# UNIVERSITY OF PLOVDIV "PAISII HILENDARSKI" FACULTY OF BIOLOGY

# **UNION OF SCIENTISTS IN BULGARIA - PLOVDIV**



# SECOND BALKAN CONFERENCE ON BIOLOGY

21-23 May 2010, Plovdiv, Bulgaria

# PROGRAM & BOOK OF ABSTRACTS

May 2010 Plovdiv

# The Second Balkan Conference on Biology is sponsored by:











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# **VENUE**

Union of Scientists in Bulgaria - Plovdiv, House of Scientists

Address: 6 Mitropolit Paisii Str., Plovdiv 4000

# REGISTRATION

Union of Scientists in Bulgaria, House of Scientists

# OPENING CEREMONY

Union of Scientists in Bulgaria, House of Scientists Plenary Hall

# **ORAL PRESENTATIONS**

Union of Scientists in Bulgaria, House of Scientists Plenary Hall & Hall №1

# **POSTER PRESENTATIONS**

Union of Scientists in Bulgaria, House of Scientists Lobby on level 1 & 2

# **WELCOME PARTY**

Puldin Restaurant

Address: 3 Knyaz Tzeretelev Str., The Old Town, Plovdiv



The House of Scientists – Plovdiv (Union of Scientists in Bulgaria - Plovdiv)

# **ORGANIZING COMMITTEE**

Prof. Mima Nikolova, DSc

Assoc. Prof. Anelia Stojanova, PhD

Assoc. Prof. Balik Dzhambazov, PhD

Assoc. Prof. Evgeniya Ivanova, PhD

Assoc. Prof. Iliana Velcheva, PhD

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Evelina Georgieva

Ivanka Popova

Ivelin Mollov

Vesela Kalavdzhieva

Viktor Ivanov

# Official Language:

The official languages of the conference will be English.

#### **Presentations:**

Both oral presentations and posters will be presented. Oral presentations will be 15 minutes long including questions and answers. Posters must fit in a space not exceeding 80x60 cm. Poster material must be prepared in advance and should be large enough to be viewed from a distance of one meter. Each poster must have a label at the top that indicates the title of the paper, the name(s) of the author(s) and their affiliation(s). Drawings and charts should be clear and simple. All posters should be attached at their designated place (see the program below) in advance – all posters should be on their places before the beginning of the poster session. Authors are expected to be at their poster during the assigned time periods. Authors are obligated to remove their posters after the poster session is finished. If you delivered a full text article associated with your presentation, it will be published as a full paper in the special online supplement of the journal "Biotechnology & Biotechnological Equipment". Only the works presented at the conference will be included in this supplement!

#### Sections:

Sessions at the Second Balkan Scientific Conference on Biology will cover the following topics:

- 1. Botany
- 2. Cell and Molecular Biology
- 3. Ecology and Environmental Conservation
- 4. Education in Biology
- 5. Functional morphology and Anthropology
- 6. Genetics and Selection
- 7. Microbiology and Biotechnologies
- 8. Zoology

# Dear colleagues and friends,

On behalf of the Faculty of Biology and the Organization Committee it is my great pleasure to welcome you in the most beautiful ancient city of Plovdiv, the southern capital of Bulgaria. The Second Balkan Scientific Conference on Biology is organized on the occasion of the 50<sup>th</sup> anniversary of the University of Plovdiv.

Facing the jubilee anniversary the organizers of the Second Balkan Scientific Conference on Biology wish to facilitate networking among Balkan scientists and the exchange of thoughts and ideas in the field of Biology.

The collaboration among scientists from various Balkan universities and institutions will contribute to our professional development, uniting our efforts in developing joint scientific projects and contracts.

We hope the conference organized by us will yield positive results for all participants. We wish you fruitful discussions, success and pleasant stay in our beautiful city dating back to Roman times.

Dean of the Faculty of Biology Assoc. Prof. Rumen Mladenov, PhD

# 12:00-16:30 Registration 17:00 **Opening Ceremony** 17:00-17:10 Welcome and Opening address 17:10-17:20 Words of Welcome Plenary presentation "Functional ecology as a scientific basis of environmental biotechnology" 17:20-17:40 by Prof. Raycho Dimkov, DSc Plenary presentation "Reconstruction of the head - methods and 17:40-18:00 results in Bulgaria" by Corresponding Member Yordan Yordanov, DSc presentation "Incapsulation Plenary enzymes for biotransformation of highly hydrophobic substrates" 18:00-18:20 by Prof. Albert Krastanov, DSc 18:20-18:30 Closing of the ceremony 18:30-19:00 Group photo 19:30 Welcome party

Friday, May 21, 2010

# **Saturday, May 22, 2010**

# **Plenary Hall**

Plenary presentation "Blood-group and Lysosomal Glycoproteins in

09:00-09:20 Tumor Progression"

by Prof. Victoria Sarafian, DSc

# SECTION "GENETICS & SELECTION"

**Plenary Hall** 

Chair of the section: Assoc. Prof. Evgeniya Ivanova, PhD

Secretary: Penka Vasileva, PhD

#### ORAL PRESENTATIONS

09:20-09:30 Technical time for preparation of section "Genetics & Selection"

Author(s): A. Hoda, Y. Biçoku and M. Cara

**09:30-09:45 Title:** Polymorphism of microsatellite loci in MHC complex for two populations of Shkodrane sheep breed in Albania

Author(s): A. Hoda, M. Vegara and V. Bozgo

**09:45-10:00 Title:** Genetic diversity of Recka sheep breed in Albania based on 15 microsatellite markers

Author(s): F. Gjurgji and L. Sena

**10:00-10:15 Title:** Genetic variation degree for meat production traits in pure-bred pigs

**Author(s):** <u>T. A. Staykova</u>, E. N. Ivanova, P. I. Tzenov, Y. B. Vasileva, D. B. Arkova-Pantaleeva and Z. M. Petkov

Title: Acid phosphatase as a marker for differentiation of silkworm (*Bombyx mori* L.) strains

10:30-11:00 Coffee break

Author(s): P. Petrov

**11:00-11:15 Title:** Organization and principles of queen selection and rearing in Bulgaria

Author(s): E. N. Ivanova, T. A. Staykova and P. P. Petrov

11:15-11:30 Title: Allozyme variability in populations of local Bulgarian honey bee

Author(s): E. N. Ivanova

**11:30-11:45 Title:** Investigation on genetic variability in honey bee populations from Bulgaria, Greece and Serbia

**Author(s):** P. Michailova, N. Atanasov, <u>J. Ilkova</u>, T. Chassovnikarova, M. Duran and E. Karadurmus

**11:45-12:00 Title:** Genome response of model invertebrates and vertebrates species to stress agents in the environment

**Author(s):** T. Staykova, E. Ivanova, <u>G. Panayotova</u>, I. Cvetkova, S. Dzhonglov and B. Dzhambazov

**12:00-12:15 Title:** General toxicity and genotoxicity of *Nodularia moravica* (*Cyanoprokariota, Nostocales*)

12:30-14:00 Lunch

# **POSTER SESSION (14:00 – 16:00)**

Author(s): A. Korubin-Aleksoska, V. Nikova and J. Aleksoski

- **Title:** Regression analysis of the inheritance of leaf size in F1 and F2 progenies in various tobacco genotypes
- 2 Author(s): M. Dimitrieski and G. Miceska
  Title: A new and more productive variety of Prilep tobacco

Author(s): J. Aleksoski

- **Title:** Estimation of the heterotic effect in F1 generation of various tobacco genotypes and their diallel crosses
- Author(s): G. Dimeska, M. Vasilevska, Ž. Sekovski, L. Cvetanovska
   Title: Aberation frequency during mitosis and meiosis in *Vicia faba* L., after the treatments with <sup>131</sup> I.

Author(s): A. Georgijeva and S. Rasic

**Title:** Analysis of spring development of some selection lines of honeybee in Eastern Serbia

Author(s): M. Mladenović and R. Radoš

**Title:** Correlation between the strength of colony, the honey area and pollen area of the observed lines of yellow honey bee in Vojvodina.

Author(s): M. Mladenović and V. D. Simeonova

- 7 Title: The variability of wing nervature angles of honey bee from the North Kosovo area
  - Author(s): L. Sena, S. Sena, F. Gjurgji and M. Nikolla
- **Title:** Seasonal Aplication of Jenter's Method for a Successful Queen Bees' Rearing in Albania

**Author(s):** L. Velkova-Jordanoska, V. Kostov, S. Stojanovski, G. Kostoski

Title: Use of RAPD fingerprinting for study and conservation of fish populations.

# **Saturday, May 22, 2010**

# SECTION "BOTANY, ECOLOGY & ZOOLOGY"

**Chair of the section:** Assoc. Prof. Iliana Velcheva, PhD **Secretaries:** Assoc. Prof. Ivanka Dimitrova-Dyulgerova, PhD

Assoc. Prof. Anelia Stojanova, PhD

#### ORAL PRESENTATIONS

09:20-09:30 Technical time for preparation of section "Botany, Zoology & Ecology"

**Author(s):** L. Cvetanovska, <u>I. Klincharska-Jovanovska</u>, G. Dimeska, M. Srbinoska and A. Cvetanoska

**09:30-09:45 Title:** Analysis of the Organic Production in Tobacco Raw Material (*Nicotiana tabacum* L.) after Treatment with Heavy Metals and Fungicide Ridomil gold

**Author(s):** <u>I. Klincharska-Jovanovska</u>, L. Cvetanovska, M. Srbinoska and A. Cvetanoska

**Title:** Anatomic and Physiological Disorder after Intoxication with Heavy Metals in Tobacco (*Nicotiana tabacum* L.)

**Author(s):** <u>G. Gecheva</u>, L. Yurukova, S. Cheshmedjiev and A. Ganeva

10:00-10:15

Title: Distribution and Bioindication Role of Aquatic Bryophytes in Bulgarian Rivers

Author(s): L. Mihajlov, F. Trajkova and V. Zlatkovski

**10:15-10:30 Title:** Climate Change and the Impact on Agriculture in Republic of Macedonia

10:30-11:00 Coffee break

Author(s): <u>I. Velcheva</u>, A. Arnaudov, E. Georgieva and A. Tsekov

11:00-11:15

Title: Influence of Zinc on breathing and gill morphology of Gibelio carp (*Carassius gibelio*)

Author(s): P. Simonović, V. Nikolić and S. Grujić

**Title:** Amazon Sailfin Catfish *Pterygoplichthys pardalis* (Castellnnau, 1855) (Loricariidae, Siluriformes), A New Fish Species Recorded in the Serbian Section of the Danube River

**Author(s):** Ts. Chassovnikarova, N. Atanassov, <u>V. Kalaydzhieva</u> and H. Dimitrov

11:30-11:45 Title: Micronucleus Test from Free Living Rodents as a Biomarker for Environmental Stress *in situ* 

Author(s): Y. Öztürk and M. A. Tabur

**11:45-12:00** Title: Seasonal and Daily Activity Pattern in Griffon Vulture in Sütçüler (Isparta-Turkey)

**Author(s):** <u>Sv. Cheshmedjiev</u>, D. Belkinova, R. Mladenov, I. Dimitrova-Dyulgerova and G. Gecheva

12:00-12:15

Title: Phytoplankton Based Assessment of the Ecological Status and Ecological Potential of Lake Types in Bulgaria

**Author(s):** <u>Sv. Cheshmedjiev</u>, R. Mladenov, D. Belkinova, G. Gecheva, I. Dimitrova-Dyulgerova, P. Ivanov and S. Mihov

**12:15-12:30 Title:** Development of Classification System and Biological Reference Conditions for Bulgarian Rivers and Lakes According to the Water Framework Directive

12:30-14:00 Lunch

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# **POSTER SESSION (14:00 – 16:00)**

**Author(s):** <u>I. Teneva</u>, D. Belkinova, I. Dimitrova-Dyulgerova, M. Vlaknova and R. Mladenov

Title: Composition and toxic potential of Cyanoprokaryota in Vacha Dam (Bulgaria)

Author(s): H. Anastasov

**Title:** Influence of oxyfluoreen on some anatomic indices in the leaves of large-leaf tobacco plant (*Nicotiana tabacum* L.)

Author(s): T. Ganeva and K. Uzunova

**Title:** Leaf epidermis structure in *Amelanchier ovalis* Medic. (Rosaceae)

**Author(s** M. Marin, L. Ascensao, S. Budimir, D. Janosevic, S. Duletic-Lausevis and P. Marin

Title: The histochemical analysis of *Thymus malyi* glandular trichomes.

**Author(s):** M. Dimitrova, Zh Yordanova, D.Dragolova and V. Kapchina-Toteva

**Title:** Influence of indole butyric acid on the micropropagation of Lamium album L.

**Author(s):** E. Yankova-Tsvetkova, G. Baldjiev, M. Petrova, E. Zayova and P.Yurukova

**Title:** Analysis on pollen and seed productivity and effectiveness in *Gentiana lutea* L..

**Author(s):** <u>G. Georgiev</u>, A.Ivanova, P.Mechkarova, A.Ivanova and L.Popova

**Title:** Rate and forms of mineral nutrition can influence dry matter accumulation and saponin content of puncture vine (*Tribulus terrestris* L.)

# Author(s): G. Petkov

**Title:** Could microalgae enhance the germination of *Tribulus terrestris* L. seeds?

# Author(s): I. Semerdjieva and L. Evstatieva

**Title:** Distribution and resources evaluation of *Tribulus terrestris* L. (Zygophyllaceae) population in Thracian floristic region.

# Author(s): D. Peev and N. Valyovska

**Title:** Distribution and recource evaluation of the *Tribulus terrestris* L. – Maltese cross (Zygophyllaceae) in populations from South Bulgaria

# Author(s): B. Sidjimova and M. Nikolova

**Title:** Distribution and resource evaluation of *Tribulus terrestris* in North Bulgaria

**Author(s):** L. Maslenkova, V.Peeva, L. Brankova, I. Lazarova and L. Evstatieva

- **Title:** Screening by thermoluminescens method the quantity of biologically active compounds from *Tribulus terrestris* plants with defferent origin
  - **Author(s):** M. Stanković, M. Topuzović, A. Marković, D. Pavlović, S. Soluić, N. Niciforović and V. Mihailović
- Title: Antioxidant activity, phenol and flavonoid contents of different Teucrium chamaedrys L. exstracts
  - **Author(s):** N. Sahakyan, M. Petrosyan, Yu. Popov, V. Volodin, N. Matistov, I. Gruzdev and T. Shirshova
- Title: Content of neutral lipids and fatty acids in callus cultures and leaves of intact plants of *Ajuga genevensis* and *Ajuga chia*

#### **Author(s):** A. Tosheva and I. Traykov

- **Title:** New chorological data of some submerged macrophytes in Bulgaria
- **Author(s):** <u>J. Matejić</u>, Z. Šarac and V. Ranđelović **Title:** Pharmacological activity of sesquiterpene lactones

# Author(s): M. Nikolova Ch. Gussev and T. Nguyen

**Title:** Evaluation of the antioxidant action and flavonoid composition of *Artemisia* species extracts

- **Author(s):** <u>G. Gecheva</u>, S. Cheshmedjiev, I. Dimitrova-Dyulgerova, D. Belkinova and R. Mladenov
- Title: Implementation and Adaptation of Macrophyte Indication System: Assessment of Ecological Status of Rivers in Bulgaria According to the Water Framework Directive
- Author(s): S. Krivokapić, B. Pestoric, D. Drakulović and N. Vuksanović
  - Title: Subsurface Chlorophyll A Maxima in the Boka Kotorska Bay
- **Author(s):** <u>S. Georgiev</u>, K. Koev and D. Kalacheva **Title:** Floristic Characteristics of Chirpanskata Gora Preserve
  - Author(s): K. Koev, D. Kalacheva and S. Georgiev
- **Title:** Floristic Characteristics and Ecological Evaluation of Debelata Koria Preserve, Chernozemen Village
  - **Author(s):** E. Daskalova, S. Dontcheva, G. Yahubyan, I. Minkov and V. Toneva
- **Title:** Ecological Characteristics and Conservation of the Protected Resurrection Species *Haberlea rhodopensis Friv.* as *in vitro* Plants Through a Modified Micropropagation System
- Author(s): <u>G. Dimeska</u> and L. Cvetanovska
  Title: Polen Fertility of *Vicia faba* L., After Treatments with X-Rays
  - **Author(s):** S. Branković, D. Pavlović-Muratspahić, M. Topuzović, R. Glišić and M. Stanković
- Title: Concentration Of Some Heavy Metals in Aquatic Macrophytes in Reservoir Near City Kragujevac (Serbia)
- **Author(s):** M. Borišev, S. Pajević, N. Nikolić, B. Krstić and A. Pilipović **Title:** Influence of Cd and Ni on content of N, P, K, Nitrates and Activity of Nitrate Reductase in Clones of *Salix spp.*
- **Author(s):** M. Stanković, M. Topuzović, A. Marković and D. Pavlović **Title:** Influence of Zinc (Zn) on Germination of Wheat (*Triticum aestivum* L.)
- Author(s): V. Bogoev, A. Kenarova, I. Traykov, R. Tzonev, R. Tzekova, <u>T. Stoyanova</u>, S. Boteva and N. Parleva

  Title: Natural Communities of Uranium Mining Impacted Area in the
  - Author(s): S. Mihov

Vicinity of the Senokos Village

- **Title:** Development of Fish Based Index for Assessing Ecological Status of Bulgarian Rivers (BRI)
- Author(s): <u>I. Mollov</u> and A. Stojanova

  Title: Diet and Trophic Niche Overlap of Three Toad Species
  (Amphibia, Anura) from Poland

- **Author(s):** A. Simeonova, J. Bekyarova, R. Chuturkova and D. Toneva
- Title: Investigation of the Organic Pollution and Contaminants'
  Biological Destruction of the River Kamchiya
  - Author(s): S. Stamenković and M. Cvijan
- **Title:** Determination of Airpolution Zones in Knjaževac (South Eastern Serbia) by Using Epiphytic Lichens
  - **Author(s):** Sv. Cheshmedjiev, T. Karagiozova, M. Michailov and V. Valev
- **Title:** Revision of River and Lake Typology within Ecoregion 12 Pontic Province and Ecoregion 7 Eastern Balkans in Bulgaria According to the Water Framework Directive
  - Author(s): L. Yurukova, S. Petrova and N. Shopova
- **Title:** Inorganic Characteristics of Polyfloral Honey in Plovdiv (Bulgaria)
  - Author(s): B. K. Temelkov
- **Title:** Guanduella podensis n. sp. and Psammosphaera sp. Foraminifera from the Bulgarian Black Sea Coast
  - **Author(s):** S. Stojanovski, N. Hristovski, P. Cakic, M. Hristovski, L. Velkova-Jordanoska and D. Blazekovic
- Title: Monogenean Trematods of Chub Leuciscus cephalus albus Bonaparte, 1838 from the Lake Ohrid (Macedonia)
  - **Author(s):** <u>T. Milutinović</u>, S. Avramović, S. Pešić, B. Blesić, M. Stojanović and A. M. Bogdanović
- Title: Contribution to the Knowledge of Pedofauna in Šumadija (Central Part of Serbia)
  - Author(s): A. Zawal, S. Stojanovski and S. Smiljkov
- **Title:** Preliminary Investigations on Odonata from the Lake Ohrid (Macedonia)
  - Author(s): A. Savić, V. Randjelović and J. Krpo-Ćetković
- **Title:** Seasonal Variability in Community Structure and Habitat Selection of Mayflies (Ephemeroptera) in the Nišava River (Serbia)
  - **Author(s):** S. Pavković-Lučić, V. Kekić, T. Obradović, L. Lučić and D. Miličić
- Title: Mating Latency and Copulation Duration in *Drosophila melanogaster* (Diptera: Drosophilidae)
- **Author(s):** S. Pavković-Lučić, V. Kekić, D. Miličić and L. Lučić **Title:** Sex Combs and Sexual Selection in *Drosophila melanogaster* (Diptera: Drosophilidae)

Author(s): A. Stojanova

- **Title:** Seed Beetle *Bruchidius terrenus* (Sharp) (Coleoptera: Chrysomelidae: Bruchinae) New Invasive Species to the Bulgarian Fauna
  - **Author(s):** <u>G. Popgeorgiev</u>, N. Tzankov, Y.V. Kornilev, B. Naumov, and A. Stojanov
  - **Title:** Species Diversity of Amphibians and Reptiles in the Special Protected Area "Besaparski Ridove", Southern Bulgaria

Author(s): Y. Koshev

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- **Title:** Interspecific Aggressive Behaviour of European Ground Squirrel (Spermophilus citellus L.)
- **Author(s):** <u>V. Racheva</u>, D. Peshev, D. Zlatanova, Z. Zaharieva And G. Gavrilov
- Title: Accommodation into the Wild of Captive Badgers (*Meles meles*, L.)
  - Author(s): D. Radmanovic, J. Lujic, D. Kostic, S. Blazic
- **Title:** The Variation of Morphometric Characters of Tarsal Bones in Species *Cervus elaphus* L., 1758 (Mammalia: Artiodactyla) Taken from Neolitic Sediments at Balkan Peninsula
  - **Author(s):** D. Kojić, J. Purać, <u>Ž. Popović</u>, E. Pamer and G. Grubor-Lajšić
- **Title:** Importance of the Body Water Management for Winter Cold Survival of the European Corn Borer *Ostrinia nubilalis* Hübn. (Lepidoptera: Pyralidae)

# Sunday, May 23, 2010

# SECTION "ZOOLOGY"

**Plenary Hall** 

Chair of the section: Assoc. Prof. Anelia Stojanova, PhD

Secretary: -

#### **ORAL PRESENTATIONS**

09:15-09:30 Technical time for preparation of section "Zoology".

