

Natural
resources
green technology
& sustainable
development

4

Zagreb,
14th-16th
September
2022
ZAGREB
CROATIA



BOOK OF ABSTRACTS



**BOOK OF
ABSTRACTS**
2022.

ECCO

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Faculty of Forestry and Wood Technology, University of Zagreb
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Croatian Society of Biotechnology
Academy of Forestry Sciences
Croatian Chamber of Forestry and Wood Technology Engineers
"Zeleni prsten" Public Institution of Zagreb County

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Dear colleagues and

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We are honored and delighted to welcome you for the 4th time to the International scientific and expert conference "Natural resources, green technology and sustainable development/4-GREEN2022" organized by Croatian Forest Research Institute and Faculty of Food Technology and Biotechnology, University of Zagreb and supported by Faculty of Forestry and Wood Technology, University of Zagreb, Institute for Adriatic crops and karst reclamation, Croatian Society of Biotechnology, Croatian Chamber of Forestry and Wood Technology Engineers, Academy of Forestry Sciences and "Zeleni prsten" Public Institution of Zagreb County.

We are pleased that the Conference is taking place once more in Zagreb, the capital city of the Republic of Croatia.

We feel very proud to organize this Conference with the support of International organizations IUFRO, EFI and EBTNA.

leagues friends,

The conference is dedicated to challenges, risks and opportunities in environment and ecosystem management, while emphasizing potential of plant extracts, functional food and useful products coming from nature as well as implementation of green technology and biomass in general.

Beyond any expectations, more than 150 abstracts written by experts from 19 countries have been acknowledged for the presentation at GREEN2022. Researchers from eminent institutions will present their recent achievements, give their valuable insights and provide predictions for the future. This sharing of cutting edge knowledge will serve to help fighting challenges, reduce risks and enlighten the best way to capitalize on the opportunities which await us. The multidisciplinary approach will bring together scientists and experts to exchange and discuss the latest achievements in science, illustrate new policies, demonstrate innovative techniques and

outline sustainability of natural resources and new challenges rising with the climate change.

We use this opportunity to express gratitude to our patrons and Auspices, International Scientific and Organizing Committee as well as to all of you for your scientific involvement which will certainly contribute to the success of the Conference. Special thanks are addressed to sponsors who enabled the preparation of this event.

Thank you for joining us!

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CONFERENCE**
Sanja Perić



**CHAIR OF ORGANIZATION
COMMITTEE**
Tamara Jakovljević



**CHAIR OF SCIENTIFIC
COMMITTEE**
Ivana Radojčić Redovniković



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CONFERENCE HALL GRAND SALON

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International

8.00-9.00 Registration
9.00-9.30 OPENING CEREMONY

CHAIRS: T. Jakovljević, I. Radojčić Redovniković

9.30-10.15 **Alessandra De Marco:** Strategic roadmap to assess forest vulnerability under air pollution and climate change, IUFRO, Coordinator of Unit 8.04.00 - Impacts of air pollution and climate change on forest ecosystems, Italian National Agency for New Technologies, Energy and the Environment, Italy

10.15-10.45 **COFFEE BREAK**

10.15-10.45 **Poster presentations (SESSIONS A&B)**

Lectures & Parallel sessions

CONFERENCE HALL GRAND MEDITERAN

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SESSION A: **ENVIRONMENT AND CLIMATE CHANGE**

CHAIRS: L. Butorac, A. Paletto

10.45-11.30 **Giorgio Alberti:** Linking tree species richness and functional diversity to the carbon cycle in the context of climate change, Department of Agri-food, Environmental and Animal Sciences, University of Udine, Italy

11.30-11.45 **Hrvoje Marjanović:** Challenges in long term monitoring CO₂ Fluxes: The Case of Jastrebarsko Oak Forest, Croatian Forest Research Institute, Jastrebarsko, Croatia

11.45-12.00 **Vojislav Dukić:** Tree-Ring Chronology of Sessile Oak (*Quercus petraea* (Matt.) Liebl.) in the Northern Part of Bosnia and Herzegovina, Faculty of Forestry Banja Luka, Bosnia and Herzegovina

12.00-12.15 **Ernest Goršić:** Growth dynamics of Pedunculate oak influenced by spreading of oak lace bug in Republic of Croatia, Faculty of Forestry and Wood Technology, Zagreb, Croatia

12.15-12.30 **Doroteja Bitunjac:** Density and carbon concentration of downed dead wood by decay classes for ten tree species in Croatia, Forest Research Institute, Jastrebarsko, Croatia

12.30-12.45 **Silvija Zec:** Forests in women's hands, Croatian Chamber of Forestry and Wood Technology Engineers, Zagreb, Croatia



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SESSION B: **PLAN EXTRACTS – VALUE ADDED PRODUCTS**

CHAIRS: M. Brnčić, G. Cravotto

10.45-11.30 **Giancarlo Cravotto:** The industrial green revolution in plant extraction and downstream, Department of Drug Science and Technology, University of Turin, Italy

11.30-11.45 **Anastasia Loukri:** A green approach for the recovery of bioactive compounds from cornelian cherry (*Cornus mas* L.*) fruits, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Greece



11.45-12.00 **Manuela Panić:** Application of natural deep eutectic solvents-based plant extracts in cosmetic industry – from academia to business, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

12.00-12.15 **Irene Gómez Cruz:** Vine shoots, a natural resource with resveratrol: evaluation of extraction parameters, Department of Chemical, Environmental and Materials Engineering, Center for Advanced Studies in Earth Sciences, Energy and Environment (CEACTEMA), University de Jaén, Jaén, Spain



12.15-12.30 **Emilie Isidore:** Optimization of supercritical carbon dioxide extraction of rosmarinic acid from clary sage, URD Agro-Biotechnologies Industrielles, CEBB, AgroParisTech, Pomacle, France
Sponsored lecture - AlphaChrom Ltd.

12.45-14.00 **LUNCH**

Lectures & Parallel sessions

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SESSION A: **ENVIRONMENT AND CLIMATE CHANGE - continues**

CHAIRS: N. Potočić, A. De Marco

14.00-14.30 **Anikó Kern:** Climate data for 1951-2100 for scientific, societal and policy purposes in Central Europe: the FORESEE database, ELTE Eötvös Loránd University, Institute of Geography and Earth Sciences, Budapest, Hungary

14.30-14.45 **Renata Sokol Jurković:** Bias corrected precipitation and temperature from regional climate models, Croatian meteorological and hydrological service, Croatia

14.45-15.00 **Luka Basrek:** Ensuring benefits for more than 1 Million people through restoring the Sava river, Zeleni prsten Public Institution of Zagreb County, Croatia

15.00-15.15 **Mia Marušić:** The response of beech (*Fagus sylvatica* L.) saplings to drought in a fertilization experiment, Croatian Forest Research Institute, Jastrebarsko, Croatia



15.15-15.30 **Damir Drvodelić:** Influence of photosselective netting on growth of cherry laurel (*Prunus laurocerasus* L.) seedlings, Faculty of Forestry and Wood Technology, University of Zagreb, Croatia

15.30-15.45 **Lucija Lovreškov:** How high is ozone in Mediterranean forest ecosystems?, Croatian Forest Research Institute, Jastrebarsko, Croatia



15.45-16.00 **Ivan Limić:** Assessment of ions concentration in aleppo and black pine forests in the mediterranean part of Croatia; Institute for Adriatic Crops and Karst Reclamation, Split, Croatia



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SESSION B: PLAN EXTRACTS – VALUE ADDED PRODUCTS - continues

CHAIRS: K. Kovačević Ganić, V. Dragovic-Uzelac

14.00-14.15 **María del Mar Contreras:** Obtainment of bioactive compounds from the extractive and protein fraction of exhausted olive pomace, Ambientally de los Materiales, Universidad de Jaén, Spain

14.15-14.30 **Andrea Casas González:** Formulation of natural active compounds with antifungal applications: construction of pseudo-ternary phase diagrams and study of the release mechanism, BioEcoUVa Research Institute, PressTech Group, University of Valladolid, Spain

14.30-14.45 **Petronela Nechita:** The food packaging performances of paper coated with xylan hemicelluloses, Dunărea de Jos University of Galați, Department of Environmental, Applied Engineering and Agriculture, Engineering and Agronomy Faculty in Brăila, Romania

14.45-15.00 **Katarina Tumpa:** Comparison of the Kjeldahl and Dry Combustion Methods for the Determination of Nitrogen and Protein Content in 'Lovran Marron' Fruits from Croatia, Faculty of Forestry and Wood Technology, University of Zagreb, Croatia



15.00-15.15 **Morad Chadni:** Improvement of sinapine extraction from mustard seed meal by application of pre-treatment technologies, URD Agro-Biotechnologies Industrielles, CEBB, AgroParisTech, Pomacle, France

15.15-16.15 **COFFEE BREAK**

15.15-16.15 **Poster presentations (SESSIONS A&B)**

15.30-17.00 **Development of Green technologies at Faculty of Food Technology and Biotechnology, University of Zagreb through the work of prof. Jasna Vorkapić Furač (on Croatian)**

18.00 **ZAGREB GUIDED TOUR**

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8.00-9.00 **Registration**

SESSION C: BIOMASS FOR BIO-BASED INDUSTRY

CHAIRS: J. A. P. Coutinho, M. Rogošić

9.00 – 9.45 **João A. P. Coutinho**, Water-based solutions for biorefineries, Department of Chemistry, University of Aveiro, Aveiro, Portugal



9.45-10.00 **Marina Tišma**: All colors of *Trametes versicolor*, Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

10.00-10.15 **Hung Anh Le Duong**: Experimental study of thermal resistance values of natural fiber insulating materials under different mean temperatures, University of Sopron, Hungary



10.15-10.30 **Mario Novak**: Purple non-sulphur bacteria in development of sustainable bioprocesses for production of high value bio-chemicals and usage in bio-remediation, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

10.30-10.45 **Theresa Rücker**: Advanced analysis of high value-added products from a pilot scale lignin depolymerization plant, SINTEF Industry, Trondheim, Norway

10.45 -11.15 **COFFEE BREAK**

10.45-11.15 **Poster presentations (SESSION D)**

SESSION C: BIOMASS FOR BIO-BASED INDUSTRY
-continues

CHAIRS: V. Živković, T. Rezić

11.15-11.30 **Elaine G. Mission**: Microwave-subcritical hydrolysis: from model compound to complex biomass, PressTech, Instituto de Bioeconomia de la Universidad de Valladolid, Spain

11.30-11.45 **Neven Voća**: Sewage sludge management via energy crop production, Faculty of Agriculture, University of Zagreb, Croatia

11.45-12.00 **Zsófia Kóczán**: Modification of cellulose sheet properties with plantago psyllium seed husk, University of Sopron, Hungary



12.00-12.15 **Sanja Jakopec**: Effect of abrasive grain size on the abrasion resistance of black locust (*Robinia pseudoacacia*) in three characteristic section, Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Croatia



12.15-12.30 **Branimir Šafran**: Influence of moisture content on the calorific value of wood raw material, Department of Processes Engineering, Faculty of Forestry and Wood Technology, University of Zagreb, Zagreb, Croatia

12.30-12.45 **Branko Ursić**: Selected physical and chemical properties of wood chips produced in selective and salvage feelings of Norway spruce (*Picea abies* (L.) Karst.)

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Workshop: FINAL PRESENTATION THE RESULTS OF THE PROJECT "Isolation and encapsulation of bioactive molecules of wild and cultivated nettle and fennel and effects on organism physiology" funded by Croatian Science Foundation, grant number IP-01-2018-49244

11.15-11.30 **Maja Repajić**: Isolation of fennel essential oil by conventional and advanced extraction techniques, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

11.30-11.45 **Ena Cegledi**: The influence of extraction and environmental parameters on the isolation of bioactive compounds from nettle (*Urtica dioica* L.), Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

11.45-12.00 **Ivona Elez Garofulić**: Spray-drying encapsulation of nettle and fennel bioactive molecules, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

12.00-12.15 **Igor Palčić**: Influence of fertilization on wild nettle (*Urtica dioica* L.) and fennel (*Foeniculum vulgare* Mill.) yield

12.15-12.30 **Domagoj Đikić**: *Urtica dioica* (Stinging Nettle) extracts and its effect on Ppara, PPARγ and metabolic markers of lipid and glucose metabolism, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

12.30 -13.30 **LUNCH**

Lectures & Parallel sessions

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SESSION D: GREEN TECHNOLOGY

CHAIRS: M. Cvjetko Bubalo, W. Kroutil

13.30-14.15 **Wolfgang Kroutil:** Using biocatalysis to green organic chemistry, University of Graz, Institute of Chemistry, BioTechMed Graz, Field of Excellence BioHealth, NAWI Graz, Austria

14.15-14.30 **James H. Clark:** Phytocat: plant-based metals as catalysts, Green Chemistry Centre of Excellence, Circa Renewable Chemistry Institute, Centre for Novel Agricultural Products, University of York, UK

14.30-14.45 **Anita Šalić:** Advances of enzymatic microreactors – successful examples of integrated systems for process intensification, Faculty of Chemical Engineering and Technology, University of Zagreb, Croatia

14.45-15.00 **Kristina Radošević:** Deep eutectic solvents – promising green solvents for pharmaceutical application, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

15.00-15.15 **Thanos Andreou:** On liminality: between stability and function, VIO Chemicals AG

15.15-15.30 **Natela Dzebisashvili:** Treatment of the Wastewater from Ammonia and Microbiological Components by using Carbon Materials

15.30-15.45 **Vlatka Petravić Tominac:** Production of Arabitol from sugar beet pulp, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

15.30 -16.15 **COFFEE BREAK**

15.30-16.15 **Poster presentations (SESSION D)**

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Workshop: Issues of Coppice Management in the area of Lika

14.00-14.15 **Martina Dodan:** Project presentation: Issues of coppice management in FA Gospić, Croatian Forest Research Institute, Jastrebarsko, Croatia

14.15-14.30 **Valeriu-Norocel Nicolescu:** Coppice forests vs. High forests: a political, technical, and social issue in Romanian forestry, Faculty of Silviculture and Forest Engineering, Transylvania University of Brasov, Romania

14.30-14.45 **Darko Smerdel:** Coppice management in Croatian forests Ltd., Croatian Forests Ltd, Zagreb, Croatia

14.45-15.00 **Darijan Prugovečki:** Preliminary results of quality and growth of Lika coppices – Perušić case study, Croatian Forest Research Institute, Jastrebarsko, Croatia

15.00-15.15 **Jasnica Medak:** Plant composition dynamics in different forest types of fa Gospić area, Croatian Forest Research Institute, Jastrebarsko, Croatia

15.15-15.30 **Ivan Pilaš:** Spatial data analysis for typological characterization of the beech coppice forests in Gospić forest administration, Croatian Forest Research Institute, Jastrebarsko, Croatia

15.30 -16.15 **COFFEE BREAK**

15.30-16.15 **Poster presentations (SESSION D)**

20.00 **GALA DINNER**



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8.00-9.00 **Registration**

SESSION E: ECOSYSTEM MANAGEMENT AND MODELLING

CHAIRS: H. Marjanovic, A. Kern

9.00-9.45 **Katarina Merganičová:** Modelling Ecosystem dynamics under changing environmental conditions

9.45-10.00 **Alessandro Paletto:** A Decision Support System (DSS) to assess the performance of forest-wood supply chain according to the principles of circular bioeconomy, Reseach Centre for Forestry and Wood, Trento, Italy

10.00-10.15 **Maša Zorana Ostrogović Sever:** Soil Organic carbon modelling in Croatia: Needs and Challenges, Croatian Forest Research Institute, Jastrebarsko, Croatia

10.15-10.30 **Želimir Kurtanjek:** Causal modelling of the northern Adriatic sea ecosystem, Faculty of food technology and biotechnology, University of Zagreb, Croatia

10.30 -11.00 **COFFEE BREAK**

10.30-11.00 **Poster presentations (SESSIONS C&E)**

SESSION E: ECOSYSTEM MANAGEMENT AND MODELLING -continues

CHAIRS: D. Vuletić, M. Avdibegović

11.00-11.15 **Ines Hrdalo:** Urban green infrastructure: Past, present, future, Faculty of Agriculture, University of Zagreb, Croatia

11.15-11.30 **Damir Ugarković:** Microclimate of urban forest ecosystems of the city of Zagreb, Faculty of Forestry and Wood Technology, University of Zagreb, Croatia

11.30-11.45 **Martina Kičić:** Assessing hotspots of cultural ecosystem Services and disservices in the city of Zagreb, Croatian Forest Research Institute, Jastrebarsko, Croatia

11.45-12.00 **George N. Zaimes:** Land Use Change impacts on the kato nevrokopi torrent in Greece, Laboratory of Geomorphology, Edaphology and Riparian Areas (GERI Lab), International Hellenic University, Greece

12.45 -13.45 **LUNCH**

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SESSION E: ECOSYSTEM MANAGEMENT AND MODELLING -continues

CHAIRS: Krunoslav Indir, M.Z. Ostrogović Sever

13.45-14.00 **Mersudin Avdibegović:** Forest governance perspectives of wood biomass for energy in the federation of Bosnia and Herzegovina, Faculty of Forestry, University of Sarajevo, Bosnia and Herzegovina

14.00-14.15 **Karlo Beljan:** Integral Approach to private forestry and game management - investment analysis from Croatian Dinarides, Faculty of Forestry and Wood Technology, University of Zagreb, Croatia

14.15-14.30 **Sanja Bogunović:** Establishment of micropropagation protocol for the narrow-leaved ash in Croatia - Preliminary report, Croatian Forest Research Institute, Jastrebarsko, Croatia

14.30-14.45 **Anđelina Gavranović Markić:** Variability of height growth and survival of European Beech (*Fagus sylvatica* L.) in provenance trial "Vrbosko" - First results, Croatian Forest Research Institute, Jastrebarsko, Croatia

14.45-15.00 **Ivana Sirovica:** Invasive capacity of *Prunus serotina* and potential occurrence of *Rhagoletis Cingulata* - A Case Study in Croatia, Croatian Forest Research Institute, Jastrebarsko, Croatia



15.00-15.15 **Andro Kokeza:** Accuracy assessment of hand-held personal laser scanning for individual tree attributes estimation in old even-aged pedunculate oak forest, Croatian Forest Research Institute, Jastrebarsko, Croatia



15.15-15.30 **Zoltan Pásztor:** Revolutionary new forest measuring technology mobileforester, Faculty of Wood Engineering and Creative Industries, University of Sopron, Hungary

15.30-15.45 **Mislav Vedriš:** Estimating forest stand structure based on re-measurement on concentric circular sample plots, Faculty of Forestry and Wood Technology, Croatia

15.45-16.00 **Ivan Pilaš:** Floodplain forests mapping using earth observations and artificial intelligence, Croatian Forest Research Institute, Jastrebarsko, Croatia

16.00 **CLOSING CEREMONY**





Poster Presenta



tions

Session A

Wednesday,
14th September
2022

- AP1** **Anđelina Gavranović Markić, Miran Lanščak, Zvonimir Vujnović, Sanja Bogunović, Mladen Ivanković:** Dynamic of fructification and conservation of genetic resources of pedunculata oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) in light of climate changes (cropforclim) - ip-2018-01-8189
- AP2** **Monika Kamenečki, Dora Tomić Reljić, Aneta Mudronja Pletenac, Petra Pereković:** Defining the requirements for green roof guidelines in Croatia
- AP3** **Damir Drvodelić:** Influence of extreme air temperatures on seed germination of sweet wormwood (*Artemisia annua* L.)
- AP4** **Jolita Kruopienė, Inga Gurauskienė, Aušra Randė:** The Lithuanian phosphorus budget as a basis for resource optimization
- AP5** **Krunoslav Sever, Antonia Vukmirović, Daniel Krstonošić, Saša Bogdan, Ida Katičić Bogdan, Marko Bačurin, Tomislav Karažija, Željko Škvorc:** Influence of phosphorus nutrition on leaf dry matter content and leaf mass per area of common beech and sessile oak saplings
- AP6** **Ivona Kerkez Janković, Marina Nonić, Mirjana Sijačić-Nikolić:** Species diversity boosting - present challenge, issue of the future
- AP7** **Nevenka Čelepirović, Monika Karija Vlahović, Sanja Novak Agbaba:** DNA barcoding of pathogenic fungi on forest trees from Učka nature park
- AP8** **Marija Gradečki – Poštenjak, Nevenka Čelepirović, Monika Karija Vlahović, Sanja Novak Agbaba:** Beechnut quality by seed regions in Croatia
- AP9** **Sanja Novak Agbaba, Nevenka Čelepirović:** Investigation of foliage diseases of forest trees in the Učka nature park
- AP10** **Sanja Novak Agbaba, Marta Kovač:** Biological control using natural beaveria sp. for the control of the oak pest corythucha arcuata - laboratory and field experiment
- AP11** **Tamara Jakovljević, Ivana Vladimira Petric, Katarina Bulešić, Goran Stjepić, Lucija Lovreškov:** Monitoring of heavy metals in the soil of protected area in Istria

- BP3** **Natka Čurko, Anita Pušek, Ana Jurinjak Tušek, Marina Tomašević, Katarina Lukić, Mihaela Šmic, Ivana Radojić Redovniković, Karin Kovačević Ganić:** Application of supercritical CO₂ as green technology for oil extraction from Graševina grape seed pomace
- BP4** **Filip Dujmić, Sven Karlović, Marko Marelja, Roko Marović, Marija Badanjak Sabolović, Matija Pejčković Preksavec, Mladen Brnčić:** Influence of ultrasonic pre-treatment on the energy consumption of pumpkin (*Cucurbita moschata*) drying
- BP5** **Silvija Šafranko, Marija Banožić, Ina Čorković, Martina Jakovljević, Krunoslav Aladić, Stela Jokić:** Separation of bioactive compounds from mandarin peel citrus unshui using subcritical water extraction
- BP6** **Maja Dent, Lucija Nikin:** The influence of ultrasonic pretreatment prior hydrodistillation of rosmarinus on the yield of essential oil
- BP7** **Maja Dent, Anđela Miljanović, Dorotea Grbin, Zoran Zorić, Sandra Pedišić, Ana Bielen:** Water hydrodistillation residues of bay laurel, rosemary and sage as a source of polyphenols
- BP8** **Romana Popović, Antonela Ninčević Grassino, Jasmina Lapić, Senka Djaković:** Isolation of carotenoid from dried tomato peel waste by using different solvents
- BP9** **Mirella Žanetić, Marin Čagalj, Tatjana Klepo, Maja Jukić Špika, Ivica Ljubenković, Barbara Soldo:** Characterization of fatty acids and phenolic profile of olive oils from millennial wild olive trees (*Olea oleaster*) grown in olive gardens of Lun, island of Pag
- BP10** **Vanja Todorović, Nevena Dabetić, Sladjana Sobajic:** Flours from sunflower and pumpkin seeds after cold-pressed oils production as sources of nutritionally valuable proteins
- BP11** **Ana Bego, Filipa Burul, Maja Jukić Špika, Marijana Popović, Tonka Ninčević, Marija Mandušić, Jakša Rošin, Marin Čagalj, Mirella Žanetić, Katja Žanić, Slavko Perica, Valerija Dunkić, Marija Nazlić, Tanja Gotlin Čuljak, Elda Vitanović:** New methods in olive pests controlling using plant volatiles
- BP12** **Maria del Mar Contreras, A. Segura-Carretero, E. Abdel-Sattar, R. H. Mekky:** Linseed cake, a source of antioxidant compounds
- BP13** **Mia Ivanov, Tomislava Vukušić Pavičić, Višnja Stulić, Jasenka Gajdoš Kljusurić, Zoran Herceg:** High voltage electric plasma discharge application in environmental preservation

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Session B

Wednesday,
14th September
2022

- BP1** **Filip Šupljika, Mojca Čakić Semencić, Marko Iveša, Martina Vučilovski, Marinela Nutrizio, Anet Režek Jambrak:** Antioxidant capacity of the oregano and rosemary extracts obtained by high voltage discharge treatment
- BP2** **Nataša Mikulić, Ksenija Marković, Nada Vahčić:** Quality parameters of different types (honeydew, floral, chestnut, meadow, amorphous, mandarin, willow, wild cherry, forest, linden, sage) of honey

Session C

Friday,
16th September
2022

- CP1**  **Ivan Horvatić, Martina Kovačević, Zlatko Svečnjak, Darko Uher:** Green biomass of maize with climbing bean and cowpea as a sustainable source of protein
- CP2** **Nikolina Barlović, Stjepan Pervan, Miljenko Klarić, Nikola Španić:** Spectrometric analysis of lignin from abonos and oak wood (*Quercus robur* L.)
- CP3** **Tonči Rezić, Magdalena Anđelini, Ivan Perković, Roland Ludwig:** Effect of amino acids on LPMO activity

- CP4** ★ **Antonio Vidaković, Marilena Idžojić, Zlatko Liber, Igor Poljak:** Morphological variability of the leaves of european wild pear (*Pyrus pyraeaster* (L.) Burgsd.) populations from continental and mediterranean parts of Croatia
- CP5** **Nikola Španić, Ivana Plazonić, Tomislav Podvorec:** Cellulose acetate and waste paper based bio nano composites - optical and thermal properties
- CP6** **Danijela Petrović, Vojislav Dukić, Srđan Bilić:** Bark thickness of serbian spruce from natural stands and plantations
- CP7** ★ **Josip Klanac, Helena Lukšić, Vera Rede:** Influence of wood cell orientation on the spruce wood hardness
- CP8** **Aleksandra Gavarić, Senka Vidović, Jelena Rodić, Zorana Mutavski, Nataša Nastić:** Integrated processes effect on polyphenols content of peppermint
- CP9** **Marijan Logarušić, Igor Slivac, Kristina Radošević, Višnja Gaurina Srček:** Biological potential of hempseed and flaxseed protein hydrolysates in cho cell culture during oxidative stress
- CP10** **Ivana Lukic, Jelena Pajnik, Stoja Milovanovic, Vanja Tadić:** Supercritical CO₂ extraction of bilberry (*vaccinium myrtillus*) fruit
- CP11** **Stoja Milovanovic, Agnieszka Dębczak, Katarzyna Tyskiewicz, Marcin Konkol:** Supercritical CO₂ extraction from dandelion: the effect of pressure on extracts yield and composition
- CP12** **Carmen Padilla-Rascón, Juan Miguel Romero-García, Encarnación Ruiz, Inmaculada Romero, Eulogio Castro:** Use of olive stones to obtain furfural in a single-phase system
- CP13** **Carmen Padilla-Rascón, Juan Miguel Romero-García, Encarnación Ruiz, Inmaculada Romero, Eulogio Castro:** Two-stage sequential pretreatment of olive stones for cellulose recovery
- DP7** **Juan Miguel Romero-García, María Ruiz-Martínez, Ximena Valles-Novoa, Inmaculada Romero, Encarnación Ruiz, Eulogio Castro:** Optimization of xylose production from almond tree pruning
- DP8** **Juan Miguel Romero-García, Carmen Padilla-Rascón, Sergio Moreno-Moreno, Inmaculada Romero, Encarnación Ruiz, Eulogio Castro:** Olive stone as raw material for the production of levulinic acid
- DP9** **Josipa Martinović, Jasmina Lukinac Čačić, Marko Jukić, Gabriela Perković, Gordana Šelo, Mirela Planinić, Marina Tišma, Ana Bucić-Kojić:** Influence of different coatings and drying of wet microbeads in vitro release of phenolic compounds
- DP10** **Natalija Velić, Mateja Kamenjaš, Janez Gorešek, Marija Stjepanović, Indira Kosović, Darko Velić, Saša Despotović:** Biosorptive removal of the cationic dye malachite green from water by inactive biomass of fomitopsis pinicola
- DP11** **Filip Vranješević, Maria Kolympadi-Markovic, Valerije Vrček, Dean Marković:** Useful compounds from CO₂ by in silico directed catalytic synthesis
- DP12** ★ **Martina Bagović, Manuela Panić, Željko Jakopović, Senka Djaković, Jasmina Lapić, Kristina Radošević, Ivana Radojčić Redovniković:** Ciprofloxacin derivative – improvement of its solubility, permeability and antibacterial activity by deep eutectic solvent
- DP13** ★ **Anja Damjanović, Klara Pavić, Valentina Rožić, Manuela Panić, Marina Cvjetko Bubalo, Kristina Radošević, Ivana Radojčić Redovniković:** Industrial application of Graševina grape pomace extracts in natural deep eutectic solvents
- DP14** **Filipovic A., Dzambaski Z. , Bondzic B.:** Microreactor technology for green and sustainable photo- and organo-catalytic synthesis
- DP15** **Ana Dobrinčić, Ena Cegledi, Erika Dobroslavić, Daniela Cvitković, Jasna Mrvčić, Verica Dragović-Uzelac:** Green synthesis of silver nanoparticles using seaweed fucus vireoides and cystoseira barbata extracts
- DP16** **Mladen Pavlecic, Dominik Piškor, Mario Novak, Antonija Tronel, Nenad Mardetko, Marina Grubišić, Blanka Ljubas Didak, Vlatka Petravić Tominac, Božidar Šantek:** The influence of cane sugar and artificial sweeteners on water kefir production
- DP17** ★ **Mia Radović:** Mimicking nature: osmolytes-based deep eutectic systems
- DP18** **Tea Sokač, Maja Benković, Davor Valinger, Jasenka Gajdoš Kljusurić, Tamara Jurina, Ivana Radojčić Redovniković, Ana Jurinjak Tušek:** Global sensitivity analysis of agrigultural waste composting model
- DP19** **Muhammad Mohsin, Mir Md Abdus Salam, Nicole Nawrot , Erik Kaipainen , Daniel J. Lane, Ewa Wojciechowska, Niko Kinnunen, Mikko Heimonen , Arja Tervahauta, Sirpa Peräniemi, Olli Sippula , Ari Pappinen , Suvi Kuittinen:** Uptake of rare earth elements by willow (*salix spp*) from hydroponic solution
- DP20** **Ena Cegledi, Erika Dobroslavić, Ana Dobrinčić, Daniela Cvitković, Jasna Mrvčić, Verica Dragović-Uzelac:** Green synthesis of silver nanoparticles using nettle (*Urtica dioica* L.), myrtle (*Myrtus communis* L.) and bay laurel (*Laurus nobilis* L.) leaf extracts

Session D

Thursday,
15th September
2022

- DP1** **Ines Cindrić, Jasna Halambek:** Potential of waste tea as effective alternative adsorbent
- DP2** **Zorana Mutavski, Nataša Nastić, Senka Vidović, Stela Jokić, Aleksandra Gavarić:** A comparison of efficiency between ultrasound and microwave-assisted extraction of black elderberry pomace
- DP3** **Jasna Halambek, Ines Cindrić, Kristijan Perić, Elizabeta Zandona:** Corrosion resistance of aluminium in solutions of organic acids with the addition of carob powder
- DP4** **Darja Barić, Ana Rajković, Katarina Štefanac, Antonela Ninčević Grassino:** Influence of microwave-assisted extraction on polyphenols recovery from mandarin peel waste
- DP5** **Alisa Hasanbegović, Karla Johman, Josipa Kapitanović, Antonela Ninčević Grassino:** Exploitation of mandarin peel waste as a source of value-added compounds
- DP6** **Maria Kolympadi Markovic, Filip Vranješević, Valerije Vrček, Dean Marković:** Green synthesis of cyclic carbonates from carbon dioxide

- EP1** **Kristijan Tomljanović, Dario Biondić, Krešimir Krapinec, Ivica Medarić, Josip Tomljanović, Marijan Grubešić:** Correlation of spatial density and trophy value of mouflon (*Ovis aries musimon* pall.) in the period 1980 - 2020 in the coastal part of north Velebit
- EP2** **Ivana Zegnal, Jasnica Medak, Marija Gradečki-Poštenjak, Nevenka Čelepurović, Ivica Čehulić, Tamara Jakovljević, Sanja Novak-Agbaba, Anton Brenko:** Controlled germination and inoculation of holm oak (*Quercus ilex* L.) with two species of the genus tuber
- EP3** **Krunoslav Teslak, Marijana Andabaka, Karlo Beljan, Jura Čavlović:** Long-term stand structure dynamics of managed and unmanaged fir-beech forests in the Croatian Dinarides
- EP4** **Tomislav Dubravac, Dijana Vuletić, Damir Barčić:** Influence of different silvicultural works' intensities on growth and development of young pedunculated oak stands (*Quercus robur* L.)
- EP5** **Ivana Antolović Smoljan:** Educational trail 'Lovrin'
- EP6** **Mario Božić, Marko Klem, Ernest Goršić, Ivan Bazijanec, Milivoj Franjević, Antonija Kolar:** Influence of oak lace bug on radial increment of pedunculate oak trees withing different age classes in Spačva basin area
- EP7** **Marko Vucelja, Linda Bjedov, Anamarija Miškulin, Milan Pernek, Tomislav Dubravac, Dinka Matošević, Darko Pleskalt, Josip Margaletić:** Monitoring of small rodents in Croatian state forests from 2017 to 2020 – are we getting any wiser?





