* species was observed in ecological samples: *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. sydowii*, *Emericella nidulans*, *Eurotium amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*, *Eurotium* sp., *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. corylophilum*, *P. crustosum*, *P. griseofulvum*, *P. viridicatum*, *P. solitum* and *Penicillium* sp. Out of 18 isolates screened for *in vitro* production of mycotoxins 11 produced at least one mycotoxin and production was vague in 2 isolates. One isolate (out of one) produced sterigmatocystin, 6 (out of 11) cyclopiazonic acid (production was vague in 2 isolates), and 3 patulin (out of 3). Conversely, none of potentially aflatoxinogenic isolates (*Aspergillus flavus*) tested in this study produced aflatoxins. Two isolates were tested for production of ochratoxin A, *Aspergillus niger* did not produce ochratoxin A and in *A. ochraceus* was production unclear.

Acknowledgement

This study was financially supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA no. 1/3456/06) and KEGA no. 3/2080/07.

References

[1] Santin E.: *Mould growth and mycotoxin production*, [in:] The Mycotoxin Blue Book. Diaz, University Press, Nottingham 2005, 225–234.

[2] Samson R.A., Hoekstra E.S., Frisvad J.C. and Filtenborg O.: Introduction to Food- and Airborne Fungi. Centraalbureau voor Schimmelcultures, Utrecht 2002, 389 p.

[3] Perrone G., Suscal A., Cozzil G., Ehrlich K., Varga J., Frisvad J.C., Meijer M., Noonim P., Mahakarnchanakul W. and Samson R.A.: Stud. Mycol. 2007, **59**, 53–66.

[4] Filtenborg O., Frisvad J.C. and Samson R.A.: *Specific association of fungi to foods and influence of physical environmental factors*, [in:] Introduction to food- and airborne fungi. Samson–Hoekstra–Frisvad–Filtenborg, Centraalbureau voor Schimmecultures, Utrecht 2002, 306–320.

[5] Frisvad J.C.: *Mycotoxins and Mycotoxigenic Fungi in Storage*, [in:] Stored-Grain Ecosystems. Jayas–White–Muir, Marcell Dekker, Inc., New York 1995, 251–288.

[6] Varga J., Juhász A., Kevei F. and Kozakiewicz Z.: Eur. J. of Plant Pathol. 2004, **110**, 627–640.

[7] Andersen B. and Thrane U.: *Food-borne fungi in fruit and cereals and their production of mycotoxins*, [in:] Advances in Food Mycology. Hocking–Pitt–Samson et al, Springer, Berlin 2006, **571**, 137–171.

[8] Lund F. and Frisvad J.C.: J. of Appl. Micotoxin. 2003, **95**, 1117–1123.

[9] Pitt J.I. and Hocking A.D.: Fungi and food spoilage, London 1997, 593 p.

[10] Klich M.A.: Identification of common *Apsergillus* species. Centraalbureau voor Schimmelcultures, Utrecht 2002, 116 p.

[11] Frisvad J.C.: Lett. Appl. Microbiol. 1985, **1**, 109–113.

[12] Pitt J.I.: *Nomenclatorial and taxonomic problems in the genus Eurotium*, [in:] Advances in *Penicillium* and *Aspergillus* systematics, Samson–Pitt, Plenum Press, New York and London 1985, 383–396.

[13] Kubátová A.: http://www.botany.natur.cuni.cz/cz/lide/kubzatova_atlas.php 2006.

[14] Ramirez C.: Manual and atlas of the Penicillia. Elsevier Biomedical Press, Netherlands 1982, 874 p.

[15] Samson R.A. and Frisvad J.C.: Stud. in Mycol. 2004, **49**, 1–173.

[16] Samson R.A., Hoekstra E.S., Lund F., Filtenborg O. and Fisvad J.C.: *Methods for the detection, isolation and characterisation of food-borne fungi*, [in:] Introduction to food- and airborne fungi. Samson–Hoekstra–Frisvad–Filtenborg, Centraalbureau voor Schimmecultures, Utrecht 2002, 283–297.

[17] Labuda R. and Tančinová D.: Ann.Agric. Environ. Med. 2006, **13**, 193–200.

[18] Frisvad J.C., Thrane U., Samson R.A. and Pitt J.I.: *Important mycotoxins and the fungi which produce them*, [in:] Advances in Food Mycology. Hocking–Pitt–Samson et al, Springer, Berlin 2006, **571**, 1–31.

[19] Abramson D., Hulasare R., York R.K., White N.D.G. and Jayas D.S.: J. Stored Prod. Res. 2005, **41**, 67–76.

[20] Čonková E., Laciaková A., Styriak I., Czerwiecki L. and Wilczynska G.: Czech J. Food Sci. 2006, **24**, 33–40.

[21] Tančinová D. and Labuda R.: *Mykotická kontaminácia vybraných surovín rastlinného pôvodu*, [in:] Výživná a technologická kvalita rostlinných produktov a ich potravinárské využitie. SPU, Nitra 2006, 167–194.

[22] Frisvad J.C. and Filtenborg O.: Mycology 1989, **81**, 837–861.

[23] Baliukoniene V., Bakutis B. and Stankevicius H.: Ann. Agric. Environ. Med. 2003, **10**, 223–227.

[24] Piecková E. and Jesenská Z.: Czech Mycol. 2001, **53**, 229–235.

[25] Tančinová D., Dovičičová M. and Labuda R.: *Aspergillus Sekcia Flavi – potenciálny producent mykotoxínov*, [in:] Zborník z medzinárodnej konferencie Rizikové faktory potravového reťazca. SPU, Nitra 2007, 219–223.

[26] Langseth W., Stenwing H., Song L. and Mo E.: Acta Agric. Scand. B Soil Plant Sci. 1993, **43**, 32–37.

