



**University of Plovdiv "Paisii Hilendarski"**

**THIRD NATIONAL YOUNG  
SCIENTISTS CONFERENCE  
ON BIOLOGY**

**BOOK OF ABSTRACTS**

1<sup>st</sup> November 2022, Plovdiv, Bulgaria



**PROGRAMME & ABSTRACTS**

- APPLIED BIOLOGY AND EDUCATION PRACTICES
- ECOLOGY, BIODIVERSITY AND CONSERVATION
- GENETICS, CELL AND MOLECULAR BIOLOGY
- MICROBIOLOGY, BIOCHEMISTRY AND BIOTECHNOLOGIES

## **Third National Young Scientists Conference on Biology – Book of Abstracts**

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## FOREWORD

*Dear Authors, Esteemed Readers,*

*It is a great pleasure to write this foreword for the Book of abstracts of the Third National Young Scientists Conference on Biology, Plovdiv, Bulgaria, 1<sup>st</sup> November 2022.*

*It is hosted by the Faculty of Biology at the Plovdiv University and is devoted to early-stage scientists in Biology and their scientific researches. Its mission is to encourage the interaction of research students in Bulgaria to present and to discuss new and current work.*

*We express our gratitude to the invited lecturers, who will motivate young scientists and will give an additional emotional charge needed for their further studies.*

*We would like to thank the authors for their active participation.*

*We also would like to express our heartily gratitude to everyone who helped us to make the Conference a success.*

***Yours sincerely,***

*Gana Gecheva (Chair of the Organizing Committee)*

## COMMITTEES

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## SCIENTIFIC PROGRAMME

### Third National Young Scientists Conference on Biology

Tuesday, November 1<sup>st</sup> 2022, Hotel Ramada Trimontium, Plovdiv

09:00 – 10:00

**Registration**

10:00 – 10:30

**Opening Ceremony,**

**Prof. Sonya Kostadinova, Dean of the Faculty of Biology**

#### PLENARY SESSION

*Chair: Gana Gecheva*

10:30 – 11:00 **Love and parting at the cellular level**

**Prof. Evgeniya N. Ivanova, PhD, D.Sc.** (*Faculty of Biology, University of Plovdiv “Paisii Hilendarski”*)

11:00 – 11:30 **Water – living environment for aquatic organisms and valuable resource for humans**

**Assoc. Prof. Emilia Varadinova, PhD** (*South-West University “Neofit Rilski”; Institute of Biodiversity and Ecosystem Research, BAS*)

11:30 – 12:00 **Sustainability – theory and perceptions vs. practice and reality**

**Boyan Rashev, MSc** (*Environmental and Resource Management, Managing Partner at Denkstatt*)

12:00 – 12:30 **How to make undreamable dreams come true?**

**Viktoriya Beshliyska** (*Writer: Glina (Clay) – Book of the Year 2021; Crystal Award for Debut of the European Awards Eurocon 2022*)

12:30 – 13:30 **Lunch**

#### SESSION I – ECOLOGY, BIODIVERSITY AND CONSERVATION

*Chair: Dilian Georgiev*

13:30 – 13:50 **Honey production and mortality rate of honey bees in the regions of Southeastern and Central Southern Bulgaria during the period 2007-2020**

**Nadezhda Shopova, Vesselin Alexandrov, Nikola Tsaikin**

13:50 – 14:10 **Assessment of the main ecological factors of the environment expressed by agrometeorological indices in the region of Southeastern Bulgaria**

**Gergana Takucheva, Nikola Tsaikin, Vesselin Alexandrov, Nadezhda Shopova**

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14:10 – 14:30 **Current floristic diversity and vegetation characteristics of habitat 2340 \*Pannonic inland dunes in Bulgaria**

*Magdalena Valcheva, Iva Apostolova, Desislava Sopotlieva*

14:30 – 14:50 **Daily and seasonal activity patterns of the Red fox (*Vulpes vulpes*) and the Stone marten (*Martes foina*) in protected area "Zlatiyata" (Northwestern Bulgaria)**

*Alexander Petrov, Desislava Tincheva*

14:50 – 15:10 **Status of the population of *Orchis provincialis* (Orchidaceae) in the Protected Site "Locality of Provanski salep – Lozengradtsi", Eastern Rhodopes, Bulgaria**

*Vladimir Trifonov, Vladimir Vladimirov*

15:10 – 15:30 **Macrophyte-based assessment of upland rivers: bioindicators vs. biomonitors**

*Silviya Stankova, Evelina Varbanova, Lidiya Kaynarova, Deyana Georgieva, Violeta Stefanova, Gana Gecheva*

15:30 – 16:10 **Coffee break and Poster session, Chair: Elena Apostolova**

**SESSION II – MICROBIOLOGY, BIOCHEMISTRY AND BIOTECHNOLOGIES  
GENETICS, CELL AND MOLECULAR BIOLOGY**

*Chair: Vesselin Baev*

16:10 – 16:30 **Effect of *Crocus sativus* extract evaluated in mouse model of osteoarthritis**

*Blagovesta Boneva, Nikolina Mihaylova, Andrey Tchorbantov*

16:30 – 16:50 **Enzyme bag for lactose-free milk obtaining**

*Donika Bivolarska, Reni Syarova, Zlatina Chengolova*

16:50 – 17:10 **Lactose-free nutrition plan for people with lactose intolerance**

*Reni Syarova, Petar Shentov, Zlatina Chengolova*

17:10 – 17:30 **Looking at the relationship between human Rh factor and basic personality characteristics**

*Stanislav Ivanov, Evgeniya Ivanova*

17:30 – 17:50 **DNA sequencing**

*Nedyalka Atsenova*

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3	Records of <i>Stictis</i> and <i>Naemacyclus</i> ( <i>Ascomycota</i> ) from Bulgaria	Zornitsa Stoyanova, <b>Dimitar Stoykov</b>
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6	Preliminary results of the composition, numbers and density of breeding birds in the Western Upper Thracian lowland	<b>Polina Hristova</b> , Georgi Popgeorgiev, Dimitar Plachiyski, Vladimir Dobrev, Hristo Dimitrov
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# PLENARY LECTURES



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## Love and parting at the cellular level

*Prof. Evgeniya Neshova Ivanova, D.Sc.*

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**Abstract:** The philosophical categories of "love" and "parting" could also be considered on a social, psychological and biological level. Genetics, as a science of the heredity and changeability of living organisms, could also look unconventionally at the questions of love and separation.