Plenary Talks



STRATEGIC ROADMAP TO ASSESS FOREST VULNERABILITY UNDER AIR POLLUTION AND CLIMATE CHANGE

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KEYWORDS:
*Fair pollution,
climate change,
forest ecosystem,
forest nutrients,
forest research
roadmap,
forest vulnerability,
radioactivity*

This work was outlined in the framework of the Research Group 8.04.00 "Air Pollution and Climate Change" under the International Union of Forest Research Organizations (IUFRO). IUFRO is the largest international network of forest scientists, promoting global cooperation in forest-related research and enhancing the understanding of the ecological, economic, and social aspects of forests and trees. Although it is an integral part of global change, most of the research addressing the effects of climate change on forests have overlooked the role of environmental pollution. Similarly, most studies investigating effects of air pollutants on forests have generally neglected impacts of climate change.

We review the current knowledge on combined air pollution and climate change effects on global forest ecosystems and identify several key research priorities as a roadmap for the future. Specifically, we recommend 1) establishment of much denser array of monitoring sites, particularly in the South Hemisphere; 2) further integration of ground and satellite monitoring; 3) generation of flux-based standards and critical levels taking into account the sensitivity of dominant forest tree species; 4) long-term monitoring of N, S, P cycles and base cations deposition together at global scale; 5) intensification of experimental studies, addressing combined effects of different abiotic factors on forests by assuring a better representation of taxonomic and functional diversity across the ~73,000 tree species on Earth; 6) more experimental focus on phenomics and genomics; 7) improved knowledge on key processes regulating the dynamics of radionuclides in forest systems; and 8) development of models integrating air pollution and climate change data from long-term monitoring programs.

LINKING TREE SPECIES RICHNESS AND FUNCTIONAL DIVERSITY TO THE CARBON CYCLE IN THE CONTEXT OF CLIMATE CHANGE

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KEYWORDS:
*carbon
sequestration
tree,
ecosystem
services,
species diversity*

The human alteration of the global environment has caused, and is still causing, widespread changes in the global distribution of species and habitats, with possible large impacts on biodiversity levels. Forests are among the least modified terrestrial ecosystems and harbour a large proportion of global biodiversity: recent estimates report that there are 73,000 tree species globally, among which 9,000 tree species are yet to be discovered, 40% of which in South America. A long history of ecological experimentation and theories supports the idea that ecosystem functions (EFs) and services (ESs) are strongly related to biodiversity: several studies have shown that increasing species richness positively affects productivity, biogeochemical cycles and soil carbon (C) sequestration, resilience and resistance to climatic extremes, microbial biomass and pedofauna diversity. However, the effects of tree species richness or diversity on EFs and ESs depend largely on the type and intensity of ecological interactions that occur within particular tree species mixtures, by site characteristics and by the particular attributes of species or their functional traits. The presentation will review the most recent findings about the existing relationship between tree species diversity and carbon storage/fluxes with a particular focus on the role of tree diversity in enhancing forest resistance (i.e. the ability to withstand harsh events) and/or resilience (i.e. the ability to regain the pre-disturbance growth rates) to climatic extremes. In fact, some evidences exist on how tree species diversity may eventually contribute in reducing the ecosystem's vulnerability to these stresses through resources partitioning, facilitation and selection effects.

THE INDUSTRIAL GREEN REVOLUTION IN PLANT EXTRACTION AND DOWNSTREAM

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technologies,
process
intensification,
subcritical water
extraction,
2-methyloxolane*

Conventional solid/liquid extraction procedures suffer from several drawbacks, such as prolonged extraction times and relatively low yields, the formation of by-products, and the use of flammable or toxic organic solvents. In other words, the extraction of natural products under conventional procedures cannot be considered a green process. Over the last two decades, the literature has highlighted the considerable effort made by researchers to find efficient and environmentally friendly extraction processes that comply with the principles of green extraction. Improved heat and mass transfer lead to lower solvent and energy consumption, increasing yields and extract quality while minimizing degradation. Relevant advances have been made with the use of non-conventional technologies and green solvents. Among the most effective extraction technologies, we can list supercritical fluid extraction (sc-CO₂) and extractions assisted by microwaves, ultrasound, hydrodynamic cavitation, pulsed electric fields, ohmic heating and by enzymatic treatments. Besides process intensification via the abovementioned energy sources, the new paradigms in plant extraction are mainly related to procedures in continuous flow, which is in contrast with the typical batch methods. One of the most versatile and eco-friendly method exploits the unique features of subcritical water extraction over the boiling point up to 150°-160°C (pressure 5-6 bar). In these conditions, hydrogen bonds are broken, and water changes polarity and dielectric constant, improving the extraction power and mimicking the behaviour of hydroalcoholic mixtures. As regard lipids extraction, a variety of alternatives to hexane have been recently investigated, among them 2-methyloxolane, which has emerged as a safe and effective bio-based alternative. Several enabling technologies have been also exploited in downstream processing (concentration, filtration, drying, crystallization, emulsification, pasteurization etc.) with a remarkable process intensification and an overall improvement of product quality.

WATER BASED SOLUTIONS FOR BIOREFINERIES

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KEYWORDS:
biorefinery,
biosolvents,
eutectic
solvents,
hydrotropy

On the quest for more sustainable processes of biomass fractionation water should, whenever possible, be the solvent of choice. Its limitations as a solvent towards a large number of compounds that range from the hydrophobic lignin and lignin fractions, phenolics and dyes to the most hydrophilic compounds such as cellulose, require that water properties are modified by physical means (e.g. temperature) or chemical additives (e.g. salts, hydrotropes, surfactants...). Novel solvents such as ionic liquids, eutectic systems or biosolvents all benefit of being used in aqueous solution, which minimizes their economic and environmental impact on the process, but also often improves their solvation ability and transport properties.

In this communication we will show how aqueous solutions can be used for biomass fractionation, and aqueous biphasic systems as stimuli-responsive media for biocompounds purification or conversion. We will present examples addressing both lignocellulosic and marine biorefineries, showing how not only wood, but also algae and fisheries waste streams can become the basis for a biorefinery, and a source of valuable natural compounds. These examples will highlight the potential of aqueous solution of green solvents, and will allow to discuss the molecular mechanisms behind their enhanced solvation performance when compared to their pure forms.

USING BIOCATALYSIS TO GREEN ORGANIC CHEMISTRY

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ELISA LANFRANCHI, SOMAYYEH
GANDOMKAR, EMMANUEL CIGAN,
FRIEDA SORGENFREI, JÖRG
H. SCHRITTWIESER, STEFAN E.
PAYER, JULIA PITZER, JÖRG H.
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KEYWORDS:
biocatalysis,
biotransformations,
light, regioselectivity,
stereoselectivity

Without doubt, organic synthesis has a major impact on our standard of living. Nevertheless, economic, environmental and social concerns of our society still urge chemistry to make synthetic routes even more efficient, more easily scalable, and more cost-effective. Using biocatalysts, thus, catalytically active proteins, has become an alternative for selected transformations. Thereby biocatalysis can contribute to green organic chemistry by various options:

(a) the outstanding regio- and stereo-selectivity enables reactions not feasible by established organic methods, like the asymmetric mono-amination of diketones. Using such transformations lengthy synthetic routes can significantly be shorted to a few steps. Another example is the C-H oxidation to chiral alcohols: using peroxygenases is becoming a powerful tool to access optically pure alcohols.

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PT

(b) Combining light and biocatalysis enables new options for transformations as well as cofactor recycling.

(c) Biocatalysts enable reactions with reduced amount of reagents under mild conditions like for instance the amidation of L-proline in organic solvents, thereby avoiding stoichiometric amounts of thionylchloride and maintaining optical purity.

(d) Several catalysts can be combined in one pot, thereby shortening processes, like the vinylation of phenols.

Examples like the ones mentioned above and other will be discussed in the lecture.

MODELLING ECOSYSTEM DYNAMICS UNDER CHANGING ENVIRONMENTAL CONDITIONS

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KEYWORDS:

*dynamic
vegetation
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empirical
models, gap
models,
process-
based models,
structural
models*

Information technologies have been developing fast over the last decades. This development has opened new computer-aided opportunities for researchers to investigate the causes and reasons of varying ecosystem dynamics. Computational modelling has been rapidly evolving and currently, a number of models that simulate natural processes in ecosystems are available across the globe. They can provide us with the predictions of ecosystem development within a short simulation time, which is considered one of the greatest advantages particularly when studying forests that live for several decades. Recently, the relevance of model usage has been increasing due to the ongoing climate change that creates novel environmental conditions. Here we discuss how the principles applied in model constructions determine their ability to answer specific questions on ecosystem dynamics. We also present how models can help us to increase our understanding of ecosystem functioning and to reveal potential risks by testing various trajectories of its future development driven by different site conditions and/or management practices.

CLIMATE DATA FOR 1951-2100 FOR SCIENTIFIC, SOCIETAL AND POLICY PURPOSES IN CENTRAL EUROPE: THE FORESEE DATABASE

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KEYWORDS:
*bias correction,
E-OBS, Euro-
CORDEX,
impact studies,
Meteorological
datasets*

The FORESEE (Open Database for Climate Change Related Impact Studies in Central Europe) is an open access, climatological database containing observed and projected daily meteorological data for Central Europe (41.5–51.5°N, 9.0–30.0°E), covering the 1951–2100 period. The FORESEE is a unique combination of observations and climate model projections that was constructed to support impact assessment in various sectors where observation based reconstruction of the past climate and reliable climate projections are needed. FORESEE mainly focuses on the essential climate variables like maximum/minimum temperature and precipitation, and is disseminated on a regular grid with 0.1° × 0.1° spatial resolution. For the 1951–2020 time period the latest FORESEE (v4.0) provides observation-based, interpolated meteorological fields derived from the E-OBS dataset. The future climate (2021–2100) is represented by the results of 28 bias- and discontinuity-corrected combinations of different Regional Climate Models (RCM) driven by Global Climate Models (GCM), representing a selection from the RCP 4.5 and 8.5 scenarios that were retrieved from the Euro-CORDEX database. Daylight average temperature, vapour pressure deficit and downward shortwave radiation flux were calculated from the base data on the same grid using the widely validated MT-CLIM model, supplementing the basic dataset. The practical application of the FORESEE datasets has been extensively growing during the last decade emphasizing the importance of the freely available climate datasets providing bias-corrected meteorological data for a large diversity of impact studies in the region. The lecture presents the correction technique that also addressed precipitation frequency. An overview of the simulated temperature and precipitation trends in the region is presented. Modelling possibilities are also discussed.



Session A:

CHALLENGES IN LONG TERM MONITORING CO₂ FLUXES: THE CASE OF JASTREBARSKO OAK FOREST

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KEYWORDS:
*dead wood,
decomposition,
eddy covariance,
forest, long-term
monitoring*

Trees live at time-scale which can span centuries, making forest ecosystems extremely complex. The ongoing change in climate, in combination with new pests and diseases, affects forest and its carbon cycle. To understand the processes in forests, as well as the effects of the various disturbances, long term monitoring is needed.

Measurement of the exchange of CO₂ between the forest and the atmosphere, coupled with the meteorological and other environmental measurements, offers profound insight into the functioning of the ecosystem. The CO₂ and water vapour flux measurements, obtained with the eddy covariance (EC) technique, enable the estimation of net ecosystem exchange (NEE), gross primary productivity (GPP), and ecosystem respiration (Reco) at half-hourly time scale. These data can then be used in the assessment of the impacts of management, pests, climate extremes on forest, as well as for calibration of the process-based models.

Setting up and maintaining an eddy covariance system functioning for years is challenging. In this presentation we will present the results of 15 years of EC flux measurements in the pedunculate oak forest near Jastrebarsko. We will reflect on the challenges we faced during that time, from technical (power and instrument failure, increasing tree height), faunal (ants, wasps, hornets, mice, wild bores), to the unforeseen impact of the COVID-19 pandemic and decomposing dead wood.



TREE-RING CHRONOLOGY OF SESSILE OAK (*Quercus petraea* (Matt.) Liebl.) IN THE NORTHERN PART OF BOSNIA AND HERZEGOVINA

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KEYWORDS:
*Bosnia and
Herzegovina,
sessile oak,
SPEI, tree-ring
chronology*

The paper presents the tree-ring chronology of sessile oak in the northern part of Bosnia and Herzegovina, which belongs to the continental biogeographical region of Europe. The required data were taken at five sites. The sample included a total of 115 trees (from 15 to 33 trees per site). Standard dendrochronological data processing was performed by sites using Cofecha and Arstan software. The determined values of correlation coefficient (from 0.40 to 0.68) and the Gleichläufigkeit (from 0.680 to 0.742) between the formed chronologies by sites showed that there is a significant match or consistency of the formed chronologies, so the master chronology of sessile oak for continental territory of Bosnia and Herzegovina was made. The first year of the tree-ring series is 1852, and the last year is 2008, ie the length of the obtained chronology is 157 years. If we take the year in which at least four tree-ring series are present as the beginning of the chronology, the first year of the chronology is 1861, that is, the length of the obtained chronology is 148 years. The obtained values of expressed population signal (EPS), which are higher than 0.85, indicate the possibilities of using the obtained chronology for dendroclimatological analysis. Correlation analysis of the relationship between the obtained master chronology of sessile oak and quarterly standardized precipitation evapotranspiration index (SPEI3) for the period from 1950 to 2008 showed that there is a statistically significant dependence ($p < 0.05$) of radial growth on dry periods, for May ($r = 0.27$), June ($r = 0.33$) and July ($r = 0.35$).



GROWTH DYNAMICS OF PEDUNCULATE OAK INFLUENCED BY SPREADING OF OAK LACE BUG IN REPUBLIC OF CROATIA

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KEYWORDS:
*climate, oak
lace bug spread,
pedunculate oak,
radial growth,
tree ring*

Oak lace bug *Corythucha arcuata* (Say, 1832) was first recorded in 2013 in eastern Slavonia and until now it has spread along the whole lowland pedunculate oak forests distribution range in Croatia. The aim of this research was to determine influence of *C. arcuata* infestation and compare trends in radial growth of pedunculate oak trees in the period before and after oak lace bug appearance in old stands distributed along Sava river and its confluents. For this research five pedunculate oak-hornbeam (*Carpino betuli-Quercetum roboris*) stands within age class of 120-140 years were chosen within regional forest offices Lipovac, Cerna, Lipovljani, Veliki Grđevac and Karlovac. In all stands sampling was conducted on 15 visually healthy pedunculate oak trees belonging to dominant or codominant tree layer to minimize influence of competition. Two core samples per tree were taken at the breast height using Pressler increment borers and tree ring width in the period from 2000 to 2019 was measured. It was expected that radial growth will steadily decrease in all sampled stands after occurrence of *C. arcuata* infestation thus making possible to track spatial spreading of the insect. Results only partially correspond to the expectations. Expected average growth trend reduction in analysed stands is not clearly visible before year 2017 despite presence of *C. arcuata* in some of them. In the year 2017, radial increment started to decline in all analysed stands. Growth reduction was on average 19,78% in relation to average growth rate prior to this period. In the period before 2017 trees show normal growth response mostly related to various stand conditions. High radial increment in 2013 and 2016 was observed in most stands and is related to favourable climate conditions. Since meteorological factors, especially air temperature and precipitation, are decisive in the insect biology (influencing metabolic activity, abundance rate, and dispersion), an explanation could be that *C. arcuata* prefers or performs better in certain conditions (high temperatures, low humidity) and also feeds on leaves that grow under low or no water deficit. Based on the obtained results it is obvious that the effect of *C. arcuata* on reduction of radial growth of adult pedunculate oak trees is present but it is tied to complex relations of insect and plant biology in relation to climate. Further, longer period of systematic monitoring of the oak lace bug population, radial growth of pedunculate oak trees and climate is required.



DENSITY AND CARBON CONCENTRATION OF DOWNED DEAD WOOD BY DECAY CLASSES FOR TEN TREE SPECIES IN CROATIA

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KEYWORDS:
*coarse
woody debris,
decomposition,
density reduction
pattern, volume
conversion
factors*

Dead wood (DW) is an important component of forest ecosystem and in the context of climate change and carbon (C) accounting policy under UNFCCC and EU regulations it is recognized as a long-term C pool. The aim of our study was to provide national DW volume conversion factors, i.e. dead wood densities (DWD) and C concentrations (CC) by decay classes, which could be used for accounting of C stocks and C stock changes in DW pool in the National GHG Inventory Report (NIR). We hypothesized that investigated traits for specific tree species group (ring-porous, diffuse-porous, non-porous) will differ with respect to different biogeographical region (BGR).

Research was conducted on ten tree species from three BGRs, Continental, Alpine and Mediterranean. Stem discs were sampled from downed DW and categorized into five decay classes, from 0 (raw wood) to 4 (very decayed DW). In total, we collected 446 DW samples evenly distributed between tree species and decay classes. Samples were analysed for DW density (DWD) and C concentrations (CC). DWDs showed decreasing trend with respect to decay class, while for CC no trend regarding decay classes was observed. For each tree species group, DWDs by decay classes were compared between different BGR. In ring-porous group, a significant difference was observed in DWD between samples collected in Mediterranean and Continental BGR for decay classes 0-2, while in non-porous group DWDs significantly differ between samples collected in Alpine and Mediterranean BGR for decay classes 2-4. By using obtained volume conversion factors from this study, we provided new estimate of downed DW C stocks in Croatia. Also, we performed a comprehensive literature search on DWDs by decay classes for genera investigated in our study and observed differences in DWDs reduction patterns.



FORESTS IN WOMEN'S HANDS

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KEYWORDS:
*forestry,
gender equality,
sustainability,
women*

Fem4Forest is an acronym of the Interreg Danube Project Forests in Women's Hands with the participation of 14 project partners from 10 Danube region states. Croatian partners are the Croatian Union of Private Forest Owners Associations and the Croatian Chamber of Forestry and Wood Technology Engineers. The main goal of the project is to strengthen the forestry sector in the Danube region at the local, regional and interregional levels through increased involvement and the ability of women, supporting their equality and market competencies. The project offers a new and innovative approach to education and mentoring, which will enable the active role of women in the forestry sector.

A survey of forest experts and private forest owners, conducted as part of the project, highlighted the main obstacles that women in the forestry sector face, related to roles and stereotypes in the sector. As part of the Fem4Forest project, a training program is planned for the second half of 2022 - education and workshops to strengthen skills in various fields and raise self-confidence for easier achievement of career goals. This will enable women in forestry to more actively contribute to sustainable forest management and better ecosystem modelling.

The goal is to emphasize to the decision-makers, managers, and employees the importance of gender equality at all organizational levels in order to be able to recognize and exploit the potential of a gender perspective in forestry in the future.



BIAS CORRECTED PRECIPITATION AND TEMPERATURE FROM REGIONAL CLIMATE MODELS

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KEYWORDS:
*Adriatic region,
bias correction,
copula,
RCM ensemble*

Analysis of bias-corrected precipitation and temperature of the regional climate model (RCM) ensemble in the wider Adriatic area is conducted. The analysis was based on a set of 12 combinations, three RCMs and four Global Climate Models (GCMs) from 1971 to 2004, divided into parts for calibration and validation. Correction of climate models was performed according to E-OBS data on the 0.1 ° network. A comparison of one univariate and three versions of bivariate methods of correction of summer and winter monthly air temperature and precipitation bias was performed. To examine the impact of bias correction methods, we used different marginal distributions and interdependence distributions (copulas). Bivariate bias correction was performed using parametric and empirical marginal distributions. Gamma (precipitation) and normal (temperature) distributions were used in the quantile mapping (univariate (QM)) and parametric bivariate method, and Gaussian and Student t copulas were additionally used in the bivariate method. The bias and impact of correction methods on precipitation and temperature, including climate change signals in historical data, have been documented. The considered methods retained the spatial distribution of trends from the uncorrected ensemble. Two experiments were also conducted on how bias correction methods affect the statistical measures of the considered variables and their relationships. It has been shown that the bivariate method with empirical distributions (eeG) is somewhat more successful in maintaining the relationship between variables detected in the measured data.



ENSURING BENEFITS FOR MORE THAN 1 MILLION PEOPLE THROUGH RESTORING THE SAVA RIVER

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KEYWORDS:
*biodiversity, green
infrastructure,
flood protection,
nature-based
solutions,
Sava*

The Sava River is one of the most interesting cultural and ecological phenomena in Europe. It originates in the Slovenian mountains and flows into the Danube River near Belgrade. Much of the river still flows freely, and compared to the Western Europe's rivers, large areas of vast floodplains and forests have been preserved to this day. On the other hand, the river has been regulated for flood protection, exploitation of energy potential and increase of transport capacities. Despite all previous attempts to promote nature-based flood defense solutions, such methods are constantly being overlooked. The gray infrastructure narrows the space of the river and compresses it into a narrow strait that does not leave enough space for lateral erosion, which results in the vertical incision of the river into its own bed.

Due to the incision of the riverbed, we are witnessing declining groundwater levels with consequences on drinking water supply, agriculture, forestry and ecological processes in the river and the floodplain. Gray infrastructure has also proven to be an inadequate solution to prevent extreme water waves and often implies huge maintenance costs.

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In the scope of the project „Sava Parks II – Freedom for Sava“, the team consisting of EuroNatur Foundation, Croatian Society for Bird and Nature Protection and Zeleni prsten Public Institution of Zagreb County, coordinated the development of a feasibility study for restoring the Sava River, that was developed by Austrian experts from the REVITAL company. The study area consists of the river stretch from the last hydropower plant Brežice in Slovenia to the border of the Zagreb County in Rugvica Municipality, and the entrance to the Sava-Odra channel. It proposes a solution that combines two river ramps with the measures for river widening that would make the river Sava safer, related to flood protection or water supply, and more alive, concerning biodiversity and recreational use.

The study not only seeks to promote nature-based flood defense solutions but aims to encourage a comprehensive strategic reassessment of the national and international strategic documents, as well as to encourage all key stakeholders to support sustainable regional development in the Sava River Basin for the benefit of local communities and in long-term to increase biodiversity.

THE RESPONSE OF BEECH (*Fagus sylvatica* L.) SAPLINGS TO DROUGHT IN A FERTILIZATION EXPERIMENT

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KEYWORDS:
common beech,
drought,
fertilization,
nutrition,
photosynthesis

The increased frequency of droughts caused by climate change is a challenge to the survival of common beech trees. Photosynthesis and growth are sensitive to changes in environmental conditions and the hydraulic status of plants. Depending on stress severity and other factors hydraulic and metabolic changes can persist or be reversed upon stress release. In this context, the potential role of mineral nutrition in alleviating the negative impacts and recovery from drought stress on *F. sylvatica* saplings has not been sufficiently studied. To determine these effects, one-year-old beech saplings were placed in a greenhouse and exposed to different watering and fertilization treatments: CH (regular watering, higher fertilization dose), CL (regular watering, lower fertilization dose), DH (drought, higher fertilization dose), DL (drought, lower fertilization dose). In the drought treatment saplings were regularly watered until mid-July when watering was stopped until the pre-dawn water leaf potential values reached -2.0 MPa, after which watering was resumed. The other half of saplings were watered regularly. Saplings were treated with complex mineral fertilizer with 4g/l in high fertilization treatment and 2 g/l in low fertilization treatment. We measured chlorophyll fluorescence (Fv/Fm, Plabs) every week, during drought and throughout the drought release period. Samples for foliar analyses were collected before drought, at the peak of drought, and after re-watering in the recovery phase. The effect of fertilization on foliar concentrations of N was significant, regardless of availability of water, but more pronounced in combination with regular watering. The most prominent effects were detected in the post-drought phase. Fertilization had a pronounced positive effect on foliar P concentrations regardless of imposed water limitation, both in the drought and recovery phases. Only in the recovery phase, photosynthetic performance expressed as Plabs was higher in DH saplings than in DL saplings, but values for both were still lower than in regular watering treatments.



INFLUENCE OF PHOTOSELECTIVE NETTING ON GROWTH OF CHERRY LAUREL (*Prunus laurocerasus* L.) SEEDLINGS

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KEYWORDS:
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photoselective
nets, vegetative
growth

Cherry laurel (*Prunus laurocerasus* L.) is an ornamental shrub used mainly for hedges. It is tolerant of pruning and air pollution, making this species ideal for urban horticulture. Cherry laurel is propagated commercially by stem cuttings, which are rooted in the period from June to April. Nets have long been used in horticulture against hail, wind and excessive sunlight. Recently, a new technology of photoselective nets has appeared. They are not only used for plant protection, but also stimulate the desired physiological plant responses by spectral manipulation and improve light utilization by scattering. This study was conducted in 2019 and 2020 in the nursery garden of the Faculty of Forestry and Wood Technology, Zagreb, Croatia. Rooted cherry laurel cuttings from a heated greenhouse were transplanted into outdoor beds on June 28, 2019, at 10 x 20 cm spacing in rows with a north-south orientation. Three photoselective nets were used: a white, yellow, and red net (AGRITECH S. r. l., Eboli, Italy) and a standard green shade net as control. In each treatment, 30 rooted cuttings plus grafts were transplanted, for a total of 120 cuttings. The following morphological variables of the seedlings were measured: Height, root collar diameter, root collar cross-sectional area (TCSA), slenderness coefficient, number of first-stage branches in the lower third of the plant, total number of first-stage branches, total length of first-stage branches in the lower third of the plant, total length of all first-stage branches, and average length of first-stage branches in the middle part of the plant. The measurement was performed on June 9, 2021. Data were statistically analyzed using SAS Ver. 9.4 statistical software (SAS Institute, NC) using ANOVA and Tukey's HSD test ($P \leq 0.05$). The main objective of this study was to investigate the possible effects of different colored photoselective nets on the vegetative characteristics of cherry laurel. The results show that there are no statistically significant differences in all the above vegetative traits of cherry laurel seedlings grown under different types of nets. Therefore, green shade netting can be used for cherry laurel seedlings in nurseries. Further studies are needed to test this technology under other agroecological conditions.



HOW HIGH ARE OZONE CONCENTRATIONS IN MEDITERRANEAN FOREST ECOSYSTEMS?

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AOT40,
ozone metrics,
Pinus forests,
PODY,
Quercus forests

Ozone is strong oxidative air pollutant produced in photochemical reaction by precursor compounds (CH₄, CO, NO_x, VOC) at high temperatures and sunlight. This air pollutant is the most damaging to forests among other air pollutants. Ground-level ozone is of particular concern for forest ecosystems in Mediterranean region. The reason is that regional road traffic and industrial emissions combined with high temperatures and sunlight in Mediterranean region result in higher ozone concentrations than in other European regions. The aim was to measure ozone concentrations and determine whether these concentrations represent a threat to Mediterranean forest ecosystems in Croatia. Ozone concentrations were measured with passive Ogawa samplers in four forest ecosystems in two regions: *Quercus ilex* and *Quercus pubescens* forests in Istria, *Pinus halepensis* and *Pinus nigra* forests in Dalmatia. From measured data, two different ozone metrics for forest protection were calculated; accumulated ozone exposure (AOT40) and phytotoxic stomatal ozone fluxes (PODY) with an hourly threshold of uptake (Y) to represent the detoxification capacity of trees used for forest protection. Measured ozone concentrations were high on all four plots and were close to or reached 100 ppb recommended as an upper plausible limit for passive monitoring measurements at forest sites. Higher average concentrations were measured in Istria than in Dalmatia. The values of the calculated metrics based on accumulated exposure (AOT40) were higher than limit for forest protection. The highest values for PODY metrics were found in Dalmatian plot and the lowest values were found on Istrian plot. RFA analyses underlined that the most important predictors affecting tree growth of *Q. pubescens* and *Q. ilex* were AOT40 and for *P. nigra* plot was PODY. Ozone concentrations were high enough to cause symptoms indicative of oxidative stress found on ground-level plants in the oak forest in Istria but not on main forest species.

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ASSESSMENT OF IONS CONCENTRATION IN ALEPPO AND BLACK PINE FORESTS IN THE MEDITERRANEAN PART OF CROATIA

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KEYWORDS:
open-field bulk,
pinus halepensi,
pinus nigra,
precipitation,
throughfall

Atmospheric deposition is considered one of the controlling factors that determine the circulation of matter in forest ecosystems. Also, is a very important transport process for gases and particles from the atmosphere to terrestrial and aquatic surfaces. The chemistry of atmospheric deposition and its transformation in contact with vegetation are of great importance for understanding its effects on forests. In order to better understand the chemical transformation of rainfall after it has passed through the canopy, throughfall and open-field bulk precipitation were measured, during 2020. The study was conducted in different Mediterranean forest ecosystems along the eastern Adriatic coast on the most dominant conifer species: black pine (*Pinus nigra*) and Aleppo pine (*Pinus halepensis*). Sampling, measurements and concentration analyses were carried out according to the ICP methodology. Ion chromatography was used to determine the concentrations of ions, i.e. chloride, nitrate, sulphate, ammonium, sodium, potassium, calcium and magnesium. The results showed that total precipitation was lower in throughfall than in open-field bulk during the studied period. The ions concentration of open-field bulk precipitation and throughfall in the Aleppo pine forest were many times higher than the concentrations observed in the black pine forest. After passing the canopy, the pH of the throughfall in both forests were decreased. The difference between two samples indicates leaching of dry deposited particles and exchange with leaf surfaces. Our results will fill the knowledge gap in understanding how Mediterranean pine forests modify the chemical properties of precipitation.



DYNAMIC OF FRUCTIFICATION AND CONSERVATION OF GENETIC RESOURCES OF PEDUNCULATE OAK (*Quercus robur* L.) AND EUROPEAN BEECH (*Fagus sylvatica* L.) IN LIGHT OF CLIMATE CHANGES (CropForClim) - IP- 2018-01-8189

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KEYWORDS:
genetic diversity,
provenance trials,
seed quality testing,
seed yield

Global climate change, rising temperatures, precipitation and the occurrence of extreme weather conditions are affecting the stability and survival of forests. The basis of the stability of forest ecosystems is the ability of a certain population to adapt to changing environmental conditions, and the main role in this process is played by the genetic diversity of species. The loss of genetic resources of forest trees can be caused by climate change as a direct factor and various indirect factors such as attacks by various pests, forest fires and others. Human activity in the direction of preserving genetic diversity and proper use of forest reproductive material is extremely important. The importance of preserving the genetic diversity of forest trees was recognized by the Croatian Science Foundation within the project IP-2018-01-8189. The main goal of the project is to ensure the conservation of gene pool the most important species of deciduous trees in the area of Croatia, pedunculate oak and european beech. The project is being implemented at the sites of Croatian Forests Ltd, and the research team consists of scientists from the Croatian Forest Research Institute and the Faculty of Forestry and Wood Technology, University of Zagreb.

The key objectives of the project are to:

1. Determine seed crop dynamics of the project species in changing environmental conditions of the local climatic region,
2. Establish a methodology for more accurate estimation of their seed crops,
3. Determine correlation between their masting (quantity and quality of the seed crops) and various environmental factors,
4. Create a protocol for optimal seed storage of the project species,
5. Establish field trials for ex situ conservation of their genetic resources,
6. Increase knowledge about adaptive genetic diversity of the project species in the local region,
7. Disseminate results to various users/stakeholders (forestry sector, legislators, private owners, etc.).

This work has been fully supported in part by Croatian Science Foundation under the project (IP-2018-01-8189).



DEFINING THE REQUIREMENTS FOR GREEN ROOF GUIDELINES IN CROATIA

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KEYWORDS:
green roof
system directives,
maintenance,
plant and
substrate
selection

Green technologies and their possible applications lately promote the installation of green roofs. In Croatia, there is lack of legislation or regulation for the construction of green roofs. It covers a When planning green roofs, the most attention is paid to the impact of the system on the building; leakage / drainage, load on the structure, fire protection, etc. Thus living elements such as substrate and vegetation remain on the margins. The rationale for the research was to identify elements that can ensure an integrated approach to planning and implementation of green roof systems. Defined by specific climatic conditions, the possibility of using local materials and soil to create special substrates for green roofs, it is important to determine optimal soil dept level for specific plant categories and the use of indigenous species in the planting plan. The general categorisation of special substrates for green roofs is divided into three main types; (1) substrate for intensive, (2) simple intensive and (3) extensive green roof systems. They are defined in accordance with the FLL regulations ("Richtlinien für die Ausführung und Pflege von Dachbegrünungen" / "Directives for Green Roof Planning, Implementation and Maintenance") through: pH value, balanced provision of nutrients, water and air, water holding capacity, drainage and technical suitability. Within these parameters, it is still possible to make a mistake in selecting an adequate system. The microclimatic conditions of the site define the selection of plant species. So, the selected species must be correlated with specific substrate parameters. Plant selection is crucial especially because of long-term maintenance (nutrient content, pest and disease problem, effective drainage, weed reduction) of the green roof system since it is an unrealistic option to expect completely maintenance-free green roof. Also, this research proposes additional expert studies that will propose guidelines adapted to the local climate. It would contribute to the quality and simplicity of installation of green roof systems and give an overview of available options when resources are limited; economic, technical or plant based.