ENDOGENNE ZANIECZYSZCZENIA PSZENICY PRZEZ GATUNKI RODZAJU *Aspergillus* I *Penicillium*

Abstrakt: Celem badań było rozpoznanie endogennych grzybów ze szczególnym uwzględnieniem rodzajów *Aspergillus* i *Penicillium* na ziarnach pszenicy poddanych powierzchownej sterylizacji. Próbki pszenicy (*Triticum aestivum* L.) pochodziły ze zbiorów z 2006 r. ze Słowacji. Sześć próbek pszenicy pochodziło z upraw konwencjonalnej, a dwanaście próbek z upraw ekologicznych. Rozpoznano 17 rodzajów endogennych

grzybów wyhodowanych na DRBC (*Dichloran Rose Bengal Chloramphenicol* agar) i DYSG (*Dichloran Yeast Extract 18 % Glycerol* agar). Gatunki z *Aspergillus* i *Penicillium*, tj.: *A. candidus*, *A. flavus*, *A. niger*, *Emericella nidulans*, *Eurotium amstelodami*, *E. chevalieri*, *Eurotium* sp., *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. corylophilum*, *P. crustosum*, *P. griseofulvum*, *P. viridicatum* i *Penicillium* sp. wykryte na DRBC pochodziły wyłącznie z upraw ekologicznych. Na DYSG stwierdzono gatunki z rodzaju *Eurotium* (*E. amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*) oraz z rodzaju *Penicillium* (*P. griseofulvum*, *P. hordei*). Znajdowały się one na ziarnach z obu typów badanych upraw. Więcej gatunków grzybów wyizolowano DYSG z ziarna pochodzącego z upraw ekologicznych niż z ziarna pochodzącego z upraw konwencjonalnych. Były to: *A. flavus*, *A. ochraceus*, *A. sydowii*, *Emericella nidulans*, *E. amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*, *Eurotium* sp. *P. aurantiogriseum*, *P. crustosum*, *P. solitum* i *Penicillium* sp. Wyizolowane gatunki z rodzajów *Aspergillus*, *Emericella* i *Penicillium* zbadano *in vitro* pod kątem produkcji toksycznych metabolitów, tj. mykotoksyn metodą chromatografii cienkowarstwowej (TLC). Wszystkie badane próbki pochodziły z upraw ekologicznych. Spośród 18 próbek wyizolowanych grzybów w 11 stwierdzono obecność przynajmniej jednej mykotoksyny. W 2 próbkach wyizolowanych grzybów obecność mykotoksyny była wątpliwa, w jednej stwierdzono obecność sterigmatocystyny, w 6 (spośród 11) odnotowano obecność kwasu cyklopiazonowego (obecność w dwóch próbkach była wątpliwa), a w 3 próbkach obecna była patulina. W żadnej z wyizolowanych próbek grzybów nie stwierdzono obecności aflatoksyn. Dwie próbki wyizolowanych grzybów zostały poddane testom na obecność ochratoksyny A. *Aspergillus niger* nie produkował ochratoksyny A, natomiast w próbkach *A. ochraceus* obecność ochratoksyny A była wątpliwa.

Słowa kluczowe: grzyby, *Aspergillus, Penicillium*, *Triticum aestivum* L., pszenica

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 1 2010

Alena VOLLMANNOVÁ[1], Ján TOMÁŠ
and Tomáš TÓTH

RISK ELEMENTS' INPUT INTO THE FOOD CHAIN IN OLD LOADED LOCALITIES

METALE CIĘŻKIE W ROŚLINACH UPRAWNYCH UPRAWIANYCH W POBLIŻU DAWNYCH ŹRÓDEŁ ZANIECZYSZCZEŃ

Abstract: The aim of this work is to evaluate the potential influence of three various sources on soil hygiene and plant production in their vicinity. In the soil of the first observed area in the vicinity of the former Nickel Smelter in Sered, the contents of Cd, Ni, Cu and Co were higher than the background values. Agricultural production grown in this area does not pose any risk to human organism with the exception of Cd in barley grain grown in one locality. The soil of the second observed area in the vicinity of Iron Ore Mines in Rudnany in loaded area of Middle Spis, can be evaluated as highly contaminated with As, Cu and Hg. Potatoes are at the highest risk in this area, but determined contents of risky metals in cereals are also higher than the legislative limits of the Food Codex (FC) of the Slovak Republic. In the third observed locality, Zahorska Lowland, in the vicinity of 5 municipal landfills, the determined total Cu, Cd and Ni soil contents were higher than the background values and the metal contents in grain of cereals were higher than the legislative limits. The results suggest that the old loaded localities present potential danger of risky elements input into the human food chain.

Keywords: old loaded areas, soil contamination, risky elements, human food chain

The old loaded localities present potential danger of risky elements input into the human food chain. The old mines, scrapheaps, landfills, industrial and municipal waste are important sources of environmental contamination. The aim of this study is to evaluate the potential influence of three various sources on soil hygiene and plant production in their vicinity. In the past these localities were intensively contaminated by metallic emissions from metallurgical factories as a dominant source of environmental contamination.

The first locality in the wider vicinity of the former Nickel Smelter in Sered is situated in the region of Lower-Povazie, one of the 8 loaded regions in the Slovak Republic [1]. The Nickel Smelter Sered began its activity in 1964. It was oriented at electrolytic nickel and cobalt production, using special steel and nickel salts produced

[1] Department of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic, email: alena.vollmannova@uniag.sk

mainly for the chemical industry. The Albanian iron-nickel ore was the basic raw material for this production. The nickel production appeared to be inefficient because of low quality and high cost of Albanian ore treatment [2]. The nickel production ended in February 1993 and the cobalt production ended in June 1993 [3]. During its activity, the Nickel Smelter produced waste which was cummulated in the form of leached scrap (mineral residuum of nickel ore in nickel and cobalt production) in the neighbourhood of the factory. The scrapheap is to be found in Sered and Dolna Streda territory on the right riverbank of the Vah. It has triangular configuration, its extent is about 32 hectares. According to the calculations, the scrapheap's cubature is 3 950 000 m^3, the weight equals 84 million Mg, the height is estimated to be 20 m. The weight of NH_4^+–N in the scrapheap measures aroud 1 848 000 kg. [4]. The black colouring of the leach scrap caused waste overheating, especially in the summer. Because of the second dusting this waste is the source of potential risky input of heavy metals into the soil near the factory. The environmental risk includes the potential contamination of the underground water in the alluvial gravel of the river Vah. The ash from the heating of the Nickel Smelter is another source of environmental contamination. The waste dump is situated in the territory Dolna Streda in the area of approximately 26 hectares. Both dumps can be the potential sources of underground water contamination by the enhanced ammonium and sulphate ion content.

The leach scrap composition is presented in Tables 1 and 2 [5].

Table 1

Chemical composition [%] of the Albanian leach scrap

Fe_{total}	Fe^{2+}	Fe^{3+}	Fe_{metal}	SiO_2	Al_2O_3	CaO	MgO	Cr_2O_3	NiO
45.89	17.60	26.29	1.32	15.03	4.80	3.54	2.21	1.06	0.17

Table 2

Mineralogical composition [%] of the Albanian leach scrap

Magnetite	Quartz	Wustite	Calcite	Ferrochromiumpicotite
54.09	13.15	8.02	6.32	5.51

Currently, the experiments with biological recultivation of scrapheap are realised. To prevent dusting of scrap with wind water is applied on the surface of the dumping site. Wetting of the black mud surface lowered the dusting, however, it is not sufficient for the elimination of emission transportation by wind. Gradual recultivation has been developed on the hills of the dump or in the vicinity of this area with the aim to hide the view on the dump and to prevent dust dispersion, waste spreading, hill fixing and erosion processes. The aim of the recultivation is to utilize the recultivated area for agricultural purposes and afforestation. Agricultural utilization requires the elimination of dump impact on crops, ie the safety of surface waste layers and cover, degassing, dewatering of the dump and the leachate from the dump. Currently, there is about 1/3 of dump covered with grass and weeds, the attempts to recultivate the whole area have not been very successful.