The current narrative-presentation has the characteristics of a scientific essay, the main purpose of which is to look philosophically at biologically significant phenomena, revealing details of the cell functioning, the cell division mechanisms and the chromosome behavior, the features of the gametogenesis and the process of fertilization. It examines how various environmental factors affect these processes and how the love and separation that occur at the cellular level influence: the cell as a functioning organism; the individual organism as a synchronous system of action; the populations (including those of humans) as a source and manifestation of enormous diversity.

Everything in nature, by its essence, turns out to be a part of an entirety and that at the same time it has a huge potential for personal realization. In this lies the idea for the love, which is principle and realization (deployment) of the life, as well as the idea for the separation/parting, which is always about the striving for and finding of love.

And it's understandable why the word "separation/parting" is plural in the explanatory dictionary of the Bulgarian language, and the word "love" is only in the singular.

**Key words:** cell division, chromosome behavior, gametogenesis, fertilization

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## **Water – living environment for aquatic organisms and valuable resource for humans**

*Emilia Varadinova*

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**Abstract:** During the recent years, humanity has increasingly felt a shortage of freshwater. As a vital resource, water come under pressure, especially when its demand exceeds supply or its poor quality limits its use. Climatic changes and water demand are the two main factors causing water scarcity. These pressures adversely affect freshwater resources, both their quantity (overexploitation or drought) and their quality (pollution and eutrophication). As a result, the rivers reduce their outflow until they dry up completely, the characteristics of the habitats change, sensitive, stenobiont species disappear, adaptive eurybiont invade, including invasive ones, and a loss of biodiversity is registered. These processes lead to habitat degradation and changes in the composition and structure of the aquatic communities which reflect in negative aspect on the integrity of the aquatic ecosystems.

Can a reasonable solution be found and a balance achieved between preserving the biodiversity and the stability of the aquatic ecosystems, and meeting the ever-increasing human needs for fresh water? The sustainable use of water resources and preservation of their quality will be challenge faced by future generations.

**Key words:** water resources, aquatic ecosystems, ecological status, water quality.

## **Sustainability – theory and perceptions vs. practice and reality**

*Boyan Rashev*

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**Abstract:** In 1972, “The Limits to Growth” projected a dire future for the world – overpopulation, resource depletion and environmental degradation caused by economic growth. The global debate led to: “sustainable is a development that meets the needs of the present without compromising the ability of future generations to meet their own needs.”

Today, the world is several times more populated. Despite all predictions and widespread perception, our lives have improved by any possible measure. We live longer and healthier, eat better, learn and travel more and this trend is clearly visible on all continents as expressed by the Human Development Index. We consume much more goods and services without having depleted any single resource. Moreover, the reserves and availability of all major resources – fossil fuels, metals and minerals, water and food – have increased.

It has been our job to enable the continuous advance of living standards. I will try to explain how this works.

**Key words:** sustainability, environmental transition, cycle of progress, growth vs. degrowth

## How to make undreamed dreams come true?

*Viktoriya Beshliyska*

Writer

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**Abstract:** There are two kinds of dreams – those that we chase and others that chase us. I'm saying this talking from my personal experience as a writer. We humans, make extraordinary efforts to achieve the first kind of dreams. Sometimes we even succeed, but often – don't. The second kind "just" happens. The aim of this lecture is to provoke young scientists to explore themselves and their own possibilities, including the various ways of making their undreamed dreams come true.

The undreamed dreams are the invisible in us all, our unseen perfect manifestation. The small bit, which according Aristotle (the father of science, the first zoologist) is planted in us as a fifth element, as a secret ingredient. Turning those dreams into reality means seeking and achieving the invisible in ourselves as well as in the world that we explore through the visible. To achieve the undreamed dream, young scientists must take the doubt, the systematicity, and the analytical thinking that are so essential to them and must upgrade those virtues with faith and curiosity to the invisible.

**Key words:** dream, achieve, young scientist, fifth element, secret ingredient



# APPLIED BIOLOGY AND EDUCATION PRACTICES



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## Correlation between retained primitive reflexes and insufficient convergence

*Neli Fialkovska, Galina Yaneva*

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**Introduction:** The role of retained primitive reflexes is well established in regards to neuro-developmental disruptions and associated psycho-motor problems in children. The direct influence of primitive reflexes on the function of vestibular system in maintaining stable posture and vestibulo-ocular reflexes has not been investigated.

Visual stabilization of posture is dependent on the performance of the visual system. Insufficient convergence can cause significant postural issues.

**Objectives:** The purpose of the present investigation is to gather qualitative data on the influence of retained primitive reflexes on the function of oculomotor system.

**Materials:** The study involved 10 participants aged 10-12 years with at least 3 retained primitive reflexes out of 4 tested. The sequence of reflexes was selected considering their impact on the oculomotor development: Retained Moro reflex known to lead to eye movement problems, Retained Tonic neck reflex related to poor binocular vision, Retained ATNR causing insufficient eye tracking and difficulty crossing visual midline, and Retained STNR associated with poor eye/hand coordination.

**Methods:** The degree of integration of reflexes was assessed using tests developed by S. Goddard on a scale 0-4. From the eye movement examination was conducted the test of Convergence.

**Conclusions:** In order to prevent developmental delay in oculomotor function in children, it is necessary to conduct an examination of the degree of reflexes integration in them and if necessary, to apply reflex therapy. It seems reasonable to introduce reflexes integration therapy in children with postural instability and poor oculomotor skills.