INFLUENCE OF EXTREME AIR TEMPERATURES ON SEED GERMINATION OF SWEET WORMWOOD (*Artemisia annua* L.)

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KEYWORDS:
Artemisia annua,
artemisinin,
climate changes,
seed germination,
sweet wormwood

About 520 species of the genus *Artemisia* L. are known in the world, while only 17 species and subspecies grow in Croatia. Sweet wormwood is an annual herbaceous plant 50 to 150 cm tall. The medicinal properties of sweet wormwood have long been known and medically proven, especially in the treatment of malaria in Africa, Asia and Europe. Sweet wormwood artemisinin brought researchers the 2015 Nobel Prize in Medicine. In Croatia, sweet wormwood belongs to the group of non-native flora, naturalized species with numerous fortified sites from east to south. The seeds are very small, weighing an average of 2.7×10^{-5} g. It belongs to the group of microbotic, Orthodox, positive photoblastic seeds with epigeic germination and a germination percentage of 79%. Almost all seeds germinate within 7 days. Given the global climate change and the increasing occurrence of extreme temperatures and their duration, the aim of the study was to determine the impact of extremely low (-40 °C) and extremely high ($+60$ °C) air temperatures on seed germination. The seeds were treated at a temperature of -40 °C and $+60$ °C for 72 hours before germination in distilled water in PVC Petri dishes. Seed germination was recorded every day, for a total of seven days. Seeds treated at high temperatures after two days began to germinate in terms of penetrating the radicals through the seed coat in a higher percentage compared to seeds treated at low temperatures. The results of this study show greater adaptability of sweet wormwood seeds to higher temperatures and, accordingly, greater potential of habitat suitability for growth in the event of an increase in average temperatures, temperature extremes and heat waves. On the seventh day, the seeds treated at high temperatures had the same germination as on the fourth day in the amount of 90%. All seedlings were regular developed. There were 6.67% of brown rotted seedlings and 3.33% of non-germinated seedlings. On the seventh day, seeds treated at low temperatures had a germination of 83.33% or 6.66% more than on the fourth day. The germination of control seeds was 90%, with all seeds germinating on the third day. Considering that some authors write about the weed and invasive character of sweet wormwood in some areas, which has not been recorded in Croatia so far, the results of this research are in favor of increasing the habitat suitability for this species in the future.



THE LITHUANIAN PHOSPHORUS BUDGET AS A BASIS FOR RESOURCE OPTIMIZATION

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flow analysis,
phosphorus
flows,
phosphorus
recycling*

Phosphate rock is one of the critical raw materials in EU, largely used to produce phosphorus (P) fertilizers. P is essential for agriculture; nevertheless, its resources are finite. Even more, phosphate rock is available only in a small amount within EU itself. Lithuania is a country, which ranked among five top producers for diamonium phosphate, while relying on imported raw material. The future of the further production is uncertain due to the current geopolitical situation.

Thus, P flows in Lithuania were analysed with the aim to find out what are the possibilities for resource optimization. With the help of material flow analysis we have analysed P inputs and outputs to industry and agriculture in the country, consumption, waste treatment, including waste water treatment plants and treatment of biowaste, as well as phosphorus losses to environment, in particular water bodies.

The analysis revealed that the highest flow value of P is coming from the import of phosphate rock, forming nearly 32% of total P flows and >86% of imports. Regarding exports, the highest flow is related to fertilizers (nearly 87% of exported flows). As for internal flows, agricultural subsystem is the main consumer of P in the country and the highest flow is related to P uptake by crops: a large part of crop production in Lithuania is related to cereals, which accumulate a significant amount of P. As a large proportion of cereals are exported from the country, with P being exported at the same time, even if full recovery of P from domestic sources such as sewage and sewage sludge and biodegradable compost is achieved, P stocks would still have to be replenished from the outside.

In line with the requirements of Urban Waste Water Treatment Directive, the territory of Lithuania was classified as a sensitive area due to the eutrophication of the Baltic Sea, which is sensitive to emissions of nutrients. Lithuania has achieved a high degree of P removal at waste water treatment plants (WWTPs). P recovery technologies are not implemented at WWTPs in the country, but the most popular methods for sludge use, namely use in agriculture and composting, allow P returning to the economy. Still, over 1000 tonnes of P is lost to the Baltic Sea from point and non-point sources.



INFLUENCE OF PHOSPHORUS NUTRITION ON LEAF DRY MATTER CONTENT AND LEAF MASS PER AREA OF COMMON BEECH AND SESSILE OAK SAPPLINGS

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KEYWORDS:

Fagus sylvatica L.,
LDMC,
saplings,
SLA,
Quercus petraea
(Matt.) Liebl,

This research was conducted on a five-year-old common beech and sessile oak saplings originating from two mature mixed common beech and sessile oak stands (provenance) from the Republic of Croatia (HR). Provenances differ in annual amount of precipitation, which in provenance Slavonski Brod (SB) from the eastern part of the HR is about 770 mm (dry provenance), and in Karlovac (KA) from the north-western part of the HR is about 1112 mm (wet provenance). During year 2021, after the establishment of the experimental trail, the transplanted saplings grew into two soil types, one was characterized by high (0.30 g P₂O₅/L soil = +P treatment) and the other by low (0.16 g P₂O₅/l soil = -P treatment) phosphorus (P) concentration. In September 2021, the saplings of both species in the +P treatment had a higher concentration of P in the leaves than the samplings from the -P treatment. The aim of this study was to examine the effect of different soil P concentration and/or phosphorus nutrition on the leaf dry matter content (LDMC = dry leaf mass/fresh leaf mass) and leaf mass per area (LMA = dry leaf mass/leaf area) of common beech and sessile oak saplings originated from dry and wet provenances. Results of factorial ANOVA with main effects of treatment (+P vs -P), provenances (dry BS vs. wet KA) and species (common beech vs. sessile oak) indicate that LDMC and LMA in +P treatment were significantly higher than in -P treatment, dry SB and wet KA provenance had similar LDMC and LMA, whilst the common beech had significantly lower LDMC and LMA than the sessile oak. Significant interaction between main effects "treatment x species" indicate thad LDMC and SLA of sessile oak were similar in both +P and -P treatments, whilst LDMC and SLA of common beech in -P treatment were significantly lower than in +P treatment.



SPECIES DIVERSITY BOOSTING - PRESENT CHALLENGE, ISSUE OF THE FUTURE

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species diversity,
survival rate*

Climate change influences every aspect of forestry, especially species diversity as one of the main factors of ecosystem stability. Enrichment in planting native species is one of the measures of boosting biodiversity and its conservation, but also one of the green solutions to mitigate climate change. In the north part of Serbia (45.502059N, 19.917268E), on an area of 7.9 ha, overall 4045 seedlings were planted. Depending on microclimate, seven species were represented by a different number of seedlings: *Fraxinus angustifolia* Vahl. (970), *Quercus robur* L. (559), *Salix alba* L. (1361), *Populus alba* L. (576), *Ulmus laevis* Pall. (146), *Tilia tomentosa* Moench. (172) and *Betula pendula* Roth. (261). This area is characterized by less than 1% of forest coverage, uneven distribution, degradation, as well as inadequate maintenance of existing areas under trees. To preserve and improve the area's diversity, in mid-November 2020 bare-root seedlings of the seven above-mentioned native species were planted. The survival rate was measured in mid-May 2021, as well as at the end of the vegetation period, in mid-October 2021. During the vegetation period, two very dry periods were present: June (average temperature 29.8°C, max temperature 39°C, average precipitations 23.9 mm) and September (average temperature 25.4°C, max temperature 31.9°C, average precipitations 16.4 mm). In July and August maximum daily temperatures reached over 37°C. The entire vegetation period was characterized by an uneven distribution of precipitation. The overall average survival rate in May 2021 was 98.6% (*F. angustifolia* 99.3%, *Q. robur* 98.0%, *S. alba* 98.8%, *P. alba* 98.8%, *U. laevis* 97.3%, *T. tomentosa* 98.3% and *B. pendula* 96.6%). Due to a lack of human and technical resources, watering was done only once (in mid-June). The overall average survival rate in mid-October 2021 was 9.8% (*F. angustifolia* 32.4%, *Q. robur* 2.9%, *S. alba* 0.3%, *P. alba* 2.8%, *U. laevis* 17.8%, *T. tomentosa* 11.0% and *B. pendula* 0.8%). *F. angustifolia* and *U. laevis* showed the best survival rate, which can be the base point for increasing the diversity of species in these and similar environmental conditions. These results are the consequence of the combined action of climate change and the negative anthropogenic factor. To mitigate climate change, the efforts of the wider society must be strengthened. Raising environmental awareness, and increasing the funds in sectors related to the practical work in preservation and improvement of the environment and biodiversity can be one of the solutions for the future.



DNA BARCODING OF PATHOGENIC FUNGI ON FOREST TREES FROM UČKA NATURE PARK

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KEYWORDS:
forest trees,
fungi, molecular
analyses,
GenBank,
ITS region

This study is aimed to identify fungi from infected forest tree species collected in Učka Nature Park. The fungal DNA was isolated from plant parts (twigs and leaves) or cultured fungi from twigs and leaves and fruiting body. The identification of fungi at the genus or species level was carried out by using macroscopic and microscopic examinations and molecular analyses. The ITS region/TEF1 gene and the ITS region/rbcl gene were used to barcode the DNA of forest trees and fungi, respectively. The PCR products were sequenced and compared with the other related sequences in GenBank (NCBI). A total of 28 fungi species were detected on forest trees belonging to several genera: genus *Acer*: *Alternaria alternate*, *Colletotrichum lineola*, *Coprinellus micaceus*, *Phoma* sp., *Rhytisma acerinum*, *Venturia tremulae*; genus *Carpinus*: *Dothiorella symphoricarposicola*, genus *Corylus*: *Fusarium avenaceum*, Genus *Fagus*: *Aureobasidium pullulans*, *Biscogniauxia mediterranea*, *Cladosporium cladosporioides*, *Diatrypella* sp., *Digitodochium rhodoleucum*, *F. avenaceum*, *Hymenopellis radicata*, *Inonotus aff. Radiate*, *Jackrogersella cohaerens*, *Melanops fagicola*, *Neonectria* sp., *Phaeosphaeria fuckelii*, *Pholiota aurivella*, *Trichoderma deliquescens*, *Trichothecium roseum*, *A. alternate*; genus *Pinus*: *Cyclaneusma minus*, *Diplodia sapinea*, *Epithamnolia xanthoriae*, *Sydowia polyspora*; genus *Ostrya*: *F. avenaceum*; genus *Quercus*: *Sordariomycetes* sp., *F. avenaceum*, and genus *Salix*: *Rhytisma salicinum*.

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Beechnut QUALITY BY SEED REGIONS IN CROATIA

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beechnut,
European beech,
health status,
pathogen fungi,
seed quality

The quality of the seeds used in production of forest reproductive material is the cornerstone of successful regeneration, reforestation or afforestation programs. In addition, climate change, which is becoming more and more visible, requires a new approach to forest regeneration and management and seeks new insights into quality of forest reproductive material. The high quality seeds possess genetic, physiological and physical characteristics that are adapted to the site conditions. Seed quality includes genetic, physiological and physical properties of seeds such as genetic purity, analytical purity, germination capacity, viability, moisture content, thousand-seed weight and health status of seeds. The aim of the research was to determine the qualitative properties and health status of beechnut by seed regions. The most recent year for beechnuts was 2016. Seed quality was tested according to ISTA methods. The fungi were determined by classical morphological analysis using macro and micro methods and molecular method. The beechnuts from the seed region Kapela – Velebit had the highest viability - 88%, and the beechnuts from the seed region Žagorje – Bilogora had the smallest viability - 69%. The results of 1000 seed weight ranged from 362.8 g in the seed region Dilj - Psunj to 183.3 g in the seed region Istra. The number of viable seeds in 1 kg ranged from 2143 pcs in the seed region Dilj - Psunj to 4038 pcs in the seed region Istra. The smallest beechnuts were from the seed region Istra and the largest one from the seed region Dilj - Psunj. Several species of pathogenic fungi were detected: *Fusarium* spp., *Alternaria alternata* (Fr.) Kisler, *Penicillium* spp., *Phomopsis* sp., *Trichothecium roseum* Link., *Mucor* sp., *Botrytis cinerea* Pers., *Phoma* sp., *Trichoderma viride* Pers., *Chetonium* sp. The most common in all seed regions were *Fusarium* spp., *A. alternata* (Fr.) Kisler and *Mucor* sp.



INVESTIGATION OF FOLIAGE DISEASES OF FOREST TREES IN THE UČKA NATURE PARK

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KEYWORDS:
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Učka Nature Park

Učka Nature Park includes the area of the Učka Mountain and part of the Ćićarije area, located along the northern Adriatic coast. Učka connects the Mediterranean and continental parts of Croatia and is rich in plant species biodiversity. The largest part of Učka is covered with beech forest, black and white hornbeam, oak, black pine, dwarf pine, and in some place Mediterranean chestnut. Many diseases occur on the leaves of deciduous and evergreen trees. Leaf diseases cause damage, drying and falling of leaves and needles. The aim of this paper is to determine the symptoms of leaf diseases and their fungal agents on forest trees in the Učka Nature Park. Transect from Veprinac via Poklon to Vojak peak, forest trees were examined and samples of diseased leaves were collected. Twenty-five species of fungal agents of leaf diseases were found. The identified fungi belong to the division Ascomycota, classis Sordariomycetes, Leotiomycetes, and Dothideomycetes. The greatest damage was caused by *Apiognomonium errabundum*, *Rhytisma acerinum*, *Sphaeropsis sapinea*, and *Mycosphaerella* spp.

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BIOLOGICAL CONTROL USING NATURAL BEAVERIA SP. FOR THE CONTROL OF THE OAK PEST CORYTHUCHA ARCUATA - LABORATORY AND FIELD EXPERIMENT

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KEYWORDS:
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Beauveria sp.,
oak lace bug,
Quercus

The oak lace bug (*Corythucha arcuata*, Heteroptera, Tingidae) is an invasive type of bug that damage oak leaves by sucking sap. The damage leads to a decrease in photosynthetic activity, drying and falling leaves. The consequence of the damage is the physiological weakening of the oaks. The use of chemical agents in forest protection is limited in order to protect the environment. Biological control of pests is an environmentally acceptable solution. Research in the world, especially in Turkey, has indicated the possibility of using the entomopathogenic fungus *Beauveria bassiana* for the biological control of forest pests as well as oak lace bug. The aim of this paper is to investigate the application and action of the native fungus *Beauveria sp.* on the mortality of the oak lace bug in the laboratory conditions and in the field. In the area of Jastrebarsko in the peduculed oak forest before the movement of the vegetation, samples of moss with hibernating bugs were collected from the base of the trees. In the laboratory, the fungus *Beauveria sp.* was isolated from naturally infected oak lace bugs. Live, vital bugs were placed in petri dishes along with moss. The bugs in the moss were sprayed with a spore suspension of *Beauveria sp.* and water. In the field, the leaves of an oak tree infected with oak lace bugs were sprayed with the same suspension. Control samples were sprayed with water. After 5 and 7 days, the samples were analyzed and dead, infected and live bed bugs were recorded. The results showed a positive effect of spraying with natural autochthonous *Bauveria sp.* spores on mortality and infection of oak lace bug. Research on the use of natural *Beauveria sp.*, culture cultivation methods, methods of preparation of suspension and application of treatments in practice should be continued.



MONITORING OF HEAVY METALS IN THE SOIL OF PROTECTED AREA IN ISTRIA

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PI Kamenjak,
zinc

The significant increase in negative anthropogenic impacts, mainly due to the increasing number of motor vehicles during the summer season in Istria, is mainly reflected in the content of soil pollutants such as copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn), which can potentially negatively affect the entire food chain, including human health. In Istria, the public institution "Kamenjak" is the owner of protected area with very important natural and public ecosystem functions. Considering the extraordinary importance of the landscape as a protected area for the daily life of people, flora and fauna, the aim of this study was to determine the presence of these heavy metals and potentially toxic elements in the soil. The study was conducted at 5 locations in the period from 2018 to 2021. We used a general linear model for the statistical analysis. The comparison was performed on 242 soil samples, from 5 locations in 4 years. The concentration of 4 metals (Zn, Cu, Pb, Cd) was measured at 2 depths (0-5 cm, 5-10 cm). The results revealed that there were statistically significant influence of year and location on concentration of the studied heavy metals in the soil.



Session B:

PLAN EXTRACTS

– Value-added products



A GREEN APPROACH FOR THE RECOVERY OF BIOACTIVE COMPOUNDS FROM CORNELIAN CHERRY (*Cornus mas* L.) FRUITS

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green solvent,
response surface
methodology

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Cornelian cherry (*Cornus mas* L.) fruits, usually deep red in color, of oval shape and sour or sweet taste, can be consumed either as whole fresh fruits or in the form of liquors, jams, vinegars etc. They constitute a rich source of bioactive compounds such as phenolic acids, flavonoids, anthocyanins and iridoids with levels that depend on the cultivar, the stage of maturity as well as the cultivation practices. Numerous biological actions have been attributed to cornelian cherry fruits, including antioxidant, antimicrobial, anti-inflammatory and neuroprotective. Natural bioactive compounds have been explored extensively as food ingredients towards the development of innovative functional products. The classical extraction techniques usually involve the use of harmful organic solvents. Based on the principals of green chemistry, alternative green solvents, such as the aqueous solutions of cyclodextrin, can reduce the negative health and environmental impact. More specifically, cyclodextrins, can be used as extraction enhancers through the formation of inclusion complexes with a variety of bioactives, such as phenolic compounds. In this view, the aim of the present study was the development of a green approach for the recovery of bioactive compounds from cornelian cherry fruits with the use of aqueous solutions of cyclodextrin. To the best of our knowledge, extremely limited are the data regarding the extraction of bioactives from cornelian cherries with aqueous solutions of β -cyclodextrin (β -CD). The extraction parameters, i.e. duration (t), temperature (T), solvent to solid ratio (L/S) and concentration of β -cyclodextrin (β -CD) were optimized in terms of total phenolic content and antioxidant activity using Response Surface Methodology. The presence of β -cyclodextrin was found to have a positive effect on the recovery of the bioactives of cornelian cherry fruits compared to water. Our results are expected to contribute to the utilization of cornelian cherry fruits in food, pharmaceutical and cosmeceutical applications through the development of ready-to use extracts.

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APPLICATION OF NATURAL DEEP EUTECTIC SOLVENTS- BASED PLANT EXTRACTS IN THE COSMETIC INDUSTRY – FROM ACADEMIA TO BUSINESS

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human keratinocyte
cell line,
natural deep
eutectic solvent,
polyphenols*

Growing awareness towards sustainability is pushing industry nowadays to change their technologies to be as “green” as possible. Since solvents are inevitable in various industries, the utilization of conventional, organic solvents is responsible for more than 40 % of CO₂ emissions. Natural deep eutectic solvents (NADES) are proven to be a great alternative to organic solvents. NADES is based on a mixture of cheap components that are non-toxic, non-volatile, chemically stable, and biodegradable. NADES are especially desirable when used for the extraction of certain biologically active molecules. The strength of NADES lies in the fact that countless possibilities of NADES component combinations can be made. That being said, NADES are easy to formulate, custom-designed solvents used for specific extraction to obtain extracts with physicochemical properties for particular purposes. In this work, we used NADES to produce white grape pomace plant extract for potential application in the cosmetic industry. We began with testing selected NADES for a maximum loading capacity of desirable bioactive compounds. Then, the optimization for optimal extraction conditions was made. Betaine: sucrose in the molar ration of 4:1 with 50 % of water was used as selected NADES. After 60 minutes of extraction at 60 °C, extracts were filtrated and the content of polyphenols was measured. Different concentrations of polyphenols were used to test if extracts have an inhibitory effect on collagenase activity. Finally, biological activity and safety were tested in vitro using a human keratinocyte cell line (HaCaT). The SCRATCH test was also made to evaluate the wound-healing property of obtained extracts. According to the results, grape pomace extracts have the potential to be applied in various products in the cosmetic industry.

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VINE SHOOTS, A NATURAL RESOURCE WITH RESVERATROL: EVALUATION OF EXTRACTION PARAMETERS

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ultrasound-
assisted extraction,
valorisation, vine
shoots*

Vine shoots are an agricultural residue generated in the wine sector as a consequence of vine pruning. Annually, between 1.5-2 tons of vine shoots are generated per hectare (Sánchez-Gómez et al., 2017). Recent studies have shown that this residue is a source of antioxidant compounds (phenolic compounds), which can be employed in the food, cosmetic, oenological, and pharmaceutical industries. The main objective of this work was the valorization of vine shoots through the extraction of antioxidant compounds. In this way, two technologies were evaluated, maceration with constant agitation and ultrasound-assisted extraction (UAE). Maceration was carried out at 150 rpm for 24 h and at a solid loading of 10% w/v. The effect of the solvent (water, 40% and 80% v/v ethanol) and biomass particle size (5 mm, 1 mm and 0.5 mm) was studied. Then, the solid loading and ethanol concentration was fixed to 10% w/v and 80% v/v, respectively, to evaluate UAE. In this case, the influence of the particle size (5 mm and 1 mm) and the ultrasound amplitude (20 and 80%) was evaluated using an extraction time of 5 min. The total phenolic content (TPC) and the resveratrol content of the extracts were determined by the Folin-Ciocalteu method and high-performance liquid chromatography (HPLC) with a diode array detector, respectively. The antioxidant capacity of the extracts was also determined by the FRAP assay. Using maceration, it was observed that the use of 80% ethanol and a reduced particle size increased the extraction of phenolic compounds, including resveratrol, and the antioxidant activity. Then, fixing 80% ethanol v/v as solvent, the highest solubilization of antioxidant compounds, including resveratrol, was obtained using UAE with the highest amplitude, 80%, and a particle size of 1 mm. In this case, the TPC was 7.11 mg gallic acid equivalents/g vine shoots, the antioxidant activity was 8.03 mg Trolox equivalents/g vine shoots and the resveratrol content was 1.21 mg/g vine shoots. Moreover, resveratrol was identified as one of the main phenolic compounds in vine shoots. Therefore, UAE is an effective "green" technique for the extraction of phenolic compounds from vine shoots, which led to obtain 170.82 mg phenolic compounds/g extract (dry weight basis), with around a 17% of resveratrol.

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OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ROSMARINIC ACID FROM CLARY SAGE

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Rosmarinic acid,
Supercritical carbon dioxide

This study investigates the extraction of bioactive compounds of Clary sage (*Salvia sclarea* L.) with carbon dioxide in supercritical conditions (SC-CO₂). The target component is rosmarinic acid, a natural phenolic compound, with strong antioxidant activity. The aim is to determine the optimal operating conditions to extract rosmarinic acid (RA) from sage using the SC-CO₂ extraction technique. Response surface methodology was applied to optimize the operating temperature and pressure as well the co-solvent composition. Indeed, the polar nature of RA requires the addition of a co-solvent to increase its affinity and solubility. The extraction was performed on the clary sage residue obtained after distillation. Extractions were carried out using supercritical fluid extraction system (SFE PROCESS, Nancy, France). 25 g of clary sage were placed into a 500 ml cell. The SC-CO₂ had a downward flow in the extraction vessel and the flow rate was kept constant at 60 g/min during the experiments. The pressure and temperature ranged between 100 and 600 bar, and 40 and 100 °C respectively. The co-solvent ranged between 0 and 100% ethanol in water (v/v), added to the supercritical fluid at 10% (w/w). The separator temperature was set at 60 °C. A Box-Behnken experimental design was employed to evaluate the effect of the studied factors, and determine the optimal conditions to obtain a high RA yield (quantified by HPLC) and antioxidant activity (AA) (measured using DPPH assay). Higher RA yields were reached at low pressures (100-420 bar) and moderate temperatures (40-85°C). The extraction rate is mainly influenced by the solvent composition. The presence of water in the co-solvent is required to achieve important yield of RA. AA is affected by pressure and ethanol. Increasing pressures (>200 bar) induced a lower AA. Ethanol in the co-solvent have a significant effect, the use of 15-60% allowed to reach the highest AA. Optimal conditions for maximizing both responses are a pressure of 100 bar, a temperature of 65 °C and 38% ethanol in co-solvent. The RA yield reached 7.02 mg/gDM with an AA of 31.01 TE/gDM. The second-order polynomial model fit the experimental data to predict the two responses. The SC-CO₂ extraction of RA from clary sage residue is not interesting compared to conventional extraction techniques. However SC-CO₂ is a good solution for the sequential extraction of the essential oil followed by the phenolic compound extraction. This way would provide a sustainable extraction and an additional valorization of clary sage.



OBTAINMENT OF BIOACTIVE COMPOUNDS FROM THE EXTRACTIVE AND PROTEIN FRACTION OF EXHAUSTED OLIVE POMACE

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exhausted
olive pomace,
hydroxytyrosol,
mannitol*

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Olive oil is a significant agroindustrial product in the Mediterranean basin and Spain is the major producer, contributing to about 40% of the world production. Particularly, in Spain, the most critical and massive residue is the olive pomace formed during oil production, which consists of around 80% of the olive weight. Currently, this bioresource is partially destoned and processed to extract the residual oil, and the rest (exhausted olive pomace or EOP) burnt. However, this residue contains a high content of extractives and also protein, whose extraction can be a source of new incomes to the sector and favour the usefulness of this residue for combustion and sugar recovery in a biorefinery platform. In this work, bioactive compounds from the extractive fraction (phenolic compounds and mannitol) and bioactive peptides were recovered in a two-step extraction scheme. First, the extractive fraction of EOP was partially removed through aqueous extraction at 85 °C, 90 min, and 10% w/v solid loading. Then, the extracted solid (3.5% w/v) was subjected to enzyme-assisted extraction at pH 8.5 using Alcalase and trypsin and 37 °C and 60 °C (1–24 h), respectively. The total phenolic content, the protein content and the antioxidant activity were determined using the Folin-Ciocalteu, Bradford and ABTS methods, respectively. Moreover, the profile of the extracts was obtained using a high-performance liquid chromatography with diode array detection. The aqueous extraction removed a large part of the extractives and ash from EOP, and thus this led to an extracted EOP solid with lower percentages of these components. Alternatively, it contained higher percentages of cellulose, hemicellulose, lignin, and protein. Moreover, this step solubilised per g of EOP: 44.5 mg gallic acid equivalents, 6.3 mg hydroxytyrosol, 95.4 mg Trolox equivalents, and 55.2 mg mannitol (extract 1). Since the protein content was increased, the solid was subjected to enzyme-assisted extraction with two peptidases to generate antioxidant peptides. The results showed that Alcalase promoted the highest release of peptides and with higher antioxidant properties. For example, after 24 h of extraction, Alcalase solubilised 75.7 mg BSA and 99.2 mg Trolox equivalents per g of EOP (extract 2). In conclusion, this novel valorisation scheme provides various valuable compounds for the food, pharmaceutical and cosmetic industries, reinforcing the potentiality of EOP use within a biorefinery context.

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FORMULATION OF NATURAL ACTIVE COMPOUNDS WITH ANTIFUNGAL APPLICATIONS: CONSTRUCTION OF PSEUDO-TERNARY PHASE DIAGRAMS AND STUDY OF THE RELEASE MECHANISM

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Pseudoternary
diagram,
terpenes,
volatilization*

The interest in the development of an environmentally sustainable crop production system, has prompted the research of new formulations from two points of view: the use of active components of natural origin (essential oil compounds), and the development of new systems that allow controlling the release of the active component to improve its performance. Moreover, it has been shown that absorption and penetration at the leaf surface play an important role in the application of fungicides. Understanding the transport mechanism through the surface with which the fungicide comes into contact is a tool used to assess the effect of foliar-type formulations; since not only the interaction between the active component and the pathogen plays an important role, but the release mechanism of the active component and its transport mechanisms on the surface and through the plant tissues are as well factors that influence in its bioavailability and efficacy. The present work seeks to identify and study the conditions for low energy emulsification in systems containing eucalyptol and g-terpinene (terpenes from essential oils), soybean lecithin and glycerol or isoeugenol, through the construction of pseudoternary diagrams by water titration at room temperature; and to optimize its formulation process. As well as valuing the volatilization reduction of the active component at the application concentration respect a control solution of the active compound, and the effect of the surfactant/co-surfactant on the surface tension of the leaf surface. Homogeneous and stable phases were identified in the pseudoternary diagrams for the concentration ranges: 15% to 13% of eucalyptol and 17% to 9% of g-terpinene, where soybean lecithin was used as surfactant and glycerol or isoeugenol as co-surfactant. It was found that a possible influence of the structures and composition of the emulsion components on the sample viscosity and stability was detected. Regarding the optimization of the emulsification process at low energy of the systems studied, it was detected that the order of addition of the phases and the stirring time have a greater impact on the stability of the samples than the temperature of the medium and stirring speed. In relation to the study of the volatilization reduction of the active component and the surface tension, a protocol is being developed to quantify the traces of the selected active component (GC-MS) and the behavior of a sessile drop (contact angle) after its application and exposure periods.

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THE FOOD PACKAGING PERFORMANCES OF PAPERS COATED WITH XYLAN HEMICELLULOSES

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*barrier properties,
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hemicellulose,
paper coatings,
xylan*

The packaging industry is nowadays under a great pressure from both authorities and consumers to improve its environmental and sustainability credentials in the coming years. So there is a need for the development of new generation of packaging that has at least the same technical performances as today's mono or multi-materials but with a demonstrated recyclability and biodegradability. In this context, cellulosic based materials (paper and board) are generally considered cost-effective and most promising candidates largely because of its inherent advantages, being cheaper than other materials, high recyclable and biodegradable, easy to convert into containers with specified strength and stiffness; are more resilient than glass/plastic over a wider temperature range; are lighter and far more easily printed comparatively with other materials. As food package, the paper/board has inherent poor barrier properties such as an insufficient barrier against water vapour, oxygen or flavours and microbial attack, due to its porous structure and hydrophilic character of cellulose fibers, which is its basic structural material.

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In this paper are presented some investigations regarding the utilisation of xylan biopolymer in native and cross-linked with Alkyl ketene dimers (AKD) to improve the barrier and strength properties of paper. A biopolymer coating based on hardwood xylan hemicellulose with and without AKD was used for surface treatment of paper using rod Mayer laboratory coating system. The obtained results were emphasized the improving of strength (i.e. bursting strength which is an important property of packaging) and water/oil absorption properties of coated papers. Based on the obtained results it can conclude that xylan hemicellulose is a promising biopolymer to substitute the synthetic polymers in treatment of papers for food packaging application.

COMPARISON OF THE KJELDAHL AND DRY COMBUSTION METHODS FOR THE DETERMINATION OF NITROGEN AND PROTEIN CONTENT IN 'LOVRAN MARRON' FRUITS FROM CROATIA

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KEYWORDS:
dry combustion method, Kjeldahl method, marrons, nitrogen content, protein content

Chestnut cultivars grown for high-quality fruits are known as marrons. 'Lovran Marron' is the only known traditional Croatian variety of the sweet chestnut. It is grown on private estates in the Lovran surroundings, on the eastern slopes of Mount Učka, where the oldest plantations are several hundred years old. The aim of this study was to compare two methods of nitrogen and protein analysis in 'Lovran Marron' fruits. Fruits were collected in October 2019, in the environs of Lovran (Croatia). In order to compare total nitrogen (N) content in marron fruits by the Kjeldahl and dry combustion methods (ISO 13878, 1998), 70 samples of 'Lovran Marron' fruits were analyzed. Protein content was calculated by multiplying nitrogen content by factor of 5.30. The Kjeldahl method was performed using Kjeltec 8200 (FOSS, Denmark), using 1 g of grounded raw fruits, previously frozen. The dry combustion method was performed on the NC Soil Flash 2000 Thermo Scientific device (Thermo Fisher Scientific, USA), using 20 mg of grounded and dried raw fruit.

Descriptive statistics and analysis of variance were applied to evaluate the differences between the results obtained by these two analytical methods.



