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ICABB 2020 CONGRESS

4th International Congress on Advances in Bioscience and Biotechnology

Book of Abstracts

SEPTEMBER 30, 2020

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İlker Camkerten
Güzin Camkerten

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Dear Scientist,

Fourth International Congress on Advances in Bioscience and Biotechnology **(ICABB)** organized by the Journal of Advances in **VetBio** Science and Techniques, and co-organized by Universiti Teknologi Malaysia UTM-Institute Of Bioproduct Development (IBD) (Malaysia) and Avicenna International College (Budapest, Hungary), and GREEN (Global Research, Education and Event Network) held **ONLINE**, in coordination from the Sarajevo / Bosnia and Herzegovina headquarters of the organization team on **September 30, 2020.**

We wanted to make this conference little bit special by bringing scientist together from different disciplines of Biology area and also to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country. The total number of submission were 32 and after a careful evaluation 17 submissions were accepted by our scientific committee and 1 of them were accepted as poster presentation and, 16 of them were accepted as oral presentation and all those presentation was taken place in the conference booklet.

We thank our academic sponsors Universiti Teknologi Malaysia (UTM) and International University of Sarajevo (IUS) and our congress partner GREEN Global Research, Education & Event Network.

We would like to send our special thanks to Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers for their special efforts. And finally the most importantly I would like to thank to all the participants individually who came from far away to join this conference.

Chair of ICABB

Dr. Güzin Camkerten

Note for Turkish Participants: Kongremizin ONLINE düzenlenmesinde YÖK'ün 06.03.2020 tarihli tavsiye kararı dikkate alınmıştır.

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Keynote Address

Probiotics: The Small Biofactoties in Our Body "Science and Business"

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Probiotics are very important component in the body of almost all living organisms. These group of natural microbes are associated with human and animal bodies and located in all internal organs as well as body surface. They play crucial role for sustainable healthy growth by different direct and indirect mechanisms. This based on their potential to control other pathogenic microbes as well as the capacity to get maximal use of our diets by their enzymatic systems and through the produce many essential bioactive metabolites. For many years, it was believed that probiotics are only limited to lactic acid bacteria (LAB) group. However, nowadays, the list of microbes of proven probiotic properties includes many non-LAB as well as yeast from different groups. In the first part of this presentation, an overview will be given about the types and beneficial effects of microbes in human body and how can they play a crucial role in the life of healthy individuals. The second part of the presentation, will provide comprehensive information about the industrialization process of probiotic yeast (*Saccharomyces boulardii*) as model for large scale production platform from cell bank up to probiotic powder production. Another novel platform technology will be also presented for the large scale production of Selenium yeast (Se-yeast) as high potential biotherapeutic product.

Keywords: Probiotics, science, business

Overview upon the Biological Properties of Quercetin. Bee Products as Rich Sources of this Important Bioflavonoid

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Bee products (honey, polen, beebread, propolis, royal jelly) are important natural products, rich in nutrients which also possess high biologically active properties. Due to the complex chemical composition, they are highly appreciated both as value-added foods, and as important ingredients used in medicine, pharmacy and/or cosmetics. Phenolic fraction of bee products is minor, but very important in establishing their value, and for this reason, different modern analytical techniques are used to determine their presence and amount in the composition. Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most ubiquitous flavonoids, and it is found in all bee products in different amounts. Numerous experiments have shown that quercetin exerts antiproliferative, antioxidative, antibacterial, anticancer and antiviral activity or severe complications associated with the pandemic influenza AH1N1 or even SARS-CoV-2 virus infection effects. The mentioned effects will be reviewed and connected with the presence of this bioflavonoid in bee products. In the context of the global health pandemic (COVID-19), safe, cheap interventions, biological rationale should be prioritized for experimental use both in prevention and in treatments, and bee products are definitively such products.

Keywords: antiviral effects, bee products, bioactive properties, quercetin

Rapid Detection of Food-Borne Pathogens Bacteria Staphylococcus aureus in Contaminated Meat and Milk Product by Real Time PCR

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The development of rapid, sensitive, and specific detection methods has increasingly become the center of attention in handling food poisoning cases. In this study, the methods of Real Time PCR with nuc gene targets were developed for the detection of Staphylococcus aureus in food samples of meat and milk. The purpose of this study is to find a specific, sensitive, and rapid detection method for Staphylococcus aureus bacteria. The results showed that the primer nuc gene with Gradient PCR could detect Staphylococcus aureus bacteria from pure culture at 57-61 oC by producing amplicons measuring 135 base pairs. Amplification by Real Time PCR in the same sample at annealing temperature of 58oC and a concentration of 53 ng/µL gave a Ct value of 15,10. The primer specificity test of the nuc gene shows that the primer can recognize the target bacteria with a very strong fluorescent signal at the Melting Curve of 79.18 oC, whereas non-target bacteria give low fluorescent signals and different Tm values. The primer sensitivity of the nuc gene has a detection limit (LOD) of 98.4 pg/µL or 5,26 x 10-2 CFU / mL. Based on these results, the Real Time PCR method with the nuc gene has the potential to be a method of detecting specific, sensitive, and rapid for Staphylococcus aureus bacteria in a food sample.

Keywords: Real-time PCR, Staphylococcus aureus, food borne pathogens, nuc gene, rapid detection

Acknowledgements: Ministry of Research, Technology and Higher Education (Indonesia) by the scheme PDUPT under the contract agreement number 2/SP2H

Bottom up approach for capped silver nanoparticles and their antibacterial activity

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Problem: Bacterial resistance is increasing with the adaptation of microbes regularly. Current medical strategies are unable to address many of such questions for well-being related to antibacterial and anti-inflammation diseases.

Objective: Silver nanoparticles are the tiny particles of silver from 1 to 100 nm in size. The current study is based on developing capped silver nanoparticles (AgNPs) by trisodium citrate (TSC) bottom up approach. Silver nanoparticles are majorly applied in medicine from diagnostic applications to therapeutic, apart from its antimicrobial activity. In most of the therapeutic applications, the antimicrobial and anti-inflammatory properties are mostly considered and explored.

Research Methods: Turkevich method was used to prepare silver precursor i.e. silver salt (AgNO3). The ions of silver are reduced by reducing agent for their non-valent metal atom for this research, Trisodium Citrate (Na3C6H5O7) was used as the reducing agent in this study. However, capping agent used was Adipic acid. Characteristic of the AgNPs/AA were analysed by UV-Vis spectrophotometer and FT-IR for the analysis of functional groups. AgNPs limit their applications due to their nanotoxicity.

