BioX Croatia 2024 - Conference & Scientific Mission : proceedings & book of abstracts

CONFERENCE & SCIENTIFIC MISSION

May 28th to June 4th
Zagreb and Zlarin, CROATIA

PROCEEDINGS & BOOK OF ABSTRACTS

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SPONSORS

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May 28th

Visit to CROATA shop, history of a necktie & Welcome reception at Hrvatska kuća, Zagreb

You may, or may not know that necktie, or somewhere known as cravat, originates from Croatia. And so, we started our BioX journey by visiting the famous Croata shop in the city center. As one of the sponsors, the Croata donated a necktie and a scarf, as a prizes in conference lottery, later to be won by dr. Mateja Jagic and Prof. Marisa Manzano!
Next, we headed for Hrvatska kuća „Materina priča" (Croatian House, a mother's story), a informal museum decorated to represent traditional houses from different parts of Croatia, as they once were. By crossing its threshold, the visitor will be "catapulted" to the time when linen cloth was produced on the loom for sewing clothes, grains were ground in a stone grinder, and children fell asleep in zips... The aim and task of the Croatian House is to present the beauty and richness of Croatian national culture, ethnic heritage and customs to our people, young generations and tourists. The BioX participants had the oportunity to enjoy not only the time travel to different parts of Croatia, but also many traditional foods and drinks…

The Croatian House

Traditional drinks – Rakija, red wine Plavac mali and white wine Žlahtina

Professors and Dr's enjoying the drinks, and PhD students enjoying the food. They still have much to learn…

May 29th

Conference at the Faculty of Science of University of Zagreb

The Main conference holded 16 lectures (including 3 online), 13 short Student's presentations, and participants from 8 countries; Albania, Croatia, France, Israel, Italy, Netherlands, Poland and Serbia. In the end, it was a marathon undertake, starting at 9:00 and finishing at 20:00 h. Congratulations to everyone for the persistence!

Ready to start

Dr. Eltzov and Prof. Leljak Levanic chairing the session..

..and later refreshing with sponsored drinks.

Dr. Vunduk, Dr. Nuijten and Prof. Sola chairing the last session

May 30th

Forest sampling at Kupinečki Kraljevec, Workshop – fungi determination/inoculation and preparation, City tour Zagreb, Gemišt tasting, Gala dinner

The expedition team at the forest near Zagreb / nice catch with many mushroom species / inoculating the samples at Microbiological laboratory at the Faculty of Science in Zagreb

Millennial photo, from left to right; Sandra Vitko, Mateja Jagic, Gal Carmeli, Michela Maifreni, Robert Marks, Marisa Manzano, Abraham Paul, May Portman, Anxhela Kamberaj, Nadav Bachar, Tomislav Ivankovic, Evgeni Eltzov, Fredric Narcross, Hana Breyer, Dunja Leljak Levanic, Lucija Beluzic, Jovana Vunduk and Iva Silla, the tour guide.

May 30th

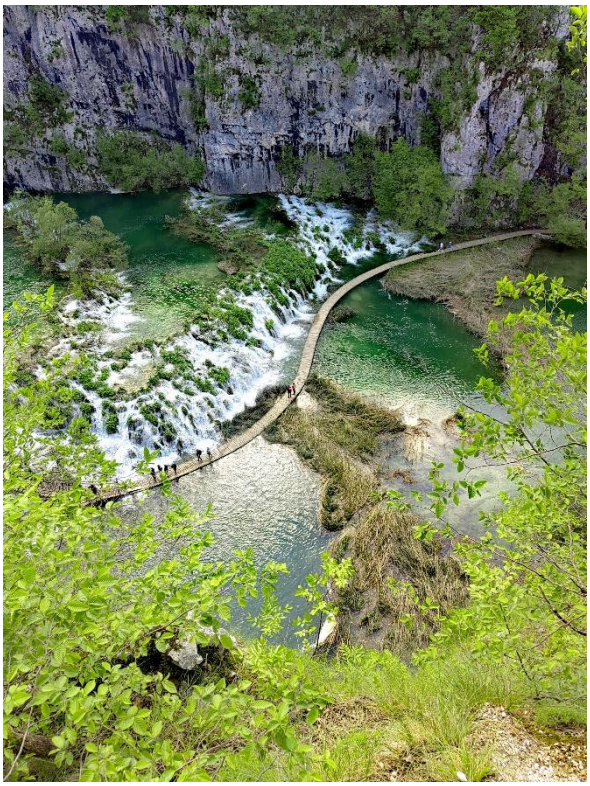
Forest sampling at Kupinečki Kraljevec, Workshop – fungi determination/inoculation and preparation, City tour Zagreb, Gemišt tasting, Gala dinner

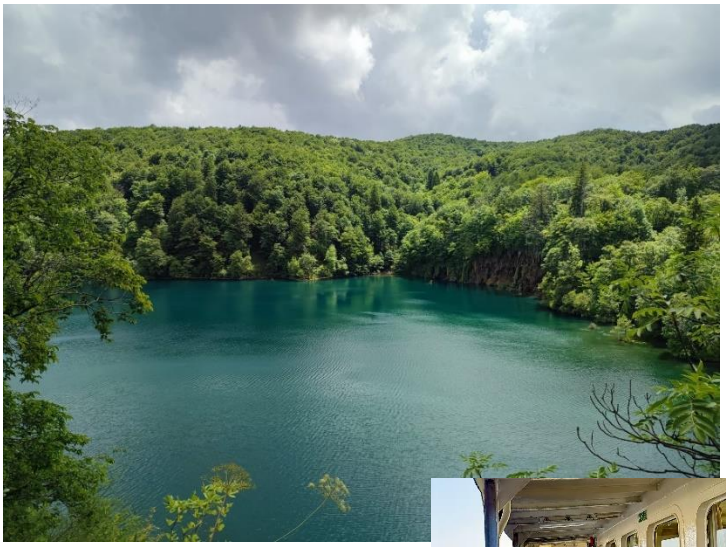
Inspired by a famous Croatian movie – A song a day takes the mischief away, the BioX expedition went for a Gemišt tasting, a traditional Zagreb drink, half white wine - half sparkling water, and from a specific glass caled the „gemištarka".

Dr. Rudnicka prepared the apetizers with the mushrooms picked earlier in the morning, and Prof. Marks giving a speech at the gala dinner.

May 31st
Departure to Zlarin island & Visit to Plitvice National Park

The Plitvice Lakes National Park area is part of the Dinarides karst region, one of the most impressive karst landscapes in the world, marked by specific geological, geomorphological and hydrological properties. The karst relief is primarily tied to carbonate rock (limestone and dolomite rock) due to its strong sensitivity to chemical and mechanical wear, and the influence of tectonics (faults, wrinkles, fissures, etc.). Carbon dioxide enriched water penetrates through fissures in the carbonate rock, dissolving the rock as it flows, creating various surface (such as funnels, depressions, karst fields, towers, columns and more) and subsurface (caves and pits) karst forms. At the same time, the carbonate rich water creates barriers as it flows, making cascade waterfally and lakes. In Plitvice, by this mechanism, river Korana formed 16 cascade lakes and numerous waterfalls over the period of 2 million years, making Plitvice one of the most famous National Park in Croatia.





Networking, as a goal of the BioX Conference, is best seen in nature…

Ferry ride to Zlarin island

June 1st
Visit to Knin fortress & vine tasting

Knin Fortress is located near the tallest mountain in Croatia, Dinara, and near the source of the river Krka. It is the second largest fortress in Croatia and most significant defensive stronghold during the medieval ages. The construction of the fortress started as early as 9th century, while the current state was brought up in 17th and 18th centuries. Fortress reached its peak during the reign of Demetrius Zvonimir, King of Croatia from 1076, as it served as a political center of the Croatian Kingdom under him. Today, the area of the stronghold is a museum of Croatian history throughout the centuries and also the biggest museum of the Croatian Homeland War.

On the left, river Krka, and on the right, view of the Dinara mountain..

A few Knin expedition members, and the view from the Fortress

For centuries the sheeps were the only livestock adjusted to very harsh weather conditions in these parts. So even now, a lamb on a spit is a tradinitional meal to have when in Knin!

June 2nd
Sampling at Zlarin island

The sampling at the island took place on the coast and on the location of a pond on the top of a small hill, called "Lokvica". Lokvica is a manmade water tank that is constantly filled with rainwater, and as a freshwater pond in the middle of the island, represents a unique and interesting micro-ecosystem. The water samples were used for a workshop "Mobile microbiological laboratory" the next day.



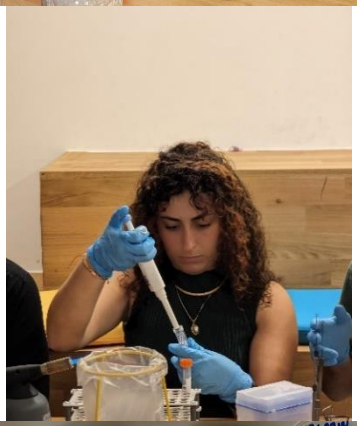
Lokvica on the left and traditional house water tank on the right



Sampling, and more importantly, overseeing the sampling…

June 2nd
Sampling at Zlarin island

The 'Mobile microbiological laboratory' workshop intended to demonstrate how a fully functional laboratory, respecting all the aseptic measures, can be fitted in a single suitcase, transported and get set-up on any location. In this case, the water samples were inoculated onto Potato Dextrose Agar and incubated at room temperature (22-25°C) for 72 h. Unexpectedly, school children attended the workshop during their visit to the Coral Center.

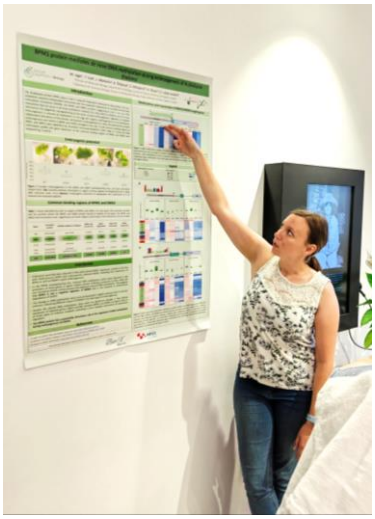


Plates after the inoculation and incubation:
Top – water from one of the public taps on the island
Middle – soil from the Lokvica site
Bottom – water from the Lokvica

June 3rd

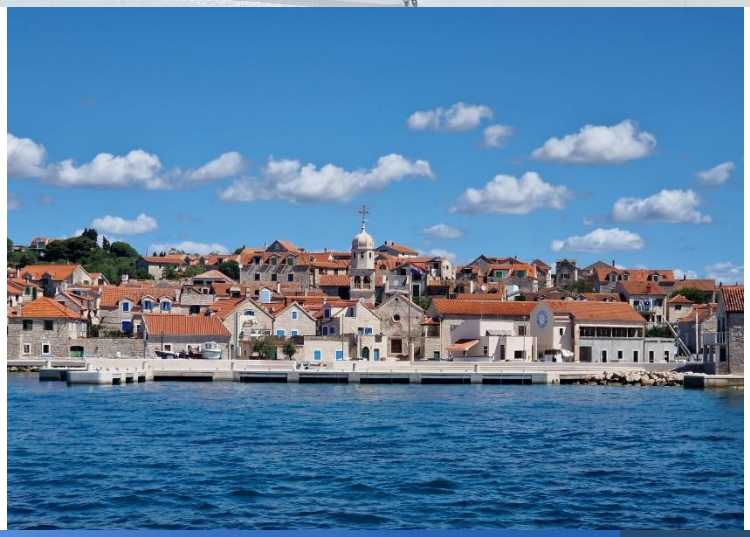
Poster session at the Coral Center Zlarin

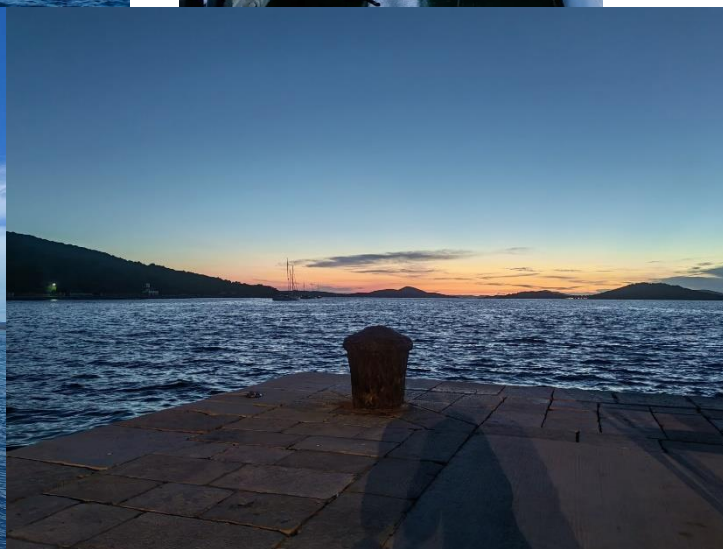
The Poster session took part at the Coral Center Zlarin since the area around the island was a very known location for coral harvesting since the 15th century. Now, the tradition of coral harvesting has almost disappeared but the Center keeps the memory and it's historical legacy. During the session, the best poster, as well as the winner of art competition, were awarded!



June 4th

End of the BioX Croatia 2024 and return to home





CONFERENCE TIMETABLE

WEDNESDAY 29th May, Zagreb, Faculty of Science

Morning Session I Chairs: Tomislav Ivankovic, Robert Marks		
Prof. Donald Martin, UGA, France *Making biotechnology from nanostructured systems*	Plenary lecture	9:15 – 9:45

Donald Martin is a full Professor in the Faculty of Pharmacy at the University Grenoble Alpes (UGA), France. He previously had the position as Chaire d'Excellence (research professor) in nanobiotechnology at UGA. He received his B.Optometry degree, a Masters degree in biomedical engineering and a PhD in biophysics of the eye and contact lenses, all from the University of NSW in Australia. He was awarded the inaugural postdoctoral fellowship from the Medical Foundation at the University of Sydney for postdoctoral studies in electrophysiology of secretory epithelia, then moved to St Vincent's Hospital in Sydney to work on electrophysiology of cardiac tissue. His career continued in Australia until 2009 when he moved to France with the award of a Chaire d'Excellence in Grenoble at UGA. In 2013 he was appointed to the Faculty of Pharmacy in UGA as a full professor. He is the head of the research team SyNaBi in the Laboratory TIMC (UMR 5525) at UGA. The scientific activities of his team SyNaBi include the development of ion-transporting systems for neuromorphic architectures capable of interfacing with excitable tissues. These architectures are included in novel microfluidic systems for interfacing to living cells. He has authored more than 130 publications, 17 patents, and is a co-founder in several startup companies in Australia and France, including 2 French startups that are commercialising medical diagnostic and therapeutic devices.

Prof. Dunja Leljak Levanic, PMF Zagreb, Croatia *"Healthy grapevine" – first patent at the Faculty of Science in Zagreb*	Keynote lecture	9:45 – 10:10

Dunja Leljak-Levanić graduated Molecular Biology at Faculty of Science at University of Zagreb. She obtained a PhD degree in 2001 on the topic of epigenetcs during plant somatic embryogenesis. After the PhD, Dunja continued scientific carreer on the topic of plant reproductive development and epigenetics. During her postdoctoral specialization at the University of Hamburg and Regensburg in Germany, her researches have expanded to plant functional genomics during reproductive development. Her scientific interests today are genetic and epigenetic mechanisams involved in plant reproduction and development. Dunja Leljak-Levanić is a full professor at Biology Department, Faculty of Science in Zagreb with courses Developmental Biology, Mechanisms of Plant Development, Scientific communication and Scientific Methodology in Biology.

Dr. Lucija Beluzic, Breyer Clinic, Zagreb, Croatia *Essential and additional strategies to achieve and maintain quality in genetic laboratories: Insights from a high-throughput diagnostic laboratory*	Invited lecture	10:10 – 10:25
Prof. Ariola Bacu, UT, Albania *Natural and primed HT tolerance at wheat – can we speak of cultivar-specific primed response?*	Invited lecture, ***online***	10:25 – 10:40

WEDNESDAY 29th May, Zagreb, Faculty of Science

Morning Session II Chairs: Marisa Manzano, Karolina Rudnicka		
Prof. Jasna Hrenovic, PMF Zagreb, Croatia *Carbapenem resistance in the environment*	Invited lecture	11:00 – 11:15
Prof. Marin Jezic, PMF Zagreb, Croatia Cryphonectria hypovirus 1 *spread across its host populations in Europe*	Invited lecture	11:15-11:30
Dr. Evgeni Eltzov, Volcani Institute, Israel *Rapid identification of quiescent* C. gloeosporoioides *in fruits: a new approach to minimize postharvest losses*	Invited lecture	11:30 – 11:45
Prof. Ivana Sola, PMF Zagreb, Croatia *Phytochemical adaptations of broccoli to elevated and decreased environmental temperatures: Implications for its nutritional value*	Invited lecture	11:45 – 12:00

Morning Student's Session, 5-7 min. Talks Chairs: Vanja Jurisic, Donald Martin, Michela Maifreni
Fredric Narcross, BGU, Israel *Footprints - social media meets knowledge discovery in a data sharing environment*
Abraham Paul, BGU, Israel *Structurally reinforced alginate Microbeads as versatile Biosensors for the detection of Quorum Sensing Molecules*
Karlo Spelic, PMF Zagreb, Croatia *Biogas production from* Miscanthus x giganteus *in a continuous anaerobic digestion system*
Blanka Dadic, PMF Zagreb, Croatia *Changes in microbial consortium composition during the cell immobilization on natural carriers*
Krisida Ciko, UT, Albania *In house validation of an LC-MS/MS multi method for the determination of mycotoxins*
Marina Drcelic, PMF Zagreb, Croatia *'Candidatus Phytoplasma solani': Chasing for protein interactions*
Xhensila Omeri, UT, Albania *Physical-biological indicators and single-cell toxicity sensing as a new monitoring model applied at Lake Butrinti, Albania (**online**)*
Gal Carmeli, BGU, Israel *Polypropylene (PP) and Polylactic acid (PLA) Biodegradation*

Afternoon Session I Chairs: Dunja Leljak Levanic, Evgeni Eltzov		
Prof. Robert S. Marks, BGU, Israel *From academic innovation in diagnostics to startup attempts*	Plenary lecture	14:15 – 14:55

Prof. Robert S. Marks is a Full Professor at the Ben-Gurion University of the Negev, Israel, at the Department of Biotechnology Engineering, where he created the interdisciplinary Biosensors Laboratory, and has affiliations there at The National Institute for Biotechnology in the Negev and the Ilse Kats Centre for Nanotechnology. In his PhD he worked on an oral vaccinate candidate to cholera. Robert has co-founded, and is the originator of the technologies, for the several companies in Israel, such as Biosensing Technologies Ltd, Biopixel Ltd. The present startups are Life Matters Ltd (Israel), Biosensorix Pte Ltd (Singapore) and Eclipse diagnostics (USA). Prof. Marks work includes developing new biosensors including chemiluminescent-based optical immunosensors to pathogen-elicited antibodies, amperometric immunosensors etc. His work also includes environmental toxicology, such as monitoring water pollution via fiber-optic probes glowing in the presence of toxicants through their associated luminescent bacteria He is the author of more than 200 papers and has 8 issued patents. Most of his developed biosensors were published and validated with real life samples.

Prof. Mustafa Culha, Augusta University, USA *Nanomaterials in cancer diagnosis and treatment*	Keynote lecture, ***online***	14:55 – 15:20

Prof. Culha is currently a Professor of Chemistry at Augusta University. He obtained his MS degree from Wake Forest University, NC, in 1997 and his Ph.D. at the University of Tennessee-Knoxville in 2002. Then, he joined in Advanced Biomedical Research Group as a post-doctoral researcher at Oak Ridge National Laboratory (2002-2003) before joining to Schering-Plough Corporation, NJ as an investigator. He accepted a faculty potion at Yeditepe University, Istanbul, Turkey, in 2004 and he involved in research and teaching at Genetics and Bioengineering Department more than 14 years. Then, he spent one and half years at The Knight Cancer Institute's Cancer Early Detection Advanced Research center (CEDAR) at Oregon Health and Science University as a visiting scientist. His current research interest includes elements from chemistry, medicine, material science, photonics, and nanoscience and nanotechnology. One of his major research directions is the biomedical applications of surface-enhanced Raman scattering (SERS). He and his colleagues have authored of more than 130 papers in refereed international journals, several book chapters and patents in the areas of analytical and bioanalytical chemistry, and nanobiotechnology. He is as Society for Applied Spectroscopy Fellow and also the president elect for The Federation of Asian Chemical Societies (FACS) for the 2023-2025 term.

Prof. Karolina Rudnicka, UniLodz, Poland *How to foster university collaboration with external partners – a case study of the Science Hub UL*	Invited lecture	15:20 – 15:35
Dr. Jovana Vunduk, IGPC Belgrade, Serbia *The role of biotechnology in psychedelic revival*	Invited lecture	15:35 – 15:50

WEDNESDAY 29th May, Zagreb, Faculty of Science

Afternoon Session II **Chairs: Jasna Hrenovic, Marin Jezic**		
Prof. Vanja Jurisic, AGR Zagreb, Croatia *Enhancing biogas production from agricultural energy crops by introducing ensiling and bioaugmentation processes*	Invited lecture	16:10 – 16:25
Prof. Marisa Manzano, UNIUD, Italy Listeria monocytogenes *detection: from plate count to an electrochemical biosensor method*	Invited lecture	16:25 – 16:40
Dr. Mark Nuijten, A2M- Minerva HE Network, Netherlands *Valuation of digital health: A comparison with innovative drugs*	Invited lecture	16:40 – 16:55
Dr. Michela Maifreni, UNIUD, Italy *Photo Dynamic Inactivation (PDI) technology: a new antimicrobial strategy against* Listeria monocytogenes	Invited lecture	16:55 – 17:10
Afternoon Student's Session, 5-7 min. talks **Chairs: Jovana Vunduk, Mark Nuijten, Ivana Sola**		
Eni Meli, UT, Albania *Assessments on the growth performance of Mediterranean mussel (*M. galloprovincialis *Mollusca, Bivalvia) reared in Butrinti Lake (South-Western Albania), according to evaluation of the parameters in VBGF*		
Nadav Bachar, BGU, Israel *Purification of water containing complex pollution using a biofilm system*		
Tomislav Mamic, PMF Zagreb, Croatia *The effects of mutations in the antiCas transcript and deletion of anti-cas gene on resistance to phage infection*		
Anxhela Kamberaj, UT, Albania *Estimation of growth parameters for a stock of Ohrid trout (*Salmo letnica *Karaman, 1924) based on the assessments of commercial catches in the Albanian part of Ohrid Lake.*		
Stela Pepa, UT, Albania *Length frequency distribution, growth parameters and mortality rates for a stock of bleak (*Alburnus scoranza *Heckel and Kner, 1857) from Ohrid Lake.*		
Ivan Brandic, AGR Zagreb, Croatia *Estimation of the HHV of biomass using the regression model of artificial neural networks*		
May Portman, BGU, Israel *Differentiating between diseases based on the chemiluminescent signature of phagocytes in peripheral blood*		
Closing of the Symposium in Zagreb		

ABSTRACTS

arranged according to the Conference Timetable

MAKING BIOTECHNOLOGY FROM NANOSTRUCTURED SYSTEMS

Donald Martin

Faculty of Pharmacy, University Grenoble Alpes, Grenoble, France

In this presentation I will explore the design and assembly of nanostructured systems with inspiration from biology. Here we consider the biological inspiration as not the approach typically adopted to construct synthetic materials inspired from nature. In contrast, the approach discussed in this presentation utilizes biological components in the assembly of smart nanostructured systems. The term “biological engineering” is used to describe a discipline that embodies this approach. Indeed, in 1970, this term was introduced formally with the intention to integrate engineering with biological systems to move beyond single disciplinary areas such as medicine, agriculture, or fermentation engineering. In this presentation we further refine the discipline of “biological engineering” to be one that utilizes biological proteins, molecules and lipids in combination with synthetic materials to assemble smart nanostructural systems. I will illustrate this discipline with the assembly of nanostructured systems that are targeted for applications such as diagnostic, therapeutic, biofuel cell, or tissue enhancement/replacement in the body.

„HEALTHY GRAPEVINE" – FIRST PATENT AT THE FACULTY OF SCIENCE IN ZAGREB

Dunja Leljak-Levanic, Nenad Malenica

Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Grapevine (*Vitis vinifera* L.) is one of the most important fruit species globally, offering both economic and nutritional value to numerous countries, including Croatia. The grapevine hosts about 80 different types of viruses, some of which cause serious economic problems including lower yield, shorter lifespan of vinyards and lower wine quality. Due to uncontrolled propagation of infected planting material in the past decades the majority of Croatia's cultivars are infected with some of the most harmful viruses: grapevine leafroll-associated virus 1 and 3 (GLRaV-1 and GLRaV-3), arabic mosaic virus (ArMV), grapevine fleck virus (GFkV) and grapevine fanleaf virus (GFLV). The most important elimination technique of viruses and other pathogens from infected plants is apical meristem culture. However, apical meristem isolation is very demanding, since the size of the explant must be below 0.5 mm for *V. vinifera* in order to reduce the risk of contact with the vascular system and the virus. In addition, a rather low survival rate of such small explants in tissue culture further decreases the overall success of the method. An alternative method used for virus elimination is somatic embryogenesis. Here, we report the results of a study in which somatic embryogenesis was initiated and standardised for a set of seven indigenous Croatian cultivars. Field-grown donor plants and SE-derived plantlets were analysed for the presence of 6 typical viruses GFLV, ArMV, GLRaV-1 -2, -3 and GFkV. The results showed that viruses GFLV, GLRaV-1, -3 and GFkV present in donor plants were successfully eliminated by the somatic embryogenesis. With this patented procedure it is possible to obtain a large number of healthy individuals from infected plant material in a relatively short time.