IMPROVEMENT OF SINAPINE EXTRACTION FROM MUSTARD SEED MEAL BY APPLICATION OF PRE-TREATMENT TECHNOLOGIES

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This work focuses on the valorization of mustard bran through the recovery of sinapine. This phenolic molecule can be used in many fields such as food, medicine, cosmetics or fine chemistry owing to its antioxidant properties. Extraction is the key step of the overall recovery process. Indeed, the choice of the most appropriate extraction technology is essential to maximize its selectivity for the target molecule and simplify the purification step. Thus, the application of physical pretreatments upstream or during the extraction can be necessary. These lead to (i) an improvement of the extraction process efficiency, and (ii) the reduction of extraction times, energy and solvent flows. In this work, the application of physical pretreatments (ultrasound and high voltage electrical discharge (HVED)) were investigated to intensify the sinapine extraction. This work is part of an approach to produce a bio-based molecule of interest (sinapine) that is economically viable and environmentally friendly. The extraction was performed on mustard bran defatted by supercritical CO₂. This recovered 19% (w/w) of mustard oil. The HVED assisted extraction of sinapine was performed in two steps; the first step consists of the application of HVED for a variable time (0-4 ms), and the second step corresponds to the diffusion of sinapine into the extraction solvent. The ultrasound-assisted extraction was implemented using a sonotrode (Hielscher, 400 W) and optimized by response surface methodology. A three-factor Box-Behnken design with three levels was carried out : temperature (25-50-75 °C), ultrasound amplitude (0-50-100%) and percentage of ethanol in water (0-35-70%). The pretreatment with HVED showed a destructive effect on sinapine. Indeed, the application of HVED on sinapine model solutions and on mustard bran induced a significant degradation of sinapine. This result, allowed us to conclude that HVED are not suitable for sinapine extraction. Optimization of ultrasound-assisted extraction showed that temperature, ethanol, amplitude, ethanol*ethanol quadratic effect and temperature*amplitude interaction have a significant effect on the extraction yield of sinapine. The optimal operating conditions for ultrasound-assisted extraction of sinapine are 70°C, 70% ethanol and 100% amplitude allowing the extraction of 6.3 mg/g DM of sinapine. These conditions also made it possible to maximize the antioxidant activity of the extract.

ANTIOXIDANT CAPACITY OF THE OREGANO AND ROSEMARY EXTRACTS OBTAINED BY HIGH VOLTAGE DISCHARGE TREATMENT

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capacity, r
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Due to their complex composition in bioactive compounds known to act as antioxidant and antimicrobial agents, oregano and rosemary are considered medicinal plants. Extraction of bioactive components from plant materials using high voltage discharge (HVED) leads to higher yields, less time, energy and solvent consumption compared to conventional extraction methods and is therefore considered as a “green” extraction method. In this work, the effect of high voltage discharge extraction technique on the antioxidant capacity of the oregano and rosemary extracts was investigated using the Oxygen Radical Absorbance Capacity (ORAC) assay. In extraction experiments, dry samples of oregano and rosemary were suspended in water or water:ethanol mixtures (25 % ethanol and 50 % ethanol) and treated with HVED in the presence of neon or argon for three or nine minutes. Untreated control samples were suspended in the same solvents and stirred for the same period of time as for the HVED treatment. From the studies performed, the following can be concluded: (i) longer treatment time in general leads to higher ORAC values, (ii) the highest ORAC values were determined for the extracts with the highest ethanol content (iii) the extracts obtained by HVED treatment have higher antioxidant activity than the control samples obtained by conventional extraction. This work has been fully supported by Croatian Science Foundation under the project IP-2016-06-1913.



QUALITY PARAMETERS OF DIFFERENT TYPES (HONEYDEW, FLORAL, CHESTNUT, MEADOW, AMORPHOUS, MANDARIN, WILLOW, WILD CHERRY, FOREST, LINDEN, SAGE) OF HONEY

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Honey is a pure product, the oldest natural food, comprised of wide variety of different compounds. Carbohydrates are the most common constituents of honey, followed by water, and other ingredients that contribute to the characteristic sensory and nutritional properties such are proteins, minerals and vitamins, organic acids, phenolic compounds, aroma compounds. To guarantee its authenticity and to protect consumers, honey must meet certain compositional criteria. During this study, different types of honey (n = 41) produced in Croatia, such as honeydew, floral honey, chestnut honey, meadow honey, amorphous honey, mandarin honey, willow honey, wild cherry honey, forest honey, linden honey, and sage honey, were analysed for water content, acidity, electrical conductivity, hydroxymethylfurfural (HMF) content, reducing sugars, and sucrose content. All the analysed honey samples, according to the obtained results, meet the prescribed regulation requirements regarding the value of acidity, HMF content, and sucrose content. 2.44 % of the analysed samples do not meet the current prescribed requirements for water content, 29.27 % of the samples do not meet the requirements for electrical conductivity, and 14.63 % of honey samples do not meet the prescribed requirements for reducing sugar content. The results indicate the need to study the regulatory criteria with regard to different specific types of honey, and the development of reliable analytical approaches that would guarantee its authenticity.

APPLICATION OF SUPERCRITICAL CO₂ AS GREEN TECHNOLOGY FOR OIL EXTRACTION FROM GRAŠEVINA GRAPE SEED POMACE

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methodology,
supercritical CO₂
extraction*

Grape seed fraction of the pomace represents valuable wine waste by-product, not just as a source of polyphenolic compounds, but also since it contains 7-22% of oil. Compared to solvent extraction methods, supercritical CO₂ extraction (SC CO₂) represents promising alternative, eco-friendly technique of extraction, since it involves green, low-cost, non-toxic and non-flammable CO₂ as solvent/extraction fluid. Consequently, low processing temperatures make this technique suitable for preservation of thermosensitive compounds. In addition, higher extraction yields were obtained compared to cold pressing, but impact on the overall quality of grape seed oil was not profoundly studied. The aim of this study was to investigate the impact of different SC CO₂ extraction variables (pressure, temperature, and CO₂ flow rate) on the oil yield (%) and oil antioxidant capacity (ORAC). The experiments were performed on Graševina grape seed pomace according to Box-Behnken design, while optimization of SC CO₂ extraction was conducted by response surface methodology (RSM). The pressure of the extraction showed to be the most significant variable influencing the oil yield ($p < 0.01$). In addition, besides the pressure ($p < 0.05$), CO₂ flow rate ($p < 0.001$), as well as interaction among flow rate and temperature ($p < 0.05$) significantly influenced the ORAC values. The optimal conditions obtained by RSM from SC CO₂ extraction of grape seed oil from pomace were 450 bar, at 40 °C, with CO₂ flow rate at 45 g/min, in order to achieve maximum extraction yield (7.82%) and ORAC value (415.24 μM Trolox equivalent/100 g oil). Experimental data were in accordance with the predicted ones since the obtained data were not significantly different within the 95% confidence interval.



INFLUENCE OF ULTRASONIC PRE-TREATMENT ON THE ENERGY CONSUMPTION OF PUMPKIN (*Cucurbita moschata*) DRYING

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vacuum drying*

Energy consumption during drying operations has great potential to be improved using novel non-thermal processing technologies. Technologies such as high-intensity ultrasonics can be used as pre-treatment to drying operations, which due to mechanical and thermal effects of cavitation can improve mass diffusion and consequently shorten drying times. Shortening the drying time could potentially lead to a decrease in energy consumption and waste production, which improves the ecological and economic aspects, increasing the sustainability of the whole process. This study investigates the effect of pre-treatment of pumpkin in an ultrasonic bath on the drying time and total energy consumption for the drying process. Square pumpkin pieces were processed using 30 %, 60 %, and 90 % of maximal ultrasonic power (380 W at 37 kHz) for 30 min, 45 min, and 60 min. Drying is conducted under atmospheric (1 bar) and low (0.1 bar) pressure at 60 °C. Obtained results show a significant difference in total used energy during conventional and vacuum drying ($2,04 \pm 0,17$ kWh kg⁻¹ compared to $3,92 \pm 0,11$ kWh kg⁻¹). An increase of ultrasonic power leads to a significant shortening of the drying time from 365 min to 320 min for conventional and from 265 to 190 min for vacuum drying. Pre-treatment using 90 % of maximal power combined with drying at 1 bar uses $2,75 \pm 0,21$ kWh kg⁻¹, while the same treatment at 0.1 bar uses 4,88 kWh for drying 1 kg of fresh pumpkin material. An increase in ultrasonic processing time exhibits the same behavior, as there is a significant increase in total energy consumption (3,75 kWh kg⁻¹ for conventional and 5,41 kWh kg⁻¹ for vacuum) while there is still a significantly shorter drying time. However, vacuum-dried pretreated samples had much better sensory properties such as texture and color. In conclusion, while pre-treatment using an ultrasonic bath did not have any influence on the decreasing of total energy consumption, it contributes to significantly shorter drying times. The combination of pre-treatment with vacuum drying has the potential to contribute to the sustainability of the drying process, as total energy consumption can be further improved using larger batches of material or possibly using much shorter processing times. Such processes would be much more beneficial using high powered ultrasound with an immersion probe, as it can process the surface of the material with the same amount of mechanical energy in a much shorter amount of time.

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SEPARATION OF BIOACTIVE COMPOUNDS FROM MANDARIN PEEL Citrus unshiu USING SUBCRITICAL WATER EXTRACTION

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Mandarin peel contains significant amounts of bioactive and high-value components, among which phenolic acids and flavonoids are the most persistent groups of plant phenolics exhibiting health-related properties including antioxidant, anticancer and anti-inflammatory. Subcritical water extraction (SWE) technique is considered as an promising green alternative to conventional extraction methods, being also efficient for extraction of variety types of bioactive compounds from plant material. The efficiency of supercritical CO₂ (SC-CO₂) extraction for obtaining high-quality essential oil from citrus peel is well-known, however the utilization of generated remain free of non-polar components is not commonly studied for obtaining extracts rich in phenolic compounds. The primary aim of this study is to investigate the possibility of using mandarin peel of the Citrus unshiu variety using subcritical water extraction (SWE) technique, and to evaluate phytochemicals, total phenolic content and antioxidant activity of the prepared extracts. After SC-CO₂ extraction, the exhausted citrus waste was subjected to SWE in a wide temperature range (130 – 220 °C) using solvent-solid ratio (10 – 30 mL/g) in periods from 5 to 15 min. Identification and quantification of individual bioflavonoids, of which hesperidin (0.16 – 15.07 mg/g of plant) was determined as the most abundant flavanon in mandarin peel, and also other polyphenolic compounds as possible products of thermal degradation, was performed using high performance liquid chromatography with a diode array detector (HPLC-DAD). At higher temperatures the presence of 5-HMF and chlorogenic acid was detected. Antioxidant activity and total phenolic content in extracts were determined using spectrophotometric methods. Process optimization was performed by response surface methodology (RSM) using Design Expert® software. Acknowledgments: This work has been supported by Croatian Science Foundation under the project

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THE INFLUENCE OF ULTRASONIC PRETREATMENT PRIOR HYDRODISTILLATION OF ROSMARINUS ON THE YIELD OF ESSENTIAL OIL

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essential oil,
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rosmarinus,
ultrasound

In recent years, new pre-treatments prior hydrodistillation of plant material have been intensively investigated, with the aim of increasing their yield. The aim of this study was to investigate whether ultrasonic pretreatment prior Clavenger hydrodistillation could increase the yield of rosemary (*Rosmarinus officinalis* L.) essential oil. The hydrodistillation was preceded by ultrasonic pretreatment (ultrasonic output power of 200 W, a probe diameter of 14 mm) for 5, 10, 20, 30 and 40 minutes of extraction time. Ultrasonic pretreatments increased the yield of rosemary essential oil for 50 % compared to the negative control after

5 and 10 minutes, while with a longer time of ultrasonic pretreatment there was no increase in the yield of rosemary essential oil. After ultrasonic pretreatment of rosemary, enzymatic pretreatment with the addition of cell-degrading enzymes xylanase-pectinase-cellulase (1:1:1) was performed at 40 °C for 4 h. However, enzymatic pretreatment did not increase oil yield compared to ultrasonic pretreatment and negative control. This suggests that the increase in oil yield is caused by ultrasonic pretreatment after only 5 and 10 minutes, and not the enzymatic activity that did not show an increase in the yield of essential oil. In the production of essential oils, hydrolate is formed as a by-product, which has a strong antioxidant capacity determined by the FRAP method. In conclusion, the results of this study showed that ultrasonic pretreatment prior hydrodistillation can be useful in the production of rosemary essential oil, the main advantage is an increase in oil content by 50% after only 5 and 10 minutes of ultrasonic pretreatment.



WATER HYDRODISTILLATION RESIDUES OF BAY LAUREL, ROSEMARY AND SAGE AS A SOURCE OF POLYPHENOLS

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sage, water
hydrodistillation
residue*

Water hydrodistillation residues produced in parallel with bay laurel, rosemary and sage essential oils contain a remarkable source of bioactive molecules. Despite rich bioactive content, these fractions considered as waste and are often underexploited. To date, there are no studies reporting on the chemical composition of water residues remaining after hydrodistillation of bay laurel and sage leaves, while there are only few reports demonstrating the valuable composition of other hydrodistillation co-products of these plants as a valuable secondary source of polyphenol components. We have used different pretreatments prior to hydrodistillation, i.e. reflux extraction, ultrasound and enzyme assisted extraction. It was described the effect of these pretreatments on the composition of polyphenolic compounds in the water hydrodistillation residues. HPLC analysis showed that the water hydrodistillation residues of bay laurel, rosemary and sage were richest in polyphenols. In bay laurel water hydrodistillation residues, procyanidins represented the dominant components, with procyanidin dimer I and II (up to 4.65 mg/g), tetramer II (up to 5.43 mg/g), trimer II, III and IV (up to 4.37, 4.92, 13.15 mg/g, respectively) as major compounds. Among other components, epicatechin-hexoside, epicatechin and epicatechin-3-O-gallate (up to 3.56, 1.60, 1.07 mg/g, respectively) were also detected in significant quantities. In sage hydrodistillation water residues, rosmarinic acid (up to 5.39 mg/g) was major compounds, followed by caffeic acid methyl ester (up to mg/g) and epicatechin (up to 2.06 mg/g). Finally, in rosemary hydrodistillation water residues rosmarinic acid, galocatehin, p-coumaric acid and syringic acid (up to 7.57, 4.83, 2.19, 3.25 mg/g, respectively) were most represented components. In general, the pretreatments didn't significantly affect the phenolic composition of water hydrodistillation residues ($p < 0.05$), i.e. the composition was comparable to the no-pretreatment control, as confirmed by Spearman's test. Our results point out that water residues remaining after hydrodistillation of bay laurel, rosemary and sage are valuable source of polyphenols and can find their application in many fields of food industry.



ISOLATION OF CAROTENOID FROM DRIED TOMATO PEEL WASTE BY USING DIFFERENT SOLVENTS

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IR-spectroscopy,
tomato

Tomato waste is considered as a rich nutrient source containing phytochemicals, among which are carotenoids pigments, therefore it can be recycled for the uses of biologically active compounds. This thesis carried out and compared carotenoid isolation efficiency from dried tomato peel waste by applying two different extraction methods, classical extraction or refluxing and Soxhlet extraction, using a series of solvents of different polarity. Larger amounts of extracts were obtained by refluxing, as well as with more polar solvents indicating that temperature and solvent polarity are important extraction parameters. The extracts were purified by preparative thin layer and column chromatography, then characterized by IR-spectroscopy which confirmed the presence of carotenoids.



CHARACTERIZATION OF FATTY ACIDS AND PHENOLIC PROFILE OF OILVE OILS FROM MILLENNIAL WILD OLIVE TREES (*Olea oleaster*) GROWN IN OLIVE GARDENS OF LUN, ISLAND OF PAG

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wild olives

Peculiar nutraceutical and health impact of virgin olive oil (VOO) is due to its particular chemical configuration, namely fatty acid (monounsaturated oleic acid, essential fatty acids - linoleic and α -linolenic) and phenolic (secoiridoid derivatives oleocanthal and oleacein, oleocanthal) composition. Yet, the composition of VOO from wild origin has been poorly studied. Wild olives (*Olea oleaster*) represent an immense unexplored pool of rich genetic potential that can be used in breeding programs. In this paper, we investigated the olive oils obtained from wild millennial olives from the Lun Olive Grove Gardens, which are located in the northern part of the island of Pag. In this unique locality, more than eighty thousand wild millennial olive trees create the largest wild olive park in the world. The aim of this research is to characterize the olive oils produced from wild olives based on their fatty acid composition and phenolic profile, as a foundation for the specifications for label protective designation of origin. Olive oil samples were collected in 2018 directly on Pag Island from olive farmers who have their olive groves within the Lun Olive Gardens. The fatty acids composition were determined by gas chromatography separation of prepared methyl esters according to ISO method (5508). Phenolic extracts were prepared using liquid-liquid extraction in n-hexane with a water/methanol mixture (60:40, w/w). The phenolic compounds of the extracts were analyzed by Perkin Elmer high-performance liquid chromatography HPLC system (Waltham, MA, USA) equipped with a variable UV/VIS detector at 280 nm and the TotalChrom Workstation software package. The results for fatty acid composition show the oleic acid content from 72.02 to 74.50%, while the content of linoleic and α -linolenic acid was from 9.14 to 10.52% and 0.31 to 0.37%, respectively. The phenolic composition shows the highest variability in the concentrations of hydroxytyrosol and tyrosol between certain oil samples (from 1.19 up to 9.44 mg kg⁻¹, and 1.80 up to 11.89 mg kg⁻¹, respectively). Oleuropein and ligstroside derivatives were the main and the most abundant phenolic fractions in all analyzed samples. Overall results indicated significant variability of phenolic compounds in VOOs from wild olives, thus indicating possible superior therapeutic features from certain analyzed oils.



FLOURS FROM SUNFLOWER AND PUMPKIN SEEDS AFTER COLD- PRESSED OILS PRODUCTION AS SOURCES OF NUTRITIONALLY VALUABLE PROTEINS

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KEYWORDS:
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protein, sunflower*

Press cakes obtained in the process of oil production, are the byproducts that have becoming very attractive sources of nutritional and essential compounds. Taking into account sunflower and pumpkin as the extraordinary material for added protein value products, this study was mainly designed to evaluate total protein content and to assess the amino acid profile of flours from cold-pressed oilseed cakes. In addition, the materials were compared with respect to the aforementioned properties. The study was conducted on flours from sunflower and pumpkin press cakes available on the Serbian market, and they were analyzed in triplicate. Total protein content was determined by the Kjeldahl method, while amino acids identification and quantification were done using ion chromatography followed by electrochemical detection. Obtained results indicated that flours obtained by grinding cake from the production of cold-pressed sunflower and pumpkin oils differ nutritionally in protein aspect between each other. Namely, protein content was above 30 % in sunflower and even above 60 % in the case of pumpkin flour, and these values are many times higher in comparison with the most common wheat flour (approximately 10 %). Regarding protein composition, the most abundant amino acid was glutamine, while within essential amino acids leucine and phenylalanine predominated in both flour samples. Around one third of all amino acids were essential ones as well in sunflower (35 %) as in pumpkin (34 %) flour. To sum up, flours from sunflower and pumpkin press cake from cold-pressed oils production are excellent dietary sources of proteins with high biological value. Therefore, by processing press seed cakes into flours and their integration into many products of food industry, these nutritionally valuable byproducts would be made valuable to the consumers.

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NEW METHODS IN OLIVE PESTS CONTROLLING USING PLANT VOLATILES

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L., plant protection,
volatile compounds

The olive fruit fly (*Bactrocera oleae*, Rossi) and olive moth (*Prays oleae*, Bern.) are economically the most important olive pests, causing yield losses in all olive growing areas where detected. For years, the use of pesticides was the main method for their controlling, which intensive use had a negative effect on the environment. Therefore, the EU is seeking to reduce pesticide use by 50 % by 2030 and 100 % by 2050. Due to all the above, more effective non-pesticide lures for *B. oleae* and *P. oleae* monitoring and/or controlling are needed. Current knowledge suggests that different insect species are attracted by volatile natural compounds, including a pheromones, host-plant, yeasts and bacterial volatiles. Since the interaction between olive tree and olive pests has been purely investigated, the aim of this study was to identify the olive tree volatiles that could be responsible for *B. oleae* and *P. oleae* attracting, and to test them in olive orchards.

Plant material was sampled on selected olive cultivars and volatiles were identified using HS-SPME-GC-MS. During investigation, around 70 different volatiles were identified from olive flower buds, fruits 4–6 mm and leaves, and 100 different volatiles from olive fruits in three ripening stages (green, half ripe and ripe), mostly belonging to the groups of esters, saturated hydrocarbons, aldehydes, alcohols, terpenes and sesquiterpenes. These results, as a pull of data will serve for identifying volatiles that could be responsible for olive moth attracting, thus selected volatiles will be tested in delta traps in multiple olive orchards. Volatiles that could be responsible for olive fruit fly attracting will be also selected and tested on yellow sticky traps in other olive orchards.

The results of our research could provide answers to many concerns about the olive/pests relationship. Finding volatiles that could be responsible for attracting harmful species may lead to the development and improvement of new attractants, that could be a useful tool for monitoring and/or controlling of *B. oleae* and *P. oleae* in the future.



LINSEED CAKE, A SOURCE OF ANTIOXIDANT COMPOUNDS

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KEYWORDS:
*Antioxidant
peptides,
bioactive
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linseed,
phenolic profiling*

Linseed (*Linum usitatissimum* L.) is an economically important crop worldwide for its oil and fiber (linen) content. Particularly, after the oil extraction, the cake is generated as a by-product. Since linseeds possess a unique nutritional profile, they have been incorporated for fortification of many food products. In addition, there is a growing interest to enhance the utilization of the linseed cake, which has interesting nutritional features as its precursor. It is also a potential source of functional bioactive metabolites aiming at the sustainable development of the linseed oil industry.

Therefore, to give new insights into the bioactive composition of this by-product, this study carried out an untargeted profiling of the cakes of four linseed cultivars via liquid chromatography coupled to quadrupole-time-of-flight-mass spectrometry. Moreover, the antioxidant properties have been evaluated by chemical methods.

The cakes presented a complex and rich phenolic composition composed of hydroxybenzoic acids (20), hydroxycinnamic acids (20), flavonoids (59), lignans (9), and coumarins (1). There were differences in this qualitative and quantitative profile between the cultivars and they also presented different total phenolic content (292.6–531.1 mg gallic acid equivalents/100 g) and antioxidant properties (717–1181 mmol Trolox equivalents/100 g). Undesirable compounds have also been detected such as cyanogenic glycosides and linatine. Therefore, the utilization of linseed cake and derived extracts to fortify food products should consider that they can enhance the phytochemical and antioxidant properties, but linatin and cyanogenic glycosides should be monitored.

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HIGH VOLTAGE ELECTRIC PLASMA DISCHARGE APPLICATION IN ENVIRONMENTAL PRESERVATION

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KEYWORDS:
HVED,
OMWW,
polyphenol
degradation

High concentrations of polyphenols, in combination with other components present in olive mill wastewater, make a big environmental problem when released into nature due to high phytotoxicity and antimicrobial properties as well as genotoxicity, which limits the biodegradation of wastewater. The aim of the work was a degradation of model solutions by high voltage electrical plasma discharge (HVED). The model solution was composed of polyphenols commonly present in the highest concentrations in olive mill wastewater hydroxytyrosol, tyrosol, vanillic acid and oleuropein. Plasma treatment was operated at a frequency of 60 Hz, a voltage of 40 kV and through time 10, 20 and 30 minutes. The initial samples were adjusted to a pH value of 7 and 10. During the treatment nitrogen (N₂) gas was bubbled into the liquid to improve treatment properties. Sample temperature, electrical conductivity, pH value and polyphenol reduction were monitored during the treatment and samples were also analysed by NIR spectroscopy. In all samples, polyphenol degradation is noticeable already after 10 minutes of treatment with a reduction of over 50% for hydroxytyrosol, tyrosol and vanillic acid and a reduction of over 99% for oleuropein.



Session C:

ALL COLORS OF *Trametes versicolor*

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KEYWORDS:
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lignocellulosic
materials,
Trametes versicolor

Trametes versicolor belongs to the Phylum Basidiomycota of the Kingdom Fungi. In Western cultures, *Trametes versicolor* is known as turkey tail, while in Eastern cultures it is known as yun-zhi (China) and kawaratake (Japan). Beside being known for its health benefits, *Trametes versicolor* is widely explored for environmental purposes.

In this work, the basic characteristics of *Trametes versicolor* and the main aspects and differences between submerged fermentation (SmF) and solid-state fermentation (SSF) which are commonly used for biovalorization of widely abundant lignocellulosic materials by *Trametes versicolor*, are presented. An overview of the *Trametes versicolor* enzymatic system involved in the degradation of lignocellulosic biomass is provided, with emphasis on the production and application of laccase in waste and wastewater treatment. Finally, the cascade production of feed, biogas and biofertilizer from lignocellulose using *Trametes versicolor* is presented and critically analyzed as a possible contribution to the development of a circular and sustainable biorefinery based on lignocellulose.



EXPERIMENTAL STUDY OF THERMAL RESISTANCE VALUES OF NATURAL FIBER INSULATING MATERIALS UNDER DIFFERENT MEAN TEMPERATURES

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KEYWORDS:
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mean
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natural fiber,
thermal
conductivity,
thermal
resistance
value*

The purpose of this paper is to experimentally study the thermal resistance values (R-value or RSI value) of building insulation materials mainly made from natural fibers. Natural fibrous materials or renewable resources and their reinforcement composites are currently used in the building field as a potential solution to reduce significantly thermal load and energy consumption. RSI value is used in describing the thermal efficiency of insulating material and in an analysis of heat transfer through the structural components of a building (such as walls, roofs, and windows) under steady-state conditions. In this study, the thermal resistance value of several samples made from raw coconut fiber, rice straw/energy reed fiber, and coconut wood is calculated from the thermal conductivity which was measured at room temperature (20 °C) using the mean of heat flow apparatus. The lowest RSI value was recorded from the phenol-formaldehyde polymeric composites reinforced by coir fiber and rice straw fiber (0.11 to 0.19 m²K/W) due to the relatively small thickness of the tested samples (8 and 12 mm). However, these samples can be used as an additional layer employing in multi-layered wall structures because of the low thermal conductivity value. The highest RSI value was reported on the raw coconut fiber specimen (0.95 m²K/W) at 50 mm of thickness, which was similar to organic insulation material such as polyurethane foam at 25.4 mm (0.97 m²K/W). Besides, the RSI value per mm was also scored to highlight a strong dependence of thermal resistant performance on the thickness factor. Another investigation is to examine the relationship between RSI value and operating temperature to observe how the influence of ambient temperature on the heat resistance of building insulation materials. Practical data showed the decreased linear proportion between the thermal resistance values and specific mean temperatures increase from 0 to 40 °C. It is apparent that increasing simultaneously the interior and exterior temperature of a building has reduced the thermal resistance of insulation materials. Based on the experimental study, once the thermal conductivity coefficient of each sample was determined, the calculated R-value is a valuable parameter to evaluate the thermal resistant effectiveness of a multi-layered installation, which allows to investigate the effect of additional layers from different insulating materials used in building envelopes.



PURPLE NONSULPHUR BACTERIA IN DEVELOPMENT OF SUSTAINABLE BIOPROCESSES FOR PRODUCTION OF HIGH VALUE BIO-CHEMICALS AND USAGE IN BIO-REMEDICATION

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purple non-sulphur
bacteria,
sustainable
development*

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The shortage of petroleum and other non-renewable material and energy resources for production of fuels and chemicals, is a very certain scenario in the near future. Demand for new technologies and energy management concepts as well as need for new socio-economic models and changes in consumers' behaviour, make new challenges on daily basis that have never been greater. One of the solutions is sustainable development of bioprocesses, that convert biomass into food, food ingredients, livestock feed, chemicals, materials, fuels, and energy through a broad spectrum of technologies and integrated conversion processes into more value-added products. In order to establish ecological and economic sustainable bioprocesses, it is necessary to select adequate working microorganisms, raw materials and cultivation conditions. Development of bioprocesses based on the renewable raw materials usage (e.g., lignocellulose containing feedstocks) are a good example. The use of photosynthetic microorganisms such as purple non-sulfur bacteria (PNSB), is generally considered one of the potential methods to reduce the cost of production and enable the maintenance of ecological balance through the use of solar energy and the ability to fix CO₂ and nitrogen from the atmosphere. Purple non-sulphur bacteria are interesting from ecological and economical point of view in sustainable biotechnological production of biofuels, biochemicals, biopolymers and biomass, as well as specific compounds such as carotenoids, vitamins, biological cofactors, and pigments. The biotechnological potential of PNSB lies in their exceptional physiological diversity, ease of adaptation to a wide range of environmental conditions, and ability to biosynthesize multiple products with possible commercial application. The intensification of research and the development of bioprocesses have led to the discovery of various biomass products that have found their application in the fields of medicine, bioenergy, food industry and agriculture. In recent years, the application of biomass of purple non-sulfur bacteria in the processes of soil and wastewater treatment from various industries has been extensively investigated where biodegradation of organic and inorganic compounds is used for simultaneous biosynthesis of high-value bioproducts.

ADVANCED ANALYSIS OF HIGH VALUE-ADDED PRODUCTS FROM A PILOT SCALE LIGNIN DEPOLIMERISATION PLANT

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KEYWORDS:
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high value
products, lignin,
pilot plant*

Lignin is the largest regenerative source of aromatic organics. In current wood pulping industries, the lignin is underutilized and widely considered a waste stream only exploited for its energy content. The LIBERATE project aims to extract valuable chemical products from this existing waste stream (lignin). A pilot plant has been built to demonstrate the commercial potential of converting low-cost lignin feedstocks into high-value chemicals. A key technology in the conversion process is the use of electrochemical methods. The conversion system consists of two reactor sections: An electrochemical reactor, producing sodium peroxydicarbonate (PODIC®) from an aqueous mixture of sodium carbonate. The PODIC® product stream is a strong oxidizer that triggers depolymerization reactions when mixed with Kraft or Organosolv Lignin in a heated plug flow reactor where the lignin is further depolymerized at elevated temperature. The reaction product is further separated and valorised by ion-exchange absorption columns and several evaporation units. The focus is on the yield of the aroma substance vanillin and other phenolic compounds. A factorial experimental design (main factors: lignin feed concentration, PODIC to Lignin ratio, PODIC feed distribution, temperature profile) is used to investigate the effect of oxidative depolymerization of lignin with PODIC in the heated plug flow reactor. A quantitative and qualitative analysis is done using GC-MS and LC-ESI-MS. Samples are taken out along the plug-flow reactor. With these data, the reactor design and process settings can be evaluated and optimized with respect vanillin and other phenols.

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MICROWAVE-SUBCRITICAL HYDROLYSIS: FROM MODE COMPOUND TO COMPLEX BIOMASS

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*cellulose, cork,
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microwave,
subcritical water*

Biomass conversion processing is key to providing suitable and renewable molecules for chemicals, cosmetics, medicals and fuels applications in a sustainable manner. The recent years have witnessed flourishing of microwave (MW) chemistry as an alternative to conventional heating for organic syntheses. MW has been concomitant with selective heating, reduced reaction times, increased product yield, enhanced product purity and better material properties. For instance, it has been demonstrated to facilitate the occurrence of hydrolysis in model natural biopolymers, namely cellulose and fucoïdan (a marine biomass representative) in synergy with graphene oxide (GO) catalyst and subcritical water. In that body of work, microcrystalline cellulose was efficiently hydrolyzed into glucose with a yield 61% at 180°C, 200 W and 60 min in a CEM MARS 6 closed reactor. Meanwhile, fucoïdan was depolymerized into fucose with a 54% yield at fucoïdan: GO: water ratio of 5:5:1, 15 min reaction time and 600 W under reflux conditions. In addition, the individual and synergistic roles of graphene-based catalysts, microwave and water were also identified.

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Banking on that knowledge, we have embarked on the microwave assisted fractionation of cork. We have fractionated cork at 235°C from 30-90 min using water as the sole solvent. It was found that the dissolved cork fractions in subcritical water increased as a function of time. Extractives and cellulosic fractions were detected in the aqueous products while lignin and suberin was enriched in the solid products. The aqueous phase was further extracted with chloroform and the GC-MS analysis confirmed the presence of various alkanes for the 30-min run which were not evident in the 90-min samples. The key message in this talk is that overall, microwave-assisted processing provided an important strategy for polysaccharide depolymerization which in turn, would help understand complex biomass processing.

SEWAGE SLUDGE MANAGEMENT VIA ENERGY CROP PRODUCTION

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Miscanthus,
sludge

The use of sewage sludge in agriculture is regulated by strict rules and directives in both the EU and Croatia. Nevertheless, there is great potential for progress in the application of sewage sludge to soils in larger than specified quantities when applied to non-food crops. Indeed, the Regulation on the Management of Sewage Sludge from Wastewater Treatment Plants when Used in Agriculture (OG 38/2008) in Croatia stipulates that a maximum of 1.66 t/ha dry matter of sewage sludge may be applied to the soil per year. However, it is possible to use a larger amount of sludge in agriculture by cultivating energy crops that also phytoremediate the soil. This method of sewage sludge application could improve poorer quality soils that are not competitive in conventional food production. Namely, only on such soils, crops for energy production can be grown, in order to avoid undesirable competition between energy and food production. Taking into account Croatia's agro-climatic conditions and the obligation to grow energy crops on marginal soils, the rhizomatic grass *Miscanthus x giganteus* is the optimal solution.

