

## 6. simpozij studenata doktorskih studija PMF-a : knjiga sažetaka

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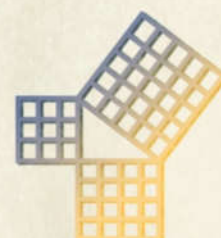


6. Simpozij studenata  
doktorskih studija PMF-a  
KNJIGA SAŽETAKA

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*6<sup>th</sup> Faculty of Science PhD  
Student Symposium  
BOOK OF ABSTRACTS*

Zagreb, 2022



Simpozij studenata doktorskih studija PMF-a

*Faculty of Science PhD Student Symposium*

2022

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Knjiga sažetaka

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*Faculty of Science, University of Zagreb*

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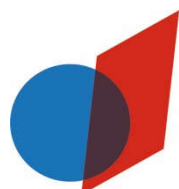
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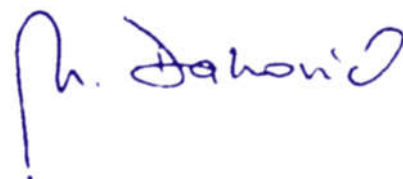
Drage doktorandice i dragi doktorandi,

Izrazito mi je zadovoljstvo pozdraviti vas na šestom Simpoziju doktorskih studenata Prirodoslovno-matematičkog fakulteta. Veseli me što smo unatoč svim izazovima s kojima smo se susreli tijekom prošle godine uspjeli organizirati još jedan u nizu Simpozija doktorskih studenata.

Isto tako, izrazito me veseli što smo ove godine uspjeli učiniti još jedan korak ka povećanju vidljivosti i prepoznatljivosti Simpozija. Predstavljanjem vaših rezultata na engleskom jeziku, u vidu kratkih usmenih priopćenja, mikro izlaganja te postera, Simpozij poziva i doktorande iz inozemstva da vam se pridruže i podijele s vama zadovoljstvo izlaganja svojih znanstvenih rezultata te čari istraživanja i bavljenja znanosti. Posljedično to povećava i vidljivost vašeg istraživanja te omogućuje da vaši rezultati dopru do šire znanstvene zajednice.

Osim toga, izlaganje na engleskom jeziku ne samo da nadograđuje Simpozij, već vama daje dodatnu razinu izobrazbe te vas potpunije priprema za izazove koji vas čekaju na znanstvenom putu kojim ste upravo kročili. Ovaj put očito nije ravan, već pun uspona i padova s možda samo nekoliko brzih cesti i prečaca, a kako biste ga učinili bržim i ugodnijim, putujte širom otvorenih očiju i oslonite se na svoju mladenačku znatiželju, vjerujte u svoje ideje i ideale i slušajte savjete onih koji su tim putem prošli prije vas. Iskoristite svaku priliku za stjecanjem znanja i iskustva jer su se upravo oni pokazali najvjernijim suputnicima na tom putu. I ubrzo ćete shvatiti da ovo putovanje donosi obilje nagrada i ispunjenja što vas osnažuje i budi želju za osvajanjem novih vrhunaca!

I na kraju želim čestitati vašim kolegama u Organizacijskom odboru koji su nakon više mjeseci neumornog i predanog rada doveli ovaj Simpozij do njegove realizacije. Čestitam i svima vama na entuzijazmu i uloženom trudu koji je rezultirao vrijednim rezultatima zbog kojih ste se danas ovdje i činite ovaj Simpozij još većim i značajnijim. U ime Znanstvenog i Organizacijskog odbora Simpozija želim vam uspješan i ugodan znanstveni vikend na Prirodoslovno-matematičkom fakultetu.



Izv. prof. dr. sc. Marijana Đaković  
prodekanica za znanost i doktorske studije



*Dear PhD Students,*

*It is a great pleasure to welcome you to the sixth Faculty of Science PhD Student Symposium. I am thrilled that despite all the challenges we encountered during the last year, we managed to organize another edition of the Symposium.*

*I am pleased that this year one more step forward was taken towards enhancing the visibility and recognition of the Symposium. By inviting all the presentations in English, the Symposium opens to PhD students from abroad to join you in sharing the excitement of doing science and presenting research findings. Moreover, it also enhances the visibility of your research and enables your research results to reach a wider scientific community.*

*More importantly, presenting in English offers an additional level of practice and prepares you for challenges that wait for you on the scientific path you just stepped on. This path is apparently not a straight one but rather full of ups and downs, with maybe only a few opportunities for hitting fast roads or shortcuts. To make the journey quicker and more enjoyable, travel it with eyes wide open and rely on your youthful curiosity, believe in your ideas and ideals, and listen to the advice of those who walked the path before you. Moreover, take every opportunity to gain knowledge and experience as they proved to be the most helpful and faithful companions on this trip. And soon, you will realize that this journey brings plenty of rewards and fulfilment and make you want to climb even higher and conquer new heights!*

*In the end, I congratulate your colleagues at the Organizing Committee who, after several months of tireless and dedicated work, brought this Symposium to its realization and to all of you who gathered to make this Symposium happen. On behalf of the Scientific and Organizing Committee of the Symposium, I wish you a successful and pleasant scientific weekend at the Faculty of Science.*



*Assoc. Prof. Marijana Đaković  
Vice-Dean for Science and Doctoral Studies*

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## Program simpozija / *Symposium Programme*

	<b>Subota</b> <i>Saturday</i>	<b>Nedjelja</b> <i>Sunday</i>	
8:30----	Registracija <i>Registration</i>	Registracija <i>Registration</i>	9:00----
9:00-9:15	Otvaranje <i>Opening</i>	<b>IL 3</b>	9:30-10:00
9:15-9:45	<b>IL 1</b>	<b>Sekcije 4A i 4B: Usmena izlaganja</b> <i>Sections 4A and 4B: Oral Presentations</i>	10:00-10:50
9:45-10:00	Embassy of France		10:50-11:20
10:00-10:10	Sponzor <i>Sponsor</i>	<b>IL 4</b>	11:20-11:50
10:10-11:05	<b>Sekcije 1A i 1B:</b> Usmena i mikro-izlaganja <i>Sections 1A and 1B:</i> <i>Oral and Flash Presentations</i>	U.S. Embassy	11:50-12:05
11:05-11:35		<b>Sekcija 5: Usmena izlaganja</b> <i>Section 5: Oral Presentations</i>	12:05-12:45
11:35-12:45	<b>Sekcije 2A i 2B:</b> Usmena i mikro-izlaganja <i>Sections 2A and 2B:</i> <i>Oral and Flash Presentations</i>	Pauza za ručak <i>Lunch Break</i>	12:45-13:45
12:45-13:45	Pauza za ručak <i>Lunch Break</i>	<b>Radionica</b> <i>Workshop</i>	13:45-15:45
13:45-14:15	<b>IL 2</b>		15:45-16:15
14:15-14:25	Sponzor <i>Sponsor</i>	<b>Sekcija 6: Usmena izlaganja</b> <i>Section 6: Oral Presentations</i>	16:15-17:05
14:25-15:05	<b>Sekcije 3A i 3B: Usmena izlaganja</b> <i>Sections 3A and 3B: Oral Presentations</i>	Sponzori <i>Sponsors</i>	17:05-17:35
15:05-15:35		Zatvaranje <i>Closure</i>	17:35
15:35-17:35	Posterska sekcija <i>Poster Section</i>		
17:35-19:00	Kviz <i>Quiz</i>		
19:00-21:00	Afterparty		

 Pauza za kavu  
*Coffee break*









IL - predavanja pozvanih predavača  
*Invited Lecturers*


## Detaljni program Simpozija *Detailed Symposium Programme*

SUBOTA 23. travnja 2022. g.  
*SATURDAY April 23, 2022*



8:30	Registracija – <i>Registration</i>	
9:00–9:15	Svečano otvaranje Simpozija – <i>Symposium Opening</i>  Predavaonica – <i>Lecture Hall: A1</i>	
9:15–9:45	<b>Pozvano predavanje 1 – <i>Invited Lecture 1</i></b> <b>prof. dr. sc. MIRKO ORLIĆ</b> KAKO JE METODA RAZVIJENA ZA ISTRAŽIVANJE JADRANA POSTALA KORISNA EKONOMISTIMA / <i>HOW A METHOD DEVELOPED FOR THE ADRIATIC-RELATED RESEARCH          BECAME USEFUL TO ECONOMISTS</i>  Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: doc. dr. sc. Kristina Pikelj</i>	
9:45–10:00	Predstavljanje Francuskog veleposlanstva u Zagrebu <i>Presentation of Embassy of France</i>  Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: Zuzana Redžović</i>	
10:00–10:10	Predstavljanje zlatnog sponzora (INETEC) <i>Golden Sponsor Promotion (INETEC)</i>  Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: Zuzana Redžović</i>	
10:10–11:05	<b>Sekcija 1A: Usmena i mikro-izlaganja</b> <i>Section 1A: Oral and Flash Presentations</i>  Predavaonica – <i>Lecture Hall: A1</i> Moderator – <i>Moderator: Ivan Biočić</i>	<b>Sekcija 1B: Usmena i mikro-izlaganja</b> <i>Section 1B: Oral and Flash Presentations</i>  Predavaonica – <i>Lecture Hall: A2</i> Moderatorica – <i>Moderator: Mateja PISAČIĆ</i>
10:10–10:20	 <b>LUCIJA DONČEVIĆ</b> IDENTIFIKACIJA DEGRADACIJSKIH PRODUKATA ROSUVASTATINA POMOĆU VEZANOG SUSTAVA nanoUPLC I nanoESI-QTOF	 <b>OZANA MIŠURA</b> MEHANIČKA SAVITLJIVOST KRISTALA KOORDINACIJSKIH POLIMERA BAKRA(II) / <i>MECHANICAL BEHAVIOUR OF</i>





	<p>SPEKTROMETRA MASA /  IDENTIFICATION OF ROSUVASTATIN  DEGRADATION PRODUCTS USING  <i>nano</i>UPLC COUPLED TO THE <i>nano</i>ESI-  QTOF MASS SPECTROMETER SYSTEM  (O-K1)</p>	<p>CRYSTALS OF COPPER(II)  COORDINATION POLYMERS (O-K2)</p>
10:20-10:30	<p> <b>ANA RAPLJENVIĆ</b>  VOLTAMMETRIJA I DIFERENCIJALNA  PRETRAŽNA KALORIMETRIJA ZA  ODREĐIVANJE METALA U TRAGOVIMA  I VRSTA POLIMERA NA MORSKOJ  (MIKRO)PLASTICI / <i>VOLTAMMETRY</i>  <i>AND DIFFERENTIAL SCANNING</i>  <i>CALORIMETRY FOR TRACE METALS</i>  <i>AND POLYMER TYPE DETERMINATION</i>  IN MARINE MICROPLASTICS (O-O1)</p>	<p> <b>ZORAN KIRALJ</b>  RAZLIKE U UNUTARSTANIČNOJ  RASPODJELI TOKSIČNIH METALA AG I  CD U PROBAVNIM ŽLIJEZDAMA TRIJU  VRSTA ŠKOLJKAŠA IZ PORODICE  UNIONIDAE / <i>DIFFERENCES IN THE</i>  <i>SUBCELLULAR DISTRIBUTION OF THE</i>  <i>TOXIC METALS AG AND CD IN THE</i>  <i>DIGESTIVE GLANDS OF THREE MUSSEL</i>  <i>SPECIES FROM THE FAMILY UNIONIDAE</i>  (O-B1)</p>
10:30-10:40	<p> <b>MARIN MIĆUNOVIĆ</b>  PRECIZNOST TEHNIKA DALJINSKIH  ISTRAŽIVANJA U GEOMORFOLOŠKIM  ISTRAŽIVANJIMA ŽALA – PRIMJER  OTOKA HVARA / <i>ACCURACY OF</i>  <i>REMOTE SENSING TECHNIQUES IN</i>  <i>GEOMORPHOLOGICAL</i>  <i>INVESTIGATIONS OF BEACHES –</i>  <i>EXAMPLE OF THE ISLAND OF HVAR</i> (O-  GG1)</p>	<p> <b>LUCIJA KNEŽEVIĆ</b>  INTERAKCIJA V(V) S HUMIČNOM  KISELINOM I ŽELJEZOVIM(III) IONIMA U  VODENOJ OTOPINI / <i>INTERACTION OF</i>  <i>V(V) WITH HUMIC ACID AND FERRIC(III)</i>  <i>IONS IN AQUEOUS SOLUTION</i> (O-O2)</p>
10:40-10:45	<p> <b>NIKOLA JAKUPEC</b>  TERMIČKI KONTROLIRANA  MEHANOHEMIJA ZA SELEKTIVNE  REAKCIJE INTERZEOLITNE PRETVORBE  / <i>APPLYING THERMALLY CONTROLLED</i>  <i>MECHANOCHEMISTRY FOR SELECTIVE</i>  <i>INTERZEOLITE CONVERSION</i>  REACTIONS (M-K1)</p>	<p> <b>KRISTIAN BODULIĆ</b>  SEKVENCIJANJE TRANSKRIPTOMA  OGULINSKE ŠPIJSKE SPUŽVICE  (<i>Eunapius subterraneus</i>) DAJE UVID U  ŠIROK SPEKTAR DUGIH  NEKODIRAJUĆIH RNA SPUŽVI  (PORIFERA) / <i>SEQUENCING OF ENDEMIC</i>  <i>CAVE SPONGE (Eunapius subterraneus)</i>  <i>TRANSCRIPTOMES REVEALS A</i>  <i>COMPREHENSIVE SET OF LONG NON-</i>  <i>CODING RNAs IN SPONGES (PORIFERA)</i>  (M-B2)</p>
10:45-10:50	<p> <b>FILIP KLIČEK</b>  OPTIMIZACIJA METODE ZA  PROČIŠĆAVANJE SLOBODNIH TE  FLUORESCENTNO OBLIJEŽENIH N-  GLIKANA / <i>OPTIMISATION OF CLEAN-</i>  <i>UP PROCEDURE OF RELEASED AND</i>  <i>FLUORESCENTLY LABELED N-</i>  <i>GLYCANS</i> (M-K2)</p>	<p> <b>LUCIJA JUKIĆ</b>  PRAĆENJE DONOR SPECIFIČNIH  ANTITIJELA SUSTAVA HLA U  TRANSPLANTACIJI BUBREGA /  <i>MONITORING OF HLA DONOR SPECIFIC</i>  <i>ANTIBODIES IN KIDNEY</i>  TRANSPLANTATION (M-B3)</p>

10:50-10:55	 <b>PETRA MALEŠ</b> VOLE LI MIJELINSKI BAZIČNI PROTEIN I FOSFOTIDILKOLINSKI LIPIDI PLESATI ZAJEDNO? / <i>DO THE MYELIN BASIC PROTEIN AND PHOSPHATIDYLCHOLINE LIPIDS LIKE TO DANCE TOGETHER?</i> (M-K3)	 <b>BRUNO ŠLAUS</b> FUNKCIJE LUMINOZITETA AKTIVNIH GALAKTIČKIH JEZGRI UNUTAR BAYESOVOG FORMALIZMA / <i>LUMINOSITY FUNCTIONS OF ACTIVE GALACTIC NUCLEI WITHIN THE BAYESIAN FRAMEWORK</i> (M-F1)
10:55-11:00	 <b>LEA PAŠALIĆ</b> UTJECAJ NH <sub>4</sub> <sup>+</sup> I Gdm <sup>+</sup> KATIONA NA TERMIČKA SVOJSTVA DPPS LIPIDNIH MEMBRANA / <i>IMPACT OF NH<sub>4</sub><sup>+</sup> AND Gdm<sup>+</sup> CATIONS ON THERMAL PROPERTIES OF DPPS LIPID MEMBRANES</i> (M-K4)	 <b>EDI GLJUŠIĆ</b> PONOVRNO OTKRIĆE VRSTE <i>Digenea simplex</i> (WULFEN) C. AGARDH 1822 (RHODOMELACEAE, CERAMIALES) U SJEVERNOM JADRANU / <i>REDISCOVERY OF Digenea simplex (WULFEN) C. AGARDH 1822 (RHODOMELACEAE, CERAMIALES) IN THE NORTHERN ADRIATIC</i> (M-O1)
11:00-11:05	 <b>IVA KOKOTOVIĆ</b> UPOMOĆ, JA SAM U OKRUŽENJU VIŠESTRUKOG STRESA! / <i>HELP ME OUT, I'M IN A MULTISTRESS ENVIRONMENT!</i> (M-B1)	 <b>IVONA BANIČEK</b> 45000 GODINA KLIMATSKIH PROMJENA NA PODRUČJU KORNATA – PALINOLOŠKA I GEOKEMIJSKA ISTRAŽIVANJA / <i>45000 YEARS OF KORNATI ISLANDS' CLIMATE CHANGE – PALYNOLOGICAL AND GEOCHEMICAL INVESTIGATION</i> (M-GL1)

11:05-11:35	Pauza za kavu <i>Coffee break</i>
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11:35-12:45	<b>Sekcija 2A: Usmena i mikro-izlaganja</b> <b>Section 2A: Oral and Flash Presentations</b>  Predavaonica – <i>Lecture Hall: A1</i> Moderator – <i>Moderator: Ivan Biočić</i>	<b>Sekcija 2B: Usmena i mikro-izlaganja</b> <b>Section 2B: Oral and Flash Presentations</b>  Predavaonica – <i>Lecture Hall: A2</i> Moderatorica – <i>Moderator: Zuzana Redžović</i>
11:35-11:45	 <b>VALENTINA MARTINEZ</b> SELEKTIVNA PRETVORBA CO <sub>2</sub> U METANOL PREKO BIMETALNOG MOF-74 KATALIZATORA / <i>SELECTIVE CONVERSION OF CO<sub>2</sub> TO METHANOL OVER BIMETALLIC MOF-74 CATALYST</i> (O-K3)	 <b>LUKA OZDANOVAC</b> DERIVATIZACIJA LINAKLOTIDA I GOVEDEG SERUMSKOG ALBUMINA S AKRILAMIDOM I 4-VINILPIRIDINOM UZ ANANLIZU MALDI-TOF/TOF I VEZANIM SUSTAVOM nanoUPLC-ESI-QTOF / <i>DERIVATISATION OF LINACLOTIDE AND BOVINE SERUM ALBUMIN WITH ACRYLAMIDE AND 4-VINYLPYRIDINE ANALYZED BY MALDI-TOF/TOF AND nanoUPLC COUPLED TO THE ESI-QTOF</i> (O-K4)



11:45-11:55	 <b>MARIJA KOVAČEVIĆ</b> SVEUBOHVATNA PROCJENA UTJECAJA TRI STROBILURINSKA FUNGICIDA NA VRSTU <i>Enchytraeus albidus</i> / <i>COMPREHENSIVE ASSESSMENT OF THE INFLUENCE OF THREE STROBILURIN FUNGICIDES ON Enchytraeus albid</i> (O-B2)	 <b>TAMARA ĐERĐ</b> MODELIRANJEM KA SMANJENJU UPOTREBE INSEKTICIDA U KONTROLI POPULACIJA KOMARACA / <i>MODELLING FOR REDUCED INSECTICIDE USE IN MOSQUITO POPULATION CONTROL</i> (O-B3)
11:55-12:05	 <b>RAFAELA RADIČIĆ</b> LASERSKA SINTEZA NANOČESTICA CINKOVOG OKSIDA DOPIRANIH SA SREBROM, ZLATOM I PLATINOM / <i>LASER SYNTHESIS OF SILVER, GOLD, AND PLATINUM DOPED ZINC OXIDE NANOPARTICLES</i> (O-F1)	 <b>NIVES NOVOSEL</b> POVRŠINSKA SVOJSTVA I PONAŠANJE STANICA ALGA KAO MARKERI STRESA U AKVATIČKIM EKOSUSTAVIMA / <i>ALGAL CELL SURFACE PROPERTIES AND BEHAVIOUR AS STRESS MARKERS FOR AQUATIC ECOSYSTEMS</i> (O-O3)
12:05-12:15	 <b>MATTEO MRAVIĆ</b> POTRAGA ZA EKSTREMALNIM SAMODUALNIM Z <sub>4</sub> -KODOVIMA / <i>SEARCH FOR EXTREMAL SELF-DUAL Z<sub>4</sub>-CODES</i> (O-M1)	 <b>JURICA SABOL</b> MIKROPALAEONTOLOŠKA ISTRAŽIVANJA MIOCENSKIH NASLAGA LOKALITETA BUKOVA GLAVA (NAŠICE) / <i>MICROPALAEONTOLOGICAL RESEARCH OF THE MIOCENE DEPOSITS FROM BUKOVA GLAVA (NAŠICE) LOCALITY</i> (O-GL1)
12:15-12:20	 <b>MARINA ČANČAR</b> ODABIR VELIČINSKE FRAKCIJE ZA ANALIZU EOCENSKE PLANKTONSKE FORAMINIFERSKE ZAJEDNICE IZ NASLAGA DINARSKOG PREDGORSKOG BAZENA / <i>SELECTION OF A SIZE FRACTION FOR THE ANALYSIS OF THE EOCENE PLANKTONIC FORAMINIFERAL ASSEMBLAGE FROM THE DINARIC FORELAND BASIN DEPOSITS</i> (M-GL2)	 <b>RAJKO ROLJIĆ</b> MORFOMETRIJSKE KARAKTERISTIKE KAMENIH CRVENIČIJA IZ RAZLIČITIH VODENIH TOKOVA ISTOČNE HERZEGOVINE (M-B5)
12:20-12:25	 <b>ANA MIKELIĆ</b> MODELI INHIBICIJSKE AKTIVNOSTI FLUORIRANIH <i>Cinchona</i> ALKALOIDA DOBIVENI STROJNIM UČENJEM / <i>MACHINE LEARNING DETERMINED MODELS OF INHIBITORY ACTIVITIES FOR FLUORINATED Cinchona ALKALOIDS</i> (M-K5)	 <b>SANDRA VITKO</b> ODGOVOR NA SOLNI I OSMOTSKI STRES U UROČNJAKA ( <i>Arabidopsis thaliana</i> (L.) Heynh.) S PROMIJENJENOM EKSPRESIJOM PROTEINA BPM I DMS3 / <i>SALT AND OSMOTIC STRESS RESPONSE IN THALE CRESS (Arabidopsis thaliana (L.) Heynh.) WITH MODIFIED EXPRESSION OF BPM AND DMS3 PROTEINS</i> (M-B6)

12:25-12:30	 <b>ZLATAN SPAHIĆ</b> MEHANOKEMIJSKA SINTEZA O-SUPSTITUIRANIH OKSIMA KINUKLIDIN-3-ONA / <i>MECHANOCHEMICAL SYNTHESIS OF O-SUBSTITUTED QUINUCLIDIN-3-ONE OXIMES (M-K6)</i>	 <b>KRISTINA KRIŽNJAK</b> ANALIZA DINAMIKE RASTA ŠKOLJKAŠA <i>Pecten jacobaeus</i> (LINNAEUS, 1758) / <i>GROWTH DYNAMICS ANALYSIS OF THE BIVALVE Pecten jacobaeus (LINNAEUS, 1758) (M-O2)</i>
12:30-12:35	 <b>SARA MARIJAN</b> STRUKTURNA I ELEKTRIČNA STUDIJA NATRIJEVE FOSFATNE STAKLO-(KERAMIKE) S OKSIDIMA NIOBIJA(V) I VANADIJA(V) / <i>STRUCTURAL AND ELECTRICAL STUDY OF SODIUM PHOSPHATE GLASS-(CERAMICS) CONTAINING NIOBIUM(V) AND VANADIUM(V) OXIDES (M-K7)</i>	 <b>KARLA ORLIĆ</b> PRISUTNOST ANTIBIOTSKE REZISTENCIJE U AKVAKULTURI ŠKOLJKAŠA NA PODRUČJU ISTOČNOG JADRANA / <i>PRESENCE OF ANTIBIOTIC RESISTANCE IN SHELLFISH AQUACULTURE IN THE EASTERN ADRIATIC (M-O3)</i>
12:35-12:40	 <b>LEONARDA VUGRIN</b> ZNAČAJNA POVEZANOST KINETIKE I PRIJENOSA ENERGIJE U MEHANOKEMIJSKIM REAKCIJAMA / <i>THE DEEP LINK BETWEEN ENERGY TRANSFER AND KINETICS IN MECHANOCHEMICAL REACTIONS (M-K8)</i>	 <b>IGOR PEJNOVIĆ</b> ODABIR EFIKASNE LABORATORIJSKE METODE PRIPREME UZORAKA: PRIMJER SREDNJE EOCENSKIH LAPORA (UVALA PODSTINE, OTOK HVAR) / <i>SORTING LABORATORY TECHNIQUES OF SAMPLE PREPARATION BY EFFICIENCY: AN EXAMPLE OF A MIDDLE EOCENE MARL (PODSTINE COVE, ISLAND OF HVAR) (M-GL3)</i>
12:40-12:45	 <b>LUKA POLOVIĆ</b> SVEOBUH VATNO ISTRAŽIVANJE SVOJSTVA OTPORNOSTI I IMUNOSTI NA ISUŠIVANJE RAZLIČITIH BIOTA U POVREMENIM RIJEKAMA I BUJIČNIM POTOCIMA / <i>COMPREHENSIVE RESEARCH OF DRYING RESISTANCE AND RESILIENCE TRAITS BY DIFFERENT BIOTA IN INTERMITTENT RIVES AND EPHEMERAL STREAMS (M-B4)</i>	 <b>SEBASTIJAN HORVAT</b> BISIMULACIJE ZA GENERALIZIRANU VELTMANOVU SEMANTIKU / <i>BISIMULATIONS FOR GENERALISED VELTMAN SEMANTICS (M-M1)</i>
12:45-13:45	Pauza za ručak <i>Lunch Break</i>	

13:45–14:15	<p><b>Pozvano predavanje 2 – <i>Invited Lecture 2</i></b></p> <p><b>prof. dr. sc. MLADEN VICTOR WICKERHAUSER</b></p> <p>SUPERPOZICIJA I ORTOGONALNOST OD POLINOMA DO VALIĆA / <i>SUPERPOSITION AND ORTHOGONALITY FROM POLYNOMIALS TO WAVELETS</i></p> <p style="text-align: right;">Predavaonica – <i>Lecture Hall: A1</i></p> <p style="text-align: right;">Moderator – <i>Moderator: prof. dr. sc. Eduard Marušić-Paloka</i></p>
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14:15–14:25	<p>Predstavljanje zlatnog sponzora (IN2 grupa) <i>Golden Sponsor Promotion (IN2 Group)</i></p> <p style="text-align: right;">Predavaonica – <i>Lecture Hall: A1</i></p> <p style="text-align: right;">Moderatorica – <i>Moderator: Zuzana Redžović</i></p>
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14:25–15:05	<b>Sekcija 3A: Usmena izlaganja</b> <i>Section 3A: Oral Presentations</i>  Predavaonica – <i>Lecture Hall: A1</i> Moderator – <i>Moderator: Ivan Biočić</i>	<b>Sekcija 3B: Usmena izlaganja</b> <i>Section 3B: Oral Presentations</i>  Predavaonica – <i>Lecture Hall: A2</i> Moderator – <i>Moderator: Mateo Kruljac</i>
14:25–14:35	 <p><b>MATEA ZUBOVIĆ</b> KONSTRUKCIJE USMJERENIH REGULARNIH GRAFOVA IZ GRUPE / <i>CONSTRUCTIONS OF DIRECTED REGULAR GRAPHS FROM GROUPS (O-M2)</i></p>	 <p><b>SANJA RENKA</b> KAKO LITIJEVA FOSFATNA STAKLA UČINITI BOLJIM ELEKTRIČNIM VODIČIMA? – UČINAK STRUKTURNIH PROMJENA / <i>HOW TO MAKE LITHIUM PHOSPHATE GLASSES BETTER IONIC CONDUCTORS? – EFFECT OF STRUCTURAL MODIFICATION (O-K6)</i></p>
14:35–14:45	 <p><b>VALENTINA EVIĆ</b> ULOGA MISTRANSLACIJE U ODGOVORU NA OKSIDACIJSKI STRES U BAKTERIJI <i>Escherichia coli</i> / <i>THE ROLE OF MISTRANSLATION IN OXIDATIVE STRESS RESPONSE IN BACTERIA Escherichia coli (O-K5)</i></p>	 <p><b>MARIJA BAJICA</b> SKUSTVO U RAZVOJU ALATA ZA PROCJENU ODRŽIVOSTI PROJEKTA / <i>EXPERIENCES WITH DEVELOPING PROJECT SUSTAINABILITY ASSESSMENT TOOLS (O-B5)</i></p>
14:45–14:55	 <p><b>MARIJA ROZMAN</b> MOLEKULARNA RAZNOLIKOST VIRUSA EPSTEIN-BARR / <i>MOLECULAR DIVERSITY OF EPSTEIN-BARR VIRUS (O-B4)</i></p>	 <p><b>FILIP MATKOVIĆ</b> RAZLIKE FIZIKALNIH SVOJSTAVA IZMEĐU KORONINIH SVIJETLIH TOČAKA UNUTAR I IZVAN KORONINIH ŠUPLJINA / <i>DIFFERENCES IN PHYSICAL PROPERTIES BETWEEN CORONAL BRIGHT POINTS WITHIN AND OUTSIDE CORONAL HOLES (O-F3)</i></p>

14:55–15:05	 <b>NAVEEN SINGH DHAMI</b> <i>HIGH-PRESSURE STUDIES IN STRONGLY CORRELATED SYSTEMS (O-F2)</i>	 <b>LEA RUŽANOVIĆ</b> KOLONIZACIJA SUHOG RIJEČNOG KORITA SEMIAKVATIČKIM I TERESTRIČKIM BESKRALJEŽNJACIMA (TSAI) / <i>DRY RIVERBED COLONISATION BY TERRESTRIAL AND SEMIAQUATIC INVERTEBRATES (TSAI) (O-B6)</i>
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15:05–15:35	Pauza za kavu <i>Coffee Break</i>
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15:35–17:35	Posterska sekcija <i>Poster Section</i>  Popis izlagača na stranicama 12–19. <i>Presenters listed on pages 12–19.</i>	Moderatorice – <i>Moderators:</i> Anđela Bačinić Katarina Pavlek
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17:35–19:00	Kviz <i>Quiz</i>
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19:00–21:00	Afterparty
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NEDJELJA 24. travnja 2022. g.  
 SUNDAY April 24, 2022

9:00	Registracija – <i>Registration</i>
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9:30–10:00	Pozvano predavanje 3 – <i>Invited Lecture 3</i> <b>prof. dr. sc. SANJA FAIVRE</b> RELATIVNE PROMJENE MORSKE RAZINE DUŽ ISTOČNE OBALE JADRANA TIJEKOM KASNOG HOLOCENA / <i>RELATIVE SEA-LEVEL CHANGES ALONG THE EASTERN ADRIATIC COAST DURING LATE HOLOCENE</i> <div style="text-align: right;">           Predavaonica – <i>Lecture Hall: A1</i>            Moderatorica – <i>Moderator: Katarina Pavlek</i> </div>
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10:00–10:50	<b>Sekcija 4A: Usmena izlaganja</b> <i>Section 4A: Oral Presentations</i> Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: Marija Petrović</i>	<b>Sekcija 4B: Usmena izlaganja</b> <i>Section 4B: Oral Presentations</i> Predavaonica – <i>Lecture Hall: A2</i> Moderatorica – <i>Moderator: Zuzana Redžović</i>
10:00–10:10	 <b>KLAUDIJA IVANKOVIĆ</b> RAZVOJ MULTIREZIDUALNE LC/MS METODE ZA ODREĐIVANJE KONCENTRACIJA FARMACEUTIKA U VODENOM OKOLIŠU / <i>DEVELOPMENT OF MULTIREZIDUAL LC-MS/MS METHOD FOR THE ASSESSMENT OF TRACE LEVELS OF PHARMACEUTICALS IN AQUATIC ENVIRONMENT (O-K7)</i>	 <b>LAURA HULJEK</b> GEOKEMIJSKA ANALIZA MANGANSKE JALOVINE IZ ŠIBENSKOG ZALJEVA (HRVATSKA) I NJENI OKOLIŠNI UTJECAJI / <i>GEOCHEMICAL ANALYSIS OF MANGANESE TAILINGS FROM ŠIBENIK BAY (CROATIA) AND THEIR ENVIRONMENTAL IMPACT (O-GL2)</i>
10:10–10:20	 <b>KARLA PERANIĆ</b> VERTIKALNI PRIJENOS VIRUSA CRYPHONECTRIA HYPOVIRUS 1 DOVODI DO EFEKTA USKOG GRLA / <i>LONG READ SEQUENCING REVEALS MUTATIONAL BOTTLENECK AFTER VERTICAL TRANSMISSION OF CRYPHONECTRIA HYPOVIRUS 1 (O-B7)</i>	 <b>IVAN IVIĆ</b> REGIONALNE RAZLIKE UČINKOVITOSTI NASTAVE GEOGRAFIJE U REPUBLICI HRVATSKOJ / <i>REGIONAL DIFFERENCES ON THE EFFICIENCY OF GEOGRAPHY TEACHING IN THE REPUBLIC OF CROATIA (O-GG2)</i>
10:20–10:30	 <b>MARIN SPAIĆ</b> ISTRAŽIVANJE LOKALNE STRUKTURE DOPIRANOG BIZMUTATA $Ba_{1-x}K_xBiO_3$ / <i>INVESTIGATING THE LOCAL STRUCTURE OF DOPED BISMUTHATE <math>Ba_{1-x}K_xBiO_3</math> (O-F4)</i>	 <b>ANDREA BILAJAC</b> RASPROSTRANJENOST, MORFOMETRIJSKE KARAKTERISTIKE I SASTAV EPIFITA VRSTE <i>Gongolaria Barbata</i> U LAGUNI ŠĆUZA / <i>DISTRIBUTION, MORPHOMETRIC CHARACTERISTICS AND EPIPHYTE COMPOSITION OF <i>Gongolaria Barbata</i> IN THE ŠĆUZA LAGOON (O-O4)</i>







10:30-10:40	 <b>ANA TERLEVIĆ</b> PRIMJENA INTEGRATIVNOG PRISTUPA ZA RAZJAŠNJENJE RAZNOLIKOSTI VRSTE <i>Dianthus sylvestris</i> Wulfen s.l. NA BALKANSKOM POLUOTOKU / <i>DISENTANGLING THE DIVERSIFICATION OF Dianthus sylvestris Wulfen s.l. ON THE BALKAN PENINSULA USING AN INTEGRATIVE APPROACH</i> (O-B8)	 <b>ANA RAMLJAK</b> UTVRĐIVANJE EKOLOŠKOG STANJA MORSKIH SEDIMENATA U SVRHU PREDLAGANJA LISTE MIKROBNIH POKAZATELJA ZA POSTIZANJE DOBROG STANJA OKOLIŠA / <i>DETERMINATION OF THE ECOLOGICAL CONDITION OF MARINE SEDIMENTS FOR THE PURPOSE OF PROPOSING A LIST OF MICROBIAL INDICATORS FOR ACHIEVING GOOD ENVIRONMENTAL STATUS</i> (O-B10)
10:40-10:50	 <b>GABRIJELA MATIJEVIĆ</b> EKOTOKSIKOLOŠKA PROCJENA UTJECAJA KARDIOVASKULARNIH FARMACEUTIKA PRISUTNIH U VODENIM EKOSUSTAVIMA NA EMBRIJE RIBE ZEBRICE <i>Danio rerio</i> (Hamilton, 1822) / <i>ECOTOXICOLOGICAL ASSESSMENT OF THE IMPACT OF CARDIOVASCULAR PHARMACEUTICALS PRESENT IN AQUATIC ECOSYSTEMS ON ZEBRAFISH EMBRYOS Danio rerio (Hamilton, 1822)</i> (O-B9)	 <b>ARBEN BERIŠA</b> STEREOSELEKTIVNA ARILACIJA DERIVATA IZOINDOLINONA U ORGANOKATALITIČKIM UVJETIMA / <i>ORGANOCATALYTIC STEREOSELECTIVE ARYLATION OF ISOINDOLINONE DERIVATIVES</i> (O-K8)

10:50-11:20	Pauza za kavu <i>Coffee Break</i>
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11:20-11:50	Pozvano predavanje 4 – <i>Invited Lecture 4</i> <b>prof. dr. sc. DOMINIK CINČIĆ</b> KRISTALNO INŽENJERSTVO METALOORGANSKIH KOKRISTALA TEMELJENIH NA HALOGENSKIM VEZAMA / <i>CRYSTAL ENGINEERING OF HALOGEN-BONDED METAL-ORGANIC COCRYSTALS</i> <div style="text-align: right;">           Predavaonica – <i>Lecture Hall: A1</i>            Moderatorica – <i>Moderator: izv. prof. dr. sc. Marijana Đaković</i> </div>
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11:50-12:05	Predstavljanje Veleposlanstva Sjedinjenih Američkih Država u Hrvatskoj <i>Presentation of U.S. Embassy in Croatia</i> <div style="text-align: right;">           Predavaonica – <i>Lecture Hall: A1</i>            Moderatorica – <i>Moderator: Zuzana Redžović</i> </div>
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12:05–12:45	<b>Sekcija 5: Usmena izlaganja</b> <b>Section 5: Oral Presentations</b>	Predavaonica – <i>Lecture Hall</i> : A1 Moderatorica – <i>Moderator</i> : Petra Stanić
12:05–12:15	 <b>DOMAGOJ GAJSKI</b> PRIRODNA PREHRANA ZIMSKO-AKTIVNIH PAUKOVA NA STABLIMA KRUŠAKA / <i>THE NATURAL DIET OF WINTER ACTIVE SPIDERS ON PEAR TREES (O-B11)</i>	
12:15–12:25	 <b>KATARINA PAVLEK</b> PROMJENE U BOČNOM KRETANJU KORITA RIJEKE ORLJAVE OD SREDINE 20. STOLJEĆA / <i>CHANGES IN LATERAL CHANNEL MIGRATION ON THE ORLJAVA RIVER SINCE THE MID-20TH CENTURY (O-GG3)</i>	
12:25–12:35	 <b>LUKA BAREŠIĆ</b> SINTEZA NOVIH OKSANORBORNANA SUPSTITURIJANIH GVANIDINOM I NJIHOVA KOKATALITIČKA AKTIVNOST U ALDOLNOJ REAKCIJI / <i>SYNTHESIS OF NOVEL GUANIDINIUM- SUBSTITUTED OXANORBORNANES AND THEIR COCATALYTIC ACTIVITY IN ALDOL REACTION (O-K9)</i>	
12:35–12:45	 <b>NARMIN BEYDIZADA</b> PERSONALITY PREDICTS MODE OF ATTACK IN A GENERALIST GROUND SPIDER PREDATOR (O-B12)	

12:45–13:45	Pauza za ručak <i>Lunch Break</i>
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13:45–14:25	Radionica – <i>Workshop</i> <b>SeaCras: dr. sc. STIPE LUKIN, TOMISLAV STOLAR, mag. chem., dr. sc. MARIO ŠPADINA</b> <i>PITCHING</i>	
14:25–15:05	<b>dr. sc. ERNEST MEŠTROVIĆ</b> JASAN I NEDVOSMISLEN IZRAŽAJ – PUT PREMA ZNANSTVENOJ IZVRSNOSTI / <i>CLEAR AND UNAMBIGUOUS EXPRESSION – THE PATH TOWARDS SCIENTIFIC EXCELLENCE</i>	
15:05–15:45	<b>prof. dr. sc. VERNESA SMOLČIĆ</b> KAKO NAPISATI USPJEŠNU ZNANSTVENU PRIJAVU / <i>HOW TO WRITE A SUCCESSFUL GRANT PROPOSAL</i>	Predavaonica – <i>Lecture Hall</i> : A1 Moderatorica – <i>Moderator</i> : izv. prof. dr. sc. Petra Korać

15:45–16:15	Pauza za kavu <i>Coffee Break</i>
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16:15–17:05	<b>Sekcija 6: Usmena izlaganja</b> <b>Section 6: Oral Presentations</b>	Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: Katarina Pavlek</i>
16:15–16:25	 <b>MARINO BEBIĆ</b> ANTROPOGENI UTJECAJI NA KRŠKE VODONOSNIKE NA PRIMJERU BLATSKOG VODONOSNIKA NA OTOKU KORČULI / <i>ANTHROPOGENIC INFLUENCES ON A KARST ISLAND AQUIFER, THE BLATO AQUIFER ON THE ISLAND OF KORČULA, CROATIA (O-GL3)</i>	
16:25–16:35	 <b>MATEJ BUBAŠ</b> MODULIRANJE STVARANJA VRUĆIH NOSIOCA NABOJA LEGIRANJEM: IMPLIKACIJE ZA PLAZMONIČKU FOTOKATALIZU / <i>MODULATING HOT CARRIER GENERATION BY ALLOYING: IMPLICATIONS FOR PLASMONIC PHOTOCATALYSIS (O-K10)</i>	
16:35–16:45	 <b>MARINA VESELI</b> BIOAKUMULACIJA I BIOAMPLIFIKACIJA FARMACEUTSKI AKTIVNIH SPOJEVA I ENDOKRINIH DISRUPTORA U VRSTI TULARA <i>Silo nigricornis</i> / <i>BIOACCUMULATION AND BIOAMPLIFICATION OF PHARMACEUTICALS AND ENDOCRINE DISRUPTING COMPOUNDS IN CADDISFLY Silo nigricornis (O-B13)</i>	
16:45–16:55	 <b>MARINELA PILJ VIDAKOVIĆ</b> UPRAVLJANJE VIBRACIJSKIH SUSTAVA U SLUČAJU KONAČNOG VREMENSKOG HORIZONTA / <i>FINITE TIME HORIZON MIXED CONTROL OF VIBRATIONAL SYSTEMS (O-M3)</i>	
16:55–17:05	 <b>IVAN BALKOVIĆ</b> PROSTORNO-VREMENSKA VARIJABILNOST ZAJEDNICA MAKROZOOBENTOSA PRIJELAZNIH VODA NERETVE, JADRA I RJEČINE / <i>SPATIO-TEMPORAL VARIABILITY OF MACROZOOBENTHIC ASSEMBLAGES IN TRANSITIONAL WATERS OF NERETVA, JADRO AND RJEČINA RIVERS (O-O5)</i>	
17:05–17:35	Predstavljanje zlatnih sponzora (Ericsson, Selvita, Oikon) <i>Golden Sponsors Promotion (Ericsson, Selvita, Oikon)</i>	Predavaonica – <i>Lecture Hall: A1</i> Moderatorica – <i>Moderator: Zuzana Redžović</i>
17:35	Zatvaranje Simpozija i dodjela nagrada <i>Closure of the Symposium and Award Ceremony</i>	Predavaonica – <i>Lecture Hall: A1</i>

**POSTER SEKCIJA - LISTA IZLAGAČA**

SUBOTA 23. TRAVNJA 2022. G., 15:35–17:35

**POSTER SECTION - PRESENTERS LIST**

SATURDAY, APRIL 23, 2022, 15:35 –17:35

Moderatorice – *Moderators:* Anđela Bačinić  
Katarina Pavlek

**BIOLOGIJA – BIOLOGY**

-  **DORA BJEDOV**  
PROCJENA BIOMARKERA U KRVI PTIČA BIJELE RODE S RAZLIČITO ZAGAĐENIH PODRUČJA U HRVATSKOJ / *NO HARM DONE: BIOMARKER ASSESSMENT IN WHITE STORK NESTLINGS' BLOOD FROM DIFFERENTLY POLLUTED AREAS IN CROATIA (P-B1)*
-  **KRISTIAN BODULIĆ**  
SEKVENCIJANJE TRANSKRIPTOMA OGULINSKE ŠPILJSKE SPUŽVICE (*Eunapius subterraneus*) DAJE UVID U ŠIROK SPEKTAR DUGIH NEKODIRAJUĆIH RNA SPUŽVI (PORIFERA) / *SEQUENCING OF ENDEMIC CAVE SPONGE (Eunapius subterraneus) TRANSCRIPTOMES REVEALS A COMPREHENSIVE SET OF LONG NON-CODING RNAs IN SPONGES (PORIFERA) (M-B2)*
-  **DIANA CULEJ**  
MODULIRANJE EKSPRESIJE PROTEINA SUSTAVA UROKINAZNOG PLAZMINOGENSKOG AKTIVATORA I NJIHOV UTJECAJ NA OTPORNOST NA KEMOTERAPEUTIKE / *MODULATION OF UROKINASE PLASMINOGEN ACTIVATOR SYSTEM AND RESISTANCE TO CHEMOTERAPY (P-B2)*
-  **JOSIPA ČONKAŠ**  
ULOGA ANDROGENA I RECEPTORA ANDROGENA U METASTAZIRANJU TUMORA GLAVE I VRATA / *THE ROLE OF ANDROGEN AND ANDROGEN RECEPTORS IN METASTASIS OF HEAD AND NECK TUMORS (P-B3)*
-  **ERIKA GAMULIN**  
USPOSTAVA OVČJEG EKSPERIMENTALNOG MODELA KAO PREDUVJET ZA ISTRAŽIVANJE FARMAKOKINETIKE BIOTERAPEUTIKA / *ESTABLISHMENT OF SHEEP EXPERIMENTAL MODEL AS A POSTULATE FOR RESEARCH OF PHARMACOKINETICS OF BIOTHERAPEUTICS (P-B4)*
-  **IVANA GRGIĆ**  
POD STRESOM - MOLEKULARNI ODGOVORI VODENIH KUKACA I MAHOVINE NA IZLAGANJE VIŠESTRUKOM STRESU / *UNDER PRESSURE – MOLECULAR RESPONSES OF AQUATIC INSECTS AND MOSS TO MULTIPLE STRESSORS (P-B5)*
-  **DOMAGOJ HACKENBERGER KUTUZOVIĆ**  
DUBOKO I PODRŽANO UČENJE U KONTROLI POPULACIJA KOMARACA / *DEEP AND REINFORCEMENT LEARNING IN MOSQUITO POPULATION CONTROL (P-B6)*
-  **LARA IVANKOVIĆ TATALOVIĆ**  
UTJECAJ IZLAGANJA TIAMOTOKSAMOM NA HRANJENJE, KRETANJE I METABOLIZAM VRSTE *Abax parallelus* (COLEOPTERA: CARABIDAE) / *THE IMPACT OF THIAMETHOXAM EXPOSURE ON FEEDING, LOCOMOTOR ACTIVITY, AND METABOLOMICS OF Abax parallelus (COLEOPTERA: CARABIDAE) (P-B7)*
-  **LUCIJA JUKIĆ**  
PRAĆENJE DONOR SPECIFIČNIH ANTITIJELA SUSTAVA HLA U TRANSPLANTACIJI BUBREGA / *MONITORING OF HLA DONOR SPECIFIC ANTIBODIES IN KIDNEY TRANSPLANTATION (M-B3)*
-  **IVANA JURIC**  
AKTIVNOST ERAVACIKLINA NA ENTEROBAKTERIJE OTPORNE NA KARBAPENEME U UVJETIMA *IN VITRO* – PRVI IZVJEŠTAJ IZ HRVATSKE / *IN VITRO ACTIVITY OF ERAVACYCLINE ON CARBAPENEM-RESISTANT Enterobacterales – THE FIRST REPORT FROM CROATIA (P-B8)*

-  **TOMISLAV KRALJ**  
STRANI I AUTOHTONI PERAKARIDNI RAKOVI U ZAJEDNICI MAKROSKOPSKIH BESKRALJEŠNJAKA U VELIKIM RIJEKAMA HRVATSKE / *ALIEN AND NATIVE PERACARIDA IN MACROINVERTEBRATE ASSEMBLAGES OF CROATIAN MAJOR RIVERS (P-B9)*
-  **IVA KOKOTOVIĆ**  
UPOMOĆ, JA SAM U OKRUŽENJU VIŠESTRUKOG STRESA! / *HELP ME OUT, I'M IN A MULTISTRESS ENVIRONMENT (M-B1)*
-  **MAJA LEDINSKI**  
UTJECAJ ČVRSTIH LIPIDNIH NANOČESTICA S UGRAĐENIM ASKORBIL PALMITATOM NA MATIČNE STANICE SARKOMA / *THE EFFECT OF ASCORBIL PALMITATE-INCORPORATED SOLID LIPID NANOPARTICLES ON SARCOMA STEM CELLS (P-B10)*
-  **ANAMARI MAJDANDŽIĆ**  
ANTIOKSIDACIJSKI ODGOVOR MAHOVINA NA SUŠNI STRES DUŽ VISINSKOG GRADIJENTA / *ANTIOXIDATIVE RESPONSES IN MOSSES TO DROUGHT STRESS ALONG AN ALTITUDINAL GRADIENT (P-B11)*
-  **IVA MARKOVIĆ**  
ANTIOKSIDACIJSKI ODGOVOR U SA-DEFICIJENTNIM BILJKAMA I BILJAKA DIVLJEG TIPA KRUMPIRA (*Solanum tuberosum L.*) NA INFEKCIJU VIROIDOM VRETENASTOG GOMOLJA KRUMPIRA, PSTVd / *ANTIOXIDATIVE RESPONSE OF SA-DEFICIENT AND WILD TYPE POTATO PLANTS (Solanum tuberosum L.) TO INFECTION WITH POTATO SPINDLE TUBER VIROID (P-B12)*
-  **MARTINA MILOLOŽA**  
BIORAZGRADNJA MIKROPLASTIKE PRIMJENOM *Bacillus licheniformis* / *BIODEGRADATION OF MICROPLASTICS BY Bacillus licheniformis (P-B13)*
-  **TINA MILOŠ**  
PREVENCIJA I LIJEČENJE VASKULARNE DEMENCIJE: *IN VITRO* ISTRAŽIVANJE POTENCIJALNOG NEUROZAŠTITNOG UČINKA DEHIDROEPIANDROSTERONA (DHEA) I NJEGOVA SULFATA (DHEAS) / *PREVENTION AND TREATMENT OF VASCULAR DEMENTIA: EXPLORING THE NEUROPROTECTIVE POTENTIAL OF DEHYDROEPIANDSTERONE (DHEA) AND DHEA SULPHATE (DHEAS) IN VITRO (P-B14)*
-  **MARIJA NAZLIĆ**  
KLASIČNA I ZELENA EKSTRAKCIJA HLAPIVIH SPOJEVA: USPOREDBA PODATAKA ZA PET VRSTA ČESTOSLAVICA IZ HRVATSKE / *CLASSICAL AND GREEN EXTRACTION OF VOLATILE COMPOUNDS: COMPARING DATA FOR FIVE CROATIAN VERONICA SPECIES (P-B15)*
-  **IVANA PAVIČIĆ**  
PROMJENA SIGNALNIH PUTOVA PROUZROČENIH DEFICIJENCIJOM S-ADENOZILHOMOCISTEIN-HIDROLAZE U STANIČNOJ LINIJI HEK293T / *SIGNALING PATHWAYS CHANGES CAUSED BY S-ADENOSYLHOMOCYSTEINE-HYDROLASE DEFICIENCY IN HEK293T CELLS (P-B16)*
-  **ENA PEŠUT**  
RAZVOJ NOVE METODE MASENE CITOMETRIJE U PREKANCEROZONOM CERVICALNOM PROBIRU / *DEVELOPMENT OF NEW METHOD MASS CYTOMETRY IN PRECANCEROUS CERVICAL SCREENING (P-B17)*
-  **LUKA POLOVIĆ**  
SVEOBUHVAATNO ISTRAŽIVANJE SVOJSTVA OTPORNOSTI I IMUNOSTI NA ISUŠIVANJE RAZLIČITIH BIOTA U POVREMENIM RIJEKAMA I BUJIČNIM POTOCIMA / *COMPREHENSIVE RESEARCH OF DRYING RESISTANCE AND RESILIENCE TRAITS BY DIFFERENT BIOTA IN INTERMITTENT RIVES AND EPHEMERAL STREAMS (M-B4)*
-  **ZUZANA REDŽOVIĆ**  
DINAMIKA POPULACIJA VRSTE *Synurella ambulans* (MÜLLER, 1846) (CRUSTACEA, AMPHIPODA) U HIPOREIČKOJ ZONI RIJEKE SAVE (HRVATSKA) / *POPULATION DYNAMICS OF Synurella ambulans*

(MÜLLER, 1846) (CRUSTACEA, AMPHIPODA) IN THE HYPORHEIC ZONE OF THE SAVA RIVER (CROATIA) (P-B18)



**IVANA STANIĆ**

USPOREDBA MIKROBNIH ZAJEDNICA DVAJU JEZERSKIH SUSTAVA U HRVATSKOJ / *MICROBIAL COMMUNITY COMPARISON OF TWO LAKE SYSTEMS IN CROATIA* (P-B19)



**ELA ŠARIĆ**

MORFOLOŠKE PROMJENE PELETA I STANIČNA SMRT KORELIRAJU S PROIZVODNOM ANTIBIOTIKA U TEKUĆOJ KULTURI BAKTERIJE *Streptomyces Rimosus* / *MORPHOLOGICAL CHANGES OF PELLETS AND CELL DEATH COINCIDE WITH ANTIBIOTIC PRODUCTION IN LIQUID CULTURE OF Streptomyces Rimosus* (P-B20)



**SARA ŠARIRI**

OMJER IZOTOPA  $87\text{Sr}/86\text{Sr}$  KAO PRIRODNI GEOKEMIJSKI MARKER U RIJECI KRKI /  *$87\text{Sr}/86\text{Sr}$  ISOTOPE RATIO AS A NATURAL GEOCHEMICAL MARKER IN THE KRKA RIVER* (P-B21)



**ENA ŠIMUNIĆ**

ANALIZA EKSPRESIJE I BIOKEMIJSKA KARAKTERIZACIJA S-ADENOSILHOMOCISTEIN-HIDROLAZE S NOVOOTKRIVENOM DELECIJOM / *ANALYSIS OF EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE WITH A NOVEL DELETION* (P-B22)



**ANA TUTIĆ**

PROČIŠĆAVANJE OTPADNE VODE KOKSNE INDUSTRIJE / *OVERVIEW OF COKING WASTEWATER TREATMENT METHODS* (P-B23)



**MIRELA UZELAC**

INVAZIVNE BILJNE VRSTE KAO POTENCIJALNI IZVOR FITOFARMACEUTIKA / *INVASIVE PLANTS SPECIES AS POTENTIAL SOURCE OF PHYTOPHARMACEUTICALS* (P-B24)



**DAMIRA VESELJAK**

KARAKTERIZACIJA SATELITOMA KUKCA BRAŠNARA *Tribolium madens* / *SATELLITOME CHARACTERIZATION OF THE BLACK FLOUR BEETLE Tribolium madens* (P-B25)



**SANDRA VITKO**

ODGOVOR NA SOLNI I OSMOTSKI STRES U UROČNJAKA (*Arabidopsis thaliana* (L.) Heynh.) S PROMIJENJENOM EKSPRESIJOM PROTEINA BPM I DMS3 / *SALT AND OSMOTIC STRESS RESPONSE IN THALE CRESS (Arabidopsis thaliana (L.) Heynh.) WITH MODIFIED EXPRESSION OF BPM AND DMS3 PROTEINS* (M-B6)



**MARIN VOLARIĆ**

NANOPORE SEKVENCIRANJE KUKACA RODA *Tribolium* S TVRDIM EGZOSKELETOM / *NANOPORE SEQUENCING OF HARD-CUTICLED Tribolium BEETLES* (P-B26)



**BARBARA VUIĆ**

POTENCIJALNE TERAPIJSKE INTERVENCIJE PROTIV TOKSIČNOSTI AMILOIDA BETA (IN VITRO MODEL ALZHEIMEROVE BOLESTI) / *POTENTIAL THERAPEUTIC INTERVENTIONS TARGETING AMYLOID BETA TOXICITY (IN VITRO MODEL OF ALZHEIMER'S DISEASE)* (P-B27)

#### FIZIKA – PHYSICS



**SAFER GRBIĆ**

GENEZA PRIRODOZNANSTVENIH ISTRAŽIVANJA U DJELU IMMANUELA KANTA / *GENESIS OF NATURAL SCIENCE RESEARCHES IN THE WORKS OF IMMANUEL KANT* (P-F1)



**SARAH IVUŠIĆ**

KLIMATSKE PROMJENE U POLJU OBORINE NA PODRUČJU JADRANA I DINARIDA / *FUTURE PRECIPITATION CHANGES OVER EASTERN ADRIATIC AND DINARIC ALPS* (P-F2)



**BRUNO ŠLAUS**

FUNKCIJE LUMINOZITETA AKTIVNIH GALAKTIČKIH JEZGRI UNUTAR BAYESOVOG FORMALIZMA / *LUMINOSITY FUNCTIONS OF ACTIVE GALACTIC NUCLEI WITHIN THE BAYESIAN FRAMEWORK (M-F1)*

### GEOLOGIJA – GEOLOGY



**IVONA BANIČEK**

45000 GODINA KLIMATSKIH PROMJENA NA PODRUČJU KORNATA – PALINOLOŠKA I GEOKEMIJSKA ISTRAŽIVANJA / *45000 YEARS OF KORNATI ISLANDS' CLIMATE CHANGE – PALYNOLOGICAL AND GEOCHEMICAL INVESTIGATION (M-GL1)*



**MARINA ČANČAR**

ODABIR VELIČINSKE FRAKCIJE ZA ANALIZU EOCENSKE PLANKTONSKE FORAMINIFERSKE ZAJEDNICE IZ NASLAGA DINARSKOG PREDGORSKOG BAZENA / *SELECTION OF A SIZE FRACTION FOR THE ANALYSIS OF THE EOCENE PLANKTONIC FORAMINIFERAL ASSEMBLAGE FROM THE DINARIC FORELAND BASIN DEPOSITS (M-GL2)*



**IGOR PEJNOVIĆ**

ODABIR EFIKASNE LABORATORIJSKE METODE PRIPREME UZORAKA: PRIMJER SREDNJEEOCENSKIH LAPORA (UVALA PODSTINE, OTOK HVAR) / *SORTING LABORATORY TECHNIQUES OF SAMPLE PREPARATION BY EFFICIENCY: AN EXAMPLE OF A MIDDLE EOCENE MARL (PODSTINE COVE, ISLAND OF HVAR) (M-GL3)*



**NATALIA ŠENOLT**

EVOLUCIJA ESTUARIJA RIJEKE KRKE TIJEKOM HOLOCENA / *HOLOCENE EVOLUTION OF THE KRKA RIVER ESTUARY (P-GL1)*

### KEMIJA – CHEMISTRY



**LEA BARBARIĆ**

EKSPRESIJA I PROČIŠĆAVANJE C-TERMINALNE DOMENE PROTEINA SH2D3C U BAKTERIJI *Escherichia coli* / *OVEREXPRESSION AND PURIFICATION OF THE C-TERMINAL DOMAIN OF THE PROTEIN SH2D3C IN Escherichia coli (P-K1)*



**NEA BAUS TOPIĆ**

KOKRISTALI S METALOORGANSKIM GRAĐEVNIM JEDINICAMA KAO DONORIMA HALOGENSKE VEZE / *COCRYSTALS WITH METAL-ORGANIC BUILDING BLOCKS AS HALOGEN BOND DONORS (P-K2)*



**NIKOL BEBIĆ**

ISTRAŽIVANJE UTJECAJA IZVORA ALUMINIJA I KATIONA NA NASTANAK ZEOLITA / *EXPLORING THE EFFECT OF ALUMINUM SOURCES AND CATIONS ON THE FORMATION OF ZEOLITES (P-K3)*



**KRISTINA BULE**

BIORAZGRADNJA MIKROPLASTIKE POLISTIRENA I POLI(VINIL-KLORIDA) PRIMJENOM KVASCA *Saccharomyces cerevisiae* / *BIODEGRADATION OF POLYSTYRENE AND POLYVINYL CHLORIDE MICROPLASTICS USING Saccharomyces cerevisiae YEAST (P-K4)*



**ANDREA ČAČKOVIĆ**

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# Pozvana predavanja

## *Invited Lectures*





## **prof. dr. sc. Mirko Orlić**

Mirko Orlić, redoviti profesor na Prirodoslovno-matematičkom fakultetu Sveučilišta u Zagrebu i redoviti član Hrvatske akademije znanosti i umjetnosti. Doktorirao je na Sveučilištu u Zagrebu, posjetio je nekoliko europskih oceanografskih institucija, radio je kao gostujući znanstvenik na Scripps Institution of Oceanography (SAD). Vodio je niz domaćih i međunarodnih projekata te je sudjelovao u istraživačkim krstarenjima Jadranskim morem, na hrvatskim, američkim i njemačkim istraživačkim brodovima. Istražuje fizikalne procese u moru, međudjelovanje atmosfere i mora te metode analize podataka. Kao autor i koautor objavio je stotinjak znanstvenih radova, većim dijelom u međunarodnim časopisima, više od stotinu pedeset kongresnih priopćenja kao i knjigu o vremenu i klimi jadranskog područja. Njegovi su znanstveni radovi do sada citirani više od 2300 puta prema bazi podataka WoS. Obnašao je funkcije u brojnim sveučilišnim i državnim tijelima, sudjelovao je u organizaciji domaćih i međunarodnih znanstvenih skupova, bio je urednik časopisa *Geofizika*, tri znanstvena zbornika i dvije knjige te gostujući urednik američkog časopisa *Journal of Geophysical Research* i europskog časopisa *Journal of Marine Systems*. Član je različitih strukovnih udruga, primjerice American Geophysical Union i The Oceanography Society. Dobio je Fulbrightovu nagradu, Državnu nagradu za znanost, Nagradu HAZU za najviše znanstveno dostignuće i Nagradu 'Andrija Mohorovičić'

### ***Prof. Mirko Orlić, PhD***

*Mirko Orlić, full professor at the Faculty of Science, University of Zagreb, and fellow of the Croatian Academy of Sciences and Arts. Obtained Ph. D. at the University of Zagreb, visited several oceanographic institutes in Europe, worked as a visiting scholar at the Scripps Institution of Oceanography. Led a number of national and international projects and participated in research cruises in the Adriatic Sea, aboard Croatian, American and German research vessels. Major research interests include physical processes in the sea, atmosphere-sea interaction and methods of data analysis. Authored and co-authored about 100 refereed publications, mostly in international journals, more than 150 conference communications and a book on weather and climate of the Adriatic area. The publications have up to now received more than 2300 citations according to the Web of Science. Served in numerous boards at the university and state levels, participated in the organization of national and international conferences, was editor of Geofizika, of three conference proceedings and of two books, served as guest editor of Journal of Geophysical Research and of Journal of Marine Systems. Member of various professional societies, for example the American Geophysical Union and The Oceanography Society. Obtained the Fulbright Award, the Croatian State Science Award, the Croatian Academy Science Award and the Andrija Mohorovicic Award.*

## KAKO JE METODA RAZVIJENA ZA ISTRAŽIVANJE JADRANA POSTALA KORISNA EKONOMISTIMA

Mirko Orlić<sup>1,2,\*</sup>

<sup>1</sup> Geofizički odsjek, Prirodoslovno-matematički fakultet, Horvatovac 95, Zagreb

<sup>2</sup> Hrvatska akademija znanosti i umjetnosti

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U predavanju se prikazuju rezultati hrvatsko-američkog projekta ITHACA, koji je proveden u srednjem Jadranu. U okviru eksperimenta obavljena su mjerenja CTD-sondama, termistorima i akustičkim strujomjerima, a dobiveni su rezultati uspoređeni sa stalnim mareografskim i meteorološkim mjerenjima. Najvažniji su nalaz bili unutarnji valovi dnevnog perioda, koji kruže oko otoka Lastova te povremeno dovode do osciliranja termokline u rasponu od oko 30 m. Da bi se objasnilo porijeklo tako velikih valova, oscilacije termokline uspoređene su s plimnim djelovanjem s jedne strane te s vjetrovnim utjecajem s druge strane. Radi toga je razvijena nova statistička metoda, tzv. parcijalna valična analiza, koja omogućuje da se dva uzroka povežu s jednom posljedicom. Pokazalo se da unutarnji valovi dnevnog perioda mogu biti rezonantno pobuđeni bilo dnevnim plimnim oscilacijama bilo dnevnom izmjenom vjetra s kopna i vjetra s mora. Taj je nalaz potvrđen rezultatima numeričkog modeliranja. Postoje naznake da unutarnji valovi vezani uz Lastovo potiču primarnu proizvodnju u tom području, budući da vertikalni pomaci povezani s takvim valovima dovode do prijenosa hranjivih tvari iz pridnenog u površinski sloj gdje Sunčevo zračenje omogućuje stvaranje organske materije. Što se tiče parcijalne valične analize, nju su zaokružili i prikazali široj znanstvenoj zajednici istraživači sa Sveučilišta u Hong Kongu pa je našla primjenu u različitim znanstvenim područjima – primjerice, u ekonomiji.

## HOW A METHOD DEVELOPED FOR THE ADRIATIC-RELATED RESEARCH BECAME USEFUL TO ECONOMISTS

Mirko Orlic<sup>1,2,\*</sup>

<sup>1</sup>Department of Geophysics, Faculty of Science, Horvatovac 95, Zagreb, Croatia

<sup>2</sup> Croatian Academy of Sciences and Arts

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In the lecture, results obtained in the framework of the Croatian-US project ITHACA, carried out in the middle Adriatic, are presented. Experimental work encompassed measurements with the CTD-probes, thermistors and ADCPs, and the results obtained were compared to the tide-gauge and meteorological data routinely collected in the area. The most important finding represented diurnal internal waves, which travel around the island of Lastovo and occasionally bring about thermocline oscillations having a range of about 30 m. With the aim of explaining the origin of waves that are so large, thermocline oscillations were compared to tidal forcing on the one hand and to wind influence on the other hand. In order to perform the comparison, a new statistical method, called partial wavelet analysis, has been developed, thus enabling two inputs to be connected with an output. It turned out that diurnal internal waves could be resonantly driven either by diurnal tidal oscillations or by diurnal exchange of land and sea breezes. The finding has been corroborated by results of numerical modeling. There are indications that the Lastovo-trapped internal waves support primary production in the area, because the wave-related vertical displacements result in nutrients being transported from the bottom to the surface layer where solar radiation enables organic matter to be produced. As for the partial wavelet analysis, it had been rounded off and presented to a wider scientific community by researchers from the University of Hong Kong and has subsequently found application in several fields of science – for example, in economics.



## prof. dr. sc. Mladen Victor Wickerhauser

Mladen Victor Wickerhauser, rođen u Zagrebu 1959. g., završio je preddiplomski studij matematike 1980. g. na sveučilištu California Institute of Technology, diplomski studij matematike 1982. g. na Sveučilištu Yale, te je 1985. g. stekao titulu doktora znanosti iz polja matematike također na Sveučilištu Yale.

Trenutno je profesor matematike i statistike te profesor biomedicinskog inženjerstva pri Sveučilištu Washingtonu u Saint Louisu u državi Missouri (SAD). Posjeduje šest patenata te je objavio više od 100 publikacija, uključujući i monografiju "Adapted Wavelet Analysis" (1994.) kao i udžbenik "Mathematics for Multimedia" (2003. i 2009.). Također se bavio i razvojem i publiciranjem matematičkih softvera još od 1979. g. Njegovo istraživanje već je dugo fokusirano na brze aproksimacijske algoritme za računanje na velikim skupovima podataka, uz primjenu teorije valića. Istraživanja su mu pronašla primjene u kompresiji slika (WSQ za otiske prstiju), onkološkoj radijaciji (Monte Carlo planiranje doziranja), i u pedijatriji (slušni pregled EEG-om). Mentorirao je 6 doktorskih studenata koji su potom i sami imali još 22 doktorska studenta.

Profesor Wickerhauser bio je urednik-suradnik u seriji knjiga "Applied Harmonic Analysis" kao i u pet časopisa, uključujući i Glasnik Matematički (od 1995.). Član je društava: American Mathematical Society (AMS), Society for Industrial and Applied Mathematics (SIAM), i SPIE - The International Society for Optics od koje je 2002. g. dobio i nagradu Wavelet Pioneer Award.

### *Prof. Mladen Victor Wickerhauser, PhD*

*Mladen Victor Wickerhauser, born in Zagreb, Croatia, in 1959, holds the degrees of B.S. with Honor (1980) from the California Institute of Technology, and M.S. (1982) and Ph.D. (1985) from Yale University, all in mathematics.*

*He is currently Professor of Mathematics and Statistics and Professor of Biomedical Engineering at Washington University in Saint Louis, Missouri. He has six U.S. patents and more than 100 publications, including the monograph "Adapted Wavelet Analysis" (1994) and the textbook "Mathematics for Multimedia" (2003, 2009). He has also been developing and publishing mathematical software since 1979. His research has long focused on fast approximate algorithms for computation with large datasets, specifically using wavelet analysis. It has found applications in image compression (WSQ for fingerprints), radiation oncology (Monte Carlo dose planning), and pediatrics (auditory screening by EEG). He has advised 6 doctoral students who among themselves have advised another 22 PhDs.*

*Professor Wickerhauser has been an associate editor for the "Applied Harmonic Analysis" book series as well as five journals, including Glasnik Matematički (since 1995). He has been a member of the American Mathematical Society (AMS), the Society for Industrial and Applied Mathematics (SIAM), and SPIE - The International Society for Optics, from which he received the 2002 Wavelet Pioneer Award.*

## SUPERPOZICIJA I ORTOGONALNOST OD POLINOMA DO VALIĆA

Mladen Victor Wickerhauser<sup>1,\*</sup>

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Aproksimacije funkcija superpozicijom imaju dugu povijest u matematici te su uz pomoć njih razvijene moćne metode za rješavanje linearnih problema i efikasnih kodiranja rješenja. Geometrijski pojam ortogonalnosti u funkcijskim prostorima doveo je i do brzih algoritama za takve aproksimacije. Spajanjem tih ideja rezultiralo je raznolikim familijama baznih funkcija od ortogonalnih polinoma, trigonometrijskih redova, pa sve do valića i vremensko-frekvencijskih atoma. Danas su dobro poznate konstrukcije koje daju željenu kombinaciju glatkoće, kompaktnog nosača, i lagane izračunljivosti funkcija. U ovom predavanju izložit će se neformalni pregled razvoja dekompozicija koje se danas koriste u kompresiji slika i u onkološkom planiranju ubrzanog zračenja. Izlaganje će biti prilagođeno svima koji su zainteresirani za kvantitativne i računalne znanosti.



# SUPERPOSITION AND ORTHOGONALITY FROM POLYNOMIALS TO WAVELETS

Mladen Victor Wickerhauser<sup>1,\*</sup>

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Approximation of functions by superposition has a long history in mathematics, leading to powerful methods for solving linear problems and encoding the solutions efficiently. The geometric notion of orthogonality in function spaces leads to fast algorithms for such approximation. Work in combining these ideas has led to diverse families of basic functions from orthogonal polynomials, to trigonometric series, to wavelets and similar time-frequency atoms. There are now well understood constructions that produce desirable combinations of smoothness, compact support, and low computational complexity. This talk will present an informal survey of the development of adapted decompositions currently used for image compression and accelerated radiation oncology planning. It is intended to be accessible to nonmathematicians who are nonetheless interested in quantitative and computational sciences.



### **Prof. dr. sc. Sanja Faivre**

Prof. dr. sc. Sanja Faivre redovita je profesorica u trajnom zvanju na Geografskom odsjeku PMF-a Sveučilišta u Zagrebu. Diplomirala je 1991., a magistrirala 1994. g. na Geografskom odsjeku. Kao stipendist Francuske vlade 1995. stječe *Diplôme d'études approfondies* na Sveučilištu Blaise Pascal u Francuskoj, a 1995. dobiva stipendiju Francuske vlade za izradu doktorske disertacije *en co-tutelle* te 1999. stječe diplomu doktora znanosti na oba sveučilišta. Područje znanstvenog istraživanja prof. Faivre je geomorfologija krša, glaciokrš, strukturna geomorfologija i geomorfologija obala. Posljednjih se godina intenzivno bavi istraživanjima relativne promjene morske razine, paleoklime i paleopotresa temeljem geomorfoloških i bioloških indikatora te razvojem potencijala algnih vijenaca kao markera morske razine visoke razlučivosti. Objavila je 64 znanstvena rada (u posljednjih 5 god. 12 Q1 i Q2 od kojih je na 6 prvi autor) koji su prema Google Scholar citirani više od 1100 puta (h-indeks 19). Do sada je bila voditeljica triju znanstvenih projekata, a suradnica na 10-tak međunarodnih i domaćih znanstvenih projekata. Sudjelovala je na više od 80 znanstvenih skupova te održala više pozvanih predavanja na međunarodnim skupovima i inozemnim sveučilištima. Obavljala je brojne funkcije na Geografskom odsjeku kao i izvan njega. Bila je dopredsjednica radne grupe za Geoarheologiju Međunarodne geomorfološke unije (IAG). Od 2017. je Dopredsjednica radne grupe Geomorphology and Society (IAG i IGU) a od 2019. i članica savjetodavnog odbora INQUA Komisije za obalne i marinske procese. Deset godina bila je glavna urednica časopisa Hrvatski geografski glasnik, a bila je i gost urednik časopisa Quaternary International (WoSCC) te je zajedno s kolegama (Antonioli, F., Ferranti, L., Monaco, C.) 2011. g. uredila broj: Tectonic contribution to relative sea-level change. Dobitnica je nagrade za znanost Federic Grisogono Hrvatskog geografskog društva Zadar 2014., te godišnje nagrade za znanost Hrvatskog geografskog društva Zagreb 2020. g..

### ***Prof. Sanja Faivre, PhD***

*Prof. dr. sc. Sanja Faivre is full professor at the Faculty of Science, University of Zagreb. She graduated in 1991. and received her Master's degree in 1994. She was awarded the same year a scholarship from the French government, enrolled in a postgraduate study programme at the University Blaise Pascal, and received the Diplôme d'études approfondies in 1995. In 1995, she received a scholarship from the French government for her doctoral dissertation en co-tutelle and in 1999 received a doctorate from both universities. Prof. Faivre's research interests are karst geomorphology, glaciokarst, structural and coastal geomorphology. Over the past 10 years, she has been intensively engaged in the study of relative sea-level change, paleoclimate, and paleoearthquakes using geomorphological and biological indicators developing the use of algal rims as a high resolution relative sea-level marker. She has*

*published 64 scientific papers (in the last 5 years 12 Q1 and Q2, in 6 of which she is first author) which are according to Google Scholar cited more than 1100 times (h-indeks 19). To date, she has led three scientific projects and cooperated on ten international and Croatian scientific projects. She has participated in over 80 scientific conferences and has held several invited lectures at international conferences and foreign universities. She has held a number of functions at the Department of Geography and outside the Department. She was Vice-chair of the Geoarcheology Working Group of the International Geomorphology Union (IGU). Since 2017, she has been Co-chair of the Geomorphology and Science Working Group (IAG and IGU) and since 2019, a member of the Advisory Board on INQUA for the Committee on Coastal and Marine Processes. Additionally, she was Editor-in-chief of the journal Hrvatski Geografski Glasnik for ten years and in 2011, she was a guest editor for journal Quaternary International (WoSCC), and edited with colleagues (Antonioli, F., Ferranti, L., Monaco, C.) an issue entitled "Tectonic contributions to relative sea level change". In 2014, she won the Federic Grisogono science award from the Croatian Geographical Society in Zadar, and in 2020, the science award from the Croatian Geographical Society in Zagreb.*

## RELATIVNE PROMJENE MORSKE RAZINE DUŽ ISTOČNE OBALE JADRANA TIJEKOM KASNOG HOLOCENA

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Relativna promjena morske razine rezultat je procesa koji djeluju na lokalnoj, regionalnoj i globalnoj razini. Kako bi se poboljšalo razumijevanje pojedinih procesa potrebni su pouzdani podaci visoke razlučivosti iz različitih dijelova svijeta koji pokrivaju različita vremenska razdoblja. Kada istražujemo procese koji su se odvijali prije instrumentalnog perioda, koristimo zamjenske podatke koje kod istraživanja relativne promjene morske razine dobivamo temeljem različitih pokazatelja. Jedan od najboljih pokazatelja relativne promjene morske razine na stjenovitim obalama Mediterana, u mikroplimnom okolišu, su biokonstrukcije koje gradi alga *Lithophyllum byssoides*. U povoljnim uvjetima, alga gradi vijence u zoni biološke srednje razine mora u vrlo uskom rasponu te je vertikalna greška u istraživanjima mala. Kronološku preciznost postigli smo metodom <sup>14</sup>C budući da smo marinski rezervoar efekt, ili efekt spremnika, prethodno detaljno analizirali. Naime, za kalibraciju <sup>14</sup>C rezultata kod karbonata marinskog podrijetla poznavanje rezervoar efekta od iznimne je važnosti. Rezultati analiza omogućili su konstruiranje geokronologija visoke razlučivosti na različitim lokacijama istočne jadranske obale što je nadalje omogućilo razlikovanje i kvantificiranje lokalnih nelinearnih, regionalnih linearnih i globalnih procesa. U okviru predavanja predstaviti ću primjere istraživanja relativne promjene morske razine koje smo kao interdisciplinarni tim napravili na području sjevernog i južnog Jadrana.

## RELATIVE SEA-LEVEL CHANGES ALONG THE EASTERN ADRIATIC COAST DURING LATE HOLOCENE

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Relative sea-level (RSL) change is the sum of effects of processes acting on local, regional and global scales. In order to improve understanding of driving processes of relative sea-level change, reliable high-resolution records from different parts of the world covering different time periods are needed. Studies of preinstrumental periods are based on indicators of relative sea-level change which can provide reliable proxy records. One of the best indicators of relative sea-level change on rocky coasts in the microtidal environment of the Mediterranean are bioconstructions built by alga *Lithophyllum byssoides*. Under favourable conditions, alga *L. byssoides* can build rims in a very narrow range at around biological mean sea-level providing good vertical precision. Chronological precision was obtained by the <sup>14</sup>C method since the marine radiocarbon reservoir effect, needed for precise calibration of <sup>14</sup>C results obtained from carbonates of marine origin, have been previously analysed. High resolution geochronologies obtained for different locations along the eastern Adriatic coast allowed distinction and quantification of local non-linear, regional linear and global scale processes. In the talk, I will present examples of studies of relative sea-level change that we conducted as an interdisciplinary team in the northern and southern Adriatic.



