

SZEGEDI TUDOMÁNYEGYETEM

Program and abstracts

19th DKMT Euroregional Conference
on Environment and Health

June 9-10, 2017
Szeged, Hungary

UNIVERSITY OF SZEGED





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University of Szeged

Szent-Györgyi Albert Education Centre

Issekutz Béla Lecture Room



**19th DKMT Euroregional Conference
on Environment and Health**

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Szeged, Hungary

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GENERAL INFORMATION

Conference venue:

Szent-Györgyi Albert Education Centre, Dóm tér 13, Szeged

The opening and the oral sessions, and the general assembly will be in

Issekutz Béla Lecture Room (4th floor, room number 49).

The posters will be presented in the Lobby on the ground floor.

Coffee and the welcome reception will be served on the 4th floor.

Signs will guide the participants from the building entrance to the sites.

Registration and information desk is open:

12:00–17:30 on Friday

8:30–10:00 on Saturday

Poster session (Lobby, ground floor)

When entering the exhibit hall, please look for your number (check your poster number in the list of posters) on the tack board.

Poster mounting material will be available for your convenience.

Poster hanging time: 14:00-16:50 on Friday

Poster removing time: 18:00-21:00 on Friday

Stand by your poster for the duration of the poster session.

The posters will be evaluated and rewarded by the national representatives of DKMT:

Prof. Dr. Biljana Škrbić, Serbia

Dr. Daniela Cirnatu, Romania

Dr. András Papp, Hungary

The E-book of the conference, containing the complete program and abstracts of oral and poster presentations, is available at

<http://web.med.u-szeged.hu/puhe/DKMT2017/abstract-book.pdf>

The Organizing Committee

PROGRAM

FRIDAY, June 9, 2017

13:30-13:50

OPENING AND WELCOME

Ferenc Bari

Dean of Faculty of Medicine, University of Szeged

Edit Paulik

Head of Department of Public Health, Faculty of Medicine, University of Szeged

13:50-15:10

ORAL SESSION: FOOD AND HEALTH

Chairs: Škrbić, B., Papp, A.

- 13:50 HEALTH RISK ASSESSMENT OF THE CROPS CONSUMPTION AFTER SOIL IRRIGATION WITH THE RECLAIMED WATER
Škrbić, B., Đurišić-Mladenović, N., Milić, N. – Novi Sad, Serbia
- 14:10 HEAVY METALS IN DRINKING AND BOTTLED WATER IN ARAD COUNTY
Cîrnațu, D., Răducanu, D., Tarcea, M. – Arad, Romania
- 14:25 ACRYLAMIDE IN FOOD: FOOD SAFETY ASPECTS
Rácz, T., Krisch, J. – Szeged, Hungary
- 14:40 DIETARY HABITS OF PRIMARY AND SECONDARY SCHOOL AGED CHILDREN IN HUNGARY
Szabó A., Molnár R., Paulik E. – Szeged, Hungary
- 14:55 TOO HIGH, TOO LOW, AND SELDOM OPTIMAL: HEALTH EFFECTS OF SOME NATURAL CONSTITUENTS IN DRINKING WATER
Papp A. – Szeged, Hungary

15:10-15:30:

COFFEE BREAK

15:30-16:50

ORAL SESSION: ENVIRONMENT AND HEALTH

Chairs: Cîrnațu, D., Páldy A.

- 15:30 LEADING ENVIRONMENTAL HEALTH PROBLEMS IN HUNGARY
Páldy, A. – Budapest, Hungary
- 15:50 WATER AND THE ENVIRONMENT
Tihon, A. – Chisinau, Moldova
- 16:05 BACTERIAL COMMUNICATION (QUORUM SENSING) – IMPACT ON ENVIRONMENT AND HEALTH
Kerekes, E.B., Vidács, A., Takó, M., Hargitai, F., Komáromi, L., Vágvölgyi, Cs., Krisch, J. – Szeged, Hungary
- 16:20 ASSESSMENT OF PAH DIAGNOSTIC RATIOS FOR IDENTIFYING SOIL POLLUTION SOURCES – A CASE STUDY
Škrbić, B., Đurišić-Mladenović, N. – Novi Sad, Serbia
- 16:35 NEUROTOXIC EFFECTS OF WELDING FUME METAL NANOPARTICLES AFTER SUBACUTE INTRATRACHEAL EXPOSURE IN RATS
Máté, Zs., Horváth, E., Szabó, A., Tombácz, E., Nesztor, D., Papp, A. Szeged, Hungary

17:00-18:00

POSTER SESSION

Chairs: Škrbić, B., Cîrnațu, D., Papp, A.

18:00

GENERAL ASSEMBLY, POSTER PRICING

18:30

CONFERENCE DINNER

SATURDAY, June 10, 2017

9:00-10:15

ORAL SESSION: MICROBIOLOGICAL TOPICS

Chairs: Krisch, J., Vágvölgyi Cs.

- 9:00 LIPOPEPTID PROFILING OF A *BACILLUS SUBTILIS* STRAIN
Bartal, A., Kecskeméti, A., Bóka, B., Kadaikunnan, Shine, Naiyf, S. Alharbi, Jamal, M. Khaled, Vágvölgyi, Cs., Szekeres, A.
Szeged, Hungary; Riyadh, Saudi Arabia
- 9:15 INVESTIGATION OF THE INITIAL STEPS OF THE NICOTINIC ACID DEGRADATION PATHWAY IN *ASPERGILLUS NIDULANS*
Bokor, E., Ámon, J., Vágvölgyi, Cs., Hamari, Zs. – Szeged, Hungary
- 9:30 WATER RISK ASSESMENT - LEGIONELLA CONTROLL IN HUNGARY - RESULTS AND EXPERIENCES IN OUR DAYS
Gömöri, Cs., Nacsa-Farkas, E., Kerekes, E. B., Vidács, A., Barna, Zs., Róka, E., Mészáros Basics, B., Póda, T., Vágvölgyi, Cs., Krisch, J.
Budapest, Hungary; Szeged, Hungary
- 9:45 TARGETED GENOME EDITING VIA THE CRISPR/CAS9 SYSTEM IN *MUCOR CIRCINELLOIDES*
Szebenyi, Cs., Nagy, G., Vaz, A., Tóth, E., Kiss, S., Vágvölgyi, Cs., Papp, T.
Szeged, Hungary
- 10:00 ESSENTIAL OIL BASED DISINFECTANTS FOR BIOFILM ELIMINATION FROM STAINLESS STEEL AND PLASTIC SURFACES
Vidács, A., Rajkó, R., Vágvölgyi, Cs., Krisch, J. – Szeged, Hungary

10:15-10:45

COFFEE BREAK

10:45-12:00

ORAL SESSION: HEALTH AND BEHAVIOUR

Chairs: Paulik, E., Lukács, A.

- 10:45 TELEMEDICINE
Tihon, A. – Chisinau, Moldova
- 11:00 THE PERINATAL OUTCOME OF TEENAGE PREGNANCY
Karai, A., Gyurkovits, Z., Sári, T., Nyári, T., Paulik, E., Németh, G., Orvos, H.
Szeged, Hungary
- 11:15 EFFECT OF CARBOHYDRATE HYDROLYZING ENZYME TREATMENT ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF FRUIT POMACE EXTRACTS
Zambrano, C., Kotogán, A., Kerekes, E., Vágvölgyi, Cs., Krisch, J., Takó, M.
Szeged, Hungary
- 11:30 HEAVY ALCOHOL CONSUMPTION AND ASSOCIATED FACTORS AMONG HUNGARIAN STUDENTS
Lukács, A., Szabó A., Horváth E., Molnár, R., Paulik, E. – Szeged, Hungary
- 11:45 MATERNAL CIGARETTE SMOKING AND USE OF VITAMIN SUPPLEMENTS BEFORE AND DURING PREGNANCY
Paulik E., Barka N., Rénes L., Gyurkovits, Z., Németh G., Orvos H.
Szeged, Hungary
- 12:00 KNOWLEDGE OF AND ATTITUDES TOWARD HUMAN PAPILLOMAVIRUS INFECTION AND VACCINATION
Erdős, Cs., Molnár R., Müller A., Paulik E. – Szeged, Hungary

12:15

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3.	Bosnyákovits, T., Rákos, N., Rózsa, Á., Sziveri Sz., Gáspár, G.	MEASLES OUTBREAK IN CSONGRÁD COUNTY FROM 29 JANUARY 2017 UNTIL 10 MARCH 2017
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5.	Vuković, G., Bursić, V., Đurović, A., Kravić, S., Stojanović, Z., Popović A., Petrović A.	LC-MS/MS PESTICIDE RESIDUES DETERMINATION IN SURFACE WATER RESOURCES USING FOR DRINKING WATER SUPPLY
6.	Gajdács M., Handzlik J., Sanmartín C., Domínguez-Álvarez E., Spengler G.	SELENOCOMPOUNDS AS COMBINATIONAL AGENTS IN CANCER CHEMOTHERAPY: AN <i>IN VITRO</i> PERSPECTIVE
7.	Gömöri, Cs., Nacsá-Farkas, E., Kerekes, E. B., Vidács, A., Barna, Zs., Róka, E., Mészáros Basics, B., Póda, T., Vágvölgyi, Cs., Krisch, J.	PRESENCE OF LEGIONELLA IN WATER SAMPLES FROM COMMUNITY AND INDUSTRIAL FACILITIES
8.	Horváth, K., Winkler, Zs., Kuti, D., Kővári, D., Polyák, Á., Ferenczi, Sz., Kovács, J.K.	STRESSFUL LIFETIME RESULTS IN NEUROINFLAMMATION
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10.	Kartali, T., Shahab, D., Ráduly, O.-Cs., Nyilasi, I., Hatvani, L., Kredics, L., Vágvölgyi, Cs., Papp, T.	SCREENING OF dsRNA ELEMENTS IN FUNGI INFECTING <i>AGARICUS BISPORUS</i>
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12.	Koledin, I., Kovač, R., Matavulj, M.	SINGLE ACRYLAMIDE DOSE AFFECTED MAST CELL NUMBER IN RAT COLON
13.	Koledin, I., Kovač, R., Matavulj, M.	SUBCHRONIC ACRYLAMIDE TREATMENT REDUCED MUC2 SECRETION IN RAT COLON
14.	Kovac, R., Koledin, I., Rajkovic, V., Matavulj, M.	ACRYLAMIDE-INDUCED TOXICITY IN LIVER NON-PARENCHYMAL CELLS
15.	Markó-Kucsera, M., Paulik, E.	A NEW OPPORTUNITY IN REHABILITATION: SELF-HELP CLUBS FOR PARKINSON'S DISEASE

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18.	Škrbić, B., Marinković, V., Petrović Gegić, A.	HEALTH RISK ASSESSMENT OF ORGANOCHLORINE COMPOUNDS IN STREET DUST OF NOVI SAD, SERBIA
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23.	Vaz, A., Takó, M., Szebenyi, Cs., Vágvölgyi, Cs., Nagy, G., Papp, T.	CHARACTERIZATION OF NOVEL TYPE SURFACE PROTEINS IN MUCORALES
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27.	Vuković, G., Bursić, V., Đurović, A., Kravić, S., Stojanović, Z., Popović A., Petrović A.	LC-MS/MS PESTICIDE RESIDUES DETERMINATION IN SURFACE WATER RESOURCES USING FOR DRINKING WATER SUPPLY
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ABSTRACTS

ORAL SESSION

LIPOPEPTID PROFILING OF A *BACILLUS SUBTILIS* STRAIN

**Bartal, A.¹, Kecskeméti, A.¹, Bóka, B.¹, Shine K.², Alharbi N. S.²,
Khaled J. M.², Vágvolgyi, Cs.^{1,2}, Szekeres, A.¹**

¹Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

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Surfactins are cyclic lipopeptide-type biosurfactants consisting of a β -hydroxide fatty acid chain of various lengths and a peptide ring of seven amino acids connected by a lactone bridge, forming the ring-like structure of the heptapeptide. The amino acid sequence of the firstly described surfactin molecule is Glu-Leu-Leu-Val-Asp-Leu-Leu, in which it was found later Leu/Val, Ala/Val, Ile/Val and Ile/Leu changes in the second, fourth and seventh positions and the aliphatic chain lengths were observed to be between C₁₂ – C₁₅. These compounds are produced mainly by the *Bacillus* species and they have numerous biological activities like antiviral and anti-inflammatory effects.