**Key words:** retained reflexes, convergence

## Resource assessment of some popular species of medicinal plants in the region of the Northern Black Sea coast (Bulgaria)

*Petya Boycheva, Djeni Cherneva, Dobri Ivanov*

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The study presents a resource assessment of medicinal plants – *Hypericum perforatum* L., *Cichorium intybus* L., *Rosa canina* L., *Agrimonia eupatoria* L. and *Matricaria chamomilla* L., naturally distributed in the municipalities of Varna and Aksakovo (Northeastern Bulgaria). This is the first targeted study to determine the resource assessment of popular medicinal plants in the study area.

**Aim:** The aim of the present study is to establish the resource potential of some widely used medicinal plants in Varna region.

**Materials and methods:** In the period 01.06.2021 – 30.06.2022 field studies of the stocks of predetermined medicinal plants were carried out on the territory of 5 settlements in the Varna region. The yield of fresh herb was calculated from 15 pre-designated control sites (for each species) with an area of 1x1m<sup>2</sup> for herbaceous species and 4x4m<sup>2</sup> for shrubs. The operational stocks and the annual yield of the target medicinal plants have been established. The number and density of the populations are taken into account.

**Results:** The control sites are located along deciduous forests, grasslands and near settlements. The established operational reserves (and annual yield) for the growing season of 2021 are: *Hypericum perforatum* – 950 kg / dca (670 kg / dca); *Cichorium intybus* – 550 kg / dca (440 kg / dca), *Rosa canina* – 350 kg / dca (245 kg / dca), *Agrimonia eupatoria* – 710 kg / dca (500 kg / dca) and *Matricaria chamomilla* – 270 kg / dca (190 kg / dca).

**Conclusion:** The natural reserves of medicinal plants in the Varna region show high resource and restoration potential.

**Key words:** operational stock, annual yield, medicinal plants

**Acknowledgements:** The survey was conducted with the assistance of Project №20014/2020 to the Science Fund of the Medical University – Varna.

# ECOLOGY, BIODIVERSITY AND CONSERVATION



## **Assessment of the main ecological factors of the environment expressed by agrometeorological indices in the region of Southeastern Bulgaria**

***Gergana Takucheva, Nikola Tsaikin, Vesselin Alexandrov, Nadezhda Shopova***

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**Abstract:** In recent years, scientists have focused on the increase in air temperature and the number and intensity of extreme events of meteorological nature. Do the hydro-thermal conditions change significantly or they remain the same? In Southeastern Bulgaria the average temperature does not change equally in different regions and seasons. This publication aims to analyze the temperature and precipitation through indicators used in agro meteorology such as the De Martonne Aridity Index and BAH (Balance of Atmospheric Humidity). Using meteorological data from representative stations in the region, the averages of the minimum and maximum values of the air temperature were calculated. The presence of significant trends was sought through the non-parametric Mann Kendall test.

**Key words:** De Martonne Aridity Index, BAH (Balance of Air Humidity), climate change

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## Current floristic diversity and vegetation characteristics of habitat 2340 \*Pannonic inland dunes in Bulgaria

***Magdalena Valcheva, Iva Apostolova, Desislava Sopotlieva***

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**Abstract:** The aim of this study was to extend the knowledge on the floristic diversity, vegetation, ecology and threats of habitat 2340 \*Pannonic inland dunes in Bulgaria. Fieldwork was conducted in 2018 and data were sampled at 35 phytosociological plots from 5 localities along the Danube in NW Bulgaria. A complete list of vascular plants and bryophytes, percentage cover of each vascular plant species, total vegetation cover and separately cover of bryophytes, herbs, litter and bare soil were recorded within each plot, and a soil sample was also collected. A total number of 132 vascular plants and 7 bryophytes were registered. Each vascular plant was assigned to one of two groups: (1) typical plants – 39 species, and (2) non-typical plants – 93 species. The total vegetation cover in the relevés was generally high, and the share of typical and non-typical plants was quite similar. Values for species richness ranged between studied localities from 17 to 23 species per plot, for total vegetation cover – between 69 and 84%, and for cover of bryophytes – between 1 and 7%. The association *Bassio laniflorae-Brometum tectorum* was reported for the first time for Bulgaria and it was presented in all localities. The substantial presence of non-typical plants registered in this study revealed a successional trend towards ruderalization, which has significantly affected the autochthonous flora and vegetation of inland dunes in Bulgaria. We believe that results from our study will be useful in order to reveal the current diversity, distribution and conservation status of European inland dune vegetation.

**Key words:** Autochthonous plant species, *Bassio laniflorae-Brometum tectorum*, *Koelerio-Corynephoretea canescentis*, Natura 2000, psammophytes, ruderalization

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## Daily and seasonal activity patterns of the Red fox (*Vulpes vulpes*) and the Stone marten (*Martes foina*) in protected area "Zlatiyata" (Northwestern Bulgaria)

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**Abstract:** The diurnal activity patterns of the sympatric Red fox (*Vulpes vulpes*) and Stone marten (*Martes foina*) have been poorly studied in the intensive agricultural conditions of Northwestern Bulgaria. Our study aimed to establish the circadian and seasonal activity of the two predators in the protected area "Zlatiyata", situated in an agricultural landscape. The study was conducted from September 2021 to August 2022 by camera trapping. It provides new data on the behavioral ecology of the target species contributing to the knowledge of their co-existence in croplands.

**Key words:** mesopredators, camera trapping, competition

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## Effects of microplastics (MPs) on common carp (*Cyprinus carpio* Linnaeus, 1758)

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**Abstract:** Microplastics (MPs) are highly diverse and vary in size, shape, colour, polymer type and their constituent chemicals that all affect how they behave in the environment. Although well studied in marine systems, data on MPs effects in freshwater ecosystems are relatively scarce. In order to better understand the effects of MPs contamination on biota, studies using biomarkers are essential to complement environmental monitoring in order to control pollution effects in aquatic ecosystems. The purpose of this study is to apply integrated biomarkers on economically important fish species such as common carp (*Cyprinus carpio* L.) in order to assess the contamination with MPs in water and its negative effects. Data generated through this laboratory-based study, which will be the first reported in Bulgaria, could be develop towards a more quantitative risk assessment and incorporated into freshwater monitoring programmes.