### **Izv. prof. dr. sc. Dominik Cinčić**

Dominik Cinčić je izvanredni profesor na Kemijskom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu gdje je 2009. g. obranio doktorsku disertaciju (mentor: B. Kaitner). Tijekom izrade doktorske disertacije 2007. g. kao znanstvenik gostovao je na Sveučilištu u Cambridgeu, UK (voditelj: William Jones i Tomislav Friščić). Znanstveno istraživanje D. Cinčića usmjereno je na područje kristalnog inženjerstva, supramolekulske kemije u čvrstom stanju te mehanokemije. Ono uključuje organsku, metaloorgansku i supramolekulsku sintezu te termalnu, spektroskopsku i strukturnu analizu materijala metodom difrakcije rentgenskih zraka. Objavio je više od 65 znanstvenih radova te je koautor više od 180 priopćenja na znanstvenim skupovima od kojih je više od 70 bilo međunarodnih. Od 2007. g. sudionik je popularno znanstvenih događanja Kemijskog odsjeka PMF-a (Čarolije u kemiji, Otvoreni dan KO). Od 2013. do 2017. g. bio je koordinator manifestacije Otvoreni dan Kemijskog odsjeka, a 2016. g. bio je koordinator manifestacije Dan i noć na PMF-u. Za svoja znanstvena dostignuća u području supramolekulskih struktura određenih halogenskom vezom odlukom Hrvatskog sabora nagrađen je 2018. g. državnom nagradom za znanost u kategoriji Godišnja nagrada za značajno znanstveno dostignuće iz područja prirodnih znanosti, polje kemija. U šest navrata bio je dobitnik nagrade studentskog zbora PMF-a Brdo za najboljeg profesora na Kemijskom odsjeku PMF-a u Zagrebu (2013., 2014., 2016., 2017., 2020. i 2021. g.). 2009. g. bio je dobitnik Godišnje nagrade Društva sveučilišnih nastavnika i drugih znanstvenika u Zagrebu mladim znanstvenicima i umjetnicima za znanstveni rad iz područja kemije.

### ***Assoc. Prof. Dominik Cinčić, PhD***

*Dominik Cinčić is an Associate Professor at the University of Zagreb, Faculty of Science, Department of Chemistry. He received his Ph.D. (2009) at the University of Zagreb with Professor Branko Kaitner. In 2007, he was a visiting researcher at the University of Cambridge under the supervision of Professor William Jones and Professor Tomislav Friščić. He is experienced in crystallization of molecular solids, polymorphism, cocrystal and salt screening of pharmaceuticals, mechanochemical and solvent-free synthesis of diverse organic and metal-organic materials, and crystallography. Current research in his group is focused on the study of halogen bonding synthons and their flexibility as well as crystal engineering of multicomponent halogen-bonded organic and metal-organic materials. He has authored over 65 scientific publications and over 180 oral and poster presentations at national and international conferences. He has also been active in science*

*popularization since 2007, participating in the annual "The Magic in Chemistry" and "Open day of the Chemistry Department at the Faculty of Science" events. He was the recipient of the Croatian National Science Award for outstanding scientific discoveries in the field of supramolecular chemistry – halogen-bonded solids (2018), the annual award 'Brdo' for the best teacher at the Department of Chemistry, administered by the Student Association at the Faculty of Science (2013, 2014, 2016, 2017, 2020 and 2021), and the Annual Young Scientist and Artist's Award, administered by the Society of University Teachers, Scholars, and Other Scientists in Zagreb (2009).*

# KRISTALNO INŽENJERSTVO METALOORGANSKIH KOKRISTALA TEMELJENIH NA HALOGENSKIM VEZAMA

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Halogenska veza intenzivno se proučava posljednjih nekoliko desetljeća u svim područjima kemije a ponajviše u kristalnom inženjerstvu.<sup>[1]</sup> U posljednjih dvadesetak godina za istraživanje halogenskih veza u binarnim krutinama najčešće su primjenjivani organski sustavi s perhalogeniranim spojevima kao donorima halogenske veze, dok su najčešće korišteni akceptori halogenskih veza brojni organski spojevi koji sadrže različite funkcijske skupine s dušikom ili kisikom.<sup>[2]</sup> Sinteza višekomponentnih kristala temeljenih na halogenskim vezama s metaloorganskim građevnim jedinicama ili akceptorima koji sadrže teže elemente 15. i 16. skupine, do sada je manje bila u središtu istraživanja. Naša grupa usredotočena je na halogenske veze kao alat u kristalnom inženjerstvu višekomponentnih kristala uporabom novih akceptora i donora a posebice na ugradnju metala u strukture. Ovo predavanje dat će pregled nedavno objavljenih rezultata istraživanja kokristala metaloorganskih spojeva. Prikazat će se potencijal kelatnih i anionskih liganada koordinacijskih spojeva kao akceptora halogenske veze te iznalaženje načinâ sastavljanja metaloorganskih supramolekulskih arhitektura halogenskom vezom.<sup>[3]</sup>

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# CRYSTAL ENGINEERING OF HALOGEN-BONDED METAL-ORGANIC COCRYSTALS

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Over the past 20 years, research into halogen bonding has been intensified in all fields of chemistry, especially in crystal engineering.<sup>[1]</sup> Studies on halogen-bonded cocrystals have mostly focused on organic systems with perfluorinated compounds as halogen bond donors, and a variety of organic acceptors involving nitrogen atoms and oxygen.<sup>[2]</sup> The use of halogen bonding to direct the assembly of coordination compounds or building blocks containing heavier elements of Groups 15 and 16 as acceptors in cocrystals remains largely unexplored. Our group is focusing on developing halogen bonding as a tool in crystal engineering of multi-component solids through the involvement of new acceptor and donor types, notably the incorporation of metals into halogen-bonded structures. This presentation will provide an overview of some of the work related to metal-based halogen-bonded cocrystals that we have pursued recently – the potential of chelating and anionic ligands as halogen bond acceptors in crystal engineering based on coordination compounds.<sup>[3]</sup>

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# Usmena izlaganja

## *Oral presentations*





## IDENTIFIKACIJA DEGRADACIJSKOH PRODUKATA ROSUVASTATINA POMOĆU VEZANOG SUSTAVA nanoUPLC I nanoESI-QTOF SPEKTROMETRA MASA

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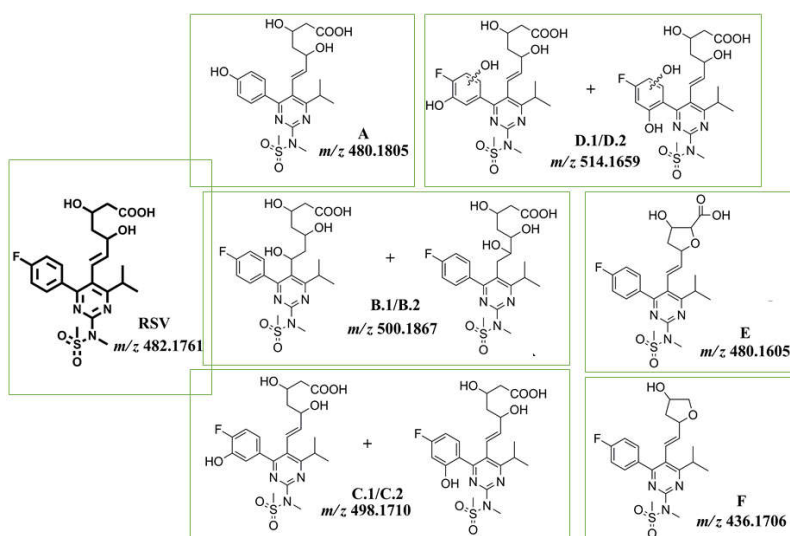
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Rosuvastatin je lijek skupine statina koji se koristi za regulaciju visoke razine kolesterola u ljudskom tijelu. Isto tako, rosuvastatin i ostali statini pokazuju zaštitnu ulogu protiv oksidativnog stresa uzrokovanog slobodnim radikalima. Cilj ovog istraživanja bio je identificirati krajnje produkte koji su nastali radikalnom razgradnjom rosuvastatina. Kako bi se potaknula radikalna razgradnja, vodena otopina rosuvastatina ozračena je različitim dozama gama zračenja (50-1000 Gy) pri oksidativnim uvjetima. Rosuvastatin i srodni degradacijski produkti odvojeni su nanoC18 kolonom gradijentnom elucijom, a identifikacija je provedena pomoću vezanog sustava nanoUPLC i nanoESI-QTOF. Pomoću točno mjenjenih masa, zajedno s algoritmom usporedbe izotopnih raspodjela, provedena je elementna analiza kojom je identificirano devet degradacijskih produkata. U ovom je istraživanju po prvi puta provedena gama-inducirana razgradnja rosuvastatina te detaljno opisana kemijska struktura, MS/MS fragmentacija te mehanizam nastanka pojedinog degradacijskog produkta. Priloženi rezultati doprinose razumijevanju razgradnog puta rosuvastatina i ostalih statina pri gama zračenju.



**Slika 1.** Strukture i pripadajuće molekulske mase u Da molekule RSV i degradacijskih produkata induciranih dozom zračenja do 1000 Gy: A, B.1/B.2, C.1/C.2, D.1/D.2, E i F.



## IDENTIFICATION OF ROSUVASTATIN DEGRADATION PRODUCTS USING nanoUPLC COUPLED TO THE nanoESI-QTOF MASS SPECTROMETER SYSTEM

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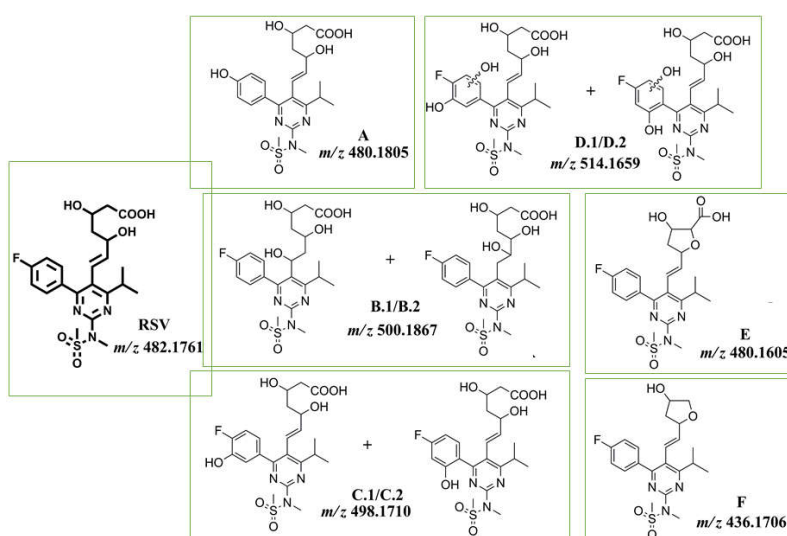
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Rosuvastatin is a member of statin drugs used to regulate high cholesterol levels in the human body. Moreover, rosuvastatin and other statins show a protective role against oxidative stress caused by free radicals. Our study aimed to investigate the end products of free radical induced rosuvastatin degradation. To promote radical degradation, aqueous rosuvastatin solution was irradiated with various doses of gamma radiation (50-1000 Gy) under oxidative conditions. Rosuvastatin and related degradation products were separated on nanoC18 column with gradient elution, and identification was performed on hyphenated system nanoUPLC and nanoESI-QTOF. Elemental composition analysis using high accurate mass measurements together with the isotope fitting algorithm identified nine main degradation products. This is the first study of gamma-induced degradation of rosuvastatin, which describes in detail the chemical structures, MS/MS fragmentation pathways and mechanisms of formation of the resulting degradation products. Presented results contribute to the understanding of degradation pathways of rosuvastatin and possibly other statins under gamma radiation conditions.



**Figure 1.** Structures and belonging molecular weights in Da of RSV molecule and degradation products induced by radiation dose up to 1000 Gy: A, B.1/B.2, C.1/C.2, D.1/D.2, E, and F.



# VOLTAMMETRIJA I DIFERENCIJALNA PRETRAŽNA KALORIMETRIJA ZA ODREĐIVANJE METALA U TRAGOVIMA I VRSTA POLIMERA NA MORSKOJ (MIKRO)PLASTICI

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Plastični otpad koji u ogromnim količinama dopijeva u morski okoliš rastući je i višeslojni problem. Utjecanjem različitih faktora, prvenstveno UV-zračenja, polimeri se degradiraju i fragmentiraju. Termoplastičnim polimerima, koji su najzastupljeniji, može se promijeniti stupanj kristalizacije. Plastične čestice djeluju i kao vektor za transport različitih tvari adsorbirajući ih na svoju površinu, među njima i potencijalno ekotoksične metale. Na vezanje metala na plastične čestice utječu brojni fizikalno-kemijski parametri, među kojima su vrsta i stupanj degradacije polimera [1]. Za određivanje metala u tragovima uzorkovane su neke od najčešćih vrsta morskog plastičnog otpada naplavljenog na obalama hrvatskog dijela srednjeg i južnog Jadrana. Izabrani uzorci su predproizvodne granule koje se svrstavaju u primarni mikroplastični otpad (< 5 mm) i higijenski štapići. Razlog takvog odabira je sveprisutnost tih vrsta otpada u svjetskim morima. Količina metala u tragovima (Zn, Cd, Pb, Cu, Ni, Co) na plastičnom otpadu te u morskoj vodi određena je elektroanalitičkom metodom voltammetrijom, a vrsta polimera diferencijalnom pretražnom kalorimetrijom (DSC). DSC je metoda termičke analize koja omogućuje brzu analizu vrlo malih pa i heterogenih uzoraka, određivanje vrste polimera i/ili njegovog stupnja degradacije. Manje količine površinskih zagađenja iz okoliša, koje mogu zakomplicirati identifikaciju osnovnog polimera nekim drugim tehnikama, kod DSC metode ne smetaju [2]. U sklopu ovog ispitivanja utvrđeno je da je maseni udio metala na površini granula na razinama njihovog udjela u sedimentu, a usporediv je s podacima iz literature [1], dok su količine metala na štapićima bile nešto niže. Za štapiće relevantna istraživanja nisu pronađena pa time ni usporedba nije moguća. DSC metodom je utvrđeno da su štapići po sastavu polipropilen (PP), dok su granule bile raznih sastava: polietilen visoke gustoće (HD-PE), polietilen niske gustoće (LD-PE) i PP. Više čimbenika utječe na međudjelovanje mikroplastike s metalnim ionima u morskom okolišu, od kemijskog sastava mora do karakteristika polimera, a za njihovo bolje razumijevanje potrebni su modelni eksperimenti koji će dati uvid u dinamiku vezanja i količine metala na površini mikroplastike.

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# VOLTAMMETRY AND DIFFERENTIAL SCANNING CALORIMETRY FOR TRACE METALS AND POLYMER TYPE DETERMINATION IN MARINE MICROPLASTICS

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Plastic litter ending up in the marine environment in massive amounts is a growing and multilayered problem. Different environmental factors, especially UV-radiation, can lead to degradation and fragmentation of polymers. That affects crystallinity of thermoplastic polymers - which are the most abundant. Plastic particles can adsorb various compounds, including potentially ecotoxic trace metals. Different physicochemical parameters, including polymer type and degree of its degradation, can influence metal adsorption [1]. To determine trace metal amounts some of the most frequent marine plastic litter types has been sampled from Croatian part of middle and south Adriatic Sea. Preproduction plastic pellets, which classifies as primary microplastic (<5 mm) and cotton bud sticks were chosen for their ubiquitous presence in oceans. The amounts of trace metals (Zn, Cd, Pb, Cu, Ni, Co) adsorbed on microplastic surfaces and in seawater have been analysed by electroanalytical method voltammetry and polymer types by differential scanning calorimetry (DSC). DSC is a thermal analysis method which can be used for quick analysis of polymer type of even very small, heterogeneous particles and/or its degree of degradation. Smaller amounts of surface contaminants from the environment, which can complicate the identification of the base polymer by some other techniques, do not matter with the DSC [2]. This study has showed that the mass fraction of metals from pellets surfaces were in the range of those found in marine sediment, and comparable with the published data [1]. The amounts of metals on bud sticks were somewhat lower compared to pellets. No relevant research has been found for sticks, so no comparison is possible. DSC analysis showed that bud sticks were made from polypropylene (PP), while pellets were of different types: high density polyethylene (HD-PE), low density polyethylene (LD-PE) and PP. Numerous factors from the marine environment can affect interactions of microplastics and metal ions, from seawater chemistry to the polymer characteristics. For better understanding model experiments are needed, as they will give insight into dynamics of bonding and amounts of metals on microplastic surface.

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## PRECIZNOST TEHNIKA DALJINSKIH ISTRAŽIVANJA U GEOMORFOLOŠKIM ISTRAŽIVANJIMA ŽALA – PRIMJER OTOKA HVARA

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Žala su dinamični obalni geomorfološki oblici. Prirodni procesi, kao i sve veći antropogeni pritisci, utječu na konstantne promjene njihovih morfoloških obilježja [1]. Osim prirodnog, žala danas imaju i važno socioekonomsko značenje u turizmu, te je potrebno brinuti o stabilnosti žala i njima održivo upravljati [2]. Monitoring žala pokazao se kao vrlo dobra metoda, koji je na otoku Hvaru započeo 2016. godine [1]. Od 2018. godine istraživanje smo nastavili uz pomoć satelitskih i ortofoto snimaka, a od 2020. godine uz pomoć bespilotne letjelice. Daljinska istraživanja se danas sve češće koriste u istraživanjima žala [3]. U ovom radu ispitat će se kvaliteta i preciznost podataka prikupljenih daljinskim istraživanjima u desetogodišnjem razdoblju (2011.-2021.) na 20 žala otoka Hvara.

Mjerenja osnovnih morfoloških obilježja žala (površina i duljina) napravljena su na temelju arhivskih satelitskih (Google Earth Pro desktop) i arhivskih digitalnih ortofoto snimaka (Geoportal – Državna Geodetska Uprava). Odabrane su snimke najbolje rezolucije (prostorne i vremenske) te su uspoređene s recentnim mjerenjima napravljenim pomoću bespilotne letjelice (DJI Phantom 4 Pro) i GNSS prijarnika (Trimble). Lokacije žala snimane su bespilotnom letjelicom tijekom 2020. i 2021. godine te su obrađene fotogrametrijskim metodama. Kako bi se izračunala preciznost mjerenja korišteni su RMSE – Root Mean Square Error (korijen srednje kvadratne pogreške) i postotno odstupanje. S obzirom da su modeli izrađeni pomoću bespilotne letjelice vrlo visoke rezolucije i terenski su mjereni, oni su korišteni kao referentna vrijednost. Usporedbom mjerenja svih korištenih izvora izračunata je jaka korelacija ( $r^2=0,98$ ), što pokazuje da sva mjerenja omogućuju daljnje analize. Prosječni RMSE površina žala iznosi 7,2%, a duljina 2,5%, dok je izračunata devijacija -2,3% do 5,6% za površine žala, a -1% do 2,7% za duljine žala. Na temelju izračunatih vrijednosti vidljiv je trend smanjenja greške na recentnim podacima. Ovaj rad je pokazao da se dostupni podaci prikupljeni metodama daljinskih istraživanja mogu koristiti u geomorfološkim istraživanjima žala, uzimajući u obzir izračunatu grešku.

### ZAHVALE

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# ACCURACY OF REMOTE SENSING TECHNIQUES IN GEOMORPHOLOGICAL INVESTIGATIONS OF BEACHES – EXAMPLE OF THE ISLAND OF HVAR

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Beaches are dynamic coastal geomorphological features. Both natural processes and increasing anthropogenic pressures influence their morphological changes [1]. Apart from natural importance, beaches nowadays also have an important socio-economic significance for tourism, so it is necessary to take care of their stability and manage beaches in a sustainable way. [2]. Beach monitoring has proven to be a very good method, which we started in 2016 on the island of Hvar [1]. From 2018 we continued our research with satellite imagery and orthophotos, and from 2020 with UAVs. Remote sensing techniques are today increasingly used in beach surveys [3]. In this paper, we will investigate the quality and accuracy of data collected by remote sensing techniques over a ten-year period (2011-2021) on 20 beaches on the island of Hvar.

Measurements of basic morphological characteristics of beaches (area and length) were made using archival satellite imagery (Google Earth Pro desktop) and archival digital orthophotos (Geoportal - State Geodetic Administration). Images were selected based on their resolution (spatial and temporal) and compared with recent measurements made by UAV (DJI Phantom 4 Pro) and GNSS receiver (Trimble). Beaches were recorded during 2020 and 2021 and further analysed using photogrammetric methods. The RMSE - Root Mean Square Error and the percentage deviation were used to calculate the measurements accuracy. Since the UAV models were created with a very high resolution and measured in the field, they were used as a reference value. Comparing measurements from all sources, a strong correlation was calculated ( $r^2=0.98$ ), showing that all measurements can further be used for analyses. The average RMSE of beach area is 7.2% and of length 2.5%, while the calculated deviation for beach areas is -2.3% to 5.6% and for beach lengths -1% to 2.7%. Based on the calculated values, decrease in error is noted for the most recent data. This work has shown that the available data collected by remote sensing can be used in beach geomorphological surveys, taking into account the calculated error.

## ACKNOWLEDGMENTS

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## MEHANIČKA SAVITLJIVOST KRISTALA KOORDINACIJSKIH POLIMERA BAKRA(II)

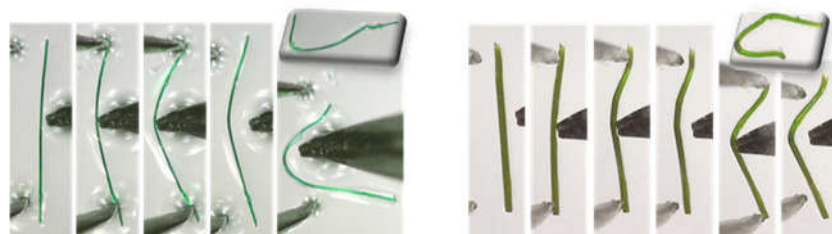
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Brzi razvoj naprednih tehnologija nameće sve veće zahtjeve na pripremu materijala unaprijeđenih svojstava gdje od željenih svojstava fleksibilnost danas zauzima vodeću ulogu. Kristalni materijali, unatoč svim prednostima koje im daje urednost dugog dometa, dugo nisu bili smatrani kandidatima za primjenu u novim tehnologijama.<sup>1</sup> Krtost, kao osnovni ograničavajući čimbenik, učinkovito ih je diskreditirala dugi niz godina. No, nedavno otkriće fleksibilnosti kristala uočene na kristalima organskih<sup>2</sup>, a potom i metalo-organskih<sup>3</sup> molekulskih krutina učinilo je važan zaokret u percepciji kristalnih materijala. Do danas su ispitani brojni kristali organskih ali i metalo-organskih spojeva te je ustanovljen njihov elastičan i plastičan mehanički odziv, no još uvijek razumijevanje strukturnih značajki koje oplemenjuju kristale željenim mehaničkim odzivom nije zadovoljavajuće.

Kako bismo pridonijeli razumijevanju fleksibilnog odziva kristala te strukturnih karakteristika koje ga omogućuju, priredili smo kristale koordinacijskih polimera bakrovih(II) halogenida s derivatima piridina,  $[\text{CuBr}_2(3\text{-Cl-py})]_n$  (**1**),  $[\text{CuBr}_2(3\text{-Br-py})]_n$  (**2**) i  $[\text{CuBr}_2(3\text{-I-py})]_n$  (**3**). Kristali spojeva **1** i **2** pokazali su tipičnu plastičnu savitljivost (slika 1) dok je kod kristala spoja **3** uočena nedavna opisana elastično->plastična savitljivost.<sup>4</sup> Strukturna analiza spojeva **1**, **2** i **3** te korelacija strukturnih karakteristika s mehaničkim odzivom ukazala je na međumolekulske interakcije kao kritični strukturni čimbenik koji usmjerava mehanički odziv.



**Slika 1.** Plastična savitljivost kristala  $[\text{CuBr}_2(3\text{-Cl-py})]_n$  (**1**), lijevo, i kristala  $[\text{CuBr}_2(3\text{-Br-py})]_n$  (**2**), desno.

### ZAHVALE

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# MECHANICAL BEHAVIOUR OF CRYSTALS OF COPPER(II) COORDINATION POLYMERS

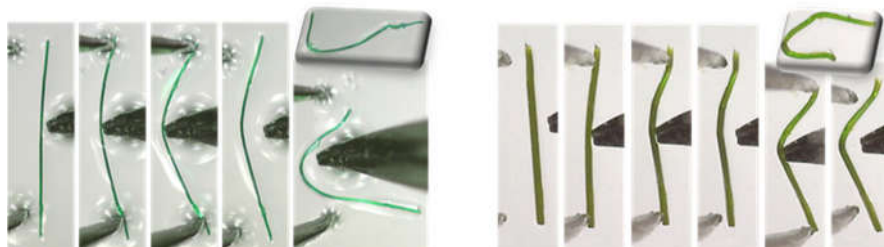
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The rapid development of advanced technologies is continuously requiring materials with improved properties, among which flexibility and adaptability are playing a central role. Crystalline materials, due to their brittleness, have for long not been considered as candidates for application in new technologies, despite all the advantages resulting from the long-range order.<sup>1</sup> But, the recently discovered flexibility of organic<sup>2</sup> and metal-organic<sup>3</sup> molecular crystals has drastically changed our perception of crystalline materials and their potential application. So far, a substantial number of organic and metal-containing crystals with flexible responses have been reported, but our understanding of structural features required for engendering crystalline materials with desired mechanical responsiveness is still far from being complete.

In order to get an insight into the flexible responsiveness of crystals as well as structural features required to equip the crystals with flexible responses, we prepared a series of crystalline coordination polymers of copper(II) halides with pyridine derivatives, namely,  $[\text{CuBr}_2(3\text{-Cl-py})]_n$  (**1**),  $[\text{CuBr}_2(3\text{-Br-py})]_n$  (**2**) and  $[\text{CuBr}_2(3\text{-I-py})]_n$  (**3**). Crystals of **1** and **2** displayed typical plasticity (Figure 1) while **3** showed recently described elastic→plastic flexible response.<sup>4</sup> In-depth structural analysis together with the correlation of structural features with the observed mechanical responses pointed at intermolecular interactions as the key structural requirement that guided a particular mechanical response.



**Figure 1.** Plasticity in crystals of  $[\text{CuBr}_2(3\text{-Cl-py})]_n$  (**1**), left, and crystals of  $[\text{CuBr}_2(3\text{-Br-py})]_n$  (**2**), right.

## ACKNOWLEDGMENTS

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## RAZLIKE U UNUTARSTANIČNOJ RASPODJELI TOKSIČNIH METALA Ag I Cd U PROBAVNIM ŽLIJEZDAMA TRIJU VRSTA ŠKOLJKAŠA IZ PORODICE UNIONIDAE

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Procesi sekvestracije metala pomoću citosolskih proteina i/ili netopljivih staničnih depozita (granule) ovise o vrsti školjkaša i prisutnom elementu [1]. Cilj ovog istraživanja bio je utvrditi postoje li razlike u unutarstaničnoj raspodjeli (raspodjela između topljive citosolske i netopljive frakcije tkiva, raspodjela među citosolskim biomolekulama različitih molekularnih masa) toksičnih metala Ag i Cd u probavnim žlijezdama triju slatkovodnih vrsta školjkaša (*Anodonta anatina*, *Unio crassus*, *Unio pictorum*) iz porodice Unionidae. Dodatni cilj bio je utvrditi važnost toplinski stabilnih citosolskih biomolekula u vezivanju dvaju navedenih elemenata. Školjkaši su uzorkovani na lokaciji niskog antropogenog utjecaja na rijeci Mrežnici. Utvrđivanje raspodjele navedenih elemenata među citosolskim biomolekulama različitih molekularnih masa ostvareno je kromatografskim razdvajanjem uz isključenje po veličini, a mjerenja metala provedena su pomoću spektrometrije masa visoke rezolucije s induktivno spregnutom plazmom.

Koncentracije Ag bile su više u netopljivoj frakciji, dok je Cd bio gotovo u potpunosti zastupljen u citosolskoj frakciji tkiva probavnih žlijezda svih triju vrsta školjkaša. Citosolsko Ag bilo je primarno vezano za biomolekule niskih molekularnih masa (NMM: 10-40 kDa) u svim školjkašima u području koje odgovara metalotioneinima. U vrstama *A. anatina* i *U. pictorum*, Ag je dodatno eluiralo u području visokih molekularnih masa (> 300 kDa). Kod svih školjkaša, eluiranje Cd zabilježeno je u NMM području te se podudaralo s eluiranjem Ag. Toplinskom obradom citosola probavnih žlijezda te usporedbom absorbanci kromatografskih profila pri valnim duljinama 254 i 280 nm dobiveni su rezultati koji ukazuju na prisutnost toplinski stabilnih metalotioneina s malim udjelom aromatskih aminokiselina.

Zaključno, rezultati ove studije ukazuju na postojanje značajnih razlika u unutarstaničnoj raspodjeli Ag i Cd, kao i na različito sudjelovanje metalotioneina u detoksikaciji ova dva toksična elementa. Potrebna su daljnja istraživanja s višim koncentracijama metala kako bi se bolje razumjele razlike u toleranciji i mehanizmima detoksikacije toksičnih metala u različitim vrstama školjkaša.

### ZAHVALE

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## DIFFERENCES IN THE SUBCELLULAR DISTRIBUTION OF THE TOXIC METALS Ag AND Cd IN THE DIGESTIVE GLANDS OF THREE MUSSEL SPECIES FROM THE FAMILY UNIONIDAE

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The patterns of sequestration of metals with cytosolic proteins and/or "insoluble" cellular deposits (granules) are species and metal specific [1]. Therefore, the aim of this study was to determine whether there are differences in the intracellular distribution (i.e. between cytosolic and insoluble tissue fractions, distribution among cytosolic biomolecules of different molecular masses) of the toxic metals Ag and Cd in the digestive glands of three freshwater mussel species (*Anodonta anatina*, *Unio crassus* and *Unio pictorum*) from the family Unionidae. Another objective was to determine the importance of heat-stable cytosolic biomolecules in the binding of the two mentioned elements. Mussels were collected at a site with low anthropogenic influence in the Mrežnica River. The distribution of elements among cytosolic biomolecules was determined by size-exclusion liquid chromatography (SEC HPLC), and the measurement of metals was performed by high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS).

Higher concentrations of Ag were measured in the insoluble fraction, whereas Cd was almost completely present in the cytosolic fraction of the digestive glands of all three bivalve species. Cytosolic Ag was mainly bound to biomolecules eluting in the low molecular mass (LMM; 10-40 kDa) range in all three species presumably containing metallothioneins. *A. anatina* and *U. pictorum* cytosolic profiles of Ag showed additional peak in the high molecular mass range (> 300 kDa). All bivalve species had a similar cytosolic Cd profile with a peak coinciding with Ag peak in the LMM region. The results of additional heat treatment of cytosolic fraction of digestive gland tissue and comparison of chromatogram absorbances at 254 and 280 nm wavelengths, suggested the presence of heat-stable metallothionein molecules with low aromatic content in the LMM region.

In conclusion, the results of this study suggest that there are significant differences in the intracellular distribution of Ag and Cd, as well as different involvement of metallothioneins in the detoxification of these two toxic elements. Further studies with higher metal concentrations are needed to better understand the differences in tolerance and detoxification mechanisms of toxic metals in different mussel species.

### ACKNOWLEDGMENTS

This work has been fully supported by Croatian Science Foundation under the project METABIOM (IP-2019-04-2636).

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## INTERAKCIJA V(V) S HUMIČNOM KISELINOM I ŽELJEZOVIM(III) IONIMA U VODENOJ OTOPINI

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Vanadij(V) je nedavno prepoznat kao potencijalno opasan zagađivač, sa sličnim toksičnim utjecajem na okolnu biotu kao živa, olovo i arsen [1]. Dok rastuća industrijska potražnja za V povećava rizik od onečišćenja prirodnih vodenih sustava, stvarni toksični utjecaj V se može procijeniti samo kroz određivanje distribucije kemijskih vrsta V u promatranom okolišu [1,2]. Anorganske slobodne vrste V(V) predstavljaju najtoksičniji i najmobilniji oblik V u prirodnim vodenim sustavima. Općenito, raspodjela V vrsta u vodenom sustavu regulirana je različitim fizikalno-kemijskim čimbenicima [3,4]. Različiti organski ligandi sadržani u prirodnoj otopljenoj organskoj tvari (*engl.* Dissolved organic matter, DOM) mogu sudjelovati u redoks reakcijama u prirodnom sustavu gdje posljedično mogu mijenjati distribuciju kemijskih vrsta V [5,6].

Interakcija V(V) s humičnom kiselinom proučavana je korištenjem ultrafiltracijskih i/ili filtracijskih jedinica povezanih s anion-izmjenjivačkom kromatografijom. Istraživanje pokazuje izraženu pH ovisnost te sposobnost redukcije V(V) u prisustvu humične kiseline. Specijacija V u otopljenoj i koloidnoj fazi implicira vezanje V(V) u koloidnoj frakciji nakon čega slijedi redukcija i formiranje V(IV) kemijskih vrsta koje su uglavnom prisutne u otopljenoj frakciji. Reducirane kemijske vrste pokazuju stabilnost prema oksidaciji, što je indikativno njihovoj prisutnosti u obliku otopljenih organskih spojeva. Nadalje, opisana interakcija se proučavala u prisustvu Fe(III) iona te je dokazano da njihova prisutnost u modelnim otopinama promiče V(V) vezanje za koloidnu fazu. Uočena promjena u distribuciji kemijskih vrsta V u vodenoj otopini pri korištenim eksperimentalnim uvjetima jasno dokazuje značaj humičnih spojeva u distribuciji kemijskih vrsta V te indicira važnost opisanih interakcija na mobilnost i biodostupnost V u prirodnim sustavima.

### ZAHVALE

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# INTERACTION OF V(V) WITH HUMIC ACID AND FERRIC(III) IONS IN AQUEOUS SOLUTION

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Vanadium has recently been recognized as a potentially hazardous pollutant for surrounding biota in the same class as mercury, lead and arsenic [1]. While the growing industrial demand for V increases the contamination risk of natural aquatic systems, real hazardous consequences should be considered with respect to V speciation [1,2]. Inorganic free V(V) species present the most toxic and mobile form of V. Distribution of V species in the aquatic mediums is governed by various physico-chemical factors [3,4]. Various organic ligands in natural dissolved organic matter (DOM) can participate in redox reactions thus influencing V speciation, as well [5,6].

The interaction of vanadium (V) with humic acid (HA) was studied after applied size separation (ultrafiltration method and/or filtration) followed by anion exchange ion chromatography. The present study demonstrates the pH-dependent reducing ability of humic acid towards V(V). Speciation of V in both the dissolved and colloidal fractions of model solutions showed that V(V) is first bound by the colloidal fraction and then reduced, while reduced species were mainly present in the dissolved fraction. Reduced V species show stability upon oxidation, indicating their presence in the form of apparent soluble organic complexes in the dissolved form. The effect of the above interaction was further investigated in the presence of Fe (III) ions. Our study shows that Fe(III)-humic acid promotes the binding of V(V) in the colloidal phase. The observed change in V species in aqueous solutions under the conditions used represents a valuable study that can contribute to a better understanding of V mobility and bioavailability in aqueous natural environments rich with organic matter content.

## ACKNOWLEDGMENTS

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## SELEKTIVNA PRETVORBA CO<sub>2</sub> U METANOL PREKO BIMETALNOG MOF-74 KATALIZATORA

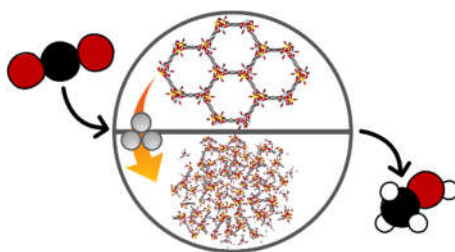
Valentina Martinez,<sup>1</sup> Tomislav Stolar,<sup>1</sup> Anže Prašnikar,<sup>2</sup> Bahar Karadeniz,<sup>1</sup> Gregor Mali,<sup>2</sup>  
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Industrijski katalitički procesi za pretvorbu stakleničkog plina CO<sub>2</sub> u vrijedne proizvode aktivno se istražuju posljednjih godina.[1] U ovom procesu obično se koriste katalizatori poput Cu/ZnO/Al<sub>2</sub>O<sub>3</sub>. Međutim, njihova je selektivnost prema proizvodnji metanola niska. Stoga se neprestano razvijaju novi i efikasniji katalizatori pri čemu metal-organske mreže pokazuju ogroman potencijal zbog velike prilagodljivosti njihovih fizikalno-kemijskih svojstava kroz strukturne promjene. Mljevenje u vibracijskom mlinu nedavno je korišteno za stehiometrijski kontrolirano formiranje bimetalnih MOF-74 [2] i amorfizaciju MOF-74 materijala. [3] Kako bismo istražili kako unošenje defekata i kolaps porozne strukture MOF-a uslijed amorfizacije utječe na njegovu aktivnost i selektivnost prema metanolu, proučavali smo katalitička svojstva kristalnog i amornog ZnCu-MOF-74 materijala. Usporedili smo ga s monometalnim Cu-MOF-74 i industrijskim Cu/ZnO/Al<sub>2</sub>O<sub>3</sub> katalizatorom. Utvrđeno je da bimetalni MOF-74 ima bolje katalitičke performanse od monometalnog, dok su i katalitička aktivnost i selektivnost bimetalnog katalizatora poboljšane mehanokemijskom amorfizacijom. [4]



**Slika 1.** Kristalni i mehanokemijski amorfizirani ZnCu-MOF-74 materijal za katalitičku hidrogenaciju CO<sub>2</sub> u metanol.

### ZAHVALE

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## SELECTIVE CONVERSION OF CO<sub>2</sub> TO METHANOL OVER BIMETALLIC MOF-74 CATALYST

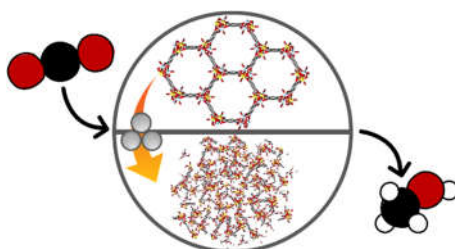
Valentina Martinez,<sup>1</sup> Tomislav Stolar,<sup>1</sup> Anže Prašnikar,<sup>2</sup> Bahar Karadeniz,<sup>1</sup> Gregor Mali,<sup>2</sup>  
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Industrial catalytic processes for converting greenhouse gas CO<sub>2</sub> into valuable products have been actively explored in recent years. [1] Catalysts such as Cu/ZnO/Al<sub>2</sub>O<sub>3</sub> are commonly used in this process. However, their selectivity towards methanol production is low. Therefore, there is a constant search for new and more efficient catalysts, with metal-organic frameworks (MOFs) showing enormous potential due to the high tunability of their physicochemical properties through structural changes. Ball milling has recently been used for the stoichiometrically controlled formation of bimetallic MM'-MOF-74 [2] and amorphization of MOF-74 materials. [3] To find out how the introduction of defects and the collapse of the porous structure of MOF by amorphization affects its activity and selectivity towards methanol, we studied the catalytic performance of crystalline and amorphous ZnCu-MOF-74 material. We compared it with monometallic Cu-MOF-74 and industrial Cu/ZnO/Al<sub>2</sub>O<sub>3</sub> catalysts. Bimetallic MOF-74 was found to have better catalytic performance than monometallic one, while both the catalytic activity and selectivity of the bimetallic catalyst were improved by mechanochemical amorphization. [4]



**Figure 1.** Crystalline and mechanochemically amorphized ZnCu-MOF74 material for catalytic hydrogenation of CO<sub>2</sub> to methanol.

### ACKNOWLEDGMENTS

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## SVEUBOHVATNA PROCJENA UTJECAJA TRI STROBILURINSKA FUNGICIDA NA VRSTU *Enchytraeus albidus*

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Prekomjerna primjena fungicida na poljoprivrednim površinama može imati negativan učinak na organizme tla, poput enhitreja, te posljedično utjecati na kvalitetu tla. Enhitreje su prepoznate kao pokazatelji kvalitete tla [1], a u ekosustavima tla obavljaju istu funkcionalnu ulogu kao gujavice [2]. Na površinama s intenziviranom obradom dolazi do smanjenja brojnosti ili potpunog izostanka gujavica, dok se aktivnost enhitreja povećava [3]. Međutim, njihova uloga u tlu ih čini lakim metama onečišćenja okoliša. Postojeća znanja o učincima strobilurinskih fungicida stečena su prvenstveno na temelju ispitivanja toksičnosti aktivnih sastojaka, dok su učinci komercijalnih preparata i dalje nejasni [4]. Stoga je cilj ovog rada pridonijeti novim saznanjima o učincima tri komercijalna preparata strobilurinskih fungicida na vrstu *Enchytraeus albidus*. Testirani su fungicidi Retengo® (piraklostrobin - PYR), Zato WG 50® (trifloksistrobin - TRI) i Stroby WG® (kresoksim-metil - KM). Dobiveni rezultati ukazali su kako na preživljavanje i reprodukciju enhitreja najviše djeluje PYR ( $LC_{50}=7,57 \text{ mg}_{a.i./kg_{tla}}$ ,  $EC_{50}=0,98 \text{ mg}_{a.i./kg_{tla}}$ ), a zatim TRI ( $LC_{50}=72,98 \text{ mg}_{a.i./kg_{tla}}$ ,  $EC_{50}=16,93 \text{ mg}_{a.i./kg_{tla}}$ ) i KM ( $LC_{50}=73,12 \text{ mg}_{a.i./kg_{tla}}$ ,  $EC_{50}>30 \text{ mg}_{a.i./kg_{tla}}$ ). Nakon sedam dana izlaganja, aktivnost mehanizma multiksenobiotske rezistentnosti (MXR) je inhibirana pri najvišim koncentracijama svih testiranih fungicida. Nadalje, pojava oksidativnog stresa (indukcija enzima SOD, CAT i GST) uočena je nakon sedam dana izlaganja, dok je povećanje lipidne peroksidacije uočeno nakon 21. dana. Izloženost fungicidima dovela je do promjena u raspoloživoj energiji, te izazvala promjene u omjeru glavnih metabolita (ugljikohidrata, lipida i proteina). Nakon izlaganja KM smanjenje raspoložive energije bilo je posljedica prekomjernog trošenja energije zbog istovremene obrane od oksidativnog stresa i reprodukcije, dok su enhitreje izložene TRI nedostatak energije kompenzirale izostankom reprodukcije. Visoka toksičnost PYR uzrokovala je smanjenje energetske zaliha bez obzira na izostanak reprodukcije. Iako su svi testirani komercijalni proizvodi pokazali negativan učinak na enhitreje, ozbiljnost i način djelovanja su se razlikovali, naglašavajući važnost temeljite procjene više krajnjih točaka u različitim vremenskim razdobljima.

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## COMPREHENSIVE ASSESSMENT OF THE INFLUENCE OF THREE STROBILURIN FUNGICIDES ON *Enchytraeus albidus*

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Excessive application of fungicides in crop fields can have a negative effect on soil organisms, such as enchytraeids, and consequently affect soil quality. Enchytraeids are recognized as indicators of soil quality [1] and perform the same functional role as earthworms, only at a lower scale [2]. Furthermore, under tillage pressure, a decrease in earthworm populations or their complete absence has been observed, while the activity of enchytraeids has increased [3]. However, their role makes them an easy target of environmental pollution. Existing knowledge on the effects of strobilurin fungicides was acquired primarily based on toxicity tests with active ingredients, while the effects of commercial products remain unclear [4]. Therefore, this work aims to contribute with new knowledge on the effects of three commercial products of strobilurin fungicide on the enchytraeid *Enchytraeus albidus*. The commercial products tested were Retengo® (pyraclostrobin - PYR), Zato WG 50® (trifloxystrobin - TRI) and Stroby WG® (kresoxim-methyl - KM). The results obtained showed the highest impact of PYR on survival and reproduction of enchytraeids ( $LC_{50}=7.57 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ,  $EC_{50}=0.98 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ), followed by TRI ( $LC_{50}=72.98 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ,  $EC_{50}=16.93 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ) and KM ( $LC_{50}=73.12 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ,  $EC_{50}>30 \text{ mg}_{a.i.}/\text{kg}_{soil}$ ). After seven days of exposure, activity of multixenobiotic resistance mechanism was inhibited at the highest concentration of all tested fungicides. Furthermore, oxidative stress (estimated with SOD, CAT, and GST activities) was induced after seven days of exposure, while increased lipid peroxidation was observed after 21 days. Moreover, changes in available energy occurred. Exposure to fungicides induced a shift in the proportion of carbohydrates, lipids, and proteins. In KM treatments, the decrease in available energy was the consequence of excessive energy expenditure due to simultaneous defence against oxidative stress and reproduction. In the case of PYR it was due to high toxicity and intensive defence against stress. Furthermore, although oxidative stress was observed after TRI treatment, the lack of reproduction compensated for the energy and avoided its reduction. Although all commercial products tested showed a negative effect on enchytraeids, severity and mode of action differed, highlighting the importance of thoroughly testing multiple endpoints in different time periods.

### ACKNOWLEDGMENTS

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## LASERSKA SINTEZA NANOČESTICA CINKOVOG OKSIDA DOPIRANIH SA SREBROM, ZLATOM I PLATINOM

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U nanoznanosti i nanotehnologiji, nanočestice imaju značajnu ulogu zbog različitih magnetskih, električnih, mehaničkih, optičkih i elektronskih svojstava u odnosu na njihove masivne (*engl.* bulk) materijale [1]. Cink oksid (ZnO) je jedan od najčešće korištenih nanomaterijala s jedinstvenim fizikalno-kemijskim svojstvima i direktnim energijskim procijepom od 3.37 eV [2]. Uvođenjem novog elementa u kristalnu strukturu ZnO-a dolazi do poboljšanja električnih i optičkih svojstava te proširuje područje njegove primjene [3]. Pulsna laserska ablacija u tekućini (PLAL) je dobila veliku pozornost zbog svoje jednostavnosti, učinkovitosti, visoke čistoće i brze proizvodnje nanočestica [4] te ima mogućnost optimiziranja mnogih parametara [5,6]. U ovome radu, razvijena je jednostavna metoda u dva koraka za sintezu nanočestica cinkovog oksida dopiranih srebrom (Ag), zlatom (Au) i platinom (Pt). Metoda se temelji na sintezi meta pomoću pulsne laserske depozicije (PLD) gdje se tanki slojevi metala (Ag, Pt, Au) deponiraju na ZnO supstratu. Nastale dvoslojne strukture su korištene kao mete za proizvodnju dopiranih nanočestica cinkovog oksida (ZnO: Ag, ZnO: Au, ZnO: Pt) putem pulsne laserske ablacije u vodi. Optička svojstva, kristalna struktura, elementarni sastav, morfologija i raspodjela veličine nanočestica proučene su putem UV-VIS spektrofotometrije, rendgenske difrakcije (XRD), rendgenske spektroskopije (XPS), skenirajuće elektronske mikroskopije (SEM) i mikroskopije atomskih sila (AFM), respektivno.

### ZAHVALE

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# LASER SYNTHESIS OF SILVER, GOLD, AND PLATINUM DOPED ZINC OXIDE NANOPARTICLES

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In nanoscience and nanotechnology, nanoparticles have a significant role due to their unique magnetic, electrical, mechanical, optical, and electronic properties with respect to the bulk materials [1]. Zinc oxide (ZnO) is one of the most widely used materials with unique physicochemical properties and direct band-gap energy of 3.37 eV [2]. Introducing a new element in the crystal structure of ZnO leads to enhancement of the electrical and optical properties and broadens the area of its application [3]. Pulsed laser ablation in liquid (PLAL) gained a lot of attention due to the simplicity, effectiveness, high purity, and fast production of nanoparticles [4], while a wide range of parameters can be optimized [5,6]. In this work, a simple two-step method for the synthesis of silver (Ag), gold (Au), and platinum (Pt) doped ZnO nanoparticles have been developed. The method is based on the fabrication of targets using pulsed laser deposition (PLD) technique where thin layers of metals (Ag, Pt, Au) have been deposited on a ZnO substrate. Such formed two-layer structures were used as a target for the production of doped nanoparticles (ZnO: Ag, ZnO: Au, and ZnO: Pt) by pulsed laser ablation in water. The optical properties, crystalline structure, elemental composition, morphology, and nanoparticle size distribution were studied using UV-VIS spectrophotometer, X-ray diffraction (XRD), X-ray spectroscopy (XPS), scanning electron microscope (SEM), and atomic force microscope (AFM), respectively.

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## POTRAGA ZA EKSTREMALNIM SAMODUALNIM $\mathbb{Z}_4$ - KODOVIMA

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$\mathbb{Z}_4$  kod duljine  $n$  je  $\mathbb{Z}_4$  podmodul modula  $\mathbb{Z}_4^n$ . Na modulu  $\mathbb{Z}_4^n$  definiran je uobičajeni unutarnji produkt modulo 4. S obzirom na taj produkt, za  $\mathbb{Z}_4$ -kod  $C$  definira se njemu dualan kod  $C^\perp$ .  $\mathbb{Z}_4$ -kod  $C$  je samodualan ako je  $C = C^\perp$ . Svakom  $\mathbb{Z}_4$ -kodu pridružena su dva binarna koda. To su rezidualni i torzijski kod. U [1] je opisana metoda konstrukcije samodualnog  $\mathbb{Z}_4$ -koda iz zadanog rezidualnog tj. torzijskog koda. Za riječ  $\mathbb{Z}_4$  koda  $x$ , definirana je euklidska težina od  $x$  kao  $n_1(x) + 4n_2(x) + n_3(x)$ , gdje je  $n_i(x)$  broj koordinata od  $x$  jednakih  $i$ .  $\mathbb{Z}_4$  kod  $C$  duljine  $n$  je ekstremalan ako je minimalna euklidska težina riječi tog koda jednaka  $8 \lfloor \frac{n}{24} \rfloor + 8$ . U ovom izlaganju opisati ćemo algoritam koji omogućava bolje pretraživanje prostora samodualnih  $\mathbb{Z}_4$ -kodova s ciljem pronalaska novih ekstremalnih  $\mathbb{Z}_4$ -kodova i nove ekstremalne kodove dobivene primjenom tog algoritma.

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## SEARCH FOR EXTREMAL SELF-DUAL $\mathbb{Z}_4$ -CODES

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A  $\mathbb{Z}_4$ -code  $C$  of length  $n$  is a  $\mathbb{Z}_4$  sub-module of  $\mathbb{Z}_4^n$ . With respect to the standard inner product modulo 4, the dual code  $C^\perp$  of the  $\mathbb{Z}_4$ -code  $C$  is defined. The code  $C$  is self-dual if  $C = C^\perp$ . There are two binary codes associated with a  $\mathbb{Z}_4$ -code  $C$  called a residue code and a torsion code. These two codes are a starting point in the construction of self-dual  $\mathbb{Z}_4$ -codes by the method given in [1]. For  $\mathbb{Z}_4$ -codes, the Euclidean weight of codeword  $x$  is defined by  $n_1(x) + 4n_2(x) + n_3(x)$ , where  $n_i(x)$  is the number of components of  $x$  which are equal to  $i$ . A  $\mathbb{Z}_4$ -code  $C$  of length  $n$  is said to be extremal if its minimal Euclidean weight is  $8 \left\lfloor \frac{n}{24} \right\rfloor + 8$ . In this talk, we will discuss an algorithm that improves the search for extremal self-dual  $\mathbb{Z}_4$ -codes which we used to obtain some new extremal codes.

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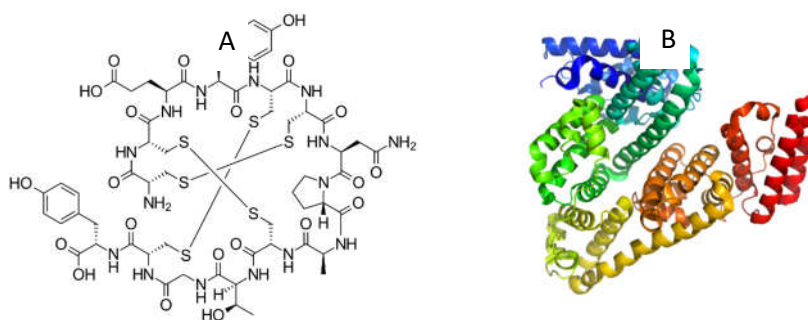
# DERIVATIZACIJA LINAKLOTIDA I GOVEĐEG SERUMSKOG ALBUMINA S AKRILAMIDOM I 4-VINILPIRIDINOM UZ ANALIZU MALDI-TOF/TOF I VEZANIM SUSTAVOM nanoUPLC-ESI-QTOF

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Proteomske identifikacijske i kvantifikacijske tehnike često uključuju postupak derivatizacije proteina. Vežanje različitih iona ili molekula na specifične dijelove proteina koristi se u svrhu promjene strukturnih, ionskih, hidrofobno-hidrofilnih i drugih svojstava proteina. Promjena strukturnih svojstava omogućuje bolju kromatografsku ili ionsku separaciju proteina ili proteinskih fragmenata. U konačnici, derivatizacija se provodi radi povećanja ukupne osjetljivosti analize. U svrhu unaprjeđenja i ubrzanja tehnika proteinske derivatizacije cisteinskih ogranaka peptida ili proteina, korištena su dva derivatizacijska reagensa, akrilamid i 4-vinilpiridin [1]. Reakcije su optimizirane korištenjem peptida Linaklotida, koji u svojoj strukturi sadrži šest cisteina međusobno povezanih s tri disulfidna mosta (Slika 1. A). Prije analize spektrometrijom masa, derivatizacija je provedena na goveđem serumskom albuminu nakon čega je uslijedila kvalitativna i kvantitativna analiza derivatiziranih triptičkih fragmenata (Slika 1. B). Selektivno alkiliranje bočnih ogranaka cisteina postignuto je nakon redukcije disulfidnih mostova. Kako bi se ubrzala reakcija alkilacije korišteno je mikrovalno zračenje tijekom 8 minuta sa snagom zračenja od 180W. Analiza peptidnih reakcijskih smjesa provedena je korištenjem MALDI-TOF/TOF i nanoUPLC-ESI-QTOF spektrometara masa. Dobiveni spektri obrađeni su programskim pretražnim paketom ProteinLynx Global SERVER™ (PLGS).



**Slika 1.** Strukturna formula Linaklotida A i 3D strukturni model goveđeg serumskog albumina B[2].

## ZAHVALE

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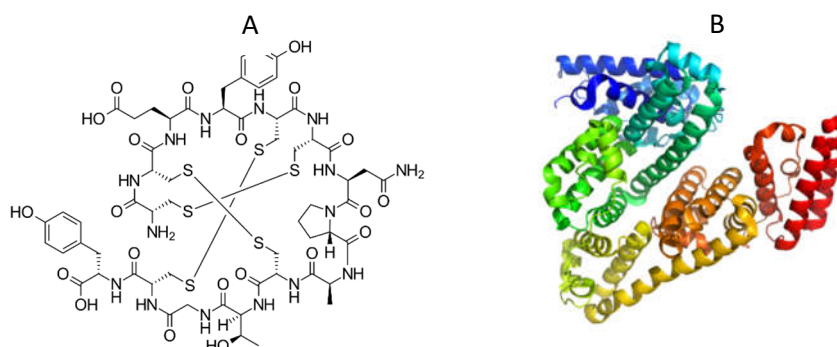
# DERIVATISATION OF LINACLOTIDE AND BOVINE SERUM ALBUMIN WITH ACRYLAMIDE AND 4-VINYLPYRIDINE ANALYZED BY MALDI-TOF/TOF AND nanoUPLC COUPLED TO THE ESI-QTOF

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Proteomics identification and quantification techniques often involve the process of protein derivatization. Binding of different ions or molecules to specific sites of the protein is used to modify structural, ionic, hydrophobic-hydrophilic, and other protein properties. Changes in structural properties allow better chromatographic or ionic separation of protein or protein fragments. At the end, derivatization is carried out to increase overall sensitivity of the analysis. To improve and accelerate protein derivatization procedures of cysteine branches of peptides or proteins, two derivatization reagents, acrylamide and 4-vinylpyridine, were applied [1]. Reactions were optimized using Linaclotide peptide that contains six cysteines interconnected with three disulfide bridges (Figure 1. A). Prior to mass spectrometric analysis derivatization procedure was conducted on bovine serum albumin protein followed by qualitative and quantitative analysis of derivatized tryptic fragments (Figure 1. B). Selective alkylation of the cysteine side branches was achieved after reduction of disulfide bridges. In order to speed up alkylation reaction microwave irradiation was utilized for 8 minutes with radiation power of 180 W. The analysis of peptide reaction mixtures was performed using MALDI-TOF/TOF and nanoUPLC-ESI-QTOF mass spectrometers. Collected spectra were processed with the software search package ProteinLynx Global SERVER™ (PLGS).



**Figure 1.** Structural formula of Linaclotide A and 3D model of bovine serum albumin structure, B[2]

## ACKNOWLEDGMENTS

This research was funded by European Regional Development Fund, KK.01.1.1.07.0023 Qua/Qua project.

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## MODELIRANJEM KA SMANJENJU UPOTREBE INSEKTICIDA U KONTROLI POPULACIJA KOMARACA

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Uslijed klimatskih promjena očekuje se intenziviranje kontrole populacija komaraca i s time povezanog zagađenja okoliša [1]. U ovom radu istražena je učinkovitost larvicidnih, adulticidnih i kombiniranih tretmana korištenjem validiranog matričnog modela populacije komaraca [2] i povijesnih okolišnih podataka za grad Osijek. Računalne simulacije izrađene su na temelju 33 strategija kontrole populacija komaraca, s različitim brojem uzastopnih dana tretmana, dana bez tretmana i različitim brojem ponavljanja obrasca tretiranja. Prema rezultatima simulacija, ovisno o vremenu provedbe tretmana, larvicidni tretmani mogu rezultirati značajnim smanjenjem brojnosti komaraca, ali mogu imati i izrazito negativne ishode. S druge strane, ishodi kombiniranih larvicidnih i adulticidnih tretmana slabije ovise od vremena njihove provedbe, te je vjerojatnost pozitivnog ishoda provedbe ovog tipa tretmana velika i u slučaju kada okolišni uvjeti nisu poznati i/ili predviđivi. Na temelju rezultata ovog istraživanja, ističemo prednosti adaptivne kontrole populacija komaraca, pri kojoj se za optimizaciju provedbe mjera kontrole populacija komaraca koriste prediktivni populacijski modeli temeljeni na prognostici okolišnih uvjeta.

### ZAHVALE

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# MODELLING FOR REDUCED INSECTICIDE USE IN MOSQUITO POPULATION CONTROL

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Due to climate change, mosquito control and related environmental pollution are expected to intensify [1]. The aim of this research was to explore performance of larvicidal, adulticidal, and combined treatments using a validated matrix mosquito population model [2] and historical environmental data for the city of Osijek. A total of 33 treatment strategies have been considered, with varying number of consecutive treatment-days, pause-days and different number of treatment pattern repeats. According to the simulation results, depending on treatment timing, larvicidal treatments can significantly reduce the mosquito population, or have extremely negative outcomes that increase overall mosquito population. On the other hand, the outcomes of combined larvicidal and adulticidal treatments depend much less on timing, and therefore give the greatest chance of a positive outcome if environmental conditions are not known or could not be predicted. Based on the results, we argue for adaptive mosquito population control, in which mosquito population models driven by weather forecast data are used for optimizing the implementation of mosquito population control measures.

## ACKNOWLEDGMENTS

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## POVRŠINSKA SVOJSTVA I PONAŠANJE STANICA ALGA KAO MARKERI STRESA U AKVATIČKIM EKOSUSTAVIMA

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Mikroalge predstavljaju najosjetljiviji dio trofičkog lanca prehrane i stoga pouzdano i brzo ukazuju na klimatske promjene u vodenim ekosustavima. Cilj ovog rada bio je povezati odgovor stanica alga u smislu njihovih površinskih svojstava i ponašanja na pojedinačne abiotičke stresore, kako bi se bolje razumjele strategije preživljavanja i prilagodbe stanica u vodenom ekosustavu. Tri široko rasprostranjene vrste alga, s različitom složenošću stanične barijere, uzgajane su na odabranim temperaturama i salinitetima kako bi se izazvao stres. Odgovor stanica istraživan je u smislu dinamike rasta, površinskih svojstava, fiziološke aktivnosti i pokretljivosti, korištenjem površinskih metoda i softvera otvorenog koda ICY. Rezultati pokazuju da su se istraživane vrste stanica uspješno prilagodile odabranim uvjetima. Sniženje temperature i saliniteta izazvalo je kemijski, mehanički i bihevioralni odgovor stanica mikroalga. Sve vrste stanica postale su tvrđe, kretanje stanica je minimalno, te je uočena razlika u hidrofobnosti i fiziološkoj aktivnosti [1-4]. Alge čiju staničnu barijeru čini glikokaliks pokazale su se osjetljivima na temperaturni stres i tolerantnima na stres salinitetom, dok se alge čiju staničnu barijeru čini teka obložena kalcitom ponašaju suprotno. Alge koje posjeduju organosilikatnu stijenku dobro su se prilagodile na oba stresora. Dobiveni rezultati ukazuju da stanice alga pokazuju promjene koje se mogu detektirati, specifične su za vrstu i ovisne o stresoru, a mogu se povezati sa strukturnim svojstvima stanične barijere. Ovaj rad pokazao je da su površinska svojstva i ponašanje alga ovisni o stresoru, te daju uvid u razumijevanje mehanizama prilagodbe alga na razini jedne stanice, i mogu ukazati na poremećaj u ekosustavu, cvjetanje alga, te za pronalazak odgovarajućih komercijalnih primjena.

### ZAHVALA

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# ALGAL CELL SURFACE PROPERTIES AND BEHAVIOUR AS STRESS MARKERS FOR AQUATIC ECOSYSTEMS

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Microalgae represent the most sensitive part of the trophic food chain and therefore reliably and rapidly indicate climate changes in aquatic ecosystems. The aim of this work was to link the response of algal cells in terms of their surface properties and behavior to individual abiotic stressors to better understand the survival and adaptation strategies of cells in an aquatic ecosystem. Three widely distributed algal species with varying cell barrier complexity were cultured at selected temperatures and salinities to provoke stress. The response of the cells was studied in terms of growth dynamics, surface properties, physiological activity, and motility behavior using the surface methods and open-source software ICY. The results show that all cell species successfully adapted to the selected conditions. A decrease in temperature and salinity triggered a chemical, mechanical and behavioral response in the microalgae. All cell species become stiffer, cell movement is minimal, and they differ in their hydrophobicity and physiological activity [1-4]. Algae surrounded by a glycocalyx layer appear to be temperature sensitive and salt tolerant, while algae surrounded by calcite-coated theca behave in the opposite manner. Algae surrounded by an organosilicate wall seem to adapt well to both stressors. The results obtained show that algal cells exhibit detectable, species-specific, and stressor-dependent changes that can be related to the structural properties of the cell envelope. This study has shown that stressor-dependent surface properties and behavior provide insights into understanding algal adaptation mechanisms at the single-cell level, that can be used to indicate ecosystem disturbances, algal blooms and find appropriate commercial applications.

## ACKNOWLEDGMENT

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## MIKROPALAEONTOLOŠKA ISTRAŽIVANJA MIOCENSKIH NASLAGA LOKALITETA BUKOVA GLAVA (NAŠICE)

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Lokalitet Bukova glava nalazi se na sjevernim padinama planine Krndija, u blizini grada Našice, unutar kamenoloma Našicecement d.d. Tijekom miocena to područje bilo je dio interkontinentalnog Paratethys mora koje se rasprostiralo većim područjem današnje Europe i Azije. U travnju 2019. izvršena su terenska istraživanja, snimljen je geološki stup kroz najgornje otvorene etaže kamenoloma i obavljeno detaljno uzorkovanje naslaga. Duž stupa, ukupne debljine 58 metara, izmjenjuju se lapori sivih nijansi s tufovima i vapnencima uz rijetke proslojke glina. Uzorkovano je ukupno 67 uzoraka, od čega 43 uzorka lapora, koji su u laboratoriju obrađeni metodom muljenja te pripremljeni za mikroskopiranje. Do sada je mikroskopskom analizom obrađeno šest uzoraka lapora iz donjeg dijela stupa, i pronađena je bogata i dobro očuvana mikrofosilna zajednica (bentičke i planktonske foraminifere, spikule spužvi, bodlje ježinaca te rjeđe ostrakodi). Cilj ovih mikropaleontoloških istraživanja je odredba starosti naslaga i paleoekološka rekonstrukcija okoliša (dubina taložnog okoliša, salinitet vode, količina kisika pri morskom dnu, količina hrane). U tu svrhu analizirane su foraminiferske zajednice na standardiziranim uzorcima i određene jedinice bentičkih foraminifera na nivou vrsta. Izdvojene su dominantne i srednje zastupljene vrste, proučeni njihovi ekološki/paleoekološki zahtjevi (dubinski raspon, način života, način ishrane), izračunati su indeksi bioraznolikosti, te indeks kisika pri morskom dnu [2]. Ukupno je određeno 19 rodova i 28 vrsta bentičkih foraminifera te je na osnovu nalaza vrsta *Anomalinoidea dividens* (LUCZKOWSKA 1967), *Elphidium hauerinum* (d'ORBIGNY 1846) i *Bolivina sarmatica* (DIDKOVSKY 1957) određena starost srednjeg miocena – sarmat. Odnos planktonskih i bentičkih foraminifera [1] kreće se u rasponu 10,66% - 49,53% i ukazuje na oscilacije u dubini taložnog okoliša od unutrašnjeg do srednjeg šelfa. Ova preliminarna istraživanja foraminiferskih zajednica iz srednjemiocenskih naslaga lokaliteta Bukova glava će zasigurno dati potpuniju sliku miocenskih zbivanja i paleoekoloških uvjeta u jugozapadnom dijelu Centralnog Paratethysa kao i cjelokupnog Panonskog bazenskog sustava.

### ZAHVALE

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# MICROPALAEONTOLOGICAL RESEARCH OF THE MIOCENE DEPOSITS FROM BUKOVA GLAVA (NAŠICE) LOCALITY

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Bukova glava locality is situated on the northern slopes of the mountain Krndija, near the town of Našice, within the quarry Našicecement d.d. During the Miocene, this area was part of the intercontinental Paratethys Sea, which covered a vast proportion of present-day Europe and Asia. During April 2019, field research was carried out, and geological column (with detailed sampling) was recorded through the upper open floors of the quarry. Through the geological column, with a total thickness of 58 meters, gray marls alternate with tuffs and limestones with rare clay interlayers. A total of 67 samples were sampled, of which 43 marl samples were processed in the laboratory by the wet-sieving method and prepared for microscopy. So far, six marl samples from the lower part of the column have been microscopic analyzed, and a rich and well-preserved microfossil assemblages has been determined (benthic and planktonic foraminifera, sponges spicule, sea urchin spines and, less frequently, ostracods). The aim of these micropaleontological research is age determination of studied sediments and palaeoecological reconstruction of the environment (depth of sedimentary basin, water salinity, bottom water oxygen content, nutrient content). For this purpose, foraminiferal assemblages were analyzed on standardized samples and benthic foraminifera are determined at species level. Dominant and common species were determined, their ecological/palaeoecological requirements were studied (depth range, mode of life, food preferences), and diversity indices and Benthic foraminifera oxygen index were calculated [2]. A total of 19 genera and 28 species of benthic foraminifera have been identified, and the age determination of the Middle Miocene – Sarmatian is based on the findings of the species *Anomalinoidea dividens* (LUCZKOWSKA 1967), *Elphidium hauerinum* (d'ORBIGNY 1846) and *Bolivina sarmatica* (DIDKOVSKY 1957). The planktonic/benthic foraminifera ratios (P/B) [1] range from 10.66% to 49.53% and indicate oscillations in the depth of the sedimentary environment from the inner to the middle shelf. This preliminary research of benthic foraminifera assemblages from the Middle Miocene deposits of the Bukova glava locality will certainly give a more complete picture of Miocene events and palaeoecological conditions in the southwestern part of the Central Paratethys as well as the entire Pannonian Basin System.

## ACKNOWLEDGMENTS

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# KONSTRUKCIJE USMJERENIH REGULARNIH GRAFOVA IZ GRUPE

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Regularan usmjeren graf  $G$  stupnja  $k$  s  $n$  vrhova je usmjereni jako regularan graf,  $DSRG(n, k, \lambda, \mu, t)$ , ako je broj usmjerenih putova duljine dva od svakog vrha  $v$  do svakog vrha  $w$  jednak  $\lambda$  ako postoji usmjereni brid  $v \rightarrow w$ ,  $t$  ako je  $v = w$  i  $\mu$  ako ne postoji brid  $v \rightarrow w$ . [1] Pojam usmjerenih jako regularnih grafova uveo je 1988. godine Art Duval.

Uzimajući za osnovni blok uniju  $G_\alpha$ -orbita tranzitivne permutacijske grupe može se konstruirati 1-dizajn. Koristeći navedeno, konstruiramo usmjerene regularne i jako regularne grafove iz tranzitivnih grupa.

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# CONSTRUCTIONS OF DIRECTED REGULAR GRAPHS FROM GROUPS

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Regular directed graph  $G$  of degree  $k$  with  $n$  vertices is directed strongly regular graph,  $DSRG(n, k, \lambda, \mu, t)$ , if number of directed paths of length two from every vertex  $v$  to every vertex  $w$  is  $\lambda$  if there exists directed edge  $v \rightarrow w$ ,  $t$  if  $v = w$  and  $\mu$  if there is no edge  $v \rightarrow w$ . In [1] directed strongly regular graphs were introduced by Art Duval in 1988.

One can construct 1-design by defining a basic block as union of  $G_\alpha$ -orbits of the transitive permutation group. Using that, we construct directed regular and strongly regular graphs from transitive groups.

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## ULOGA MISTRANSLACIJE U ODGOVORU NA OKSIDACIJSKI STRES U BAKTERIJI *Escherichia coli*

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Translacija je osnovni stanični proces u biosintezi proteina. Ključnu ulogu imaju enzimi aminoacil-tRNA sintetaze (aaRS) koji povezuju tRNA s pripadnom aminokiselinom. Reakcija aminoacilacije se odvija u dva koraka: aktivacija aminokiseline uz pomoć ATP-a pri čemu nastaje aminoacil-adenilat te prijenos aminoacilne skupine na odgovarajuću tRNA. Zbog strukturne i kemijske sličnosti nekih aminokiselina, neke aaRS razvile su mehanizam popravka pogreške nakon prijenosa koji omogućava hidrolizu misaminoacilirane tRNA kako bi se smanjio udio mistranslacije. Mistranslacija ima štetne učinke na stanicu te može dovesti i do umiranja stanice, no u nekim slučajevima pokazao se pozitivan utjecaj mistranslacije u odgovoru na drugi stres [1].

Kako bi se istražio utjecaj mistranslacije na oksidacijski stres, u istraživanjima je korišten soj bakterije *Escherichia coli* koji eksplicira mutiranu inačicu izoleucil-tRNA-sintetaze (IleRS) u kojoj je inaktivirana domena za popravak pogreške. Različite razine mistranslacije inducirane su dodatkom različitih koncentracija (0.25, 0.5, 0.75 and 1 mM) neprirodnog proteinogenog valina (Val) ili neproteinogenog norvalina (Nva) u medij [2]. Oksidacijski stres je induciran dodatkom 1 mM H<sub>2</sub>O<sub>2</sub>. Svjetlosna i transmisijska elektronska mikroskopija pokazale su morfološke promjene stanica, odnosno formiranje filamenata, te promjene u ultrastrukturi bakterijskih stanica izazvane stresom. Testovi preživljenja pokazali su značajno bolje preživljenje u oksidacijskom stresu ako su bakterije prethodno uzgajane s 0,5, 0,75 i 1 mM Val ili Nva. Krivulje rasta pokazale su da bakterije prethodno inkubirane s Val ili Nva rastu bolje u uvjetima oksidacijskog stresa. Rast s Val pokazao je koncentracijsku ovisnost, dok je koncentracija od 0,5 mM imala najizraženiji učinak za Nva. Ako su bakterije istodobno izložene i mistranslacijskom i oksidacijskom stresu, veće razine mistranslacije pokazale su povoljni učinak, no samo u ranoj fazi nakon izlaganja oksidacijskom stresu. Rezultati upućuju da postoji adaptivni utjecaj mistranslacije u uvjetima oksidacijskog stresa. Sljedeća istraživanja bit će usmjerena na analizu proteoma kako bi se identificirali stanični mehanizmi koji omogućavaju bolje preživljenje i rast u oksidacijskom stresu uslijed ugradnje valina ili norvalina umjesto izoleucina u proteinima.

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## THE ROLE OF MISTRANSLATION IN OXIDATIVE STRESS RESPONSE IN BACTERIA *Escherichia coli*

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Translation is an essential cellular process in protein biosynthesis. Enzymes aminoacyl-tRNA synthetases (aaRS) have a key role as they attach tRNAs with their cognate amino acids. Aminoacylation is a two-step reaction consisting of: activation of amino acid utilizing ATP to form an aminoacyl-adenylate intermediate and transfer of aminoacyl moiety to cognate tRNA. Due to the structural and chemical similarity of some amino acids, a group of aaRSs developed post-transfer editing mechanism that facilitates hydrolysis of misaminoacylated tRNA to reduce mistranslation rate. Mistranslation has deleterious effects on a cell leading even to cell death, but in some cases beneficial effect of mistranslation was found in response to other stress conditions [1].

To investigate effect of mistranslation on oxidative stress, *Escherichia coli* strain expressing mutant variant of isoleucyl-tRNA synthetase (IleRS) with inactivated editing domain was used in experiments. Variable mistranslation rates were induced by adding different concentrations (0.25, 0.5, 0.75 and 1 mM) of noncognate proteinogenic valine (Val) or nonproteinogenic norvaline (Nva) in the medium [2]. Oxidative stress was induced by adding 1 mM H<sub>2</sub>O<sub>2</sub>. Light and transmission electron microscopy showed morphological changes, namely formation of filaments, as well as ultrastructural changes in bacteria under stress. Survival assays revealed a significant increase in the survival of bacteria in the presence of 1 mM H<sub>2</sub>O<sub>2</sub> if they are grown with 0.5, 0.75 and 1 mM Val or Nva prior to induction of oxidative stress. Growth curve measurements showed that bacteria preincubated with Val and Nva grow better in oxidative stress conditions. Better growth with Val was concentration dependent, while 0.5 mM concentration had the most prominent effect for Nva. If bacteria were exposed simultaneously to both mistranslation and oxidative stress, higher mistranslation rates have shown to be beneficial as well, however, the effect was only observed during early exposure to oxidative stress. The results show that there is mistranslation-induced adaptation to oxidative stress. Further work will focus on proteome analysis in order to clarify cellular mechanisms that increase survival and growth under oxidative stress due to misincorporation of valine or norvaline at isoleucine positions in proteins.

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## MOLEKULARNA RAZNOLIKOST VIRUSA EPSTEIN-BARR

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Virus Epstein-Barr (EBV, od eng. *Epstein-Barr virus*) je sveprisutan virus koji pripada porodici *Herpesviridae*. Akutna infekcija EBV-om u adolescenata i mlađih odraslih osoba često se prezentira kao infektivna mononukleoza (IM, od eng. *Infectious Mononucleosis*). Replikacijski ciklus EBV-a uključuje lizogenu fazu koju karakterizira latentna infekcija te litičku fazu tijekom koje nastaju novi virioni. Latentna infekcija EBV-om klasificira se u pet tipova (0, I, IIA, IIB, III) i povezuje se s nastankom malignih bolesti koje su posljedica neoplastične transformacije epitelnih stanica, limfocita te mezenhimalnih stanica [1]. Tijekom latencije tipa IIA i III eksprimiran je gen *LMP-1* koji je ujedno i najznačajniji onkogen EBV-a. Protein LMP-1 oponaša biološku aktivnost molekule CD40 što u konačnici dovodi do transformacije limfocita B u proliferirajuće limfoblastoidne stanice. Izolati EBV-a razvrstani su u šest varijanti tj. *LMP-1* podtipova koji su definirani specifičnim mutacijama u C-terminalnoj regiji gena *LMP-1*: China1, China2, North Carolina, Alaskan, Mediterranean s delecijom i Mediterranean bez delecije [2]. Cilj ovog istraživanja bila je analiza molekularne raznolikosti EBV-a s obzirom na polimorfizme *LMP-1* u djece oboljele od IM. U istraživanju su korišteni uzorci periferne krvi 34 djece oboljele od IM (medijan dobi 12 godina) s > 1,000 kopija EBV DNA po mL. Primjenom posebno dizajniranih početnica i lančane reakcije polimerazom umnožene su ciljne sekvence C-terminalnog kraja gena *LMP-1* i analizirane metodom Sangerovog sekvenciranja. Dobivene sekvence uspoređene su s referentnom sekvencom (soj: B95-8, GenBank pristupni broj: NC\_007605) i analizirane bioinformatičkim alatima poravnanja. Ukupno 14 od 34 sekvenci pripadale su divljem tipu EBV-a, 6/34 podtipu North Carolina, a 3/34 podtipu Mediterranean bez delecije. Koinfekcije divljim tipom i podtipom North Carolina detektirane su u 4/34 ispitanika, dok je u 7 uzoraka otkrivena koinfekcija divljim tipom i podtipom China1. Mann-Whitneyev U-test korišten je za utvrđivanje odnosa između varijanti *LMP-1* i odabranih imunoloških parametara. Koncentracija biomarkera upale, C-reaktivnog proteina, bila je značajno veća u bolesnika s koinfekcijom različitim *LMP-1* podtipovima (medijan 13 mg/L) u usporedbi s bolesnicima koji su bili zaraženi jednim podtipom EBV-a (medijan 9,25 mg/L, p=0,048). Omjer CD4+/CD8+ limfocita T bio je veći u bolesnika s koinfekcijom (0,3) u usporedbi s pacijentima zaraženima s jednom varijantom (0,2; p=0,05). Rezultati ovog istraživanja pokazali su širok raspon genetičke raznolikosti gena *LMP-1* EBV-a u IM, te ukazali na moguću povezanost koinfekcije različitim podtipovima gena *LMP-1* s parametrima staničnog i upalnog imunskog odgovora.