Results and Conclusion: The silver nanoparticles are colloidal, stable, uniform and polydisperse in nature. Their size is less than 100nm. These silver nanoparticles were checked against six antibiotics, against Gram-positive (Enterobacteriaceae, Escherichia coli, Staphylococcus aureus) and Gram-negative bacteria, (Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae) ATCC 8885, ATCC 2881, ATCC 8868, ATCC 9353 by disc susceptibility test. The best resistance was shown against Azithromycin for K. pneumoniae.

Keywords: Nanoparticles, bottom up approach, Adipic acid, Azithromycin, K. pneumoniae

Acknowledgements: The authors would like to thankful to Department of Chemistry, University of Karachi, Dr. Irshad Hussain of (Professor SBASSE, LUMS), for analysis of DLS and Surface Potential and Dr. Sana Shamim (Professor, Department of Pharmacy, DUHS)

Estimation of alpha and beta diversity in different forest communities based on Sentinel S2 spectral species in natural monument "Tajan“

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The research aim was to analyse alpha and beta diversity of forest communities in sampled area of natural monument “Tajan” using method of spectral diversity recorded on satellite image. Alpha diversity refers to the frequency of occurrences of different species within forest communities while beta diversity refers to different composition of species between forest communities.

The research area covered part of the natural monument “Tajan” in central Bosnia. Twenty-four circular plots with radius of 25 meters were established in broadly classified six most present forest communities selected by species dominance in tree layer (pine, oak, beech, fir, mixed beech-fir with beech dominance and mixed fir-beech with fir dominance). Sample plots were geolocated, recorded, and dominant tree species were identified. The main remote sensing data source was Sentinel S2 satellite image captured in June 2019.

Spatial prediction of distribution frequency for twenty spectral species on spatial unit 400 m^2 size using principal coordinate analysis (PCoA) was applied. The statistical significance of divergent species composition between analysed forest communities was assessed with permutational analysis of variance. The model validity was evaluated with analysis of variance on Bray – Curtis distances.

The mapping of predicted spectral alpha and beta diversity is conducted on forest research area. Predicted alpha diversity is represented with Shannon (H') index with range values from 0.63 to 1.56. High beta diversity and statistically highly significant differences in predicted species composition between investigated forest communities were registered ($p < 0.01$).

Alpha and beta biodiversity prediction using spectral species is giving promising insight in spatial distribution of biodiversity on broader forest areas. The results can contribute to better understanding of spatial characteristics of diversity in natural monument for the purpose of conservation and improvement planning measures. More detailed knowledge of the spatial distribution of alpha and beta diversity could be provided by more intensive field sampling and multi-temporal satellite images in future.

Keywords: Alpha and beta diversity, forest communities, spectral species, Sentinel S2A, natural monument

Use of *Vitreoscilla* Hemoglobin Technology for biodesulfurization of a model organosulfur compound

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Dibenzothiophene (DBT), an organic, sulfur containing component of petroleum based fuels, has been used as a model compound for microbial desulfurization studies. In the past, a number of biotechnological approaches have been investigated as ways to improve sulfur removal from DBT. The present study focused on enhancement of DBT desulfurization through VHb technology. For this, biodesulfurization (BDS) of DBT was carried using the BDS competent moderate thermophile *Paenibacillus* 32O-Y and this strain bearing the *Vitreoscilla* hemoglobin gene, *vgb* (strain 32O-Y*vgb*). Both strains were incubated in media containing DBT as sulfur source over a range of initial concentrations. Within this concentration range, the presence of *vgb* was correlated with enhanced growth at 0.1 and 0.5 mM initial DBT concentrations. The use of VHb technology may provide a useful increment to BDS when combined with other biotechnological approaches.

Keywords: Bacterial Hemoglobin, DBT, *Paenibacillus*, biodesulfurization

Acknowledgements: This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, 118Y416)

Platelet hyperreactivity related with COVID-19 disease severity

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Objectives: A hypercoagulability status has been reported in SARS-CoV-2 infection. As platelets are referred as immune cells, the purpose of the study was to examine platelet activation and aggregation in COVID-19.

Material Method: This case–control study comprised 61 patients with SARS-CoV-2 infection and 18 healthy individuals. The patients were separated into groups with respect to the need of treatment in intensive care unit (ICU). CD41, CD61, CD42a and CD42b were determined as platelet activation markers and platelet aggregation tests were analyzed in all groups.

Results: Platelet CD41, CD61, CD42a and CD42b expressions were significantly elevated in ICU patients compared to non-ICU patients and healthy donors. Patients in ICU group had increased platelet aggregations than those in non-ICU patients and controls. Additionally, platelet activation and platelet function tests strongly correlated with inflammatory and coagulation markers involving C-reactive protein (CRP), Interleukin-6 (IL-6), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and monocyte to lymphocyte ratio (MLR) and D-dimer and fibrinogen concentrations.

Conclusion: Enhanced platelet activity and faster platelet aggregation were observed in ICU COVID-19 patients. It is possible that platelet hyperreactivity may contribute to the progression of SARS-CoV-2 infection. The intense relationships between platelet activation and functions tests shows that systemic inflammation and cytokines may trigger the hypercoagulability in COVID-19 patients in ICU or hyperactivated platelets could augment the inflammation and be involved in COVID-19 pathophysiology.

Keywords: Platelet aggregation, COVID-19, flow cytometry, inflammation, platelet activation, SARS-CoV-2.

Cytotoxic effects of palladium complexes with pyridine derivative ligands on hepatocellular carcinoma cell line

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Cis-diamminedichloroplatinum (II) was synthesized in 1962, later known as cisplatin. It was used in the treatment of several types of cancer. Some palladium(II) complexes were tested in animals bearing transplanted tumors. Although the activity of palladium complexes was shown to be lower than that of platinum analogues with similar structures. Moreover, many palladium(II) and palladium (I) neutral complexes were found to exhibit antitumor activity. The biological activities of pyridine derivatives were studied for various biological actions. Metal halide complexes of pyridine derivatives were extensively studied. However, the antitumor activities of these halide complexes have received less attention in the literature. This report describes the synthesis and cytotoxicity of Dichlorobis (4-(4-nitrobenzyl) pyridinepalladium(II) (PdCl2L21) K1, Dichlorobis (2-amino-5-bromopyridine) palladium(II) (PdCl2L22) K2, Dichlorobis (1,3-dimethylpyridine) palladium(II) (PdCl2L23) K3, Dichlorobis (3,4-dimethylpyridine) palladium(II) (PdCl2L24) K4 complexes on HepG2 human hepatocellular carcinoma cell line.