ESSENTIAL AND ADDITIONAL STRATEGIES TO ACHIEVE AND MAINTAIN QUALITY IN GENETIC LABORATORIES: INSIGHTS FROM A HIGH-THROUGHPUT DIAGNOSTIC LABORATORY

Lucija Beluzic

Polyclinic Breyer, Zagreb, Croatia

The delivery of high-quality laboratory medical services empowers physicians to make confident decisions regarding disease prevention, diagnosis, and treatment. Several factors are crucial for ensuring the effective functioning of a laboratory, as they intersect and influence one another. Based on our experience, any faults in these factors can have negative impact on result quality.

Careful planning and optimal space design are considered to be a foundation of any laboratory. The layout of the space must align with appropriate biohazard standards to protect both personnel and the environment, while also maintaining sample integrity. Moreover, efficient lab design will also limit unnecessary workflow steps, enhancing personnel productivity while minimizing errors. We have implemented three variations of space design, customized to specific lab protocols and expected sample volumes, each of which will be presented in more detail.

Another important aspect affecting quality is the laboratory personnel. Therefore, prioritizing investment in staff training is fundamental. Basic training in laboratory protocols is mandatory, complemented by specialized education tailored to the specific diagnostic procedures and individual team members.

Investing in automation and digitalization can further reduce error rates and enhance quality. Our laboratory management has adopted various levels of automation, balancing cost-effectiveness with rapid sample turnaround times. Examples ranging from manual to fully automated protocols will be showcased.

Molecular diagnostic laboratories require additional precautions to prevent sample cross-contamination, given the abundance of DNA fragments generated during amplification procedures. We will explore approaches that combine different strategies to prevent nucleic acid contamination, along with internally derived protocols for quality monitoring.

To ensure constant delivery of high-quality results over time, every laboratory must assess the necessary investments in space, personnel, automation, and contamination prevention measures.

NATURAL AND PRIMED HT TOLERANCE AT WHEAT - CAN WE SPEAK OF CULTIVAR-SPECIFIC PRIMED RESPONSE?

Ariola Bacu[1,2] , Krisida Ciko[3], Eniada Reci[1], Enia Rama[1], Vjollca Ibro[4]

[1] Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania
[2] Centre for Biotechnology & Genetics, Academy of Sciences of Albania
[3] Departament of Toxicology, Food Safety and Veterinary Institute-ISUV, Albania
[4] Department of Agricultural Sciences, Agricultural University of Albania

Despite the continuous global interest on the creation of transgenic abiotic stress resistant wheat cultivars, yet there are not approved lines for consumption and trade. This has triggered the need to analyse the molecular basis of natural resistance, as well as to find out possible trans-generational primed stress memory mechanisms. Wheat cultivars produced via selective breeding, and others of foreign origin, in use in Albania are being studied in order to discriminate the ones which display tolerance to environmental stresses (HT, draught, salinity), and to test conditions which may trigger plant's epigenetic memory. Here are presented data on the cultivar-specific differential response of 19 wheat cultivars (*Triticum aestivum* L.) to two subsequent HT treatments of 30ºC during early development (before anthesis), based on morphometric parameters, physiological phenomena (fine root cells death evaluated via fluorescence microscopy), biochemical synthesis (chlorophyll pigments, carbohydrates) and Relative Water Content (RWC). Based on the group's results, the possibility that the modified response of plants to repeated stress conditions could be considered as epigenetically regulated (primed) stress memory, is also discussed.

Acknowledgements:
The authors would like to acknowledge the support of this work by the project "Use of molecular tools for the early detection of Fusarium infection & Evaluation of tolerance towards abiotic stresses of wheat (*Triticum aestivum* L.) local cultivars in use in Albania", which is implemented under the National Program for Research & Development of Albania 2023-2024, funded by the Albanian National Agency for Scientific Research & Innovation (AKKSHI). Presentation's content is the responsibility of the authors, the opinions expressed in it are not necessarily the opinion of AKKSHI.

CARBAPENEM RESISTANCE IN THE ENVIRONMENT

Jasna Hrenovic

Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Bacterial resistance to β-lactam class of antibiotics, carbapenems, is found as intrinsic in environmental autochthonous species. Emergence of bacteria with acquired resistance to carbapenems gains much attention in clinics during the past 20 years. In Croatia, carbapenem resistance of two clinically important bacteria increased from 2008 to 2022 from 10 % to 99 % for *Acinetobacter baumannii* and from 0.3 % to 24 % for *Klebsiella pneumoniae*. During the last decade One Health approach opened the problem of the presence of clinically relevant carbapenem-resistant bacteria in environment. Here, the overview of the presence of these problematic bacteria in natural environment in Croatia is given.

Clinically relevant carbapenem-resistant gram-negative bacteria were recovered from polluted environment influenced by human solid and liquid waste, but not from the pristine environment. Dissemination of carbapenem-resistant bacteria was found via untreated hospital wastewaters, to urban sewage, wastewater treatment plant and Sava River as natural recipient of treated wastewater. Input of untreated urban and hospital wastewater resulted in the presence of carbapenem-resistant bacteria in water and sediment of Krapina River. Carbapenem-resistant bacteria were found in soils at illegal dump sites, swine manure and agricultural soils fertilized with manure.

Clinically relevant bacteria with acquired resistance to carbapenems in the environment are the source of community-acquired and consequently, nosocomial infections. Disinfection of potentially infective waste prior to its discharge in environment is obligatory to avoid a public health risk.

CRYPHONECTRIA HYPOVIRUS 1 SPREAD ACROSS ITS HOST POPULATION IN EUROPE

Marin Ježić[1], Mirna Ćurković-Perica[1], Lucija Nuskern[1], Karla Peranić[1], Deborah Marie Leigh[2], Daniel Rigling[2], Simone Prospero[2], Igor Poljak[3], Marilena Idžojtić[3]

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Invasive species are usually a significant stressor for the native species in an ecosystem. When these alien species are parasitic or even pathogenic, invasive species cause direct harm to the native ones, instead of just outcompeting and replacing them. A sac fungus *Cryphonectria parasitica* has been a prime example of an unintentionally introduced phytopathogenic species that has devastated native chestnut forests across North America and Europe since the beginning of the 20th century. It causes a disease called chestnut blight characterised by the appearance of the cankers: wounds and necrotic lesions appear on the bark of the trees and can cause dieback of the affected branches or entire stems. Fortunately, an infectious RNA, later determined to be an unencapsidated virus named *Alphahypovirus cryphonectriae* (CHV1) was discovered in 1960s and shown to have an attenuating effect on its fungal host's virulence, making the blight disease less severe. Several subtypes and many unique genotypes of CHV1 have been detected across Europe with highly variable effects on *C. parasitica*. Subtype F1 (French) usually has a severe effect on the host's physiology (e.g. growth rate) and fertility (sporulation), while the effect caused by the infection with subtype I (Italian) usually has milder symptoms. This is reflected in the population structure of CHV1 in Europe – the much more "severe" F1 subtype has been found thus far only in a few locations in France, despite being often artificially introduced by humans as a means for biological control of the disease. On the other hand, the "milder" I subtype is widespread across many *C. parasitica* populations in Europe, as the virus is often transmitted via the conidia, the production of which is not as strongly affected by the I subtype.

RAPID IDENTIFICATION OF QUIESCENT *COLLETOTRICHUM GLOEOSPORIOIDES* IN FRUITS: A NEW APPROACH TO MINIMIZE POSTHARVEST LOSSES

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The problem of postharvest food losses is a major issue, having been estimated at 40% to 50% of harvested crops worldwide, mostly due to rots caused by fungi. After penetrating the unripe fruit, *C. gloeosporioides* (e.i., pathogenic fungi) remain quiescent ("sleeping") until the fruit ripens. The quiescent infections are microscopic and cannot be visually detected during packaging or subsequent transport. Thus, there is a need to design assays that allow the identification of the fungi at an initial quiescent stage of infection to prevent potential fruit decay during the supply chain and consumer storage. A rapid and easy-to-use paper-based LAMP assay was designed for detecting the enoyl CoA hydratase quiescent marker of *C. gloeosporioides*. The developed method requires a cheap cellulose membrane and heat block, enabling this method to be employed in resource-limited settings. The paper-based LAMP assay evinces superior specificity as it effectively prevented the formation of spurious products during amplification. The assay was found highly specific for the quiescent stage of *C. gloeosporioides* with an analytical sensitivity of 0.5 pg of total extracted RNA. The developed assay generated the results within 40 min and hence can be efficiently employed for identifying *C. gloeosporioides* presence and pathogenicity states in resource-limited settings. The unique ability of the proposed system to detect and recognize the fungus during the quiescent (latent) stage will decrease food losses by allowing improved postharvest management. For example, fruit with a high inoculum rate will be sold to the local market or as processed food, whereas fruit with low inoculum rates can be stored for long periods or exported.

PHYTOCHEMICAL ADAPTATIONS OF BROCCOLI TO ELEVATED AND DECREASED ENVIRONMENTAL TEMPERATURES: IMPLICATIONS FOR ITS NUTRITIONAL VALUE

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This work aimed to investigate the impact of low (LT) and high (HT) temperatures on the phytochemical composition of broccoli microgreens (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.) and to assess the biological effects of their extracts. Our goal was to identify sensitive phytochemical parameters as potential markers of LT/HT stress and provide insights for optimizing temperature conditions to enhance the concentration of specific compounds and antioxidant effects for producers and consumers of microgreens. We measured the effects of LT and HT on different groups of phenolics, total glucosinolates, proteins, and soluble sugars, photosynthetic pigments, plant hormones, vitamin C, and individual phenolic acids and flavonoids, and on antioxidant capacity of broccoli extracts. The data collected were statistically analyzed using one-way analysis of variance, Pearson's correlations, and principal component analysis to evaluate differences between samples and visualize relationships among parameters. The results showed that LT increased total phenolics and tannins in broccoli. Total glucosinolates were also increased by LT; however, they were decreased by HT. Soluble sugars, known osmoprotectants, were increased by both types of stress, considerably more by HT than LT, suggesting that HT causes a more intense osmotic imbalance. Both temperatures were detrimental for chlorophyll, with HT being more impactful than LT. HT increased hormone indole-3-acetic acid, implying an important role in broccoli's defense. Ferulic and sinapic acid showed a trade-off scheme: HT increased ferulic, while LT increased sinapic acid. We suggest that parameters responsive to one type of temperature stress, but not the other could be potential mediators crucial for plants' adaptation to LT/HT stress. These findings contribute to understanding the physiological responses of broccoli microgreens to temperature stress and could aid in optimizing growing conditions to enhance their phytochemical composition and bioactivity.

"*FOOTPRINTS*" – SOCIAL MEDIA MEETS KNOWLEDGE DISCOVERY IN A DATA SHARING ENVIRONMENT

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Three trends appear ripe for consolidation and mutual benefit: use of social media in daily life, use of artificial intelligence technology for knowledge discovery in data repositories, and the sharing of raw data to promote advancement in research. The advantages to consolidating these three trends under a single umbrella are significant.

- Reusing raw data for the knowledge discovery potential that is within it promotes growth and development of vital research areas like biotechnology and biomedicine. It further promotes advancement in these areas for diagnostics and remediation and enables more researchers to perform more research without the expense of new, time-consuming and expensive experimental procedures.
- The application of artificial intelligence technology is proving to be transformative across many industries especially in biotechnology and biomedicine. Tools, like AlphaFold that can predict protein folding patterns from amino acid sequences, as well as molecular AI models like Umol promise to expedite drug design and discovery. There are many further tools in use to perform chemical analysis of compounds, sequence strands of RNA and DNA, and to perform enzyme studies. Cancer therapeutics, and visual diagnostics of radiological images are also standout examples.
- Surrounding these tools and data with an easy-to-use social media interface places the knowledge discovery potential of the tools within a framework that is common, easy to use, and familiar. It further enables widespread sharing of useful techniques within groups of users who share similar interests and on a global scale. It also encourages a 24/7/365 conference-like environment where users can turn for help, assistance, or can share their own techniques and tools that have benefitted them.

"*Footprints*" is a platform, which brings these three trends together and holds promise to reinvent how research work might be supplemented and advanced within a social media user supported environment, where data sharing supports knowledge discovery.

REPORTER BACTERIA STRAINS ENCAPSULATED WITHIN POLY-LYSINE-REINFORCED ALGINATE MICROBEADS AS VERSATILE BIOSENSORS FOR THE DETECTION OF QUORUM SENSING MOLECULES

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Human *Pseudomonas aeruginosa* infection remains a significant public health challenge due to its extensive antibiotic resistance. The pathogenesis of *P. aeruginosa* is closely linked to quorum sensing (QS), which involves the communication of bacteria through signaling molecules called autoinducers. The QS molecules have emerged as important biomarkers for detecting *P. aeruginosa*. To this end, a whole-cell biosensor was developed to detect a broad range of bacterial autoinducer (homoserine lactone, HSL) concentrations. The bioreporter bacteria-based biosensor was developed by immobilizing two genetically modified strains (LasR and RhlR) of *P. aeruginosa* within poly-lysine-reinforced alginate microbeads. When the immobilized reporter bacteria were incubated with bacterial culture or growth media spiked with synthetic autoinducer or quorum sensing inhibitor (QSI, furanone-C-30), HSL and QSI molecules could diffuse into the microbeads, eliciting a dose-dependent biological response. This whole-cell bacteria biosensor offers a direct and quantitative detection of sub-nanomolar concentrations of synthetic C_4-HSL and C_{12}-HSL that are common in the QS signaling molecules of *P. aeruginosa*. Notably, the biosensor is versatile and can detect bacterial QS molecules in real-life samples (such as different stages of biofilms and bacterial cultures) without requiring additional sample preparation steps. The microbeads exhibit high operational and storage stability for over 40 days at 4 °C. Upon 30 minutes of preincubation at 37 °C, the previously stored (-80 or 4 °C) biosensor microbeads are ready to use, making it a robust on-demand biosensor for the detection of *P. aeruginosa* with potential applications in clinical and environmental settings.

BIOGAS PRODUCTION FROM MISCHANTUS X GIGANTEUS IN A CONTINUOUS ANAEROBIC DIGESTION SYSTEM

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This study investigates the potential of *Miscanthus x giganteus* as a sustainable feedstock for biogas production in a continuous anaerobic digestion system. Given the increasing need for renewable energy sources, miscanthus presents a viable alternative due to its high yield potential and low maintenance. The experiment measured biogas yield and quality comparing it with traditional corn silage feedstock. Results indicate that *Miscanthus* can produce a comparable amount of biogas with a significant methane content, suggesting its potential as a sustainable biogas source.

*Full paper for this abstract is available

CHANGES IN MICROBIAL CONSORTIUM COMPOSITION DURING THE CELL IMMOBILIZATION ON NATURAL CARRIERS

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The aim of this study was to determine which bacterial species from microbial consortium, previously isolated from activated sludge, have a greater affinity for immobilization on different natural carriers. A microbial consortium was isolated and conditioned for bioaugmentation of biogas-producing reactors using *Miscanthus X giganteus* as a substrate, and chosen carriers were natural zeolitized tuff, ZeoSand®, perlite, and crushed corncob. The identification of grown colonies from the bacterial suspension and the colonies obtained after immobilization was achieved through the cultivation on nutrient agar plates and subsequent usage of MALDI-TOF mass spectrometry method. The results indicate that certain bacterial species from the bacterial suspension did not exhibit a strong affinity for specific carriers, whereas *Enterobacter cloacae* demonstrated an ability to be immobilized in largest numbers on each tested carrier. Other dominant species in the consortium were *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter asburiae*, *Leclercia adecarboxylata* and *Exiguobacterium indicum*.

Furthermore, the goal was to determine the number of immobilized bacteria on each natural material. The highest rate of immobilization of microbial consortium was obtained on perlite, followed by ZeoSand®, crushed corncob and natural zeolitized tuff. Due to their porous structure, suitable surface for immobilization, and non-toxicity, all natural materials could be appropriate carriers of the microbial consortium.

IN HOUSE VALIDATION OF AN LC-MS/MS MULTI METHOD FOR THE DETERMINATION OF MYCOTOXINS IN WHEAT

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Mycotoxins are secondary metabolites secreted by many fungal species present in plants during their pre-and post-harvest, transportation, processing and storage, and often found in food and feed. In particular wheat grains are susceptible to contamination with various *Fusarium* mycotoxins such as Deoxynivalenol (DON), Zearalenone (ZEN), T-2 toxins, HT-2 toxin, Fumonisin B1, Fumonisin B2 etc, As their presence can cause disease and death both in humans and livestock, a fundamental step for ensuring public health is the development of highly sensitive, robust, selective, quick, easy, and multi-analyte extraction and detection method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was the method of choice for the quantification of the main mycotoxins, inclusive of some mycotoxins that are currently regulated acc to EU 2023/915, and emerging mycotoxins such as Enniatins (not included in the previous EU regulation), in plant material (wheat seeds of 20 *Triticum aestivum* L. cultivars in use in Albania). Extraction of mycotoxins was performed using a modified QuEChERS extraction in the presence of the acidified aqueous extraction and organic solvent. Matrix-matched calibration curves were established, and limits of quantification were below the maximum levels (0.5 µg/kg (each of the total Aflatoxins) to 50 µg/kg (DON). According to 2782/2023_EU, LOQ shall be ≤0.5*ML or should preferably be ≤0.2*ML). Recoveries ranged between 70 and 120%, fulfilling the EU legislation (SANTE 11312-2021). Results demonstrated that the procedure was suitable for determining 23 mycotoxins in wheat grains.

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'*Candidatus* Phytoplasma solani': CHASING FOR PROTEINE INTERACTION

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'*Candidatus* Phytoplasma', formally known as phytoplasmas, are diverse, pleomorphic pathogen bacteria that live inside plant phloem sieve cells and cause various plant diseases. They can infect a wide variety of plants, including many economically important crops around the world. These bacteria rely on plants and insects for their survival and are spread by insects - vectors that feed on plant sap. Despite efforts, phytoplasma *in vitro* cultivation has not been established yet, which makes studying them difficult.

'*Ca.* P. solani', has a large and highly variable genome compared to other phytoplasmas. Understanding how '*Ca.* P. solani' interacts with its hosts on protein level and what are the protein effectors that contribute to its ability to adapt to different environments and hosts, requires application of different biotechnological scientific approaches. Combining different methods such as floral dip, yeast-2-hybrid and agroinfiltration could provide valuable insights into management and assessment the risks associated with this pathogen. By studying its genetic diversity and how it spreads, we can develop better strategies for controlling and preventing its impact on crops.

PHYSICAL-BIOLOGICAL INDICATORS AND SINGLE-CELL TOXICITY SENSING AS A NEW MONITORING MODEL APPLIED AT LAKE BUTRINT, ALBANIA

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The assessment of surface and underground water quality in Albania has been carried out for years based on standardized methods for measuring physical-chemical indicators, trophic level (ISO 17025), and bacteriological indicators (ISO16649-3:2015), and lately is implemented the use of cellular biosensors to assess the cytotoxicity of waters (ISO 16649-3:2015).This paper will refer to the results on the quality of waters of Butrint Lake in Albania and the problems encountered, using conventional standardized methods (ISO) and advanced methods of biotechnology (single-cell biosensors, CARD-FISH, fluorescence microscopy, Flow Cytometry, Factorial analysis of bacterial DNA to environmental factors, Genetic diversity of phytoplankton under conditions of pollutants of different categories, etc.).The quality of waters is among the most discussed issues in the context of climate change, and so do the methodologies used to assess it. In this context, we believe that a combined use of monitoring protocols with those of scientific research, can provide more complete and reliable results on water quality and their complex relationship with the geological content of soils, hydrology, climatic conditions and human interactions.

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*Full paper for this abstract is available

DEVELOPMENT OF A RAPID ASSESMENT METHOD FOR EVALUATING THE BIODEGRADATION OF POLYPROPYLENE AND POLYLACTIC ACID

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The persistence of synthetic polymers like Polypropylene (PP) and Polylactic Acid (PLA) in the environment poses significant ecological threats due to their resistance to natural degradation. Our laboratory, which has been actively involved in studying the biodegradation of different types of plastics, has successfully isolated three bacterial strains that have demonstrated biodegradation capabilities toward plastics such as polyethylene (PE) and Polyethylene Terephthalate (PET). These strains include *Rhodococcus ruber* (C208), *Brevibacillus agri* (712), and *Brevibacillus borstelensis* (707).

In this research, we focus on developing a rapid assessment method to evaluate the biodegradation of PP and PLA using these strains. The method involves a short incubation period of polymer samples, including those coated with various additives, in a medium low in carbon sources under controlled conditions. Analytical techniques such as Fourier-transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) were utilized to evaluate the biodegradation of these polymers.

The results revealed that after a short 21-day incubation period, we were able to observe significant changes in the polymers' FTIR analysis. After incubating PP samples with *R. ruber* (C208) alone, no significant changes were detected. However, by using a bacterial mix of all three strains, we observed substantial changes, with up to a 26.49% change in the FTIR peak ratios for algae-coated PP samples. For PLA, the bacterial mix also demonstrated enhanced degradation effects compared to *R. ruber* (C208) alone.

SEM results showed adhesion of the bacteria to the polymers and biofilm formation, which are crucial steps in the biodegradation process. However, no significant changes in the polymers' surface structure were observed, suggesting that a longer incubation period may be required to detect surface-level changes.

These findings underscore the potential of our rapid assessment method as an effective tool for evaluating microbial degradation of plastics.

FROM ACADEMIC INNOVATION IN DIAGNOSTICS TO STARTUP ATTEMPTS

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An academic career innovating in the field of biosensors and diagnostics has led to the usual output in publications (https://orcid.org/0000-0002-9697-3805) and patents, most of which were abandoned by the institution, save those that led to the creation of the start-ups I founded. Some companies closed usually for lack of funding or team complications, some still in operation, but no serious investments nor buyouts. In short no true successes, so far. The lecture will cover the landscape and issues the academic encounters in making the jump into the cut-throat world of business. It is a worthwhile challenge for some, but the academic must be aware: he is on his own with truly little from his own institution save cosmetic help.

NANOMATERIALS IN CANCER DIAGNOSIS AND TREATMENT

Mustafa Culha

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There is an enormous effort to utilize nanomaterials and nanostructures for disease diagnosis and treatment. In this presentation, first I will discuss our research effort for label-free cancer diagnosis using surface-enhanced Raman scattering, a very sensitive vibrational spectroscopic technique, where gold and silver nanostructures are used as substrates. Then, I will summarize our effort to utilize DNA-origami based nanostructures, gold nanoparticles, BNNTs and hBNs as drug and antisense oligonucleotide carriers.

HOW TO FOSTER UNIVERSITY COLLABORATION WITH EXTERNAL PARTNERS – A CASE STUDY OF THE Science Hub UL

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The presentation explores the development and implementation of Science Hub (SH) at the University of Lodz in Poland. Science Hub aims to foster university collaboration with external partners, including local communities, NGOs, cities, and private entities, which is an example of cooperation between the University and its socio-economic environment, in line with the concept of the knowledge-based economy. The initiative builds upon the model of science shops, defined as structures that facilitate science–society collaborations to address civil society concerns. In Science Hub, the collaboration with external partners is extended beyond public and social partners and includes the industry partners. Thus, SH bridges academia with the public, private and third sectors. The operational framework of SH focuses on fostering communication, securing partnerships for collaborative projects, and appointing faculty ambassadors to champion and sustain the integration of SH within our institutional framework. A pivotal element of this initiative was the launch of an open competition aimed at financing mini-projects that involve collaboration between external partners, students, and researchers. A critical requirement for participation was an initial consultation to align the external partner's needs with achievable research objectives under expert mentorship. We successfully funded 40 diverse projects, yielding practical solutions, experimental results, expert analyses, and evaluative studies that not only fulfilled but often exceeded our partners' expectations. Twelve of these projects originated from the Faculty of Biology and Environmental Protection. During my presentation, I will explain the process of mini-projects implementation, highlighting challenges, solutions and benefits for all parties: students, scientists, and partners. I will speak about the development and implementation of the various elements of the Science Hub operational framework. I aim to emphasise profound benefits it brings to our university, our external collaborators, and our students, thus exemplifying a proven solution resulting in the impact on society.

Overall, I will present the SH initiative as a valuable case study, offering insights and reference material for organizations considering similar socially engaged endeavors.

The project is co-financed by the state budget under the program of the Minister of Education and Science "Science for Society", project number NdS/543803/2021/2022.

THE ROLE OF BIOTECHNOLOGY IN PSYCHEDELIC REVIVAL

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The Anthropocene became Artificialinteligencene while humanity indulged in technology. While we progress towards an ever more efficient, fast, and technologically developed society we encounter the severe burden of mental illnesses, led by depression and anxiety. The initial promise, that antidepressants will be the solution given by the pharmaceutical industry in the middle of the 20th century, failed. According to the World Health Organization, today 280 million people have depression while 700 000 die of it every year. Seems like we lost our souls and reason while artificial intelligence is steadily taking our place. Psychedelics became the new solution proposed by Western science and businesses. Known for millennia by ancient native communities and practiced by their shamans, psychedelics like hallucinogenic mushrooms, are now forbidden and classified as Schedule I drugs.

This prevented research for decades but only recently their potential as novel antidepressants came to the fore. However, it should not be assumed that the research community went back to the tribal practices and performed experiments with raw mushrooms. Instead, synthesized psilocybin entered the scientific arena and became its focal point. Around 100 natural producing mushroom species are not reliable enough since the active compound cannot be controlled as a pure substance. Yield in classical chemical synthesis is not sufficient and expensive. The solution came in the form of genetically modified organisms paired with chemical modifications. This enabled the production of a satisfactory amount of psilocybin within 72 h. The question is if synthetic psilocybin will bring us the promised depression-free new world.