**Author(s):** G. Markov and H. Dimitrov

**09:30-09:45** Title: Habitat Fragmentation and its Implications for Abundance of Guenther's Vole in Southeastern Bulgaria (Strandzha Mountain

Region)

Author(s): P. Genov and A. Dzhindzhieva

09:45-10:00 Title: Damages of Gray Wolf (Canis lupus L.) During Ten Year Period

in Bulgaria

Author(s): P. Genov, A. Dzhindzhieva and A. Mircheva

**10:00-10:15** Title: Dynamic of Distribution and Number of Gray Wolf (Canis lupus

L.) During Ten Year Period in Bulgaria

10:15-10:30 Coffee break

## SECTION "FUNCTIONAL MORPHOLOGY & ANTROPOLOGY"

Hall №1

**Chair of the section:** Prof. Mima Nikolova, DSc **Secretary:** Chief. Assist. Slavi Tineshev, PhD

#### ORAL PRESENTATIONS

Technical time for preparation of section "Functional Morphology & Anthropology", section "Education in Biology",

09:00-09:15 section "Microbiology & Antimopology", section "Education in Biology", section "Cell &

Molecular Biology".

Author(s): Y. Gluhcheva, V. Atanasov, R. Zhorova, M. Madzharova,

J. Ivanova and M. Mitewa

09:15-09:30 Title: Cobalt Bioaccumulation in Mouse Blood Plasma and Liver

# **POSTER SESSION (10:30 – 12:00)**

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**Author(s):** E. Pavlova, M. Madzharova, N. Atanassova and R. Sharpe

**Title:** Quantification of Rat Spermatogenesis in Late Puberty After Neonatal Hormonal Manipulation

**Author(s):** M. Madzharova, Y. Gluhcheva, E. Pavlova and N. Atanassova

**Title:** Effect of Cobalt on Male Reproductive Organs During Puberty

Author(s): T. Pavlica, V.Bozic-Krstic and R. Rakic

**Title:** Correlation of Vital Lung Capacity with Body Weight, Longitudinal and Circumference Dimensions

Author(s): M. Nikolova and Sl. Tineshev

**Title:** Comparison of the Body Mass Index to Other Methods of Body Fat Assessment in Bulgarian Children and Adolescent

Author(s): SI. Tineshev and M. Nikolova

**Title:** Anthropological Characteristics of Body Composition in Children and Adolescents from Plovdiv

Author(s): E.Andreenko and M.Nikolova

**Title:** Topical Distribution of the Subcutaneous Fat Tissue on Some Parts and Regions of the Body in Children and Adolescents from South Bulgaria

Author(s): S. Mladenova and D. Kodgebasheva

- **Title:** Changes in Components of Body Mass and Their Relation During Period of Growth in Girls From Smolyan Region, Bulgaria (Anthropometric Study)
- **Author(s):** A. Baltadjiev, G. Baltadjiev **Title:** Body Composition Of Children Suffering From Diabetes Type 1

Author(s): A. Baltadjiev

**Title:** Growth Dynamics Of The Chest In Children With Type 1 Diabetes

# SECTION "EDUCATION IN BIOLOGY"

Hall №1

Chair of the section: Assoc. Prof. Margarita Panayotova, PhD

Secretary: Senior Assist. Teodora Kolarova, PhD

#### **ORAL PRESENTATIONS**

Author(s): V. Vasilev, T. Kolarova and I. Hadjiali

Title: Innovative Didactical Means For Developing And Assessing

09:30-09:45 Students' Intellectual Reflection In The High School Education Of

Genetics

# **POSTER SESSION (10:30 – 12:00)**

Author(s): S. Pavković-Lučić, L. Radenović and V. Kekić

**Title:** Behavioral Science at the Faculty of Biology, University of Belgrade, Serbia

**Author(s):** D. Miličić, T. Karan-Žnidaršič, S. Pavković-Lučić, L. Lučić and S. Jokić

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**Title:** Teaching in Biological Sciences at Primary Schools in Serbia – An Application of the *Hands On* Method

## SECTION "CELL & MOLECULAR BIOLOGY"

Hall №1

Chair of the section: Assoc. Prof. Balik Dzambazov, PhD

Secretary: Assoc. Prof. Tsanko Gechev, PhD

# **ORAL PRESENTATIONS**

Author(s): T. Batsalova, M. Vestberg, R. Holmdahl and B.

Dzhambazov

09:45-10:00 Title: MOG79-90 Peptide in Complex with Recombinant MHC Class II

Molecules Ameliorates Experimental Autoimmune Encephalomyelitis

Author(s): V. Radeva, V. Petrov, I. Minkov, V. Toneva and T. Gechev

10:00-10:15 Title: Effect of Cadmium on Arabidopsis thaliana Mutants Tolerant to

Oxidative Stress

## **POSTER SESSION (10:30 – 12:00)**

**Author(s):** M. Draganova-Filipova, M. Nikolova, A. Mihova, L. Peychev and V. Sarafian

**Title:** A Pilot Study on the Immunomodulatory Effect of Bulgarian Propolis

Author(s): M. Kazakova, T. Deneva, V. Uzunova and V. Sarafian Title: YKL-40 in Healthy Subjects

Author(s): A. Ivanova, I. Lazarova, P. Mechkarova and B.

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**Title:** HPLC Method for Screening of Steroidal Saponins and Rutin as Biologically Active Compounds in *Tribulus terrestris* L.

**Author(s):** <u>I. Sainova</u>, I. Vavrek, V. Pavlova, T. Daneva, S. Manchev and E. Nikolova

Title: Experimental Model for Safe Gene Transfer by Recombinant Gene Constructs

**Author(s):** M. Draganov, G. Miloshev, N. Popov, M. Murdjeva, D. Staneva, M. Kostova, D. Draganov and N. Tomlekova

Title: Molecular and Cytogenetic Criteria for Identification of Serum-Free Cell Cultures

**Author(s):** D. Ivanova, T. Vachev, V. Baev, I. Minkov and M. Gozmanova

Title: Identification of Potato Spindle Tuber Viroid Small RNA in Orobanche ramosa by Microarray

Author(s): S. Peykov and S. Dimov

**Title:** An Optimized Procedure for DNA Silver Staining in Polyacrylamide Gels

Author(s): V. Georgiev, I. Ivanov and A. Pavlov

**Title:** Obtaining and Selection of *Pancratium maritimum* L. *in vitro* Cultures with Acetylcholinesterase Inhibitory Action

# SECTION "MICROBIOLOGY & BIOTECHNOLOGIES"

#### Hall № 1

**Chair of the section:** Assoc. Prof. Ilia Iliev, PhD **Secretary:** Chief. Assist. Velizar Gochev, PhD

#### **ORAL PRESENTATIONS**

**Author(s):** <u>I. Simenonov,</u> E. Choruкova, V. Mamatarkova and L. Nikolov

**10:15-10:30 Title:** Biogas Production from Organic Wastes in Suspended Cell Cultures and in Biofilms

#### 12:00 Closing of the conference

# **POSTER SESSION (10:30 – 12:00)**

20	<b>Author(s):</b> M. I. Georgiev, K. I. Alipieva and P. Denev <b>Title:</b> Antioxidant Activity and Bioactive Constituents of the Aerial Parts of <i>Harpagophytum procumbens</i> Plants
21	Author(s): I. Movsesyan, N. Ahabekyan, <u>I. Bazukyan</u> , R. Madoyan, M. Dalgalarrondo, J. Chobert, Y. Popov and T. Haertlé Title: Properties and Survival under Simulated Gastrointestinal Conditions of Lactic Acid Bacteria Isolated from Armenian Cheeses and Matsuns
22	Author(s): A. Margaryan, H. Panosyan and Yu. Popov Title: Isolation and Characterization of New Metallotolernat Bacilli Strains
23	Author(s): T. Mihajilov-Krastev, D. Radnović and D. Kitić Title: Satureja L. Essential Oils in Prevention and Phytotherapy of Salmonella Infection
24	<b>Author(s):</b> K. Karapetyan, N. Huseynova, R. Arutjunyan, <u>F. Tkhruni</u> and Th. Haertle <b>Title:</b> Perspective of Using New Strains of Lactic Acid Bacteria for Biopreservation
25	Author(s): D. Dimitrijević, Z. Stojanović-Radić, M. Stanković, V. Ranđelović, D. Lakušić Title: Antimicrobial Activity, Total Phenol and Flavonoid Contents of Jovibarba heuffelii (Schott.) A Löve & D. Löve Extracts
26	Author(s): A. Kenarova, G. Radeva, I. Danova, S. Boteva and I. Dimitrova Title: Soil Bacterial Abundance and Diversity of Uranium Impacted Area in North Western Pirin Mountain
27	Author(s): V. Y. Petrova and A. V. Kujumdzieva Title: Robustness of Saccharomyces cerevisiae Genome to Antioxidative Stress
28	<b>Author(s):</b> <u>Ts. Ignatova-Ivanova</u> , R. Ivanov, I. Iliev and I. Ivanova <b>Title:</b> Influence of Galactooligosaccharides on the Growth and Antimicrobial Activity of <i>Lactobacillus fermentum</i> I-5
29	<b>Author(s):</b> D. I. Koleva, G. P. Docheva, V. Y. Petrova and A. V. Kujumdzieva
	<b>Title:</b> Superoxide Dismutase Enzymes in Oxidative Type Yeast <i>H. polymorpha</i>

30	and A. V. Kujumdzieva <b>Title:</b> Effect of Cd <sup>2+</sup> on the Antioxidant Status of <i>Shizosaccharomyces</i> pombe
31	<b>Author(s):</b> E. I. Pisareva and A. V. Kujumdzieva <b>Title:</b> Influence of Carbon and Nitrogen Sources on Growth and Pigment Production by <i>Monascus pilosus</i> C <sub>1</sub> Strain
32	Author(s): S. Rusinova-Videva, K. Pavlova, I. Panchev, K. Georgieva and M. Kuncheva  Title: Effect of Different Factors on Biosynthesis of Exopolysaccharide from Antarctic Yeast
33	Author(s): V. Gochev, A. Dobreva, T. Girova and A. Stoyanova Title: Antimicrobial Activity of Essential Oil from Rosa alba
34	Author(s): V. Ivanova Title: Immobilization of Cyclodextrin Glucanotransferase from Paenibacillus macerans ATCC 8244 on Magnetic Carriers and Production of Cyclodextrins
35	Author(s): P. Petrova, V. Ivanova  Title: Perspectives for the Production of Bioethanol from Lignocellulosic Materials
36	Author(s): T. Girova, V. Gochev, L. Jirovetz, G. Buchbauer, E. Schmidt and A. Stoyanova Title: Antimicrobial Activity of Essential Oils from Spices against Psychrotrophic Food Spoilage Microorganisms
37	<b>Author(s):</b> T. Vasileva, I. Ivanova and I. Iliev <b>Title:</b> Glucooligosaccharides Synthesized by Glycosyltransferases from Mutant Strain <i>Leuconostoc mesenteroides</i> M2860 and Their Prebiotic Potential
38	Author(s): I. A. Ivanova, S. Kambarev, R. A. Popova, E. G. Naumovska, K. B. Markoska and C. D. Dushkin  Title: Determination of <i>Pseudomonas putida</i> Live Cells with Classic Cultivation and Staining with "Live/Dead Baclight Bacterial Viability Kit"
39	<b>Author(s):</b> T. Vasileva, <u>V. Bivolarski</u> , I. Ivanova and I. Iliev <b>Title:</b> Some Aspects of Carbohydrate Metabolism and Production of Glycosyltransferases from Mutant Strain <i>Leuconostoc mesenteroides</i> M2860
40	Author(s): R. Shukla, I. Iliev and A. Goyal  Title: Purification and Characterization of Dextransucrase from

Author(s): E. I. Pisareva, M. V. Kostova, T. S. Nedeva, A. I. Angelov

Leuconostoc mesenteroides NRRL B-1149

Author(s): V. Gjinovci, B. Bijo, K. Sulaj, A. Musaj and R. Keqi 41 Title: Check on the Presence of Enterobacteria and Salmonella spp. in Sausage Author(s): Ts. Paunova, R. Ivanova and S. Stoitsova 42 Title: Growth Temperature-Related Cell Surface Changes of Escherichia coli O157:H-Author(s): M. Marhova, S. Kostadinova and S. Stoitsova 43 Title: Biofilm-Forming Capabilities of Urinary Escherichia coli Isolates Author(s): G. Chikov, V. Kalichuk, N. Kirilov and S. Dimov 44 Title: Characteristic of Two Bacteriocin-Producing Enterococcus Strains Author(s): S. Dimov, S. Stojanovski, R. Stoyanova, N. Kirilkov, S. Antonova-Nikolova and I. Ivanova 45 Title: Molecular Typing of Lactobacilli Isolated from Dry Sausage "Lukanka": Comparison of Whole Cell Protein (WCP) Versus DNA-**Based Methods** Author(s): S. Kostadinova and M. Marhova 46 Title: Purification and Properties of Alkaline Phosphatase from Bacillus cereus Author(s): V. Gochev, Z. Velkova and M. Stoytcheva 47 Title: Biosorption of Cooper (II) By Immobilized Dead Biomass of Saccharomyces cerevisiae Author(s): Y. Evstatieva, D. Nikolova, S. Ilieva, L. Getov, V. Savov 48 **Title:** Identification and Characterization of A-Amylase and Endoxylanase, Produced by Aspergillus Mutant Strains **Author(s):** N. Hristova, V. Baloutzov 49 Title: Researches on the Possibilities for Selection of Streptomyces

ambofaciens High Producing Strain

# **BOOK OF ABSTRACTS**

#### PLENARY PRESENTATIONS

# FUNCTIONAL ECOLOGY AS A SCIENTIFIC BASIS OF ENVIRONMENTAL BIOTECHNOLOGY

## Raycho Dimkov

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**ABSTRACT.** According to its historical and conventional definition ecology deals with the distribution and number of organisms, migration and structure of communities. Traditionally the accent is put on the interaction and interrelation between organisms and also between them and the environmental factors. It is a matter of discussion whether functional ecology is an inseparable part, a sub-division, a continuation or an up-grading of general ecology. But without doubt it represents one of scientific fundamentals of environmental biotechnology.

This key statement of principle is emerging from interdisciplinary association of biology (in its classical, i.e. at the organism's level, and molecular versions), biotechnology and engineering sciences for environment protection. From today's point of view it is recommendable to be held a constructive dialogue among various experts in order to find the most suitable and relevant solutions, certainly – on the basis of an integrated approach. Therefore we have to take into account the need for opening of a scientific discussion on the topics relating to ecology (on the one hand) and to its logical conjugation with biochemistry, enzymology, molecular biology, microbial physiology and environmental biotechnology (on the other hand). It means to have a holistic view on the biosphere, man and environment considered altogether.

This paper presents author's concepts related to:

- selected aspects of metabolism of ecosystems;
- dynamics of functional parameters;
- design and application of environment friendly processes and new materials;
- assessment of risks concerning biosafety and biosecurity;
- influence of GMO on the biodiversity and ecosystem's sustainability;
- monitoring and management of processes for bioremediation;
- education, qualification and motivation of young generation, resp. insertion of elements of functional ecology into bachelor's and master's curricula in universities.

Briefly, the goal of the author is to expose and argue some ideas about the potential, intellectual capacity and economic impact of functional ecology as a reliable bridge connecting biotechnology, ecology and environment protection.

#### RECONSTRUCTION OF THE HEAD – METHODS AND RESULTS IN BULGARIA

#### Yordan Yordanov

**ABSTRACT.** Restoration of the head on the basis of the skull is a scientific method which utilizes the interrelations between soft tissues of the face and head and the underlying cranial bones in man. The method of the head restoration by the skull is particularly relevant for the anthropologic science (anthropology, the branch of science dealing with races), archaeology and history.

In our country the results of the application of the method for head restoration by the skull comprise 65 plastic portraits.

# INCAPSULATION OF ENZYMES FOR BIOTRANSFORMATION OF HIGHLY HYDROPHOBIC SUBSTRATES

#### Albert Krastanov

University of Food Technologies, Plovdiv, Bulgaria

**ABSTRACT.** The potential possibilities for application of enzymes incapsulated in linear-dendric co-polimeres for biotransformation of highly hydrophobic substrates in water solutions, polymeraze reactions, structural modifications of macromolecule substrates and bioremediation of highly toxic compounds were demonstrated. The totally new conception for multiply transformation of insoluble substrates in water solutions with the studied enzyme complex is proposed.

# BLOOD-GROUP AND LYSOSOMAL GLYCOPROTEINS IN TUMOR PROGRESSION

#### Victoria Sarafian

Department of Biology, Medical University – Plovdiv, Bulgaria

Human blood-group antigens (BGA) ABH are conserved glycoproteins with role in haemotransfusions and transplantations, forensic expertise and serve as anthropological markers. The lysosome-associated membrane glycoproteins (LAMP-1, LAMP-2) ensure the stability of lysosomal membranes. The precise function of both molecules is still obscure.

They are intensively examined for a possible function in cell proliferation, differentiation and death and intercellular communications. The participation in the interaction with pathogens, predisposition to diseases and tumorigenesis is still undefined.

Our investigations prove that BGA serve as markers for endothelial differentiation of embryonal mesenchyme, as markers for keratinocyte differentiation and neonagiogenesis in granulation tissue. Probably they participate in intercellular adhesion during tissue remodeling. The accumulation of LAMP in the apical part of endothelial cells suggests that they might be a possible

intracellular factor directly involved in the process of lumenization of newly formed capillaries, most likely by autophagy of parts of the apical cytoplasm.

LAMP mRNA expression in the course of keratinocyte differentiation *in vitro* upon treatment with NaBu and in autonomous growth reveals that LAMP-1 could be regarded as a novel keratinocyte differentiation marker.

The enhanced expression of BGA and LAMP in acute thymic involution and in infantile hemangioma implies a possible activation of autophagy, augmented lysosomal activity of nonprofessional macrophages in florid apoptosis in which these molecules are implicated.

BGA and LAMP contribute to the metastatic phenotype of tumor cells by interactions with lectins thus providing matrix-independent survival of malignant cells. These surface carbohydrate antigens are regarded as end products of tumor progression and serve as prognostic and diagnostic markers and therapeutic targets. The interaction BGA-lectins triggers a signal cascade regulating the transcriptional activity of metastasis-associated genes. A reprogramming of gene transcription by antimetastatic synthetic glycoamines reverses the metastasis-associated gene profile of tumor cells. BGA are the morphologic endpoint of clonal evolution and clonal selection during tumor progression.

Our studies of skin carcinoma prove that BGA and LAMP modulate their expression, probably related to the altered differentiation and malignant potential of the tumor. The tumor cell lines examined show different level and localization of of LAMP expression suggesting a role in cell locomotion, invasion and metastasis. Our flowcytometry analysis proves for the first time that LAMP might be expressed on cell membranes of tumor cells but not on lysosomes only and serve as ligands for galectin-3. This finding highlights a new role of LAMP in tumor progression.

BGA are also involved in intercellular communications through the dialogue epithelial cells - lymphocytes thus maintaining the tissue architectonics and acting as an element of dynamically altering microsurrounding within the thymic gland.

In conclusion, BGA do not serve for blood typing only and lysosomes are not just cell garbage stores. BGA and LAMP participate in intercellular communications, cell proliferation, differentiation and death and hence contribute to tumor progression.

# **SECTION** "BOTANY"

# ANATOMIC AND PHYSIOLOGICAL DISORDER AFTER INTOXICATION WITH HEAVY METALS IN TOBACCO (NICOTIANA TABACUM L.)

- L. Cvetanovska<sup>1</sup>, I. Klincharska-Jovanovska<sup>1</sup>, G. Dimeska<sup>1</sup>, M. Srbinoska<sup>2</sup>, A. Cvetanovska<sup>1</sup>
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ABSTRACT. Continuous intoxication with heavy metals seems to be the major problem of the modern societies, which results most of the morphologic-physiological, as well as genetic anomaly in plants. The aim of this study is to analyze intoxicated tobacco material (*Nicotiana tabacum* L.) with heavy metals (copper, cadmium, lead) and overdoses of fungicide (Ridomil gold) for enzymatic (catalase activity) and biopigment activity (chloroplast pigments) and to make morpho-anatomic examinations (lateral cut of stem and leaf). The plants were from the crop 2008 of two oriental (half oriental) types: PRILEP (P-156) and JAKA (Jv-125/3). They were cultivated in controlled experimental conditions in the Botanical Garden by the Department of Botany, Institute of Biology, Faculty of Natural Sciences and Mathematics-Skopje. Analysis material was taken from leaves in the first insertion or lower leaves (I phase).

# ANALYSIS OF THE ORGANIC PRODUCTION IN TOBACCO RAW MATERIAL (NICOTIANA TABACUM L.) AFTER TRETMAN WITH HEAVY METALS AND FUNGICIDE RIDOMIL GOLD

- I. Klincarska-Jovanovska<sup>1</sup>, L. Cvetanovska<sup>1</sup>, M. Srbinoska<sup>2</sup>, A. Cvetanovska<sup>1</sup>
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ABSTRACT. The issue of this study refers to the organic production (concentration of carbohydrates, antocyanins and organic acids) in tobacco (*Nicotiana tabacum* L.) from the crop 2008 of two oriental types: PRILEP (P-156) and JAKA (Jv-125/3). The plants were cultivated in controlled experimental conditions in the Botanic garden of Department of Botany, Institute of Biology, Faculty of Natural Sciences and Mathematics-Skopje. Material is treated with heavy metals, applicated in four different concentrations: copper, cadmium, lead and fungicide Ridomil gold. Analyses were made on the first insertion, also named as a phase of lower leaves. Protective antioxidative answer was examined (content of antocyanins). The results show the organic production in tobacco culture (content of carbohydrates and organic acids), which directly reflects the quality characteristics of fermented tobacco and changes physical as well as tasteable performances of tobacco raw material. Conclusions affirmed that noticeable dysfunctions in secondary metabolism were caused, as a result of toxicological effect of heavy metals.