**Key words:** microplastics, water, contamination, fish, biomarkers

**Acknowledgments:** The authors also thank the Science Research Fund at the University of Plovdiv "Paisii Hilendarski" for the financial support for project FP21 BF008.



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## Histopathological effects in *Cyprinus carpio* L. gills caused by propamocarb hydrochloride fungicide

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**Abstract:** Water pollution is mainly due to contamination with hazardous chemicals from agricultural runoff and waste water from household and industries. In environmental biomonitoring studies, it is important to use sentinel species, and thus, fish have proved to be sensitive organisms for assessing the condition and functioning of aquatic ecosystems. The general aim of the present study was to investigate the negative short-term effects of different concentrations of propamocarb hydrochloride in common carp (*Cyprinus carpio* Linnaeus, 1758) under laboratory conditions. The fish were exposed to the fungicide for 96 h and the histological biomarkers were investigated in the gills. We found mainly intense proliferative and, to a lesser extent, degenerative changes and alterations in the circulatory system, such as necrosis and vasodilation. The results obtained from this experiment could help us to further understand the toxicity of pesticides on non-target organisms and to better serve plant protection practices and environmental safety.

**Key words:** pesticides, water, contamination, fish, biomarkers

**Acknowledgments:** The "Vita Plus" project financed by Science Research Fund at the University of Plovdiv "Paisii Hilendarski" under the "Green Technologies" project line, is highly appreciated. The authors also thank the Science Research Fund at the University of Plovdiv "Paisii Hilendarski" for the financial support for project FP21 BF008.

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## **Honey production and mortality rate of honey bees in the regions of Southeastern and Central Southern Bulgaria during the period 2007-2020**

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**Abstract:** Honey production and mortality rate of honey bees in the regions of Southeastern and Central Southern Bulgaria in the period 2007-2020. Honey bees have a unique ecological function to maintain diversity in plant societies. They are directly related to crop production and crop productivity. In the conditions of climate change and increased use of pesticides, bees are exposed to many stressors that affect their life status and honey yield. This publication aims to assess honey production and mortality from various causes in the agricultural regions of Southeastern and Central Southern Bulgaria. Data from the Ministry of Agriculture and the non-parametric Mann-Kendall test were used for the analysis. There is a negative trend in winter mortality in the region of Southeastern Bulgaria and a positive trend in pesticide poisoning in the same region.

**Key words:** honeybee (*Apis mellifera* L.), honey yield, pesticides, mortality, South Bulgaria

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## Knowledge of wild edible mushrooms depending on educational level in the region of Varna

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**Purpose:** Numerous wild edible mushrooms are relatively well-known in Bulgaria and many countries all over the world. The objective of this inquiry study was to analyze some peculiarities of local knowledge of such mushrooms depending on inhabitants' educational level in the region of Varna, Bulgaria.

**Material/Methods:** We performed a study of 200 adult individuals, 100 males and 100 females, on the territory of the region of Varna using anonymous inquiry with a set of 12 concrete items dealing with respondents' knowledge of wild edible mushroom and focusing on the role of their educational level for this knowledge. Statistical data processing was carried out using descriptive, variation and correlation analysis. Statistical significance was considered at the level of  $p < 0.05$ .

**Results:** There was prevalence of the respondents with higher education. A total of 122 respondents (61% of the cases) could recognize wild edible mushrooms in the region of Varna. The respondents with secondary education are most informed about these mushrooms. Numerous mushroom nominations of single concrete mushroom species were reported by the respondents. The edible boletus *Boletus edulis* Bull was the most commonly recognized wild edible mushroom. Respondents' elementary educational level correlated statistically significantly with knowledge acquisition of wild mushrooms (Pearson's coefficient = 30.032;  $p < 0.001$ ).

**Conclusion:** This inquiry study demonstrated a satisfactory extent of awareness of these respondents with secondary, elementary and higher education concerning the wild edible mushrooms in the region of Varna.

**Key words:** wild edible mushrooms, knowledge, inquiry, education, region of Varna

## Macrophyte-based assessment of upland rivers: bioindicators vs. biomonitors

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**Abstract:** For the first time, macrophyte-based assessment of ecological status was related to the accumulated in aquatic plants trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn). Three moss and two vascular plant species were tested: *Fontinalis antipyretica* Hedw., *Leptodictyum riparium* (Hedw.) Warnst., *Platyhypnidium riparioides* (Hedw.) Dixon, invasive *Elodea canadensis* Michx. and *Myriophyllum spicatum* L.

Three streams were assessed in good to high ecological status which correlated with low contamination based on calculated contamination factors (CF) and metal pollution index (MPI). Two sites evaluated in moderate status revealed to be in heavy trace element contamination. The most significant was the accumulation in moss sample from Chepelarska River (bridge at Kemera). Mercury exceeded the environmental quality standard (EQS) for biota in 3 of the studied upland river sites.

**Key words:** aquatic macrophytes, mosses, ecological status, EQS

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## Preliminary results of the composition, numbers and density of breeding birds in the Western Upper Thracian lowland

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**Abstract:** The plain forests in the Upper Thracian lowland are significantly fragmented and located within areas with intensively managed agrosystems. They harbour high diversity of bird's taxa. There is lack of in-depth studies of the avifauna in these habitats, as well as the knowledge of their significance for the biodiversity. The study present large dataset on the composition, number and density of bird species within 15 plain forest fragments of the Upper Thracian lowland. Birds were censused by point counts in 69 sample plots from 2020 to 2021. Each point was counted twice during the breeding season (between April and June), and each count lasted at least 15 minutes. The birds were vocally or visually registered. Number and density of the most abundant species is calculated using Distance 7.3 Software. In total of 57 bird species were registered. The greatest number of bird's taxa registered per forest fragment was 37 species. The smallest number of bird taxa registered per forest patch was 15 species. The most numerous species for all study plots is the great tit, *Parus major* (N = 9919; D = 3.199), and with lowest number the hoopoe, *Upupa epops* (N = 1502; D = 0.551).