ENHANCING BIOGAS PRODUCTION FROM AGRICULTURAL ENERGY CROPS BY INTRODUCING ENSILING AND BIOAUGMENTATION PROCESSES

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Biogas production is a key technology for reducing greenhouse gas emissions and increasing energy independence, with agricultural energy crops recognized as a sustainable alternative to traditional feedstocks such as corn silage. The research investigates the increase of biogas production from agricultural energy crops through ensiling and bioaugmentation. The aim of the research was to determine the production of biogas and biomethane in bioaugmented bioreactors in batch and continuous processes. The energy crops used were *Miscanthus x giganteus*, *Arundo donax* and *Panicum virgatum* grown on marginal land and natural habitats. In batch bioaugmentation process, bacteria were isolated from the anaerobic sludge and immobilized on perlite, which improved the enzymatic degradation of the biomass. The research highlights the potential of energy crops to replace maize silage and achieve EU targets to reduce greenhouse gas emissions by 2030 and 2050 and increase energy independence.

LISTERIA MONOCYTOGENES DETECTION: FROM PLATE COUNT TO AN ELECTROCHEMICAL BIOSENSOR METHOD

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Listeria monocytogenes is a high-risk food pathogen that can cause infections in weak and immunocompromised individuals. Listeriosis is a foodborne invasive disease, which occurs following ingestion of contaminated food (RTE products, dairy products, meat, raw ham, fish, smoked fish, and vegetables). The currently recommended ISO 11290-1:2017 standard method (horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.) although sensitive and able to ensure the compliance with microbiological criteria, requires a long time, up to 7 days for identification confirmation and is not quantitative. Molecular methods generally are more cost-effective than conventional culture-based methods, due to the reduced detection time, are more specific, but requires the extraction of DNA from food samples before the utilization of specific primer for application in PCR.

Nowadays, specific, rapid and sensitive detection for pathogens is possible by the utilization of biosensors, devices which combine fundamental biological, chemical, and physical sciences with engineering and informatics to satisfy needs of a wide range of sectors, including food safety. Food industries require rapid protocols that can provide results in short times to avoid recalls with economic losses. Among the various bioreceptors used for target detection biosensors which utilize ssDNA probes are called genosensors and are successfully used in food analyses. Moreover, other ssDNA short sequences of DNA able to fold in a 3D- structure (aptamers) can be used to build aptasensors, biosensors used for the specific detection of proteins, toxins, metals and whole cells.

PHOTO DYNAMIC INACTIVATION (PDI) TECHNOLOGY: A NEW ANTIMICROBIAL STRATEGY AGAINST *LISTERIA MONOCYTOGENES*

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Antimicrobial Blue Light (aBL) has been recently discovered as a non-thermic technology for sanitising food-related environments. This technique is based on the combined action of Blue Light (400-480 nm), oxygen, and endogenous or exogenous Photosensitizers (PS), which lead to the formation of Reactive Oxygen Species (ROS), responsible for microbial death. aBL efficacy has been performed against different pathogens and its microbial inactivation capacity depends on Light Dose (D), wavelength, and microbial species.

This study proposed to exploit the antimicrobial activity of Blue LED Lights at 405, 420 and 450 nm, against *L. monocytogenes*, exploiting its endogenous PS.

L. monocytogenes is a food-ubiquitous pathogen which could be present in food-related contexts in its planktonic form or biofilm. For this concern, the study aims to understand the inactivation capacity of this food pathogen in both its forms.

The microbial inactivation was assessed on agar plates and in liquid medium. The direct assessment of cells in agar plates evidenced that a D > 300 J cm-2 was able to inactivate the tested microbe at all the wavelengths completely.

Decreasing the D (< 300 J cm-2), results differed based on the wavelength used.

In the liquid medium, the application of D = 300 J cm-2 showed a different *L.monocytogenes* behaviour of inactivation at the three tested wavelengths. During all the experiments, the temperature was monitored every.

The second part of the study was focused on the antibacterial activity of mature biofilm after exposure under blu light. The 13-day biofilm was analysed related to viable cells and the biofilm structure was observed by Confocal Laser Microscope (CLSM). Both the cell counts, and the microscope images evidenced an effect of the Blue Light treatment on the mature biofilm of L. monocytogenes.

The results of this research highlighted that aBL is a powerful technology for microbial inactivation in food-related environments; however, the inactivation efficiency depends on the wavelength, the microbial species, and the dose.

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ASSESSMENTS ON THE GROWTH PERFORMANCE OF MEDITERRANEAN MUSSEL (*M. GALLOPROVINCIALIS* MOLLUSCA, BIVALVIA) REARED IN BUTRINTI LAKE (SOUTH-WESTERN ALBANIA), ACCORDING TO EVALUATION OF THE PARAMETERS IN VBGF

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In Albania, since the 60s of the last century, the commercial aquaculture of Mediterranean mussel (*M. galloprovincialis*) is located only in Butrinti Lake. In the period between April and December 2023 we have estimated the parameters in the VBGF, the instantaneous rate of natural mortality (*M*), the predicted length at first maturity (*L*m), and some growth performance indices for mussels reared in the panel-structures of aquaculture system. In April the average value of shell length was *SL*=34.91±1.821 mm, while in December this parameter has the value *SL*=63.11±4.273 mm. The maximum value of *SL*, found during sampling in the aquaculture farm was *SL*max=66.9 mm. The average value of specific growth rate index was *SGR*=0.210±0.039. The following values were estimated by as for the parameters in VBGF: asymptotic length $L\infty$ = 76.2 mm; annual growth coefficient *K* = 0.23/yr; theoretical or expected age at length zero *t*o = -0.566 yr; the theoretical lifespan *t*max = 7.04 yr. For the shell length was found this Von Bertalanffy's growth function: $Lt = 76.20\ [1- e^{-0.230\ (t+0.566)}]$. The value of the predicted length at first maturity was *L*m = 45.15 mm. The value of instantaneous rate of natural mortality was *M* = 0.457/yr. The overall growth performance index had the value *OGP* = 5.008, while the value of ϕ'-index, or the growth performance index, was ϕ' = 3.12. The value for theoretical age at which the length achieved 50% of $L\infty$ (*t*50%) was 2.54 yr. This study marks the start of the involvement of stock assessment procedures in Albanian aquaculture.

Acknowledgements:
The authors would like to acknowledge the support of this work by the project "Evaluation of the quality and citotoxicity of waters at Vlora Bay for a clean Mariculture environment.", which is implemented under the National Program for Research & Development of Albania 2023-2024, funded by the Albanian National Agency for Scientific Research & Innovation (AKKSHI). Presentation's content is the responsibility of the authors, the opinions expressed in it are not necessarily the opinion of AKKSHI.

*Full paper for this abstract is available

PURIFICATION OF WATER CONTAINING COMPLEX POLLUTION USING A BIOFILM SYSTEM

Nadav Bachar,Osher Gueta, Engineers without bordersBGU brunch
Acknowledge: Saar David, Micha Mirkin, Noam Polani, Shaked Partush

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In 2023, a delegation from Engineers Without Borders (EWB) BGW embarked on a pivotal initiative, targeting the Massai villages in northern Tanzania. This endeavor was aimed at the enhancement of living conditions, with a specific focus on improving water quality and accessibility—a cornerstone for sustainable community development. The approach adopted was comprehensive, integrating a variety of water purification methodologies to address this critical issue.

The interventions employed were multifarious, incorporating sedimentation processes using ash and limestone, alongside the deployment of slow sand filtration systems. The latter technique is characterized by its dual mechanism of action: mechanical purification achieved through percolation of water through sand beds, and biological purification via the establishment of a biofilm on the surface of the filtration medium. This synergistic process significantly ameliorated the water's quality, evidenced by a dramatic reduction in turbidity levels from in excess of 10,000 Nephelometric Turbidity Units (NTU) to below the threshold of 5 NTU. Such an outcome not only denotes a remarkable improvement in water clarity but also signifies compliance with the World Health Organization's (WHO) potability standards for developing regions.

Looking forward, the commitment of EWB BGW extends beyond the scope of initial interventions. There is a concerted effort to foster ongoing collaboration with the Massai communities. Future endeavors will revisit these villages to disseminate the employed methodologies, while also facilitating a bilateral exchange of knowledge. This initiative aims to integrate indigenous insights with scientific advancements, thereby co-developing more
refined and efficacious water purification solutions. This strategy is anticipated to underpin sustainable enhancements in local water infrastructure, thereby catalyzing a profound and lasting uplift in the community's standard of living and overall well-being.

THE EFFECTS OF MUTATIONS IN THE *antiCas* TRANSCRIPT AND DELETION OF *anti-cas* GENE ON RESISTANCE TO PHAGE INFECTION

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The CRISPR-Cas adaptive immune system protects many bacteria and most archaea from invading DNA. In *E. coli,* the CRISPR-Cas system is silenced by the global repressor H-NS under normal laboratory growth conditions. However, in cells lacking H-NS and containing anti-lambda spacers, CRISPR-Cas-mediated resistance to phage λ*vir* is highly temperature dependent. It is active at 30 °C and inactive at 37 °C. A short anti-cas transcript of 373 nt, which is controlled by the divergently oriented anti-Pcas promoter, is also regulated by the H-NS repressor. When this gene is overexpressed from the plasmid, it inhibits CRISPR-Cas-mediated resistance to phage λ*vir* at 30 °C by an unknown mechanism. In this study we investigated the effects of different mutations in the *antiCas* transcript and deletion of the first 153 nt of the *anti-cas* gene on CRISPR-Cas-mediated resistance to λ*vir* infection. The indel mutations in the *antiCas* transcript had a slightly reduced inhibitory effect on CRISPR-Cas activity. Deletion of the *anti-cas* gene resulted in smaller plaque size and lower plaque forming units (PFU) count at 37 °C, suggesting that *antiCas* transcript contributes in part to reduced CRISPR-Cas activity at elevated temperature.

ESTIMATION OF GROWTH PARAMETERS FOR A STOCK OF OHRID TROUT (*SALMO LETNICA* KARAMAN, 1924) BASED ON THE ASSESSMENTS OF COMMERCIAL CATCHES IN THE ALBANIAN PART OF OHRID LAKE

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Ohrid trout (*Salmo letnica*) is a endemic fish species from Ohrid Lake (South-East of Balcan Penisula). In this paper are presents some informations about growth features of this species, based on assessments of the commercial catches in the Albanian part of the Lake. Were assessed the coefficients *a* and *b* in the allometric relationship between total length (TL,cm) and total weight (W,g) (LWR), the allometric condition factor (*K*') and the parameters ($L\infty$,*K*,*t*o and *t*max) in the Von Bertalanffy's growth function (VBGF). The values of coefficients in LWR were: initial growth coefficient *a*=0.0161 and the slope *b*=2.956. The value for allometric condition factor was *K*'=1.624. For the parameters of VBGF were calculatet these values: asymptotic length $L\infty$=53.46 cm, annual growth coefficient *K*=0.192/year, the "age" at length 0 *t*o=-1.41 years and the maximum theoretical age *t*max=15.6 years. The obtained values for LWR coefficients shows almost isometric growth for the assessed stock of Ohrid trout. The walues for VBGF shows a greater similarity with the limnophilic species of the genus Salmo than with the species that live in the rivers.

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The authors would like to acknowledge the support of this work by the project "Evaluation of the quality and citotoxicity of waters at Vlora Bay for a clean Mariculture environment.", which is implemented under the National Program for Research & Development of Albania 2023-2024, funded by the Albanian National Agency for Scientific Research & Innovation (AKKSHI). Presentation's content is the responsibility of the authors, the opinions expressed in it are not necessarily the opinion of AKKSHI.

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LENGTH FREQUENCY DISTRIBUTION, GROWTH PARAMETERS AND MORTALITY RATES FOR A STOCK OF BLEAK (*ALBURNUS SCORANZA* HECKEL AND KNER, 1857) FROM OHRID LAKE.

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The bleak (*A. scoranza*) is a native fish species within the Ohrid-Drin-Skadar drainage. Aiming the bleak's stock assessment in the Ohrid Lake, we have studied the length-frequency distribution and have estimated some growth parameters from VBGF, the theoretical age at which the total length achieves 50% of $L\infty$ (t50%), and the instantaneous rate of natural mortality (M). The average values for total length and total weight were: TL, cm=11.71±2.45 (Var%=20.92) and W, g=12.87±6.58 (Var%=51.11). The values of growth parameters in VBGF were: the asymptotic length $L\infty$=20.76 cm; the annual growth coefficient K=0.198/yr and the hypothetical age the fish would have had at zero length to=-1.023 yr. The value of t50% was 2.4 yr and the value of natural mortality parameter was M = 0.484/yr. It resulted that in comparison to other studies, carried out for the stocks of *A. scoranza* in the Skadar Lake, the stock of Ohrid Lake is distinguished by smaller size individuals as well as by the lowest values of growth parameters.

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The authors would like to acknowledge the support of this work by the project "Evaluation of the quality and citotoxicity of waters at Vlora Bay for a clean Mariculture environment.", which is implemented under the National Program for Research & Development of Albania 2023-2024, funded by the Albanian National Agency for Scientific Research & Innovation (AKKSHI). Presentation's content is the responsibility of the authors, the opinions expressed in it are not necessarily the opinion of AKKSHI.

ESTIMATION OF THE HIGHER HEATING VALUE OF BIOMASS USING THE REGRESSION MODEL OF ARTIFICIAL NEURAL NETWORKS

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The conducted study investigates the application of artificial neural networks (ANN) to estimate the higher heating value (HHV) of different biomass types based on input data from the ultimate and proximate analysis. The results show that the developed ANN model trained by 100,000 cycles achieves high performance in training (R^2=0.93) and testing (R^2=0.99) with a low degree of error. This approach highlights the possibility of using machine learning techniques to improve the planning and optimization of biomass applications and suggests a scalable model for wider applications.

*Full paper for this abstract is available

DIFFERENTIATING BETWEEN DISEASES BASED ON THE CHEMILUMINESCENT SIGNATURE OF PHAGOCYTES IN PERIPHERAL BLOOD

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We have innovatively designed a sensitive diagnostic tool for differentiating viral and bacterial infections. This tool is based on the unique chemiluminescent pattern of circulating phagocytes in peripheral blood. Phagocytes, including neutrophils and monocytes, are immune cells that are pivotal in both the early and late stages of immune responses. Their primary function is to circulate and migrate through tissues to ingest and destroy both microbes and cellular debris. The destruction of ingested material is facilitated by the generation of reactive oxygen species inside the phagolysosomes of the phagocytes. They are the key players in the organism's innate non-adaptive immune response to infection, forming the first line of defense against intruders in the human body.

During an infection, the host's whole-blood phagocytes are 'primed', meaning they are pre-tuned for 'future tasks', reflecting the host's readiness for defense. This unique characteristic of peripheral blood phagocytes holds high predictive value, making them a sensitive infection marker. This predictive value can be harnessed to distinguish between viral and bacterial infections, making our diagnostic tool a promising avenue for future research and application.

Upon interaction and phagocytosis of harmful invading microorganisms, peripheral blood phagocytes produce large amounts of toxic oxygen radicals, known as reactive oxygen species (ROS). This occurs through the activation of the NADPH-oxidase complex enzyme, in a process commonly referred to as respiratory burst. In the presence of luminol, used as a substrate amplifier, the ROS generation process is accompanied by detectable and measurable light emission. This light emission is a result of a process known as Luminal Chemiluminescence (LCL), which is a key component of our diagnostic tool.

Our research has revealed that the innate cellular defense mechanism is inherently abnormal or altered by an acquired disease (i.e., infection), leading to detectable changes in the phagocytes' cellular chemiluminescence pattern. This means the cellular chemiluminescence pattern is different after the immune system encounters a bacterial versus a viral infection. To fully harness this knowledge and recognize the cellular chemiluminescence pattern specific to the disease-causing phylum, we have innovatively developed a pattern recognition algorithm. This algorithm, based on LCL readings from a whole-blood system, will enable us to differentiate between viral and bacterial infections, marking a significant advancement in our understanding and diagnosis of infectious diseases.

POSTER PRESENTATIONS

COVALENTLY SYNTHESIZED ALGINATE-PYRROLE HYDROGEL AS A 3D PRINTABLE ELECTROCONDUCTIVE INK

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Electrically conductive hydrogels are investigated actively for potential applicability in biosensing, cellular interface, and tissue engineering. Because conventional hydrogels lack electrical conductivity, efforts are being made to incorporate conductive materials into such hydrogels in a facile but stable manner. Hydrogels of alginate composite are particularly attractive because of their tunable viscoelasticity, excellent biocompatibility, and ease of preparation. In this study, the alginate-pyrrole composite was covalently synthesized via EDC/NHS mediated conjugation between the carboxyl group of the alginate and amino group of the synthesized aminopropyl pyrrole monomer, followed by the spectroscopic characterization of the composite using UV-visible, NMR and FTIR spectroscopies. The hydrogels having variable alginate/pyrrole ratio were prepared by physical crosslinking using Ca^{2+} ion, while $FeCl_3$, $(NH4)_2S_2O_{8,}$ and H_2O_2 were evaluated for oxidative chemical synthesis of polypyrrole from the pyrrole monomer. The resulting hydrogels were subjected to rheological assessment, electrical conductivity study, and morphological characterization using a rheometer, 4-point probes, and SEM. Finally, the composite hydrogels were processed and used as 'ink' for extrusion-based 3D printing in optimized chemical composition, partial crosslinking, printing speed, and pressure conditions. The obtained composite hydrogel exhibited excellent electrical conductivity and high printability with optimal extrusion pressure and rheological properties. Thus, the alginate-pyrrole composite has potential applicability in the tissue engineering of excitable cells and in the biofunctionalization of electrodes in developing electrochemical biosensors.

UTILIZING ENVIRONMENTAL SAMPLING, BLANKS AND ASSAY CONTROLS FOR RAPID TROUBLESHOOTING IN GENETIC LABORATORIES

Lucija Beluzic, Julijana Marinac, Matea Katic, Tonci Milardovic, Tea Mejovsek, Antonija Crnecki, Doroteja Berakovic, Sara Kevic Desic, Ivana Marusic, Maja Fijan, Nikolina Katalenic

Polyclinic Breyer, Zagreb, Croatia

Next-generation sequencing (NGS) laboratories demand stringent cleanliness levels to avoid contamination events. External contaminants can lower enzymatic activity and affect sensitive reactions. Additional sources of nucleic acid, such as foreign material introduced via testing materials or laboratory personnel, pose additional contamination risks. Cross-contamination may occur when DNA fragments from previous sample analyses contaminate subsequent samples.

From January 2019 to January 2024, NGS laboratory at Polyclinic Breyer issued over 75,000 reports for noninvasive prenatal testing (NIPT). Each analysis, conducted in a 96-well plate format, included blank and procedural assay controls (positive and negative). Regular environmental monitoring, involving air and surface sampling every 1.5 months, was implemented. Blank and environmental samples were assessed midway through the protocol, while positive and negative controls were evaluated post-completion of the laboratory procedure and bioinformatic analyses.

Data obtained from control wells facilitated fast troubleshooting in cases where deviations were noticed: values were over recommended limits or outliers when compared to average laboratory values. First example included elevated concentrations of positive control that prompted investigation and subsequent communication with the reagent manufacturer. Second issue appeared with elevated blank values, persistently increasing despite several rounds of cleaning procedures. A couple of isolated instances of unusually high blank values were also detected.

Despite elevated positive control levels, real sample results remained unaffected, permitting continued use of the reagent lot. On the other hand elevated blank values were traced to human DNA contamination in a specific reagent lot, leading to its immediate discontinuation by the manufacturer. Isolated events of high blanks were resolved through thorough cleaning of automated liquid handling machine, preventing further cross-contamination.

Regular monitoring of environmental cleanliness and reagent quality enables prompt and informed decision-making, saving time and mitigating potential expenses. Preparedness, through the development of standardized operating procedures (SOPs) for cleaning and addressing adverse events, significantly enhances response times.

ASSESING HEAT STRESS TOLERANCE OF ARABIDOPSIS SEEDLINGS WITH ALTERED *DMS3* EXPRESSION

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The DEFECTIVE IN MERISTEM SILENCING 3 (DMS3) protein plays a pivotal role in the RNA-directed DNA methylation (RdDM) mechanism. RdDM is essential for maintaining genome stability and regulating gene expression, ensuring adaptation to environmental challenges throughout the plant's life cycle. To investigate the role of DMS3 in the response of *Arabidopsis thaliana* (L.) Heynh. to heat stress, the wild type (wt), a line overexpressing the *DMS3* gene (*oeDMS3*), and a line with a mutated *DMS3* gene (*dms3-1*) were exposed to 37 °C for 6 hours. Photosynthetic efficiency, proline content, and HSP90 protein level were evaluated immediately after the treatment and after 24-hour recovery at optimal temperature. All three lines showed reduced photosynthetic rate immediately after the treatment, with the *dms3-1* line displaying a decline even after recovery. Proline content decreased in all three lines immediately after the treatment and returned to control levels in the wt and *oeDMS3* line. However, the *dms3-1* line showed significantly increased proline content after recovery compared to the corresponding control. HSP90 protein accumulated in treated seedlings of all three lines at both time points, with the *dms3-1* line showing the lowest basal expression under control conditions and the highest accumulation after the treatment. These results suggest that heat stress had a stronger effect on the *dms3-1* line than on the wt and *oeDMS3* line, highlighting the importance of a functional DMS3 protein for heat tolerance at the seedling stage.

BPM1 PROTEIN MEDIATES *DE NOVO* DNA METHYLATION DURING EMBRYOGENESIS OF *ARABIDOPSIS THALIANA*

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The Arabidopsis BPM1 protein is a member of the widespread MATH-BTB protein family. MATH-BTB proteins participate in numerous plant developmental processes, including stress responses and embryogenesis. In addition to the important role of some MATH-BTB proteins in ubiquitin-dependent proteasomal degradation of specific proteins involved in flowering, seed development, embryogenesis and abiotic stress response, BPM1 establishes ubiquitin-independent interactions with the important components of the RdDM mechanism, the RDM1 and DMS3 proteins, suggesting its possible role in de novo DNA methylation. RNA-directed DNA methylation (RdDM) is one of the key mechanisms for epigenetic reprogramming during the onset of plant embryogenesis, regardless of whether embryogenesis is induced by fertilization of the egg cell (zygotic embryogenesis) or by fertilization-independent stimulation of the somatic cell (somatic embryogenesis, SE). In this work, we have shown that the potential for SE is mediated by the presence of BPM1 and DMS3. Furthermore, common binding regions of BPM1 and DMS3 in the Arabidopsis genome were identified by chromatin immunoprecipitation. Of the identified target genes, FBW2 and RKP were selected for further analysis, while CML41, a gene known to be regulated by RdDM, was selected as a control gene. DNA methylation profile and gene expression were analyzed in zygotic and somatic embryos of lines overexpressing BPM1 (oeBPM1) or DMS3 (oeDMS3), in line with downregulated BPM1, 4, 5 and 6 (amiR-bpm) and in line with impaired RdDM function (*dms3-1*). The results suggested a stimulatory role of the BPM1 protein in RdDM, and the mechanism was more pronounced in zygotic embryogenesis than in somatic embryogenesis.

FULL PAPERS

BioX Conference & Scientific Mission
May 28th to June 4th Zagreb and Zlarin

NATURAL AND PRIMED HT TOLERANCE AT WHEAT - CAN WE SPEAK OF CULTIVAR-SPECIFIC PRIMED RESPONSE?

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Abstract

Despite the continuous global interest on the creation of transgenic abiotic stress resistant wheat cultivars, yet there are not approved lines for consumption and trade. This has triggered the need to analyse the molecular basis of natural resistance, as well as to find out possible trans-generational primed stress memory mechanisms. Wheat cultivars produced via selective breeding, and others of foreign origin, in use in Albania are being studied in order to discriminate the ones which display tolerance to environmental stresses (HT, draught, salinity), and to test conditions which may trigger plant's epigenetic memory. Here are presented data on the cultivar-specific differential response of 19 wheat cultivars (*Triticum aestivum* L.) to two subsequent HT treatments of 30ºC during early development (before anthesis), based on morphometric parameters, physiological phenomena (fine root cells death evaluated via fluorescence microscopy), biochemical synthesis (chlorophyll pigments, carbohydrates) and Relative Water Content (RWC). Based on the group's results, the possibility that the modified response of plants to repeated stress conditions could be considered as epigenetically regulated (primed) stress memory, is also discussed.

Keywords: *HT-High Temperature, apoptosis, fluorescence microscopy, RWC*

Acknowledgements: The authors would like to acknowledge the support of this work by the project "Use of molecular tools for the early detection of *Fusarium* infection & Evaluation of tolerance towards abiotic stresses of wheat (*Triticum aestivum* L.) local cultivars in use in Albania", which is implemented under the National Program for Research & Development of Albania 2023-2024, funded by the Albanian National Agency for Scientific Research & Innovation (AKKSHI). Presentation's content is the responsibility of the authors, the opinions expressed in it are not necessarily the opinion of AKKSHI.