# PHYTOPLANKTON BASED ASSESSMENT OF THE ECOLOGICAL STATUS AND ECOLOGICAL POTENTIAL OF LAKE TYPES IN BULGARIA

S. Cheshmedjiev<sup>1</sup>, D. Belkinova<sup>2</sup>, R. Mladenov<sup>2</sup>, I. Dimitrova-Dyulgerova<sup>2</sup>, G. Gecheva<sup>2</sup>

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ABSTRACT. Research has been carried out of the main characteristics of phytoplankton communities in order to assess the ecological status and ecological potential of the types of lakes in Bulgaria, according to the requirements of WFD 2000/60/EC. Eighty lakes/reservoirs have been researched on the territory of the Republic of Bulgaria. The assessment was made on the basis of four main metrics (phytoplankton biovolume; Algae Groups Index; transparency, chlorophyll a) and three additional metrics (% Cyanobacteria; intensity of algal "bloom" and presence of toxic species). More than half of the researched lakes in Bulgaria are in compliance with the WFD requirements for good ecological state (high and good ecological status, maximum and good potential). A classification system for assessment of ecological status or potential has been developed using above-mentioned phytoplankton metrics. The existing 17 types of lake in Bulgaria are classified in two main groups: oligotrophic lake types and mesotrophic lake types.

# COMPOSITION AND TOXIC POTENTIAL OF CYANOPROKARYOTA IN VACHA DAM (BULGARIA)

I. Teneva, D. Belkinova, I. Dimitrova-Dyulgerova, M. Vlaknova, R. Mladenov

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**ABSTRACT.** Some species of Cyanoprokaryota produce toxins that affect animals Most of the freshwater basins in Bulgaria, including dams, are relatively well studied in terms of the phytoplankton composition, but the data for presence of cyanotoxins are limited. The aim of our study was to evaluate the diversity, distribution and quantitative development of the phytoplankton as well as the presence of cyanotoxins in the public reservoir Vacha. We have collected water and phytoplankton samples from Vacha reservoir at different time points. All water samples were analyzed for presence of cyanotoxins by ELISA, and tested for cytotoxicity on cell cultures in vitro. Physicochemical parameters, including water temperature, pH, total nitrogen and total phosphorus were measured. Algae, belonging to seven divisions (Cyanoprokaryota, Chlorophyta, Xantophyta, Dinophyta, Euglenophyta, Bacillariophyta and Criptophyta) were identified. A potentially toxic Aphanizomenon flos-aquae cvanoprokarvote was detected concentrations in July and August 2008 as well as in July 2009 together with Microcystis aeruginosa. The water sample collected in August 2008 contained 0.25 ppb microcystins/nodularins. The total microcystins/nodularins concentration in the water samples collected in September 2009 was 0.5 ppb. The viability of HeLa cells was affected mainly after 48 h of exposure to the collected water samples.

# INFLUENCE OF OXYFLUORFEN ON SOME ANATOMIC INDICES IN THE LEAVES OF VIRGINIA TOBACCO PLANT (NICOTIANA TABACUM L.)

#### H. Anastasov

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ABSTRACT. The oxyfluorfen was applied at a dose of 80ml/dka and 100ml/dka 72 hours before the process of tobacco planting. During the vegetation period some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, deformation of leaves and vegetation tip, weak chlorosis, etc. For the purpose of determining the herbicide influence on the tobacco leaf anatomy, several fixed samples from the leaves' middle sections were taken as well. The following indices were taken into consideration – stomata number/mm2 and stomata size (µm) from the upper and lower epidermis, size of assimilation parenchyma (mesophyll) in leaf. It was established that oxyfluorfen caused considerable changes in the tobacco leaf anatomy, which found expression in reduction of stomata number/mm2, as well as increase the thickness of leaf lamina (blade), compared those in the non-treated control plants.

# LEAF EPIDERMIS STRUCTURE IN AMELANCHIER OVALIS MEDIC. (ROSACEAE)

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ABSTRACT. The leaf epidermis structure of *Amelanchier ovalis* Medic. was studied by light and scanning electron microscopy. Rodlets of crystalloid epicuticular waxes and papillae were observed. Simple single trichomes and cyclocytic stomatal type were described. An attempt was made to estimate all features of taxonomic value which are relevant for further clarification of the relationships within subfamily Maloideae. The epidermal structure shows adaptation to various environmental conditions which explains the broad geographical distribution of the species.

# THE HISTOCHEMICAL ANALYSIS OF *THYMUS MALYI* RONNINGER GLANDULAR TRICHOMES

M. Marin<sup>1</sup>, L. Ascensao<sup>2</sup>, S. Budimir<sup>3</sup>, D. Janošević<sup>1</sup>, S. Duletić-Laušević<sup>1</sup>, P. Marin<sup>1</sup>

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**ABSTRACT.** The genus *Thymus* L. belongs to the family Lamiaceae, and comprises numerous aromatic species which are used for medical purposes and also as culinary herbs. *Thymus malyi* Ronninger is an endemic species from central Balkan which grows on serpentine hills. Histochemical analyses of the peltate and capitate glandular trichomes of *Thymus malyi* were carried out using light microscopy. Results of histochemical tests showed positive reaction to phenol compounds in the secretory heads of peltate trichomes. Positive reaction for terpenes was obtained in capitate trichomes and in the subcuticular spaces of peltate trichomes. Lipid reaction was positive in the stalk cell of the capitate trichomes, and in the peltate trichomes heads. Positive reaction for polysaccharides was observed in the secretory head of both types of glandular trichomes, with more intensive reaction in the capitate trichomes.

# INFLUENCE OF INDOLE BUTYRIC ACID ON THE MICROPROPAGATION OF LAMIUM ALBUM L.

M. Dimitrova, Zh. Yordanova, D. Dragolova, V. Kapchina-Toteva

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**ABSTRACT.** The genus *Lamium* L. (Lamiacea) comprises about 40 species of annuals and perennials distributed in Europe, Asia and Africa. *Lamium album* L. possesses astringent, antispasmodic, anti-inflammatory, antibiotic and bacteriostatic properties. The effect of auxin IBA (Indole-3-butyric acid) on micropropagation of *Lamium album* L. has been investigated. On IBA-supplemented MS medium all concentrations stimulated the length of shoots, average number of leaves and roots. IBA did not influence number of shoots, callusogenesis and dry weight of in vitro propagated plants. To the best of our knowledge, this is the first report on in vitro multiplication of *Lamium album* through direct plant regeneration technique offers an effective alternative method of propagation for this important multipurpose medicinal plant.

# ANALYSYS ON POLLEN AND SEED PRODUCTIVITY AND EFFECTIVENES IN GENTIANA LUTEA L.

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**ABSTRACT.** A study on the viability of pollen grains and embryos in *Gentiana lutea* distributed in Bulgarian flora has been carried out. Degenerative processes predominantly of the mature pollen were observed. As a result of that, in the studied populations, the effective mature pollen grains in the anthers reduce in different amount. The used tetrazolium test shows that in the mature seeds the viability of embryos reduced progressively during the three consecutive years of the present study.

# RATE AND FORMS OF MINERAL NUTRITION CAN INFLUENCE DRY MATTER ACCUMULATION AND SAPONIN CONTENT OF PUNCTURE VINE (TRIBULUS TERRESTRIS L.)

G.I.Georgiev<sup>1</sup>, A.Ivanova<sup>2</sup>, P.Mechkarova<sup>2</sup>, A.Ivanova<sup>1</sup>, L.Popova<sup>1</sup>

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 2 - Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria
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ABSTRACT. Effect of mineral nutrition (soil or foliar supply of nutrients) on the growth, dry matter and saponin content of the shoot biomass of Bulgarian variety puncture vine (*Tribulus terrestris* L.) grown on soil as pot experiment in green house were studied. Soil fertilization rate of 100mgN/kg or 90P/kg of dry soil, oppositely to the results obtained from the foliar fed plants (0.3 % solution of liquid fertilizer AgroleafR (Scotts Co, USA) with formulation N12P52K5, increased shoot total N and P without significant change of dry matter. Changes of total reducing sugars, amino acids, phenolics and flavonoids and activity of leaf photosynthetic apparatus (chlorophyll (Chl) a, b and carotenoids content and parameters of chlorophyll a prompt fluorescence) were found to relate to the variation of individual saponin content analysed by HPLC technique. Soil fertilized plants in contrast to the foliar fed plants showed more protodioscin, prototribestan and dioscin than control but contained less of flavonoid glycoside rutin.

# COULD MICROALGAE ENHANCE THE GERMINATION OF TRIBULUS TERRESTRIS L. SEEDS?

#### G. Petkov

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**ABSTRACT.** Seeds of *Tribulus terrestris* L. were sown in transparent plastic leaky boxes, at a depth of 4-5 mm in sand. The transparent pots were put on plates, where water has been added through the bottom holes and capillary ascending to the surface. The temperature of soil was 20-37 0C, temperature of air 20-40 0C. Either the soil microalgae, or preliminary grown microalgal suspension, single added with the water has been thriving at these conditions. At all experiments, the first seeds germinated exactly 3 days after they have been sown. Above 90 % of the seeds germinated to 7-9 day and single of them to 20 day. Inside, on the wall of the pots, green collour appeared due to microalgae and significantly later mosses appeared, too. Having worked with seeds from Pazardzhik a germination of 31  $\pm$  12 % was achieved (74 seeds). Seeds gathered from nature, Black Sea coast, Zarevo, have germinated 7 of 10 in a single experiment. The manner of work is in consistency with a possible breeding of the plant as greenhouse seedlings.

# DISTRIBUTION AND RESOURCES EVALUATION OF *TRIBULUS TERRESTRIS* L. /ZYGOPHYLLACEAE/ POPULATION IN THRACIAN FLORISTIC REGION

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**ABSTRACT.** *Tribulus terrestris* is an annual medicinal plant. Its row material is collected for industrial uses from the nature. The distribution of its populations as well as the quantity of the drug production (productivity) in specific sites and conditions were studied. Thirty three economically significant localities were established in the Thracian floristic region during 2009, distributed on area of 1314 dka, which could give about 90 tons of dry drug annually. The biomass production of every area was affected by the change in the environmental conditions. The best populations for collection and production of pharmaceutical drug and international trade were determined.

# DISTRIBUTION AND RECOURCE EVALUATION OF THE TRIBULUS TERRESTRIS L. – MALTESE CROSS (ZYGOPHYLLACEAE) IN POPULATIONS FROM SOUTH BULGARIA

D. Peev, N. Valyovska

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**ABSTRACT.** During the vegetation season june - september 2009 a investigation on the distribution and resource evaluation of the 55 *Tribulus terrestris* populations in South Bulgaria has been organized. They belongs to the 6 florobotanical regions of the country. As an element of the wild flora, and in the same time as a weed, that species inhabits abandoned places, vineyards, agricultural terrains, coastal and inland sands from 0 to 800 m.s.l.a. The populations developed on slightly humid, with good aeration soils, formed the most highest green mass yields in the limits of 5.25 kg to 89 tons. per dk. The total amount of the recourses from the investigated territory can be evaluated in the limits of some 615 tons fresh mass.

# DISTRIBUTION AND RESOURCE EVALUATION OF TRIBULUS TERRESTRIS L. IN NORTH BULGARIA

#### B. Sidjimova and M. Nikolova

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**ABSTRACT.** The present study examined the distribution and resource capacity of *T. terrestris* populations in North Bulgaria. Thirty three populations of four floristic regions – Danube plain, North Black sea coast, Balkan foothill region and North Eastern Bulgaria were confirmed by our investigations. Some populations had areas that exceed 10 ha. The results of resource assessment showed that 29 305 kg dry mass of Herba *Tribulus terrestris* may be collected every year from these areas.

# SCREENING BY THERMOLUMINESCENCE METHOD THE QUANTITY OF BIOLOGICALLY ACTIVE COMPOUNDS FROM TRIBULUS TERRESTRIS PLANTS WITH DIFFERENT ORIGIN

- L. Maslenkova<sup>1</sup>, V. Peeva<sup>1</sup>, L. Brankova<sup>1</sup>, I. Lazarova<sup>2</sup> and L. Evstatieva<sup>3</sup>
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ABSTRACT. *Tribulus terrestris* L. (Zygophyllaceae) is an annual prostrate medicinal plant that is widely used for treatment of sexual deficiency, as an affrodiziak. Steriodal saponins and rutin are among the basic compounds responsible for the biological activities of *T. terrestris* extracts. In the present study a comparative analysis of thermoluminescence (TL) emission parameters and the content of steriodal saponins and rutin from three different origins of *Tribulus terrsetris* (Turkey, Hungary and Bulgaria) was presented. The plants were cultivated at identical conditions on the field near Sofia and the samples were collected in the stage of full blossoming and seedling. The observed correlation between TL properties of the leaves and the content of the dominating biologically active compounds of the samples - furostanol saponins, can be consider as an possibility TL method to be used for early screening the quality of the herb.

# ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF DIFFERENT TEUCRIUM CHAMAEDRYS L. EXSTRACTS

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ABSTRACT. The paper presents the results of antioxidant activity of water, methanolic, ethyl-acetate, acetone and petroleum ether extract from plant species *Teucrium chamaedys* L. (Lamiaceae). Antioxidative activity was determined in vitro, using the DPPH reagent by spectrophotometric method. Experimental values are expressed as IC50 values (mg /ml), and show that the tested extracts have high antioxidant activities, which range in scope from 341.08 mg/ml to 29.46 mg/ml. In the tested extracts of plant species *Teucrium chamaedys* L. quantitative composition of total phenols and flavonoids were determined by spectrophotometric method. Total phenols determined by Folin-Ciocalteu reagent and their amounts in the range of 30.39 mg/g to 169.50 mg/ g (expressed as gallic acid equivalent, mg GA/g of extract). The amounts of flavonoids in plant extracts of *Teucrium chamaedys* L. are in the range of 16.67 mg/g to 87.17 mg / g (expressed as rutin equivalent, mg RU/g of extract).

# CONTENT OF NEUTRAL LIPIDS AND FATTY ACIDS IN CALLUS CULTURES AND LEAVES OF INTACT PLANTS OF *AJUGA GENEVENSIS* AND *AJUGA CHIA*

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**ABSTRACT.** The comparative study of quantity and composition of neutral lipids and their fatty acids in the callus cultures and leaves of intact plants of *Ajuga genevensis* and *Ajuga chia* was carried out. The dependence of composition and content of lipids and fatty acids on the species belonging, the origin of calli and the culture age (number of subcultures) was revealed.

# NEW CHOROLOGICAL DATA OF SOME SUBMERGED MACROPHYTES IN BULGARIA

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ABSTRACT. The data on the distribution of some submerged macrophytes from different types of limnetic water bodies from the ecoregions of Bulgaria were summarized. New chorological data for 11 species were found: Ceratophyllum demersum, Ceratophyllum submersum, Elodea canadensis, Hydrocharis morsus-ranae, Myriophyllum spicatum, Najas marina, Nymphaea alba, Potamogeton gramineus, Potamogeton pectinatus, Potamogeton pusillus, Trapa natans. The distribution of Potamogeton berchtoldii Fieb. has been confirmed for the territory of Bulgaria.

#### PHARMACOLOGICAL ACTIVITY OF SESQUITERPENE LACTONES

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**ABSTRACT.** Sesquiterpene lactones belong to the large group of plant terpenoids. In nature, sesquiterpene lactones play an important role in plant defense, as antibacterials, antivirals, antifungals, insecticides and by reducing the herbivores' appetites for such plants. They also have some allelopathic potential. In recent years there is an increasing interest in sesquiterpene lactones, mostly because of their cytotoxic and anticancer activity. This paper will summarize current knowledge about sesquiterpene lactones in plants.

# EVALUATION OF THE ANTIOXIDANT ACTION AND FLAVONOID COMPOSITION OF ARTEMISIA SPECIES EXTRACTS

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**ABSTRACT.** The antioxidant potential of methanol extracts and ethyl acetate fractions of 10 Artemisia species was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The screening of all extracts showed that the ethyl acetate fractions possessed the highest antioxidant activity. The fractions of *A. vulgaris, A. campestris, A. annua, A. alba, A. austriaca* were the most active and their scavenging potencies as indicated in IC50 values, being 11.96, 12.50, 15.85, 30.79, 37. 35  $\mu$ g/ml, respectively. Among the methanol extracts these of *A. pontica* and *A. annua* (Bulgarian origin) exhibited the strongest antioxidant capacity – 61.27 and 67.45  $\mu$ g/ml. Five flavonoid glycosides were identified by TLC analysis.

## SECTION "CELL & MOLECULAR BIOLOGY"

# MOG79-90 PEPTIDE IN COMPLEX WITH RECOMBINANT MHC CLASS II MOLECULES AMELIORATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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ABSTRACT. The recent advancement in the field of biomedical technologies has opened up new possibilities in the treatment of autoimmune disorders such as multiple sclerosis (MS). Here, we report the production of soluble complexes between the myelin oligodendrocytic glycoprotein (MOG) 79-90 peptide and a genetically engineered murine MHC class II molecule Aq. Using mouse model of MS, we demonstrate that the generated complexes are functional and able to ameliorate the clinical signs and reduce the incidence of experimental autoimmune encephalomyelitis (EAE). Our findings offer a new possibility for the treatment of chronically active autoimmune inflammation such as multiple sclerosis.

# EFFECT OF CADMIUM ON ARABIDOPSIS THALIANA MUTANTS TOLERANT TO OXIDATIVE STRESS

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**ABSTRACT.** In this paper we investigate the changes in fresh weight, chlorophyll content, catalase and guiacol peroxidase activities of nine oxidative stress-tolerant *atr* (AAL-toxin resistant) Arabidopsis thaliana mutants subjected to cadmium stress. We show that *atr*2, *atr*7 and *atr*9 are more tolerant to CdCl<sub>2</sub>. There was no significant change in catalase activity under cadmium treatment in all atr lines including the parental line *loh*2 compared with untreated seedlings. All lines but *atr*9 showed reduction in guaiacol peroxidase activity. This suggests that the sensitivity to the cadmium-induced oxidative stress at least in some lines is linked to impaired reactive oxygen species detoxification.

# A PILOT STUDY ON THE IMMUNOMODULATORY EFFECT OF BULGARIAN PROPOLIS

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ABSTRACT. Propolis is a product from the honey bee A. mellifera with various pharmacological properties. Its immunomodulatory activity is in the focus of the current research. Peripheral blood mononuclear cells (PBMC) from heparinized venous blood of healthy donors (n=6) were cultured for 24 h in the presence of propolis from the Eastern Rodopi Mountain (ethanol extractions with concentration 0, 01; 1; 2,5; 5 and 10 mg/L) or CAPE in concentration 2, 4, 8 and 16 mg/L. PBMC cultured in serum free RPMI only were used as controls. The percentage of T helper/inducer (CD4+CD3+), T cytotoxic (CD8+CD3+), B (CD19+CD3-) and NK (CD56+CD16+CD3-) lymphocyte subsets, as well as the proportion of apoptotic (Annexin V+) cells within each subset were determined before and after the cultivation by flow cytometry (FACS Calibur, BD). The percentage of CD19+ cells decreased in high concentrations of both of substances, but in low concentrations they had a protective effect on the proliferation and B cell activity. Low dozes had no effect on the percentage of CD4+ and CD8+ T cells. The high concentrations of propolis (10 mg/L) and CAPE (16 mg/L) induced apoptosis in a large portion of all of cells types. All concentrations tested had no negative effect on the proliferation and vitality of NK cells. Our results evidence that high propolis concentrations are toxic for human PBMC, but low concentrations modulate cellular immunity.

#### YKL-40 IN HEALTHY SUBJECTS

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ABSTRACT. YKL-40 is a plasma protein, belonging to the chitinase protein family, but has no chitinase activity. It is expressed and secreted by macrophages, chondrocytes, activated neutrophils, differentiated monocytes, vascular smooth muscle cell and cancer cells. The objective of the present study was to determine serum YKL-40 levels in healthy subjects and to develop a valid reproducible enzymelinked immunosorbent assay. Serum YKL-40 concentrations were determined by a two-site, sandwich-type, enzyme-linked immunosorbent assay (ELISA) in 10 healthy female volunteers aged 18-50. Our investigation show a mean value 41,11 (20-59) ng/ml of serum YKL-40 in healthy women. We determined that the correlation between protein level and age is feeble, but positive. Our study is the first in Bulgaria to measure serum YKL-40 level in healthy subjects. Elucidation of YKL-40 functions in normal and pathologic processes is an important objective of future analyses.

### HPLC METHOD FOR SCREENING OF STEROIDAL SAPONINS AND RUTIN AS BIOLOGICALLY ACTIVE COMPOUNDS IN TRIBULUS TERRESTRIS L.

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**ABSTRACT.** An improvement method for extraction of steroidal saponins and rutin of Tribulus terrestris L. (Zygophyllaceae) was evaluated. It is shown that the ultrasound extraction is faster, easier, solvent-saving, more reliable and effective method than the conventional heat reflux extraction method.

Under optimized conditions, the main biologically active compounds protodioscin, prototribestin, dioscin and rutin were extracted from Tribulus terrestris with different origin and analyzed by revesed phase HPLC with UV detector.