The second locality where the survey was conducted was the wider vicinity of Iron Ore Mines in Rudnany. In the past, this enterprise belonged to one of the main sources of emission contamination of the environment in loaded area of Middle Spis. The first record about the copper ore deposit in the mentioned locality comes from 1332. The year 1874 made a breakthrough in the mining period. Mining for iron siderite ores had begun after mining for copper and silver ores. Precise records about ore mining have existed in Rudnany since 1899. In that year, the mining activities represented $114.7 \cdot 10^9$ g. The great progress of mining in Rudnany was recorded at the end of the 18th century and in the first decade of the 19th century, when copper and silver ore minig were in their maximum. As the consequence of the world economic crisis in 1932–1933 the mining was stopped. After the liberation of Slovakia and especially after 1948, there was a big boom of mining and Iron Ore Mines Rudnany became the biggest ore enterprise in the former Czechoslovak Republic and the biggest exporter of mercury. Maximal ore mining of $968.5 \cdot 10^9$ g was achieved in 1978. Gradually, the reserves of siderite, chalcopyrite, tetraedrite with the mixture of mercury, silver and antimony had been running out. After 1989, all mining activities declined. In 1992, mining of siderite ore was finished as the consequence of this social phenomenon.

Waste presents a complex of ecological, economic and also social problems. Waste disposal takes place in all stages of production and consumption cycle. At present new possibilities of waste utilization beside classical disposal in various areas of economy are studied [6]. Out of the total volumes of generated waste in the Slovak Republic, 43 % of waste was disposed, which in absolute numbers means 6 185 272 Mg of waste. Dominance of landfill waste is a historical rule, with a 91 % share of total waste disposal. As of December 31, 2006, there were 160 landfills operating in Slovakia [7].

In the third observed locality, Zahorska Lowland, the influence of the 5 former or present landfills on soil and plant heavy metal contents in their vicinity is estimated. The landfill Mokry Haj began its activity in 1992 with the assumed closure in 2013. Biela Jama arose in 1975 as the material hole. Despite its recultivation in the 1990s, at present this place is utilized as municipal landfill. The landfills in Skalicke hory and Hrudy were built in the 1970s. The first of them was utilized for dangerous industrial waste, the second for municipal waste. After recultivation of landfill Hrudy agricultural plants are grown in this area. The landfill in the vicinity of the machinery factory in Skalica was localized aside the sewage tank. Currently this area is utilized for agricultural production.

Materials and methods

The observation of risky elements in soils and in cultivated crops were realized on plots in the cadasters of the villages: Zavar, Krizovany, Vlckovce, Hoste, Mala Maca and Velka Maca, in the distance of 5 to 9 km in the northern and north-western part and in cadasters of villages Velka Maca, Sintava and Vinohrady nad Vahom in the distance of 2 km south-west, 2.8 km east and 3.5 km north-east of former emission source of the Nickel Smelter Sered.

In the Middle Spis region, the soil samples and cultivated agricultural products were taken in cadaster of Markusovce in the distance of 10–12.5 km north-west, Matejovce –

7.6 km north, Chrast nad Hronom – 10 km north-east and Poráč – 6 km east of former emission source in Rudnany.

In the third observed locality, Zahorska Lowland, the soil samples and cultivated crops in 5 observed areas were taken.

The soil samples from the surface soil layer were taken from the experimental sites with pedological probe. Samples of soil ground of fine soil I. (average 2 mm) were analysed and from this fine soil the representative sample was taken and sieved through the sieve with an average of 0.2 mm (fine soil II). The total content of risky elements was determined using the AAS method in soil extract gained after total decomposition of soil using the wet way with the mixture of acids HF + HNO_3 + $HClO_4$.

The plant material was taken in full ripeness from the same localities as the soil. The samples of plant material were dried and homogenized before the analysis. The content of risky metals in plant material was determined using the AAS method after its previous mineralization using the dry way.

Results

Table 3 presents the values of selected heavy metals soil contents in the surveyed localities in wider vicinity of the former emission source, the Nickel Smelter Sered. As regards the risky metal contents, these soils can be considered as relatively "clean", in spite of moderately enhanced contents of Cd, Cu, Ni and Co. There was no content value indicating analytical proof of soil contamination. The Cd content was in the range from 1.6 to 2.5-fold of background value in all observed plots. The Ni content exceeded the background value in all plots with the exception of the locality of Vinohrady nad Vahom, while it was enhanced from 1.1 to 1.4-fold. The copper content was increased in the localities with the exception of cadasters of Zavar and Velka Maca (1.03 to 1.93-fold of background value). The enhanced soil content of Co was observed in villages Zavar, Hoste, Velka Maca and Sintava (1.06 to 1.22-fold of background value).

Table 3

Total content of heavy metals [mg · kg^{-1}] in soil samples in the vicinity of emission source Nickel Smelter Sered

Locality No.	Cr	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Co
1	68.4	2.00*	46.4*	24900	664.8	43.2	86.0	40.0*	18.8
2	67.2	1.88*	46.4*	22440	790.0	34.4	102.4	38.8*	18.4
3	62.0	1.40*	28.4	24128	662.0	48.4	88.8	41.2*	22.8*
4	71.2	1,90*	43.0*	27720	556.0	33.2	86.0	49.2*	20.0*
5	70.4	1.68*	37.2*	25880	636.4	36.4	91.6	41.2*	19.2
6	66.0	1.28*	26.8	25088	687.2	51.2	86.0	44.0*	21.6*
7	34.0	1.92*	25.2	24640	775.6	35.6	85.2	42.8*	21.2*
8	31.2	1.32*	44.4*	22440	1062.0	33.6	63.2	38.4*	24.4*
9	22.4	1.40*	69.6*	19880	743.2	32.4	63.6	30.4	17.6

* Exceeded the background soil content of the element.

All determined values were deeply below the hygienic limit, which in the case of enhancing means soil contamination.

The situation in the wider vicinity of the second surveyed site of the former emission source was diametrically different (Table 4).