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## MOLECULAR DIVERSITY OF EPSTEIN-BARR VIRUS

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Epstein-Barr virus (EBV) is an ubiquitous virus that belongs to the *Herpesviridae* family. Acute EBV infection in adolescents and young adults often presents as infectious mononucleosis (IM). Replication cycle of EBV involves a lysogenic phase which is characterized by latent infection and a lytic phase during which new virions are produced. EBV latent infection is classified into 5 types (0, I, IIA, IIB, III) and can be associated with the development of malignant diseases as result of neoplastic transformation of epithelial cells, lymphocytes and mezenhimal cells. Latency stages type IIA and III are characterised by the expression of an *LMP-1* gene that is the most significant oncogene of EBV. LMP-1 protein imitates a biological activity of CD40 molecule which leads to transformation of B-lymphocytes into proliferating lymphoblastoid cells. EBV isolates are classified into six *LMP-1* variants defined by specific mutations in C-terminal region of *LMP-1* gene: China1, China2, North Carolina, Alaskan, Mediterranean with deletion and Mediterranean without deletion [2]. The aim of this study was to analyse molecular diversity of EBV based on *LMP-1* polymorphisms in pediatric patients with IM. In this study peripheral blood samples, from 34 children with IM (median age 12 years) with >1000 copies of EBV DNA/mL, were used. By applying specifically designed primers and polymerase chain reaction, target sequences of *LMP-1* C-terminal region were amplified and analysed with Sanger sequencing. Obtained sequences were compared with a reference sequence (strain: B95-8, GenBank accession number: NC\_007605) and analysed with bioinformatics alignment tools. In total 14/34 sequences were wild type, 6/34 North Carolina and 3/34 Mediterranean without deletion. Coinfections with wild type and North Carolina were detected in 4/34 patients while 7 samples showed coinfection with wild type and China1. Mann-Whitney U test was used to define the relationship between *LMP-1* variants and selected immunological parameters. Concentrations of an inflammatory biomarker C-reactive protein was significantly higher in patients with coinfection of *LMP-1* variants (median 13 mg/L) compared to patients infected with a single variant (median 9,25 mg/L,  $p=0,048$ ). The ratio of CD4+/CD8+ T-lymphocytes was higher in coinfecting patients (0,3) compared with patients infected with a single variant (0,2,  $p=0,05$ ). The results of this study showed a broad range of *LMP-1* genetic diversity in IM and suggested a possible association between coinfection with different *LMP-1* variants and parameters of cellular and inflammatory immunological response.

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23. – 24. travnja 2022. g



# HIGH-PRESSURE STUDIES IN STRONGLY CORRELATED SYSTEMS

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High pressure plays a prominent role in geophysics. Using a diamond anvil high-pressure cell, one can generate extreme pressures equivalent to the pressure hundreds of kilometers beneath the earth's crust. High-pressure application is not limited to geophysics but is also important for materials science. Especially among strongly correlated systems, numerous studies have been performed using pressure, such as high-pressure syntheses of high-temperature superconductors, pressure-controlled metal-insulator transitions, magnetic and structural phase transitions, and many more. In my doctoral study, I study the physical properties of strongly correlated systems under extreme physical conditions (high pressure and low temperature) using the diamond anvil cell (DAC). During my talk, I will present the details of pressure cells (mainly about DAC), their working principles, applications, and some experimental results using DAC.



## KAKO LITIJEVA FOSFATNA STAKLA UČINITI BOLJIM ELEKTRIČNIM VODIČIMA? – UČINAK STRUKTURNIH PROMJENA

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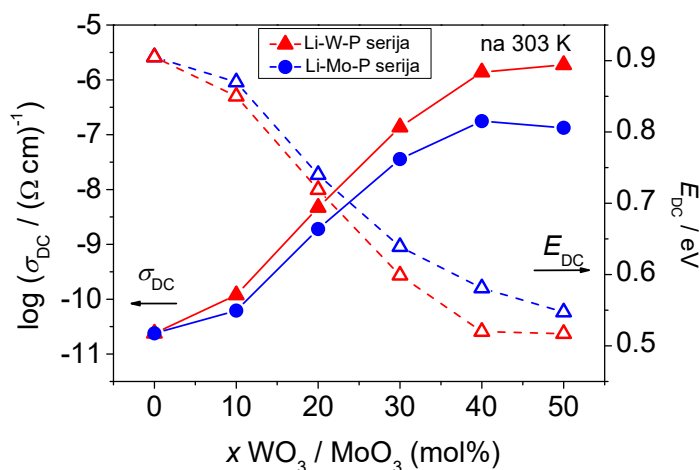
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Litij-fosfatna stakla se danas istražuju kao potencijalni katodni i elektrolitni materijali u čvrstim baterijama zbog dobre termičke i elektrokemijske stabilnosti. Ipak, većina oksidnih stakala pati od relativno niske električne vodljivosti koja se u nekim slučajevima može znatno poboljšati strukturnim promjenama izazvanim dodatkom drugog konvencionalnog ili uvjetnog staklotvorca. U ovom radu dobiveno je veliko povećanje vodljivosti litijevih iona kao posljedica zamjene  $P_2O_5$  s  $MoO_3$  i  $WO_3$  u dvije serije stakla pripremljenih metodom naglog hlađenja taline:  $40Li_2O-xMoO_3-(60-x)P_2O_5$  and  $40Li_2O-xWO_3-(60-x)P_2O_5$ ;  $x=0-50$  mol%. Električna svojstva pripremljenih stakala proučavana su impedancijskom spektroskopijom (u području od 0,01 Hz do 1 MHz i od  $-90$  °C do 240 °C), dok su strukturna svojstva određena  $^{31}P$  MAS-NMR i Ramanovom spektroskopijom. Kao što je vidljivo na slici 1. dodatkom  $WO_3$  i  $MoO_3$  u fosfatno staklo značajno se povećava DC vodljivost i to do pet redova u slučaju volframova i više od tri u slučaju molibdenova oksida. Također, povećanje je kontinuirano do 40 mol%  $WO_3$  i  $MoO_3$  dok se pri višem udjelu ovih oksida, 50 mol%, vrijednosti vodljivosti zadržavaju gotovo jednakima. Uvidom u strukturna svojstva utvrđeno je da ugradnja  $WO_6$  i  $MoO_6$  oktaedara u fosfatnu mrežu i nastajanje neuređene volframsko/molibdensko-fosfatne mreže uvelike ubrzava transport  $Li^+$  iona i poboljšava električnu provodnost ovih materijala. S druge strane, pri 50 mol%  $WO_3$  i  $MoO_3$  strukturnom mrežom dominiraju trodimenzionalni klasteri  $WO_6$  i  $MoO_6$  oktaedara koji pak ograničavaju mobilnost iona i električnu vodljivost.



**Slika 1.** Povećanje ionske vodljivosti u litijevim fosfatnim staklima dodatkom  $WO_3$  i  $MoO_3$ .





## HOW TO MAKE LITHIUM PHOSPHATE GLASSES BETTER IONIC CONDUCTORS? – EFFECT OF STRUCTURAL MODIFICATION

Sanja Renka,<sup>1,\*</sup> Tomáš Hostinský,<sup>2</sup> Petr Kalenda,<sup>3</sup> Gregory Tricot,<sup>3</sup> Petr Mošner,<sup>3</sup> Ladislav Koudelka,<sup>3</sup> Ana Šantić<sup>1</sup>

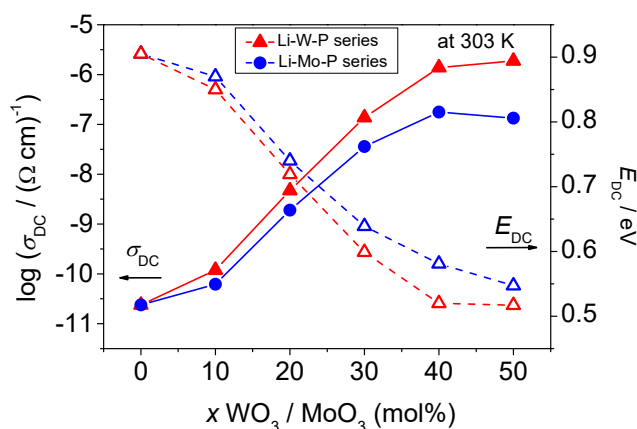
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Owning to the good thermal and electrochemical stability, lithium phosphate glasses are nowadays investigated as potential cathode and electrolyte materials in solid-state batteries. However, most of the glassy materials suffer from relatively low electrical conductivity which has been, in some cases, found to be greatly improved by the structural modification induced by the addition of second conventional or conditional glass-forming oxide. In this study, we report a huge enhancement of lithium ion conductivity arising with the replacement of  $P_2O_5$  with  $MoO_3$  and  $WO_3$  in two glass series prepared by melt quenching technique:  $40Li_2O-xMoO_3-(60-x)P_2O_5$  and  $40Li_2O-xWO_3-(60-x)P_2O_5$ ;  $x=0-50$  mol%. The electrical properties of these glasses were studied by the impedance spectroscopy (in a range of 0.01 Hz to 1 MHz and  $-90$  °C to  $240$  °C) while the structural properties were evaluated by  $^{31}P$  MAS-NMR and Raman spectroscopies. As shown in Figure 1., the addition of  $WO_3$  and  $MoO_3$  significantly increases DC conductivity for five orders of magnitude in the case of tungsten and more than three in the case of molybdenum oxide. Also, the increase is continuous up to 40 mol% of  $WO_3$  and  $MoO_3$  while the conductivity remains nearly the same value for the highest content. The obtained electrical properties were found to be structurally driven in a way that incorporation of  $WO_6$  and  $MoO_6$  octahedra in phosphate glass network forms a more disordered and opened structural network which eases up the mobility of  $Li^+$  ions. However, at the 50 mol% structural network is dominated by three-dimensional clusters of  $WO_6$  and  $MoO_6$  octahedra which tends to limit the mobility of ions and hence their ionic conductivity.



**Figure 1.** Enhancement of ionic conductivity in lithium phosphate glasses by the addition of  $WO_3$  and  $MoO_3$ .



## ISKUSTVO U RAZVOJU ALATA ZA PROCJENU ODRŽIVOSTI PROJEKTA

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Potrebu za razmatranjem održivosti razvoja istaknuo je Rimski klub već prije pedeset godina [1]. Od tada je artikulirano čitav niz deklaracija, definicija, ciljeva, propisa i standarda kako bi se definirali koraci koje bi vlade i organizacije trebale poduzeti kako bi osigurale tu održivost. Godišnji ciklusi financijskog i nefinancijskog izvješćivanja prisiljavaju organizacije da pruže dokaze o njihovim naporima u provedbi ciljeva održivosti. Svrha ove studije je istražiti mogućnost brze procjene razine usklađenosti koju projekti, kao nositelji akcijskih planova provedbenih strategija, imaju s ciljevima održivosti organizacije. Brza procjena se omogućava razvijenim alatom, upitnikom za članove projektnih timova koji su odgovorni za upravljanje projektom da procijene održivost svojih projekata kao dio rutine procesa životnog ciklusa projekta. Upitnik se sastoji od 34 pitanja o održivosti, izvučenih iz 10 širih područja definiranih „triple bottom line“ gospodarskog, okolišnog i društvenog konteksta održivosti [2], koja bi se trebala koristiti za procjenu održivosti projekata kao dio procesa statusnog izvješćivanja. U studiji slučaja provjerava se mogućnost korištenja upitnika u praksi i odražavaju li rezultati usklađenost projekta s ciljevima održivosti organizacije. Istraživanje pokazuje da se testiranje održivosti može provesti kao dio svih projektnih procesnih grupa [3] i da daje mjeru usklađenosti s postavljenim ciljevima održivog razvoja.

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## EXPERIENCES WITH DEVELOPING PROJECT SUSTAINABILITY ASSESSMENT TOOLS

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The need for considering the sustainability of development was addressed by the Club of Rome fifty years ago [1]. Since that time a plethora of declarations, definitions, goals, regulations and standards have been articulated to define the steps governments and organisations should take in ensuring that sustainability. Annual of financial and non-financial reporting cycles force organisations to provide evidence of their respective implementation efforts. The purpose of this study is to investigate the possibility of quickly assessing the level to which projects that serve as the building blocks of action plans for implementation strategies are aligned with the organisation's sustainability goals. An attempt was made in providing tool for project management practitioners to assess sustainability of their projects as part of project life-cycle process routine. 34 sustainability questions were drawn from 10 wider triple bottom line [2] reporting framework areas that should be used in questionnaire to assess sustainability of projects as part of status reporting process. In a case study it is researched if questionnaire can be used in practice and do the results reflect project alignment with the organisation' sustainability goals. The research shows that sustainability testing can be performed as part of all project process groups [3] and provide a measure of alignment with established sustainable development goals.

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# RAZLIKE FIZIKALNIH SVOJSTAVA IZMEĐU KORONINIH SVIJETLIH TOČAKA UNUTAR I IZVAN KORONINIH ŠUPLJINA

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Koronine svijetle točke osnovna su klasa solarne aktivnosti i predstavljaju skup niskokoronalnih petlji plazme malih razmjera koje spajaju fotosferne koncentracije magnetskog toka suprotnih polariteta [1]. Koristeći EUV 193 Å i magnetogram podatke prikupljene pomoću svemirskog teleskopa SDO (eng. Solar Dynamics Observatory) i podatke radiozračenja cijelog solarnog diska na valnoj duljini od 1.21 mm pomoću radioteleskopa ALMA (eng. Atacama Large Millimeter/submillimeter Array) [2], izvještavamo o mjerenjima intenziteta i veličine koroninih svijetlih točaka unutar koroninih šupljina i u području mirnog Sunca izvan koroninih šupljina. Odabrano je pet različitih koroninih šupljina za različita vremena u blizini središta solarnog diska, a njihove granice izvučene su iz EUV slika koristeći CATCH (eng. Collection of Analysis Tools for Coronal Holes) [3]. Koronine svijetle točke su zatim identificirane koristeći EUV i magnetogram podatke i provedena su mjerenja maksimalnog i srednjeg intenziteta uz promjer i površinu koroninih svijetlih točaka i za SDO EUV i za ALMA slike. Nadalje, 200 para uzoraka koroninih svijetlih točaka jednake veličine odabrana su slučajnim odabirom te je nad njima proveden t-test s nejednakim varijancama [4]. Svaki par uzoraka uključivao je jedan uzorak koroninih svijetlih točaka unutar koronine šupljine, a drugi izvan koronine šupljine. Statistička analiza izmjerenih svojstava pokazala je da koronine svijetle točke unutar koroninih šupljina u prosjeku imaju manji intenzitet i po veličini su manje od koroninih svijetlih točaka izvan koroninih šupljina. Ovo je bio slučaj i za SDO i za ALMA podatke, s nešto manjom razlikom za ALMA podatke, vjerojatno zbog loše prostorne razlučivosti. Ovisno o veličini uzorka i koroninoj šupljini, razlika intenziteta obično je iznosila oko  $3\sigma$  do  $4\sigma$ , ponekad i  $5\sigma$ , dok je za promjer i površinu razlika obično iznosila oko  $2\sigma$  do  $3\sigma$ , rijetko  $4\sigma$ . Ovim istraživanjem pokazali smo da postoji statistički značajna razlika određenih fizikalnih svojstava između koroninih svijetlih točaka unutar i izvan granica koroninih šupljina, a naše buduće istraživanje fokusirat će se na magnetsko polje koroninih šupljina kao jedan od mogućih uzroka opaženih razlika.

## ZAHVALE

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# DIFFERENCES IN PHYSICAL PROPERTIES BETWEEN CORONAL BRIGHT POINTS WITHIN AND OUTSIDE CORONAL HOLES

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Coronal bright points are a fundamental class of solar activity and represent a set of low-corona small-scale loops of plasma that connect photospheric magnetic flux concentrations of opposite polarities [1]. Using EUV 193 Å and magnetogram data obtained by SDO (Solar Dynamics Observatory) space telescope and full-disk radio emission data at 1.21 mm wavelength by ALMA (Atacama Large Millimeter/submillimeter Array) radio telescope [2], we report measurements of the intensity and size of coronal bright points within coronal holes and in the quiet Sun region outside coronal holes. Five different coronal holes at different times were chosen near the centre of the solar disk and their boundaries were extracted from EUV images using CATCH (Collection of Analysis Tools for Coronal Holes) [3]. Coronal bright points were then identified using EUV and magnetogram data and measurements of the maximum and mean intensity with diameter and area of selected coronal bright points were conducted for both SDO EUV and ALMA images. Next, 200 pairs of equal size coronal bright point samples were randomly chosen and unequal variances t-test was conducted on them [4]. Each sample pair included one coronal bright point sample within the coronal hole and another outside the coronal hole. Statistical analysis of the measured properties showed that coronal bright points within the coronal holes have on average lower intensity and are smaller in size than the coronal bright points outside coronal holes. This was the case for both the SDO and ALMA data, with a slightly smaller difference for ALMA data, possibly because of a poor spatial resolution. Depending on the sample size and coronal hole, the intensity difference was normally around  $3\sigma$  to  $4\sigma$ , sometimes even  $5\sigma$ , while for the diameter and area the difference was normally around  $2\sigma$  to  $3\sigma$ , rarely  $4\sigma$ . With this study we showed that there is a significant difference in certain physical properties between coronal bright points within and outside the boundaries of coronal holes and our future study will focus on the coronal hole magnetic field as one of the possible causes for the observed differences.

## ACKNOWLEDGMENTS

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## KOLONIZACIJA SUHOG RIJEČNOG KORITA SEMIKVATIČKIM I TERESTRIČKIM BESKRALJEŽNJACIMA (TSAI)

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U povremenim tekućicama voda periodično prestaje teći i one presuše. Povremene tekućice jedan su od najslabije proučavanih slatkovodnih ekosustava te se većina dosadašnjih istraživanja fokusirala isključivo na istraživanja funkcionalnih značajki svojiti akvatičkih organizama. Glavni pokretač ekoloških procesa ovih hidrološki dinamičnih i složenih slatkovodnih ekosustava je nestalnost protoka, [1] pri čemu se stvara mozaik lotičkih, lentičkih i terestričkih staništa. Njihova izmjena i trajanje utječu na varijabilnost zajednica semiakvatičkih i terestričkih beskralježnjaka (TSAI). Za TSAI zajednice značajno je lokalno nakupljanje i veliki obrtaj vrsta [1,2]. Iako su vrlo brojne u povremenim tekućicama te se sastoje od različitih svojiti, TSAI zajednice često su zanemarivane u dosadašnjim istraživanjima [3]. Organizmi TSAI zajednica su fiziološki i morfološki prilagođene na presušivanje, naseljavaju riparijsku zonu za vrijeme lotičke faze, a u terestričkoj fazi dolaze u suho riječno korito gdje prerađuju organske i hranjive tvari ili se hrane akvatičkim materijalom zaostalim nakon presušivanja [3].

Cilj istraživanja je utvrditi obrasce disperzije beskralježnjaka u suho riječno korito, stoga je istraživanje provedeno na rijeci Krčić, povremenoj tekućici Dinarskog krša, smještenoj podno Dinare. TSAI zajednice su bile uzorkovane u riparijskom staništu i suhom riječnom koritu pomoću križnih zamki za presretanje leta koje su pražnjene svakih 12 sati. Smjer i brzina vjetra kontinuirano su mjereni tijekom razdoblja uzorkovanja. Prikupljeni podaci analizirani su kako bi se utvrdile razlike u brzini vjetra (m/s) te smjeru vjetra (stupnjevi azimuta) između pojedinih staništa i između razdoblja uzorkovanja (dan/noć) u svakom tipu staništa. Uzorci prikupljeni zamkama sortirani su po višim taksonomskim kategorijama. Preliminarni rezultati ukazuju da semiakvatički i terestrički beskralježnjaci koloniziraju suho riječno korito iz riparijskih staništa. Također, primijetili smo razlike u obrascima aktivnosti i kolonizacije suhog korita među pojedinim svojitama letećih beskralježnjaka. Mjereni okolišni čimbenici utjecali su na proces kolonizacije.

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## DRY RIVERBED COLONISATION BY TERRESTRIAL AND SEMIAQUATIC INVERTEBRATES (TSAI)

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Intermittent rivers seasonally cease to flow and run dry. They are among the least studied freshwater ecosystems with most of the previous research focusing on the functional importance of aquatic organisms. The main driver of ecological processes in these habitats is flow intermittence [1] that creates a habitat mosaic of lotic, lentic, and terrestrial phases. The alternation and duration of phases strongly affects the variability of terrestrial and semiaquatic invertebrate (TSAI) communities, which are characterized by nestedness and species turnover [1,2]. TSAI communities were often overlooked in previous research despite being highly abundant and represented by numerous taxonomic groups in intermittent rivers [3]. These taxa are physiologically and morphologically adapted to drying events, they inhabit riparian habitats during the lotic phase and dry riverbed during the terrestrial phase, where they process organic matter or feed on residue aquatic material [3].

This research aimed to provide novel insights into invertebrate dispersal patterns in the dry riverbed. The research was conducted on the Krčić River, an intermittent river in Dinaric karst, beneath the Dinara Mountain.

TSAI communities were sampled in two focal habitat types: riparian and dry riverbed, using cross-vane window traps that were emptied every 12 hours. Wind velocity and directionality were measured continuously during the sampling period. Data collected by the loggers were analyzed to test for differences in wind velocity (m/s) and directionality (azimuth degrees) between the habitats and sampling intervals (day vs. night). TSAI samples were sorted to higher taxonomic levels (order or family). Preliminary results show that TSAI colonize the dry riverbed from adjacent riparian habitats. Moreover, we observed different activity and colonization patterns among flying invertebrate taxa. The colonization process was impacted by the measured environmental variables.

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## RAZVOJ MULTIREZIDUALNE LC/MS METODE ZA ODREĐIVANJE KONCENTRACIJA FARMACEUTIKA U VODENOM OKOLIŠU

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Farmaceutici ili aktivni farmaceutski sastojci (engl. *active pharmaceutical ingredients*; API) su kemijske tvari dizajnirane da pomognu u regulaciji specifičnih metaboličkih puteva u živim organizmima. U skladu s tim, može se očekivati da bi prisustvo tih, izrazito biološki aktivnih spojeva u vodenom okolišu, moglo dovesti do neželjenih efekata kod izloženih ne ciljnih organizama. S obzirom na osobine, kao i na kontinuirani porast njihove proizvodnje i potrošnje u svijetu, API danas predstavljaju jednu od najistaknutijih skupina tzv. novih tipova zagađivala [1-4].

Cilj ovog rada bio je razvoj i validacija multirezidualne analitičke metode za kvantitativno određivanje tragova 44 API i njihovih metabolita u različitim tipovima vodenih matrica primjenom spregnutog sustava tekućinske kromatografije i tandemne spektrometrije masa (LC-MS/MS). Kako bi se razvio robustan i pouzdan analitički protokol, pažljivo je optimiziran niz ključnih koraka uključenih u pripremu i instrumentnu analizu uzoraka. Razvijeni analitički postupak uključuje ekstrakciju uzoraka na kolonicama Oasis HLB te LC-MS/MS analizu koja se provodi uz primjenu ionizacije elektroraspršenjem u pozitivnom polaritetu te visokoselektivnu detekciju temeljenu na praćenju po 2 odabrane tranzicijske reakcije za svaki pojedini analit. Kromatografska separacija analita postignuta je na koloni Synergi Polar, dok su pokretne faze bile voda i metanol, uz dodatak 0.1% (v/v) mravlje kiseline. Točnost kvantifikacije niskih koncentracija analita osigurana je primjenom odgovarajućih izotopno označenih surogata. Validacijski parametri uključivali su određivanje linearnog raspona određivanja, analitičkih povrata ekstrakcije i uparavanja, utjecaja matrice te preciznosti i točnosti mjerenja. Razvijena analitička metoda primijenjena je za analizu uzoraka otpadne vode grada Zagreba i rijeke Save.

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# DEVELOPMENT OF MULTIREZIDUAL LC-MS/MS METHOD FOR THE ASSESSMENT OF TRACE LEVELS OF PHARMACEUTICALS IN AQUATIC ENVIRONMENT

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Pharmaceuticals or active pharmaceutical ingredients (APIs) are chemical compounds designed to help regulate the specific metabolic pathways in living organisms. Due to the high biological activity, exposure to these highly biologically active compounds in the aquatic environment could lead to adverse effects on non-target species. Consequently, having in mind a continuous increase in their production and consumption, APIs represent one of the most prominent categories of so-called emerging pollutants [1-4].

The aim of this work was to develop and validate a novel multiresidue method for the quantitative determination of trace levels of 44 APIs and their metabolites in aqueous matrices, using liquid chromatography tandem mass spectrometry (LC-MS/MS). To develop a robust and reliable analytical protocol, a careful optimization of the key steps included in the sample preparation and instrumental analysis was performed. The optimized method involved solid-phase extraction using Oasis HLB cartridges and LC-MS/MS analysis employing positive electrospray ionization and highly specific MRM detection, based on two selected precursor/product ion transitions for each analyte. The chromatographic separation of selected analytes was achieved using a Synergi Polar column with water and methanol, both containing 0.1% (v/v) formic acid, as eluting solvents. Accurate quantification of ng/L levels of target APIs was achieved using isotopically labeled surrogate standards. Validation parameters included determination of the method linearity range, extraction and evaporation recoveries, matrix effect, method precision and accuracy. The developed analytical method was applied to assess the API concentrations in wastewaters of the city of Zagreb and the Sava River.

## ACKNOWLEDGMENTS

This study was supported by the Croatian Science Foundation, Project Pharma-Risk (grant number IP-2019-04-9519), and Project Young Researchers Career Development – Education of Ph.D. Students (grant number DOK-2020-01-9250).

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## VERTIKALNI PRIJENOS VIRUSA CRYPHONECTRIA HYPOVIRUS 1 DOVODI DO EFEKTA USKOG GRILA

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*Cryphonectria parasitica* je fitopatogena gljiva, uzročnik raka kore kestena, koja je uvrštena na IUCN-ov popis 100 svjetskih najinvazivnijih alohtonih vrsta. Biokontrola ovog opasnog patogena je posredovana virusom *Cryphonectria hypovirus 1* (CHV1) koji smanjuje virulentnost gljive *C. parasitica* prema kestenu, smanjujući oštećenja na kori stabla uzrokovana ovom gljivom. CHV1 se prenosi vertikalno nespolnim sporama u potomstvo i horizontalno anastomozom hifa dvaju micelija. Prethodna istraživanja pokazuju da se, u prosjeku, virus prenese u 69% nespolnih spora (konidija) te da uspješnost prijenosa uvelike ovisi o podtipu samog virusa te o genotipu gljive [1]. U ovom istraživanju korištena su dva podtipa CHV1: podtip I, najrašireniji u Europi te prirodno prisutan u Hrvatskoj i podtip F, prisutan u nekim populacijama *C. parasitica* u zapadnoj Europi. Postotak uspješnog prijenosa virusa u konidije je bio visok (preko 75%) te nije bilo razlike u uspješnosti prijenosa između ova dva podtipa. Budući da su najbolji izvor informacija o virusima i njihovoj evoluciji analize genomskih sekvenci, analizirana je raznolikost populacija virusa CHV1 unutar domaćina. Korištena je metoda sekvenciranja PacBio (Pacific Biosciences) koja generira duga i točna očitavanja, kreira vrlo točnu konsenzus sekvencu te se odlikuje ujednačenom kvalitetom očitavanja duž čitave sekvence [2]. Procijenjene su stopa mutacije te promjena haplotipske raznolikosti između roditeljskog micelija i spora. Uočeno je značajno opadanje nukleotidne raznolikosti, ali ne i haplotipske raznolikosti, čiji pad nije bio statistički značajan. Efekt uskog grla je stoga vidljiv u smislu opadanja mutacijske raznolikosti, no čini se da se haplotipska raznolikost uglavnom prenosi u spore.

### ZAHVALE

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# LONG READ SEQUENCING REVEALS MUTATIONAL BOTTLENECK AFTER VERTICAL TRANSMISSION OF CRYPHONECTRIA HYPOVIRUS 1

Karla Peranić<sup>1,\*</sup>, Deborah Leigh,<sup>2</sup> Maja Morić,<sup>1</sup> Lucija Nuskern,<sup>1</sup> Mirna Ćurković Perica,<sup>1</sup> Quirin Kupper,<sup>2</sup> Carolina Cornejo,<sup>2</sup> Daniel Rigling,<sup>2</sup> Marin Ježić<sup>1</sup>

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*Cryphonectria parasitica*, a phytopathogenic fungus which causes chestnut blight is enlisted in the top 100 of the world's worst invasive alien species by IUCN. *Cryphonectria hypovirus 1* (CHV1) can act as a biocontrol agent of this serious pathogen, by reducing the virulence of its host fungus towards the chestnut tree, thus giving it time to heal. CHV1 is transmitted by vertical transmission through asexual spores into the progeny and by horizontal transmission via hyphal anastomosis between individuals. Previous research showed that, on average, transmission of the virus was observed into 69% of the asexual spores (conidia) and is dependant significantly on CHV1 subtype and fungal strain [1]. In this experiment two different subtypes of CHV1 were used: subtype I, the most widespread across Europe and naturally present in Croatia, and subtype F, which is present in several *C. parasitica* populations in Western Europe. The percentage of CHV1 transmission into the conidia was high (over 75%) and didn't differ between the two subtypes. As the analysis of viral genomes provide the most comprehensive source of information about their evolution and dissemination, we analysed intra-host CHV1 diversity utilising PacBio (Pacific Biosciences) sequencing which generates long reads with high consensus accuracy and a uniform coverage throughout the entire generated sequence [2]. We studied the mutational rate and change of haplotypic diversity between the parental isolates and their single spore progeny. While a significant decrease in mutational diversity was observed, however, there was no statistically significant decrease in haplotypic diversity of the CHV1 between parental mycelium and its progeny. The bottleneck effect during the vertical virus transmission reduces overall nucleotide diversity of the viral populations, but it appears that the overall haplotypic diversity i.e. number of distinct viral haplotypes, is inherited by the spores.

## ACKNOWLEDGMENTS

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## ISTRAŽIVANJE LOKALNE STRUKTURE DOPIRANOG BIZMUTATA $Ba_{1-x}K_xBiO_3$

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Kalijem dopirani perovskit  $BaBiO_3$  (BKBO) povijesno je prvi otkriveni visokotemperaturni supravodič [1,2] s temperaturom supravodljivog prijelaza ( $T_c$ ) koja seže do 35 K [2]. Ipak, važna pitanja koja se tiču lokalne strukture ovog materijala ostala su nerazriješena, dok njihovi odgovori u sebi sadrže implikacije za prirodu metalnog i supravodljivog stanja [2]. Ovdje predstavljamo istraživanje lokalne strukture bizmutata BKBO u okolini metal-izolator prijelaza pomoću kombinacije difuznog rendgenskog raspršenja i Monte Carlo modeliranja. Usporedbom eksperimentalnih i modeliranjem dobivenih podataka s visokom vjerojatnošću isključeno je postojanje dugodosežnog i kratkodosežnog vala gustoće naboja povezanim s fenomenom preskakanja valencije koji je primjećen na nižim dopiranjima. Ovaj rezultat jednoznačno pokazuje da preskakanje valencije i povezani polaroni efekti [4] nisu relevantni za razumijevanje metalnog i supravodljivog stanja u ovom materijalu, o čemu se dugo spekuliralo u literaturi. Umjesto toga, iz vektorske distribucijske funkcije parova (3D- $\Delta$ PDF) direktno dobivene iz eksperimentalnih podataka, uz lokalne „size-effect“ distorzije uzrokovane dopiranjem, uočavaju se i strukturne korelacije koje lome inverzijsku simetriju na nanoskali te bi zbog toga mogle imati dalekosežne implikacije za elektronsku fiziku u BKBO. Na posljetku, koristi se Monte Carlo modeliranje kako bi se ovaj rezultat konsolidirao te kako bi se isti doveo u vezu s međuatomskim interakcijama, kemijskim razmatranjima te tendencijama u prosječnoj strukturi [5].

### ZAHVALE

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# INVESTIGATING THE LOCAL STRUCTURE OF DOPED BISMUTHATE $Ba_{1-x}K_xBiO_3$

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The potassium doped perovskite  $BaBiO_3$  (BKBO) exhibits a superconducting transition temperature ( $T_c$ ) as high as 35 K [1] and was the first high- $T_c$  oxide to be discovered [2,3], yet pivotal questions regarding the nature of the local structure remain unresolved, with possible implications for the nature of both the superconducting and metallic state [3]. Here we present a combined diffuse x-ray scattering and Monte Carlo (MC) modeling study of the local structure of BKBO across its insulator-metal boundary. By comparing the data obtained from modeling and experiment we exclude with high probability the existence of either long- or short-range valence disproportionation. This resolves a major conundrum, as it shows that disproportionation and the related polaronic effects [4] are not relevant to the metallic and superconducting states. Instead, using the vector pair distribution function (3D- $\Delta$ PDF) obtained from diffuse x-ray scattering data we uncover nanoscale structural correlations that break inversion symmetry, which might have long-ranging implications for electronic physics in BKBO, along with substantial size-effect distortions caused by potassium doping. In the end, we use Monte Carlo modeling to confirm this insight and to investigate its possible causes and relation to interatomic interactions, crystal chemistry, and average structural tendencies [5].

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- [4] Jiang, M., Sawatzky, G. A., Berciu, M. & Johnston, S. *Phys. Rev. B* **103** (2021) 115129.
- [5] Pei, S. et al. *Phys. Rev. B* **41** (1990) 4126.



## PRIMJENA INTEGRATIVNOG PRISTUPA ZA RAZJAŠNJENJE RAZNOLIKOSTI VRSTE *Dianthus sylvestris* Wulfen s.l. NA BALKANSKOM POLUOTOKU

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*Dianthus sylvestris* s.l. je skupina morfološki vrlo varijabilnih svojite rasprostranjenih u središnjem Mediteranu, čija je najveća taksonomska raznolikost zabilježena u Italiji. Drugo središte raznolikosti je Balkanski poluotok, gdje je unutar vrste *D. sylvestris* opisano šest svojiti tretiranih na razini podvrste: subsp. *alboroseus*, subsp. *bertisceus*, subsp. *kozjakensis*, subsp. *nodosus*, subsp. *sylvestris* i subsp. *tergestinus*. Njihovo je prepoznavanje izazovno zbog složene morfološke i ekološke varijabilnosti. U tekućem istraživanju, kombiniramo niz metoda od nomenklature revizije i morfometrije, preko citologije i procjene veličine genoma, do analize okolišne niše i populacijske genetike, kako bi razriješili odnose unutar *D. sylvestris* s.l. na Balkanskom poluotoku. Morfološke analize pokazale su postojanje kontinuirane varijabilnosti kvantitativnih morfoloških osobina i nedovoljno jasno razlučivanje kvalitativnih morfoloških osobina između podvrsta. Pored toga, procjena preklapanja niša je pokazala kako je sličnost niša češća između šest istraživanih podvrsta, nego njihova divergencija. Rezultati populacijske genetike pokazuju da *D. sylvestris* subsp. *tergestinus* predstavlja odvojenu evolucijsku liniju, dok ostale populacije na Balkanskom poluotoku tvore klinu genetske diferencijacije u smjeru JI-SZ. Konačno, procijenjene vrijednosti relativne veličine genoma pomoću protočnog citometra i potvrda stupnja ploidijske na temelju prebrojanih kromosoma, pokazali su postojanje poliploidizacije u SZ dijelu Balkanskog poluotoka, gdje su tetraploidne populacije ( $2n=4x=60$ ) geografski ograničene na području Istre i Kvarnera u Hrvatskoj te u Italiji. Sve ostale balkanske populacije bile su diploidne ( $2n=2x=30$ ).





## DISENTANGLING THE DIVERSIFICATION OF *Dianthus sylvestris* Wulfen s.l. ON THE BALKAN PENINSULA USING AN INTEGRATIVE APPROACH

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*Dianthus sylvestris* s.l. is a group comprising morphologically highly variable taxa distributed in central Mediterranean, with the highest taxonomic diversity described in Italy. Another diversity centre is the Balkan Peninsula, from where six taxa mostly treated as subspecies of *D. sylvestris* have been described: subsp. *alboroseus*, subsp. *bertisceus*, subsp. *kozjakensis*, subsp. *nodosus*, subsp. *sylvestris* and subsp. *tergestinus*. Their identification is challenging due to great morphological variability and complex patterns of variation with respect to ecological preferences. In an ongoing study, we are combining an array of methods ranging from nomenclatural revision and morphometrics, over cytological and genome size estimation, to environmental niche analyses and population genetic analyses to disentangle relationships within *D. sylvestris* s.l. on the Balkan Peninsula. The morphometric analyses revealed a continuous variability of quantitative morphological characters and an absence of clear-cut qualitative morphological differences among the subspecies. In addition, the niche overlap assessment indicated that niche similarity is more common among the six subspecies, than niche divergence. Population genetics analyses suggest that *D. sylvestris* subsp. *tergestinus* presents a separate evolutionary lineage, while other populations on the Balkan Peninsula form a cline of genetic differentiation in the SE-NW direction. Finally, the relative genome size estimation using flow cytometry, with confirmatory chromosome counts, revealed polyploidisation in the NW Balkan Peninsula, as the tetraploid populations ( $2n=4x=60$ ) are restricted to the Istrian Peninsula and Kvarner in Croatia and Italy. All the remaining Balkan populations were diploid ( $2n=2x=30$ ).



# EKOTOKSIKOLOŠKA PROCJENA UTJECAJA KARDIOVASKULARNIH FARMACEUTIKA PRISUTNIH U VODENIM EKOSUSTAVIMA NA EMBRIJE RIBE ZEBRICE *Danio rerio* (Hamilton, 1822)

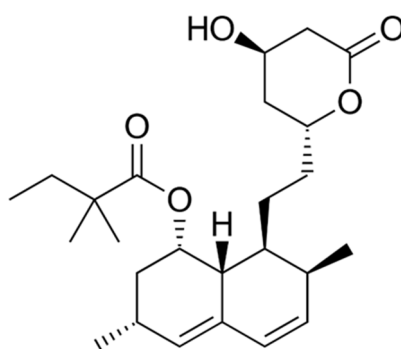
Gabrijela Matijević,<sup>1,\*</sup> Göran Klobučar,<sup>2</sup> Sanja Babić<sup>1</sup>

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Proces onečišćenja okoliša doživljava eksponencijalan i nezaustavljiv rast te je postao jedan od najvećih problema s kojima se danas čovječanstvo susreće. Napredak znanosti doveo je i novu vrstu onečišćenja, lijekovima, odnosno farmaceutski aktivnim spojevima koji su jedna od glavnih komponenti onečišćenja kopnenih voda i koji su utvrđeni u sedimentu i plazmi riba iz rijeke Save [1,2]. Na temelju dosadašnjih istraživanja izdvojeni su kardiovaskularni lijekovi: amiodaron, ramipril, simvastatin i verapamil čiji je utjecaj istražen, pojedinačno i u smjesi, na razvoj embrija ribe zebrice *Danio rerio* (Hamilton, 1822) u prvih 96 hpf. Tijekom izlaganja okolišno relevantnim koncentracijama (0,1, 1,0 i 10,0 µg/L) praćene su izazvane promjene kao što su mortalitet i stopa abnormalnosti, dužina ličinki, stopa izvaljivanja, brzina otkucaja srca, spontani pokreti prsnim perajama i oksidativni stres. Povećanjem koncentracije i vremenske izloženosti jedinki testiranim spojevima uočeni su učinci pri izlaganju svim spojevima, no najveća toksičnost zabilježena je na uzorku simvastatina. Integracijom krajnjih bioloških učinaka ispitivanih unutar ovog istraživanja, potvrđena je izvrsna uzročno-posljedična veza između izlaganja farmaceutskim spojevima i zabilježenog učinka na akvatičkom organizmu, čime je pružena podloga za biomonitoring onečišćenja okoliša, posebice slatkovodnih ekosustava.



Slika 1. Struktura simvastatina.

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# ECOTOXICOLOGICAL ASSESSMENT OF THE IMPACT OF CARDIOVASCULAR PHARMACEUTICALS PRESENT IN AQUATIC ECOSYSTEMS ON ZEBRAFISH EMBRYOS *Danio rerio* (Hamilton, 1822)

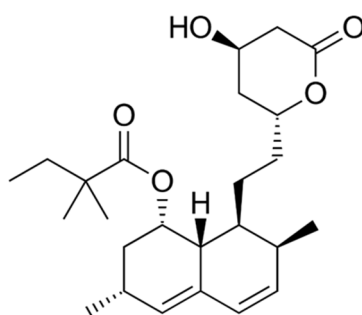
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The process of environmental pollution is experiencing exponential and unstoppable growth and has become one of the biggest problems facing humanity today. Progress in science has also brought a new type of pollution, therapeutic drugs, or pharmaceutically active compounds, which are among the main components of inland water pollution and have been found in the sediment and plasma of fish from the Sava River [1,2]. Cardiovascular drugs were selected based on previous studies: amiodarone, ramipril, simvastatin and verapamil, whose effect on the development of the zebrafish embryo *Danio rerio* (Hamilton, 1822) was studied in the first 96 hpf individually and in a mixture. During exposure to environmentally relevant concentrations (0.1, 1.0, and 10.0 µg/L), induced changes such as mortality and abnormality rate, larval length, hatching rate, heart rate, spontaneous movements of the pectoral fins and oxidative stress were monitored. With increasing concentration and duration of exposure of individuals to the tested compounds, effects on exposure to all compounds were observed, but the highest toxicity was observed in the simvastatin sample. The integration of the ultimate biological effects examined in this study confirmed the excellent cause-effect relationship between exposure to pharmaceutical compounds and the observed effect on aquatic organisms, thus providing a basis for biomonitoring of environmental pollution, especially freshwater ecosystems.



**Figure 1.** Structure of simvastatin.

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## GEOKEMIJSKA ANALIZA MANGANSKE JALOVINE IZ ŠIBENSKOG ZALJEVA (HRVATSKA) I NJENI OKOLIŠNI UTJECAJI

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Na istočnoj obali Jadranskog mora, manganska jalovina iz povijesne Tvornice elektroda i ferolegura (TEF) ostavljena je pod utjecajem ponekad vrlo nepovoljnih atmosferskih prilika nakon zatvaranja tvornice u drugoj polovici 1990-ih godina. Ovaj jalovinski materijal koji se nalazi u blizini stare jezgre grada Šibenika, povremeno je izložen vjetrovima do 130 km/h te predstavlja zdravstveni i okolišni rizik. Tvornica je otvorena 1900. godine te od tada postoji značajan unos različitih onečišćivača u okoliš. Kako bi se utvrdio utjecaj na okoliš i mogućnost ponovne ekstrakcije metala iz jalovine, provedene su detaljne fazne i geokemijske analize. Analize su uključivale rendgensku difrakciju na prahu (XRD), masenu spektrometriju (BCR sekvencijalna analiza, fuzija litijeva metaborata analizirane ICP-MS-om) te SEM-EDS analize. Analize su obavljene na uzorcima sedimenta s dvije uvale: obližnja Mala Martinska i udaljena uvala Mala Stupica na otoku Žirje; te na uzorcima prašine s krova tvornice i jalovinskog materijala. Fazna analiza utvrdila je nekoliko manganskih vrsta u jalovini i prašini (bustamit -  $(Ca, Mn)_3Si_3O_9$  i manganske okside), a manganske faze su utvrđene i u sedimentima iz uvale Mala Martinska. Geokemijske analize pokazale su 256 g/kg Mn u uzorku prašine, 1 g/kg Zn te 100 mg/kg REE. Ovakve koncentracije s napretkom tehnologije mogu postati od interesa. Oko 500 mg/kg Mn izmjereno je u sedimentima uvale Mala Martinska, dok koncentracije Mn u uvali Mala Stupica dosežu  $\approx 45$  mg/kg, što ukazuje na utjecaj jalovine na okoliš. Poznato je da mangan ima nekoliko negativnih efekata na organizme u različitim okolišima – uzrokujući promjene u normalnim funkcijama ponekih imunoloških parametara u organizmima. U okolišu mangan postaje biodostupan kroz proces eutrofikacije, a ukoliko se reduktivni uvjeti nastave, može ostati biodostupan kroz dulje vrijeme. Zbog toga, koncentracije Mn i nekih drugih elemenata od interesa u ovom istraživanju uspoređene su s maksimalno dozvoljenim koncentracijama (MDK) u sedimentu propisanim u Norveškoj i Australskoj legislativi, budući da iste nema u Europskoj Uniji. Utvrđeno je da je većina vrijednosti unutar dozvoljenih granica.

### ZAHVALE

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# GEOCHEMICAL ANALYSIS OF MANGANESE TAILINGS FROM ŠIBENIK BAY (CROATIA) AND THEIR ENVIRONMENTAL IMPACT

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On the eastern Adriatic coast, manganese tailings from a historic ferroalloy plant (TEF) were left in (sometimes very harsh) atmospheric conditions after the plant closed in the late 1990s. These tailings near the old town of Šibenik are sometimes exposed to winds of up to 130 km/h and pose a health and environmental risk. Production at the plant began in 1900, and there has been an input of different contaminants in the surrounding area ever since. To determine the environmental impact of the tailings and dust and to assess whether there was potential for reuse detailed phase and geochemical analyses were carried out. These included X-ray powder diffraction (XRD), mass spectrometry (BCR sequential analysis and lithium borate fusion followed by ICP-MS) and SEM-EDS analysis. The analyses were carried out on sediment samples from two coves: a nearby cove (Mala Martinska Cove) and a more distant cove on the island of Žirje (Mala Stupica Cove), as well as on tailings and dust samples from factory roofs. Phase analysis revealed various manganese phases in tailings and dust (bustamite -  $(\text{Ca, Mn})_3\text{Si}_3\text{O}_9$ ; and manganese oxides), and manganese phases were also detected in the sediments of Mala Martinska Cove. Geochemical analyses revealed 256 g/kg Mn in the dust sample, 1 g/kg Zn and 100 mg/kg REE. These values are lower than those currently required for reuse, but could become promising with advances in technology. About 500 mg/kg Mn was detected in the sediments of Mala Martinska Cove, while the Mn concentration in Mala Stupica Cove reaches  $\approx 45$  mg/kg, indicating the environmental impact of the tailings. Manganese is known to have several negative effects on organisms in different environments – one being changes in the regular functions of some immune parameters in organisms. Manganese becomes bioavailable through eutrophication and remains so for a long time if reducing conditions persist. Therefore, the concentrations of Mn and some other elements of interest determined in this study were compared with Norwegian and Australian sediment limit value legislation, as none exist in the European Union. Most of the values were found to be within the permissible limits.

## ACKNOWLEDGMENTS

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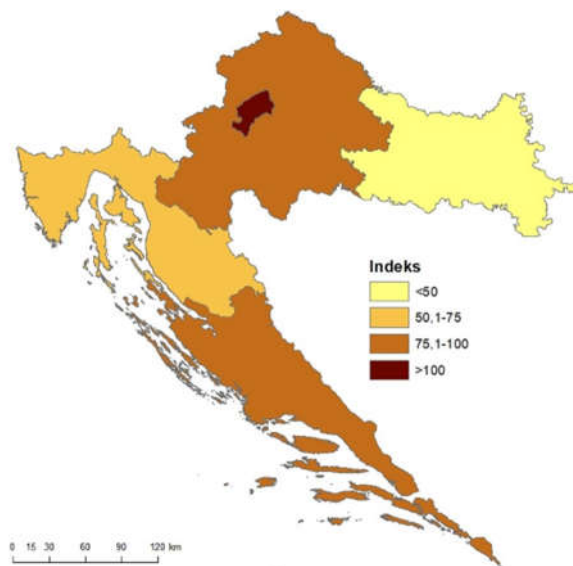
## REGIONALNE RAZLIKE UČINKOVITOSTI NASTAVE GEOGRAFIJE U REPUBLICI HRVATSKOJ

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Učinkovitost nastave geografije i razina učeničke motivacije rezultat su brojnih psiholoških i socio-ekonomskih čimbenika te obilježja škole, nastave i nastavnika [1]. S obzirom na promjenjivost tih čimbenika u prostoru, učinkovitost nastave geografije pokazuje značajne razlike i u sustavu osnovnih škola na teritoriju Republike Hrvatske. Primjenom dvaju specifičnih pokazatelja razine učinkovitosti i motivacije za nastavni predmet, a na uzorku od 139 osnovnih škola, razvidne su jasne i razmjerno velike regionalne razlike, osobito promatrajući indeks nacionalne geografske konkurentnosti (Sl. 1) [4]. Najizraženiji pol visoke razine učinkovitosti predstavlja Grad Zagreb gdje je prosječna riješenost Nacionalnog ispita iz geografije [2] za četiri postotna poena veća od nacionalnog prosjeka, a udio učenika-sudionika državnih natjecanja gotovo dvostruko veći od udjela učeničke populacije ove prostorne jedinice u ukupnoj učeničkoj populaciji Republike Hrvatske. Navedeno je svakako u razmjerno velikoj mjeri određeno socio-ekonomskim uvjetovanostima koji na ovom uzorku, slično kao i u prethodnim istraživanjima [3], objašnjavaju oko 25 % varijabiliteta školskog uspjeha iz nastavnog predmeta geografija.



Slika 1. Indeks nacionalne geografske konkurentnosti (2016.-2020.) u izdvojenim regijama

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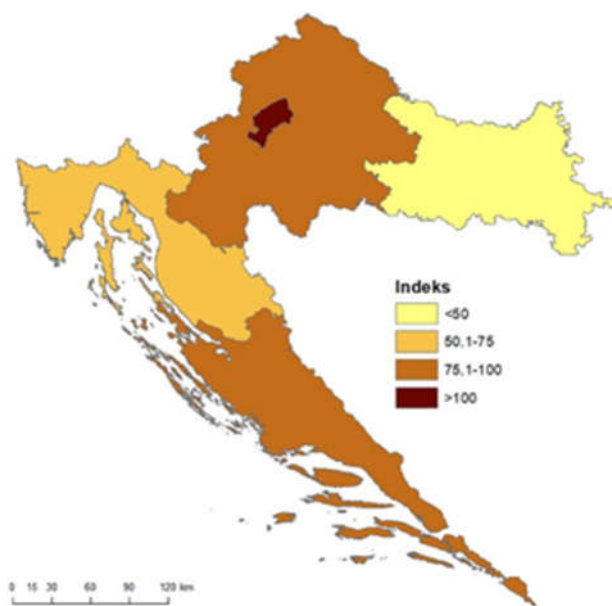


## REGIONAL DIFFERENCES ON THE EFFICIENCY OF GEOGRAPHY TEACHING IN THE REPUBLIC OF CROATIA

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The effectiveness of geography teaching and student motivation are the result of numerous psychological and socioeconomic factors, as well as school, classroom, and teacher characteristics [1]. Given the spatial variability of these factors, the effectiveness of geography teaching in the system of primary school on the territory of the Republic of Croatia shows significant differences. Using two specific indicators of the level of efficiency and motivation for the subject and a sample of 139 primary schools, significant and relatively large regional differences emerge, especially when considering the National Geographic Competitiveness Index (Fig. 1). The most pronounced pole of high efficiency is the City of Zagreb, where the average resolution of the national geography exam [2] is four percentage points higher than the national average, and the proportion of students participating in national competitions is almost double the proportion of students in the total student population of the Republic of Croatia. This is certainly determined to a relatively large extent by socioeconomic conditions, which in this sample, similar to previous studies [3], explain about 25% of the variability in school achievement in geography.



**Figure 1.** National geographic competitiveness Index (2016–2020) in selected regions [4]

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## RASPROSTRANJENOST, MORFOMETRIJSKE KARAKTERISTIKE I SASTAV EPIFITA VRSTE *GONGOLARIA* *BARBATA* U LAGUNI ŠĆUZA

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Vrsta *Gongolaria barbata* je smeđa makroalga karakteristična po formiranju gustih podmorskih šuma izrazito značajnih za bioraznolikost i integritet morskih ekosustava. Međutim, u posljednjih nekoliko desetljeća zbog negativnog utjecaja raznih stresora, brojnost vrsta iz skupine „*Cystoseira sensu lato*“, uključujući vrstu *G. barbata*, značajno opada diljem Sredozemlja [1]. Uvala Šćuza obalna je laguna na jugu Istre, smještena između mjesta Pomer i Premanture, a odvojena od Medulinskog zaljeva uskim betonsko – kamenim mostićem. Od vegetacije, u laguni dominira morska cvjetnica *Cymodocea nodosa*, dok je po brojnosti i biomasi prati makroalga *G. barbata*. Naselje vrste *G. barbata* u laguni Šćuza je najveće poznato naselje navedene vrste u priobalju Istre i jedno od posljednjih utočišta vrste u sjevernom Jadranu. U laguni osim pričvršćenih jedinki vrste *G. barbata* na hridinastom dnu, pronađena je i slobodnoživuća forma vrste, kod koje se morfometrijske karakteristike već na prvi pogled razlikuju. Ciljevi ovog rada su utvrditi rasprostranjenost vrste *G. barbata* u laguni, opisati osnovne morfometrijske karakteristike dviju zabilježenih formi i odrediti sastav epifita na njezinom talusu. Za svaku formu je kvantitativno sakupljeno 37 talusa. U laboratoriju je za svaki talus određena mokra biomasa i sljedeće morfometrijske karakteristike: dužina kauloida, ukupna dužina kauloida i broj kauloida. Talusi vrste obrasli su epifitima koji su pažljivo odstranjeni i detaljnije analizirani. Obje forme vrste *G. barbata* utvrđene su na južnom i jugozapadnom dijelu lagune, na kojem je djelomično hridinasto dno te pjeskovito i muljevito dno. Na dubini od 0,5 do 1,5 metara na hridinastom dnu utvrđena su gusta, kontinuirana naselja vrste, dok je slobodnoživuća forma bila usko lokalizirana. Kod slobodnoživućih jedinki utvrđene su statistički značajno više vrijednosti mokre biomase, dužine kauloida, ukupne dužine kauloida i broja kauloida u odnosu na pričvršćene jedinke. Najveća ukupna dužina kauloida slobodnoživućeg talusa iznosila je 575 cm, a kod pričvršćene jedinice 187 cm. Nadalje, i ukupan broj kauloida kod slobodnoživućih jedinki bio je veći u odnosu na pričvršćene jedinke. Upravo visoke vrijednosti ukupne dužine i brojnosti kauloida slobodnoživućih jedinki, upućuju na višegodišnju starost ovih jedinki. Od epifita je zabilježeno ukupno 9 svojti makroalgi i 7 svojti makrozoobentosa. Vrsta *G. barbata* u laguni Šćuza predstavlja potencijal donorske populacije, koja bi se u budućnosti mogla koristiti pri obnovi degradiranih staništa ove vrste duž zapadne obale Istre.

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# DISTRIBUTION, MORPHOMETRIC CHARACTERISTICS AND EPIPHYTE COMPOSITION OF *GONGOLARIA BARBATA* IN THE ŠĆUZA LAGOON

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*Gongolaria barbata* is a brown algal species known for forming dense underwater forests crucial for biodiversity and the integrity of marine ecosystems. In recent decades, the impact of different negative factors has resulted in the abundance of „*Cystoseira sensu lato*“ species, including *G. barbata*, being reduced to a minimum throughout the Mediterranean Sea [1]. The Šćuza lagoon is a coastal lagoon in the south of Istria, located between Pomer and Premantura, divided from Medulin bay with a narrow concrete-stone bridge. The vegetation is dominated by seagrass *Cymodocea nodosa*, followed by high abundance of *G. barbata*. The *G. barbata* population in Šćuza is the largest known population of the species along the Istrian coast, and probably one of the last refuge of the species in the northern Adriatic Sea. Besides the attached form of *G. barbata* found on the rocky bottom, free-living specimens stood out with visible morphometric differences. The aim of the present study is to describe the distribution of the species in the lagoon, inspect for differences in main morphometric characteristics between the two forms and to get an insight into the epiphyte composition. For each form, 37 individuals were quantitatively collected for further analysis. The two forms were compared based on biomass and morphometric characteristics such as: cauloid length, total cauloid length and number of cauloids. Thalli of the species were overgrown by epiphytes which were carefully removed and analyzed. The species is distributed in the southern and southwestern part of the lagoon where the substrate is rocky, sandy and muddy. A dense, homogenous population of the attached form was found on the rocky bottom at depths between 0.5 to 1.5 m, while the free-living individuals were mainly localized. Individuals characterized as free-living showed significantly higher values for biomass, as well as for the main morphometric characteristics in comparison to the attached individuals. The maximum value of total cauloid length in the free-living individuals was 575 cm, while 187 cm in the attached individuals. Furthermore, the total number of cauloid was higher in the free-living individuals. High total cauloid length values, as well as high cauloid number values, indicate perennial age of the free-living individuals. Epiphyte composition shows the total of 9 macroalgal taxa and 7 macrozoobenthos taxa. The *G. barbata* species in the Šćuza lagoon represents a high value donor population, which could serve for future restoration actions of degraded habitats along the Istrian coast.

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# UTVRĐIVANJE EKOLOŠKOG STANJA MORSKIH SEDIMENATA U SVRHU PREDLAGANJA LISTE MIKROBNIH POKAZATELJA ZA POSTIZANJE DOBROG STANJA OKOLIŠA

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S obzirom na višestruke antropogene pritiske u obalnim okolišnim zonama te na činjenicu da više od polovice stanovništva Mediterana živi upravo u tim područjima, države mediteranske regije donijele su niz inicijativa a sa svrhom zaštite morskog okoliša. Među njima se ističe Okvirna direktiva o Morskoj strategiji u sklopu koje su se države potpisnice obvezale izraditi strategiju za postizanje Dobrog stanja morskog okoliša (DSO) [1]. Međutim, među pokazateljima koji se kontinuirano prate izostaje praćenje promjena na razini mikrobnih zajednica, iako upravo mikroorganizmi imaju ključnu ulogu u funkcioniranju hranidbenih mreža, biogeokemijskim ciklusima kruženja elemenata u morskome okolišu te su odgovorni za održavanje stabilnosti morskih ekosustava [2]. Smatramo da bi upravo mikrobnе zajednice mogle biti potencijalno vrlo vrijedni indikatori stanja okoliša putem kojih bih se pravovremeno mogla izdati upozorenja o antropogeno uvjetovanim promjenama u okolišu, i donijeti mjere oporavka onečišćenih područja. Cilj istraživanja je istražiti važnost i doprinos praćenja promjena na razini mikrobnih zajednica u procjeni stanja morskog okoliša. Istraživanje se bazira na morskome sedimentu koji predstavlja mjesto dugotrajne akumulacije/koncentracije onečišćivala čime daje stvaran uvid u dugotrajne posljedice antropogenog utjecaja na okoliš. Prvi rezultati fizikalno-kemijske karakterizacije sedimenata sakupljenih u 7 luka duž istočne obale Jadrana, koji su godinama ocjenjene kao lokacije s „lošim stanje okoliša“, ukazali su na prisutnost niza različitih onečišćenja na lokacijama uzorkovanja od kojih su na nekima mjerene iznimno velike koncentracije onečišćivala. Uz pretpostavku da će struktura i funkcija mikrobnih zajednica na navedenim lokacijama pratiti odnosno odražavati sliku ekološkog stanja sedimenata na tim lokacijama ovim istraživanjem predložiti će se određeni „atributi“ mikrobnih zajednica kao potencijalno novi indikatori stanja okoliša. Ovo istraživanje predstavljati prvi korak u potencijalnoj integraciji mikrobnih zajednica kao jednog od bitnih parametara za postizanje Dobrog stanja okoliša (DSO).

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# DETERMINATION OF THE ECOLOGICAL CONDITION OF MARINE SEDIMENTS FOR THE PURPOSE OF PROPOSING A LIST OF MICROBIAL INDICATORS FOR ACHIEVING GOOD ENVIRONMENTAL STATUS

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Given the multiple anthropogenic pressures in coastal zones and the fact that more than half of the Mediterranean population lives in these areas, the countries of the Mediterranean Sea region have adopted a number of initiatives aimed at protecting their marine environment. Among them is the Marine Strategy Framework Directive, under which the countries have committed themselves to develop a strategy for achieving Good Environmental Status (GES) [1]. However, among the routinely monitored indicators, changes within the microbial community structure and function is neglected, despite the fact that microorganisms play a key role in the functioning of marine food webs, biogeochemical cycles in the marine environment and are responsible for maintaining marine ecosystem stability [2]. We suggest microbial communities could potentially be valuable indicators of environmental change which could help in issuing early warnings about anthropogenic pollution and adopting faster measures for recovery. The aim of this project is to investigate the importance and contribution of monitoring changes at the level of microbial communities for the assessment of the health status of the marine environment. The research focuses on a benthic habitat, which represents a sink for accumulation of pollutants, thus providing us with an insight into the long-term consequences of anthropogenic pressures on the environment. Our first results, based on physicochemical characterization of sediments collected in seven harbors situated along the eastern Adriatic coast, marked as sites with "poor environmental status", indicated contamination with a number of different contaminants, some of which measured in extremely high concentrations. Assuming that the structure and function of microbial communities at these sites will reflect the ecological status of the marine environment, this research will propose certain "characteristics" of microbial communities as potentially new indicators of marine environmental health. This research represents the first step in the potential integration of microbial communities as one of the essential parameters for achieving Good Environmental Status (GES).

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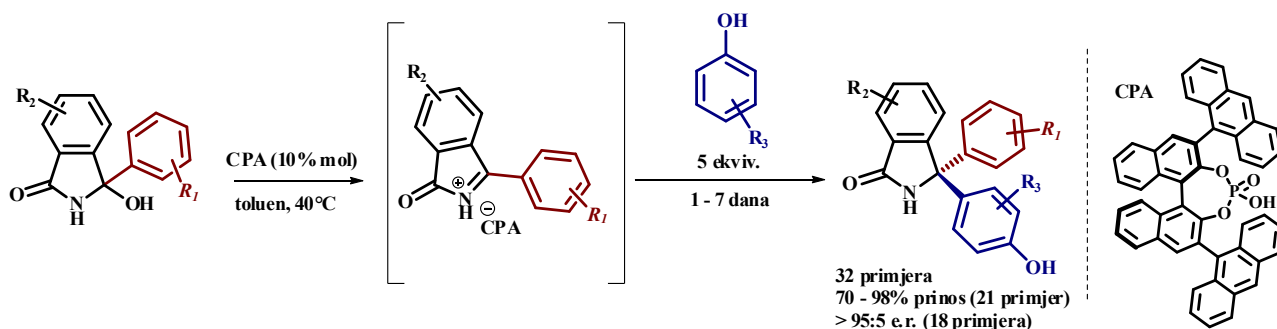


# STEREOSELEKTIVNA ARILACIJA DERIVATA IZOINDOLINONA U ORGANOKATALITIČKIM UVJETIMA

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Optički čisti 3,3-disupstituirani izoindolinonski derivati predstavljaju se kao sveprisutne strukturne podjedinice u brojnim farmakološki aktivnim molekulama čija je aktivnost uvjetovana prisutnošću određenih supstituenata oko stereogenog centra, kao i njegovoj apsolutnoj konfiguraciji.<sup>[1]</sup> Dosad je u literaturi opisan veliki broj protokola koji uključuju organokatalitičke transformacije na izoindolinonima, kao što su stereoselektivne adicije heteroarila,<sup>[2]</sup> heteroatoma<sup>[3]</sup> te nearomatskih ugljikovih nukleofila.<sup>[4]</sup> No, još uvijek nije razvijen protokol koji bi omogućio enantioselektivno uvođenje trećeg arilnog supstituenta na C3 položaj izoindolinona. U tom kontekstu uspješno smo razvili protokol koji opisuje primjenu kiralnih fosforinih kiselina (CPA) za sintezu triarilnih derivata izoindolinona s kvaternim stereogenim centrom u organokatalitičkim uvjetima. Uspjeh ove transformacije može se pripisati *in situ* stvaranju *N*-acil ketimina iz 3-hidroksisupstituiranih izoindolinona čineći ih dovoljno elektrofilnima za reakciju s fenolom (shema 1).<sup>[5]</sup> Reakcija je regioselektivna i enantioselektivna, te je tolerantna s obzirom na prisutnost različitih funkcionalnih skupina na oba reakcijska partnera. Mehanizam stereokemijske indukcije izučavan je primjenom kvantnomehaničkih proračuna, te je ustanovljeno da nukleofilni napad fenola više favoriziran preko *Re* prokiralne strane elektrofila.



**Slika 1.** Stereoselektivna arilacija *N*-acil ketimina katalizirana kiralnom fosforinom kiselinom.

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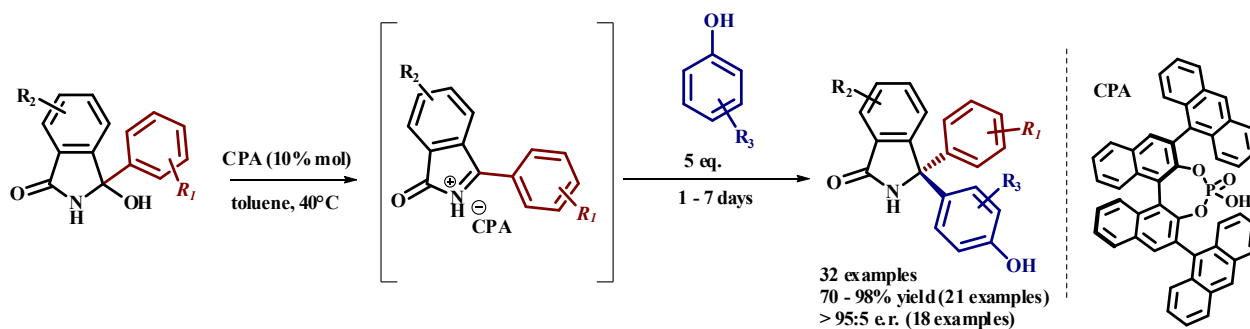


# ORGANOCATALYTIC STEREOSELECTIVE ARYLATION OF ISOINDOLINONE DERIVATIVES

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Enantiomerically pure 3,3-disubstituted isoindolinones are represented as highly valuable structural motifs found in a numerous of natural products and biologically active compounds. Their biological activities are greatly influenced by the type of substituents and absolute configuration on this position. [1] A broad range of methodologies for the synthesis of chiral isoindolinone derivatives has already been developed, including additions of heterocycles,[2] heteroatoms [3] and non-aromatic carbon nucleophiles.[4] On the other hand, there are still no reports on organocatalytic protocols for the enantioselective construction of the third phenyl ring on the isoindolinone C3 position. Herein, we report a chiral phosphoric acid-catalyzed (CPA) stereoselective reaction between diaryl ketimines and phenols for the construction of triarily substituted quaternary stereogenic center. The success of this transformation lies in the *in situ* generation of the reactive *N*-acyl ketiminium species from 3-hydroxysubstituted isoindolinones, making them susceptible for a reaction with phenols (scheme 1). [5] The reaction proceeds with high regioselectivity and enantioselectivity, and is tolerant of various functionalities on isoindolinone alcohol, as well as on phenol. Stereochemical induction model was investigated with density functional theory calculations. The calculation showed that *Re* face attack of phenols is more favorable.



**Scheme 1.** Stereoselective arylation of *N*-acyl ketimine catalyzed by chiral phosphoric acid.

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## PRIRODNA PREHRANA ZIMSKO-AKTIVNIH PAUKOVA NA STABLIMA KRUŠAKA

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Suvremeni sustavi za suzbijanje štetočina u poljoprivredi, kao što su integrirani sustavi za suzbijanje štetočina (IPM), oslanjaju se na prirodno prisutne paukove za suzbijanje štetočina [1]. Ipak, malo je istraživanja provedeno kako bi se procijenila njihova učinkovitost, osobito tijekom zimskog razdoblja kada je njihov potencijal velik [2]. U ovom istraživanju smo se usredotočili na tri roda zimsko-aktivnih paukova: *Clubiona*, *Philodromus* i *Anyphaena*, uobičajenih grabežljivaca na stablima kruřaka tijekom zime. Istražili smo njihovu zimsku aktivnost i prirodnu prehranu na stablima kruřaka te ih usporedili s obzirom na dva načina gospodarenja (IPM i organsko). Za to smo upotrijebili molekularne metode za identifikaciju plijena u utrobi. *Anyphaena accentuata* imala je najveću predacijsku aktivnost od svih skupina pauka tijekom zime, po stopi sličnoj proljetnom razdoblju, dok su vrste druga dva roda lovile dva do tri puta manjom stopom nego u proljeće. Međutim, brojnost *A. accentuata* bila je najniža od svih proučavanih vrsta paukova te su potpuno izostali iz IPM voćnjaka. Bez obzira na način gospodarenja nije bilo razlika u sastavu ulovljenog plijena paukova tijekom zime. Zimska prehrana sastojala se uglavnom od štetočina, posebno lisnih buha (Hemiptera: Psyllidae). Međutim, u rano proljeće pauci iz IPM voćnjaka su čeřće lovili indiferentan plijen (uglavnom dvokrilce), dok su oni iz organskih voćnjaka i dalje lovili štetnike. Iako je potrebno više informacija za kvantificiranje izravnih i neizravnih interakcija u složenim hranidbenim mrežama člankonožaca u voćnjacima kruřaka, dobiveni rezultati ukazuju na vrste paukova koji bi se mogli upotrijebiti za upravljanja voćnjacima u ranoj i kasnoj sezoni.

### ZAHVALE

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## THE NATURAL DIET OF WINTER ACTIVE SPIDERS ON PEAR TREES

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Modern agricultural pest management systems, such as integrated pest management systems (IPMs), rely on naturally occurring generalist spider predators to suppress pests [1]. Still, little research has been done to assess their overall effectiveness, especially over the winter period when their potential is high [2]. In this study we focused on three genera of winter active spiders *Clubiona*, *Philodromus* and *Anyphaena*, common predators on pear trees during winter. We investigated their winter activity and natural diet on pear trees and compared it considering two distinct management types (IPM and organic). For that, we utilized molecular methods to identify the prey they ate. *Anyphaena accentuata* had the highest predation activity of all spider groups during winter, at a rate similar to the spring period, while the other two genera preyed at a rate two to three times lower than in spring. However, the abundance of *A. accentuata* was the lowest of all studied spider species and it was completely absent from IPM orchards. When comparing management types, there were no differences in the prey composition of spiders during winter. The winter diet is comprised predominantly of pests, especially psyllids. However, in early spring, spiders of IPM orchards seemed to prey more frequently on indifferent prey (mostly dipterans), while the ones from organic orchards preyed on pests. Although more information is needed to quantify direct and indirect interactions in complex arthropod food webs in pear orchards, the results obtained from this research provide essential information on which spiders should be targeted for early and late season management actions.

### ACKNOWLEDGMENTS

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## PROMJENE U BOČNOM KRETANJU KORITA RIJEKE ORLJAVE OD SREDINE 20. STOLJEĆA

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Hidromorfologija brojnih rijeka značajno je promijenjena ljudskim djelovanjem kako bi se stvorili povoljniji uvjeti za različite namjene, poput zaštite od poplava, vodoopskrbe ili hidroenergije [1]. Izgradnja umjetnih nasipa, pregrada i obaloutvrda, kao i izravnavanje i kanaliziranje tekućica, mijenjaju prirodnu morfodinamiku rijeka [2]. U ovom istraživanju analizirane su promjene u bočnom kretanju korita na dionici rijeke Orljave u istočnoj Hrvatskoj koja je pod utjecajem hidrotehničkih mjera te izložena značajnim poplavama. Na dva odsječka rijeke ukupne duljine 14 km, između utoka rijeke Londže i mlina kod Dragovaca, digitalizirana je crta sredine korita na temelju dostupnih zračnih snimaka i topografskih karata iz razdoblja 1966.-2019. Godišnja stopa kretanja korita izračunata je kao površina poligona između dvije uzastopne crte sredine korita podijeljena s polovicom opsega tog poligona [3]. Na uzvodnom odsječku (do mlina kod Bučja) zabilježena je niža stopa kretanja korita, što se može povezati s manjim brojem umjetno presječenih meandara (prokopa) i blažim nagibom korita. Međutim, povećanje kretanja korita, počevši od 2011., zabilježeno je na oba odsječka. To je vjerojatno posljedica uklanjanja obalne vegetacije koje je provedeno nakon poplave 2010., povratnog perioda 12,6. Nizvodno od Bučja, najveća prosječna stopa kretanja korita na razini odsječka od 3,5 m godišnje vjerojatno je uzrokovana još jednom značajnom poplavom iz 2014., povratnog perioda 30,6. Tijekom razdoblja 2014.-2019., kretanje korita na odsječku se polako smanjivalo pod utjecajem novoizgrađene stepenice na najnižvodnijoj točki odsječka koja je smanjila nagib korita i energiju toka. Općenito, najveće stope kretanja korita na razini meandra u promatranom razdoblju zabilježene su nizvodno od prokopanih meandara te uzvodno i nizvodno od proboja stepenice kod Bučja. Čini se da je ubrzanje bočnog kretanja korita nakon 2011. u velikoj mjeri povezano s recentnim hidrotehničkim radovima, posebice s uklanjanjem vegetacije s riječnih obala, budući da velike poplave u prošlosti nisu uzrokovale tako visoke stope kretanja korita. Povećana erozija riječnih obala dovodi do gubitka okolnog poljoprivrednog zemljišta, što je važan problem za lokalno stanovništvo.

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## CHANGES IN LATERAL CHANNEL MIGRATION ON THE ORLJAVA RIVER SINCE THE MID-20TH CENTURY

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The hydromorphology of numerous rivers has been significantly modified by human interventions in order to create favourable conditions for various uses, such as flood protection, water supply or hydroenergy [1]. The construction of artificial levees, barriers, and bank revetments as well as river straightening and channelization alter the natural morphodynamics of rivers [2]. This study investigates changes in lateral channel migration of the Orłjava River in Eastern Croatia affected by engineering works and major flood events. The channel centreline on two reaches with a total length of 14 km between the confluence with the Londža River and the mill near Dragovci was digitised based on available aerial images and topographic maps from the period 1966-2019. The rate of channel migration per year was calculated as the area of the intersecting polygon between two successive centrelines divided by half of its perimeter [3]. In the upstream reach (up to the mill at Bučje), the rate of channel migration was generally lower, which can be related to fewer artificial meander cutoffs and a gentler channel slope. However, the increase in channel migration starting in 2011 was noted in both reaches after removal of riparian vegetation following a flood event in 2010 (recurrence interval 12.6). Downstream of Bučje, the largest reach-averaged migration rate of 3.5 m per year was probably induced by another major flood event from 2014 (recurrence interval 30.6). During 2014-2019, reach-scale channel migration slowly decreased, affected by the construction of a weir at the most downstream point of the reach that reduced the channel slope and stream power. In general, the largest rates of channel migration on meander level in the studied period were recorded downstream of artificial cutoffs and upstream and downstream of a weir breach near Bučje. Acceleration of lateral channel migration after 2011 seems to be highly related to recent engineering works, particularly removal of vegetation from river banks, since major flood events in the past did not cause such high migration rates. The increased erosion of the river banks leads to the loss of adjacent agricultural land, which is an important problem for the local population.

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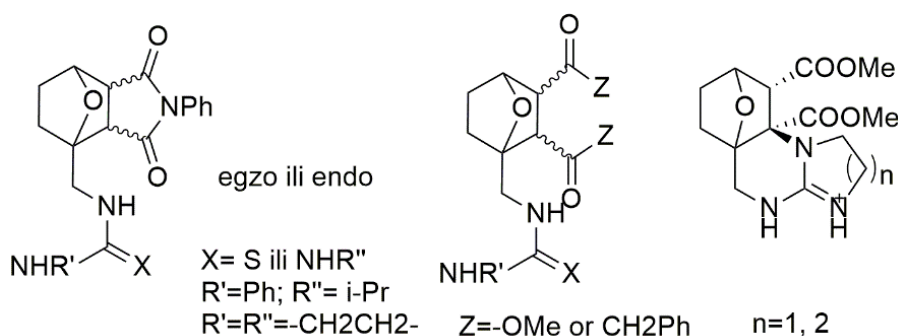
# SINTEZA NOVIH OKSANORBORNANA SUPSTITURIranih GVANIDINOM I NJIHOVA KOKATALITIČKA AKTIVNOST U ALDOLNOJ REAKCIJI

Luka Barešić,<sup>1,\*</sup> Zoran Glasovac,<sup>1</sup> Davor Margetić<sup>1</sup>

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Norbornani i njihovi oksa-derivati posjeduju krutu strukturu, svojim oblikom oponašaju sekundarne strukture polipeptida poput  $\beta$ -zaokreta i  $\beta$ -ukosnice. Norbornanski motiv može poslužiti kao model za induciranje dobro definiranih vodikovih veza u peptidima [1]. Vodikove veze igraju ključnu ulogu u katalitičkim reakcijama enzima i organskih katalizatora. Tiouree i gvanidini su jedni od primjera organskih katalizatora [2,3]. Aldolna reakcija katalizirana L-prolinom pokazuje slabu diastereoselektivnost u formaciji *anti* i *syn* adukta što se može poboljšati dodatkom tiouree ili gvanidinijevih soli kao kokatalizatora [4,5]. U ovom izlaganju bit će predstavljeni sintetski putevi u pripravi novih oksanorbornana sa tioueidnom ili gvanidinskom skupinom (Slika 1) i njihova kokatalitička aktivnost u L-prolinom kataliziranoj aldolnoj reakciji.



Slika 1. Primjeri kokatalizatora.

## ZAHVALE

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# SYNTHESIS OF NOVEL GUANIDINIUM- SUBSTITUTED OXANORBORNANES AND THEIR COCATALYTIC ACTIVITY IN ALDOL REACTION

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Norbornane structures and their oxa-derivatives have rigid scaffold and are good mimics of secondary structures in polypeptides such as  $\beta$ -turn and  $\beta$ -hairpin. Norbornane motifs can serve as the inducers of well-defined hydrogen bonding patterns in peptides [1]. Hydrogen bond plays a crucial role in enzymes and small molecule organocatalysis. Thioureas and guanidines are two types of such organocatalysts [2,3]. Aldol reactions catalysed with L-proline are known to show poor diastereoselectivity in formation of *anti* and *syn* adducts and can be improved by adding guanidinium salts or thioureas as cocatalysts [4,5]. Herein we report a synthetic route to a novel thiourea and guanidinium oxanorbornanes carrying different structural motifs (Figure 1) and their activity in L-proline catalyzed aldol reaction.

