УНИВЕРЗИТЕТ У БАЊОЈ ЛУЦИ
UNIVERSITY OF BANJA LUKA
ПРИРОДНО-МАТЕМАТИЧКИ ФАКУЛТЕТ
FACULTY OF NATURAL SCIENCES AND MATHEMATICS

SBERS 2020

ZBORNIK SAŽETAKA

BOOK OF ABSTRACTS

IV SIMPOZIJUM BIOLOGA I EKOLOGA REPUBLIKE SRPSKE

sa međunarodnim učešćem – SBERS2020

Prirodno-matematički fakultet, Univerzitet u Banjoj Luci

12-14. novembar 2020.

IV SYMPOSIUM OF BIOLOGISTS AND ECOLOGISTS OF REPUBLIC OF SRPSKA

with international participation – SBERS2020

Faculty of Natural Sciences and Mathematics, University of Banja Luka

12-14 November 2020

Banja Luka, 2020.

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Izdavač/*Publisher*:
Prirodno-matematički fakultet, Univerzitet u Banjoj Luci, Mladena Stojanovića 2, 78000 Banja Luka, Republika Srpska, B&H, https://pmf.unibl.org
Faculty of Natural Sciences and Mathematics, University of Banja Luka, Mladena Stojanovića 2, 78000 Banja Luka, Republic of Srpska, B&H, https://pmf.unibl.org

Za izdavača/*For Publisher*:
Prof. dr Goran Trbić

Urednik/*Editor*:
Prof. dr Duško Jojić

Tehnički urednik/*Technical Editor*:
Prof. dr Siniša Škondrić

Grafički dizajn/*Graphic Design*:
Divna Džombić

Način pristupa (URL)/*Available on*:
https://pmf.unibl.org/wp-content/uploads/2020/11/zbornik_SBERS2020.pdf

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BOTANY AND PLANT PHYSIOLOGY

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BEZBEDNOST HRANE I ZDRAVLJE POTROŠAČA
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EKOLOGIJA BILJAKA
PLANT ECOLOGY

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ECOLOGICAL – PHYTOGEOGRAPHICAL CHARACTERISTICS OF THE WEED FLORA OF VEGETABLES CROPS IN THE SURROUNDING OF ČENTA VILLAGE (SOUTH BANAT, SERBIA)
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VARIJABILNOST SESKVITERPENA U ETARSKOM ULJU VRSTE *Teucrium montanum* L. (LAMIACEAE) SA SERPENTINITSKIH I KREČNJAČKIH STANIŠTA
VARIABILITY OF SESQUITERPENES IN ESSENTIAL OIL OF *Teucrium montanum* L. (LAMIACEAE) FROM SERPENTINITE AND CALCAREOUS HABITATS
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ZAJEDNICE MAKROINVERTEBRATA U EKSTREMNIM STANIŠTIMA
MACROINVERTEBRATE COMMUNITIES IN EXTREME ENVIRONMENTS
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USMENA IZLAGANJA/*ORAL PRESENTATIONS*

KVALITETA ARHIVSKOG MATERIJALA ZA DNA-BARKODIRANJE
QUALITY OF ARCHIVED MATERIAL FOR DNA BARCODING
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POSTER PREZENTACIJE/*POSTER PRESENTATIONS*

STANJE TAKSONA ZOOBENTOSA KAPTIRANIH IZVORA NACIONALNOG PARKA KOZARA
STATE OF ZOOBENTHOS TAXA IN CAPTURED SPRINGS OF KOZARA NATIONAL PARK
Dejan Dmitrović, Goran Šukalo, Ana Savić & Vladimir Pešić

PRVI NALAZI *Cyzicus* sp. (CRUSTACEA, SPINICAUDATA) U BOSNI I HERCEGOVINI
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IHTIOFAUNA DONJEG DIJELA TOKA RIJEKE VRBANJE (REPUBLIKA SRPSKA, BiH)
ICHTHYOFAUNA OF LOWER COURSE OF VRBANJA RIVER (REPUBLIC OF SRPSKA, B&H)

Svjetlana Cvijić, Dragojla Golub, Goran Šukalo, Radoslav Dekić, Desanka Kostić & Branko Miljanović

REPRODUKTIVNI CIKLUS MEDITERANSKE DAGNJE (*Mytilus galloprovincialis* LAMARCK, 1819) U BOKOKOTORSKOM ZALIVU (CRNA GORA, JUGOISTOČNI JADRAN)
REPRODUCTIVE CYCLE OF THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis* LAMARCK, 1819) IN BOKA KOTORSKA BAY (MONTENEGRO, SOUTH-EAST ADRIATIC)
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NEW RECORDS OF ENDEMIC SPECIES OF EARTHWORM THE *Allolobophora mayeri* (MRŠIĆ, 1990) (LUMBRICIDAE) IN BOSNIA AND HERZEGOVINA
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NOVI NALAZI VRSTE *Ancilus recurvus* MARTENS, 1873
U BOSNI I HERCEGOVINI
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IN BOSNIA AND HERZEGOVINA
Jasminko Mulaomerović

PRILOG POZNAVANJU SLIJEPIH MIŠEVA BRDA MOŽURA
CONTRIBUTION TO THE KNOWLEDGE OF BATS OF THE MOŽURA HILL
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MOLECULAR IDENTIFICATION OF GREEN FROGS (ANURA: RANIDAE: *Pelophylax*) OF WESTERN BALKANS (BOSNIA & HERZEGOVINA AND MONTENEGRO)
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GREEN FROGS (*Pelophylax* spp.) SCREENINGS OF AMPHIBIAN EMERGING PATHOGENS *Batrachochytrium dendrobatidis* AND *Ranavirus*
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SUBBIOCODE – RAZVOJ NOVIH ALATA ZA BRZU PROCJENU PODZEMNOG BIODIVERZITETA U BOSNI I HERCEGOVINI
SUBBIOCODE – DEVELOPING NEW TOOLS FOR RAPID ASSESSMENT OF SUBTERRANEAN BIODIVERSITY IN BOSNIA AND HERZEGOVINA
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ZAŠTITA BIODIVERZITETA
BIODIVERSITY CONSERVATION

PLENARNA PREDAVANJA/*PLENARY LECTURES*

ULOGA NAUKE U ZAŠTITI BIODIVERZITETA
THE ROLE OF SCIENCE IN BIODIVERSITY CONSERVATION
Jelena M. Aleksić

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UTJECAJ VIŠESTRUKIH STRESORA NA ZAVIČAJNE ZAJEDNICE SLATKOVODNE IHTIOFAUNE
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BENTOSKE ZAJEDNICE RIJEKE NERETVE
BENTHIC ASSEMBLAGES OF THE NERETVA RIVER BASIN
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ALGAE DIVERSITY IN MOUNTAIN STREAMS IN THE AREA OF VRANICA MOUNTAIN (BOSNIA AND HERZEGOVINA)
Ermin Mašić, Nadira Likić & Amela Sarajlić

DIVERZITET MEDONOSNIH BILJAKA U UZORCIMA MEDA IZ BOSNE I HERCEGOVINE
DIVERSITY OF HONEY PLANTS IN HONEY SAMPLES ORIGINATING FROM BOSNIA AND HERZEGOVINA
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OVERVIEW OF PUBLISHED PAPERS ON MACROINVERTEBRATE ASSEMBLAGE IN SMALL WATER BODIES IN BALKAN PENINSULA
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POREĐENJE IMTA I UZGOJA MEDITERANSKE DAGNJE (*Mytilus galloprovincialis* L.) U MONOKULTURI U BOKOKOTORSKOM ZALIVU
IMTA vs. MONOCULTURE FARMING OF MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis* L.) IN BOKA KOTORSKA BAY
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KVALITET VODE REKA BANJE I POCIBRAVE
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Nikola Dukić & Milica Živković

MICROPLASTICS IN WATER AND ITS IMPACT ON THE ENVIRONMENT
Kristina Manevski, Dina Tenji, Jasna Stepanov, Vesna Teofilović, Lara Dronjak, Bojan Damnjanović & Milica Živković

BIOLOGIJA I EKOLOGIJA: POLOŽAJ U ŠKOLAMA
BIOLOGY AND ECOLOGY: POSITION IN SCHOOLS

PLENARNO PREDAVANJE/PLENARY LECTURE

NASTAVNI PROGRAMI BIOLOGIJE U OSNOVNOJ ŠKOLI
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POSTER PREZENTACIJE/*POSTER PRESENTATIONS*