Table 4

Total content of heavy metals in soil samples in the vicinity of emission source (ES) Rudnany

Locality No.	Cr	Cd	Cu	Fe	Mn	Pb	Zn	Hg	As
10	2.60	0.25	90.00*	2168	352.15	12.65	17.55	20.64***	25.00
11	1.50	0.24	73.27*	2348	324.30	12.15	12.85	38.00***	43.40**
12	2.40	0.26	29.30	2447	339.20	14.10	8.35	19.06***	15.00
13	1.40	0.45	11.40	1014	294.60	16.30	14.80	1.50*	12.00
14	1.70	0.35	16.85	1499	298.60	15.85	8.75	3.16**	22.60
15	1.90	0.23	13.75	1886	315.25	12.35	10.25	3.00**	23.70
16	2.30	0.38	14.65	1776	288.10	14.65	12.70	2.00**	18.40
17	3.40	0.25	19.30	2875	573.10	14.55	17.00	1.50*	12.00
18	3.20	0.24	14.30	2949	421.80	14.60	19.60	0.18	9.50
19	1.80	0.62	28.95	1392	918.50	25.75	27.05	9.30**	27.00
20	2.70	0.44	114.65**	3442	986.80	16.20	25.70	39.10***	32.00**
21	2.30	0.19	9.30	1890	249.60	14.70	10.25	0.40*	12.00

* Exceeded the background soil content of the element; ** Analytical proof of soil contamination by a certain element; *** Need of soil sanation with regard to the extremely high soil content of the element.

The enormly high content of Hg was found in cadaster of Markusovce in the locality Olsanske Pole, which was even exceeding the hygienic limit determining the sanation of the soil (1.9 to 3.8-fold). Similarly the extremely high content of Hg was determined in cadaster of Porac village, where the 3.2-fold higher exceeding of this limit value was determined. The contamination of soil by mercury was obvious also in other observed localities: Pod Horky, Zemkovske (Markusovce) and Na Strani (Matejovce), while the exceeding of limit value indicates analytical proof of soil contamination, and it was exceeded 4.65-fold.

Similarly, the soil contamination was proved in case of arsenic in cadasters of Markusovce village (locality Olsanske Pole), where the limit value was exceeded 1.45-fold and Porac (locality Pasienky) with 1.06-fold of limit. In Porac, the soil contamination by copper was evident (1.15-fold of limit).

The determined content of risky elements in soil samples of the observed localities in Zahorska Lowland are deeply below background values with the exception of Cd content in all localities, Cu content in Hrudy and Skalica and Ni content in Hrudy, Mokry Haj, Skalicke Hory and Skalica (Table 5). The problem of enhanced Cd content in soil is evident in many localities of the Slovak Republic and the potential risk of its input into the food stuffs of plant origin is high [7–9]. As far as other risky metal contents are concerned, the soils in the observed locality can be considered as relatively "clean".

Table 5

Total content of heavy metals [mg · kg^{-1}] in soil samples in the vicinity of landfills in Zahorska Lowland

Locality No.	Cr	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Co
22	17.6	0.97*	11.3	6843	220.5	20.3	19.7	16.7	9.5
23	73.6	1.01*	42.7*	26592	486.5	33.2	70.0	51.1*	18.5
24	55.4	0.80*	32.1	21328	755.9	32.1	53.6	38.0*	16.9
25	78.3	1.16*	35.9	29437	529.3	30.3	79.3	54.0*	18.7
26	63.5	0.95*	41.7*	21019	527.9	54.8	66.4	35.9*	15.3

* Exceeded the background soil content of the element.

The total content includes all forms of heavy metals in soil. The human health risk is determined by the bioavailable forms of the element and by the plant species and crop grown on the metal loaded soil.

The values of determined contents of risky elements in crops cultivated in the surveyed localities are presented in Tables 6–8. The content of risky metals in overground biomass (straw) was evaluated according to the valid legislation for forage, the content of metals in edible parts (grain) of crops and in foodstuffs of plant origin was evaluated according to the Food Codex of the Slovak Republic (FC SR).

Despite the relatively "clean" soils, the agricultural production grown in the investigated localities in wider vicinity of former emission source of Nickel Smelter Sered presents a potential human organism risk from the point of Cd content in barley grain grown in Vlckovce (1.25-fold exceeding of hygienic limit) and Pb in all the crop samples used for human nutrition (Table 6). The overground biomass from the point of its utilization as forage showed the enhanced content of Cr (1.54-fold) in wheat and Co (1.3-fold) in sunflower from Velka Maca in comparison with defined hygienic limits.

The situation in the second observed locality is entirely different. The cereals are characterised by a high content of metals: grain of barley from Markusovce Cd and As (the content on the level of the limit value), Hg 1.8 to 2-fold and Pb even 2.65-fold of hygienic limit; grain of rye from Markusovce Hg 1.6-fold and grain of wheat from Chrast Cd 1.5-fold and Hg 3-fold of the maximum acceptable amount for foodstuffs (Table 7). From the crops grown in wider vicinity of former emission source Iron Ore Mines Rudnany, potatoes cultivated in Markusovce (Olsanske Pole) are the most risky. The determined contents of Cd, Cu, Pb, Zn and Hg were 1.4-fold, 3.1-fold, 1.7-fold, 1.43-fold and 6-fold, respectively, higher than hygienic limits defined in the Food Codex of the Slovak Republic. The Hg content was higher also in the overground biomasss of barley from Markusovce, where it reached even 4-fold and in grassland in Porac, where the 2-fold of hygienic limit defined for forage was found.

In Table 8 the results of determined heavy metal contents in crops grown in the vicinity of landfills in Zahorska Lowland are presented. The contents of risky heavy metals in overground biomass of grown crops were deeply under the hygienic limits given by the legislation for forage. From all the tested crops the Pb content in grain in the observed localities was higher than the hygienic limit given by the Food Codex of

Table 6

Heavy metal content [mg · kg^{-1}] in samples of crops grown in the vicinity of ES Nickel Smelter Sered

No.	Crop		Cr	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Co
1	barley	s	1.60	0.260	7.10	50.4	24.9	0.70	18.5	1.20	0.80
	barley	g	1.25	0.125**	7.14	41.8	16.3	0.50**	36.9	0.29	0.22
2	barley	s	1.90	0.300	6.40	49.2	46.5	1.50	20.4	1.10	1.10
	barley	g	1.40	0.055	8.50	49.4	20.8	0.50**	38.3	0.40	0.30
3	maize	s	2.00	0.220	9.40	85.1	25.2	2.00	40.4	0.90	0.90
4	wheat	s	3.39	0.197	10.61	66.1	26.4	0.98	13.4	0.77	0.77
	wheat	g	0.50	0.085	4.10	43.6	34.3	0.35**	29.2	0.35	0.35
5	sunflower	s	2.40	0.560	23.10	112.8	59.3	3.90	42.2	2.60	2.60*
6	wheat	s	4.63*	0.375	11.25	60.9	45.9	3.53	13.0	1.10	1.32
	wheat	g	0.45	0.055	1.75	56.2	36.6	0.20**	33.2	0.35	0.20
7	paprika		0.25	0.055**	0.81	17.1	2.3	0.85**	2.3	0.08	0.04
	tomatoes		0.13	0.022	0.49	6.7	0.9	0.31**	1.5	0.13	0.05
	carrot		0.04	0.025	0.48	4.4	1.3	0.22**	2.2	0.11	0.07
	parsley		0.43	0.031	1.80	12.8	3.4	0.59**	3.9	0.27	0.09
8	sugar beet		0.04	0.035	0.91	20.7	4.8	0.12	4.0	0.20	0.08

* Exceeded hygienic limit for forage (s = straw); ** Exceeded hygienic limit for foodstuffs of plant origin (g = grain).