A mixture of surfactins extracted from the selected *Bacillus subtilis* strain was studied by HPLC-IT-MS technique for the purpose of separation and structural characterization of the components. After successful separation, the examination of the MS² spectra of the sodium adduct precursor ions ([M+Na]⁺) revealed a novel, yet unknown group of surfactins possessing different amino acid sequence in its heptapeptide. In several identified molecules the number of carbon atoms in the fatty acid chain also differs from those reported earlier.

Measurements of the relative quantity of the newly identified variants and homologues in the ferment broth resulted that almost 30% of the detected surfactin molecules produced by the microorganism deviate in peptide sequence, and the ratio of the yet unreported homologues is over 50%.

Finally, various cultivation parameters were applied using different carbon sources and metal ions in the culture medium of *B. subtilis* in order to examine their effects on the production ratios of the different surfactin isoforms. The relative quantity of the novel variants observed to be higher in all of the modified culture media.

This work was connected to the project GINOP-2.3.2-15-2016-00012. The authors extend their appreciation to the International Scientific Partnership Program (ISPP) at King Saud University for funding this research work through ISPP#0105. AS was supported through the New National Excellence Program of the Ministry of Human Capacities (UNKP-16-4).

INVESTIGATION OF THE INITIAL STEPS OF THE NICOTINIC ACID
DEGRADATION PATHWAY IN *ASPERGILLUS NIDULANS*

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Many microorganisms can utilize nicotinic acid as sole nitrogen-source; however, the degradation of nicotinic acid was studied only in a limited number of prokaryotes. In eukaryotes, the nicotinic acid catabolic process is completely unknown, only the nicotinic acid hydroxylase (Purine hydroxylase II) of *Aspergillus nidulans* had been thoroughly studied, which catalyses the first step of the nicotinic acid degradation.

As a result of our research we identified the genetic background of the nicotinate catabolic pathway of *A. nidulans*. We found the pathway specific transcription factor (HxnR) and clustered 5 genes, which we named *hxnP/S/T/Y* and *hxnZ*. These genes belong to the Nicotinic acid Degradation Cluster 1 (NDC₁) and are localized on Chromosome VI together with the NDC₂ which contains 3 genes, *hxnV*, *hxnX* and *hxnW*. Furthermore, we found two more genes, *hxnM* and *hxnN* on Chromosome I, which take part in the degradation. We named this cluster NDC₃.

In this work, we present the investigation of the initial steps of the catabolic pathway which is carried out by the *hxnS*, *hxnT* and *hxnY* genes. During our work, we carried out growth tests on different media. The result of these tests referred to the phenomenon that the nicotinic acid degradation pathway splits up to alternative routes right after the first step where nicotinic acid is converted to 6-hydroxynicotinic acid. Moreover, we revealed that these alternative routes meet at one point from where the *hxnV*, *hxnX*, *hxnW* and *hxnM* genes further carry on the process. In order to find out how the degradation gets to the point where the alternative routes meet we created different multiply deleted mutants which we analysed by further growth tests. Based on these results the initial steps of the nicotinic acid degradation process become clearer.

Acknowledgement: This research was supported by the National Research, Development and Innovation Office (NKFI-K16 119516) and by GINOP-2.3.2-15-2016-00035.

HEAVY METALS IN DRINKING AND BOTTLED WATER IN ARAD COUNTY

Cîrnațu, D.¹, Răducanu, D.², Tarcea, M.³

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Ingestion of heavy metals is associated with a large number of diseases. These elements are found naturally in the earth's crust so the concentrations in drinking and bottled water vary among different localities, resulting in spatial variations of surrounding concentrations.

Based on the analysis of the contents of heavy metals (HM) – Cd, Pb, Cu, Co, Ni, Cr, Zn – in the sources and all the assortments of the 3 operators that are bottling mineral and still water in Arad county, was evaluated health risks associated with these food category during 2016, HM concentrations were analyzed by using graphite furnace atomic absorption spectrometer in the laboratories network of Romanian Health Ministry. Health risk assessment like chronic daily intake (CDI) and hazard quotient (HQ) was calculated. The values for CDI were found in the order of Mn > Cd > Cu > Pb > Cr > Ni > Zn and the values of HQ were < 1 for all HM in drinking water samples indicating no health risk.

One sample of bottled carbonated water was found to be containing high amount of chromium. Investigating the incompliance, the possible cause of the accidental contamination was found to be the transfer for the plastic used for bottling. For the same operator, analyses of the source and of the still water sampled in the same day were in compliance with the legislation.

Monitoring the amount of heavy metals in bottled water is important in order to control the risk pose of this environmental factor to the health, considering the proportion of the population that are consuming only this type of water.

KNOWLEDGE OF AND ATTITUDES TOWARDS HUMAN PAPILLOMAVIRUS INFECTION AND VACCINATION

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Hungary

Background: Cervical cancer is an important global public health problem, the fourth most frequent cancer in women. Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. HPV is responsible for nearly all cervical cancer cases and is linked to genital, anal, and oropharyngeal cancers, too. The nature and the consequences of HPV infections are poorly understood by the general population. The aim of this study was to explore knowledge, attitudes and beliefs related to HPV and cervical cancer among Hungarian youth generation in South-East Hungary.

Methods: The sample of our cross-sectional study were chosen from three county towns of South-East Hungary including three different types of secondary schools (high school, technical school, vocational school) and six faculties of the University of Szeged. The sample of the study consists of 1029 students. The self-administered questionnaire involved a specific HPV-related block which analysed the HPV and cervical cancer related questions, additionally, sociodemographic and lifestyle factors were assessed. Statistical analysis was performed with the IBM SPSS Statistics 24 software. The study protocol was approved by the Regional Human Medical Biology Research Ethics Committee of Albert Szent-Gyorgyi Clinical Centre, University of Szeged.

Results: Generally, the teenagers, men and vocational school students had less information about HPV and HPV-related topics, in addition the willingness of vaccination was also very low in these subgroups.

Conclusion: Today, irresponsible sexual behaviour and inadequate knowledge can increase the risk of sexually transmitted diseases. The knowledge of HPV in the measured population is partial and inadequate. The results call the attention to the gap of knowledge in different subgroups, especially among men, whose role in safe sexual behaviour is equally important to that of women.

WATER RISK ASSESMENT - LEGIONELLA CONTROL IN HUNGARY - RESULTS
AND EXPERIENCES IN OUR DAYS

**Gömöri, Cs.¹, Nacsa-Farkas, E.¹, Kerekes, E. B.¹, Vidács, A.², Barna, Zs.³,
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Legionellosis is a rare form of pneumonia caused by *Legionella pneumophila* and other *Legionella* species. Except for some cases, the disease is transmitted through inhalation of *Legionella* contaminated aerosols. Most frequently encountered sources of *Legionella* infections are cooling towers, air-conditioning units, hot and cold-water networks, spa pools, high pressure cooling and cleansing equipments. In many countries of the European Union, the risk assessment of *Legionella* is covered by laws and decrees based on Directive 2000/54/EC. In Hungary, the regulation of EMMI Nr 49/2015 (XI 6) give the legal background of *Legionella* control: limit values, testing obligations, and test methods are clearly defined. Nowadays, *Legionella* risk management focuses on public facilities (*e.g.*, hotels, spas, hospitals, schools, office and industrial buildings) which operate water systems generating aerosols, and the water temperature range is between 20 °C and 50 °C. In the last decade (31/01/2007-31/01/2017) the National Public Health Institute analyzed 1327 water samples; they were collected in Hungary from hot water networks, spa pools and cooling water (from schools, office buildings, industrial facilities, and spas). The analyzes of these samples revealed that the hot water systems were colonized by *Legionella* in 42% of the cases; 3% of them showed very high (>100 000 CFU/l) *Legionella* counts. In case of pools (205 samples) similar values were obtained. Concerning the 734 cooling water samples, 78% of them were under the limit value of 100 CFU/l.

This study was connected to the project GINOP-2.3.2-15-2016-00035.

THE PERINATAL OUTCOME OF TEENAGE PREGNANCY

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Introduction: Pregnancy among teenagers is a global problem and these pregnant girls are considered as a high-risk group. Teenage pregnancies are associated with higher rate of maternal, foetal and neonatal complications.

Materials and methods: A retrospective analysis was carried out on the data of all mothers aged below 20 years who had delivered at the Department of Obstetrics and Gynaecology, University of Szeged, between 2010 and 2014. During this period, 12845 births were recorded, and 274 (2.1%) of these mothers were younger than 20 years; 275 neonates were born to these adolescent mothers. We compared the data of the adolescent mothers with the data of all mothers who delivered in Hungary in the same period.

Results: The frequency of Caesarean section was 33.5% and of the premature deliveries was 10.2%. 184 (67.2%) pregnant teenagers were primigravida. The mean birth weight was significantly lower in the teenage group than in the general Hungarian population (3110.2±564 grams vs 3247 grams). The rate of major congenital malformations (8% vs. 5%) and of transfers to the Neonatal Intensive Care Unit (12.4% vs. 8%) were significantly higher in the teenage group. The umbilical cord blood pH was less than 7.2 in 18.5% of the neonates from the teenage pregnancies.

Conclusions: Young maternal age is associated with higher risk of adverse perinatal outcome, so the prevention of early pregnancy, including the education about the potential complications, has to be a part of the sexual health education of the youth population in Hungary.

BACTERIAL COMMUNICATION (QUORUM SENSING) – IMPACT ON ENVIRONMENT AND HEALTH

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Bacteria communicate with one another via the production and detection of secreted signal molecules (autoinducers). This cell-to-cell communication process is called quorum sensing (QS) and it allows bacteria to synchronize their behavior on a population-wide scale. This leads to increased resistance against antibiotics and sanitizers making their removal and treatment difficult or even impossible. Besides healthcare where severe nosocomial infections can develop, the food industry is also affected by the negative impact of these populations; processed food can be contaminated endangering the health of consumers. QS was first characterized in the 1970s in luminescent marine *Vibrio* species and further studies showed that this phenomenon is widely spread among bacteria. The acylhomoserine lactone (AHL) QS system is specific for Gram-negative bacteria, the autoinducing peptide (AIP) QS system for Gram-positive bacteria and the autoinducer-2 (AI-2) QS system for both bacterial groups. The production of antibiotics, virulence factors, biofilm formation and resistance are regulated by QS. Resistance of bacteria has become a major problem over the years, therefore, those anti-QS agents that do not exert selective pressure, which would lead to resistance are of particular interest. Several attempts have been made to inhibit this process with antagonistic molecules, natural inhibitors like essential oils or plant extracts and some proved to be efficient in *in vitro* experiments. The identification of the most effective QS-modulators could give us the tools to influence bacterial behavior and control their pathogenicity.