**Key words:** plain forests, bird composition, bird density, Upper Thracian lowland, distance sampling

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## Records of *Naemacyclus* and *Stictis* (Ascomycota) from Bulgaria

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**Abstract:** Most species of genus *Stictis* inhabit bark of trees, wood or other plant parts, including grassy stems. *Stictis radiata* was recorded in Bulgaria on bark of *Platanus* tree in Belasitsa Mt. *Naemacyclus fimbriatus* was observed on pine cones with two collections from Vitosha region (Plana Mt) and Rila Mts. It is the second report of this species from the Balkan Peninsula after the first record from Turkey. These collections represent the first finds of *Naemacyclus* on dead cones of Scots pine and of *Stictis radiata* on tree bark in Bulgaria. The studied materials are presented with brief descriptions and colour illustrations.

**Key words:** Bulgarian mycota, Leotiomycetes, Ostropomycetes, *Platanus*, Scots pine

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## Status of the population of *Orchis provincialis* (Orchidaceae) in the Protected Site "Locality of Provanski salep – Lozengradtsi", Eastern Rhodopes, Bulgaria

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**Abstract:** *Orchis provincialis* Lam. & DC. (Provence orchid, Orchidaceae) is of high conservation concern in the Bulgarian flora. Thus, it is of interest for monitoring and research. In Bulgaria, the species was evaluated as "Critically Endangered" in the national Red List and in the Red Data Book, and is legally protected by the Biodiversity Act. In addition, it is included in Appendix I of the Bern Convention. It has been recorded in only a few localities in two floristic regions: Rhodopi Mts (Eastern – near Chakalarovo, Kirkovo and Lozengradtsi villages) and Strandzha Mts (a single collection in 1921 near Kosti village), up to 400 m a. s. l. The general distribution range of the species comprises the Mediterranean region and the neighbouring countries in Europe (to France and Switzerland northwards), North Africa and Asia Minor.

In May 2022, a study was conducted on the population of the species in the Protected Site "Locality of Provanski Salep – Lozengradtsi", Eastern Rhodopes, Bulgaria. The research is a continuation of more than 20 years of observations of the species in the locality. The area of the protected site is 7.07 ha. A permanent monitoring plot of 7 × 5 m was installed in order to reveal the population trends. In 2022, 48 flowering and 121 vegetative plants (169 in total), in 9 groups, have been counted.

Data on the species population and accompanying vascular plants are presented. Characteristics of the habitat and the threats are discussed.

**Key words:** *Orchis provincialis*, threats, monitoring, rare plant species, protected areas

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## Traditional knowledge of medicinal plants in relation to local customs and practices of the North Black Sea coast population

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**Abstract:** This comprehensive ethnobotanical study was conducted along the North Black Sea coast area to verify the extent to which indigenous knowledge of medicinal plants and its relation to Bulgarian traditions and customs is preserved. We aim to identify the tendencies of passing this knowledge to future generations. The survey was conducted in the period April – June 2015 in various urban and rural North Black Sea coast areas, using face-to-face interviews technique with random demographic selection of respondents. More than half of the respondents (61.08%) are not familiar with rituals and traditional practices related to medicinal plants. Moderate influence to respondents' answers is rendered by age only. The question "Are you familiar with customs and rituals in your area which go along with the use of herbs?" suggests the following responses: the largest percentage of negative answers belongs to respondents aged 61 to 70 years of age – 78.05%, followed by younger age groups of 20 to 30 and from 31 to 40 years of age – 61.54% and 66.67%, respectively. For the local population of the North Black Sea coast, Enyovden turns out to be the most popular holiday related to medicinal plants. Enyovden is the herbalists' feast and is cited by 20% of respondents. The same percentage of interviewees mention *Geranium* sp. and *Ocimum basilicum* L. as the most favoured herbs related to local traditions and customs. Traditional knowledge of the relation between Bulgarian rituals and customs to medicinal plants and their magical powers is fading.

**Key words:** Ethnobotany, medicinal plants, traditions and customs, North Black Sea coast



# **GENETICS, CELL AND MOLECULAR BIOLOGY**



## Age-dependent dynamics of ovarian hormones in hCG-primed endometrial cycle of healthy women

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**Abstract:** The study included 7 voluntary participants aged between 29 and 44 years. Each participant has completed a Patient Card and Declaration of Informed Consent. To make ovulation predictable and the dating of the receptive phase more accurate, human chorionic gonadotropin (hCG) was administered, which primed ovulation within 36-48 hours after subcutaneous administration. Reproductive hormones were determined at four time points within the same menstrual cycle, corresponding to the proliferative phase and at 2, 7 and 9 days after hCG administration by electrochemiluminescence immunoassay. Data showed that preovulatory follicles of approximately the same size, assessed by ultrasound and luteinizing hormone (LH) below 13 IU/L, were associated with different estradiol (E2) values. The reference levels of 130 nmol/l were not achieved in one of the patients (P7), although ovulation was reported thereafter. The application of hCG in this patient was made at a value below 130 nmol/L, considering other markers of impending ovulation – reported follicle 20 mm and LH value of nearly 6 IU/L, suggesting a recent increase in LH within hours. In all cases after LH-peak there is a tendency of decrease of E2 and increase of progesterone in hCG+7 and hCG+9 of the endometrial cycle, corresponding to the implantation window. There is a decrease in progesterone at hCG+9 in one of the patients (P7), which we believe is age-related, as well as a displacement of the implantation window in the same patient according to histological examination.

**Key words:** ovarian hormones, endometrium

**Acknowledgement:** This project has received funding from the National Science Fund of Bulgaria (KП-06 H31/2).

## **DNA sequencing**

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**Abstract:** The theme of the paper is DNA sequencing – its applications, major platforms, other applications. The paper explains Whole genome sequencing, Whole exome sequencing, Targeted region sequencing, Mutation detection in cancer research and Clinical DNA sequencing service. Paper also depicts other applications of DNA sequencing.