A solution of PdCl2 (0.025 mol) in ethyl alcohol (40 mL) was added dropwise to a stirred and heated (70°C) solution of pyridine derivatives (4-(4-nitrobenzyl) pyridine, 2-amino-5-bromopyridine, 1,3-dimethylpyridine, 3,4-dimethylpyridine) (0.05 mol) in ethyl alcohol (20 mL). The reaction mixture was refluxed for 2 h at a 70°C and then cooled to allow for the precipitation of the complexes. The complex that was obtained was filtered off, washed with ethyl alcohol and dried in a desiccator. The prepared complexes were characterized by FT-IR and 1H-NMR spectrometers. Cytotoxic effects of complexes on HepG2 cell line were investigated using MTT cell proliferation assay.

The chemical structures of the complexes were verified by using 1H-NMR and FT-IR spectrometer. Analytical and spectral results are consistent with the proposed structures. The cell viability changes were found to depend on the concentrations and type of the complexes. According to the cell viability data K4 was determined the most toxic. Further studies are needed to better understand the metabolic effects of these complexes.

Keywords: Cytotoxic activity, HepG2, Palladium complexes

Effect of dimethyl sulfoxide (DMSO) on growth of different bacteria

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Dimethyl sulfide (DMS) a volatile sulfur compound could be biologically produced in sea-surface water and realized to the atmosphere. Oxidation of DMS forms DMSO (dimethyl sulfoxide) can be consumed by bacteria as a sulfur source. This work aimed to investigate the potential effects of DMSO on growth of different bacteria. Therefore, two *Paenibacillus* strains (32O-W and 32O-Y), *B.subtilis* ATCC 6633 and *E.coli* ATCC 25922 strains were incubated in minimal liquid media including 0.1 mM DMSO for 96 hours. Although the growth (OD_{600} values) of *E.coli* and *B. subtilis* strains were found to be constant, the OD_{600} values of *Paenibacillus* strains increased throughout the 96 hour cultivation. As a result, the growth of *Paenibacillus* strains were not inhibited by DMSO and these strains can be used for biodesulfurization of organic sulfur compounds.

Keywords: Bacterial Growth, DMSO, *Paenibacillus*, *Bacillus*, *E.coli*

Acknowledgements: This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, 118Y416).

Microbial remediation in environmental pollutants cleanup: Biosorption and Bioaccumulation

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Today, some physical, chemical and biological removal methods are used to treat and control toxic organic and inorganic pollutants in different receiving environments. However, biotechnological treatment methods have many advantages when comparing with other methods. In particular, it is widely used in the removal of potential toxic pollutants due to its low cost, easily applicable, degradation of organic pollutants and an environmentally friendly method.

Biosorption and bioaccumulation mechanisms are frequently made use of in the biotechnological remediation of materials with high organic content such as agricultural and domestic wastes.

Biosorption is an extracellular adsorption process independent from metabolism. The system is passive and the biosorbents used are not alive. Bioaccumulation is an active process, bioaccumulators are made up of living cells and it is a more complex process. When all these aspects are considered, bioaccumulation is a more complex and expensive method than biosorption in the treatment of various pollutant groups. The general purpose of this study is to reveal the differences, current status and research potential in the literature of biosorption and bioaccumulation, which are microbial improvement methods used in the treatment of various contaminants such as dyes, heavy metals and drug residues.

Keywords: Bioaccumulation, Biosorption, Contaminant removal, Eco-friendly, Remediation

Evaluation of trout farms in adana in terms of occupational health and safety

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As in many other sectors, there is loss of life and property in the aquaculture sector as a result of work accidents, which is one of the most important problems of working life. Occupational health and safety are practices that should be emphasized in terms of risks and potential dangers, as the employees in the aquaculture industry generally do not have a safe and healthy working environment. In this study, it was aimed to measure the awareness levels of the employees in terms of occupational health and safety by conducting a survey of 35 items on occupational health and safety in 10 trout farming facilities in Adana and the relevant occupational health and safety legislation. According to the findings obtained, it was found that 20% of the personnel of the trout production enterprises operating in the Adana region received Occupational Health and Safety training. In addition, 30% of the operating personnel stated that they had information about the risk of carrying heavy loads, 20% of them found themselves competent in OHS and 80% of them should receive training on this subject. As a result, it has been revealed that the enterprises in the region are not equipped with this issue and the risks of accidents and losses can be reduced by training.

Keywords: Occupational Health and Safety, Adana, salmon trout

The effect of biochemical blood parameters on udder lobe with subclinical mastitis

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Subclinical mastitis; although it does not cause any visible symptoms or changes in milk or udder, serious disorders in the udder parenchyma, decrease in milk quality/quantity and biochemical changes in the living body cause economic losses. In our study, a total of 30 Holstein breed dairy cows were used during the lactation period. Groups; Group 1 (those with negative test results but no subclinical mastitis), Group 2 (those with subclinical mastitis in two udders), Group 3 (those with subclinical mastitis in four udders) and 10 animals in each group. All of the cows in the study were in the mid-lactation period (100-200 days after birth), and cows whose udder lobes appeared normal in the clinical examination but showed a positive reaction with CMT were included in the study. For biochemical analysis, 5 ml of blood was taken from V. Jugularis of all animals and serum was extracted and glucose, T-cholesterol, T-bilirubin, AST and ALT measurements were made in Arkray Spotchem EZ SP-4430 dry system biochemistry analyzer. The data obtained were analyzed with the Oneway ANOVA test. In the evaluation of glucose and total cholesterol measurements between groups; While there was no difference between group 1 and group 2 ($P>0.05$), the difference between groups 1 and 3 was significant ($P<0.001$). In total bilirubin measurements between groups, the difference between Group 1 and other groups (with subclinical mastitis) was significant ($P<0.001$), while AST and ALT measurements were found to be significantly different between all groups ($P<0.001$). As a result, considering the disease state of the udder lobes; It has been concluded that by supporting the relevant organ according to the use of drugs, determining the duration of the treatment and biochemistry results, the success of the treatment can be increased and unnecessary drug residues and costs can be prevented.