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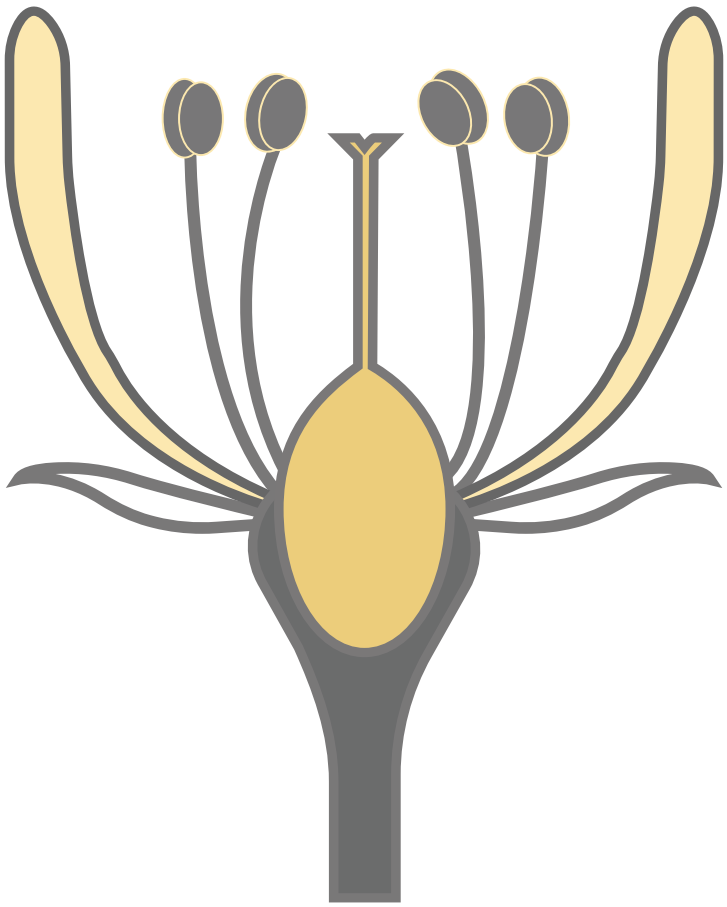
Milica Savić Knežević & Zorana Hrkić Ilić

Botanika i fiziologija biljaka

Botany and Plant Physiology



VRSTE RODA *Centaurium* (FAM. GENTIANACEAE) NA BALKANSKOM POLUOSTRVU: DIVERZITET, SPECIJALIZOVANI METABOLIZAM I EKOFIZIOLOGIJA

Danijela Mišić*, Branislav Šiler, Tijana Banjanac, Marijana Skorić, Uroš Gašić, Dragana Matekalo, Suzana Živković, Jasmina Nestorović Živković, Biljana Filipović, Slavica Dmitrović, Jelena Božunović, Milica Milutinović, Neda Aničić & Luka Petrović

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Rod *Centaurium* Hill (fam. *Gentianaceae*) sadrži oko 25 jednogodišnjih ili dvogodišnjih biljnih vrsta mediteranskog porekla, koje poseduju dobro poznata bioaktivna svojstva. U flori Balkanskog poluostrva prisutno je pet vrsta roda *Centaurium*: *Centaurium erythraea* Rafn, *C. littorale* (Turner) Gilmour, *C. tenuiflorum* (Hoffmanns. & Link) Fritsch, *C. pulchellum* (Sw.) Druce i *C. maritimum* (L.) Fritsch. Višegodišnja istraživanja vrsta roda *Centaurium* u okviru Instituta za biološka istraživanja „Siniša Stanković" - Instituta od nacionalnog značaja za Republiku Srbiju, Univerziteta u Beogradu, rezultovala je formiranjem kolekcije semena *ex situ*, koja dobro oslikava biodiverzitet roda *Centaurium* na Balkanskom poulostrvu. Pored priznatih taksona sa diploidnim i poliploidnim genomima, kolekcija semena koja broji ukupno 153 uzorka, sadrži homoploidne i alopoliploidne hibridogene jedinke različitog porekla. Ovaj neprocenjivi izvor biljnog materijala olakšao je rasvetljavanje složenih filogenetskih odnosa u okviru roda *Centaurium*, primenom morfoloških, molekularnih (EST-SSR, *trn*L-F, TRAP, etc.) i hemijskih markera, koji pokazuju visoku rezoluciju u diskriminaciji taksona. Pored toga, omogućeno je istraživanje ekoloških i evolucionih procesa (kao što su: međuvrsna hibridizacija, poliploidizacija, fragmentacija staništa, genetički drift) i njihovog efekta na biodiverzitet (genetički i hemijski) taksona koji pripadaju rodu *Centaurium.* Primena "*multi-omics*" pristupa u istraživanjima, omogućila je rasvetljavanje metabolizma sekoiridoidnih glukozida kod vrsta roda *Centaurium*, opisivanje njegovog odnosa sa celokupnom fiziologijom biljaka i njegove uslovljenosti biotičkim i abiotičkim faktorima spoljašnje sredine. Multidisciplinarni pristup istraživanjima dao je uvid u molekularne osnove i regulatorne mehanizme konstitutivne biosinteze sekoiridoida kod vrsta roda *Centaurium* pod uticajem faktora spoljašnje sredine, i na različitim nivoima organizacije: unutar- i među-vrsna varijabilnost, kao i ona na unutar-individualnom nivou.

Primenom biotehnoloških metoda u istraživanjima težimo održivoj proizvodnji bioaktivnih jedinjenja vrsta roda *Centaurium* u homologim i heterologim sistemima.

KLJUČNE REČI: *Centaurium*, sekoiridoidi, genetički diverzitet, hemodiverzitet, metabolizam sekoiridoida

Centaurium TAXA (FAM. GENTIANACEAE) IN THE BALKAN PENINSULA: DIVERSITY, SPECIALIZED METABOLISM AND ECOPHYSIOLOGY

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The genus *Centaurium* Hill (fam. Gentianaceae) comprises about 25 annual or biannual species of the Mediterranean origin possessing widely recognized bioactive properties. Five species of this genus are represented within the flora of the Balkan Peninsula: *Centaurium erythraea* Rafn*, C. littorale* (Turner) Gilmour*, C. tenuiflorum* (Hoffmanns. & Link) Fritsch*, C. pulchellum* (Sw.) Druce*,* and *C. maritimum* (L.) Fritsch. Decades of research on the *Centaurium* species within the Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, has resulted in the establishment of *ex situ* seed collection, which fully represents the biodiversity of this genus in the Balkan Peninsula. In addition to accepted taxa with diploid or polyploid genomes, homoploid and allopolyploid hybrids of different origin are stored within the *ex situ* collection, which contains totally 153 accessions. This valuable source of plant material has facilitated resolving the complex phylogenetic relationships within the *Centaurium* genus by adopting morphological, molecular (EST-SSR, *trn*L-F, TRAP, etc.) and chemical markers with high discrimination resolution within taxa. Furthermore, it enabled studying the interplay between ecological and evolutionary processes (e.g. interspecific hybridization, polyploidization, habitat fragmentation, genetic drift), and their effects on the biodiversity of *Centaurium* taxa (both genetic and chemical). By adopting multi-omics approach we study secoiridoid glucosides metabolism and its relationship to the overall plant physiology, as well as its role in plant-biotic and plant-abiotic interactions. The multidisciplinary approach of our studies provided an insight into the molecular background and regulatory networks of constitutive secoiridoids' biosynthesis in *Centaurium* taxa as influenced by environmental factors, while resolving their complex multi-level diversity at inter- and intra-species, as well as at intra-species level. Through the implementation of biotechnology tools,

our intention is to provide sustainable sources of valuable secoiridoids in homologous or heterologous systems.