This work was connected to the project GINOP-2.3.2-15-2016-00012.

HEAVY ALCOHOL CONSUMPTION AND ASSOCIATED FACTORS AMONG HUNGARIAN STUDENTS

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Despite the decreasing average consumption in adults, alcohol drinking remained a significant social and public health problem in the world. Rising tendency of alcohol use among adolescents and young adults, as well as higher rates of dangerous drinking practices such as binge drinking, was observed in certain European countries including Hungary. Binge drinking (when men consume 5 or more drinks, and women, 4 or more drinks, in about 2 hours) is associated with several health problems (cardiovascular problems, HIV infection etc.); and it is the leading cause of injury, violence and death among young adults. The aim of our study was to measure the frequency of binge drinking and its association with sociodemographic, familial and lifestyle factors among Hungarian students. The cross-sectional study was done among secondary school and university students (n=2449) studying in Csongrád County, Hungary, in 2015. Students' personal data, parents' educational level and financial status, and students' academic performance and self-reported use of tobacco, drugs and alcohol, were collected by an anonymous self-administered questionnaire. Descriptive and analytical statistics were applied; data were analyzed by SPSS 24.0 software. Nearly one third of the students were classified as binge drinker. We found a significant gender difference in binge drinking, it was more common among boys compared to girls. Among students with higher educated parents' the frequency of binge drinking was higher. High social status of the parents constituted a protective factor, the rate of binge drinking was the highest among students whose family's financial condition was the worst. Binge drinker students used tobacco and illicit drugs significantly more commonly than non-binge drinkers. The academic performance of non-binge drinkers was higher than that of binge drinkers. Problematic binge drinking among Hungarian students seems to be associated with some parental characteristics and the use of other harmful substances. Binge drinking strongly influenced the school performance of the students. Modifying alcohol consumption patterns of young adults – by education, parent interventions, and adequate political decisions – is crucial for the prevention of possible serious consequences.

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NEUROTOXIC EFFECTS OF WELDING FUME METAL NANOPARTICLES AFTER SUBACUTE INTRATRACHEAL EXPOSURE IN RATS

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Welding fumes, containing a complex mixture of metal oxides partly in nanoparticle form, are frequently encountered in occupational settings, and can cause disease, including neurological problems, among the workers. Taking into account the abundance of welding as a job and source of exposure, the possible health effects especially regarding the central nervous system, and further that it is not possible to assess the overall toxic effects of welding fumes based only on individual toxicity data of the components, our aim was to investigate the general toxicological and central nervous system electrophysiological effects of nanoparticulate Mn, alone, and in combination with Fe and/or Cr nanoparticles, in a subacute model using intratracheal application. Oxide nanoparticles of Mn, Mn + Fe, Mn + Cr and the triple combination were applied, in aqueous suspension, to the trachea of young adult male Wistar rats for 4 weeks, 5 times per week. Metal content of brain, lung and blood, measured by ICP-MS, were correlated to the observed functional alterations to see the relationship between metal load and toxic effects. The decrease of body weight gain during treatment, caused by Mn, was counteracted by Fe, but not Cr. At the end of treatment, spontaneous and evoked cortical electrical activity was recorded. Mn caused here a shift to higher frequencies, and lengthened latency of evoked potentials, which were also strongly diminished by co-application of Fe only. The interaction of the metals seen in body weight gain and cortical activity were not related to the measured blood and brain metal levels. It was supposed instead that Fe might have initiated protective (antioxidant) mechanisms with a more general effect.

LEADING ENVIRONMENTAL HEALTH PROBLEMS IN HUNGARY

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According to an assessment of 2015 6.2% of the global burden of diseases can be attributed to environmental risks in Hungary– this proportion is less than the global average (11.8%). The major environmental risk is air pollution: ambient particulate matter and ozone exposure.

PM emission was gradually decreasing in the period of 2005-2014. The main source of emission is burning fossil fuels by the inhabitants. 0.05-2.2% of all-cause mortality can be attributed to short term PM_{2.5} exposure over the daily mean concentration of 25 µg/m³ among the population >30 years. This corresponded to 300 excess death cases in Budapest in 2005, in the most polluted year. In a long term PM_{2.5} pollution over the WHO guideline value (10 µg/m³) is responsible for 3-15% of total mortality of the adult population in relation to the yearly pollution levels. Household burning of solid fuels contributes to 15-30 lung cancer cases yearly.

A special problem is mesothelioma due to occupational and environmental exposure to asbestos. The yearly mean number of reported cases was 66 between 2001-2013. While mesothelioma has a long latency period, the peak of incidence can be expected in 2020-2030. Ragweed pollen is a major airborne biological allergen, affecting 1.2 million inhabitants. The yearly pollen load showed an increasing tendency, affected by the yearly weather variability by the climatic regions of the country. The daily maximum pollen concentration (1000 pollen grains/m³) was measured in 2014.

The health effect of heat waves is also significant. In the period of 2005-2014 the yearly mean excess mortality due to heat waves were 700 cases in a range of 24 cases in 2014 and 1900 cases in 2015. Daily mean mortality increased by 15% in average during heat waves.

The most important chemical pollutant is arsenic in drinking water. In contrast to the past decade when 1.5 million inhabitants consumed drinking water with As concentration over the limit value (>10 µg/l), the number of exposed is reduced to 400 000.

TOO HIGH, TOO LOW, AND SELDOM OPTIMAL: HEALTH EFFECTS OF SOME
NATURAL CONSTITUENTS IN DRINKING WATER

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It is a common belief that natural things are naturally healthy, but this is by far not true. What mankind takes from nature, directly or indirectly, to maintain their life – that is, drinking water and food – can have more or less optimal chemical composition which means that inadequate or excessive intake of certain micronutrients, with consequences to population health, is possible and takes in fact place.

In case of drinking water, hardness a basic quality indicator. Harder than the average drinking water is a rich source of calcium and magnesium. It has been supposed, and partly verified, that waterborne magnesium or calcium contributes to lessening of the prevalence of cardiovascular diseases. On the other hand, kidney stones may be more prevalent in areas with hard drinking water but the results are contradictory and even a protective role of magnesium has been supposed.

Since the discovery of the role fluoride in dental health, the importance of sufficient fluoride intake has become public knowledge. However, for geochemical reasons, there are several “fluoride belts” on the Earth where the local chemical environment, and so, locally available water and food, contains excessive fluoride leading to endemic dental and (potentially disabling) skeletal fluorosis.

Boron was considered as unwanted component of drinking water because of reproductive toxicity in animals. This, however, was not verified in humans and the WHO guideline was raised from 0.5 mg/L (2006) to 2.4 mg/L (2011).

Iodine comes both from drinking water and solid food. The typical problem with iodine is deficiency but cases of extreme intake from drinking water have been described, with consequences partly similar to those of low intake.

The local non-living – geochemical – environment can exert major influence on the health of the population and may require attention of public health authorities.

MATERNAL CIGARETTE SMOKING AND USE OF VITAMIN SUPPLEMENTS
BEFORE AND DURING PREGNANCY

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Background: Maternal smoking is associated with adverse neonatal outcomes (e.g. preterm delivery, low birth weight, birth defects), while multivitamin/mineral supplementation in pregnancy may reduce the risk of these consequences. The aim of this study was to determine the association between regular multivitamin/mineral supplementation before and during pregnancy and smoking behavior of pregnant women.

Methods: The cross-sectional study was conducted among women having their babies in 2014 at the Department of Obstetrics and Gynecology, University of Szeged. Data collection was based on a self-administered questionnaire and the health documentation of mothers and newborns. Participation was offered to each woman who could voluntarily decide to participate. Altogether 1669 women filled in the questionnaire one or two days after the delivery; finally, due to missing data 1548 mothers were involved into the present analysis. The self-administered questionnaire comprised mothers' sociodemographic characteristics, lifestyle, dietary factors, vitamin and mineral supplementation and pregnancy-related variables of women before and during their pregnancy. Statistical analysis was performed with the IBM SPSS statistics. The study protocol was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged. Written informed consent was obtained from each participant of the study.

Results: Although the prevalence of smoking decreased during pregnancy, finally, 6.5% of women remained smoker. The percentages of women reporting multivitamin, folic acid and omega-3 fatty acid consumption before and during pregnancy were significantly lower among smokers vs. nonsmokers.

Conclusion: Today, it is well-known that adequate vitamin and mineral intake is important for the health of women of childbearing age. Our results showed that women who smoke during pregnancy are less likely to take vitamins and minerals than women who don't. These results call the attention for the importance of improved education about healthy way of life during pregnancy, especially among smokers.

ACRYLAMIDE IN FOOD: FOOD SAFETY ASPECTS

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Acrylamide which is widely used in the plastic industry can be also found in food as a processing contaminant. It is produced at high temperature cooking procedures, above 120°C, during frying and baking foods with high starch content. In the presence of reducing sugars, the amino acid asparagine can form acrylamide. Based on risk assessment performed by the European Food Safety Authority (EFSA) in 2015, acrylamide in foods is likely to be carcinogenic in humans. A questionnaire was prepared on the consumption habits of high acrylamide containing foods and drinks, like potato chips, French fries, toast, and instant coffee, and also on consumer awareness. Based on the questionnaire the most consumed foods are coffee, followed by chips and French fries. Although respondents said that fried potatoes are supposed not to be over fried, from pictures of different colored French fries they selected mostly the over fried ones. Consumption of foods with high acrylamide content was estimated by 40.4% of the respondents as high risk.

HEALTH RISK ASSESSMENT OF THE CROPS CONSUMPTION AFTER SOIL IRRIGATION WITH THE RECLAIMED WATER

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The practice of using the wastewater for vegetable crops irrigation is the widespread and growing worldwide. There have been many publications reporting results of the studies on plant uptake of the pharmaceuticals, proving that once in the agricultural environment, such micro contaminants have potential to be taken up by crops and the level of the accumulation depends on numerous factors (physicochemical nature of the compounds, soil properties, irrigation water quality, physiology of the plant, irrigation frequency and duration, etc.). The uptake of pharmaceutically active compounds by plants represents their entrance into the food chain, which should be considered as an important alternative pathway for the exposure of humans to pharmaceuticals, with potential health implications. The aims of this presentation are: to give an overview of the approaches for human health risk assessment based on consumption of crops cultivated in soil irrigated with the reclaimed water, and to present the range of the estimated intakes available in the literature taking into account different approaches for the potential exposure estimation in order to assess the human health risks through consumption of the reclaimed water irrigated crops.

Acknowledgment. The results presented here are obtained within the project "Pharmaceutical active compounds in flooded arable soil: distribution, bioaccumulation and risk assessment", (No.142-451-2640/2017-01/02) supported by Provincial Secretariat for Higher education and Scientific Research of Vojvodina.