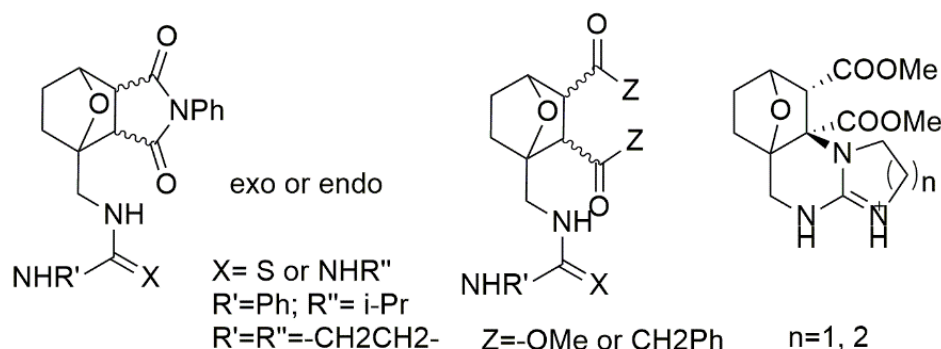


Figure 1. Example of cocatalysts.

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Sveučilište u Zagrebu, Prirodoslovno-matematički fakultet  
6. Simpozij Studenata Doktorskih Studija 2022  
23. – 24. travnja 2022. g



## PERSONALITY PREDICTS MODE OF ATTACK IN A GENERALIST GROUND SPIDER PREDATOR

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Personality traits, such as boldness and/or aggressiveness, have long been accepted to have a profound influence on many aspects of the lives of animals, [1,2], including foraging. However, little is known about how personality traits shape the use of a particular attack strategy. Ground spiders use either venom or silk attack to immobilize prey [3]. In this study, we tested the hypothesis that behavioral differences among individuals (measured as boldness and aggressiveness) drive the use of a particular attack strategy. We used a generalist ground spider, *Drassodes lapidosus*, and recorded the mode of attack on two types of prey, dangerous and safe. Moreover, we measured the size of the venom gland to test the effect of venom volume on the personality and the mode of attack. *D. lapidosus* individuals showed consistent behavioral differences in the way they attacked prey. Venom attack was significantly related to increased aggressiveness and slightly to increased boldness, while silk attack was significantly related to shyness/docile traits. We also found both personality traits to decline significantly with the increasing body size of the predator. The volume of venom was not related to the attack strategy.

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## ANTROPOGENI UTJECAJI NA KRŠKE VODONOSNIKE NA PRIMJERU BLATSKOG VODONOSNIKA NA OTOKU KORČULI

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Hrvatska obala je jedna od najrazvedenijih obala na svijetu. Na hrvatskoj strani Jadranskog mora nalazi se 781 otok te gotovo još toliko grebena i hridi. Korčula je jedan od najvećih i najrazvijenijih otoka u Jadranskom moru. Vela Luka, Blato i Korčula su tri velika naselja na otoku te većina od 18,000 stanovnika gravitira tim naseljima. Područje ovog istraživanja biti će Blatsko Polje, smješteno na zapadnom dijelu otoka te predstavlja tipičan primjer krškog polja u "Dinarskom tipu" krša [1]. Osnovna značajka vodnih resursa u kršu je njihova podložnost promjenama vezanima uz procese promjene rubnih uvjeta, u svim komponentama njihove pojavnosti i na različitim vremenskim skalama. Čovjek svojim aktivnostima i potrebama značajno zadire u hidrološki ciklus vodnih pojava u kršu te dodatno utječe na njihovu dinamiku. Teren je izgrađen od okršanih karbonatnih stijena, pretežito redne starosti. Zapadni dio otoka (Blato, Vela Luka i okolna mjesta) opskrbljuje se vodom sa 4 vodozahvata u Blatskom Polju ukupne izdašnosti od oko 60 l/s. Hidrogeološke značajke otoka odgovaraju krškom tipu vodonosnika s pukotinsko – kavernošnom poroznošću. Na otoku nema stalnih površinskih tokova, a podzemna voda predstavlja infiltrirane padaline koje podzemno otječu prema moru. Prosječna količina oborina je 850 mm/god, ali kada količine padnu ispod 700 mm/god postoji velika mogućnost pojave prodora mora u vodonosnik, pogotovo tijekom ljetne (sušne) sezone. Rizik se jasno povećava kada se sušne godine ponavljaju. Hidrokemijska istraživanja pokazuju da se u različitim hidrološkim trenutcima javljaju dva glavna izvora onečišćenja: prodor morske vode događa se u suhom ljetnom razdoblju kada je crpljenje maksimalno, a prihranjivanja vodonosnika gotovo da i nema; te ispiranje nitrata i drugih antropogenih pokazatelja onečišćenja tla tijekom kišnih perioda [2][3]. U ovako osjetljivom okolišu prilikom planiranja upravljanja i zaštite, svi čimbenici se moraju uzeti u obzir.

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# ANTHROPOGENIC INFLUENCES ON A KARST ISLAND AQUIFER, THE BLATO AQUIFER ON THE ISLAND OF KORČULA, CROATIA

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The Croatian coast is one of the most indented coasts in the world. In the Croatian part of the Adriatic Sea there are 718 islands and many rock crags and reefs. Korčula is one of the largest (sixth) and most developed islands. It has about 18,000 inhabitants in three larger settlements - Blato, Vela Luka and Korčula. The area of this research will be Blatsko Polje, located in the western part of the island and is a typical example of a karst field in the "Dinaric type" of karst [1]. The main feature of karst water resources is their susceptibility to changes related to the processes of changing boundary conditions, in all components of their occurrence and on different time scales. With its activities and needs, man significantly interferes with the hydrological cycle of water phenomena in karst and additionally influences their dynamics. The terrain is built of karstified carbonate rocks, mostly of the Cretaceous age. In the Blatsko polje there are four water supply wells with a total yield of about 60 l/s and they are used to supply water to the western part of the island (Blato, Vela Luka and surrounding places). Hydrogeological characteristics of the island correspond to the karst type of aquifer with fissure - cavernous porosity. There are no permanent surface flows on the island, and groundwater is infiltrated precipitation that flows underground to the sea. Average precipitation is 850 mm/year, but when there is less than 700 mm/year there is a high possibility of sea-water intrusion during the summer season. The risk significantly increases when dry years repeat. Hydrochemical research has shown that two main pollution sources occur at different hydrological moments: sea-water intrusion happens in the dry summer period when there is maximal extraction and almost no recharge; and the washing of nitrates and other humanly caused pollution indicators from the soil and epikarst belt during the rainy season [2][3]. All factors must be taken into account when planning management and protection of such a sensitive environment.

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## MODULIRANJE STVARANJA VRUĆIH NOSIOCA NABOJA LEGIRANJEM: IMPLIKACIJE ZA PLAZMONIČKU FOTOKATALIZU

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Interakcija svjetla i metalnih nanočestica može dovesti do kolektivnih oscilacija elektrona u nanočestici, koje nazivamo plazmonima. Trnućem plazmona dolazi do stvaranja elektrona i odgovarajućih šupljina veoma visoke energije - “vrućih” nosioca naboja. Korištenje vrućih nosioca naboja proučava se za izradu senzora i fotovoltaike najnovije generacije, kao i za otvaranje novih reakcijskih puteva pomoću plazmoničke fotokatalize [1]. Računalne metode poput DFT-a pokazale su se veoma korisnima za proučavanje utjecaja legiranja na neka od plazmoničkih svojstava ali utjecaj legiranja na stvaranje vrućih nosioca naboja trenutačno je relativno nepoznat. Štoviše, trenutačno najkorištenija metoda za procjenu distribucije energija vrućih nosioca naboja potpuno je neosjetljiva na utjecaj legiranja. U ovom izlaganju pokazujemo da metoda koju smo razvili daje uvid u efekte legiranja na distribuciju energija vrućih nosioca naboja i raspravljamo implikacije rezultata za kontrolu katalitičkih puteva.

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## MODULATING HOT CARRIER GENERATION BY ALLOYING: IMPLICATIONS FOR PLASMONIC PHOTOCATALYSIS

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Interaction of light and metal nanoparticles can cause collective oscillations of electrons in the nanoparticle, which are called plasmons. Plasmon decay brings about creation of electrons and corresponding holes of very high energy - “hot” carriers. Utilization of hot carriers is being studied for latest generation sensor and photovoltaic design, as well as for unlocking the new reaction pathways through plasmonic photocatalysis [1]. Computational methods like DFT have shown to be very useful for studying the influence of alloying on some of the plasmonic properties but the influence of alloying on the hot carrier generation is currently relatively unknown. Moreover, the currently most used method for estimating hot carrier energy distribution is completely insensitive to the influence of alloying. In this presentation we show that the method we developed provides insight into the effects of alloying on the energy distribution of hot carriers and discuss the implications of the results on the control of catalytic pathways.

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## BIOAKUMULACIJA I BIOAMPLIFIKACIJA FARMACEUTSKI AKTIVNIH SPOJEVA I ENDOKRINIH DISRUPTORA U VRSTI TULARA *Silo nigricornis*

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Onečišćenje otpadnim vodama uzrokuje prisutnost različitih farmaceutski aktivnih spojeva (PhAC) i endokrinih disruptora (EDC) u vodenom okolišu diljem svijeta. Osim njihovog utjecaja na vodenu biotu, sve više se istražuje prijenos tih spojeva iz vodenih u kopnene ekosustave. PhAC i EDC se mogu transportirati u kopneni okoliš putem hranidbene mreže, ali i emergencijom vodenih kukaca, koji mogu bioakumulirati spojeve tijekom svoje vodene faze života [1], [2]. S obzirom na to da ekološke i biološke značajke vodenih organizama utječu na dostupnost, izloženost i bioakumulaciju PhAC i EDC u organizmima, istražili smo obrasce bioakumulacije u svim životnim stadijima holometabolne vrste tulara *Silo nigricornis* (Pictet, 1834). U svrhu utvrđivanja trendova promjena koncentracija tijekom različitih faza metamorfoze, izračunati su bioamplifikacijski faktori (BAMF). Uzorci su prikupljeni na lokaciji koja je pod utjecajem ispuštanja nepročišćenih otpadnih voda te na kojoj je prisutna velika brojnost vrste *S. nigricornis*. U tkivima tulara ukupno je detektirano 17 spojeva. Utvrđene su značajne razlike u koncentracijama između životnih stadija za sedam spojeva, s većim vrijednostima u odraslih jedinki u usporedbi s ličinkama i/ili kukuljicama. Povećanje koncentracije kod odraslih jedinki rezultat je isključivo promjena koje se događaju tijekom metamorfoze, budući da se tulari ne hrane kao odrasle jedinke. Sukladno tome, BAMF-ovi su pokazali povećanje koncentracije tijekom najmanje jedne faze metamorfoze za 13 spojeva. Štoviše, za šest spojeva bioamplifikacija (BAMFs  $\geq 1$ ) je zabilježena za obje faze metamorfoze. Povećana koncentracija PhAC i EDC u tkivima odraslih tulara potencijalno može značajno utjecati na njihove riparijske predatore, kao što su pauci, ptice i šišmiši. Rezultati ovog istraživanja pružaju nove uvide u ponašanje i sudbinu PhAC i EDC na granici vodenog i kopnenog ekosustava.

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## BIOACCUMULATION AND BIOAMPLIFICATION OF PHARMACEUTICALS AND ENDOCRINE DISRUPTING COMPOUNDS IN CADDISFLY *Silo nigricornis*

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Wastewater effluent pollution causes the presence of various pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) in aquatic environments worldwide. Aside from their impact on aquatic biota, increased attention is recently given to researching the transfer of these chemicals from aquatic to terrestrial ecosystems. PhACs and EDCs can be transferred to terrestrial environments via food webs, but also with emergence of aquatic insects, which can bioaccumulate compounds during their aquatic life stage [1], [2]. Considering that the ecological and biological traits of aquatic organisms affect availability, exposure and bioaccumulation of PhACs and EDCs in organisms [3], [4], we examined bioaccumulation patterns in all life stages of the holometabolous caddisfly *Silo nigricornis* (Pictet, 1834). Furthermore, by calculating bioamplification factors (BAMFs) we aimed at determining trends in changing concentrations during different stages of metamorphosis. Samples were collected at wastewater impacted location with great abundance of the *S. nigricornis*. In total, 17 compounds were bioaccumulated in caddisfly tissues. Significant differences in concentrations between life stages were determined for seven compounds, all having highest values in adults compared to larvae and/or pupae. Concentration increase in adults is result of the metamorphosis solely, as these caddisflies do not feed as adults. Accordingly, BAMFs showed increase in concentrations during at least one stage of metamorphosis for 13 compounds. Moreover, for six compounds bioamplification was inferred (BAMFs  $\geq 1$ ) through both metamorphosis stages. All compounds with BAMFs over 1 have concentration in the tissues increased without additional exposure to the compounds. Increased body burden of PhACs and EDCs in adult caddisflies could greatly impact their riparian predators, such as spiders, birds and bats, which are consequently exposed to elevated concentrations of contaminants originating from aquatic pollution. Results of this research provide new insights on the behavior and fate of PhACs and EDCs on the aquatic-terrestrial ecosystem boundary.

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## UPRAVLJANJE VIBRACIJSKIH SUSTAVA U SLUČAJU KONAČNOG VREMENSKOG HORIZONTA

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U radu razmatramo problem upravljanja vibracijskih sustava u konačnom vremenskom horizontu. Za mjeru performanse sustava uzimamo  $p$ -mješovitu  $H_2$  normu koja generalizira standardnu  $H_2$  normu. Predstavljamo algoritam za učinkovit izračun ove norme u slučaju kada je sustav ovisan o parametrima i kada je broj ulaza i izlaza sustava značajno manji od reda sustava. Naš pristup temelji se na novom postupku koji se ne temelji na rješavanju Ljapunovljevih jednadžbi i koji uzima u obzir strukturu sustava. Koristimo karakterizaciju  $H_2$  norme danu u terminima integrala koje rješavamo korištenjem adaptivnih kvadrturnih formula. To nam omogućuje korištenje recikliranja kao i paralelizacije. Efikasnost novog algoritam omogućuje nam analizu utjecaja različitih parametara sustava i različitih konačnih vremenskih horizonata na vrijednost  $p$ -mješovite  $H_2$  norme. Naš pristup ilustriramo numeričkim primjerima koji se odnose na oscilator s  $n$  masa i jednim prigušivačem.

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# FINITE TIME HORIZON MIXED CONTROL OF VIBRATIONAL SYSTEMS

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In this talk we consider a vibrational system control problem over a finite time horizon. The performance measure of the system is taken to be a  $p$ -mixed  $H_2$  norm which generalizes the standard  $H_2$  norm. We present an algorithm for efficient calculation of this norm in the case when the system is parameter dependent and the number of inputs and outputs of the system is significantly smaller than the order of the system. Our approach is based on a novel procedure which is not based on solving Lyapunov equations and which takes into account the structure of the system. We use a characterization of the  $H_2$  norm given in terms of integrals which we solve using adaptive quadrature rules. This enables us to use recycling strategies as well as parallelization. The efficiency of the new algorithm allows us to analyse the influence of various system parameters and different finite time horizons on the value of the  $p$ -mixed  $H_2$  norm. We illustrate our approach by numerical examples concerning an  $n$ -mass oscillator with one damper.

## ACKNOWLEDGMENTS

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## PROSTORNO-VREMENSKA VARIJABILNOST ZAJEDNICA MAKROZOOBENTOSA PRIJELAZNIH VODA NERETVE, JADRA I RJEČINE

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U razdoblju od jeseni 2016. g. do ljeta 2019. g. u dva navrata je uzorkovan makrozoobentos pomičnih dna u prijelaznim vodnim tijelima Rječine, Jadra i Neretve. Za uzorkovanje na ušću Rječine korišteno je Van Veen-ovo grabilo površine zahvata 0.16 m<sup>2</sup>, a za ušća Jadra i Neretve ono površine zahvata 0.1 m<sup>2</sup>. Uzorkovano je ukupno 3024 jedinki. Koljena Mollusca i Polychaeta činila su udio u uzorcima od 72% za Rječinu, 94% za Neretvu i 97% za Jadro. Stoga, daljnje statističke analize su računate samo na temelju brojnosti vrsta ta dva koljena. Najveći broj vrsta i brojnost jedinki su zabilježeni na ušću Neretve. Općenito, broj vrsta je bio veći kasnije u sezoni (jesen, tj. kasna jesen) na ušćima Neretve i Jadra, dok su brojnosti na ušću Rječine bile iste u ljeto i jesen. nMDS analiza je na podacima transformiranima korjenovanjem pokazala razlike između postaja na granici od 40% sličnosti po Bray-Curtis-u, dok je prag od 20% dao dvije grupe. Uzorci s ušća Rječine su se jasno odvojili od onih s ušća Jadra i Neretve koji su grupirani zajedno.



## SPATIO-TEMPORAL VARIABILITY OF MACROZOOBENTHIC ASSEMBLAGES IN TRANSITIONAL WATERS OF NERETVA, JADRO AND RJEČINA RIVERS

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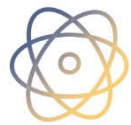
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Soft-bottom macrozoobenthos in transitional water bodies of rivers Rječina, Jadro and Neretva was sampled on two occasions in the period from autumn 2016 to summer 2019. Van Veen grab with a sampling surface of 0.16 m<sup>2</sup> was used for Rječina and the one with a sampling surface of 0.1 m<sup>2</sup> was used for Jadro and Neretva. Total of 3024 individuals were sampled. Phyla Mollusca and Polychaeta comprised 72%, 94% and 97% of sampled individuals for Rječina, Neretva and Jadro, respectively. Therefore, all subsequent statistics were performed only on species abundances from those phyla. Highest species number and abundance was recorded at Neretva. In general, number of species was higher later in the season (autumn, i.e. late autumn) at Neretva and Jadro, while the numbers were the same at Rječina in summer and autumn. nMDS on square-root transformed data showed distinction between stations on 40% Bray-Curtis similarity, while 20% similarity showed two groups. Samples from Rječina clearly separated from Jadro and Neretva, which grouped together.



# Mikro i poster izlaganja

## *Flash and poster presentations*





## TERMIČKI KONTROLIRANA MEHANOKEMIJA ZA SELEKTIVNE REAKCIJE INTERZEOLITNE PRETVORBE

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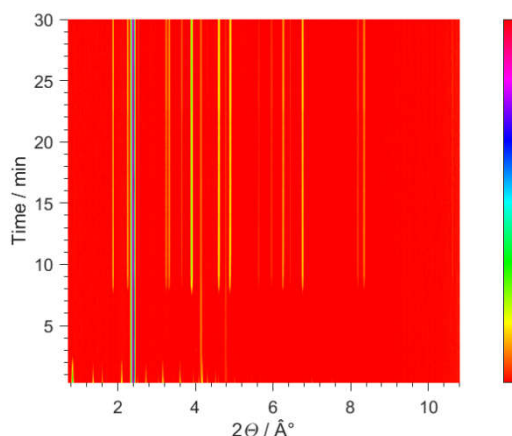
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Zeoliti su mikroporozni kristalni materijali koji se sastoje od  $\text{TO}_4$  tetraedara koji grade supramolekulske mreže s dobro definiranim kanalima i šupljinama. Danas su poznata 253 različita strukturna tipa zeolita. Našli su široku primjenu kao ionski izmjenjivači, adsorbensi i katalizatori, zbog čega su vrlo značajni materijali u industriji. Klasične metode pripreme zeolita obično uključuju korištenje solvotermalnih metoda i uvjeta koje traju nekoliko dana ili čak tjedana i troše puno resursa i vremena.[1]

S ciljem da se pronađu jeftinije i brže metode sinteze zeolita, koristili smo termički kontroliranu mehanokemiju – novo razvijenu tehniku koja omogućuje izvođenje mehanokemijskih reakcija pri povišenim temperaturama do 250 °C. [2] U ovom smo radu pokazali kako se mljevenjem zeolita Y s različitim bazama (kalijevim, natrijevim i cezijevim hidroksidom), s i bez vode, pri 110 °C produkt može dobiti u samo nekoliko minuta, dok solvotermalnim metodama takav produkt ne nastaje niti nakon dva dana. Uz pomoć *in situ* PXRD mjerenja napravljenih na Deutsches Elektronen-Synchrotronu (DESY), identificirali smo intermedijerne faze, finalne produkte te vremena njihovog nastajanja, dajući mogućnost provođenja selektivnih reakcija interzeolitne konverzije zeolita Y kako bi se priredili drugi zeoliti.



Slika 1. Waterfall dijagram *in situ* PXRD mjerenja interzeolitne pretvorbe zeolita Y s kalijevim hidroksidom.

### ZAHVALE

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# APPLYING THERMALLY CONTROLLED MECHANOCHEMISTRY FOR SELECTIVE INTERZEOLITE CONVERSION REACTIONS

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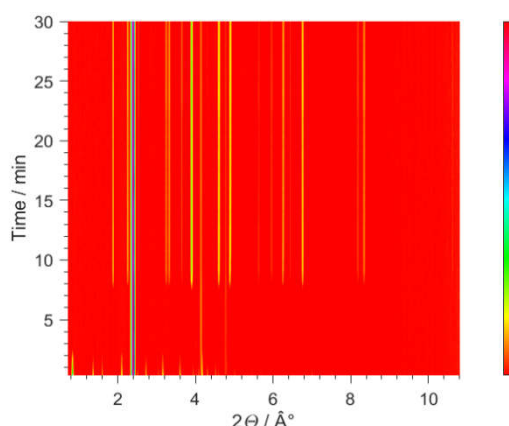
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Zeolites are microporous crystalline materials comprised of TO<sub>4</sub> tetrahedra which assemble into supramolecular networks with well-defined channels and cavities that form 253 structural types of zeolites known today. They are valuable materials in the industry and have found broad applications as ion-exchangers, adsorbents and catalysts. Classic methods of zeolite synthesis usually include solvothermal means, which take a few days or even weeks at temperatures up to 200 °C and, as such, are both time and resource-consuming. [1]

In an attempt to find cheaper, faster and above all more environment-friendly methods of zeolite synthesis, we have employed thermally controlled mechanochemistry - a newly developed technique enabling mechanochemical reactions to be conducted at temperatures up to 250 °C. [2] We have found that milling zeolite Y with different bases (sodium, potassium, and cesium hydroxide) with and without water at 110 °C yields unique products in only a few minutes, while the solvothermal reference experiments yielded no new products even after two days. Combined with *in situ* PXRD measurements performed at the Deutsches Elektronen-Synchrotron (DESY), we have identified the intermediate and final products as well as the period of their formation, giving way to selective interzeolite conversion reactions of zeolite Y that enables obtaining various zeolite materials.



**Figure 1.** Waterfall diagram of *in situ* PXRD measurement of interzeolite conversion of zeolite Y with potassium hydroxide.

## ACKNOWLEDGMENTS

The funding of the Croatian Science Foundation under the project UIP-2019-04-4977 is acknowledged.

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## OPTIMIZACIJA METODE ZA PROČIŠĆAVANJE SLOBODNIH TE FLUORESCENTNO OBLILJEŽENIH N-GLIKANA

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N-glikani su razgranati te su stoga inherentno složeni u sastavu prema strukturi.[1] Glikani se kovalentno vežu na proteine u kao ko-translacijske i post-translacijske modifikacije te kao takvi imaju važnu ulogu u funkciji, strukturi i stabilnosti proteina.[2] N-glikani se uobičajeno analiziraju tekućinskom kromatografijom (LC) uporabom kolona koje se baziraju na hidrofilnim interakcijama (HILIC) te detektiraju fluorescentnim detektorom. [3] Kako bi glikani bili primjereni za ovakav tip analize potrebno je odvojiti N-glikane od proteina te ih naknadno obilježiti fluorescentnim biljekom. Prije same LC analize, oslobođeni te fluorescentno obilježeni glikani se pročišćavaju kako bi se uklonile razne nečistoće, tako se povećava kvaliteta podataka te osigurava dugovječnost kromatografske kolone. Kao standardnu operativnu proceduru koristili smo GHP filter pločicu s 96-jažica (Pall corporation) u kojoj se pročišćavanje baziralo na HILIC ekstrakciji na čvrstoj fazi (SPE). [4] Cilj ove studije je naći alternativne i usporedive filter pločice kao bi se osiguralo rezervno rješenje. Stoga, za završno testiranje odabrane su dvije filter pločice, wwPTFE filter pločica te Supor filter pločica, obje su proizvedene od Pall-a. Sve tri pločice su testirane pomoću dva različita početna materijala, plazma iz ljudske krvi te imunoglobulin G izoliran iz te iste plazme. Varijacije u zastupljenosti N-glikanskih pikova unutar svake pločice je kvantificirana koeficijentom varijacije, dok se je Kendall korelacija koristila za mjerenje korelacija između pločica. Nadalje, testirana je ponovljivost i reproduktivnost na čin da je testiranje provedeno u dvije vremenske točke. Zaključno, uočene su vrlo male razlike među pločicama s ponovljivošću i reproduktivnošću na razini već prethodno utvrđene procedure za pročišćavanje. Dakle, wwPTFE i Supor pločice su prikladne zamjene za GHP filter pločicu.

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## OPTIMISATION OF CLEAN-UP PROCEDURE OF RELEASED AND FLUORESCENTLY LABELED N-GLYCANS

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N-glycans are branched oligosaccharides and are inherently complex in their composition and chemical structure. [1] They are covalently attached to proteins as co- and post-translational modification and as such play an important role in their function, structure, and stability. [2] N-glycans are commonly analysed by liquid chromatography (LC) using hydrophilic interaction liquid chromatography (HILIC) column and a fluorescence detector. [3] For N-glycans to be suitable for this type of analysis, it is required to detach N-glycans from proteins and subsequently label them with a fluorescent tag. Prior to LC analysis, a clean-up procedure of released and labelled N-glycans is performed to remove various impurities and thus improve data quality and ensure longer column life. As a standard operating clean-up procedure we were using 96-well GHP filter plates (Pall corporation) in which clean-up was based on HILIC solid-phase extraction (SPE) chromatography. [4] The aim of this study was to find an alternative highly comparable HILIC SPE filter plate(s) to ensure a back-up solution. In that regard, for final testing two filter plates were chosen, wwPTFE filter plate and Supor filter plate, both produced by Pall. All three plates were tested using two different starting materials, human blood plasma and immunoglobulin G isolated from that same plasma. The variations of resulting N-glycan peak abundances within tested plates were quantified by coefficients of variation, while Kendall rank correlation was used to measure correlation between the plates. Also, we tested for repeatability and reproducibility by performing the plate testing in two different time points. In conclusion, only minor differences were observed between the plates with repeatability and reproducibility being in line with already established clean-up procedure, suggesting both wwPTFE and Supor plate are suitable alternatives to GHP filter plate.

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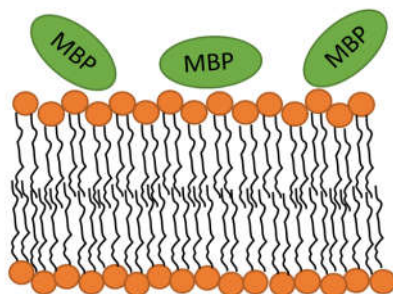


## VOLE LI MIJELINSKI BAZIČNI PROTEIN I FOSFOTIDILKOLINSKI LIPIDI PLESATI ZAJEDNO?

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Mijelin je višeslojna membrana čvrsto omotana oko aksona neurona čime omogućuje učinkovit prijenos neuralnih impulsa. Male promjene u raspodjeli lipida u mijelinskoj membrani utječu na njihova adhezivna svojstva, kao i na njihovu vezu s mijelinskim bazičnim proteinom (engl. *myelin basic protein*, MBP), jednim od najvažnijih mijelinskih proteina. Sa svojim visokim udjelom pozitivno nabijenih aminokiselinskih ostataka, nestrukturirani MBP se veže na anionske lipide citoplazmatskih slojeva, što uzrokuje promjenu ravnoteže između nabijenih lipida i promjene u bočnom slaganju i organizaciji lipida. [1] Sve te promjene javljaju se kod neurodegenerativnih bolesti kao što su multipla skleroza (MS) i encefalomijelitis (EAE). [2] Još nije poznato kakvu ulogu ima ograničavanje MBP-a na učinkovitost njegove adsorpcije na lipidni dvosloj od zwitterionskog lipida 1,2-dipalmitoil-*sn*-glicero-3-fosfokolina (DPPC), koji čini oko 40% udjela mijelinskih lipida. [3] Jednostavne spektroskopske i kalorimetrijske tehnike kao što su FTIR spektroskopija, UV/Vis spektrofotometrija i DSC mogu razotkriti utjecaj MBP-a na temperature faznih prijelaza ( $T_p$ ,  $T_m$ ) DPPC višeslojnih i jednoslojnih liposoma i na njihov strukturni raspored. Spektroskopski podaci će se analizirati odgovarajućim kemometrijskim alatom. [4]



Slika 1. Pretpostavljeni shematski prikaz MBP proteina na DPPC lipidnom dvosloju.

### ZAHVALE

Zahvaljujemo Hrvatskoj zakladi za znanost na financijskoj potpori projekta „Model demijelinizacije na molekularnoj skali pri fiziološkim i patološkim uvjetima“ (HrZZ UIP-2020-02-7669).

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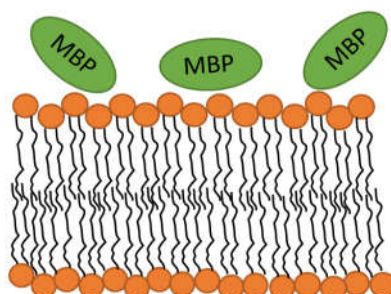


# DO THE MYELIN BASIC PROTEIN AND PHOSPHATIDYLCHOLINE LIPIDS LIKE TO DANCE TOGETHER?

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Myelin is a multilayer membrane tightly wrapped around the axon of neurons, allowing efficient transmission of neural impulses. Slight changes in distributions of lipids in myelin membranes affect their adhesive properties, as well as their bonding with myelin basic protein (MBP), one of the most important myelin-associated proteins. With its high content of positively charged residues, unstructured MBP binds to the anionic lipids of the cytoplasmic leaflets, which causes balance changes between charged lipids and alternations in their lateral organization. [1] All these changes appear in neurodegenerative diseases such as multiple sclerosis (MS) and encephalomyelitis (EAE). [2] It is not yet known what role MBP confinement plays in the efficiency of its adsorption to lipid bilayer made from zwitterionic lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), which constitutes about 40% of myelin lipids. [3] Rather simple spectroscopic and calorimetric techniques such as FTIR spectroscopy, UV/Vis spectrophotometry and DSC can unravel the impact of MBP on phase transition temperatures ( $T_p$ ,  $T_m$ ) of DPPC multilamellar and unilamellar liposomes and their structural arrangements. Spectroscopic data will be analyzed with an appropriate chemometric tool. [4]



**Figure 1.** Assumed schematic representation of MBP protein on DPPC lipid bilayer.

## ACKNOWLEDGMENTS

We thank Croatian Science Foundation for the financial support within the project „Model of demyelination on a molecular scale at physiological and pathological conditions“ (HrZZ UIP-2020-02-7669).

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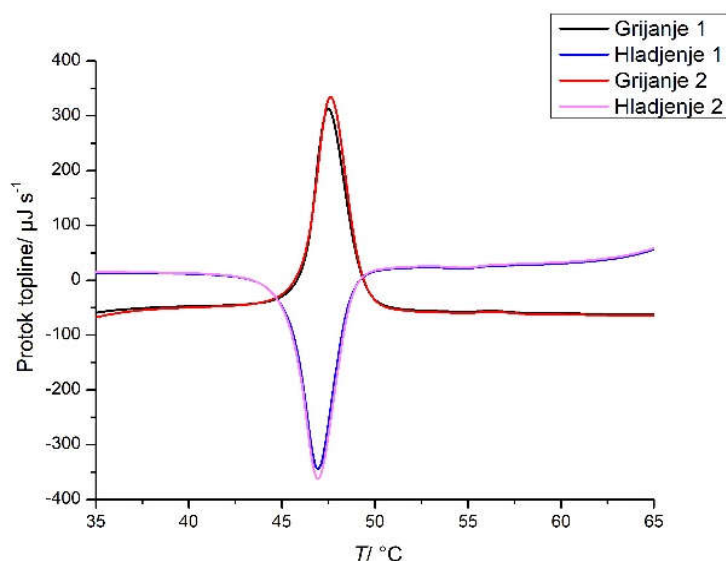
## UTJECAJ $\text{NH}_4^+$ I $\text{Gdm}^+$ KATIONA NA TERMIČKA SVOJSTVA DPPS LIPIDNIH MEMBRANA

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Fosfolipidi su neophodan dio biomembrana. Fosfatidilserin (PS) glavni je anionski fosfolipid prisutan u eukariotskim membranama i smješten je u citosolnom membranskom sloju. Među glavnim ulogama PS-a smatra se održavanje negativnog naboja u unutrašnjem membranskom sloju [1] koji je potreban za vezanje i aktivaciju različitih perifernih membranskih proteina [2]. Lipidne membrane podliježu faznim prijelazima, koji uz, temperaturu, tlak i pH, ovise i o kemijskom potencijalu iona [3]. U ovom smo radu usredotočeni na praćenje termalnih svojstava lipidnih membrana sastavljenih od 1,2-dipalmitoil-*sn*-glicero-3-fosfatidilserina (DPPS) u prisutnosti gvanidinijevih ( $\text{Gdm}^+$ ) i amonijevih kationa ( $\text{NH}_4^+$ ) što se odražava na promjenu temperature taljenja (engl. *melting temperature*,  $T_m$ ). Koristeći FT-IR spektroskopiju, UV-Vis spektrofotometriju i diferencijalnu pretražnu kalorimetriju (DSC) naš cilj je razumjeti kako navedeni kationi utječu na termalna svojstva DPPS lipidne membrane. Svi eksperimenti popraćeni su računalnim studijama.



Slika 1. DSC krivulja DPPS lipida u vodenoj otopini GdmCl-a.

### ZAHVALA

Zahvaljujemo Hrvatskoj zakladi za znanost na financijskoj potpori u sklopu projekta „Model demijelinizacije na molekularnoj skali pri fiziološkim i patološkim uvjetima“ (HrZZ UIP-2020-02-7669).

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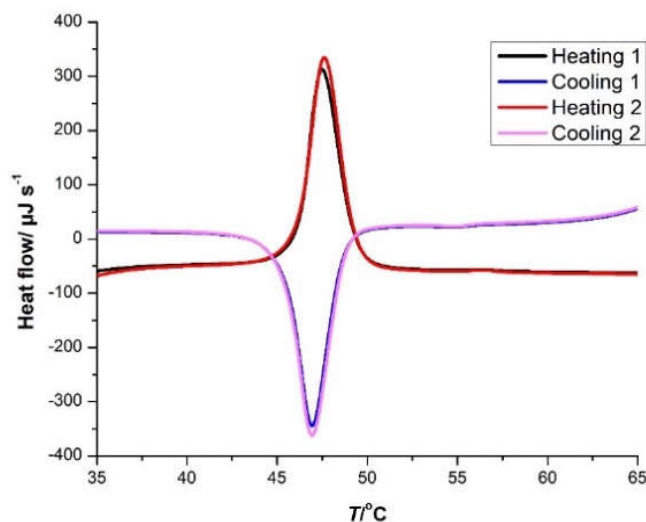
## IMPACT OF NH<sub>4</sub><sup>+</sup> AND Gdm<sup>+</sup> CATIONS ON THERMAL PROPERTIES OF DPPS LIPID MEMBRANES

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Phospholipid molecules present an essential part of biomembranes. Phosphatidylserine (PS) is the major anionic phospholipid present in eukaryotic membranes located on the cytosolic leaflet. One of its primary functions seems to be to impart a negative charge to the inner surface of the membrane lipid bilayer [1] which is required for the binding and activation of various peripheral membrane proteins [2]. Lipid-constituted membranes undergo phase transitions, which in addition to temperature, pressure, and pH also depend on the chemical potentials of ions [3]. In this work we are focused on monitoring thermal properties of lipid membranes constituted from 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylserine (DPPS) in the presence of guanidinium (Gdm<sup>+</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) cations which is manifested in a change in melting temperature ( $T_m$ ). Using FT-IR spectroscopy, UV-Vis spectrophotometry, and differential scanning calorimetry (DSC) our goal is to understand how these cations affect thermal properties of DPPS lipid membranes. All experimental results are supported by the computational study.



**Figure 1.** DSC curves of DPPS lipid in aqueous solution of GdmCl.

### ACKNOWLEDGMENTS

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## HELP ME OUT, I'M IN A MULTISTRESS ENVIRONMENT!

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The impact of water pollution and climate change on freshwater ecosystems has increased significantly over the decades and is a serious problem worldwide [1]. Various pollutants can co-occur and interact in different ways, crossing ecosystem boundaries and spreading to adjacent terrestrial habitats [2]. The aim of the current study was to investigate the response of non-model aquatic insects to individual and combined effects of emerging contaminants (ECs) such as pharmaceuticals and endocrine disruptors, and elevated temperatures. A laboratory microcosm experiment was conducted using a simplified freshwater food web containing bryophytes and Trichoptera larvae feeding mainly as shredders. Sampling included an initial and several successive collections that included all life stages (larvae, pupae, and the adult stage). Analyses such as total protein content, total lipid content, metabolite and lipidome profiling were performed. Preliminary results indicate that metabolome was more affected by ECs, while temperature had a greater effect on lipidome. Furthermore, increased water temperature affected phenology and resulted in earlier adult emergence. A multiple stressor effect was observed in treatments with a combination of ECs and increased water temperature, as evidenced by the greatest decrease in total protein content of larvae and pupae and total lipid content of adults. Additionally, identification of metabolites and quantification of ECs in caddisfly and moss tissues will be conducted. This research will provide valuable insights into the effects of ECs and increased water temperature on aquatic food webs. It will also provide detailed insights into the cross-ecosystem transport of ecological subsidies.

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The impact of water pollution and climate change on freshwater ecosystems has increased significantly over the decades and is a serious problem worldwide [1]. Various pollutants can co-occur and interact in different ways, crossing ecosystem boundaries and spreading to adjacent terrestrial habitats [2]. The aim of the current study was to investigate the response of non-model aquatic insects to individual and combined effects of emerging contaminants (ECs) such as pharmaceuticals and endocrine disruptors, and elevated temperatures. A laboratory microcosm experiment was conducted using a simplified freshwater food web containing bryophytes and Trichoptera larvae feeding mainly as shredders. Sampling included an initial and several successive collections that included all life stages (larvae, pupae, and the adult stage). Analyses such as total protein content, total lipid content, metabolite and lipidome profiling were performed. Preliminary results indicate that metabolome was more affected by ECs, while temperature had a greater effect on lipidome. Furthermore, increased water temperature affected phenology and resulted in earlier adult emergence. A multiple stressor effect was observed in treatments with a combination of ECs and increased water temperature, as evidenced by the greatest decrease in total protein content of larvae and pupae and total lipid content of adults. Additionally, identification of metabolites and quantification of ECs in caddisfly and moss tissues will be conducted. This research will provide valuable insights into the effects of ECs and increased water temperature on aquatic food webs. It will also provide detailed insights into the cross-ecosystem transport of ecological subsidies.

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## SEKVENCIJANJE TRANSKRIPTOMA OGULINSKE ŠPILJSKE SPUŽVICE (*Eunapius Subterraneus*) DAJE UVID U ŠIROK SPEKTAR DUGIH NEKODIRAJUĆIH RNA SPUŽVI (PORIFERA)

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Napretkom tehnologija sekvenciranja nove generacije, duge nekodirajuće RNA prepoznate su kao važne komponente regulacije ekspresije gena [1]. Ipak, duge nekodirajuće RNA bazalnih životinja još uvijek nisu dovoljno istražene, pri čemu jedini primjer takve studije obuhvaća analizu nekodirajućih transkriptoma spužve *Amphimedon queenslandica* [2]. U ovom istraživanju obogatili smo katalog dugih nekodirajućih RNA spužvi sekvenciranjem transkriptoma ogulinske špiljske spužvice (*Eunapius subterraneus*), endemske stigobiotske spužve karakteristične za područje Ogulina. Koristeći niz bioinformatičkih metoda identificirali smo širok, ali i pouzdan set koji obuhvaća 1981 dugu nekodirajuću RNA ove spužve. Pronađene duge nekodirajuće RNA pokazale su značajnu sličnost dugim nekodirajućim RNA kraljeznjaka, uključujući nisku razinu alternativnog prekrivanja, preferenciju za dva eksona, kraće duljine u usporedbi s transkriptima mRNA i mjesta prekrivanja slična intronima mRNA. Većina pronađenih dugih nekodirajućih RNA nalazila se unutar introna gena koji kodiraju proteine (59.3%). Potom, slijedile su duge nekodirajuće RNA bez preklapanja s genima koji kodiraju proteine (34.1%) i duge nekodirajuće RNA koje u svojim intronima sadrže gene koji kodiraju proteine (6.6%). Također, u identificiranim dugim nekodirajućim RNA pronašli smo visoku frekvenciju insercija transpozona, što upućuje na važnost transpozona u nastanku i evoluciji dugih nekodirajućih RNA spužvi. Nadalje, otkrili smo velik broj dugih nekodirajućih RNA koje pokazuju značajnu sličnost i sinteniju s dugim nekodirajućim RNA srodne spužve *Ephydatia muelleri*. Prilikom analize gena koji kodiraju proteine u susjedstvu lokusa dugih nekodirajućih RNA, pronašli smo gene koji kodiraju proteine važne u brojnim biološkim procesima, uključujući vezanje za DNA, integraciju u DNA i deubikvitilaciju proteina. Štoviše, većina dugih nekodirajućih RNA u blizini lokusa gena koji kodiraju proteine uključenih u ove procese bila je slična ili sintenična s dugim nekodirajućim RNA drugih spužvi. Ovaj rezultat naglašava potencijalnu ulogu dugih nekodirajućih RNA u regulaciji brojnih važnih bioloških procesa. Zaključno, rezultati ove studije upućuju na visok stupanj sličnosti između dugih nekodirajućih RNA spužvi i bilateralnih životinja.

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# SEQUENCING OF ENDEMIC CAVE SPONGE (*Eunapius Subterraneus*) TRANSCRIPTOMES REVEALS A COMPREHENSIVE SET OF LONG NON-CODING RNAs IN SPONGES (PORIFERA)

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With the recent advances in genomic research, long non-coding RNAs (lncRNAs) have been recognized as key players in gene expression regulation [1]. However, research on basal metazoan lncRNAs has been scarce, with a few studies exploring the non-coding transcriptomes in developmental stages of the *Amphimedon queenslandica* sponge [2]. In this study, we enriched the catalog of sponge lncRNAs by sequencing the transcriptomes of *Eunapius subterraneus*, an endemic stygobiotic demosponge found in Ogulin, Croatia. Using our lncRNA identification pipeline, we found a comprehensive yet highly confidential set of 1981 poriferan lncRNA transcripts. We discovered that identified lncRNAs exhibit many of the vertebrate lncRNA characteristics, such as relatively low levels of alternative splicing, two-exon bias, shorter length compared to mRNA transcripts and splice sites characteristic for mRNA introns. The majority of identified lncRNAs were found inside the introns of protein-coding genes (59.3%), followed by lncRNAs without overlaps with protein-coding genes (34.1%) and lncRNAs which contain protein-coding genes within their introns (6.6%). We also found an enrichment of transposable element insertions in the identified lncRNAs, highlighting the importance of transposons in poriferan lncRNA origin. Additionally, a surprisingly large number of identified lncRNAs was similar to or syntenic with lncRNAs found in *Ephydatia muelleri*, a demosponge closely related to *Eunapius subterraneus*. When analyzing protein-coding genes close to identified lncRNA loci, we found numerous protein-coding genes involved in various biological processes, including DNA binding, DNA integration and protein deubiquitination. Moreover, the majority of lncRNAs proximal to protein-coding genes involved in these processes were conserved or syntenic in multiple sponge species. This finding represents evidence for the potential importance of cis-acting sponge lncRNAs in regulating the expression of protein-coding genes involved in multiple important cellular processes. Taken together, our results demonstrate a surprising similarity between poriferan and bilaterian lncRNAs.

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## PRAĆENJE DONOR SPECIFIČNIH ANTITIJELA SUSTAVA HLA U TRANSPLANTACIJI BUBREGA

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Transplantacija bubrega je već više od pola stoljeća glavna metoda liječenja završne faze zatajenja bubrega, no još uvijek je glavno pitanje kako izbjeći imunološku reakciju koja može uzrokovati nefunkcionalnost presatka ili čak njegovo odbacivanje. Sustav HLA (od engl. *Human Leukocyte Antigen*) ima važnu ulogu u mehanizmu imunološke obrane domaćina te se iz tog razloga koristi u pronalasku najpogodnijeg donora organa. Nepodudarnosti (MM, od engl. Mismatch) u sustavu HLA između primatelja i potencijalnog donora organa imaju utjecaj na preživljenje presatka [1]. Pojava donor-specifičnih antitijela (DSA) *de novo* nakon transplantacije može povećati rizik od akutnog i kroničnog odbacivanja organa te je zato praćenje razine DSA presudno u prilagođavanju terapije nakon transplantacije [2]. Epleti su najmanje funkcionalne jedinice epitopa HLA koje mogu odrediti specifičnost antitijela. *HLAMatchmaker software* koji se inače koristi za karakterizaciju epleta se sve više primjenjuje i za određivanje podudarnosti između primatelja i donora kako bi se smanjio rizik od nastanka DSA [3]. Retrospektivnom analizom je utvrđena povezanost MM na lokusima HLA-A, -B, -DRB1 i -DQB1 s razvojem DSA kod 47 pacijenata s transplantiranim bubregom koji prije transplantacije nisu imali prisutna antitijela sustava HLA. Medijan vremena praćenja je bio 6 godina. Od ukupno 47 primatelja bubrega, njih 19 (40%) je razvilo DSA (DSA+), dok je od ukupno 28 primatelja bez DSA (DSA-), njih 7 (15%) imalo antitijela HLA drugih specifičnosti, a 21 primatelj (45%) nije imao antitijela HLA. Kod DSA+ primatelja, njih 16% je razvilo samo antitijela HLA razreda I, 21% antitijela HLA razreda I i razreda II, a čak 63% ih je razvilo samo HLA antitijela razreda II. Nepodudarnosti na svim lokusima su doprinijeli razvoju lokus specifičnih antitijela HLA, ali najveći utjecaj je uočen na lokusu HLA-DQB1 budući da je 41% primatelja koji su imali MM na HLA-DQB1 razvio DSA. Napravljena je *HLAMatchmaker* analiza MM epleta lokusa HLA-DR/DQ te je uočena statistički značajna razlika između DSA- (N=33) i DSA+ (N=14) primatelja za broj MM epleta razreda II  $\leq 15$  (24 u skupini DSA-, 3 u skupini DSA+;  $p=0.0163$ ) i za vrijednost medijana (9.1 u skupini DSA-, 17.7 u skupini DSA+;  $p=0.00039$ ). Ovi rezultati naglašavaju važnost podudarnosti primatelja i donora u sustavu HLA razreda II budući da se kod primatelja s MM HLA razreda II češće razvio DSA nego kod onih koji su primili bubreg s nepodudarnostima u HLA razredu I.

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## MONITORING OF HLA DONOR SPECIFIC ANTIBODIES IN KIDNEY TRANSPLANTATION

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Kidney transplantation has been the main treatment for end-stage renal failure for more than half a century, but the main obstacle remains how to avoid immunological rejection which then can lead to allograft dysfunction or even allograft loss. Human leukocyte antigen (HLA) system plays the critical part of the host immune defense mechanism and is used to match organ recipient with the most appropriate donor. Mismatches (MM) in HLA system between the recipient and potential donor have the impact on graft survival [1]. *De novo* formation of donor-specific antibodies (DSA) posttransplant can increase the risk for acute and chronic rejections and monitoring of these antibodies plays a crucial role in adjusting the posttransplant treatment [2]. HLAMatchmaker software commonly used for characterization of eplets (the smallest functional unit of HLA epitope that can determine antibody specificity), is becoming more and more used for matching donor and recipient to minimize DSA development [3]. We have retrospectively analyzed the relationship between MM at HLA-A, -B, -DRB1 and -DQB1 locus and the development of DSA in 47 kidney transplant patients that were negative for the presence of any HLA antibodies before transplantation. The median follow-up time was 6 years. Among 47 recipients, 19 (40%) developed DSA (DSA+), while among 28 DSA negative (DSA-) recipients, 7 (15%) had non-DSA antibodies and 21 (45%) remained negative. Among DSA+ recipients, 16% of them developed only HLA class I antibodies, 21% developed both HLA class I and class II antibodies, while most of the recipients (63%) developed only HLA class II antibodies. All HLA loci MM contributed to the development of HLA locus-specific antibodies, but HLA-DQ predominated, as 41% of recipients with HLA-DQ MM developed DSA. HLAMatchmaker analysis for HLA-DR/DQ eplet MM showed statistically significant difference between DSA- (N=33) and DSA+ recipients (N=14) for a number of HLA class II MM eplets  $\leq 15$  (24 in DSA- vs 3 in DSA+;  $p=0.00163$ ) and for a median value (9.1 in DSA- vs 17.7 in DSA+;  $p=0.00039$ ). The results are pointing the importance of HLA-class II matching in kidney transplantation as recipients of HLA-class II MM grafts developed DSA more frequently than those receiving HLA-class I incompatible grafts.

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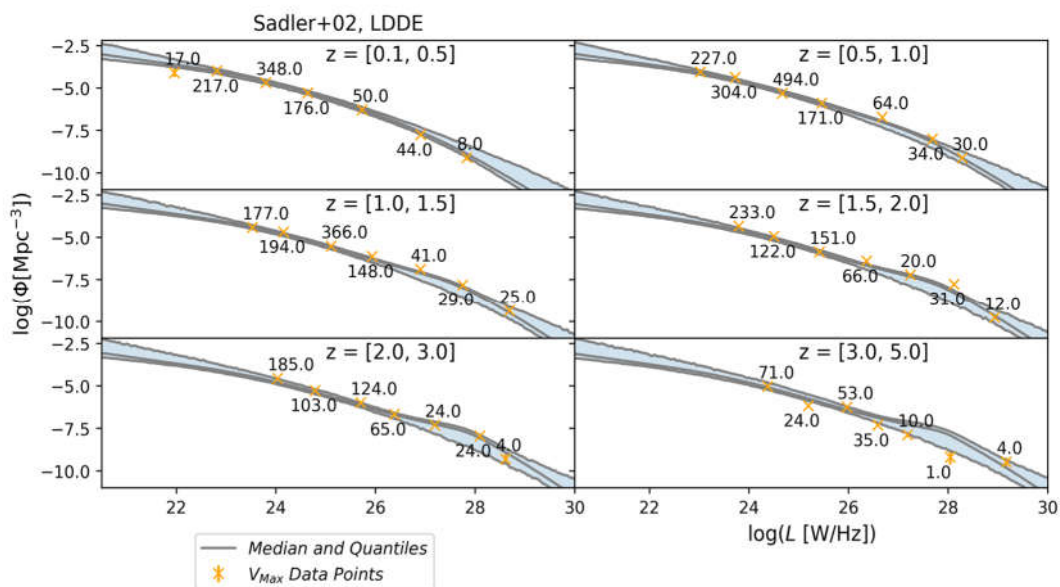
# FUNKCIJE LUMINOZITETA AKTIVNIH GALAKTIČKIH JEZGRI UNUTAR BAYESOVOG FORMALIZMA

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Gustoća i evolucija aktivnih galaktičkih jezgri kvantificira se putem funkcija luminoziteta koje prikazuju prostornu gustoću izvora kao funkciju njihovog luminoziteta. Modeliramo promjenu funkcija luminoziteta kroz kozmičko vrijeme koristeći pritom ne-parametarske i parametarske metode. Naglasak je stavljen na parametarske metode provedene unutar Bayesovog formalizma koristeći Dynesty programski paket [1]. Ova metoda je nadalje omogućila biranje najboljega modela funkcija luminoziteta. Proučili smo veliki set različitih funkcija što je rezultiralo time da je najbolji model bio model luminozitetno-ovisne evolucije gustoće. Ovaj rezultat dobiven je korištenjem većeg broja istraživanja različite dubine i površine, točnije plitkih 3CRR, 7C i 6CE polja, dubokog COSMOS polja, te srednjih XXL-South i North polja [2], [3].



Slika 1. Model luminozitetno ovisne evolucije gustoće (sive linije prikazuju median i 90% kvantile) te narančaste točke dobivene ne-parametarskom metodom.

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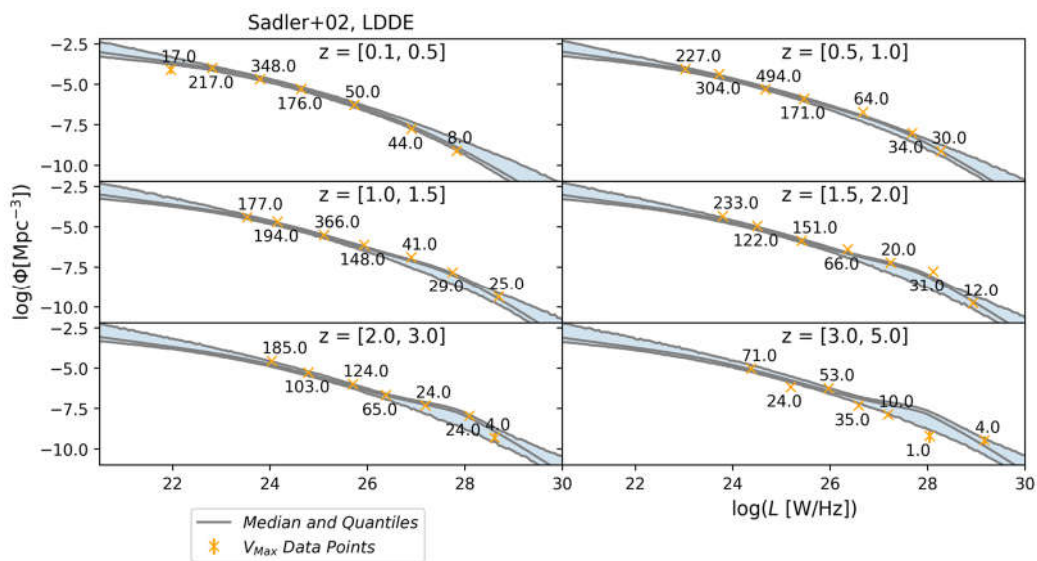
# LUMINOSITY FUNCTIONS OF ACTIVE GALACTIC NUCLEI WITHIN THE BAYESIAN FRAMEWORK

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The density and evolution of active galactic nuclei is quantified by luminosity functions, which constrain the number density of sources as a function of their luminosity. We model the change of luminosity functions through cosmic time using both non-parametric and parametric methods. The emphasis is placed on the parametric methods performed within the Bayesian framework, using the Dynesty program package [1]. This method furthermore allowed us to select a best-fitting model from a set of different functions. A wide set of different functions were tested, resulting in the best-fitting model being the one which assumed luminosity-dependent density evolution. This result was obtained using a set of surveys of varying depth and area, namely the wide 3CRR, 7C and 6CE fields, the deep COSMOS field, and the intermediate XXL-South and North fields [2], [3].



**Figure 1.** Luminosity dependent density evolution model (grey lines denoting median and 90% quantiles), and the orange data points from the non-parametric method.

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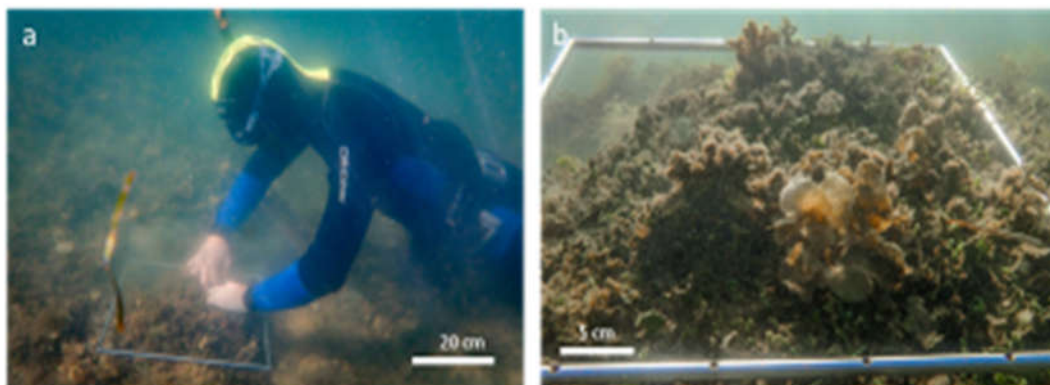
## PONOVNO OTKRIĆE VRSTE *DIGENEA SIMPLEX* (WULFEN) C. AGARDH 1822 (RHODOMELACEAE, CERAMIALES) U SJEVERNOM JADRANU

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*Digenea simplex* (Wulfen) C. Agardh 1822 (Rhodomelaceae, Ceramiales) je vrsta crvene makroalge prvotno opisane u Tršćanskom zaljevu 1803. godine iako je više tropski rasprostranjena. Nalazi ove vrste iz dvadesetog i dvadesetprvog stoljeća [1-3] spominju prisustvo vrste, iako ne prilažu nikakve sakupljene primjerke. Na temelju skupljenih povijesnih podataka [4-6] izgleda da od kraja devetnaestog stoljeća nije sakupljen niti jedan primjerak ove vrste. U ovom radu predstavljamo novootkrivenu populaciju vrste *D. simplex* na zapadnoj obali Istre, što je blizu lokacije gdje je njezin „*locus typicus*“. Populacija je otkrivena u mediolitoralu te plitkom infralitoralu, u blizini mjesta Funtana (sjeverni Jadran) tijekom 2021. godine. Sakupljeni su uzorci za potrebe zbirke te za promatranje „*in vivo*“. Nadalje, proveden je detaljniji pregled područja ronjenjem na dah kako bi se saznala rasprostranjenost i gustoća populacije vrste. U prirodi, vrsta je epilitska i raste u zajednici fotofilnih algi, često s epibiontima. Otkrivena populacija bila je izuzetno nepravilno rasprostranjena na površini od oko 5290 m<sup>2</sup> s jednim gustim naseljem veličine 20 m<sup>2</sup> te pokrovnošću od 57,5 talusa/m<sup>2</sup>, uključujući mlade i velike, puzajuće taluse. Na ostatku pregledanog područja, nalazili su se tek izolirani primjerci alge. Pošto je ovo jedina do sada nađena populacija, detaljniji pregledi povijesnih lokacija nalaza su planirani u budućnosti.



Slika 1. Određivanje gustoće naselja vrste *Digenea simplex* metodom kvadrata (a) i zajednica u kojoj dominira vrsta *D. simplex* (b).

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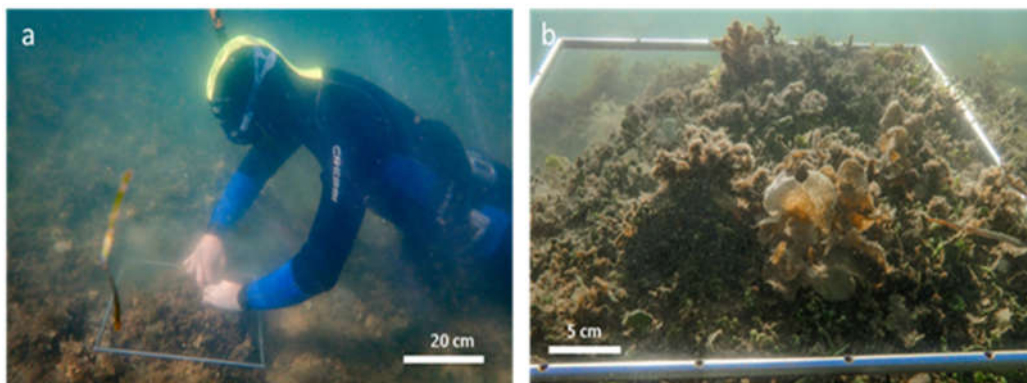
## REDISCOVERY OF *DIGENEA SIMPLEX* (WULFEN) C. AGARDH 1822 (RHODOMELACEAE, CERAMIALES) IN THE NORTHERN ADRIATIC

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*Digenea simplex* (Wulfen) C. Agardh 1822 (Rhodomelaceae, Ceramiales) is a species of red macroalgae first described in the bay of Trieste in 1803, despite being more tropically distributed. The 20<sup>th</sup> and 21<sup>st</sup> century [1-3] records all state the species as present, but do not show any collected specimens, suggesting that not a single specimen of *D. simplex* has been collected since the late 19<sup>th</sup> century, according to compiled historical data [4-6]. Here we present the recent rediscovery of an extant population on the western Istrian coast, close to the species' "*locus typicus*". An established population was discovered in year 2021 near Funtana (Northern Adriatic) in intertidal and shallow subtidal zone. Samples were collected for preservation and *in vivo* observation. Additionally, a more detailed survey of the area was performed by snorkelling to determine the distribution and population density. In the studied area, *D. simplex* is an epilithic species, growing in a photophilic community, often hosting epibionts. The discovered population was unevenly distributed over an area of approximately 5290 m<sup>2</sup> with the largest patch covering approximately 20 m<sup>2</sup>, whose density was determined to be 57.5 thalli/m<sup>2</sup>, including juvenile stages and large, creeping thalli. In the rest of the surveyed area only occasional isolated thalli were found. Since this is the only population discovered so far, a more detailed study of the historical localities across the Istrian Peninsula is planned for the future.



**Figure 1.** *Digenea simplex* patch density determination with the use of square rectangles (a) and a community dominated by *D. simplex* in Funtana (b).

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## 45000 GODINA KLIMATSKIH PROMJENA NA PODRUČJU KORNATA – PALINOLOŠKA I GEOKEMIJSKA ISTRAŽIVANJA

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Kornati su arhipelag sastavljen od 150 krških otoka, otočića i hridi raspoređenih na površini od 320 km<sup>2</sup>, što ga čini najgušćim te vrste u Sredozemnom moru [1]. Njegova specifična morfologija barijernih podmorskih uzvišenja i pragova duž rasjedne linije čini ovaj prostor izvrsnim kandidatom za proučavanje kasnokvartarnih paleookolišnih promjena povezanih s varijacijama razine mora. Navedene varijacije dovele su do razvoja različitih okoliša od jezerskih do morskih tijekom glacijalno-interglacijalnih ciklusa. Osim toga, sadašnja oskudna vegetacija otoka posljedica je antropogenog utjecaja koji je prisutan još od neolitika, što pak nudi jedinstveni uvid u klimatske promjene uzrokovane ljudskim djelovanjem. Projekt Hrvatske zaklade za znanost pod nazivom QMAD - Porijeklo i taloženje sedimenata u vrijeme kasno kvartarnih promjena morske razine: Sustav rijeke Krke i Srednjojadranske kotline, proučava paleookolišnu evoluciju istočne obale Jadrana, od Prokljanskog jezera kod estuarija rijeke Krke do Srednjojadranske kotline. Istraživanjima će se dobiti niz klimatskih i paleookolišnih podataka koji će dati uvid u potopljene krajolike kasnog kvartara i njihov utjecaj na migraciju ljudi duž obale istočnog Jadrana. U sklopu projekta, napravljena su sveobuhvatna istraživanja Kornatskog kanala. Tijekom nekoliko terenskih kampanja dobivene su detaljne batimetrijske karte te su snimljeni seizmički profili visoke razlučivosti ukupne duljine do 96,5 km. Izbušeno je i sedam sedimentnih jezgri. Detaljnije su uzorkovane dvije jezgre (KOR-1a i KOR-5) te su napravljena mjerenja magnetskog susceptibiliteta, opsežne palinofacijsne analize, <sup>14</sup>C datiranje, određivanje veličine zrna, koncentracije ukupnog i organskog ugljika i dušika. Istraživanje temeljeno na više različitih metoda pruža potpuniji pregled zamršenih odnosa koji su oblikovali proučavano područje potičući i bolju procjenu budućih klimatskih promjena.

### ZAHVALE

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### LITERATURNI IZVORI

[1] V. Skračić, *Kornati*, Forum, Zadar, 2003, 1-80.



## 45000 YEARS OF KORNATI ISLANDS' CLIMATE CHANGE – PALYNOLOGICAL AND GEOCHEMICAL INVESTIGATION

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The Kornati islands is an archipelago of 150 karst islands, isles and rocks scattered in a 320 km<sup>2</sup> area, making it the densest of its kind in the Mediterranean Sea [1]. Its specific morphological setting that consists of barrier seamounts and sills along a fault line makes this area a good candidate for studying Late Quaternary paleoenvironmental changes in relation to sea-level variability. This aspect led to the formation of different environments from lacustrine to marine during the Quaternary glacial-interglacial cycles. In addition, the islands current sparse vegetation is due to anthropogenic influence that has been present since Neolithic. The vegetational dynamics offers a unique insight into islands' climate change driven by human population. The project titled QMAD - Sediments between source and sink during a Late Quaternary eustatic cycle: The Krka river and the Mid Adriatic Deep (MAD) System, funded by the Croatian Science Foundation, is studying the paleoenvironmental evolution of the area from the Lake Prokljan in the Krka River estuary to MAD. A variety of climate and environmental proxy data will be obtained that will shed a new light onto the Late Quaternary submerged landscapes and their impact on human migration along the eastern Adriatic coast. The Kornati Channel has been extensively studied during the project as well. During several field campaigns, detailed high resolution bathymetric and backscatter maps were obtained together with high resolution seismic profiles with a total length of 96.5 km. Seven sediment cores were drilled. Two sediment cores (KOR-1a and KOR-5) were sampled with extensive geochemical analyses underway. Palynofacies analysis, <sup>14</sup>C dating and magnetic susceptibility measurements have been completed so far. Sediment samples are being analyzed for grain size, total and organic carbon and nitrogen concentrations. A multiproxy based research provides a more complete overview of the intricate relations that shaped the studied area fostering a better response for future climate change.

### ACKNOWLEDGMENTS

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# ODABIR VELIČINSKE FRAKCIJE ZA ANALIZU EOCENSKE PLANKTONSKE FORAMINIFERSKE ZAJEDNICE IZ NASLAGA DINARSKOG PREDGORSKOG BAZENA

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Odrediti vrijeme kad su naslage istaložene i paleoekološke uvjete u kojima su one nastale može se pomoću planktonskih foraminifera. Pravilno uzorkovanje i laboratorijska obrada uzoraka važna je koliko i identifikacija vrsta i rodova. Odabir veličinske frakcije na kojem će se analiza raditi je važan jer utječe na sastav foraminiferske zajednice [1]. Uzorak dubokomorskog sedimenta eocenske starosti iz uvale Podstine (otok Hvar, Dinarski predgorski bazen) obrađen je u svrhu procjene najbolje veličinske frakcije za analizu zajednice eocenskih planktonskih foraminifera. Uzorak je lapor s ~68% udjela CaCO<sub>3</sub>. Nakon što je jednaka količina uzorka podvrgnuta otapanju klasičnom metodom i metodom koja uključuje tretiranje s peroksidom i natrijevim hidrogenkarbonatom, uslijedilo je mokro prosijavanje, te su odabrane za usporedno istraživanje frakcije >63 μm i >125 μm na standardiziranim pod-uzorcima. Kriteriji za promatranje bile su morfološke osobine kućica što su i kriteriji za određivanje vrsta planktonskih foraminifera: sačuvanost kućica (cijela; nedostaje više od pola kućice), ušće (vidljivo; sa sedimentnom ispunom) i vanjski izgled površine kućice [1]. Udio cjelovitih kućica u pod-uzorku tretiranom s H<sub>2</sub>O<sub>2</sub> i Na<sub>2</sub>CO<sub>3</sub> veći je u frakciji >125 μm i iznosi 79%, dok je u frakciji >63 μm 68%. Udio kućica na kojima je bilo nemoguće odrediti ušće ili je ušće ispunjeno iznosi 92% u >125 μm i 90% u frakciji >63 μm. Za razliku od njega, uzorak tretiran samo s H<sub>2</sub>O<sub>2</sub> pokazuje sljedeće rezultate: relativna zastupljenost cjelovitih kućica iznosi 54% (>125 μm); 63% (>63 μm), ušće je vidljivo na 7% kućica (>125 μm); 13% kućica (>63 μm). Rezultati su pokazali podjednaku zastupljenost cjelovitih, dobro očuvanih kućica u obje frakcije oba uzorka, što znači da nema značajne razlike među frakcijama. Foraminifere iz frakcije >63 μm teže je izolirati, izbrojati i identificirati zbog njihove sitnije građe. Uzimajući u obzir mogućnost za sigurno prepoznavanje vrsta, možemo sugerirati da je frakcija >125 μm bolja za analizu eocenskih planktonskih foraminifera iz naslaga lapora bogatog karbonatom komponentnom koje su se taložile tijekom srednje eocenskog termalnog optimuma u području Dinarskog predgorskog bazena.

## ZAHVALE

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## LITERATURNI IZVORI

[1] M. Kucera, *Developments in Marine Geology*, Vol 1., Elsevier, Amsterdam, 2007, 213–262.





# SELECTION OF A SIZE FRACTION FOR THE ANALYSIS OF THE EOCENE PLANKTONIC FORAMINIFERAL ASSEMBLAGE FROM THE DINARIC FORELAND BASIN DEPOSITS

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Planktonic foraminifera are commonly used to determine the ages of sedimentary deposits and to interpret paleoecological conditions under which they lived. Appropriate sampling and laboratory processing of samples are as important as the identification of species and genera. Therefore, the selection of right size fraction on which the analysis will be performed due to its effects on the composition of the foraminiferal assemblage [1] matters a lot. A sample of deep-water Eocene sediment from Podstine Bay (island of Hvar, Dinaric foreland basin) was treated to estimate the best size fraction for the analysis of the Eocene planktonic foraminiferal assemblage. The sample is a marl containing ~68% CaCO<sub>3</sub>. An equal amount of the sample was dissolved in peroxide and in mixture of peroxide and sodium bicarbonate. After that, wet sieving was performed and fractions >63 μm and >125 μm in standardized sub-samples were selected for comparison. The observation criteria were the morphological characteristics of the tests, which are also criteria for determining planktonic foraminiferal species like test morphology, aperture (shape, position and secondary infillings) and wall structure, along with the preservation of the tests (whole; broken with more than half of the test is missing) [1]. The percentage of whole tests in the sub-sample treated with H<sub>2</sub>O<sub>2</sub> and Na<sub>2</sub>CO<sub>3</sub> is higher in the fraction >125 μm with 79%, while in the fraction >63 μm is 68%. The percentages of tests for which it was impossible to determine the type of the aperture are 92% in >125 μm and 90% in >63 μm. However, the sub-sample treated only with H<sub>2</sub>O<sub>2</sub> showed the following results: the relative abundance of whole tests is 54% (>125 μm); 63% (>63 μm), the aperture is visible on 7% of tests (>125 μm); 13% of tests (>63 μm). The results showed an equal abundance of the whole, well-preserved tests in both fractions of the two sub-samples, showing that there is no significant difference between the fractions. Foraminiferal tests from the >63 μm fraction are more difficult to identify due to their smaller size (younger individuals). Considering the possibility for correct species identification, we can suggest that a fraction >125 μm is better for the analysis of Eocene planktonic foraminifera from carbonate-rich marls deposited during the Middle Eocene Climate Optimum in the Dinaric foreland basin.

## ACKNOWLEDGMENTS

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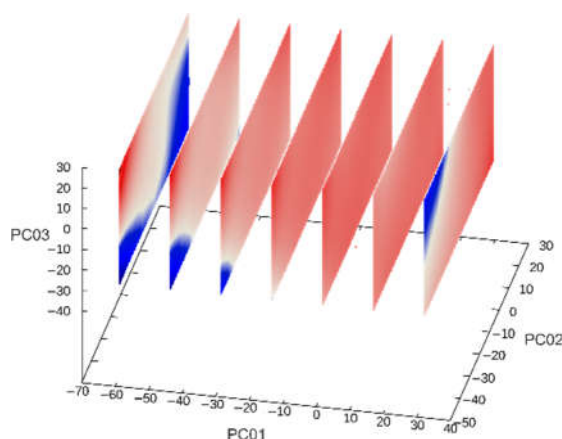
## MODELI INHIBICIJSKE AKTIVNOSTI FLUORIRANIH *CINCHONA* ALKALOIDA DOBIVENI STROJNIM UČENJEM

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Niz od 25 fluoriranih derivata *Cinchona* alkaloida teorijski je istražen izračunom ploha potencijalne energije (PES). PES svih spojeva uzorkovane su provođenjem simulacija molekularne dinamike [1] i zatim dekomponirane analizom glavnih komponentata. Svaki PES predstavljen je trima točkama u novoutvrđenom reduciranom prostoru. Ove su točke iskorištene kao neovisne varijable za uspostavljanje regresijskog modela aktivnosti/PES, dok su prethodno izmjerene inhibicijske aktivnosti prema acetyl- i butirilcolinesterazi korištene kao ovisne varijable. Multivarijantni linearni regresijski modeli izgrađeni su ekstenzivnog primjenom strojnog učenja pri čemu su korištene linearne kombinacije originalnih varijabli kao i njihovi polinomni članovi višeg reda. Unakrsna validacija uz izostavljanje po jednog člana skupa (LOO-CV) korištena je za validaciju dobivenih modela [2,3]. Optimalni modeli aktivnost/PES odabrani su na temelju prilagođenog  $R^2$ , predviđenog  $R^2$  i srednje kvadratne pogreške LOO-CV (Slika 1).



Slika 1. Model inhibicijskih aktivnosti fluoriranih *Cinchona* alkaloida u ovisnosti o glavnim komponentama ploha potencijalne energije spojeva dobiven strojnim učenjem.

### ZAHVALE

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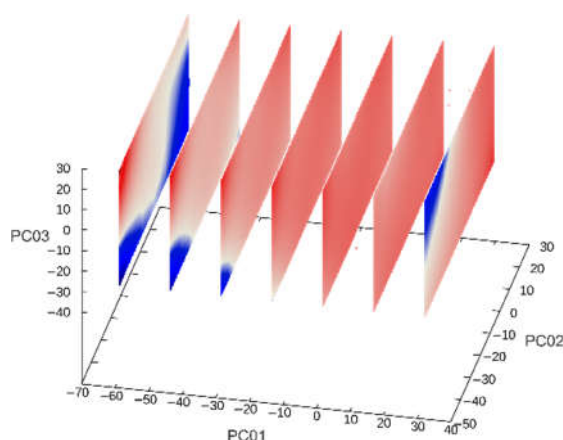
# MACHINE LEARNING DETERMINED MODELS OF INHIBITORY ACTIVITIES FOR FLUORINATED *CINCHONA* ALKALOIDS

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A series of 25 fluorinated *Cinchona* alkaloids derivatives was theoretically investigated by calculation of their potential energy surfaces (PES). PES for all compounds were sampled by performing molecular dynamics simulations [1] and then decomposed by principal component analysis. Each PES was represented by three points in the newly determined reduced space. These points were used as independent variables for establishing activity/PES regression models whereas previously measured inhibitory activities towards human acetyl- and butyrylcholinesterase were used as dependent variables. Multivariate linear regression models were built by applying an extensive machine learning protocol where linear combinations of original variables as well as their higher-order polynomial terms were used. *Leave-one-out cross-validation* (LOO-CV) was used to validate obtained models [2,3]. Optimal activity/PES models were selected based on the adjusted  $R^2$ , predicted  $R^2$  and the LOO-CV mean squared error (Figure 1).



**Figure 1.** Machine learning determined model of inhibitory activities for fluorinated *Cinchona* alkaloids derivatives dependent on the principal components of compounds' PES.

## ACKNOWLEDGMENTS

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## MEHANOKEMIJSKA SINTEZA *O*-SUPSTITUIRANIH OKSIMA KINUKLIDIN-3-ONA

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Jedan od rjeđe korištenih pristupa za sintezu oksima je i mehanokemijska sinteza [1] koja je privukla značajan interes istraživačke zajednice zbog mogućnosti provođenja kemijskih reakcija bez upotrebe toksičnih i/ili skupih otapala, ali i mogućnosti da se dobiju različiti glavni produkti takvim reakcijama u odnosu na one koje se izvode u otopini [2]. U okviru ovog rada bit će opisana priprava *O*-supstituiranih oksima kinuklidin-3-ona. U reakciji kinuklidin-3-ona ili kinuklidin-3-on-hidroklorida te odgovarajućih *O*-supstituiranih hidroksilamin-hidroklorida pripremljeni su metilni, benzilni, *tert*-butilni i fenilom supstituirani oksimi kinuklidin-3-ona. Uspoređeni su rezultati sinteze u otopini i mehanokemijske sinteze koji ukazuju na prisutnost oksima (*E*) i (*Z*) konfiguracije. Obzirom na dobivene rezultate dodatno je istraženo kako promjena reakcijskih uvjeta i korištenja različitih baza utječe na omjere dobivenih stereoisomera. ATR mjerenjima tijekom eksperimenata praćena je kinetika kemijskih reakcija. Identifikacija i kontrola čistoće pripremljenih spojeva ispitana je klasičnim analitičkim postupcima koji su uključivali ATR i <sup>1</sup>H NMR spektroskopiju.

### ZAHVALE

Rad je izrađen u okviru projekata Hrvatske zaklade za znanost: "Projekt razvoja karijera mladih istraživača - izobrazba novih doktora znanosti" ESF-DOK-01-2018 te "*Aktivnošću i in silico usmjeren dizajn malih bioaktivnih molekula*" (IP-2016-06-3775).