The aim of the project "Sewage Sludge Management via Energy Crop Production", funded by the Croatian Science Foundation, is to analyse the properties of soil and sewage sludge and to determine the effects of fertilising with sewage sludge on the yield and the energetic properties of *Miscanthus* biomass. The main objective of the research was to investigate the effects of the application of sewage sludge from the Zagreb WWTP in three different rates of application 1.66, 3.22 t/ha and 6.44 t/ha on soil properties and on the yield and quality of *Miscanthus* biomass in the two growing seasons. The analysis of the sewage sludge quality showed that the concentration of heavy metals was below the maximum permissible concentrations and that sludge was without pathogenic bacteria. According to organic matter and valuable nutrients content the sewage sludge can be classified as an organic soil improver. After the application of the largest amount of sewage sludge, the standard biomass yields were observed without a significant soil heavy metal accumulation and energetic properties of the *Miscanthus* biomass suitable for the process of direct combustion.



MODIFICATION OF CELLULOSE SHEET PROPERTIES WITH PLANTAGO PSYLLIUM SEED HUSK

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Plantago psyllium,
seed husk

The improvement of the mechanical properties of a cellulose sheet, which made from secondary fiber was investigated by the addition of *Plantago psyllium* seed husk flour. The *psyllium* husk was used because it has high hemicellulose (arabinoxylan) content. Arabinoxylan shows good compatibility with cellulose furthermore it has film-forming properties. The *psyllium* husk was added in 5wt% to the cellulose suspensions. The handsheets were formed at the end of different absorption time. So we measured the mechanical properties after 10, 30, 60 and 120 minutes absorption time. The treated handsheets' results was compared with the results of the control handsheet which was not included the additives. According to the results can be determined that the *psyllium* husk added to the secondary fiber causes a significant improvement even with an absorption time of 10 minutes. However, an absorption time of 60 minutes was considered optimal depending on the improvement of the properties. The use of *Plantago psyllium* seed husk as an additive improves the mechanical properties of paper in an environmentally friendly way. For the improvement of mechanical properties of recycled paper, the *Plantago psyllium* seed husk could be a promising additive.



EFFECT OF ABRASIVE GRAIN SIZE ON THE ABRASION RESISTANCE OF BLACK LOCUST (*Robinia pseudoacacia*) IN THREE CHARACTERISTIC SECTIONS

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abrasion resistance,
black locust

Black locust (*Robinia pseudoacacia* L.) is a widespread hardwood tree originating from North America that can be found in all temperate regions. Its ability to succeed in different soil types and develop a strong branched root system was the key to its naturalization in many European countries since it was brought to the continent in the 17th century. Alike all wood species, Black Locust has a hierarchically complex structure. Specific cells are used for mechanical strength (fibers) and for water transportation (vessels), combining micro and nano-sized elements. Anisotropy highly influences its properties. Microstructure and properties vary between three main wood sections: tangential, radial, and cross section. The aim of this article is to determine how the change of section affects abrasive wear resistance, and how the abrasive grain size affects abrasive volume loss. Samples from each section were exposed to abrasive wear on Taber abraser using different grain-sized paper. After examination, the mass loss was measured and recalculated into the volume loss to indicate differences between sections. The results showed that the orientation of the wood structure and the size of abrasive grains have a significant influence on the abrasion resistance. The highest abrasion resistance was observed in cross section. The abrasion resistance of radial and tangential sections were similar and lower than abrasion resistance in cross section. On all the tested samples, a phenomenon of critical abrasive grain size was observed. By increasing the size of the abrasive grains to a critical size, at the same time, the abrasive volume loss also increased. After the value of the critical size of the abrasive grain, the volume loss ceased to grow or even began to decrease.



INFLUENCE OF MOISTURE CONTENT ON THE CALORIFIC VALUE OF WOOD RAW MATERIAL

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moisture content,
wood moisture
uptake, wood raw
material*

Combustion of wood releases a certain amount of heat, or wood has a certain calorific value. As the moisture content of wood increases, its calorific value decreases. In this paper, the influence of moisture content of 4 types of wood (oak, beech, fir and black locust) on their calorific value is analyzed. The wood raw material was previously dried to an absolutely dry state, after which it was exposed to defined microclimatic conditions (relative humidity 80 % and temperature 30 °C) for 111 hours. According to the available literature, the equilibrium moisture content for these microclimatic conditions is $\approx 15.6\%$, so long-term exposure of wood raw material would achieve the stated moisture content. In this paper, the moisture uptake of wood raw material in the time period and its impact on the calorific value are observed. The measurement determined the intensive moisture uptake of wood raw materials in the initial 7 hours of exposure and amounted to 10–11 % for all types of raw materials, while this content was further raised to 12 % in the next 8 hours of exposure. Slightly lower moisture uptake was recorded for acacia raw materials, and moisture content of $\approx 11\%$ was recorded at 15 hours of exposure. By further exposure to these conditions, the raw material receives moisture very slowly, and after the final 111 hours of exposure, the increase in moisture content in all species was below 1 % of the moisture content. By measuring the calorific value, a linear decrease in the gross calorific value with an increase in the moisture content of the raw material was determined. The raw material of hardwoods (oak, beech and black locust) with a moisture content of 0.5 % has an gross calorific value of ≈ 19.5 kJ/g, while the raw material of fir has a slightly higher calorific value of 20 kJ/g. The highest moisture uptake and decrease in calorific value was recorded in oak raw material. In the case of black locust raw material, regardless of lower moisture uptake, a significant decrease in calorific value was also recorded compared to other types of wood.



SELECTED PHYSICAL AND CHEMICAL PROPERTIES OF WOOD CHIPS PRODUCED IN SELECTIVE AND SALVAGE FELLINGS OF NORWAY SPRUCE (*Picea Abies* (L.) KARST.)

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elemental analyses,
wood decay

After the tree dies, wood degradation starts, caused by different biological organisms, which results in changes in wood's physical and chemical properties. The physical and chemical properties of solid biofuels are correlated with the material type they were produced of. For example, the content of some chemical elements depends on tree species; ash content depends on bark content, etc. This research aims to determine the correlation between physical and chemical wood chips properties and material types. Wood chips were produced from two material types, from the area of the Forest Administration Delnice. The first material type was pulpwood produced from dead Norway spruce (*Picea abies* (L.) Karst.) trees from salvage felling, and the second material type was pulpwood produced from healthy Norway spruce trees from selective felling. Laboratory analyses were done according to the HRN EN ISO standards. Values of moisture, ash, carbon, hydrogen, nitrogen and sulphur content and particle size distribution were determined. The moisture content was 44.4% for healthy spruce trees, which is significantly higher than the 27.3% that was determined for dead spruce trees. The ash content of dead trees was 0.44% (due to the lower bark content) which is more favourable (because of lower bark content) than the significantly higher ash content of 0.75% of healthy trees. The average share of carbon content was 50.54 ± 0.13 % for dead trees and 50.78 ± 0.38 % for healthy trees. The absence of statistically significant differences for some parameters is likely caused by in-material variability. In addition, for some purposes, that would be more useful to present results in absolute values (for example, carbon content), including possible wood matter losses.



GREEN BIOMASS OF MAIZE WITH CLIMBING BEAN AND COWPEA AS A SUSTAINABLE SOURCE OF PROTEIN

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KEYWORDS:
*biomass, climbing
bean, cow pea,
maize, protein*

Maize forage is poor in protein content which shows its low quality and nutritive value. Regarding to high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content and, thus, providing cost savings. Maize (*Zea mays* L.), climbing bean (*Phaseolus vulgaris* L.) and cow pea (*Vigna unguiculata* L.) intercropped in different sowing densities and pure maize crop were evaluated to determine the best intercropping system with respect to green biomass and protein yield. The highest dry matter yield (21.4 t ha⁻¹) over two years was produced by intercropping maize population 75 000 plants ha⁻¹ and climbing bean population 75 000 plants ha⁻¹, whereas the lowest yield (18.3 t ha⁻¹) produced intercrop consisting for the monocrop maize population 75 000 plants ha⁻¹. The highest dry matter yield (20.6 t ha⁻¹) over two years was produced by intercropping maize population 75 000 plants ha⁻¹ and cow pea population 75 000 plants ha⁻¹, whereas the lowest yield (18.3 t ha⁻¹) produced intercrop consisting for the monocrop maize population 75 000 plants ha⁻¹. The highest crude protein yield (2.33 t ha⁻¹) over two years was produced by intercropping maize population 75 000 plants ha⁻¹ and climbing bean population 75 000 plants ha⁻¹, whereas the lowest yield (1.39 t ha⁻¹) produced intercrop consisting for the monocrop maize population 75 000 plants ha⁻¹. The highest crude protein yield (2.13 t ha⁻¹) over two years was produced by intercropping maize population 75 000 plants ha⁻¹ and cow pea population 75 000 plants ha⁻¹, whereas the lowest yield (1.39 t ha⁻¹) produced intercrop consisting for the monocrop maize population 75 000 plants ha⁻¹. The results of this study clearly showed that among all intercropped forages the maize population 75 000 plants ha⁻¹ and climbing bean population 75 000 plants ha⁻¹ was better performing than other intercropped, and may contribute to more efficient green biomass and protein production on dairy farms, and future green biorefinery needs under particular agroecological conditions in Croatia.



SPECTROMETRIC ANALYSIS OF LIGNIN FROM ABONOS AND OAK WOOD (*Quercus robur* L.)

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KEYWORDS:
abonos,
bog-wood,
lignin,
oak,
spectrophotometric

Lignin, alongside cellulose, is the main macromolecular substance of the cell walls of wood, whose content varies significantly between wood species. Literature given values of lignin content in wood are around 20-40 %, depending on the wood species. However, lignin is prone to degrading in most of the processes that include treating of wood with various chemicals. Therefore, several methods of lignin quantification were developed. Three of those methods were employed in this paper (i.e. acetyl bromide, Klasons lignin and Brauns lignin determination) to quantify the lignin contents in abonos (bog oak-wood) and oak wood (*Quercus robur* L.). The soluble part of all three lignins was determined by means of UV/Vis spectrophotometry, with additional determination of insoluble part of Klasons lignin by ignition. The obtained results showed differences between lignin contents of two examined samples, alongside expected difference of lignin contents due to method used. Additional FT-IR analysis of Brauns lignin confirmed the difference between examined species.



EFFECT OF AMINO ACIDS ON LPMO ACTIVITY

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amino acids,
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hydrocoerulignone,
lytic polysaccharide
monoxygenases

Enzymatically catalyzed processes for the production of cellulose have numerous advantages over chemical ones, and the group of enzymes that have attracted special interest are lytic polysaccharide monoxygenases (LPMO). These enzymes play an important role in the breakdown of lignocellulose because they act synergistically with other enzymes by increasing the availability of cellulose for cellulases. As a result of their C1-oxidizing activity, LPMOs disrupt the cellulose fibers by the introduction of carboxyl groups thus increasing the yield of nanocellulose. A new method has been developed for measuring the LPMO activity based on hydrocoerulignone as substrate and hydrogen peroxide as cosubstrate which allows reliable detection of LPMO in complex matrices like culture media. Influence of the amino acids on the determination of LPMO activity by hydrocoerulignone method will be closely discussed.

MORPHOLOGICAL VARIABILITY OF THE LEAVES OF EUROPEAN WILD PEAR (*Pyrus pyraeaster* (L.) Burgsd.) POPULATIONS FROM CONTINENTAL AND MEDITERRANEAN PARTS OF CROATIA

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*biogeographical
structure, leaf
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morphometric analysis,
population variability,
wild pear*

European wild pear (*Pyrus pyraeaster* (L.) Burgsd.) is a rare, noble hardwood species belonging to the Rosaceae family, with high-quality wood and great importance for biodiversity and ecosystems. It is native in almost all of Europe, and along with *P. caucasica* Fed., is considered to be the ancestor of the cultivated European pear (*P. communis* L.). In this study, we evaluate the morphological variability of the wild pear in Croatia. The inter- and intrapopulation variability was determined on the basis of ten measured morphological characteristics of leaves, using descriptive and multivariate statistical methods. The conducted research included 12 populations from both continental and Mediterranean parts of Croatia, and each population was represented by ten trees. The differences between the trees within populations, as well as the differences between populations, were confirmed for all studied characteristics. Biogeographical structuring of populations with a high level of among-tree variation within the populations was identified. In general, populations from the Mediterranean and the continental regions differed in the majority of the studied characteristics, with the Mediterranean populations possessing generally smaller leaves. This study provided insights into the variability of the European wild pear populations in Croatia, which is an important part of the efforts towards producing guidelines for the breeding and conservation of genetic resources of this rare and endangered noble hardwood species.



CELLULOSE ACETATE AND WASTE PAPER BASED BIO NANO COMPOSITES - OPTICAL AND THERMAL PROPERTIES

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composites,
cellulose acetate,
filler, properties,
waste paper*

Recycling of waste materials and production of new products with more added values are defined by the principles of the circular economy. Given that the circular economy imply also the continual use of resources and raw materials, this paper deals with the possibility of waste paper use as nano sized filler for cellulose acetate based bio composites. Potential use of multiple times recycled, waste packaging (Kraft) paper and laboratory filter paper as starting materials for nano sized cellulose based filler preparation was determined. As for cellulose acetate polymeric matrix synthesis, Black alder (*Alnus glutinosa* (L.) Gaertn.) wood and commercial microcrystalline cellulose (Avicel) were used. The optical (UV spectroscopy) and thermal (DSC) properties of prepared bio-nano composites were determined, with supplementary FT-IR analysis of synthesised cellulose acetates. The test results indicated the exceptional importance of the purity of the cellulose base both for the synthesis of nano particles and for the synthesis of the polymeric base of bio- nano composites.

BARK THICKNESS OF SERBIAN SPRUCE FROM NATURAL STANDS AND PLANTATIONS

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KEYWORDS:
*bark thickness, bark
share, Bosnia and
Herzegovina, Serbian
spruce*

From the point of view of wood processing and the use of biomass, bark thickness and the share of bark are the two most important characteristics of the bark. The paper presents the determined average values of bark thickness and its share in fifteen trees of Serbian spruce. Nine spruce trees come from natural stands in the vicinity of Visegrad (eastern part of Bosnia and Herzegovina), and six trees from plantations (three trees from a plantation in the vicinity of Banja Luka and three trees from the plantation in the vicinity of Srebrenica). The thickness of the bark and its share were determined at different heights, starting from 0.3 m, 1.3 m, 3.3 m and further at every two meters of tree height. It was found that with increasing tree height, the thickness of the bark decreases, while the share of bark in the lower parts of the trunk is mostly constant, and in the part of the canopy where there is a large taper, it increases. Trees from plantations have a higher average bark thickness (4.35 mm) than trees from natural stands (4.15 mm). Also, the share of bark is higher for trees from plantations. Regression analysis showed that with increasing diameter, the bark thickness increases, and the share of bark decreases.



INFLUENCE OF WOOD CELL ORIENTATION ON THE SPRUCE WOOD HARDNESS

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KEYWORDS:
*hardness,
spruce wood,
structure orientation*

This paper presents the influence of the orientation of wood cells on the hardness of spruce wood. The hardness measurement was carried out by the measuring method by Brinell, from cross-section to tangential section, in sequences of 15° each. The hardness was measured 10 times for each angle. The results showed that the hardness of wood largely depends on the orientation of the structure. In the cross-section, the highest hardness values were obtained and amounted to 50.0 HB, while the lowest ones were obtained in the tangential section and amounted to 26.1 HB. In the tests carried out, there are relatively large scatterings of results, but the variation coefficients are within the allowable values (10.7 % to 19.8 %). The test precision index ranges between 6.67 % and 12.56 %. The measured hardness values match relatively well with the hardness values calculated by Hankinson's formula.



INTEGRATED PROCESSES EFFECT ON POLYPHENOLS CONTENT OF PEPPERMINT

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KEYWORDS:
*maceration, Mentha
piperita, polyphenols,
ultrasound assisted
extraction*

The peppermint (*Mentha piperita* L.) represents one of the most studied plant species due to wide spectrum of pharmacological activities ascribed to its extracts and essential oil. According to EMA, major constituents of essential oil are menthol (30-55%) and menthone (14-32%). Since about 75% of the polyphenolic compounds present in the peppermint leaves are extracted in an infusion, conventional extraction can serve as a pretreatment step prior to ultrasound assisted extraction (UAE) by ultrasonic probe. The aim of this study was to investigate which integrated process would deliver the optimal peppermint extract so that it could be scaled up in an industrial environment. Integrated processes consisted of 30 min maceration prior to UAE (10 min), 60 min maceration prior to UAE, UAE prior to 30 min maceration and UAE prior to 60 min maceration. In all experiments, the solid/liquid ratio was 1:20 and 50% ethanol was selected as a solvent. The highest contents of total phenols (263.07 mg GAE/g DW) and total flavonoids (220.81 mg CE/g DW) were obtained in integrated process which consisted of 60 min maceration prior to UAE (10 min). This integrated process provided peppermint extract with higher total phenols content than extract obtained by 24 h maceration at room temperature.

BIOLOGICAL POTENTIAL OF HEMPSEED AND FLAXSEED PROTEIN HYDROLYSATES IN CHO CELL CULTURE DURING OXIDATIVE STRESS

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KEYWORDS:
*CHO cells,
hydrolysates, oil
cake, oxidative
stress*

The oil cakes are by-products of edible oil extraction process from certain industrial plant seeds. They were treated until recently as a low-value agro-waste despite their high protein content. Oil cakes of hempseed and flaxseed contain around 30% proteins, as well as many other bioactive compounds, and as such, they can be processed into a nutritive supplement for animal cell cultivation. The majority of high-value therapeutic proteins today are produced with CHO (Chinese hamster ovary) cells. The use of plant protein hydrolysates as growth media supplements in CHO cell culture has two main purposes: enhancing cell growth and increasing the recombinant protein production. Reactive oxygen species (ROS), represented by hydrogen peroxide, nitric oxide, and free radicals, are regular metabolic products of cells grown in vitro. They affect diverse cellular functions and cause damage of biological macromolecules such as lipids, proteins and nucleic acids. In this work, we investigated the protective effects of flaxseed and hempseed protein hydrolysates (FPH and HPH) obtained by various microbial proteases (Alcalase®, Neutrase®, Protamex®) on hydrogen peroxide-induced oxidative stress in CHO cells producing IgG. Cell cytotoxicity was evaluated through the aspects of cell viability determined by the MTS assay. The results showed that the cell culture supplementation with FPH and HPH, produced by different enzymes, has a mild proliferation effect on CHO cells. When oxidative stress in CHO cells was induced by hydrogen peroxide, FHP obtained by Neutrase® (0.5 g/L), and HPH obtained by Protamex® (2.5 g/L) showed a protective effect. All this indicates benefits of FHP and HPH as cell-protecting agents, but more profound analysis is required to identify molecular species contributing to the observed effects.



SUPERCRITICAL CO₂ EXTRACTION OF BILBERRY (*Vaccinium myrtillus*) FRUIT

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KEYWORDS:

*bilberry, extraction,
supercritical co₂, total
phenolic content*

Bilberries (*Vaccinium myrtillus*) are believed to have one of the highest antioxidant levels of all common fruits and vegetables. As a source of antioxidants, they have been used in traditional medicine to reduce inflammation and protect against diseases associated with oxidative stress, such as cardiovascular disease, diabetes, and age-related cognitive decline, as well as a remedy for eye conditions. The biological activity of bilberries is associated with the high content of anthocyanins, a large group of water-soluble flavonoids. Besides the complex anthocyanin profile, bilberries also contain other phenolic compounds including flavonols, tannins, and phenolic acids. Supercritical fluid extraction (SFE) is a green and environmentally friendly process based on the use of supercritical fluids, most commonly CO₂, as an alternative to organic solvents, to obtain solvent-free and highly valuable plant extracts. Utilization of supercritical CO₂ (scCO₂) extraction also enables the preservation of thermally labile compounds working at low temperatures, and good selectivity accomplished by the appropriate choice of operating pressure and temperature. In this study, scCO₂ extraction was investigated as a method for isolating the extract from bilberry dried fruit. Extractions were performed at temperature of 70 °C and pressures of 15 and 30 MPa, with and without the ethanol as co-solvent, with the aim to optimize the yield of the obtained extract based on the composition. The obtained extraction yields ranged from 1.2 to 7% and the highest yield was obtained, as expected, at a higher pressure of 30 MPa. The effect of ethanol as co-solvent in the scCO₂ extraction was studied with the goal to modify scCO₂ selectivity and enhance the solubility of polar substances. The results showed a positive effect on the extraction yield, while total phenolic content (TPC) analysis revealed that extracts obtained using co-solvent have higher TPC values. Chemical analysis of the obtained extracts was performed in order to qualitatively evaluate their composition.

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SUPERCRITICAL CO₂ EXTRACTION FROM DANDELION: THE EFFECT OF PRESSURE ON EXTRACTS YIELD AND COMPOSITION

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KEYWORDS:
*fatty acid
composition,
supercritical carbon
dioxide extraction,
taraxacum
officinale,
total flavonoid
content,
total phenolic
content*

Dandelion (*Taraxacum officinale*) is becoming an industry valuable crop due to the increase in its utilization in the food and phytopharmaceutical industry. Nonetheless, its application is still limited especially considering its extracts. Therefore, this study was aimed at exploring dandelion seeds as a source of bioactive compounds. For this purpose, an environmentally friendly supercritical CO₂ extraction technique was employed and extracts were separated at pressures of 10–45 MPa and a temperature of 313 K. Obtained extracts were characterized using a gas chromatograph equipped with mass spectroscopy (GC-MS), a gas chromatograph equipped with flame ionization detector (CG-FID), as well as by Folin–Ciocalteu and DPPH assays using a UV/VIS spectrophotometer. It was shown that the selection of process pressure determined both extract yield and extract composition. An increase in pressure increased extraction yield from 7.4 to 25.2% and the content of dominant linoleic and oleic fatty acids (from 536.3 to 658.3 mg/g and 125.8 to 161.7 mg/g, respectively). Total phenolic and flavonoid content in obtained extracts ranged from 5.5 to 9.0 mg GAE/g and from 208.6 to 497.5 mg QE/g, respectively. The strongest DPPH radical scavenging activity with inhibition of 64.4% was recorded for extract obtained at 10 MPa for extract solution concentration of 20 mg/mL. Obtained results confirmed that extracts obtained from dandelion seeds are a valuable source of bioactive compounds.

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USE OF OLIVE STONES TO OBTAIN FURFURAL IN A SINGLE-PHASE SYSTEM

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KEYWORDS:
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furfural,
olive stones,
xylose dehydration

Furfural is a platform molecule of great interest, from which a wide variety of chemical compounds can be obtained, including those used in petroleum refining, plastics production, food, pharmaceutical and agricultural industries. Interest in the production of furfural from renewable energy sources has grown in recent years, gaining much attention within the biorefinery concept. Lignocellulosic biomass is a source of value-added chemicals, including furfural. It is obtained by dehydration of pentoses and is of particular interest when obtained from biomasses with a high pentose content, in particular xylose, which is usually the most abundant pentose in these biomasses.

In this work, the recovery of the xylose contained in the olive stone (OS) was maximized using a central composite rotatable experimental design, at varying temperatures (170–200 °C) and sulfuric acid concentrations (0.5–2%), in which the results obtained were statistically analyzed and the xylose concentration of the hydrolysate was maximized. The production of furfural from xylose can be facilitated by the addition of catalysts, in this case sulfuric acid was used as Brønsted acid (the one contained in the hydrolysate after acid pretreatment) and ferric chloride as Lewis acid. The hydrolysate obtained under optimum conditions (194 °C and 0.5%w/v H₂SO₄), with a concentration of 67 g/L xylose, was used for the production of furfural. Different experimental conditions were proposed in a microwave reactor at 200 °C, varying the concentration of ferric chloride (0.1–0.4 M) to maximize the furfural yield. The maximum concentration of furfural obtained was 26.5 g/L with 0.3 M ferric chloride.

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TWO-STAGE SEQUENTIAL PRETREATMENT OF OLIVE STONES FOR CELLULOSE RECOVERY

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KEYWORDS:
*biorefinery,
cellulose,
olive stones,
pretreatment*

Olive cultivation and the industry associated with olive oil production generate a large amount of by-products and waste, including olive stones (OS), pomace, pruning, leaves... Specifically, the OS represents between 10-15% by weight of the olive, which is approximately 750,000 tons per year. OS is mainly composed of cellulose, hemicellulose (mainly xylose) and lignin.

This work focused on the recovery of the cellulosic fraction. A first stage of acid pretreatment was performed according to a central composite rotatable experimental design, at varying temperatures (170-200 °C) and sulfuric acid concentrations (0.5-2%). The results obtained were statistically analyzed and two working conditions were optimized. On the one hand, xylose recovery by acid hydrolysis and glucose recovery by enzymatic hydrolysis were maximized (194 °C and 0.5%w/v H₂SO₄) and on the other hand, only xylose recovery was maximized (170 °C and 2%w/v H₂SO₄). The solid obtained under these optimized conditions was subjected to a second delignifying step with alkaline peroxide at different concentrations 1-7%, to increase the enzymatic digestibility of the cellulose. Glucose recovery was evaluated after enzymatic hydrolysis without delignification and after delignification. A maximum enzymatic hydrolysis yield of 70% was achieved after a first acid step at 194 °C and 0.5%w/v H₂SO₄, followed by delignification with 7% alkaline peroxide.

We can confirm that the recovery of cellulose contained in OS is efficient after acid pretreatment and delignification with alkaline peroxide.

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Workshop 1

FINAL PRESENTATION THE
RESULTS OF THE PROJECT

W

“Isolation and encapsulation of
bioactive molecules of wild and
cultivated nettle and fennel and
effects on organism physiology”
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ISOLATION OF FENNEL ESSENTIAL OIL BY CONVENTIONAL AND ADVANCED EXTRACTION TECHNIQUES

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KEYWORDS:
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hydrodistillation,
steam distillation,
supercritical CO₂
extraction*

Fennel (*Foeniculum vulgare* Mill.) is a perennial aromatic and medicinal plant belonging to the Apiaceae family. It originates from the Mediterranean area, but it has spread worldwide through naturalization and cultivation. The use of fennel in culinary purposes and traditional medicine is known from ancient times, but nowadays its application has been extended to the pharmaceutical, cosmetic and food industries. Although different parts of fennel (leaves, young shoots, roots and seeds) can be utilized, fennel seeds are mostly exploited due to their abundance of essential oil (up to 5-6%) which is characterized by a strong and pleasant aroma. Fennel essential oil has been recognized to possess antimicrobial, antifungal and antioxidant properties, therefore it is widely used in various areas. The chemical composition of fennel essential oil showed up to 80 volatile compounds, among which trans-anethole, fenchone and estragole are major compounds, being the key compounds of the characteristic fennel flavor. Besides these compounds, the presence of other compounds such as α - and β -pinene, myrcene, α -phellandrene, limonene and p-anisaldehyde have also been reported. The isolation of essential oil can be accomplished by various extraction techniques, such as hydrodistillation (HD) and steam distillation (SD) which are known as conventional ones and still are the most common in use. They are effective for the extraction of essential oils from spices and herbs from which such oils are difficult to isolate and they do not include the use of chemical solvents. On the other hand, their main drawbacks are long duration, difficult regulation of heat and possible loss of thermolabile and light volatiles. Therefore, in order to overcome these deficiencies, advanced extraction techniques have been developed, including microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), subcritical water extraction (SWE) and supercritical fluids extraction (SFE), namely extraction with supercritical carbon dioxide (SC-CO₂) which is considered "green" showing the most promising beneficial effects due to being sustainable, environmentally friendly and cost-effective. However, regardless of the selected extraction technique, it is necessary to define optimal process conditions taking into account all process parameters in accordance with the raw material used, and optimize the extraction process with the intention to achieve the maximum yield of essential oil of the best quality and unaltered composition.

THE INFLUENCE OF EXTRACTION AND ENVIROMENTAL PARAMETERES ON THE ISOLATION OF BIOACTIVE COMPOUNDS FROM NETTLE (*Urtica dioica* L.)

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KEYWORDS:
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extraction, habitat,
phenological stage,
Urtica dioica*

Nettle (*Urtica dioica* L.) is one of the medicinal plant species that, due to its chemical composition and content of bioactive components, is an excellent basis for obtaining products with high biological potential. It is a perennial wild plant, known in folk medicine since ancient times, widely distributed and adapted to different climate zones. Nettle products are mostly used in industry as liquid extracts, so it is important to apply and optimize a suitable extraction method to obtain higher yields and ensure the stability of targeted bioactive molecules during extraction. Therefore, various advanced extraction techniques such as microwave assisted extraction (MAE) and accelerated solvent extraction (ASE) were used throughout this study and compared with conventional techniques. One of them - ASE, as a green extraction technique, was investigated as potential for the recovery of the antioxidant molecules (polyphenols, pigments and phytosterols) from all parts of the nettle (leaves, stalks, roots). Also, changes in chemical composition and distribution of compounds depend on the climate and habitat of the plant and can occur as the plant matures, with bioactive molecules being present in different ratio during different phenological stages. So, the aim of this study was to optimize the extraction of bioactive molecules from nettle using ASE and to examine the influence of phenological stage and habitat on their accumulation during vegetation. The optimal conditions for the extraction of polyphenols and pigments from nettle leaf extract were 110 °C, 10 min of static time and three or four extraction cycles. On the other hand, for the efficient isolation of phytosterols from nettle root extract, the optimal conditions were 150 °C, 10 min of static time during four extraction cycles. Furthermore, ASE showed better performance in comparison with conventional extraction techniques. In addition, comparing the proportion of bioactive molecules in the leaves and stalks of nettle, more natural antioxidants were accumulated in the leaves and should be collected during the early phenological period (before and at the flowering stage). Moreover, the amounts of polyphenols and pigments from nettle greatly differed based on the natural habitat, as samples from the seaside region were characterized with elevated accumulation of pigments, while higher polyphenols amounts were present in habitats located in continental and mountain areas.

SPRAY-DRYING ENCAPSULATION OF NETTLE AND FENNEL BIOACTIVE MOLECULES

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fennel,
nettle,
polyphenols

Both nettle and fennel are valuable plants with high content of bioactive molecules. Beneficial effects of nettle are mainly attributed to polyphenols, while fennel is recognized for its essential oil (EO) content. Although chemically different, both nettle extract and fennel EO are characterized with loss of stability and bioactivity during prolonged storage and processing. Spray drying, as a method for encapsulation of both hydrophilic and lipophilic compounds, can provide stabilization, preservation as well as controlled release and delivery of both nettle bioactives and fennel EO. Therefore, the aim of this study was to evaluate and optimize the spray drying encapsulation of nettle leaves extract and fennel EO using different carrier materials and drying temperatures. Nettle leaves extract was obtained by microwave-assisted extraction and encapsulated using maltodextrin (MD) and β -cyclodextrin (CD) as single carriers or in mixtures with gum arabic (GA) in proportion 1:1 and 3:1, respectively. Carriers were used in sample:carrier ratios 1:1, 1:2 and 1:3, while spray drying was performed at 120, 160 and 200 °C. Fennel EO was spray dried at same temperatures and emulsified with following carrier mixtures, MD:CD, MD:GA and CD:GA in proportions 1:1, 1:3 and 3:1 at fixed EO:carrier ratio of 1:3. The highest yield and encapsulation capacity for nettle leaves extract was achieved with addition of MD as a carrier agent in ratio 1:3 at 120 °C, while optimal encapsulation conditions for fennel EO were mixture of CD:GA in proportion 3:1 at 200 °C which resulted in the highest EO retention in microcapsules and the highest encapsulation efficiency. The differences in obtained encapsulation conditions arise from the differences in chemical properties of encapsulated materials, showing that carrier agents such as CD and GA are suitable for non-polar constituents of EO, while MD is more efficient in encapsulation of polar compounds such as polyphenols. The encapsulation of nettle extract and fennel EO provided preservation and stabilization of respective bioactive compounds and increased their bioavailability in encapsulated form, indicating the great potential of such microcapsules for further application in food industry.

INFLUENCE OF FERTILIZATION ON WILD NETTLE (*Urtica dioica* L.) AND FENNEL (*Foeniculum vulgare* Mill.) YIELD

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climate change,
fertilizers,
vegetation index,
wild species

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Climate change and the growing world population are affecting every human activity, especially agricultural production. The main goal is to produce adequate yields of high-quality plant material rich in nutritive value. One of the possible solution to solve the above-mentioned problems is the introduction of wild species into agricultural production. Wild species such as stinging nettle (*Urtica dioica* L.) and fennel (*Foeniculum vulgare* Mill.) are plant species that have a strong biological potential and nutritive value whether wild or grown under controlled conditions (cultivated). The aim of the present research was to test the influence of different fertilization treatments on the quality of wild nettle and fennel. A split-plot experiment was set with four fertilization treatments as factors – control (no fertilization), mineral fertilizer, organic fertilizer and a combination of mineral and organic fertilizers, set on both stinging nettle and fennel. Fennel experiment results showed a significant increase in the vegetation index under mineral fertilizer application, compared to other treatments. Both mineral and combination (mineral and organic fertilizer) resulted in higher yield. Stinging nettle experiment results showed a significant increase in vegetation index under mineral, organic and combination treatments application, compared to control treatment. Furthermore, the same results were obtained regarding stinging nettle yield. It can be concluded that the introduction of wild species such as stinging nettle and fennel into agricultural production can be successfully achieved and fertilizer application showed a positive effect on both vegetation index and yield.