ASSESSMENT OF PAH DIAGNOSTIC RATIOS FOR IDENTIFYING SOIL
POLLUTION SOURCES – A CASE STUDY

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Understanding the impact of particular emission sources on different areas or ecosystems is crucial for appropriate risk assessment and management. In this presentation, source identification of polycyclic aromatic hydrocarbons (PAHs) detected in soil and street dust samples from 30 sites throughout the Novi Sad city (Vojvodina, Serbia) was performed using the so-called diagnostic ratios or fingerprints. On the base of the contents of 16 EPA PAHs analyzed in soil samples taken in two seasons (winter and summer), seven ratios were calculated, e.g. anthracene/sum of anthracene and phenanthrene ($\text{Ant}/(\text{Ant}+\text{Phe})$), fluoranthene/sum of fluoranthene and pyrene ($\text{Fla}/(\text{Fla}+\text{Pyr})$), benz[a]anthracene/sum of benz[a]anthracene and chrysene ($\text{BaA}/(\text{BaA}+\text{Chr})$), indeno[1,2,3-*cd*]pyrene/sum of indeno[1,2,3-*cd*]pyrene and benzo[ghi]perylene ($\text{IndP}/(\text{IndP}+\text{BghiP})$), benz[a]pyrene/benzo[ghi]perylene (BaP/BghiP), sum of combustible PAHs/sum of 16 PAHs ($\Sigma\text{COMB}/\Sigma\text{PAHs}$), and sum of the light molecular weight PAHs/sum of the heavy molecular weight PAHs ($\Sigma\text{LMW}/\Sigma\text{HWM}$). Comparing the calculated ratios with the relevant, well-known reference values, qualitative distinguishing of petrogenic and pyrogenic sources of PAHs in the Novi Sad soil was done. Moreover, the information obtained taking into account different ratios was assessed in order to overcome shortcomings of the diagnostic ratios applicability in the source determination. The results indicated dominance of the pyrogenic sources, as well as the necessity to use three or more PAH ratios simultaneously in order to avoid the source misinterpretation.

DIETARY HABITS OF PRIMARY AND SECONDARY SCHOOL AGED CHILDREN IN HUNGARY

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Dietary habits in Hungary is traditionally not healthy that is represented in the dietary behaviour of the young ones. Healthy nutrition of the children would be essential for healthy growing and it determines the manifestation of future chronic diseases (cardiovascular diseases, obesity, diabetes, cancer, etc.) Nutritional habits are developed and ingrained in childhood and adolescence. At the time of primary school parental role pattern is very important, while at the time of secondary school parental control slackens and teenagers tend to follow peers' and media mediated habits, therefore monitoring of dietary habits of school-aged children is essential. Health Behaviour in School-aged Children is the largest international study, involving Hungary, which observes – among others – nutritional habits of 11, 13 and 15 years old children. The picture is unfavourable (just half of the children eats breakfast on every weekday, 14% of students eats together with the family on every day, one third eats fruit or vegetable at least once every day, 30% consume sweets every day, while 25% drink sugary beverages at least daily once), habits became worse and worse by increasing age groups, but some positive tendencies can be seen over time. Dietary behaviour of the secondary school aged children is shown by a cross-sectional study done at Csongrád county involving 864 students. As a result, 68% of students have breakfast regularly, 75% drink at least 1.5 litre fluid per day (mainly boys), and 68% snacks daily (predominantly girls). The most frequently consumed drink is unhealthy in 8% (soft drinks) and 5% (tea with sugar) of the respondents (especially in case of boys). Just 24% of the students eat fresh vegetables or raw fruits several times per day. 41% of them pay attention to healthy diet. Thanks to the health political decisions made in the recent years in Hungary, the dietary habits of the school aged children will be improving in the future.

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TARGETED GENOME EDITING VIA THE CRISPR/CAS9 SYSTEM IN *MUCOR CIRCINELLOIDES*

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A CRISPR-Cas9 system has been developed and established as a robust and versatile genetic modification tool for site-specific mutagenesis of *Mucor circinelloides*. Our aim was to optimize a CRISPR-Cas9 system to disrupt genes *CotH1* and *CotH2*, encoding spore coat protein H in *M. circinelloides*. During the transformation, the crRNA and trans-activating crRNA (tracrRNA) were used together (gRNA) to guide CRISPR-associated (Cas) nuclease to create double-strand breaks in the targeted DNA sequence. In this transformation strategy, a linearized deletion cassette was used as a template DNA for HR repair constructed by PCR.

M. circinelloides is one of the most studied carotene producing Mucoromycotina species widely used as model organisms in different genetic and molecular biological studies. Even if genetic transformation of *M. circinelloides* has been reported before, stable integration of the introduced DNA into the host genome have remained a great challenge in this fungus and the mitotic stability of transformants considered low.

Previously, directed mutagenesis was achieved by various transformation strategies using the components of the CRISPR-cas9 system in different filamentous fungi. In this study, CRISPR-Cas9 was used to disrupt the *CotH1* and *CotH2* genes in *M. circinelloides*. *CotH1* and *CotH2* genes have important role during invasion of endothelial cells by acting as fungal ligands that bind to GRP78. The Cas9 nuclease and a synthesized gene specific gRNA with a deletion cassette was introduced by PEG mediated protoplast transformation.

Double-strand breaks of DNA were achieved by co-transformation of the Cas9 and gRNA with the deletion cassette repaired by own homologous recombination system of *Mucor circinelloides*. The presence of the *pyrG* gene was revealed by molecular analysis as selection marker in the coding sequence of *CotH1* and *CotH2* genes.

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TELEMEDICINE

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Telemedicine, a term coined in the 1970s, which literally means “healing at a distance”, signifies the use of ICT to improve patient outcomes by increasing access to care and medical information. Recognizing that there is no one definitive definition of telemedicine – a 2007 study found 104 peer-reviewed definitions of the word – the World Health Organization has adopted the following broad description: “The delivery of health care services, where distance is a critical factor, by all health care professionals using information and communication technologies for the exchange of valid information for diagnosis, treatment and prevention of disease and injuries, research and evaluation, and for the continuing education of health care providers, all in the interests of advancing the health of individuals and their communities”.

The terms ‘telemedicine’, ‘telehealth’ and ‘e-health’ are often used interchangeably. We examined the occurrence of these terms in the Scopus database. A total of 11,644 documents contained one of the three terms in the title or abstract. Telemedicine was the most common term, with 8028 documents referring to it, followed by e-health (n =2573) and then telehealth (n =1679). Telemedicine was referred to in documents from 126 countries; the terms telehealth and e-health were found in publications from 55 and 99 countries, respectively.

Telemedicine involves the provision of health care and sharing of medical knowledge using telecommunications technologies. Preventive, diagnostic, and therapeutic services, as well as patient education and assistance with self-management of health, can be provided via telemedicine. Telemedicine applications can be classified into two basic types, according to the timing of the information transmitted and the interaction between the individuals involved—be it health professional-to-health professional or health professional-to-patient. Store-and-forward, or asynchronous, telemedicine involves the exchange of pre-recorded data between two or more individuals at different times. These two basic approaches to telemedicine are applied to a wide array of services in diverse settings, including teledermatology, telepathology, and teleradiology. At the level of the individual, telemedicine can support improvements in a patient’s health and quality of life, particularly for those with chronic diseases, by enabling safer monitoring at home and reducing the number of hospital visits. For example, a review of 14 random controlled trials involving 4 264 patients showed that remote monitoring programmes reduced rates of admission to hospital for chronic heart failure by 21% and all causes of mortality by 20%. Telemedicine is gaining popularity due to the provision of ubiquitous health care services that is a fundamental need for every socialized society.

WATER AND THE ENVIRONMENT

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Managing the provision of safe drinking water has a renewed focus in light of the new World Health Organization (WHO) water safety plans. Risk analysis is a necessary component to assist in selecting priority hazards and identifying hazardous scenarios, be they qualitative to quantitative assessments. An increasing drive for water efficiency is leading to a corresponding growing interest in a variety of water recycling initiatives to assist in water efficiency efforts. While available knowledge and technologies exist to provide suitable fit-for-purpose recycled water, concern exists relating to water quality issues, particularly microbial pathogens and chemicals-of-concern. At times these concerns result in the requirement for over treatment of recycled water, which can lead to potentially valuable water reuse projects becoming too costly and inefficient to be viable. The risks from both microbial pathogens and chemicals-of-concern can vary depending on the type of recycled water, the type of pathogen or chemical present and their numbers or concentration, the water treatment employed and the resulting use of the recycled water. For any approach, acute diarrhoeal pathogens are often the higher risk issue for municipal water supplies, no matter how health burden is assessed. Furthermore, potential sequelae (myocarditis, diabetes, reactive arthritis and cancers) only further increase the potential health burden of pathogens; despite the enormous uncertainties in determining pathogen exposures and chemical dose-responses within respective microbial and chemical analyses.

Pathogens and chemicals-of-concern can vary in their ability to resist treatment and their survival in receiving environments. In addition, the risks associated with these contaminants depend on their ability to cause illness in susceptible members of the community. The presence of pathogens and chemicals-of-concern in recycled waters, the effectiveness of treatment methods to remove them and their persistence in the environment were examined.

ESSENTIAL OIL BASED DISINFECTANTS FOR BIOFILM ELIMINATION FROM
STAINLESS STEEL AND PLASTIC SURFACES

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Development of new, natural disinfectants is a challenge in the food industry. In our study, optimization of essential oils (cinnamon, marjoram, and thyme) based disinfectants was investigated. The essential oils (EOs) are natural, have antimicrobial effect and are Generally Recognized as Safe (GRAS). The Response Surface Box-Behnken Design (RSD) with three variables and 17 assays was used to optimize concentration of essential oils (EOs) (1.1–15.8 mg/ml), disinfection time (10 min), and level of pH (4.5-7.5) in the EO-based disinfection solutions. The optimized disinfectants were used against 24-, and 168-hours old *Escherichia coli* and *Listeria monocytogenes* biofilms formed on stainless steel and plastic surfaces. The EO based disinfectants eliminated the immature and mature biofilms, except cinnamon EO for *E. coli* where the killing time was over 30 minutes, which is too long for industrial application. The disinfectant effect of the EO-based natural solutions was in most cases equivalent or better compared to industrial sanitizers.

EFFECT OF CARBOHYDRATE HYDROLYZING ENZYME TREATMENT ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF FRUIT POMACE EXTRACTS

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Synthetic preservatives in foods provoke serious concern in consumers. Therefore, there is a need to develop new bioprocesses to produce natural antioxidative agents with antimicrobial properties. The phenolic compounds present in fruits can enhance the stability and shelf life of food products by increasing their antioxidant capacity, and inhibiting microbial growth. However, most of these phenolics are in glycosylated forms resulting in limited bioavailability. Carbohydrate-cleaving enzymes can release the phenolic aglycones. Here, enzymatic pomace treatment of oven dried and lyophilized black grape, apple and pitahaya samples was carried out using a cellulolytic cocktail produced from a *Rhizomucor miehei* isolate. To decrease cell wall integrity, *Aspergillus niger* pectinase was added to the reaction mixtures. After enzyme treatment, the antioxidant and antimicrobial potential of the extracts was examined against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Escherichia coli* and *Listeria monocytogenes*. Enzymatic treatments enhanced the antioxidant and antimicrobial activity of the fruit pomaces. Generally, total phenolic content and ferric reducing antioxidant power of lyophilized grape and apple pomace showed significant difference between S1 (cellulase treated) and S2 (cellulase/pectinase treated) samples. Extracts from oven dried and lyophilized grape pomace samples showed similar antimicrobial activity. In the case of lyophilized apple and pitahaya residues, however, higher antimicrobial activity was detected, as compared to oven dried samples. Treatment S2 increased the antimicrobial activity of grape and pitahaya pomace extracts, while treatments S1 and S2 showed similar performance for apple. For all extracts the resistant bacteria were *L. monocytogenes* and *E. coli* and the sensitive ones were *S. aureus* and MRSA.