In the beginning, it starts with a brief history of sequencing and principles of sequencing platforms.

Second, different types of mutations are explained – somatic, germline and driver. Driver mutations have roles in cancer pathways.

In the end, other applications are described like low-pass whole genome sequencing, mitochondrial DNA sequencing, metagenomic next generation sequencing, epigenome sequencing.

**Key words:** DNA sequencing, major applications, different mutations

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## Looking at the relationship between human Rh factor and basic personality characteristics

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**Abstract:** The present study aimed to analyze the relationships between the five basic characteristics of the personality Extraversion, Agreeableness, Conscientiousness, Emotional stability, Intellect/Imagination and the human Rh factor. More than 900 individuals with an average age of 32.3 years were included in the study. The basic characteristics of personality were studied through the Goldberg's "Big-Five factor markers, International Personality Item Pool - IPIP" questionnaire. Rh blood groups were reported by the participants themselves after having conclusions from a clinical laboratory. The results were analyzed by usage of Descriptive statistics and SPSS software.

Mean calculated values of the five studied personality traits showed that Agreeableness was most pronounced, followed by Conscientiousness and Intellect/Imagination and Emotional stability. Among the studied group, 79% of the persons were with Rh+ and 21% – with Rh-.

A statistically significant relationship has been found between emotional stability and human Rh factor. The data showed lower levels of the emotional stability in people with positive Rh factor.

As the present study successfully reveals statistically significant relationships between various human traits on the base of a complex approach, it could be used as an appropriate model for other future investigations of human phenotypic diversity.

**Key words:** blood groups Rh, human personality traits, phenotypic variability

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## Prognostic markers in quadruple negative breast cancer

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**Abstract:** Quadruple negative breast cancer (QNBC) presents with negative expression of estrogen, progesterone and androgen receptors (AR) and of human epidermal growth factor receptor 2. This breast cancer subtype has the worst prognosis. In QNBC, there is a greater paucity of prognostic biomarkers than in AR-positive triple negative breast cancer (TNBC). Integration of the knowledge of QNBC-specific microRNA regulatory networks enables to study microRNA dysregulation. There is increased microRNA-135b expression as it promotes QNBC pathogenesis. MicroRNAs interact with their target mRNAs and modulate their expression. Long non-coding RNAs act alone or interact with microRNAs or other molecules through various signaling pathways in QNBC. Non-coding RNAs are explicitly implicated in breast cancer regulation and in cross-talk between TNBC and QNBC. MicroRNAs play important roles in breast cancer proliferation, progression and metastasis and are used as biomarkers for future diagnostic applications. In African American women, QNBCs have an enriched basal and immune signature. These patients have an odds ratio of 66.60 of being basal-like compared to other PAM50 subtypes. This is associated with longer time to progression and decreased overall survival. A lack of AR expression confers a more aggressive disease course and correlates with the expression of cancer stem cell phenotype, COX-2 and basal markers such as CK5 and nestin. Basal-like phenotype correlates with QNBC and is significantly associated with adverse prognostic markers including high KI-67, COX-2 expression and cancer stem cell phenotype. Thymidylate synthase serves as valuable prognostic biomarker in QNBC.

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## Therapeutic challenges in quadruple negative breast cancer

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**Abstract:** Quadruple negative breast cancer (QNBC) is a recently identified subtype of triple negative breast cancer (TNBC) presenting with negative androgen receptor expression. QNBC characterization and treatment is fraught with many challenges. A pathologic complete response (pCR) is achieved in 35.86% of TNBC patients treated by neoadjuvant chemotherapy as 86.21% of them have a QNBC. The rate of neoadjuvant chemotherapy in patients with QNBC phenotype is 37.6%. High Ki-67 values (>50%) are observed in 76.8% of QNBC cases. Higher Ki-67 expression, higher differentiation grade, and lymph node involvement correlate significantly with the pCR rate in QNBC. QNBC is diagnosed in 67.42% of TNBC patients. The Ki-67 expression is 86.7% in QNBC. The rate of pathologic complete response of 60% in QNBC is higher than that of 24% in TNBC. Nine epigenetically altered genes/differentially expressed proteins in QNBC are associated to its aggressiveness. Restoring the normal expression of these genes via epigenetic reprogramming is beneficial to QNBC patients. QNBC expresses unique proteins amenable to use in the development of effective targeted therapies by using ACSL4, SKP2, immune checkpoint inhibitors, EGFR, MicroRNA signatures and Engrailed 1. Molecular pathways dysregulated specifically in QNBC and the global regulatory networks surrounding them represent therapeutic targeting opportunities. Imatinib, cabozantinib, dasatinib, lucitanib, sunitinib and thymidylate synthase are effective in QNBC. Restoring T-cell responses such as PD-1, PD-L1 and CTLA-4 are promising immunotherapies. These genes are increased in QNBC and such patients are candidates for this therapeutic class.

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## Toxic effects of thiamethoxam investigated on the *Allium cepa* root meristem

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**Abstract:** Neonicotinoids are neurotoxic insecticides widely used in modern agriculture to control pests. Their negative impact on various natural components and nature in general is an object of many researches during last years. The active substance thiamethoxam is a component of pesticides with different trade names. Based on a survey conducted in 2021 among beekeepers in Bulgaria, this agrochemical has been identified as a possible cause of poisoning of honey bees in some areas. This fact motivates the purpose of the present study – to analyze the toxic potential of the thiamethoxam insecticide "Actara WG" by the usage of the *Allium cepa* root meristem as a model system.

Anaphase analysis and micronucleus mutagenicity test have been applied in order to study the genotoxic effect of the pesticide. A control and experimental samples (insecticide solutions with different concentrations) have been compared. Approximately 2 000 cells per individual and five individuals per sample have been analyzed. The comparative analysis of MI demonstrates a negative effect of the pesticide on the cell division rate. Thiamethoxam demonstrates also a genotoxic effect on the *Allium cepa* test system. Chromosomal anomalies such as pulverized chromosomes, diagonal anaphases, chromosome fragments, anaphase and telophase bridges – alone and in combination with fragments, wandering and lagging chromosomes and micronuclei have been detected in meristem cells after the treatment.