### EXPERIMENTAL MODEL FOR SAFE GENE TRANSFER BY RECOMBINANT GENE CONSTRUCTS

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**ABSTRACT.** For gene transfer in laboratory-cultivated mouse embryonic stem cells (mESCs), previously designed recombinant gene constructs with respective genes inserted in them are necessary. For this aim, recombinant DNA-genomes from adeno-associated virus (AAV) (Parvoviridae), containing promoter of gene, coding Elongation Factor 1-alpha (EF1-α); isolated from 3T3 fibroblasts of adult laboratory mice Balb/c inserted oncogene Dcn1, (in its role of regulator on the tumor-suppressor gene p53 by specific pathways of indirect inhibition), as well as gene for neomycin resistance, isolated from bacterial DNA-plasmid, are used for gene transfection by electroporation. On the other hand, eventual subsequent super-activation of tumor-suppressor genes both in vitro and in vivo is also necessary.

### MOLECULAR AND CYTOGENETIC CRITERIA FOR IDENTIFICATION OF SERUM-FREE CELL CULTURES

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**ABSTRACT.** The information about the authenticity of newly or already established animal cell cultures is essential. By using DNA analysis six cell lines has been investigated: 3T3, HeLa, McCoy, HEp-2, McCoy-Plovdiv and HEp-2-Plovdiv E. For the last four was made cytogenetic analysis confirmed that serum-free cell strains McCoy-Plovdiv and HEp-2-Plovdiv E are originally derived from McCoy and HEp-2 cell lines. PCR - analysis demonstrated that 3T3 cells are mouse originally, HeLa, HEp-2 and HEp-2-Plovdiv E are with the human origin and McCoy and McCoy-Plovdiv are hybrid cells carrying mouse and human genes in their genome.

### IDENTIFICATION OF POTATO SPINDLE TUBER VIROID SMALL RNA IN OROBANCHE RAMOSA BY MICROARRAY

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**ABSTRACT.** Post transcriptional gene silencing (PTGS) in plants is reported as a defence mechanism against pathogens, like invading viruses and viroids, transposons and transgenes. The processing of double stranded RNAs to 21-24 nt duplex RNAs (small interfering RNAs) by RNAse III—type nuclease homologs- Dicerlike (DCL) enzymes is a key step of this process.

We were interested whether the viroid RNA can trigger silencing in parasitic plant O.ramosa. Therefore we infected O.ramosa attached to tomato with Potato Spindle Tuber Viroid (PSTVd). The presence of small interfering RNAs derived from PSTVd has been detected by miRNA microarray.

### AN OPTIMIZED PROCEDURE FOR DNA SILVER STAINING IN POLYACRYLAMIDE GELS

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**ABSTRACT.** An optimized procedure for staining of DNA in polyacrylamide gel electrophoresis was created. It was found that picogram quantities of DNA can be detected in about one hour. The major advantages of the method proposed are to be relatively simple and fast, as well as enough sensitive for some of the most common applications.

# OBTAINING AND SELECTION OF PANCRATIUM MARITIMUM L. IN VITRO CULTURES WITH ACETYLCHOLINESTERASE INHIBITORY ACTION

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**ABSTRACT.** Two types of plant in vitro systems - calli and shoot cultures were obtained from ovaries of sea daffodil (Pancratium maritimum L.) – a Bulgarian threatened plant belonging to the Amaryllidaceae family. Using TLC method, ten different alkaloids were separated in extracts from in vitro cultures, as well as their abilities to inhibit the acetylcholinesterase (EC. 3.1.1.7) were evaluated by the same techniques. All investigated lines from both type in vitro systems exhibited high levels of somaclonal variability concerning the number and the levels of alkaloids produced. However, the callus cultures produced alkaloids in significantly lower levels compared to the shoot cultures. Two shoot lines (H6L7 and H6L6) producing four alkaloids with high acetylcholinesterase inhibitory action (with Rf values of 0.47, 0.43, 0.25 and 0.12) they were selected as prospective source for further screening of new acetylcholinesterase inhibitors.

### SECTION "ECOLOGY & ENVIRONMENTAL CONSERVATION"

# REVISION OF RIVER & LAKE TYPOLOGY IN BULGARIA WITHIN ECOREGION 12 (PONTIC PROVINCE) AND ECOREGION 7 (EASTERN BALKANS) ACCORDING TO THE WATER FRAMEWORK DIRECTIVE

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ABSTRACT. A revision of river and lake typology in Bulgaria has been done within the ecoregion No. 12 Pontic province and ecoregion No. 7 Eastern Balkan according to the Water Framework Directive 2000/60/EC. Certain geographic changes between the ecoregion No. 12 and ecoregion No. 7 have been proposed based on existing biogeographic data, at the beginning. The whole part of the Danube River Basin and Black Sea River Basin District have been associated to the Pontic Province (ER No. 12), as well as the rest part of the Southern Bulgaria (Maritza River basin, Mesta RB and Struma RB) has been allied to the Eastern Balkan (ER No. 7). A serious reduction of the total number of river types (from 33 to 16 types) was justified using clear hydromorphological and biological criteria, e.g. ecoregion, vertical factors (4 altitude zones, main substratum characteristics, slope, and other supplementary factors such as fish and vegetation zonation, climate maps) and horizontal factors (calcareous geology, salinity, size category). Similar reduction of the number of lake types (from 33 to 17 types) was proposed in parallel with a significant modification of the lake typology method. Lake typology in Bulgaria was based on the obligatory factors (4 altitude zones, size typology based on surface area, depth, salinity and geology) and optional factors (residence time, mixing characteristics, e.g. monomictic, dimictic and polymictic, presence of profundal zone, reference trophic status). Seven lake types were identified as "reservoir types", which were only presented by artificial water bodies or heavily modified water bodies without any possibility for natural lake equivalent within the country or region. Four coastal lake types with various salinity (from freshwater <0.5 % to hypersaline >40%) have been reviewed as belonging to the category "transitional waters".

### DEVELOPMENT OF CLASSIFICATION SYSTEM AND BIOLOGICAL REFERENCE CONDITIONS FOR BULGARIAN RIVERS AND LAKES ACCORDING TO THE WATER FRAMEWORK DIRECTIVE

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ABSTRACT. The study focused on sampling procedures and analysis of biological and physico-chemical quality elements (according to the EU Water Framework Directive (WFD)) and aimed at defining reference conditions and sites, maximum ecological potential, specific physico-chemical and hydromorphological conditions for assessed surface water types of "rivers" and "lakes" categories. Biological quality elements (BQE) and their metrics were selected in compliance with WFD requirements and its additional guidelines. All five compulsory BQEs were surveyed (phytoplankton, macrophyte flora, phytobenthos, macrozoobenthos, fish fauna) towards establishing rivers and lakes ecological status and potential. Current research indicates a certain necessity for integration of all assessments and analysis of ecological status/potential and their direct link to the measurement and monitoring programmes in Bulgaria.

### CLIMATE CHANGE AND THE IMPACT ON AGRICULTURE IN REPUBLIC OF MACEDONIA

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**ABSTRACT.** The agriculture sector is one of the most important sectors in Macedonian economy and it is the second source of gas emission in the country. The agriculture sector will face serious impact of global climate change which will alert on climate significantly. According to the climate scenarios, the most vulnerable agricultural areas to climate change in Republic of Macedonia are Povardarie, southeastern part of the country, south Vardar valley, valley of Skopje and Kumanovo, Ovche Pole, Pelagonija, Plog and Prespa/Ohrid region. In this paper analysis of climate change and evaluation of its impact on agriculture in Republic of Macedonia are presented.

# INFLUENCE OF ZINC ON GILL MORPHOLOGY AND BREATHING OF GIBELIO CARP (CARASSIUS GIBELIO)

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**ABSTRACT.** The influence of increasing concentration of zinc (0.5; 1.0  $\mu$  2.0 mg.l<sup>-1</sup> ZnSO<sub>4</sub>x7H<sub>2</sub>0) on gill morphology and breathing of Gibelio carp (*Carassius gibelio*) was investigated *ex situ*. The presence of zinc in the water causes both destructive and hyperplasic alterations in the gills. With the increasing of the zinc concentration, the hyperplasic alterations prevail over the destructive ones.

It was ascertained and a disturb of the breathing intensity was, manifested as 31- 48% reducing of oxygen consumption depending on the concentration of the zinc. All experimental specimens possess less hardiness to oxygen deficit as compared to fish of control group (40-71.4 % mortality depending on the concentration of  $ZnSO_4$ ).

### DISTRIBUTION AND BIOINDICATION ROLE OF AQUATIC BRYOPHYTES IN BULGARIAN RIVERS

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**ABSTRACT.** The distribution and abundance of aquatic bryophytes, and 10 physicochemical parameters of river water have been studied in 204 river sites in Bulgaria, 15 river types. Forty-nine bryophyte species were registered at 51 sites. The commonest species were *Platyhypnidium riparioides, Fontinalis antipyretica, Brachythecium rivulare* and *Leptodictyum riparium*. Principal component analysis revealed that the sites with higher abundance of *Leptodictyum riparium* were in inverse correlation with sites where the rest 3 species occurred. Canonical correspondence analysis indicated that 9 of the 10 selected environmental parameters significantly influenced bryophytes distribution. The study comprised and analyzed data from macrophyte surveys in Bulgaria undertaken as a part of two EUfunded projects during 2009.

# IMPLEMENTATION AND ADAPTATION OF MACROPHYTE INDICATION SYSTEM: ASSESSMENT OF ECOLOGICAL STATUS OF RIVERS IN BULGARIA ACCORDING TO THE WATER FRAMEWORK DIRECTIVE

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ABSTRACT. The legal requirements stated in the EU Water Framework Directive (WFD) have led to increased activity concerning macrophyte indicator metrics in Europe. Nevertheless in Bulgaria such indexes are still under consideration because of the lack of official methodologies. The macrophyte surveys undertaken as a part of two EU-funded projects are a unique resource allowing aquatic plant communities to be studied. The Reference Index (RI) was chosen as macrophyte assessment method since classifies rivers by using regional approach and reflects different kinds of environmental pressures. RI was implemented on 73 sampling sites in Bulgaria. On the basis of this dataset, our attempt to redesign the RI by the addition of further species, and the re-grouping of existing species, resulted in a considerable improvement in relation with regional conditions. The chosen macrophyte method can be applied at Bulgarian rivers in order to assess their ecological status.

#### SUBSURFACE CHLOROPHYLL a MAXIMA IN THE BOKA KOTORSKA BAY

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ABSTRACT. Subsurface chlorophyll maximum (SCM) are common phenomena in variety of environmental, from fresh water, brackish water, estuaries, shelf areas, the coastal zone to open ocean. It is now known that the chlorophyll maximum at the subsurface layer occurs widely in water where the water column is stratified. In permanent stratified tropical water, therefore, the SCM is expected to be a year round phenomenon. In higher latitudes, where there are occasional vertical mixing of the water column due to seasonal surface cooling or strong winds, the SCM will be restricted during a particular period of the year, such as in the summer or between spring and autumn. The Boka Kotorska Bay is semi-enclosed basin, located in the south-easter Adriatic Sea (Mediterranean Sea). The weekly changes in chlorophyll a and physico-chemical parameters were investigated in the period September 2008 to March 2009. Maximum phytoplankton biomass (11.13 mg m-3 chlorophyll a) was observed on 2m depth in February nearly the pycnocline. The good correspondence of SCM with occurrence of nitrate clearly indicates that the phytoplankton that make up the SCM depended highly on nitrate, which is supplied mainly by diffusional process from depth below the SCM.

#### FLORISTIC CHARACTERISTICS OF CHIRPANSKATA GORA PRESERVE

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ABSTRACT. A research was conducted, prospecting the vegetation in Chirpanskata Gora Preserve. A list of the determined species of supreme plants was prepared based on this. The taxonomic structure of the flora and the ecological and biological characteristics were defined. The relicts, endemics and medicinal plants on the researched territory were mapped as result of the research. The availability of taxa of conservative importance, according to the Bulgarian legislation, was analyzed. The observed anthropogenic influence was analyzed and based on this conclusions were drawn and recommendations were made, which guarantee the resilient presence of Chirpanskata Gora Preserve in perspective.

## FLORISTIC CHARACTERISTICS AND ECOLOGICAL EVALUATION OF DEBELATA KORIA PRESERVE, CHERNOZEMEN VILLAGE

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**ABSTRACT.** A research was conducted, prospecting the vegetation and flora in Debelata Koria Preserve, located along the Valley of Stryama River, which is part of Kraishtensko –Tundzhanska interim zone in the region of Gornotrakiyska lowland. The taxa of the supreme seed vegetation, the ecological and biological structure, the elements of conservative importance and the negative anthropogenic influences were determined as result of the research. Two species of high preservative value and two species that have not been mentioned before for this floral region were discovered.

# ECOLOGICAL CHARACTERISTICS AND CONSERVATION OF THE PROTECTED RESURRECTION SPECIES HABERLEA RHODOPENSIS FRIV. AS IN VITRO PLANTS THROUGH A MODIFIED MICROPROPAGATION SYSTEM

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ABSTRACT. Among the especially interesting and rare plants on the Balkan Peninsula are tertiary relics and endemics belonging to the tropical family Gesneriaceae: Haberlea rhodopensis Friv (Rhodope silivryak, Orpheus' flower) and representatives of the genus Ramonda. Haberlea rhodopensis Friv. is included in the European list of rare, in danger of extinction and endemic plants and in the Bulgarian list of endangered plants. In recent years, our team has extended in a new directions the traditions of many years' investigations of this interesting species at the Dept. of Plant physiology and Molecular biology. We started a thorough exploration of the various habitats and ecological characteristics of Haberlea rhodopensis Friv. Simultaneously, at the Plant Biotechnology laboratory at the University of Plovdiv we started the establishment of a live collection of in vitro Haberlea rhodopensis Friv. from various populations and habitats in Bulgaria. Such in vitro live collection with the aim of conservation and investigation of the natural population is created for a first time in Bulgaria. This is accomplished through an in vitro system for regeneration and propagation, modified by our research group. The live collection of in vitro Haberlea rhodopensis Friv. plants will be a donor for conservation and reintroduction of adapted in vitro plants in their natural endangered habitats and also for physiological studies of drough tolerance, and multidisciplinary comparative analyses.

# CONCENTRATION OF SOME HEAVY METALS IN AQUATIC MACROPHYTES IN RESERVOIR NEAR CITY KRAGUJEVAC (SERBIA)

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**ABSTRACT.** In this study, concentration of metals (Fe, Mn, Cu and Pb) were investigated in some aquatic macrophytes (5 plants), mud and water in artificial lake Gruža near city Kragujevac (Serbia). The results obtained indicate important role of macrophytic vegetation in aquatic ecosystems, with respect of bioremediation and bioindication, and confirm presumption that chemical analysis of test-species can give very important data, which offer complete picture of ecological status of investigated aquatic ecosystem, and identify plant species that are well hyperaccumulators and test-species for remediation of mentioned pollutants. Aquatic macrophytes can be use in the study of ecological status of water ecosystems and in monitoring of metals and other pollutants, and theirs application can be possible in finding of solutions for problems of protection, sanation and revitalization of those areas.

### POLEN FERTILITY OF *VICIA FABA* L., AFTER TREATMENTS WITH X-RAYS

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ABSTRACT. An attempt has been made to examine the fertility of polen from the Vica faba L., after short-term exposure to low dosages of X – rays (1X - 16,2 cGy; 2X - 32,4 cGy; 3H - 48,6 cGy; 4X - 64,8 cGy). The material has been analyzed during three consecutive generations M1, M2 and M3. Dose-effect dependence was determined at the level of fertility and the morphology of the polen grains (the results are shown in tables in the text below). The irregularities in grain's normal form are result of the irregularities during the microsporogenesis in all the treatments. The filled grains with generally unchanged shape that differ from the typical ones in volume have been separated from the defective grains with lower vitality. Beside the empty (sterile) grains, a number of triangular grains with considerably enlarged volume have been detected, as well as grains with spherical shape in miniature dimension and in very low percentage grains with polygonal shape, squashed and with wrinkled surface. The decreased fertility of the polen grain and the possible abnormalities in the flower's constitution result with decrease in the total number of plants in the experimental groups especially in M3 generation.

# INFLUENCE OF Cd AND Ni ON CONTENT OF N, P, K, NITRATES AND ACTIVITY OF NITRATE REDUCTASE IN CLONES OF Salix spp.

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**ABSTRACT.** Influence of excessive amounts of heavy metals to plant nutrients and metabolism of mineral elements is usually regarded as inhibiting. Results of this paper are only partially in correspondence with this hypothesis. Levels of investigated nutrients were determined in four willow clones exposed to two concentrations of Cd and Ni in water culture solutions (10-4 M and 10-5 M). Nitrogen and nitrate uptake was unaffected. Activity of nitrate reductase was significantly decreased only by Ni. Contents of K and P were highly dependent on genotype and level of applied heavy metals. Possible causes of these variations are discussed.

# INFLUENCE OF ZINC (Zn) ON GERMINATION OF WHEAT (TRITICUM AESTIVUM L.)

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ABSTRACT. The paper presents the results of research of negative effects of zinc (Zn) on seed germination of wheat (Triticum aestivum L.). Besides the impact on the percentage of germination, the toxic effect on some morphometric characteristics was followed, too. Ripe seeds of wheat were exposed in standard laboratory conditions to the influence of zinc, in form of zinc chloride (ZnCl2), at different values of concentration. For each value of concentration it was determined the percentage of germinationed seeds, as well as the length of root and shoot. It as found the value of the concentration of ZnCl2, which inhibited completely germination of wheat. The mean values of the length of root and shoot for each concentration were compared with values obtained for the control group of seeds, which were not treated with ZnCl2. In addition to causing of inhibition of seed germination, the presence of zinc in the medium affects disorder of the physiological - biochemical processes during the growth and development of vegetative organs that it indicates the difference in the length of the root and shoot of treated seeds in relation to the control group of untreated seeds.

## NATURAL COMMUNITIES OF URANIUM MINING IMPACTED AREA IN THE VICINITY OF THE SENOKOS VILLAGE

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ABSTRACT. Uranium containing wastes accumulated during mine activities have resulted in a multitude of contaminated sites in Bulgaria. The lack of biomonitoring programs limits the environmental impact assessment of uranium impacted areas. The aim of the study was to investigate the health of natural terrestrial (vegetation, soil bacteria and millipedes) and freshwater (macrozoobenthos) communities in the impacted area of former uranium mine Senokos. The mine is one of the forty-nine former uranium mines in Bulgaria, reclaimed in the beginning of the 90's, but the reclamation was compromised due to surface erosion of the protective layers. The vegetation is typical for the region and uranium pollution has not caused any significant adverse affects on it. Adverse affects on soil bacterial communities are recorded only to their activity, but not to the abundance. Soil millipedes are in low density dominated by *Pachyiulus cattarensis* (Latzel 1884). The benthic community of Luda River is influenced by both uranium loaded sediments and infiltrate water from the mine.

# DEVELOPMENT OF FISH BASED INDEX FOR ASSESSING ECOLOGICAL STATUS OF BULGARIAN RIVERS (BRI)

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ABSTRACT. An index has been elaborated to define the ecological status of rivers in Bulgaria, using the fish as indicator for human impact. The index is based on classical approach for biological integrity, employing nine biotic variables and more than 70 freshwater fish species. The main purpose is intended to determine ecological status of Bulgarian rivers according the requirements of the EU Water Framework Directive. The method is fast, simplified and adapted to fishing methods that do not require colecting of specimens. The advantages are that BRI has been especially elaborated for Bulgarian rivers and its sensitivity directed to indication of antropogenic pressures to which the other biological elements are not so sensitive. The methods of the index elaboration have been explained and hints for its practical implementation given.

# DIET AND TROPHIC NICHE OVERLAP OF THREE TOAD SPECIES (AMPHIBIA, ANURA) FROM POLAND

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**ABSTRACT.** During our study we identified 53 prey items in the trophic spectrum of *Bombina bombina*, 124 prey items in the diet of *Bufo bufo* and 95 prey items in the diet of *Epidalea viridis*. The average number of prey items per stomach is as follows: *Bombina bombina* - 7.57, *Bufo bufo* - 9.0 and *Epidalea viridis* - 13.57. In all studied species the most important prey category is Coleoptera. Other important prey animals are Hemiptera, Hymenoptera and Dermaptera as well as non-insect invertebrates (Gastropoda and Arachnida) which also play significant role. All toads consume almost only terrestrial prey. The trophic niche breadths for the three species are as follows: *Bombina bombina* - 5.40, *Bufo bufo* - 4.47 and *Epidalea viridis* - 2.42. The estimated trophic niche overlap between the species is moderate (58.82% - 63.72%) and probably there is no or insignificant competition for food resources between them in the places with sympatric distribution. All studied species are polyphagous zoophages, like other amphibian species and they are probably consuming all mobile objects which they come in contact with and can swallow.

# DETERMINATION OF AIRPOLUTION ZONES IN KNJAŽEVAC (SOUTH EASTERN SERBIA) BY USING EPIPHYTIC LICHENS

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ABSTRACT. Airpollution investigations have not been done in Knjaževac until now. In this work, method of bioindication by lichens has been used. On 18 investigated points it has been found 22 lichen taxa from 12 genera. By calculating Index of Atmospheric Purity (IAP) 2 zones with different air pollution ("lichen desert" and "transitional" zone) has been found. The most sensitive species in Knjaževac are Buelia punctata, Melanelia acetabulum, M. exasperata, and Parmelia tiliacea and most tolerant are Lecanora alophana, L. intumescens, L. pulicaris, Lecidella elaeochroma and Phaeophyscia orbicularis.