Urtica dioica (STINGING NETTLE) EXTRACTS AND ITS EFFECT ON PPAR α , PPAR γ AND METABOLIC MARKERS OF LIPID AND GLUCOSE METABOLISM

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transcription factors,
Urtica dioica

The peroxisome proliferator-activated receptors have a pivotal role in lipid and glucose homeostasis. Synthetic activators of peroxisome proliferator-activated receptor alpha (PPARalpha - fibrates) and proliferator-activated receptor gamma (PPARgamma - litozones) are therefore widely used for treatment of dyslipidaemia and diabetes, respectively. However, there is experimental evidence for herbal whole plant consumption of *Urtica dioica* (stinging nettle) have effect on the named nuclear receptor signalling and that this plant has the ability of restoring lipid balance and blood glucose levels. In mice there is evidence that *U. dioica* vegetable diet protects against diet induced obesity through mechanisms involving lipid accumulation and glucose metabolism in skeletal muscle, liver, and adipose tissue. Notable genes that impact lipid or glucose metabolism and whose expression is modulated by *U. dioica* diet are fasting induced adipocyte factor (FIAP) in adipose and skeletal muscle, peroxisome proliferator-activated receptor- α (PPARalpha) and forkhead box protein O1 (FOXO1) in muscle and liver, and carnitine palmitoyltransferase I (CPT-1) in liver after, dietary intake of whole plant. The ethanolic extracts of *U. dioica* were tested before in in human volunteers where only systemic effects were measured and the extracts improved blood dyslipidaemia or blood glucose. Under in vitro conditions in hepatic cell culture, the levels of PPAR transcription factors were assayed and *U. dioica* extracts modulated PPAR levels in hepatocyte. Following those previous studies we organized a 10-day dietary intervention study, where male and female C57BL/6 mice were daily given *U. dioica* water extract in a dose of 20 mg/kg of total polyphenols in extract. The effects of the extract were evaluated to establish whether the mouse liver PPAR's, other transcription factors such as NrF2, ACOX and carnitine palmytoil synthetase enzymes and blood glucose could be modulated after such treatment.

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Session D:

GREEN TECHNOLOGIES



PHYTOCAT: PLANT-BASED METALS AS CATALYSTS

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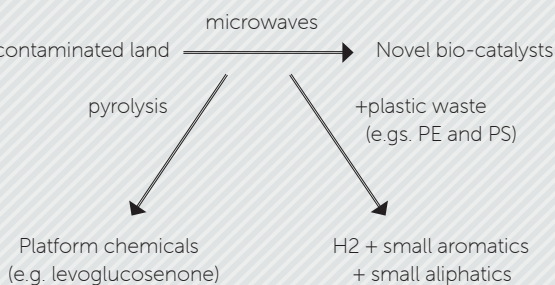
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KEYWORDS:
bio-resources,
catalysis,
metal recovery,
phytocat,
plastic recycling

Traditionally, metal resources have been sourced in relatively accessible places in different regions of the planet. But easily accessible minerals are finite and mining is becoming more difficult, expensive and polluting. Used devices and materials containing metals are rarely recycled and in some cases the metals are becoming scarce with governments identifying “critical metals” that are vital to local industry but with uncertain medium-term availabilities. However, there are increasing volumes of metallic wastes being generated by modern society and these wastes largely end up in landfill sites representing both a wasted resource and a pollution problem. There are also large areas of land contaminated with toxic metals both naturally and man-made which cannot be used to grow food adding to the existing pressures on agriculture and the supply of food to a growing population. These wasted and polluting sites are widely distributed and should be seen as resources.

We have developed two green and bio-based technologies to capture metals from such sites. The most recent of these uses plants to capture metals and then uses the metal-doped plants either as a source of useful chemicals or as heterogeneous catalysts that can be used as alternatives to virgin metals in important chemical processes. Among the processes we will report, the recycling of waste plastics holds the most promise for making a major contribution to environmental protection and waste valorisation. Our key results can be summarized as:



ADVANCES OF ENZYMATIC MICROREACTORS – SUCCESSFUL EXAMPLES OF INTEGRATED SYSTEMS FOR PROCESS INTENSIFICATION

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KEYWORDS:
biodiesel,
enzyme catalysis,
hexanal,
microreactor,
process
intensification

Enzymes are widely recognized as desirable catalysts in applied catalysis. The main goal of many catalytic reactions is to develop an economical, efficient, and environmentally friendly process. One of the main limitations in the application of enzymes is usually the low reaction performance whether it is the duration or the low productivity of the process. In order to intensify the process, there is a constant search for new and efficient catalytic technologies. One of the possible solutions for intensification of enzymatic reactions is the use of microreactors. In this presentation, two successful examples of process intensification by the use of microreactor will be presented.

The first example is the development of an integrated microsystem for biodiesel production and purification. In the developed integrated system, a biodiesel yield of 94% and a glycerol content of less than 0.02% (w/w) were obtained with a residence time of only 20 min. In comparison, when the same reaction (without purification step) was carried out in a batch reactor with the same enzyme and substrate, a yield of 96% was obtained after 48 h.

The second example is the production of the "green note" component hexanal since conventional methods (fermentation, extraction from plants and enzyme-catalysed reactions in a batch reactor) cannot provide a sufficient amount of hexanal. Hexanal was produced in a microreactor by the oxidation of hexanol using NADH-dependent alcohol dehydrogenase (ADH) with integrated coenzyme regeneration. To find the optimal catalyst, different ADH sources were investigated (suspended and immobilized enzyme ADH and permeabilized baker's yeast cells). The best results were obtained with the suspended enzyme, where 100% conversion of coenzyme was achieved with a very short residence time ($\tau = 0.8$ s). Regenerated coenzyme was reused by recirculation for three days without addition of fresh coenzyme.



DEEP EUTECTIC SOLVENTS – PROMISING GREEN SOLVENTS FOR PHARMACEUTICAL APPLICATIONS

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KEYWORDS:
*active
pharmaceutical
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DES,
permeability,
solubility*

The breakthrough of deep eutectic solvents (DESs) was in 2004 when Abbott et al. stated that they are multilateral alternatives to ionic liquids, in terms of green solvents which might replace harmful organic solvents in different processes. Since then, their application potential as green solvents grows exponentially. Unique physico-chemical properties, green character, favorable prices, and the possibility to design DESs for specific purposes ensure their position as unique solvents with very promising applications in various fields. The application of DESs in life science assumes that DESs mimic the natural conditions, since their existence in living cells as a third medium in the body, along with water and lipids, is indicated by recent research. Therefore, DESs could be of great importance in biotechnology, biomedicine, and the pharmaceutical industry. Adequate and sufficient solubility of active pharmaceutical ingredients (APIs) is one of the greatest challenges in drug development. Improvement of solubility for different APIs by simple dissolution in deep eutectic solvents or by obtaining therapeutic deep eutectic systems (THEDES) is already shown by some examples. Additionally, DESs could also have a beneficial effect on the stability, permeability, and bioavailability of APIs which makes them very attractive for further research & development. Nevertheless, some issues remain to be investigated. How to speed up and narrow down the search for an ideal solvent? Or, are the hydrophobic DESs, which proved to be a good solution for poorly water and/or lipid-soluble drugs, really a good choice for such application? Last, but not least, for implementation in the real sector, it is necessary to assess the possibility of transferring such an approach on a larger scale as well as the cost-effectiveness of the use of DESs for the improvement of pharmaceutical formulations.

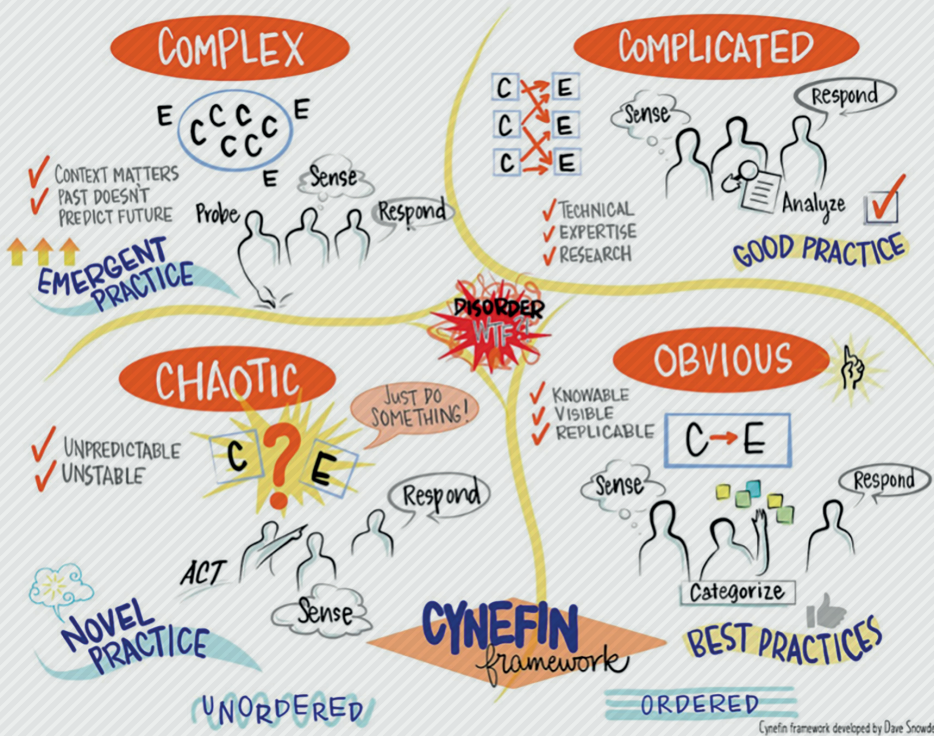


ON LIMINALITY: BETWEEN STABILITY AND FUNCTION

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KEYWORDS:
complexity,
cynefin,
deep eutectic
systems,
liminality,
osmoregulation

Observing liminal states between solid and liquid, within the liminal spaces between macromolecules and the soup of numerous small compounds we call "their microenvironment", can provide us with new ways to probe and sense complex biological systems. We may then respond by acknowledging the interdependences and become increasingly aware of the microenvironment context wherein interactions occur.



Once we sense possible directions and choose a desired one, we can then analyze context-specific interactions and engineer energetically favorable pathways to eventually navigate the liminal phase between the unordered domains of homeostasis and the ordered ones of scalable practice. Image copyright: Dave Snowden's Cynefin framework as drawn by Danae West in Medium

TREATMENT OF THE WASTEWATER FROM AMMONIA AND MICROBIOLOGICAL COMPONENTS BY USING CARBON MATERIALS

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KEYWORDS:
*Wastewater,
Sorbents,
Treatment,
Ammonia,
Microbiology*

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The natural water one is of the main wealth is of such and the important natural resource of Georgia. In general, the surface water quality of Georgia is satisfactory but the main problem is the increased content of nitrogen-forms in the water, including ammonium nitrogen and microbiology indicators. The concentration of ammonia nitrogen and microbiological indicators in most of the rivers exceeds the maximum allowable levels, the cause of which is called municipal, agricultural and industrial wastewater. The aim of our research was to obtain new - alternative sorbents from organic polymer fraction of municipal solid waste, study their sorption potential and determine the possibility of their use for wastewater treatment for removal of harmful substances.

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Within the research received sorbents from plastic waste (polypropylene) by low temperature thermochemical processing (up 400 °C) and investigated sorption potential of the new carbon material (activated/inactivated). The carbon sorbents obtained from plastic waste were tested for the extraction of E.coli, Total Coliforms and Ammonia ions. Studies have shown that obtained carbon materials characterized up to 70% sorption capacity for ammonia ions. The carbon materials showed high antimicrobial activity, moreover as the size of carbon particles decreases down to nanoscale range their antimicrobial activity increases because of their larger surface area per unit volume.

PRODUCTION OF ARABITOL FROM SUGAR BEET PULP

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arborariae,
Spathaspora
passalidarum,
sugar beet pulp,
sustainable
bioprocess
configuration

Sucrose production from sugar beet results in a large amount of sugar beet pulp (SBP) rich in pectin. After the appropriate pretreatment, this by-product of sugar industry is suitable for the production of sugar alcohols. One of the interesting sugar alcohols is arabitol, which is used as a low-calorie sweetener and is therefore applicable in the pharmaceutical and food industries. One of the possibilities of sugar alcohols production is by applying specific non-Saccharomyces yeasts such as yeasts of the genus *Spathaspora*. Liquid phase rich in arabinose can be obtained as a result of a simple one-step pretreatment of the SBP in a high-pressure reactor, using a weak sulphuric acid (0.5% w/w). The weak acid pretreatment of the SBP was tested in temperature range from 160 to 200 °C, at reactor retention times between 1 and 10 minutes. Optimal conditions were achieved at 200 °C/1 minute, resulting with the maximum yield of total carbohydrates in liquid hydrolysate (calculated as a sum of glucose, xylose, and arabinose), containing 80.60 % of arabinose in total carbohydrates. The obtained liquid acidic hydrolysate can be used as a nutrient medium for arabitol production by cultivation of *Spathaspora arborariae* CBS 11463 and *Spathaspora passalidarum* CBS 10155. Specific growth rate of *Sp. arborariae* CBS 11463 was lower than that of *Sp. passalidarum* CBS 10155 in the same media and agitation speed conditions during all cultivations. When grown on the weak acid hydrolysate of SBP, yeast *Sp. passalidarum* CBS 10155 produced 8,4810 g L⁻¹ of arabitol (YP/S = 0.6038 g g⁻¹, Pr = 0.1767 g L⁻¹ h⁻¹) while *Sp. arborariae* CBS 11463 produced 2.1759 g L⁻¹ (YP/S = 0.5570 g g⁻¹, Pr = 0.0453 g L⁻¹ h⁻¹). On the basis of the obtained results, a sustainable bioprocess configuration for arabitol production was established.



POTENTIAL OF WASTE TEA AS EFFECTIVE ALTERNATIVE ADSORBENT

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KEYWORDS:
*activate carbon,
methylene blue,
waste tea*

Efficiency of wasted black tea were investigated as a low-cost adsorbent for removal of methylen blue dye from aqueous solution. Different methods and chemical activators were used to produce activated carbon. Batch experiments were conducted to determine the factors affecting adsorption and kinetics of the process. This study demonstrated the ability of waste tea as an effective, low-cost and sustainable adsorbent for removal organic pollutant.

A COMPARISON OF EFFICIENCY BETWEEN ULTRASOUND AND MICROWAVE-ASSISTED EXTRACTION OF BLACK ELDERBERRY POMACE

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KEYWORDS:
*black elderberry
pomace,
microwave-assisted
extraction,
phenolic
compounds,
ultrasound-assisted
extraction*

One of the main directions in the fight against the accumulation of organic waste is its use in order to obtain high-quality products. The question is how it is possible to use these by-products after industrial production and how to isolate the desired compounds from them. The aim of this work was to isolate phenolic compounds from the black elderberry (*Sambucus nigra* L.) pomace, a by-product left after juice processing. Elderberry pomace has been characterized as an efficient biomaterial with the potential to be converted into various nutrients and bioactive phytochemicals, such as phenolics, anthocyanins, etc., using different valorization approaches. The increasing interest in waste valorization has resulted in increased attention for different extraction techniques and their optimization. In the present study, the comparison of two green-based extraction techniques, ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) was carried out. UAE was performed using an ultrasonic probe on sonication amplitude 100%, using 30% ethanol as solvent, and by varying temperature from 40-80 °C. During the extraction, changes in temperature, power, and energy consumption depending on time were monitored. As in the case of the UAE, the same solvent and extraction temperature range was used for MAE during 10 min. The extraction efficiency was evaluated in terms of extraction yield (EY), and the contents of total phenols (TPC) and flavonoids (TFC). UAE at the highest temperature (80 °C) provided the highest EY (31.10%) and energy consumption, while lower temperature proved to be better for phenolics isolation (TPC=130.43 mg/g DE). TFC decreased with increasing temperature and extraction time. Two-fold lower EY (16.33%) was achieved by using MAE at a temperature of 50 °C, being the optimal for MAE. The maximum TPC (168.54 mg/g DE) and TFC (51.83 mg/g DE) in MAE were attained at a temperature of 60 °C. Both extraction techniques have been shown to be suitable for further processing of elderberry pomace, in fact, for the isolation of phenolic compounds. Further research could determine the influence of extraction parameters on the content of individual phenolic compounds.



CORROSION RESISTANCE OF ALUMINIUM IN SOLUTIONS OF ORGANIC ACIDS WITH THE ADDITION OF CAROB POWDER

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KEYWORDS:
*aluminium,
corrosion, carob,
organic acids*

Although aluminum has good corrosion resistance to a number of aggressive environments and many food products, aluminum can corrode in the food industry, which in this case has an impact on the final food product. To stabilize the metal surface and prevent food contamination with aluminum, the most commonly used method in the food industry is the use of coatings or additives in the food product that can act as corrosion inhibitors. Instead of organic coatings, there is great interest in the use of various plant polysaccharides, i.e. biopolymers such as pectin, carrageenan, etc. as well as their use in the production of corrosion inhibitors. The potential application of plant polysaccharides as corrosion inhibitors is based not only on their chemical structure, but also on the ability to form passivation layers on the metal. In this paper, the influence of often used organic acids in the food industry (3% lactic, acetic and citric acid) on the corrosion of aluminum without and with the addition of different concentrations of carob powder was investigated. Aluminum corrosion rates were determined by gravimetric method at 4°C and 25°C after 30 days of exposure of aluminum samples in the tested solutions, while the surfaces of aluminum samples were recorded with an optical metallographic microscope.



INFLUENCE OF MICROWAVE-ASSISTED EXTRACTION ON POLYPHENOLS RECOVERY FROM MANDARIN PEEL WASTE

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KEYWORDS:
*flavonoids,
mandarin peel waste,
microwave-assisted
extraction,
phenols,
UV/Vis
spectrophotometry*

With increasing of interest in green chemistry, a more attention is being devoted to findings environmentally-friendly extraction method, and one of such is microwave-assisted extraction, which significantly reduced the time of sample treatment with uses of low volume of solvents. In that connection this work presents application of microwave-assisted extraction as an innovative technique for isolation of polyphenols from mandarin peel waste.

Effect of solvents (ethanol and acetone) and its volume fraction (25, 50 and 70 %, v/v), temperature (30, 50 and 80 °C) and time (3, 7, 11 and 22 min) of extraction were evaluated with regard to total phenols and flavonoids content.

The results revealed that all samples contained 52.52 to 110.58 mg/g of phenols and 8.26 to 18.13 mg/g of flavonoids with variations due to different time, temperature and solvent. The recovery of phenols from almost all samples enhanced by using of ethanol and rising the temperature from 30 °C (52.52 - 64.17 mg/g) to 50 °C (57.01 - 76.37) and 80 °C (63.99 - 93.58 mg/g). On the other hand, by using acetone the yield of phenols decreased from 50 °C (74.10 - 99.42 mg/g) to 80 °C (58.07 - 96.28 mg/g). Similar to phenols, the low mass fractions of flavonoids are obtained at temperature of 80 °C, particularly by using 25 % ethanol (6.40 - 9.60 mg/g) and acetone (4.13 - 8.90 mg/g) for 3, 7, 11 and 22 min.

Although the various parameters were involved in microwave-assisted extraction of mandarin peel, it should be emphases that temperature of 50 °C, time of 7 and 11 min, and 50 and 70 % ethanol and acetone presented the optimal, as well as compromise conditions between phenols and flavonoids isolation.

Since the polyphenols were isolated in great amounts with minimal expenditure of time, applying eco-friendly microwave-assisted extraction, this technique could be utilized in further polyphenols production from sustainable, low cost mandarin peel.



EXPLOITATION OF MANDARIN PEEL WASTE AS A SOURCE OF VALUE-ADDED COMPOUNDS

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KEYWORDS:
analytical methods,
extraction,
mandarin peel
waste,
value-added
compou

Re-use of wastes and by-products generated from food processing industry has gained a large interest due to increasing of awareness for waste reduction and environmental protection. Besides these the residues of food industry are also interesting from the economic standpoints of view due to production of natural compounds of added value.

In that connection, the present study was undertaken to exploit the under-utilised mandarin peel for production of value-added compounds. Moisture, ash, fat, cellulose, lignin, sugars, proteins, phenols and flavonoids as main constituents of mandarin peel were estimated using gravimetric and UV/Vis spectrophotometric methods.

The results showed that mandarin peel contains 73 % of water, 1.03 % of ash and 0.63 % of sulphated ash. Regarding other constituent mandarin peel contains high values of lignin (29.13 %) and proteins (10.44 %), and lower values of fat (0.05 %), cellulose (0.11 %) and sugars (1.29 %). Additionally, mandarin peel has a high quantities of total phenols, which are lied in the range of 82.2 to 126.2 mg/g, depending on volume fraction of ethanol (25, 50 and 70 %, v/v) used in extraction under reflux (1,5 h). In comparison with these results and that obtained after extraction of peel with water, the mandarin peel extracted with acetone (25, 50 and 70 %, v/v) contained much higher values of total phenols (112.9 to 209.8 mg/g). Regarding the content of flavonoids seems that ethanol provides its better recovery then acetone and water. The obtained values are 6.14 mg/g, 7.4 to 11.9 mg/g and 10.4 to 29.26 mg/g for water, acetone and ethanol, respectively.

Overall, the obtained findings revealed that mandarin peel as by-products gained after processing and manufacturing of fruit juice could be effectively re-used for production of value-added compounds, providing benefits, not only for producers, but also for consumers, e.g. decreasing the waste disposal costs and take opportunity that some of isolated compounds could be reintroduced into food.



GREEN SYNTHESIS OF CYCLIC CARBONATES FROM CARBON DIOXIDE

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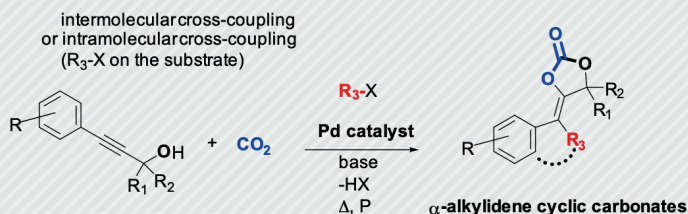
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KEYWORDS:
 carbon dioxide,
 catalysis,
 cyclic carbonates,
 in-silico studies

Although carbon dioxide (CO₂) is a nontoxic and inert gas, it represents an environmental hazard because it is mainly responsible for the greenhouse effect, and furthermore for the global warming. On the other hand, being the final product of every hydrocarbon combustion, CO₂ is an abundant and renewable carbon source. It is considered as an attractive C1 building block in modern organic synthesis for producing highly valued and biologically active chemicals, such as carbonates and carbamates, following the principles of green chemistry. To overcome the thermodynamic and kinetic stability of CO₂, we have envisaged energetically favored Pd-catalyzed inter- or intramolecular C-C cross-coupling reactions on suitable propargylic alcohol substrates. Notably, calculations based on density functional theory (DFT) method predict that these reactions are exergonic owned to product stabilization through the formation of additional C-C bonds. Our combined studies -experimental and computational- enable the rational design of new CO₂ trapping substrates and the preparation of useful fine chemicals such as cyclic carbonates.



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OPTIMIZATION OF XYLOSE PRODUCTION FROM ALMOND TREE PRUNING

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KEYWORDS:
*almond tree
pruning,
biorefinery,
optimization,
xylose*

Almond tree pruning is generated annually by the necessary removal of the old branches of the almond trees. Its current disposal is performed burning it, which contributes to the global warming. The valorization of this lignocellulosic biomass could have important environmental and socioeconomic advantages with the development of a new industry. The production of this product in Spain is more than 0.8 million tons/year. The Almond tree pruning as a lignocellulosic material, is a renewable raw material interesting for production of 2nd generation bioethanol and other added value compounds such as, oligosaccharides, antioxidants, xylitol, etc., under a biorefinery approach.

The aim of this work was to study the optimal conditions to solubilize the xylose of almond tree pruning with the maximum concentration, which could be later fermented to produce ethanol using unconventional microorganisms capable of assimilating pentoses, or xylitol, or other products; leaving a cellulose-rich solid which could be subject to a pretreatment or enzymatic hydrolysis to obtain glucose. Crushed almond tree pruning were treated in a reactor (with liquid to solid ratio 20 %w/v) according to a central composite experimental design, temperature (170–200 °C) and phosphoric acid concentration (0.5–1.5 %w/v) as variables. The analysis of results with Response Surface Methodology indicates that the maximum xylose concentration in liquors is obtained at 185 °C and 1.5% phosphoric acid concentration, with an estimated value higher than 25 g/l.

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OLIVE STONE AS RAW MATERIAL FOR THE PRODUCTION OF LEVULINIC ACID

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KEYWORDS:
*biorefinery,
levulinic acid,
olive stone,
optimization*

Olive stones constitute an important byproduct generated in the olive oil extraction process and in pitted table olive industries. The production of olive stones in Spain is more than 0.5 million tons/year. The main use of this byproduct is as a fuel to produce electricity or heat. As a lignocellulosic material, whose main components are hemicellulose (mainly consisting of xylose), cellulose and lignin, olive stone has been proposed as raw material for the production of 2nd generation bioethanol and other bioproducts (xylitol, furfural, levulinic acid, etc.), particularly under the biorefinery concept. In addition, the low costs of manipulation and transport of this material make it attractive for biorefineries.

The aim of this work was to study the optimal conditions to produce levulinic acid in the liquids; the remaining cellulose-rich solid could be subjected to a pretreatment or enzymatic hydrolysis to obtain glucose. Crushed olive stones were treated in a reactor (10 min, with liquid to solid ratio 40 %w/v) according to a central composite experimental design, temperature (170–210 °C) and sulfuric acid concentration (1–4 %w/v) as variables. The analysis of results with Response Surface Methodology indicates that the maximum levulinic acid concentration in liquors was obtained at 210 °C and 4% sulfuric acid concentration, with an estimated value greater than 25 g/l.

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INFLUENCE OF DIFFERENT COATINGS AND DRYING OF WET MICROBEADS IN VITRO RELEASE OF PHENOLIC COMPOUNDS

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KEYWORDS:
drying,
encapsulation,
geometry
parameters and
texture,
in vitro
release,
phenolic
compounds

The aim of this study was to investigate the influence of different coatings and drying of wet microbeads on their parameters of shape (circularity, roundness, solidity), size (area, perimeter, feret), and texture (hardness), as well as on the in vitro release of phenolic compounds from dried microbeads. First, hydrogels (wet microbeads) were prepared by encapsulating polyphenol-rich extracts from grape pomace Cabernet Sauvignon variety by ionic gelation with different natural coatings. Sodium alginate (SA) and combinations of SA with maltodextrin DE 4-7 (SA+MD1), maltodextrin DE 16.5-19.5 (SA+MD2), gum arabic (SA+GA), gum tragacanth (SA+GT), or chitosan (SA/CHIT) were used. The prepared hydrogels were air dried, vacuum dried, or freeze dried to produce various dried microbeads. Geometry parameters (shape and size) were analysed using image analysis, while texture parameter (hardness) were analysed using a texture analyzer. The in vitro release of phenolic compounds was monitored for 250 minutes. SA/CHIT hydrogels had the highest values for all size parameters tested, while SA hydrogels had the lowest values. Drying causes the expected shrinkage of the hydrogels. It was found that the size of the hydrogels decreased the most during air drying (83.5-88%), except for the beads coated with SA+GT, which shrank the most during vacuum drying (85.5%). The results show that the vacuum-dried SA+GT microbeads had the smallest size but the most spherical shape. Freeze-drying was causing the smallest change in surface area compared to other drying methods (32.1-62.2%). Considering the hardness of hydrogels and dried microbeads, the results show that hydrogels have the lowest hardness (0.311-0.445 N) and air-dried microbeads have the highest hardness (28.6-37.9 N). A difference was also observed between the hardness of the dried microbeads, with the freeze-dried ones having the lowest hardness (0.5-3.4 N). In general, in vitro release showed that most phenolic compounds were released from freeze-dried microbeads, with the exception of SA+MD2 microbeads, where most phenolic compounds were released from vacuum-dried microbeads. The highest concentrations of phenolic compounds were released from SA/CHIT freeze-dried microbeads (43.27 mgTPC/gMB), but it was observed that their concentration started to decrease after 143 min of release. A similar behavior was observed in SA and SA+MD1 freeze-dried microbeads, while in freeze-dried microbeads prepared with SA+GA and SA+GT, a steady increase in concentration was observed during release.

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BIOSORPTIVE REMOVAL OF THE CATIONIC DYE MALACHITE GREEN FROM WATER BY INACTIVE BIOMASS OF *Fomitopsis pinicola*

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KEYWORDS:
biosorption,
Fomitopsis pinicola,
malachite green,
removal of dyes,
wastewater

The aim of this study was to investigate the biosorptive potential of the inactive biomass of the medicinal mushroom *Fomitopsis pinicola* (fruiting body and mycelial biomass) to remove the synthetic dye malachite green (MG) from water. Various factors affecting the biosorption process were investigated in a batch procedure, namely the concentration of biosorbent (0.5 - 10 g/L), contact time (1 - 180 min), initial dye concentration (10 - 100 mg/L), and pH (4 - 10). The percentage of dye removal increased with increasing biosorbent concentration and contact time between biosorbent and MG. A decrease in the percentage of dye removal (from 96 to 58%) and an increase in the amount of dye adsorbed on biomass (from 4.33 to 19.61 mg/g) were observed when the concentration of MG was increased from 10 to 100 mg/L. The removal of MG from synthetic wastewater with the addition of MG was as efficient as the removal from MG model solutions, with the percentage of dye removal ranging from 62.6 to 96.75%. The pH > 7 favoured the efficiency of biosorption. The data from the model MG solution experiments were analysed using adsorption equilibrium models (Freundlich and Langmuir). The results showed that the Freundlich model described the process of MG biosorption on inactive *F. pinicola* biomass slightly better. The results suggest that the extracted inactive biomass of the medicinal mushroom *F. pinicola* has potential for remediation of wastewater contaminated with the synthetic dye malachite green. synthetic dyes.

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USEFUL COMPOUNDS FROM CO₂ BY IN SILICO DIRECTED CATALYTIC SYNTHESIS

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KEYWORDS:
carbon dioxide,
catalysis,
in silico

The fine-tuning of synthetic pathways based on in silico considerations represents the future of organic chemistry. Likewise, the use of CO₂ as a green, cheap and readily available carbon source in synthetic chemistry has been an area of increased interest. In this project, we have modelled the ability of certain unsaturated alcohols and amines to undergo a double cyclisation with CO₂ using transition metal-based catalysts. Several substrates have been selected and synthesized. Their CO₂ absorbing abilities are currently being investigated under a range of conditions and with different transition-metal based catalysts. While cyclic carbonate and carbamate formation using CO₂ is a well-established synthetic procedure, the carboxylation-aromatic substitution double cyclisation is proving more challenging. The scope of this reaction will also be probed. The compounds obtained by this process – bicyclic carbonates and carbamates – may prove to be biologically active compounds and will be tested for potential anti-bacterial activity. Finally, the mechanism of this reaction will be examined both using in silico and experimental methods. This will help to further refine catalysts for such processes.

CIPROFLOXACIN DERIVATIVE – IMPROVEMENT OF ITS SOLUBILITY, PERMEABILITY AND ANTIBACTERIAL ACTIVITY BY DEEP EUTECTIC SOLVENT

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KEYWORDS:
*antimicrobial
activity,
ciprofloxacin
derivative,
DES,
PAMPA,
solubility*

Deep eutectic solvents (DES) have emerged as a promising option in the search for novel solvents that comply with green chemistry guidelines. As they are non-toxic, non-flammable, and non-volatile they represent a good replacement for standardly used organic solvents. They are made of two or more renewable compounds that are found in nature, which when combined in a specific molar ratio and heated become homogenized liquid, stable at room temperature. With more than a million options for their synthesis as well as proven antibacterial activity, in the present work, we have used them as a solvent to improve some of the pharmaceutically relevant characteristics of ciprofloxacin derivate. As it is known antibacterial resistance is a global problem in medicinal treatments and healthcare. It is pivotal to find new alternatives to commonly known antibiotics, in order to overpass this problem. Ciprofloxacin is a clinically used antibiotic, from a group of fluoroquinolones. To bridge the resistance problem N-acylated chiral derivate of ciprofloxacin with CH(CH₃)CH₂CH₂CH₃ group was synthesized (1-Cyclopropyl-6-fluoro-7-[4-(2-methyl-pentanoyl)-piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid). To further improve potential of herein synthesized ciprofloxacin derivate we aimed to design it as a THEDES (Therapeutic Deep Eutectic Solvent), which is a mixture of chosen API and forming compounds commonly used for the synthesis of DES. Solubility of ciprofloxacin derivate in DES was tested in silico using Cosmo Therm program. Options with the lowest $\ln\gamma$ value, which indicates the best solubility, were tested experimentally. We also tested permeability by using in vitro model of passive permeation the Parallel Artificial Membrane Permeability Assay (PAMPA). The antimicrobial potential of this novel ciprofloxacin derivate formulation was assessed by disk diffusion assay on different microbial cultures. According to the obtained results, the chosen ciprofloxacin derivate in a form of THEDES shows many benefits and seemingly overpasses current problems in more than one way.