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# MECHANOCHEMICAL SYNTHESIS OF *O*-SUBSTITUTED QUINUCLIDIN-3-ONE OXIMES

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One of the less commonly used approaches for oxime synthesis is the mechanochemical approach [1]. Mechanochemical synthesis has attracted significant interest of research community due to the possibility of conducting chemical reactions without the use of toxic and/or expensive solvents, and the possibilities to obtain different main products in such reactions compared to those performed in the solution [2]. The preparation of series of *O*-substituted quinuclidin-3-one oximes will be described by this work. Methyl, benzyl, *tert*-butyl, and phenyl substituted oximes of quinuclidin-3-one were prepared in the reaction of quinuclidin-3-one or quinuclidin-3-one hydrochloride with the corresponding *O*-substituted hydroxylamine hydrochlorides. Obtained results for the synthesis in solution and mechanochemical synthesis were compared, and the presence of (*E*) and (*Z*) stereoisomers of oximes identified. Furthermore, it was investigated to what extent the change of the reaction conditions and use of different bases can affect ratio of the obtained stereoisomers. Kinetics of chemical reactions were monitored by recording ATR spectra. Identification and control of the purity of prepared compounds was examined by classical analytical methods such as ATR and <sup>1</sup>H NMR spectroscopy.

## ACKNOWLEDGMENTS

The study was prepared within the projects of the Croatian Science Foundation: "Young researchers' career development - Training New Doctoral Students" ESF-DOK-01-2018 and "Activity and *in silico* guided design of bioactive small molecules" (IP-2016-06-3775).

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## STRUKTURNA I ELEKTRIČNA STUDIJA NATRIJEVE FOSFATNE STAKLO-(KERAMIKE) S OKSIDIMA NIOBIJA(V) I VANADIJA(V)

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Natrijeva fosfatna stakla privukla su veliku pozornost u istraživanju i razvoju baterija u čvrstom stanju u kojima mogu poslužiti kao elektroliti ili elektrodni materijali, nudeći obećavajuću alternativu uobičajeno korištenim skupocjenim litijevim materijalima. Premda u odnosu na litijeve materijale spomenuta stakla imaju slabiju električnu vodljivost, nova istraživanja pokazuju da je značajan porast ionske vodljivosti moguće postići dodatkom drugog staklotvorca (tzv. *učinak miješanih staklotvoraca*) [1]. Od posebnog su interesa sustavi u kojima se klasični staklotvorac ( $P_2O_5$ ) zamjenjuje uvjetnim staklotvorcima kao što su oksidi prijelaznih metala ( $Fe_2O_3$ ,  $MoO_3$ ,  $WO_3$ ,  $V_2O_5$  i  $Nb_2O_5$ ). Osim porastom ionske vodljivosti uzrokovane strukturnim promjenama, oksidi prijelaznih metala mogu doprinijeti ukupnoj električnoj vodljivosti dodatnom elektronskom komponentom ako je prijelazni metal prisutan u dva oksidacijska stanja [2].

U ovom radu, klasičnom metodom hlađenja taline sintetizirana su stakla kvaternog sustava  $Na_2O-V_2O_5-P_2O_5-Nb_2O_5$ . S ciljem da se istraži *učinak miješanih staklotvoraca* i područje nastajanja stakla, udijeli  $Na_2O$  i  $V_2O_5$  su konstantni, dok je klasični staklotvorac ( $P_2O_5$ ) postupno zamjenjivan uvjetnim staklotvorcem ( $Nb_2O_5$ ). Amorfni karakter priređenih stakala potvrđen je PXRD metodom, dok su djelomično kristalizirani uzorci, dobiveni spontanom kristalizacijom pri visokim udjelima  $Nb_2O_5$ , kvalitativno i kvantitativno analizirani. (Mikro)struktura dobivene staklo-(keramike) ispitana je SEM-EDS analizom te IR-ATR spektroskopijom, dok su njihova termička svojstva proučavana metodom DTA. Električna svojstva dobivenih produkata detaljno su istražena impedancijskom spektroskopijom u čvrstom stanju (SS-IS) u širokom frekvencijskom (0,01 Hz–1 MHz) i temperaturnom području (-90 °C–240 °C). Rezultati ovog istraživanja rasvijetlit će odnos između sastava, (mikro)strukture i električnih svojstava odabranog sustava čije je razumijevanje nužno za pripremu materija s mogućnošću ugađanja električnih svojstava.

### ZAHVALE

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# STRUCTURAL AND ELECTRICAL STUDY OF SODIUM PHOSPHATE GLASS-(CERAMICS) CONTAINING NIOBIUM(V) AND VANADIUM(V) OXIDES

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Sodium phosphate-based glasses offer a promising alternative to the commonly used, high-cost lithium materials for the development of solid-state batteries for use as electrolytes and electrode materials. Although these glasses show lower electrical conductivity than the lithium ones, recent research demonstrates that a significant increase in ionic conductivity can be achieved by the addition of another glass-forming oxide (the so-called *mixed glass-former effect*, **MGFE**) [1]. Of particular interest is the study of glass systems in which the conventional glass-forming oxide ( $P_2O_5$ ) is replaced by transition metal oxides (TMO) such as  $Fe_2O_3$ ,  $MoO_3$ ,  $WO_3$ ,  $V_2O_5$  and  $Nb_2O_5$ , known as conditional glass formers. In addition to the enhancement of ionic conductivity due to induced structural changes, TMOs also contribute to the overall electrical conductivity by electron hopping between TM ions in different oxidation states [2].

In this study, glasses of the quaternary system  $Na_2O-V_2O_5-P_2O_5-Nb_2O_5$  are prepared by the conventional melt-quenching method. In order to investigate the MGFE and glass-forming tendency, the content of  $Na_2O$  and  $V_2O_5$  is constant, while  $P_2O_5$  is gradually replaced by  $Nb_2O_5$ . The amorphous character of prepared glasses is confirmed by PXRD technique, whereas the partially crystallized samples, obtained by spontaneous crystallization which occurs at high  $Nb_2O_5$  content, are qualitatively and quantitatively analysed. The (micro)structure of the obtained glass-(ceramics) is investigated by SEM-EDS analysis and IR-ATR spectroscopy, while their thermal properties are determined by DTA. The electrical properties of studied samples are investigated in detail by solid-state impedance spectroscopy (SS-IS) in a wide frequency (0.01 Hz–1 MHz) and temperature range (-90 °C–240 °C). The results of this research shed light on the relationship between the composition, (micro)structure and electrical properties of the selected system, which is of the utmost importance for the development of materials with the possibility of fine-tuning the desired electrical properties.

## ACKNOWLEDGMENTS

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## ZNAČAJNA POVEZANOST KINETIKE I PRIJENOSA ENERGIJE U MEHANOKEMIJSKIM REAKCIJAMA

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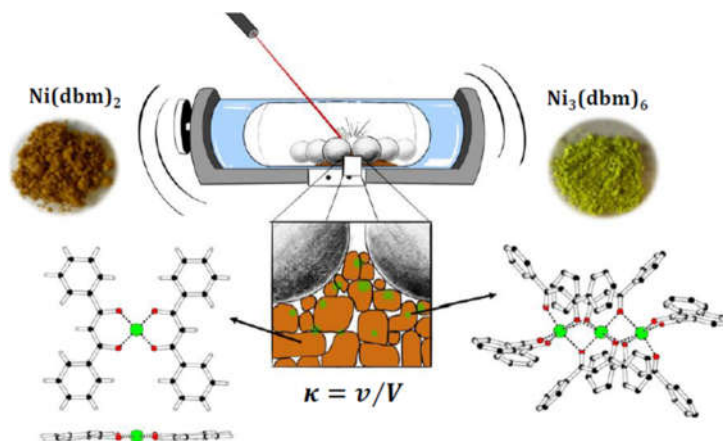
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Kemijske reakcije u čvrstom stanju zelena su alternativa otopinskim sintezama, ali za širu primjenu mehanokemijskih reakcija unutar okvira zelene kemije potrebno je detaljno ispitivanje reakcijskih mehanizama na molekularnoj razini [1]. Jedan od trenutnih nedostataka mehanokemije je nedovoljno poznavanje utjecaja parametara mljevenja na prijenos energije tijekom kugličnog mljevenja. U ovom radu, jednostavna jednostupanjska trimerizacija niklovog(II) dibenzoilmetanata poslužila je kao modelni sustav za sistematično istraživanje utjecaja frekvencije mljevenja, materijala i mase kuglica za mljevenje na brzinu mehanokemijske oligomerizacije u realnom vremenu Ramanovom spektroskopijom *in situ* (slika 1). Mijenjanjem parametara mljevenja, modificirana je ulazna količina energije potrebna za kemijsku transformaciju što je rezultiralo različitim reakcijskim profilima. Numeričkim modeliranjem procesa mljevenja, ustanovljena je linearna povezanost između brzine reakcije i doze energije koju apsorbira uzorak. Posljedično, količina energije raste s frekvencijom mljevenja, masom i prosječnom brzinom kuglice, ali se smanjuje s tvrdoćom mljevenog materijala do minimuma koji je potreban za inicijaciju kemijske transformacije.



**Slika 1.** Ilustracija procesa mljevenja i transformacije monomera (smeđi prah, lijevi prikaz) u oligomer (zeleni prah, desni prikaz) nakon ostvarivanja kritičnih uvjeta opterećenja (CLC).

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# THE DEEP LINK BETWEEN ENERGY TRANSFER AND KINETICS IN MECHANOCHEMICAL REACTIONS

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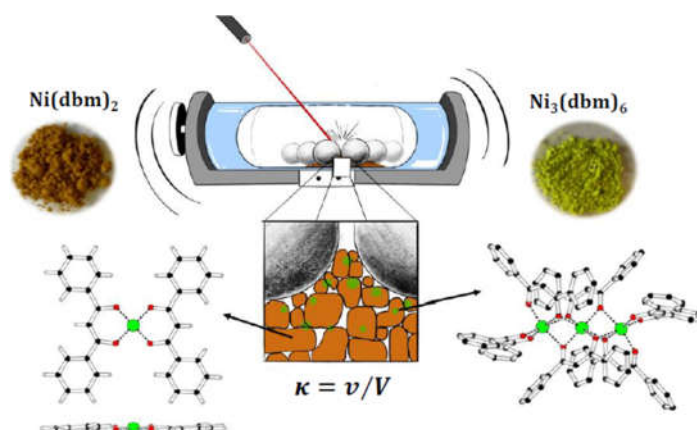
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Solid-state synthesis by mechanochemistry serves as an alternative to solution synthesis, but for a wider application within the framework of green chemistry, detailed investigations about reaction mechanisms at the molecular level are crucial [1]. One of current disadvantages of mechanochemistry is insufficient understanding of the influence of milling parameters on energy transfer during ball milling. In this paper, simple one-step trimerization of nickel(II) dibenzoylmethane was used as a model system to systematically examine by *in situ* Raman spectroscopy the effect of the milling frequency, and the material and mass of milling balls on the mechanochemical oligomerization reaction rate (Figure 1). By varying milling parameters, we have modified energy input required for the chemical transformation leading to different reaction profiles. Based on numerical simulations of the milling processes, a linear relationship was established between the reaction rate and the energy dose absorbed by the sample. Consequently, the energy dose increases with the frequency of impacts, mass and the average ball velocity, but decreases with the hardness of milling media to the minimum energy needed to initiate chemical conversion.



**Figure 1.** Illustration of a ball-milling process where the starting monomer (brown powder, left) transform to the oligomer (green powder, right) after the critical loading conditions (CLC) achievement.

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## SVEOBUHVAATNO ISTRAŽIVANJE SVOJSTVA OTPORNOSTI I IMUNOSTI NA ISUŠIVANJE RAZLIČITIH BIOTA U POVREMENIM RIJEKAMA I BUJIČNIM POTOCIMA

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Posljednjih godina klimatske promjene su sve prisutniji faktor stresa na okoliš. Nisu sva područja, aktivnosti, staništa ili ekosustavi pogođeni na isti način ili istim intenzitetom, ali predviđa se kako su pod utjecajem klimatskih promjena slatkovodna staništa vrlo ranjiva. Zbog toga brzo reagiraju na klimatske promjene te su stoga izvrstan model za istraživanje. Najosjetljivije među slatkovodnim ekosustavima su povremene rijeke i bujični potoci (eng. intermittent rivers and ephemeral streams - IRES), kao i rijeke u vrućim klimama koje sve učestalije iz trajnih postaju povremene. Zbog porasta globalne prosječne temperature mijenjaju se ciklusi i duljine perioda sušnih i vlažnih razdoblja. Kao sredstvo koncentriranja napora na istraživanje, razumijevanje, predviđanje i ublažavanje utjecaja klimatskih promjena na IRES, pokrenut je Horizon 2020 Project DRYvER. Napori unutar projekta uključuju rad stručnjaka s 4 kontinenta koji surađuju i imaju interdisciplinarni pristup cilju razumijevanja, predviđanja i zaštite od utjecaja klimatskih promjena na biološku raznolikost, funkcioniranje ekosustava i usluge IRES-a. Jedan od tih napora je razvoj dinamičkog meta-sustavnog okvira koji će katalizirati naše razumijevanje učinaka isušivanja na funkcije riječne mreže. Izrada spomenutog okvira uključuje stvaranje baze podataka slatkovodnih zajednica iz Europe i Južne Amerike uz fokusiranje na nove podatke prikupljene iz presušnih riječnih mreža (eng. Drying River Network - DRN) i određivanje svojstava bakterija, gljiva, dijatomeja, makrobekralješnjaka i riba koje imaju najvažniju ulogu u njihovoj otpornosti ili imunosti na isušivanje. Osim toga, te osobine se moraju odnositi na sposobnost svojiti da izbjegnu sušu, zadrže vodu, osiguraju preživljavanje potomstva, razviju fazu mirovanja itd. Ta vrsta rada uključuje doprinose stručnjaka po pitanju njihovih područja istraživanja te biota kojime proučavaju, prikupljanje postojećih i brojnih lako dostupnih podataka, te istraživanje literature primjenjivo na svojte koje se očekuju u DRN-ovima. Do sada smo obavili mnoge razgovore sa stručnjacima te pokrenuli mnoge rasprave i suradnje, prikupili uzorke i podatke iz 9 DRN-ova diljem Europe i Južne Amerike te nastavljamo prikupljati postojeće podatke. Količina informacija koja se može prikupiti je neograničena, ali postoji ograničenje vremena. Problem koji se može pojaviti tijekom opsežnog istraživanja kao što je ovo je određivanje dosega, preciznosti i vremenskih ograničenja s kojima moramo raditi, a to je problem s kojim se znanstvenici često susreću. Stoga je jedan od najvećih problema s kojima se suočavamo je odrediti kako prikupiti i iskoristiti najveću količinu informacija u dodijeljenom vremenu.





## COMPREHENSIVE RESEARCH OF DRYING RESISTANCE AND RESILIENCE TRAITS BY DIFFERENT BIOTA IN INTERMITTENT RIVES AND EPHEMERAL STREAMS

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During the recent years, climate change has been an increasingly pervasive environmental stressor. Not all areas, activities, habitats or ecosystems are affected in the same way or severity, but under the influence of climate change, freshwater habitats have been projected to be highly vulnerable. This can make them react to climate changes quickly and therefore they are a great model for research. Most sensitive among freshwater ecosystems are intermittent rivers and ephemeral streams (IRES), as well as perennial rivers in hot climates which are increasingly becoming intermittent. Because of the increase of global average temperature, the cycles between dry and wet periods are changing. As a means to concentrate efforts to research, understanding, predicting and mitigating the influences of climate change on IRES, Horizon 2020 Project DRYvER was initiated. Different efforts inside the project include the work of experts from 4 continents that collaborate and have an interdisciplinary approach to the goal of understanding, predicting and protecting from the influence of climate change on biodiversity, ecosystem functioning and services of IRES. One of these efforts is developing a dynamic meta-system framework to catalyze our understanding of the effects of drying on river network functions. Creating of a mentioned framework includes creating a database of freshwater communities from Europe and South America while focusing on new data gathered from drying river networks (DRN) and determining the traits of bacteria, fungi, diatoms, macroinvertebrates and fish that have the most important role in making them resistant or resilient to drying. In addition, the traits must pertain to the ability of taxa to evade droughts, sustain water, ensure survival of progeny, develop dormant phase etc. That type of work includes inputs by experts regarding their respective fields and biota, gathering of existing and abundant readily available data and research of literature applicable to the taxa expected across the DRNs. So far, we have conducted many interviews and initiated many discussions and collaborations, gathered samples and data from 9 DRNs around Europe and South America and continue to gather existing data. The amount of information to be gathered is unlimited, but there is the limitation of time. The problem that can occur while making a comprehensive research such as this is determining the reach, accuracy and time scale we have to work with, and it is the problem met often by scientists. So, as such, one of the biggest problems we are tackling is to determine how to gather and use the biggest amount of information in the allotted time.



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## MORPHOMETRIC CHARACTERISTICS OF STONE CRAYFISH FROM DIFFERENT WATERCOURSES OF EASTERN HERZEGOVINA

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The indigenous species *Austropotamobius torrentium* (stone crayfish) inhabiting freshwaters of Eastern Herzegovina and a larger number of streams in the area of Foča. In recent decades, conditions in most habitats of this species have changed significantly. Watercourses in the area of Foča differ in the quality of the environment, physico-chemical and biological aspect. Some watercourses are active all year round, with lower or higher flows, while some are temporary. The research included the examination of basic morphometric parameters (according to [1]), including the mass of individuals and Fulton's coefficient of condition in crayfish from three watercourses in the area of Foča (Bistrica, Miljevka, Govza). Relevant statistical methods were used to process the obtained data and compare the monitored characteristics of individuals from different watercourses. The results of statistical data processing show the existence of significant differences in the values of some individual parameters. The highest values were found in individuals from the Govza River, while the lowest, except for the Fulton's coefficient of condition, were found in crayfish from the Bistrica watercourse. Namely, according to the research Govza River, greater diversity of bottom substrate composition has positive impact on condition of stone crayfish.

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## ODGOVOR NA SOLNI I OSMOTSKI STRES U UROČNJAKA (*Arabidopsis thaliana* (L.) Heynh.) S PROMIJENJENOM EKSPRESIJOM PROTEINA BPM I DMS3

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Proteini BPM iz porodice MATH-BTB dio su ubikvitin-proteasomalnog puta (UPS) – regulatornog mehanizma koji omogućuje selektivnu degradaciju proteina u stanici [1]. Novija istraživanja sugeriraju da su proteini BPM uključeni u mehanizam metilacije DNA posredovane malim molekulama RNA (RdDM) kroz interakciju s proteinom DMS3 [2]. Oba mehanizma, UPS i RdDM, važna su za razvojne procese, ali i u prilagodbi rasta i razvoja biljke tijekom promjenjivih okolišnih uvjeta [3, 4]. Cilj ovog rada bio je istražiti utjecaj solnog i osmotskog stresa u klijanaca uročnjaka s promijenjenom ekspresijom funkcionalnih proteina BPM i DMS3. U istraživanju su korišteni klijanci divljeg tipa, linije s pojačanom ekspresijom gena *BPM1* (*oeBPM1*), linije s utišanim genima *BPM1*, *4*, *5* i *6* (*amiR-bpm*), linije s pojačanom ekspresijom gena *DMS3* (*oeDMS3*) te linije s nefunkcionalnim proteinom DMS3 (*dms3-1*). Pokazatelji stresa bili su fotosintetska učinkovitost ( $PI_{abs}$ ), sadržaj prolina, stupanj lipidne peroksidacije izražen kao sadržaj MDA te aktivnost dvaju antioksidacijskih enzima – askorbat peroksidaze (APX) i katalaze (CAT). Za istraživanje solnog i osmotskog stresa, klijanci su od 5. do 12. dana uzgajani na podlozi koja je sadržavala  $75 \text{ mmol dm}^{-3}$  NaCl-a, odnosno  $150 \text{ mmol dm}^{-3}$  manitola. Usporedbom kontrolnih klijanaca te klijanaca tretiranih NaCl-om ili manitolom, linija *oeBPM1* pokazala se osjetljivijom na tretman manitolom koji je uzrokovao povećanje sadržaja prolina, MDA i aktivnosti CAT te pad  $PI_{abs}$ . S druge strane, linija *amiR-bpm* pokazala se osjetljivijom na solni stres pri čemu je utvrđena povećana aktivnost CAT te smanjenje parametara MDA, APX i  $PI_{abs}$ . U liniji *oeDMS3* oba tretmana uzrokovala su nakupljanje prolina i smanjenje  $PI_{abs}$ . Međutim, nije utvrđena razlika u sadržaju MDA te aktivnosti APX i CAT u odnosu na kontrolu. U klijanaca linije *dms3-1* nakupljanje prolina i MDA bilo je najveće nakon tretmana manitolom. Pojačana aktivnost APX i CAT zabilježena je nakon tretmana NaCl-om, ali ne i nakon tretmana manitolom. Oba tretmana uzrokovala su pad  $PI_{abs}$ , u usporedbi s kontrolom. Razlike u pokazateljima stresa u liniji *oeBPM1* i *amiR-bpm* ukazuju na moguće specifične uloge pojedinih proteina BPM u odgovoru uročnjaka na solni i osmotski stres. Usto, dobiveni rezultati impliciraju da protein DMS3 ima ulogu u toleranciji solnog i osmotskog stresa u uročnjaka.

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## SALT AND OSMOTIC STRESS RESPONSE IN THALE CRESS (*Arabidopsis thaliana* (L.) Heynh.) WITH MODIFIED EXPRESSION OF BPM AND DMS3 PROTEINS

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BPM proteins from the MATH-BTB family are part of the ubiquitin-proteasomal pathway (UPS) - a regulatory mechanism that enables the selective degradation of proteins in the cell [1]. Recent research suggests that BPM proteins are involved in RNA-directed DNA methylation (RdDM) through direct interaction with DMS3 protein [2]. Both mechanisms, UPS and RdDM, are important for developmental processes, but also for the adaptation of plant growth and development in changing environmental conditions [3, 4]. The aim of this study was to investigate the effect of salt and osmotic stress in *Arabidopsis thaliana* seedlings with modified expression of the functional proteins BPM and DMS3. Seedlings with overexpressed *BPM1* gene (*oeBPM1*), downregulated *BPM1*, *4*, *5* and *6* genes (*amiR-bpm*), overexpressed *DMS3* gene (*oeDMS3*), nonfunctional DMS3 protein (*dms3-1*), or wild type seedlings were used for the study. Stress indicators were photosynthetic efficiency ( $PI_{abs}$ ), proline content, level of lipid peroxidation expressed as MDA content, and activity of two antioxidant enzymes – ascorbate peroxidase (APX) and catalase (CAT). To induce salt or osmotic stress, seedlings were cultured from day 5 to day 12 on medium containing 75 mmol dm<sup>-3</sup> NaCl and 150 mmol dm<sup>-3</sup> mannitol, respectively. When comparing control seedlings and seedlings treated with NaCl or mannitol, *oeBPM1* line proved to be more sensitive to mannitol treatment, which caused an increase in proline content, MDA and CAT activity, and decrease in  $PI_{abs}$ . On the other hand, *amiR-bpm* line showed higher sensitivity to salinity, with increased CAT activity and a decrease in MDA, APX, and  $PI_{abs}$  parameters. In *oeDMS3* line, both treatments resulted in proline accumulation and a decrease in  $PI_{abs}$ . However, no difference was observed in MDA content, and activities of APX and CAT compared to the control. Seedlings of *dms3-1* line showed greatest accumulation of proline and MDA after mannitol treatment. Increased activity of APX and CAT was observed after NaCl treatment but not after mannitol treatment. Both treatments caused a decrease in  $PI_{abs}$  compared with control. The differences in stress response in *oeBPM1* and *amiR-bpm* lines suggest a possible specific role of individual BPM proteins in the response to salt and osmotic stress. In addition, the results suggest that DMS3 protein has a role in the tolerance of salt and osmotic stress in thale cress.

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## ANALIZA DINAMIKE RASTA ŠKOLJKAŠA *PECTEN JACOBÆUS* (LINNÆUS, 1758)

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U sklopu izrade doktorske disertacije provoditi će se sklerokronološko istraživanje na ljušturama vrste *Pecten jacobæus* (Linnaeus, 1758) iz porodice Pectinidae. Vrsta *P. jacobæus*, kao i drugi školjkaši, ima periodičan rast kroz cijeli život, koji se manifestira taloženjem novonastalog materijala na rub ljušture [1]. Novonastale strije, jasno vidljive na vanjskoj strani lijeve (ravne) ljušture, formiraju se periodično te omogućuju analizu dinamike rasta i procjene starosti jedinke [2].

Na prethodno prikupljenim uzorcima provedeno je uzastopno fotografiranje vanjske površine ljušture u visokoj rezoluciji pod stereoskopskom lupom, duž osi maksimalnog rasta. Prikupljene fotografije za svaku jedinku su naslagane u kompozitnu fotografiju na kojoj je provedeno brojanje strija i mjerenje udaljenosti između njih koristeći Image-Pro program.

U daljnjem istraživanju, provodit će se geokemijska analiza ljušturnog materijala, uključujući analizu stabilnih izotopa kisika ( $\delta^{18}\text{O}$ ), s ciljem validacije periodičnosti polaganja strija. Analiza vrijednosti stabilnih izotopa kisika omogućit će rekonstrukciju dinamike rasta tijekom godine [3]. Dobiveni rezultati dat će jasniji uvid u ekologiju ove gospodarski važne vrste te mogućnost njene prilagodbe na promjene u okolišu.

### ZAHVALE

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## GROWTH DYNAMICS ANALYSIS OF THE BIVALVE *PECTEN JACOBÆUS* (LINNÆUS, 1758)

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Sclerochronological research will be conducted on shells of the bivalve *Pecten jacobæus* (Linnaeus, 1758) from the family Pectinidae as part of the preparation of the doctoral dissertation. *Pecten jacobæus*, like other bivalves, has periodic growth throughout its lifetime, which is manifested by the deposition of newly formed material on the edge of the shell [1]. Newly formed striae, clearly visible on the external part of the left (flat) valve, are formed periodically and allow the analysis of growth dynamics and age assessment of the individual [2].

On previously collected samples, successive imaging of the external surface of the shell was performed in high resolution under a stereoscopic microscope, along the axis of maximum growth. Collected photographs for each individual were stacked into a composite image on which counting of striae and measuring of the distance between them was performed, using the Image-Pro program.

In further research, a geochemical analysis of the shell material, including the analysis of stable oxygen isotopes ( $\delta^{18}\text{O}$ ), will be performed, with the aim of validating the periodicity of striae deposition. Analysis of the values of stable oxygen isotopes will enable the reconstruction of growth dynamics during the year [3]. The obtained results will give a clearer insight into the ecology of this commercially important species and the possibility of its adaptation to changes in the environment.

### ACKNOWLEDGMENTS

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## PRISUTNOST ANTIBIOTSKE REZISTENCIJE U AKVAKULTURI ŠKOLJKAŠA NA PODRUČJU ISTOČNOG JADRANA

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Uzgoj školjkaša u Hrvatskoj se u potpunosti odvija u prirodnoj sredini upotrebom tradicionalnih metoda. Zbog načina prehrane putem filtracije, školjkaši nakupljaju veliki broj tvari i mikroorganizama iz okoliša. Jedan od najčešćih rodova koji se javljaju u vodenom stupcu je rod *Vibrio*, rod kojem pripadaju brojni oportunistički patogeni koji izazivaju bolesti vibroze [1]. Jedan od rastućih problema u morskim okolišima je pojava antibiotske rezistencije zbog prekomjernog korištenja antibiotika te antropogenih ispusta u morski okoliš [2]. Međutim, antibiotska rezistencija u akvakulturi školjkaša je slabo istražena.

Ovo istraživanje je provedeno na dvije lokacije poznate po tradicionalnom uzgoju školjkaša – Mali Ston i Limski zaljev. Uzeti su uzorci vode, sedimenta i tkiva dvije vrste školjkaša koji se komercijalno uzgajaju u Hrvatskoj – europska kamenica, *Ostrea edulis* Linneaus, 1758 i mediteranska dagnja, *Mytilus galloprovincialis* Lamarck, 1819. Uzorci su nasadeni na TCBS hranjivim podlogama selektivnim za rod *Vibrio* na 22°C i 35°C, te su izolirane pojedinačne kolonije. Pripadnost rodu *Vibrio* je određena MALDI-TOF masenom spektrometrijom [3], a antibiotska rezistencija putem difuzije na disku na Muller Hinton agaru za odabranih 13 antibiotika. Projekt je financirala Hrvatska zaklada za znanost u sklopu projekta „Osjetljivost komercijalno važnih školjkaša u akvakulturi istočnog dijela Jadrana na promjene okolišnih uvjeta” - Best Adria (IP-2019-04-1956).

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## PRESENCE OF ANTIBIOTIC RESISTANCE IN SHELLFISH AQUACULTURE IN THE EASTERN ADRIATIC

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In Croatia, traditional bivalve farming is dominated, which takes place entirely in the natural environment. As bivalves are filter feeders, they accumulate a large quantity of particles, including microorganisms from the environment. One of the most common genera that occurs in the water column is the genus *Vibrio*, known for numerous opportunistic pathogens that cause diseases - vibriosis. One of the growing problems in marine environments is the emergence of antibiotic resistance due to the excessive use of antibiotics and anthropogenic discharges into the marine environment. However, antibiotic resistance in bivalve aquaculture has been poorly studied.

This research was conducted at two traditional bivalve aquaculture locations - Mali Ston Bay and the Lim Bay. Water, sediment and tissue samples were collected from two bivalve species of commercially grown in Croatia - the European oyster, *Ostrea edulis* Linnaeus, 1758 and the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819. Samples were inoculated on nutrient media TCBS selective for genus *Vibrio* at 22°C and 35°C, and individual colonies were isolated. Belonging to the genus *Vibrio* was determined by MALDI-TOF mass spectrometry, and antibiotic resistance by disk diffusion on Mueller Hinton agar for 13 selected antibiotics. Project was funded by Croatian Science Foundation as part of project „Sensitivity of commercially important bivalves from eastern Adriatic aquaculture to variations in environmental conditions” - Best Adria (IP-2019-04-1956).

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## ODABIR EFIKASNE LABORATORIJSKE METODE PRIPREME UZORAKA: PRIMJER SREDNJEEOCENSKIH LAPORA (UVALA PODSTINE, OTOK HVAR)

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Lapori, miješane glinovito-karbonatne stijene, važna su sastavnica dubokomorskih naslaga (fliš). Bogate mikrofosilima (osobito planktonskim foraminiferama) pružaju brojne podatke za rekonstrukciju paleokoliša. Predmet ovog rada je odabir optimalne laboratorijske metode pripreme uzoraka srednjeocenskih laporovitih naslaga. Uzorkovanje je provedeno u uvali Podstine (otok Hvar) i uzorak je na terenu opisan kao kalcitni lapor. Kalcimetrijom smo utvrdili da stijena sadrži 67,78% kalcita (kalcijevog karbonata,  $\text{CaCO}_3$ ) i da pripada podvrsti lapora zvanj marlit [1]. Uzorak je razdijeljen na tri pod-uzorka, svaki mase 200 g. Dva pod-uzorka obradili smo standardnim metodama pripreme laporovitih stijena [2]. Prvi smo tretirali otopinom 50 ml 30-postotnog vodikova peroksida ( $\text{H}_2\text{O}_2$ ) u 2 l vode u trajanju od 24 sata, a drugi otopinom 150 ml vodikova peroksida iste koncentracije u 1 l kipuće vode uz dodatak 10 g natrijevog hidrogenkarbonata ( $\text{NaHCO}_3$ ) u trajanju od 48 sati. Treći pod-uzorak tretirali smo metodom višestrukog zamrzavanja i naglog otapanja, ukupnog trajanja 48 sati, što je testirano kao potencijalna zamjena za standardnu metodu usitnjavanja konsolidiranog uzorka tekućim dušikom. Pod-uzorke smo standardizirali pomoću mikrosplitera i pregledali pod stereoskopskom lupom. Odielili smo cijele kućice foraminifera s otvorenom površinom koje su pogodne za morfološku identifikaciju rodova i vrsta od kućica koje su bile oštećene ili oblijepljene sedimentom. U pod-uzorku obrađenom vodikovim peroksidom udio foraminiferskih kućica pogodnih za identifikaciju iznosio je 47,26%, dok je kod pod-uzorka obrađenog reakcijom vodikova peroksida i natrijevog hidrogenkarbonata taj udio iznosio 53,28%. U pod-uzorku tretiranom metodom zamrzavanja i otapanja udio očuvanih kućica iznosio je 28,49%. Mali postotak cjelovitih kućica ovu metodu je eliminirao kao dobru za marlite. Usporedimo li vrijeme koje je trebalo uložiti za usitnjavanje pod-uzoraka i broj kućica foraminifera temeljem kojih će se raditi rekonstrukcija paleokoliša, preporučili bismo metodu otapanja samo vodikovim peroksidom. Ta metoda je vremenski kraća, a tek je neznatna razlika u zastupljenosti kućica planktonskih foraminifera.

### ZAHVALE

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## SORTING LABORATORY TECHNIQUES OF SAMPLE PREPARATION BY EFFICIENCY: AN EXAMPLE OF A MIDDLE EOCENE MARL (PODSTINE COVE, ISLAND OF HVAR)

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Marls are mixed carbonate-clay rocks. They are an important component of deep-water flysch deposits. Being rich in microfossils, planktonic foraminifera in particular, they can provide a great variety of data useful for palaeoecological reconstructions. Laboratory preparation of marls is a key step to get good data. In this study, we were looking for the most efficient laboratory techniques. Sample collected at Podstine cove (Hvar is.), was described as a calcite-rich marls. Calcimetry analysis reveals that marls contain 67.78% of calcite ( $\text{CaCO}_3$ ), placing it within the realm of marlites [1]. The sample was split into three sub-samples, each weighing 200 g. Two sub-samples were treated by standard procedures for marls [2]. One sub-sample was soaked in a mixture of 50 ml of 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) with 2 l of water for 24 hours, while the other one was soaked in a mixture of 150 ml of 30% hydrogen peroxide, 1 l of boiling water and 10 g of sodium hydrogen carbonate ( $\text{NaHCO}_3$ ), for the next 48 hours. The third sub-sample was repeatedly frozen and then rapidly thawed. This method took 48 hours to be completed. After being wet sieved through a set of meshes, the fraction  $>125 \mu\text{m}$  was dried and standardised, using microsplitter, into aliquots of about 300 foraminiferal tests. We identified pristine tests with undamaged wall structure suitable for morphological identification of species from those tests that were damaged or have diagenetically altered wall structure. In the sub-sample treated with hydrogen peroxide the proportion of pristine foraminiferal tests was 47.26%, while in the sub-sample treated by mixture of hydrogen peroxide and sodium hydrogen carbonate 53.28% of the tests were pristine. In the sub-sample treated by freezing and thawing the intact tests made 28.49% of all tests. The low proportion of intact tests obtained by this method makes it unsuitable for studying marlites. Comparing the time required to treat the sub-samples with the amount of foraminiferal tests suitable for further study, we recommend the method of using hydrogen peroxide only. This method is less time-consuming, while producing only a slightly lesser number of suitable tests.

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## BISIMULACIJE ZA GENERALIZIRANU VELTMANOVU SEMANTIKU

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Bisimulacije u logici možemo shvatiti kao opise (nedeterminističkih) pobjedničkih strategija za jednog igrača u odgovarajućim igrama za usporedbu modela. U slučaju bisimulacija za osnovnu modalnu logiku to je ilustrirano u [2]. U slučaju bisimulacija za logiku dokazivosti to se može primjerice vidjeti u [1]. Čačić i Vrgoč su u [1] iskoristili igre na Veltmanovim modelima kako bi pokazali da modalna ekvivalencija ne povlači bisimuliranost.

Prvo ću spomenuti korištenje bisimulacijskih igara u poznatim rezultatima za Veltmanovu semantiku. S obzirom da Veltmanova semantika nije dovoljna za određene aplikacije, pojavio se pojam generalizirane Veltmanove semantike. Time su dobiveni neki važni rezultati, a također su pokazana njihova dobra svojstva (primjerice u [3]). Zbog sve veće važnosti takve semantike, definirati ću bisimulacijske igre (te njihove konačne aproksimacije –  $n$ -bisimulacijske igre) za generaliziranu Veltmanovu semantiku. Također ću komentirati i dobiveni standardni rezultat – ekvivalenciju između postojanja pobjedničke strategije u bisimulacijskoj igri i postojanja bisimulacije u slučaju generalizirane Veltmanove semantike.

### ZAHVALE

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# BISIMULATIONS FOR GENERALISED VELTMAN SEMANTICS

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Bisimulation relations in logic may be understood as descriptions of (non-deterministic) winning strategies for one player in corresponding model comparison games. In case of bisimulations for basic modal logic that was illustrated in [2]. In case of bisimulations for provability logic that can be found e.g., in [1]. Čačić and Vrgoč in [1] used games on Veltman models to show that modal equivalence does not imply bisimilarity.

First, I will mention the use of bisimulation games in known results for Veltman semantics. Since Veltman semantics is not fine-grained enough for certain applications, the notion of generalised Veltman semantics emerged to obtain certain non-derivability results. It has turned out that this semantics has various good properties, as shown in [3]. Because of that, I will define bisimulation games (and their finite approximation - n-bisimulation games) for generalised Veltman semantics. I will also comment on the standard result - equivalence between the existence of a winning strategy in the bisimulation game and the existence of a bisimulation in case of generalised Veltman semantics.

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# Posterska izlaganja

## *Poster presentations*





## PROCJENA BIOMARKERA U KRVI PTIĆA BIJELE RODE S RAZLIČITO ZAGAĐENIH PODRUČJA U HRVATSKOJ

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Bijela roda (*Ciconia ciconia*) rasprostranjena je gnijezdeća vrsta u kontinentalnoj Hrvatskoj, vršni predator koji nastanjuje poljoprivredna polja, močvare i livade. Ptici bijele rode dobri su bioindikatori pružajući informacije o stanju okoliša. Zbog promjena u okolišu uzrokovanih antropogenim aktivnostima i intenzivne poljoprivrede, bijele rode napuštaju svoja gnijezdeća područja što ukazuje na pad prirodne vrijednosti. Tijekom sezone gniježđenja 2021., u sklopu projekta prstenovanja bijelih roda, uzorkovana je krv (4 mL) iz brahijalne vene ptića ( $n = 106$ ) za analizu biomarkera, počupkano je nekoliko perja za određivanje spola te je izmjerena duljina kljuna za određivanje redoslijeda izlijeganja. Područja istraživanja nalaze se u blizini tri velike rijeke u Hrvatskoj: Save, Drave i Dunava, a jedno područje uzorkovanja nalazi se u Zagrebu, odlagalište otpada Jakuševac. Analiziran je skup biokemijskih biomarkera: acetilkolinesteraza (AChE), karboksilesteraza (CES), glutation S-transferaza (GST) i glutation reduktaza (GR), te molekularni biomarkeri: glutation (GSH) i reaktivne kisikove jedinice (ROS) u dvije frakcije krvi – plazma i homogenat krvnih stanica (S9). Analizom specifičnih i nespecifičnih biomarkera u dvije frakcije krvi dobili smo potpuniji pregled fiziološkog stanja ptića. Niža aktivnost AChE bilježi se u Podunavlju, području poznatom po velikoj upotrebi gnojiva i intenzivnoj poljoprivredi, što ukazuje na prisutnost kemijskih stresora u okolišu. S druge strane, biomarkeri oksidativnog stresa pokazuju nedostatak statistički značajnih razlika u odnosu na područje uzorkovanja. Zbog složenog antioksidativnog odgovora, mogu se uključiti i drugi antioksidativni mehanizmi, stoga se ne može zaključiti o zagađivalima iz okoliša i/ili njihovom mogućem učinku na ptiće bijele rode.



## NO HARM DONE: BIOMARKER ASSESSMENT IN WHITE STORK NESTLINGS' BLOOD FROM DIFFERENTLY POLLUTED AREAS IN CROATIA

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The white stork (*Ciconia ciconia*) is an abundant breeding species in continental Croatia, a top predator inhabiting mostly agricultural fields, wetlands, and meadows. White stork nestlings are a good bioindicator species providing information regarding the local environment. Due to environmental transformation and intensive agriculture, white storks leave their breeding areas indicating a decline in natural value. During breeding season 2021, as a part of white stork ringing scheme, blood samples (4 mL) were collected from the nestling brachial vein ( $n = 106$ ) for enzyme analysis, few feathers were plucked for sex determination, and beak length was measured for determining hatching order. Study areas are in the proximity to three major rivers in Croatia: Sava, Drava and Danube, and one sampling area is located in Zagreb, landfill Jakuševac. A set of biochemical biomarkers: acetylcholinesterase (AChE), carboxylesterase (CES), glutathione S-transferase (GST), and glutathione reductase (GR), as well as molecular biomarkers: glutathione (GSH) and reactive oxygen species (ROS) were analysed in two blood fractions – plasma and blood cell homogenate (S9). By analysing a set of specific and non-specific biomarkers in two blood fractions we obtained a more complete overview of the nestlings' physiological condition. Lower AChE activity is recorded in Podunavlje, an area known for high fertiliser use and intensive agriculture, indicating the presence of chemical stressors in the environment. On the other hand, oxidative stress biomarkers show the lack of statistical difference in regard to sampling areas. Due to complex antioxidative responses, other antioxidative mechanisms may be included, thus no conclusion could be made about pollutants from the environment and/or their possible effect on the nestlings.



# MODULIRANJE EKSPRESIJE PROTEINA SUSTAVA UROKINAZNOG PLAZMINOGENSKOG AKTIVATORA I NJIHOV UTJECAJ NA OTPORNOST NA KEMOTERAPEUTIKE

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Urokinazni plazminogeni aktivator (urokinaza, uPA) visokospecifična je serinska proteaza koja se kao zimogen luči iz stanice, a ekstracelularnom aktivacijom cijepa plazminogen i omogućuje nastanak plazmina [1]. Plazmin je širokorasprostranjena proteaza koja sudjeluje u remodeliranju izvanstaničnog matriksa, procesima migracije i invazije, zarastanju rana, otapanju krvnih ugrušaka itd [2]. Urokinazna aktivnost je ovisna i o ekspresiji i sekreciji specifičnog urokinaznog inhibitora, PAI-1. uPA se može vezati za membranski receptor, uPAR, te se tako lokalizira urokinazna aktivnost. Ekspresija i aktivnost urokinaze strogo je regulirana brojnim signalnim putovima aktiviranim faktorima rasta, hormonima, kemoterapeuticima, oštećenjem DNA i dr. i ovisna o staničnom tipu [1]. Cilj projekta je bio istražiti ulogu elemenata sustava urokinaznog plazminogenog aktivatora u staničnoj migraciji i preživljenju nakon obrade kemoterapeuticima kod tumorskih staničnih linija u kulturi. Konstruirani su eukariotski ekspresijski vektori sa sekvencama za uPA, PAI-1 i uPAR i njima transfecirane stanične linije glioblastoma i humanog embrionalnog bubrega. Uzgojeni su klonovi povećane i smanjene urokinazne aktivnosti. Analizirana je proliferacija i migracija klonova, u odnosu na kontrolne linije, te njihova otpornost na adriamicin i N-metil-N'-nitro-N-nitrozoguanidin.

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## MODULATION OF UROKINASE PLASMINOGEN ACTIVATOR SYSTEM AND RESISTANCE TO CHEMOTHERAPY

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Urokinase plasminogen activator (urokinase, uPA) is a highly specific serine protease that is secreted from the cell as a zymogen, by extracellular activation cleaves plasminogen and enables the formation of plasmin [1]. Plasmin is a widespread protease involved in extracellular matrix remodeling, migration and invasion processes, wound healing, blood clot dissolution, etcetera [2]. Urokinase activity also depends on the expression and secretion of a specific urokinase inhibitor, PAI-1. uPA can bind to membrane receptor, uPAR, thus localizing urokinase activity. The expression and activity of urokinase is strictly regulated by numerous signaling pathways activated by growth factors, hormones, chemotherapeutics or DNA damage and is dependent on cell type [1]. The aim of the project was to enquire into the role of urokinase plasminogen activator system in cell migration and survival after chemotherapeutic treatment in tumor cell lines. Eukaryotic expression vectors with sequences for uPA, PAI-1 and uPAR were designed and transfected into glioblastoma and human embryonic kidney cell lines. Clones of increased and decreased urokinase activity were grown. The proliferation and migration of clones, relative to the control lines, and their resistance to adriamycin and N-methyl-N'-nitro-N-nitrosoguanidine were analyzed.

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## ULOGA ANDROGENA I RECEPTORA ANDROGENA U METASTAZIRANJU TUMORA GLAVE I VRATA

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Tumori glave i vrata imaju ishodište bilo gdje na sluznici gornjeg dišnog i probavnog trakta. Šesti su po incidenciji, s učestalošću od 5% kod muškaraca i 2% kod žena [1]. Najčešće se pojavljuju kod starijih od 50 godina, uzrok su značajnog morbiditeta i mortaliteta, a petogodišnje preživljenje iznosi oko 50%. Glavni uzročnici su konzumacija alkohola i pušenje te infekcije humanim papiloma virusom [2]. Liječenje je prvenstveno kirurško, uz adjuvantnu kemo i radioterapiju. Preko 40% slučajeva u trenutku dijagnoze već ima metastazu u regionalnom limfnom čvoru [3]. Obzirom da su ovi tumori češći kod muškaraca, želja nam je istražiti koja je uloga androgena i receptora androgena u metastaziranju tumora glave i vrata. Kao stanični model koristit ćemo trajne stanične linije FaDu (primarni karcinom) i Detroit 562 (metastaza), kupljene od tvrtke ATCC (TCP-1012™, Manassas, VA, SAD). Za aktivaciju receptora androgena koristit ćemo dihidrotosteron (DHT), a za depleciju androgena simvastatin. Migratornu sposobnost netretiranih i tretiranih stanica provjerit ćemo pomoću testa zacjeljivanja rana i testa stvaranja kolonija. U navedenim stanicama te uzorcima tkiva primarnih tumora, metastatskih limfnih čvorova i zdrave sluznice odredit ćemo ekspresiju gena za nuklearni (*AR*) i membranske (*CACNA1C*, *OXER1* i *SLC39A9*) receptore androgena pomoću metode qPCR. Dosadašnji rezultati su pokazali da tretman DHT-om povećava, a simvastatinom smanjuje migracijski potencijal stanične linije Detroit 562, dok se u stanicama FaDu primjećuje samo pozitivan utjecaj simvastatina. Također, u usporedbi sa zdravom sluznicom, ekspresija gena *AR* je snižena, gena *OXER1* i *SLC39A9* povišena, a gena *CACNA1C* jednaka u metastazama u odnosu na primarne tumore. Ovi preliminarni rezultati ukazuju na moguću važnost membranskih receptora androgena u metastaziranju tumora glave i vrata, kao i na terapijski potencijal statina za liječenje tog tipa tumora.

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## THE ROLE OF ANDROGEN AND ANDROGEN RECEPTORS IN METASTASIS OF HEAD AND NECK TUMORS

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Head and neck tumors originate anywhere on the mucosa of the upper respiratory and digestive tracts. They are sixth by incidence, with a frequency of 5% in men and 2% in women [1]. They most often occur in people over 50 years of age, cause significant morbidity and mortality, and five-year survival is about 50%. The main causes are alcohol consumption and smoking, and human papillomavirus infection [2]. Treatment is primarily surgical, with adjuvant chemo and radiotherapy. Over 40% of cases at the time of diagnosis already have a metastasis in the regional lymph node [3]. Since these tumors are more common in men, we want to investigate the role of androgen and androgen receptors in the metastasis of head and neck tumors. As a cell model, we will use the cell lines FaDu (primary cancer) and Detroit 562 (metastasis), purchased from the ATCC (TCP-1012™, Manassas, VA, USA). We will use dihydrotestosterone (DHT) to activate androgen receptors and simvastatin for androgen depletion. We will assess the migratory ability of untreated and treated cells using wound healing assay and colony forming assay. In these cells and tissue samples of primary tumors, metastatic lymph nodes and healthy mucosa, we will determine the expression of genes for nuclear (*AR*) and membrane (*CACNA1C*, *OXER1* and *SLC39A9*) androgen receptors using the qPCR method. Present results have shown that treatment with DHT increases, while simvastatin decreases migration of Detroit 562 cells, while in FaDu cells only inhibitory effect of simvastatin is observed. In addition, compared to a healthy mucosa, gene expression of *AR* is decreased, *OXER1* and *SLC39A9* increased, while *CACNA1C* is the same in metastases compared to primary tumors. These preliminary results indicate a potential role of androgen membrane receptors in metastasis of head and neck tumors, as well as therapeutic potential of statins for this type of tumors.

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## USPOSTAVA OVČJEG EKSPERIMENTALNOG MODELA KAO PREDUVJET ZA ISTRAŽIVANJE FARMAKOKINETIKE BIOTERAPEUTIKA

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Prilikom otrovanja venomi se u tijelo žrtve unose *s.c.* ili, rjeđe, *i.m.* putem nakon čega se oslobađaju u intersticijski prostor te apsorpcijskim procesom, putem krvnih i/ili limfnih kapilara, dospijevaju u krvotok [1]. Venomi zmija iz porodice *Viperidae* najvećim dijelom se apsorbiraju putem limfnog sustava koji služi kao izvor za njihovu kontinuiranu dostavu u sistemsku cirkulaciju [1]. Parenteralna primjena antivenoma je okosnica terapije otrovanja izazvanih zmijskim ugrizom [2]. Prevladava mišljenje da njihova primjena *i.m.* putem ne predstavlja optimalan izbor za uspješno liječenje otrovanja te se preporuča *i.v.* terapijski princip kao primjereniji za usklađivanje farmakokinetičkog profila antivenoma s onim ciljnog venoma, a koji je dugi niz godina bio praćen isključivo u sistemskoj cirkulaciji [2]. Međutim, nedavno je pokazano da bi neutralizacija venoma u limfnom sustavu također mogla biti važna za klinički ishod. Stoga se nametnula potreba za preispitivanjem prednosti i mana *i.v.*, odnosno *i.m.* primijenjenog antivenoma praćenjem njegovog utjecaja na razinu venoma u oba relevantna tjelesna sustava. Cilj istraživanja je bio na ukupno četiri ovce uspostaviti animalni model kako bi se razvijene procedure i kirurške tehnike za biološko uzorkovanje krvi i limfe, u idućoj fazi, mogle implementirati u studiju farmakokinetike na eksperimentalno otrovanim i liječenim životinjama. Kao netoksični, modelni protein je korišten ovalbumin potkožno apliciran u dozi koja odgovara količini venoma injektiranoj u tipičnom otrovanju. Krv je prikupljena iz jugularne vene, a limfa kontinuiranim dreniranjem iz prsnog limfovoda. Razvijen je ELISA test za kvantifikaciju ovalbumina, potvrđeno je da je aplicirana doza dostatna za pojavu mjerljivih koncentracija u sistemskoj i limfnoj cirkulaciji te je definiran optimalni protokol uzorkovanja za pouzdan izračun farmakokinetičkih parametara. Razdoblje od 14 dana bilo je dovoljno za uzorkovanje seruma. Za dvije životinje određeno je poluvrijeme eliminacije (22,9 h i 59,7 h), volumen distribucije (94,8 L i 144,7 L) i klirens (47,7 mL min<sup>-1</sup> i 28,5 mL min<sup>-1</sup>). Limfu, u kojoj je oporavljeno 11% primijenjene doze, treba uzorkovati najmanje 4 h kako bi se postigao plato apsorpcije.

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## ESTABLISHMENT OF SHEEP EXPERIMENTAL MODEL AS A POSTULATE FOR RESEARCH OF PHARMACOKINETICS OF BIOTHERAPEUTICS

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In envenomation distribution of *s.c.* or *i.m.* injected venom into the interstitial space occurs. An absorption process, by blood or lymphatic capillaries, is necessary before it reaches the bloodstream [1]. Viper venoms are mostly absorbed through the lymphatic system which serves as a depot for their delivery into the systemic circulation [1]. Antivenoms constitute the mainstay in the snakebite envenoming therapy [2]. There is a prevailing opinion that their application by *i.m.* route has poor efficacy and that they should be administered *i.v.* [2]. Venom/antivenom interplay for many years has been monitored in the bloodstream exclusively. Recently, it was concluded that neutralization in the lymphatic system might be of great importance for clinical outcome. Therefore, need to reconsider (dis)advantages of each therapeutic principle has emerged by monitoring the antivenom's effect on venom levels in both body compartments. We aimed to establish an animal model on four sheep in order to implement the developed procedures and surgical techniques for blood and lymph sampling in the pharmacokinetic study that will furtherly be performed on experimentally envenomed and antivenom-treated animals. Each received *s.c.* injected non-toxic protein, ovalbumin, that was administered in the dose corresponding to the amount of venom injected in a typical envenoming. Blood samples were collected from the jugular vein, and lymph by continuous drainage from *d. thoracicus*. An ELISA test for ovalbumin quantification was developed. The applied dose was confirmed to be sufficient for the occurrence of measurable ovalbumin concentrations. The period of 14 days was long enough for serum sampling. For two animals, the elimination half-life (22,9 h and 59,7 h), volume of distribution (94,8 L and 144,7 L) and clearance (47,7 mL min<sup>-1</sup> and 28,5 mL min<sup>-1</sup>) were determined. The lymph, in which 11% of the administered dose was recovered, should be sampled for at least 4 h in order to attain the absorption plateau.

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## POD STRESOM - MOLEKULARNI ODGOVORI VODENIH KUKACA I MAHOVINE NA IZLAGANJE VIŠESTRUKOM STRESU

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Slatkovodni ekosustavi svrstavaju se među najugroženije ekosustave na globalnoj razini zbog brojnih negativnih posljedica ljudskih djelatnosti, kao što su zagađenje i onečišćenje, pretjerano iskorištavanje vodnih resursa, unošenje invazivnih vrsta te klimatske promjene [1]. Stresni čimbenici u prirodi obično ne djeluju samostalno, nego u kombinaciji jedni s drugima, a njihov međuodnos može biti vrlo složen, s raznolikim posljedicama na žive organizme. Učinci višestrukih stresora mogu biti aditivni (učinak je jednak zbroju djelovanja dvaju ili više stresora) ili bez aditivnog djelovanja. U posljednjem slučaju razlikujemo pozitivno (sinergizam) i negativno (antagonizam) djelovanje jednog stresora na drugi [2]. Praćenje utjecaja stresa vrlo se često promatra na razini zajednice (npr. u vodenim staništima prilikom redovnog procesa monitoringa), ali najbrže i najpreciznije praćenje neželjenih posljedica postiže se analizom odgovora na molekularnoj razini. Stoga je ovo istraživanje usmjereno na praćenje promjena u metaboličkim odgovorima te u produkciji proteina u vodenih kukaca (Trichoptera, Insecta) i mahovine (Bryophyta) prilikom izlaganja realnim koncentracijama farmaceutika, mikroplastike te njihovoj kombinaciji. Analizom ukupne koncentracije proteina te strukture metabolita uočili smo da mahovina i vodeni kukci imaju različit mehanizam odgovora na stres u okolišu. Proteini, kao i metaboliti, u mahovina pokazuju sličan trend povećanog stresa u uzorcima izloženim farmaceuticima. S druge strane, vodeni kukci pokazuju približno jednaku razinu stresa u svim tretmanima, no ona postaje manje naglašena s vremenom, što može biti rezultat prilagodbe na stres.

### ZAHVALE

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## UNDER PRESSURE – MOLECULAR RESPONSES OF AQUATIC INSECTS AND MOSS TO MULTIPLE STRESSORS

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Freshwater ecosystems are among the most endangered ecosystems in the world. This is due to the numerous negative consequences of human activities, such as contamination and pollution, overexploitation of water bodies, introduction of invasive species, and climate change [1]. Stressors in nature usually do not act in isolation, but in combination with each other, and these relationships can be very complex, with different consequences for living organisms. Effects caused by multiple stressors can be additive (the effect is the sum of the effects of two or more stressors) or non-additive. In the latter case, we distinguish between positive (synergism) and negative (antagonism) effects of one stressor on another [2]. Monitoring the effects of stress is very often done at the community level (e.g., in aquatic habitats as part of regular monitoring), but the most rapid and accurate monitoring of negative effects is achieved by analyzing responses at the molecular level. Therefore, this research aims to monitor changes in metabolic responses and protein production of aquatic insects (Trichoptera, Insecta) and mosses (Bryophyta) when exposed to real concentrations of pharmaceuticals, microplastics, and their combination. By analyzing total protein concentration and metabolite structure, we found that mosses and aquatic insects have different mechanisms of response to environmental stress. In mosses, proteins and metabolites in samples exposed to pharmaceuticals show a similar pattern of increased stress. In aquatic insects, however, stress levels are roughly the same across treatments, but the response becomes less pronounced over time, possibly due to stress adaptation.

### ACKNOWLEDGMENTS

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## DUBOKO I PODRŽANO UČENJE U KONTROLI POPULACIJA KOMARACA

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Zbog klimatskih promjena, zabilježen je povećani rizik pojave bolesti koje prenose vektori. [1] Najvažniji vektori zaraznih bolesti su komarci. Stoga je brzo i efikasno praćenje dinamike populacija komaraca od iznimne važnosti. U ovom radu opisan je okvir primjene višeslojnog sustava za podršku u odlučivanju pri kontroli populacije komaraca. Slojevi okvira primjene kombiniraju znanje biologije, Internet of Things (IoT), velikih skupova podataka i moderne tehnike umjetne inteligencije, kao što su duboko i podržano učenje, te modeliranje populacija [2], za izradu sustava za podršku u odlučivanju sposobnog automatski analizirati dinamiku populacije komaraca i osmisliti strategiju za kontrolu populacija komaraca.

### ZAHVALE

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# DEEP AND REINFORCEMENT LEARNING IN MOSQUITO POPULATION CONTROL

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Because of climate change, an increase in the risk of vector-borne diseases has been reported [1]. Mosquitoes are dominant vectors of most of these diseases. Therefore, the rapid monitoring of mosquito population dynamics it is of the utmost importance. In this work, the framework of a multi-layered decision support system for the control of the mosquito population is described. Layers of the framework combine biological knowledge, internet of Things (IoT), big data, and state-of-the-art AI techniques, such as deep and reinforcement learning, and population modeling [2], to make a decision support system that will be able to automatically survey mosquito population dynamics and devise a strategy for population control.

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## UTJECAJ IZLAGANJA TIAMOTOKSAMOM NA HRANJENJE, KRETANJE I METABOLIZAM VRSTE *Abax parallelus* (COLEOPTERA: CARABIDAE)

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Trčci su rašireni grabežljivci koji služe u biološkom suzbijanju nametnika na biljkama u poljoprivredi i šumarstvu [1]. Istraživanja su pokazala da pesticid imaju subletalni utjecaj na njihovo ponašanje i fiziologiju, s potencijalno negativnim učinkom na grabežljivu učinkovitost [2]. Ovdje istražujemo utjecaj neonikotinoida tiametoksama na hranjenje, pokretanje i metabolizam grabežljive vrste *Abax parallelus*. Trčci su uronjeni u otopinu tiametoksana različitih koncentracija te ostavljeni da se hrane preko noći. Za svakog trčka izračunata je masa pojedene hrane kao postotak njegove tjelesne mase. Sposobnost pokretanja udova promatrana je 48 sati nakon tretmana. Metabolički profil i koncentracija superoksid-dismutaze (SOD) u tkivu izmjereni su za svaku jedinku. Grupe tretirane s višim koncentracijama tiametoksama hranile su se značajno manje i imali viši udio jedinki s vidljivim znakovima trovanja i uginulih jedinki. Koncentracije sukcinata i d-glukoze značajno su se razlikovale između tretiranih grupa i kontrolne grupe, što ukazuje na poremećaj energetske proizvodnje. Nasuprot tome, nije izmjerena značajna razlika u aktivnosti SOD-a u tkivu trčaka ovisno o tretmanu. Zaključujemo da kratkotrajno izlaganje tiametoksamu može izazvati negativne učinke na grabežljivu aktivnost, ponašanje, i energetske zalihe, ali učinci dugotrajnog izlaganja nižim koncentracijama trebaju se dodatno istražiti.

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## THE IMPACT OF THIAMETHOXAM EXPOSURE ON FEEDING, LOCOMOTOR ACTIVITY, AND METABOLOMICS OF *Abax parallelus* (COLEOPTERA: CARABIDAE)

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Carabids are widespread predators in ecosystems that serve as biocontrol agents in agroecosystems and forestry [1]. Pesticides have been proven in studies to have sub-lethal effects on their behavior and physiology, which can affect predation efficiency [2]. The impact of neonicotinoid thiamethoxam on feeding rate, locomotion, metabolomics, and oxidative stress in a predatory carabid, *Abax parallelus*, is investigated in this study. Beetles were dipped in increasing quantities of thiamethoxam and allowed to feed overnight. For each beetle, the mass of the ingested food was calculated as a percentage of its body weight. After the treatment, the locomotor capacity was monitored for 48 hours. The researchers looked at their metabolomics profile as well as the levels of superoxide dismutase (SOD) in their tissue. Individuals treated with higher concentrations had significantly lower feeding rates and a higher percentage of intoxicated and moribund individuals. Succinate and d-glucose had statistically significant differences in concentrations between treatment and control group, indicating a disruption in energy production. No statistically significant changes in SOD activity were detected between the groups. To summarize, short-term thiamethoxam exposure can cause unfavorable sub-lethal consequences in predatory activity, behavior, and energy budget, but long-term exposure to lower dosages need more research.

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## AKTIVNOST ERAVACIKLINA NA ENTEROBAKTERIJE OTPORNE NA KARBAPENEME U UVJETIMA *IN VITRO* – PRVI IZVJEŠTAJ IZ HRVATSKE

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Liječenje infekcija uzrokovanih enterobakterijama otpornima na karbapeneme (KRE) je ograničeno, stoga su novi antimikrobni agensi, izuzev beta-laktama, s aktivnošću koja ne ovisi o klasi beta-laktamaza, posebno važni. Među najproširenijim karbapenemazama nalazi se enzim OXA-48 iz skupine D koji hidrolizira oksacilin, te metalo-beta-laktamaze IMP, VIM i NDM iz skupine B. Eravaciklin (ERV) je prvi potpuno sintetski fluorociklin indiciran za liječenje kompliciranih intraabdominalnih infekcije u odraslih. Nakon identifikacije bakterijskih sojeva MALDI-TOF masenom spektrometrijom, odabrani geni detektirani su metodom lančane reakcije polimerazom (engl. polymerase chain reaction, PCR) i elektroforezom na agaroznom gelu. Osjetljivost osamdeset izolata KRE na ERV ispitivano je metodom disk difuzije i određivanjem minimalne inhibicijske koncentracije (MIK). Ukupno su 54 (54/80; 67,5%) izolata bila osjetljiva na ERV s vrijednostima MIK<sub>50</sub> ≤ 0.5 µg/mL i MIK<sub>90</sub> od 4 µg/mL. Osjetljivost izolata koji sintetiziraju enzim OXA-48 nije bila značajno veća u usporedbi s izolatima koji sintetiziraju NDM (P=0,539) i VIM (P=0,7805). ERV je zbog drugačijeg mehanizma djelovanja od onog u karbapenema moguća alternativa novim kombinacijama inhibitora beta-laktamaze za liječenje KRE infekcija temeljeno na ispitivanju osjetljivosti fenotipskim metodama, molekularnim određivanjem vrste karbapenemaze, težini infekcije i osobitostima svakog pacijenta.

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## ***IN VITRO* ACTIVITY OF ERAVACYCLINE ON CARBAPENEM-RESISTANT *ENTEROBACTERALES* – THE FIRST REPORT FROM CROATIA**

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Treatment of carbapenem-resistant *Enterobacterales* (CRE) infections is limited, so the novel antimicrobial agents other than beta-lactams with activity not being dependent on beta-lactamase class are especially important. Among the most widespread carbapenemases are the enzyme OXA-48 from group D which hydrolyzes oxacillin and IMP, VIM and NDM-type metallo-beta-lactamases from group B. Eravacycline (ERV) is the first fully synthetic fluorocycline indicated for the treatment of complicated intra-abdominal infections in adults. After identification of bacterial strains by MALDI-TOF mass spectrometry, genes of interest were detected by polymerase chain reaction (PCR) and agarose gel electrophoresis. Eighty CRE isolates were examined for susceptibility to ERV by disc diffusion method and minimal inhibitory concentration (MIC). Total of 54 (54/80; 67.5%) isolates were susceptible to ERV with MIC<sub>50</sub> of ≤ 0.5 µg/mL and MIC<sub>90</sub> of 4 µg/mL. Susceptibility of OXA-48 enzyme-synthesizing isolates was not significantly higher in comparison with NDM (P=0.539) and VIM (P=0.7805) enzyme-synthesizing isolates. Due to a different mechanism of action than that of carbapenems, ERV is a possible alternative to novel beta-lactamase inhibitor combinations for treatment of CRE infections based on susceptibility testing by phenotypic methods, molecular determination of carbapenemase type, severity of infection and characteristics of each patient.

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## STRANI I AUTOHTONI PERAKARIDNI RAKOVI U ZAJEDNICI MAKROSKOPSKIH BESKRALJEŠNJAKA U VELIKIM RIJEKAMA HRVATSKE

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Rakovi iz nadreda Peracarida (redovi: Amphipoda, Isopoda i Mysida) predstavljaju jednu od najvažnijih skupina invazivnih beskralješnjaka u europskim slatkovodnim ekosustavima jer su značajno doprinijeli homogenizaciji zajednice makroskopskih beskralješnjaka dna [1]. Unatoč tome, njihovo širenje i utjecaji su još uvijek nedovoljno istraženi u Hrvatskoj. Ciljevi ovog istraživanja su utvrditi rasprostranjenost i gustoću autohtonih i stranih Peracarida u velikim rijekama Hrvatske te njihov udio u ukupnoj gustoći makroskopskih beskralješnjaka. Sakupljeni su kvantitativni uzorci ( $20 \times 0.0625 \text{ m}^2$ ) na 48 lokacija na četiri velike rijeke (Dunav-4 postaje, Sava-21, Drava-20, Mura-3) u dva navrata (2015 i 2016/2017) prema AQEM protokolu. Ukupno je pronađeno 16 vrsta, 5 autohtonih (Amphipoda-4, Isopoda-1) i 11 stranih vrsta (Amphipoda-8, Isopoda-1, Mysida-2). U Dunavu su pronađene samo strane vrste (10 spp.), dok su u Muri i najuzvodnijim postajama na rijekama Savi i Dravi pronađene samo autohtone vrste. U Savi (5 vrsta) i Dravi (6 spp.) je pronađen podskup stranih vrsta pronađenih u Dunavu. Najuzvodnija vrsta u rijeci Dravi je *D. villosus* (270 rkm), a u rijeci Savi *D. haemobaphes* (631 rkm). Najrasprostranjenija vrsta u ovom istraživanju je *Jaera istri* (29 postaja) i rakušci *Dikerogammarus villosus* (24) i *Chelicorophium curvispinum* (22). Analizom nemetričkog multidimenzionalnog skaliranja (NMDS) zajednice Peracarida na 47 postaja (postaja DR7 je isključena zbog izostanka Peracarida) pokazuje jasno odvajanje postaja sa samo autohtonim vrstama od onih na kojima su prisutne strane vrste. Na postajama na kojima su prisutne strane vrste postoji statistički značajna razlika između rijeka (PERMANOVA, pseudo-F = 23,83 df = 2,  $p = 0,001$ ). Karakteristične vrste za rijeke Dravu i Dunav su *D. villosus* i *C. curvispinum*, a za rijeku Savu su *D. haemobaphes* i *C. sowinskyi*. Udio stranih Peracarida u ukupnoj gustoći makroskopskih beskralješnjaka ima najveću srednju vrijednost i najveće maksimume u rijeci Dravi (24,4%, 90,1%), a najmanju srednju vrijednost u rijeci Savi (17,7%). Rakušci rodova *Chelicorophium* i *Dikerogammarus* imali su najveće gustoće i udjele među stranim svojatama. Mjere koje bi usporile daljnje uzvodno širenje invazivnih perakaridnih rakova, poput edukacije ribiča i voditelja plovila, potrebno je hitno uvesti kako bi se zaštitila bioraznolikost autohtonih beskralješnjaka u najuzvodnijim dijelovima velikih hrvatskih rijeka.

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## ALIEN AND NATIVE PERACARIDA IN MACROINVERTEBRATE ASSEMBLAGES OF CROATIAN MAJOR RIVERS

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Peracarid crustaceans (orders Amphipoda, Isopoda and Mysida) represent one of the most important group of invaders in European freshwaters that largely contribute to the homogenization of macroinvertebrate fauna [1], but their spread and impacts are still understudied in Croatia. The aims of this study were to investigate distribution, density and assemblages of native and alien Peracarida in Croatian large rivers and their proportion in total density of benthic macroinvertebrates. Quantitative samples (20×0.0625 m<sup>2</sup>) were collected at 48 locations on four major large rivers (Danube-4 sites, Sava-21, Drava-20, Mura-3) on two occasions (2015 and 2016/2017) following the AQEM sampling protocol. In a total, 16 species were recorded, five native (Amphipoda-4, Isopoda-1) and 11 alien species (Amphipoda-8, Isopoda-1, Mysida-2). In the Danube River only alien species were found (10 spp.), while in the Mura River and at the most upstream sites of Sava and Drava Rivers only native species were found. The Sava (5 species) and Drava (6 spp.) Rivers had subset of alien species found in the Danube River. The most upstream species were *D. villosus* in the Drava (270 rkm) and *D. haemobaphes* in the Sava (631 rkm). The most widespread species found in this study were isopod *Jaera istri* (29 sites) and amphipods *Dikerogammarus villosus* (24) and *Chelicorophium curvispinum* (22). Non-parametric multidimensional scaling (NMDS) analysis of peracarid assemblages at 47 sites (site DR7 excluded due to absence of peracarids) showed a clear separation of sites with only native species from those inhabited by alien species. For group of sites with alien species there was significant difference between rivers (PERMANOVA, pseudo-F = 23.83 df = 2, *p* = 0.001). Characteristic species for the Drava and Danube were *D. villosus* and *C. curvispinum* and for the Sava *D. haemobaphes* and *C. sowinskyi*. Proportions of alien Peracarida in total density of benthic macroinvertebrate had the highest average and maximum values in the Drava River (24.4%, 90.1%) and lowest average in the Sava River (17.7%). The two amphipod genera, *Chelicorophium* and *Dikerogammarus* had the highest densities and proportions among the alien taxa. Measures that would slow down the upstream spread of invasive peracarids, such as education of anglers and boat drives, are urgently needed to protect the remaining native macroinvertebrate biodiversity in the uninvaded upstream segments of Croatian large rivers.

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# UTJECAJ ČVRSTIH LIPIDNIH NANOČESTICA S UGRAĐENIM ASKORBIL PALMITATOM NA MATIČNE STANICE SARKOMA

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Iskorjenjivanje tumorskih matičnih stanica (engl. *cancer stem cells*, CSC) jer vrlo važno jer su one odgovorne za otpornost na konvencionalnu kemoterapiju, formiranje metastaza i povratak tumora nakon liječenja. Potvrđeno je kako askorbinska kiselina (engl. *ascorbic acid*, AA) ima citotoksični učinak na CSC, ali to još nije istraženo u sarkoma. Zbog nestabilnosti AA, često se koriste njezini derivati. Askorbil palmitat (AP) je stabilniji lipidni derivat AA, međutim, kako bi se mogao primjenjivati u vodenim okruženjima, mora biti pakiran u nosač.

Zato smo sintetizirali čvrste lipidne nanočestice s ugrađenim askorbil palmitatom (SLN-AP). Karakterizirali smo ih mjerenjem veličine, polidisperzitetu, zeta potencijala i evaluirali njihovu morfologiju TEM-om. Kako bismo ispitali stanični unos SLN-AP inkubirali smo stanične linije HEK 293, U2OS i CSC dobivene izolacijom iz biopsije pacijenta sa SLN-coumarin-6 i vizualizirali unos na fluorescencijskom mikroskopu. Citotoksični učinak SLN-AP i AA na HEK 293, U2OS i CSC smo ispitali MTT testom.

SLN-AP imaju promjer 414 nm, zeta potencijal 0 mV te polidisperzitet od 0,228. Vizualizacija na TEM-u je potvrdila njihovu okruglu morfologiju i promjer. Fluorescentnom mikroskopijom je pokazano kako SLN postupno ulaze u stanice. Također, vizualizacijom CSC je uočena jaka aktivnost egzosoma odnosno vezikula što sugerira potencijalno izbacivanje SLN. Ispitivanje citotoksičnosti je pokazalo kako su CSC osjetljivije na tretman SLN-AP u odnosu na tretman AA u slobodnom obliku.

Zaključno, uspješno smo sintetizirali i karakterizirali čvrste lipidne nanočestice s ugrađenim askorbil palmitatom. Potvrđen je ulazak SLN-AP u stanice, a ispitivanje citotoksičnosti je pokazalo njihov potencijal za ciljanje CSC sarkoma.

## ZAHVALE

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# THE EFFECT OF ASCORBYL PALMITATE-INCORPORATED SOLID LIPID NANOPARTICLES ON SARCOMA STEM CELLS

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Eradicating cancer stem cells (CSC) is of great importance since they are responsible for resistance to conventional chemotherapy, metastasis formation and cancer relapse. Ascorbic acid (AA) has proven to have a cytotoxic effect on CSC, however, it has not yet been investigated in sarcomas. Because of the instability of AA, its derivatives are often used. Ascorbyl palmitate (AP) is a more stable lipophilic derivative, however, for application in aqueous environments, it has to be applied in a lipid carrier. Therefore, we have synthesized solid lipid nanoparticles with incorporated ascorbyl palmitate (SLN-AP) [1]. We have characterized them by measuring their size, polydispersity, zeta potential and evaluating their morphology by visualization on TEM. To test cellular uptake of SLN-AP, we have incubated HEK 293, U2OS and CSC derived from the patients' sarcoma sample with SLN-coumarin-6 and visualized the uptake on a fluorescent microscope during 48h. Cytotoxic effects of SLN-AP and AA on HEK 293, U2OS and CSC was evaluated by MTT test.

SLN-AP have a diameter of 414 nm, zeta potential of 0 mV and polydispersity of 0,228. TEM revealed they have a round morphology and confirmed the size of nanoparticles. Fluorescent microscopy showed SLN are gradually uptaken by the cells. However, visualization of CSC revealed they have high exosome/vesicle activity indicating potential efflux of SLN. Cytotoxicity assays revealed that CSC derived from patients' biopsies are more sensitive to treatment with SLN-AP than AA in free form.

To conclude, we have successfully synthesized and characterized ascorbyl palmitate-incorporated solid lipid nanoparticles. Their cellular uptake was confirmed. Also, cytotoxicity assays show their potential in targeting sarcoma CSC.

## ACKNOWLEDGMENTS

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## ANTIOKSIDACIJSKI ODGOVOR MAHOVINA NA SUŠNI STRES DUŽ VISINSKOG GRADIJENTA

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Planine su jedan od najosjetljivijih ekosustava kojeg klimatske promjene pogađaju brže nego ostala kopnena staništa. Zbog specifične građe i osjetljivosti na sušu, neke vrste mahovina prikladne su za praćenje učinka klimatskih promjena na ekosustave. Cilj ovog istraživanja je procijeniti sposobnost prilagodbe na sušu u mahovina vrste *Brachythecium rutabulum* i *Hylocomium splendens*. U tu svrhu biti će istražene sezonske promjene u fiziološkom i antioksidacijskom odgovoru u mahovina raslim duž visinskog gradijenta u NP Risnjak (5 lokacija: Leska, Izvor Kupe, Južna strana NP Risnjak - Horvatova staza, Vrh Risnjaka (Snježnik) i Sjeverna strana NP Risnjak – Lazac), te utvrditi postoji li korelacija između aktivnosti antioksidacijskih enzima i tolerancije na sušu. Rezultati istraživanja mogu pridonijeti boljem razumijevanju mehanizama prilagodbe ovih vrsta mahovina na sušni stres te pomoći razvoju sustava za praćenje učinka klimatskih promjena na planinske ekosustave primjenom mahovina kao bioindikatora. Prelimiarni rezultati pokazuju, tijekom sušnog razdoblja (lipanj-kolovoz 2016) uočeno je značajno smanjenje sadržaja vode u tkivu uzoraka obje vrste na svim lokacijama, osim na lokaciji 2 (Izvor Kupe). Uočene su razlike u fiziološkom odgovoru između ispitanih vrsta mahovina. Tako npr. u kolovozu na lokaciji 4 (Vrh Risnjaka, Snježnik) u vrste *Brachythecium* izmjereno je smanjenje sadržaja ukupnih klorofila, a u vrste *Hylocomium* lagani porast. Sušno razdoblje (lipanj-kolovoz 2016) uzrokovalo je značajan pad u sadržaju MDA (uM/ g s.t.) na lokaciji 4 (Vrh Risnjaka, Snježnik) u obje vrste mahovina. Kao dobar biokemijski pokazatelj stresa pokazala se aktivnost POX, CAT i GR. Od dvije ispitane vrste, osjetljivijom se pokazala vrsta *Hylocomium*. Zbog većih razlika u vrijednostima za ispitane parametre, a i zbog veće biomase ova vrsta mogla bi biti pogodna za buduće dugoročno praćenje (monitoring) učinka klimatskih promjena na brdsko-planinsku vegetaciju.

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## ANTIOXIDATIVE RESPONSES IN MOSSES TO DROUGHT STRESS ALONG AN ALTITUDINAL GRADIENT

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Mountains are among the most sensitive ecosystems to climate change and are being affected at a faster rate than other land habitats. Due to specific architecture and sensitivity to water loss, some mosses are suitable for monitoring the impact of climate change on ecosystems. The goal of this study is to assess drought tolerance in moss species *Brachythecium rutabulum* and *Hylocomium splendens*. Therefore, I will investigate seasonal variations in the physiological and antioxidative responses in mosses obtained from an altitudinal gradient in NP Risnjak (5 locations: Leska, Spring of the Kupa, South side of NP Risnjak – Horvat path, Peak of Risnjak (Snježnik) and North side of NP Risnjak - Lazac), and to determine whether there is correlation between antioxidant enzyme activities and drought tolerance. Results can contribute to a better understanding of mechanisms of drought stress responses in examined mosses, and help the development of a system for monitoring the effects of climate change on mountain ecosystems using moss as a bioindicator. Preliminary results show that during the dry season (June-August 2016) a significant decrease in water content in the tissue of samples of both species was observed at all locations except location 2 (Spring of Kupa). Differences in the physiological response between the examined moss species were observed. Thus, for example, in August at location 4 (Peak of Risnjak, Snježnik) in the species *Brachythecium* a decrease in the content of total chlorophyll was measured, and in the species *Hylocomium* a slight increase. The dry season (June-August 2016) caused a significant decrease in the content of MDA (µM / g s.t.) at location 4 (Peak of Risnjak, Snježnik) in both types of moss. The activity of POX, CAT and GR proved to be a good biochemical indicator of stress. Of the two species tested, *Hylocomium* proved to be the most sensitive. Due to larger differences in values for the examined parameters, and also due to higher biomass, this species could be suitable for future long-term monitoring of the effect of climate change on mountain vegetation.