The results of the present study reveal the cyto- and genotoxic effect of the neonicotinoid thiamethoxam on the *Allium cepa* model system, which could be accepted as a potential danger for the honey bees. Future studies with *Apis mellifera* in situ would give more clarity on this problem.

**Key words:** Neonicotinoids, thiamethoxam, genotoxicity, *Allium cepa* model system, honey bee colony losses

**Acknowledgments:** This study was supported by the National Research Fund of Bulgaria through the contract KP-06-H5112/2021 "Complex assessment of genetic and environmental factors related to the losses of honey bees (*Apis mellifera* L.) in Bulgaria".

# **MICROBIOLOGY, BIOCHEMISTRY AND BIOTECHNOLOGIES**





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## Current options in the treatment of inflammation caused by *Staphylococcus aureus* biofilm with bioactive glass S53P4

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**Abstract:** Implant-related infections in surgical and dental practices are among the most severe complications following surgical procedures. The ability of microorganisms to adhere and form biofilms on the implant surface is one of the major causes of treatment failure in infections. When implanted into the human body, biomaterials can provide a suitable adhesive substrate for cell attachment and bacterial colonization. One of the most common microorganisms found in hospital infections is *Staphylococcus aureus* with most infections due to biofilm formation. When biofilm forms, bacteria achieve an arsenal of properties that allow them to survive in adverse environments, increasing their resistance to antimicrobial agents. Bioactive glass has revolutionized medicine. Initially discovered and applied for its extraordinary properties to stimulate osteogenesis by releasing biologically active ions, and then as a substance with antibacterial properties on a range of antibiotic resistant bacteria due to an increase in osmotic pressure and environmental pH without affecting the host tissue. Taken together, these properties make bioactive glass very suitable for the treatment of bone infections characterized by bone necrosis and destruction of bone structure. The aim of this study is to review the results reported in the worldwide literature on the antimicrobial efficacy of S53P4 bioactive glass on *Staphylococcus aureus* biofilm.

**Key words:** bioactive glass, inflammation, biofilm, *Staphylococcus aureus*

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## Effect of *Crocus sativus* extract evaluated in mouse model of osteoarthritis

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**Aim:** Osteoarthritis affects a high percentage of the population over the age of 60, and the disease leads to a decline in quality of life and disability. The disease is age-related, it's detected at a late stage of progression and there is no adequate treatment. The aim of our research is to study and observe the effect of *Crocus sativus* extract and the possibility of its long-term administration on collagenase- induced mouse model of osteoarthritis (CIOA).

**Materials and methods:** Inbred BALB/C mouse strain, collagenase type IA and *Crocus sativus* extract have been used to develop a sufficiently effective model that allows the monitoring of the stages of the osteoarthritis, an optimal period for the treatment of the experimental animals and a subsequent set of studies to track the pathogenesis and changes in cell populations by histology, and flow cytometry. Sera were collected from the animals, and changes in the knee joint were monitored during the disease stages. We studied also the characteristics and toxicity of *Crocus sativus* extract, and we decided to work with concentrations of 25, 50 and 100 mg/kg.

**Results:** After per os supplementation of Saffron, we observed changes in the cell populations of cells isolated from the synovium of the knee joint. The population of neutrophils and monocytes cells was measured, where the most significant changes were found. This model allows us to follow up on the pathogenesis and the multiple histological changes in the joints. In the Saffron – treated animal group, some of the disease symptoms are either reduced in scope or not detectable.

**Conclusion:** At this stage of the research, the therapy with the Saffron extract showed positive effects on the control and restriction of the disease progression, and improved disease symptoms in the treated groups.

**Key words:** Osteoarthritis, CIOA, *Crocus sativus* extract, mouse model, synovium

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## **Enzyme bag for lactose-free milk obtaining**

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**Abstract:** Lactose is an important component in mammalian milk. It is a disaccharide also called “milk sugar”. In healthy individuals, lactose enters the intestinal lumen and is being hydrolyzed to glucose and galactose by the enzyme lactase. There are people with reduced lactase activity and the disaccharide is hydrolyzed by the microorganisms in their gut. This leads to lactose malabsorption which leads to symptoms such as diarrhea, nausea, gas and abdominal pain. The condition is called lactose intolerance. Those people with less or no amount of the enzyme lactase are forced to avoid lactose-containing foods or pre-process them in order to hydrolyze the milk sugar before consuming the product. This paper presents a method by which anyone could get lactose-free milk at home. The so-called “enzyme bag” resembles a tea bag which is dunked into a glass of milk. It uses the biodegradable, biocompatible and non-toxic polysaccharide – chitosan as an inert and harmless carrier, and the immobilized enzyme beta-galactosidase. The process does not require special equipment – is performed at room temperature and is fast, easy and convenient. The future application of this method allows with a few tools and a few easy steps to solve the problem of a large number of people with lactose intolerance.

**Key words:** lactose, milk, lactose intolerance, enzyme immobilization

## Immobilization of high-molecular glucosyltransferase URE 13-300 and characterization of the synthesized products

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**Abstract:** *Leuconostoc mesenteroides* URE 13 produces high-molecular-weight glucosyltransferase (300 kDa), which was cloned and expressed in *Escherichia coli* BL21. It is an  $\alpha$ -transglucosylase that synthesizes gluco-oligosaccharides and branched  $\alpha$ -glucan. Designing different methods for improving enzyme functional properties is key, hence an immobilization of URE 13-300 with low-molecular chitosan and sodium tripolyphosphate was performed.

The aim of the present work is to study enzyme reaction of immobilized glucosyltransferase URE 13-300 by glucan and oligosaccharide synthesis.