Table 7

Heavy metal content [$mg \cdot kg^{-1}$] in samples of crops grown in the vicinity of ES Iron Ore Mines Rudnany

No.	Crop	Cr	Cd	Cu	Fe	Mn	Pb	Zn	Hg	As
10	colza	0.75	0.20	7.9	88.6	34.0	2.16	37.7	0.05	0.20
11	barley s	1.22	0.22	7.4	92.6	10.9	2.97	13.2	0.40*	0.30
	barley g	0.74	0.10**	7.0	54.3	14.4	0.90**	25.7	0.10**	0.20**
12	rye s	0.13	0.01	5.5	33.8	33.9	0.85	28.5	0.09	0.20
	rye g	0.13	0.01	4.8	31.3	33.6	0.73**	24.4	0.08**	0.15
13	grassland	1.11	0.19	6.6	161.9	36.2	1.69	21.1	0.05	0.40
14	clover	1.27	0.27	13.1	120.8	28.7	2.29	20.9	0.06	0.40
15	barley s	0.64	0.09	5.9	66.9	14.1	1.32	20.2	0.10*	0.16
	barley g	0.26	0.06	4.9	31.6	34.8	0.77**	22.3	0.09**	0.12
16	barley s	1.11	0.17	6.4	49.9	10.1	0.72	20.2	0.20*	0.30
	barley g	0.59	0.07	4.6	40.9	15.4	2.65**	23.3	0.09**	0.10
17	potatoes	0.50	0.14**	9.4**	28.8	5.5	1.70**	14.3**	0.12**	0.10
18	grassland	1.27	0.27	13.8	136.3	26.0	2.51	23.5	0.05	0.30
19	grassland	1.31	0.21	8.3	127.2	44.0	1.47	22.2	0.05	0.40
20	grassland	1.00	0.23	11.6	168.0	44.0	1.53	39.6	0.10*	0.30
21	wheat g	0.43	0.15**	4.7	43.6	55.1	0.94**	29.4	0.15**	0.15

* Exceeded hygienic limit for forage (s = straw); ** Exceeded hygienic limit for foodstuffs of plant origin (g = grain).

Table 8

Heavy metal content [mg · kg^{-1}] in samples of crops grown in the vicinity of landfills in Zahorska Lowland

No.	Crop		Cr	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Co
22	barley	s	1.70	0.270	2.00	53.8	18.1	1.60	14.1	0.80	0.70
	barley	g	1.20	0.065	10.15**	42.3	14.9	0.60**	32.4	0.35	0.35
23	wheat	s	1.90	0.250	3.40	88.7	10.3	0.90	33.7	1.00	0.80
	wheat	g	1.05	0.160	4.05	208.2	36.0	0.35**	29.8	0.95	0.30
24	barley	s	2.30	0.160	2.60	106.9	21.5	1.20	13.6	1.10	0.40
	barley	g	1.50	0.135**	4.55	55.8	14.0	0.45**	25.1	0.40	0.30
25	wheat	s	2.50	0.230	2.70	85.0	16.1	1.80	12.3	1.10	0.90
	wheat	g	0.70	0.120	4.65	54.9	14.2	0.45**	28.8	0.75	0.20
26	maize	s	1.80	0.140	8.60	88.9	80.9	1.30	24.5	1.10	1.00
	maize	g	0.60	0.035	1.45	26.2	6.50	0.30**	18.6	0.55	0.10

** Exceeded hygienic limit for foodstuffs of plant origin (g = grain).

the Slovak Republic. The grain of barley from the locality Mokry Haj contained an enhanced content of Cd and the same crop from the locality Biela Jama contained enhanced content of Cu in comparison with the hygienic limits**.** The negative influence of waste deposition in the observed landfills can be the potential cause of enhanced heavy metal contents in agricultural plants cultivated near the landfills.

The heavy metal mobility in soil depends on the soil reaction, the content of organic matter, the soil sorption capacity and it can be affected by the change of these factors [10]. The transfer of risky elements from the soil into the agricultural plants is influenced by soil properties as well as by plant species or variety [11, 12].

Conclusion

The results suggest that the investigated soils in the area of the former Nickel Smelter and in the area of the former or present landfills in Zahorska Lowland can be considered as "clean" from the point of heavy metal contents. In these areas, only weakly enhanced soil contents of some risky elements in relationship to their background values were found. Despite this fact, a high Pb content in the grown crops was confirmed. In this respect, lead can be considered as the most risky heavy metal with possible negative influence on human health.

Our results also suggest causal connection between the enhanced content of risky heavy metals, especially Hg, in soils and crops grown in the vicinity of former emission source in Rudnany and the former metallurgical activity in this area. Residual metal load of soil and possible contamination of the food chain in the area of Middle Spis presents a serious potential risk to human health. Therefore, it is important to control the waste treatment, to strictly follow the legislative directives and to monitor the risky matters input into the environment with the aim to inhibit the non-controlled pollution of the environment and to ensure food safety.

Acknowledgement

The work was financed by the projects: VEGA No. 1/3455/06 and KEGA No. 3/5081/07.

References

[1] State of the Environment Report – Slovak Republic 2006. SEA, Banská Bystrica 2007, 242 pp.

[2] Vrabcová E. and Petrovič R.: Sereď, dejiny mesta. Sereď 2002, 369 pp.

[3] Burger F. and Čelková A.: *Prognózy šírenia znečisťujúcich látok z bodových zdrojov vo zvodnenom systéme v zóne vplyvu navrhovaného vodného diela Sereď – Hlohovec.* J. Hydrol. Hydromech. 2001, **49**(2), 125–146.

[4] Polák R., Drtil M. and Hutňan M.: *Changes in underground water quality in the surrounding of nickel dump in NH Sered (in Slovak).* Proc. Conference Sludge and Waste 2001, Tatranské Zruby, Slovak Republic, April 26.–27.2001, 163–171.

[5] Pyszková M. et al: *Chemická stabilita mikrovlnne vitrifikovaných odpadov*. Acta Montanist. Slovaca 2004, **9**(4), 410–413.

[6] Kováčik P.: *Riziká použitia popol – popolčekovej zmesi pri pestovaní jačmeňa jarného.* Risk Factors of Food Chain. Nitra 2006, 175–179.