KEYWORDS: *Centaurium*, secoiridoids, genetic diversity, chemodiversity, secoiridoid metabolism

MORPHOLOGICAL VARIABILITY OF FOLLICLE AND SEED TRAITS IN YELLOW-FLOWERED *Sempervivum* (CRASSULACEAE) SPECIES FROM THE BALKAN PENINSULA

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Based on morphological differences several yellow-flowered *Sempervivum* species from the Balkan Peninsula could be distinguished as *S. ciliosum* and *S. ruthenicum* complexes. Taxonomical position of the species from these two groups is interpreted in various ways in scientific literature since the boundaries for morphological characters are still not clearly defined. To the best of our knowledge, studies dealing with morphological variability of ripened fruits and seeds were not conducted within the members of these groups, regardless of their general taxonomical importance in Crassulaceae. The objective of this study is the quantification of fruit and seed morphological variability and potential use of their traits in delimitation between *S. ciliosum* (*S. ciliosum, S. galicicum, S. octopodes*) and *S. ruthenicum* (*S. ruthenicum, S. zeleborii, S. leucanthum*) aggregates. Applying descriptive statistics, Univariate analysis of variance (ANOVA), Principal component analysis (PCA), and Canonical discriminant analysis (CDA), four quantitative traits of follicles and seeds were analyzed. Additionally, variation in nine qualitative traits was also determined. The results indicated a low degree of variability of all selected traits, while multivariate analysis (PCA, CDA) couldn't prove clear segregation of any of the analyzed species, except to a certain extent *S. galicicum*. A major difference among the species was not found within the qualitative traits of their follicles and seeds either. Although recognized as important in other genera of the family, morphological characters of follicles and seeds are not suitable for delimitation of the taxa within *S. ciliosum* and *S. ruthenicum* complexes to such extent as it was expected.

KEYWORDS: *Sempervivum ciliosum*, *S. ruthenicum*, follicles, seeds, variability, taxonomy

MORFOLOŠKA VARIJABILNOST VRSTE *Crataegus monogyna* JACQ. (ROSACEAE) U GORNJEM DIJELU DOLINE RIJEKE CVRCKE (BOSNA I HERCEGOVINA)

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Najšire rasprostranjena vrsta roda *Crataegus* u Bosni i Hercegovini je *Crataegus monogyna*. Vrsta *C. monogyna* karakteriše se izraženom varijabilnosti morfoloških karaktera, koji su pod značajnim uticajem spoljašnih faktora. U sistematici roda *Crataegus* najpouzdaniji su karakteri listova i plodova. Pored izražene intraspecijske varijabilnosti, vrsta *C. monogyna* hibridizuje sa najmanje pet drugih vrsta datog roda u Evropi. Ta pojava dodatno usložnjava komplikovanu taksonomiju roda *Crataegus.* Cilj ovog rada je analiza morfološke varijabilnosti karaktera lista i ploda vrste *C. monogyna* u gornjem dijelu doline rijeke Cvrcke. Materijal za morfološku analizu sakupljen je na području gornjeg toka rijeke Cvrcke (Kostići, Kneževo). Sakupljeni su kratki izdanci sa listovima i plodovima sa ukupno 33 jedinke. Morfološka analiza listova i plodova vrste *C. monogyna* urađena je na osnovu 15 morfoloških karaktera od kojih četiri karaktera predstavljaju odnos ili količnik dva originalna mjerenja. Na ukupnom uzorku urađena je deskriptivna statistika i analiza korelacije morfoloških karaktera. Karakteri lista pokazuju slabu do umjerenu varijabilnost, dok karakteri ploda pokazuju slabu varijabilnost. Analiza korelacije pokazala je da svi karakteri pokazuju statistički značajnu korelaciju, a koja je slabije izražena kod karaktera ploda. Rezultati su poređeni sa sličnim studijama na području Banj brda (Banja Luka), Bardače i ostalih literaturnih podataka. Ovo istraživanje doprinosi boljem poznavanju morfološke varijabilnosti vrste *C. monogyna* u Bosni i Hercegovini.

KLJUČNE RIJEČI: *Crataegus monogyna*, morfologija, Cvrcka

MORPHOLOGICAL VARIABILITY OF *Crataegus monogyna* JACQ. (ROSACEAE) IN THE UPPER PART OF THE CVRCKA VALLEY (BOSNIA AND HERZEGOVINA)

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The most widespread species of the genus *Crataegus* in Bosnia and Herzegovina is *Crataegus monogyna*. *C. monogyna* is characterized by pronounced variability of morological characters, which are highly influenced by environmental factors. The characters of leaves and fruits are the most informative in the taxonomy of the genus *Crataegus*. In addition to pronounced intraspecific variability, *C. monogyna* hybridizes with at least five other European species. This phenomenon further complicates the taxonomic complexity of the genus *Crataegus*. The aim of this paper is to analyze the morphological variability of leaf and fruit characters of *C. monogyna* in the upper part of the Cvrcka valley. Material for morphological analysis was collected in the area of the upper part of the Cvrcka valley (Kostići, Kneževo). Short shoots from 33 individuals were collected with leaves and fruits. Morphological analysis of *C. monogyna* was performed on 15 morphological leaf and fruit characters, of which four characters represent the ratio or quotient of the two original measurements. Descriptive statistics and correlation analysis of morphological characters were performed on the total sample. Leaf characters showed low to moderate variability, while fruit characters showed low variability. Correlation analysis showed that all characters are statistically significant correlated, which is less pronounced in fruit characters. The results were compared with similar studies in the area of Banj brdo (Banja Luka), Bardača and other literature data. This research contributes to a better understanding of morphological variability of *C. monogyna* in Bosnia and Herzegovina.

KEYWORDS: *Crataegus monogyna*, morphology, Cvrcka

UTICAJ URBANOG ZAGAĐENJA NA MORFOMETRIJSKE KARAKTERISTIKE LISTA VRSTE *Tilia platyphyllos* SCOP. U BANJA LUCI I DOBOJU

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Saobraćaj, industrija i toplane su najčešći izvori zagađenja teškim metalima i drugim polutantima u urbanim sredinama. Dendroflora ima značajnu ulogu kao bioindikator zagađenja urbanog zemljišta, vode i vazduha. U ovom radu su predstavljeni rezultati procjene nivoa zagađenja gradskih sredina Banja Luke i Doboja metodom biološkog monitoringa. Izmjereni su morfometrijski parametri listova lipe (*Tilia platyphyllos* Scop.), česte drvenaste vrste koja se gaji u parkovima i drvoredima ovih gradova. Da bi se istražio značaj listova kao bioindikatora urbanog zagađenja, izvršeno je poređenje sa kontrolnim uzorkom, sakupljenim na lokalitetu gdje je isključeno zagađenje. Analizirano je pet morfometrijskih karakteristika. Mjerenja su vršena na skeniranim listovima pomoću programa Digimizer Image Analysis Software Version 4.0.0.0. Statistička analiza je pokazala značajne razlike između vrijednosti analiziranih morfometrijskih karaktera listova skupljenih na istraženim gradskim lokalitetima i kontrolnom lokalitetu. Rezultati ukazuju na značaj primjenjenih analiza u biološkom monitoringu stepena zagađenja gradskih sredina.

KLJUČNE RIJEČI: urbano zagađenje, Banja Luka, Doboj, lipa, morfometrijske karakteristike

IMPACT OF THE URBAN POLLUTION ON THE MORPHOMETRIC TRAITS OF THE LEAVES OF *Tilia platyphyllos* SCOP. IN THE CITIES OF BANJA LUKA AND DOBOJ

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Traffic, industry, and heating plants are the most common sources of pollution with heavy metals and other pollutants in the urban environments. Dendroflora plays a significant role as a bioindicator of urban soil, water and air pollution. This paper presents the results of the assessment of the pollution levels in the urban areas of Banja Luka and Doboj by the method of biological monitoring. The morphometric parameters of linden leaves (*Tilia platyphyllos* Scop.), a common woody species grown in the parks ad alleys of these cities, were measured. In order to investigate the importance of leaves as a bioindicator of urban pollution, a comparison was made with a control sample, collected at the site where pollution was excluded. Five morphometric traits were analyzed. Measurements were performed on the scanned leaves by Digimizer Image Analysis Software Version 4.0.0.0. Statistical analyzis was shown significant differences between the values of the analyzed morphometric traits of the leaves collected at the investigated urban sites and the control site. The results indicate the importance of applied analyzes in the biological monitoring of the pollution leven of urban environments.