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ABSTRACTS

POSTER SESSION

ADOLESCENT PERCEPTIONS OF THE RECENTLY IMPLEMENTED CLEAN AIR
LAW IN ROMANIA

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Background and main objective: In March 2016, an amended Romanian tobacco control law prohibits smoking in all enclosed public spaces. The aim of this study was to assess the perceptions of the new tobacco control law among adolescents in Romania.

Methods: A cross-sectional, self-administered questionnaire was administered among 8th grade students from three Transylvanian counties (Mures, Covasna, Harghita) during June 2016, approximately 2 months after the new law was implemented. The sample includes 695 students from 21 localities, 21 schools, and 55 classes. We report descriptive statistics regarding respondent perceptions of the new law.

Results: Almost half (46.2%) of the respondents reported that they had been informed about the clean air law in school. 26.7% students were not aware that smoking is not allowed near school. 46.9% of students were skeptical about the law. According to the students' perceptions, 2.5% of the smoking teachers and 8.6% of their friends stopped smoking. 13.8% of nonsmokers and 29% of smokers believed that the new law promoted secretive smoking ($p < 0.001$), such as smoking in various private places (e.g. street, hidden places, home, inside). 58.3% of occasional smokers and 16.7% of daily smokers indicated that they plan to reduce the amount smoking after law, while 12.5% and 5.6% plan quitting smoking, respectively ($p = 0.007$).

Conclusion: Overall, the law appears to have had a positive influence about their tobacco use perceptions among adolescents in Romania. There is an opportunity for additional efforts to promote cessation among adolescents who smoke daily and to discourage secretive smoking.

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STRUCTURE AND FUNCTION ANALYSIS OF THE NICOTINIC ACID CATABOLIC
PATHWAY SPECIFIC TRANSCRIPTION FACTOR, HXNR IN *ASPERGILLUS*
NIDULANS

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Despite many microorganisms can utilize nicotinic acid as sole nitrogen-source, only the prokaryotic catabolic pathways were studied so far. Because of the industrial potential of the pathway enzymes the research topic of prokaryotic nicotinic acid degradation became popular. The pathway enzymes can serve as bioconversion tools for processing heterocyclic precursors to obtain biological active drugs and compounds for pharmaceutical- and agricultural chemicals. Four different catabolic routes were described in prokaryotes but according to our research the eukaryotic pathway differs from the prokaryotic routes.

The investigation of the first eukaryotic nicotinic acid catabolic pathway has begun in our laboratory on the model organism *Aspergillus nidulans*. We aimed to unravel the genetic and biochemical background of the nicotinic acid degradation and we have identified three geneclusters, NDC1 (Nicotinic acid Degradation Cluster 1 – hxnS, hxnR, hxnP, hxnT, hxnY, hxnZ) and NDC2 (hxnX, hxnV) and NDC3 (hxnN, hxnM) involved. All cluster genes are induced by nicotinic acid or 6-hydroxynicotinic acid metabolite and the pathway specific, Cys(2)His(2) binuclear transcription factor, HxnR, which occurs only in fungi.

To study the relation of structure and function of HxnR we obtained constitutive hxnR mutants by UV mutagenesis. After the mutagenesis we have isolated thirty constitutive mutants on selective media, which produced constitutively the purine hydroxylase II enzyme, HxnS. In the thirty constitutive mutants we have identified the mutations by sequence analyses, and investigated putative HxnR orthologues and constructed a CONSURF profile of the protein. All constitutive mutations mapped in highly conserved regions in two patches which are necessary to maintain the protein in its physiological inactive state.

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MEASLES OUTBREAK IN CSONGRÁD COUNTY FROM 29 JANUARY 2017 UNTIL
10 MARCH 2017

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Measles is a serious disease caused by a virus in the paramyxovirus family. In 1980, before widespread vaccination, measles caused an estimated 2.6 million deaths each year. The disease remains one of the leading causes of death among young children globally, despite the availability of a safe and effective vaccine. Approximately 134 200 people died from measles in 2015, mostly children under the age of 5. The virus infects the respiratory tract, then spreads throughout the body. It is characterized by a prodrome of fever and malaise, cough, coryza, conjunctivitis and pathognomonic enanthema (Koplik spots) followed by maculopapular rash. The rash usually appears about 14 days after a person is exposed. The rash spreads from the head to the trunk to the lower extremities. Patients are considered to be contagious from 4 days before to 4 days after the rash appears. Measles is one of the most contagious of all infectious diseases; approximately 9 out of 10 susceptible persons with close contact to a measles patient will develop measles. The virus is transmitted by direct contact with infectious droplets or by airborne spread when an infected person breathes, coughs, or sneezes. Measles virus can remain infectious in the air for up to 2 hours after an infected person leaves an area.

From 29 January 2017 until 10 March 2017 we experienced a measles outbreak with the first known morbilli patient (from Romania) treated for fever, catarrh and rash (which was at first diagnosed as drug hypersensitivity) on 13 February 2017 at the emergency department of the hospital in Makó. Totally 13 persons were involved in this outbreak in Makó and Szeged (11 health care professionals and 2 persons not belonging to this group with the following age distribution: 20-29 years: 1 female, 30-39 years: 3 female + 2 male, 40-49 years: 6 female + 1 male). Based on sequencing of viral RNA genome 5 cases revealed B3 genotype. In consequence of our enormous efforts and interventions including active measles surveillance, quarantine, isolation, aspecific preventive measures (medical examination, education, usage of protective equipment), observing in-patients, epidemiological monitoring of health care workers, immunological screening, post-exposure vaccination (N=334) we could successfully terminate the occurrence of further measles cases in Csongrád county.

DETERMINATION OF PESTICIDE RESIDUES IN THE STARA TISA

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Stara Tisa meander lies within the borders of Nature Park Stara Tisa and is under level III of protection. The meander is surrounded by arable land and fields; thus, water potentially contains high levels of agrochemicals (pesticides and fertilizers), but it is intensively used for irrigation. The simple validated (according to SANTE/11945/2015) multiresidue method was used for the determination of 70 pesticides by LC-MS/MS with ESI. The water sample was cleaned up and concentrated by solid phase extraction on OASIS HLB cartridges. The obtained LOQs for all the pesticides investigated were 0.020 µg/L. The accuracy and precision were determined via recovery experiments, spiking reagent water at 0.02, 0.1 and 0.2 µg/L, in three replicates per level and the mean recoveries were 64.9 – 109.5% with the RSD of 1.7 – 14.2% for all the compounds. The developed LC-MS/MS chromatographic procedure exhibits the linearity ($R^2 > 0.99$) in the range from 10 to 200 ng/mL with RSD less than 15%. The carbofuran-D3, atrazine-D5 and isoproturon-D6 were used as internal standards. The obtained results were compared with the MACs according to the Directive 2008/105/EC and Commission Implementing Decision 495/2015/EC. Detected pesticides were terbuthylazine-desethyl and terbuthylazine in the concentration of 0.009 µg/L, propazine (0.008 µg/L), clothianidin (0.007 µg/L) and clomazone in the concentration of 0.0038 µg/L. The pesticide levels were under the MACs in all the samples.

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SELENOCOMPOUNDS AS COMBINATIONAL AGENTS IN CANCER
CHEMOTHERAPY: AN *IN VITRO* PERSPECTIVE

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Introduction: The importance of combination chemotherapy in the treatment of various malignancies has been described in detail both in the clinical practice as well as in a research setting. The significant problem of cancer multidrug resistance (MDR) and tumour cell heterogeneity further confirms the relevance of multiple drug treatment. Organoselenium compounds as anticancer agents have attracted reasonable attention both as monotherapy and as adjuvants in combination chemotherapy. It has been described that they can influence inflammatory processes, reactive oxygen species production, immune response, and they can induce various programmed cell death pathways.

Aim: The aim of our study is to evaluate the efficacy of cytotoxic selenocompounds in combination chemotherapy using an *in vitro* model system.

Materials and methods: A checkerboard microplate method was applied to evaluate the effect of drug interactions between the tested compounds (1: cyclic selenoanhydride; 2-11: selenoesters; 12-15: chalcogen derivatives) and the reference drugs (doxorubicin, cisplatin, 5-fluorouracil, topotecan). The assay was carried out using multidrug-resistant mouse T-lymphoma cells overexpressing the ABCB1 transporter. The nature of drug interactions was expressed as combination index (CI) values using the Chou-Talalay method.

Results and conclusions: The tested compounds showed favourable interactions with doxorubicin, topotecan (topoisomerase-inhibitors) and 5-fluorouracil (antimetabolite). The reference chalcogens also presented synergism with some of the anticancer drugs. In contrast, antagonism was generally observed with cisplatin (alkylating agent). The methyl-ketone selenoesters (9-11) were active in small concentrations, while the compound containing a thiophene ring (2) proved to be the most effective, as it showed positive interactions with all tested drugs. The tested compound showed promising properties as combinational agents. The design of further experiments is needed to fully assess their potency as adjuvants.

PRESENCE OF LEGIONELLA IN WATER SAMPLES FROM COMMUNITY AND INDUSTRIAL FACILITIES

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Legionellosis is a rare form of pneumonia caused by *Legionella pneumophila* and other *Legionella* species. Except for some cases, the disease is transmitted through inhalation of *Legionella* contaminated aerosols. Most frequently encountered sources of *Legionella* infections are cooling towers, air-conditioning units, hot and cold water networks, spa pools, high pressure cooling and cleansing equipments. Nowadays, *Legionella* risk management focuses on public facilities (e.g., hotels, spas, hospitals, schools, office and industrial buildings) which operate water systems generating aerosols, and the water temperature range is between 20°C and 50°C. In the last decade (31.01.2007-31.01.2017) the National Public Health Institute analyzed 1327 water samples; they were collected in Hungary from hot water networks, spa pools and cooling water (from schools, office buildings, industrial facilities, and spas). Analysis of these samples revealed that the hot water systems were colonized by *Legionella* in 42% of the cases; 3% of them showed very high (>100 000 CFU/L) *Legionella* counts. In case of pools (205 samples) similar values were obtained. Concerning the 734 cooling water samples, 78% of them were under the limit value of 100 CFU/L.

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STRESSFUL LIFETIME RESULTS IN NEUROINFLAMMATION

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Modern stressful lifestyle poses psychosocial and physical stressors, thereby induces centrally mediated complex adaptive changes referred to as systemic stress response. In addition to changes in metabolism and immune regulation, chronic stress results in significant rearrangements in the neuroendocrine system. Microglia play a main role in generating neuroinflammation, and contribute to the formation, operation and adaptation of neuronal circuitry in health and disease. Fractalkine is a key regulator of microglial activity and neuron-microglia communication. Its receptor (CX₃CR₁) is mainly expressed in microglial cells. We aimed to characterize chronic variable stress (CVS)-induced metabolic changes, and associated microglial alterations in metabolic-related brain regions.

Experiments were performed on C57Bl6 (control) and transgenic mice CX₃CR₁^{-/-} in which fractalkine signaling is impaired. Half of the animals from each genotype were exposed to chronic variable stress for four weeks (n=10/9, CVS). Control animals were not stressed.

Body weight of the animals and the main metabolic parameters were analyzed by indirect calorimetry. Iba-1 immunohistochemistry followed by morphometrical analysis was used to visualize morphological changes of microglia in the arcuate nucleus.

Changes were detected in body composition, home cage locomotor activity and metabolic parameters after CVS. Chronic stress resulted in increased respiratory exchange ratio (RER) in wild type but not in CX₃CR₁^{-/-} animals. CVS procedure significantly increased the weight of the adrenal glands and plasma corticosterone. Furthermore, CVS resulted in microglia activation in the arcuate nucleus, the main energy sensor in the brain, but this activation was not seen in stressed CX₃CR₁^{-/-} mice.