[7] Hegedüsová A., Hegedüs O. and Musilová J.: Riziká kontaminácie pôd kadmiom. Monografia**.** UKF, Nitra 2006, 89 pp.
[8] Vollmannová A., Tóth T., Lahučký L., Musilová J. and Jomová K.: *Kumulácia medi a kadmia alternatívnymi plodinami.* Chem. listy 2006, **100**(8), 720–721.
[9] Stanovič R. and Bystrická J.: *Vplyv kadmia ako karcinogénneho prvku na úrodu láskavca*, [in:] Výživa a potraviny pre tretie tisícročie "Výživa a nádorové ochorenia". SPU, Nitra 2006, 230–233.
[10] Vollmannová A., Lahučký L., Tomáš J., Hegedüsová A. and Jomová K.: *The arrangement of extremely acid soil reaction in relationship to Cd, Pb, Cr and Ni intake by the plants.* Ekológia (Bratislava) 2002, **21**(4), 442–448.
[11] Musilová J., Bystrická J. and Timoracká M.: *Odrodová afinita ľuľka zemiakového k rôznym hladinám záťaže pôdy kadmiom a olovom*, [in:] Bezpečnosť a kvalita surovín a potravín, Nitra 2008, 382–385.
[12] Peltznerová L., Musilová J. and Harangozo Ľ.: *Vplyv odrody na obsah vitamínu C v ľuľku zemiakovom (Solanum tuberosum L.).* Risk Factors of Food Chain. Nitra 2007, 172–175.

METALE CIĘŻKIE W ROŚLINACH UPRAWNYCH UPRAWIANYCH W POBLIŻU DAWNYCH ŹRÓDEŁ ZANIECZYSZCZEŃ

Abstrakt: Celem pracy było zbadanie wpływu trzech różnych źródeł zanieczyszczeń na jakość gleby oraz produkcję roślinną. Zaobserwowana została zwiększona zawartość Cd, Ni Cu i Co w glebie w okolicy byłej huty niklu. Uprawy rolne na tym terenie nie stanowią jednak zagrożenia dla zdrowia ludzi z powodu stosunkowo małej zawartości metali ciężkich. Wyjątek stanowiło ziarno jęczmienia zawierające wysoki poziom kadmu. Na drugim badanym stanowisku – Kopalnia Rudy Żelaza Rudnany, Środkowy Spisz – stwierdzono dużą zawartość As, Cu i Hg w glebie. Największą ilość metali na tym terenie kumulowały ziemniaki. Ponadto w ziarnach zbóż przekroczone zostały obowiązujące w Republice Słowackiej normy zawartości metali ciężkich. Ostatni z badanych terenów to Nizina Zahorska. Na obszarze tym zbadano zawartość metali w glebie pól położonych w pobliżu pięciu składowisk śmieci. Uzyskane wyniki wskazały zwiększoną zawartość Cu, Cd i Ni w glebie oraz przekroczone limity dla zawartości tych metali w ziarnie zbóż. Przeprowadzone badania wykazały, że dawna emisja metali ciężkich do środowiska nadal stanowi źródło zanieczyszczenia żywności.

Słowa kluczowe: nieaktywne źródła zanieczyszczeń, zanieczyszczenie gleby, czynniki ryzyka, żywność

Reviews
Recenzje

Piotr KONIECZKA i Jacek NAMIEŚNIK

"QUALITY ASSURANCE AND QUALITY CONTROL IN THE ANALYTICAL CHEMICAL LABORATORY. A PRACTICAL APPROACH" TAYLOR & FRANCIS, BOCA RATON, LONDON, NEW YORK 2009, 233 SS, ISBN 978-1-4200-8270-8

Na początku 2009 roku nakładem Wydawnictwa CRC Press Taylor & Francis Group w Analytical Chemistry Series ukazała się bardzo wartościowa monografia "Quality Assurance and Quality Control in the Analytical Chemical Laboratory. A Practical Approach". Książka ta jest bardzo ważną pozycją, wypełnia on na analitycznym rynku wydawniczym istotną lukę. Jest napisana w języku angielskim, co ma duże znaczenie dla szerokiego grona pracowników nauk ścisłych, ponieważ używa obecnie obowiązujacą terminologię angielską, w którym to języku jest publikowana zdecydowana większość prac naukowych z zakresu chemii analitycznej.

Katedra Chemii Analitycznej Politechniki Gdańskiej, kierowana od wielu lat przez Prof. zw. dr hab. inż. Jacka Namieśnika, ma ważne osiągnięcia w zakresie badań dotyczących oceny i kontroli jakości wyników pomiarów analitycznych. Niedawno została wydana przez Wydawnictwa Naukowo-Techniczne w Warszawie na ten temat książka zespołu pod kierunkiem Profesora, która okazała się bestsellerem. W katalogu Wydawnictwa jest ona określana jako jedna z paru hitów wydawniczych. I istotnie ta książka jest niezbędna każdemu polskiemu chemikowi analitykowi i nie tylko analitykowi, ponieważ dobra znajomości chemii analitycznej jest niezbędna praktycznie rzecz biorąc, każdemu kto zajmuje się badaniami doświadczalnymi w obszarze nauk przyrodniczych, niekoniecznie będącemu chemikiem z wykształcenia.

Natomiast celem omawianej książki było, zgodnie z jej tytułem, dostarczenie praktycznych informacji dotyczących **zapewnienia** odpowiedniej **jakości** oraz **kontroli** analiz chemicznych. W pierwszym rozdziale książki przedstawiono rozkłady zmiennych losowych, miary i testy statystyczne wraz z literaturą. Po tym wprowadzeniu podstawowych pojęć statystyki, kolejne rozdziały opisują jakość wyników analitycznych, spójność pomiarową, niepewność, materiały odniesienia, badania międzylaboratoryjne i metody walidacji. Każdy z rozdziałów po wprowadzeniu definicji i podstawowych wiadomości

teoretycznych zawiera ćwiczenia praktyczne wraz z literaturą. Jest to bardzo użyteczna część książki, umożliwiająca pełną aplikację przedstawionej wcześniej wiedzy, kończy się ona załącznikiem z 15 tablicami rozkładów statystycznych. Śledzenie tych zapisów ułatwia na jego wstępie spis akronimów. Bardzo cenną stroną tej książki jest rozbudowany skorowidz. Nowością jest płyta CD ułatwiająca łatwy dostęp do niej większej liczbie osób w każdych warunkach przy wykorzystaniu komputera. Praca Piotra Konieczki i Jacka Namieśnika charakteryzuje się prostą prezentacją treści w sposób łatwy edukacyjnie. Materiały graficzne (rysunki i tabele) zostały dobrze dobrane, co ułatwia szybkie przyswajanie niełatwego, a precyzyjnego problemu oceny jakości wyników analitycznych.