KEYWORDS: urban pollution, Banja Luka, Doboj, linden, morphometric traits

NOVI NALAZ MAHOVINE *Buxbaumia viridis* (MOUG. EX LAM. & DC.) BRID. EX MOUG. & NESTL. (BUXBAUMIACEAE) U BOSNI I HERCEGOVINI

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Uslijed dugogodišnjeg odsustva brioloških istraživanja u Bosni i Hercegovini, borealno-montana mahovina *Buxbaumia viridis* (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl. poznata je samo sa nekoliko starijih nalazišta i jednog novijeg nalazišta u Nacionalnom parku Sutjeska. Ova vrsta je zakonski zaštićena i nalazi se na Prilogu II Direktive o staništima i Dodatku I Bernske konvencije. Vrsta *B. viridis* ima status najmanje zabrinjavajuće (LC) na trenutnoj Crvenoj listi mahovina Evrope, dok je na prethodoj listi imala status ranjive vrste (V). Cilj ovoga rada je upotpunjavanje podataka o distribuciji vrste *B. viridis* u Bosni i Hercegovini. Tokom florističkih terenskih istraživanja planine Orjen 2019. godine, pronašli smo novo nalazište mahovine *B. viridis.* Populacija je pronađena na području Parka prirode Orjen, između Pirine poljane i Dobrog dola, u pojasu bukovih šuma. Jedinke su naseljavale površinu palog stabla koje se nalazilo u poodmakloj fazi truljenja. Za svoj razvoj, vrsta *B. viridis* zahtijeva staništa sa humidnim mikroklimatom, tako da se može očekivati da je vrsta rasprostranjena i na ostalim pogodnim staništima na planini Orjen i ostalim prašumskim područjima Bosne i Hercegovine.

KLJUČNE RIJEČI: Bryophyta, Orjen, Dinaridi, Bernska konvencija, distribucija

NEW RECORD OF MOSS *Buxbaumia viridis* (MOUG. EX LAM. & DC.) BRID. EX MOUG. & NESTL. (BUXBAUMIACEAE) IN BOSINA AND HERZEGOVINA

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Due to the long absence in bryophyte explorations in Bosnia and Herzegovina, a Boreal-montane moss species *Buxbaumia viridis* (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl. is known only from a few older findings and one newer finding in the Sutjeska National Park.. This species is legally protected and listed in the Annex II of the Habitats directive and Appendix I of the Bern Convention. The status of *B. viridis* on the current European Red List of bryophytes is LC (Least Concern), while in previous European Red List of bryophytes it was considered as V (Vulnerable). The aim of this paper is to contribute to distribution data of *B. viridis* in Bosnia and Herzegovina. During the floristic field investigations of the Mt Orjen in 2019, we discovered a new locality of *B. viridis*. The population of *B. viridis* occurred in the area of Nature Park Orjen, in a beech forest belt between Pirine poljane and Dobri do. The individuals inhabited the surface of fallen logs in advanced stages of decay. For its ontogeny, *B. viridis* requires habitats with a humid microclimate, so it can be expected that the species is distributed in other suitable habitats on Mt Orjen and other primeval forests of Bosnia and Herzegovina.

KEYWORDS: Bryophyta, Orjen, Dinaric Mountains, Bern Convention, distribution

NOVI NALAZ VRSTE *Pontechium maculatum* (L.) BÖHLE & HILGER (BORAGINACEAE) U BOSNI I HERCEGOVINI

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Pontsko-panonska vrsta *Pontechium maculatum* (L.) Böhle & Hilger odlikuje se ograničenim arealom u Bosni i Hercegovini. Raste na različitim geološkim podlogama i pripada grupi fakultativnih metalofita. Do sada je poznato samo nekoliko lokaliteta u Bosni i Hercegovini na kojima je pristutna vrsta *P. maculatum*: planina Varda, Vardište, te planine Ozren i Borja. Cilj ovog rada je dopuna podataka o distribuciji vrste *P. maculatum* u Bosni i Hercegovini. Tokom terenskih floristićkih istraživanja ultramafita u maju 2019. godine, zabilježeno je novo nalazište vrste u istočnom dijelu Bosne i Hercegovine. Biljke su rasle na strmim ultramafitskim padinama jugoistočnih ekspozicija vrha Vidkovac prema rijeci Lim (Mioče, Rudo). Populacija je brojala do 100 jedinki. Literaturni podaci o lokalitetima koji se nalaze u blizini novokonstatovanog potiču iz 1912. godine. Vrsta *P. maculatum* nalazi se na popisu Priloga II i IV Direktive o staništima, kao i na revidiranom Prilogu I Rezolucije 6 Bernske konvencije. Takođe, uvrštena je u Spisak biljnih vrsta za Crvenu knjigu Bosne i Hercegovine u kategoriji rijetke ili potencijalno ugrožene vrste. Probijanje puteva, kamenolomi i požari moguće su prijetnje koje u velikoj mjeri doprinose degradaciji specifičnih ultramafitskih staništa na kojima ova biljka raste. Potrebno je vršiti stalno praćenje populacija date vrste u skladu sa regulativima vezanim za očuvanje njenih staništa u Bosni i Hercegovini. Novi nalaz vrste *P. maculatum* doprinosi boljem poznavanju njene distribucije u Bosni i Hercegovini.

KLJUČNE RIJEČI: *Pontechium maculatum*, ultramafiti, flora

NEW RECORD OF *Pontechium maculatum* (L.) BÖHLE & HILGER (BORAGINACEAE) IN BOSNIA AND HERZEGOVINA

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The Pontic-Pannonian species *Pontechium maculatum* (L.) Böhle & Hilger has limited area in Bosnia and Herzegovina. It inhabits different geological substrates as facultative metallophyte. So far there has only been known a few localities in Bosnia and Herzegovina where *P. maculatum* is present: mountain Varda, Vardište, and mountains Ozren and Borja. The aim of this paper is to complement the knowledge of distribution of *P. maculatum* in Bosnia and Herzegovina. During the field floristic investigations of ultramafic bedrocks in May 2019, new locality of the species was recorded in the eastern part of Bosnia and Herzegovina. Plants were growing on southeastern expositions of steep ultramafic slopes of summit Vidkovac towards river Lim (Mioče, Rudo). Population counted less than 100 individuals. Literature data about localities near the new one date from 1912. *P. maculatum* is listed on Annexes II and IV of Habitats Directive as well as on revised Annex I of Resolution number 6 of the Berne Convention. It is also on the List of plant species for the Red Book of Bosnia and Herzegovina in the category of rare or potentially endangered species. The possible threatening factors as road penetration, stone pits and fires contribute greatly to degradation of the species' specific ultramafic habitats. Further population monitoring is needed in accordance with regulations related to the conservation of its habitats in Bosnia and Herzegovina. New finding of *P. maculatum* contributes to better knowledge of its distribution in Bosnia and Herzegovina.

KEYWORDS: *Pontechium maculatum*, ultramafics, flora

ALELOPATSKI UTICAJ AMBROZIJE NA KUKURUZ *(Zea mays* L.) I PŠENICU (*Triticum aestivum* L.)

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Ambrosia artemisiifolia L. (ambrozija) kao izuzetno invazivna vrsta koja brzo nalazi optimalne uslove za rast nije samo jak alergen već značajno snižava i ugrožava kvalitet biljne zajednice. Alelopatski uticaj između ambrozije i drugih vrsta biljaka još uvijek nije dovoljno razjašnjen, što otežava razumijevanje komatibilnost sa drugim biljkama, kako u poljoprivrednim, tako i u prirodnim uslovima. Stoga, u ovom radu smo pratili alelopatski uticaj vodenog ekstrakta ambrozije različitih koncetracija (koncentrovani ekstrakt 1:2, 1:4 i 1:8) na klijavost i rast kukuruza i pšenice. Vodeni ekstrakti pripremljeni su od svježih, potom osušenih listova ambrozije prema standardnoj metodi. Obe ispitivane vrste su bile osjetljive na ekstrakt ambrozije, ali je procenat klijavosti bio manji kod kukuruza (za 40%) u odnosu na pšenicu, pa se može reći da je ova vrsta bila osjetljivija. Dužina korijenčića i ponika obe ispitivane vrste se smanjivala proporcionalno sa povećanjem koncentracije ekstrakta. Dobijeni rezultati su pokazali da je potrebno pravovremeno suzbijati ovu korovsku vrstu u usjevima gajenih biljaka, uslijed inhibitornog uticaja na klijavost i rast kukuruza i pšenice.