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## ANTIOKSIDACIJSKI ODGOVOR U SA-DEFICIJENTNIM BILJKAMA I BILJAKA DIVLJEG TIPA KRUMPIRA (*Solanum tuberosum* L.) NA INFEKCIJU VIROIDOM VRETNASTOG GOMOLJA KRUMPIRA, PSTVd

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Viroidi su jednolančane, kružne, nekodirajuće RNA molekule kojima nedostaje protein omotača ili drugi protein, te su kao takvi najmanji poznati biljni patogeni. Najizrazitiji simptomi infekcije viroidom vretenastog gomolja krumpira (engl. *potato spindle tuber viroid*, PSTVd) su smanjenje rasta i deformacija gomolja krumpira, dok su simptomi na listovima i stabljikama slabi i pojavljuju se u kasnoj fazi infekcije, što može dovesti do kasne dijagnoze i velikih gubitaka prinosa. Patogenost nekodirajućih viroidnih RNA uglavnom je posljedica kombiniranih učinaka mehanizma za utišavanje RNA i drugih komponenti imunog odgovora biljke domaćina, uključujući hormonsku signalizaciju i antioksidativne odgovore. Salicilna kiselina (SA) je fitohormon važan za indukciju obrambenih odgovora dvosupnica protiv širokog spektra patogena. Kako bismo istražili moguću biološku važnost endogene SA u interakciji krumpir-PSTVd, koristili smo biljke krumpira koje ekspimiraju NahG transgen koji sprječava nakupljanje endogenog SA, i kontrolne biljke divljeg tipa. Prvo smo kvantificirali viroidnu RNA tijekom infekcije u lišću biljaka krumpira koristeći real-time PCR u jednom koraku. Transgene biljke s nedostatkom SA pokazale su povećanu osjetljivost na PSTVd u usporedbi s biljkama divljeg tipa, što je dokazano ranim ispoljavanjem simptoma i povećanom akumulacijom viroidne RNA 4 do 5 tjedana nakon inokulacije (wpi). Simptomi na NahG biljkama zaraženim PSTVd uključivali su male i vretenaste mlade listove, te klorozu i propadanje starih listova, što sugerira da infekcija izaziva oksidativni stres u sistemski zaraženim listovima transgenih biljaka. Drugo, spektrofotometrijskom metodom odredili smo koncentraciju vodikovog peroksida ( $H_2O_2$ ) u svježe sakupljenim listovima PSTVd- i mock-inokuliranih biljaka. Histokemijske promjene u  $H_2O_2$  analizirane su metodom bojenja 3,3'-diaminobenzidinom (DAB). Rezultati su pokazali povećanu akumulaciju  $H_2O_2$  u sistemski zaraženim listovima NahG biljaka 6 i 8 wpi u usporedbi s listovima zaraženih biljaka divljeg tipa. Treće, analiza ekspresije gena koji kodiraju antioksidativne enzime kao što su peroksidaza, katalaza i askorbat peroksidaza provedena je metodom PCR u stvarnom vremenu. Ekspresijska analiza pokazala je povećanu transkripciju peroksidaze u sistemskim listovima zaraženih NahG biljaka, ali ne i biljaka divljeg tipa, 6 wpi. U ovoj studiji pokazali smo da inaktivacija SA signalnog puta povećava virulentnost PSTVd u osjetljivim biljkama krumpira. Rezultati sugeriraju da je SA važna komponenta bazalne obrane biljaka krumpira od PSTVd.

### ZAHVALE

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## ANTIOXIDATIVE RESPONSE OF SA-DEFICIENT AND WILD TYPE POTATO PLANTS (*Solanum tuberosum* L.) TO INFECTION WITH POTATO SPINDLE TUBER VIROID

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Viroids are single-stranded, circular, noncoding RNA molecules that lack an envelope protein or other protein, and as such they are the smallest known plant pathogens. The most prominent symptoms of Potato spindle tuber viroid (PSTVd) infection are growth reduction and deformation of potato tubers, whereas symptoms on leaves and stems are weak and appear at a late stage of infection, which can lead to delayed diagnosis and severe yield losses. The pathogenicity of noncoding viroid RNAs is mainly due to the combined effects of the RNA-silencing machinery and other components of host plant immunity, including hormone signaling and antioxidative responses. Salicylic acid (SA) is a phytohormone important for the induction of defense responses of dicotyledons against a wide range of pathogens. To investigate the possible biological relevance of endogenous SA in the potato-PSTVd interaction, we used potato plants expressing the NahG transgene, which prevents the accumulation of endogenous SA, and wild-type control plants. First, we quantified viroid RNAs during infection in the leaves of potato plants using one-step real-time PCR. Transgenic SA-deficient plants showed increased susceptibility to PSTVd compared with wild-type plants, as evidenced by both early symptom expression and increased viroid RNA accumulation 4 to 5 weeks after inoculation (wpi). Symptoms on PSTVd-infected NahG plants included small and spindle-shaped young leaves, and chlorosis and decay of old leaves, suggesting that the infection induces oxidative stress in systemically infected leaves of transgenic plants. Second, we determined hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentration in freshly collected leaves of PSTVd- and mock-inoculated plants using a spectrophotometric method. Histochemical changes of H<sub>2</sub>O<sub>2</sub> were detected by 3,3'-diaminobenzidine (DAB) staining method. The results showed increased H<sub>2</sub>O<sub>2</sub> accumulation in systemically infected leaves of PSTVd-infected NahG plants at 6 and 8 wpi compared with the leaves of PSTVd-infected wild-type plants. Third, expression analysis of genes encoding antioxidant enzymes such as peroxidase, catalase and ascorbate peroxidase was examined by real-time PCR. Expression analysis showed increased transcription of a peroxidase in systemic leaves of infected NahG plants, but not wild-type plants, at 6 wpi. We show here that inactivation of the SA signaling pathway increases the virulence of PSTVd in susceptible potato plants. Taken together, these results suggest that SA is an important component of the basal defense of potato plants against PSTVd.

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## BIORAZGRADNJA MIKROPLASTIKE PRIMJENOM *Bacillus licheniformis*

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Mikroplastika predstavlja globalno onečišćenje te izaziva zabrinutost znanstvenika s obzirom da je pronađena u svim sastavnicama okoliša. Mikroplastikom se smatraju čestice veličinom manje od 5 mm. Ove čestice dugo zadržavaju polimerni oblik, te se dugo i zadržavaju u okolišu pa je potrebno ispitivati njihov utjecaj na vodene organizme. Iako se mikroplastika smatra teže (bio)razgradiva, potrebno je istraživati postupke uklanjanja čestica mikroplastike iz okoliša. U navedene svrhe se istražuju fizikalni, kemijski, ali i biološki procesi [1]. Bioremedijacija je učinkovit, ekonomičan i jednostavno izvediv postupak koji obuhvaća primjenu mikroorganizama. U ovome je radu ispitivana biorazgradnja čestica polistirena i polivinil klorida primjenom Gram pozitivne bakterije *Bacillus licheniformis*. Bakterija je izolirana iz uzorka sedimenta obogaćenog mikroplastikom. Tijekom 30 dana provedbe pokusa ispitano je 7 čimbenika na dvije razine prema Taguchi eksperimentalnom planu. Čimbenici koji su se ispitivali su obuhvaćali pH-vrijednost, temperaturu, veličinu čestica mikroplastika, koncentraciju mikroplastika, broj okretaja rotacijske tresilice, optičku gustoću bakterijske suspenzije te dodatak glukoze pri minimalnoj i maksimalnoj vrijednosti. Tijekom istraživanja praćen je ukupan broj živih stanica bakterije (CFU) *Bacillus licheniformis*, a za vodenu fazu je određivana masena koncentracija ukupnog (TC), organskog (TOC) i anorganskog (TIC) ugljika. Nakon provedenog pokusa, čestice mikroplastika su analizirane FTIR-ATR spektroskopijom. U svrhu određivanja značajnih čimbenika za biorazgradnju polistirena i polivinil klorida, kao odziv je korišten CFU. Rezultati su pokazali da bakterija *Bacillus licheniformis* ima sposobnost razgradnje ispitivanih čestica mikroplastika.

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## BIODEGRADATION OF MICROPLASTICS BY *Bacillus licheniformis*

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Microplastics represent global pollution and are a cause of concern to scientists as it is found in all components of the environment. Particles less than 5 mm in size are considered microplastics. These particles retain their polymer form and remain in the environment for a long time, so it is necessary to examine their impact on aquatic organisms. Although microplastics are considered more (bio) degradable, it is necessary to investigate the procedures for removing microplastic particles from the environment. For these purposes, physical, chemical and biological processes are investigated [1]. Bioremediation is an efficient, economical and easily feasible procedure that involves the use of microorganisms. In this work, the biodegradation of polystyrene and poly(vinyl) chloride particles was investigated using Gram-positive bacterium *Bacillus licheniformis*. The bacterium was isolated from a sediment sample enriched with microplastics. During the 30 days of the experiment, 7 factors were examined at two levels according to the Taguchi experimental plan. Factors that were examined included pH-value, temperature, microplastic particles size, microplastics concentration, agitation speed, optical density of the bacterial suspension, and glucose addition at minimum and maximum values. During the research, the total number of living cells (CFU) of *Bacillus licheniformis* was monitored, and the mass concentrations of total (TC), organic (TOC) and inorganic (TIC) carbon were determined for the aqueous phase. After the experiment, the microplastic particles were analyzed by FTIR-ATR spectroscopy. In order to determine significant factors for the biodegradation of polystyrene and poly(vinyl) chloride, CFU was used as a response. The results showed that the bacterium *Bacillus licheniformis* has the ability to degrade the tested microplastic particles.

### ACKNOWLEDGMENTS

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## PREVENCIJA I LIJEČENJE VASKULARNE DEMENCIJE: *IN VITRO* ISTRAŽIVANJE POTENCIJALNOG NEUROZAŠTITNOG UČINKA DEHIDROEPIANDROSTERONA (DHEA) I NJEGOVA SULFATA (DHEAS)

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Ishemijska ozljeda mozga i cerebrovaskularna bolest su najčešći uzroci kognitivnog pada i demencije kod starijih osoba [1]. Vaskularna demencija (VaD) drugi je najčešći uzrok demencije kod starije populacije nakon Alzheimerove bolesti. VaD je posljedica oslabljenog protoka krvi u različite regije mozga, što dovodi do smanjenog dotoka kisika i hranjivih tvari u te regije, uzrokujući propadanje neurona i staničnu smrt [2]. Neurosteroidi, dehidroepiandrosteron (DHEA) i njegov sulfat (DHEAS) su najzastupljeniji steroidni hormoni u mozgu te se sintetiziraju u mozgu *de novo* [3]. Uključeni su u modulaciji neurogeneze, metabolizam neurona i neurozaštitu [4]. Dosadašnja istraživanja pokazala su da ovi neurosteroidi ublažavaju posljedice ekscitotoksičnosti i oštećenja prouzrokovanih oksidativnim stresom te imaju potencijalno zaštitno djelovanje u slučaju ishemijske ozljede mozga [5]. Cilj ovog istraživanja bio je ispitati potencijalni zaštitni učinak DHEA i DHEAS primjenom modela deprivacije kisika i glukoze te reperfuzije (OGD/R) koji oponaša ishemijske uvjete i s tim povezana patološka stanja. U svrhu dobivanja *in vitro* modela vaskularne demencije, na SHSHY-5Y humanim stanicama neuroblastoma te primarnoj kulturi neurona izoliranih iz C57BL/6 miševa izazvana je ozljeda deprivacijom glukoze i kisika. Obje stanične kulture tretirane su lijekovima DHEA i DHEAS 24 sata prije ozljede (predtretman) ili 24 sata nakon ozljede (posttretman), nakon čega je ispitana vijabilnost stanica i razina oksidativnog stresa. Dobiveni rezultati istraživanja pokazuju pozitivan učinak tretmana DHEA i DHEAS na stanično preživljavanje i vijabilnost, što upućuje na to da ispitivani neurosteroidi imaju potencijalni neurozaštitni učinak u liječenju VaD.

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## PREVENTION AND TREATMENT OF VASCULAR DEMENTIA: EXPLORING THE NEUROPROTECTIVE POTENTIAL OF DEHYDROEPIANDSTERONE (DHEA) AND DHEA SULPHATE (DHEAS) *IN VITRO*

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Ischemic brain injury and cerebrovascular disease are common causes of cognitive decline and dementia in the elderly [1]. Vascular dementia (VaD) is the second most common cause of dementia in the elderly population after Alzheimer's disease. VaD is caused by reduced blood flow to the various brain regions, depriving them of oxygen and nutrients and causing neurodegeneration and cell death [2]. Neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are the most abundant steroid hormones and can be synthesized *de novo* in the brain [3]. They have been shown to be potent modulators of neurogenesis, neuronal metabolism and neuroprotection [4]. Previous research has shown that these neurosteroids alleviate the effects of excitotoxicity and damage caused by oxidative stress and have potentially protective effects in the case of ischemic brain injury [5]. The aim of this study was to investigate the potential neuroprotective effects of DHEA and DHEAS using an oxygen and glucose deprivation and reperfusion (OGD/R) model that mimics ischemic injury and related pathological conditions. Oxygen-glucose deprivation (OGD) was performed in primary mouse neurons derived from C57BL/6 mice, and human SH-SY5Y neuroblastoma cells, as an *in vitro* model of VaD. Both cell cultures were treated with DHEA and DHEAS 24 hours before injury (pretreatment) or 24 hours after injury (posttreatment) and cell viability and oxidative stress parameters were determined. The obtained results demonstrated beneficial effects of DHEA and DHEAS treatment on cell survival and viability, suggesting potential neuroprotective actions of these neurosteroids in VaD.

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## KLASIČNA I ZELENA EKSTRAKCIJA HLAPIVIH SPOJEVA: USPOREDBA PODATAKA ZA PET VRSTA ČESTOSLAVICA IZ HRVATSKE

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Rod *Veronica* L. (čestoslavice) iz obitelji Plantaginaceae obuhvaća oko 500 vrsta koje su nešto više rasprostranjene po sjevernoj hemisferi [1]. Najviše predstavnika roda *Veronica* raste u područjima s mediteranskom klimom, od razine mora do visokog alpskog područja [2]. Ovo istraživanje opisuje fitokemijsku karakterizaciju hlapivih komponenti izoliranih klasičnom hidrodestilacijom (HD) i zelenom mikrovalnom ekstrakcijom (ME) iz pet vrsta *Veronica* rasprostranjenih u Hrvatskoj, sa staništa s umjerenom količinom raspoložive vode. Proučavane su vrste: *V. acinifolia*, *V. chamaedrys*, *V. serpyllifolia*, *V. urtifolia* i *V. polita*. Cilj ovog rada je dobiti nove podatke za nedovoljno proučene hlapive tvari te steći uvid u razlike i sličnosti u sadržaju hlapivih spojeva među vrstama dobivenih različitim metodama izolacije. E-kariofilen, kariofilen oksid, heksahidrofarnezil aceton, fitol,  $\beta$ -ionon, heksadekanska kiselina i dokosan identificirani su u svih pet izolata dobivenih HD. *V. acinifolia* je bogata  $\beta$ -iononom (17,01%), zatim spojevima heksahidrofarnezil acetonom (15,37%) i kariofilen oksidom (7,71%). Vrste *V. chamaedrys*, *V. polita*, *V. serpyllifolia* i *V. urticifolia* bogate su fitolom, posebno *V. urtifolia* koja sadrži 47,55% navedene tvari. Iste glavne komponente identificirane u izolatima pomoću HD također dominiraju u sastavu hlapivih komponenti izolata dobivenih ME. Rezultati ovog istraživanja pokazuju da su heksahidrofarnezil aceton, heksadekanska kiselina, fitol, E-kariofilen i kariofilen oksid komponente identificirane u obje metode ekstrakcije. Kako su ovi spojevi izolirani u svim vrstama, mogli bi se smatrati kemofenetskim markerima za rod *Veronica*. Buduća istraživanja koja uspoređuju skupine temeljene na hlapljivim spojevima i skupine proizašle iz genetičkih istraživanja mogla bi razriješiti ovu hipotezu. Gledajući rezultate za izolirane hlapljive spojeve za rod *Veronica* i činjenicu da se svi glavni spojevi ekstrahiraju s obje metode, pri ekstrakciji hlapljivih spojeva treba uzeti u obzir mikrovalnu ekstrakciju jer je to prihvatljiv izbor manje štetan za okoliš koji koristi manje vode i energije.

### ZAHVALE

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## CLASSICAL AND GREEN EXTRACTION OF VOLATILE COMPOUNDS: COMPARING DATA FOR FIVE CROATIAN VERONICA SPECIES

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The genus *Veronica* L. (speedwell) from family Plantaginaceae includes about 500 species distributed slightly more over the Northern Hemisphere [1]. The most representatives of the genus *Veronica* grow in areas with a Mediterranean climate, from the sea level to high alpine regions [2]. This research describes the phytochemical characterization of volatile compounds obtained by classical hydrodistillation (HD) and green microwave extraction (ME) from five *Veronica* species distributed in Croatia, from habitats with moderate water availability. The species studied were: *V. acinifolia*, *V. chamaedrys*, *V. serpyllifolia*, *V. urticifolia* and *V. polita*. The aim of this work is to obtain new data for the insufficiently studied volatiles of *Veronica* species and to gain insight into the differences and similarities in volatile compounds content between species obtained by different isolation methods. E-caryophyllene, caryophyllene oxide, hexahydrofarnesyl acetone, phytol,  $\beta$ -ionone, hexadecanoic acid, and docosane were identified in all five isolates obtained by HD. *V. acinifolia* is rich in  $\beta$ -ionone (17.01%) followed by hexahydrofarnesyl acetone (15.37%) and caryophyllene oxide (7.71%) compounds. Species *V. chamaedrys*, *V. polita*, *V. serpyllifolia* and *V. urticifolia* are rich in phytol, especially *V. urticifolia* with 47.55% of the compound present in the extracts. The same major components identified in the isolates using HD also dominate in the composition of the volatile isolates by ME. The results of this study show that hexahydrofarnesyl acetone, hexadecanoic acid, phytol, E-caryophyllene and caryophyllene oxide are components identified in both methods of extraction. As these compounds are isolated in all species they might be considered as a chemophenetic markers for the genus *Veronica*. Future research comparing clusters based on volatile compounds and clusters resulting from genetic investigations might resolve this hypothesis. Looking at the results for the isolated volatile compounds for the genus *Veronica* and the fact that all main compounds are extracted with both methods, microwave extraction should be considered when extracting volatile compounds because it is environmentally friendlier choice that uses less water and energy.

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# PROMJENA SIGNALNIH PUTOVA PROUZROČENIH DEFICIJENCIJOM S-ADENOZILHOMOCISTEIN-HIDROLAZE U STANIČNOJ LINIJI HEK293T

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Deficijencija S-adenozilhomocisteinske hidrolaze (AHCY, od eng. *S-adenosylhomocysteine hydrolase*) je poremećaj uzrokovan mutacijama u kodirajućoj regiji gena *AHCY* čime je smanjena aktivnost proteina AHCY. AHCY ima ključnu ulogu u pravilnom odvijanju ciklusa aminokiseline metionina u stanici, stoga nedostatak njegove funkcije uzrokuje težak metabolički, multisistemski poremećaj [1]. Klinička slika ovog potencijalno letalnog oboljenja je karakterizirana kombinacijom mišićnih, neuroloških i jetrenih poremećaja te je uzrokovana inhibicijom transmetilacijskih reakcija ovisnih o S-adenozil-metioninu [2]. Cilj ovog istraživanja bio je provesti analizu RNA-seq na stabilnoj staničnoj liniji HEK293T kojoj je utišan gen *AHCY* uz pomoć programa IPA (Ingenuity Pathway Analysis). Napravljena je analiza Core i utvrđeno je koji su stanični putevi diferencijalno eksprimirani. Utvrđeno je 25 diferencijalno eksprimiranih signalnih puteva. Možemo zaključiti da AHCY direktno i indirektno utječe na veliku mrežu signalnih molekula što nije iznenađujuće s obzirom da se radi o multisistemskom poremećaju.

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## SIGNALING PATHWAYS CHANGES CAUSED BY S-ADENOSYLHOMOCYSTEINE-HYDROLASE DEFICIENCY IN HEK293T CELLS

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S-adenosylhomocysteine hydrolase (AHCY) deficiency is a disorder caused by lowered enzymatic activity of AHCY protein due to the mutations in coding region of *AHCY* gene. AHCY has a key role in proper functioning of the methionine cycle in the cell, therefore the lack of AHCY function causes severe metabolic and multisystem disorder [1]. Clinical presentation of this potentially lethal disorder includes a combination of muscular, neurological and hepatic disorders due to inhibition of S-adenosyl methionine-dependent trans methylation reactions [2]. The aim of this research was the analysis and understanding of molecular and cellular roles of AHCY in HEK293T deficient cells. RNA-seq analysis was performed on HEK293T deficient cells. The results were analyzed by IPA (Ingenuity Pathway Analysis) Software and Core analysis was performed. 25 differentially expressed signaling pathways were identified. We can conclude that AHCY directly and indirectly affects a large network of signaling molecules, which is not surprising given that it is a multisystem disorder.

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## RAZVOJ NOVE METODE MASENE CITOMETRIJE U PREKANCEROZNOJ CERVIKALNOJ PROBIRI

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Rak vrata maternice jedan je od najčešćih malignih bolesti među ženama unatoč postojećim mogućnostima prevencije. Nastanku raka vrata maternice prethode prekancerozne promjene nazvane skvamozne intraepitelne lezije (SIL) koje se obično razvijaju tijekom nekoliko godina (1). SIL je podijeljen u tri stupnja: skvamozne intraepitelne lezije niskog stupnja (LSIL), atipične skvamozne stanice neodređenog značaja (ASCUS) i skvamozne intraepitelne lezije visokog stupnja (HSIL) ovisno o težini (1). Rak vrata maternice i SIL povezani su s visokorizičnom infekcijom humanim papiloma virusom koja se spolno prenosi i odgovorna je za više od 95% slučajeva raka vrata maternice među ženama (2). Iako se rak vrata maternice može spriječiti cijepljenjem, citološki i molekularni probir spolno aktivnih žena, nedostatak organiziranih programa probira i/ili neodlučnost o cjepivu ograničavaju primarne preventivne mjere u nekim populacijama. Zbog navedenog, rak vrata maternice će u bliskoj budućnosti ostati značajan javnozdravstveni problem. Masena citometrija omogućuje paralelnu identifikaciju markera na pojedinačnim stanicama kombinacijom protočne citometrije i masene spektrometrije s izotopima teških metala (3). Omogućuje istovremenu analizu do 40 različitih biomarkera, kao i barkodiranje uzoraka (3). U ovoj studiji uzorci brisa cerviksa prikupljeni su četkicom i pohranjeni u mediju za tekućinsku citologiju NovaPrep. Iskusni citolog klasificirao je uzorke kao normalne, LSIL, ASCUS i HSIL. HPV genotipizacija pomoću Multiplex PCR-a provedena je na izoliranoj DNA. NovaPrep fiksirane stanične linije i cervikalni uzorci analizirani su na prisutnost nekoliko markera masenom citometrijom. Preliminarni podaci upućuju na to da se NovaPrep fiksirane stanične linije i cervikalni uzorci mogu pouzdano bojiti analiziranim markerima na masenoj citometriji. Daljnji planovi su analizirati više cervikalnih uzoraka i biomarkera te uključiti informacije prikupljene tijekom 2-godišnjeg razdoblja praćenja kako bi se pronašli oni povezani s postojanošću ili progresijom bolesti ili virusa.

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## DEVELOPMENT OF NEW METHOD MASS CYTOMETRY IN PRECANCEROUS CERVICAL SCREENING

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Cervical cancer is one of the most common malignancies among women despite existing prevention options. The development of cervical cancer is preceded by precancerous changes called squamous intraepithelial lesions (SIL) that usually develop over several years (1). SIL is divided into three stages: low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells of undetermined significance (ASCUS) and high-grade squamous intraepithelial lesions (HSIL) depending on severity (1). Cervical cancer and SILs are associated with high-risk human papillomavirus (HR HPV) infection that is sexually transmitted and responsible more than 95% of cervical cancer cases among women (2). Although cervical cancer can be prevented by vaccination, cytological and molecular screening of sexually active women, lack of organized screening programs and/or vaccine hesitancy limit primary preventive measures in some populations. Due to the above, cervical cancer will remain a significant public health issue in the near future. Mass cytometry enables parallel identification of markers on individual cells by combination of flow cytometry and mass spectrometry with heavy metal isotopes (3). It allows simultaneous analysis of up to 40 different biomarkers and also barcoding of samples (3). In this study, cervical samples were collected with cytobrush and stored in NovaPrep liquid-based cytology (LBC) medium. Samples were classified as normal, LSIL, ASCUS and HSIL by an experienced cytologist. HPV genotyping by Multiplex PCR was performed on the isolated DNA. NovaPrep fixed cell lines and cervical samples were assessed for the presence of several markers by mass cytometry. Preliminary data suggest that NovaPrep fixed cell lines and cervical samples can be reliably stained with the analyzed markers on mass cytometry. Further plans are to assess more cervical samples and biomarkers and include the information collected through a 2-year follow up period to find those associated with disease or virus persistence or progression.

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# DINAMIKA POPULACIJA VRSTE *Synurella ambulans* (MÜLLER, 1846) (CRUSTACEA, AMPHIPODA) U HIPOREIČKOJ ZONI RIJEKE SAVE (HRVATSKA)

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Poznavanje strukture i dinamike populacije rakova ključno je za razumijevanje ekologije populacija i zaštitu vodenih ekosustava. Hiporeička zona (HZ) je jedinstveni dinamičan ekoton koji se nalazi na granici površinske i podzemne vode [1]. Ovo stanište pripada ekosustavima povezanim s podzemnim vodama koji su ugroženi ljudskim aktivnostima zbog akumulacije onečišćujućih tvari u vodonosnicima. Vrsta *Synurella ambulans* je mali slatkovodni stigofilni rakušac koji nastanjuje relativno širok raspon vodnih tijela, uključujući HZ. Cilj istraživanja bio je utvrditi populacijsku dinamiku i reproduktivnu biologiju u jedinkama *S. ambulans* iz HZ rijeke Save (lokacije uzorkovanja: Medsave i Jarun) uzorkovanim u prosincu 2018., travnju, srpnju i listopadu 2019. Analizirana su sljedeća obilježja životnog ciklusa: duljina tijela i omjer spolova (ženke:mužjaci). Sezona razmnožavanja vrste *S. ambulans* započinje u rano proljeće (od ožujka do travnja) jer se juvenilne jedinke pojavljuju tijekom proljeća (travanj 2019.). Roditeljska generacija je nestala iz populacije nakon proljeća (od svibnja do lipnja) te je zamijenjena juvenilnim jedinkama koje su se počele razmnožavati početkom sljedećeg proljeća (ožujak). Na Jarunu je ukupna duljina tijela (UDT) odraslih ženki bila između 3,38 i 6,00 mm (srednja vrijednost = 5,08 mm; SD = 0,67), dok je na lokaciji Medsave bila između 4,13 i 6,38 mm (srednja vrijednost = 5,22 mm; SD = 0,49). ), u svim godišnjim dobima. UDT odraslih mužjaka bila je između 3,00 i 4,50 mm (srednja vrijednost = 3,57 mm; SD = 0,32) na Jarunu, te između 3,00 i 5,00 mm (srednja = 3,68 mm; SD = 0,40) na lokaciji Medsave. Rezultati su pokazali da je srednja duljina tijela ženki veća od mužjaka. Ove su vrijednosti usporedive s literaturnim podacima, gdje je maksimalna duljina tijela ženki i mužjaka bila 7,0 i 4,7 mm [2]. Podaci o omjeru spolova *S. ambulans* pokazuju da su mužjaci bili brojniji od ženki u svim godišnjim dobima, dok je suprotno utvrđeno u populacijama iz Poljske [2]. Uočen je izražen sezonski trend u omjeru spolova tijekom cijelog razdoblja uzorkovanja, u rasponu od 0,44:1 u listopadu do 0,08:1 u srpnju. Navedeni rezultati o životnom ciklusu i razmnožavanju *S. ambulans* pružaju nove spoznaje o ovoj slabo istraženoj vrsti i njezinoj vremenskoj dinamici u osjetljivom staništu povezanom s podzemnim vodama.

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# POPULATION DYNAMICS OF *Synurella ambulans* (MÜLLER, 1846) (CRUSTACEA, AMPHIPODA) IN THE HYPORHEIC ZONE OF THE SAVA RIVER (CROATIA)

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Knowledge of population structure and dynamic in crustaceans is fundamental for the understanding of population ecology and protection of the aquatic ecosystems. The hyporheic zone (HZ) is a unique dynamic ecotone that is located at the interface of surface water and groundwater [1]. This habitat belongs to the groundwater connected ecosystems (GWCEs) which are threatened by human activities due to accumulation of pollutants in aquifers. Species *Synurella ambulans* is a small freshwater stygophilous amphipod inhabiting a relatively wide range of water bodies, including the HZ. The objective of the study was to determine population dynamic and reproductive biology in *S. ambulans* from HZ of the Sava River (sampling sites: Medsave and Jarun) sampled in December 2018, April, July and October 2019. The following life history traits were analysed: body length of individuals and sex ratio (female:male). The reproductive season of *S. ambulans* was determined to be in early spring (from March to April) because juveniles appeared during the spring (April 2019). The parenting generation died out after the spring season (May to June) and was subsequently replaced by juveniles which started to reproduce at the beginning of the following spring (March). In the Jarun study site the total body length (TBL) of adult females ranged between 3.38 and 6.00 mm (mean = 5.08 mm; SD = 0.67), whereas in Medsave ranged between 4.13 and 6.38 mm (mean = 5.22 mm; SD = 0.49), in all seasons. Adult male TBL ranged between 3.00 and 4.50 mm (mean = 3.57 mm; SD = 0.32) in Jarun, and between 3.00 and 5.00 mm (mean = 3.68 mm; SD = 0.40) in Medsave. Results revealed that mean body length of females was greater than that of males. These values were comparable to the literature data, where the maximum body length of females and males was found to be 7.0 and 4.7 mm, respectively [2]. Data on the sex ratio of *S. ambulans* indicate that males were more abundant than females in all seasons, while the opposite was found in populations from Poland [2]. Distinct seasonal trend in sex ratio throughout the sampling period was observed, ranging from 0.44:1 in October to 0.08:1 in July. Current results concerning the life cycle and reproduction of *S. ambulans* shed light on this poorly investigated species and its temporal dynamics in the vulnerable groundwater-connected habitat.

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## USPOREDBA MIKROBNIH ZAJEDNICA DVAJU JEZERSKIH SUSTAVA U HRVATSKOJ

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Osim što su glavni izvori pitke vode, jezera su osjetljivi indikatori promjene okoliša i antropogenog utjecaja. Poznavanje prostorne i vremenske varijabilnosti mikrobnih zajednica jezerskih sustava važno je za utvrđivanje raznolikosti jezera te praćenje stanja okoliša [1]. S tim ciljem, za ovo istraživanje odabrana su dva jezerska sustava s različitim klimom, stupnjem trofije te režimom miješanja vode - Plitvička jezera i Baćinska jezera. Uzorkovanje je provedeno od svibnja do studenog 2021. godine, a parametri okoliša mjereni su *in situ* višeparametarskom sondom (EXO2, YSI, USA). Za taksonomsko određivanje mikrobne zajednice korištena je metoda 16S rDNA sekvenciranja, a za statističku analizu podataka korišten je R programski jezik (R Core Team, 2021). Utvrđene najzastupljenije mikrobne zajednice u oba jezerska sustava su Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria i Verrucomicrobiota. Zabilježena je sezonska razlika u zastupljenosti zajednica unutar pojedinih jezera te su utvrđene zajednice karakteristične za svaki jezerski sustav. Red Fibrobacterota se pojavljuju samo u Plitvičkim jezerima, a redovi Nitrospinota, Nitrospirota te SAR324, karakteristični za morske ekosustave, pojavljuju se u Baćinskim jezerima.

### ZAHVALE

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## MICROBIAL COMMUNITY COMPARISON OF TWO LAKE SYSTEMS IN CROATIA

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Freshwaters are not only the most important drinking water resources, but also sensitive indicators of environmental change. Lake ecosystems have been exposed to strong environmental and anthropogenic influence, making them highly vulnerable. Understanding the spatial and temporal variability of the microbial community in lakes is necessary to determine diversity patterns and monitor the environment [1]. In this study, two lake systems with different climate, trophic status and lake type were investigated – Plitvice Lakes and Baćina Lakes. Samples were collected from May to November 2021. Environmental parameters were measured *in situ* using a multiparameter sonde (EXO2, YSI, USA). For taxonomic determination of the microbial community, the 16S rDNA pair-end sequencing method was used. Statistical analysis was performed using R software (R Core Team, 2021). The most abundant microbial communities in both lakes were Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria and Verrucomicrobiota. We determined seasonal changes in microbial communities in all lakes, but also identified some site characteristic communities: Fibrobacterota for Plitvice Lakes and the marine type phyla Nitrospirota, Nitrospirota and SAR324 for Baćina Lakes.

### ACKNOWLEDGMENTS

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## MORFOLOŠKE PROMJENE PELETA I STANIČNA SMRT KORELIRAJU S PROIZVODNOM ANTIBIOTIKA U TEKUĆOJ KULTURI BAKTERIJE *Streptomyces Rimosus*

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Streptomiceti su višestanične gram-pozitivne bakterije koje karakterizira složena morfološka diferencijacija. Ove bakterije sintetiziraju veliku većinu prirodnih bioaktivnih spojeva, uključujući klinički važne antibiotike (tetracikline, streptomicine ili  $\beta$ -laktame), imunosupresive (rapamicin) i lijekove protiv raka (doksorubicin). *Streptomyces rimosus* jedan je od najbolje okarakteriziranih industrijskih streptomiceta poznat kao primarni izvor oksitetraciklina (OTC), jednog od klinički najvažnijih antibiotika koji se koristi protiv širokog spektra gram-pozitivnih i gram-negativnih bakterija. Do sada su razvoj peleta i fiziološka diferencijacija koja dovodi do proizvodnje antibiotika primjenom raznih 'omics' metoda istraživani samo za modelni organizam, *S. coelicolor*. Uočili smo da fragmentacija peleta *S. rimosus* tijekom rasta u tekućoj kulturi koji korelira s proizvodnjom OTC-a nije usporediva s diferencijacijom micelija uočenom za *S. coelicolor* [1]. U ovom istraživanju smo pratili morfološke promjene micelija i sintezu antibiotika. Na razini proteoma otkrili smo niz proteina koji predstavljaju fiziološka stanja mladih i starih peleta, kao i stanični odgovor na iscrpljivanje hranjivih tvari i nakupljanje antibiotika. Usporedili smo naše rezultate s proteomom *S. coelicolor* [2, 3, 4] kako bismo dobili uvid u globalne stanične trendove te otkrili neke specifične procese koji karakteriziraju bakteriju *S. rimosus*, često korištenu vrstu u industriji.

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# MORPHOLOGICAL CHANGES OF PELLETS AND CELL DEATH COINCIDE WITH ANTIBIOTIC PRODUCTION IN LIQUID CULTURE OF *Streptomyces Rimosus*

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Streptomycetes are multicellular gram-positive bacteria characterized by complex morphological differentiation. These bacteria synthesize the vast majority of natural products, including clinically important antibiotics (tetracyclines, streptomycins, or  $\beta$ -lactams), immunosuppressants (rapamycin), and cancer drugs (doxorubicin). *Streptomyces rimosus* is one of the best characterized industrial streptomycetes known as the primary source of oxytetracycline (OTC), one of the most clinically important antibiotics used against a wide range of gram-positive and gram-negative bacteria. So far, pellet development and physiological differentiation leading to antibiotic production have been investigated, with many omics studies, only for the model organism, *S. coelicolor*. We observed that fragmentation of *S. rimosus* pellets during submerged growth coinciding with OTC production was not comparable to mycelial differentiation observed for *S. coelicolor* [1]. Here, we monitored morphological changes in mycelium and antibiotic synthesis. At the proteome level, we discovered a number of proteins that well represent the physiological phases of young and old pellets and cellular response to nutrient depletion and antibiotic accumulation. We compared our results with proteome of *S. coelicolor* [2, 3, 4] in order to gain insight into global cellular trends as well as to detect some specific processes that characterize the bacterium *S. rimosus*, a widely exploited industrial species.

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## OMJER IZOTOPA $^{87}\text{Sr}/^{86}\text{Sr}$ KAO PRIRODNI GEOKEMIJSKI MARKER U RIJECI KRKI

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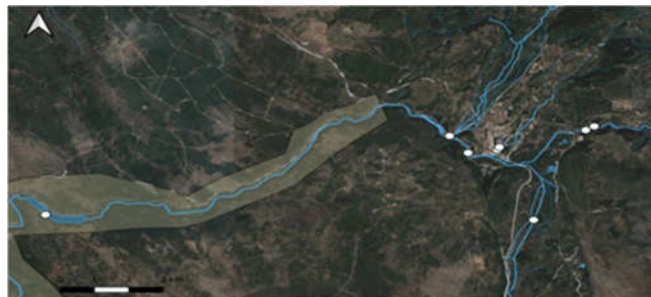
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Zbog svoje prostorne i vremenske stabilnosti, omjer izotopa  $^{87}\text{Sr}/^{86}\text{Sr}$  sve se više koristi kao geokemijski marker u okolišnim istraživanjima [1]. U ovom je istraživanju procijenjena prirodna varijabilnost omjera izotopa  $^{87}\text{Sr}/^{86}\text{Sr}$  i omjera masenih udjela Sr/Ca u vodi rijeke Krke i njezinih pritoka, kako bi se omogućila njihova primjena u istraživanju geokemijskog otiska i migracija riba u riječnom sustavu. Iako je donji dio toka rijeke Krke proglašen nacionalnim parkom, uzvodno područje već je desetljećima opterećeno neadekvatno pročišćenim otpadnim vodama te bi omjer izotopa Sr mogao biti vrijedan prirodni marker u budućim istraživanjima. Riječna voda je uzorkovana u lipnju 2021., na osam lokacija: tri u glavnom toku rijeke Krke, po jedna na svakoj od četiri uzvodne pritoke i jedna na bazenima s industrijskom otpadnom vodom (Slika 1.). Nakon odvajanja Sr od matriksa ionskom kromatografijom, omjeri izotopa Sr izmjereni su MC-ICP-MS-om. Vrijednosti omjera izotopa  $^{87}\text{Sr}/^{86}\text{Sr}$  bile su u skladu s očekivanjima s obzirom na geološku starost istraživanog područja (0.70741 - 0.70771). Vrijednosti omjera Sr/Ca kretale su se u rasponu od 0.00151 to 0.0162 i bile posebno visoke u pritoci Butišnici. Uzorak iz Brljanskog jezera upućivao je na miješanje vode iz Butišnice s glavnim tokom Krke, ukazujući na značajan utjecaj ove pritoke na rijeku Krku. Prikazani rezultati predstavljaju prve podatke, korisne za buduća istraživanja u rijeci Krki, osobito njezinom donjem toku, koji kao jedinstven prirodni fenomen treba posebno zaštititi.



Slika 1. Mapa istraživanja s označenom granicom nacionalnog parka Krka i lokacijama uzorkovanja.

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## $^{87}\text{Sr}/^{86}\text{Sr}$ ISOTOPE RATIO AS A NATURAL GEOCHEMICAL MARKER IN THE KRKA RIVER

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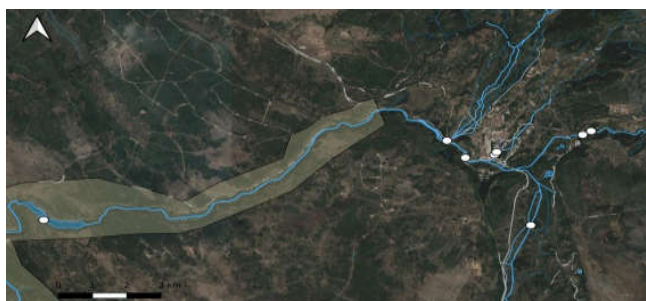
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Due to its local and temporal stability, the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio is increasingly used as a geochemical tracer in environmental science [1]. In the present study, we analyzed the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio and Sr/Ca elemental mass fraction ratio in water from the karst Krka River and its tributaries to assess the natural variability of these ratios for applications such as geochemical fingerprinting and migrations of fish. Although the lower part of the Krka River watercourse was proclaimed a national park, its upstream watercourse has been impacted by inappropriately treated wastewaters for decades and Sr isotopes could be used as natural tracer in future studies. Water was sampled in July 2021, at eight locations: three along the main Krka River watercourse, one at each of the four upstream tributaries, and one from the basins with industrial wastewater (Fig. 1). After Sr/matrix separation by ion exchange chromatography, Sr isotope ratios were measured by MC-ICP-MS. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were consistent with the expected signatures according to the geological age of the study area (0.70741 - 0.70771), while the Sr/Ca ratios ranged from 0.00151 to 0.0162 and particularly high ratio was determined in the Butišnica tributary. The sample from the Brljan Lake showed mixing of the Butišnica River with the main Krka River, indicating the influence of this tributary on the Krka River. Those results represent initial data for future studies on the Krka River, especially on its lower course, which is a unique natural phenomenon and needs strict protection.



**Figure 1.** Map of the study area with the Krka National Park border and water sampling sites.

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## ANALIZA EKSPRESIJE I BIOKEMIJSKA KARAKTERIZACIJA *S*-ADENOZILHOMOCISTEIN-HIDROLAZE S NOVOOTKRIVENOM DELECIJOM

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*S*-adenozilhomocistein-hidrolaza (SAHH) katalizira reakciju hidrolize *S*-adenozilhomocisteina (SAH) na adenzin i homocistein. Budući da je SAH produkt svih reakcija transmetilacije ovisnih o adenzilmetioninu i njihov kompetitivni inhibitor, njegovo uklanjanje ključno je za održavanje normalnog metilacijskog potencijala stanice te za normalnu funkciju organizma. [1] Do sada je pronađeno nekoliko mutacija koje uzrokuju smanjenje aktivnosti SAHH (R49C, R49H, G71S, D86G, A89V, Y143C, Y328D i W112Ter). U nedavnom istraživanju, uz ove poznate mutacije, kod češkog pacijenta otkrivena je delecija 4pb u kodirajućoj regiji SAHH, koja dovodi do pomaka okvira čitanja i preuranjene terminacije translacije na poziciji aminokiseline 403 (c.1207-1211del, p.N403Ter; neobjavljeni rezultati). Preliminarna istraživanja pokazuju oko 20% aktivnosti SAHH u eritrocitima te varijabilno povećanje omjera SAH/SAM (*S*-adenozilmetionina) kod braće, majke i majčinog oca. Općenito, nedostatna aktivnost SAHH dovodi do inhibicije reakcija transmetilacije ovisnih o adenzilmetioninu što uzrokuje teške metaboličke poremećaje te patološka stanja kod ljudi. Ova metabolička bolest prvi put je opisana u Hrvatskoj 2004. godine [2]. U ovom istraživanju SAHH s novootkrivenom mutacijom prekomjerno je eksprimirana u bakterijskom soju *E.coli* BL21, u trajanju od 4h na 30°C, te je potom izolirana i pročišćena afinitetnom kromatografijom Ni-NTA. Za potvrdu izolacije prekomjerno eksprimiranog proteina i dobivanje uvida u njegovu strukturu korištene su SDS i nativna poliakrilamidna gel elektroforeza. Enzimska aktivnost mutiranog proteina izmjerena je spektrometrijski, uz dodatak 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolijevog bromida (MTT), na temelju promjene apsorbancije uslijed nastajanja formazana u reakciji. Iz dobivenih podataka, kinetički parametri izračunati su korištenjem jednadžbe Michaelis-Menten, pomoću linearizacije Lineweaver-Burk. Također, izmjerena je enzimska aktivnost novootkrivenog mutiranog proteina uz dodatak 5,5'-ditio-bis-(2-nitrobenzojeve kiseline) (DTNB), reagensa koji reagira s tiolnim skupinama i ditiotreitola (DTT). Kod novootkrivenog mutiranog proteina, kao i kod divljeg tipa i nekih od do sada poznatih mutiranih varijanti primijećen je povrat aktivnosti enzima nakon dodatka DTT-a.

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## ANALYSIS OF EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE WITH A NOVEL DELETION

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S-adenosylhomocysteine hydrolase (AHCY) is an enzyme that catalyzes hydrolysis of S-adenosyl-L-homocysteine (SAH) to adenosine and homocysteine. Since SAH is the product and competitive inhibitor of all S-adenosyl methionine-dependent transmethylation reactions, its removal is crucial for maintaining cells' methylation potential and normal organism function. [1] So far, several mutations that lead to reduced AHCY activity have been found (R49C, R49H, G71S, D86G, A89V, Y143C, Y328D and W112Ter). In a recent study, along these known mutations, a new 4bp deletion in the coding sequence of AHCY has been identified in a Czech patient, leading to frameshift and subsequently premature termination of translation at amino acid position 403 (c.1207-1211del, p.N403Ter; unpublished results). Preliminary results show approx., 20% activity of AHCY in erythrocytes, and with variable increase in SAH/SAM (S-adenosyl methionine) in two siblings, their mother and the maternal father. In general, reduced AHCY activity leads to inhibition of S-adenosyl methionine-dependent transmethylation reactions which leads to severe metabolic disorders and pathological conditions in humans. This metabolic disease was described for the first time in Croatia in 2004. [2] In this study, AHCY with novel mutation was overexpressed in bacterial strain *E.coli* BL21, for 4h at 30°C, followed by isolation and purification using Ni-NTA affinity chromatography. SDS-PAGE and native-PAGE electrophoresis were used to confirm isolation of overexpressed protein and gain insight into its structure. Enzymatic activity of mutant AHCY was measured spectrophotometrically, with addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), based on change in absorbance resulting from formation of formazane in the reaction. Kinetic parameters were calculated from obtained data using Michaelis-Menten equation and Lineweaver-Burk linearization. Furthermore, enzymatic activity of this novel mutant was measured with addition of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), a reagent that reacts with thiol groups, and dithiothreitol (DTT). Return of impaired enzymatic activity after the addition of DTT has been observed in novel mutant as well as in wild type enzyme and other known mutants.

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## PROČIŠĆAVANJE OTPADNE VODE KOKSNE INDUSTRIJE

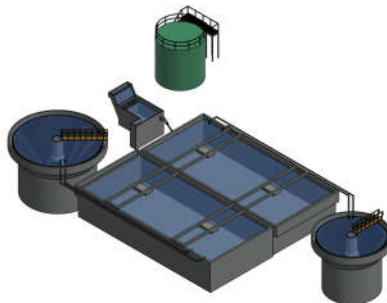
Ana Tutić<sup>1\*</sup>, Viktorija Prevarić<sup>2</sup>, Martina Miloloža<sup>2</sup>, Matija Cvetnić<sup>2</sup>, Šime Ukić<sup>2</sup>, Dajana Kučić Grgić<sup>2</sup>

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Kokсна otpadna voda kompleksan je industrijski efluent, generiran u postrojenjima za proizvodnju čelika. Industrija željeza i čelika ključna je za razvoj svjetske ekonomije. U takvim industrijskim postrojenjima, koriste se velike količine koksa proizvedenog destruktivnom destilacijom ugljena na visokim temperaturama, u odsustvu zraka. Vrući koks hladi se vodom, prilikom čega nastaje otpadna voda okarakterizirana crnom bojom, alkalnim pH, visokim organskim opterećenjem (izraženim preko kemijske potrošnje kisika (KPK)), visokom koncentracijom suspendiranih čestica, amonijaka, fenola, cijanida, tiocijanata i ostalih toksičnih spojeva te je kao takva teška za rukovanje. Zbog kompleksnog sastava i toksičnosti koksne otpadne vode, u svrhu učinkovitog pročišćavanja, potrebno je kombinirati fizikalne, kemijske i biološke postupke. Najčešće primjenjivani postupci su koagulacija s flokulacijom, flotacija, adsorpcija, Fentonov proces i drugi, implementirani kao predtretman ili završno poliranje, nakon biološkog postupka pročišćavanja. U ovom radu prikazan je pregled fizikalno-kemijskih procesa pročišćavanja primijenjenih u tretiranju koksne otpadne vode, u kombinaciji s najučinkovitijim, najjeftinijim i ekološki prihvatljivim – biološkim postupcima.



**Slika 1.** Primjer postrojenja za biološko pročišćavanje otpadne vode koksne industrije

### ZAHVALE

Ovo istraživanje potpomognuto je projektom „*Optimizacija postojećeg biološkog uređaja za pročišćavanje otpadnih voda koksne industrije*“. Posebno zahvaljujem svim suradnicima, uključujući Fakultet kemijskog inženjerstva i tehnologije te BP group.

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## OVERVIEW OF COKING WASTEWATER TREATMENT METHODS

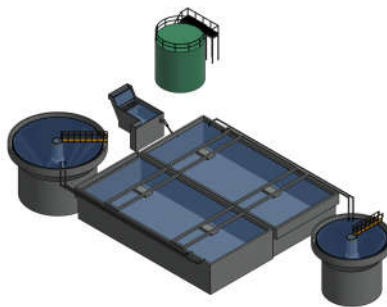
Ana Tutić<sup>1</sup>, Viktorija Prevarić<sup>2</sup>, Martina Miloloža<sup>2</sup>, Matija Cvetnić<sup>2</sup>, Šime Ukić<sup>2</sup>, Dajana Kučić Grgić<sup>2</sup>

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Coking wastewater is a complex industrial effluent, generated in steel production plants. The steel industry is crucial for the development of the world economy. In such industrial plants, large quantities of coke are produced by destructive distillation of coal at high temperatures and in the absence of air. The hot coke is then cooled with water. This produces wastewater characterized by a black color, alkaline pH, high organic load (expressed by chemical oxygen demand (COD)), high concentration of suspended particles, ammonia, phenol, cyanide, thiocyanate and other toxic compounds. Such wastewaters are difficult to handle. Due to the complex composition and toxicity of coking wastewater, it is necessary to combine physical, chemical and biological methods for effective treatment. The most commonly used methods are coagulation with flocculation, flotation, adsorption, Fenton process, and others, used as pretreatment or final treatment, after biological treatment. This article overviews physicochemical treatment processes used in the treatment of coking wastewater in combination with the most efficient, cost-effective and environmentally friendly - biological processes.



**Figure 1.** Example of biological wastewater treatment plant in coke industry

### ACKNOWLEDGMENTS

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## INVAZIVNE BILJNE VRSTE KAO POTENCIJALNI IZVOR FITOFARMACEUTIKA

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U Hrvatskoj gotovo da ne postoji stanište na kojemu nisu prisutne invazivne strane biljne vrste (ISBV). Takve vrste uzrokuju gubitak biološke raznolikosti, gospodarsku štetu, a često i zdravstvene probleme kod čovjeka. Unatoč brojnim negativnim osobinama ISBV-a, poznato je da one mogu biti vrijedan izvor sekundarnih metabolita koji se mogu koristiti u medicini, farmaceutici, poljoprivredi i fitoremedijaciji. Glavni cilj projekta Hrvatske zaklade za znanost NATURALLY (IP-2020-02-6899) je istražiti fitofarmaceutski potencijal ekstrakata četiriju invazivnih stranih biljnih vrsta: *Ailanthus altissima* (Mill.) Swingle, *Solidago canadensis* L., *Helianthus tuberosus* L. i *Robinia pseudoacacia* L. Područje istraživanja je Istarska županija (Republika Hrvatska) gdje su tijekom vegetacijske sezone u 2021. godini prikupljeni uzorci ISBV-a, sušeni na zraku, samljeveni i pohranjeni u hladnjak na +4°C. Prvi korak u istraživanju fitofarmaceutskog potencijala bila je provedba spektrofotometrijskih analize ukupnog sadržaja fenola (TP), ukupnih flavonoida (TF) i ukupnih neflavonoida (TNF) u 90%-tnom metanolnom i 70%-tnom etanolnom ekstraktu listova. Antioksidativni kapacitet (AC) oba ekstrakta ispitan je s tri standardna testa: ABTS, DPPH i FRAP. Preliminarni rezultati pokazali su najveće vrijednosti TP i TNF u ekstraktu *A. altissima*, a slijedila je vrsta *H. tuberosus*. Vrijednosti AC također su potvrdile da ispitivane vrste predstavljaju biljni materijal bogat antioksidansima, s potencijalom uporabe u obliku novih fitofarmaceutskih pripravka.





## INVASIVE PLANTS SPECIES AS POTENTIAL SOURCE OF PHYTOPHARMACEUTICALS

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Invasive alien plant species (IAPS) have become widespread in Croatia causing loss of biodiversity, economic damage and often health issues in humans. Despite numerous negative traits of IAPS, it is known that invasive alien plants can be a valuable source of secondary metabolites which can be used in medicine, farmaceutics, agriculture and phytoremediation as novel ecosystem services. The main goal of the Croatian Science Foundation project NATURALLY (IP-2020-02-6899) is to explore the phytopharmaceutical potential of extracts of four invasive alien plant species: *Ailanthus altissima* (Mill.) Swingle, *Solidago canadensis* L., *Helianthus tuberosus* L. and *Robinia pseudoacacia* L. The research study area is the Istria Region in Croatia. During the vegetation season in 2021, the samples were collected, air dried, grinded and stored at +4 °C. The first step in researching the phytopharmaceutical potential was the spectrophotometric evaluation of total phenolic content (TP), total flavonoids (TF), and total non-flavonoids (TNF) in 90% methanol and 70% ethanol leaf extracts. The antioxidant capacity (AC) in both extracts was examined by three standard assays: ABTS, DPPH, and FRAP. The preliminary results have shown the highest TP and TNF values in the extracts of *A. altissima*, followed by *H. tuberosus*. The AC values also confirmed that IAPS represent antioxidant-rich plant material, with a potential of use as novel phytopharmaceuticals.





## KARAKTERIZACIJA SATELITOMA KUKCA BRAŠNARA *TRIBOLIUM MADENS*

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U heterokromatinskim regijama eukariotskih kromosoma nalazi se značajna količina ponovljenih elemenata DNA. Među ponovljenim elementima DNA dominiraju satelitne DNA (satDNA), uzastopno ponovljene nekodirajuće sekvence koje prevladavaju u (peri)centromernim i (sub)telomernim kromosomskim regijama [1]. Iako je u prošlosti detekcija satDNA bila zahtjevna, razvojem novih visokoprotočnih metoda sekvenciranja postalo je moguće karakterizirati satelitome - cjelokupne kolekcije satDNA pojedine vrste.

Kukac brašnar *Tribolium madens* pripadnik je roda *Tribolium*. U genomima vrsta roda *Tribolium* dominiraju veliki blokovi satDNA, a u kukcu *T. madens* do sada su definirane dvije satDNA koje zajedno čine 34% ukupne genomske DNA [2]. Međutim, postojanje drugih satDNA u genomu ove vrste dosad nije bilo dokazano.

U ovom radu korištene su metode visokoprotočnog sekvenciranja kako bi se karakterizirao satelitom kukca brašnara *T. madens*. Sekvenciranje cijelog genoma provedeno je korištenjem dviju različitih tehnologija. Sekvenciranje sintezom obavljeno je na platformi Illumina, a sekvenciranje pojedinih molekula u stvarnom vremenu na platformi PacBio. Pomoću kratkih neposloženih očitavanja s platforme Illumina računalni je algoritam TAREAN [3] definirao konsenzuse monomernih sekvenci satDNA. Mapirajući dobivene konsenzusne sekvence satDNA na duga i točna očitavanja s platforme PacBio HiFi, istražena je ponavljajuća organizacija detektiranih satDNA. Na ovaj je način definirano deset novih satDNA. Budući da svaka pojedina novootkrivena satDNA čini manje od 0,05% genoma, može ih se definirati kao niskozastupljene satDNA. Fluorescencijskom hibridizacijom *in situ* otkriveno je da su niskozastupljene satDNA raspršene na različitim kromosomima unutar komplementa ( $2n=20 +$  prekobrojni kromosomi). Kako bi se došlo do novih saznanja o evoluciji satDNA vrste *T. madens*, potražili smo slične sekvence u genomima srodnih vrsta *Tribolium castaneum* i *Tribolium freemani*. Ortologne sekvence u genomima ovih vrsta evoluirale su slijedeći princip usklađene evolucije. Pomoću komparativnih analiza satelitoma kongeneričkih vrsta rasvjetljuje se način evolucije repetitivnih sekvenci, ali i produbljuje znanje o genetičkim razlikama koje mogu dovesti do specijacije.

### ZAHVALE

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## SATELLITOME CHARACTERIZATION OF THE BLACK FLOUR BEETLE *TRIBOLIUM MADENS*

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Heterochromatic regions of eukaryotic genomes consist of large portions of repetitive DNA elements. Repetitive DNA elements are often dominated by satellite DNAs (satDNAs), tandemly repeated non-coding sequences located primarily in the (peri)centromeric and (sub)telomeric chromosomal regions [1]. Although detecting these sequences in a genome used to be difficult, high-throughput sequencing technologies now allow the disclosure of entire satellitomes, i.e., comprehensive collections of satDNAs in a single genome.

The black flour beetle *Tribolium madens* belongs to the genus *Tribolium*, whose species are known for a large share of satDNAs in the genome content. So far, the two satDNAs have been detected in *T. madens*, comprising together 34% of the genomic DNA [2]. However, there is no evidence as yet of other *T. madens* satDNAs.

In this work, we addressed the *T. madens* satellitome by applying high-throughput sequencing approach. First, we sequenced the whole *T. madens* genome by two different technologies - NGS based on sequencing by synthesis performed by Illumina and single-molecule, real-time sequencing performed by PacBio. Based on the unassembled short Illumina reads, the computational pipeline TAREAN [3] defined the putative satDNA consensus repeat units. By mapping the consensus sequences to the long and highly accurate PacBio HiFi reads we explored their tandem organization. In this way we discovered ten new satDNAs. As each of newly detected satDNA comprises less than 0.05% of the genome, these sequences represent low copy satDNAs. Fluorescence *in situ* hybridization revealed that the low-copy satDNAs are scattered on different chromosomes of the complement (2n=20 + supernumerary chromosomes). To gain insight into the evolution of *T. madens* satDNAs, we further investigated whether there were similar sequences in the genomes of closely related species *Tribolium castaneum* and *Tribolium freemani*. The satDNAs, which have the orthologous sequences in the related genomes, have been shown to evolve according to the principles of concerted evolution. Comparative analyses of the satellitomes of the congeneric species not only illuminate the evolution of repetitive sequences, but also might further our understanding of the genetic differences that could lead to speciation.

### ACKNOWLEDGMENTS

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## NANOPORE SEKVENCIRANJE KUKACA RODA *Tribolium* S TVRDIM EGZOSKELETOM

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Napredak u području sekvenciranja genoma, a posebno razvoj tehnologije Nanopore, omogućili su u zadnje vrijeme uspješno sastavljanje složenih genoma i analizu regija bogatih ponavljanjima. Jedan od najvažnijih čimbenika za uspješno sekvenciranje i slaganje genoma je izolacija visokomolekularne DNA, odnosno DNA velike prosječne duljine, prikladne čistoće u dovoljnoj količini. Izolacija DNA s takvim karakteristikama je zahtjevna prilikom primjene na tkivima, a postaje još zahtjevnija ukoliko je prisutan tvrdi egzoskelet kod kukaca. Također, komercijalno dostupni kompleti za izolaciju DNA često nisu testirani niti optimizirani za insekte. U tu svrhu, razvijena je reproducibilna metoda za izolaciju visokomolekularne DNA iz kukaca roda *Tribolium* te uporabu u sekvenciranju tehnologijom Nanopore. Ukratko, izolacijski protokol sastoji se od izolacije staničnih jezgri pomoću pufera optimiziranog za izolaciju jezgri sa što manjim gubicima te naknadnog pročišćavanja DNA na kromatografskoj koloni baziranoj na izmjeni aniona. Krajnji rezultat ovog postupka je visokomolekularna DNA odgovarajućih svojstava za pripremu DNA knjižnice koja se pokazala u dovoljnoj količini za više nanošenja na jednu MinION-pločicu za sekvenciranje, dajući do 13 Gb podataka. Utvrđeno je i da blago fragmentiranje DNA povećava prosječne vrijednosti N50očitavanja do 26 kb te poboljšava životni vijek pora tijekom sekvenciranja. Iako je ovaj protokol testiran na kukcu *T. castaneum* i dvije blisko srodne vrste, opisani koraci u optimizaciji mogli bi biti korisni za primjenu na bilo kojem organizmu koji predstavlja izazov prilikom korištenja dostupnih konvencionalnih metoda za izolaciju DNA.

### ZAHVALE

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## NANOPORE SEQUENCING OF HARD-CUTICLED *Tribolium* BEETLES

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Recent advances in the field of genome sequencing, especially Nanopore technology, have allowed assembly of complex genomes and analysis of repeat-rich regions. One of the most important factors for successful sequencing and assembly is isolation of high molecular weight (HMW) DNA of adequate purity in sufficient quantity. Isolation of DNA with these qualities proves to be a hard task for tissues but becomes even more complicated when hard exoskeletons are involved. In addition, commercially available kits are often not tested and optimized for insects. For that purpose, we developed reproducible method for the isolation of HMW DNA from *Tribolium* beetles and its use in Nanopore sequencing. In short, the isolation protocol consists of isolation of cell nuclei using a specific buffer and subsequent purification of DNA on an anion-exchange chromatography column. This procedure results in HMW DNA of adequate properties for library preparation which showed to be in sufficient amount for multiple runs on a single Nanopore MinION sequencing flow cell, yielding up to 13 Gb of output. We have also found that DNA shearing increased average N50 read values up to 26 kb together with better overall pore health during sequencing experiments. Even though this protocol was tested on *T. castaneum* and two closely related species, described optimization steps could be beneficial when applied to any organism that poses a challenge using conventional DNA isolation methods.

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## POTENCIJALNE TERAPIJSKE INTERVENCIJE PROTIV TOKSIČNOSTI AMILOIDA BETA (*IN VITRO* MODEL ALZHEIMEROVE BOLESTI)

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Alzheimerova bolest (AB) je progresivni neurodegenerativni poremećaj koji čini oko 70% svih slučajeva demencije. Patogenezu AB karakterizira agregacija amiloidnih  $\beta$  ( $A\beta$ ) peptida u izvanstanične senilne plakove i stvaranje unutarstaničnih neurofibrilarnih snopića od hiperfosforiliranog proteina tau. Iako se smatra da obje strukture uzrokuju značajan gubitak neurona i sinapsi,  $A\beta$  hipoteza prevladavajuće je objašnjenje više od 25 godina [1]. Nakupljanje  $A\beta$  peptida u neuronima može prethoditi stvaranju  $A\beta$  plakova i formiranju neurofibrilarnih snopića. Poznato je da se  $A\beta$  peptidi sami slažu u dimere, trimere i oligomere višeg reda, za koje se vjeruje da su glavni izvor toksičnosti koji izaziva smrt neurona [2]. Budući da je trenutna terapija (inhibitori kolinesteraze donepezil, galantamin i rivastigmin, kao i memantin, antagonist N-metil D-aspartatnog receptora) još uvijek simptomatska, potencijalne strategije liječenja koje modificiraju bolest opsežno se istražuju [3]. Osim zbog pozitivnih učinaka na cjelokupno ljudsko zdravlje, neurosteroidi dehidroepiandrosteron (DHEA) i njegov sulfatni oblik dehidrepiandrosteron sulfat (DHEAS) [4], kao i neurotrofin moždani neurotrofni čimbenik (BDNF) [5] od velikog su interesa kao potencijalne terapijske mete za AB, zbog njihove uključenosti u neurogenezu, sinaptičku plastičnost, rast, diferencijaciju, zaštitu i preživljavanje neurona. U našem laboratoriju, kultivirani primarni neuroni dobiveni od C57BL/6 miševa bili su izloženi raznim  $A\beta$  preparacijama u svrhu optimiziranja *in vitro* modela AB. Kako bi se istražilo potencijalno neuroprotektivno djelovanje protiv  $A\beta$  toksičnosti, primarne kulture neurona tretirane su s DHEA/S, BDNF, i njihovom kombinacijom i njihov učinak na vijabilnost stanica i mehanizmi djelovanja proučavani su različitim staničnim i biokemijskim testovima. Naši rezultati koji upućuju na neuroprotektivne učinke DHEA/S, BDNF i njihove kombinacije mogli bi ukazivati na nove terapijske intervencije za ovu još uvijek neizlječivu bolest. Međutim, naše bi nalaze trebalo potvrditi daljnjim *in vitro* istraživanjima, kao i *in vivo* eksperimentima na životinjskim modelima AB i uzorcima ljudske krvi.

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## POTENTIAL THERAPEUTIC INTERVENTIONS TARGETING AMYLOID BETA TOXICITY (*IN VITRO* MODEL OF ALZHEIMER´S DISEASE)

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Alzheimer´s disease (AD) is a progressive neurodegenerative disorder that accounts for about 70% of all dementia cases. The AD pathogenesis is characterized by the aggregation of amyloid  $\beta$  ( $A\beta$ ) peptides into extracellular senile plaques, and the formation of intracellular neurofibrillary tangles by the hyperphosphorylated tau protein. Although both structures are considered to cause a significant loss of neurons and synapses, the  $A\beta$  hypothesis has been the dominant opinion for more than 25 years [1]. The intraneuronal accumulation of  $A\beta$  peptides may precede the generation of  $A\beta$  plaques and neurofibrillary tangles formation. The  $A\beta$  peptides are known to self-assemble into dimer, trimer and higher-order oligomers, which are believed to be the main source of toxicity by causing the death of neurons [2]. Since current therapy (cholinesterase inhibitors donepezil, galantamine and rivastigmine, as well as memantine, an N-methyl D-aspartate receptor antagonist) is still symptomatic, potential disease-modifying treatment strategies have been extensively investigated [3]. In addition to positive effects on overall human health, neurosteroids dehydroepiandrosterone (DHEA) and its sulfated form dehydroepiandrosterone sulfate (DHEAS) [4], as well as neurotrophin brain-derived neurotrophic factor (BDNF) [5], are of a great interest as potential therapeutic targets for AD, due to their involvement in neurogenesis, synaptic plasticity, neuronal growth, differentiation, protection, and survival. In our laboratory, cultured primary neurons derived from C57BL/6 mice were exposed to various  $A\beta$  preparations to optimize the *in vitro* model of AD. To investigate the potential neuroprotective actions against  $A\beta$  toxicity, primary neuronal cultures were treated with DHEA/S, BDNF and their combination and their effects on the cell viability and mechanisms of action were studied using various cell-based and biochemical assays. Our results suggesting neuroprotective effects of DHEA/S, BDNF and their combination could point to the new therapeutic interventions for this still incurable disease. However, our findings should be confirmed by further *in vitro* research, as well as by *in vivo* experiments using AD animal models and human blood samples.

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## GENEZA PRIRODOZNAJSTVENIH ISTRAŽIVANJA U DJELU IMMANUELA KANTA

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Kritika čistoga uma kao glavnoga djela Immanuela Kanta preuzela je primat nad njegovim cjelokupnim opusom i suvremenost ne pridaje toliku važnost njegovim djelima iz prirodnoznanstvenog perioda koliko pridaje važnost njegovim djelima iz kritičkoga perioda, pa ipak Kantov doprinos prirodnoj znanosti ne bi smio biti tek tako skrajnut zbog njegovog iznimnog doprinosa tadavremenim prirodnoznanstvenim istraživanjima. Na tomu tragu ovaj rad ima cilj izložiti genezu prirodnoznanstvenih istraživanja u djelu Immanuela Kanta od djela iz 1749. godine do 1781. godine i objavljivanja Kritike čistoga uma. Hipoteza ovoga rada se ogleda u dokazivanju važnosti prirodnoznanstvenih istraživanja Immanuela Kanta i utjecaju njegovih istraživanja na druge tadavremene prirodnoznanstvenike kao i na samo njegovo glavno djelo oslovljeno kao Kritika čistoga uma čime se zapravo želi pokazati geneza prirodnoznanstvenih istraživanja u djelu Immanuela Kanta temeljnom za Kantova filozofska istraživanja. Na tomu tragu, osnovna literatura koju ćemo koristiti pri izradi rada jeste: 1) Immanuel Kant, *Kritika čistog uma*, BIGZ, 1970., 2) Immanuel Kant, *Metafizička načela prirodne znanosti*, Veselin Masleša, Sarajevo, 1990., 3) Peter Plaass, *Kant's theory of natural science*, Springer Netherlands, Boston, 1994.

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## GENESIS OF NATURAL SCIENCE RESEARCHES IN THE WORKS OF IMMANUEL KANT

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The Critique of the Pure Reason as the main work of Immanuel Kant has taken precedence over his entire oeuvre, and modernity does not give as much importance to his works from the natural science period as it does to his works from the critical period, having in mind his exceptional contribution to contemporary scientific research. In this regard, this paper aims to expose the genesis of natural science research in the work of Immanuel Kant from the work from 1749 to 1781 and the publication of the Critique of the Pure Reason. The hypothesis of this paper is reflected in proving the importance of Immanuel Kant's scientific research and the impact of his research on other contemporary naturalists as well as his main work entitled Critique of Pure Reason. So, the basic literature for this research will be: 1) Immanuel Kant, *Kritika čistog uma*, BIGZ, Beograd, 1970., 2) Immanuel Kant, *Metafizička polazna načela prirodne znanosti*, Veselin Masleša, Sarajevo, 1990., 3) Peter Plaass, *Kant's theory of natural science*, Springer Netherlands, Boston, 1944.

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## KLIMATSKE PROMJENE U POLJU OBORINE NA PODRUČJU JADRANA I DINARIDA

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Regionalni klimatski modeli (RKM-i) su alat za sveobuhvatnu analizu klime na regionalnoj skali čija je svrha prikazati regionalne procese i forsiranja (kompleksna topografija, zemljišni pokrov, rijeke, obalna područja itd.) na malim skalama. RKM-i se intenzivno koriste u svrhe istraživanja, posebice u istraživanjima budućih klimatskih promjena. Cilj rada je procijeniti buduće klimatske promjene u polju oborine nad topografski kompleksnim obalnim i gorskim područjem istočnog Jadrana i Dinarida, koji su posebno osjetljivi na klimatske promjene. Koristimo do sada najveći ansambl RKM-e iz inicijative EURO-CORDEX, koji se sastoji od ~140 simulacija buduće klime kako bi obuhvatili sve izvore nepouzdanosti i što više mogućih budućih uvjeta. Ansambl čine simulacije s prostornim korakom mreže od ~12.5 km za tri scenarija koncentracije stakleničkih plinova, RCP2.6, RCP4.5 i RCP8.5, koji pretpostavljaju različite putanje koncentracija do kraja stoljeća. Istražujemo signal klimatskih promjena za nekoliko budućih tridesetogodišnjih perioda u usporedbi s povijesnim periodom. Rezultati pokazuju promjene u ukupnoj oborini koja se znatno smanjuje ljeti, dok je zimi prisutan gradijent između porasta oborine nad južnim i smanjenja nad sjevernim dijelovima regije. Do kraja stoljeća broj kišnih dana će značajno opasti, dok će intenzitet oborine porasti. Ovo će dovesti do intenziviranja događaja s obilnom oborinom u svim sezonama, a koji mogu uzrokovati opasne bujične poplave, klizišta, prekide u prometu, probleme u opskrbi čiste vode i električne energije, pa čak i ljudske žrtve.



## FUTURE PRECIPITATION CHANGES OVER EASTERN ADRIATIC AND DINARIC ALPS

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Regional climate models (RCMs) are a tool for comprehensive climate analysis at regional scales aiming to represent regional processes and forcings (complex topography, land cover, rivers, coastal areas, etc.) at a fine scale. RCMs are extensively used for research purposes, especially for investigations of future climate changes. The study aims to estimate the future climate change of precipitation over the topographically complex coastal-mountainous region of the eastern Adriatic and Dinaric Alps, which is particularly vulnerable to climate change. We use an unprecedented ensemble of ~140 RCM simulations of future climate from the EURO-CORDEX initiative to cover all sources of uncertainty and as many possible future conditions. The ensemble includes simulations at a spatial resolution of ~12.5 km for three greenhouse gas concentration scenarios, RCP2.6, RCP4.5 and RCP8.5, which assume different concentration pathways by the end of the century. We examine the climate change signal for several future thirty-year periods compared to the historical period. The results show changes in total precipitation, with a considerable reduction in summer and a south-north gradient in winter (an increase in northern parts of the region and a reduction in southern parts). By the end of the century, the number of rainy days will significantly decrease, however, the projections show a precipitation intensity increase. The latter will lead to an intensification in extreme precipitation events in all seasons, which pose a risk of severe flash floods, landslides, traffic interruptions, water and electricity supply problems and even human casualties.



## EVOLUCIJA ESTUARIJA RIJEKE KRKE TIJEKOM HOLOCENA

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Početak holocena obilježen je naglim porastom morske razine koje je rezultiralo potapanjem mnogih obalnih okoliša i formiranjem raznih sedimentnih tijela, uključujući i estuarije. Estuariji su obalna vodena tijela pod snažnim kopnenim i morskim utjecajem i kao takvi su predmet brojnih istraživanja promjena morske razine. Estuarij rijeke Krke predstavlja jedan jedinstveni sustav koji omogućuje rekonstrukciju kvartarnih paleookolišnih promjena i geoloških procesa povezanih s promjenama morske razine. Estuarij Krke je krški vrlo stratificirani estuarij koji se nalazi u središnjem dijelu istočne jadranske obale. Istraživan je primjenom koncepta visoko rezolucijske seizmičke stratigrafije u kombinaciji s podacima analiza sedimentnih jezgara i datiranjem metodom radioaktivnog ugljika (<sup>14</sup>C). Seizmički podaci pokazali su jedinstveni potopljeni paleookoliš te ispunu bazena s više od 16 m sedimenata. Identificirane seizmičke jedinice u korelaciji su s podacima sedimentnih jezgara iz estuarija. Istraživanje je pokazalo da su se početkom postglacijalne transgresije stvorili pogodni uvjeti za rast sedrenih naslaga i akumulaciju riječnih i jezerskih naslaga na ovom području. S napredovanjem porasta morske razine, more je počelo potapati područje istraživanja te time onemogućilo daljnji rast sedre, a omogućeno je taloženje estuarijskih naslaga. Potpuni vrlo stratificirani estuarijski uvjeti uspostavljeni su prije otprilike 7500 godina prije sadašnjosti. Istraživanje je pokazalo vrlo dinamičnu paleookolišnu evoluciju estuarija, koja je bila pod utjecajem naglog porasta morske razine, geomorfologije i hidrodinamike.

### ZAHVALE

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## HOLOCENE EVOLUTION OF THE KRKA RIVER ESTUARY

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The beginning of the Holocene is marked by rapid sea level rise resulted in the flooding of many coastal environments and formation of various sedimentary bodies, including estuaries. Since estuaries are coastal water bodies under the influence of the land-sea interface, thus, they are a very important subject for the studies of sea level change. The Krka River estuary represents such a unique example and opportunity to reconstruct Quaternary paleoenvironmental changes and geological processes related to sea level changes. It is a karstic salt-wedge estuary located in the central part of the Eastern Adriatic coast. The estuary was studied by applying concepts of high-resolution seismic stratigraphy coupled with sediment core analysis and radiocarbon <sup>14</sup>C data. The seismic analysis revealed a unique submerged paleolandscape and more than 16 m of sedimentary infill. The identified seismic units correlate with the sediment cores extracted from the estuary. Our data revealed that at the beginning of the postglacial transgression, a favorable conditions were developed for growing of the tufa deposits and accumulation of the fluvial and lacustrine sediments. With further sea level rise, the sea started to flood the studied area and prevent further development of tufas with the deposition of the estuarine deposits. The fully estuarine salt wedge conditions were established at approx. 7500 y BP. This study revealed a very dynamic paleoenvironmental evolution of the estuary controlled by a rapid sea level rise, geomorphology and hydrodynamics.

### ACKNOWLEDGMENTS

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## EKSPRESIJA I PROČIŠĆAVANJE C-TERMINALNE DOMENE PROTEINA SH2D3C U BAKTERIJI *ESCHERICHIA COLI*

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Interakcije protein-protein ključne su za mnoge stanične procese te nam njihovo proučavanje omogućava razumijevanje tih procesa s biokemijskog gledišta. S obzirom da promijene u regulaciji signalnih puteva u stanici često dovode do nastanka raznih bolesti, proučavanje interakcija protein-protein može nam pomoći da bolje razumijemo njihov nastanak, razvoj te utjecaj na ljudsko zdravlje.

SH2D3C ima ulogu proteina adaptera koji sudjeluje u signalnim putevima uključenim u adheziju i migraciju stanica, organizaciju tkiva i regulaciju imunološkog odgovora. [1] Protein sadrži Ras GEF-like C-terminalnu domenu koja nema značajnu GEF aktivnost, [2] ali može tvoriti interakcije s drugim proteinima.

Analizom staničnog proteoma metodom SILAC-MS nađena je potencijalna interakcija SH2D3C s dipeptidil-peptidazom III (DPP III). DPP III sudjeluje u regulaciji oksidacijskog stresa kompetitivno se vežući na protein KEAP1 koji je ključan sudionik signalnog puta Nrf2/KEAP1. [3] To bi značilo da njihova interakcija predstavlja moguću poveznicu između signalnog puta Nrf2/KEAP1 i regulacije stanične migracije.