INDUSTRIAL APPLICATION OF GRAŠEVINA GRAPE POMACE EXTRACTS IN NATURAL DEEP EUTECTIC SOLVENTS

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KEYWORDS:
*biological activity,
gastrointestinal
system,
grape pomace,
natural deep
eutectic solvents,
polyphenols*

Grape pomace, a wine industry by-product, consists of skins, seeds, and stems, and is considered a valuable plant material because grapes are highly rich in polyphenols. Polyphenols are secondary plant metabolites known for their antioxidant, anti-inflammatory, and antimicrobial properties. Their particular biological activity makes them an important part of human nutrition. Using grape pomace as a source of polyphenols gives new value to otherwise undisposed plant waste. Natural deep eutectic solvents (NADES) are becoming solvents of choice for the extraction of polyphenols from various plant materials. NADES are proven to increase the efficiency and sustainability of the extraction process. In addition to having GRAS status, NADES extracts are considered safe for human consumption. The biological activity of polyphenols in food depends greatly on their stability through the digestion process. However, according to the available data, the biological activity and stability of active compounds found in plants are much higher in NADES compared to conventional solvents. Therefore, the objective of this work was to examine the stability of polyphenol in NADES when used as extracts in food or as a dietary supplement. Firstly, COSMOtherm software was used to select and design preferred NADES for polyphenols extraction to obtain ready-to-use extracts from grape pomace. Betaine: sucrose was selected as optimal NADES for polyphenol extraction. Then, the absorption and digestion of polyphenols in NADES were analyzed through in-vitro simulation of the gastrointestinal system. The simulation was based on mimicking physiological conditions of the digestive process in the mouth, stomach, and small intestines. Obtained results showed that NADES could preserve polyphenols' chemical and physical stability and provide better adsorption of polyphenols when orally consumed.

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MICROREACTOR TECHNOLOGY FOR GREEN AND SUSTAINABLE PHOTO- AND ORGANO-CATALYTIC SYNTHESIS

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KEYWORDS:
green synthesis,
microfluidic
chemistry,
organocatalysis,
photoredox
catalysis,
sustainable
synthesis

In recent years, the application of microfluidic devices demonstrated significant promise as a novel method in organic chemistry. One of the research fields in which microfluidics have shown a great potential is visible light photoredox catalysis. The implementation of microreactors offers considerable advantages over the batch reactor as follows: a more predictable reaction scale-up, decreased safety hazards, preserves atom economy, improved reproducibility and yields, and decreased energy consumption. The high surface-area-to-volume ratios provide more efficient irradiation of a reaction mixture, reduction of irradiation times, and hence prevention of undesired side reactions. As a result, enhanced selectivity, product purity, and lower catalyst loading are achieved, which leads to overall more sustainable and greener processes. Even though significant progress has been achieved, greener alternatives to many common industrial processes still remain elusive, especially in the fine chemicals industry. To perform processes greener and cheaper, catalysis is a key tool to reduce energy consumption and develop more atom-economical transformations. To show the potential use of microdevices in organic synthesis, we have applied microfluidic chemistry in mutual photoredox and organocatalytic synthesis of the functionalized Tetrahydroisoquinolines, a biologically active compound with interesting pharmaceutical properties. The results obtained in microreactor devices were compared with those obtained in batch reactions and it was demonstrated that microreactors can achieve superb yields and decreased waste generation. Thus, microflow photochemistry unambiguously has demonstrated its superiority over conventional reactor systems and its potential as green technology in synthesis processes.

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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SEAWEED *Fucus virsoides* AND *Cystoseira barbata* EXTRACTS

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KEYWORDS:
antimicrobial activity,
green synthesis,
seaweed extracts,
silver nanoparticles

The synthesis of silver nanoparticles (AgNPs) is an important area of research in nanotechnology due to their unusual size- and shape-dependent properties, strong antimicrobial activity and their attractive applications in medicine, catalysis, optoelectronics, and biotechnology. Various methods can be used for the synthesis of AgNPs, such as the sol-gel method, chemical vapour deposition, or thermal decomposition, but these methods are often expensive and employ toxic chemicals. To overcome these drawbacks, biosynthetic approaches using water extracts from different organisms, including fungi, plants and algae, have recently emerged as simple and viable substitutes because they are simple, cheap, and environmentally friendly. Marine macroalgae or seaweeds, such as *Fucus virsoides* and *Cystoseira barbata*, are abundant source of secondary metabolites such as polyphenols, polysaccharides, carotenoids, vitamins, minerals, amino acids, and proteins. Their aqueous extract can act as an efficient metal reducing agent as well as a capping agent to provide excellent stability to the formed nanoparticles. UV-visible spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles because the conducting electrons in the outer orbital of AgNPs resonate with specific wavelengths. Reduction of silver nitrate to AgNPs is confirmed with a peak in the range from 335 nm to 560 nm, while the absence of peaks indicates the absence of NP aggregation. In the present study, the water extracts of *F. virsoides* and *C. barbata* were used to prepare AgNPs by reducing Ag⁺ ions from a 1 mM silver nitrate solution to Ag⁰. Nanoparticle formation was confirmed by UV/Vis spectroscopy, with peaks detected at 380 nm for both algae. The antimicrobial activity of the synthesised nanoparticles against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella thyphimurium*, *Enterococcus faecalis*, *Escherichia coli* and *Listeria monocytogenes* was investigated and compared with the antioxidant activity of the seaweed extracts.



THE INFLUENCE OF CANE SUGAR AND ARTIFICIAL SWEETENERS ON WATER KEFIR PRODUCTION

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KEYWORDS:
acane sugar,
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water kefir

Kefir drink represents a product which is obtained by fermentation of sugary medium, at room temperature, by different types of microorganisms contained within water kefir grains. It has been shown that water kefir has positive effects on human health including antihyperglycemic, antimutagenic, and antioxidative. In spite of these facts, the industrial production is still very limited because of varying fermentation conditions, and poorly explored and complex microorganism interaction present in water kefir grains. So far it was proven that water kefir drink can be produced using different initial sugar concentrations together with different types of fruit which are added as an additional carbon and nitrogen source for the working microorganisms. In this study, we tested the possibility of usage of non refined brown sugar from sugar cane as carbon source and its effect on the dynamics of water kefir production. In the first part of investigation the influence of two different initial sucrose concentrations (40 and 60 g L⁻¹) on the composition of water kefir dring was tested, while in the second part the experiments were conducted with the addition of sweeteners (erythrol, xylitol and steviol). In this case, the initial sucrose concentration (cane sugar) was lower (20 g L⁻¹) and the media contined the same concentration of added artificial sweetener. Obtined results show that water kefir production can be obtained with higher intitial sucrose concentrations and that it is possible to use cane sugar with no negative effects. Also, the addition of artificial sweeteners at the beginning of fermentation doesnt have any negative impacts on the fermentation process, respectively.



MIMICKING NATURE: OSMOLYTES-BASED DEEP EUTECTIC SYSTEMS

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KEYWORDS:
*deep eutectic
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osmoDES,
osmolytes*

Looking at the way living creatures cope with changes in the environment, and by understanding the functions provided by organisms or processes in nature, could help us shape and create new products, processes, and systems. We can learn from organisms frequently exposed to harsh environments, such as extremophilic bacteria, marine organisms, sporulating microorganisms and plants, to living systems functioning in complex microenvironments, such as the human kidney. All of these living systems share a similar mechanism for coping with the stresses of a harsh environment that implies the accumulation of solutes commonly called osmolytes - versatile organic compounds that have several biological functions, among which an increase in thermodynamic stability of macromolecules, without compromising their native functional activities, is the most pronounced one. For this reason, osmolytes induced stability of biomacromolecules has attracted considerable attention in various industrial fields. Meanwhile, neoteric systems that effectively mimic the innate environment for various biomolecules, the so-called deep eutectic solvents systems (DESs), has been intensively studied as a nontoxic and highly tunable solvent in food, agrochemicals, cosmetics, and pharmaceuticals production.

Here, we hypothesize that osmolytes, when accumulated inside a cell, act as eutectic systems that help maintain the native conformation and functionality of proteins and other biomolecules under the adverse conditions to which cells are often exposed to. Furthermore, this new point of view provides an excellent opportunity to learn from nature and create new, osmolyte-based solvents and systems (osmoDES) that affectively mimic the macromolecules' natural microenvironment. We believe that osmoDES can be a remarkable new tool to study and understand cell functioning and organization in complex systems, along the proposed "probe-sense-respond" approach towards such systems. Further on, osmoDES may function as an enabling constraint and help sense and analyse these systems in higher granularity. We can then respond by engineering tunable parameters of the microenvironment in order to exploit favourable iterations and evaluate new ones and to optimize the resulting processes ("good practices") for efficiency and sustainability at scale.



GLOBAL SENSITIVITY ANALYSIS OF AGRICULTURAL WASTE COMPOSTING MODEL

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KEYWORDS:
*composting proces,
mathematical model
Fourier Amplitude
Sensitivity Test (FAST)*

The scientific community has paid close attention to composting since it is closely tied to proper waste management and is associated with the generation of usable and stable soil fertilizer. The right design and control of the composting process may result in the desired end compost quality. Numerous process variables and conditions like substrate composition, oxygen concentration, pollutant concentrations, composting duration, temperature, etc. have an effect on the composting process and all of them, as well as their interactions, have to be taken into consideration for process improvement. Therefore, mathematical models of the composting process assist in getting information on how different effects on the compost quality during the process and are a useful tool for forecasting the operation of the composting technique and may be used as a guide in creating and analyzing the circumstances that will result in good compost quality. But there is still a lack of information about the kinetic parameters values that could precisely describe the composting process.

In this work, non-stationary Fourier Amplitude Sensitivity Test (FAST) global sensitivity analysis was used to identify the most important parameters of the model describing the olive waste composting process (Vasiliadou et al., 2015). The model includes 15 balances and 16 kinetics parameters and describes the three-phase composting process. The FAST technique ensures that the entire set of model parameters is sensitive to a large and parallel change. The basic idea behind this approach is to turn a multidimensional space of model parameters into a one-dimensional space with a single parameter. The results of the FAST analysis can be used to identify and optimize critical parameters in a model that describes the composting process.



UPTAKE OF RARE EARTH ELEMENTS BY WILLOW (SALIX SPP.) FROM HYDROPONIC SOLUTION

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KEYWORDS:
deep eutectic systems, mimicking nature, osmoDES, osmolytes

Rare earth elements (REEs) have significant importance in modern technologies such as automobiles, computers, electronic equipment, wind turbines, precision-guided weapons, electric vehicles and audio equipment. Industrial and agricultural activities can release REEs into the surrounding environment which leads to deteriorating the plants and human health. Phytoremediation is a green technology and sustainable solution to remediate metals contaminated water using plants. To date, the phytoremediation potential of willow species for REEs has not been explored. In this study, two different willow cultivars (Klara and Karin) were grown hydroponically in different concentrations of six-REEs for a period of 4-weeks in the greenhouse at the school of forest sciences. The treatments were: T1 (Control: tap water), T2 (La: 1g) and T3 (La: 230 mg + Y 220 mg + Nd 210 mg + Dy 200 mg + Ce 240 mg + Tb 230 mg). REEs effect on the two willows growth (height, biomass, shoot diameter and root length) and their accumulation in tissues were investigated. Results have revealed that under single-La and six-REEs doses, the willow height growth, dry biomass, shoot diameter and root length of Karin remained similar to the control treatment except Klara which showed an increment in all growth parameters. Furthermore, among six-studied REEs, the highest La accumulation (10548 µg) were observed in the Klara roots. REEs translocation to willow above ground tissue was found <1 which indicates their phytostabilisation potential. Results have indicated that willow could be an optimal candidate for remediation of REEs contaminated sites or wastewater.



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING NETTLE (*Urtica dioica* L.), MYRTLE (*Myrtus communis* L.) AND BAY LAUREL (*Laurus nobilis* L.) LEAF EXTRACTS

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KEYWORDS:
antimicrobial activity,
green synthesis,
plant extracts,
silver nanoparticles

In recent years, silver nanoparticles (AgNPs) have gained interest in the research community due to their unique physico-chemical and biological properties which allow for their strong antimicrobial activity against a variety of bacteria, viruses and fungi. Various methods such as sol-gel method, chemical vapour deposition or thermal decomposition can be applied for the synthesis of AgNPs, however they often include the use of toxic chemicals. Therefore, biogenic synthesis using plant extracts has emerged as an environmentally friendly method where different bioactive molecules oxidize the Ag⁺ to Ag⁰, leading to the formation of nanoparticles. Medicinal plants such as nettle (*Urtica dioica* L.), myrtle (*Myrtus communis* L.) and bay laurel (*Laurus nobilis* L.) contain a variety of bioactive molecules such as phenolic compounds, organic acids, tocopherols and terpenoids which give them potential for use in the green synthesis of AgNPs. The synthesized nanoparticles are most often monitored by UV-visible spectroscopy since the conducting electrons in the outer orbital of AgNPs oscillate in resonance with certain wavelengths. The peaks in the area near 335–560 nm generally confirm the reduction of silver nitrate into AgNPs, while the absence of peaks indicates the absence of NP aggregation. In the present research, nettle, myrtle and bay laurel leaf water extracts were used to prepare silver nanoparticles by reducing the Ag⁺ ions from a 1 mM silver nitrate solution to Ag⁰. The formation of nanoparticles was confirmed by UV/Vis spectroscopy where peaks were detected at 320 nm, 400 nm and 480 nm for nettle, myrtle and bay laurel extracts, respectively. Antimicrobial activity of the synthesized nanoparticles against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella thyphimurium*, *Enterococcus faecalis*, *Escherichia coli* and *Listeria monocytogenes* was investigated and compared to the antimicrobial activity of the plant extracts.



Workshop 2

ISSUES OF COPPICE
MANAGEMENT IN
THE AREA OF LIKA

w2

PROJECT PRESENTATION: ISSUES OF COPPICE MANAGEMENT IN FA GOSPIĆ

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KEYWORDS:
*increase of value,
indirect conversion,
initiated activities,
natural
regeneration,
priority areas.*

Coppices on the area of FA Lika are similar to majority of European coppices in respect to drivers forming them. Nevertheless, management of Lika coppices stand out in Croatian forestry by its complexity, which arises from entangled historical, socio-economic and exceptional bio-ecological contex. Due to their area, significance and related management challenges issues of Lika coppices is often regarded as one of the most complex issue of Croatian forestry. Variety of site conditions determined by karst geological formations and specific climate conditions, often with distinct limitations, variety of forest communities, depopulation and the lack of manpower, poverty and underdevelopment of the region together with other related complexities create demanding circumstances for management of Lika coppices. Furthermore, Lika region is expected to be mostly affected by changing climate out of all continental areas of the country. Thus, during the year 2020 joint scientific and expert efforts started between forest practitioners and researchers as a first step to meet these complex, but also newly emerging challenges. Activities are initiated under the project funded by Croatian Forests Ltd. and led by Croatian Forest Research Institute under the title "Issues of coppice management in FA Gospić". Paper describes initiated expert and scientific activities specifically aiming at describing state-of-the-art of Lika coppices, possibilities of their natural regeneration, priorities for coppice conversion and enhancement of coppice managment and coppice condition in general. The main activities include insight into available data on coppices, creation of database, questionnaires on the forest office level, establishment of comparative trial plots, data analysys (Stat. soft., descriptive statistics, repeated measures ANOVA), SWAT analysys, possibilities of the use of satellite images, phytocoenological measurements. To enhance management of Lika coppices is long lasting process, but these activities present first step in that direction with preliminary expertt and scientific guidelines.

COPPICE FORESTS VS. HIGH FORESTS: A POLITICAL, TECHNICAL, AND SOCIAL ISSUE IN ROMANIAN FORESTRY

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KEYWORDS:
*coppice,
forest legislation,
forest policy,
forest ownership,
high forest*

High forest (regenerated generatively; with long rotations, to produce large trees for timber production; producing maximum amount of industrial wood and minimum amount of firewood) and coppice (regenerated vegetatively; with maximum rotation age depending on the potential of stumps to coppice vigorously or abundantly; wood used mostly for firewood) are the two fundamental regeneration methods used worldwide. In Romania, a country of broadleaves and with forests mostly non-state-owned before 1948 (nationalization of all forests up to 1989), coppice forests (including coppice-with-standards) were found at that time on ca. 30 per cent of national forestland, compared to only about 5 per cent nowadays. This tremendous drop is the output of a politically-driven process, including (i) the forbidding of application of coppice-with-standards and (ii) the conversion of the majority of coppice forests towards high forests, based on mandatory provisions of Forest Laws, Technical Norms, Ministerial Orders, etc. In technical terms, the process of conversion was achieved (1) by ageing (natural regeneration by seed), in healthy, vigorous, and productive simple coppice stands, rich in valuable tree species and where the soil conditions were favourable to natural regeneration by seed, and (2) by replacement/restoration (artificial regeneration by planting), in degraded simple coppice stands that had a low proportion of valuable tree species, low productivity, old stumps and low potential of natural regeneration by seed. In many situations, the conversion (by ageing) process failed, as it has begun from too old coppice stands, and the rotation ages have been too long, much longer than the age when forest species start producing seeds abundantly and the application of regeneration cuttings (e.g. shelterwood systems) can start. After 1989 and three waves of forest restitution to the pre-WWII owners, about 34 per cent of national forest land became privately-owned, with over 750,000 small forest owners and a mean size of their holdings of only 1.1 ha. In such fundamentally changed social context, two important questions should be asked in the coppice vs. high forest debate: (1) What about changing the Forest Law and adjoining Technical Norms to give the private forest owners formally the freedom to return from high forest to simple coppice or coppice-with-standards, and (2) If they will accept to re-convert their coppices to high forests, what about providing them financial incentives?

COPPICE MANAGEMENT IN CROATIAN FORESTS LTD.

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KEYWORDS:
*current and future
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of forest types,
new dimension
of coppice
management,
variety of
management
activities*

Croatian Forests Ltd. is a public company in charge of management of forests and forest land in possession of the Republic of Croatia on its entire territory. On relatively small area a high diversity of forest communities, terrain configuration and site conditions can be found, thus resulting with high diversity of ways of forest management. Among variety of challenges are certainly issues of coppice management, which in present bio-ecological and social context assume a completely new dimension. Coppices differ significantly due a number of conditions, which require special attention when primary and secondary forest roads are build, tree marking for thinning activities or other silvicultural and protection activities are done. The paper presents short overview of coppice management currently applied by Croatian Forests Ltd. in publicly owned coppices in Croatia.

PRELIMINARY RESULTS OF QUALITY AND GROWTH OF LIKA COPPICES – PERUŠIĆ CASE STUDY

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statistical analysis,
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trial plots*

Silvicultural activities such as thinning are crucial for development of quality and healthy high forests, but in coppices of Lika region they are neglected due to high cost, lack of manpower and other limitations linked to terrain features (e.g. high slopes, rocky terrain), low number of forest roads, etc. Extent of expert knowledge and available research results on influence of thinning regimes on coppices have been limited so far, but there are initial results pointing into direction of significant benefits of thinnings on composition, quality and growths of Lika coppices. Paper presents results of comparative research on the influence of thinning in three different types of coppices (typical coppice, coppice with standards, coppice in transition phase – beech and sessile oak dominated stands). A set of trial plots was established in previously thinned vs. unthinned coppices (research initiated already in 2002) on three localities (FA Gospić, forest administration Perušić). Activities are initiated under the project funded by Croatian Forests Ltd. and led by Croatian Forest Research Institute under the title "Issues of coppice management in FA Gospić". Trial plots and trees have been permanently marked with unique plot/tree numbers, first measurements have been done (tree species, d1,3, total tree heights, tree/stem origin, number of stems per stump) as well as assessment of tree/stem quality (6 features). A total of 1.404 trees (have been marked and measured, while a total of 5 trial plots (50 x 50 m) established on the area of 1,25 ha. Descriptive statistics and ANOVA have been done with STATISTICA 8.2, StatSoft Inc. 2007. Preliminary statistical analysis reveals clear difference between treatments (thinning, control) and underline beneficial influence of thinnings on tree growth on all trial plots, which supports initial results acquired in this area. In the course of the project repeated measures will be conducted to analyse DBH, tree heights increment per tree and treatment, but further research and monitoring will be needed.

PLANT COMPOSITION DYNAMICS IN DIFFERENT FOREST TYPES OF FA GOSPIĆ AREA

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*Croatian Forest
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Institute (CFRI),
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(CF), managed
seed stands,
phytosociology,
unmanaged
coppice forests*

This research represents part of the basic scientific activities in the Lika area which have been continuously implemented since 2020 as the result of cooperation between Croatian Forest Research Institute (CFRI) and Croatian Forests Ltd. within the project: "Problems of coppice management in the Forest Administration of the Gospić area". Coppice management represents one of the most complex forest management issue of both European and Croatian forestry. This especially refers to a specific and complex historical, social and economic circumstances as well as natural constraints of Lika coppice forests. Considering variability between well-managed seed stands and unmanaged coppice stands, the aim of this study was to compare their floristic composition and habitat characteristics. The study area included 3 pre-selected experimental plots (50 x 50 m) of managed seed stands in subcompartment 21a and 3 plots of unmanaged coppice stands in subcompartment 55, both located in the „Mrsinj” management unit (Korenica forest office). In order to investigate current habitat characteristics phytosociological approach is applied. The survey was conducted on an area of 625 m². The results indicate great variability of both plant cover and their abundance which provides great insight about floristic diversity of different forest forms as well as contribution of improving their management and vitality in the future.

SPATIAL DATA ANALYSIS FOR TYPOLOGICAL CHARACTERIZATION OF THE BEECH COPPICE FORESTS IN GOPSIĆ FOREST ADMINISTRATION

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*Beech coppice,
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Forest
administration
Gospić,
typological
properties*

In the area of Forest administration (UŠP) Gospić, during the project so far, the preparation of spatial analyses using the Geographic Information System (GIS) and the collected vector and raster layers has begun. The initiated analyses are aimed at gathering knowledge about the distinctive ecosystem properties of the beech coppice, and their differences in relation to other beech silvicultural types in the area. In addition to examination of the quantitative and qualitative characteristics, the possibility of determining their productivity on the basis of stochastic relationships was also examined, on the basis of which, certain recommendations can be made for their improved management. The GIS analysis included three main steps: (1) Collection of available spatial subsets, (2) transformation of layers and their aggregation within the spatial database, and (3) Determining the interrelationships using basic statistical methods. The first step in the analysis was the collection and consolidation of the forest inventory data i.e. management units, departments and sections with the primary goal of isolating beech coppice stands. As the basic basis for this purpose, the available spatial data of the forest distribution, provided by the company Hrvatske šume was used, which were taken over for this purpose from the investor. Vector data with the spatial distribution of structural and other characteristics of stands, up to the forest compartement level, formed the basis for all further steps in the analysis.

From raster subsets we used products from Copernicus Land Service, namely:

- Corine Land Cover (2018) to analyze the general condition of forest and other cover
- Dominant Leaf Type (2012 and 2018) - for precise determination of the distribution of conifers and deciduous trees
- Tree Cover Density (2012 and 2018) to determine the density of forest stands, respectively change in the period under review.
- Water and wetness (surface cover moisture, 2018)
- Product of Net Primary Production (NPP) of MODIS satellite (MOD17A3, annual data 2010 - 2020), taken from the NASA-USGS portal.

In addition to the above, spatial subsets with a 30-year average for the period 1960-1990 of temperatures and precipitation (State Hydrometeorological Service) and digital terrain model in a resolution of 25m (State Geodetic Administration) have also been collected.

Session E:

ECOSYSTEM MANAGEMENT AND MODELLING



A DECISION SUPPORT SYSTEM (DSS) TO ASSESS THE PERFORMANCE OF FOREST-WOOD SUPPLY CHAIN ACCORDING TO THE PRINCIPLES OF CIRCULAR BIOECONOMY

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spatial analysis,
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The circular bioeconomy is a key concept in solving many societal challenges such as managing natural resources sustainably, reducing dependence on fossil-fuel, mitigating climate change, creating green jobs, and improving the competitiveness of eco-friendly companies. The forest-based sector plays a key role in ensuring a sustainable and balanced environmental, economic, and social development using bio-based resources in accordance with the Sustainable Development Goals (SDGs) and the European Union (EU) Bioeconomy Strategy. The contribution of forest-based sector to achieve the objectives of the SDGs is due to the versatility of wood to produce multiple products, from high value raw materials to bioenergy production. The main aim of the present study is to develop a Decision Support System (DSS) capable to assess the performance of forest-wood supply chain according to the principles of circular bioeconomy. To achieve the aforementioned aim, the study was structured in five phases: (1) literature review on circular bioeconomy related to forest-based sector; (2) review-based identification of a set of indicators suitable to assess the forest-based sector; (3) weighing of the circular bioeconomy indicators considering experts' opinions; (4) development of a DSS using the indicators previously identified and weighted; (5) implementation and testing of the DSS in a pilot area in Italy (Unione di Comuni Valdarno e Valdisieve, Tuscany region). At the end of the first two phases, a set of 14 indicators was developed considering the three pillars of sustainability (environmental, economic, and social) and the 4R (Reduce, Reuse, Recycle, Recover) of circular economy. In the third phase, the indicators were assessed and weighed by 30 experts of the forest-based sector. Experts indicated as the most important indicators the ratio between annual value and annual mean volume harvested (Reduce), the time of use of products (Reuse), the ratio between the potential economic value of the wood assortment and the real value earned (Recycle); and the percentage of wood waste for bioenergy production (Recover). In the last two phases, a DSS – freely available in add-on repository of Quantum GIS and GRASS GIS software – was developed and tested in the Italian case study. The open-access structure and the modular composition of the DSS facilitate its applicability and replicability in other contexts as-well-as the accounting of forest chains' impacts from financial and socio-environmental viewpoint.



SOIL ORGANIC CARBON MODELLING IN CROATIA: NEEDS AND CHALLENGES

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national inventory
report*

Soil organic carbon (SOC) is the largest terrestrial C pool and at the same time very susceptible to environmental changes. There are evidences that warmer climate can stimulate SOC losses and possibly cause a positive feedback loop between the carbon cycle and climate warming. Therefore, SOC is a mandatory pool in national inventory reports on greenhouse gas (GHG) emissions and removals.

In order to provide an estimate of C emissions or removals from soil pool a continuous soil measurements, performed according to methodology prescribed by International Panel on Climate Change (IPCC), are needed. Measuring of SOC changes is challenging due to high spatial variability of soil C and slow process of soil C accumulation or loss. Unless there is a high number of sample plots within the soil inventory, estimates of SOC changes can be highly uncertain, even more if cumulative changes of C stocks with time are not large enough. Alternative method is SOC modelling which is a cost-effective method and is already in use for national GHG inventory reporting in some countries.

In this research we tested a suitability of process-based model Biome-BGCMuSo for modelling of soil carbon. We simulated soil organic carbon stocks down to 30 cm (SOC30) for four different land use categories (Deciduous/Coniferous Forest, Grassland and Annual Cropland) distributed in three biogeographical regions (Alpine, Continental and Mediterranean) and compared them with results of a national soil survey. In total a 573 plot level simulations were performed and we evaluated model performance at three levels (land use, land use x biogeographical region and plot). Only at the level of land use the model showed good performance and reproduced the overall country mean of SOC30 with no overall bias. Nevertheless, disaggregation of the results to the level of land use x biogeographical region and to a plot level showed worsening of the agreement between the modelled results and field measurements. Further model calibration, improvement and testing, as well as repeated soil survey are needed in order to assess the changes in SOC30 and to evaluate the potential of the Biome-BGCMuSo model for use in GHG reporting.



CAUSAL MODELLING OF THE NORTHERN ADRIATIC SEA ECOSYSTEM

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KEYWORDS:
*Adria, causality,
kernel,
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structural*

This work is focused on the discovery of structural causal relations from the database of longterm ecological research (LTER) in the northern Adriatic sea. The data cover the period of 50 years from 1965-to 2015 from 25 research parent sites and is available from the official site and data registry for the International LTER network, DEIMS-SDR repository (Dynamic Ecological Information Management). The database is composed of observations on abiotic parameters and phytoplankton and zooplankton abundances, collected during 299 cruises in different sampling stations in the broader region of the northern Adriatic sea. It is a big-data base that contains time series of 108685 records of physical and chemical parameters and 21 of abiotic, phyto, and zooplankton abundances data. Applied is artificial intelligence (AI) methodology for discovering causal relationships between the abiotic parameters and the biotic measurements. The model variables are mapped by Gaussian kernel to high dimensional artificial feature space. Conditional correlations are evaluated by Hilbert-Schmidt matrices accounting for nonlinear functional dependencies. The obtained significant correlations are integrated as a structural causal model (CMS) and depicted as a directional acyclic graph (DAG). Evaluation of binary causality coefficients between abiotic and biotic features are deconfounded of covariate variables by determination of the adjustment sets. Applied are Bayes neural networks (BNN) for the estimation of nonlinear functional dependencies by marginal distributions as partial dependency plots. For causal analysis applied is software support by Microsoft available from <https://microsoft.github.io/dowh>. stations, in the System – Site and Dataset Registry.

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URBAN GREEN INFRASTRUCTURE: PAST, PRESENT, FUTURE

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urban planning*

Urban green infrastructure is term mainly used in recent decade due to Green Infrastructure Strategy realised in 2013th by European commission. Research of evolution of historical towns give us many insights in Urban green infrastructure development because town planning from the past had shown that systematics approach in development of urban open spaces has been conducted many centuries ago. Present situation indicates many formal recommendations of its implementation emphasised with Green Plan from 2020th year. This research shows what kind of changes can be expected in urban planning in future and in which direction our towns are going to be developed. Sustainability, as a key concept of the future urban spaces, needs to envision many changes in urban planning and in policies connected to it. This changes in traditional, less flexible, spatial and urban planning processes needs to accommodate changes in a way of thinking, conceptualising while publicly participating in development of plans. In these transformational processes concerns in lack of environmental empathy of society in hole (including different stakeholders – planners, local authorities, citizens etc.) is going to be crucial in Croatia. There are highly visible needs for education, transparency and accessibility of different stakeholders. In these processes knowledge based planning, mainly in bioecological and urban morphology premises is going to be crucial. Experiences in landscape studies of different towns are giving us insights of diverse problematic agenda which needs to be addressed in this transformation.

MICROCLIMATE OF URBAN FOREST ECOSYSTEMS OF THE CITY OF ZAGREB

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ecosystems*

Urban forest ecosystems have their own specific climate or microclimate in relation to other parts of cities. The aim of the research was to determine and analyse the microclimate of urban forest ecosystems in the city of Zagreb. Microclimatic conditions were measured at five different locations in the urban forests of the city of Zagreb. The locations where the measurements were performed were the park of the Faculty of Forestry, University of Zagreb, Forest Park Maksimir, meadow in Forest Park Maksimir, second Maksimir Lake, and avenue of wild chestnuts (*Aesculus hippocastanum* L.) in Maksimirska Street. At 7 h, 14 h and 21 h for ten consecutive days in July, air temperature (° C), relative air humidity (%), wind speed (m / s), soil temperature (° C) and soil volumetric water content were measured. The comfort index is calculated based on the values of air temperature, relative air humidity and wind speed. During the ten days of July, the mean values of air temperature (24.0 ° C), soil temperature (24.9 ° C) and wind speed (1.0 m/s) were the highest in the tree line in Maksimirska Street. The mean value of relative air humidity was highest in the Forest Park Maksimir, and the soil volumetric water content along the lake in the Park Forest Maksimir. In contrast, mean air and soil temperature values were lowest in the Forest Park Maksimir. The mean values of relative air humidity were the lowest in the tree line in Maksimirska Street, and the soil volumetric water content in the Forest Park Maksimir. According to the comfort index for the human body, the park, forest park and lake were "comfortable", while the meadows and tree-lined avenues in Maksimirska Street were "fresh" for the human body. Wind speeds were higher in more open locations such as park, meadow and tree-lined avenue in Maksimirska Street, while they were smaller in locations with a complete set of canopies, such as in the Forest Park Maksimir and by the lake. The significance of these measurements is in determining the contribution of forest vegetation to the modification of the microclimate of urban ecosystems, which contributes to a more pleasant stay and recreation of residents in these locations.