KLJUČNE RIJEČI: alelopatija, ambrozija, kukuruz, pšenica

ALLELOPATHIC INFLUENCE OF AMBROSIA ON MAIZE (*Zea mays* L.) AND WHEAT (*Triticum aestivum* L.)

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Ambrosia artemisiifolia L. (ragweed) as an extremely invasive species that quickly finds optimal conditions for growth is not only a strong allergen but also significantly lowers and threatens the quality of the plant community. The allelopathic influence between ragweed and other plant species is still not sufficiently elucidated, making it difficult to understand compatibility with other plants, both in agricultural and natural conditions. Therefore, in this paper, we monitored the allelopathic effect of ragweed aqueous extract of different concentrations (concentrated extract 1:2, 1:4 and 1:8) on germination and growth of corn and wheat. Aqueous extracts were prepared from fresh, then dried ragweed leaves according to the standard method. Both tested species were sensitive to ragweed extract, but the germination percentage was lower in maize (by 40%) compared to wheat, so it can be said that this species was more sensitive. The length of roots and ponies of both examined species decreased in proportion to the increase of the extract concentration. The obtained results showed that it is necessary to timely control this weed species in crops of cultivated plants, due to the inhibitory effect on germination and growth of corn and wheat.

KEYWORDS: allelopathy, ragweed, corn, wheat

UTICAJ DEFICITA VODE IZAZVANOG MANITOLOM NA KLIJAVOST SJEMENA I RAST KUKURUZA

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Suša je jedan od ključnih abiotičkih faktora koji utiče na smanjenje rasta, razvoja i produktivnosti biljaka širom svijeta. S obzirom da je klijanje prva faza rasta koja u velikoj mjeri određuje kvalitet i prinos biljke, od velike je važnosti praćenje uticaja različitih faktora na odvijanje ovog procesa. Stoga je u ovom radu praćen uticaj stresa izazvanog sušom na klijavost sjemena i rast ponika kukuruza (sorte Šećerac i hibrida Pioneer B23) tretiranih različitim koncentracijama manitola: 5%, 10% i 20%. Rezultati istraživanja pokazali su značajne razlike između ispitivane sorte i sjemena. Pri tretiranju sjemena hibrida Pioneer b23 sa 5% rastvorom manitola uočava se da je klijavost sjemena bila jednaka kao u kontroli, dok je pri 10% rastvoru manitola klijavost sjemena za 6.67% bila manja u odnosu na kontrolu dok je najveća koncentraciji manitola upotpunosti inhibirala klijanje sjemena. Procenat klijanja pri tretmanu 5% manitolom bio smanjen za 8.89%, pri 10 % manitolom za 17.78%, dok je pri uticaju 20% manitola za 37.78% % u odnosu na kontrolu. Rezultati statističke analize pokazuju signifikantnu razliku između svježe i suve mase i dužine korijena i stabla u odnosu na različite koncentracije manitola, Uočena zavisnost svakako nije samo posljedica povećane koncentracije manitola nego i razlike u vododržećoj sposobnosti ispitivanih sorti. Upoređujući dobijene rezultate zaključujemo da se sjeme kukuruza Pioneer B23 pokazalo kao toleratnije i otpornije na sušu u odnosu na sortu Šećerac te ga možemo preporučiti za gajenje u uslovima vodnog deficita.

KLJUČNE RIJEČI: kukuruz, klijanje, manitol, rast, stres

IMPACT OF WATER DEFICIT INDUCED BY MANNITOL ON SEED GERMINATION AND SEEDLING GROWTH OF MAIZE

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Drought is one of the major abiotic factors leading to diminishing growth, development, and productivity of plants worldwide. Considering that germination is the first phase of growth which in large measure determines plant quality and yield, knowing the effects of different factors on this process is of major importance. This paper studies the effect of drought-induced stress on seed germination and seedling growth of maize (the Sweet corn variety and the hybrid Pioneer B23) treated with different concentrations of mannitol: 5%, 10%, and 20%. The results show significant differences between the variety and hybrid examined. Seed germination of the hybrid Pioneer B23 treated with the 5% mannitol solution was the same as in the control, while treatment with 10% mannitol solution resulted in seed germination 6.67% lower than the control, and the highest concentration of mannitol completely inhibited seed germination. Seed germination of the Sugar corn variety treated with the 5% mannitol solution was reduced by 8.89%, at 10% mannitol by 17.78%, and at 20% mannitol by 37.78% compared to the control. The results of the statistical analysis show a significant difference between fresh and dry mass and the length of the roots and the stem at different concentrations of mannitol. The observed difference is certainly not just a consequence of higher mannitol concentrations, but also a difference in the water-retention capability of the variety and hybrid studied. Comparing the results, we conclude that the seed of Pioneer B23 corn has proven more drought tolerant and resistant.

KEYWORDS: germination, growth, maize, mannitol, stress

SEED PRIMING EFFECTS ON THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN RADISH, BASIL, AND TOMATO

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The priming method is one of the most effective methods for improving seed germination and plant growth in unfavourable environmental conditions. The research aimed to determine the effects of different priming methods (10^{-3} GA_3, 10^{-3} IAA, 2,5% KNO_3, 1% $MgSO_4$, 1% H_2O_2 and 0.01% ascorbic acid (AA)) on the concentration of photosynthetic pigments in radish (*Raphanus sativus* L.), basil (*Ocimum basilicum* L.) and tomato (*Solanum lycopersicum* L.). From each plant species at least 30 seeds per treatment (three replicas) were treated for 24 h and then exposed to dessication process for 48 h, while non-primed seeds were regarded as a control. The effect of these treatments on the concentration of chlorophyll and carotenoids was determined by the spectrophotometric method. The results of the study showed that the application of priming solutions had different effects on the concentration of photosynthetic pigments of the tested species. Comparing the efficiency of the applied treatments it is noticed that the treatment with KNO_3 had the most significant effect on the concentration of photosynthetic pigments of tomatoes, while in radish and basil it causes a decrease in the values of these pigments compared to the control (non-primed seeds). The most favourable effect on the pigment concentrations was observed in the treatment with $MgSO_4$ in radish and in the treatment with AA in basil. In addition, less statistically significant differences of carotenoids were found. It may be concluded that the appropriate priming method could improve plant photosynthetic potential and primary production.

KEYWORDS: priming, chlorophyll, carotenoids, concentration, cultivated plants

EFFECT OF DIFFERENT PRIMING METHODS ON RELATIVE WATER CONTENT OF SELECTED CROPS

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Priming is a treatment of seeds by various agents, which enable fast and synchronized germination and improve the performance of grown plants. Leaf relative water content (RWC) is an important indicator of plants water status since reflects the balance between water supply to the leaf tissue and transpiration rate. This research aimed to determine the influence of hormo-, halo-, hydro- and chemo priming treatments on the RWC of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.). From each plant species at least 30 seeds per treatment (three replicas) were treated with 10^{-3} GA_3, 10^{-3} IAA, 2.5% KNO_3, 1% $MgSO_4$, 1% H_2O_2, 0.01% ascorbic acid (AA) and H_2O for 24 h and then exposed to dessication process for 48 h, while non-primed seeds were regarded as a control. The obtained results indicate that applied treatments significantly affected the leaf RWC of treated compared to untreated plants, but treatments differed in efficacy, and statistically significant difference was found between the applied treatments. Analysis of RWC of wheat and oats shows a stimulating effect for each of the applied treatments. The highest RWC values in barley leaves were recorded during treatment with H_2O_2, KNO_3, and H_2O. These results confirm the assumption that the applied priming treatments could have a stimulating effect on the RWC of selected plants. This is important from the aspect of crop quality improvement since the plants with higher RWC values are known to have a higher ability to avoid dehydration under water stress conditions.