### INVESTIGATION OF THE ORGANIC POLLUTION AND CONTAMINANTS' BIOLOGICAL DESTRUCTION OF THE RIVER KAMCHIYA

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ABSTRACT. The subject of the present work is an investigation of the water quality of the river Kamchiya with regard to the organic pollution and contaminants' biodegradability. Nutrients content (ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphate), Dissolved oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxigen Demand (COD) were analyzed in 10 monitoring stations downstream for three years period. The river was characterized with good oxygen status. The nitrates corresponded to the national water quality standards. Concentrations of ammonium nitrogen, nitrite nitrogen and phosphates over the threshold limits were determined in most of the monitoring stations. The average annual concentrations of nitrite pollution at the nearest to the river mouth monitoring stations Poda exceeded the threshold limits 4 times for 2006, 9 times for 2007 and 5 times for 2008 year. The highest concentration measured at this station during June 2007 year was 0, 91 mg/dm3 at 0,04 mg/dm3 threshold limit. The highest levels of ammonia and nitrites exhibited disturbance of the nitrification processes. The coefficient of conservatism of the organic pollutants was calculated by the correlation between BOD and COD values. In most of the cases the coefficient value was under 0,5 which determined the organic pollutants as highly conservative with regard to biological destruction.

# INORGANIC CHARACTERISTICS OF POLYFLORAL HONEY IN PLOVDIV (BULGARIA)

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**ABSTRACT.** Representative honey samples, harvest of 2009, from different places of the town of Plovdiv and its district (Bulgaria), were characterized on the basis of their physico-chemical parameters and inorganic chemical data. Water content, pH, electrical conductivity, macroelements – Ca, K, Mg, P, S and microelements – Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, Zn were determined after the Harmonised Methods of the International Honey Commission, and ICP-AES method in a certified laboratory. The results were discussed in order to evaluate the existence of data patterns and the possibility of differentiation of Plovdiv honey samples according to their site characteristics.

### SECTION "EDUCATION IN BIOLOGY"

# TEACHING IN BIOLOGICAL SCIENCES AT PRIMARY SCHOOLS IN SERBIA – AN APPLICATION OF THE HANDS ON METHOD

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ABSTRACT. The project Hands on has being implemented in Serbia since 2001 in order to uplift and revitalize teaching of the natural sciences in Primary Schools and enhance a personal engagement by the pupils in different topics. In 2003, the Serbian Ministry of Education decided to put forward an optional course "Hands on – Discovering the World", for children from 6 to 8 years old. Resources related to the biology have been occurred through seven modules, as follows: Plants, Food and digestion, Five senses, Growing and aging, Ecosystems, Environment. Knowing that topics in systematic biology are usually considered difficult and demanding, here we present the new, interesting, simple and creative way to teach systematics and classification since the early age, based on the Hands on method. New module refers to the classification of living beings, when teachers encourage their pupils to observe similar attributes, arrange the hierarchy of nested groups and classify animals discovering the evolutionary relationships of taxa.

# BEHAVIORAL SCIENCE AT THE FACULTY OF BIOLOGY, UNIVERSITY OF BELGRADE, SERBIA

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ABSTRACT. Whether behavior is present in the science for many years, it is still young and very attractive discipline that needs interdisciplinar approach for studying. Large scale of experimental models makes experimental and scientific work more exciting. At the Faculty of Biology, University of Belgrade (Serbia), we have behavioral courses at all three levels of studies (bachelor, master and PhD). At the basic level, we have Animal Behavior course, than, at the master level, we offer to our students two courses, Biology of Behavior and Introduction into Behavioral Genetics. Finally, for PhD students of Neuroscience we have two courses – Cellular Basis of Behavior and Neurobiology of Behavior, while PhD students of Genetics attend course Behavior Genetics. In this way, all aspects and levels of understanding behavioral mechanisms are covered.

# INNOVATIVE DIDACTICAL MEANS FOR DEVELOPING AND ASSESSING STUDENTS' INTELLECTUAL REFLECTION IN THE HIGH SCHOOL EDUCATION OF GENETICS

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ABSTRACT. This article has substantiated the need to use innovative didactical means - cognitive tests with a reflective close for the formation, development and diagnostics of the intellectual reflection in the study of classical and molecular genetics in the 9th and 10th grade. There are presented authors' versions of tests with a reflective close to activate and to measure two properties which characterize intellectual reflection skills - productivity of reflective thinking and awareness of their own mental activities of 15-16-year-old students. Each of the developed tests is structured in two subtests. The first subtest is composed by following the model of criteria-oriented tests and it contains tasks designed to update the pupils' reflection acts over their own cognitive activity. The second subtest is marked with the term "reflective close" (end) and it includes questions that require students' reasoning on their methods of action applied to solving the tasks of the first subtest. With the results from solving the first subtest we measure the productivity of the reflective thinking, and the results from the second test are used for judging the level at which students are aware of the reasons of their own cognitive actions. We determined the qualities reliability and validity of the constructed tests by the expert evaluation and testing them among a sample of 170 students in 9th and 10th grade. The results of statistical processing of empirical data were performed with the program SPSS 13.00 and they give us the confidence to say that the overall set of tasks with the additional to them a reflective close are reliable and valid means for development and diagnostics of the intellectual reflection in school teaching in genetics.

### SECTION "FUNCTIONAL MORPHOLOGY & ANTHROPOLOGY"

#### COBALT BIOACCUMULATION IN MOUSE BLOOD PLASMA AND LIVER

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ABSTRACT. Heavy metals such as cobalt are shown to accumulate in various organs of humans and animals. Oral exposure of immature mice to cobalt compounds (cobalt chloride and cobalt-EDTA) led to significant increase in cobalt (II) concentration in blood plasma and liver. Pregnant balb/c mice in late gestation were subjected to cobalt chloride (CoCl<sub>2</sub>.6H<sub>2</sub>O) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg/kg or 125 mg/kg which continued until day 30 of the newborn mice. Cobalt salts were dissolved and obtained from drinking tap water. Pure tap water was used as control. Mice were maintained in individual standard hard bottom polypropylene cages to ensure that all experimental animals obtained the required dose of cobalt salts. The newborn pups were sacrificed on days 18, 25 and 30 which correspond to different stages of development. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. Blood plasma and liver were used for measuring cobalt bioaccumulation. Cobalt (II) compounds showed differential bioaccumulation: higher concentrations were measured in the plasma compared to those measured in the liver. The effect depended on the type of compound used, dose, time duration as well as on the age of the experimental animals. Higher metal concentrations were detected in samples of mice treated with cobalt chloride compared to the samples exposed to Co-EDTA. The results indicate that day 18 mice are more sensitive to chronic exposure to cobalt compounds in high doses. Cobalt(II) concentrations in blood plasma may be used as a useful marker for diagnosing chronic exposure to cobalt compounds.

# QUANTIFICATION OF RAT SPERMATOGENESIS IN LATE PUBERTY AFTER NEONATAL HORMONAL MANIPULATION

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ABSTRACT. It has been known for many years that estrogens administration to experimental animals during the neonatal period or adulthood can impair sperm production and maturation. In earlier studies the negative effects of E were explained only as a result of suppression of gonadotropin secretion during the treatment. We aimed to assess GC development on day 35 (different GC types) in tandem with Sertoli cell support toward GCs (efficiency of spermatogenesis) by complex systems of quantitative criteria. We used experimental model for manipulation of neonatal hormonal environment by treatment with DES-10 µg, DES-1 µg, DES-0.1 µg or GnRHa. DES-10 greatly affected testis development and spermatogenesis in rat whereas GnRHa was quite less effective in producing negative impact. In contrast during the onset of puberty (d 18) both treatments exerted similar negative effect, and this time dependent response of the testis corresponds to different hormonal profiles. Quantitative evaluation of late pubertal spermatogenesis demonstrate that the most differentiated GC population- spermatides was the most sensitive to hormonal manipulation compared to spermatogonia (Sg) and spermatocytes (Sc). Different mechanisms are probably involved in mediation of the effect of E and gonadotropins and direct E action on GC differentiation is suggested. Our finding would elucidate our understanding about the hormonal regulation of different germ sell steps of spermatogenesis.

# ANTHROPOLOGICAL CHARACTERISTICS OF BODY COMPOSITION IN CHILDREN AND ADOLESCENTS FROM PLOVDIV

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**ABSTRACT.** This article studies the development of body weight components on the basis of anthropometric determination of the dynamics of the amount of fat, muscles and bones in 2094 children and adolescents aged 7 to 17 years (1054 girls and 1040 boys). The data were transversely collected at some schools in Plovdiv. The results were obtained for the differentiation in the change of body building, both in individuals of the same age and in the process of growth and development. Our results indicate that namely in the puberty period there is a change in the developmental processes of fat and muscle mass, which determines the further architecture of the male or female body. In girls the development of skeletal muscles and bone tissue practically stops after the puberty period (14-15 years), while in boys these two components of body composition continue to increase after this age with relatively high intensity.

#### EFFECT OF COBALT ON MALE REPRODUCTIVE ORGANS DURING PUBERTY

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**ABSTRACT.** Cobalt is an essential oligoelement for mammals. It is not a cumulative toxin but chronic exposure induces negative effects on the organism. Data from the literature evidenced that in experimental animals cobalt impaired male reproductive organs and fertility when applied chronically. The aim of our study is to follow the effect cobalt on pubertal male progeny of female mice treated with cobalt in late pregnancy and during suckling period. Macroscopic parameters as weight of male reproductive organs and organ/body weight ratio were established. Significant reduction in body weight and 20% decrease (non significant) of testicular and epididymal weight as well as in testis/body weight index was found. The impact of cobalt on male progeny could be explained with transplacental route of exposure and with possible transfer of cobalt into mothers' milk. The negative effect of cobalt was not seen in mid puberty (day 25) with the exception of epididymal weight which was not compensated suggesting that epididymis is more sensitive to cobalt treatment. In conclusion, our data indicate that exposure to cobalt during perinatal and postnatal period affected body weight during puberty but not significantly reduced reproductive organs growth. However, negative impact of cobalt on later life could not be rule out and cobalt might be considered as possible risk factor for male reproductive health.

# TOPICAL DISTRIBUTION OF THE SUBCUTANEOUS FAT TISSUE ON SOME PARTS AND REGIONS OF THE BODY IN CHILDREN AND ADOLESCENTS FROM SOUTH BULGARIA

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ABSTRACT. The purpose of this paper is to assess, in intersexual and interage aspects, the topical distribution of SFT on the various body parts and areas of children and adolescents from different territorial regions of southern Bulgaria. We transversally examined 1491 children and adolescents aged 10 to 17 years (753 girls and 738 boys) from schools in the municipalities of Plovdiv, Pazardjik, Haskovo, Svilengrad. The subjects were divided into 8 one-year age groups. We measured calliper-metrically the thicknesses of 9 SF on the body and limbs. The topical SFT distribution on different body parts and areas was estimated basing on the following ratios: Ratio of SFT - torso/limbs of 4 SF; Ratio of SFT - torso/limbs of 6 SF; Ratio of SFT - upper/lower torso - 4 SF; Two Ratio of SFT - upper /lower limbs: (1) and (2). The results show that in students from both genders, the processes of redistribution of SFT on the upper and lower parts of the body are most intensive in the period 15-16 years. In boys there is a tendency towards an increase of the SFT on the upper torso - chest, while in girls - on the lower part the abdomen. Throughout the period 10-17 years, in both genders, the thickness of SFT is higher on the lower limbs in comparison with the upper limbs.

### COMPARISON OF THE BODY MASS INDEX TO OTHER METHODS OF BODY FAT ASSESSMENT IN BULGARIAN CHILDREN AND ADOLESCENT

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**ABSTRACT.** The purpose of this study is to assess the body composition and body nutritional status of children and adolescents with the values of BMI and the percentage of body fat. We transversally examined 2269 children and adolescents from South Bulgaria /1114 boys and 1155 girls/, aged 7 to 17 during 2008-2009. We applied the anthropometric and bioimpedance-metric methods. The results show that throughout the observed age period, boys have a bigger amount of fat-free body mass per unit height, and girls accumulate greater amount of fat mass per unit height. According to the BMI data, the average frequency of occurrence of underweight is 5% higher in girls, while the frequency of occurrence of overweight is on average 9% higher in boys. The comparison of the results for body nutritional status from both methodical approaches shows that according to the percentage of body fat, the average frequency of underweight occurrence is higher than the average frequency of overweight and obesity occurrences, but according to the discriminatory BMI values, the frequency of overweight occurrence is much higher. This is probably due to the fact that the discriminatory values of BMI were developed for each age and gender groups, while those for the percentage of body fat, although developed for both sexes, were the same for the entire age period. The results obtained confirm the need the body nutritional status of children and adolescents to be assessed through at least the two components of the body weight.

#### BODY COMPOSITION OF CHILDREN SUFFERING FROM DIABETES TYPE 1

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ABSTRACT. The aim of this study is to determine the indicator of % body fat tissue of children suffering from Diabetes type 1, boys and girls aged 7 to 18 years, and to present its dynamics in the aspect of age and gender. We examined 37 boys and 36 girls suffer-ing from Diabetes type 1, they were divided into two age groups: 7 to 12 years and 12 to 18 years. The body fat % was deter-mined by bioelectrical impedance analysis with the apparatus "Tanita". The obtained quantitative data was processed through variation analysis. The results show that the indicator is higher in girls than in boys. There is a statistically significant difference of high extent (P<0.001) in the senior age (12-18) between boys and girls. A reliable difference of low extent (P<0.05) also exists in the interage comparison in boys. Conclusion: There is a characteristic interage and intersexual dynam-ics of the indicator of % body fat in children with Diabetes type 1. Bioelectrical impedance gives accurate data on the components of body composition.

# CORRELATION OF VITAL LUNG CAPACITY WITH BODY WEIGHT, LONGITUDINAL AND CIRCUMFERENCE DIMENSIONS

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ABSTRACT. Vital lung capacity is an indicator of body constitution type and functional ability of individuals. Since the vital capacity is affected by a number of factors, the limiting values of the volume and capacity are rather broad. The objective of the study was to determine the changes in vital capacity in relation to the age and its correlation with the educational status and anthropological traits. Material and method: A cross-section anthropological study of adult population of Backa and Banat region was carried out in the period of 2001-2006. In total, 4504 individuals took part in the study, including 1965 males and 2539 females. The mean age of male and female subjects was 40.10±11.84 and 41.12±10.75 years, respectively. The correlation of vital capacity with the age, educational level and morphological traits of the subjects was obtained by regression analysis and Pearson correlation coefficient (r) at the level of significance p<0.01 and p<0.05. Results: The average vital capacity in males is 3269±733.65ml and in females 2000±528.64ml. From the age of 20 to 39, the vital capacity of both sexes remains at the same level, while later it decreases considerably. The vital capacity correlates with longitudinal dimensions, particularly with the height, while the correlation with the weight is considerably lower. A negative correlation is obtained in relation to all circumference dimensions of females and the waist circumference of males. Conclusion: The vital capacity is affected by the sex, age, height and level of education. Education reflects the socioeconomic status, which in turn affects the life quality. Good living conditions can provide development of higher values of morphophysiological traits. These traits tend to change with aging.

#### **GROWTH DYNAMICS OF THE CHEST IN CHILDREN WITH TYPE 1 DIABETES**

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ABSTRACT. Diabetes depresses the growth process in diabetic children. The purpose of this study is to determine the pace of chest growth, in the age and gender aspects, in children suffering from type 1 diabetes. 71 boys and 69 girls, aged 7 to 18 years, suffering from type 1 diabetes were examined. They are divided into two age groups: junior - 7 to 12 and senior - 12 to 18 years. Healthy children were measured as a control, divided into the same age groups. All children investigated are of Bulgarian ethnicity. The sagittal and transverse chest diameters and the chest circumference at respiratory pause were measured. The data were processed through variation analysis and calculated with Student's t-test criterion. Data analysis indicates that diabetic patients' chest grows more in transversal direction than in sagittal. There were observed significant differences in growth rate in the senior age group in both sexes, which is probably due to the puberty occurrence. Growth rate of the chest in children with diabetes and controls did not differ significantly.

# CHANGES IN COMPONENTS OF BODY MASS AND THEIR RELATION DURING PERIOD OF GROWTH IN GIRLS FROM SMOLYAN REGION, BULGARIA (ANTHROPOMETRIC STUDY)

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**ABSTRACT.** The aim of this study was to investigate the development of body mass components and their relations in the period of growth of girls. In the present work we study the development and variability of body mass components and their relation during the growth period of the girls.

The sample included 768 girls from region of Smolyan, aged 7-17, and measured in period 1998-2010. By Martin-Saller's method of each person two total body measurements, eight skinfold, four circumferences and four epicondilar diameters of lower and upper limbs were measured. For characteristics of the fat development of each person by means of GPM caliper with constant pressure of 10g/mm2 eight skinfolds were measured. Using formula body mass index (BMI, kg/m2) was calculated. The all observed persons have normal physical development and body nutritional status. The body nutritional status of each persons were defined using of International cut -off points of BMI, created by Cole et al. The development of body mass components was based on anthropometric assessment of the fat, muscle, bone and residual mass. They were calculated using Matiegka method (by Veskler) and Mc Ardle, Katch and Katch formulas. The data were processed implementing descriptive and correlation analysis.

The results show that the total growth of body mass during childhood have been different importance, structure and mechanism. It is base are the qualitative changes in body composition, which occur at certain stages of girls development and characterized with different relation between the fractions, constituting body mass. The age interval of 9-10 is crucial in girls development. At this age the puberty growth jump occurs- the height increases by 10 cm on the average and weight- by 6 kg. Changes in the development of body mass components occur at this age determinate the future female architechtonics of the body. The fat component of girls is characterized by highest increase rate throughout the examined growth period, followed by the muscle, bone and residual component's increase rate. Body mass changes in girls of normal physical development and nutritional status are associated to a different higher degree with changes in muscle, bone and fat tissue mass. In this connection when diagnosing obesity it is necessary to use not only overweight signs as BMI, but also methods of fractioning body mass, which allow analyzing the development of its components, the fat component in particular.

### POLYMORPHISM OF MICROSATELLITE LOCI IN MHC COMPLEX FOR TWO POPULATIONS OF SHKODRANE SHEEP BREED IN ALBANIA

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ABSTRACT. Major Histocompatibility complex (MHC) plays a key role in immune response. We intend to study the polymorphism in two microsatellite loci of MHC complex, for two populations of Shkodrane sheep breed, whose location is North of Albania. The study was carried out in 113 unrelated individuals. Both markers were highly polymorphic. The analysis revealed a great number of alleles 14 and 40 per OMHC1 and OLADRB respectively. The level of heterozygosity was very high: 80% for OMHC1 and 60% OLADRB. This can be explained with the participation of the molecules encoded by MHC complex in the immune response, and with the balanced selection which act on antigen recognition site. PIC values were higher than 0.5. Therefore, both loci can be used as genetic markers.

### GENETIC DIVERSITY OF RECKA SHEEP BREED IN ALBANIA BASED ON 15 MICROSATELLITE MARKERS

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**ABSTRACT.** Recka is an autochtonous sheep breed in Albania. We intend to analyze the genetic diversity of Recka sheep breed, by the use of 15 microsatellite markers. Allelic and genotypic frequencies, heterozygosities and gene diversity were estimated. A total of 143 alleles were distinguished by the 15 microsatellite markers used. All the microsatellites were highly polymorphic, with mean allelic number of 9.53, ranging 5-15 per locus. The observed heterozygosity ranged between 0.625 to 0.968, with mean of 0.785, indicating high genetic variation in this breed. PIC values were higher than 0.5, indicating that the set of used markers was highly informative. It was noticed a low rate of inbreeding within breed (FIS = 0.013). The results suggest that this set of loci was very effective for testing genetic variation. This is the first report of microsatellite variation in Recka sheep breed in Albania.

### GENETIC VARIATION DEGREE FOR MEAT PRODUCTION TRAITS IN PURE-BRED PIGS

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ABSTRACT. The genetic variation degree for the meat production traits (traits of rearing, carcass and meat quality) were considered and evaluated in 4 pure-breeds (Durok, Hampshire, Yorkshire and Landras) totaling some 120 heads, coming from various farms which were bred in trial-out farms. The pigs slaughtered weighed some 105 kg each, which later were subject to further evaluation for various parts of the carcass. A mixed model was used for each farm the pigs of mixed breeds were coming from and the following factors were looked at and closely considered: herd origin, litter, error as random effects, breed, season and the fixed effects. For some of the afore-mentioned traits it proved that the variation among breeds of the same herd (pigs pertaining to a certain farm and of various breeds) were much bigger when compared with pigs of the same breed but which belonged to various herds. While with regard to meat quality traits (marbling appearance, color, structure) were relatively bigger among breeds of various herds. The differences for these traits when compared among herds are far smaller. For all of the traits, the variation concerning the genetic value of livestock within the herds and breeds of the same herd is much broader compared with that among various breeds and breeds belonging to the same herds. For most traits, it is more important to choose the best source of breeding stock than the best breed.

# ACID PHOSPHATASE AS A MARKER FOR DIFFERENTIATION OF SILKWORM (BOMBYX MORI L.) STRAINS

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**ABSTRACT.** This study was carried out on twelve silkworm strains maintained by the Sericulture and Agriculture Experiment Station germplasm bank, located in Vratza, Bulgaria. The polymorphism of acid phosphatase from larval haemolymph was investigated by method of electrophoresis in polyacrylamide gel. Five fundamental types of this enzyme were found in the studied strains of various origins. The acid phosphatase isozymes were considered to be controlled by five codominant alleles. It was found out that the acid phosphatase is very suitable marker for analyzing the inter- and intra-strain diversity and the strain differentiation.