Keywords: Subclinical mastitis, biochemistry, lactation

The Effects of the Addition of Inactivated Yeast Metabolites (*Saccharomyces cerevisiae*) to Rations Prepared with Different Roughage Materials on Nutrient Digestibility and Rumen Parameters of Lambs

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This study is planned in order to determine the effects of the supplementation of inactivated yeast metabolites (IYM) (*Saccharomyces cerevisiae*) to rations containing alfalfa hay (AH), meadow hay (MH) and wheat straw (WS) on lambs in terms of nutrient digestibility and some rumen parameters. In this study where 36 singleton born male Anatolian Merino lambs of 26.22±1.07 kg average weight, weaned at age of 2,5 months were used; 6 groups were formed for each roughage, 3 with IYM additive and 3 without IYM additive. Lambs were kept in individual partitions, were offered roughage in constant amounts (150 g/day) while concentrated feed and water were offered ad libitum. Extra IYM were added in constant amounts (7 g/day) to the roughage of the groups with supplement. Concentrate feed (2730 Mcal/kg ME, 17.41% protein) were kept in the feeders after roughage and/or roughage + IYM mixture were consumed. At the end of the period of stool collection (last 10 days of fattening) rumen liquid samples were extracted using rumen catheter and the pH, ammonia nitrogen (NH3-N) and volatile fatty acids (acetic acid, buritic acid and propionic acid) amounts were determined. At the end of the research, it has been seen that crude cellulose ($P<0.05$) and hemicellulose ($P<0.01$) digestibilities were effected from the different roughage used in ration and that ADF (acid detergent fiber) ($P<0.01$) and cellulose ($P<0.001$) digestion increased with IYM addition. The effect of feed x additive interaction on acetic acid, propionic acid and total volatile fatty acids concentrations were found significant ($P<0.01$). The concentration of NH3-N in rumen was effected by roughage type while acetic acid: propionic acid ratio was effected by additive ($P<0.05$). It is concluded that the addition of IYM to lamb rations results in positive effects on rumen fermentation and some cell wall components digestibility.

Keywords: Lamb fattening, digestibility, rumen parameters, inactive yeast

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Histology of retina in the Nannospalax xanthodon (Rodentia: Spalacidae)

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Blind mole rats (Nannospalax xanthodon) are wild subterranean rodents of the Spalacidae family. These animals are strictly fossorial rodents with various specific features that emphasize their adaptation to underground life. Blind mole rats have underdeveloped, non-functional subcutaneous eyes and are not able to detect light stimuli. There is no definite information about the retina histology of blind mole rats owing to the spreading these animals to only a certain habitat. The present study aimed to investigate of the histological structures of the retina in Nannospalax xanthodon. For this purpose, eye tissue samples were taken from five blind mole rats (Nannospalax xanthodon). The samples were fixed in 10% neutral buffered formalin, processed using routine histological methods and mounted in paraffin blocks. Six micrometer-thick sections were cut and stained with Crossmon's trichrome, H&E and periodic acid Schiff method. This study has shown even though the eye in Nannospalax xanthodon is severely regressed, the presence of a well-defined and functional retina. The lens greatly degenerated and the stratification of the retinal layers was very clear. The eyes studied had no pupil, which seemed to be collapsed by the accumulated heavily pigment. Retinal layers were observed to be composed of retinal pigmented epithelium, receptor layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, and nerve fibre layer. The mammalian eye is an essential intermediate in processes like reproduction, thermoregulation and hibernation, which require information about changes in photoperiod. In conclusion, Nannospalax xanthodon is blind but the retina may still be photosensitive and functionally may play a role in photoperiodic perception. These findings are important in having a better understanding of their evolution and adaptation to subterranean life.

Keywords: Histology, Retina, Nannospalax xanthodon

Effects of Hypericum Perforatum on Serum and Tumor Tissue Total Antioxidant Capacity, Total Oxidant Capacity and Nitric Oxide levels in Experimental Breast Cancer

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Hypericum perforatum is one of the plants whose effects are investigated in cancer treatment. Nitric oxide is a modulator involved in many physiological and pathological processes. The imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system that detoxifies ROS results in oxidative stress. Excessive accumulation of ROS can cause damage to biomolecules, resulting in lipid peroxidation, mutagenesis and cancer. The aim of the study is to determine the effects of Hypericum perforatum on serum and tumor tissue total antioxidant capacity, total oxidant capacity and nitric oxide levels in Ehrlich ascites carcinoma (EAC) in mice.

In the present study forty adult female Balb-c mice were divided into five groups as fallowed; Control-tumor negative group, Control Tumor positive group, low-dose H. Perforatum, high-dose H. Perforatum and doxorubicin groups. All animals (except Group 1) were introduced 2.5 x 106 EAC cells. Water extract of H. Perforatum was applied tumor bearing mice at different doses orally once every other day, for 10 days.

Compared with Group 1, there was a significant decrease in serum TAS level in Group 5 and Group 2. There were no significant differences between the groups in tumor tissue TAS levels. Serum TOS levels were decreased in Group 2 and Group 4 and increased in Group 5 and Group 3 compared to Group 1 ($p < 0.05$). However, these changes are not statistically significant. But there were significant decreased in Group 2 and 4 compare to group 5 in serum TOS level. There were no significant differences between the groups in tumor tissue TOS levels.

When the serum NO levels were evaluated, no significant difference was found between the groups. But significant reduction was observed in tumor tissue NO levels in Group 3, 4 and 5 compare to group 2.

Keywords: Hypericum Perforatum, Total Antioxidant Capacity, Total Oxidant Capacity, Nitric Oxide, Breast Cancer

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Evaluation of the shear bond strength of different cement types to dentin surface after magnetic resonance imaging

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Aim: The aim of this in vitro study is to evaluate the effect of magnetic resonance imaging on the shear bond strength of Co-Cr metal specimens cemented with different cement types to dentin surface.

Materials and Methods: Ninety human molar teeth were prepared until sufficient circular area of dentin was exposed and were assigned into 4 groups for the application of 1 of 3 types of cement: (1) Glass ionomer, (2) resin-modified glass ionomer, and (3) resin (n=30). Each group then was subdivided into 4 subgroups according to the duration of magnetic resonance application: control (no application), 15, and 30 min (n=10). Co-Cr metal specimens were fabricated using laser sintering system in the final dimensions of 7 mm in diameter and 3 mm in thickness. Magnetic resonance imaging application was performed after their cementation according to randomization. Shear bond strength test was performed to assess the effect of magnetic resonance imaging on the adhesion of metal specimens to dentin surface.

Results: In the glass ionomer cement; the value of the shear bond strength of 15 min group was significantly lower than that of the control group ($p<0.05$). In the same vein, the value of the 30 min group was significantly lower than the value of the 15 min group ($p<0.05$). In the resin cement and resin-modified glass ionomer cement; the value of 15 min group was lower than that of the control group ($p>0.05$). The value of the 30 min group was lower than the value of the 15 min group, but these differences are not reached the significance level ($p>0.05$).

Conclusions: In patients using prosthesis made of Co-Cr alloy, resin or resin-modified glass ionomer cement are recommended for patients with a history of frequent exposure to magnetic resonance imaging.