In conclusion, we provided evidence for chronic stress induced metabolic changes in mice which were parallel with activation of microglia in the arcuate nucleus, in which fractalkine signaling had an important role.

DETECTION OF NEUROTOXICITY OF TITANIUM DIOXIDE NANOPARTICLES *IN VIVO* AND *IN VITRO* MEASUREMENTS

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Due to scientific and technological development, an increasing number of industrial processes and products are based on NPs, that is, particles with <100 nm typical diameter, involving the risk of environmental release and human exposure. The physical, chemical and biological properties of nanoparticulate substances differ from those seen in other physical states, leading to biological interactions and possible toxicological consequences not seen with more conventional materials.

Widespread application of titanium dioxide nanoparticles (TiO₂ NPs) raises the question of potential occupational, environmental and/or intentional human exposure and health hazard, including nervous system damage. Up to now, contradictory findings have been published on absorption and migration, including penetration to the brain, of TiO₂ NPs after pulmonary, dermal or oral exposure. That nervous system effects can be expected is indicated by the oxidative stress generating potency of TiO₂ NPs and by biochemical, histological and functional alterations observed mostly in mice.

In the present work, toxicity of TiO₂ nanorods of 10x100 nm size, suspended in a vehicle of 1% polyacrylic acid in phosphate-buffered saline, was investigated. General toxicity at cellular level was tested on lung-derived A594 cultured cells while functional neurotoxicity was investigated in young adult male Wistar rats exposed by the TiO₂ NPs via intratracheal instillation for 28 days.

On A594 cells, concentration- and treatment time-dependent decrease of viability and increase of free radical generation was observed.

In the treated rats, deposition of NPs, and increased activation of phagocytes, was seen in the lung interstitium.

Changes, partly significant, of the numeric parameters of electrophysiology, e.g. latency of cortical responses or nerve conduction velocity, indicated functional damage in the treated rats. Also in the climb test, treated rats' performance was weaker than the control ones'.

Our results provided some more arguments to the neurotoxic character of TiO₂ NPs which is relevant also in terms of human health.

SCREENING OF dsRNA ELEMENTS IN FUNGI INFECTING *AGARICUS BISPORUS*

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Mycoviruses (fungal viruses) are typically symptomless, however, some of them can cause smaller or greater changes in the phenotype of their fungal host as well as they can reduce or enhance the fungal virulence causing hypovirulence and hypervirulence effects. Currently, there are more than 90 known mycoviruses and most of them have linear double stranded RNA (dsRNA) genome, but there are some fungal viruses which have single stranded RNA or DNA genome. Mycoviruses have a unique characteristic: they are transmitted only intracellularly, by horizontal (through hyphal anastomoses and protoplast fusion) and vertical routes (through sexual or asexual spores). Mycoviruses have been found in almost all major phyla of fungi. They were described in Ascomycota and Basidiomycota species as well as in the different groups of the former Chytridiomycota and Zygomycota phyla.

The dry and wet bubble, caused by fungal pathogens *Lecanicillium fungicola* and *Mycogone perniciosa* respectively, are two detrimental diseases that affect the commercial cultivation of white button mushroom (*Agaricus bisporus*). This problem becomes more and more serious, as 40% of commercially produced mushrooms come from *A. bisporus* in the entire world. Both pathogenic fungi tend to parasitize on fruiting bodies (edible part) of *A. bisporus*, deteriorate them, and ultimately cause huge economic losses to the growers.

The aim of this research was the screening of *Lecanicillium* and *Mycogone* species for the presence of dsRNA elements.

During our research, we have screened 19 *Lecanicillium* and 2 *Mycogone* strains. dsRNA elements were isolated with CF-11 cellulose chromatography from a total nucleic acid extract. The nature of the dsRNA elements has been verified by their resistance to DNase I and S1 nuclease digestions. In some of the isolates we found a characteristic pattern with 3 discrete fragments of dsRNA molecules with different sizes (approximately 3.5, 3.1 and 2.8 kb).

Our long-term goals are the precise identification of the viruses isolated from these fungi and the examination of the effect of their presence on the host phenotype. If these viruses are able to cause hypovirulence, then they might be used as biocontrol agents against the fungal pathogens of economically important mushrooms.

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FUMONISIN PRODUCTION OF *BIPOLARIS MAYDIS*

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Bipolaris species are plant pathogen cosmopolitan filamentous fungi that occur frequently in regions with warmer climate. *Bipolaris maydis* (*Cochliobolus heterostrophus*) is an economically important species responsible for the southern corn leaf blight (SCLB) causing serious crop losses worldwide. In the USA (e.g. in Illinois State) in 1970 this fungus destroyed more than 15% of maize crop. Preharvest fungal infection can lead to the accumulation of mycotoxins in food and feeds including fumonisins. These compounds are a large group of carcinogenic mycotoxins that were originally identified in *Fusarium verticillioides*, but several other species are also able to produce these mycotoxins. Recently, a polyketide synthase (PKS) gene, which showed high similarity to the PKS taking part in fumonisin biosynthesis in *Fusarium verticillioides* has been found in the genome of *C. heterostrophus*.

During our work, we identified several other fumonisin biosynthetic genes in the available genomes of *C. heterostrophus* isolates C4 and C5. To investigate the fumonisin producing ability, *B. maydis* CBS 130.26 was cultured on different media at 25 °C and 30 °C. By HPLC-MS technique we were able to detect the presence of fumonisin isomer-like compounds except FB1, however further investigations are needed to clarify these results.

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SINGLE ACRYLAMIDE DOSE AFFECTED MAST CELL NUMBER IN RAT COLON

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The detection of acrylamide (AA) in various food commodities has triggered a myriad of studies investigating the potential biological effects of this chemical substance. The main toxic endpoints of AA in humans and animals are: neurotoxicity, developmental and reproductive toxicity, genotoxicity and carcinogenicity.

Aim of the present study was to investigate if single oral dose of AA affected the mast cell number in young rat colon.

Animals were gavaged with different AA concentrations (0, 25, 50 mg/kg/day) on 23rd postnatal day, and sacrificed 24 hours after treatment. Colon tissue samples were processed for routine paraffin embedding and Toluidine blue staining protocol.

The experimental groups showed no significant decrease in the number of mast cells compared to the controls. Despite that dose-dependent reduction of mast cell number after single AA exposure was noticed.

The results of present study showed immunosuppressive effect of AA on young rats colon mast cells only 24 h after treatment. These findings provoke an extension of the study to better clarify the risk of AA for human immune system.

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SUBCHRONIC ACRYLAMIDE TREATMENT REDUCED MUC2 SECRETION IN RAT COLON

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Dietary acrylamide (AA) is derived from heat-induced reactions between the amino acid asparagine and reducing sugars at elevated cooking temperatures and in low moisture conditions. AA has been found primarily in plant-based foods, notably potato products. AA is also a known human neurotoxicant and animal reproductive toxicant.

Aim of the present study was to determine the effect of subchronic AA treatment on secretion of MUC2 mucus, the major secreted mucin in colon.

Animals were gavaged with different AA concentrations (0, 25, 50 mg/kg/day) from 23 to 42 postnatal day. Colon tissue samples were processed for routine paraffin embedding and immunohistochemistry staining protocol with the polyclonal rabbit anti-rat MUC2.

Dose-dependent reduction of MUC2 positive goblet cells was noticed between groups in both crypt regions. Prominent changes was revealed in the upper crypt region, where the MUC2 positive goblet cells was significantly decreased ($p < 0.05$) in both treated groups, while in the lower crypt part the MUC2 positive goblet cells was significantly decreased ($p < 0.05$) only in high-dose group comparing to the control group.

Goblet cells represents first-line defenders of the mucosa. The stability of mucus layer is essential in preserving the integrity of the intestinal epithelium. Scientist shown that chemical irritants applied to the luminal surface of the mucosa can elicit local mucus release from goblet cells. Our study shown that AA affects mucins exocytosis from goblet cells, so we can conclude that AA is a chemical irritant for colon goblet cells. Based on our research we must pay full attention on AA and its content in our diet.

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ACRYLAMIDE-INDUCED TOXICITY IN LIVER NON-PARENCHYMAL CELLS

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As a monomer, acrylamide (AA) is known for its toxicity. AA is often present in considerable amounts in baked, fried, grilled and roasted starch-based food. It is spontaneously formed during Maillard browning reaction, between amino acids (asparagine), and reducing sugars (glucose or fructose) at high temperatures (≥ 120 °C) with a presence of water.

Processed carbohydrate-rich food containing AA is consumed in a greater extent by the children and young population, in general, placing them into a risk of being more exposed to AA than the adults.

After entering the body, AA is metabolized in the liver. Toxic effects of AA on hepatocytes were previously reported. Nevertheless, its impact on non-parenchymal liver cells has not been investigated, although these cells are important participants in maintaining liver homeostasis. The study examined apoptotic effect of AA on non-parenchymal cells in rat liver. Juvenile male Wistar rats received oral application of 50 mg/kg per body weight of AA, 5 days a week, for 21 day. The control animals received distilled water in the same way. Following the experiment, liver samples were formalin-fixed and paraffin-embedded. Subsequently, tissue sections were immunohistochemically stained for Caspase 3 (marker for apoptosis) and microscopically examined.

Histological analysis detected an increase of immunoreactivity in non-parenchymal cells for Caspase 3 in AA-treated group compared to the control. Immunopositivity was mainly present in cytoplasm of Kupffer and endothelial cells of liver sinusoids.

Obtained results in this study suggest AA potential for apoptosis induction in non-parenchymal liver cells. Present findings provide new insight regarding AA mode of action as well as its toxic outcome in the liver. In order to fully understand the whole spectrum of these implications, further investigation is required.

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A NEW OPPORTUNITY IN REHABILITATION: SELF-HELP CLUBS FOR
PARKINSON'S DISEASE

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The number of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (PD), is increasing throughout the world in parallel with the aging of population. PD affects approximately ten million people globally and the prevalence of the disease rises. Today, approximately 20,000 people with PD live in Hungary; it is estimated that the number will be doubled by 2040. PD is important not only in medical but also social and economic point of view, and these aspects can be analyzed in the framework of studies on quality of life (QoL). The aim of the current study was to measure the QoL of individuals with PD attending self-help clubs in Hungary. Our survey was delivered in 2013-2014; the 39-item Parkinson's Disease Questionnaire examined the daily living factors influencing the QoL along eight dimensions. The questions were scored from 0 to 4 (0=never, 1=rarely, 2=half the time, 3=frequently, 4=always), from which the scores of dimensions were calculated (0= perfect health, never have difficulty; 100=worse health, always have difficulty); higher scores representing worse QoL. All respondents (n=101) were active members of Parkinson clubs from different parts of the country. Descriptive statistics (percentage, mean and standard deviation) and one-way ANOVA tests were applied; $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using IBM SPSS version 23. The study protocol was approved by the Human Institutional and Regional Biomedical Research Ethics Committee, University of Szeged. Sex distribution showed a female dominance (61.4%), the mean age was 67 years. The concept of QoL is multidimensional and many factors may have an effect on it, such as those connected to the individual, social networks, environment and society, but health status is a key factor in determining how good a person's QoL is. Among the eight dimensions the highest score was found in "bodily discomfort", while the lowest was in "social support". Our results seem to be correlated with the attendance of self-help clubs providing mental and social health support. The operation of the patient organizations such as the patient clubs seems to be an excellent way of the rehabilitation among PD participants.