# ORGANIZATION AND PRINCIPLES OF QUEEN SELECTION AND REARING IN BULGARIA

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ABSTRACT. In recent years, beekeeping in Bulgaria has expanded because of the available diversity of flora offering possibilities for production of different types of honey. The local Bulgarian honeybee, consideres as an own variety, is of scientific and beekeeping interest for selection. During the past years, this local type was endangered by many activities. A major threat is importation of foreign gueens. For more than three decades in the past Apis mellifera ligustica, A. m. carnica and A. m. caucasica have been reared in Bulgaria modifying local bees through hybridization. Further, queen rearing contributes to the reduction of effective population sizes. Since 1999 efforts have been made towards an implementation of a new national program for breeding and improving the work with Bees. Its purpose is the conservation of the gene pool of the local Bulgarian honey bee. In 2001 in Bulgaria the National Bee Breeding Association was registered. It performs the activities of honeybee breeding and reproduction and the realization of the approved selection program. There are about 750000 bee colonies in Bulgaria at the moment. The National Bee Breeding Association monitors the bee colonies from the National gene fund and of breeding bases with more than 14 000 bee colonies in total.

# ALLOZYME VARIABILITY IN POPULATIONS OF LOCAL BULGARIAN HONEY BEE

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ABSTRACT. Genetic variation of honey bee populations from six different locations corresponding to tree geographic regions: North-western, North-central and North-eastern of Bulgaria was studied on 6 enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 genetic loci. Allozyme analysis revealed that all loci studied were polymorphic in almost all populations studied. The mean number of alleles per locus varied from 1.8 to 2.5. The estimated percentage of polymorphic loci was between 50% and 100%. The observed and expected heterozygosities (Ho and He) ranged from 0.17 to 0.221 and 0.250 to 0.315, respectively. There are not significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations (0.99 > P > 0.1). The estimated mean FST value from allozyme data was 0.0443 which shows that 4.43% of the overall genetic diversity observed was among populations, as opposed to 95.57% within populations. The values of genetic distance range from 0.002 to 0.036. UPGMA dendrograms were constructed.

# INVESTIGATION ON GENETIC VARIABILITY IN HONEY BEE POPULATIONS FROM BULGARIA, GREECE AND SERBIA

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ABSTRACT. Genetic variation of honey bee populations from Bulgaria (local type A. m. rodopica), Greece (A. m. macedonica) and Serbia (A. m. carnica) was studied on 6 enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 genetic loci. Allozyme analysis revealed that all loci studied were polymorphic in most of the populations studied. Four alleles were detected at MDH-1 locus, three – at ME, five – at EST-3, three – at ALP, two – at PGM and three – at HK. The observed and expected heterozygosities ranged from 0.163 (Serbia) to 0.236 (Bulgaria) and from 0.248 (Greece) to 0.263 (Serbia), respectively. Nei's genetic distances range from 0.012 (between Greece and Bulgaria) to 0.157 (between Serbia and Bulgaria). In UPGMA dendrogram there are two clusters formed. Bulgarian and Greek populations are clustered together in first branch and Serbian population is grouped in the second cluster.

### GENOME RESPONSE OF MODEL INVERTEBRATES AND VERTEBRATES SPECIES TO STRESS AGENTS IN THE ENVIRONMENT

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**ABSTRACT.** The genome response of model invertebrate and vertebrate species to stress agent in the environment was studied. The both group of species were collected from contaminated water stations along Marisa (Kemera) and Chaya (Asenovgrad) Rivers and terrestrial areas near Asenovgrad. The structural and functional alterations of the salivary gland chromosomes of Chironomus riparius as well as the aberrations of mitotic chromosomes of Apodemus flavicollis and Microtus arvalis were analyzed. In the polytene chromosomes of C. riparius a high spectrum of somatic aberrations were detected, which appeared in significantly higher frequency in comparison of the control (Kemera: G=37.99, df =1, P<0.001; Asenovgrad, G= 42.82, df = 1, P < 0.001). Also, the key structure of the polytene chromosomes: Balbiani rings and Nucleolar Organizer decreased their normal function activity which indicates that they are direct target for the stress of contaminants in the environment. The frequency of aberrant cells of small rodents (Apodemus flavicollis and Microtus arvalis) was significantly higher than the control, well expressed in M. arvalis (G = 4.54, df =1, P < 0.05). In both groups of studied species genome instability were found, realized by many structure chromosome rearrangements which can be used as a cost-effective indicator of genotoxicity, and hence suitable markers of potential environmental stress. However, the studied species show differences in their response which might be depended on their biology.

# GENERAL TOXICITY AND GENOTOXICITY OF NODULARIA MORAVICA (CYANOPROKARYOTA, NOSTOCALES)

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ABSTRACT. General toxicity and genotoxicity of cyanoprokaryote Nodularia moravica was investigated on the base of Allium root meristem in vivo test system. Seeds sprouted in dechlorinated tap water were analyzed as a control sample. Seeds sprouted in the water-Dimethyl sulfoxide (9:1, v:v) extract, diluted 50 and 100 times (D1-797 and D2-797, respectively) were used as test samples. It was found that different concentrations of dilutions of the initial extract of cyanoprokaryote Nodularia moravica induced damages such as micronuclei (MNi), fragments, anaphasic and telophasic bridges and laggards with higher total frequency than in the control. Germination percentage and root length were found to be in positive correlation with percentage of chromosome aberrations and depend on the extract concentration. It was concluded that Nodularia moravica extract in water-DMSO solution induces general and genotoxycity in Allium cepa root meristem cells. There were not found data about cytotoxicity.

#### A NEW AND MORE PRODUCTIVE VARIETY OF PRILEP TOBACCO

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**ABSTRACT.** The need to create and introduce new and more productive oriental varieties of tobacco with better quality than the existing ones permanently increases. As a result of scientific and research work on this problem during the last two decades, the Department of genetics and breeding in Tobacco Institute-Prilep created a great number new lines of Prilep tobacco with significantly better quality compared to the standard. Some of them were recognized as varieties, among which Prilep 66-9/7 should be especially emphasized for its productivity.

### REGRESSION ANALYSIS OF THE INHERITANCE OF LEAF SIZE IN F1 AND F2 PROGENIES IN VARIOUS TOBACCO GENOTYPES

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**ABSTRACT.** Investigations were made on the inheritance of length, width and middle belt leaf area in progenies of six F1 and six F2 diallel crosses from four parental varieties (oriental Prilep P12-2/1, Pobeda P-2 and Yaka YV 125/3 and semi-oriental Forchheimer Ogrodowny - FO). Crossing was made in 2004 and 2005, and the trial with parents and hybrids was set up in 2006, in the field of Tobacco Institute-Prilep, at randomized block system with four replications.

The aim of the investigations was to present a comprehensive picture on the genetic system of leaf size, through regression analysis.

Values of the major genetic components required for graphic presentation were calculated from the average values of the investigated characters.

The regression analysis reveals partially dominant mode of inheritance of leaf size and lack of interallelic interaction. According to the position of points along the regression line, there is higher number of dominant genes in P 12-2/1 and of recessive genes in P-2 for inheritance of leaf size in both generations. In YV 125/3 higher frequency of dominant genes was observed in F1 and of recessive genes in F2 for inheritance of leaf length; of recessive genes in F1 and dominant genes in F2 for leaf width; of dominant genes in F1 and equal number of dominant and recessive genes in F2 for inheritance of middle belt leaf area. In FO, higher frequency of dominant genes for inheritance of leaf length was observed in both generations, of recessive genes in F1 and dominant genes in F2 for leaf width and equal number of dominant and recessive genes in F1 and of recessive in F2 for inheritance of middle belt leaf area.

## USE OF RAPD FINGERPRINTING FOR STUDY AND CONSERVATION OF FISH POPULATIONS

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ABSTRACT. This paper analysed a total of 76 fish individuals collected at four different localities in Republic of Macedonia. The following were encompassed: River Strumica, River Vardar and two natural lakes-Ohrid Lake and Prespa Lake. The samples affiliated with the four previously mentioned barbel species on the territory of Republic of Macedonia: Barbus cyclolepis Kar., Barbus prespensis Kar., Barbus macedonicus Kar. and Barbus peloponnesius Val. The molecular RAPD method was used for investigation of the population structure of the Barbus genus. A total of ten oligonucleotide primers was used to obtain various RAPD profiles in different species. The established genetic markers allow an opportunity for exact identification of the species and populations of barbel and for detection of interspecies hybrids.

### ESTIMATION OF THE HETEROTIC EFFECT IN F1 GENERATION OF VARIOUS TOBACCO GENOTYPES AND THEIR DIALLEL CROSSES

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**ABSTRACT.** Two-years trial (2007 - 2008) was set up in the field of Tobacco Institute-Prilep, to study the mode of inheritance and heterotic effect for the characters: height of the stalk with inflorescence, height of the stalk without inflorescence, leaf number, middle belt leaf area, green mass yield and dry mass yield per stalk. The investigations included four parental genotypes (the oriental pink flowered Suhum S1, white flowered Suhum S2 and red flowered Prilep-84, and the large-leaf variety Burley B-2/93 in CMS form) and their six diallel F1 hybrids. The trial was set up in randomized blocks with four replications and traditional cultural practices were applied in tobacco growing.

The analysis of variance revealed statistically justified differences between parents and their progenies for the characters investigated. Positive heterosis with poor heterotic effect was recorded in S1 x S2 hybrid for stalk height with and without inflorescence and for green/dry mass yields in S2 x P-84, while in S2 x P-84 only for height of the stalk without inflorescence. Negative heterosis with poor heterotic effect was recorded in hybrids S1 x P-84 for leaf number and for green/dry mass yields and S2 x P-84 for leaf number and green mass per stalk. The low heterotic effect indicates that application of heterosis is economically unjustified, but in the same time it points out to the eventual breeding activities for creation of new and more superior varieties.

### ABERRATION FREQUENCY DURING MITOSIS AND MEIOSIS IN *VICIA FABA L.*, AFTER THE TREATMENTS WITH 131 I

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**ABSTRACT.** The influence of  $\gamma$ - rays on genetic variability is in direct correlation with the condition of irradiated material. Here we tried to increase the variability of Vicia faba L. var. major karyotype, after contamination with 131I (a kind of radioactive rain) in elongation phase in ten plant groups. The effects of irradiation were observed not only by controlling mitotic activity and calculating mitotic index in control and treated groups, but also by examination of every phase of mitotic and meiotic division. The values for normal and defect dividing cells are given in percentages. The most common types of aberrations were recorded during M1 generation.

### ANALYSIS OF SPRING DEVELOPMENT OF SOME SELECTION LINES OF HONEYBEE IN EASTERN SERBIA

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**ABSTRACT.** The aim of this paper is to determine the cause of variation in quantity of bees and broods. This work covers quantitative characteristics of honeybees from 4 selection lines belonging to selection center "TIMOMED" from Knjaževac (eastern Serbia.)

The research includes the data collected in two years of selection study, which demonstrates the intensity of spring development of a colony before the main honeyflow in eastern Serbia. Using statistical methodology we showed the difference between the examined lines of honeybee.

The degree of successful wintering and the influence of cluster size to spring development were analyzed.

# CORRELATION BETWEEN THE STRENGTH OF COLONY, THE HONEY AREA AND POLLEN AREA OF THE OBSERVED LINES OF YELLOW HONEY BEE IN VOJVODINA

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**ABSTRACT.** The aim of this work is to determine correlation connection between the strength of colony, the honey area and pollen area in the four observed lines of yellow honey bee in Vojvodina. Technique is based on an assessment of the amount of bees, honey and pollen at 20 bee's collonies, three times per year, in the two years. Based on the results it can be concluded that the greatest degree of interaction is achieved between the quantity of bees and quantity of pollen (R  $^2$  = 0,48); while the lowest interaction was recorded between the quantity of pollen and the amount of honey (R  $^2$  = 0,13).

### THE VARIABILITY OF WING NERVATURE ANGLES OF HONEY BEE FROM THE NORTH KOSOVO AREA

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ABSTRACT. Honey bee's wings are on dorsal side and are attached to the second segment of the thorax. The system of chitin nerves runs trough them. The wing nervature is variable, but the layout of cells on the wings is not random, however, and is used for the determination of bees.

Honey bee is one of the most studied insects with a total of 42 morphometric parameters used for the purpose of racial determination and taxonomy.

The aim of this paper is to determine the morphometric parameters of the angles between the nerves of the front wing, which are: A4, B4, D7, E9, G18, J10, J16, K19, L13, N23 and O26, as well as to analyze the morphometric parameters on the rear wing of the honey bee - angles W1, W2 and W3).

The bee samples were taken from the area of north part of Kosovo and Metohija on the sites Lešak, Sočanica and Kosovska Mitrovica. The samples were comprised of 10 bees each, and are indigenous material.

Also, the variation coefficient and maximum and minimum value of the researched parameters were determined in this study.

### SEASONAL APLICATION OF JENTER'S METHOD FOR A SUCCESSFUL QUEEN **BEES' REARING IN ALBANIA**

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ABSTRACT. This research tested the rearing method of Queen Bees according to Jenter's. Two consecutive planting were realized, respectively on June 24 and July 12, each of these time-periods representing one stage of the experiment.

To implement this research, the strongest bee-colonies of the apiary were used. During each stage of experiment 10 Queen Bees were produced. Their quality was judged and decided based on their growth parameters and performance.

During each stage, the percentage of larvae's sealing, young Queen-Bees emergence, their time of copulation, their body size, the number of laid eggs were surveyed and measured. At the end of the research, it was concluded that Queen-Bees rearing parameters were better on the first stage of the study, all this due to the sufficient food availability and favourable climate conditions during this period of time. No significant differences were observed for the copulation time between two stages. During the second stage, the egg production is 27.7% lower than the first stage. The differences in value for the number of laid eggs in each stage are significant. The biggest size of the body-length and the maximum body weight of the Queen - Bees were reached during the June-July period (respectively 271.5 mg and 2.01mm). However, these parameters have been within the limits during the period of study.

### SECTION "MICROBIOLOGY & BIOTECHNOLOGY"

### ANTIOXIDANT ACTIVITY AND BIOACTIVE CONSTITUENTS OF THE AERIAL PARTS OF HARPAGOPHYTUM PROCUMBENS PLANTS

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**ABSTRACT.** Harpagophytum procumbens is an herbaceous plant with high medicinal value that grows in the Kalahari Desert region of Africa. Devil's claw plant tubers have been used since time immemorial by the native population of Southern Africa for treating a huge number of human ailments, including fever, diabetes, diarrhea and blood diseases. More recently, extracts of the secondary roots of the species have been found to be effective in the treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation and heart disease. Therefore, Harpagophytum procumbens has been increasingly considered an alternative to non-steroidal anti-inflammatory drugs. The antioxidant activities of pure metabolites, as well as of total methanol extracts of the Devil's claw plants were evaluated in 2,2′-diphenyl-1-picrylhydrazyl (DPPH•), oxygen radical absorbance capacity (ORACFL) and hydroxyl radical averting capacity (HORACFL) assays. The crude methanolic extract may be attractive for various commercial purposes since it displayed antioxidant activity and it can be conveniently and economically prepared.

# PROPERTIES AND SURVIVAL UNDER SIMULATED GASTROINTESTINAL CONDITIONS OF LACTIC ACID BACTERIA ISOLATED FROM ARMENIAN CHEESES AND MATSUNS

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**ABSTRACT.** More than 30 lactic acid bacteria strains were isolated from different sources (matsuns, cheeses) collected in various regions of Armenia. Four of them inhibit the growth of different test-organisms. Treatment of antibacterial agent with various enzymes established their nature of inhibition by protein bacteriocin or by hydrogen peroxide production. The isolated strains were evaluated according to their growth, acidity and antibacterial activities as well as according to their survival under simulated gastrointestinal conditions. All tested strains during both "stomach" phase and in gastro-intestinal tract phase kept large number of viable cells.

### ISOLATION AND CHARACTERIZATION OF NEW METALLOTOLERNAT BACILLI STRAINS

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**ABSTRACT.** A total of ten mesophilic and thermotolerant metallotolerant bacilli were isolated from water and soil samples and identified up to species. The morphological, physiological and biochemical properties of metallotolerant strains were described, as well as 16S rDNA sequence analyses were carried out. The stability of isolates was demonstrated in the presence of different concentrations of Cd2+, Cu2+, Zn2+ and Ni2+ in growth media. The ability of isolates to accumulate mentioned metals was studied.

### SATUREJA L. ESSENTIAL OILS IN PREVENTION AND PHYTOTHERAPY OF SALMONELLA INFECTION

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ABSTRACT. In wide regions of the Balkan Peninsula, plant species of the genus Satureja L. (Lamiaceae) are traditionally used as natural preservatives for meat products, and as antimicrobial agents in phytotherapy of food borne diseases. The present study describes the antimicrobial activity of eight Satureja species essential oils against Salmonella enteritidis. The aerial parts of wild growing plant material of eight Satureia species were collected from the central part of Balkan Peninsula. After drying, essential oils were produced by hydrodistillation in a Clavenger-type apparatus. The antimicrobial activities of the tested essential oils were evaluated using the following two methods: agar disc diffusion method and broth micro-well dilution method. The results of disc diffusion method showed especially high activity of S. subspicata ssp. subspicata, S. montana ssp. montana and S. hortensis essential oils. The minimum inhibitory/bactericidal concentration (MIC/MBC) of the essential oils was in the range from 0.20 – 6.25 µl/ml. S. horvatii, S. hortensis and S. montana ssp. montana exibited the highest antimicrobial activity. Also, it has been recorded that each essential oil, except S. subspicata, in the same concentration had both inhibitory and bactericidal effect (MIC=MBC).

### PERSPECTIVE OF USING NEW STRAINS OF LACTIC ACID BACTERIA FOR BIOPRESERVATION

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**ABSTRACT.** The strains of lactic acid bacteria (LAB) exhibiting antimicrobial activity against the spore-forming microflora of dairy and meat products have been isolated from salted cheese and researched. Decrease of general contamination in the technology of processed cheese production has been observed when 0.5-1% solutions of purified supernatants of L. acidophilus 1991 and P. pentosus 28 were used. Employment of purified supernatants of L. acidophilus 1991, L. plantarum 109 in meat stuff also caused decrease in the level of common contamination as compared with control samples. It can be concluded that the isolated strains can be properly used in the technologies of processed cheese and meat stuff production.

# ANTIMICROBIAL ACTIVITY, TOTAL PHENOL AND FLAVONOID CONTENTS OF JOVIBARBA HEUFFELII (SCHOTT.) A LÖVE & D. LÖVE EXTRACTS

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ABSTRACT. The extract was prepared from fresh leaves of *Jovibarba heuffelii* (Schott.) A Löve & D. Löve and used to evaluate the antimicrobial activity against a panel of Gram-positive and Gram- negative bacteria as well as against fungi. Antimicrobial activity was evaluated using broth microdilution method. For determination of the total phenolic and flavonoid contents methanolic, ethyl acetate, acetone and diethyl ether extracts were prepared. The phenolic content of the extracts was determined spectrophotometrically using Folin–Ciocalteu reagent and the results are expressed as gallic acid equivalents (GAE). *Jovibarba heuffelii* extract showed moderate antibacterial activity, while its antifungal activity was significantly higher. The high contents of total phenolic compounds and total flavonoids indicated that these compounds contribute to the antimicrobial activity.

### SOIL BACTERIAL ABUNDANCE AND DIVERSITY OF URANIUM IMPACTED AREA IN NORTH WESTERN PIRIN MOUNTAIN

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ABSTRACT. The former uranium mine Senocos (Blagoevgrad district, Bulgaria) has been exploited until 1991 when it was closed and later (1994-1997) reclaimed. Recently, the reclamation is compromised due to the erosion of protective layers and the mine wastes continue to affect the area resulting in increased radiation and uranium concentration in mine area. Soil bacterial abundance in more radioactive environment of mine remains unchanged (on average ((8.52±5.1) x 108 cell g-1) compared to the control ((8.76±3.5) x 108 cell g-1) in contrast to their dehydrogenase activity which decreases more than two times. Physiological and species diversity of bacterial mine communities are also affected by the pollution as it is expressed by lower values of Shannon indices compared to the control communities. All carbon sources in BIOLOG assay except 2-hydroxy benzoic acid are available for control bacterial communities and the number of unavailable carbon sources increases linearly with increasing the concentration of uranium in soil. The high levels of radionuclide pollution decrease the biodiversity to 3-4 species (66% of total) in the most polluted point of the mine territory (SPS(5)).

## ROBUSTNESS OF SACCHAROMYCES CEREVISIAE GENOME TO ANTIOXIDATIVE STRESS

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ABSTRACT. Recent advances in experimental and computational biology allow addressing of complex questions regarding the evolution of biological adaptation and stress tolerance in yeast cell. Since enzymatic antioxidant defense metabolism in yeast Saccharomyces cerevisiae has been well studied at biochemical and genetic levels, it represents an excellent system for evaluating the relative roles of duplicate genes and alternative metabolic pathways as possible mechanisms for the stability of antioxidant metabolism against null mutations. In this work, the specific role of key antioxidant enzymes for adaptation to oxidative stress in S. cerevisiae was evaluated by screening of a wide selection of gene specific disruption mutants ( $\square$ sod 1-2,  $\square$ ctt 1, cta1,  $\square$ gpx1-3,  $\square$ gtt1-2,  $\square$ ure2,  $\square$ cp1,  $\square$ sa1-2,  $\square$ prx1,  $\square$ rr1-2,  $\square$ glr1). It was shown that yeast cells employ a variety of mechanisms to ensure functional robustness against stress conditions. One of the strategies appeared to be gene duplication events that have produced a number of isoenzymes functioning under variable environmental and physiological conditions. However, emergency of alternative pathways represents the most significant mechanism for increasing the robustness of this system.