Keywords: Glass ionomer cement, resin-modified glass ionomer cement, resin cement, magnetic resonance imaging, shear bond strength

Clustering of high forest types based on spectrally characterized vegetation and environmental conditions using Sentinel S2 data

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Native mixed high forest types are the most present forest cover in central Bosnia. They appear in different forest species composition and environmental conditions. Forest type classification is based on typology criteria considering vegetation and soil components terrestrially observed. Recent interest is related to possibility to characterize wider areas of different forest types using satellite data. The objective of this research was to analyse similarities between different forest types regarding Sentinel S2 spectrally based indicators of forest biomass and environmental conditions.

Research area of 240.8 ha covered private native mixed high forests distributed in Ilidža municipality. Here is used time-series (seven images) of Sentinel S2 images from vegetation season in 2017. Images are pre-processed, generated normalized difference vegetation indices (NDVI) and performed tasselled cap transformations (greenness, brightness, wetness). Six different native high forest types were analysed. Clustering was based on Ward's method (squared Euclidian distance) for transformed values averages on management class level. Discriminant analysis was used to confirm differentiation.

Clustering resulted in three clusters. The first cluster contains secondary fir-spruce, oak and secondary beech forest on deep soils. The beech-fir-spruce and secondary fir-spruce forests on shallow soils belong to the second cluster while black alder forest on river valleys is separated in the third cluster. Discriminant analysis revealed consistent results with two significant functions (eigenevalues of 10 and 1) with 99% cumulative discrimination. It seems that similar environmental conditions are present for different forest species compositions. Soil type appears as differential attribute for clustering which confirms correlation of remotely sensed area characterisation with terrestrial soil type identification.

Obtained clusters indicate environmental similarities for diverse forest species composition what could be used in further consideration related to forest type typology classification. Additional forest type characteristics and satellite data could be integrated in further research for forest management planning purposes.

Keywords: forest types, Sentinel S2A image, normalized difference vegetation index, tasselled cap transformation

Estimation of alpha and beta diversity in different forest communities based on Sentinel S2 spectral species in natural monument „Tajan“

Research Paper

ABSTRACT

The research aim was to analyse alpha and beta diversity of forest communities in sampled area of natural monument “Tajan” using method of spectral diversity recorded on satellite image. The research area covered part of the natural monument “Tajan” in central Bosnia. Twenty-four circular plots with radius of 25 meters were established in broadly classified six most present forest communities selected by species dominance in tree layer (pine, oak, beech, fir, mixed beech-fir with beech dominance and mixed fir-beech with fir dominance). Sample plots were geo-located, recorded, and dominant tree species were identified. The main remote sensing data source was Sentinel S2 satellite image captured in June 2019. Spatial prediction of distribution frequency for twenty spectral species on spatial unit 400 m^2 size using principal coordinate analysis was applied. The statistical significance of divergent species composition between analysed forest communities was assessed with permutational analysis of variance. The model validity was evaluated with analysis of variance on Bray–Curtis distances. The mapping of predicted spectral alpha and beta diversity is conducted on forest research area. Predicted alpha diversity is represented with Shannon (H') index with range values from 0,63 to 1,56. High beta diversity and statistically highly significant differences in predicted species composition between investigated forest communities were registered ($p < 0,01$). Alpha and beta biodiversity prediction using spectral species is giving promising insight in spatial distribution of biodiversity on broader forest areas. The results can contribute to better understanding of spatial characteristics of diversity in natural monument for the purpose of conservation and improvement planning measures. More detailed knowledge of the spatial distribution of alpha and beta diversity could be provided by more intensive field sampling and multi-temporal satellite images in future.

Keywords: Diversity prediction, forest communities, natural monument, satellite image, spectral species

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INTRODUCTION

Forests are the most diverse land ecosystems with the majority of the world's terrestrial biodiversity. Forest biodiversity is a more levelled topic referring to the variety of all life forms found within forest ecosystems with their population interactions and associated genetic diversity (CBD, 1993; Innes and Koch, 1998). accelerates enzymatic reactions as well as antibacterial and antifungal effects, and the elements in its structure work like enzymes (Rakmai, 2009). The biodiversity assessments, especially for different forest communities, are increasingly important for appropriate management strategies for conservation planning, forest sustainability and ecosystem services. In most cases the term biodiversity is used in a complementary context with species diversity or species richness combined with species evenness. Whittaker (1972) suggested partition of biodiversity into three measures: alpha, beta and gamma diversity. Alpha diversity (α-diversity) is defined as the species diversity within communities or particular area. It is usually expressed by the number of species i.e., species richness in that community and represented with Shannon index (Shannon, 1948) as the most used indicator of tree diversity (Whittaker, 1977).

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Beta diversity (β-diversity) compares the species composition variation and detected differences across communities. Jointly, alpha and beta diversity determine gamma diversity (γ-diversity) as an overall diversity across large area or region. Usually, field surveys are the main source for biodiversity assessments. Beside "traditional" species diversity assessment, the remote sensing data are rapidly becoming more applicable in biodiversity analysis, using variations in the spectral heterogeneity, especially for broader spatial scales (Innes and Koch, 1998; Palmer et al., 2000, 2002; Gould, 2000; Rocchini et al. 2004; Rocchini et al. 2010, Meng et al. 2016; Rocchini et al., 2016; Rocchini et al., 2017; Laliberté et al., 2019). Currently available remote sensing systems have many advantages: improved instrumental characteristics, free image and easier accessibility, lower costs compared to field and resource costs, and high spatial accuracy estimates with adequate spatial and spectral resolution. Sentinel S2 as a high spatial coverage multispectral satellite now provides accessible multispectral imagery and data to differentiate species or habitats (Hoffmann, 2018; Féret and de Boissieu, 2019) and to analyse their interactions (Starčević et al., 2019). Those data availability is an opportunity to analyse relation spectral – ecological diversity (Innes and Koch, 1998). Alpha and beta diversity prediction and analysis by remote sensing is possible due accordance and relation of spectral diversity (remotely sensed signal information) and environmental/habitat diversity which implies that high habitat diversity indicates high species diversity within it. The explained principle is defined with Spectral Variation Hypothesis (Palmer et al., 2000, 2002; Rocchini et al., 2004; Hoffmann, 2019). Accordingly, the remotely sensed estimates could be very convenient for showing diversity patterns and monitoring biodiversity changes in protected areas as key biodiversity habitats with aim to prevent and reduce biodiversity loss through conservation strategies and specific conservation objectives (CBD, 1993; CBA - B&H, USAID/ B&H (2016)). Based on Country biodiversity analysis (CBA - B&H, USAID/ B&H (2016)), Bosnia and Herzegovina have particularly rich and significant biodiversity but systematic, efficient system of data collection and biodiversity monitoring is very scarce. Developing reports related to forest and mountain ecosystems and protected areas is an important goal defined by CBD and Strategy and action plan for projection of biological diversity in Bosnia and Herzegovina (2015 – 2020), (2016). So far, to our knowledge, no research has been done to estimate and evaluate the diversity in different forest communities using remote sensing data in central Bosnia. The research aim was to identify alpha and beta spectral diversity in sampled area of protected monument Tajan using Sentinel S2 image data and to evaluate differences in spectral species diversity in defined forest communities.