KEYWORDS: priming method, RWC, *Triticum aestivum*, *Hordeum vulgare*, *Avena sativa*

KLIJANJE SEMENA OZIMIH SORTI PŠENICE, RAŽI I TRITIKALE U USLOVIMA STRESA IZAZVANOG $NiCl_2$

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Degradacija zemljišta teškim metalima je sve češći faktor koji ograničava biljnu proizvodnju. Iz tog razloga, od velike važnosti je ispitivanje tolerantnosti vrsta, sorti i hibrida na stresne uslove spoljašnje sredine izazvane teškim metalima. Cilj istraživanja je da se utvrdi procenat klijavosti, porast korenka i pojava hipokotila ozimih sorti pšenice (*Triticum aestivum* L. sorta Takovčanka), raži (*Secale cereale* L. sorta Raša) i tritikalea (*Tritikalea* sorta КГ 20) u uslovima stresa izazvanog prisustvom $NiCl_2$. U eksperimentu su korišćeni rastvori $NiCl_2$ koncentracija 10^2 mol/m^3, 10 mol/m^3, 1 mol/m^3, 10^{-1} mol/m^3, 10^{-2} mol/m^3, 10^{-3} mol/m^3. Semena su površinski sterilisana sa 0,01% rastvorom $HgCl_2$ u trajanju od 1 minuta a zatim su dobro isprana u destilovanoj vodi. Nakon toga po 50 semena je postavljeno u sterilne petri kutije obložene filter papirom i natopljena su sa 5ml rastvora $NiCl_2$ određene koncentracije. Procenat klijavosti i porast klice zabeleženi su petog dana od početka eksperimenta. Rezultati istraživanja ukazuju da se inhibitorno dejstvo Ni na ispitivane parametre povećava sa povećanjem koncentracije. Koncentracija 10^2 mol/m^3 je delovala letalno na semena jer u toj koncentraciji u laboratorijskim uslovima ne dolazi do klijanja. Ozima sorta Tritikalea je tolerantnija na prisustvo Ni u odnosu na sorte pšenice i raži.

KLJUČNE RIJEČI: klijanje, pšenica, raž, tritikale, $NiCl_2$

GERMINATION OF SEEDS OF WINTER VARIETIES OF WHEAT, RYE AND TRITICALE UNDER CONDITIONS OF STRESS CAUSED BY $NiCl_2$

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Soil degradation by heavy metals is an increasingly common factor limiting plant production. For this reason, it is of great importance to examine the tolerance of species, varieties, and hybrids to stressful environmental conditions caused by heavy metals. The aim of research was to determine the percentage of germination, root growth, and occurrence of hypocotyl of winter wheat cultivars (*Triticum aestivum* L. cultivar Takovčanka), rye (*Secale cereale* L. cultivar Raša), and triticale (*Tritikalea* cultivar KG 20) under conditions of stress caused by the presence of $NiCl_2$. In this experiment were used $NiCl_2$ solutions of 10^2 mol/m^3, 10 mol/m^3, 1 mol /m^3, 10^{-1} mol/m^3, 10^{-2} mol/m^3, and 10^{-3} mol/m^3.The seeds were surface sterilized with 0.01% $HgCl_2$ solution for 1 minute and then washed well in distilled water. After that, 50 seeds were placed in sterile Petri dishes lined with filter paper and soaked with 5 ml of $NiCl_2$ solution of a certain concentration. Germination percentage and germ growth were recorded on the fifth day from the beginning of the experiment. The results of the research indicate that the inhibitory effect of Ni parameters testing increases with increasing concentration. The concentration of 10^2 mol/m^3 had a lethal effect on the seeds because no germination occurs in laboratory conditions at that concentration. The winter variety Triticale is more tolerant to the presence of Ni compared to the varieties of wheat and rye.

KEYWORDS: germination, wheat, rye, triticale, $NiCl_2$

ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS FROM RHIZOME OF SPECIES *Bolboschoenus laticarpus* MARHOLD HROUDOVA, ZAKRAVSKY & DUCHAČEK

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Bolboschoenus laticarpus (Cyperaceae) is a common species in river floodplains and temporarily flooded terrestrial habitats in Europe. The related species *Bolboschoenus maritimus* has been studied for antioxidant activity and used therapeutically as an astringent and a diuretic, while for *B. laticarpus* such data are completely absent. The aim of this study was to determine and compare antioxidant activity and total phenol and flavonoid content in various rhizome extracts of *B. laticarpus.* This study compared solvents with different polarity values. Methanol, chloroform, ethanol and ethyl-acetate extracts were tested at 250, 500, 625, 750, 825 and 1000 µg/ml of dry extract concentrations. Antioxidant activity was evaluated using the DPPH method, the total flavonoid content by the $AlCl_3$ (aluminum chloride) method and the total phenolic content by the Folin-Ciocalteau method. The results revealed that ethanol extract had the highest antioxidant capacity (IC_{50}, 0.981 mg/ml) while the chloroform extract has shown the lowest activity (IC_{50}, 11.78 mg/ml). Analysis of total phenolic content in different extract types showed that ethyl-acetate extract had the highest concentration of phenols (80 mgGAE/g extract), while chloroform extracts had the lowest values (7 mgGAE/g extract). The highest concentration of flavonoids was noticed in ethyl-acetate extract (539 mgQuE/g extract), while the lowest concentration was observed in methanol extract (103 mgQuE/g extract). This study confirmed that rhizome of *B. laticarpus* has significant antioxidant capacity and is good source of phenols and flavonoids. Further research is necessary in order to identify its chemical composition and bioactive compounds responsible for the antioxidant properties.

KEYWORDS: *B. laticarpus*, antioxidant activity, phenols, flavonoids

EFFECT OF TEMPERATURE STRESS ON ANTHOCYANIN CONTENT IN BASIL SEEDLINGS

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Variation of abiotic factors directly affects the synthesis of secondary metabolites in plants, and increased synthesis of secondary metabolites is among the most important adaptations to unfavourable environmental conditions. This study aimed to examine the total anthocyanin content (TAC) in basil seedlings (*Ocimum basilicum* L. cv. Genovese) under the conditions of low-temperature stress. After one month of growth under controlled conditions, the basil seedlings were exposed to positively low temperature (4 ˚C and 10 ˚C) for four hours, while the control group of plants was grown at 25 ˚C. The TAC in basil leaves and roots were determined spectrophotometrically and expressed as cyanidin-3-glucoside equivalent (mg cy3-GE/mg DW). The highest TAC was measured in basil leaves exposed to 4 ˚C (1.39 ± 0.06 mg cy3-GE/mg DW), followed by the values measured in leaves exposed to 10 ˚C (1.17 ± 0.18 mg cy3-GE/mg DW), which was higher compared to the TAC in leaves of control plants (0.93 ± 0.22 mg cy3-GE/mg DW). For basil roots, the highest TAC was measured under the treatment of 4 ˚C (1.00 ± 0.13 mg cy3-GE/mg DW), followed by 10 ˚C treatment (0.55 ± 0.16 mg cy3-GE/mg DW). Both values were higher compared to the TAC content in roots from control plants (0.42 ± 0.16 mg cy3-GE/mg DW). Based on the results of this research whereas the increased synthesis of anthocyanins under conditions of low-temperature stress can be seen, it could be concluded that anthocyanins as basil secondary metabolites have a significant protective role under stressful conditions.

KEYWORDS: anthocyanins, basil, temperature stress, secondary metabolites

ACTIVITY OF CLASS I AND CLASS III PEROXIDASES IN BASIL SEEDLINGS UNDER LOW-TEMPERATURE STRESS CONDITIONS

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During their life cycle plants are exposed to the various stressful conditions which resulting in lower plant productivity. Plants have adapted to the action of abiotic factors by developing different protection mechanisms including enzyme protection against oxidative damage caused by reactive oxygen species. Ascorbate peroxidase (A-POX) belongs to the Class I peroxidase and catalyzes the degradation of H_2O_2 using ascorbate as a reducing agent. Class III peroxidases including various isoforms of hem containing oxidoreductases that catalyze the oxidation of different substrates in the presence of H_2O_2. In the present work guaiacol peroxidase (G-POX, using guaiacol as an electron donor) and pyrogallol peroxidase (P-POX, using pyrogallol as an electron donor) were investigated. This study aimed to examine the effect of low-temperature stress (4 ˚C and 10 ˚C) on the activity of A-POX, G-POX, and P-POX in basil (*Ocimum basilicum* L.) leaves. After five weeks of seedling growth under controlled conditions, treatments with low temperature (4 ˚C and 10 ˚C) was carried out for four hours, whereas as control plants seedlings were grown under 25 ˚C. The A-POX activity was inhibited at low temperature (both under 4 ˚C and 10 ˚C, respectively). However, at the same temperature conditions, the activity of G-POX and P-POX was increased, but only under 10 ˚C, while under the 4 ˚C activity of these enzymes was lower compared to the control plants. The results obtained in this study suggest that in the basil seedlings different peroxidase activities were included under the conditions of unfavourable temperatures.