INVESTIGATION OF FATTY ACIDS FROM *ASPERGILLUS* STRAINS

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The isolates belonging to the genus *Aspergillus* have an importance in fermentation industry as the producers of various organic acids, enzymes and secondary metabolites. Among these mycotoxins have a serious impact on the health of humans and animals. The *Aspergillus* genus includes 344 species, which could be characterized both with morphological and molecular taxonomical techniques. Besides these, the characterization of cellular fatty acid composition also seems to be a useful approach for the identification of these filamentous fungi. *Aspergillus* isolates representing different sections of the genus were grown under standard conditions without agitation for 7 days. Fatty acids were extracted from the fungal mycelia by a five-step sample preparation procedure involving harvest, saponification, methylation, extraction and final wash. After the sample pretreatment the recovered fatty acid esters were analysed by gas-chromatography. The similarities were quantified among the species on the basis of peak area ratio observed on the chromatograms. In the tested *Aspergillus* isolates the most common fatty acids were the hexadecanoic-, octadecanoic-, 9-octadecanoic-, and 9,12-octadecanoic-acid. The main objective of this study was to determine a characteristic fatty acid composition for various members of the *Aspergillus* genus and to reveal the relationship between the taxons and fatty acid profiles.

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EPIDEMIOLOGY AND CHALLENGES OF OSTEOPOROSIS IN HUNGARY

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Several compiled studies have shown that osteoporosis is a significant disease in Hungary, after current and future populations were analyzed and projected. Not only does osteoporosis affect both sexes, but lifestyle choices made early have shown to have an impact later on in bone life. Thankfully, screening procedures are in place that first came about years ago in the country. After the definition of the disease itself was implemented, investigations were carried out in order to better prepare Hungary for what is a real issue which has long-term ramifications. Trends and results began to take effect in the country's protocols and realistic improvements were witnessed. Over the years, doctors and researchers (both Hungarian and otherwise) have worked to further our understanding. More and more was being discovered about how and why the disease arises.

Screening initiatives and several clinical risk factors for both primary and secondary osteoporosis have been brought forth. Factors such as genetics, nutrition, background diseases, lifestyle, and the impact on men were developed. More was also being learned about how the disease could be prevented. The influence of patient compliance remains a challenge today.

HEALTH RISK ASSESSMENT OF ORGANOCHLORINE COMPOUNDS IN STREET DUST OF NOVI SAD, SERBIA

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Organo-chlorinated compounds (OCCs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are an important groups of organic pollutants. Their high chemical stability, lipophilicity and persistency give them the ability of long term persistence in the environment. Their persistence is particularly due to their strong affinity for particulate matter leading to slow degradation. Four decades after national governments began to ban their production and usage, PCBs and OCPs remain a major concern to human health and the natural environment. Street dust can be considered a valuable indicator of environmental pollution as the main source of street dust is atmospheric depositions before its incorporation into the soil matrix. Thus, the extent of atmospheric contamination may be better revealed by street dust than by bulk soils. Street dust is easily re-suspended or adhered to human skin, being an advantage to assess health risk, and also has received much attention due to its potential health risk to children through hand-to-mouth activities. Street dust samples (n=30) were collected from different types of locations: schools, recreational areas, residential and industrial zones in order to investigate 6 indicator PCBs and 18 OCPs content in Novi Sad, and to compare them with national guidelines. The total lifetime span carcinogenic risks (TLCR) were calculated for PCBs and DDTs, covering ingestion, dermal contact, and inhalation of the re-suspended dusts. The total concentrations of PCBs in street dust samples were in the range <0.008 ng/g -3.3 ng/g, with a mean value of 1.9 ng/g. PCBs were detected only at four sampling sites, whereas at all other were below limit of detection. Within the investigated PCBs (28, 52, 101, 138, 153 and 180) PCBs 153, 138 and 180 were only detected. Among examined OCPs, DDTs were only detected. The total concentrations of DDTs were in the range <0.010 ng/g – 249 ng/g with a mean value of 48.6 ng/g. The concentration of PCBs and DDTs were compared with corresponding target and intervention values according to the Serbian national limits for PCBs and DDTs in soil. At all sampling sites, concentrations of PCBs were below target value (20 ng/g) sets by the Serbian legislation. The concentrations of DDTs at 27 from 30 sampling sites were higher than target value (10 ng/g), but was much lower than intervention value (1000 ng/g for DDTs), so no further action is necessary. The TLCR at all sampling sites were bellow 10^{-6} for PCBs and DDTs, indicating that the lifetime cancer risk was acceptable. The highest risks for PCBs and DDTs were from the ingestion, followed by dermal contact and the inhalation.

SELECTIVE TOXICITY OF NEW SYNTHETIC ANIONOPHORES

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Antibiotic resistance is an emerging and continuously increasing problem in human healthcare. Clinical practice encounters a growing proportion of multidrug resistant strains of human pathogenic bacteria and fungi. This situation sometimes makes the known antibiotics unusable and the infections caused by multidrug-resistant strains complicated or even impossible. Therefore, there is great demand to find new potent antibiotics or synthesize drugs against these pathogens.

During this study, the antimicrobial activities of three synthetic anionophores were investigated against pathogenic yeasts and bacteria. The compounds selectively inhibited the growth of Gram positive bacteria; but none of the examined Gram negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, *P. fluorescense*, *Xanthomonas campestris*, *Salmonella enteritidis*) were sensitive to them.

All the three compounds proved effective against the human pathogenic yeast, *Cryptococcus neoformans*, however, the minimum inhibitory concentrations were different. One compound had moderate effect on *Candida parapsilosis* the other investigated *Candida* species were resistant.

The effective compounds exerted cidic effect either on Gram positive bacteria or *Cr. neoformans*. Paradoxical growth of *Cr. neoformans* was observed in the respond to compound ACF508 at higher concentration of the drug. This phenomenon is known for *C. albicans* in response to echinocandin treatment as well. As echinocandins inhibit the fungal cell wall synthesis, presumably this compound acts in the same way on *Cr. neoformans*.

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BULK PRODUCTION OF THE ANTI-YEAST PROTEIN NFAP₂ IN *PENICILLIUM CHRYSOGENUM*

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Neosartorya fischeri antifungal protein 2 (NFAP₂) secreted by *N. fischeri* NRRL 181 represents a new member within the group of cysteine-rich antifungal proteins from Ascomycetes. This thermostable protein exerts high antifungal activity on clinically relevant yeasts, possibly due to its plasma membrane disruption ability. These features render NFAP₂ a highly interesting and promising compound for further structure-function studies and the development of a new antifungal strategy against yeast pathogens. One of the limiting factors of these studies on NFAP₂ is its low-yield expression by the native producer. Recently, we developed a *Penicillium chrysogenum*-based expression system, which proved to be appropriate to produce high amounts of cysteine-rich antifungal proteins from Ascomycetes for structural and functional investigations. We adopted this system for bulk production of NFAP₂. Based on mass spectrometry analysis, this system produced NFAP₂ in a correctly processed form. The average yield of NFAP₂ was 40-times higher than in the native producer. The antifungal efficacy of recombinant NFAP₂ was comparable to the native protein; it also showed high anti-yeast activity against different *Candida* species in broth microdilution assays. These results further evidenced the versatility of the *P. chrysogenum*-based expression system, and allowed us to produce sufficient amounts of antifungal active NFAP₂ for studies focusing on antifungal mechanism, toxicity and *in vivo* activity.

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SCREENING OF RHIZOMORPH-ASSOCIATED SOIL SAMPLES FOR POTENTIAL BIOCONTROL AGENTS AGAINST FOREST-DAMAGING *ARMILLARIA* SPECIES

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Various *Armillaria* species (honey fungus) are important inhabitants of forest soils. They belong to the white-rot fungi playing an essential role in nutrient production required for terrestrial life. Plant pathogenic members of the genus *Armillaria* are able to attack and destroy mostly woody, dicotyledonous plants, causing necrotic lesions beneath the root bark. These species have been suggested behind the devastation of forests in the Keszthely-hill region (Hungary) during the last decade. Chemical pesticides are applied world-wide in agricultural production; however, soil biocontrol agents might elicit the harmful chemicals. There is an emerging need for an efficient, environment-friendly approach to prevent the damage caused by these pathogens.

The aim of our study was to screen for potential biocontrol agents among bacteria and fungi associated with *Armillaria* rhizomorphs. Four rhizomorph samples were collected from Keszthely-hills and identified as *Armillaria gallica*. *Pseudomonas* and *Trichoderma* isolates were also purified from the samples and identified by sequence-based molecular tools. Frequently encountered *Pseudomonas* species were the *P. mandelii*, *P. fluorescens* and *P. putida*; while the *Trichoderma* isolates belonged to the *T. virens*, *T. harzianum* and *T. citrinoviride*. *In vitro* antagonism and antibiosis tests were performed to evaluate the potential of the *Pseudomonas* and *Trichoderma* isolates to control *A. gallica*. According to these results, *Trichoderma* isolates seem to be remarkable candidates for biological control against plant pathogenic *Armillaria* species.

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DETECTION OF ANCIENT TB INFECTION FROM BONES ON THE BASE OF
SPECIFIC LIPID BIOMARKERS

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As tuberculosis is still causes the highest number of deaths among infectious diseases, the research on its pathomechanism and evolution of its infectious agent are surpassingly important to achieve better understanding. This disease is mainly caused by *M. tuberculosis* belonging to the *Mycobacterium tuberculosis* complex. The complex includes several species with the ability to cause tuberculosis. Despite tuberculosis being primarily known because of the large-scale destruction in the beginning of the 20th century, this disease has been present at least for 10000 years in the history of mankind.

Since the fast and accurate recognition of the disease has a great priority, several detection methods were developed to diagnose TB infection in the clinical practice. Some of these methods were sensitive enough to be adapted to historical anthropology, for example DNA and lipid biomarker examinations. These molecular and analytical techniques can complement the macromorphological investigations with great efficiency. One of the analytical techniques is based on specific lipids that build up the Mycobacterial cell wall. We can use mycolic acids, mycocerosates and mycolipenates as biomarkers. Several samples from the osteological collection of the Department of Biological Anthropology were examined with a lipid biomarker based protocol optimized for the detection of mycolic acids with LC-MS technique from ancient bones.

In the present study, the most interesting cases will be presented from the mentioned collection, which were examined to diagnose ancient tuberculosis infection.

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CHARACTERIZATION OF NOVEL TYPE SURFACE PROTEINS IN MUCORALES

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Hydrophobic surface binding protein A (Hsb-A) is a small secreted protein which was first isolated from culture broth of *Aspergillus oryzae* RIB40. These protein could recruit lytic enzyme CutL I to the surface of hydrophobic solid material and promote their degradation or directly interact with the enzyme and increase their activity. This protein also participates in fungal resistance to stress that could be caused due to toxicity of some aromatic compound or reactive oxygen species released during the degradation process. During infection of MH-S macrophages, *Lichtheimia corymbifera* expressed Hsb-A like protein in high level. Hsb-A protein is functionally uncharacterized in Mucorales, hence could pose as a potential therapeutic target.

We have found six *hsb-A* genes, encoding hypothetic Hsb-A proteins in *M. circinelloides* genome, which are homologous to *L.corymbifera* Hsb-A. Two genes (*hsb-A1* and *hsb-A2*) are highly expressed during the life cycle of the fungus.

The objective of the current study is to isolate and characterize Hsb-A proteins in *L. corymbifera* and *M. circinelloides* by constructing an expression system in *Pichia pastoris* and purifying the expressed proteins for further analysis.