MATERIALS and METHODS

Study area

The research was conducted on the part of natural monument Tajan bordering municipalities Zavidovići, Kakanj, Žepče, Zenica and Vareš in central Bosnia. The research was conducted within the area of the natural monument Tajan in central Bosnia (Fig. 1. - red rectangle). According to the map of biogeographical regions of Europe, the area belongs to the alpine biogeographical region (EEA, 2016). The mountain area of Tajan was designated as a protected area in 2008 under the category of Natural Monument. The total area of the natural monument is 4948,35 ha with highest altitude up to 1297 m. The unique natural richness is represented with abundant water streams, canyons, speleological objects with archaeological findings, lake, the water springs, geological, animal, and forest diversity with associated endemic flora. The terrain configuration is defined by a series of steeper and gentler slope sides with bays and creeks undercrossed influencing different aspect orientations. The climate is temperate continental with mountain climate features.

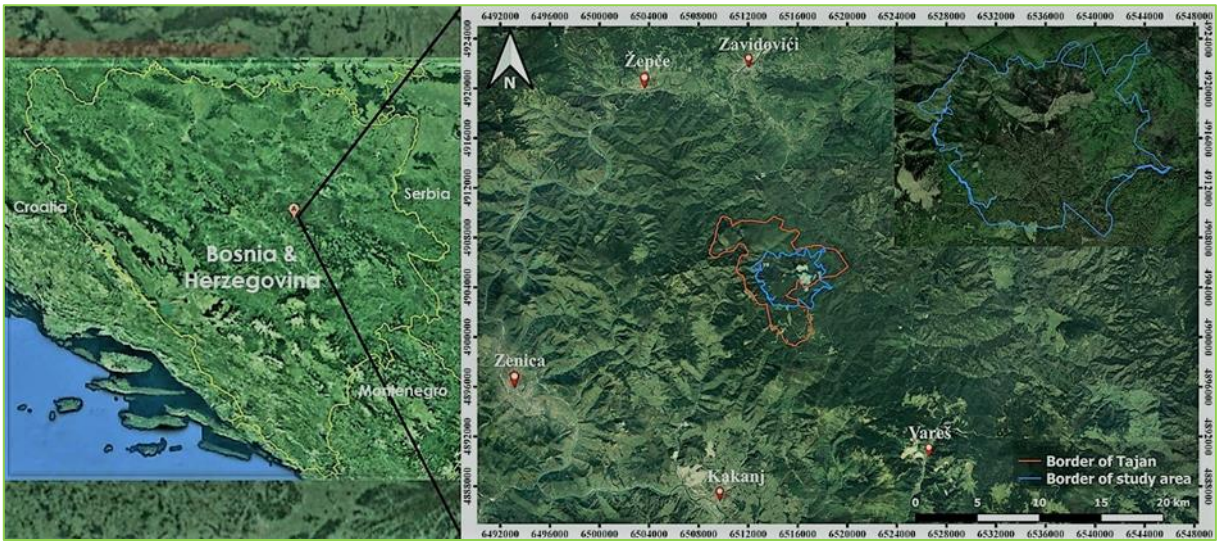


Figure 1: Study area.

The regular cutting measures and heavy mechanisation use are forbidden to protect high biodiversity of this area. According to the forest management plans (Forest management plans for forest management area "Krivajsko" for 2017 - 2026 and Management unit "Gostović") and recent description (Vojniković, 2017), in this area are mostly present acidophilic communities of beech and fir (with spruce) (*Fago – Abietetum*), neutrophilic forest communities of beech and fir on limestone and gabbro (*Abiety – Fagetum illirycum*), different forests of black and white pine on peridotite and serpentinite (*Pinetum nigrae-sylvestrys serpentinicum, Erico – pinetum nigrae serpentinicum, Erico – Pinetum sylvestrys serpentinicum*), oak forest on peridotite and serpentinite (*Erico – Quercetum petrae seprentinicum, Ostryo – Quercetum petraeae seprentinicum*).

Sample plots

On the most diverse part of Tajan area (between latitudes 44º16'22'' - 44º18'21'' N and longitudes 18º9'0'' – 18º10'53'' W) terrestrial observations were conducted and identified six representative forest communities related to unique tree species dominance (pine, oak, beech, fir) and dominant tree species mixtures (mixed beech-fir with beech dominance and mixed fir-beech with fir dominance). Here were geo-located 24 circular sample plots with radius of 25 meters in July 2019 and belonging to the tree species community was recorded. The dominant pine and oak sample plots were located at lower altitudes (approximately 600 m) at steeper slope side. The location of other plots was in the higher mountain altitude range, 800 – 1100 m with slopes between 15-20° included in the beech forests and mixed beech and fir forest on dystric cambisols, luvisols on silicate and silicate - carbonate sediments and acidic eruptive rocks.

Spectral Sentinel S2 data

The remote sensing data source was Sentinel S2 satellite image captured in June 2019 (Identifier: S2B_MSIL1C_20190619T094039_N0207_R036_T33TYK_20190619T142705) with coordinates system: CRS WGS 84 UTM Zone 33, downloaded from the ESA Sentinel data hub (https://scihub.copernicus.eu). Downloaded satellite image was geometrically and radiometrically corrected (TOA reflectance) Level-1C system product. We used channels: Band 2 (blue, 10 m), Band 3 (green, 10 m), Band 4 (red, 10 m), Band 5 (red edge, 20 m), Band 6 (red edge, 20 m), Band 7 (red edge, 20 m), Band 8 (near-infrared [NIR], 10 m), Band 8a (red edge, 20 m), Band 11 (shortwave infrared [SWIR], 20 m) and Band 12 (shortwave infrared [SWIR], 20 m). Spatial resolution of the

selected channels was resampled to a unique pixel size of 10 m. Spatial sub-setting was made on the framed study area and subset image was exported in ENVI hdr format. Satellite image pre-processing was done using SNAP Desktop 5.0.