KEYWORDS: basil, temperature stress, ascorbat peroxidase, guaiacol peroxidase, pyrogallol peroxidase

MAKROFITSKA FLORA CERSKOG OBODNOG KANALA (ŠABAC, SRBIJA)

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Veštačka vodna tela, kao što su kanali, mogu predstavljati značajne prostore florističkog bogatstva u urbanim ekosistemima. Makrofite su važni indikatori prilikom monitoringa površinskih voda, dok makrofitska vegetacija predstavlja staništa za razvoj ostalih grupa akvatičnih organizama. Cerski obodni kanal je najveći veštački vodotok na teritoriji grada Šapca, ukupne dužine toka 30 km, uliva se u reku Savu. Cilj ovog rada je određivanje recentne flore akvatičnih makrofita, kao i određivanje ekološkog potencijala na osnovu diverziteta makrofita jednog dela Cerskog obodnog kanala. Istraživanje je vršeno tokom letnjih meseci 2020. godine na deonici toka kroz grad Šabac, ukupne dužine 5 km uzvodno od ušća. Podaci o makrofitskoj vegetaciji prikupljani su na svakih 1000 m prema standardnoj UKTAG LEAFPACS metodi. Na pet sektora sačinjeno je ukupno 100 vegetacijskih snimaka i zabeleženo je 13 vrsta makrofita. Najvećom relativnom pokrovnošću izdvajaju se vrste: *Lemna minor* L., *Sparganium erectum* L., *Ceratophyllum demersum* L. i *Nuphar lutea* (L.) Sm. Među konstatovanim tri vrste imaju konzervacioni značaj: *Nuphar lutea* ima status strogo zaštićene vrste, a *Iris pseudacorus* L. i *Potamogeton nodosus* Poiret. status zaštićenih vrsta na teritoriji Srbije. Prema broju vrsta makrofita (SR=13) i Shannon–Weaver indeksu diverziteta (SW=2,26) ekološki potencijal istraživanog dela toka ocenjen je kao dobar i bolji. Na osnovu rezultata može se zaključiti da Cerski obodni kanal predstavlja optimalno stanište za razvoj akvatične vegetacije, samim tim i drugih hidrobionata, kao i za razvoj retkih i ugroženih vrsta makrofita. Rezultati potvrđuju činjenicu da veštačka vodna tela mogu imati značajnu ulogu u očuvanju biodiverziteta.

KLJUČNE RIJEČI: cerski kanal, makrofite, konzervacioni značaj, ekološki potencijal

MACROPHYTE FLORA OF CERSKI BOUNDARY CHANNEL (ŠABAC, SERBIA)

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Artificial water bodies can represent significant places of floristic richness in urban ecosystems. Macrophytes are important indicators in monitoring of surface water bodies, while macrophyte vegetation represents habitats for other groups of aquatic organisms. Cerski boundary channel is the largest artificial watercourse on the territory of the town Šabac. The aim of this paper was determination of the recent flora of aquatic macrophytes and assessement of ecological potential based on the macrophytes richness and diversity of the Cerski boundary channel. The research was carried out during the summer months in 2020. Macrophyte vegetation data were collected according to the standard UKTAG LEAFPACS method. A total of 100 vegetation records were taken at five sectors and thirteen macrophyte species were recorded. The most abundant species with the highest relative cover were: *Lemna minor* L., *Sparganium erectum* L., *Ceratophyllum demersum* L. and *Nuphar lutea* (L.) Sm. Among the recorded, three species are of conservation interest for the territory of Serbia: *Nuphar lutea* have status of strictly protected species, while *Iris pseudacorus* L. and *Potamogeton nodosus* Poiret. have status of protected species. According to species richness (SR=13) and Shannon – Weaver diversity index (SW=2.26) ecological potential was assessed as good and better. It can be concluded that the Cerski boundary channel represents an optimal habitat for development of aquatic vegetation, and therefore other organisms, as well as for the development of rare and endangered macrophyte species. The results confirm the fact that artificial water bodies can play a significant role in biodiversity conservation.

KEYWORDS: Cerski channel, macrophytes, conservation value, ecological potential

WILD RASPBERRY FROM THE SERBIA – ANATOMICAL VARIABILITY OF THE LEAF

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Rubus idaeus belongs to subgenus *Ideobatus* which comprises approximately 200 species. In the Balkans, the wild raspberry mostly grows at altitudes above 1000m a.s.l. The aim of this study was to describe differentiations among populations based on multivariate statistics on characters related to the leaf anatomy. Anatomical analysis was carried out on plant samples from seven populations (30 specimens per each population) collected in the mountain region of Serbia. Observed anatomical parameters includes: height of adaxial and abaxial epidermis, thickness of palisade and spongy tissue, length and width of the main vascular bundles, length and width of the vascular bundles. The epidermis of adaxial and abaxial sides of the leaves were single-layered. Observed anatomical variability represented an adaptive response to different geographical and environmental factors. Multivariate statistics showed that the Mt. Golija population is distinguished on the basis of all the tested characters. In these individuals, the higher values for almost all anatomical examined characters was observed. Mt. Ozren population is characterized by the thinner leaves, as well as the smallest size of other tested parameters. The results obtained in this study indicate the great ecological plasticity of the wild raspberry populations from Serbia.

KEYWORDS: wild raspberry, anatomical variation, Serbia, multivariate statistics

WILD RASPBERRY FROM THE SERBIA – MORPHOLOGICAL VARIABILITY

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Wild raspberry is a member of the Rosaceae family, which have about 3000 species, distributed in about 90 genera. The aim of this study was to quantify morphological variation among populations and to describe differentiations among populations based on multivariate statistics on characters related to the leaf morphology. Morphological analysis was made for 30 samples per each population. Height and latitude of bush was carried out directly, on study sites, while the length and width of the leaves were measured on herbarium samples. Sudy locations were: Mt. Studena planina, Mt. Željin, Mt. Goč, Mt. Golija, Mt. Kopaonik, Mt. Ozren and Mt. Stara Mountain. The highest average height had individuals from the Goč (123.40 cm), while the lowest average height had individulas from Mt. Kopaonik study site (77.00 cm). This study showed that the longest and the widest leaves had representatives of the population Mt. Studena planina (9.20 and 9.25 cm). The smallesst leaves had the representatives from population P5 (length – 6,32 cm; width – 5.11 cm). One way Anova showed that there was a statistically significant difference between all populations for all morphological characters examined ($p<0.05$). PCA showed that the Mt. Studena planina population is distinguished on the basis of all the tested characters. In these individuals, was recorded the longest and widest leaves, as well as individuals with the largest latitude. The results obtained in this study represent a significant contribution to the knowledge of the morphological characteristics of wild raspberry populations from Serbia.

KEYWORDS: wild raspberry, morphological variation, Serbia, multivariate statistics

ANATOMSKE KARAKTERISTIKE LISTA VRSTA RODA *Polygonum* s. str. SA PROSTORA CENTRALNOG BALKANA I JUŽNOG OBODA PANONSKE NIZIJE

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Dosadašnja istraživanja lista vrsta roda *Polygonum* (Polygonaceae) odnose se na morfologiju i mikromorfologiju. Međutim anatomskim istraživanjima, do sada, su obuhvaćene samo pojedine vrste. Cilj rada je da se utvrdi da li postoje anatomski parametri u građi lista koji bi bili od koristi u razdvajanju blisko srodnih vrsta roda *Polygonum* s. str. Istraživanjem je obuhvaćeno pet vrsta i jedna podvrsta iz 31 populacije sa prostora centralnog Balkana i južnog oboda Panonske nizije. Poprečni preseci listova napravljeni su metodom kriotehnike. U anatomskom pogledu, liska ispitivanih taksona roda *Polygonum* je izolateralne građe, sa hlorenhimom izdiferenciranim na palisadno i sunđerasto tkivo. *Listovi su amfistomatični.* Glavni provodni snopić kod svih taksona formira na abaksijalnoj strani liske jasno ispupčenje u vidu rebra. Prisutno je, pored njega, i više bočnih snopića različite veličine. Kod taksona bliskih vrsti *P. arenarium,* glavni nerv je jasno izražen i na adaksijaloj strani. Grupe ćelija sklerenhimskog tkiva prisutne su subepidermalno u zoni rebara i na obodu lista, kao i uz floemski i ksilemski deo provodnih snopića. Bočna rebra su kod vrsta *P. arenastrum* i *P. aviculare* jasno vidljiva na abaksijalnoj strani, kod taksona bliskih vrsti *P. arenarium* rebra su manje ili više jasno vidljiva i na adaksijalnoj strani, dok kod *P. graminifolium* bočna rebra nisu izražena. Na osnovu datog opisa anatomske građe lista moguće je razdvojiti analizirane taksone u tri grupe, ali je za definitnivno donošenje zaključaka neophodno uključiti i rezultate merenja odgovarajućih anatomskih karaktera lista.