Introduction

Heat stress threatens agriculture worldwide, however, it is already proven that plants can acquire heat stress tolerance through priming, which establishes stress memory during mild or severe transient heat stress (1, 5). The heat tolerance has been linked to increased thermo-tolerance of the photosynthetic apparatus (1), maintenance of better membrane thermo-stability, low level of ROS accumulation due to improved antioxidant capacity, as compared with the non-primed plants (1). The impact of heat priming during early vegetative stages (before anthesis) (1, 2, 4), during the stem-elongation stage, booting and anthesis (3) on the post-anthesis phases resulted in higher grain yield under a subsequent high temperature stress. Furthermore, the positive effect of heat priming on the response to heat stress during grain filling was pronounced differently in plants primed at the booting stage than in those primed at the stem-elongation or anthesis stage (3). Another study direction related to priming has to do with the differentiated tolerance displayed by winter wheat plants after low heat priming (LP) than moderate heat priming (MP). LP at early growth stage was shown to be beneficial to sustain to heat stress during flowering stage, while MP at early growth stage helped winter wheat to better adapt to heat stress at booting stage (2); Also, effects of heat priming applied to the first generation on tolerance of the successive generation to post-anthesis high temperature stress were investigated, and thermo-tolerance was reported to be induced through heritable epigenetic alternation and signaling transduction (4). Proteome analysis indicated that the proteins involved in photosynthesis, energy production and protein destination and storage were up-regulated in the primed versus non-primed plants. Another approach used to explain the impact of priming under severe heat stress was through metabolomic analysis. It seems that altered energy pathways, and the so-called crosstalk between carbohydrate metabolism and tyrosine metabolism, can bring increased production of branched-chain amino acids, raffinose family oligosaccharides (RFOs), lipolysis products, and tocopherols that are responsible for the improved tolerance (5). Following previous reports the described impact of priming may be attributed to the effectively alleviated photosynthetic and oxidative damage, and enhanced carbohydrate remobilization (1); significantly higher photochemical efficiency (1); a better redox homeostasis as exemplified by the higher activities of superoxide dismutase (SOD) in chloroplasts and glutathione reductase (GR), and of peroxidase (POD) in mitochondria (1); maintenance of better membrane thermo-stability, and low level of ROS accumulation due to improved antioxidant capacity (1); higher sucrose contents and sucrose-phosphate activity in leaves and greater above-ground dry matter; increased stomatal conductance and chlorophyll (3); Genes related to signal transduction, transcription, energy, defence, and protein destination and storage, respectively, were also differently expressed according to the transcriptome profile (4). Among the last, the gene encoding the lysine-specific histone demethylase 1 (LSD1), which was involved in histone demethylation related to epigenetic modification was found to be up-regulated in the PH compared with NH (4); The metabolomic analysis speaks of a crosstalk between two glycerophospholipid pathways (the biosynthetic pathways of the thermo-memory metabolite S-adenosyl-L-homocysteine and the terpenoid backbone) and the δ-tocopherol (chloroplast lipid) pathway, which favours the production of glycine betaine and other essential tocopherols, respectively, compounds which are essential for abiotic stress tolerance in plants (5).

Materials & Methods

Plant material and Germination

Seeds of 19 cultivars of winter wheat (*Triticum aestivum* L.) were planted in plastic pots in soil with the following content: organic soil material (95%), and the rest limestone fertilizer, clay, perfit, nitrogen, phosphorus, and potassium (NPK) fertilizers, and other secondary materials, pH value: 5.5, salt content 1.2 g/l potassium chloride. After planting, seeds were allowed to germinate and grow under normal

lighting conditions and temperatures ranging from 15°C to 22°C (control conditions), while being periodically watered with tap water for three weeks.

HT Treatment

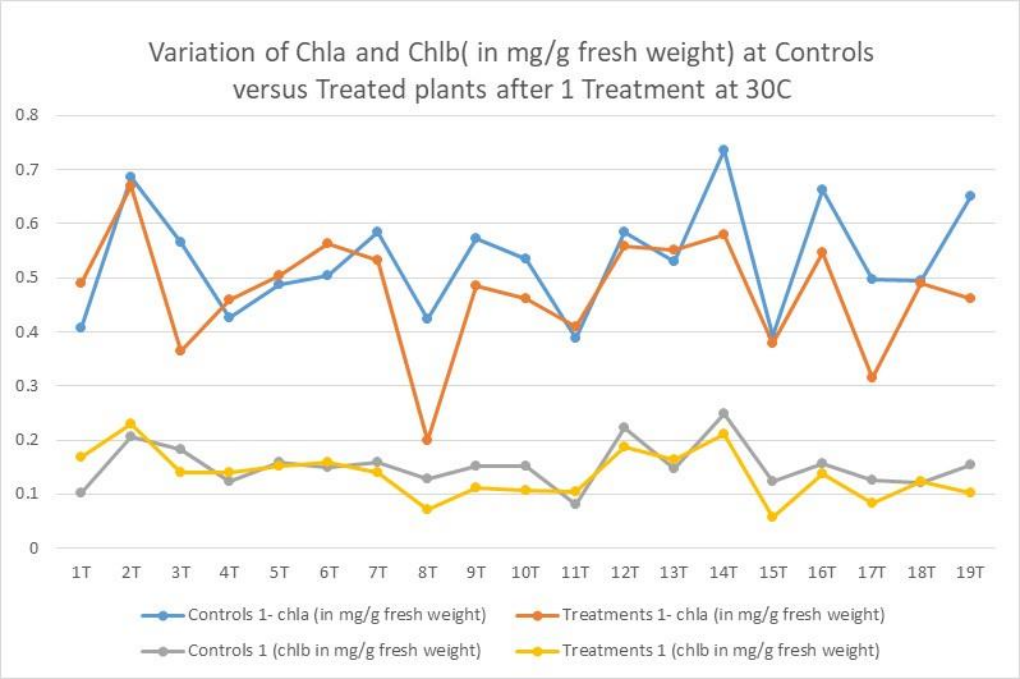
Three weeks old plantlets were exposed at 30°C for 1 hour, then kept in control conditions for 48 hrs, and subsequently exposed to a second HT treatment of 30°C for 1 hour.

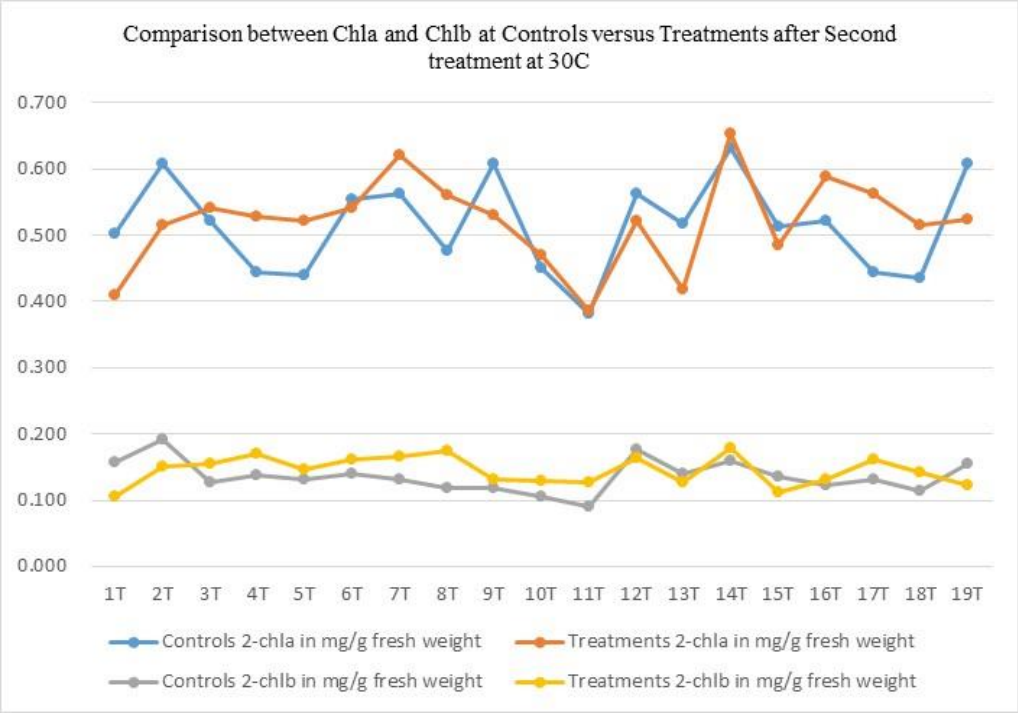
Parameters measured

- Morphometric traits (total plant length, root length, stem length, and lengths of the first three leaves) were measured manually 7 days after each treatment;
- Relative Water Content (RWC) was measured following [7] 48hrs after each treatment;
- Leaf pigment content was evaluated according to [6] 48hrs after each treatment;
- Total carbohydrates: were determined by standard anthrone method 48hrs after each treatment.
- Fine root cell mortality was determined (72hrs after each treatment) by counting dead cells based on staining the root cell chromosomes with DAPI, and visualizing via fluorescence microscope (OPTICA) equipped with an Optikam PRO6 Digital Camera. For each sample measurements were repeated 5 times in different square areas of the same dimensions.

Results & Discussions

Photosynthetic pigments (chla, chlb, carotenoids)





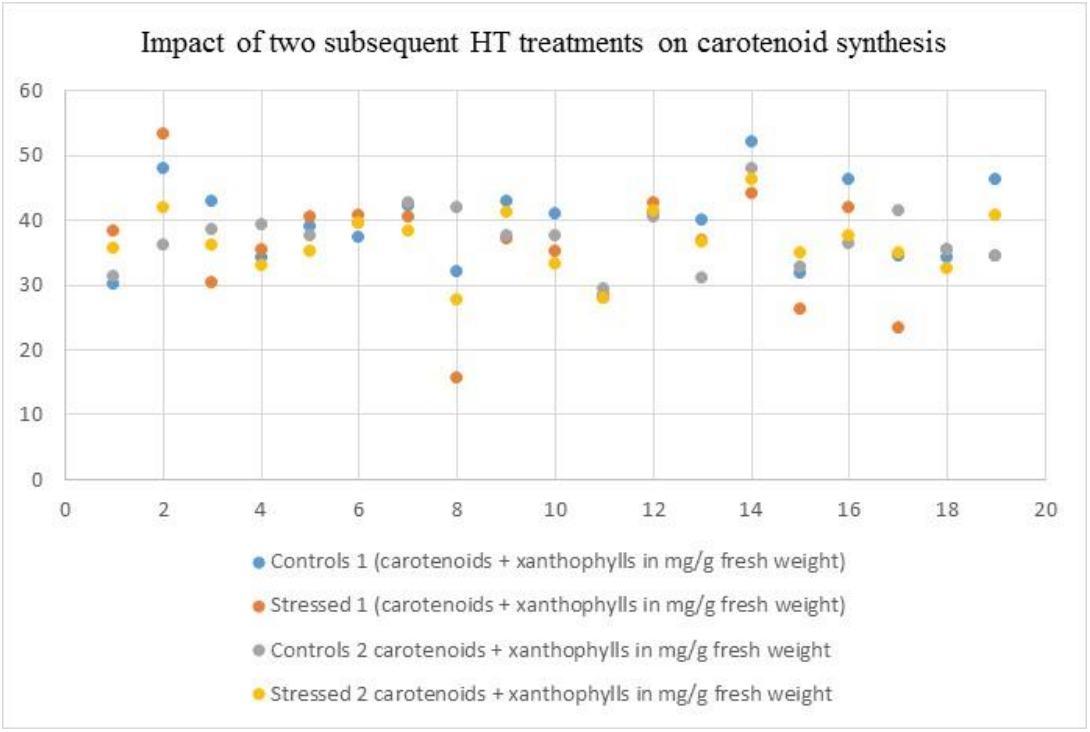
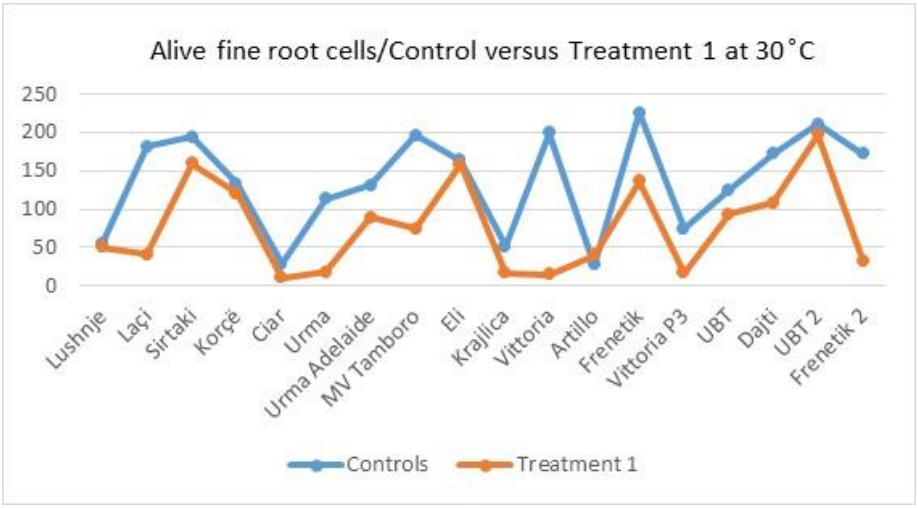


Figure 1. Impact on pigment synthesis of two subsequent HT applications at wheat cultivars. a. Upper graphic: Results of first treatment; 11/19 cultivars synthesize less chla and chlb after the first treatment; b. Middle graphic: Results of second treatment. 5/19 synthesize less chla and chlb after the second treatment. C. Lower graphic: Carotenoid synthesis, 10/19 cultivars synthesize less carotenoids and xantophylls after the second treatment compared to the first.

Fine root cell mortality:

After the second treatment with HT the number of cells which do survive is higher than after the first treatment (Fig 2). This speaks on a triggered epigenetic response.



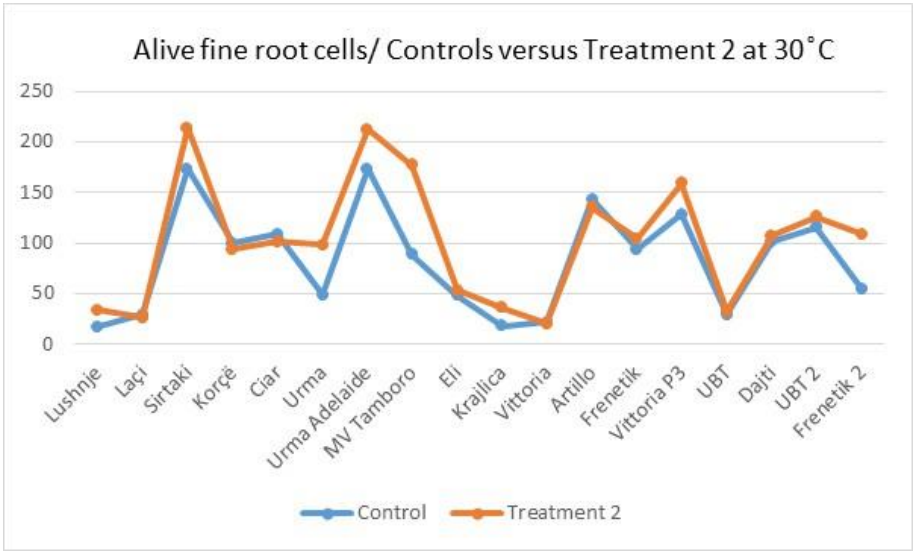


Figure 2. Results on the mortality of fine root cells exposed to two subsequent HT treatments based on staining the chromosomes with DAPI, and visualizing via fluorescence microscopy.

Total Carbohydrates:

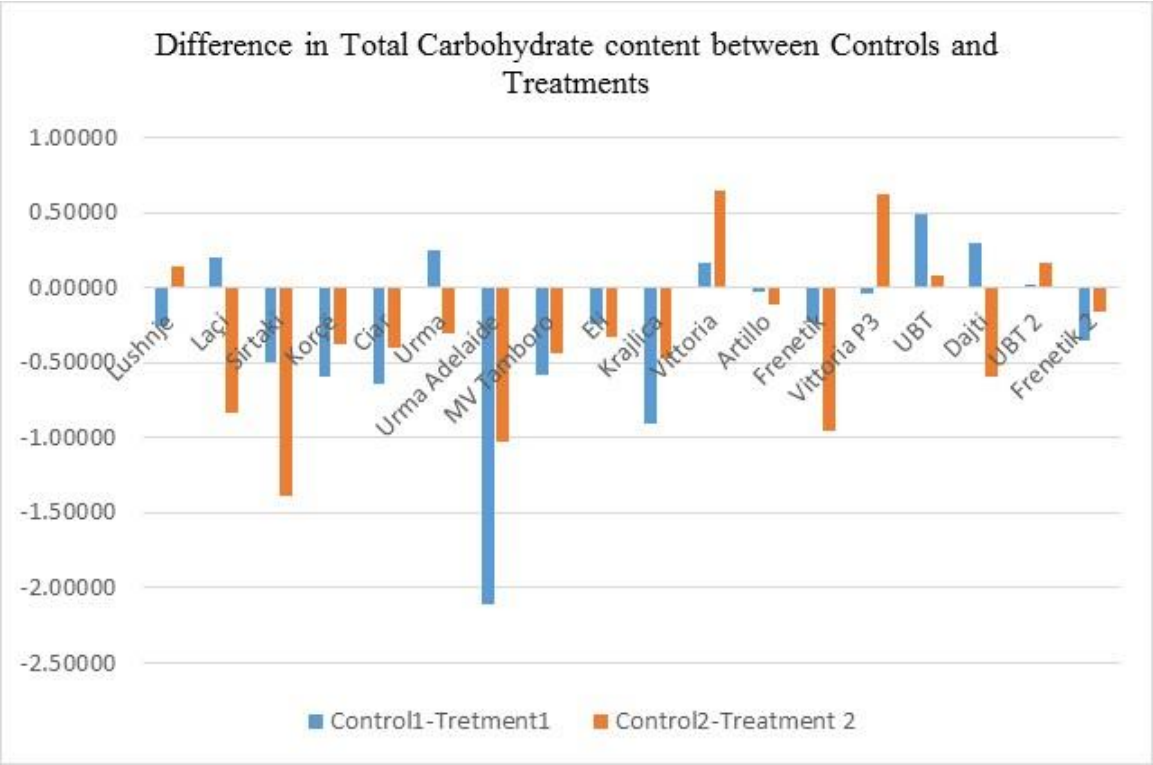


Figure 3. Impact of two subsequent HT applications on total carbohydrates at wheat cultivars before anthesis: Positive values show that controls have higher carbohydrate content compared to stressed plants.

The difference in the amount of total carbohydrates between control and treated plants shows that for 7/19 cultivars the amount is higher after the second treatment (Fig3a/3b).

Relative Water Content (RWC)

At 11/19 cultivars the RWC after the 2nd treatment is decreased compared to after the 1st treatment. Based on the results the response to HT treatment is cultivar-specific, but, in any case the RWC values do not remain the same after the two subsequent treatments.

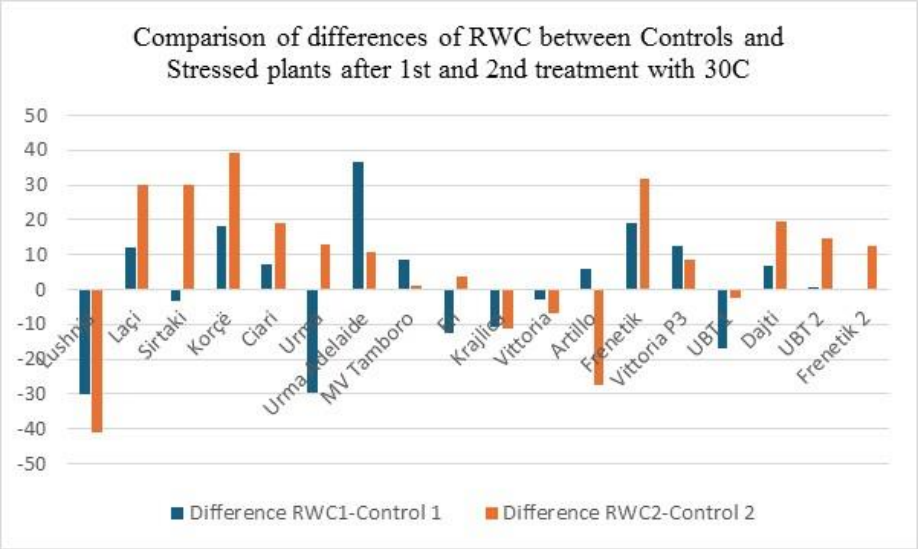


Figure 4. Impact of two subsequent treatment with 30C on RWC at wheat cultivars. Positive values prove that RWC at controls is higher than at treated plants, and vice versa for negative values.

Morphometric parameters

Impact of HT on root (Fig 5) and stalk length was cultivar-specific.

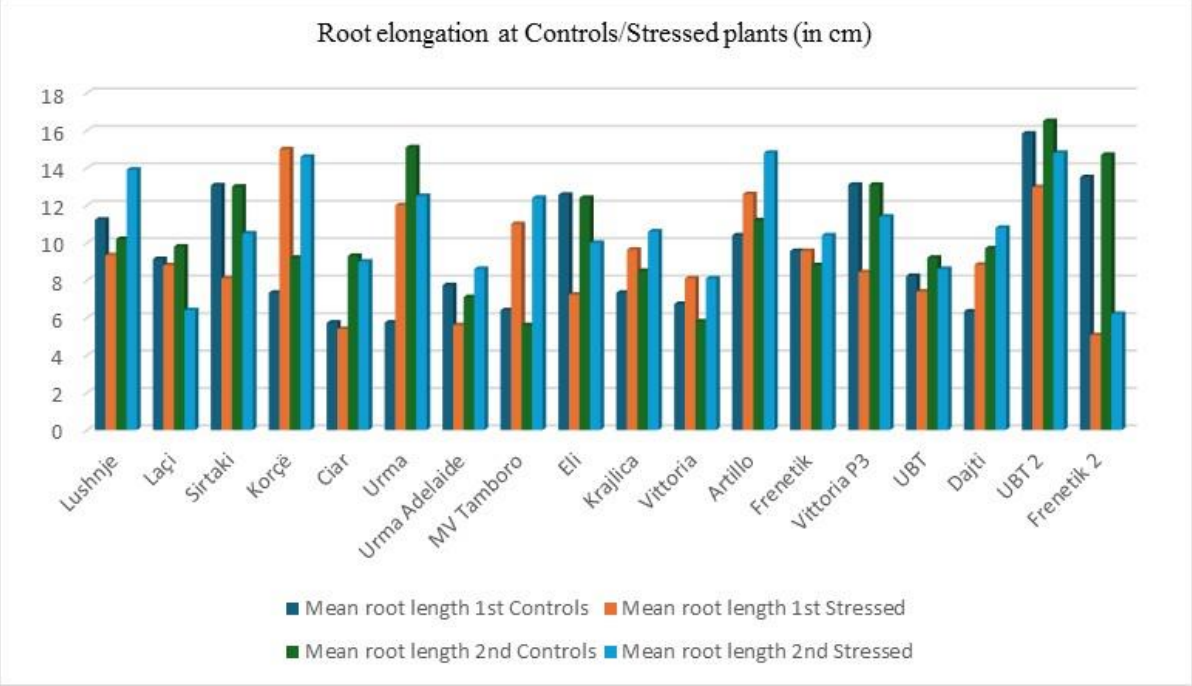


Figure 5. Impact of two subsequent treatment with 30C on morphometric parameters.

In conclusion, the impact of two consecutive treatments with HT (30C) at wheat seedlings before anthesis on morphometric parameters, pigment synthesis, total carbohydrate metabolism, fine root cells mortality, and RWC is cultivar-specific; However, in a group of 19 cultivars (*Triticum aestivum* L.) it was evidenced that the number of cultivars which displayed impaired pigment synthesis after the first treatment, was lowered after the second treatment; All cultivars survived better the fine root cells mortality at 2nd treatment; The total carbohydrates were higher at 7/19 cultivars after 2nd treatment; and RWC decreased at 11/19 cultivars after the 2nd treatment. These results speak on a different response of plants to the two consecutive treatments of the same intensity (30C), which could be considered as epigenetically regulated cultivar-specific primed stress memory.

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ESTIMATION OF THE HIGHER HEATING VALUE OF BIOMASS USING THE REGRESSION MODEL OF ARTIFICIAL NEURAL NETWORKS

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Abstract

The conducted study investigates the application of artificial neural networks (ANN) to estimate the higher heating value (HHV) of different biomass types based on input data from the ultimate and proximate analysis. The results show that the developed ANN model trained by 100,000 cycles achieves high performance in training (R^2=0.93) and testing (R^2=0.99) with a low degree of error. This approach highlights the possibility of using machine learning techniques to improve the planning and optimization of biomass applications and suggests a scalable model for wider applications.

Keywords: Modelling , analysis, calorific value, energy properties.

Introduction

Biomass is one of the most important and abundant renewable energy resources in Europe, and it is used in several ways: it can be used directly through combustion, by converting it into electricity, and as a fuel for transportation. In addition, biomass serves as a key ingredient in the production of biofuels such as bioethanol and biodiesel, which further contributes to reducing dependence on fossil fuels and promotes sustainability in the energy sector (Antar et al., 2021). One of the most important property of biomass is the higher heating value (HHV), which represents the total amount of energy released during combustion and is expressed in MJ kg $^{-1}$ (Hosseinpour et al., 2017). For biomass to be successfully used in energy applications, the process needs to be planned, modeled, and optimized. Recently, machine learning approaches have been increasingly used, especially artificial neural network (ANN) models (Kargbo et al., 2021). ANNs represent mathematical models that are inspired by the structure and functions of the human brain and are designed to solve complex problems through the ability to learn from a database. ANNs use sets of interconnected artificial neurons that process information using algorithms to recognize patterns and make decisions based on them (Islam et al., 2019). Based on all of the above, this research aims to create an ANN model for estimating the HHV of different categories of biomass about the input data of the ultimate and proximate analysis, and to assess the possibility of modeling, thus creating a more universal model for estimating the calorific value.

Materials and Methods

The data were taken from a published scientific paper (Yin et al., 2011) and describe the variables of the ultimate (C, H, N, O, and S), proximate (VM, FC, and ash) analysis as well as the HHV. After the data underwent a cleaning process (Li et al., 2021), data processing was performed in the *Python programming language* (Lutz, 2009). The data processing included calculations of the mean and standard deviation. In addition, an analysis of variance (ANOVA) was performed to determine the differences between the

observed groups. The principal component analysis (PCA) graphically shows the direction of movement of the variables (as vectors) concerning the numbered group. In addition, a correlation matrix was created to determine the relationship between the analyzed variables. The final step of the study involved the creation of an ANN model to estimate the HHV based on the input data from the ultimate and proximate

analyses. All data is split into 70% for learning and 30% for testing (Brandić et al., 2022). The ANN was trained in 100,000 cycles, ensuring a sufficient number of iterations to obtain reliable results. To solve the numerical optimization, the Broyden-Fletcher-Goldfab-Shanno (BFGS) algorithm was used, which has proven its worth in the problem of high computational complexity (Gao et al., 2023).