Methods

Mapping the estimation of alpha and beta diversity of spectral species using Sentinel S2 images on chosen area was based on methodology proposed in Féret and de Boissieu, 2019. We specified a size of two pixels (400 m^2) as the spatial mapping unit and twenty clusters for k-means clustering of spectral species (k=20). The mean alpha diversity index estimate was mapped based on the spectral species distribution of the spatial unit for the partition of n. Beta diversity was estimated based on subsets of image spatial units. The Bray-Curtis dissimilarity matrix was determined, and the ordination method was applied. For each spatial unit, the coordinate in the ordination space was calculated as the distance weighted average of the three nearest neighbours using principal coordinate analysis (PCoA). The raster for three ordination components for all spatial units was presented. The sample plot data (geo-position, forest community type) were used for further analysis. The estimates of alpha and beta diversity were extracted to geo-positions of sample plots in point vector. The alpha diversity expressed as Shannon index was used to calculate and compare statistical estimates between (across) forest communities. Also, the estimated values were compared with Shannon index values in similar studies. For beta diversity analysis the PCoA was applied analogous to ordination method for beta diversity mapping. The similarity analysis of spectral species in different forest communities was carried out based on the interpretation of graphical representations of beta diversity. The statistical significance of spectral species composition differences was tested using permutational analysis of variance (PermANOVA). The homogeneity of spectral species composition variability within different forest communities was verified by analysis of Bray-Curtis distance variance (ANOVA). The analysis was performed using the R package for α- and β- diversity mapping using remotely sensed images (Féret and de Boissieu, 2019).

RESULTS

Results of alpha and beta diversity estimates of spectral species on the chosen area are presented in cartographic, tabular and graphical forms. Resulted cartographic estimates of twenty spectral species, alpha and beta diversity maps are presented in Figure 2.

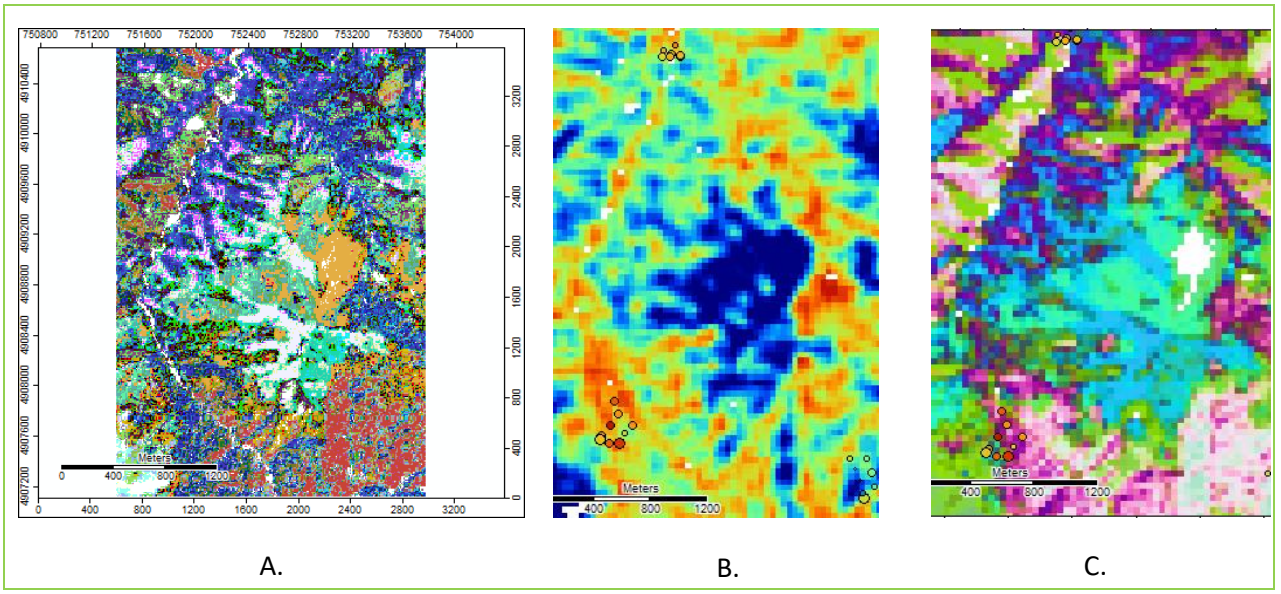


Figure 2: Estimated spectral species (A.), alpha (B.) and beta diversity maps (C.).

Figure2A provides estimates of spatial distribution of spectral species and it represents differentiation of study area considering the composition of the spectral species. Figure2B present estimates of alpha diversity represented with blue-orange colours. Blue tones represent low estimated alpha diversity and orange tones represent high estimated alpha diversity. Here are included estimated Shannon index values on sample plots presented with different size and scaled colour circles. Figure2C includes estimates of beta diversity. Surfaces with different composition of spectral communities represented by contrasting colours are shown here.

Estimates of spectral species alpha diversity

Estimates of spectral species alpha diversity expressed as Shannon index related to sample plots in chosen forest communities are presented in Table 1.

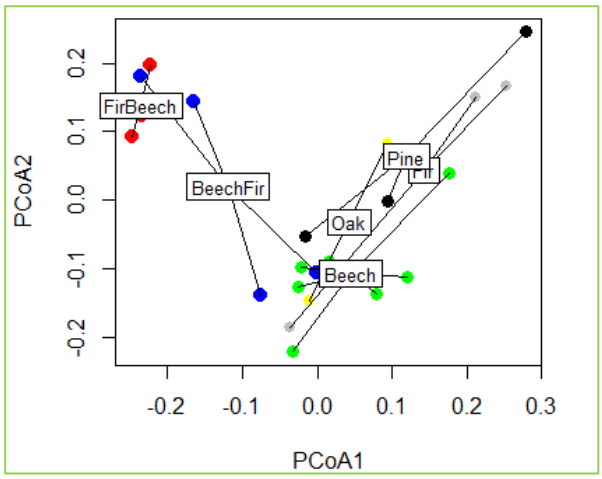
Table 1: Descriptive statistics for Shannon index H for sample plots in forest communities

Forest community	Minimum	Maximum	Average	Standard deviation	Coefficient Variation (%)
Beech	0,63	1,56	1,18	0,34	28,8
Beech with fir	1,09	1,26	1,15	0,07	6,1
Fir	1,30	1,50	1,41	0,10	7,1
Fir with beech	1,25	1,36	1,29	0,06	4,7
Oak	1,26	1,33	1,30	0,04	3,1
Pine	1,30	1,40	1,34	0,04	3,0

Estimation of alpha diversity expressed as Shannon diversity index of spectral species on field plots ranges from 0,63 to 1,56. The lowest average index of alpha diversity is estimated in beech-fir community (1,15), and the fir community has the highest average index of alpha diversity estimate (1,41). The highest coefficient of variation of alpha diversity is estimated for beech community (28,8%) while other forest communities are more homogenous. PermANOVA of estimated alpha diversity between forest communities resulted in statistically significant differences ($p<0,01$). Also, ANOVA confirmed homogeneity of species composition variability between forest communities which means that conclusions on the significance of differences obtained by applying PermANOVA can be considered valid ($p>0,05$).