### INFLUENCE OF GALACTOOLIGOSACCHARIDES ON THE GROWTH AND ANTIMICROBIAL ACTIVITY OF LACTOBACILLUS FERMENTUM I-5

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**ABSTRACT.** Oligosaccharides may alter the antimicrobial activity of lactobacilli strains cultivated in whey media. The utilization of galactooligosaccharides in whey media by Lactobacillus fermentum I-5 was performed. The quantity of the used galactooligosaccharides had a pronounced effect on the growth rate. It was found that concentrations of 15% galactooligosaccharides and 2% of whey proteins contribute to higher growth rate. The obtained results revealed again that the higher percent of oligosaccharides stimulate higher antimicrobial activity.

### SUPEROXIDE DISMUTASE ENZYMES IN OXIDATIVE TYPE YEAST H. POLYMORPHA

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ABSTRACT. Hansenula polymorpha CBS 4732, cultivated on media containing glucose, glycerol or methanol as carbon source has been investigated for specific activity of Superoxide dismutase (SOD) enzymes during batch – wise cultivation. Furthermore cell free extract from the analyzed strain cultivated on different carbon sources were subjected to 8% native PAGE. One band corresponding to sensitive of KCN Cu/Zn SOD with Rm 0.29 is inhibiting in all samples. After inhibition of cyanide sensitive Cu/Zn SOD, three bands specifically stained for SOD activity, corresponding to Mn SOD are observed in samples obtained by H. polymorpha cultivated on medium containing methanol. Two bans are retaining in glucose and glycerol grown cells. This data suggest that probably in H. polymorpha yeasts appears multiple enzyme forms of Mn SOD enzyme. Multiple enzyme forms become more stable and do not disappear after 1 min at 75°C. One band with Rm 0.34 in all yeast samples cultivated on methanol, glucose and glycerol, remains till heating at 75°C for 10 minutes.

### EFFECT OF CD2+ ON THE ANTIOXIDANT STATUS OF SHIZOSACCHAROMYCES POMBE

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Cd<sup>2+</sup> ABSTRACT. The of on influence the antioxidant svstem Shizosaccharomyces pombe DSMZ 70576 was studied during batch cultivation on YPD medium containing 1.0 mM Cd(NO<sub>3</sub>)<sub>2</sub> versus a control variant without Cd(NO<sub>3</sub>)<sub>2</sub>. The Cd<sup>2+</sup> was added to the medium on the 12th h of the process. Samples for determination of the cells' growth, carbon source consumption, ethanol production and antioxidant system's status, represented by the enzymes superoxide dismutase (SOD) and catalase (CAT), and the intracellular principal antioxidant glutathione, were taken along the 48 h cultivation. The presence of 1.0 mM Cd<sup>2+</sup> under the test conditions repressed the cell growth and resulted in less production of ethanol. The SOD and CAT specific enzyme activities and glutathione concentration were estimated as 11.8 U mg-1, 1.7 U mg-1 and 0.57 mM mg-1 respectively. The SOD and glutathione values were 1,6 and 1.7 fold higher than the corresponding ones measured during growth without Cd2+, while the CAT activity decreased by 40%. These results indicate that glutathione obviously had scavenged efficiently the H2O2 produced, thus compensated the diminished CAT activity and probably maintained the H<sub>2</sub>O<sub>2</sub> homeostasis of the culture under the stress conditions. Electrophoretical analysis of SOD and CAT in cell free extracts obtained from the strain cultivated in the presence of Cd<sup>2+</sup> and in norma indicated the absence of Mn SOD enzyme under the stress conditions. Apparently, the presence of 1.0 mM Cd(NO<sub>3</sub>)<sub>2</sub> leaded to inhibition of mitochondrial functions and Mn SOD operation in the cells of Shizosaccharomyces pombe.

#### ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM ROSA ALBA

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**ABSTRACT.** Antimicrobial activity of two trade lots of essential oils from Rosa alba L. against Gram-positive bacteria, belonging to genera Staphylococcus and Bacillus, Gram-negative bacteria, belonging to genera Escherichia, Pseudomonas, Salmonella and Citrobacter and yeasts, belonging to genera Candida was investigated. It was determined that Gram-positive bacteria were more sensitive and Gram-negative bacteria, especially Pseudomonas spp., were more resistible to essential oils and pure components. The major pure constituents citronellol, geraniol and nerol demonstrated higher antimicrobial activity in comparison with essential oil samples.

## INFLUENCE OF CARBON AND NITROGEN SOURCES ON GROWTH AND PIGMENT PRODUCTION BY MONASCUS PILOSUS C1 STRAIN

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ABSTRACT. Pigments produced by Monascus fungi are widely used in food and biotechnology as natural colorants, flavors and preservatives. There are various factors influencing the pigments productions, among which are the nutrient media composition, pH, ambient temperature, mode of cultivation. In the present study the influence of different carbon and nitrogen sources on the pigments biosynthesis by Monascus pilosus C1 strain was investigated during batch cultivation at the following conditions: T = 30°C, pH 6.0, agitation 300 rpm and modified Chapec - Dox medium with different carbon (a range of sugars and alcohols) or nitrogen sources for 7 days. Some process kinetic parameters - YX/C, YP/C, Vav, were calculated and analized. It was found that the micelial growth, expressed as dry weight (DW) and the specific pigment production (SPP) were strongly stimulated by glucose as a carbon source. The DW and SPP reached values of 10.89 g DW I-1, and 1.97 OU mg DW-1 and 1.01 OU mg DW-1 for the total red and yellow pigments, respectively. Sodium glutamate combined with glucose increased effectively the pigments production up to 2.29 OU mg DW-1 and 1.67 OU mg DW-1 respectively for the total red and yellow pigments. The obtained yield coefficient of the studied processes indicated the feasibility of Monascus pilosus C1 strain for pigments production. None of the investigated carbon or nitrogen sources provoked citrinin biosynthesis.

## EFFECT OF DIFFERENT FACTORS ON BIOSYNTHESIS OF EXOPOLYSACCHARIDE FROM ANTARCTIC YEAST

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ABSTRACT. Psychrophilic yeast Isolate 100 was selected as a producer of exopolysaccharide. A laboratory scheme was created for obtaining of biopolymer. Biotechnological and physicochemical factors influencing its biosynthesis were investigated. It was determined that the inocolum quantity from 6-10%, cultivated for 48 hours at 22°C, is suitable for maximum synthesis. The exopolysaccharide that was synthessed in the culture liquid was thermostable at 50° C to 70°C and it degrades at 80°C. The quantity of the sedimentary polymer, that was synthesis on environment with arabinose and mannose from Isolate 100, after staying at the time of 120 hours in ethanol at 4°C, was increased with 40%. The influence of the temperature over drying of the exopolysaccharide at 65°C and 105°C was studied. At the low temperature of drying the polymer contained near 20% humidity. The effect of the experimental conditions (temperature, time, concentration of 2%, 3%, 4%, 5%) on the apparent viscosity values of the cultural liquid was studied. It was found to reveal behaviour of non-Newtonian liquid. The power law equation of Oswald- de Waale describing the change of the apparent viscosity was derived.

# IMMOBILIZATION OF CYCLODEXTRIN GLUCANOTRANSFERASE FROM PAENIBACILLUS MACERANS ATCC 8244 ON MAGNETIC CARRIERS AND PRODUCTION OF CYCLODEXTRINS

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ABSTRACT. The extracellular enzyme cyclodextrin glucanotransferase produced by Paenibacillus macerans ATCC 8244 cells was immobilized by covalent linking via glutaraldehyde to silanized magnetic nanoparticles treated with PEI and on cellulose-coated magnetite microparticles in order to study the immobilization capacities of the carriers, the activity recovery and some biochemical characteristics of the enzyme after immobilization. These biocatalysts were used for an efficient production of cyclodextrins from starch in repeated-batch runs, retained more than 52-83% of their initial activity and were stable after 60 day storage at 4oC. No significant increase of pH stability profile was observed, whereas the thermal stability of immobilized GTase was superior to that of free CGTase.

### PERSPECTIVES FOR THE PRODUCTION OF BIOETHANOL FROM LIGNOCELLULOSIC MATERIALS

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ABSTRACT. The most common renewable fuel today and suitable alternative to replace fossil fuels is ethanol that can be blended with petrol or used as neat alcohol in engines. Ethanol is currently produced from sugar (Brazil) or grain (starch, USA). However, this raw material base will not be sufficient because the increasing demand for fuel ethanol and the lower than expected reduction of greenhouse gases. An alternative is the production of bioethanol from agroindustrial wastes containing abundant cellulosic fibers and carbohydrates such as grape pomace, sugar beet pomace, barley and rice straw, corncobs, sunflower stalks and heads, cotton waste, brewer's spent grain, forest residues etc. Lignocellulosic raw materials and agroindustrial wastes minimize the potential conflict between land use for food (and feed) production and energy feedstock production. This review summarizes recent developments in the bioconversion processes, the new technologies required and the advances achieved in recent years to bring agricultural feedstock and lignocellulosic ethanol towards industrial production.

## ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM SPICES AGAINST PSYCHROTROPHIC FOOD SPOILAGE MICROORGANISMS

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**ABSTRACT.** At current study the antimicrobial activity of *Origanum vulgare, Satureja montana, Thymus vulgaris, Pimenta dioica* and *Syzygium aromaticum* against psychrotrophic microorganisms, isolated from spoiled chilled meat products was investigated. MIC, MBC and MFC of the essential oils were determined both at 37° and 4°C. Antimicrobial activity of the essential oils retained unchanged at both temperatures. Among the tested psychrotrophic microorganism Gram-positive bacteria *Brochothrix thermosphacta* was the most sensitive strain and Gram-negative bacteria *Pseudomonas aeruginosa* was the most resistible. The results obtained expanded the possibilities for application of studied oils not only as flavour enhancers, but even as natural antimicrobials in chilled meat products.

#### GLUCOOLIGOSACCHARIDES SYNTHESIZED BY GLYCOSYLTRANSFERASES FROM MUTANT STRAIN LEUCONOSTOC MESENTEROIDES M2860 AND THEIR PREBIOTIC POTENTIAL

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**ABSTRACT.** Functional foods, of which probiotic- and prebiotic-containing foods are a subset, have recently justified the efforts of health authorities in many countries. strains of Leuconostoc mesenteroides synthesize transglucosydases (GTFs) capable of producing dextrans and related glucans from sucrose. In the presence of sucrose and an acceptor like maltose, they synthesize glucooligosaccharides. It was shown that the extracellular GTFs from the mutant strain Leuc. mesenteroides M2860 catalyze acceptor reactions and transfer the alucose unit from sucrose onto maltose to produce glucooligosaccharides. By increasing the sucrose/maltose ratio (S/M), it was possible to catalyze the synthesis of oligosaccharides of increasing degree of polymerisation. For an S/M ratio of 7, both linear oligosaccharides (only composed of -(1-6) linkages and a maltose residue at the reducing end) and branched oligosaccharides were produced. Some of the glucooligosaccharides synthesized contain  $\alpha$ -(1-6)osidic linkages and were completely hydrolyzed by dextranase. The other glucooligosaccharides synthesized, resisted the action of this enzyme. It could be concluded that the catalytic properties of the extracellular GTFs, as well as their pH optima and products formed are of great importance for the synthesis of different types of oligosaccharides. Branched oligosaccharides produced by GTFs coming from Leuc. mesenteroides M2860 were readily catabolized by lactobacilli but not by Escherichia coli and Listeria innocua strains, pointing toward their application in intestinal microflora modification.

### BIOGAS PRODUCTION FROM ORGANIC WASTES IN SUSPEDED CELL CULTURES AND IN BIOFILMS

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ABSTRACT. The results of a comparative study of two biogas production bioprocess systems are presented. The systems submitted to comparison are based on the suspended cells cultures and the biofilm formed on solid inert support. A comprehensive research concept is formulated and discussed. It includes the main considerations regarding the choice of substrate, bioagent as mixed microbial society, type of bioreactors, regimes of functioning, analytical determinations and method of comparison. The main requirements for efficient experimental activity in comparative investigations are formulated. Their satisfaction can grant correctness of the experimental design and data acquisition. On this basis the key parameter of comparison of the two systems is defined as the specific productivity of the bioprocess systems. Under these conditions series of preliminary experiments are carried out for testing the readiness of experimental set ups for long time stable functioning and monitoring devices capabilities to maintain the bioprocess parameters at the determined intervals. These tests grant continuous incessant experimentation with the investigated bioprocess systems.

The results obtained show that biofilm bioprocess systems possess up to two and half time higher specific productivity in comparison with the bioprocess systems with the suspended cells. Some visions about the future developments of comparative research on the influence of additional parameters like the mixer rotation steed, organic loads, and higher values of dilution rates are outlined.

# DETERMINATION OF PSEUDOMONAS PUTIDA LIVE CELLS WITH CLASSIC CULTIVATION AND STAINING WITH "LIVE/DEAD BACLIGHT BACTERIAL VIABILITY KIT"

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ABSTRACT. **ATCC** Periodic culture of Pseudomonas putida strain 12633=NBPMCC1090 was investigated in a 24 h experiment for sensitivity to zinc oxide thin films constructed under different conditions. The growth of bacteria was followed at every three hours and three methods were used to determine live cells. Optical density, cultivation and microscopic methods were applied and compared. To distinguish active and inactive cells LIVE/DEAD BacLight Bacterial Viability Kit staining was compared with classical cultivation methods. Bacterial quantity determinated on nutrient media, appear higher than those of live cells counted on epifluorescent microscope.

# SOME ASPECTS OF CARBOHYDRATE METABOLISM AND PRODUCTION OF GLYCOSYLTRANSFERASES FROM MUTANT STRAIN LEUCONOSTOC MESENTEROIDES M2860

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ABSTRACT. The production of glycosyltransferases (GTFs) from constitutive mutant strain Leuconostoc mesenteroides M2860 was studied. When grown in glucose medium in the absence of sucrose Leuc. mesenteroides M2860 produced low, but detectable GTF activity. Much of the GTF activity (81%) in sucrose grown cultures was located in the cell pellets. Extracellular and cell associated activities were determined when run on SDS-PAGE for in situ activity detection by periodic acid-Schiff 's staining. In situ analysis showed single band corresponding to 180 kDa molecular size in supernatant and enzyme concentrate, received by cultivation on glucose media. The enzyme concentrate and supernatant fraction obtained by fermentation on sucrose media showed three bands corresponding to 180 kDa, 120 kDa and 86 kDa.

### PURIFICATION AND CHARACTERIZATION OF DEXTRANSUCRASE FROM LEUCONOSTOC MESENTEROIDES NRRL B-1149

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ABSTRACT. Leuconostoc mesenteroides NRRL B-1149 produces extracellular dextransucrase which in this study was purified using different concentrations of polyethylene glycol (PEG). The dextran produced by this enzyme is unique in that it contains α-(1□6) and α(1□3) linkages which have clinical applications. The cell free supernatant with 0.9 U/mg enzyme specific activity was subjected to fractionation by PEG-400 and PEG-1500. The 33% PEG-400 gave dextransucrase with specific activity of 9.2 U/mg and 10 fold purification and the 15% PEG-1500 gave dextransucrase with maximum specific activity of 15 U/mg and 17 fold purification in a single step. The purified enzyme showed multiple molecular forms on denaturing SDS-PAGE with three prominent bands. The purified dextransucrase confirmed the presence of glucan, after in-situ activity detection by Periodic acid Schiff's staining after running under denaturing SDS-PAGE. The three bands that appeared on denaturing SDS-PAGE stained with silver nitrate solution, corresponded to the three activity bands.

# CHECK ON THE PRESENCE OF ENTEROBACTERIA AND SALMONELLA SPP. IN SAUSAGE

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ABSTRACT. Sausage production in Kosovo has started years ago, a product which has a very high use, as in family kitchens as well as in restaurants. This product is very liked and known by local consumers, but is also known in the Balkan countries. Sausage is prepared by different types of meat but the most favorite remains by the bovine meat, from the bovine minced meat sausage produced under receptures of producers, where they add water, nitrites, nitrates, spices and salt. During processing the temperature used is 65 - 72°C for 60 minutes, which after processing is considered usable for consumption within 60 days. During the production process and post production process analytical inspections and controls of meat processing industry were attentive and rigorous in order of misusing this product, which is very favorite of customers. But despite the high care, occasionally the cases of toxyalimentary infections were recorded by sausage consumption. This fact served as a reason to allow a more detailed study in relation to microbiological control and analytical indicators; Enterobacteria and pathogen Salmonella spp. This study was undertaken in a meat processing factory in Kosovo. Samples of the final product were collected for microbiological analysis during the period January - September 2009 and their total reached 60 samples. Their analysis was conducted by Institute of Food Safety and Veterinary in Tirana, Albania. From the results of laboratory analysis were confirmed 60 samples of sausages or 15 % of them have over-presence of enterobacteria, above the limit (100 – 500 cfu/g). By the microbiological spectrum in the same samples wasn't identified the presence of Pathogen Salmonella spp.

#### BIOFILM-FORMING CAPABILITIES OF URINARY ESCHERICHIA COLI ISOLATES

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**ABSTRACT.** The aim of the present study was to investigate biofilm-forming capabilities of clinically isolated strains Escherichia coli, associated with different urinary tract infections. Biofilm production was detected in 36% of the isolates from UTI. Additionally some of virulence factors are estimated to find correlation between antibiotic resistance, hemolysins, morfotypes and biofilm production. Our data indicate that no combination of VFs was highly associated with biofilm production.

## GROWTH TEMPERATURE-RELATED CELL SURFACE CHANGES OF ESCHERICHIA COLI 0157:H-

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ABSTRACT. Escherichia coli O157 is a foodborne pathogen. We have previously shown that when grown on agar at 37°C but not 20°C it is capable to attach to its surface a glycoconjugate that is reactive with the lectin concanavalin A (ConA) but is poorly recognized by specific antiserum. The aim of the present study is to check whether, and to what extent may such growth-temperature related ConA reactivity interfere with immunoreactivities of the bacterial population and of individual bacterial cells. The results show that there is no strict correlation between ConA reactivity and immunoreactivities of selected single colonies as revealed by immunofluorescence and immunogold for electron microscopy. One important observation was the pronounced cell-to-cell variability regarding access at the surface of immunoreactive sites. This may be due to several factors among which cell-to-cell differences in O157 lipopolysaccharide production dependent on individual cell growth, or differences in amounts of other surface components like the enterobacterial common antigen.

#### CHARACTERISTIC OF TWO BACTERIOCIN-PRODUCING ENTEROCOCCUS STRAINS

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**ABSTRACT.** Two newly isolated strains from yellow cheese identified as *Enterococcus* was screened for bacteriocin activity. It was found that the tested strains displayed a broad spectrum activity, even against Gram-negative bacterial species. The agents were proven to be of protein nature by treatment with proteinase K. High temperature treatment revealed strong thermostable properties of the molecule.

# MOLECULAR TYPING OF LACTOBACILLI ISOLATED FROM DRY SAUSAGE "LUKANKA": COMPARISON OF WHOLE CELL PROTEIN (WCP) VERSUS DNA-BASED METHODS

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ABSTRACT. A collection of forty-two Lactobacillus isolates from "Lukanka" sausage, previously determined as belonging to the species L. plantarum, L. pentosus and L. paraplantarum, was subjected to phylogenetic analyses by protein- and DNA- based methods in order to investigate the diversity within the Lactobacillus microflora participating in the ripening of the final product, as well to compare the discriminatory powers of the methods used. Similar clusterings were obtained by all the four methods (whole cell protein electrophoresis, RAPD-PCR, Rep-PCR and ERIC-PCR), and consequent UPGMA analysis of the results showed that some of the methods are more applicable for differentiating closely related strains, while others are for species differentiation.

### PURIFICATION AND PROPERTIES OF ALKALINE PHOSPHATASE FROM BACILLUS CEREUS

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**ABSTRACT.** Extracellular and membrane-bound alkaline phosphatases were produced at the middle stationary phase of growth by a strain Bacillus cereus. Twenty two percent of the enzyme activity was secreted into the culture media. An extracellular alkaline phosphatase (AP I) and a membrane-bound alkaline phosphatase (AP II) were purified 282-fold and 70-fold, respectively by a combination of chromatographic methods. Enzyme activity of alkaline phosphatase preparations was maximal at pH 9.5. Both enzymes were inhibited by EDTA and were reactivated by addition of Ca2+. The molecular weight of AP I was estimated to be 43 ± 1 kDa, and that of AP II was estimated to be 44 ± 1 kDa. Alkaline phosphatase activity of both enzyme preparations was completely lost by heating at 80°C.

### BIOSORPTION OF COOPER (II) BY IMMOBILIZED DEAD BIOMASS OF SACCHAROMYCES CEREVISIAE

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ABSTRACT. At present study the biosorption potential of dead biomass of Saccharomyces cerevisiae immobilized in Ca-alginate and co-immobilized in Ca-alginate and bentonite and Ca-alginate and activated carbon for removal of Cu (II) from model solutions was investigated. The highest biosorption potential demonstrated the biosorbent of S. cerevisiae co-immobilized in Ca-alginate and activated carbon. The effect of pH, biosorbent concentration, contact time and initial metal concentration on the Cu (II) removal was studied. The optimal pH value for metal removal was 4,0 and biosorption equilibrium was reached for about 30 min. The increasing of biosorbent concentration increased metal removal by the selected biosorbent. At the equilibrium maximum metal uptake qmax 47,04 mg/g was reached. The biosoption data fitted better to the Langmuir adsorption model.