ASSESSING HOTSPOTS OF CULTURAL ECOSYSTEM SERVICES AND DISSERVICES IN THE CITY OF ZAGREB

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spatial analysis,
urban green
spaces*

PPGIS (Public Participation GIS) is a tool and methodological approach for collecting spatial data primarily from non-experts about different aspects concerning places of interest. It is proven that this approach is highly useful when dealing with intangible and subjective aspects of places such as cultural ecosystem services. In the city of Zagreb, the PPGIS questionnaire was employed as a data collection method to assess and quantify the spatial distribution of perception toward cultural ecosystem services and disservices provided by urban green spaces. Precise spatial data in a point shape was collected and employed for spatial analyses. Knowing the locations and types of urban green spaces that are perceived as providers of specific service or disservice can enhance our understanding of those places and their general importance for the beneficiaries. Spatial analysis results with, among others, cartographic representation of ecosystem services' manifestation in an urban landscape, that is, hotspot maps. Within this work, hotspot maps are produced for 19 selected attributes of cultural ecosystem services (15) and disservices (4) throughout the city of Zagreb. Hotspot maps are further enhanced by calculating spatial autocorrelation between collected spatial points for each perception attribute separately. Results emerged with different maps of point spatial distribution, hence indicating diverse usage patterns and perceptions people in Zagreb hold toward urban green spaces. While some attributes such as Cultural Identity are spatially clustered revealing locations in Zagreb perceived to be more important for providing particular cultural ecosystem service or disservice, others such as Running resulted in a more dispersed spatial pattern. Results of calculating spatial autocorrelation between points further enhanced interpretation and provided numerical values that can be used to describe produced hotspot maps. Maps are powerful and effective tools when communicating spatial information to stakeholders and policymakers in being easy to comprehend. Perception-based assessment and quantification of the spatial distribution of cultural ecosystem services and disservices in Zagreb provided useful and understandable information. Hotspots represent locations that are highly recognised, however the resulting coldspots should be acknowledged too. PPGIS questionnaire employed in the city of Zagreb proved to be an effective tool for gathering meaningful spatial data which, properly analysed, can be used as a valuable citizen's input for enhancing planning and management practices regarding urban green spaces in the city of Zagreb.



LAND USE CHANGE IMPACTS ON THE KATO NEVROKOPI TORRENT IN GREECE

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solutions,
UAV,
water management*

Managing water resources is crucial for the welfare of all Mediterranean countries. This will require the sustainable management of not just the larger water bodies e.g., rivers and lakes but also smaller water bodies like torrents. Torrents are the most frequently abundant running water body in the Mediterranean region. If managed properly they can provide many ecosystem services such as water supply or electricity. In contrast their improper management can lead them to cause flash flood events that can have catastrophic impact. Greece has experienced such flash flood events in the last years. These flash floods are the result of the impending climate change impacts (higher intensity rainfall events) but also other anthropogenic changes. One of these, are the major land-use changes that happen in a watershed. Alteration in land-uses at the watershed scale alter the hydrologic regime of a torrent and thus can lead to greater peak discharges. This study focused on Kato Nevrokopi torrent that crosses the Drama regional unit in Northern Greece. The watershed has forested areas but also several agricultural areas. In addition, it runs through the city of Kato Nevrokopi that has almost flooded in the last years. This led to the study of the hydrologic characteristics of the watershed. Specifically, land use changes of CORINE 2018 in relation to CORINE 2012 were analyzed through the Geographic Information System (GIS). Orthophoto maps of the area were utilized to digitize changes due to the continued urbanization in the riparian area of the main channel within the settlement of Kato Nevrokopi. Free satellite imagery was also used to capture flood-prone and sensitive areas developing vegetation and water indices. In addition, unmanned aerial vehicles (UAV) were used to capture the current state of the Kato Nevrokopi torrent in combination with terrestrial topographic measurements. The purpose of the measurements is to understand the hydrologic regime of the torrent and suggest nature-based solutions to allow to implement a sustainable water management plan.



FOREST GOVERNANCE PERSPECTIVES OF WOOD BIOMASS FOR ENERGY IN THE FEDERATION OF BOSNIA AND HERZEGOVINA

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the Federation
of Bosnia and
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The term “forest governance” refers to a specific approach to forest management, with changed roles of all actors and institutions, while respecting the basic principles of this concept such as participation, cooperation and transparency in decision-making. The aim of this paper is to identify and analyse existing practices in the forestry sector of the Federation of Bosnia and Herzegovina, and to propose recommendations for improving the mix of forest policy instruments aimed at intensifying the use of wood biomass for energy. The methodological approach included a review and analysis of the current political, strategic and legislative framework, financial mechanisms, institutional solutions and other relevant aspects related to the mobilization of wood biomass for energy in the Federation of Bosnia and Herzegovina. Through direct contact with representatives of relevant institutions (face-to-face interviews) the effects of existing forestry policy instruments were determined and the possibility of applying good international practices for wood biomass mobilization was identified. The results show the current state and perspective of mobilization of wood biomass for energy, according to the following mechanisms of “forest governance” concept: (i) financial incentives, (ii) regulatory framework and application of legal provisions, (iii) forestry planning and (iv) access to information and coordination. Changes in the socio-political and economic environment require institutions in the forestry sector to adapt and simultaneously implement activities aimed at meeting the requirements for renewable energy sources and climate change mitigation. The results of this work can be useful to policy makers and other actors and institutions in the forestry sector of the Federation of Bosnia and Herzegovina, interested in the mobilisation of wood biomass as a renewable energy source and multifunctional use of forest resources.



INTEGRAL APPROACH TO PRIVATE FORESTRY AND GAME MANAGEMENT- INVESTMENT ANALYSIS FROM CROATIAN DINARIDES

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forest management
planning,
game management,
private forests*

Investing capital in renewable resources like forests represents spatial and temporal management which is significantly limited by the natural potential of a certain habitat (e.g. volume increment, the quantity of food for wildlife, etc.). In other words, the rate of return which is expected by the investor is quite fixed and the only tool by which the investor can increase it is to add additional business activities and/or extent present added-value-chain. In the Republic of Croatia only forests which can be bought by the individual or institutional investors, and in which it is possible to establish commercial hunting ground, are the private forests. Based on these insights we have investigated the characteristics of capital investing in a large-scale private forest, in which besides extensive forest management, the game management takes place. Among 1,104 hunting grounds the one with the greatest share of forest cover (94%) and the share of private ownership (92%) is taken to be the focus of this case-study (name of the hunting ground: VIII/120 Permani (10,017 ha)). The theoretical approach is used in which the investor buys all private forests (predominantly consisting of common beech), conducts forest management activities and makes a profit by selling timber. Furthermore, the hunting segment consists of game management for red deer, roe deer, wild boar and brown bear. Both forestry and game management businesses include all relevant costs which are essential for sustainable development and in the end outcoming with the incomes from selling timber and commercializing the hunting right. In the time period of the next 30 years the internal rate of return, net present value and discounted payback period are calculated in order to quantify the investing potential.



ESTABLISHMENT OF MICROPROPAGATION PROTOCOL FOR THE NARROW-LEAVED ASH IN CROATIA - PRELIMINARY REPORT

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gene bank,
tissue culture

Narrow-leaved ash (*Fraxinus angustifolia* Vahl.) is one of the most important tree species in the lowland floodplain forests of the Republic of Croatia. In recent years, increased dieback of narrow-leaved ash has been noticed in Europe, but also in the entire territory of the Republic of Croatia. Tree dieback in forest stands of all ages in some localities creates major environmental and economic problems and classifies this species as one of the most endangered at the moment. Climate change and groundwater and flood disturbance have led to physiological weakening of narrow-leaved ash. After the appearance of the pathogenic fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya in interaction with other pathogens, rapid dieback of this tree species has occurred. The aim of this research was to develop protocol for micropropagation of narrow-leaved ash and conservation of potentially resistant individuals *in vitro*. This would enable fast and successful propagation of the most resistant and high-quality narrow-leaved ash trees, which would contribute to the conservation of this species due to climate change and attacks of pathogens and pests. Some potentially resistant trees have been found in forest stands and they served as a source of plant material for micropropagation. Several protocols have been tested and three of them have shown promising results. The resistance of cloned seedlings will be tested in the continuation of the research. The developed technology may be used for mass propagation of common ash superior trees.



VARIABILITY OF HEIGHT GROWTH AND SURVIVAL OF EUROPEAN BEECH (*Fagus sylvatica* L.) IN PROVENANCE TRIAL „VRBOVSKO“ – FIRST RESULTS

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KEYWORDS:
adaptive genetic variability,
genetic field trial,
quantitative phenotypic traits

European beech is the most widespread forest species in Croatia and has an important role in economic and environmental terms. Knowledge of the genetic structure for such a significant forest tree species is the fundamental prerequisite for its future preservation. Specifically, the knowledge of the genetic structure allows more advanced use of forest reproductive material in order to increase the adaptability of beech populations to stressful environmental conditions. Seed material was collected during autumn of 2016 from four seed stands in Croatia (Bjelovar, Karlovac, Ogulin and Požega). After collection, seed was transfer to Croatian Forest Resaearch Institute, where the seedlings were grown and later distributed for the purpose of trial establishment. With 2-year-old seedlings and collected natural beech saplings from Vrbovsko, in 2020 a genetic field trial was established in the area of the Delnice Forest administration. It was planted over a total area of 0.18 ha in 20 blocks (17 blocks of 72 m² and 3 blocks of 30 m²), with five families (the family consists of progeny of one mother tree) per population and two plants per family in larger blocks and one in smaller blocks. In total, 925 saplings were planted with 2.0 x 1.0 spacing. Around trial, a buffer strip of one rows was planted to avoid edge effects. All plants within the trial, as well as plants of the buffer strip were protected immediately after planting with tree shelters (so-called Tuley tubes). In addition, an archive of 250 plants was planted in order to evaluate the early development of beech seedlings with and without tree shelters. The height growth was measured and the survival and height increment were determined through three consecutive years (2020–2022). The mean survival of measuring plants within trial was extremely good (98.4% in 2022). The total mean height of all plants in 2020 was 35.8 cm, in 2021 60.0 cm and in 2022 87.4 cm.

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INVASIVE CAPACITY OF PRUNUS SEROTINA AND POTENTIAL OCCURRENCE OF RHAGOLETIS CINGULATA - A CASE STUDY IN CROATIA

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KEYWORDS:
*Jastrebarsko
forest office,
regeneration area,
Tephi-Trap,
UAV*

Previous findings of Black cherry (*Prunus serotina* Ehrh.) species affected during its growing season (2018-2021) in Pedunculate oak (*Quercus robur* L.) forest regeneration area of Jastrebarsko forest management unit have shown a considerable spread and alteration in current vegetation. The continuation of research is focused on monitoring its spreading area as well as its impact on the current habitat characteristics. In addition, this research includes defining potential occurrence of Cherry fruit fly (*Rhagoletis cingulata*) species, naturally associated with the *P. serotina*'s presence. With the aim of sampling both infected and uninfected parts of the monitored area, our survey consists of 20 polygon network plots equally distributed into polygon of the regeneration area (15 plots), as well as the surrounding forest area (5 plots). In order to investigate current habitat characteristics, phytosociological approach is applied. The area is surveyed by the use of the unmanned aerial vehicles (UAV) DJI Mavic 2 Pro and DJI Matrice 600, both equipped with multispectral cameras and DJI Ground Station Pro. Monitoring of *Rh. cingulata* includes 20 yellow Chromotrap and 8 Tephri-Traps located on the edges of the regeneration area. The results confirm invasive capacity of *P. serotina* indicating a concerning potential of its subsequent expansion in the near future. In this regard, *P. serotina*'s progression monitoring is highly recommended as one of the control measures of its development.



ACCURACY ASSESSMENT OF HAND-HELD PERSONAL LASER SCANNING FOR INDIVIDUAL TREE ATTRIBUTES ESTIMATION IN OLD EVEN-AGED PEDUNCULATE OAK FOREST

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KEYWORDS:
*diameter at breast
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forest inventory,
mobile laser
scanning,
tree height*

The emergence and availability of hand-held personal laser scanning (PLSHH) systems in recent years resulted in an initial research on the possibility of its application in forest inventory, primarily for the estimation of the main tree attributes (e.g. stem position, diameter at breast height, tree height, etc.). Up to now, a limited number of studies have been conducted; therefore, further research is needed to test the possibilities for eventual inclusion of PLSHH into forestry practice. The aim of this study is to assess the accuracy of PLSHH for individual tree attributes (diameter at breast height, tree height, tree volume) estimation in old even-aged pedunculate oak forest. For this purpose, PLSHH data were compared with three different datasets, i.e. with data collected by statical terrestrial laser scanning (TLS), conventional (traditional) field measurements (FMC) and by detailed field measurements (FMD). The latest (FMD) served as ground truth-data. FMD included measurements of tree circumference at 1.30 m above ground using a measuring tape and calculation of diameter with assumption that tree's stem is circular, as well as measurements of length of felling trees using range finder and stumps' height to obtain reference tree height. The following results are presented by mean absolute error. For diameter at breast height, PLSHH produced estimates of just slightly lower accuracy (2.2 cm) than TLS (1.6 cm) and FMC (1.2 cm). PLS and TLS estimated tree height with almost identical accuracy (0.4 m) which was considerably higher than by FMC (1.4 m) conducted using Vertex instrument. The obtained results confirmed great potential of PLSHH for operational use in forest inventory. Further research should be focused on forest of different ages and forest types, as well as on testing various scanning schemes and algorithms for PLSHH data processing.



REVOLUTIONARY NEW FOREST MEASURING TECHNOLOGY MOBILEFORESTER

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KEYWORDS:
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MobileForester,
photo optical
measurement*

Precise and fast digital data is a basement of modern forestry management. These are the information on the basis of which forest holdings can carry out an efficient operation activity. The data collection is a time and cost consuming task, which emphasizes the importance to develop effective technologies and devices. In the last decades, different methods have been developed for measuring trees, forest and wood and wood stack parameters. Taking into consideration the amount and market value of wood, the accuracy of applied measurement is of high importance, as the price is calculated according to the measured volume. On the other hand the effectivity of measurement forced the user to find the best and most effective technology for perform the task. The third important aspect is the prompt digital data on the site which can be connected to a central data base server in the appropriate form. MobileForester is a newly developed high tech solution for integrating almost all measuring task in one digital tool. Photo optical technologies highly developed and used in the practice by the intelligent algorithm combined with the available technical level. The digital relascope provides the results data promptly in the site, the tree diemater can be measured even 50 meter far without moving to the tree, the solid content of the stacked wood can be determined only be photo technology. The accuracy of the new souldtions can be much lower than that of the former maunal measureing technologies e.g. the stack solid volume can be measured less than 3% accuracy, the diamer can be determinde less the 1 cm error. The measued data are available in digital form and can be integrated into the company data management systems.



ESTIMATING FOREST STAND STRUCTURE BASED ON RE-MEASUREMENT ON CONCENTRIC CIRCULAR SAMPLE PLOTS

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KEYWORDS:
Forest inventory, field plots, repeated measurement, sampling, stand structure

Field plots serve as a basic data source on forest stand structure – number of trees (N), basal area (G) and tree volume (V). Concentric circular sample plots for measuring trees of different size are designed to balance work efficiency and precision of results. However, these type of plots present difficulties when used as permanent plots for repeated measurements, because of ingrowth trees and change of plot size with tree size. The aim of this research was to evaluate the outcomes of stand structure (N, G, and V) from re-measurement on concentric circular plots. Tree data from 29 field plots with 20 m radius measured in selection fir-beech stand in Gorski kotar, Croatia, were used as reference values. Concentric plots (7, 13 and 20 m radii with dbh thresholds 10, 30 and 50 cm) were generated at measurement time 1 (T1). Modelling increment and ingrowth resulted in stand structure at time 2 (T2) for both plot types. Differences in N, G, V between plot types were examined on stand, plot and tree level and tested on stand level using paired t-test with 0,05 significance level.

The concentric plots slightly underestimated fixed plot results on stand level at T1: N by -38,0 stems/ha (7,7%), G by -0,9 m²/ha (-2,5%) and V by -7,4 m³/ha (1,6%), and resulted with higher variability, especially for number of stems. The differences at plot level ranged from 18,8 to 320,8 stems/ha (N), 0,5 to 9,9 m²/ha (G), and 0,0 to 131,5 m³/ha (V).

At T2 concentric plots statistically significantly overestimated N by 88,0 stems/ha (15,6%, p=0,01) on stand level, and gave very close estimates of G (+0,6 m²/ha) and V (+1,2 m³/ha), with higher variability than fixed plots. Individual differences at plot level ranged from 1,1 to 377,4 stems/ha (N), 0,0 to 14,6 m²/ha (G), and 0,5 to 194,4 m³/ha (V).

The re-measurement on concentric plots showed reliable estimates of G and V, likely resulting from two opposite effects that cancel each other at stand level. The inclusion of new trees that reach dbh thresholds 30 and 50 cm in second measurement increases, while larger plot area for trees that shift to higher dbh class decreases the results. Number of stems is apparently more influenced by ingrowth than reduction of tree factor and its estimate is not found reliable. These effects should be taken into account when using this plot type and investigated in different stand conditions.



FLOODPLAIN FORESTS MAPPING USING EARTH OBSERVATIONS AND ARTIFICIAL INTELLIGENCE

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Sentinel

Earth Observation data in combination with Machine learning (ML) algorithms present a very powerful tool for accurate mapping and updating the state of the vegetation cover. The Sentinel 1 and 2 space missions, as part of the EU Copernicus Earth observation program, enable the monitoring of the vegetation cover in almost real-time, in high spatial resolution (10 m). On the other hand, advanced ML algorithms are very data-intensive approaches; for the construction, validation, and testing of the ML model, a large number of points or samples (in the range of 1000-10000) is required. The problem with mapping vegetation at the scale of the forest communities or phytocenoses is often the lack of quality "Ground truth" data for training ML algorithms. Existing phytocenological maps are often outdated (at the level of several decades) and often do not accurately reflect the current situation. Also, the boundaries between different types are most often determined by field assessments and therefore quite unreliable. In addition, the existing methods of field determination of floral composition require significant professional expertise and are thus very expensive and time-consuming. The new project "Earth Observations And Artificial Intelligence For the NATURA2000 Floodplain Forests Mapping" approved for funding by the European Space Agency (ESA) aims to bridge the gap between "Ground truth" requirements for ML applications. The main objective of the project is to tailor and prototype suitable Artificial Intelligence (AI) pipeline for the rapid high-resolution mapping of the Natura2000 floodplain forest habitats based on the Copernicus EO information i.e. to demonstrate the capabilities of EO-based AI approaches as a powerful alternative to traditional and widely used field survey methods. A particular emphasis of this concept is related to the rapid generation of the ground truth information from variable external data sources thus reducing the required time-consuming field survey to a minimum (only necessary field locations for the final testing of the algorithm). In this way, the idea is to establish the concept of fast and reliable habitat mapping, using a combination of EO and expert domain knowledge, which could be applicable for the circular periodic mapping and monitoring of similar forest habitats in the entire territory of the Republic of Croatia.



CORRELATION OF SPATIAL DENSITY AND TROPHY VALUE OF MOUFLON (*Ovis aries musimon* Pall) IN THE PERIOD 1980 - 2020 IN THE COASTAL PART OF NORTH VELEBIT

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KEYWORDS:

*hunting**management,**mouflon,**North Velebit,**population,**trophy development*

The mouflon was inhabited on the southern part of Northern Velebit in the 1980s. Good habitat conditions, lack of predator pressure, relatively small interspecies competition and economic measures have led to fast population progress in the first years after settlement. This research analyzed and compared population and trophy development of mouflon within three hunting grounds, in the research period 1980 – 2020, in order to determine the connection between spatial density and trophy values. Altogether 630 graded trophies have been collected and processed for this research. Population dynamics has been analyzed based on information from the Central hunting records and hunting chronicles. Mouflon management in this area during 38 years of research shows significant population progress in numbers, however throughout the same research period average trophy values show a negative trend. Individually, the biggest cull of trophy heads has been recorded in 2005, when 85 trophy mouflon's have been shot. The average cull age of analyzed trophies for the research period is $4,43 \pm 1,51$ years, which is relatively small considering that the regression analysis shows culmination only after eight years of age. Rated trophies range from 86.00 - 225.85 CiC points with a mean of 137.19 ± 37.46 . In the mid-1980s, the average trophy value of shot mouflons was 200 CiC points (only capital trophy were shot), while for the last research year this average was about 180 CiC points. From the measured elements, the most pronounced is the decrease in average lengths (1985 = 84.2 cm; 2018 = 72.75 cm). Population dynamics do not follow the trend of trophy values. The population was in strong progression in the 1980s and 1990s. During the period of 2005 – 2015, there was an abundance culmination and it reached over 500 heads. After that, abundance decreases and follows the downward trend of trophy values. The results that have been reached indicate that the management measures that have been conducted in the researched area contribute to population development and abundance progress, however no correlation has been found through regression analysis between average value of trophies and population density.



CONTROLLED GERMINATION AND INOCULATION OF HOLM OAK (*Quercus ilex* L.) WITH TWO SPECIES OF THE GENUS TUBER

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non-wood
forest products,
plantations,
reforestation

Truffles are hypogeous mushrooms that are intensively cultivated due to their gastronomic value and the decline of natural production. Modern truffle cultivation is based on planting inoculated seedlings on suitable soils of appropriate ecological conditions to complete the life cycle of the fungus. The purpose of the research is to find the optimal inoculation method that will ensure a satisfactory degree of mycorrhization. Holm oak was chosen as the host plant because it is a natural symbiont of black and summer truffles, our indigenous specie and ecologically suitable for the habitat of planned plantations. The use of inoculated seedlings with truffle mycelium is very wide, such as raising plantations, reforestation and recuperation of degraded habitats or their free sale. The research tested three methods of storage and germination of acorns, two methods of inoculation (inoculation with substrate and inoculation by injection into substrate) with different doses of mineral fertilizer and different treatments with fulvic acid. 19 different experiments were set up with a total of 1320 holm oak seedlings inoculated with two species of the genus Tuber, black truffle (*Tuber melanosporum* (Vittad.)) and summer truffle (*Tuber aestivum* (Wulfen) Spreng.).



LONG-TERM STAND STRUCTURE DYNAMICS OF MANAGED AND UNMANAGED FIR-BEECH FORESTS IN THE CROATIAN DINARIDES

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KEYWORDS:
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protected forests,
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selection harvest,
stand growth
simulator*

Stand structure dynamics in Central European fir-beech forests from the mid-20th century, characterized by the accumulation of large-diameter firs, fir dieback, and poor regeneration, are well documented. In the Croatian Dinarides, in addition to environmental factors, light harvesting was suggested as one of the main causes for such discrepancies of the stand structure according to the theoretical selection structure. There are high requirements and challenges to promote stand regeneration and achieving balanced selection structure in selection forest management nowadays. Further, maintaining an old-growth stand structures that sustain biodiversity and mitigate climate change, are of great importance. Based on the established monitoring system (permanent plots) in the Croatian Dinarides fir-beech forests and MOSES 3.0 stand simulator, long-term projections of a stand structure were performed. The aim was to explore influence of management regime on the stand dynamics. One-ha "virtual" sample plot for actively managed state forests (35 plots of 1,257 m² each; average standing volume of 459.3 m³/ha), and for forests within national parks that were out of management (harvests) during last 3-4 decades (18 plots of 1,257 m² each; average standing volume of 653.3 m³/ha), were generated in the stand simulator as the initial average stand structures for the two forest strata. Long-term stand structure dynamics (75 years) and regeneration with respect to theoretical values, using three management regimes differing by the applied selection harvesting intensity (traditionally applied, theoretical intensity, and no harvest approach) were simulated under the three management scenarios. The management scenarios were validated by four management variables (amounts and deviations according to the theoretical) and by ten indicators of achieved stand structure vs. the theoretical at the end of the simulation period. Results showed a positive influence of harvest intensity on stand regeneration. Two harvest-approach scenarios predicted a decrease in stand volume in both, managed (22% and 38%, respectively) and in unmanaged (41% and 58%, respectively) forests. Due to the initial lack of small diameter trees and poor regeneration, 75-year period was too short to achieve a balanced selection structure. The theoretical intensity scenario was validated as the better management scenario in terms of management and stand structure variables. No-harvest approach would maintain and improve old-growth structures both forest strata: accumulation of standing volume (93% and 61%, respectively), large trees (520% and 121%, respectively) and standing dead wood during 75-year period (265 and 281 m³/ha, respectively). The obtained results can be useful in the Dinaric region to support the conversion of high-stocked stands allocated for economic use to a balanced structures and, on the other hand, to achieve old-growth structure aimed to maintain ecological forest functions.



INFLUENCE OF DIFFERENT SILVICULTURAL WORKS' INTENSITIES ON GROWTH AND DEVELOPMENT OF YOUNG PEDUNCULATED OAK STANDS (*Quercus robur* L.)

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KEYWORDS:
*daptation
measures,
silvicultural work,
pedunculated oak,
stand age, stand
structure*

Forest ecosystems are increasingly exposed to different biotic and abiotic pressures in recent decades, which will significantly affect future forest management especially in lowland pedunculated oak forests. The management and restoration of these stands will be a challenge in the future for Croatia and for Europe, as well. The most important measures of adaptation to changed habitat conditions are timely and adequate silvicultural works of cultivation of stands by clearing and first thinning implementation. The aim of this paper is to contribute to a better understanding of within stand relationships in young pedunculated oak stands, and to find out how different intensities of cultivation work affect the structure, dynamics and development of these stands.

The research was conducted in the stands of pedunculated oak and hornbeam at the age of 11 and 16 years in the area of the Spačva basin where we set up 8 permanent test plots with different intensities of silvicultural works in two repetition. Data were collected on 240 marked modal trees (trees of the future) in two surveys (2011th and 2016th). Selection of the modal trees was based on spatial and phenotypical criteria. All basic structural indicators were measured as follows: diameter at breast height (dbh), tree height, crown radius and trunk length, while crown length was calculated as the difference between three high and trunk length. Data analysis consists of descriptive statistics and analysis of variance (ANOVA) between individual intensities of breeding work, and statistical data processing was performed in Excel and Statistica (StatSoft Inc 2007).

Results indicates the interdependence of different intensities of silvicultural works and their impact on the growth and development of young pedunculated oak trees. Stronger intensities of silvicultural works lead to the largest increase in the growth and development of diameter at breast height, three height and radius of the crown, but also strongly affect the quality of the trunk and canopy.

Obtained insight to structural features and within stand relationships of young pedunculated oak stands can be useful for the future management when deciding on the time, manner and intensity of silvicultural works, and forestry practice can benefit from the findings to streamline their works.



EDUCATIONAL TRAIL 'LOVRIN'

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KEYWORDS:
*educational trail,
forest,
recreation, sweet
chestnut*

Educational trail 'Lovrin' is situated in the Lovrin forest near the city of Pazin in central Istria, Croatia. The forest has 19,5 ha and is managed by Hrvatske šume d.o.o. It has a rich biodiversity and is specific because of its sweet chestnut trees (*Castanea sativa* Mill.). Sweet chestnut is heavily affected with disease and its vitality is endangered worldwide. Sweet chestnut has an important part in the traditional life of local people due to its fruit (marun) and in the past it was a source of very valuable construction wood. Today, the value of chestnut forests lies in their ecological functions as is the case with Lovrin forest – it is managed as a protective forest. This means that it primarily serves to preserve public functions and to protect soil, water, air, settlements and other property.

The educational trail 'Lovrin' has a round concept and is 2,3 km long. It contains several seating areas with benches, educational boards, 'amphitheater' zone for outdoor learning, 'sweet chestnut giants' zone, small info-boards for plant names and entrance zone with a parking area for visitors. There are seven educational boards, each covering a specific theme like chestnut trees, plants and animals relevant to location, the importance of forest protection, ecology, climate change. The main and overall theme of the trail is sweet chestnut and all the urban equipment and zones are shaped in the form of a leaf, symbolising the leaf of sweet chestnut.

The main goal of the project is to create a recreational space in the natural forest environment that has educational contents suitable for young children with the possibility of interdisciplinary learning. Local and regional schools and kindergartens are the main target group of the project, as well as families with children. Another important aim of the project is to make Lovrin forest one of the most important green areas of the city in the form of a forest park and an important part of the green system of the city. The project was realised in 2022 and is 100% funded from EU funds.



INFLUENCE OF OAK LACE BUG ON RADIAL INCREMENT OF PEDUNCULATE OAK TREES WITHING DIFFERENT AGE CLASSES IN SPAČVA BASIN AREA

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*climatic factors,
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radial increment,
stand age*

In the last few years, in the pedunculate oak forests, there has been a wide spread of an alien invasive species *Corythucha arcuata* Say, 1832 (Heteroptera: Tingidae), the oak lace bug (OLB). OLB attack is manifested in leaf sap sucking, which results in the gradual leaf mass deterioration throughout the vegetation period. This paper investigates the oak lace bug influence on the pedunculate oak radial increment in stands of different ages. The research was conducted in a young, middle-aged and old pedunculate oak stand, in the area of Spačva basin, where the OLB first appeared in Croatia. Increment trends from fifteen trees sampled on each plot were analyzed. The increment trend after 2014 is negative in all three stands. The increments in 2016, and especially in 2014, were significantly higher than the increments before the appearance of oak lace bug (2013). By comparing annual increments with climatic factors, a significant positive correlation of increments with precipitation and a negative one with average temperatures were observed. The extremely low increment in 2012 is probably due to the extremely low rainfall combined with the above-average high mean temperature in July and August of that year compared to the 20-year average. Significantly higher increments in 2014 and 2016 were due to high rainfall and lower mean temperatures during July and August, as well as weaker impact of the oak lace bug on oak physiology due to sufficient nutrients and lower temperatures at which there is no intensive feeding with leaf sap. These results indicate that the OLB has a negative impact on the radial growth of oak trees, but that the dominant influence for now are climatic factors. In the negative trend of growth after the OLB appearance, it is currently difficult to distinguish which part falls on climatic factors and which on OLB, especially since they interact with each other.



MONITORING OF SMALL RODENTS IN CROATIAN STATE FORESTS FROM 2017 TO 2020 – ARE WE GETTING ANY WISER?

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rodents,
seeds

Monitoring of small rodents (Murinae:mice; Arvicolinae:voles) in the state forests of Croatia has been carried out systematically for over 40 years by Croatian Forest Research Institute (CFRI) and Croatian forests, limited liability company, that is a public enterprise for forest and woodland management in the Republic of Croatia. From the beginning of the 1980s to 2016, monitoring included estimating the intensity of damage caused by rodents (%) and recording the size of forest areas (ha) with noticeable damage to seeds and saplings of economically important tree species. Since 2017, the rodent monitoring has been extended to determine the relative abundance (RA; %) by trapping rodents, as well as to determine the share of damaged seeds and tree saplings (%). The aim of this work was to analyze the trends and results of rodent monitoring carried out from 2017 to 2020. Data was provided by CRFI (stetnici.sumins.hr) and analysed using Microsoft Excel 365. In that four year period overall damage caused by rodents in Croatian state forests were recorded on 16.506,2 ha (avr. 4.1236,55 ha) and rodents were treated using rodenticides (API Zinc phosphide) on 13.343,41 ha (avr. 3.335,85 ha). Monitoring included 11 (out of 17) (avr. 8) Forest Administrations – Subsidiaries (FAS) (Vinkovci, Zagreb, Sisak, Našice, Nova Gradiška, Osijek, Bjelovar, Požega, Koprivnica, Slatina, Karlovac), 40 (out of 169) regional forest offices (RFO) and 214 trapping locations average per year. Overall 14.263 (avr. 3.566) small rodents (mice: 11.976; avr. 2.994; voles: 2.020; avr. 505; not deremined: 267, avr. 67) were trapped. Average relative abundance (RA) in all FAS varied from 7,4 % to 23% per year with maximum RA 92% that was recorded in FAS Osijek in year 2017. Average share of seedlings with damaged bark varied annually from 0,2 % to 9,5 % (avr. 5,0 %; max. 66,7 %) and of those with damaged root from 0,2 % to 3,1 % (avr. 0,9%; max. 23,3 %). Damage on forest seeds varied overall from 0,3 % to 4,8 % (avr. 2,6 %; max. 36,6 %). Eventhough the average annual relative abundances, nor the damage on seedlings or seeds don't indicate rodent mass occurrence at FAS level, it is important to approach and interpret rodent monitoring and provide protection measures for every regional forest office, and further more for every specific microlocation on which monitoring takes place. Can this relatively low damage on samplings be explained by the high share of mice (> 80 %) recorded continuously during all four years? Shouldn't we expect more severe damaged on the seeds if the species from Murinae subfamilies are so dominant over Arvicolinae? Do we need to reconsider the monitoring methodology in years to come? Continuous monitoring should provide further answers.



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