Enzyme reactions with immobilized glucosyltransferase URE 13-300 along with control reaction, catalyzed by free enzyme solution were carried out. About 50% loss of activity was measured after immobilization. Oligosaccharide synthesis was performed with sucrose as donor of glucosyl units and maltose as an acceptor with ratio of 0.5. Samples at the beginning of the reaction and at fixed time frames were analyzed by HPLC. The synthesized oligosaccharides with degree of polymerization (DP) of 6 were present in the sample not until the 24<sup>th</sup> hour, unlike the reactions performed with the unbound enzyme. In that case oligosaccharides with DP = 3 and higher are present even thirty minutes after initialization. Moreover, the maltose derived oligosaccharides with DP = 4 and higher were at considerably diminished amount as opposed to control reactions. Sucrose consumption was decreased as well, with values higher than 30% at the end of the reaction time.

Glucan synthesis was performed as well and its structure was analyzed by <sup>1</sup>H NMR spectroscopy. The obtained results from this immobilization method show significantly altered enzyme function and it should be object of further examination and optimization.

**Key words:** glucosyltransferase, immobilization, chitosan nanoparticles, glucooligosaccharides, glucan

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## **Lactose-free nutrition plan for people with lactose intolerance**

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**Abstract:** A large number of the world population suffer from lactose intolerance. Lactose intolerance occurs when the small intestine does not produce enough of the lactase enzyme, which digests the lactose. People with lactose intolerance make the decision to reduce or eliminate milk and dairy products out of their diet as well as some of the modern industry food – so-called “hidden lactose”. Lactose is also found in some medicines as an accompanying ingredient to the active substance. Therefore, people with symptoms of lactose intolerance should be extremely careful when choosing their food and supplements. It is well known that complete cutoff of milk and dairy products may reduce the levels of calcium and some vitamins in the body. In order to create a balanced nutrition plan which contains all the essential vitamins and minerals needed for the human body, the composition of cow’s milk has been examined. In this paper, discussions about alternative foods containing the essential vitamins and minerals found in milk are made. Different plant-based milks were studied and analyzed, considered to be milk substitutes. Furthermore, different organically derived supplements, suggested for people with lactose intolerance, were discussed in order to prevent deficiencies. The studies are in line with the foods and supplements that are available worldwide, but attention is also paid to those available on the market in Bulgaria. In this regard, the paper presents an exemplary nutritional plan for people with lactose intolerance, providing wholesome food and important nutrients.

**Key words:** lactose intolerance, nutrition plan, foods and supplements

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## Relationship between the water microbiome and the ecological status of heavily modified water bodies in Bulgaria

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**Abstract:** This study comprises the first attempt to describe the physiological and taxonomic diversity of the planktonic bacterial communities of heavily modified water bodies (HMWB) in Bulgaria with developed net-cage aquaculture applying Biolog EcoPlate™ and next-generation sequencing (NGS) technologies. Such systematic studies in HMWBs with a well-established physico-chemical status could shed a light on the role of the water microbiome as an environmental driver, and the possible application of microbiological indicators as additional biological quality elements. Community-level physiological profiling (CLPP) was used to determine metabolic differences, and the activity was expressed, as average well color development (AWCD). The lowest AWCD was calculated for reservoirs with bad ecological status based on the physico-chemical parameters (Ovcharitsa, Aheloy, Studen Kladents, Ivaylovgrad), where communities are dominated by a limited number of fast-growing species with p-strategies. Reservoirs with good to moderate ecological potential (Dospat, Alexander Stamboliyski, Zhrebchevo, Koprinka, Ogosta) had higher AWCD values and CLPP favoring the development of slow-growing k-strategies. Next generation sequencing revealed that > 95% of the total number of sequences refer to 10 Divisions including *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Fusobacteria*. The communities are formed by a similar dominant complex comprising >200 OTUs, with about 75% of the taxonomic composition common to the nine water bodies and unique sequences are <20%. Cluster analysis revealed significant spatial differences within the water bodies, where the area near the net-cage farms forms a separate cluster characterized by a reduced relative abundance of *Cyanobacteria* and an increased abundance of saprophytic members of *Bacteroidetes*. The results were confirmed by the analysis of similarities (ANOSIM) and principal coordinate analysis (PCoA) based on the identified operational taxonomic units (OTUs).

**Key words:** reservoirs, next generation sequencing, Biolog EcoPlates, community level physiological profile, multivariate analysis

## Study of the influence of organic solvents on the activity of the enzyme laccase

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**Abstract:** Laccases (EC 1.10.3.2) are multicopper oxidases found in plants, fungi, and bacteria. Laccases oxidize a variety of phenolic substrates, performing one-electron oxidations, leading to crosslinking. For example, laccases play a role in the formation of lignin by promoting the oxidative coupling of monolignols, a family of naturally occurring phenols. Other laccases produced by fungi can facilitate the biosynthesis of melanin pigments. Laccases catalyze ring cleavage of aromatic compounds. Monitoring phenolic compounds is critical in the environmental, food, and medical sectors. Among many recent advanced detection platforms, laccase-based biosensing platforms gave very rapid, effective, online, and in situ sensing of phenolic compounds. In laccase-based biosensors, laccase immobilization techniques have a vital role.

The aim of our work is to determine the influence of different organic solvents such as ethanol, methanol, dimethyl sulfoxide and acetone on the enzyme activity of laccase.

In our study one commercial enzyme – laccase from *Trametes versicolor* was used to measure the enzyme activity in heterogenic catalysis in presence of organic solvents, compared with enzyme reaction in optimal conditions.

We have studied enzyme activity after treatment of laccase in presence of 10% organic solvent for two hours. The experimental results of the enzyme activity in presence of 10% organic solvents was compared with the native enzyme activity and shows that enzyme is stable for two hours and keeps activity up to 50% with methanol, 40 % with ethanol, 40% with acetone and 30% with DMSO.

The experimental results shows that the laccase could be used for determination of polyphenols in presence of ethanol and methanol.

**Key words:** laccase, organic solvent, enzyme activity, ABTS

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