# IDENTIFICATION AND CHARACTERIZATION OF $\alpha$ -AMYLASE AND ENDOXYLANASE, PRODUCED BY ASPERGILLUS MUTANT STRAINS

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**ABSTRACT.** Filamentous fungi are widely used for the production of homologous and heterologous proteins. Recently, there has been increasing interest in Aspergillus oryzae PP and Aspergillus awamori K-1 because of its ability to produce heterologous proteins in submerged (liquid) cultures. The goal of this investigation was to determine the  $\alpha$ -amylase and endoxylanase enzyme production ability and molecular characteristics of fungal strains Aspergillus oryzae PP and Aspergillus awamori K-1 and its mutant strains R5 and A45. The strains were cultivated in liquid culture and maximum enzyme production of parent and mutant strains was determined after 72-96 h of cultivation. Extracellulars  $\alpha$ -amylase and endoxylanase were partially purified from the culture filtrates, using molecular sieve chromatography with Gel permeation chromatography. The molecular weight of the partial purified enzymes has been estimated to be 57 kDa for  $\alpha$ -amylase and 31 kDa for endoxylanase on SDS-polyacrylamide gel electrophoresis. The temperature optimum of the enzyme  $\alpha$ -amylase was 30°C, respectively for endoxylanase was 40°C and the pH optimum was 4.7 and 4.0.

### RESEARCHES ON THE POSSIBILITIES FOR SELECTION OF STREPTOMYCES AMBOFACIENS HIGH PRODUCING STRAIN

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ABSTRACT. After a preliminary genotype optimizing of Streptomyces ambofaciens ATCC 15154, the most active variant has been isolated, which has been further subjected to a multiple mutagenic treatment with determined optimal doses of N-methyl - N'-nitro - N-nitrosoguanidine and gamma radiation (separately and in a combined variant). A stable, multifold more active mutant has been obtained, in which population in comparison with the original strain, less number morphological types have been found, differing not only in quantitative presence, but also in stability and biosynthetic activity. From the composition of the most stable and most active morphological type a variant has been isolated, which is an object of our present studies, with the aim of obtaining high producing and morphologically homogeneous strain of S. ambofaciens.

## GUANDUELLA PODENSIS N. SP. AND PSAMMOSPHAERA SP. - FORAMINIFERA FROM THE BULGARIAN BLACK SEA COAST

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**ABSTRACT.** Two new unilocular foraminifers are described, having spherical shape, flexible, organic, partially agglutinated test – *Guanduella podensis* n. sp. (Allogromiidae) and *Psammosphaera* sp. (Psammosphaeridae). The benthos samples from which they were isolated were collected respectively from Lake Poda and the bay of the Ropotamo River mouth (Bulgarian Black Sea sector).

#### MONOGENEAN TREMATODS OF CHUB LEUCISCUS CEPHALUS ALBUS BONAPARTE, 1838 FROM THE LAKE OHRID (MACEDONIA)

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**ABSTRACT.** During the parasitological investigations on the gills of *Leuciscus cephalus albus* from Lake Ohrid (Macedonia), are found 4 monogenean species: *Dactylogyrus sphyrna, Dactylogyrus folkmanovae, Dactylogyrus vistulae* and *Paradiplozoon ergensi.* The total prevalence of infestation is 58,0% and the highest prevalence is of *Dactylogyrus sphyrna* (found in 26,0% of chubs). The average intensity of infestation is 5,50, and the highest level is that of *Paradiplozoon ergensi.* All monogenean species mentioned in this study represents a first record for the fishes from natural lakes in Macedonia, with the exception of *Paradiplozoon ergensi,* previously found in the Lake Ohrid. Among the monogenean species, found out in Lake Ohrid, the greatest pathological influence is associated with *Dactylogyrus vistulae* and *Paradiplozoon ergensi.* 

# CONTRIBUTION TO THE KNOWLEDGE OF PEDOFAUNA IN ŠUMADIJA (CENTRAL PART OF SERBIA)

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ABSTRACT. Intensive investigations of some groups of soil fauna in Šumadija were permanent more than 20 years. This central part of Serbia has an interesting geological history. It's interesting flora and fauna partially is result of continuous anthropogenic influences in the last centuries. In these investigations were included: (Insecta, Coleoptera), Lumbricidae (Oligochaeta), and Protura and Diplura (Insecta, Apterygota). The studieed ecosystems were natural (forests and meadows) and anthropogenous (orchards, gardens, fiels and artificial meadows). Earthworms from family Lumbricidae, were established 14 genera with 30 taxa and 6 new species for Šumadija: Dendrobaena alpina, Helodrilus cernosvitovianus, Octolasion cyaneum, Octodrilus complanatus, Serbiona serbica and Serbiona paratuleskovi. From the subfamily Cryptorhynchinae (Curculionidae) founded 5 genera, 12 species and three dominant species: Ruteria hypocrita, Echinodera behnei and Acallocrates colonnellii. New records of five weevils: Acalles (Acalles) aubei, Acalles papei, Acelles petryszaki, Echinodera behnei, Echinodera valida are given for Serbia. From Protura founded 3 families, 7 genera with 16 species, from Diplura founded 2 families which belong to 7 genera with 16 species.

# PRELIMINARY INVESTIGATIONS ON ODONATA FROM THE LAKE OHRID (MACEDONIA)

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ABSTRACT. There is much information about odonates in lakes in the whole Europe. The lack of data about this group of invertebrates in the Lake Ohrid - the UNESCO natural and cultural heritage is unusual. The aim of our paper is to cover that field. Material included 17 species and 476 specimens of only adult Odonata, which was collected in June 2009 year on 21 stations of the Lake Ohrid. Such number of species is not very high and appears in low differential of phyto-littoral of the lake. The most numerous species is *Enallagma cyathigerum*, which is widespread and associated with nymphaeid and elodeid plant species. Such big number of individuals the species is supported by a large belt of elodeids (*Potamegeton perfoliatus*) occurring in the Lake Ohrid. Four odonates belong to South-European species; the other ones are widespread European species. Probably the reason is position of the Lake Ohrid in the mountains, which made climate more moderate.

# SEASONAL VARIABILITY IN COMMUNITY STRUCTURE AND HABITAT SELECTION OF MAYFLIES (EPHEMEROPTERA) IN THE NIŠAVA RIVER (SERBIA)

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ABSTRACT. The sedentary nature of many members of the river macroinvertebrate community allows spatial and temporal analyses of disturbance effects. This characteristic also implicates their strong affinity towards certain types of microhabitats. The results of the mayfly (Ephemeroptera) community structure analysis in the Nišava River (Serbia) have shown a clear dependence of the mayfly community on habitat type (solid vs. loose bottoms), physical and chemical parameters (nutrient content), and season. Over a one-year period, on a monthly basis, 28 species of mayfly were identified at 12 localities along the 151 km long stretch of the Nišava River. The community structure changed longitudinally with the changes of physical and chemical parameters and habitat type. The lowest Shannon's diversity index was estimated for the localities with the lowest percentage of solid bottoms (rock, pebble). The highest diversity index was estimated for localities with the highest percentage of solid bottoms and the lowest average content of phosphorus and nitrogen.

# SEED BEETLE BRUCHIDIUS TERRENUS (SHARP) (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) – NEW INVASIVE SPECIES TO THE BULGARIAN FAUNA

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**ABSTRACT.** The East Palaearctic seed beetle *Bruchidius terrenus* (Coleoptera: Chrysomelidae: Bruchinae) is recorded for the first time to the Bulgarian fauna. Larvae of the bruchid infest mature seeds of introduced mimosa *Albizia julibrissin* (Fabacea), an ornamental tree in many countries. The level of damage on seeds caused by bruchid larvae was examined.

# MATING LATENCY AND COPULATION DURATION IN *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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**ABSTRACT.** Sexual behavior of *Drosophila melanogaster* is a sequence of fixed action patterns including several courtship steps that culminate in copulation. Mating latency and copulation duration represent two important behavioral traits included in mating of *D. melanogaster*, since they are closely linked with different fitness components. Mating latency is usually defined as a measure of female receptivity and male courtship intensity and efficiency. In laboratory, it is measured as time between introduction of the flies into mating vial until initiation of copulation. Duration of copulation is species-specific trait: it is scored as time from initiation to the termination of copulation.

In this paper, mating latency and duration of copulation in *D. melanogaster* were tested in laboratory under different conditions. In the first experiment, different developmental temperatures did not contribute to examine behavioral traits. Similarly, in the second experiment, no difference in mating latency and duration of copulation among flies developed on different diets was observed. The only one parameter that significantly induced both behavioral traits was female mating experience, since, in the third experiment, experienced females expressed significantly longer latencies and copulated shorter in comparing with naïve females. These results will be discussed in the context of sexual selection and mating strategy of this dipteran species.

# AMAZON SAILFIN CATFISH *PTERYGOPLICHTHYS PARDALIS* (CASTELLNNAU, 1855) (LORICARIIDAE, SILURIFORMES), A NEW FISH SPECIES RECORDED IN THE SERBIAN SECTION OF THE DANUBE RIVER

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ABSTRACT. Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) (Loricariidae, Siluriformes) is a new non-indigenous fish species recorded in the Serbian section of the Danube River, being reported for the first time in inland waters of Europe, as well. A single, female fish was ripe and in good shape, although considering its original neotropical dispersal area and recording of occurrence in summer, with the only single female individual, its acclimatization is not likely. However, both its estimated invasive potential after the FISK protocol scoring of 26 and the risk of its introduction and establishment in the recipient area in concern after the IFRA protocol scoring of 61 should be considered moderate. That, together with the invasive history of this sailfin catfish and its congeners in south-eastern Asia and Meso-American region imposes a need for precaution.

# SEX COMBS AND SEXUAL SELECTION IN *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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**ABSTRACT.** In past twenty years, developmental instability and its role in sexual selection has been the subject of many investigations, but the results are still contradictory. The most common measure used to detect and describe the magnitude of developmental instability is fluctuating asymmetry (FA), which refers about subtle departures from identical expression of a trait across an axis of symmetry. There are numerous and often different data concerning the relationship among mating success in males and bilateral symmetry of certain characteristics in *Drosophila*.

The male sex combs is bilateral and highly variable secondary sexual trait which is present in the melanogaster and obscura species groups of the subgenus Sophophora. It is represent as an array of specialized mechanosensory bristles on the male forelegs. Sex comb morphology (position, size, shape, color, number of teeth) as well as its function varies greatly among *Drosophila* species. In *Drosophila melanogaster*, males use sex combs for grasping of extruded female genitalia before mounting. Experimental removal of sex combs as well as genetic ablation technique confirmed previously that this morphological structure contributes to male mating success in this species.

In this paper, sexual selection in *D. melanogaster*, related to number and FA in sex comb teeth in males was tested both in laboratory and natural conditions. The greater number of sex comb teeth does not appear to favour male mating success in this species, since mating and non-mating males did not differ in mean number of sex comb teeth both in laboratory and nature. On the other hand, in field samples, single males had greater asymmetry in number of sex comb teeth than their mating counterparts. It looks like that symmetry of this bilateral trait plays a role in sexual selection in this dipteran species in nature, as greater symmetry in number of sex comb teeth was associated with male mating success and/or females detect the higher level of FA through their mechanosensory organs, and reject males in which FA in this traits persists.

# IMPORTANCE OF THE BODY WATER MANAGEMENT FOR WINTER COLD SURVIVAL OF THE EUROPEAN CORN BORER OSTRINIA NUBILALIS HÜBN. (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT. Winter diapause, a common strategy of many insect species occupying temperate regions, is usually closely related to and coincides with their cold hardiness. Freezing of body fluids represents one of the major obstacles for sub-zero temperatures survival and thus the body water management is an important part of cold hardiness. In this study, we examined some cryobiological parameters, as well as content of glycerol and trehalose in non-diapausing and freeze tolerant diapausing larvae of the European corn borer, Ostrinia nubilalis. Diapausing larvae were divided into two experimental groups – a group exposed to field temperatures (which were in average above 0°C) and a group exposed to -8°C for ten days. Contents of the total body water, osmotically active (OA) and inactive (OI), as well as the supercooling point (SCP) of hemolymph and fat body, were measured by differential scanning calorimetry (DSC). The content of glycerol and trehalose was analysed by gas chromatography. Compared to diapausing groups, non -diapausing larvae had higher SCP, lower content of trehalose and glycerol in both tissues. The content of total and OA water in both tissues of diapausing larvae had changed with low temperatures exposure. At -8°C, the amount of total and OA body water was decreased in hemolymph and increased in fat body while the content of OI water was slightly increased in hemolymph but remained unchanged in fat body. Mean SCPs of both tissues were significantly different - for hemolymph it was around -21°C, which was almost two times lower than for fat body (-10°C). However, the SCPs of fat body and hemolymph had not significantly changed after the exposure to low temperature. The content of glycerol and trehalose was far greater in hemolymph than in fat body for all groups, which is in accordance with the difference between the SCPs of these tissues. Furthermore, exposure of diapausing larvae to sub-zero temperatures (-8°C) had simultaneously provoked an increase in glycerol/trehalose concentration in hemolymph and the decrease in fat body. These adjustments of water and cryoprotectors distribution are an important part of cold hardiness mechanisms.

## SPECIES DIVERSITY OF AMPHIBIANS AND REPTILES IN THE SPECIAL PROTECTED AREA "BESAPARSKI RIDOVE", SOUTHERN BULGARIA

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**ABSTRACT.** We present briefly the distribution of amphibians and reptiles in the Special Protected Area "Besaparski ridove" in southwestern Bulgaria, based on a 2x2 km UTM grid. Between 1998 and 2008, we identified 24 species, and failed to verify the literature data only for a fossorial boa, *Eryx jaculus*. We documented five new amphibian species for the region (*Salamandra salamandra, Triturus karelinii, Bombina variegata, Bufo bufo, Rana dalmatina,* and *R. graeca*) and four species of reptiles (*Testudo graeca, T. hermanni, Ablepharus kitaibelii,* and *Zamenis longissimus*). The least common amphibians in the protected area were *S. salamandra, R. graeca, and T. karelinii* (number of squares in which these species are present from the total, A = 1.85%) and the most common were *Pelophylax ridibundus* (A = 35.19%), *B. bufo* (A = 20.37%), and *Epidalea viridis* (A = 18.52%). The least common reptiles were *T. graeca, T. hermanni, Z. longissimus, Platyceps najadum* (A = 1.85%) and *A. kitaibelii* and *E. jaculus* (A = 3.7%). The most common reptiles were *Podarcis tauricus* (A = 44.44%), *Lacerta trilineata* (A = 42.59%), and *L. viridis* (A = 29.63%).

# SEASONAL AND DAILY ACTIVITY PATTERN IN GRIFFON VULTURE IN SÜTÇÜLER (ISPARTA-TURKEY)

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ABSTRACT. Gyps fulvus is an important raptor to control for some agricultural pest (i.e. reptiles, amphibians, insects, rodents) and epidemic invasion in habitats, to breed and distribute for plant species, and to recycle organic material. In this study, daily and seasonal activities with nest selection, behavior and morphologic characters of Griffon Vulture Gyps fulvus were investigated between October 2007-July 2008 in Sütçüler (Isparta-TURKEY) having suitable for breeding and resting areas. Counts in the observation stations were performed twice a month. It was conducted in the three periods of day that morning (06:00-11:00), midday (11:00-15:00) and afternoon (15:00-19:00). It was determined that the only active nest was observed in Yazılıkanyon National Park while the others (11) were abandoned in the area, to be found daily and seasonally differences among individuals. Marble-quarries and use of chemicals on agricultural lands around the area effects individuals negatively. If the factors were removed by protecting efforts, it could be said that the number of vulture can increase there. So, it must be done to conserve both local and international protection for this species.

# INTERSPECIFIC AGGRESSIVE BEHAVIOUR OF EUROPEAN GROUND SQUIRREL (SPERMOPHILUS CITELLUS L.)

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**ABSTRACT.** Interspecific behaviour of European ground squirrel (*Spermophilus citellus*) is still poorly studied. During behavioural studies of free-ranging European ground squirrels tree kinds of aggression to other vertebrate species: reptile, bird and mammal (*Lacerta trilineata, Corvus frugilegus, Mustela nivalis*) have been described. To our knowledge, this is the first field study describing interspecies aggressive behaviour in S. citellus. The description of this behaviour may have important implications for interpreting studies on interspecies competition and interactions, behaviour activity, and predation in this rear and threatened semi-fossorial rodent.

### MICRONUCLEUS TEST FROM FREE LIVING RODENTS AS A BIOMARKER FOR ENVIRONMENTAL STRESS IN SITU

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**ABSTRACT.** In vivo MN test in peripheral erythrocytes of free-living rodents, chronically exposed to heavy metal pollution, was used for detection of genotoxic agents and species at higher risk. Yellow-necked mice (*Apodemus flavicollis*), Common vole (*Microtus arvalis*) and Algerian mice (*Mus spretus*) were collected in areas displaying low or high environmental pollution. Mean frequencies of MN observed in the rodents from the impact region were significantly higher compared to the frequencies from the same species in the background region. The comparative analysis of results confirms, that the Apodemus flavicollis species may be a suitable species for biomonitoring studies using MN frequencies. The results obtained demonstrate that the in vivo MN test may be a sensitive end-point for the detection of genotoxity that may result from the simultaneous action of several metals and may be useful as a biomarker of environmental stress in situ.

# HABITAT FRAGMENTATION AND ITS IMPLICATIONS FOR ABUNDANCE OF GUENTHER'S VOLE IN SOUTHEASTERN BULGARIA (STRANDZHA MOUNTAIN REGION)

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ABSTRACT. The effect of landscape structure on distribution of the Œinthers vole in typical and mostly widespread habitats in the region of Strandja Mountain was studied. In the studied region, which represents the most northeastern part of the range of voles of guentheri group in Europe, general trends in distribution and biotopic adherence of M. g. strandzensisin have been outlined. The established distribution of *Microtus guentheri* in the studied biotopes revealed the presence of Günther's vole only in wet habitats with hydrophilic vegetation. The established relative numbers of the Günther's vole in natural and semi-natural habitats and agricultural areas under autumn crops determined this species as rare in Strandja region, while in the preferred habitat in natural wet zones along Veleka River it was presented as common species. It was recommended to carry out regular examinations of M. g. strandzensis in the region, focused particularly in clover and wheat fields and their neighbouring roadsides; species numbers in these habitats should be used as indicator of its population state.

## DAMAGES OF GRAY WOLF (CANIS LUPUS L.) DURING TEN YEAR PERIOD IN BULGARIA

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ABSTRACT. For ten year period (1990-1998), were no evidences about wolf damages of domestic animals. According incomplete information from 1999 to 2009 wolfs killed domestic animals (1207 sheep's, 519 goats, 175 cows and calves, 123 horses and foals, 134 mules and donkeys and 110 dogs) and wild animals (62 red deers, 35 fallow deers, 183 roe deers, 85 wild boars, 31 mouflons and 9 wild goats). Based on this data looks that the wolf is serious pest for domestic and wild animals, but this is unavoidable, because it is predator. How much is its sanitary role in nature prey population in Bulgaria is unknown, because there are no investigations about age and physical condition of victims. On the other hand its damage on domestic animals is indisputability. It is necessary to make serious investigations on wolf ecology in Bulgaria and its interactions with local economy and society. The results from such investigations will supply the scientific data for compensation of owners of domestic animals, as the practice in other EU countries.

## DYNAMIC OF DISTRIBUTION AND NUMBER OF GRAY WOLF (CANIS LUPUS L.) DURING TEN YEAR PERIOD IN BULGARIA

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ABSTRACT. After 2000 year was started to collect statistical data about number of wolfs (*Canis lupus* L.) in Bulgaria. The number during ten years period was above 2000 ind. (with maximum 2479 ind. during 2008). In our opinion these data are not correctly. According to our estimates, based on suitable areas (forest area) in country, home range size of the pack (about 18.4 ha) established with radiotelemetry in Europe, in the country inhabit average 918 wolfs before opening of the hunting season and about 498 ind. after that. For one period of ten years 92.9-95.2 % of wolfs were shooting from October to March. From April to September were shooting from 4.8 - 7.1 % of wolfs. We propose one part of this six month period to be protected (for example April-June, when flocks don't carry out in the summer pastures). This protected period (without shooting) will be beneficial for species, without conflicts with local economics and society.

# ACCOMMODATION INTO THE WILD OF CAPTIVE BADGERS (MELES MELES, L.)

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**ABSTRACT.** This study follows the process of releasing back to the wild of captive badgers (*Meles meles* L.) considered as unsuitable for zoo purposes. The animals were marked with ear tags and were equipped with radio transmitters. The stages of accommodation are traced by taking into account the parameters of the acquisition of new habitats.

# THE VARIATION OF MORPHOMETRIC CHARACTERS OF TARSAL BONES IN SPECIES CERVUS ELAPHUS L., 1758 (MAMMALIA: ARTIODACTYLA) TAKEN FROM NEOLITIC SEDIMENTS AT BALKAN PENINSULA

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ABSTRACT. The researches on the skeletal elements astragalus and calcaneus have been made in order to determine differences among deer (*Cervus elaphus* L) populations. Specimens were taken from several Neolithic localities on the territory of Balkan Peninsula. Possible differences among the populations are possible due to different geographic and ecologic factors at the research localities, and due to influence of artificial selection. The artificial selection is evidenced by the choice of game, taking into consideration usability of deer as a hunting game for Neolithic people. Statistic analysis has shown that there is variability among individuals of the species *Cervus elaphus* L. at the localities. However, there are no statistically significant differences among the populations. The individual variations among wild populations have been confirmed, while the most significant anthropogenic influence is evidenced by prominent selection of hunting game. Scatter analysis has shown that there is significantly larger number of male individuals, in comparison to female ones, in the specimen. This data is in correlation with the already mention fact that usability of male individuals is greater.