Results and Discussion

Table 1 shows the average values of the proximate and ultimate analysis of the different types of biomass after statistical data processing.

Table 1. Mean values of the variables of the proximate and ultimate analysis of the different types (categories) of biomass

No.	Category	Proximate analysis (%)			Ultimate analysis (%)					MJ kg $^{-1}$
		VM	FC	Ash	C	H	N	O	S	HHV
1	Fruit and Nut Shell Residue	73.92± 7.73	21.62± 8.06	3.64± 3.5	50.43± 2.73	6.2± 0.4	3.96± 11.35	39.85± 3.24	0.11± 0.08	19.8± 1.63
2	Agricultural Biomass	76.3± 7.45	16.7± 4.5	6.92± 6.29	45.81± 3.95	5.88± 0.52	4.14± 11.03	38.3± 9.52	0.18± 0.19	18.23± 1.69
3	Woody Biomass	78.1± 6.41	16.98± 3.46	4.92± 3.99	48.99± 0.5	6.24± 0.44	1.82± 1.15	39.38± 5.43	0.41± 0.47	19.39± 0.72
4	Other Biomass	75.96± 5.93	18.43± 4.44	4.06± 3.79	47.33± 4.18	6.3± 0.75	1.89± 1.39	41± 3.07	0.45± 0.34	19.42± 1.96
	Statistical significance	ns	ns	ns	*	ns	ns	ns	ns	*

No. Represents category name for reading the PCA diagram (Figure 1.); VM – Volatile matter; FC – Fixed carbon; C – Carbon; H – Hydrogen; N – Nitrogen; O – Oxygen; S – Sulphur; HHV – Higher heating value; Statistical significance * $p<0.01$; ns – not significant.

Table 1 shows that there is no difference between the variables in the proximate analysis when categorizing the data. In the ultimate analysis, however, there are statistically significant differences in C, where the highest proportion is found in the categories fruit and nutshell residues, while the lowest average value is found in agricultural biomass. The HHV is also statistically significantly different at $p<0.01$ for the same sample.

Figure 1 shows the principal component analysis (PCA) of the observed values. PCA is defined as a mathematical method used to reduce the number of dimensions of the entire data set to simplify visualization for a better understanding of the occurrence of patterns (Beattie & Esmonde-White, 2021).

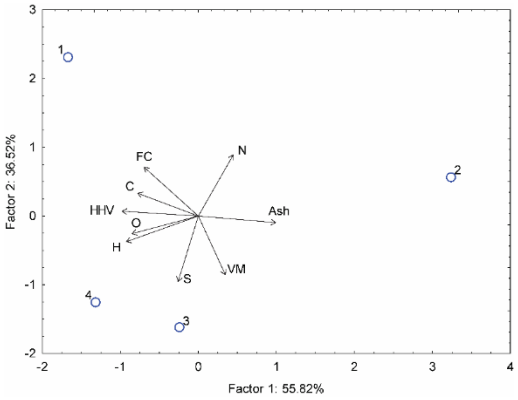

Figure 1. PCA of the observed variables used to create the ANN model.

Figure 1 shows that sample 2 has the highest ash content, while sample 1 has the highest FC content, as can be seen in Table 1.

Figure 2 shows the correlation matrix of the observed variables in the study. A correlation matrix is a graph that shows the correlation coefficients (Pearson) between two variables from -1 to 1 (Graffelman & de Leeuw, 2023). The most important correlations in the study are printed in bold and framed in red and their p-values are displayed.

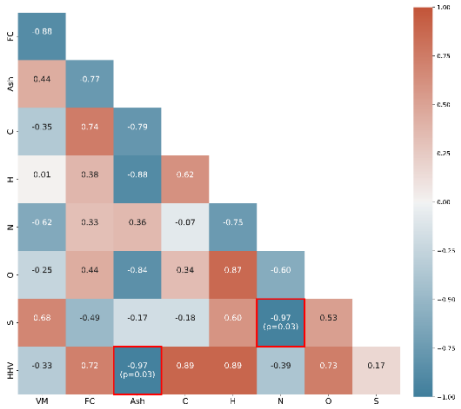

Figure 2. Correlation matrix of observed variables in the research

Figure 2 shows that the variable ash correlates significantly (r=-0.97) with the HHV, as do the variables N and S with the same negative correlation coefficient.

Table 2 summarises the ANN model developed to estimate the HHV.

Table 2. Summary of the developed 8-10-1 ANN model for HHV estimation

Net. Structure	Training perf.	Test perf.	Training error	Test error	Training algorithm	Error function	Hidden activation	Output activation
MLP 8-10-1	0.93	0.99	0.09	0.04	BFGS 20	SOS	Exponential	Logistic

MLP – Multilayer perceptron; BFGS – Broyden Fletcher Goldfarb Shanno algorithm; SOS – Sum of Squares. Training and testing performance are shown as the coefficient of determination R^2.

Table 2 shows that the ANN model has a high performance in estimating HHV biomass, as determined by the high R^2 for both training (0.93) and testing (0.99). On the other hand, the model also has a relatively low modeling error.

Figure 3 shows a scatter plot illustrating the overlap of real and modeled data.

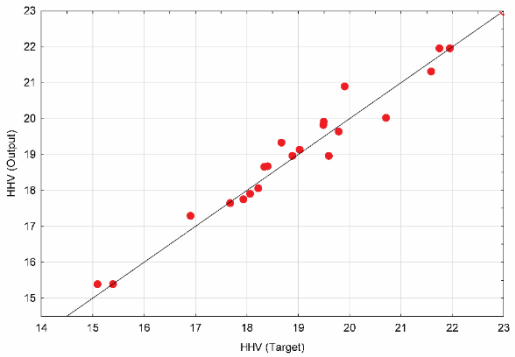

Figure 3. Scatterplot HHV (Output) vs. HHV (Target)

Conclusions

The application of ANN in the estimation of HHV biomass was found to be effective, with a high degree of data overlap (R^2) of 0.93 for learning and 0.99 for testing, indicating high efficiency and reliability of the model. The model using the BFGS algorithm has minimal error in the estimates, confirming the potential for optimizing biomass energy use through ANN models.

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IN HOUSE VALIDATION OF AN LC-MS/MS MULTI METHOD FOR THE DETERMINATION OF MYCOTOXINS IN WHEAT

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Abstract

Mycotoxins are secondary metabolites secreted by many fungal species present in plants during their pre-and post-harvest, transportation, processing and storage, and often found in food and feed. In particular wheat grains are susceptible to contamination with various *Fusarium* mycotoxins such as Deoxynivalenol (DON), Zearalenone (ZEN), T-2 toxins, HT-2 toxin, Fumonisin B1, Fumonisin B2 etc, As their presence can cause disease and death both in humans and livestock, a fundamental step for ensuring public health is the development of highly sensitive, robust, selective, quick, easy, and multi-analyte extraction and detection method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was the method of choice for the quantification of the main mycotoxins, inclusive of some mycotoxins that are currently regulated acc to EU 2023/915, and emerging mycotoxins such as Enniatins (not included in the previous EU regulation), in plant material (wheat seeds of 20 *Triticum aestivum* L. cultivars in use in Albania). Extraction of mycotoxins was performed using a modified QuEChERS extraction in the presence of the acidified aqueous extraction and organic solvent. Matrix-matched calibration curves were established, and limits of quantification were below the maximum levels (0.5 µg/kg (each of the total Aflatoxins) to 50 µg/kg (DON). According to 2782/2023_EU, LOQ shall be ≤0.5*ML or should preferably be ≤0.2*ML). Recoveries ranged between 70 and 120%, fulfilling the EU legislation (SANTE 11312-2021). Results demonstrated that the procedure was suitable for determining 23 mycotoxins in wheat grains.

***Keywords*:** Mycotoxins, Contamination, Multi-method, LC-MS/MS, Validation, EU regulation

INTRODUCTION

Mycotoxins are secondary metabolites secreted by a wide variety of fungal species (Assefa et al., 2018). The most well-known mycotoxins are: Aflatoxins and some *Fusarium* toxins. Given that their toxicological effects can be acute, legislation on the permitted levels in food has been established by European Commission (EU_2023/915) (Giannoti et al., 2023). In addition to the regulated mycotoxins, there is also a group of currently non-regulated mycotoxins produced by *Fusarium spp,* also known as "emerging mycotoxins", which includes beauvericin (BEA) and enniatins (ENNs). The objective of this work was to optimize and validate a LC-MS/MS method capable of measuring 23 mycotoxins in wheat grain.

Material and Methods

Mycotoxin standard solutions

Primary stock standard solutions, intermediate and working mix standard solutions were prepared according to the table 1 in a 10 ml volumetric flask and diluting to volume with the same solvent like their stock solution.

Table 1. Intermediate and working Standard solution preparation

Standard solution	Active substance	Standard stock solutions (µg/ml)	Target concentration (µg/kg)	Volume to be taken from the stock solution (µl)	Solution concentration (0.1*ML) (µg/ml)
MIX st 1 (in Methanol)	15-acetyl DON	100	100	1000	10
	3-acetyl DON	100	100	1000	10
	Enniatine A	100	100	1000	10
	Anniatine A1	100	100	1000	10
	Enniatin B	100	100	1000	10
	Enniatin B1	100	100	1000	10
	Fusarenon X	100	100	1000	10
	HT-2	88.8	50	563	5
	Zearalenone	112	100	893	10
	T-2	88.8	50	562	5
MIX st 2 (in Acetonitrile)	DON	500	1250	2500	125
	Ergocornine	100	100	1000	10
	Ergocorninine	100	100	1000	10
	Ergocristine	100	100	1000	10
	Ergocristinine	100	100	1000	10
	Ergometrine	100.5	100	995	10
	Ergometrinine	50	100	2000	10
MIX st 3 (in Acetonitrile/H2O)	Fumonisin B1	49.22	50	1016	5
	Fumonisin B2	50.87	50	983	5
MIX st 4 (in Acetonitrile)	Aflatoxin B1	1	1	1000	0.1
	Aflatoxin B2	1	1	1000	0.1
	Aflatoxin G1	1	1	1000	0.1
	Aflatoxin G2	1	1	1000	0.1

Calibration curve

Five point extracted matrix calibration curve were prepared by fortifying negative samples (5g) with the appropriate volume from working standard solutions (see table nr.2). A calibration line was fitted using linear least squares regression with 1/X fit.

Table 2. Calibration curve

	Volume from MIX st.1 (µl)	Volume from MIX st.2 (µl)	Volume from MIX st.3 (µl)	Volume from MIX st.4 (µl)
Control	0	0	0	0
1*LOQ	50 µl (from standard 0.01ML)	20 µl (from standard 0.01ML)	50 µl (from standard 0.01ML)	25 µl (from standard 0.01ML)
2*LOQ	100 µl (from standard 0.01ML)	40 µl (from standard 0.01ML)	100 µl (from standard 0.01ML)	50 µl (from standard 0.01ML)
5*LOQ	250 µl (from standard 0.01ML)	100 µl (from standard 0.01ML)	250 µl (from standard 0.01ML)	125 µl (from standard 0.01ML)
10*LOQ	50 µl (from standard 0.1ML)	200 µl (from standard 0.01ML)	50 µl (from standard 0.1ML)	25 µl (from standard 0.1ML)

Extraction

5 g ±0.02 g milled wheat grain was mixed with nine ml of 0.1M HCl using a multi vortex for 5 min and allowed to stand in the dark for 10 min. 10 ml acetonitrile was added in each tube and were shaken for 10 min in high speed. The extraction salts (containing 1g NaCl and 4g Na_2SO_4), were added to samples which were vortexed and shaken for 2 min (Mcelhinney *et al., 2015).* After the centrifugation for 10 min at 5000 rpm at 4^0C, a portion of supernatant was transferred to a new centrifuge tube. The extraction solution was evaporated at 40^0C under N_2 gas (Mbisana *et al*., 2023). The reside was dissolved with ACN: H_2O: Ac. Ac (35:64.5:0.5), filtered with 0.2µm PTFE membrane filter and placed in a pp-vial.

LC-MS/MS condition

Liquid chromatography separation was carried out on a Shimadzu 8040 liquid chromatography system. The analytical column was Acquity HSS C18 (100 mm × 2.1 mm, 1.8 µm particle size). Separation was achieved using a binary gradient comprising of 0.5% acetic acid aqueous solution with 5 mM ammonium acetate (solvent A), and Methanol with 0.5% acetic acid and 5 mM ammonium acetate (solvent B) at a flow rate of 0.35 ml/min (SSH EN 17194:2019). The eluent gradient profile was as follows: 0 min: 10% B; 0.05 min: 10% B; 1 min: 10% B; 6 min: 100% B, 9 min: 100% B, 9.01 min: 10% B and 12 min: 10% B (*Liao et al., 2013*). The temperature of the column was 40 °C and the injection volume was 2 µL.

RESULTS

1. Evaluation of the Matrix Effect

Matrix effects were assessed at the present method validation stage. This was done by comparison of the response arising from solvent standards and from matrix-matched standards. A maximum 20% difference is considered as acceptable according to SANTE 11312/2021/v2 for using solvent standards as calibration standards. Matrix Effects were calculated according to the equation:

$$\text{Matrix effects (\%)} = (100 * \frac{A}{B}) - 100$$

Where A is the response of the analyte in the matrix and B is the response of the same analyte, in the same concentration in the solvent. Matrix effects results are shown in the Table 4.

2. Optimization of LC-MS/MS method

Initially, a full scan and MS/MS spectra of all mycotoxins were obtained by injecting individual standard solutions (with concentration 1ppm) into the mass spectrometer. The precursor and products ions, retention time, collision energy (CE), Dwell time, Q1 PreBias (voltage promotes the ionization of the precursor ion) and Q3 PreBias (voltage promotes the ionization of the product ion) were optimized as well for all the compounds and are shown in the Table 3.

Table 3. Mass spectrometric parameters

Retention time	Analyte	Precursor ion	Product ion	Q1 PreBias	CE	Q3 PreBias	Dwell	Polarity
5,003	Aflatoxin B1	313,1	241	-23	-39	-25	4	Positive
			285	-12	-24	-30	4	
			128	-12	-55	-23	4	
4,741	Aflatoxin G1	329,2	243	-16	-26	-26	4	Positive
			200	-12	-41	-19	4	
			115	-16	-55	-21	4	
4,613	Aflatoxin G2	331,1	313	-13	-24	-21	4	Positive
			189	-15	-42	-19	4	
			245,1	-16	-31	-25	4	
4,896	Aflatoxin B2	315,1	287,1	-12	-26	-20	4	Positive
			259	-15	-31	-27	4	
			115	-15	-55	-29	4	
5,809	T-2	489,21	245	-23	-16	-23	8	Positive
			327,1	-11	-14	-21	8	
6,159	Ergocristinine	610,2	305,1	-28	-27	-15	4	Positive
			223,1	-28	-34	-24	4	
			268,1	-30	-27	-28	4	
5,55	HT-2	447,2	345,1	-12	-12	-28	8	Positive
			285,1	-12	-13	-15	8	
3,662	Ergometrine	326,1	223,1	-12	-23	-23	8	Positive
			208,1	-12	-29	-21	8	
5,549	Ergocristine	610,3	223,1	-30	-36	-23	8	Positive
			268,1	-30	-26	-29	8	
5,919	Ergocorninine	562,3	223,1	-28	-38	-23	8	Positive
			277,1	-28	-28	-30	8	
5,357	Ergocornine	562,2	223,1	-26	-34	-23	8	Positive
			268,1	-26	-25	-19	8	
6,881	Enniatin B1	671,4	196,1	-32	-32	-21	8	Positive
			210,1	-32	-32	-22	8	
6,812	Enniatin B	657,4	196,1	-32	-31	-21	8	Positive
			214,1	-32	-32	-22	8	
6,977	Enniatin A1	685,4	210,1	-34	-33	-22	8	Positive
			228,1	-32	-33	-24	8	
7,078	Enniatin A	699,4	100,1	-20	-55	-19	8	Positive
			210,1	-20	-33	-23	8	
6,165	FB2	706,4	318,3	-32	-44	-22	8	Positive
			512,4	-32	-35	-22	8	
4,216	Ergometrinine	326,1	208	-12	-27	-21	8	Positive
			223,1	-12	-24	-23	8	
5,686	FB1	722,4	352,3	-34	-40	-25	8	Positive
			528,2	-20	-33	-38	8	
4,377	15-acetyl DON	356,2	321,1	-10	-14	-22	8	Positive
			137	-17	-17	-24	8	
3,804	FuX	413,3	353	11	11	23	4	Negative
			59,1	11	24	20	4	
			263,2	11	16	11	4	
6,009	ZEN	317,3	175,1	15	26	17	4	Negative
			131,1	14	30	12	4	
			272,5	14	26	24	4	
3,161	DON	355,2	295,2	16	11	19	4	Negative
			59	16	24	20	4	
			265,2	16	16	17	4	
4,375	3-acetyl DON	397,2	337,2	18	10	24	4	Negative
			59	18	23	18	4	
			307	18	17	19	4	

3. Method validation

This analytical method was validated according to the guidelines in SANTE 11312/2021/v2 and 2782/2023_EU. In table 4 is summarized the calibration range, coefficient of determination, LOD, LOQ, Matrix effects, RSDr and recovery. Satisfactory coefficients of determination (R2 > 0.95) were obtained for all the analytes. The limits of quantification established in this method are below the limits specified in the EU legislation 2782/2023. Two fortification levels were assessed and replicated six times across each fortification level in two different days. RSDr were less than 20%, indicating that this method can be used for the routine analysis of mycotoxins in grains. Overall, the recovery rates varied from 72% to 110%.

Qualitative analytical criteria were also monitored during the validation process. Each compound was characterized by its retention time and its two product ions. Based on the Document N°SANTE/11312/2021 the most intense product ion was used as a quantitative ion, while the second product ion was used for confirmation. Retention time relative to the standard chromatographic peaks of analytes were consistent at ±2.5 % and S/N values were >10 for each transition during each validation run.

Table 4. Results of the validation

Analyte	Calibration range µg/kg	Matrix effects	Level 1 (1*LOQ)		Level 2 (5*LOQ)		LOD	LOQ
			Accurancy %	RSDr (%)	Accurancy %	RSDr (%)		
Aflatoxin B1	0.5 - 5	15.4	74	2.98	73	13.87	0.15	0.5
Aflatoxin G1	0.5 - 5	12.6	72	2.72	75	10.45	0.15	0.5
Aflatoxin G2	0.5 - 5	13.6	76	5.54	78	9.36	0.15	0.5
Aflatoxin B2	0.5 - 5	12.2	72	2.7	73	15.28	0.15	0.5
T-2	5- 50	9.4	80	7.03	82	2.87	1.5	5
Ergocristinine	4 - 40	10.3	94	9.4	100	4.24	1.2	4
HT-2	5- 50	16.4	83	8.2	86	4.11	1.5	5
Ergometrine	4 - 40	15.3	99	3.79	103	5.38	1.2	4
Ergocristine	4 - 40	16.4	97	4.15	100	14.67	1.2	4
Ergocorninine	4 - 40	13.4	99.4	3.82	104	8.48	1.2	4
Ergocornine	4 - 40	8.6	101	6.23	94	3.56	1.2	4
Enniatin B1	10 - 100	4.7	106	5.34	100	7.59	3	10
Enniatin B	10 - 100	9.5	104	11.7	98	3.76	3	10
Enniatin A1	10 - 100	13.7	94	5.53	102	9.04	3	10
Enniatin A	10 - 100	12.6	90	2.33	99	10.46	3	10
FB2	5- 50	16.5	109	8.91	97	6.48	1.5	5
Ergometrinine	4 - 40	9.3	95	4	89	2.57	1.2	4
FB1	5- 50	14.7	110	4.16	100	7.48	1.5	5
15-acetyl DON	10 - 100	16.7	85	4.38	79	15.4	3	10
FuX	10 - 100	18.3	89	2.75	93	13.85	3	10
ZEN	10 - 100	16.8	96	6.3	89	9.47	3	10
DON	50 - 500	15.9	83	1.98	96	6.58	15	50
3-acetyl DON	10 - 100	13.7	82	7.6	97	5.39	3	10

4. Carryover

The acetonitrile/water (50:50, v/v) solution was injected directly after the highest matrix-matched calibration standard to check for sample carryover. No analyte carryover was observed.

CONCLUSIONS

Matrix-matched calibration curves and limits of quantification were below the maximum levels (0.5 µg/kg (each of the total Aflatoxins) to 50 µg/kg (DON). According to 2782/2023_EU, LOQ shall be ≤0.5*ML or should preferably be ≤0.2*ML). Recoveries ranged between 70 and 120%, fulfilling the EU legislation (SANTE 11312-2021). In conclusion, results demonstrated that the procedure was suitable for determining 23 mycotoxins in wheat grains.

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ESTIMATION OF GROWTH PARAMETERS FOR A STOCK OF OHRID TROUT (*SALMO LETNICA* KARAMAN, 1924) BASED ON THE ASSESSMENTS OF COMMERCIAL CATCHES IN THE ALBANIAN PART OF OHRID LAKE.

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Abstract

Ohrid trout (*Salmo letnica*) is a endemic fish species from Ohrid Lake (South-East of Balcan Penisula). In this paper are presents some informations about growth features of this species, based on assessments of the commercial catches in the Albanian part of the Lake. Were assessed the coefficients *a* and *b* in the allometric relationship between total length (TL,cm) and total weight (W,g) (LWR), the allometric condition factor (*K*') and the parameters ($L\infty$,*K*,*t*o and *t*max) in the Von Bertalanffy's growth function (VBGF). The values of coefficients in LWR were: initial growth coefficient *a*=0.0161 and the slope *b*=2.956. The value for allometric condition factor was *K*'=1.624. For the parameters of VBGF were calculatet these values: asymptotic length $L\infty$=53.46 cm, annual growth coefficient *K*=0.192/year, the "age" at length 0 *t*o=-1.41 years and the maximum theoretical age *t*max=15.6 years. The obtained values for LWR coefficients shows almost isometric growth for the assessed stock of Ohrid trout. The walues for VBGF shows a greater similarity with the limnophilic species of the genus Salmo than with the species that live in the rivers.

Keywords: Balkan trout, length-weight relationship, isometric growth, allometric condition factor, Von Bertalanffy growth parameters, feeding activity.

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Introduction:Two salmonid fishes,Ohrid trout (*Salmo letnica*) and Ohrid belvica (*Acantholingua ohridana*) are endemic for Ohrid Lake.In addition to the faunistic values, these two species also have economic values,as they account for about 40% of the fish catches (by weight) that are realized by commercial fishing in the last ten years.The studies that were carried out for the Ohrid trout were mainly focused on the problems of taxonomy (Karaman,1924;Filipi,1959; Rakaj and Flloko,1995), analysis of factors that control the dynamics of populations of this species (Filipi,1959;Spirkovski,1991;Spirkovski,2003;Palluqi et al.,2017),physiology (Beqiraj-Kalamishi et al.,2006) and genetics (Wilson,2004;Sell and Spirkovski,2004).

The taxonomic and genetic studies have pointed out the existence of three ecological populations of Balcan trout in the Ohrid Lake;the typical form or "winter koran" (*Salmo letnica typicus*) (Wilson,2004),the "river koran" (*Salmo letnica lumi*) (Filipi,1959) and the "summer koran" (*Salmo letnica aestivalis*) (Filipi,1959;Rakaj and Flloko,1995;Wilson,2004;Sell and Spirkovski,2004).Most researchers express the opinions that the main risks for the endemic salmonids of the Ohrid Lake are generated by overfishing, abusive fishing, urban pollution and damage to breeding sites (Filipi,1959;Spirkovski,2003;Palluqi at al.2017).

Materials and Methods: The samples of Ohrid trout for assessments about the growth characteristics were taken from commercial catches during the period September-November 2023.

The Ohrid Lake is located in the South-eastern part of Albania,at an altitude of 695 m and has a surface area of 348.8 km² (229.9 km² are part of North Macedonian Republic and 118.9 km² are part of Republic of Albania).The fish fauna of this lake is composed of 19 indigen species and subspecies as well as six alien species.Five are endemic fish species.The fish production capacity of the Ohrid Lake is from 15 to 20 kg/ha (Filipi,1958;Shegani,2007).Is about 9 the number of target species for commercial fishing.

Each sample that was included in the measurements for the estimation of one growth parameter consisted of more than 30 fishes.The measured biological indicators were total length (TL,cm) and live weight (W,g).The estimation of growth parameters was performed according to the following methodical protocols.

i.The coefficients *a* and *b* in the allometric relationship total length-live weight (LWR):

The relationship $W = aTL^b$ (Kuriakose,2017) was applied for the calculation of coeficient's values.The interval values for the intercept *b* is 2.5-3.0-3.5.In the case when the value of *b* = 3.0 the growth was considered isometric.In the cases when the values of *b* were *b*<3.0 or *b*>3.0 the growth is considered allometric (respectively,negative allometric values,which confirm poor growth and positive allometric values which confirm good growth) (Ricker,1975).

ii.The allometric condition factor (*K*'):

The value estimated for intercept *b* in the LWR was used as exponent in the expression proposed by Bagenal and Tesch (1978) to calculate the allometric condition factor:$K' = (W/TL^b)*100$.

iii.The parameters in the Von Bertalanffy's growth function (VBGF):

The method most commonly used for estimating the parameters $L\infty$ and *K* of the VBGF is the "Ford-Walford Plot" which essentially consists of a rewritten version of VBGF in the form:

$Lt+1 = a + bLt$

where:$L\infty = a/1-b$ and $K = -\ln b$ (Pauly,1983).

The theoretical age at the length 0 (*Lo*),was estimated using the empirical formula by Dong et al.(2019):

Log10(-*t*0) = - 0.392 – 0.275 Log10(*L*∞) – Log10(*K*)

The following expression (Pauly,1983),was used to estimate theoretical maximum age of Balcan trout:

*t*max = 3/*K*

were *K* in the annual growth parameter.