Da bi se interakcija potvrdila drugim metodama poput termoforeze na mikro skali (MST), C-terminalna domena proteina SH2D3C nadeksprimirana je u bakteriji *E. coli* s dva različita privjeska, GST i MBP. Proteini s GST, odnosno MBP privjeskom pročišćeni su afinitetnom kromatografijom te su uspoređeni prinosi i čistoća pročišćenih proteina.

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## OVEREXPRESSION AND PURIFICATION OF THE C-TERMINAL DOMAIN OF THE PROTEIN SH2D3C IN *ESCHERICHIA COLI*

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Protein-protein interactions are crucial for many cellular processes and their research allows us to understand these processes from a biochemical point of view. Since changes in the regulation of signaling pathways in the cell often lead to the development of various diseases, studying protein-protein interactions can help us better understand their origin, development and impact on human health.

SH2D3C acts as an adapter protein in signaling pathways involved in cell adhesion and migration, tissue organization, and regulation of the immune response. [1] The protein contains a Ras GEF-like C-terminal domain that has no significant GEF activity, but may interact with other proteins [2].

Analysis of the cellular proteome by SILAC-MS revealed a potential interaction of SH2D3C with dipeptidyl peptidase III (DPP III). DPP III is involved in the regulation of oxidative stress by competitively binding to the KEAP1 protein which is a key participant in the Nrf2/KEAP1 signaling pathway. [3] This would mean that their interaction represents a possible link between the Nrf2/KEAP1 signaling pathway and cell migration regulation.

To confirm the interaction by other methods such as micro-scale thermophoresis (MST), the C-terminal domain of SH2D3C was overexpressed in *E. coli* with two different tags, GST and MBP. GST- and MBP-tagged proteins were purified by affinity chromatography, and yields and purities of the proteins were compared.

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# KOKRISTALI S METALOORGANSKIM GRAĐEVNIM JEDINICAMA KAO DONORIMA HALOGENSKE VEZE

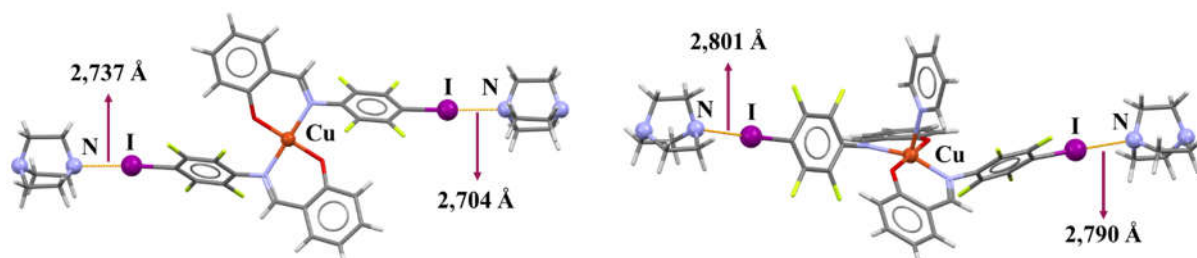
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Koordinacijski spojevi proučavani su kao akceptori i donori u dizajnu metaloorganskih višekomponentnih kristala temeljenih na halogenskoj vezi [1]. Odabirom funkcijskih skupina liganda omogućuje se nastanak specifičnih međumolekulskih interakcija. U dosadašnjim istraživanjima, koordinacijski spojevi daleko su češće izučavani kao akceptori halogenske veze [2].

U ovom istraživanju po prvi put pripremljeni su kokristali u kojima su donori halogenske veze iminski bakrovi(II) kompleksi. Imini korišteni kao ligandi pripremljeni su kondenzacijom 2,3,5,6-tetrafluor-4-jodanilina i salicilaldehida (**itfasal**) ili *ortho*-vanilina (**itfaovan**). Nadalje, pripremljeni su bakrovi(II) kompleksi Cu(**itfasal**)<sub>2</sub>, Cu(piridin)(**itfasal**)<sub>2</sub>, Cu(**itfaovan**)<sub>2</sub> i Cu(piridin)(**itfaovan**)<sub>2</sub>. Kompleksi su kokristalizirani s ditopičnim akceptorima halogenske veze koji sadrže dušikove atome 4,4'-bipiridinom (**bpy**), 1,2-bis(4-piridil)etanom (**bpean**) i 1,4-diazabiciklo[2.2.2]oktanom (**dabco**) u stehiometrijskom omjeru 1:1 (Slika 1). Mehanokemijskom sintezom i kristalizacijom iz otopine pripremljeno je pet kokristala koji su okarakterizirani metodama difrakcije rentgenskog zračenja na jediničnom kristalu i praškastom uzorku. Strukturna analiza pokazala je da je u svim pripremljenim kokristalima ostvarena ciljana halogenska veza I...N između atoma joda perhalogenirane periferije metaloorganskih jedinica i dušikovih atoma akceptora.



**Slika 1.** Halogenske veze u kokristalima kompleksa Cu(**itfasal**)<sub>2</sub> · **dabco** (lijevo) i Cu(pyridine)(**itfasal**)<sub>2</sub> · **dabco** (desno).

## ZAHVALE

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# COCRYSTALS WITH METAL-ORGANIC BUILDING BLOCKS AS HALOGEN BOND DONORS

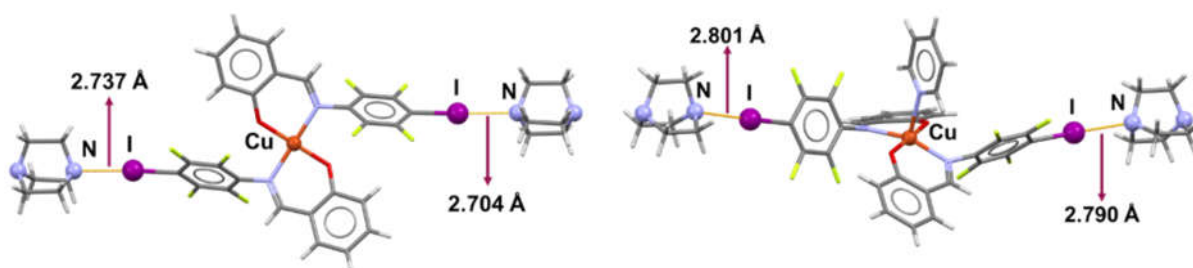
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Metal-organic complexes are studied as both halogen bond acceptors and donors in the design of halogen-bonded [1] metal-organic multi-component crystals. Choosing the appropriate functional groups in ligands can enable the formation of specific intermolecular interactions. Most of the research in this field was focused on investigating metal-organic complexes as halogen bond acceptors [2].

In this research, we have prepared the first known cocrystals in which imine copper(II) complexes are halogen bond donors. Imines used as ligands were prepared by condensation of 2,3,5,6-tetrafluoro-4-iodoaniline and salicylaldehyde – **itfasal** or *ortho*-vanillin – **itfaovan**. Furthermore, copper(II) complexes Cu(**itfasal**)<sub>2</sub>, Cu(pyridine)(**itfasal**)<sub>2</sub>, Cu(**itfaovan**)<sub>2</sub> and Cu(pyridine)(**itfaovan**)<sub>2</sub> were synthesized. Those complexes were cocrystallized with ditopic nitrogen-containing halogen bond acceptors 4,4'-bipyridine (**bpy**), 1,2-bis(4-pyridyl)ethane (**bpean**) and 1,4-diazabicyclo[2.2.2]octane (**dabco**) in 1:1 stoichiometric ratio (Figure 1.). Five novel cocrystals were prepared mechanochemically and by crystallization from solution and were characterized by diffraction methods (single-crystal X-ray diffraction and X-ray powder diffraction). Structure analysis revealed that in all cocrystals the desired I⋯N halogen bond was formed between iodine atoms from peripherally perhalogenated metal-organic units and nitrogen atoms of acceptor molecules.



**Figure 1.** Halogen bonds in cocrystals of complexes Cu(**itfasal**)<sub>2</sub> · **dabco** (left) and Cu(pyridine)(**itfasal**)<sub>2</sub> · **dabco** (right).

## ACKNOWLEDGMENTS

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## ISTRAŽIVANJE UTJECAJA IZVORA ALUMINIJA I KATIONA NA NASTANAK ZEOLITA

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Zeoliti su kristalni materijali nastali polimerizacijom oksoaniona silicija i aluminijskih jona. Jedna od glavnih značajki zeolita su uređeni sustavi kanala i šupljina zbog čega se primjenjuju kao sorbenti, katalizatori i ionski izmjenjivači [1]. Kapacitet ionske zamjene, stupanj hidrofobnosti, broj i priroda kiselih mjesta te specifična površina zeolita se mogu modificirati prilagodbom omjera silicija i aluminijskih jona, položajem atoma aluminijskih jona u strukturi kao i oblikom i veličinom kristala [2,3]. Kako bi se stekao uvid u parametre koji kontroliraju nastanak različitih materijala strukturnih tipova BEA i MFI, ispitan je utjecaj različitih izvora aluminijskih jona, i/ili natrijevih ili kalijevih kationa pri različitim temperaturama provođenja reakcija. Prethodno spomenuti parametri utjecali su na položaj i količinu atoma aluminijskih jona u strukturi, morfologiju, te fazni sastav produkata. Istraživanje je provedeno na sustavima početnog molarnog sastava: 1 SiO<sub>2</sub> : 0,3 TEAOH : 6,8 H<sub>2</sub>O, (TEAOH = tetraetilamonijev hidroksid), pri čemu je prvi dio izveden tako što su varirani izvori aluminijskih jona držeći udio aluminijskih jona u sustavu konstantnim. Neki od izvora aluminijskih jona ujedno su bili i izvori natrijevih ili kalijevih kationa. Drugi dio istraživanja proveden je na sustavima koji su umjesto izvora aluminijskih jona sadržavali natrijev nitrat ili kalijev nitrat kao izvore kationa. Produkti su okarakterizirani difrakcijom rendgenskog zračenja na polikristalnom uzorku, pretražnom elektronskom mikroskopijom, termogravimetrijskom analizom, raspršenjem laserske svjetlosti pri niskim kutovima, atomskom apsorpcijskom spektrofotometrijom, te spektroskopijom nuklearne magnetske rezonancije uzoraka u čvrstom stanju. Proizišli rezultati upućuju na to da je za dane uvjete pri nižoj temperaturi (150 °C) za nastanak zeolita strukturnog tipa BEA ključna prisutnost aluminijskih jona. Kada je hidrotermička sinteza provedena pri 180 °C, iz reakcijskih smjesa gdje su korišteni aluminijski izopropoksid i aluminijski hidroksid su nastale smjese dviju faza, točnije materijala strukturnih tipova MFI i BEA. Ovisno o korištenom izvoru aluminijskih jona, nastale su različite količine svakog materijala. U slučaju kada je kao izvor aluminijskih jona korišten natrijev aluminat (kao izvor i aluminijskih jona i natrijevih kationa) nastale su dvije faze: kvarc i zeolit strukturnog tipa MFI. To se može dovesti u korelaciju s rezultatima dobivenim iz sustava koji su sadržavali samo kalijev ili natrijev nitrat.

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## EXPLORING THE EFFECT OF ALUMINUM SOURCES AND CATIONS ON THE FORMATION OF ZEOLITES

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Zeolites are crystalline materials formed by the polymerization of oxoanions of silicon and aluminum. One of the main features of zeolites is the ordered system of channels and cavities that enables them to act as sorbents, catalysts, and ion-exchangers [1]. Ionic-exchange capacity, degree of hydrophobicity, number and nature of acid sites, and specific surface area of zeolites can be modified by the adjustment of silicon to aluminum ratio, the position of the framework aluminum atoms as well as by the size and shape of crystals [2,3]. To gain insight into the parameters that control the formation of different BEA- and MFI-type zeolite materials, the effect of various aluminum sources and/or sodium or potassium cations, at different reaction temperatures, was investigated. Varied parameters were found to affect the framework aluminum content and position, morphology, as well as the phase composition of the final products. The present research was conducted by studying the systems of the initial molar composition 1 SiO<sub>2</sub> : 0.3 TEAOH : 6.8 H<sub>2</sub>O (TEAOH = tetraethylammonium hydroxide) whereby the first part of research was conducted by varying the aluminum source while maintaining the aluminum content constant. Some of the aluminum sources were also the sources of sodium and potassium cations. The second part of the research was conducted on systems that contained sodium nitrate or potassium nitrate as cation sources instead of aluminum sources. Products were characterized by powder X-ray diffraction, scanning electron microscopy, thermogravimetric analysis, low-angle laser light scattering, atomic absorption spectrometry, and solid-state nuclear magnetic resonance spectroscopy. Results indicate that at the given conditions at lower temperature (150 °C) the presence of aluminum is crucial for the formation of BEA-type zeolite. In the case when the hydrothermal treatment is carried out at 180 °C, reaction systems with aluminum isopropoxide or aluminum hydroxide, a mixture of two phases, namely MFI- and BEA-type zeolites, was obtained. The amount of each material varied depending on the aluminum source used. In the case when sodium aluminate was used (as both the source of aluminum and sodium cations), the formed phases were quartz and MFI-type zeolite which can be correlated with the results obtained from the solely potassium and sodium nitrate-containing systems.

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## BIORAZGRADNJA MIKROPLASTIKE POLISTIRENA I POLI(VINIL-KLORIDA) PRIMJENOM KVASCA *SACCHAROMYCES CEREVISIAE*

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Biorazgradnja je proces u kojem mikroorganizmi, uključujući bakterije, kvasce i plijesni, razgrađuju organske tvari na komponente jednostavnije strukture. Željeni stupanj biorazgradnje je mineralizacija, koja podrazumijeva potpunu promjenu strukture početne molekule do CO<sub>2</sub>, H<sub>2</sub>O, uz oslobađanje topline, te nastanak biomase.[1] Aktualna problematika koja zabrinjava znanstvenu zajednicu je problematika čestica mikroplastika, čije dugotrajno zadržavanje u okolišu uzrokuje globalno onečišćenje. Mikroplastikom (MP) se nazivaju čestice plastike veličine u rasponu od 5 mm do 1 μm.[2] Dosadašnja istraživanja pokazala su da određeni mikroorganizmi mogu razgraditi različite vrste čestica MP-a, no stupanj biorazgradnje ovisi o vrsti, veličini i koncentraciji MP-a, kao i o vrsti mikroorganizama te uvjetima provođenja pokusa biorazgradnje.[1]

U ovome radu ispitana je biorazgradnja MP-e polistirena (PS) i poli(vinil-klorida) (PVC) primjenom kvasca *Saccharomyces cerevisiae* u uvjetima bez i uz dodatak glukoze. Biorazgradnja se provela tijekom 30 dana, a pratio se ukupan broj živih stanica kvasca (CFU), udio ugljika u sustavu (TC/TOC/TIC) te je provedena LC-MS analiza vodene faze. Čestice MP-a okarakterizirane su, prije i nakon provedbe procesa biorazgradnje, FTIR-ATR spektroskopijom. Rezultati pokazuju da *Saccharomyces cerevisiae* ima sposobnost biorazgradnje obje vrste ispitivanih MP-a. Dodatak glukoze pogoduje procesu, a s obzirom na porast CFU-u tijekom provedbe pokusa, viši stupanj biorazgradnje uočen je za čestice PS-a. Navedeno je potvrđeno FTIR spektroskopijom s obzirom na veće smanjenje intenziteta pikova za spomenutu vrstu čestica.

### ZAHVALA

Rad je izrađen u sklopu projekta „Primjena naprednih tehnologija obrade voda za uklanjanje mikroplastike“ (IP-2019-04-9661) Hrvatske zaklade za znanost na Fakultetu kemijskog inženjerstva i tehnologije Sveučilišta u Zagrebu.

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# BIODEGRADATION OF POLYSTYRENE AND POLYVINYL CHLORIDE MICROPLASTICS USING *SACCHAROMYCES CEREVISIAE* YEAST

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Biodegradation is a process in which microorganisms, including bacteria, yeasts and molds, break down organic matter into components of simpler structure. The desirable degree of biodegradation is mineralization. Mineralization involves a complete change in the structure of the initial molecule to CO<sub>2</sub>, H<sub>2</sub>O, with the release of heat, and the formation of biomass.[1] The current issue of concern to the scientific community is the issue of microplastic particles, whose long-term retention in the environment causes global pollution. Microplastics (MP) are plastic particles ranging in size from 5 mm to 1 μm.[2] Previous research has shown that certain microorganisms can degrade different types of MP particles, but the degree of biodegradation depends on the type, size and concentration of MP, as well as the type of microorganisms and the conditions of the biodegradation experiment.[1]

In this work, the biodegradation of MP of polystyrene (PS) and polyvinyl chloride (PVC) was investigated using the yeast *Saccharomyces cerevisiae* in conditions without and with the addition of glucose. Biodegradation was performed 30 days, and the total number of living yeast cells (colony-forming unit, CFU), the carbon content of the system (TC/TOC/TIC) was monitored, and the LC-MS analysis of the aqueous phase was performed. MP particles were characterized, before and after the biodegradation process, by FTIR-ATR spectroscopy. The results show that *Saccharomyces cerevisiae* has the ability to biodegrade both types of tested MPs. Glucose supplementation favors the process, and given the increase in CFU during the experiment, a higher degree of biodegradation was observed for PS particles. This was confirmed by FTIR spectroscopy due to the greater reduction in peak intensity for the mentioned type of particles.

## ACKNOWLEDGMENTS

This paper was prepared within the project "Application of advanced water treatment technologies for the removal of microplastics" (IP-2019-04-9661) of the Croatian Science Foundation at the Faculty of Chemical Engineering and Technology, University of Zagreb.

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- [2] Hu, Y., Gong, M., Wang, J., Bassi, A., *Rev. Environ. Sci. Biotechnol.* **18** (2019) 207–230.





## SEZONSKE PROMJENE MIKROBIOLOŠKE ZAJEDNICE U PLITVIČKIM JEZERIMA

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Plitvička jezera su najveći, najpoznatiji i najposjećeniji Nacionalni park u Hrvatskoj. Najistaknutija atrakcija parka, kaskadna jezera, nastala su stalnim biodinamičkim procesom stvaranja i rasta sedrenih barijera. Bioraznolik vodeni ekosustav Nacionalnog parka uključuje i velik broj potoka koji jezera opskrbljuju vodom. Mikrobna zajednica ima važnu ulogu u procesu stvaranja sedre [1]. Zbog velike posjećenosti Nacionalnog parka, ali i klimatskih promjena, mikrobna zajednica vodenih staništa sklona je promjenama. Promjene mikrobne zajednice duž slivnog toka mogu ugroziti proces formiranja sedre, a time i sam izgled jezera. Cilj ovog istraživanja bio je utvrditi strukturu i promjene raznolikosti mikrobne zajednice u vodenim ekosustavima Nacionalnog parka Plitvička jezera. Stoga smo proveli istraživanje duž slivnog područja Plitvičkih jezera u dva različita doba godine. Uzorci vode prikupljeni su u svibnju i rujnu 2019. godine na 24 mjesta koja su uključivala potoke koji spajaju jezera i sve površinske vode potoka koji pripadaju slivnom području Plitvičkih jezera. Također, uzorkovana je i rijeka Korana, koja predstavlja glavni izlazni tok iz cijelog jezerskog sustava. Za *in situ* mjerenje okolišnih parametara korištena je multisenzorska sonda (EXO2, YSI, USA). Za taksonomsko određivanje mikrobne zajednice upotrijebljena je metoda sekvenciranja spojenih parova 16S rDNA. Sve statističke analize provedene su u R sučelju. Najzastupljenije bakterijske zajednice na istraživanom području u oba godišnja doba su predstavnici koljena Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria i Verrucomicrobiota. U potocima koji međusobno spajaju jezera koljeno Actinobacteria je općenito bio brojniji, i to s većom brojnošću u rujnu. U ostalim površinskim tokovima pronađene su inače rijetke vrste iz koljena Bdellovibrionota, s većom brojnošću u svibnju, te vrste iz koljena Patescibacteria, s većom brojnošću u rujnu. U svim uzorcima, koljena Bacteroidota i Cyanobacteria pokazali su veću brojnost u svibnju, a koljeno Verrucomicrobiota u rujnu. Uglavnom su uzorci iz potoka koji međusobno povezuju jezera pokazali drugačiju mikrobnu zajednicu, a zajednica rijeke Korane bila je slična upravo tim uzorcima.

### ZAHVALE

Zahvaljujemo Nacionalnom Parku Plitvička jezera na podršci tijekom istraživanja.

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## SEASONAL CHANGES OF MICROBIAL COMMUNITY IN PLITVICE LAKES

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Plitvice Lakes are the largest, best known and most visited national park in Croatia. The most prominent attraction of the park, the cascading lakes, are formed by the constant biodynamic process of formation and growth of tufa barriers. The National Park's biodiverse aquatic ecosystem also includes a large number of streams that supply water to the lakes. The microbial community plays an important role in the process of tufa formation [1]. Due to the high anthropogenic pressure in the National Park, but also due to climate change, the microbial community of aquatic habitats is prone to change. Changes in the microbial community along the watercourse can jeopardize the tufa formation process and the appearance of the lakes themselves. The aim of this study was to establish the structure and diversity changes of the microbial community in the aquatic ecosystems of the Plitvice Lakes National Park. For this reason, we conducted research within the Plitvice Lakes catchment area at two different times of the year. Water samples were collected in May and September 2019 from 24 sites on streams connecting the lakes and from all surface waters in the Plitvice Lakes catchment area. The Korana River was also sampled, as it represents the main outflow of the entire lake basin. A multisensor probe (EXO2, YSI, USA) was used for *in situ* measurement of environmental parameters. The pair-end sequencing method of 16S rDNA was used for taxonomic determination of the microbial community. All statistical analyses were performed in the R environment. The most abundant bacterial communities in the study area in both seasons belonged to phyla Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria and Verrucomicrobiota. In streams connecting the lakes phylum Actinobacteria was more abundant, with higher abundance in September. In other surface streams, not so common phyla Bdellovibrionota, with higher abundance in May, and Patescibacteria in September, were found. Phyla Bacteroidota and Cyanobacteria had higher abundance in May and the phylum Verrucomicrobiota in September, in all samples. In particular, the samples from the streams connecting the lakes had a different microbial community and the Korana River community was similar to these very samples.

### ACKNOWLEDGMENTS

We thank the Plitvice lakes National Park for providing support during research.

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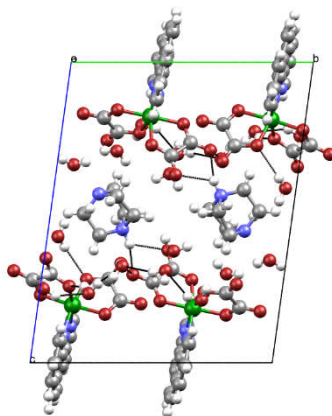
## STRUKTURNE PROMJENE U KROM(III) KOMPLEKSNOJ SOLI UZROKOVANE PROMJENOM RELATIVNE VLAŽNOSTI

Marko Dunatov,<sup>1,\*</sup> Andreas Puškarić,<sup>1</sup> Lidija Androš Dubraja<sup>1</sup>

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Vlažnost je bitan čimbenik u mnogim područjima, kao što su elektronika i sustavi upravljanja kakvoćom [1]. Strukturne pretvorbe koje su izravno povezane s dehidracijom/rehidracijom u pravilu se očituju kao pomaci atoma u kristalnoj rešetki, što rezultira promjenom svojstava materijala [2]. Takvi materijali mogu biti pogodni kandidati za molekulske senzore. Predstavljene su priprava i karakterizacija metaloorganske kompleksne soli koja se sastoji od bicikličkog amonijevog kationa (DABCO)<sup>2+</sup> (1,4-diazabicyclo[2.2.2]oktan-1,4-diium) i bis(oksalato)kromat(III) aniona. Obzirom na slabu topljivost jednog od reaktanata, ali i prateći načela zelene kemije, sinteza je provedena mehanokemijski. Jedinični kristali naknadno su dobiveni iz vodene otopine priređene kompleksne soli, u obliku nonahidrata (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)<sub>2</sub>·9H<sub>2</sub>O. Kristali pod polariziranim svjetlom mijenjaju boju iz ružičaste u narančastu, kao posljedica pleokroizma. Kada su kristali izloženi zraku s niskom relativnom vlažnošću, pretvaraju se u bezvodni oblik (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)<sub>2</sub>. Pretvorba između mokre i suhe faze proučavana je difrakcijom rendgenskih zraka na polikristalnom uzorku, infracrvenom spektroskopijom, termičkom analizom i kronoamperometrijskim mjerenjima.



**Slika 1.** Prikaz pakiranja molekula kompleksnog spoja (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)<sub>2</sub>·9H<sub>2</sub>O duž osi a.

### ZAHVALE

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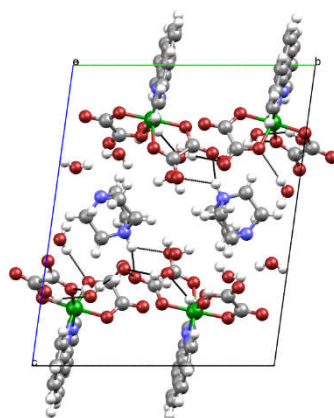
# HUMIDITY INDUCED STRUCTURAL TRANSFORMATIONS IN CHROMIUM(III) COMPLEX SALT

Marko Dunatov,<sup>1,\*</sup> Andreas Puškarić,<sup>1</sup> Lidija Androš Dubraja<sup>1</sup>

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Humidity is an essential parameter in many fields, such as electronics and process control systems [1]. Structural changes related directly to the dehydration/rehydration process usually result from minor displacements of atoms in the crystal lattice, leading to a change in material properties [2]. Such materials are potential candidates for use as molecular sensors. Herein we report the synthesis and characterization of metal-organic complex salt containing a bicyclic amine cation (DABCO)<sup>2+</sup> (1,4-diazabicyclo[2.2.2]-1,4-dium) and a bis(oxalato)chromium(III) anion [Cr(phen)(C<sub>2</sub>O<sub>4</sub>)]<sup>-</sup> (phen = 1,10-phenanthroline). The synthesis was carried out mechanochemically due to the low solubility of the reactants, as well as following the green chemistry principles. Single crystals were subsequently grown from the aqueous solution of the prepared complex salt as nonahydrate (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)]<sub>2</sub>·9H<sub>2</sub>O. These crystals show pleochroism when viewed under polarized light, changing color from pink to orange. When the crystals are exposed to air with low relative humidity, they transform to an anhydrous form (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)]<sub>2</sub>. The transformation between the wet and dry forms was followed by powder X-ray diffraction, IR spectroscopy, thermal analysis, and chronoamperometric measurements.



**Figure 1.** Crystal packing of (DABCO)[Cr(phen)(C<sub>2</sub>O<sub>4</sub>)]<sub>2</sub>·9H<sub>2</sub>O along *a* axis.

## ACKNOWLEDGMENTS

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## UTJECAJ IZBORA REAKCIJSKIH PARAMETARA I VRSTE KATALIZATORA NA SINTEZU IZOBUTILNIH I IZOPENTILNIH ESTERA MASNIH KISELINA

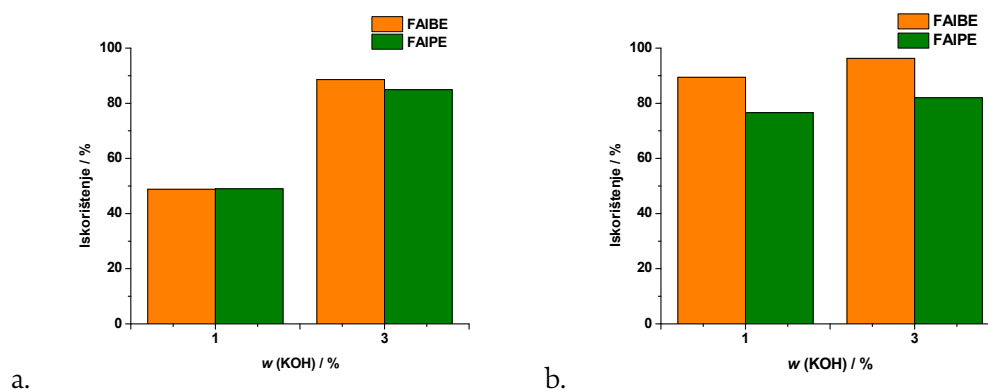
Mia Gotovuša,<sup>1\*</sup> Paula Huzjak,<sup>1</sup> Marta Krasić,<sup>1</sup> Martina Zdravec,<sup>1</sup> Ivana Zdravec,<sup>1</sup> Jelena Parlov-Vuković,<sup>2</sup> Lucija Konjević,<sup>2</sup> Fabio Faraguna<sup>1</sup>

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Izobutilni i izopentilni esteri masnih kiselina (FAIBE, odnosno FAIPE), kao vrste biodizela, mogu se sintetizirati reakcijom transesterifikacije u prisutnosti različitih tipova katalizatora. Najčešće korišteni su kemijski bazični ili kiseli katalizatori, iako reakcije transesterifikacije može biti i enzimatska. U ovom istraživanju biodizeli su sintetizirani korištenjem različitih vrsta biosirovina (suncokretovog ulja, repičinog ulja, kokosovog ulja, životinjske masti i otpadnog ulja) te kemijskih katalizatora (kalijeveg hidroksida i sumporne kiseline). Proučavan je utjecaj temperature, vremena reakcije, molarnog omjera reaktanata te masenog udjela katalizatora na iskorištenje reakcije transesterifikacije. Rezultati su pokazali da su najviša iskorištenja od 95 do 100 % dobivena u sustavima s alkoholom izobutanolom i kalijevim hidroksidom kao katalizatorom, dok su u slučaju izopentanolu ona iznosila oko 85 %. U cilju mogućeg poboljšanja iskorištenja FAIPE-a, provedeni su dodatni eksperimenti sa sumpornom kiselinom. Općenito, najznačajnijim reakcijskim parametrima za sintezu ovih biodizela pokazali su se molarni omjer reaktanata i maseni udio katalizatora.



**Slika 1.** Utjecaj masenog udjela KOH na iskorištenje reakcije sinteze FAIBE-a i FAIPE-a, kada je molarni omjer reaktanata 6:1 (a) i 12:1 (b).

### ZAHVALE

Istraživanje provedeno u sklopu Projekta Hrvatske zaklade za znanost Razvoj funkcionalnih biogoriva i (bio)aditiva te ispitivanje primjenskih svojstava mješavina s mineralnim gorivima (FunBioFA, UIP-2019-04-5242).



## INFLUENCE OF THE REACTION PARAMETERS AND CATALYST SELECTION ON THE SYNTHESIS OF FATTY ACID ISOBUTYL AND ISOPENTYL ESTERS

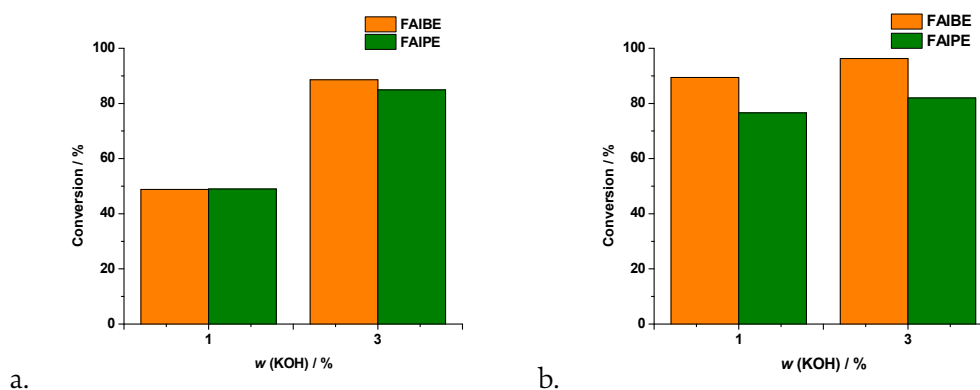
Mia Gotovuša,<sup>1,\*</sup> Paula Huzjak,<sup>1</sup> Marta Krasić,<sup>1</sup> Martina Zdravec,<sup>1</sup> Ivana Zdravec,<sup>1</sup> Jelena Parlov-Vuković,<sup>2</sup> Lucija Konjević,<sup>2</sup> Fabio Faraguna<sup>1</sup>

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Fatty acid isobutyl and isopentyl esters (FAIBE and FAIPE, respectively), as types of biodiesel, can be synthesized via transesterification reaction in the presence of different types of catalysts. The most commonly used are chemical alkaline or acidic catalysts, however the transesterification reaction can be enzymatic as well. Here, the biodiesels were synthesized with the use of different sorts of feedstock (sunflower oil, rapeseed oil, coconut oil, animal fat and waste cooking oil) and chemical catalysts (potassium hydroxide and sulphuric acid). The influence of the temperature, time, molar ratio of the reactants and mass fraction of the catalyst on the reaction conversion was studied. The results showed that the highest reaction conversions of 95-100 % were obtained with the use of alcohol isobutanol and potassium hydroxide as a catalyst, whereas in the case of isopentanol, the highest achieved conversions were around 85 %. To possibly further improve the conversion of FAIPE, additional experiments with sulphuric acid were conducted. Overall, the most significant reaction parameters for the synthesis of these biodiesels were the molar ratio of the reactants and the mass fraction of the catalyst.



**Figure 1.** The influence of the mass fraction of KOH on reaction conversion of FAIBE and FAIPE, when the molar ratio of the reactants is 6:1 (a) and 12:1 (b).

### ACKNOWLEDGMENTS

This research was conducted within the Project of the Croatian Science Foundation Development of functional biofuels and (bio)additives and characterization of blends with mineral fuels (FunBioFA, UIP-2019-04-5242).



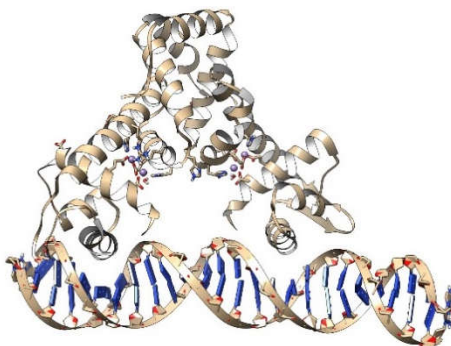
## MODELIRANJE PROTEN-DNA KOMPLEKSA TRANSKRIPCISKOG FAKTORA MntR IZ BAKTERIJE *Bacillus subtilis*

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Porodica bakterijskih transkripcijskih faktora MntR sudjeluje u regulaciji homeostaze metalnih iona u velikom broju bakterijskih vrsta. Transkripcijski faktor MntR iz bakterije *Bacillus subtilis* je manganov metalosenzor koji veže manganove ione u binuklearna vezna mjesta kada stanična koncentracija manganovih iona prijeđe optimalnu vrijednost, što ga aktivira za vezanje na DNA i represiju ekspresije gena za unos manganovih iona [1]. Iako je ovaj protein dobro istražen [2,3], niti u literaturi, niti u dostupnim bazama podataka ne postoje strukture ovog proteina u kompleksu s ciljnom DNA sekvencom. U svrhu detaljnijeg istraživanja proteina MntR, provedene su simulacije molekulske dinamike proteina u kompleksu s DNA te same ciljane DNA sekvence. Na temelju klaster analize dobivenih trajektorija izabrane su strukture za izgradnju protein-DNA kompleksa homolognim modeliranjem na temelju protein-DNA strukture DtxR proteina iz MntR proteinske obitelji.



**Slika 1.** Kompleks proteina MntR iz bakterije *Bacillus subtilis* i DNA.

### ZAHVALE

Istraživanje je napravljeno u sklopu HRZZ projekta “Manganovi metalosenzori“ IP-2020-02-3446.

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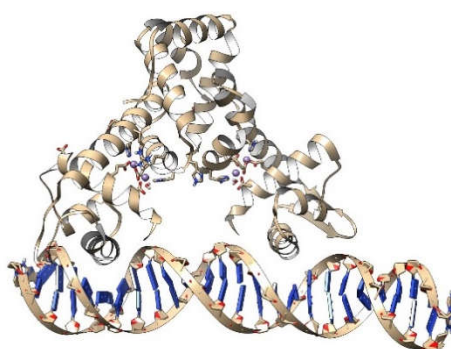
# MODELLING THE PROTEIN-DNA COMPLEX BETWEEN THE TRANSCRIPTION FACTOR MntR FROM *Bacillus subtilis* AND ITS TARGET DNA SEQUENCE

Zoe Jelić Matošević,<sup>1,\*</sup> Branimir Bertoša<sup>1</sup>

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The MntR family of bacterial transcription factors regulate metal ion homeostasis across a wide range of bacterial species. The transcription factor MntR from *Bacillus subtilis* is a manganese metallosensor. When cellular concentration of manganese exceed a certain limit, MntR binds manganese ions in binuclear binding sites, which activates it for DNA binding and repression of genes for manganese ion import [1]. Although MntR is well researched [2, 3], a structure of MntR in complex with DNA is not available, neither in the literature nor in the PDB. We performed molecular dynamics simulations of MntR in complex with manganese ions as well as simulations of its target DNA sequence. Using cluster analysis, the most representative conformations were identified and used as starting structures for building protein-DNA complexes. Homology modelling using protein-DNA structures of the homologous DtxR protein as a template, was applied.



**Figure 1.** The protein-DNA complex of the MntR protein from the bacterium *Bacillus subtilis*.

## ACKNOWLEDGMENTS

This work was supported by the Croatian Science Foundation project “Manganese metallosensors” IP-2020-02-3446.

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# ADSORPCIJA ŠIROKOG IZBORA ANTIBIOTIKA NA NANOMATERIJALE TEMELJENE NA GRAFENU: STUDIJA MODELIRANJEM

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Postojeće spoznaje o ponašanju antibiotika prilikom sorpcije na nanomaterijale su ograničene, pogotovo u pogledu mehanizma adsorpcije na površinu ugljikovih nanomaterijala [1], koji može imati odlučujući utjecaj na kapacitete adsorpcije i efikasnost regeneracije grafenskih adsorbensa. U ovom radu koristili smo molekulsko modeliranje kako bi generirali (do sada) najpotpuniji skup podataka o adsorpciji 8  $\beta$ -laktamskih, 3 makrolidska, 12 kinolonskih, 4 tetraciklinska, 15 sulfonamidnih, 2 linkosamidska, 2 fenikolska, 4 nitroimidazolna antibiotika, trimetoprima i transformacijskih produkata na grafen i funkcionalizirani grafen u vodi i n-oktanolu. Iz rezultata je vidljivo da više nekovalentnih interakcija, poput van der Waals ovih interakcija,  $\pi$ -interakcija, hidrofobnih interakcija i vodikovih veza, djeluju istovremeno omogućujući adsorpciju. Svojstva molekula antibiotika, grafena / grafen oksida te druga svojstva otopine reguliraju snagu i odnos navedenih interakcija. Pokazujemo da je, od ovdje istraženih, najefikasnija metoda uklanjanja antibiotika iz vodenog okoliša korištenje grafena pri očekivanom pH. Naknadno, uvjeti koji su indicirali najefikasniju regeneraciju adsorbensa su nepolarna otapala blago lužnatog pH (8–10). Ova, teoretskim metodama dobivena, saznanja proširuju i nadopunjuju eksperimentalna opažanja te pružaju značajan doprinos daljnjem istraživanju adsorpcijskih svojstava materijala temeljenih na grafenu te razvoju prediktivnih modela adsorpcije.

## ZAHVALE

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# ADSORPTION OF A WIDE VARIETY OF ANTIBIOTICS ON GRAPHENE-BASED NANOMATERIALS: A MODELLING STUDY

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The knowledge on the sorption behaviour of antibiotics on nanomaterials is limited, especially regarding the reaction mechanism on the surface of carbon nanomaterials [1], which may determine both the adsorptive capacity and regeneration efficiency of graphene adsorbers. In this work, we used molecular modelling to generate the most comprehensive (to date) adsorption dataset for pristine and functionalised graphene interacting with 8  $\beta$ -lactams, 3 macrolide, 12 quinolone, 4 tetracycline, 15 sulphonamide, trimethoprim, 2 lincosamide, 2 phenicol and 4 nitroimidazole antibiotics, and their transformation products in water and n-octanol. Results show that various non-covalent interactions that operate simultaneously, including van der Waals dispersion forces,  $\pi$ -interactions, hydrophobic interaction and hydrogen bonding, facilitate adsorption. The molecular properties of antibiotics and graphene/graphene oxide, as well as the composition of the background solution regulate the magnitude of these interactions. Our findings demonstrate that the most efficient method for the removal of antibiotics from aquatic environments is the use of graphene at environmental pH. The subsequent regeneration of the sorbent is best achieved through washing with slightly basic (pH 8–10) non-polar solvents. The obtained theoretical insights expand and complement experimental observations and provide important information that can contribute to further exploration into the adsorbent properties of graphene-based materials, and towards the development of predictive adsorption models.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the computing resources and support provided by The University Computing Centre (SRCE) in Zagreb. Proofreading by Katarina Cetinić (Ruđer Bošković Institute, Zagreb) is gratefully acknowledged. This work was supported by the Croatian Science Foundation project no. IP-2018-01-2298.

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## KINETIČKA REZOLUCIJA MONO I 2,2-DISUPSTITUIRANIH PROPARGILNIH EPOKSIDA

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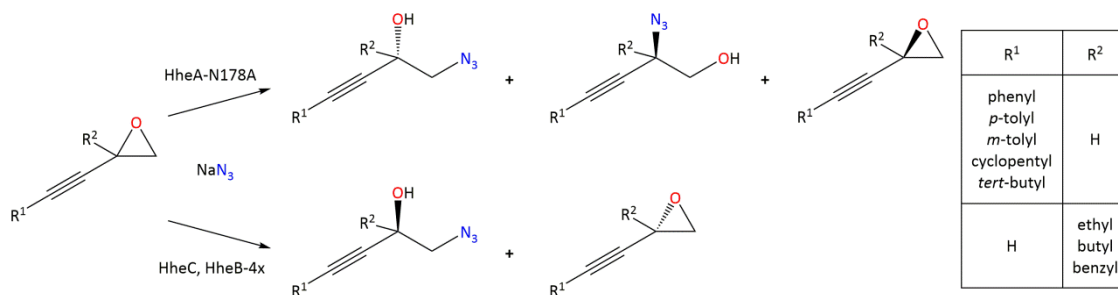
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Propargilni epoksidi i alkoholi, zahvaljujući trostrukoj vezi, podilaze različitim inter- i intramolekulskim transformacijama. [1,2]. Upotrebom halohidrin halogenaza mogu se iz racemičnih propargilnih epoksida pripremiti enantiomerno čisti polazni spojevi za transformacije trostruke veze i/ili nukleofila.[3] Stoga, opisana je priprava mono- i disupstituiranih propargilnih epoksida te enantio selektivno otvaranje prstena halohidrin dehalogenazama (HheC, HheA-N178A and HheB-4x mutant) (shema 1).

Interno propargilni epoksidi pripremljeni su iz odgovarajućih terminalnih acetilena uvođenjem i epoksidacijom dvostruke veze. Također, *p*- and *m*-tolilni derivati pripremljeni su sličnim putem, polazeći od odgovarajućeg jodtoluena i trimetilsililacetilena. 2,2-Disupstituirani epoksidi pripremljeni su iz odgovarajućih metil ketona, njihovim selektivnim  $\alpha$ -halogeniranjem, uvođenjem trostruke veze Grignardovom reakcijom i ciklizacijom Williamsonovog tipa.

Kinetičke rezolucije u prisutnosti natrijevog azida katalizirane su trima HHDH. Reakcijama monosupstituiranih supstrata s HheC i HheA-N178A dobiveni su enantiomerno čisti sekundarni azido alkoholi (*ee* > 99%, *E* > 200). Dok je pomoću HheC dobiven gotovo isključivo (*R*)- $\beta$ -azido alkohol (do 99:1), pomoću HheA-N178A dobiven je uglavnom (*S*)- $\beta$ -azido alkohol ( $\beta$  :  $\alpha$  omjer između 90:10 i 54:46). Kod disupstituiranih epoksida, reakcijama s HheC i HheB-4x tercijski azido alkoholi dobiveni su gotovo regiospecifično ( $\beta$  :  $\alpha$  omjer >99:1) s odličnim do vrlo dobrim enantio selektivnostima (*ee* između >99 i 91%, *E* između >200 i 60).



**Schema 1.** Kinetic resolution of synthesized propargylic epoxides with sodium azide catalysed by halohydrin dehalogenases HHeC, HheA-N178A and HheB-4x mutant

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[3] Z. Findrik Blažević, N. Milčić, M. Sudar, M. Majerić-Elenkov, *Adv. Synth. Catal.* **363** (2021) 388–410.



# KINETIC RESOLUTION OF MONO- AND 2,2-DISUBSTITUTED PROPARGYLIC EPOXIDES

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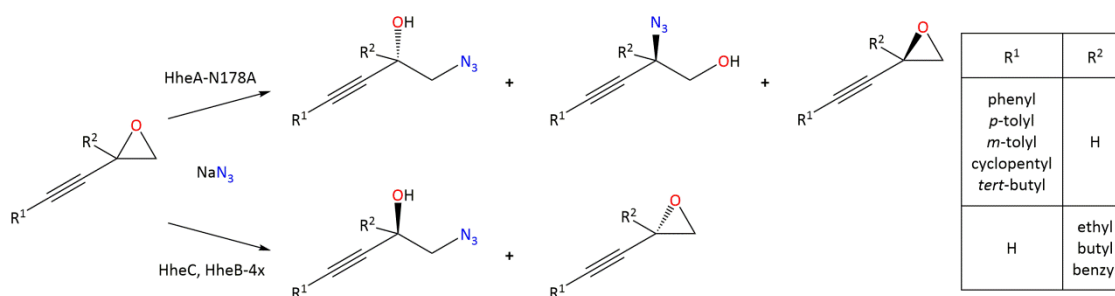
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Propargylic epoxides and alcohols, owing to the presence of a triple bond, undergo various intermolecular and intramolecular reactions. [1,2]. Halohydrin dehalogenases can be used to obtain enantiomerically pure starting compounds from racemic propargylic epoxides for triple bond and/or nucleophile transformations. [3] Therefore, the synthesis of mono- and disubstituted propargylic epoxides and subsequent enantioselective ring opening by halohydrin dehalogenases (HheC, HheA-N178A and HheB-4x mutant) was described (Figure 1).

Internal propargylic epoxides were synthesized from the corresponding terminal acetylenes by introduction and epoxidation of a double bond. Also, *p*- and *m*-tolyl derivatives were synthesized in a similar reaction sequence, starting from the corresponding iodotoluene and trimethylsilylacetylene. 2,2-Disubstituted epoxides were prepared from corresponding methyl ketones, starting with selective  $\alpha$ -halogenation, followed by triple bond introduction through a Grignard reaction and Williamson-type cyclisation.

Kinetic resolution reactions in the presence of sodium azide were catalyzed by three HHDHs. Reactions of monosubstituted substrates with HheC and HheA-N178A yielded enantiomerically pure secondary azido alcohols ( $ee > 99\%$ ,  $E > 200$ ). While HheC yielded almost exclusively (*R*)- $\beta$ -azido alcohol (up to 99:1), HheA-N178A gave mostly (*S*)- $\beta$ -azido alcohol ( $\beta : \alpha$  ratio between 90:10 and 54:46). For disubstituted epoxides, reactions with HheC and HheB-4x yielded tertiary azido alcohols almost regiospecifically ( $\beta : \alpha$  ratio  $>99:1$ ) with excellent to very good enantioselectivities ( $ee$  between  $>99$  and 91%,  $E$  between  $>200$  and 60).



**Figure 1.** Kinetic resolution of synthesized propargylic epoxides with sodium azide catalysed by halohydrin dehalogenases HHeC, HheA-N178A and HheB-4x mutant

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## UTJECAJ ANIONA NA SUPRAMOLEKULSE INTERAKCIJE U *EGZO*-KOORDINIRANIM SREBROVIM KOMPLEKSIMA SA N<sub>2</sub>O<sub>2</sub> MAKROCIKLIČKOM SCHIFF-OVOM BAZOM

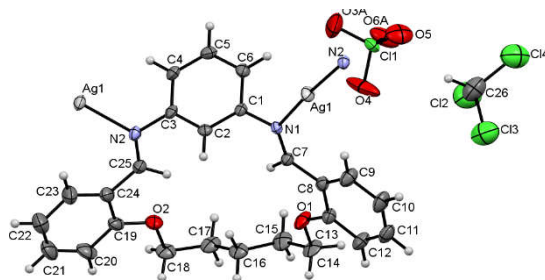
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Metalni ioni mogu se vezati unutar (*endo*) ili izvan (*egzo*) šupljine makrocikla, što najviše ovisi o fleksibilnosti molekule, veličini šupljina te o obliku makrocikličkog liganda [1]. U prethodnim istraživanjima je otkriveno da određena razina dizajniranja liganda za pripremu *egzo*-koordiniranih spojeva može postići prisustvom rigidne C=N veze koja je u blizini aromatskog sustava [2]. *Egzo*-koordinirani makrociklički spojevi su dosta neuobičajeni, ali mogu biti zanimljivi za istraživanje, zbog svoje izvanredne strukturne raznolikosti i potencijalnog stvaranja 3D poroznih struktura. *Egzo*-koordinirani kompleksi s makrociklima se najčešće pripremaju supstitucijom liganda u postojeći *endo*-makrociklički kompleks ili primjenom specifično dizajniranih makrocikličkih liganada. Anioni mogu biti ključan čimbenik u stvaranju kompleksnih spojeva, kao i u konačnom topološkom obliku *egzo*-koordiniranih makrocikličkih spojeva. Otkriveno je da za srebrove(I) oksa-tia makrocikličke koordinacijske spojeve, odabir određenog aniona može uzrokovati stvaranje *egzo*-koordiniranih (PF<sub>6</sub><sup>-</sup> anion) i *endo*-koordiniranih (ClO<sub>4</sub><sup>-</sup> anion) kompleksnih spojeva [3]. Priprema kompleksa izvedena je uz odgovarajući ligand L (L = 1,5-diaza-2,4:7,8:16,17-tribenzo-9,15-dioksa-ciklooktadeka-1,5-dien) i srebrovu sol (AgX; X=ClO<sub>4</sub><sup>-</sup>, PF<sub>6</sub><sup>-</sup>, SbF<sub>6</sub><sup>-</sup> and BF<sub>4</sub><sup>-</sup>) u U-cijevi uz diklormetan i metanol kao otapalo, dok je kao barijera između dva otapala korišten kloroform. Uspješno su riješene 4 nove kristalne strukture. Dobiveni spojevi su karakterizirani IR spektroskopijom (FTIR), elementarnom i termičkom analizom te rendgenskom difrakcijom.



Slika 1. ORTEP prikaz spoja (AgLClO<sub>4</sub>)<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub>

### ZAHVALE:

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# ANION INFLUENCE ON SUPRAMOLECULAR INTERACTIONS IN *EXO*-COORDINATED SILVER(I) COMPLEXES WITH N<sub>2</sub>O<sub>2</sub> SCHIFF BASE MACROCYCLE

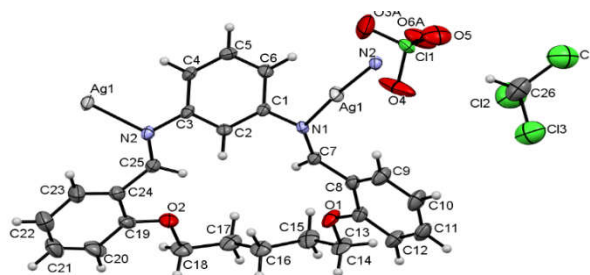
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Metal ion can be bound inside (*endo*) or outside (*exo*) macrocyclic cavity depending upon the flexibility of a molecule, cavity size (interdonor distance), and design of macrocyclic ligand [1]. As we have found in our previous investigations some level of ligand preorganization for the preparation of *exo*-coordinated species can be achieved by the presence of a rigid C=N bond in close proximity to an aromatic system [2]. *Exo*-coordinated macrocyclic species are quite uncommon, but these compounds could become very attractive due to remarkable structural diversity and potential formation of 3D porous structures. *Exo*-coordinated complex compounds with macrocycles are usually prepared by the substitution of stronger ligand in existing *endo*-macrocyclic complex or by an employment of a specifically designed macrocyclic ligand. Anions can also be a major factor in the formation and overall topology of *exo*-coordinated macrocyclic species. It was found for silver(I) oxathia macrocyclic coordination compounds that choice of an anion can induce formation of *exo*-coordinated (presence of ClO<sub>4</sub><sup>-</sup>) and *endo*-coordinated complex (presence of PF<sub>6</sub><sup>-</sup>) [3]. Silver(I) complexes with aza-oxa macrocyclic Schiff bases L (L = 1,5-diaza-2,4:7,8:16,17-tribenzo-9,15-dioxacyclooctadeca-1,5-dien) were prepared by the reaction of the corresponding macrocycle with four different silver salts (AgX; X=ClO<sub>4</sub>, PF<sub>6</sub>, SbF<sub>6</sub> and BF<sub>4</sub>). Four new crystal structures were obtained. Structural characterization was conducted with FTIR spectroscopy, thermal analysis (TGA) and with X-ray diffraction analysis.



**Figure 1.** ORTEP plot of (AgLClO<sub>4</sub>)<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub> with displacement ellipsoids of non-hydrogen atoms drawn at the 50 % probability level.

## ACKNOWLEDGMENTS

This work was carried out with financial support from *Josip Juraj Strossmayer University of Osijek* (Project: UNIOS-ZUP 2018-112).

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# HETEROPOLINUKLEARNI [Mn<sup>II</sup>Cr<sup>III</sup>] KOORDINACIJSKI SPOJEVI S PREMOŠĆUJUĆOM OKSALATNOM SKUPINOM I DIMETILETIL AMONIJEVIM KATIONOM – UTJECAJ N- DONORSKOG LIGANDA

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U posljednje se vrijeme naveliko istražuje protonska vodljivost metal-organskih spojeva, zbog kristaliničnosti i velike poroznosti, kao i mogućnosti dizajniranja i podešavanja njihove strukture i svojstava. Najjednostavnija metoda za uvođenje protonskih nosača je ugradnja protuiona kao što su hidronijevi (H<sub>3</sub>O<sup>+</sup>) odnosno amonijevi kationi [NH<sub>4</sub><sup>+</sup>, (CH<sub>3</sub>)<sub>2</sub>NH<sub>2</sub><sup>+</sup>,...] ili anioni (SO<sub>4</sub><sup>2-</sup>), što rezultira nabijenim spojevima. Protuioni tvore vodikove veze s vodom kao molekulom gosta ili s drugim dijelovima spoja, stvarajući protonski vodljive puteve građene od mreža vodikovih veza. Vodljivost se također može postići uvođenjem funkcionalnih strukturnih komponenata kao što su nekoordinirane skupine u organskim ligandima (e.g., -OH, -NH<sub>2</sub>, -COOH or -SO<sub>3</sub>H) ili koordiniranjem metalnih centara s funkcionalnim molekulama kao što su H<sub>2</sub>O, EtOH i imidazol [1]. Vrlo važnu ulogu u dizajnu i sintezi multifunkcionalnih materijala ima oksalatna skupina, C<sub>2</sub>O<sub>4</sub><sup>2-</sup>, zbog različitih mogućnosti koordiniranja na metalne centre i njezine sposobnosti posredovanja u električnom prijenosu između paramagnetskih metalnih iona. Protonski vodljivi materijali trebaju sadržavati vodu i biti kemijski postojani pod utjecajem vlage i temperature, a oksalatni sustavi općenito imaju uređene strukture i stabilne mreže. Za pripremu heteropolinuklearnih spojeva koristi se pristup "kemija građevnog bloka", u kojem se molekulski anionski ligand, najčešće tris(oksalato)metalatni anion [M<sup>III</sup>(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>]<sup>3-</sup> koristi kao ligand prema drugom metalnom kationu, posebno za dobivanje dvodimenzijских (2D) i trodimenzijских (3D) proširenih sustava. Nadalje, ovi sustavi su uglavnom negativno nabijeni i stoga se mogu kombinirati s funkcionalnim molekulskim kationima kako bi se dobile složene soli u kojima se kooperativni magnetizam kombinira s drugim svojstvom od interesa, posebno električnim [2].

Motivirani prethodnim rezultatima [2,3], priređena su dva nova heterometalna [Mn<sup>II</sup>Cr<sup>III</sup>] koordinacijska spoja koja sadrže premošćujuće oksalatne skupine i alkil-amonijeve katione, u reakcijama u kojima je [Cr(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>]<sup>3-</sup> korišten kao građevni blok prema ionima mangana(II), u prisutnosti dimetiletilamonijevih kationa: trinuklearni [NH(CH<sub>3</sub>)<sub>2</sub>(C<sub>2</sub>H<sub>5</sub>)][{MnCl(H<sub>2</sub>O)(bpy)}<sub>2</sub>Cr(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>] (**1**; bpy = 2,2'-bipiridin) i 2D {[NH(CH<sub>3</sub>)<sub>2</sub>(C<sub>2</sub>H<sub>5</sub>)]<sub>8</sub>[Mn<sub>4</sub>Cl<sub>4</sub>Cr<sub>4</sub>(C<sub>2</sub>O<sub>4</sub>)<sub>12</sub>]}<sub>n</sub>. Uvođenje aromatskog N-donora u reakcijsku smjesu značajno utječe na strukturno uređenje spoja **1**.

## ZAHVALA

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# HETEROPOLYNUCLEAR [Mn<sup>II</sup>Cr<sup>III</sup>] OXALATE-BRIDGED COORDINATION COMPOUNDS WITH DIMETHYLETHYLAMMONIUM CATION – INFLUENCE OF *N*-DONORS LIGAND

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Proton conductivity has recently been regarded as a new functionality of the metal-organic compounds due to their crystallinity and high porosity as well design ability and tunability in structure and property. The simplest method to introduce proton carriers is to include a counterion such as hydronium (H<sub>3</sub>O<sup>+</sup>), ammonium [NH<sub>4</sub><sup>+</sup>, (CH<sub>3</sub>)<sub>2</sub>NH<sub>2</sub><sup>+</sup>,...] or anion (SO<sub>4</sub><sup>2-</sup>), resulting in the charged compounds. The counterions form the hydrogen-bonding with the guest water or other constituents of compound, making proton-conducting pathways composed of hydrogen-bond networks. Conductivity can also be achieved by introducing of functionalized structural components such as non-coordinated groups in organic ligands (e.g., -OH, -NH<sub>2</sub>, -COOH or -SO<sub>3</sub>H) or by coordinating a metal center with functional molecules as H<sub>2</sub>O, EtOH, and imidazole [1].

A very important role in the design and synthesis of multifunctional materials belongs to the oxalate moiety, C<sub>2</sub>O<sub>4</sub><sup>2-</sup>, due to its various possibilities of coordination to metal centres and its ability to mediate electronic effects between paramagnetic metal ions. The proton conductive materials must have good water and chemical durability under the influence of humidity and temperature, and in general, oxalate-based systems have regular structures and stable frameworks. The synthetic strategy for preparation of (hetero)polynuclear species is "building block chemistry", in which a molecular anionic ligand, very often the tris(oxalato)metalate anion [M<sup>III</sup>(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>]<sup>3-</sup>, is used as a ligand towards other metal cation, especially for getting two-dimensional (2D) and three-dimensional (3D) extended systems. Furthermore, these systems are mostly negatively charged and therefore can be combined with functional molecular cations in order to afford complex salts combining cooperative magnetism with a second property of interest, especially electrical ones [2].

Motivated by previous results [2,3], two novel heterometallic [Mn<sup>II</sup>Cr<sup>III</sup>] oxalate-bridged trinuclear [NH(CH<sub>3</sub>)<sub>2</sub>(C<sub>2</sub>H<sub>5</sub>)][{MnCl(H<sub>2</sub>O)(bpy)}<sub>2</sub>Cr(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>]} (**1**; bpy = 2,2'-bipyridine) and 2D {[NH(CH<sub>3</sub>)<sub>2</sub>(C<sub>2</sub>H<sub>5</sub>)]<sub>8</sub>[Mn<sub>4</sub>Cl<sub>4</sub>Cr<sub>4</sub>(C<sub>2</sub>O<sub>4</sub>)<sub>12</sub>]}<sub>n</sub> coordination compounds with cationic alkyl ammonium components were obtained in reactions in which [Cr(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>]<sup>3-</sup> in the presence of dimethylethylammonium cations was used as a building block towards manganese(II) ion. The introduction of an aromatic *N*-donor into the reaction mixture significantly affects the structural arrangement of compound **1**.

## ACKNOWLEDGMENTS

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## RAČUNALNA EVALUACIJA NOVIH KANDIDATA ZA CJEPIVO PROTIV GRIPE

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Po principu racionalnog dizajna odgovarajući kandidat za cjepivo protiv određenog patogena trebao bi uspješno inkorporirati antigen, sustav isporuke i adjuvant, što bi rezultiralo izazivanjem predvidljivog imunološkog odgovora [1]. Nukleoprotein (NP) RNA virusa je najimunogeničniji strukturni virusni protein (antigenska funkcija) i ima ključnu ulogu u zaštiti i prezentaciji virusnog genoma tijekom transkripcije i replikacije. Sadrži RNA-vezujuću i oligomerizacijsku domenu te formira filamentoznu česticu u nano veličini (funkcija isporuke i adjuvantna funkcija) [2]. Naš pristup razvoja novog cjepiva uključuje stvaranje rekombinantnih proteina umetanjem prirodnih i/ili dizajniranih epitopa virusnih patogena u strukturu nukleoproteina virusa humane parainfluence tipa 2 (HPIV2). Nukleoprotein se koristi kao neinfektivni nosač antigenskih determinanti. Korištenjem računalnih metoda istraživali smo konformacijsko ponašanje epitopa virusa gripe fuzioniranih unutar rekombinantnog HPIV2 NP [3]. Odabrano je nekoliko epitopa virusa gripe, što uključuje epitope domene šiljka proteina hemaglutinina i epitope cijele ekstracelularne domene proteina M2. HPIV2 NP ima najveću aminokiselinsku sličnost s nukleoproteinom virusa parainfluence tipa 5 (PIV5 NP) za koji je poznata 3D struktura. Komparativnim modeliranjem dobiven je model 3D strukture HPIV2 NP, korištenjem PIV5 NP strukture za predložak. Analizom strukturnog modela HPIV2 NP, odabrano je pet mjesta za ugradnju epitopa, s najvećom izloženosti za interakciju s imunološkim sustavom. Komparativnim modeliranjem stvoreni su i modeli 3D struktura svakog od fuzioniranih proteina. Simulacija molekularne dinamike omogućila je bolji uvid u konformacijsku dinamiku umetnutih epitopa i općenito u stabilnost proteinske strukture. *In silico* eksperiment pokazao je da globalni nabor ostaje stabilan tijekom simulacije u svim strukturama. Računalne metode su dokazano vrijedne tijekom pretraživanja i razvoja najpogodnijih kandidata za cjepivo.

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## COMPUTATIONAL EVALUATION OF THE NEW VACCINE CANDIDATES AGAINST INFLUENZA INFECTION

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Following the rational design premises, suitable vaccine candidates protecting against a particular pathogen should successfully integrate antigen, delivery system, and adjuvant functions to elicit a predictable immune response [1]. The RNA virus nucleoprotein (NP) is the viral most immunogenic structural protein (antigen function), which plays an essential role in protecting and presenting the viral genome during transcription and replication. It comprises the RNA binding and oligomerization domains, forming filament shape nano-sized particles (delivery system and adjuvant function) [2]. In our approach, new vaccine development involves creating recombinant protein(s) by incorporating natural and/or designed epitopes of the viral pathogens into an NP structure from human parainfluenza virus type 2 (HPIV2) using it as an adaptive noninfectious carrier of antigenic determinants. Here, we investigate the conformational behaviour of the incorporated influenza epitopes within the fused recombinant protein using computational methods [3]. Several influenza epitopes are selected, including those from the stalk domain of the hemagglutinin (HA) protein and the whole extracellular domain of the M2 protein. HPIV2 NP has the highest amino acid sequence similarity with nucleoprotein of parainfluenza virus type 5 (PIV5) for which 3D structure is known. Comparative modelling was carried out to obtain HPIV2 NP 3D structure model using PIV5 NP structure as a template. Analyzing the HPIV2 NP structure model, five spots were selected for inserting pathogen epitopes, maximizing their exposure for interaction with the protective immune system. The same comparative modelling approach was employed to obtain 3D structures for each of the fused proteins. Molecular dynamics (MD) simulations analysis provided closer insight into conformational dynamics behaviour of the inserted epitope and overall protein structure stability. In silico study showed that the global fold remained stable during simulation in all predicted structures. Computational methods are proven valuable in the screening step toward developing the most promising vaccine candidates.

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## MAGNETIZAM Cu(II)-MOF-74 I NJEGOVA TRI MIJEŠANA ANALOGNA SPOJA S Zn(II)

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Metallo-organske mreže (metal-organic frameworks, MOFs) intenzivno se istražuju zbog potencijalne primjene u ionskoj izmjeni, dostavi lijekova, pohrani vodika, razdvajanju smjese plinova, itd. Ovi su porozni koordinacijski polimeri građeni od metalnih iona/klastera koji predstavljaju čvorove mreže te organskih liganada koji predstavljaju veze između njih. [1] M(II)-MOF-74 materijali, gdje je M(II) metalni ion prijelaznih elemenata, istraživani su zbog moguće primjene kao posljedice postojanja otvorenih metalnih mjesta i dobro definiranih velikih pora u sačastoj strukturi, kao i zbog njihovih zanimljivih magnetskih svojstava koja proizlaze iz složene povezanosti metalnih iona. Nadalje, adsorpcija, stabilnost i katalitička aktivnost M1(II)-MOF-74 povećava se djelomičnim uvođenjem drugog metalnog centra M2(II) u njegovoj strukturi, tj. postojanjem heterometalnih čvorova.[2] Istraživali smo magnetska svojstva bakra(II)-MOF-74 (Cu-MOF-74) te njegova tri miješana analogna spoja s cinkom(II). CuZn-MOF-74 i ZnCu-MOF-74 dobiveni su korištenjem različitih intermedijera prilikom sinteze: magnetskog Cu-INT i nemagnetskog Zn-INT. Alloyed-MOF-74 dobiven je mehanokemijskim miješanjem amorfizirane smjese Cu-MOF-74 i Zn-MOF-74 koji su uslijed ubrzanog starenja zajedno izrasli u MOF-74.[3,4] Magnetska su svojstva ovih spojeva istražena mjerenjem magnetizacije, spektroskopijom elektronske paramagnetske rezonancije (EPR) u X-području, višefrekventom EPR (HF-EPR) spektroskopijom kao i teorijskim modelima.

### ZAHVALE

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## MAGNETISM OF Cu(II)-MOF-74 AND ITS THREE MIXED ANALOGOUS COMPOUNDS WITH Zn(II)

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Metal-organic frameworks (MOFs) are the subject of intensive study due to their potential applications in ion exchange, drug delivery, hydrogen storage, gas separation, etc. These porous coordination polymers are comprised of metal ions/clusters that represent framework nodes and organic ligands as bonds between them. [1] M(II)-MOF-74 materials, where M(II) is a transition metal center, are widely researched for applications related to open metal sites, well-defined large channels in their honeycomb structure and also for their interesting magnetic properties arising from the complex connectivity of metal ions. Furthermore, it was shown that adsorption, stability and catalytic activity of M1(II)-MOF-74 were increased by the partial incorporation of a second metal center M2(II) in its structure, i.e. the existence of heterometal nodes.[2] We investigated magnetic properties of copper(II)-MOF-74 (Cu-MOF-74) and its three mixed analogous compounds with zinc(II). CuZn-MOF-74 and ZnCu-MOF-74 were obtained through different intermediate complexes, from magnetic Cu-INT and non-magnetic Zn-INT, respectively. The Alloyed-MOF-74 was obtained by mechanical alloying, growing the MOF-74 structure from mechanically amorphized Cu-MOF-74 and Zn-MOF-74 mixture by accelerated aging process.[3,4] Magnetic properties of these compounds were studied by static magnetization measurements, X-band electron paramagnetic resonance (EPR) spectroscopy, multifrequency high-field high-frequency EPR (HF-EPR) spectroscopy as well as theoretical models.

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## DIZAJN I KARAKTERIZACIJA AZODIOKSIDNIH VIŠESLOJEVA NA POVRŠINI ZLATA

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Adsorpcija organskih molekula na metalne površine i njihovo slaganje u uređene samoudružene jednoslojeve (engl. *self-assembled monolayers*, SAM) i/ili višeslojeve omogućuje dizajn različitih funkcionalnih materijala sa širokom potencijalnom primjenom. Aromatski C-nitrozo spojevi mogu se međusobno povezivati preko azodioksidnih veza u dimerne i polimerne strukture. Nedavna istraživanja su pokazala da adsorpcijom aromatskih C-nitrozo derivata na površinu zlata (111) preko veze S-Au dolazi do reakcija nitrozo skupina na međupovršini jednosloja sa slobodnim nitrozo molekulama u otopini i nastajanja azodioksidnih dvoslojeva [1–3].

U ovom radu ispitana je mogućnost nastanka samoudruženih azodioksidnih višeslojeva na površini zlata (111) korištenjem polimerizacijskih svojstava aromatskih dinitrozo spojeva. Azodioksidni višeslojevi pripremljeni su uranjanjem supstrata zlata (111), na koji je prethodno vezan molekularni jednosloj derivata nitrozobenzena preko tiocijanatne funkcionalne skupine [3], u otopinu odgovarajućeg dinitrozo derivata poznate koncentracije na određeno vrijeme. Kako bi se ispitaio utjecaj strukturnih i eksperimentalnih parametara, sintetizirani su različiti dinitrozo derivati te su korištena različita vremena adsorpcije za pripremu višeslojeva. Morfološke karakteristike i debljina azodioksidnih višeslojeva na površini zlata (111) istražene su mikroskopijom atomskih sila (engl. *atomic force microscopy*, AFM) te elipsometrijom.

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## DESIGN AND CHARACTERIZATION OF AZODIOXY MULTILAYERS ON GOLD SURFACE

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Adsorption of organic molecules on metal surfaces and their arrangements into ordered self-assembled monolayers (SAM) and/or multilayers offer a way for design of various functional materials with wide potential applications. Aromatic C-nitroso compounds can interact through azodioxy bonds to form dimeric and polymeric structures. Recent studies indicated that adsorption of aromatic C-nitroso derivatives on gold (111) surface through S–Au bond leads to interactions of nitroso groups at the monolayer interface with free nitroso molecules in solution and formation of azodioxy bilayers [1–3].

Here we investigated the possibility of the formation of azodioxy multilayers on gold (111) surface by using the polymerization properties of aromatic dinitroso compounds. Azodioxy multilayers were prepared by immersing the gold (111) substrate, to which the molecular monolayer of the nitrosobenzene derivative was previously attached through thiocyanate functional group [3], in a solution of the corresponding dinitroso derivative of known concentration for a defined time. To examine the influence of structural and experimental parameters, different dinitroso derivatives were synthesized and various adsorption times were used to prepare multilayers. Morphological characteristics and thickness of azodioxy multilayers on gold (111) surface were studied by atomic force microscopy (AFM) and ellipsometry.

### ACKNOWLEDGMENTS

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# SAMOUDRUŽIVANJE NUKLEINSKIH BAZA POBOLJŠANIM UZORKOVANJEM MD: RAZUMIJEVANJE BIOLOŠKE TVARI IZ PERSPEKTIVE MOLEKULSKIH GRADIVNIH BLOKOVA

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Svojstva molekularnih sustava, poput agregata u otopinama ili molekularnih kristala, kontrolirana su međumolekularnim interakcijama. U ovom kontekstu, primjer su optička svojstva. Evolucija optičkih odziva, od izolirane molekule do supramolekularnog udruživanja i molekularnog kristala, izravno je povezana s interakcijama između gradivnih jedinica, a to će uvelike ovisiti o strukturnom obilježju meke materije [1]. S ciljem procjene svojstava (bioloških) materijala, fokus ovog rada je na strukturnim svojstvima udruživanja nukleinskih baza u vodi. Štoviše, nukleinske baze i njihovo specifično sparivanje predstavljaju paradigmu za formiranje većih sustava poput nukleinskih kiselina [2]. Hipoteza se, u kontekstu prebiotičke kemije, bazira na odabiru kojeg diktira prirodni odabir, u smislu strukturnih svojstava kao i stabilnosti prilikom izlaganja UV svjetlosti. Modeliranje može pomoći u razumijevanju relacija između navedenih elemenata, iz fundamentalne i mikroskopske perspektive.

Računalno predviđanje strukture molekularnog samoudruživanja nije jednostavno i moramo se osloniti na tehnike poboljšanog uzorkovanja. Istražena su formiranja  $N$ -mera ( $N=2-8$ ) molekularnih udruženja u vodi koristeći metadinamiku s klasičnim potencijalom. Korištene su dvije kolektivne varijable definirane kao koordinacijski broj za pi interakcije i Watson-Crick vodikove veze između jedinica [3]. Preliminarni rezultati pokazuju ključnu ulogu ove dvije kolektivne varijable za dobivanje strukture udruženja nukleinskih baza.

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# NUCLEOBASE ASSEMBLIES VIA ENHANCED-SAMPLING MD: UNDERSTANDING BIOLOGICAL MATTER FROM ITS MOLECULAR BUILDING BLOCKS

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The properties of molecular systems, such as aggregates in solutions or molecular crystals, are controlled by interactions between the molecular units. In this context, an example is optical properties. The evolution of the optical responses from the isolated molecule to a supramolecular assembly and large crystal is directly connected to the interactions between the units, which in turn depends on the structural key feature of the soft matter [1]. With the aim of assessing the properties of (biological) material, we have focused on the structural properties of nucleobases assemblies in water. Besides, nucleobases and their specific base pairing is the paradigm of molecular recognition in nature [2]. The hypothesis, in the context of prebiotic chemistry, focuses on the selection operated by nature, in terms of their structural properties, but also in respect of their stability under UV light. Modeling techniques can help in understanding the relationship between these elements from a fundamental and microscopic perspective.

The computational prediction of the molecular assemblies structure is not an easy task and we must rely on the usage of enhanced sampling techniques. We have explored the formation of  $N$ -mer ( $N=2-8$ ) assemblies in water by combining metadynamics with classical potential. We have biased two collective variables defined as coordination numbers for  $\pi$ -stacking and Watson-Crick hydrogen bonding [3]. Our preliminary results show the crucial role of these two collective variables for obtaining the structure and properties of nucleobase assemblies.

## ACKNOWLEDGMENTS

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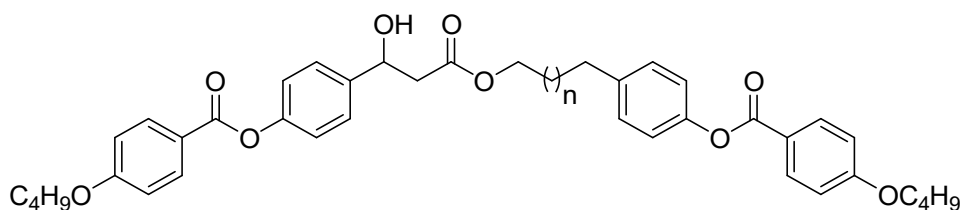
## EFEKT DULJINE RAZMAKNICE NA MEZOGENA SVOJSTVA DIMERA SVIJENE GEOMETRIJE

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Pojam tekućih kristala (LC) odnosi se na tvari koje uz tri osnovna agregacijska stanja posjeduju i stanje u kojem molekule imaju veći stupanj uređenosti i orijentacije od onog u tekućinama, ali manji od onog u kristalima [1]. Takvo stanje nazvano je mezofazom, a spojevi koji se organiziraju u različite mezofaze mezogenima. LC dimeri sastoje se od dviju mezogenih jedinica povezanih fleksibilnom razmaknicom čiji paritet utječe na geometriju i mezogena svojstva molekule [2]. Kod dimera s neparnom duljinom razmaknice mezogene jedinice su međusobno nagnute što rezultira svijenom geometrijom molekule, a takvi dimeri su posebno zanimljivi jer se mogu organizirati u različite helikalne strukture [3]. U fokusu našeg istraživanja su dimeri koji u svojoj strukturi sadrže 3-hidroksi propanoatni ester kao dio razmaknice, a razlikuju se po duljini i paritetu razmaknice (slika 1). U radu će biti prezentiran utjecaj razmaknice na mezogena svojstva pripremljenih dimera.



Slika 1. Općenita struktura dimera svijene geometrije s različitom duljinom razmaknice.

### ZAHVALE

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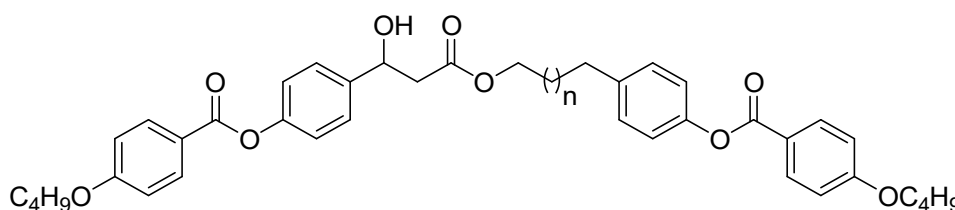
# THE EFFECT OF SPACER LENGTH ON MESOGENIC PROPERTIES OF BENT-SHAPED DIMERS

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The term liquid crystals (LC) refers to substances that, in addition to the three basic aggregation states, also have a state in which molecules have a higher degree of order and orientation compared to liquids, but less compared to crystals [1]. Such a condition is called the mesophase, and compounds that organize into different mesophases are called mesogens. LC dimers consist of two mesogenic units connected by a flexible spacer whose parity affects the geometry and mesogenic properties of the molecule [2]. In dimers with an odd spacer length, mesogenic units are inclined to each other, resulting in a bent-shaped geometry of the molecule, and such dimers are particularly interesting because they can be organized into different helical structures [3]. In the focus of our research are dimers that contain 3-hydroxy propanoate ester moiety in the spacer, and differ in the length and parity of the spacer (Figure 1). The influence of the spacer length on the mesogenic properties of the prepared dimers will be discussed.