Z uwagi na wysoki poziom naukowo-edukacyjny książki w pełni ją rekomenduję wszystkim pracownikom nauki, doktorantom i studentom kierunków przyrodniczych na wszystkich uczelniach w kraju i za granicą. Charakteryzuje się ona interdyscyplinarnością stosowania i to w bardzo szerokim zakresie praktyki. Recenzowana monografia nie tylko wprowadza czytelnika w bardzo specyficzny nurt jakości analityki, ale w wyczerpujący sposób zaznajamia z daleko posuniętą oceną jakości wyników analitycznych w różnych laboratoriach. Książka i jej pierwsze wydanie szybko znajdzie nabywców i zapewne wkrótce zostanie wyczerpane.

Prof. zw. dr hab. Jerzy Siepak,
Uniwersytet im. Adama Mickiewicza, Poznań

Varia

Invitation for ECOpole '10 Conference

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 19th annual Central European Conference ECOpole '10, which will be held in **13–16 X 2010** (Thursday–Saturday) on Wilhelms Hill at Uroczysko in Piechowice, the Sudety Mts., Lower Silesia, PL.

The Conference Programme includes oral presentations and posters and will be divided into five sections:

The Conference Programme includes oral presentations and posters and will be divided into five sections – SI–SV:

- SI Chemical Pollution of Natural Environment and its Monitoring
- SII Environment Friendly Production and Use of Energy
- SIII Risk, Crisis and Security Management
- SIV Forum of Young Scientists and Environmental Education in Chemistry
- SV Impact of Environment Pollution on Food and Human Health

The Conference language is English.

Contributions to the Conference will be published as:

- abstracts on the CD-ROM (0.5 page of A4 paper sheet format)
- extended Abstracts (4–6 pages) in the semi-annual journal *Proceedings of ECOpole*
- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A or S.

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15.07.2010** and for the Extended Abstracts: **1.10.2010**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from 15.07.2010) on the Conference website.

The papers must be prepared according to the Guide for Authors on Submission of Manuscripts to the Journals.

The Conference fee is 300 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 170 €) for young people actively participating in the Forum of Young Scientists. But the colleague has to deliver earlier the Extended

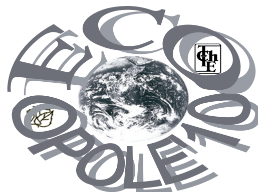
Abstract (4–6 pages) of his/her contribution (deadline is on **15.08.2010**), and a recommendation of his/her Professor.

At the Reception Desk each participant will obtain a CD-ROM with abstracts of the Conference contributions as well as Conference Programme (the Programme will be also published on this site).

Further information is available from:
Prof. dr hab. Maria Wacławek
Chairperson of the Organising Committee
of ECOpole '10 Conference
University of Opole
email: Maria.Waclawek@o2.pl
and mrajfur@o2.pl
phone +48 77 455 91 49 and +48 77 401 60 42
fax +48 77 401 60 51

Zapraszamy
do udziału w Środkowoeuropejskiej Konferencji
ECOpole '10
w dniach 13–16 X 2010

SUBSTANCJE CHEMICZNE W ŚRODOWISKU PRZYRODNICZYM



Będzie to dziewiętnasta z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w ośrodku „Uroczysko" na Wzgórzu Wilhelma w Piechowicach, koło Szklarskiej Poręby. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego.

Obrady konferencji ECOpole '10 będą zgrupowane w pięciu sekcjach:
– SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring
– SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie
– SIII Zarządzanie środowiskiem w warunkach kryzysowych
– SIV Forum Młodych (FM) i Edukacja prośrodowiskowa
– SV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi.

Materiały konferencyjne będą opublikowane w postaci:
– abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
– rozszerzonych streszczeń o objętości 4-6 stron w półroczniku *Proceedings of ECOpole*;
– artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) ser. A i S oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wystąpień upływa w dniu 15 lipca 2010 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 15 lipca 2010 r. na stronie webowej

ecopole.uni.opole.pl

Aby praca (dotyczy to także streszczenia, które powinno mieć tytuł w języku polskim i angielskim, słowa kluczowe w obydwu językach) przedstawiona w czasie konferencji

mogła być opublikowana, jej tekst winien być przygotowany zgodnie z wymaganiami stawianymi artykułom drukowanym w czasopismach *Ecological Chemistry and Engineering* ser. A oraz S, które jest dostępne w wielu bibliotekach naukowych w Polsce i za granicą. Są one takie same dla prac drukowanych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*. Zalecenia te są również umieszczone na stronie webowej konferencji.

Koszt uczestnictwa w całej konferencji wynosi 1000 zł i pokrywa opłatę za udział, koszt noclegów i wyżywienia oraz rocznej prenumeraty Ecol. Chem. Eng. (razem blisko 2000 ss.) łącznie z materiałami Konferencji. Jest możliwość udziału tylko w jednym wybranym przez siebie dniu, wówczas opłata wyniesie 650 zł i będzie upoważniała do uzyskania wszystkich materiałów konferencyjnych, jednego noclegu i trzech posiłków (śniadanie, obiad, kolacja), natomiast osoby zainteresowane udziałem w dwóch dniach, tj. w pierwszym i drugim lub drugim i trzecim, winny wnieść opłatę w wysokości 800 zł. Opłata dla magistrantów i doktorantów oraz młodych doktorów biorących aktywny udział w Forum Młodych może być zmniejszone do 600 zł, przy zachowaniu takich samych świadczeń. Osoby te winny dodatkowo dostarczyć: rozszerzone streszczenia (4–6 stron) swoich wystąpień (**do 15.08.2010 r.**). Jest także wymagana opinia opiekuna naukowego. Sprawy te będą rozpatrywane indywidualnie przez Radę Naukową oraz Komitet Organizacyjny Konferencji. Członkowie Towarzystwa Chemii i Inżynierii Ekologicznej (z opłaconymi na bieżąco składkami) mają prawo do obniżonej opłaty konferencyjnej o 25 zł. Opłaty wnoszone po 15 września 2010 r. są większe o 10% od kwot podanych powyżej. Wszystkie wpłaty powinny być dokonane na konto w Banku Śląskim:

BSK O/Opole Nr 65 1050 1504 1000 0005 0044 3825

i mieć dopisek ECOpole '10 oraz nazwisko uczestnika konferencji.

Po konferencji zostaną wydane 4–6-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesłane do **1 października 2010 r**. Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej. Wszystkie streszczenia oraz program Konferencji zostaną wydane na CD-ROM-ie, który otrzyma każdy z uczestników podczas rejestracji. Program będzie także umieszczony na stronie webowej Konferencji.