The biometric processing of the data and the corresponding calculations were performed using the computer's program IBM SPSS Statistics 21.0 (2019),the FiSAT II
(Gayanilo et al.,2005) and the 2010's version of Microsoft Office Exel Software.

Results:

i.The estimated values of coefficients *a* and *b* in the LWR.

Initially,based on biometric processing of the data from the measurements,we obtained information on the average values (M) of the total length (TL,cm) and live weight (W,g) as well as the for two statistical indicators (SD and Var%), respectively for the samples,as a whole,and especially for their male and female fractions.

The calculated values for the coefficients *a* and *b*, which resulted from the regressive and correlative analysis, were as follows:

$W = 0.0148TL^{2.9844}$,for female fraction of the samples;$W = 0.0204TL^{2.8769}$,for male fraction of the samples and $W = 0.0161TL^{2.9566}$ for mixed samples according to the sexes.The three values that resulted from the estimation of the intercept *b* results included in the order of negative allometric values (b<3.0),manifesting a tendency, especially for the female fraction and the sample as a whole,toward the isometric value (b=3.0).

ii.The sexual differences for allometric condition factor (K').

Using the estimations and theoretical comments made by Barnham and Baxter (1998) for the condition factor in the natural populations of salmonid fishes,we can affirm that the values calculated by us,for the female and male fractions in the evaluated stock of Ohrid trout,in no case prove the existence of a poor or extremely poor condition.

The lowest value of the K'-factor (1.26) was estimated for Ohrid trout's females aged 2 years.This value and the other two values (K'=1.31 and K'=1.34),respectively for females aged 5 and 6 years, inform about fish conditions at "sufficient" level (or "a fair fish").The females in other age-groups were distinguished by their "good" or "exellent" condition.For the male fraction of the Ohrid trout's stock we did not find the condition at "sufficient" level.The interval values for the K'-factor in the male fraction was from a minimum of 1.54 (fishes aged 5 years) to a maximum of 1.99 (fishes aged 9 years).These values were indicative of "good" and "exellent" condition.

The average value of allometric condition factor,estimated for all the samples was K'=1.624 ± 0.201 (Var% = 12.37).The average value of the variance (Var%=12.37) was more determined by the variability of K'-factor in the female fraction of the stock (Var%=13.54),than from respective variability in the male fraction (Var%=7.49). However,analysis of variance (ANOVA) did not confirm significant sexual differences for average values of allometric condition factor (t=0.91;P>0.05).

iii.The estimated values of VBGF parameters.

Using the values of two coefficients *a*=9.355 and *b*=0.825 in the linear regression, according to the methodical protocols presented before,we have calculated the values for the parameters of Von Bertalanffy growth function (VBGF), and in the respecive formula:

$L_t = 53.46\ [1- e^{-0.192\ (t+1.41)}]$

Discussion: With small exceptions,as was the case of the river trout (*Salmo trutta fario*),which manifested negative allometric growth for both males (b=2.634) and females (b=2.766) (Fazli et al.,2011),the order of distribution of values for intercept *b* in the LWR,in all the other studies we have consulted, including those found by as for the Ohrid trout, turns out to be the same.Thus,red spotted trout (*Salmo trutta macrostigma*) exhibited isometric growth for the males (b=3.009) and growth close to isometric value for the female individuals (b=2.971) (Alp et al.,2005).

The analysis of the results we consulted,including our results for the stock of Ohrid trout,lead to the opinion that there exist a relative stability for the growth characteristic in the species of genus Salmo.This stability is manifested by the same growth rates as for length and weight (b=3.0).We think that the unity of the growth characteristics,for the taxons of a genus,some of which are inhabitants of streams and some inhabitants of lakes,is an adaptive feature,in our case,for the genus Salmo,which may be the result of the body shape.The morphological shape of the adult fish can have a specific impact on its growth characteristics and that fusiforme shape,which is a principal feature for all the species in the genus Salmo,under favorable environmental condition,can promote the situation of balanced growth for both length and weight of the fish.

We think that the lower average value for the allometric condition factor in the female fraction (K'=1.513) of the Ohrid trout stock,compared to the corresponding value in the male fraction (K'=1.734),may be a result of metabolic differences in the pre-reproductive period,possibly caused by specific elements in the alimentary behaviours (or by differences in feeding intensity) of female and male individuals.According to Ragheb (2023) the origin of the variability for the values of allometric condition factor (k_a) were the feeding intensity and the value of allometric coefficient in LWR.The impact of the feeding intensity on the values of coefficients in the LWR as well as on the values of K'-factor was assessed by Bagenal and Tesch (1978) and by Kuriakose (2017).

Acording to Kuriakose (2017),the values for the coefficients in LWR and for the K'-factor are affected by the stage of sexual maturity of the fish.

The value estimated by as for the asymptotic length of the Ohrid trout ($TL\infty$ = 53.46 cm) result greater than the values estimated by Vicenzi et al. (2007) for the marble trout (*Salmo marmoratus*) in Slovenia ($TL\infty$ = 31.82-37.57 cm),by Pedicillo et al. (2010) for marine brown trout (*Salmo trutta trutta*) in Italy ($TL\infty$ = 44.72 cm),by Fazli et al.(2011) for river brown trout (*Salmo trutta fario*) in Iran ($TL\infty$ = 45.0 cm),and by Raikova-Petrova et al.(2018) as well as by Arslan et al.(2007) for two populations of typical brown trout (*Salmo trutta*),respectively in Bulgaria ($TL\infty$ = 23.16 cm) and in Turkey ($TL\infty$ = 32.13 cm).On the other hand,were estimated greater values of the parameter $TL\infty$,compared to our values in Ohrid trout,for red spotted trout (*Salmo trutta macrostigma*) in Turkey ($TL\infty$ = 72.80 cm) (Alp et al.,2005),for typical brown trout (*S.trutta*) in Spain ($TL\infty$ = 65.94 cm) (Lobon-Cervia et al.,1986),for Caspic sea trout (*Salmo trutta caspius*) in Iran ($TL\infty$ = 104.0 cm) (Sayyadbourani et al.,2016) and for flathead trout (*Salmo platycephalus*) in Turkey ($TL\infty$ = 60.78 cm) (Kara et al.,2011).

From the comparative summary that was performed,it seems that,with the small exeptions,according to the asymptotic length the fishes of the genus Salmo can be divided into two groups;the first group of trouts that is distinguished by its small body,which turn out to be inhabitant of

streams and the second group of trouts with the large body,some of wich,including Balcan trout,are inhabitants of lakes.

According to the data from scientific publications,the values estimated for annual growth parameter (*K*) in the VBGF,for the natural population of trouts (genus Salmo),fluctuate in the range from 0.091/year,for flathead trout (Kara et al.,2011) to 0.498/year,for typical brown trout (Raikova-Petrova et al.,2018).The corresponding value estimated by as for the stock of Ohrid trout was *K*=0.192/year.This value was close to the average value (*K*=0.185/year) obtained from the evaluations in the largest number of the salmonid species that were analyzed.We can affirm that the opinion expressed above regarding to the stabilization of the values of the intercept *b* in LWR in the range of isometric value (b=3.0) turns out to be valid also for annual growth parameter in VBGF,whose value in most freshwater species and sub-species of the genus Salmo is not greater than 0.20/year.

As a conclusion,from this study were pointed out two features of Ohrid trout (*Salmo letnica*).First,the estimations for the parameters *b* and *K* showed that as for the majority of trouts (genus Salmo) and for the Ohrid trout fusiforme shape of the body promote isometric growth,and second,according to asymptotic length ($TL\infty$) the Ohrid trout can be classified as large-bodied limnophilic trout.

At least for the Albanian part of the Lake Ohrid,the argument we discussed in this paper is addressed for the first time.We belive that the study of the analytical characteristics of the growth,for the Ohrid trout's stocks will contribute to the conservation of this endemic species.

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ASSESSMENTS ON THE GROWTH PERFORMANCE OF MEDITERRANEAN MUSSEL (*M. GALLOPROVINCIALIS* MOLLUSCA, BIVALVIA) REARED IN BUTRINTI LAKE (SOUTH-WESTERN ALBANIA), ACCORDING TO EVALUATION OF THE PARAMETERS IN VBGF.

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Abstract

In Albania, since the 60s of the last century, the commercial aquaculture of Mediterranean mussel (*M.galloprovincialis*) is located only in Butrinti Lake. In the period between April and December 2023 we have estimated the parameters in the VBGF, the instantaneous rate of natural mortality (*M*), the predicted length at first maturity (*L*m), and some growth performance indices for mussels reared in the panel-structures of aquaculture system. In April the average value of shell length was *SL*=34.91±1.821 mm, while in December this parameter has the value *SL*=63.11±4.273 mm. The maximum value of *SL*, found during sampling in the aquaculture farm was *SL*max=66.9 mm. The average value of specific growth rate index was *SGR*=0.210±0.039. The following values were estimated by as for the parameters in VBGF: asymptotic length $L\infty$ = 76.2 mm; annual growth coefficient *K* = 0.23/yr; theoretical or expected age at length zero *to* = -0.566 yr; the theoretical lifespan *t*max = 7.04 yr. For the shell length was found this Von Bertalanffy's growth function: *L*t = 76.20 [1- $e^{-0.230\ (t+0.566)}$]. The value of the predicted length at first maturity was *L*m = 45.15 mm. The value of instantaneous rate of natural mortality was *M* = 0.457/yr. The overall growth performance index had the value *OGP* = 5.008, while the value of ϕ'-index, or the growth performance index, was ϕ' = 3.12. The value for theoretical age at which the length achieved 50% of $L\infty$ (*t*50%),was 2.54 yr. This study marks the start of the involvement of stock assessment procedures in Albanian aquaculture.

Keywords: Mediterranean mussel, asymptotic length, growth performance index, aquaculture.

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Introduction:The aquaculture of Mediterranean mussel (*M.galloprovincialis*) in Butrinti Lake (or lagoon) (the Albanian coast of Ionian Sea) started in 1968,in 80 concrete "panel" structures (Peja et al.,1996).These structures were installed at the depth from 6 m to 10 m,in three areas of the lake,in the northern area (Manastiri),in the western area (Pallavraq) and in the southern area (Butrint).The maximal production was 4920 ton/yr,in 1989.

Despite the interventions to apply mussel cultivation to the Albanian coast of the Adriatic Sea, the aquaculture of Butrinti Lake has remained the only farm for the commercial production of this mollusk. An evaluation about the topics of the Albanian research,carried out in the field of mussel aquaculture,in the period between 2000-2023,reveals that the scientific publications on "public health" comprise ower 85%,while technical and biological publications,only 15%.

The aim of this publication was the information on the values of growth prameters according to Von Bertalanffy function as well as on the growth performance and natural mortality in a Mediterranean mussel population,reared in Butrinti Lake's aquaculture farm.The methodology of our study,that is included in the stock assessment practices,is applied for the first time in the study of a species produced by Albanian aquaculture.

Materials and methods:The study was carried out in the mussel "population" that was reared in the panels of a fixed suspended culture,on the Western part of the Butrinti Lake. The sampling starts in April and continue in 30-day intervals,until the beginning of December 2023 (9 months in total or from ½ to ¾ of the full mussel's production cycle).

Sampling of molluscs was done without selection,in three parts of the ropes with growing mussels;in the upper 1/3,in the middle and in the lower 1/3 of the rope.In each sampling the mussels from two panels were included in the the samples.For the estimation of each of the prameters,more than 80 molluscs were sampled.Since the study included the estimations on the changes in the length of the shell (*SL*,mm) according to the age of the mussels (Pauly,1983) (day/365,for each month of the period between April and December),we have calculated the average length and standard deviations (*SL*M±SD,mm) for each month.Before measuring of the length (*SL* or anterior-posterior distance in mm), the mussels were cleaned from detritus and fouling and were dried with absorbent paper. The measurements were carried out with a digital caliper, with an accuracy of 0.01 mm.

The calculation of different parameters was carried out according to the following protocols:

-The specific growth rate index (SGR) was estimated using the formula according to Hopkins (1992): *SGR*SL= [(ln*SL*2 – ln*SL*1) / (*t*2 – *t*1)] * 100,were,*SL*1 is the average length (mm) of the mollusks on the month of April;*SL*2 is average length in December and *t*2-*t*1 is the number of days between the two samplings.

-The parameters in VBGF (Von Bertalanffy,1938):*i*)Asymptotic length (*SL*∞ or *L*∞) and annual growth coefficient (*K*).This two parameters were estimated applying the protocol according to Ford and Walford (Pauly,1983),which uses the shell lengths,respectively in the age-groups *t* and *t*+1,to realize the graphic solution of the linear regression *L*t+1 = a + b*L*t.Using the values of the coefficients *a* and *b* we have estimated the values for the two parameters of the VBGF by means of the expresions: *L*∞ = a/1-b and *K* = -ln b. ii) The theoretical lifespan (*t*max) was estimated according to Taylor (1958):*t*max = [ln *L*95% - ln(*L*∞ - *L*95%)]/*K*,were *L*∞ dhe *K* are the parameters of VBGF and *L*95% represents 95% of the maximum shell length recorded during field sampling (Taylor,1958;Herrmann et al.,2009).*iii*)Again,according to Taylor (1958) was estimated the "initial condition parameter" or the point

in time when the organisms's length is zero (*to*) (Farghaly et al.,2022),applying the formula: $t_{max} = t_o + 3 / K$.

-Length at first maturity (*Lm*) was estimated applying the formula according to Froese and Binohlan (2000),including in the calculation the value of the asymptotic length: ln *(Lm)* = 0.8979 · ln ($L\infty$) – 0.0782.

-The instantaneous rate of natural mortality (*M*),was calculated using an empirical formula,integrated in the Pauly protocols (Pauly,1980): ln(*M*)=-0.0152 – 0.279 in($L\infty$) + 0.6543 ln(*K*) + 0.463 ln(*T*).In this expression the tree variables are,asymptotic length ($L\infty$),growth coefficient (*K*) and average annual water temperature of Butrinti Lake (T°C).

-Growth performance indicators:*i*)The overall growth performance index (*OGP*), which represents growth rate at the point of inflection of the size-growth curve (Pauly,1979),was calculated using the formula *OGP* = log[$K^*(L\infty)^3$] (Munro and Pauly,1982;Delgado et al.,2017).*ii*)To compare the growth of Mediterranean mussel from Butrinti Lake with those from other studies,was calculated the fi-prim index (ϕ') of growth performance.The formula for calculations,according to Gayanilo and Pauly (1997),was: $\phi' = \log (K) + 2 \log (L\infty)$,where *K* and $L\infty$ are the parameters in VBGF.The third performance index,theoretical age at which the length achieved 50% of $L\infty$ (*t*50%),was estimated according to Ragonese et al.(2012),using the formula *t*50%=*to*+0.6931/*K*.

FiSAT II (Gayanilo et al.,2005) was applied to solve the principal formulas.The Electronic Length Frequency Analysis (ELEFAN I) was used to estimate the values of growth parameters from VBGF.The computer program IBM SPSS Statistics 21.0 (2019) was included in the biometric data processing.The analysis of variance (ANOVA),Student's t-test and the regression and correlation analysis were performed to test the significance levels ($P<0.05$) of the differences when comparing average values for different variants of the estimated parameters (Zar,2010).

Results:

The average value of shell length,estimated in April was *SL*=34.91±1.821 mm,while in December this parameter had the value of *SL*=63.11±4.273 mm.On the basis of the scale of polymorphism in the size of the molluscs,after estimating the values of the monthly growth variance (Var%),it results that the highest values for this biometric indicator were in August (Var%=6.15) and in December (Var%=7.08).

-**Specific Growth Rate (*SGR*) index**:The average value of this indicator of growth,in the mussels reared in Butrinti Lake,was *SGR*=0.210±0.039 (Var%=18.14).Monthly values of *SGR*,in the period between April and December,ranged from a maximum of 0.285,which was characteristic for the Spring season,to a minimum of 0.174,which was recorded in July .Also,the reduction in the values of SGR was found in the Winter season (in December *SGR*=0.181).

-**Parameters in the Von Bertalanffy growth function (VBGF)**: Implementing the methodical protocol according to Ford and Walford we have estimated the values of coefficients *a* and *b*,based on the shell length data (*SL*,mm) at the respective ages *t* and *t*+1.The values for these two coefficients were used to calculate the values of the parameters in the Von Bertalanffy growth function (VBGF).

Growth function according to Von Bertalanffy,for mussel's population reared in Western zone of Butrinti Lake,had the values:

$Lt = 76.20 [1- e^{-0.230 (t+0.566)}]$.

-Growth performance indices:

-The shell length at first maturity (*L*m):The estimated value of the mussels'shell length at first maturity,using asymptotic length (*L*∞) as a variable,was *L*m = 45.15 mm.

-The instantaneous rate of natural mortality (*M*):The value of natural mortality (*M*),calculated by applying an empiricall formula,using as variables the values of asymptotic length (*L*∞),annual growth coefficient (*K*) and average annual water temperature in lake (TºC),for mussel's population reared in Butrint Lake, was *M* = 0.457/yr.

Discussion:The dynamics of the monthly values for the length of the shell confirmed that mussel growth,according to total length, has been continued from April to December. However, the value of the monthly increase in length was not constant,as it varied from 1.04 to 5.81 mm. Lok et al.(2007) had found that the monthly increase in the mollusc's length differed from 1.00 to 3.85 mm.This fact proved that althrough the mussel is a poikilotherm animal,it feeds and grows even in the cold periods of the year,as long as it finds food resources in the rearing environment.The water temperature was the most important parameter affecting the growth of Mediterranean mussels.But,the fact that mussels continue to grow during the winter months,when the water temperature can be considered low,indicates that the water in the region is rich in terms of TPM and POM (Keskin et al.,2020).However,shell growth is not necessary coupled with tissue growth in bivalves. Indeed, shell growth can remain positive even in periods when tissue growth is negative (Steffani and Branch,2003).

Specific growth rate (SGR),estimated according to shell length,was calculated as a indicator of mussel's growth rates,depending on the season.We had found that for the two months of the Spring season,April and May,the average value of SGR was 27.9%.For the three months of the Summer season the respective value of this indicator was 20.1%,for the Autumn season's months the average value of SGR was 19.8% and for the December this value was 18.1%.The significant reduction in SGR-values ($t=7.93;P<0.05$) for the July,compared to the two months of the Spring season,must be caused by the increased values of temperature and salinity and the low values of the oxygen content in the water layer where the mussels were located.These characteristics of the aquatic environment are known for the Butrinti Lake in the Summer season (Peja et al.,1996;Moisiu et al.,2016). Salinity is one of the dominant environmental factors controlling growth (Andriasoa et al.,2019). Craciun (1980) reports that 25 °C is the upper limit at which *M. galloprovincialis* can perform normal physiological activities due to the affectation of the Leydig cells.

The values that resulted from our estimations for two parameters in the Von Bertalanffy growth function (VBGF) were:asymptotic length *L*∞ = 76.2 mm and annual growth coefficient *K* = 0.23/yr.The involvement of the Von Bertalanffy parameters in the studies on mussel growth can be found in different studies,were the growth of this mollusk is treated in the condition of aquaculture farms,which practice different technologies of production,or in experimental trials.

Ramon et al.(2007) comparing the values of VBGF-parameters,had analyzed the differences in growth between two groups of mussels that were reared in a sea bay of the North-eastern coast of Spain.These two groups were distinguished by the fact that the seed was taken on two separate locations,in a sea bay,where the trials took place and in the open sea. Although there was no significant difference in final length for the two seed sources, better performance was observed for bay seed with respect to weight (both shells and soft tissues) and mortality (Ramon et al.,2007).It results that the value of asymptotic length was the same (*L*∞=85 mm) in the two groups of mussels,while the values of the growth coefficient were *K*=0.761/yr,for the sea bay's mussels and *K*=0.839/yr,for the mussels in open

sea.The respective values of the hypothetical age the molluscs would have had at zero length,were *to*=-0.347 yr and *to*=-0.426 yr.If we compare the values found for the VBGF parameters,in the mussel's population from Butrinti Lake,with the values found by Ramon et al.(2007),in the mussels reared in a sea environment,we can affirm that the cultivation in the sea guarantees better condition for the growth of this molusc,compared to the cultivation in a lagoon,such as the Butrinti Lake.

In a lagoon,compared to the sea,there may be more favorable conditions for the growth of the algae,which are the principal food for the bivalve molluscs. But, as declared Ramon et al.(2007) the seasonal pattern of *M. galloprovincialis* growth in Fangar Bay, with highest growth rates in spring and lowest growth in winter and August, was not related to the pattern of primary production.No correlations were found between the increase in shell length and chl-*a*, temperature or seston, which was probably due to the wide ranges of the environmental parameters throughout the growth cycle in Fangar Bay (Ramon et al.,2007).

The value estimated in our study for the ϕ' (fi-prim) growth performance index,in the mussels population reared in Butrinti Lake,was ϕ' = 3.12.According to the studies carried out by Ramon et al.(2007),Andriasoa et al.(2019) as well as by Amine et al.(2019),the respective values of this parameter were 3.48;3.74 and 1.096.The last value vas estimated for the mussels in a polluted natural environment from the Atlantic coast of Morocco.The value of ϕ' = 3.12,in the mussel's population from Butrinti Lake,informs about good performances of increasing in length of the molluscs.

As a conclusion,we emphasize that the values estimated for the VBGF parameters confirms a good growth of the Mediterranean mussel in the Butrinti Lake. The protection of the water environment of the lake from different sources of pollution and the creation of the opportunities for its supply with fresh water,are conditions that improves mussel production from aquaculture.

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PHYSICAL-BIOLOGICAL INDICATORS AND SINGLE-CELL TOXICITY SENSING AS A NEW MONITORING MODEL APPLIED AT LAKE BUTRINT, ALBANIA

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Abstract

The assessment of surface and underground water quality in Albania has been carried out for years based on standardized methods for measuring physical-chemical indicators, trophic state (ISO 17025), and bacteriological indicators (ISO16649-3:2015), and lately is implemented the use of cellular biosensors to assess the cytotoxicity of waters (ISO 16649-3:2015). This paper will refer to the results on the quality of waters of Butrint Lake in Albania and the problems encountered, using conventional standardized methods (ISO) and advanced methods of biotechnology (single-cell biosensors, fluorescence microscopy). The quality of waters is among the most discussed issues in the context of climate change, and so do the methodologies used to assess it. In this context, we believe that a combined use of standardized protocols with those of scientific research, can provide more complete and reliable results on water quality and their complex relationship with the geological content of soils, hydrology, climatic conditions and human interactions.

Keywords: CB-cyanobacteria, chemotaxonomy, single-cell biosensors, fluorescence microscopy, Flow Cytometry.

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INTRODUCTION

Lake Butrint is next to Butrint National Park in the municipal district, southeast of Saranda. The lake is approximately 16.3 km^2 in height, and a maximum depth of 21 m [8]. Butrint National Park became listed on the National Heritage List of Protected Monuments in 1948. The Ramsar Convention acknowledged the natural values of the Butrint Wetlands in 2002. [8,9]. It was added to the UNESCO World Heritage List in 1992 [8]. Furthermore, the Butrint area (about 40 kilometers) is on the list of key plant areas (IPA) [10]. Furthermore, cultivation of the mussel *Mytilus galloprovincialis* for human consumption is among important activities in the area [11]. During recent decades, extensive industrialization and farming, and other anthropogenic activities combined with poor waste management policies, have resulted in the release of a wide range of toxic compounds such as perfluorinated chemicals, polyaromatic hydrocarbons, polychlorinated biphenyls, pesticides, and heavy metals, into aquatic ecosystems. As a result, groundwater and surface water contamination provide a significant risk to humans and aquatic creatures. Whole cell biosensors appear to be effective replacements or complementing techniques to traditional chemical methods for monitoring and assessing the toxicological effects of water contaminants [1,2]. Recombinant DNA technology has recently been used to build a wide range of whole-cell bacterial biosensors. In principle, bacteria are genetically modified to respond to chemicals or physiological stimuli by manufacturing a reporter protein, such as luciferase, β-galactosidase, or green fluorescent protein. One of them consisted in the bioluminescence analysis of modified strains of *E.Coli* resulting from the expression of the luxCDABE operon, under the transcriptional control of specific promoters, to determine the toxicity of the tested materials using the portable biosensor kit [4]. Furthermore, the application of single-cell biosensors may provide signals that can be identified by flow cytometry, digital imaging, and/or epifluorescence microscopy [3, 5]. In general, a biosensor can be a cell-free or whole cell-based system that detects an analyte at a quantitative or qualitative level [6].

This study aims to provide a better approach towards the investigation of the quality of waters at Lake Butrinti, Albania through a combined use of ISO and research driven protocols for the determination of physical-chemical characteristics, biological indicators (chlorophyll a, TSIC), total prokaryotic cell abundance, and single-cell toxicity sensing.

Figure 1. Map showing the locations of the sampling stations at Lake Butrinti, Albania.

MATERIALS & METHODS

Water and Sediment samples were collected in

12 different stations (Fig 1), GPS locations as following:

S1- 102˚E 39˚48'47"N 20˚0'52"E';

S2- 256˚W 39˚48'36"N 20˚0'46"E;

S3- 83˚E 39˚47'21"N 20˚0'44"E;

S4- 185˚S 39˚44'37"N 20˚1'9"E;

S5- 298˚NW 39˚48'46"N 20˚0'59"E;

S6- 9˚N 39˚48'45"N 20˚1'31"E;

S7- 2˚N 39˚48'36"N 20˚1'58"E;

S8- 26˚NE 39˚48'21"N 20˚2'27"E;

S9- 264˚W 39˚48'39"N 20˚1'52"E;

S10- 25˚NE 39˚48'1"N 20˚2'56"E;

S11- 62˚NE 39˚45'21"N 20˚2'50"E

S12- 39˚46'9"N 20˚00'32.5"E

at lake Butrinti during 2023-2024. Water (~ 4 l) was collected for phytoplankton DNA isolation, for chlorophyll pigments isolation, chemical parameters measurements (2 l). Samples were kept in plastic containers, in dark, at 4ºC, until transported at the laboratories. For sediments aliquots of 5ml were taken from each sample and stored as a sediment slurry in 96% ethanol at-20°C to guarantee cell preservation for further processing.