Estimation of spectral species beta diversity

Graphic 1 provides estimated beta diversity of surveyed forest communities obtained by principal coordinate analysis (PCoA). According to Graphic 1 the distance between fir-beech community and other surveyed forest communities which indicates that fir-beech community contains the most diverse composition of spectral species. Also, this could indicate that there are some spectral species in fir-beech community that are not contained in other surveyed forest communities. The beech-fir community is positioned between the fir-beech and the beech communities. It seems that part of the spectral species present in fir-beech and beech communities are located in beech-fir community as well, what is logical.



Graphic 1: Beta diversity of different forest communities - Principal coordinate analysis (PCoA)

The pine and fir communities are close to one another, which points out that they have a similar composition of spectral species or/and similar abundance. The beech and oak communities are closer and relatively distant from others, which means that they share many similarities in the composition of spectral species that differ from the compositions in fir-beech/beech-fir forests and pine/fir communities. Also, there are overlaps of a number of spectral species, as expected, because these forest communities certainly share an abundance of spectral species such as, for example, reflection of leaf/needle litter, healthy grass and shrubs.

DISCUSSION

The aim of this research was to estimate spectral species alpha and beta diversity in different forest communities in the natural monument Tajan. Thus, the spatial prediction of alpha and beta diversity using Sentinel S2 image data with analysis of spectral species differences was done in this paper. Alpha diversity indicates predicted spectral species heterogeneity within sampled plots, and it is represented with Shannon index. The results of prediction showed the highest average alpha diversity on forest plots with fir dominance (Shannon index = 1,41), and the lowest values for mixed beech - fir forest type (Shannon index = 1,15). This indicates that the highest number of different spectral species was manifested in plots with fir dominance and plots with beech dominance had the smallest number of different spectral species. Bagnaresi (2002) reported a generally high diversity level for all stands in coniferous forests described as *Abietetum on silicate soils* dominantly with silver fir and norway spruce with Shannon values ranging from 1,5 what is in accordance with our results. The Shannon index values for pine, oak, beech, and beech – fir plots are decreasing, respectively. Similar descending Shannon index values were reported by Redowan (2015). Similar to our prediction, pine stand diversity was higher than oak and beech stand diversity, respectively (Redowan, 2015). Redowan (2015) also reported good agreement between remotely sensed data with ground information, and medium diversity for beech forests in national park in Italy on both levels. Our results for alpha diversity could be explained with spectral manifestation of other floristic and vegetation compounds such as grass, shrubs, forest floor and even spectral diversity of geomorphological and substrate-soil related surface components. We imply that the vegetation component did not manifest as dominant in spectral composition, but only as one spectral species regardless of the area or number of occurrences. Multilayer and well-developed understory could also contribute to results. Conifers usually have sparse canopy, less spread branches and higher light transmittance for each layer while deciduous species, especially beech, have dense canopy which can block the light from lower layer plants. Gazdić et al. (2018) presented in his biodiversity research that beech was actually dominant in all layers and diversity was rather low due to strong canopy shading in Montenegro's forests and higher species richness is registered with the occurrence of more light demanding tree species such as black pine. Rad (2009) reported oak communities as floristically richest and concluded that species diversity is inversely correlated with the dominance of shade tolerant species like beech. Compared to Turkis and Elmas (2018) obtained Shannon diversity values (1,68 - 1,94), and Singh (2018) obtained Shannon

diversity values (1,04 - 1,23) in oak stands for tree layer, our oak range is moderate. The period of research could also have an impact on prediction results because of the growing season, phenology, canopy cover and photosynthetic capacity of the analysed species. While alpha diversity is referred to local variability, beta diversity represents the predicted spectral heterogeneity between forest plots in defined forest communities. Statistically highly significant differences of species composition in different forest communities are registered. The PCoA showed the extraction of fir - beech forest plots considering spectral species composition which indicates that spectral species common to other communities occur less. Mixed beech – fir and fir - beech plots were plotted closer in ordination space, but dominance of the edificatory species affected the differentiability of other spectral species. Gazdić et al. (2018) reported higher alpha diversity for mixed fir-beech type compared to moderate diversity of dominantly beech forest. Beech – fir and beech forest plots are also similar in relation to spectral species composition, but fir presence and abundance, however, implies the occurrence of spectral species which do not occur or occur infrequently in pure beech communities. Turkis and Elmas (2018) reported about similar alpha diversity for beech-fir and beech forests stands. In mixed beech – fir, beech, oak, fir and pine forest communities common spectral species were less present. Knowing that spectral species are products of registered reflections from different communities of substrates, it is likely that the common spectral species were reflections of rocks, soil or grass. Terrain observations showed that canopy tree cover was not absolute on the whole plot's surfaces. Spectral prediction of beta diversity provides differentiating areas due to diversity differences but does not precisely point out the uniqueness of some habitats (Rocchini, 2016). Composition similarity of the spectral species for beech and oak communities could be the result from the dominance of the vegetation component of the main tree species (deciduous trees), as well as similarity in pine and fir communities (conifers). Also, composition similarity of the spectral species for oak and fir – beech communities could be resulted from habitat condition similarity of these communities or the structural characteristics (communities with lower degree of ground cover by tree canopies). Barbier (2008) concluded that over-story composition and structure influence understory vegetation diversity in terms of light, thermal conditions, water and soil nutrients, light transmittance, forest litter and other ecological components. The results show that the manifested relationships of alpha and beta diversity have also appeared in several studies based on intensive field surveys, which indicates the potential of Sentinel S2 for alpha and beta biodiversity prediction. Hoffmann (2018) reported up to 85% of beta diversity represented by remote sensing variables and better explanatory results for Sentinel S2 compared to other satellite sensors but pointed out the need of in-situ sampling. Also, by Nagendra (2001) review, estimations of spectral diversity may be more useful as indicators of areas with higher species diversity for regional scales and this method has limited purpose.

CONCLUSION

The estimated diversity of the research area indicates that this is an area with present differentiation of habitat species, which is a feature of protected areas and landscapes. The obtained results are partially sufficient for insight into species diversity and diversity hotspots location. The applied method seems a promising source and gives prediction of spatial alpha and beta diversity for broader areas. The research enhances biodiversity variation within forest communities, but combination of spectral data of remote sensing, and comprehensive analysis of ecological, vegetation, and other characteristics of the study area would give more reliable conclusions. So, the future research should be orientated in planning field surveys for phytocenose characterisation and ecological analysis of studied area for more precise taxonomic and structural data which could help in developing complex predictive models of tree

diversity but also biodiversity monitoring and improvement.

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