Prof. dr hab. Maria Wacławek
Przewodnicząca Komitetu Organizacyjnego
Konferencji ECOpole '10

Wszelkie uwagi i zapytania można kierować na adres:
Maria.Waclawek@o2.pl
lub mrajfur@o2.pl
tel. 77 401 60 42 i 77 455 91 49
fax 77 401 60 51

GUIDE FOR AUTHORS ON SUBMISSION OF MANUSCRIPTS

A digital version of the Manuscript addressed –

Professor Witold Wacławek
Editorial Office of monthly *Ecological Chemistry and Engineering*
(Ecol. Chem. Eng.)
Uniwersytet Opolski
ul. Oleska 48, 45–951 Opole, Poland
Tel. +48 77 452 71 34, fax +48 77 455 91 49,
Email – waclawek@uni.opole.pl

should be sent by email to the Editorial Office Secretariat – mrajfur@o2.pl

The Editor assumes, that an author submitting a paper for publication has been authorised to do that. It is understood the paper submitted to be original and unpublished work, and is not being considered for publication by another journal. After printing, the copyright of the paper is transferred to *Towarzystwo Chemii i Inżynierii Ekologicznej* (*Society for Ecological Chemistry and Engineering*). In preparation of the manuscript please follow the general outline of papers published in the most recent issues of *Ecol. Chem. Eng*., a sample copy can be sent, if requested.

Papers submitted are supposed to be written in English language and should include a summary and keywords, if possible also in Polish language. If not then the Polish summary and keywords will be provided by the Editorial Office. All authors are requested to inform of their current addresses, phone and fax numbers and their email addresses.

It is urged to follow the units recommended by the *Systéme Internationale d'Unites* (SI). Graph axis labels and table captions must include the quantity units. The use of the following commonly applied expressions is recommended: mass – m/kg, time – t/s or t/min, current intensity – I/A; thermodynamic temperature – T/K, Celsius scale temperature – t/°C or θ/°C (if both time and Celsius scale units need to be used, the symbol θ/°C for temperature is to be taken) etc.

Symbols recommended by the International Union of Pure and Applied Chemistry (Pure and Appl. Chem., 1979, **51**, 1–41) are to be followed.

Graphics (drawings, plots) should also be supplied in the form of digital vector – type files, *e.g*. Corel-Draw, Grapher for Windows or at least in a bitmap format (TIF, PCK, BMP). In the case of any query please feel free to contact with the Editorial Office.

Footnotes, tables and graphs should be prepared as separate files.

References cited chronologically should follow the examples given below:

[l] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**(3), 2080–2085.

[2] Nowak S: Chemia nieorganiczna, WNT, Warszawa 1990.

[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

Journal titles should preferably follow the Chem. Abst. Service recommended abbreviations.

Receipt of a paper submitted for publication will be acknowledged by email. If no acknowledgement has been received, please check it with the Editorial Office by email, fax, letter or phone.

ZALECENIA DOTYCZĄCE PRZYGOTOWANIA MANUSKRYPTÓW

Praca przeznaczona do druku w miesięczniku *Ecological Chemistry and Engineering A/Chemia i Inżynieria Ekologiczna A* powinna być przesłana na adres Redakcji:

Profesor Witold Wacławek
Redakcja Ecological Chemistry and Engineering
Uniwersytet Opolski
ul. Oleska 48, 45–951 Opole
tel. 077 401 60 42, fax 077 455 91 49
email: waclawek@uni.opole.pl

w postaci cyfrowej w formacie Microsoft Word (ver. 7.0 dla Windows) emailem (mrajfur@o2.pl) lub na dyskietce.

Redakcja przyjmuje, że przesyłając artykuł do druku autor w ten sposób oświadcza, że jest upoważniony do tego oraz zapewnia, że artykuł ten jest oryginalny i nie był wcześniej drukowany gdzie indziej i nie jest wysłany do druku gdzie indziej oraz, że po jego wydrukowaniu copyright do tego artykułu uzyskuje Towarzystwo Chemii i Inżynierii Ekologicznej.

W przygotowaniu manuskryptu należy przede wszystkim wzorować się na postaci najnowszych artykułów opublikowanych w *Ecological Chemistry and Engineering*, na przykład zamieszczanych na stronie webowej Towarzystwa: http://tchie.uni.opole.pl/tchie/index.php?option=content&pcontent=1&task=view&id=49&Itemid=76

Prace przesyłane do publikacji winny być napisane w języku angielskim oraz zaopatrzone w streszczenia oraz słowa kluczowe w języku angielskim oraz polskim.

Zalecamy, ażeby artykuł zawierał adresy i emaile oraz numery telefonów i faksów wszystkich autorów danej pracy, szczególnie głównego autora, którego nazwisko wyróżniamy gwiazdką.

Usilnie prosimy o stosowanie układu jednostek SI. Zwracamy uwagę, że osie wykresów oraz główki tabel powinny bezwzględnie zawierać jednostki stosownej wielkości. Polecamy symbolikę zalecaną przez PTChem (Symbole i terminologia wielkości i jednostek stosowanych w chemii fizycznej, Ossolineum, Wrocław 1989; Pure Appl. Chem. 1979, **51**, 1–41). Materiał graficzny (rysunki, wykresy), obok wersji na papierze, powinien również być dostarczony w postaci cyfrowych plików wektorowych, np. za pomocą programu: CorelDraw wersja 3.0–8.0, Grafer dla Windows lub przynajmniej bitowe (TIF, PCX, BMP). W przypadku trudności z wypełnieniem tego warunku Redakcja

zapewnia odpłatne wykonanie materiału graficznego na podstawie dostarczonego szkicu, bliższe informacje można uzyskać telefonicznie 077 401 60 42.

Przypisy i tabele podobnie jak rysunki zapisujemy jako osobne pliki.

Literaturę prosimy zamieszczać wg poniższych przykładów:

[1] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**, 2080–2085.

[2] Nowak S.: Chemia nieorganiczna, WNT, Warszawa 1990.

[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

Tytuły czasopism należy skracać zgodnie z zasadami przyjętymi przez amerykańską Chemical Abstracts Service. Autor może, jeżeli uważa to za wskazane, podawać też tytuł cytowanych artykułów z czasopism, który będzie składany kursywą oraz numer zeszytu danego woluminu (w nawiasie, po numerze woluminu).

Redakcja potwierdza emailem otrzymanie artykułu do druku. W przypadku braku potwierdzenia prosimy o interwencję: emailem, faksem, listem lub telefonicznie.

REDAKTOR TECHNICZNY
Halina Szczegot

SKŁAD KOMPUTEROWY
Jolanta Brodziak

PROJEKT OKŁADKI
Marian Wojewoda

Druk: „Drukarnia Smolarski", Józef Smolarski, 45–326 Opole, ul. Sandomierska 1. Objętość: ark. wyd. 13,50, ark. druk. 11,0.