Physical parameters were measured in water and sediment samples. For waters the physical-chemical parameters were measured in situ with the PRO DSS probe. For sediments aliquots of 5ml were taken from each sample and chemical parameters were analyzed according to the relevant ISO: COD-S SH ISO 10523:2012; BOD- APHA/AWWA 5210D/2017; NO3- ISO 7890-3:1988 and PO4- APHA/AWWA 4500-P C/ 2017.

Measurement of chlorophyll pigments

Water samples (2 L) for phytoplanktonic pigments isolation were filtered through a 47 mm diameter 0.7 µm pore size, GF/F filter under gentle vacuum. Content of chlorophyll “a”, “b”, and “c” was determined according to the acetone trichromatic methods using the equations based on the absorption maxima for each component respectively.

Total prokaryotic cell abundance in sediments was analyzed through microscopic counting. To first detach and purify cells from the sediment slurry, physical and chemical pretreatment following a protocol proposed by Amalfitano and Fazi, (2008) was performed. The number of cells on each slide was counted using a fluorescence inversion microscope (OPTICA) equipped with an Optikam PRO6 Digital Camera. Per section, a minimum of 300 cells was counted (in at least 10 randomly selected fields) to calculate overall cell abundance, taking into account the dilution from the slurry preparation.

Bacteria and Archaea Cell Abundance was determined following the *CARD-FISH* according to Fazi *et al*., 2007. For the hybridization of the filter sections, different HRP-labelled oligonucleotide probes and hybridization buffers were used to target the *Bacteria* (probes EUB338 I-III) and *Archaea* (probe ARCH915). At the end of the CARD-FISH protocol, filter sections were stained with 1.5 µ gml−1 DAPI solution and analyzed under the microscope (OPTICA) equipped with an Optikam PRO6 Digital Camera.

Water cytotoxicity testing was performed using the BioFix Lumi "Single-Shot" freeze-dried luminous bacteria kit (*Vibrio fischeri*) and a portable BioFix Lumi-10 luminometer to measure the water triggered stimulation/inhibition of the bacterial luminescence.

RESULTS

Chlorophyll Pigments and trophic state

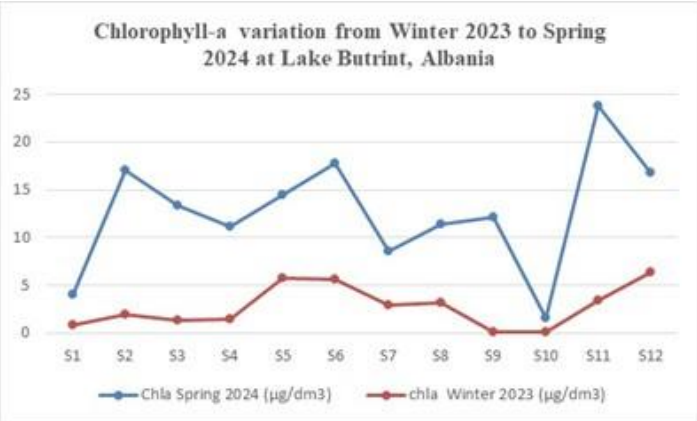

Figure 1. Chlorophyll-a content at Lake Butrint, Albania during Winter 2023-Spring 2024.

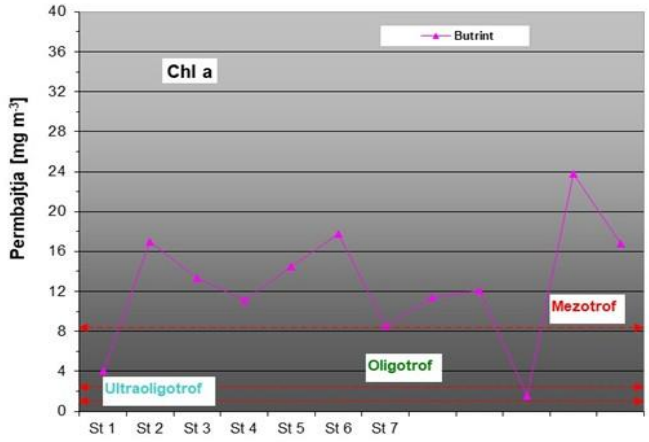

Figure 2. Trophic state at Lake Butrint (TSIC-Trophic State Index Carlson), Albania in Spring 2024. Only Stations 1 and 10 belong to oligotrophic state, the rest are typical mesotrophic.

Based on chlorophyll-a and TSIC the trophic state of waters at Lake Butrinti from winter 2023 to spring 2024 were changed from oligotrophic to mesotrophic (stations 1 and 10 from ultraoligotrophic to oligotrophic).

Physical-Chemical parameters of water samples

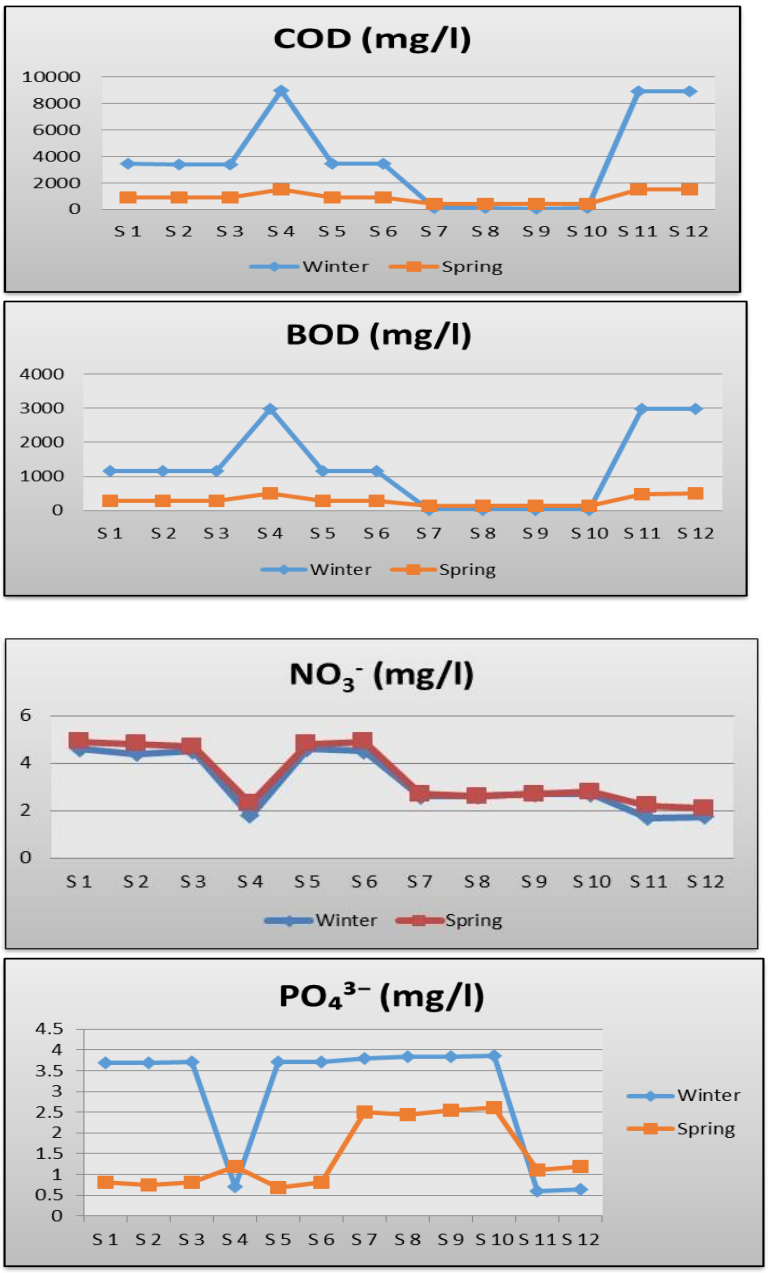

Figure 3. Chemical parameters (COD, BOD, NO3, PO4) in shallow waters at Lake Butrint, measured in 2023 - 2024.

Maximal values: The maximum value of pH was measured in January, while the maximum value of BOD was found in November. Two indicators had their maximum values in May (DO and TOC), while the other two (TSS and COD) in September. The maximum value for TDS was measured in July.

Minimal values: For the four indicators the minimum values were found in March (pH, TSS, TOC and BOD). For two indicators (DO and TDS), the minimum values corresponded to November. The minimum value for the COD variable was found in April.

Total prokaryotic cell abundance in sediments

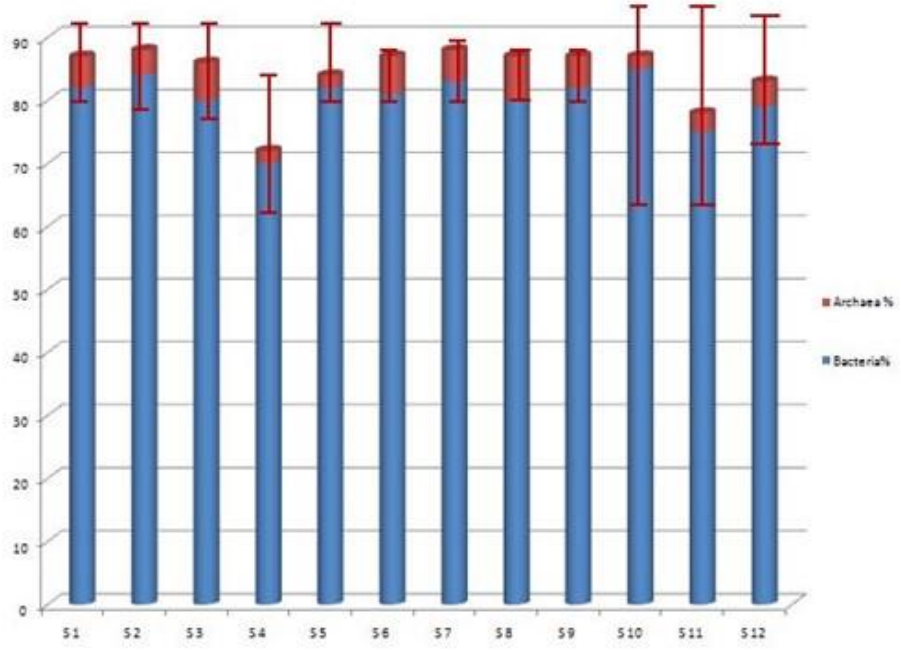

Figure 4.
Bacteria and Archaea cell abundance after CARD-FISH for sediment samples. Data are expressed as number of cells per gram of dry weight (DW).

Mean cell count after DAPI staining at sediment samples

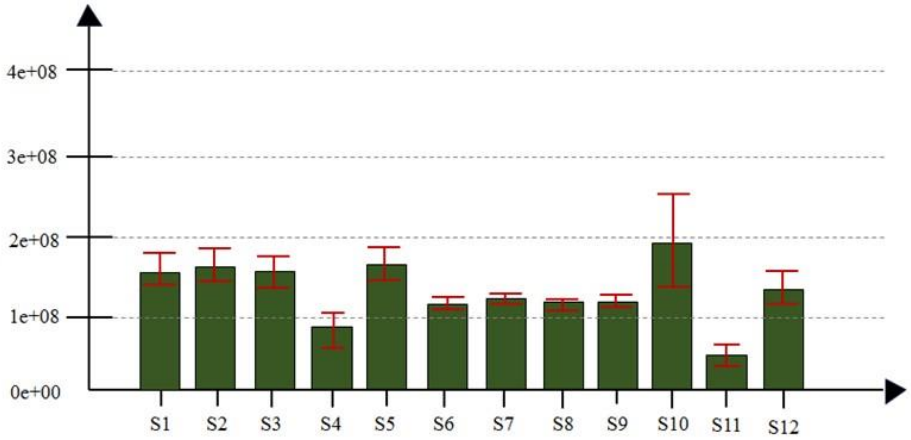

Figure 5. Mean cell count after DAPI staining. Data are expressed as number of cells per gram of dry weight (DW).

Heavy Metal and other Chemicals content at sediment samples & Cytotoxicity in water samples

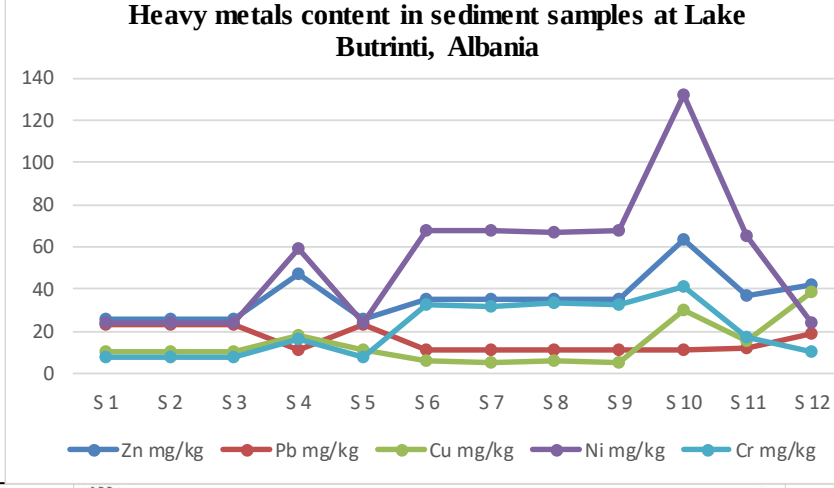

Figure 6. Heavy metals content in sediments at Lake Butrint, Albania.

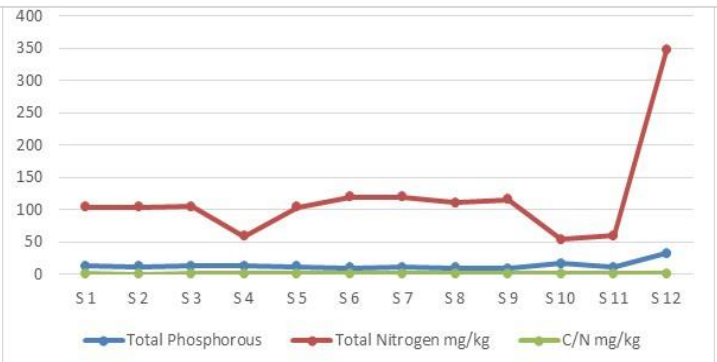

Figure 7. Chemical content in sediments at Lake Butrint, Albania.

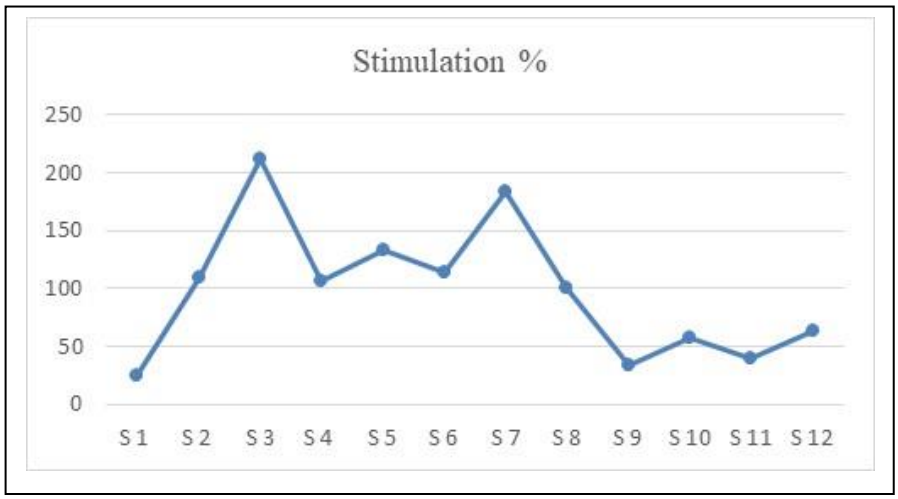

Figure 8. Cytotoxicity testing via investigation of the Stimulation / Inhibition of luminescence of bacteria in water samples.

Presence of heavy metals in sediments (Fig 6) is evidenced in all stations (especially at stations 4, 10), and high cytotoxicity (Fig 8) is present at waters in stations 2-8, where potential pollutants such as pesticides, herbicides, may be present due to intensive agricultural use of the area.

In a brief summary of the results we evidenced that:

In shallow waters:

- Chlorophyll pigments and trophic state at stations 1 and 10 correspond to clean waters (lowest phytoplankton concentrations compared to the rest of stations), however, from autumn-winter to spring season the trophic state is changing toward mesotrophy for 10/12 stations.
- Physical-chemical parameters of water help in identifying spatial and temporal quality of the waters in the lake, and contribute for a better understanding of the microbial density and composition.
- Cytotoxicity values, measured in spring 2024, reach the lowest values for stations 1 and 11, and highest values for stations 3 and 7, the last corresponding to the location of Center for mussel depuration and an intensive agricultural area, respectively.

In sediments:

- Heavy metal concentrations indicate the presence of Arsenic, Cadmium, Chromium, Copper, and Lead, suggesting strong anthropogenic impacts, especially at stations 4, 10 and 11 where Cr and Ni levels are higher compared to the rest of stations (the last correspond to Vivari Channel and an intensive agricultural area respectively).
- Mean cell count in sediment samples after DAPI staining indicate lowest values for Stations 4 and 11, which correlate with the added concentration of heavy metals.
- Cell abundance in sediment samples based on CARD-FISH also indicate a lower cell abundance at stations 4 and 11 compared to the rest.

In conclusion, different methods applied to determine the quality of waters prove to be effective in elucidating different aspects. Thus, the presence of heavy metals (especially of Cr) and possibly other pollutants of anthropogenic origin in sediments, explain the low cell abundance in stations 4 and 11. While water cytotoxicity at stations 3-7 is explained by the increased presence of organic pollutants used for agriculture in the respective areas. Meanwhile, the shift of the trophic state from oligotrophic to mesotrophic to most of the shallow waters around the lake, from autumn to spring time, speaks of the added impact the warmer climate conditions might have to the phytoplankton growth, and in general to the quality of waters. The combined use of monitoring protocols with those of scientific research, can provide more complete and reliable results on water quality and their complex relationship with the geological content of soils, hydrology, climatic conditions and human interactions.

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LENGTH FREQUENCY DISTRIBUTION, GROWTH PARAMETERS AND MORTALITY RATES FOR A STOCK OF BLEAK (*ALBURNUS SCORANZA* HECKEL AND KNER, 1857) FROM OHRID LAKE.

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Abstract

The bleak (*A. scoranza*) is a native fish species within the Ohrid-Drin-Skadar drainage. Aiming the bleak's stock assessment in the Ohrid Lake, we have studied the length-frequency distribution and have estimated some growth parameters from VBGF, the theoretical age at which the total length achieves 50% of $L\infty$ (t50%), and the instantaneous rate of natural mortality (M). The average values for total length and total weight were: TL, cm=11.71±2.45 (Var%=20.92) and W, g=12.87±6.58 (Var%=51.11). The values of growth parameters in VBGF were: the asymptotic length $L\infty$=20.76 cm; the annual growth coefficient K=0.198/yr and the hypothetical age the fish would have had at zero length to=-1.023 yr. The value of t50% was 2.4 yr and the value of natural mortality parameter was M = 0.484/yr. It resulted that in comparison to other studies, carried out for the stocks of *A. scoranza* in the Skadar Lake, the stock of Ohrid Lake is distinguished by smaller size individuals as well as by the lowest values of growth parameters.

Keywords: *bleak's stock, length frequency distribution, growth parameters, total length, asymptotic length, rate of mortality.*

Acknowledgements:

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Introduction: The genus *Alburnus* Rafinesque,1820 belongs to the largest teleost family, Cyprinidae, and comprises 38 recognized species distributed from Europe to northern parts of Southwest Asia (Freyhof and Kottelat 2007a,b). *Alburnus* species are commonly known as 'bleaks' (Buj et al., 2010). Four *Alburnus* species (*A. albidus*, *A. arborella* Bonaparte, 1841,*A. belvica* Karaman, 1924, and *A. scoranza* Bonaparte,1845) are reported to inhabit the waters of the Adriatic Basin in Italy, Switzerland, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Macedonia, Albania and Greece (Kottelat and Freyhof ,2007;Buj,2010).

Bleak is a small but commercially valuable consumable fish, with different commercial potential across the Balkan Peninsula. In the late '50s of the last century the bleak comprised 22% of the total catches in the Albanian part of Ohrid Lake (Filipi,1959).In the period between '60s and '80s the production of this fish comprised up to 60% of the total catches,as a result of the application of light fishing and the use of the purse seine as pelagic fishing gear (Shegani,2007).In recent years the bleak catches has fallen considerably (Dervishi et al.,2018).We think that the main reason for the drastic reduction of the bleack's stocks was the overexploitation of all age-groups of this fish,after the starting of the trout farming in the Pogradeci region and the large-scale use of bleak as food for the rainbow trout.

The aim of the present study was to estimate some bleak's stock parameters, including growth parameters from the VBGF, and to evaluate the structure of this species according to age-length relationships.Our assessment was multiparametric,takin into account the fact that we have to deal with a target species of commercial fisheries that is under the constant pressure of many natural and human stresors.

Material and Methods:The stock of the bleak (*Alburnus scoranza*),that is exploited by commercial fishing in the Albanian part of Ohrid Lake,was the object of this study. Ohrid Lake (40º 58' 41" N dhe 20º 41' 28" E) is located in the Balcan Penisula,on the border between the Republic of Albania and the Republic of Northern Macedonia.

Since in the period from April 20 to June 15,the bleak's fishing is prohibited by the low, sampling was started after June 20 and continue until the end of the November. All bleaks included in the samples were taken from commercial catches carried out with gillnets. For each fish were measured total length (*TL*,cm),to the nearest 0.1 cm and total weight (*W*,g),to the nearest 0.1 g.The scales,taken in the first-half part of the body,above the lateral line,are used to determine the age of the fish (Vilizzi,2018).

The parameters of VBGF ($L\infty$ - asymptotic length and K – annual growth coefficient) were estimated using the method of Ford-Walford (Pauly,1983).The hypothetical age the fish would have had at zero length (t_0 at L_0),was estimated using the empirical formula by Pauli (1979) as well as by Dong et al.(2019):$\text{Log10}(-t_0) = -0.392 - 0.275\ \text{Log10}(L\infty) - \text{Log10}(K)$.The formula: $t_{max} = 3/K$ (Pauly,1983),was used to estimate theoretical maximum age of the bleak.

The value of $t_{50\%}$ (the theoretical age at which the length achieved 50% of $L\infty$) was estimated using formula: $t_{50\%}=t_0+0.6931/K$ (Ragonese et al.,2012).Empirical formula $\ln(M)=-0.0152 - 0.279\ \ln(L\infty) + 0.6543\ \ln(K) + 0.463\ \ln(T)$,by Pauli (1980),using Von Bertalanffy growth parameters and the average annual water temperature in the lake (TºC),was used to estimate the instantaneous rate of natural mortality (M).

A difference of 1 cm is accepted as an interval between successive length-classes.The length frequency was estimated using the expression:$FTL\% = (n * 100) / N$,were n – the number of fishes in the corresponding length-classe and N – the total number of fishes in the sample.

Statistical analyses were performed with computer's program IBM SPSS Statistics 21.0 (2019),accepting a significance level of 0.05 and the Microsoft Office Exel Software version 2010.Comparison of observed mean were carried out by analysis of variance (ANOVA) (Zar,2010).

Results:

a.*The length-age relationship*:

The average values for total length and total weight were:TL,cm=11.71±2.45 (Var%=20.92) and W,g=12.87±6.58 (Var%=51.11).The smallest bleak measured aged 0+ and had a length of *TL*=4.8 cm, while the largest bleak aged 4+ and had a length of *TL*= 15.6 cm. According to the age-groups that were identified in the assessed stock,the highest variability for the total length was in two (SD=1.12;Var%=12.72) and three-year-old (SD=1.08; Var%=11.13).The smallest value for the biometric indicators, that inform about the degree of body size polimorphism.were found for individuals older than 4 to 5 years (SD=0.71;Var%=5.00%).

b.*Length-frequency distribution*:

As in all normal distributions,the smallest values of the parameter *F*% belonged to the extreme length-classes (2.29% for the first and 4.00% for the eleventh length- classes).For the black's stock evaluated by as,the maximum value of *F*% belonged to the length-class 12.5-13.4 cm (*F*% = 24.57).

c.*The values of parameters in the VBGF*.

Applying the protocol according to Ford-Walford (Pauly,1983),we estimated the values of coefficients *a* and *b*.

a = 3.7432 ; *b* = 0.8177 ; r = 0.906

These values were used to calculate the values of asymptotic length ($L\infty$) and the annual growth coefficient (*K*):

$L\infty$ = a/1-b

$L\infty$ = 20.73 cm

K = -ln b = -ln 0.8177

K = 0.198/yr

The value of the hypothetical age the fish would have had at zero length,estimated using empirical formula by Pauli (1979) as well as by Dong et al.(2019),was *to*=-1.023 yr.

The coefficients in the Von Bertalanffy growth function (VBGF),were:

$Lt = 20.73\ [1- e^{-0.198\ (t+1.023)}]$

d.*The value of the theoretical age at which the total length achieves 50% of* $L\infty$ (*t*50%):

Applying the formula $t50\% = -to + 0.6931/K$ we have estimated the value of $t50\% = 2.39 \approx 2.4$ yr for the the theoretical age at which the total length achieves 50% of $L\infty$.

e. *The instantaneous rate of natural mortality* (*M*).