Our other objective was to analyse the role of Hsb-A protein in the pathogenesis of *L. corymbifera* and *M. circinelloides* by constructing deletion and overexpression mutants. For overexpression, *hsb-A* genes of *Mucor* were isolated and cloned into pPT81 vector. These vectors are transferred into *M. circinelloides* by PEG-mediated protoplast transformation method. We have started to create a knock-out mutant from *Lichtheimia* and *Mucor hsb-A* genes therefore deletion cassettes were also built. The transformation and characterization of mutants are in progress.

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EFFECTS OF RODENTIZED MOZART'S MUSIC AND TREADMILL EXERCISE ON THE COGNITIVE BEHAVIOR IN AN *IN VIVO* RAT MODEL

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By 2050, 40% of the population will probably be over 60 years, so neurodegenerative diseases typical for old age will be more prevalent. Beyond known risk factors (pesticides, trauma) other stressors, intensified by modern lifestyle, may play there an additive or synergistic role. Systemic responses to distress include altered cognitive behavior which is also the earliest indicator of slowly developing neurodegeneration. Regular physical activity was shown to decrease incidence of ROS-associated neurodegenerative diseases. As mechanism, adaptation induced by oxidative stress can be assumed. In our experiment the effect of rhythmic music and exercise on the cognitive behavior was investigated.

Sixty rats, in 5 groups of 12, were used in the 10-week experiment where their cognitive behavior (spatial memory performance, spontaneous exploration, psychomotor, rota-rod activity as well as sensorimotor gating) was investigated. The "music" group listened to *Mozart's* piano sonata in D major (K 448) transposed higher by two octaves (rodentized sonata) at 60 dB loudness, once daily for 80 minutes, while the positive control group was exposed to white noise of identical parameters. The „trained" animals performed treadmill exercise, once daily for 12 minutes, and the fourth ("trained music") group received rodentized musical stimuli plus treadmill exercise as above. Negative controls received neither music nor treadmill. Body weight was measured daily. On the 10th week, acoustic startle response and pre-pulse inhibition as well as co-ordination performance of the rats was recorded individually. At the end of that week, peripheral blood was taken to measure corticosterone and glucose. Weight of brain and adrenals was measured during dissection.

Performance of rats in the "music" group was significantly improved vs. both controls in short- and long-term and long-lasting working memory and recall. Treadmill exercise alone had no effect, but combined with music increased long-term and long-lasting spatial memory. Body weight in "music", "trained" and "trained music" groups was significantly higher at the end of the experiment than in controls. In the same three groups, brain-related relative adrenal weights indicated no chronic distress, but elevated blood glucose and decreased stress-hormone levels in peripheral blood suggested eustress. Acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) onset latencies were significantly decreased in these animals vs. control, but the ASR peak time was attenuated only in the "trained" group vs. both controls and "music" group. PPI peak amplitude of "trained music" rats decreased vs. "music" animals. In the rats' co-ordination performance, no difference was seen.

Conclusion: Rodentized *Mozart's* sonata and moderate intensity treadmill exercise as well as their combination, applied regularly over the 10 weeks, caused no chronic stress or anxiety in the animals, but induced temporary eustress that promoted hippocampus-dependent learning process and memory fixation. Both expositions – likely acting on both hemispheres – were able to change the cholinergic innervation of ASR/PPI central area which converges at the level of the reticularis pontis caudalis with "limbic CSPP" circuitry (hippocampus, prefrontal cortex, striatum, pedunculopontine tegmental area, etc.) and enhance rewarding effect of mesolimbic and mesocortical dopaminergic projection in nucleus accumbens.

MANGANESE INDUCED BEHAVIORAL ALTERATIONS AND
NEUROPROTECTIVE MEANS IN A SUBCHRONIC RAT MODEL

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The symptoms (cognitive deficit, psychosis, movement disorders) of the Parkinson-like disease caused by manganese (Mn) exposure are related to the involvement of Mn-accumulating structures (basal ganglia, nigrostriatum, hippocampus) of the central nervous system and damage of astrocyte-neuron interaction as well as to (di)stress. Like in other neurodegenerative diseases, synaptic protection is a key element in progression of manganism. Regular physical activity and melodic, rhythmic music can reduce the progression of lifestyle- and stress-dependent chronic illnesses. The aim of our experiment was to examine the effects of regular music and light exercise on Mn-induced neurotoxicity and stress response by functional, morphometric, chemical and genetic methods.

Out of five groups of 7 rats (altogether 35), four were treated for 10 weeks with 14.8 mg/kg b.w. MnCl₂ solution per os, from which one group listened to *Mozart's* piano sonata in D major (K 448) transposed higher by two octaves („rodentized” sonata) once daily for 1.5 hours, the other performed treadmill exercise for 10 minutes, while the third group was exposed to both (epigenetic) effects. Positive controls had MnCl₂-treatment but neither music nor exercise, and negative controls were untreated. The animals' cognitive behavior (spatial memory, spontaneous exploratory activity), emotional status and attention (psychomotor performance, sensorimotor gating) as well as coordination ability was tested. Their weight gain was measured daily. Peripheral blood glucose and stress hormone levels were determined at the end of the 10th week. Measurement of organ weights, hippocampal β -actin, MAPK1 stress kinase and APP levels, and gene expression (mRNA) of the ionotropic (NR2A, NR2B) and metabotropic (mGluR2, mGluR3) glutamate receptors were performed during necropsy.

Acquisition of new information was positively influenced by both epigenetic factors (separately and together, too) while short-term memory and recall, was improved best by exercise. Musical training prolonged the latency of acoustic startle reflex and sensorimotor gating as well as the time to peak amplitude of pre-pulse inhibition. MAPK1 and APP levels decreased on all epigenetic factors (mainly on music) at mRNA level. Music and running had positive impact on body weight gain. The gene expression of examined glutamatergic receptors did not differ significantly among the groups. Music plus training diminished cortisol level, and music alone reduced MAPK1 mRNA gene expression, moderating Mn-induced stress.

In summary, regular exercise and rhythmic music can contribute to slowing of progression of mental retardation, motility deficiency, and rigidity associated with Mn exposure; as well as to more successful achievement and retention of long-lasting working ability and independent life.

A FAST METHOD FOR PRELIMINARY EVALUATION OF NITRATE CONTENT
DURING DESALINATION OF BUILDING MATERIALS

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Nitrate pollution is one of major challenges in Earth surface environment due to applications of fertilizers and fossil fuels in the growing needs for more food and energy. Furthermore, nitrate salts have also become more prominent cause of decay of cultural heritage materials which are affected by destructive influence of anthropogenic pollutants.

Numerous methods are available for measurement of nitrate (NO_3^-) content. These methods are either time consuming or require specific equipment. The idea of our work was to use infrared spectroscopy with Fourier transformation (FTIR technique) for a preliminary assessment of the nitrates content. The standard spectrophotometric measurements of nitrate content are overlong and involve brucine-sulfanilic acid reagent, while the FTIR technique includes direct testing of powders from masonry structures without any special preparation and the measurement duration takes only about 1.5 min per sample.

The FTIR technique was successfully applied during the development of biocleaning poultices for nitrate salts reduction in the affected cultural heritage objects. A large number of samples (at different heights and depths of the brick models and of the masonry structure) was needed. The implementation of a rapid screening method for the measurement of the nitrate content presented an important step, thus the nitrate content in the powders was measured by using FTIR technique. The gained spectra recorded in the ATR mode were mathematically processed: (1) by integration of the peak area characteristic for nitrates and (2) by measuring the ratio of intensity of the characteristic peaks. Both of these mathematical functions are incorporated in the OPUS software which was used in order to identify and record the samples. The obtained results based on FTIR technique were linked with the test results gained by the standard spectrophotometric measurements. Accordingly, the results from the fast (FTIR technique ATR mode without sample preparation) and those obtained by a slow and demanding spectrophotometric technique (UV-VIS spectrophotometer) were compared. This comparison showed that the proposed mathematical analysis of the FTIR spectra gives the same trend of quantitative results as the UV-VIS results promising to be a useful tool for preliminary investigation of nitrate content in the solid powdered materials.

LC-MS/MS PESTICIDE RESIDUES DETERMINATION IN SURFACE WATER
RESOURCES USING FOR DRINKING WATER SUPPLY

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This work aimed to assess the risks and provide the insight into the present pesticide contamination of surface water which is used for public water supply, from the two localities Novi Sad and Begeč, Serbia. In routine multiresidue pesticide analysis several dozen pesticide groups can be targeted in a single sample. These analytes, due to their varying physicochemical characteristics can require different chromatography and ionisation models including liquid chromatography (LC). An LC-MS/MS method was developed, optimised and validated for the determination of 70 pesticides residues in water with carbofuran-D₃, atrazine-D₅ and isoproturon-D₆ as internal standards. In the study, an Agilent 1200 HPLC system with a binary pump and Agilent 6410B Triple-Quad LC/MS system was used. The chromatograph separation was performed using Zorbax Eclipse XDB-C₁₈ column and mobile phase in gradient mode (mixture of acidified water and methanol). The pesticides were extracted on OASIS HLB. The obtained LOQs for all the pesticides investigated were 0.020 µg/L, with the average recoveries (three spiking levels: 0.02, 0.1 and 0.2 µg/L, in three replicates per level) in the range of 64.9 to 109.5% with the RSD of 1.7 – 14.2% for all the compounds. The obtained correlation coefficients (R²) of all investigated analytes were over 0.99, in the range from 10 to 200 ng/mL with RSD less than 15%. The obtained results were compared with the MACs according to the National Regulation (Off. Gazz. 42/98 and 44/99). In the water sample from Novi Sad clotianidin, metribuzin and thiophanate-methyl were detected in very low concentrations (under 0.01 µg/L), but from the locality Begeč clomazone was detected in the concentration of 0.038, thiophanate-methyl in 0.069 µg/L, and clothianidin in low concentration. It can be concluded that the concentrations of some detected pesticides were lower than the MACs. Given the fact that, sometimes, “raw” water is directly used for human consumption, thus can present hazard for human and animal health.

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AGING AND LIPOPEROXIDATION MARKERS IN VASCULAR DISEASES

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Oxidative attack of oxygen free radicals on fatty acids of cell membranes called lipoperoxidation is one of the well known mechanisms associated with aging. We have studied the involvement of lipoperoxidation by determining malondialdehyde as Thiobarbituric(ATB)-reactive acid substances in peripheral venous blood.

Material and Method . We evaluated a group of 33 elderly with cardiovascular disease and / or hypertension (Lot I) of atherosclerotic etiology. Patients were hospitalized for decompensated cardiovascular diseases, mainly for symptomatic ischemic heart disease, stable angina, aggravated angina pectoris, left ventricular failure, peripheral arterial disease. We compared this group with another group of patients without symptomatic ischemic heart disease (clinical and ECG) which we determined by the extension lipoperoxidation as ATB-reactive substances under basal conditions and in terms of hemodynamic balance (Satoh method).

Results . In the study we found the extension lipoperoxidation elevated as ATB reactive substances in the group of patients with vascular disease aged over 60 years compared with control group. The average of the ATB reactive substances was $4.54 \pm 0.62 \mu\text{mol} / \text{L}$ vs $2.69 \pm 0.54 \mu\text{mol} / \text{l}$ ($p < 0.001$).

Conclusions . The data found in this study come to support the involvement of oxygen free radicals in aging, by extension of non-enzymatic lipoperoxidation process, without being able to determine whether oxidative stress is a cause or consequence of aging.

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