**Figure 1.** General structure of bent-shaped dimers having different spacer length.

## ACKNOWLEDGMENTS

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## DIMERNI KLORIDNI PALADOCIKLI AZOBENZENA KAO KATALIZATORI SUZUKI-MIYAURA REAKCIJE U ČVRSTOM STANJU

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Metalociklički spojevi su najčešće korišteni katalizatori za procese unakrsnog spajanja kao što su Suzuki-Miyaura, Mizoroki-Heck i Sonogashira reakcije [1]. Njihova svojstva, primjenjivost i katalitički potencijal uvelike ovise o specifičnom okruženju metalnog centra i strukturnim svojstvima liganada poput prisutnosti elektron-donorskih i/ili elektron-akceptorskih supstituenata [2]. Razvoj novih katalizatora uglavnom se temelji na promjeni liganada vezanih na metalni centar, dok je utjecaj svojstava pojedinog liganada na reaktivnost katalizatora često neistražen [3]. Nadalje, novosintetizirani katalizatori često sadrže velike i složene ligande, koji smanjuju topljivost katalizatora u većini otapala. U tom aspektu, uvođenjem protokola za organsku sintezu u čvrstom stanju omogućuje se alternativni, ekološki prihvatljiv i održiv pristup koji zaobilazi korištenje otapala i izbjegava probleme vezane uz topljivost katalizatora i/ili reaktanata [4].

U ovom istraživanju provedena je Suzuki-Miyaura reakcija unakrsnog spajanja 4-bromazobenzena i fenilboronske kiseline kao arilacijskog reagensa u čvrstom stanju katalizirana s nekoliko azobenzenskih paladocikla bez fosfinskih liganada dimerne strukture s premošćujućim kloridnim ligandima. Istražen je utjecaj različitih 4,4'-supstituenata azobenzenskog liganada na katalitička svojstva paladocikličkih spojeva. Sve reakcije su praćene Ramanovom spektroskopijom *in situ* čime je omogućen uvid u katalitičku učinkovitost dimernih monopaladiranih kompleksa azobenzena.

### ZAHVALE

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# DIMERIC CHLORIDE AZOBENZENE PALLADACYCLES AS CATALYSTS FOR SOLID-STATE SUZUKI-MIYAURA REACTION

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Metalacyclic complexes are the most commonly used catalysts for cross-coupling processes, such as Suzuki-Miyaura, Mizoroki-Heck, and Sonogashira reactions [1]. Their properties, applicability, and catalytic potential largely depend on the specific surrounding of the metal center and structural properties of coordinating ligands, such as the presence of electron-donating or electron-withdrawing substituents [2]. In the development of new catalysts, different ligands were usually used. However, the correlation between ligand properties and reactivity often remained unexplored [3]. Moreover, newly synthesized catalysts often contain bulky ligands that reduce their solubility in most solvents. Therefore, the introduction of solid-state methods in organic synthesis represents an alternative, environmentally friendly, and sustainable method that bypasses the use of solvents and avoids problems with the solubility of catalysts or reactants [4].

In this work, we report a solid-state Suzuki-Miyaura cross-coupling reaction between 4-bromoazobenzene and phenylboronic acid as an arylation agent catalyzed by several phosphine-free azobenzene palladacycles with dimeric structure and bridging chloride ligands. The influence of different 4,4'-substituents of the azobenzene ligand on catalytic properties of palladacycles was investigated. All reactions were monitored by *in situ* Raman spectroscopy, which provided insight into the catalytic efficiency of the dimeric azobenzene monopalladated complexes.

## ACKNOWLEDGMENTS

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## ZAŠTITA KORODIRANIH ČELIČNIH POVRŠINA OD ATMOSFERSKE KOROZIJE PRI POVIŠENOJ TEMPERATURI

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Atmosferska korozija odvija se u vodenom kondenzatu koji nastaje na metalnoj površini zbog vlažnosti zraka i ima svojstva elektrolita. Kako bi umanjili ili čak spriječili atmosfersku koroziju u zatvorenim sustavima za zaštitu koristimo inhibitore u parnoj fazi (VCI). Hlapivi inhibitori su čvrste organske tvari čijim se parama zasićuje atmosfera ili drugi plin. Takvim zasićenjem atmosfere u kojoj je prisutan čelik dolazi do adsorpcije inhibitora na njegovoj površini i nastajanju filma, koji usporava proces anodne ili katodne korozije [1]. U praksi VCI su korišteni za zaštitu obe površine korodirale i čiste. Međutim, istraživanja učinkovitosti VCI uglavnom se provode na čistoj površini. Ovo istraživanje ima za cilj poboljšati razumijevanje utjecaja dva komercijalna praškasta hlapiva inhibitora na korodiralnoj površini ugljičnog čelika. Ispitivanja su provedena elektrokemijskim metodama EIS (elektrokemijska impedancijska spektroskopija) i Tafelovom polarizacijom u dva korozivna medija (kisela kiša pH=6.5; vodena otopina 1% NaCl) pri 40°C s dva komercijalna inhibitora pri različitim koncentracijama. Korodirala površina ugljičnog čelika dobivena je nakon sedmodnevnog naizmjeničnog uranjanja u otopinu umjetne kiše (pH=8) i sušenja. Na temelju dobivenih elektrokemijskih odziva ugljičnog čelika može se zaključiti da je učinkovitost inhibitora bolja u kiseljoj kiši (pH = 6,5), nego u vodenoj otopini 1% NaCl.



Slika 1. Izgled uzorak ugljičnog čelika prije ispitivanja

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# PROTECTION OF CORRODED STEEL SURFACES FROM ATMOSPHERIC CORROSION AT ELEVATED TEMPERATURE

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Atmospheric corrosion takes place in water condensate, which is formed on a metal surface, due to the humidity of the air, and has the properties of electrolytes. In order to reduce or even prevent atmospheric corrosion in closed systems, volatile corrosion inhibitors (VCI) are used. Volatile inhibitors are solid organic substances whose vapors saturate the atmosphere or other gas. Such saturation of the atmosphere in which steel is present leads to inhibitor adsorption on its surface and the formation of a film, that slows down anodic or cathodic corrosion process [1]. In practice VCI are applied for protection of both bare and corrodes surfaces. However, research on VCI efficiency is mainly conducted on bare surface. This study aims to improve the understanding of the influence of two commercial powder volatile inhibitors on the corroded surface of carbon steel. The tests were performed by electrochemical methods EIS (electrochemical impedance spectroscopy) and Tafel polarization in two corrosion media (acid rain pH = 6.5; aqueous solution of 1% NaCl) at 40°C with two commercial inhibitors at different concentrations. The corroded carbon steel surface was obtained after seven days of alternating immersion in simulated rain solution (pH = 8) and drying. Based on the obtained electrochemical responses of carbon steel in blank and inhibited corrosive solutions it can be concluded that effectiveness of studied corrosion inhibitors is better in acid rain (pH = 6.5) than in aqueous solution of 1% NaCl.



Figure 1. Appearance of carbon steel sample before testing

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## ISTRAŽIVANJE UTJECAJA UREE NA STRUKTURU G-KVADRUPLEKSA SPEKTROSKOPIJOM POVRŠINSKI POJAČANOG RAMANOVOG RASPRŠENJA

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G-Kvadrupleksi (G4) su uređene strukture nukleinskih kiselina koje nastaju svijanjem ponavljajućih DNA i RNA sljedova bogatih gvaninom. Građeni su od gvaninskih tetraada koje čine četiri baze gvanina međusobno povezane Hoogsteenovim vodikovim vezama, koje se slažu jedna iznad druge interakcijama  $\pi$ -slaganja i tvore četverolančane spiralne strukture. Poznate su različite topologije G4 struktura ovisno o duljini i sastavu baza nukleinskog slijeda te prisutnosti kemijskih vrsta koje ih stabiliziraju, kao što su  $\text{Na}^+$  i  $\text{K}^+$  ioni [1]. Natrijevi ioni koordinirani su karbonilnim kisikovim atomima gvanina u središnjoj šupljini G-kvadrupleksa te na taj način stabiliziraju nastalu strukturu. Za razliku od alkalijskih iona, urea je dobro poznato denaturirajuće sredstvo i koristi se desetljećima u istraživanju proteina i njihovih struktura budući da utječe na ravnotežu između razmotane i uređene strukture. G4 su globularnog oblika te imaju manji naboj u usporedbi s drugim nukleinskim kiselinama, što ih čini sličnima globularnim proteinima [2]. Cilj istraživanja bio je istražiti utjecaj uree na strukturu G4. U tu svrhu primijenjena je spektroskopija površinski pojačanog Ramanovog raspršenja (SERS) koja se temelji na pojačanju raspršenog zračenja prilikom adsorpcije ili kemijskog vezanja analita na nanostrukturiranu površinu metala [3]. SERS spektroskopija je korištena za istraživanje strukture humanog telomernog slijeda d[TTAGGG]<sub>4</sub> (Tel24) u odsutnosti i prisutnosti stabilizirajućih  $\text{Na}^+$  iona (100 mM NaCl) te nakon dodatka uree. U SERS spektrima bez  $\text{Na}^+$  iona uočene su karakteristične vrpce nasumične strukture Tel24 [4]. Spektri snimljeni nakon zagrijavanja otopine Tel24 s  $\text{Na}^+$  ionima potvrđuju očekivanu prisutnost uređene strukture G-kvadrupleksa. Omjeri intenziteta karakterističnih vrpce koje potječu od vibracija disanja prstenova adenina i gvanina dokazuju prisutnost savijenog telomernog slijeda na površini nanočestica srebra. Dodatak uree u sustav potiče razaranje uređene strukture G4 što je jasno vidljivo iz SERS spektara koji nalikuju spektrima nasumične strukture Tel24.

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# STUDY OF UREA INFLUENCE ON G-QUADRUPLEX STRUCTURE BY SURFACE-ENHANCED RAMAN SCATTERING SPECTROSCOPY

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G-Quadruplexes (G4) are ordered structures of nucleic acids that can form within repetitive guanine-rich DNA or RNA strands. They are built of G-quartets which are planar structures consisted of four guanine bases connected by Hoogsten hydrogen bonds that are stacked on top of each other by  $\pi$ -stacking interactions, forming four-chain spiral structure. Different topologies of G4 are known depending on the length and composition of the nucleic base sequence and the presence of stabilizing chemical species, such as Na<sup>+</sup> and K<sup>+</sup> ions [1]. Sodium ions are coordinated by carbonyl oxygen atoms of guanine in the central cavity of G-quadruplex and thus stabilize the resulting structure. Unlike alkali ions, urea is a well-known denaturing agent and has been used for decades in the study of proteins and their structures, given that it affects the equilibrium between unfolded and folded structure. G4 have globular shape and are less charged compared to other nucleic acids, that makes them similar to globular proteins [2]. The research objective was to investigate the effect of urea on the structure of G4. For this purpose, surface-enhanced Raman scattering (SERS) spectroscopy was applied, which is based on amplification of scattered radiation during adsorption or chemical binding of analytes to a nanostructured metal surface [3]. SERS spectroscopy was used to investigate the structure of human telomeric sequence d[TTAGGG]<sub>4</sub> (Tel24) in the absence and presence of stabilizing Na<sup>+</sup> ions (100 mM NaCl) as well as after addition of urea. Characteristic marker bands of unfolded Tel24 structure were observed in the SERS spectra without Na<sup>+</sup> ions [4]. After heating Tel24 with Na<sup>+</sup> ions the SERS spectra confirmed the expected presence of ordered G-quadruplex. The intensity ratios of the bands originating from breathing vibrations of adenine and guanine rings proved the presence of the folded telomeric sequence on the surface of silver nanoparticles. Addition of urea induces destruction of the ordered G4 structure, which was clearly visible from the SERS spectra looking like the spectra of unfolded Tel24.

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## POBOLJŠANJE RAZGRADNJE CIPROFLOKSACINA, UZROKOVANE SUNČEVIM ZRAČENJEM, FOTODEPOZICIJOM SREBRA NA POVRŠINU IZOTIPIČNOG HOMOPOVEZANOG BiVO<sub>4</sub>

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BiVO<sub>4</sub> je privukao pažnju istraživača kao jedan od obećavajućih fotokatalizatora u vidljivom spektru zračenja, zbog širine svoje zabranjene zone ( $\approx 2,4$  eV) i odgovarajuće structure vodljive i valentine vrpce za oksidaciju vode. Međutim, visoka stopa rekombinacije foto-generiranih parova elektron-šupljina kao i kratka difuzija nositelja naboja mogu dovesti do niske fotokatalitičke učinkovitosti BiVO<sub>4</sub>. Ova ograničenja mogu se prevladati korištenjem različitih strategija, uključujući dizajn izotopičnog homopovezivanja, tj. spoja između dvije različite kristalne faze istog poluvodičkog materijala. Takva struktura može učinkovito potisnuti proces rekombinacije naboja poboljšavajući fotokatalitičku aktivnost.

U ovoj studiji sintetiziran je izotopični homopovezani (monoklinsko-tetragonalni) BiVO<sub>4</sub>, a zatim modificiran tehnikom fotodepozicije korištenjem različitih udjela nanočestica Ag. Pripremljeni Ag-BiVO<sub>4</sub> materijal karakteriziran je pomoću SEM, XRD, FTIR, BET i UV-vis spektra. Utjecaj nanočestica Ag na fotoelektrokemijsku aktivnost BiVO<sub>4</sub> sustavno je evaluiran. Osim toga, elektrokemijska impedancijska spektroskopija (EIS) je provedena za sučelje poluvodič/elektrolit. Rezultati su otkrili da se otpor prijenosa naboja smanjio pod osvjetljenjem za sve pripremljene materijale, a Ag-BiVO<sub>4</sub> je pokazao niži otpor prijenosa naboja u usporedbi s netaknutim izotopičnim homopovezanim BiVO<sub>4</sub> materijalom. Fotokatalitička aktivnost pripremljenih materijala ispitana je praćenjem razgradnje ciprofloksacina (CIP) kao modelne organske onečišćujuće tvari pod Sunčevim zračenjem unutar različitog pH raspona (4 do 8) korištenjem Ag-BiVO<sub>4</sub> s različitim udjelom Ag fotodeponiranog (1 do 15%) i dodatkom H<sub>2</sub>O<sub>2</sub> (5 do 30 puta veće koncentracije od CIP-a) u usporedbi s netaknutim izotopičnim homopovezanim BiVO<sub>4</sub>.



## ENHANCEMENT OF SOLAR DRIVEN DEGRADATION OF CIPROFLOXACIN BY PHOTODEPOSITED Ag ONTO ISO-TYPE HOMOJUNCTION BiVO<sub>4</sub> SURFACE

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BiVO<sub>4</sub> has attracted attention of researchers as one of the promising candidates for visible-light photocatalysis due to its suitable bandgap ( $\approx 2.4$  eV) and appropriate band structure for the water oxidation. However, the high recombination rate of photo-generated electron-hole pairs as well as short diffusion of charge carriers may lead to low photocatalytic efficiency of BiVO<sub>4</sub>. These limitations may be overcome employing various strategies, including a design of iso-type homojunction, i.e. a junction between two different crystal phases of the same semiconducting material. Such structure can effectively suppress the charge recombination process improving the photocatalytic activity.

In this study an iso-type homojunction (monoclinic-tetragonal) BiVO<sub>4</sub> material was synthesized and then modified employing photodeposition technique using different amounts of Ag nanoparticles. The as-prepared Ag-BiVO<sub>4</sub> has been characterized using SEM, XRD, FTIR, BET, and UV-vis spectra. The impact of Ag nanoparticles on the photoelectrochemical activity of BiVO<sub>4</sub> has been systematically evaluated. In addition, electrochemical impedance spectroscopy (EIS) has been conducted for the semiconductor/electrolyte interface. The results revealed that the charge transfer resistance decreased under illumination for all as-prepared materials, and Ag-BiVO<sub>4</sub> exhibited smaller charge transfer resistance compared to pristine iso-type homojunction BiVO<sub>4</sub> material. The photocatalytic activity of as-prepared materials was examined by monitoring the degradation of ciprofloxacin (CIP) as a model organic contaminant under solar irradiation within different pH range (4 to 8) employing Ag-BiVO<sub>4</sub> with different Ag content photo-deposited (1 to 15%) and concentration of H<sub>2</sub>O<sub>2</sub> (5 to 30 times the concentration of CIP) and compared to that of pristine iso-type homojunction BiVO<sub>4</sub>.



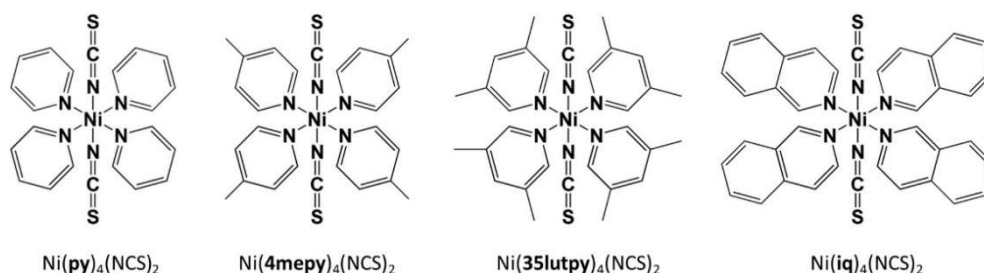
## WERNEROVI KOORDINACIJSKI SPOJEVI KAO GRAĐEVNE JEDINICE U KOKRISTALIMA TEMELJENIM NA HALOGENSKIM VEZAMA

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Wernerovi koordinacijski spojevi su skupina spojeva opće formule  $MX_2L_4$ , gdje se M odnosi na dvovalentni metalni centar, X na izotiocijanatni, cijanatni, cijanidni ili nitratni ion, dok je L supstituirani piridin ili  $\alpha$ -arilalkilamin [1,2]. Korištenje takvih koordinacijskih spojeva kao akceptora halogenske veze vrlo je slabo istraženo [3]. Pretraživanjem kristalografske baze Cambridge Structural Database (CSD) s motivom halogenske veze  $I \cdots S$  u kokristalima koji sadrže izotiocijanatne Wernerove koordinacijske pronađeno je samo četiri skupa podataka [4]. Kako ne postoji sistematično istraživanje halogenske veze u sustavima s takvim građevnim jedinicama, cilj ovog istraživanja bio je istražiti potencijal izotiocijanatnog sumpora kao akceptora halogenske veze korištenjem serije Wernerovih koordinacijskih spojeva. Sintetizirani su izotiocijanatni koordinacijski spojevi koji sadrže niklov metalni centar, ali različiti tip piridinskog liganda kako bi se proučio i sterički utjecaj na ostvarivanje halogenskih veza: piridin (**py**), 4-metilpiridin (**4mepy**), 3,5-lutidin (**35lut**) i izokinolin (**iq**) (slika 1). Kompleksi su zatim kokristalizirani s četiri različita perhalogenirana donora halogenske veze: 1,2-dijodtetrafluorbenzenom, 1,3-dijodtetrafluorbenzenom, 1,4-dijodtetrafluorbenzenom i trifluor-1,3,5-trijodbenzenom. Mehanokemijskom sintezom dobiveno je 13 novih kristalnih produkata, dok je molekulska i kristalna struktura određena za ukupno 8 kokristala dobivenih iz otopine. Kokristali su okarakterizirani difrakcijom rentgenskog zračenja na jediničnom kristalu i polikristalnom uzorku. Utvrđeno je da je u svim sintetiziranim kokristalima dominantna interakcija halogenska veza  $I \cdots S$ , dok vrsta liganda prisutna na metalnom centru utječe značajno na supramolekulsku arhitekturu.



Slika 1. Wernerovi koordinacijski spojevi korišteni u istraživanju

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# WERNER COORDINATION COMPOUNDS AS BUILDING BLOCKS IN HALOGEN-BONDED COCRYSTALS

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Werner coordination compounds are metal complexes of  $MX_2L_4$  general formula, where M is a divalent metal, X is an anionic ligand ( $NCS^-$ ,  $CN^-$ ,  $NO_3^-$ ,  $NCO^-$ ) and L is a substituted pyridine or  $\alpha$ -arylalkylamine [1,2]. The usage of this type of coordination compounds as halogen bond acceptors has been poorly studied [3]. A search of the Cambridge Structural Database (CSD) for  $I \cdots S$  halogen bonds in cocrystals containing isothiocyanate Werner coordination compounds resulted in only four data sets [4]. As there is no systematic research of this type of interaction, the aim of our research was to study isothiocyanate proclivity for halogen bonding with a series of Werner coordination compounds. To study the steric influence of ligands in halogen bond formation, coordination compounds containing nickel metal center, but a different type of pyridine ligands were synthesized: pyridine (**py**), 4-methylpyridine (**4mepy**), 3,5-lutidine (**35lut**) and isoquinoline (**iq**) (Figure 1). These compounds were then cocrystallized with different halogen bond donor molecules: 1,2-diiodotetrafluorobenzene, 1,3-diiodotetrafluorobenzene, 1,4-diiodotetrafluorobenzene and 1,3,5-trifluoro-2,4,6-triiodobenzene. Cocrystallization experiments were performed both mechanochemically and from the solution, using single-crystal and powder X-ray diffraction for characterization of products. Using mechanochemical synthesis we obtained 13 new crystal products, while crystal and molecular structure was determined for 8 obtained by crystallization from solution. Dominant interactions in all obtained cocrystals are  $I \cdots S$  halogen bonds, while the ligand in the coordination compound significantly affects the supramolecular architecture.

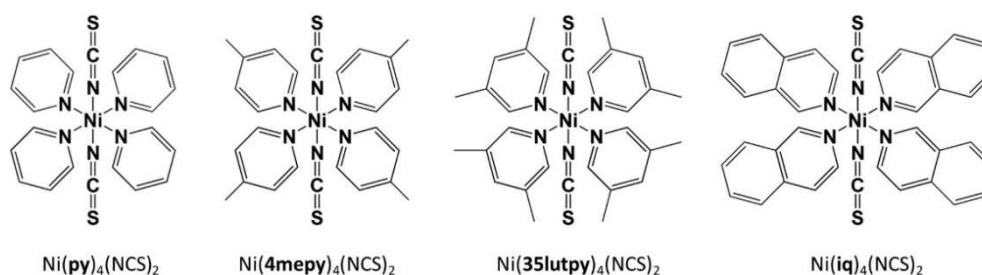


Figure 1. Werner coordination compounds used in this study

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## METAKRILATNI POLIMERNI ADITIVI ZA SNIŽENJE TECIŠTA DIZELSKOG GORIVA - UTJECAJ MOLEKULSKE MASE

Ivan Pucko,<sup>1,\*</sup> Marko Racar,<sup>1</sup> Fabio Faraguna<sup>1</sup>

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Niske temperature uzrokuju poteškoće u primjeni dizelskog goriva. To se ponajviše odnosi na kristalizaciju parafinskih komponenti dizela, koji na nižim temperaturama pretežito kristaliziraju u igličaste parafinske kristale koji se odvajaju od dizela i poprečno povezuju u trodimenzionalne mreže. Kao rezultat toga povezivanja, dolazi do začepljenja pora filtera goriva u vozilima. Kako bi se to spriječilo, dodaju se aditivi (npr. za sniženje tecišta), obično polimeri, kako bi se promijenila veličina i oblik parafinskih kristala i mehanizam njihovog nastanka. Da bi bili učinkoviti, njihova se struktura obično sastoji od nepolarnog alkilnog dijela i polarnog dijela. Osim sastava, na učinkovitost aditiva može utjecati i njihova molekulska masa. Dodavanjem prijenosnika rasta lanca tijekom polimerizacije, promijenjena je molekulska masa aditiva kako bi se proučio njen utjecaj na ponašanje formulacija aditiva s dizelskim gorivom pri niskim temperaturama. U našem radu prosječna masena molekulska masa ( $M_w$ ) određena gel permeacijskom kromatografijom (GPC) varirala je od 8 100 do 54 800 g mol<sup>-1</sup>, a prosječna brojčana molekulska masa ( $M_n$ ) od 6 200 do 30 200 g mol<sup>-1</sup>. Morfologija kristala i kristalno ponašanje formulacija aditiva s dizelskim gorivom proučavana je optičkom mikroskopijom s polarizacijom. Istraživanja su pokazala da se morfologija parafinskih kristala u formulacijama promijenila u odnosu na čisti dizel od velikih igličastih struktura prema većem broju manjih sferičnih struktura, što je spriječilo stvaranje trodimenzionalne mreže. To je pomoglo poboljšati točku filtrabilnosti (CFPP) za do 10 °C i vrijednost točke tecišta (PP) za do 30 °C, budući da pri niskim temperaturama nije došlo do začepljenja, iako je nastala slična količina parafinskih kristala.

### ZAHVALA

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## METHACRYLIC POLYMER POUR POINT DEPRESSANTS FOR DIESEL FUEL: IMPACT OF MOLECULAR WEIGHT

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Low temperatures cause difficulties in the application of diesel fuel. This is mostly related to the crystallization of paraffin components of diesel, which at lower temperatures predominantly crystallize into needle-shaped wax crystals that separate from the diesel and cross-link to form 3D networks. As a result of this cross-linking, the pores of fuel filters in vehicles become clogged. To prevent this, additives (pour point depressants), usually polymers, are added to change the size and shape of paraffin crystals and their formation mechanism. To be effective, their structure usually consists of a non-polar alkyl part and a polar part. In addition to the composition, the effectiveness of the additives could be influenced by their molecular weight. By adding the chain transfer agent during the polymerization, the molecular weight of the additives was changed to study its effect on the low - temperature behavior of additive/diesel formulations. In our work, the weight average molecular weight ( $M_w$ ) determined with gel permeation chromatography (GPC) varied from 8 100 to 54 800 g mol<sup>-1</sup>, and the number average molecular weight ( $M_n$ ) from 6 200 to 30 200 g mol<sup>-1</sup>. The crystal morphology and crystallization behavior of diesel and additive/diesel formulations was studied with optical microscopy with polarization. Our studies showed that the morphology of wax crystals in additive/diesel formulations changed compared to diesel from large needle-like structures to a larger number of smaller spherical structures, which prevented the formation of a three-dimensional network. This helped to improve the cold filter plugging point (CFPP) by up to 10°C and pour point (PP) values by up to 30 °C, as no clogging occurred at low temperatures, although a similar amount of wax crystals formed.

### ACKNOWLEDGMENTS

This research was conducted within the Project of the Croatian Science Foundation Development of Functional Biofuels and Additives and Characterization of Blends with Mineral Fuels (FunBioFA, UIP-2019-04-5242).



# COMPUTATIONAL SIMULATIONS OF THE SloR METALLOREGULATORY PROTEIN FROM THE BACTERIUM *Streptococcus mutans*

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*Streptococcus mutans*, the primary causative agent of human dental caries, is an oral pathogen that grows as a plaque biofilm on the tooth surface. Metal ion homeostasis is essential for the growth and survival of most microorganisms, including *S. mutans* [1]. Divalent metal ions, such as Fe<sup>2+</sup> and Mn<sup>2+</sup>, are important enzyme cofactors and stabilizing components of proteins in various key metabolic pathways. *S. mutans* harbors the SloR metalloregulatory protein which has an important role in maintaining homeostasis of Fe<sup>2+</sup> and Mn<sup>2+</sup> ions. As a transcriptional factor, SloR regulates metal ion transport upon binding to DNA in response to manganese availability [2]. SloR is a homodimer in solution and each subunit binds three manganese ions [3]. Molecular dynamics simulations of different forms of SloR protein were conducted using different parameters, bonded and unbonded, for describing the interactions between the protein and metal ions.

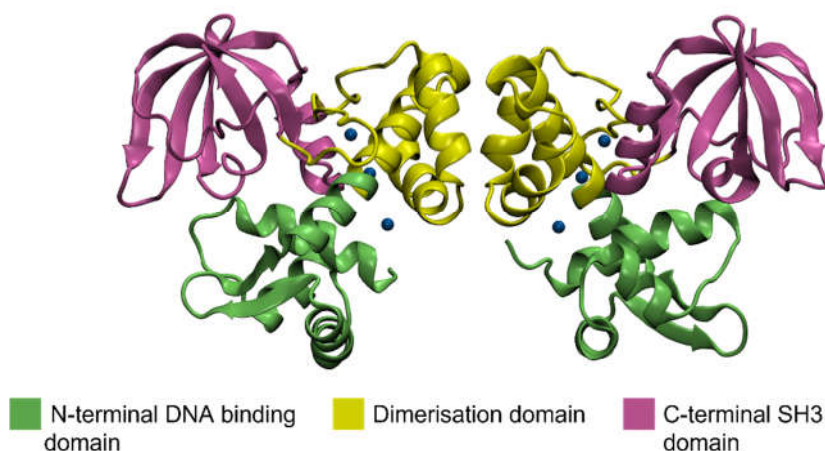


Figure 1. Structure of the SloR protein from *Streptococcus mutans*.

## ACKNOWLEDGMENTS

This work was supported by the Croatian Science Foundation project “Manganese metallosensors” IP-2020-02-3446.

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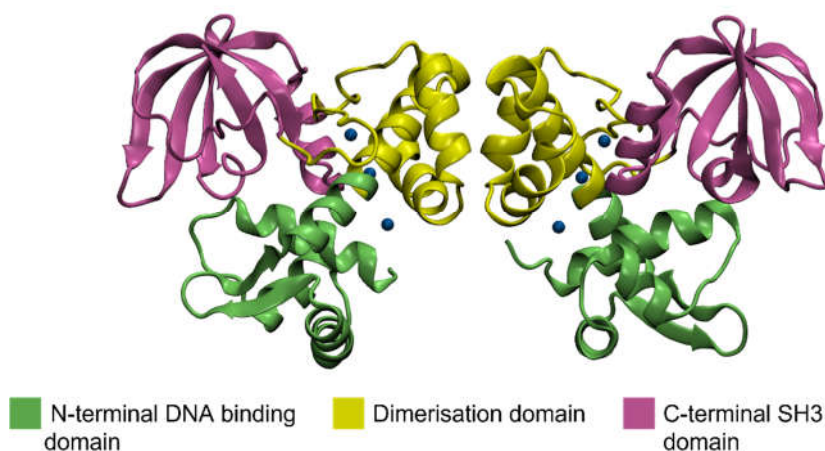


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## UČINCI $\text{MoO}_3$ I $\text{WO}_3$ NA STRUKTURU I ELEKTRONSKI PRIJENOS U VANADATNO-FOSFATNIM STAKLIMA

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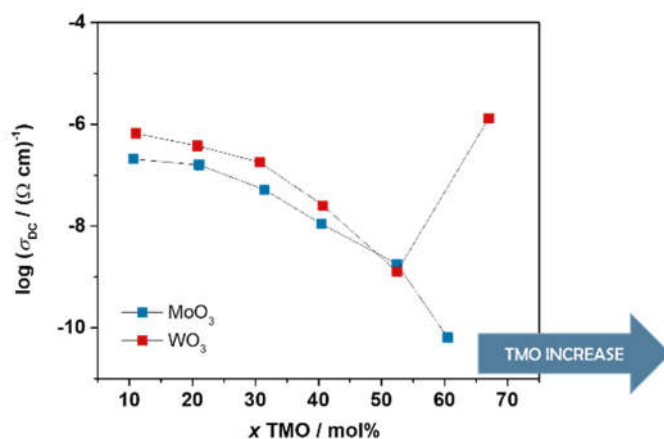
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Oksidna stakla koja sadrže okside prijelaznih metala (TMO) kao što su  $\text{V}_2\text{O}_5$ ,  $\text{MoO}_3$ ,  $\text{WO}_3$  i  $\text{Fe}_2\text{O}_3$  pokazuju elektronsku vodljivost koja je posljedica skokova malih polarona između TM iona u različitim oksidacijskim stanjima. Elektronska vodljivost u ovim staklima ovisi o količini TMO, udjelu TM iona u različitim oksidacijskim stanjima i prosječnoj udaljenosti između tih iona. U ovom radu istražen je utjecaj dodatka drugog TMO na elektronsku vodljivost vanadatno-fosfatnih stakala. U tu svrhu pripremljene su dvije serije ternarnih  $(60-x)\text{V}_2\text{O}_5-x\text{TMO}-40\text{P}_2\text{O}_5$  stakala širokog raspona sastava:  $x = 0-60$  mol% za  $\text{TMO}=\text{WO}_3$  i  $\text{MoO}_3$ , metodom naglog hlađenja taline. Struktura i električna svojstva pripremljenih stakala proučavana su Ramanovom i impedancijskom spektroskopijom, a toplinska svojstva diferencijskom termičkom analizom. Ukupni udio iona prijelaznih metala u nižem oksidacijskom stanju određen je iz temperature ovisnosti magnetizacije. Promjene električne provodnosti sa sastavom stakla pokazuju da se s porastom udjela  $\text{MoO}_3$  provodnost kontinuirano smanjuje za tri reda veličine, dok kod  $\text{WO}_3$  serije stakala pokazuje duboki minimum za  $x=53$  mol%. Uočeno smanjenje provodnosti izravno je povezano sa smanjenjem koncentracije vanadijevih iona koji imaju dominantnu ulogu u prijenosu polarona. U obje serije stakala ioni molibdena i volframa neznatno doprinose elektroničkom prijenosu te ne mogu nadoknaditi smanjenje udjela  $\text{V}_2\text{O}_5$ . S druge strane, visoka elektronska provodnost binarnog  $\text{WO}_3\text{-P}_2\text{O}_5$  stakla povezana je sa strukturom ovog stakla koju karakteriziraju klasteri volframovih jedinica unutar kojih je prijenos elektrona brz.



**Slika 1.** DC provodnost pri 30°C u ovisnosti o udjelu TMO za stakla sastava  $(60-x)\text{V}_2\text{O}_5-x\text{TMO}-40\text{P}_2\text{O}_5$ .



## EFFECTS OF MoO<sub>3</sub> AND WO<sub>3</sub> ON THE STRUCTURE AND ELECTRONIC TRANSPORT IN VANADIUM PHOSPHATE GLASSES

Marta Razum,<sup>1,\*</sup> Luka Pavić,<sup>1</sup> Damir Pajić,<sup>2</sup> Jana Pisk,<sup>3</sup> Petr Mošner,<sup>4</sup> Ladislav Koudelka,<sup>4</sup> Ana Šantić<sup>1</sup>

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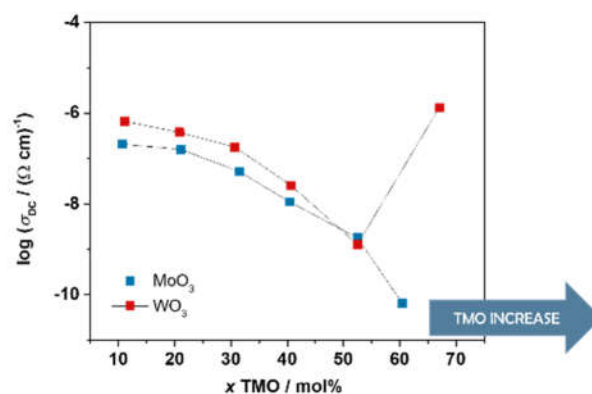
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Electronic conduction has been demonstrated in various oxide glasses containing transition metal oxides (TMO) such as V<sub>2</sub>O<sub>5</sub>, MoO<sub>3</sub>, WO<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub>. The electronic conductivity in these glasses arises from small polaron hopping between TM ions in different oxidation states and depends on the amount of TMO, the proportion of TM ions in different oxidation states, and the average distance between these ions. In this study, we investigate the influence of the addition of the second TMO on the electronic conductivity in vanadate-phosphate glasses. For that purpose, two series of ternary (60-x)V<sub>2</sub>O<sub>5</sub>-xTMO-40P<sub>2</sub>O<sub>5</sub> glasses with a wide range of compositions: x = 0-60 mol% for TMO=WO<sub>3</sub> and MoO<sub>3</sub>, were prepared by the melt quenching method. The structure and electrical properties of the prepared glasses were studied by Raman and impedance spectroscopy, and the thermal properties by Differential Thermal Analysis. The sum fraction of transition metal ions in the lower oxidation state was determined from the temperature dependence of magnetization. The results show that with the addition of MoO<sub>3</sub> conductivity continuously decreases over three orders of magnitude, whereas it shows a deep minimum at 53 mol% WO<sub>3</sub>. The observed conductivity decrease is directly related to the decrease in the concentration of vanadium ions which play a dominant role in the polaron transport in both glass series. It seems that molybdenum and tungsten ions in these glasses do not contribute to the electronic transport as much as vanadium and hence cannot compensate for the decrease in V<sub>2</sub>O<sub>5</sub> content. On the other hand, the high conductivity of binary WO<sub>3</sub>-P<sub>2</sub>O<sub>5</sub> glass is related to the structural peculiarities of this glass system evidenced in the tendency of tungsten units to form clusters within which the transport of polarons is facilitated.



**Figure 1.** DC conductivity at 30°C as a function of TMO content for glasses from (60-x)V<sub>2</sub>O<sub>5</sub>-xTMO-40P<sub>2</sub>O<sub>5</sub> series.





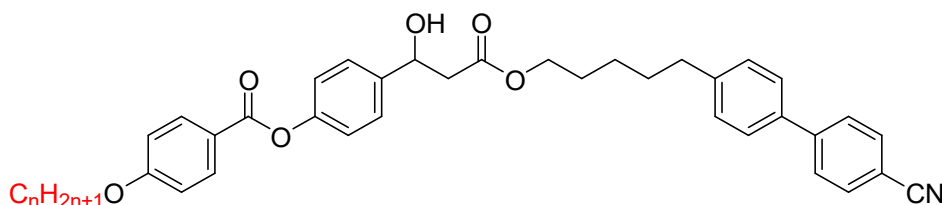
## UTJECAJ DULJINE TERMINALNOG LANCA NA MEZOGENA SVOJSTVA SVIJENIH DIMERA

Aleksandra Šimanović,<sup>1,\*</sup> Irena Dokli,<sup>1</sup> Andreja Lesac<sup>1</sup>

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Tekući kristali su organske molekule koje imaju svojstva između čvrstog kristala i konvencionalne tekućine: fluiditet tekućine te neka strukturna i optička svojstva kristala. Po temperaturi i fizikalnim svojstvima tekuće-kristalna faza nalazi se između krute i tekuće te je nazvana mezofazom, a spojevi mezoženim spojevima. Molekule tekućeg kristala mogu se samoorganizirati u različite mezofaze koje pokazuju karakteristične teksture vidljive pod polarizacijskim mikroskopom. Ovisno o slaganju i usmjerenosti molekula u prostoru najčešće se razlikuju smektička, nematička, kolesterička i kolumnarna mezofaza [1]. Primarnu ulogu u formiranju međufaza ima oblik molekule, a posebno su zanimljivi dimeri svijene geometrije jer se mogu organizirati u helikalne strukture bez kiralnosti na molekularnoj razini [2]. Kako bi se ispitaio odnos strukture i svojstva sintetizirani su dimeri svijenog oblika koji se sastoje od dvije mezogene jedinice povezane fleksibilnom neparnom razmaknicom, a razlikuju se u duljini terminalnog lanca (slika 1). Ispitana su mezogena svojstva sintetiziranih dimera, a općenito se opaža da povećanje duljine terminalnog lanca stabilizira smektičku fazu [3].



Slika 1. Struktura sintetiziranih dimera svijene geometrije;  $n = 4, 6, 8, 10$ .

### ZAHVALE

Autori zahvaljuju Hrvatskoj zakladi za znanost [broj projekta: IP-2019-04-7978] za financijsku potporu.

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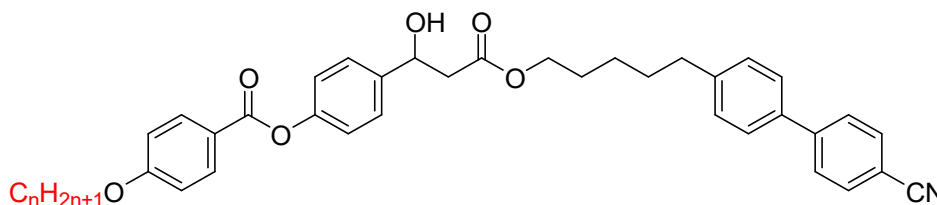
# THE EFFECT OF TERMINAL CHAIN LENGTH ON MESOGENIC PROPERTIES OF BENT-SHAPED DIMERS

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Liquid crystals are organic molecules with properties between solid crystal and conventional fluid: the fluidity of the liquid and some structural and optical properties of crystals. According to temperature and physical properties, the liquid-crystalline phase occurs between solid and liquid and therefore is called the mesophase and the compounds mesogenic compounds. Liquid crystal molecules can self-organize into different mesophases that exhibit characteristic textures visible under a polarizing microscope. Depending on the arrangement and orientation of molecules in space, smectic, nematic, cholesteric and columnar mesophases are most often distinguished [1]. The formation of mesophases is primarily governed by the shape of the molecule; bent-shaped dimers are particularly interesting because they can form helical structures without chirality at the molecular level [2]. In order to examine the structure-property relationship, bent-shaped dimers that consist of two mesogenic units connected by a flexible odd spacer and differ in the length of the terminal chain were synthesized (Fig. 1). The mesogenic properties of the prepared dimers were investigated. It is generally observed that increasing the length of the terminal chain stabilizes the smectic phase [3].



**Figure 1.** The structure of synthesized bent-shaped dimers; n = 4, 6, 8, 10.

## ACKNOWLEDGMENTS

The authors thank the Croatian Science Foundation [grant ref. IP-2019-04-7978] for financial support.

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## ISPITIVANJE EKOTOKSIČNIH UČINAKA NITROMONOAROMATSKIH SPOJEVA *IN VITRO* I *IN VIVO* BIOTESTOVIMA

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Nitromonoaromatski spojevi (engl. *nitromonoaromatic compounds*, NACs) su toksični spojevi sveprisutni u atmosferi koji mogu imati mutagena svojstva. Smatraju se indikatorima sekundarno nastalih organskih lebdećih čestica koji nastaju uslijed antropogenog zagađenja i izgaranjem biomase [1, 2, 3]. NACs predstavljaju značajnu frakciju vodotopljive organske materije lebdećih čestica koja apsorbira sunčevo zračenje te ima utjecaj na ukupnu bilancu zračenja u atmosferi [4]. Budući da NACs putem atmosferskog taloženja mogu dospjeti u vodeni okoliš cilj ove studije bio je ispitati njihove ekotoksikološke učinke na vodene organizme.

Modelni NACs (4-nitrofenol (4NP), 4-nitrokatehol (4NC), metil-nitrokateholi (MNCs), 4-nitrogvajakol (4NG) i 2,4-dinitrofenol (2,4DNP)) su analizirani *in vitro* i *in vivo* biotestovima. Akutni citotoksični učinak spojeva određen je MTT testom na PLHC-1 ribljim stanicama u trajanju od 72h izlaganja ispitivanim spojevima. Kronična toksičnost određena je AlgaeTox testom na jednostaničnoj zelenoj algi *Scenedesmus subspicatus* nakon 96h izlaganja ispitivanim NACs. Sposobnost spojeva da induciraju fazu I. staničnog detoksikacijskog mehanizma određena je EROD biotestom mjerenjem aktivnosti CYP1A1 enzima na ribljim PLHC-1 stanicama dok je interakcija s fazom 0. određena mjerenjem inhibicije transportne aktivnosti polipeptidnog prijenosnika organskih aniona Oatp1d1 ribe zebrice (*Danio rerio*) stabilno eksprimiranog na HEK293 Flp-In staničnoj liniji.

Dobiveni rezultati pokazuju da pojedini testirani NACs imaju značajan citotoksični učinak te sposobnost inhibicije proteinskog prijenosnika. Rezultati dobiveni EROD biotestom pokazali su da ispitivani spojevi nisu tipični induceri CYP1A1 detoksikacijskog enzima.

### ZAHVALE

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# EXPLORING ECOTOXICOLOGICAL EFFECTS OF NITROMONOAROMATIC COMPOUNDS BY *IN VITRO* AND *IN VIVO* BIOASSAYS

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Nitromonoaromatic compounds (NACs) are toxic compounds that are ubiquitous in the atmosphere and may have mutagenic properties. They are considered to be indicators of secondary organic aerosol formed from anthropogenic pollution and biomass burning [1, 2, 3]. NACs represent a significant fraction of the water-soluble organic matter of aerosol that absorbs solar radiation and affects the overall radiative balance in the atmosphere [4]. Since NACs can enter the aquatic environment through atmospheric deposition, the aim of this study was to examine their ecotoxic effects on aquatic organisms.

Model NACs (4-nitrophenol (4NP), 4-nitrocatechol (4NC), methyl-nitrocatechols (MNCs), 4-nitroguaiacol (4NG) and 2,4-dinitrophenol (2,4DNP) were analyzed by *in vitro* and *in vivo* bioassays. Acute cytotoxicity of the compounds was determined by MTT assay using PLHC-1 fish cells exposed for 72 hours to tested compounds. Chronic toxicity was determined by the AlgaeTox assay with the unicellular green algae *Scenedesmus subspicatus* after the 96 h exposure to the tested NACs. The ability of the compounds to induce phase I of the cellular detoxification mechanism was determined by the EROD bioassay by measuring the activity of CYP1A1 enzymes in fish PLHC-1 cells, while interaction with phase 0 was determined by measuring the inhibition of the transport activity of the zebrafish (*Danio rerio*) organic anion-transporting polypeptide Oatp1d1 stably expressed in the HEK293 Flp-In cell line.

The obtained results show that some of the tested NACs showed a significant cytotoxic effect and the ability to inhibit protein transporter activity. The results of the EROD bioassay showed that the model NACs are not typical inducers of CYP1A1 detoxification enzyme.

## ACKNOWLEDGMENTS

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## RASPODJELA KEMIJSKIH VRSTA BAKRA U MORSKOM OKOLIŠU

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Bakar (Cu) je esencijalni mikronutrijent neophodan većini vodenih organizama, no visoke koncentracije slobodnog bakra toksične su za žive organizme. Predviđa se da će pod utjecajem antropogenog zagađenja koncentracija slobodnog bakra porasti za 50% do kraja stoljeća [1]. Mnogi vodeni organizmi proizvode ligande kao obrambeni mehanizam za zaštitu od toksičnosti slobodnog bakra. Ligandi poput tiola i humičnih supstanci vezuju Cu, smanjujući njegovu biodostupnost [2]. Bakar se u oksidnim sredinama poput oceana primarno javlja kao Cu (II), stoga većina modela specijacije Cu pretpostavlja samo Cu(II) kao značajnu kemijsku vrstu Cu. Objavljena je tek nekolicina studija s određenim značajnim vrijednostima Cu(I) u estuarijima i morskoj vodi s vrijednostima Cu(I) od 5-10% u oceanima te do 80% u estuarijima [3,4,5,6]. U ovom radu biti će prezentirana redukcija bakra iz Cu(II) u Cu(I) pod različitim uvjetima poput pH i koncentracije liganda. Stabilnost Cu(I) kompleksa će također biti diskutirana. Višemetodski pristup, uključujući kromatografske i spektrofotometrijske metode, korišten je za određivanje Cu(I) i ispitivanje Cu(I) kompleksa.

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## COPPER SPECIATION IN MARINE ENVIRONMENT

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Copper (Cu) is an essential micronutrient to the most aquatic organisms. However, at high concentrations free copper ion is toxic to the most living species. It is predicted that free copper concentrations will increase due to the anthropogenic pollution by 50% by the end of the century [1]. Many aquatic organisms are producing ligands as defense mechanism against the toxicity of the free copper. Ligands such as thiols and humic substances are binding Cu, reducing its bioavailability [2]. Copper in oxygenated systems such as oceans is primarily present as Cu(II), leading to speciation models assuming only Cu(II) as significant Cu state. Only few studies reported detectable amounts of Cu(I) in estuaries and oceans, with Cu(I) concentrations ranging from 5-10% in oceans and up to 80% in estuaries [3,4,5,6]. In this work research on copper reduction from Cu(II) to Cu(I) under different conditions, such as pH and ligand concentrations, will be presented. Cu(I) complex stability will also be discussed. Multimethod approach is used for determination of Cu(I) and for studies of Cu(I) complexes, including chromatographic and spectrophotometric methods.

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# ISTRAŽIVANJE UTJECAJA NANOČESTICA BAKRA NA CITOGENETIČKE ABERACIJE U EMBRIJIMA JEŽINCA *Sphaerechinus Granularis*

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Bakar i nanočestice bakra (CuNPs) se često koriste u raznim granama industrije poput nanotehnologije, medicine, biotehnologije i u sanaciji okoliša [1]. Upotreba nanočestica bakra (CuNP) u premazima protiv obraštaja postaje sve popularnija i predstavlja izravan put u morski ekosustav gdje nanočestice mogu utjecati na organizame u svim životnim fazama, od embrija do odraslih jedinki [2,3]. U ovom istraživanju ispitani su učinci nanočestica bakra i bakrovih oksida (Cu, CuO, Cu<sub>2</sub>O) na citogenetičke anomalije, kao i promjene u mitotičkoj aktivnosti u embrijima ježinca *Sphaerechinus granularis*. Morfološke abnormalnosti koje su praćene bile su: anafazni mostovi, zaostali kromosomi, acentrični fragmenti, raspršeni kromosomi i višestruka diobena vretena. Iako je poznato da su ioni bakra toksični za organizme i često se koriste kao pozitivna kontrola u biološkim testovima, nanočestice bakra (Cu) pokazale su vrijednosti slične onima Cu<sup>2+</sup> iona, posebno veće koncentracije od 2000 i 5000 mg/L. Nadalje, utvrđeno je da su nanočestice bakrovog oksida (CuO, Cu<sub>2</sub>O) manje toksične od nanočestica bakra, a najčešće pronađene abnormalnosti bile su inhibicija mitotičke aktivnosti i anafazni mostovi. Razlike u toksičnosti među različitim vrstama nanočestica pripisane su njihovoj kinetici otapanja i specijaciji, iako omotač nanočestica također može imati važnu ulogu. U konačnici, ovaj rad može poslužiti kao pokazatelj poželjnijih kemijskih oblika nanočestica bakra u premazima protiv obraštaja tijekom duljeg vremenskog perioda.

## ZAHVALE

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# INVESTIGATING COPPER NANOPARTICLE-DERIVED CYTOGENETIC ABBERATIONS IN EARLY LIFE STAGES OF SEA URCHIN *Sphaerechinus Granularis*

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Copper and copper oxide nanoparticles (CuNPs) are widely used in a broad range of industrial applications such as nanotechnology, medicine, biotechnology and environmental remediation [1]. The use of copper nanoparticles (CuNP) in anti-fouling formulations has been gaining popularity and that presents a direct route into marine environments where they can impact a wide range of organisms at all life stages, from embryos to adults [2,3]. In this study, the effects of copper and copper oxide nanoparticles (Cu, CuO, Cu<sub>2</sub>O) on sea urchin *Sphaerechinus granularis* embryos were tested in terms of cytogenetic anomalies, as well as changes in mitotic activity. The morphological abnormalities that were scored included anaphase bridges, lagging chromosomes, acentric fragments, scattered chromosomes and multipolar spindles. While copper ions are known to be toxic to organisms and are often used as a positive control in bioassays, copper nanoparticles showed values close to those of Cu<sup>2+</sup> ions, especially at higher copper concentrations of 2000 and 5000 mg/L. Furthermore, copper oxide nanoparticles were generally found to be less toxic than copper nanoparticles, with the most common abnormalities found being inhibition of mitotic activity and anaphase bridges. Differences in toxicity among the various types of nanoparticles was ascribed to their kinetics of dissolution and speciation although nanoparticle coating may also play a role. Ultimately, this work may indicate preferred chemical forms of copper nanoparticles for tailoring ship paints to maintain anti-fouling abilities over extended time periods.

## ACKNOWLEDGMENTS

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## UTJECAJ SEZONE (TEMPERATURE, SALINITETA I DUBINA) NA POJAVU INVAZIVNOG PLAŠTENJAKA *Clavelina oblonga* U MARIKULTURI U LIMSKOM ZALJEVU: TERENSKI EKSPERIMENT

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Suzbijanje obraštaja predstavlja 14,7% godišnjih operativnih troškova u akvakulturi školjkaša [1]. Invazija plaštenjaka *Clavelina oblonga* Herdman, 1880 na uzgajalištima školjkaša započela je u ljeto 2020. godine na zapadnoj i istočnoj obali Istre, Hrvatska. Zbog brzog rasta vrsta *C. oblonga* značajno povećava operativne troškove i angažman radne snage u uzgajalištima, povećava mortalitet i stvara gubitke u proizvodnji uz smanjenje vrijednosti proizvoda [2]. Na terenu je postavljen eksperiment za praćenje invazije *C. oblonge*, njenog obraštaja i utjecaja dubine, saliniteta i temperature na rast i preživljavanje komercijalne vrste kamenice *Ostrea edulis* Linnaeus, 1758. Poše sa 100 kamenica su postavljene na 5 različitih dubina: 3, 5, 8, 10 i 12 m (3 m od dna). HOBO sonda za praćenje temperature i saliniteta je postavljena na pošu na 3 m dubine, a na sve druge dubine su postavljene temperature sonde. Od srpnja 2021. do veljače 2022. cijele instalacije sa pošama su svaki mjesec čišćene, praćen je obraštaj, mortalitet i rast kamenica. Tijekom kolovoza 2021. vrsta *C. oblonga* je tek počela formirati nove kolonije po instalacijama, što se nastavilo i doseglo svoj vrhunac u rujnu kada je prekrila 100% infrastrukture. Naši rezultati praćenja obraštaja vrste *C. oblonga* na 5 dubina, upućuju da se pojavnost, širenje, rast i biomasa mogu povezati sa sezonom (temperatura m.v. >22°C). Međutim, u isto vrijeme na njihovu pojavnost, distribuciju i biomasu ne utječe dubina i vertikalna stratifikacija temperature morske vode. Sam obraštaj sačinjen prvenstveno od vrste *C. oblonga* nije imao značajan učinak na mortalitet kamenica, kada se čistio svakih mjesec dana, ali visoke ljetne temperature (> 22°C) i stratifikacija vodenog stupca (3 i 5 m dubine) povećale su mortalitet i usporile rast kamenica.

### ZAHVALE

Ovo istraživanje je sufinancirano od strane Istarske županije i IRB-a.

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# SEASONAL (TEMPERATURE, SALINITY AND DEPTH) EFFECTS ON THE APPEARANCE OF INVASIVE TUNICATE *Clavelina oblonga* IN LIM BAY MARICULTURE: A FIELD EXPERIMENT

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Biofouling control represents 14.7% of the annual operating cost in bivalve aquaculture [1]. A recent intensive *Clavelina oblonga* Herdman, 1880 invasion occurred in shellfish farms on the western and eastern coasts of the Istrian peninsula (Croatia), beginning in summer 2020. Due to its fast growth in mariculture areas, *C. oblonga* significantly increases both operating costs and working effort, resulting in the decrease of product value, as well as production lost and increased organisms mortality [2]. An experimental field setup at an endangered aquaculture location was established to observe *C. oblonga* invasion, overgrowth and effects of depth, salinity and temperature on the growth and survival of a commercially important bivalve species, oyster *Ostrea edulis* Linnaeus, 1758. Nets with 100 oysters were hung from a rope at 5 different depths: 3, 5, 8, 10 and 12 m (3 m from the bottom). A HOBO salinity-temperature data logger was attached to the net at 3 m, while temperature data loggers were set at all the other depths. The entire installation with nets was cleaned and monitored for overgrowth, oyster mortality and growth patterns on a monthly basis, from July 2021 to February 2022. During August 2021 *C. oblonga* started forming new colonies on the installations, which continued and reached its greatest extent in September when 100% of nets and infrastructure was covered with this species. Our results and observations of *C. oblonga* overgrowth at this location on oyster nets at 5 depths, indicated that its incidence, spread, growth and biomass relate to the season (seawater temperatures >22°C). Its incidence, distribution and biomass were not found to be affected by depth and s.w. vertical temperature stratification differences at the same time. *Clavelina oblonga* overgrowths itself did not provoke significant mortality in *O. edulis*, when cleaned on monthly basis, but high summer s.w. temperatures (> 22°C) and water column stratification increased mortality and decreased the growth of oysters at 3 m and 5 m depths.

## ACKNOWLEDGMENTS

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## UTJECAJ NANOČESTICA POLISTIRENA NA BIOMARKERE EFEKTA U DAGNJE, *MYTILUS GALLOPROVINCIALIS*

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Unos plastike u okoliš predstavlja svjetski problem zagađenja, a pogoršava se razgradnjom plastičnih materijala na sve manje i manje čestice, kao što su čestice nano veličina (<100 nm), potencijalno postajući biološki dostupnije organizmima, kao i vektori za druge onečišivače [1]. Ekološki i komercijalno važni filtratori, kao što su dagnje, sve se više istražuju zbog njihovog staništa, načina ishrane i vjerojatnosti susreta s plastičnim česticama različitih veličina [2], koje se često preklapaju s veličinom čestica hrane iz prirode. Cilj ovog rada bio je sintetizirati i karakterizirati nanočestice polistirena (NČ PS) te istražiti njihov utjecaj na specifične biomarkere efekta, odnosno aktivnost acetilkolinesteraze (AChE) i glutation S-transferaze (GST) u mediteranske dagnje, *Mytilus galloprovincialis*. NČ PS s inkapsuliranim etidijevim bromidom karakterizirane su skenirajućim elektronskim mikroskopom. Dagnje su izložene različitim koncentracijama NČ PS (0 (kontrola), 10, 50 i 100 mg/L) tijekom 42 sata, a škrge i probavna žlijezda uzorkovane su za analizu. NČ PS izazvale su promjene neurotransmisije, iskazane kao inhibicija aktivnosti AChE. Paralelno, utvrđeno je povećanje aktivnosti GST nakon izlaganja nanoplastici. Općenito, pokazalo se da izlaganje jedinki vrste *M. galloprovincialis* NČ PS povećava oksidativni stres utječući na normalnu aktivnost enzima. U konačnici, ovaj je rad korak u smjeru boljeg razumijevanja toksičnosti plastičnih nanočestica na komercijalno važne školjkaše i posljedično na bioakumulaciju u drugim morskim organizmima.

### ZAHVALE

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[2] M. S. Bank, *Microplastic in the Environment: Pattern and Process*, Springer, Switzerland, 2022, 225.



# THE INFLUENCE OF POLYSTYRENE NANOPARTICLES ON BIOMARKERS OF EFFECT IN MUSSEL *MYTILUS GALLOPROVINCIALIS*

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Entry of plastics into the environment represents a worldwide pollution problem and is exacerbated by such plastic materials degrading into ever smaller particles, e.g. nano-sized particles (<100 nm), potentially becoming more bioavailable to organisms as well as a vector for other contaminants [1]. Ecologically and commercially important filter feeders such as mussels are increasingly being used as sentinels due to their habitat, feeding behaviour and probability of encountering plastic particles of varying sizes [2] which often overlap in size with natural particles of food. The aim of this work was to synthesise and characterise polystyrene nanoparticles (PS NPs) and investigate their influence on specific biomarkers of effect, *viz* acetylcholinesterase (AChE) and glutathione S-transferase (GST) activity in Mediterranean mussel *Mytilus galloprovincialis*. The size and morphology of the PS NPs with encapsulated ethidium bromide were determined by scanning electron microscopy. Mussels were exposed to a range of PS NPs concentrations (0 (control), 10, 50 and 100 mg/L) for 42 h and gills and digestive gland were sampled for analysis. The PS NPs induced effects on neurotransmission, measured as inhibition of AChE activity. In parallel, an increase in GST activity was determined after exposure to the nanoplastics. Overall, it is shown that exposure of *M. galloprovincialis* to PS NPs increases oxidative stress by impacting the normal activity of enzymes. Ultimately, this work is a step in the direction to better understanding plastic nanoparticles' toxicity on commercially important shellfish and more broadly bioaccumulation in other marine biota.

## ACKNOWLEDGMENTS

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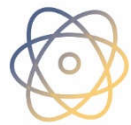
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# Radionice

## *Workshops*





## dr. sc. Mario Špadina

Mario je osnivač SeaCras-a, trenutni izvršni direktor i glavni znanstveni direktor. Magistrirao je kemiju na Sveučilištu u Zagrebu i doktorirao fizičku kemiju na Sveučilištu Montpellier i Francuskoj komisiji za alternativnu energiju i atomsku energiju (CEA). Mario je trenutno poslijedoktorand na Zdravstvenom fakultetu u Ljubljani, Slovenija. Mario je do sada objavio 13 znanstvenih radova o statističkoj mehanici organiziranih kondenziranih tekućina. Njegov životopis uključuje i dva postdoktorska ugovora, pozvana predavanja na najkonkurentnijim znanstvenim konferencijama kao što je ACS Spring Meeting 2022. u San Diegu (SAD), nekoliko velikih stipendija i nagrada, kao i više od 10 suradničkih laboratorija diljem svijeta. Mario je član EIT Climate-KIC-a gdje je 2019. godine dobio stipendiju za nadogradnju svog istraživanja analize podataka satelitskih snimaka u grupi Climatology by Satellites na Sveučilištu u Valenciji. Godine 2020. dobio je nagradu od EIT Climate-KIC za provođenje inovativnog istraživanja o zelenom aspektu baterija.

### *Dr. Mario Špadina*

*Mario is SeaCras founder, current CEO, and chief scientific officer (CSO). He holds master's degree in chemistry from University of Zagreb and PhD degree in physical chemistry from University of Montpellier and the French Alternative Energies and French Atomic Energy Commission (CEA). Mario is currently a post-doctoral researcher at faculty of Health Sciences in Ljubljana, Slovenia. Mario's academic record to the date includes 13 published scientific papers on statistical mechanics of organized condensed fluids. Furthermore, his resume includes two post-doctoral contracts, invited lectures on most competitive scientific conferences such as 2022 ACS Spring Meeting in San Diego (USA), few major grants and awards, as well as over 10 collaborating laboratories across the Globe. Mario is a member of EIT Climate-KIC where he was in 2019 awarded with scholarship to upgrade his craft in data analysis of satellite imagery at the Climatology by Satellites group, at the University of Valencia. In 2020 he was awarded by EIT Climate-KIC for conducting innovative research on green aspect of batteries.*





## dr. sc. Stipe Lukin

Stipe je jedan od suvlasnika SeaCras-a i glavni tehnološki direktor. Magistrirao je kemiju i doktorirao fizikalnu kemiju na Sveučilištu u Zagrebu. Trenutno je poslijedoktorand na Institutu Ruđer Bošković u Zagrebu. Stipina stručnost leži u spektroskopiji i analizi podataka, što je potkrijepljeno dosadašnjom 21 znanstvenom publikacijom i širokom suradničkom mrežom. Kao član EIT Climate KIC-a, Stipe je dobio stipendiju da provede mjesec dana u Tallinnu (Estonija), radeći u startup-u GelaTex, inovativnoj tvrtki fokusiranoj na kružno gospodarstvo i proizvodnju nanovlakana. Tamo je Stipe iz prve ruke iskusio unutarnje djelovanje uspješnog startup-a, što mu je dalo širu sliku onoga što je potrebno za uspjeh u takvom ekosustavu.

### *Dr. Stipe Lukin*

*Stipe is one of SeaCras co-owners and chief technology officer (CTO). He holds a master's degree in chemistry and PhD in Physical Chemistry from the University of Zagreb. Currently, he is a post-doctoral researcher at Ruđer Bošković Institute in Zagreb. Stipe's expertise lies in spectroscopy and data analysis, corroborated with 21 scientific publications to date and a broad collaboration network. As the EIT Climate KIC member, Stipe was awarded a scholarship to spend one month in Tallinn, Estonia, working in startup GelaTex, an innovative company focused on circular economy and nanofibers manufacturing. There, Stipe experienced in the first-hand the inner workings of a successful startup that provided the broader picture of what is necessary to succeed in such an ecosystem.*



## Tomislav Stolar, mag. chem.

Tomislav je jedan od suvlasnika SeaCras-a i direktor marketinga. Magistrirao je kemiju, a trenutno je upisan na doktorski studij kemije. Njegov akademski uspjeh do sada uključuje 14 znanstvenih publikacija. Tomislav je dobio stipendiju EIT Climate-KIC-a kako bi proveo mjesec dana na praksi na Tallinn Institute of Technology (TalTech) u Estoniji 2019. godine. Kao rezultat toga, Tomislav je napisao “Startup priručnik”

- vodič za početnike u startup svijetu od 23 stranice na temelju estonskog startup ekosustava kao studije slučaja. Također, Tomislav je 2020. godine dobio nagradu EIT Climate-KIC-a za provođenje akademskog istraživanja o korištenju CO<sub>2</sub> kao sirovine i pretvaranju u korisno kemijsko gorivo. U SeaCras-u Tomislav preuzima poslove sukladno organizacijskoj i marketinškoj strategiji te kemijskoj analizi komponenti baze podataka in situ.

### *MSc. Tomislav Stolar*

*Tomislav is one of SeaCras co-owners and chief marketing officer (CMO). He holds a master's degree in chemistry and is currently enrolled in PhD studies in chemistry. His academic achievements include 14 peer-reviewed publications to date. Tomislav won a scholarship from EIT Climate-KIC to spend one month on an internship at Tallinn Institute of Technology (TalTech) in Estonia in 2019. As a result, Tomislav wrote “Startup handbook” - a 23-page beginners guide to the startup world based on the Estonian startup ecosystem as a case study. Also, Tomislav won a grant from EIT Climate-KIC in 2020 to conduct academic research on using CO<sub>2</sub> as a feedstock and converting it to useful chemical fuel. In SeaCras, Tomislav takes over duties according to the organizational and marketing strategy as well as chemical analysis of in situ component database.*

## PITCHING

Stipe Lukin,<sup>1,\*</sup> Tomislav Stolar,<sup>1,\*\*</sup> Mario Špadina<sup>2,\*\*\*</sup>

<sup>1</sup> Zavod za fizičku kemiju, Institut Ruđer Bošković, Bijenička 54, Zagreb, Hrvatska

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U prvom dijelu radionice proći ćemo kroz teoriju različitih vrsta pitcha. Zatim ćemo demonstrirati 5-min pitch. Nakon toga slijede upute i vrijeme za vježbu u kojoj ćete pripremiti vlastiti elevator pitch. Naposljetku slijedi diskusija i Q&A.

## PITCHING

Stipe Lukin,<sup>1,\*</sup> Tomislav Stolar,<sup>1,\*\*</sup> Mario Špadina<sup>2,\*\*\*</sup>

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In the first part of the workshop, we will cover the theory of different types of pitch. Next, we will demonstrate a 5-min pitch. After that, you will receive instructions and have time to prepare your own elevator pitch. Finally, there will be discussion as well as Q&A.



## Prof. dr. sc. Ernest Meštrović

Prof. dr. sc. Ernest Meštrović, redoviti je profesor u naslovnom zvanju na Prirodoslovno-matematičkom fakultetu. Zaposlen je na mjestu višeg direktora za znanost i nove tehnologije u globalnom odjelu Istraživanja i razvoja farmaceutske kompanije Xellia d. o. o. Diplomirao je i doktorirao je na Kemijskom odsjeku PMF-a u Zagrebu. Dodatno se usavršavao u primjeni Sinklotronskog zračenja u istraživanju strukture i svojstva tvari u Italiji i Francuskoj.

Od 2004. g. počinje raditi u Istraživanju i razvoju farmaceutske kompanije PLIVA Hrvatska, počevši od eksperta u području kemije i analitike čvrstog stanja, zatim na mjestu voditelja

laboratorija, glavnog istraživača za analitiku djelatnih tvari, direktora odjela analitike, a od 2008. g. preuzima ulogu Višeg direktora TAPI Istraživanja i razvoja. Na tom mjestu ostaje do 2017. g., kada preuzima globalnu ulogu Višeg direktora za Nove tehnologije i životni ciklus proizvoda unutar TAPI divizije. Od 2018. g. radi u farmaceutskoj kompaniji Xelli d.o.o na poziciji Višeg direktora za znanost i nove tehnologije gdje je odgovoran za primjenu najnovijih znanstvenih spoznaja i tehnologija kako bi se na tržište plasirali inovativni proizvodi koji daju odgovor trenutno neispunjenim medicinskim potrebama i izazovima u pružanju sigurnih i učinkovitih tretmana u korist pacijenta.

Znanstvena djelatnost profesora Meštrovića temelji se na istraživanju u području strukturne kemije i kemije materijala, kristalnog inženjerstva i molekuskog prepoznavanja, te rasta kristala što je primjenjivo na istraživanje i razvoj djelatnih tvari i farmaceutskih proizvoda. Također znanstveno je aktivan u području održivih kemijskih procesa, zelene kemije i očuvanja okoliša kroz kontrolu kemijskih pretvorbi. U radu nastoji povezati istraživanja koja se provode u industriji sa fundamentalnim spoznajama do kojih dolaze istraživači na Sveučilištu ili Javnim institutima.

Autor je više od 60 znanstvenih radova, sudjelovao je u oblikovanju inovacija koje su publicirane u 12 patenata i patentnih prijava. U segmentu primjene znanosti sudjelovao je ili vodio pedesetak projekata za koje je načinjen transfer tehnologije u proizvodne kapacitete u Hrvatskoj, Izraelu, Indiji, Kini i Meksiku. Kao mentor sudjelovao je u izradi dvadesetak diplomskih radova dok je pet pristupnika uspješno obranilo doktorske disertacije.

Pored znanstvene i stručne djelatnosti aktivan je u popularizaciji znanosti, održava niz predavanja i seminara, podržava i osigurava sredstva za učeničke i studentske projekte. Za dosadašnje aktivnosti Ernest Meštrović dobio je nekoliko nagrada PLIVE kao i medalju kemijskog odsjeka PMF-a. Član je mnogih znanstvenih i stručnih društava od kojih vrijedi izdvojiti: Hrvatsko kemijsko društvo,

Američko kemijsko društvo, Hrvatska kristalografska zajednica, Hrvatska udruga kristalografa. Napisao je knjigu od dvadesetak priča pod naslovom „To si ti“.

### ***Prof. Ernest Meštrović, PhD***

*Prof. Ernest Meštrović is full professor at the Faculty of Science and works as a senior director for science and new technologies in the global research and development department of the pharmaceutical company Xellia d. o.o. Professor Meštrović graduated and received his PhD from the Department of Chemistry of the Faculty of Science in Zagreb. He further specialized in the application of synchrotron radiation in the study of the structure and properties of matter in Italy and France.*

*Since 2004 he has been working in the Research and Development of the pharmaceutical company PLIVA Croatia, starting as an expert in chemistry and solid-state analytics, then as head of the laboratory, chief researcher for API analysis, director of analytics, and since 2008 Senior Director of TAPI Research and Development. In 2017 he takes on the global role of Senior Director of New Technologies and Product Lifecycle within the TAPI Division. Since 2018, he has been working for the pharmaceutical company Xella d.o.o. as Senior Director of Science and New Technologies, where he is responsible for applying the latest scientific knowledge and technologies to market innovative products that meet current medical needs and challenges in providing safe and effective treatment for the benefit of the patient.*

*Professor Meštrović's scientific activity is based on research in the field of structural chemistry and material chemistry, crystal engineering and molecular recognition, and crystal growth, which is applicable to research and development of active substances and pharmaceutical products. He is also active in the field of sustainable chemistry processes, green chemistry and environmental protection through the control of chemical transformations. He is engaged to connect research conducted in industry with the fundamental knowledge gained by researchers at the Universities or Public Institutes.*

*He is the author of more than 60 scientific papers, and he participated in the design of innovations published in 12 patents and patent applications. In the segment of applied science, he participated in about fifty projects for which technology was transferred to production facilities in Croatia, Israel, India, China and Mexico. He mentored about twenty diploma theses and five doctoral dissertations. In addition to scientific and professional activities, he is active in science popularization, holds a series of lectures and seminars, supports and provides funds for student projects. For all his activities, professor Ernest Meštrović has received several PLIVA awards as well as a medal from the Department of Chemistry of the Faculty of Science, University of Zagreb. He is a member of the*

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*Workshops*

*Croatian Chemical Society, the American Chemical Society, the Croatian Crystallographic Society, the Croatian Association of Crystallographers. Besides all that, he wrote a book of about twenty stories entitled "To si ti".*

## JASAN I NEDVOSMISLEN IZRAŽAJ – PUT PREMA ZNAJSTVENOJ IZVRSNOSTI

Ernest Meštrović<sup>1,\*</sup>

<sup>1</sup>Zavod za opću i anorgansku kemiju, Kemijski odsjek, Prirodoslovno-matematički fakultet, Horvatovac 102a, Zagreb, Hrvatska

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Tijekom radionice prof. dr. sc. Ernest Meštrović će pokazati koliko je važna jasna i nedvosmislena komunikacija, jednako pismena kao i usmena. Činjenica je da se mnogi rezultati koji su objavljeni u znanstvenim radovima ne mogu ponoviti. To predstavlja iznimno veliki izazov za sve nas. Mnogo je razloga zašto je to tako, no jedan od osnovnih što sve manje pričamo, pišemo, što se komunikacija svodi na neku vrstu meta jezika. Sve su to izazovi s kojima se doktorandi susretnu kada dođu u neku novu sredinu, a posebno u industriju.

## CLEAR AND UNAMBIGUOUS EXPRESSION - THE PATH TOWARD SCIENTIFIC EXCELLENCE

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During the workshop prof. dr. sc. Ernest Meštrović will show how important clear and unambiguous communication is, both written and oral. The fact is that many of the results published in scientific papers cannot be replicated. This is an extremely big challenge for all of us. There are many reasons why this is so, but one of the basic ones is that we talk and write less and less, that communication is reduced to a kind of meta language. These are all challenges that doctoral students face when they come to a new environment, and especially to industry.





## Prof. dr. sc. Vernesa Smolčić

Prof. dr. sc. Vernesa Smolčić je svjetski priznata astrofizičarka te redovita profesorica na Fizičkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu. Znanstveno se usavršavala u SAD-u, Njemačkoj i Australiji, a nakon devet godina rada i usavršavanja na najboljim sveučilištima u svijetu, vratila u Hrvatsku. Fokus njezinih istraživanja su stvaranje i razvoj galaksija, proučava aktivne galaktičke jezgre, radiogalaksije te izvangalaktičko stvaranje zvijezda. Članica je brojnih međunarodnih kolaboracija, a u nekima od njih obnaša voditeljske funkcije, kao u Cosmic Evolution Survey (COSMOS) te The Ultimate XMM Extragalactic Survey. Između ostalog, koordinatorica je aktivnosti PMF-a vezanih uz Europsku svemirsku agenciju i članica Referentne skupine za Svemir (MZO). Objavila je preko 140 znanstvenih radova, a za svoj je rad primila brojne nagrade, kao što su Ernst Patzer Award, Zagrepčanka godine 2014., Priznanje Fakultetskog Vijeća PMF-a za značajno znanstveno postignuće i doprinos ugledu PMF-a, COSMO znanstvenica godine 2015., Državna nagrada za znanost za 2017. g., Nagrada HAZU za najviša znanstvena i umjetnička dostignuća u RH za 2019. g. za područje matematičkih, fizičkih i kemijskih znanosti, te je uvrštena u najuspješnije ljude generacije 2013. od Forbes magazina. Uz prestižni ERC Starting grant za projekt "Istraživanje rasta zvjezdane mase i mase supermasivnih crnih rupa u galaksijama kroz kozmičko vrijeme: Utiranje puta za sljedeću generaciju pregleda neba", vodila je brojne znanstvene projekte. Profesorica je vrlo aktivna u popularizaciji znanosti te vodi projekt nazvan Astroučionica, web platformu koja je namijenjena za popularizaciju astronomije. Astroučionica je nastala tijekom provedbe ERC projekta 2018. g. te je od tada nastavila rasti. Također, ambasadorica je kampanje „Duboko iznad svih“ prirodne mineralne vode Jana.

### *Prof. Vernesa Smolčić, PhD*

*Prof. Vernesa Smolčić is a world-renowned astrophysicist and full professor at the Department of Physics, Faculty of Science, University of Zagreb. She continued her studies in the USA, Germany and Australia, and after nine years of work and training at the best universities in the world, she returned to Croatia. Her research focuses on the formation and development of galaxies, she studies active galactic nuclei, radio galaxies and extragalactic star formation. She is a member of numerous international collaborations, and in some of them she holds leadership positions, such as in the Cosmic Evolution Survey (COSMOS) and The Ultimate XMM Extragalactic Survey. Among other things, she is the coordinator of the activities of the Faculty of Science related to the European Space Agency and a member of the Reference Group for Space (MSE). She has published over 140 scientific papers and received numerous awards for her work, such as the Ernst Patzer Award, Zagreb Woman*

*of the Year 2014, Recognition of the Faculty Council of the Faculty of Science for significant scientific achievement and contribution to the reputation of the Faculty of Science, COSMO Scientist of the Year 2015, State Award for Science for 2017, Award of the Croatian Academy of Sciences and Arts for the highest scientific and artistic achievements in the Republic of Croatia for 2019 in the field of mathematical, physical and chemical sciences, and was included in the list of most successful people of generation 2013 by Forbes magazine. In addition to the prestigious ERC Starting Grant for the project "Constraining Stellar Mass and Supermassive Black Hole Growth through Cosmic Times: Paving the Way for the Next Generation Sky Surveys", she has led numerous scientific projects. The professor is very active in popularizing science and runs a project called Astroučionica, a web platform intended for the popularization of astronomy. Astroučionica was created during the implementation of the ERC project in 2018 and has continued to grow since then. She is also the ambassador of the "Deep Above All" campaign of Jana's natural mineral water.*

## KAKO NAPISATI USPJEŠNU ZNANSTVENU PRIJAVU

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Svaki se znanstvenik/ca prije ili kasnije susretne s pisanjem prijave za financiranje projekta. Taj proces znatno se razlikuje od pisanja znanstvenog rada. U radionici ću proći kroz najvažnije aspekte takvih prijava te kako napisati uspješnu prijavu za znanstveni projekt.

## HOW TO WRITE A SUCCESSFUL GRANT PROPOSAL

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Every researcher sooner or later has to apply for science funding. The process of writing a grant proposal is quite different than writing a science paper. In the workshop I will go through the most important aspects of science funding proposals and tips and tricks on how to write a successful grant proposal.



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