The value of natural mortality parameter,estimated according to Pauly (1980) was M = 0.484/yr

Discussion:

For the Ohrid's bleak,Filipi (1959) have found the corresponding values *TL*M=9.23 cm and *TL*max = 10.9 cm,for the average and the maximum total length.Also,for this population Kolaneci et al.(2010a) had reported the average total length of *TL*M=10.6 cm,while in the publication of Milosevic and Talevski (2016) was given the value of 11.65 cm for this parameter and the value 12.9 cm for the maximum total length (*TL*max).For the samples of *A.scoranza*,from the Black Drin and Zeta Rivers in the Ohrid-Drin-Skadar drainage and the Mat River,Buj et al.(2010) had estimated the value of 7.6 cm,as the average value for standard length (*SL*,cm).

In all the studies carried out for the *A.scoranza* from the Ohrid-Drin-Skadar drainage,the Ohrid population had demonstrated the smallest values of the length and body weight. In the Ohrid Lake population the values found for average total length (*TL*M,cm) were from 9.23 cm (Filipi,1959) to 11.65 cm (Milosevic and Talevski,2016).For the maximum total length (*TL*max,cm) the smallest value was 10.9 cm (Filipi,1959) and the highest value was 15.0 cm (Talevski,1992).In the Skadar Lake population the corresponding values of the mentioned parameters were: *TL*M = 13.1 cm (Kolaneci et al.,2010b) and *TL*max = 19.6 cm (Kolaneci et al.,2010b).Also,the values we found for the *A.scoranza* stock from Ohrid Lake (*TL*M = 11.71 cm and *TL*max = 15.6 cm) turned out to be smaller than those foun by mentioned authors that had assessed the population of this species from Skadar Lake.

It was found that geographic isolation plays a key role in the evolution of reproductive isolation and divergent morphology and that divergence cannot be explained by molecular genetic variation (Worsham et al.,2017). Southern positioning (related to the distribution's area of the species *A.scoranza*),the altitude of 695 m and the average annual water temperature of 13.2ºC,are features that distinguish Ohrid Lake as a biotope from Skadar Lake,which have an altitude of 6 m and an average annual water temperature of 16.5ºC.Filipi (1959) stated that if the bleak of Ohrid Lake and that of Shkodra Lake belong to a single species,the differences in the morphometric parameters show that the environmental conditions in Ohrid Lake are less favorable.

Our results proved that the bimodality of the length-frequencies in the bleak's stock from Ohrid Lake was generated by the reduction in the number of individuals on the intermediate length-interval,from 10.5 cm to 12.4 cm.This phenomenon was also found for the population of the bleak from Skadar Lake (Kolaneci et al.,2010b),as long as after the length-class 9 cm,the length-classes 10-11 cm demonstrated the reduction of the frequencies,according to the number of individuals.The authors who have analyzed the phenomenon of bimodality in length frequency-distribution,for fish populations,have identified several generating factors and mechanisms.

The sexual maturation was a primary influence on the development of bimodality,due to the fact that sexual maturation implies diversion of resources from potential somatic growth to gonadal development and,as a conseguence,a reduced growth rate must be expected for maturing as compared with immature fishes (Thorpe et al.,1982;Hunt et al.,1982). Fish within a single population may spawn at different times over a spawning period, often producing a young-of-the-year cohort that has a bimodal or multimodal size distribution. Such disjunct spawning is usually attributed to weather events (Huston and DeAngelis,1987).

Huston and DeAngelis (1987) had affirmed that:"Changes in size distribution result from the interaction of four primary factors elated to characteristics of individuals composing the population or

cohort (1) their initial size;(2) the distribution of growth rates resulting from differences among individuals because of random genetic and/or environmental effects;(3) the size and the dependence of each individual's growth rate that results from the interaction of the life story pattern with environment;and (4) mortality that may affect size classes differentely.Each of this factors may be influenced by an individual's genetic makeup,as well as by abiotic and biotic environmental effects.We classify the biological mechanisms that may affect these factors in a two-way table as either "inherent" (i.e,genetic) or "imposed" and as either "noninteractive" or "interactive",depending on whether they require interactions among the individual organisms in order to be expressed".

The bimodality in the length-frequency distribution,in addition to the factors related to the biological characteristics of the respective species (non-simultaneous release of the gamets,differentiated mortality after the reproduction as a result of the exhaustion of energetic reserves or in the periods of insufficiency of food resources,ecc) as well as abiotic and biotic environmental factors (including the regime of temperatures during the stage of embrionic development,the photoperiod alterations,disponibility of the food,alimentary competition,predation,ecc),may also be the cause the selectivity of the fishing gear and,in the our case,the gillnet selectivity.

Kolaneci et al.(2010b) had estimated the value of 0.17/yr for the instantaneous rate of natural mortality (*M*) in the *A.scoranza* population from Skadar Lake.The value estimated by as for this species in the Ohrid Lake,was *M* = 0.48/yr.

In conclusion,we affirm that our assessments reveal a partial situation of some features of the *A.scoranza's* stock,in some areas of the Ohrid Lake,in which this fish species is exploited by commercial catches.The opinions we discussed dictate the need for a differential evaluation of some intrinsic and extrinsic factors,that promote the polymorphism of morphometric and growth parameters, the bimodality of length-frequency distribution and the inclusion of different errors in the evaluations,when comparing the populations of a single species.

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BIOGAS PRODUCTION FROM *MISCHANTUS X GIGANTEUS* IN A CONTINUOUS ANAEROBIC DIGESTION SYSTEM

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Abstract
This study investigates the potential of *Miscanthus x giganteus* as a sustainable feedstock for biogas production in a continuous anaerobic digestion system. Given the increasing need for renewable energy sources, miscanthus presents a viable alternative due to its high yield potential and low maintenance. The experiment measured biogas yield and quality comparing it with traditional corn silage feedstock. Results indicate that Miscanthus can produce a comparable amount of biogas with a significant methane content, suggesting its potential as a sustainable biogas source.

Keywords: miscanthus, biogas production, continuous anaerobic digestion

Introduction

The utilization of renewable energy sources is increasingly essential as global energy demands escalate alongside growing environmental concerns (Ozili & Ozen 2023). Among renewable options, biogas emerges as a critical component due to its ability to convert biodegradable substrate into usable energy. *Miscanthus x giganteus*, a high-yield perennial grass, offers promising prospects due to its efficient biomass conversion into biogas, providing a sustainable alternative to conventional feedstocks like corn silage (Kiesel & Lewandowski, 2014). In the context of biomass for biogas production, miscanthus has shown to have a lower environmental impact compared to traditional crops, particularly in terms of carbon and energy footprints (Whittaker et al., 2016).
Furthermore, the continuous anaerobic digestion (AD) of miscanthus not only highlights its potential in sustainable energy production but also aligns with global strategies towards enhancing renewable energy outputs while minimizing ecological footprints (Johnson et al., 2007). The exact quantity and quality of the biogas may vary depending on the feedstock used and the conditions of the AD process (Bharathiraja et al., 2018). Biogas primarily consists of methane (CH_4), the most important component of this energy production (Teng et al., 2014), i.e. the greater the quantity, the better the quality of the biogas. This study aims to evaluate and compare the biogas yield and quality from miscanthus to determine its viability as a competitive and sustainable feedstock.

Materials and methods

Samples of *Mischanthus x giganteus* were collected in September 2021 and stored at −18°C. These samples were milled to less than 10 mm before undergoing anaerobic digestion. Digestate from a nearby biogas plant was used as inoculum along with corn silage samples. Before entering the 30-L bioreactor at mesophilic temperatures (38 ± 2.0°C), the samples were

defrosted and made into a paste which was used daily as feedstock for AD. A statistical analysis was carried out after the data was collected. Data were presented as the mean for each setup of AD during the 30-day period, along with the standard deviation and Tukey's HSD test ($p < 0.05$) for comparison of means. Graphical representations have been produced to provide a better understanding of the AD process.

Results and discussion

Table 1. Biogas production during continuous anaerobic digestion

Feedstock	Biogas NL/day/vs	Methane NL/day/vs	CH_4 (%)	CO_2 (%)
Corn silage	31,82±5,12^b	16,31±2,61^b	0,51±0,01^b	0,48±0,01^a
Corn silage : miscanthus (2:1)	29,01±4,241^b	14,21±2,17^b	0,49±0,03^a	0,51±0,03^b
Corn silage : miscanthus (1:2)	22,35±8,64^a	10,97±4,43^a	0,49±0,02^a	0,51±0,02^b
Miscanthus	22,28±5,87^a	10,94±2,92^a	0,49±0,014^a	0,51±0,01^b

The Table 1. presents data on biogas and methane production per unit of volatile solids (VS) from different feedstocks used in the study. The biogas production from corn silage showed the highest yield at 31.82±5.12 NL/day/VS, with a methane production of 16.31±2.61 NL/day/VS and a methane concentration of 51%. When corn silage was mixed with miscanthus in a 2:1 ratio, both biogas and methane production slightly decreased, registering 29.01±4.241 NL/day/VS and 14.21±2.17 NL/day/VS, respectively. The concentration of methane slightly decreased to 49%, while CO_2 increased to 51%. In a 1:2 mixture of corn silage to miscanthus, there was a further reduction in both biogas and methane outputs, recording 22.35±8.64 NL/day/VS and 10.97±4.43 NL/day/VS, respectively. Pure miscanthus feedstock showed similar outputs to the 1:2 mixture, indicating a lower biogas potential compared to corn silage but maintaining consistent methane and CO_2 concentrations.

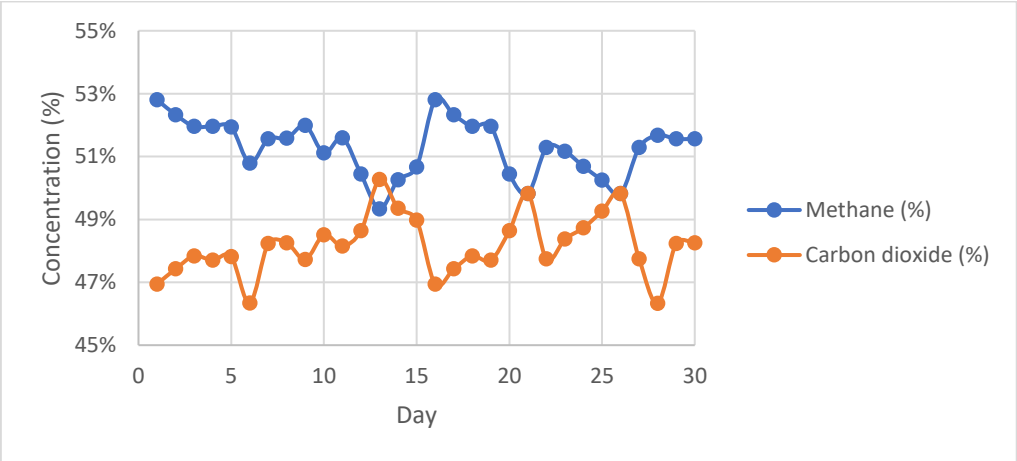

Figure 1. Biogas quality of corn silage

The graph displays the daily concentrations of methane and carbon dioxide in the biogas produced from corn silage over a 30-day period. The methane concentration, represented by the blue line, fluctuates between approximately 49% and 53%, showing a decreasing trend in the latter half of the month. In contrast, the carbon dioxide levels, illustrated by the orange line, generally range from 47% to 51%, inversely correlating with methane levels, increasing

as methane decreases. This inverse relationship is typical in biogas production, where the production of one gas can impact the concentration of another due to the fixed total gas volume.

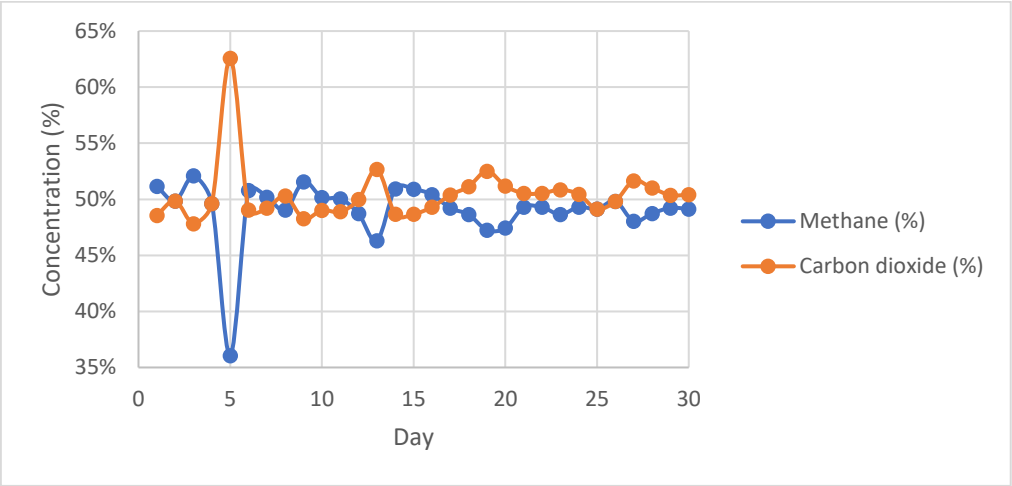

Figure 2. Biogas quality of corn silage and miscanthus (2:1 ratio)

This graph shows the concentrations of methane and carbon dioxide in the biogas produced from a mixture of corn silage and miscanthus in a 2:1 ratio. The methane concentration, exhibits significant variability early in the period, including a notable spike up to approximately 60% around day 5, followed by a sharp decline. Post this event, the methane levels stabilize around 50%. The carbon dioxide levels are hovering around 50% for most of the period. This pattern suggests a relative stabilization of biogas quality after initial fluctuations in the anaerobic digestion process.

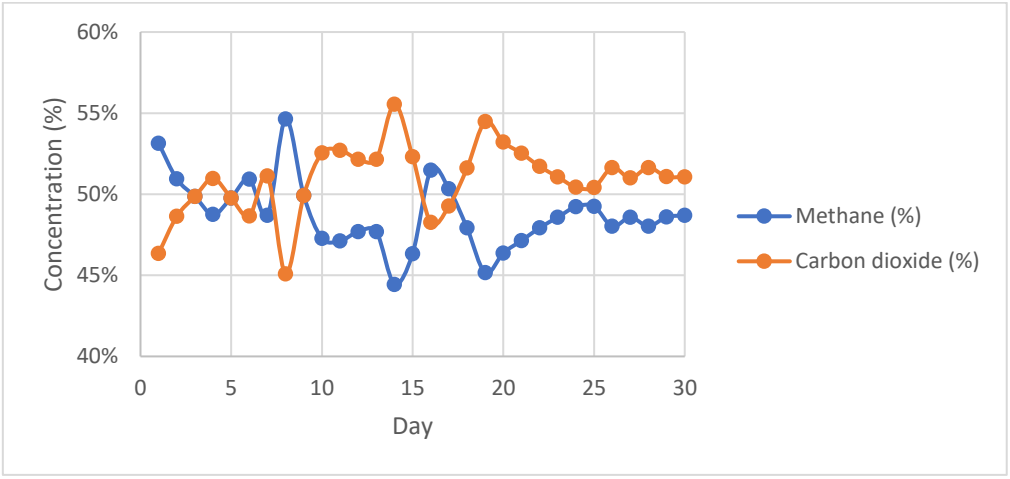

Figure 3. Biogas quality of corn silage and miscanthus (1:2 ratio)

The Fig. 3 biogas quality over a 30-day period from a mixture of corn silage and miscanthus in a 1:2 ratio. Methane concentrations, fluctuate significantly throughout the period, ranging from around 45% to just above 55%. Carbon dioxide levels, exhibit a complementary pattern to methane, typically oscillating inversely between approximately 45% and 55%. The variability in gas concentrations likely reflects the dynamic nature of microbial activity and substrate interaction within the anaerobic digestion process.

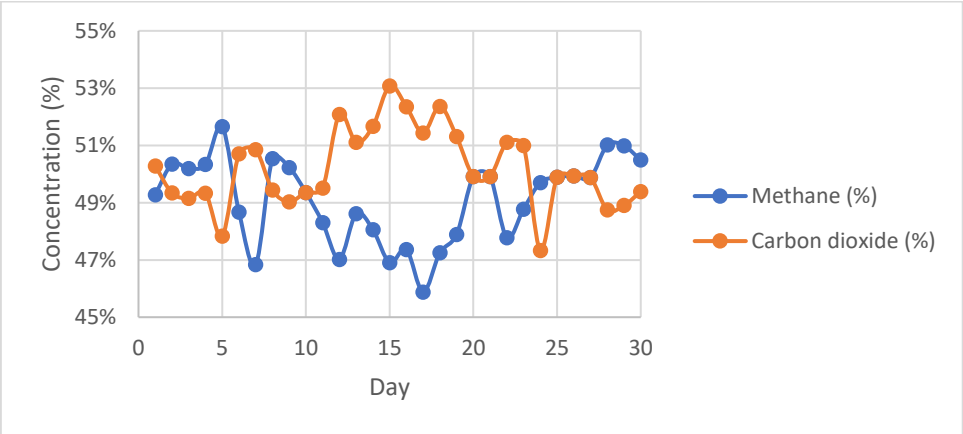

Figure 4. Biogas quality of miscanthus

The Fig. 4 shows the biogas quality using only miscanthus as the feedstock. Methane concentrations, fluctuate between 47% and 53%, as well as carbon dioxide levels. The frequent shifts in gas concentrations suggest active microbial processing and adjustment to the biogas system's internal conditions, highlighting the dynamic interaction between microbial communities and the miscanthus substrate.

Conclusion

This study reconfirmed the potential of *Miscanthus x giganteus* as a viable and sustainable feedstock for biogas production through continuous anaerobic digestion. The results show that although miscanthus produces slightly less biogas than corn silage, its sustainable cultivation methods and lower environmental impact make it an attractive alternative for the long-term renewable energy production strategy. The study highlights the effectiveness of miscanthus in maintaining a consistent biogas quality, with a remarkable methane content comparable to conventional feedstocks. Ultimately, the successful implementation of miscanthus in biogas production not only contributes to energy diversity but also enhances agricultural sustainability by providing a substitute crop for conventional corn silage.

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MUSHROOM HANDBOOK

On the May 30th the BioX expedition went for mushroom harvesting in Kupinečki Kraljevac, a forest near Zagreb (45°39'24.7"N 15°52'32.5"E). While picking mushrooms, participants from different countries found it interesting to compare common names of mushrooms in different languages. I.e. the mushroom *Leccinum scabrum* is called „Grandma" in Polish, but „Grandpa" in Croatian. Thus, the idea came to write down the common names of the mushrooms in languages of all the participants of the BioX. We therefore present the

BioX Mushroom Handbook

Below are the mushrrom names, followed by images taken onsite. The handbook was done with the help of „Kamilo Blagaić Mushrrom Society", https://www.cromushrooms.eu/ website, and ChatGPT OpenAI tool.





NUMBER	LATIN NAME	ENGLISH	ALBANIAN	CROATIAN	DUTCH
1	*Pleurotus*	Oyster Mushroom	Kërpudha e gocës	Bukovače	Oesterzwam
2	*Crepidotus epibryus*	Grass Oysterling	Krepidoti	Lisnata batrljica	Crepidotus
3	*Boletus reticulatus*	Summer Bolete	Kërpudha boletus	Vrganj	Zomereekhoorntjesbrood
4	*Leccinum scabrum*	Brown Birch Bolete	Boleti i thupërs	Dedek	Gewone berkenboleet
5	*Pluteus salicinus*	Willow Shield	Pluteu i shelgut	Vrbina krovnjača	Wilgenplooirokje
6	*Stemonitis axifera*		Stemoniti	Nitnica	Stemonitis
7	*Polyporus*	Umbrella Polypore	Polipori	Jelenovo uho	Polyporus
8	*Artomyces pyxidatus*	Coral Fungus	Kërpudha kurorë koral	Zdjeličasta capica	Kroontjeskoraalzwam
9	*Trametes gibbosa*	Lumpy Bracket	Tramete gibbosa	Guba	Gezonken elfenbankje
10	*Mycena sp.*	Bonnet	Mikena	Šljemovka	Helmmycena
11	*Lycoperdon perlatum*	Common Puffball	Kërpudha topth perlash	Puhara	Parelstuifzwam
12	*Macrolepiota procera*	Parasol	Kërpudha çadra	Sunčanica	Grote parasolzwam
13	*Hemimycena sp.*	Mosscap	Hemimikena	Šljemovka	Kleine mycena
14	*Xylaria polymorpha*	Dead Man's Fingers	Gishtat e vdekjes	Mrtvačevi prsti	Dodemansvingers
15	*Coriolopsis trogii*	Brownflesh Bracket	Coriolopsis trogii	Rupičarka	Coriolopsis
16	*Pluteus cervinus*	Deer Shield	Pluteu dreri	Jelenska krovnjača	Hertenzwam
17	*Mycena renati*	Beautiful Bonnet	Mikena e Renatit	Žutonoga šljemovka	Renati's mycena
18	*Leccinellum pseudoscabrum*	Brown Birch Bolete	Boleti i rremë	Dedek	Valse ruwe berkenboleet
19	*Agaricus bisporus*	Table mushroom	Shampinjon	Šampinjon	Champignon
20	*Cyclocybe aegerita*	Poplar mushroom	Populinëza	Jablanovača	Pioppino






NUMBER	LATIN NAME	FRENCH	ISRAELI	ITALIAN	POLISH	SERBIAN
1	*Pleurotus*	Pleurote	פטריית יער	Fungo ostrica	Boczniak	Bukovača
2	*Crepidotus epibryus*	Crépidote	קרפידוטוס	Crepidoto	Ciżmówka mchowa	Školjkarica
3	*Boletus reticulatus*	Cèpe d'été	פטריית בולט קיצית	Porcino estivo	Borowik usiatkowany	Prolećni vrganj
4	*Leccinum scabrum*	Bolete rude	לקצינום מחוספס	Porcinello grigio	Koźlarz babka	Brezov ded
5	*Pluteus salicinus*	Plutée des saules	פלוטאוס הערבה	Pluteo salicino	Drobnołuszczak zielonawowoszary	Vrbina krovnjača
6	*Stemonitis axifera*	Stémonite	סטמוניטיס	Stemonite	Październiorek rdzawy	Dlakasta sluznica
7	*Polyporus*	Polypore	פוליפורוס	Poliporo	Żagiew	Kukurijek
8	*Artomyces pyxidatus*	Clavaire couronnée	ארטומיצס	Fungo corallo	Świecznik rozgałęziony	Korlaka
9	*Trametes gibbosa*	Tramète bossue	טרמטס גבנוני	Tramete gibbosa	Wroślak garbaty	Zelenkasta ravnocevka
10	*Mycena sp.*	Mycène	מיצנה	Micena	Grzybówka	Slavkovače
11	*Lycoperdon perlatum*	Vesse-de-loup perlée	פטריית אבקית הפנינים	Vescie	Purchwka chropowata	Tikvasta puhara
12	*Macrolepiota procera*	Coulemelle	פטריית מצנפת גבוהה	Mazza di tamburo	Czubajka kania	Sunčanica
13	*Hemimycena sp.*	Hémimycène	המימיצנה	Emimicena	Białogrzybówka	Šlemovka
14	*Xylaria polymorpha*	Doigts de mort	פטריית אצבעות המתים	Dita di morto	Próchnilec maczugowaty	Đavolji prsti
15	*Coriolopsis trogii*	Coriolopsis trogii	קוריולופסיס		Włochatka jasna	Trogijeva rupičarka
16	*Pluteus cervinus*	Plutée couleur de cerf	פלוטאוס איילי	Pluteo cervino	Drobnołuszczak jeleni	Jelenska kovinjača
17	*Mycena renati*	Mycène de Renati	מיצנה של רנטי	Micena di Renati	Grzybówka złototrzonowa	Renatijeva šlemovka
18	*Leccinellum pseudoscabrum*	Bolete rude faux	לקצינלום מדומה	Porcinello falso	Koźlarz grabowy	Veliki grabov ded
19	*Agaricus bisporus*	Champignon de Paris	פטריית שמפיניון	Il prataiolo	Pieczarka dwuzarodnikowa	Šampinjon
20	*Cyclocybe aegerita*	Pholiote du peuplier	פטריית דבש הפופלר	Pioppino	Topolowy grzyb	Jablanovača

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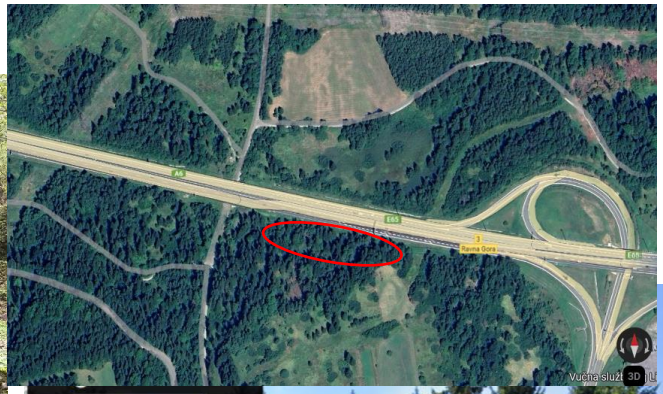
What's next?

Motivated by sucesfull sampling, and again, a very sucesfull networking, the BioX crew decided to continue research on the mushrooms. General idea is to sample them in the locations that are possibly burdened with heavy metals, and to check if mushrooms from that locations bioaccumulate heavy metals in significant amount.

Thanks to prof. Karolina Rudnicka and her colleague prof. Sylwia Rozalska from Lodz University, we already determined that on the first location, Kupinecki Kraljevec, there are elevated levels of manganese in the soil.

Also, second sampling was done by Croatian team, on two locations, one is the closed copper and iron mine „St. Barbara, Rude" near Zagreb, and the other is a mountain forest „Kupjak" just next to the main highway. Analysis of these samples is in progress. In the end, we hope to publish a BioX article in a relevant scientific journal.



Entrance to the old copper and iron mine St. Barbara.
Location: 45.763482, 15.671232
And the view on the Kupjak forest, by Google street view.
Location: 45.38441, 14.90904

Until next time....