KLJUČNE REČI: glavni nerv, *Polygonum*, rebra, sklerenhim

ZAHVALNICA: Ovo istraživanje je finansijski podržano od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije (Br. 451-03-68/2020-14/ 200125).

LEAF ANATOMICAL CHARACTERISTICS OF THE GENUS *Polygonum* s. str. SPECIES IN THE CENTRAL BALKAN AND THE SOUTHERN PART OF THE PANNONIAN BASIN

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Previous research of the leaf in the genus *Polygonum* (Polygonaceae) was focused on morphology and micromorphology, while anatomical research has so far covered only certain species. The aim of this study is to determine whether there are anatomical parameters in the leaf structure that would be useful in separation of closely related species of the genus *Polygonum* s. str. The study includes 5 species and 1 subspecies from 31 populations from the central Balkan and the southern part of the Pannonian Basin. Leaf cross-sections have been made by the cryotechnic procedure. Anatomically, the examined leaves are isolateral, with the chlorenchyma differentiated into palisade and spongy tissue. The leaves are amphistomatic. The main vein is prominent abaxially, for all taxa. Apart from it, there are numerous lateral vascular bundles of different sizes. In taxa closely related to *P. arenarium*, the midrib is clearly expressed on the adaxial side, too. Groups of cells of sclerenchyma tissue are present subepidermaly in the rib zone, on the leaf margins, and closely to the phloem and xylem. Lateral ribs are clearly differentiated abaxially in *P. arenastrum* and *P. aviculare*. In taxa closely related to *P. arenarium* ribs are also more or less apparent adaxially, while in *P. graminifolium* lateral ribs are not visible. Based on the given anatomical description of the leaf structure, it is possible to differentiate three groups of taxa. However, measured values of the appropriate anatomical characteristic of the leaf are necessary to be included so as to come to definite conclusions.

KEYWORDS: main vein, *Polygonum*, ribs, sclerenchyma

ACKNOWLEDGEMENT: This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2020-14/ 200125).

DIVERSITY OF NEEDLE VOLATILES OF NATIVE *Abies cephalonica* LOUDON POPULATIONS

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Abies cephalonica Loudon (Greek Fir) is a fir species endemic to the high mountains of central and southern Greece. In the present study, diversity of headspace needle volatiles of six *A. cephalonica* populations from the Cephalonia Island, the Peloponnese and central continental Greece was analyzed using GC-MS/FID (Gas Chromatography-Mass Spectrometry/Flame Ionization Detector) analyses. In order to determine the overall phytochemical variation and relationships among the individuals from analyzed populations, principal component analyses (PCA) was performed. The obtained scatter plot did not indicate any population segregation, as individuals from both island and continental populations generally overlapped indicating at least occasional pollen transport from the mainland to the Cephalonia Island. Furthermore, based on diversity of needle volatiles, the south-central continental population from Parnassos Mt., assigned to *A. cephalonica* var. *graeca* (Fraas) T.S. Liu showed significant resemblance to the rest of the analyzed Greek fir populations.

KEYWORDS: *Abies cephalonica*, headspace, needle volatiles, chemodiversity

ACKNOWLEDGEMENTS: Financial support of the Ministry of Education, Science and Technological Development of Serbia (Project No. 451-03-68/2020-14/200124) is gratefully acknowledged.

MORPHOLOGICAL VARIABILITY OF *Achillea millefolium* L. AND *A. collina* (BECKER EX RCHB.F.) HEIMERL FROM SERBIA

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In this study the morphological variability of *Achillea millefolium* L. and *A. collina* (Becker ex Rchb.f.) Heimerl from Serbia was analyzed. As these are two morphologically similar and related taxa from *A. millefolium* aggregate, the main objective of this study was their better morphological characterization. A total number of 75 individuals from 5 populations was compared at the level of 9 quantitative traits. According to ANOVA 6 analyzed quantitative traits can be used to distinguish the two taxa. In addition, PCA pointed out the existence of two clearly distinguished morphological units: populations of *A. millefolium* were characterized by longer rosette leaves, rosette leaf segments and stem leaf segments as well as shorter ligulate and tubular florets and involucral bracts compared to population of *A. collina*.

KEYWORDS: *Achillea millefolium*, *A. collina*, Serbia, morphometry, variability

ACKNOWLEDGEMENTS: This research was supported by Grant No 173029 by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Zoologija i fiziologija životinja

Zoology and Animal Physiology

DIVERZITET ZMIJA OKOLINE TREBINJA

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Opština Trebinje je locirana na krajnjem jugu Republike Srpske, a u literaturi je poznata kao „vruća tačka“ raznovrsnosti gmizavaca Bosne i Hercegovine. Cilj ovog rada je predstaviti raznovrsnost zmija okoline Trebinja, a na bazi rezultata terenskih istraživanja samih autora kao i literaturnih podataka. Terenska istraživanja su na navedenom području sprovedena tokom 2019. i 2020. godine. Ukupno je zabilježeno 10 vrsta zmija: jedna vrsta iz porodice Viperidae (poskok – *Vipera ammodytes*), jedna vrsta iz porodice Psammophiidae (zmajur – *Malpolon insignitus*), dvije vrste iz porodice Natricidae (bjelouška – *Natrix natrix* i ribarica – *Natrix tessellata*) i šest vrsta iz porodice Colubridae (Eskulapov smuk – *Zamenis longissimus*, leopardski smuk – *Zamenis situla*, četvoroprugi smuk – *Elaphe quatuorlineata*, balkanski smuk – *Hierophis gemonensis*, šilac – *Platyceps najadum* i crnokrpica – *Telescopus fallax*). Najveći broj pronađenih vrsta (sedam) zabilježen je tokom juna mjeseca. Najčešće su nalažene jedinke vrsta *Hierophis gemonensis* i *Malpolon insignitus*, dok su jedinke vrsta *Zamenis situla* i *Telescopus fallax* imale najmanju frekvenciju nalaženja. Zabilježene vrste, od kojih je većina zaštićena, daju istraživanom području veliki značaj u smislu zaštite i očuvanja prirodnih vrijednosti i diverziteta čitavog prostora.

KLJUČNE RIJEČI: Trebinje, fauna zmija, Viperidae, Colubridae

SNAKES DIVERSITY IN THE VICINITY OF TREBINJE

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The Municipality of Trebinje is located in the southernmost part of the Republic of Srpska. In literature, it is known as a hotspot of reptilian diversity in Bosnia and Herzegovina. This paper aimed to present the diversity of snakes in the vicinity of Trebinje, based on the authors' field investigations and literature data. Field investigations in the given area were conducted during 2019 and 2020. In total, 10 snake species were recorded: one of the family Viperidae (nose-horned viper – *Vipera ammodytes*), one of the family Psammophiidae (Eastern Montpellier Snake – *Malpolon insignitus*), two of the family Natricidae (Grass snake – *Natrix natrix* and Dice snake – *Natrix tessellata*) and six of the family Colubridae (Aesculapian snake – *Zamenis longissimus*, Leopard snake – *Zamenis situla*, Four-lined snake – *Elaphe quatuorlineata*, Balkan whip snake – *Hierophis gemonensis*, Dahl's whip snake – *Platyceps najadum* and Cat snake – *Telescopus fallax*). Majority of the recorded species (seven) were recorded during June. Most often, individuals of *Hierophis gemonensis* and *Malpolon insignitus* were found, while the frequency of observations was the lowest for *Zamenis situla* and *Telescopus fallax*. The recorded species, the majority of which are protected, provide great importance to the searched area in terms of protection and preservation of natural values and diversity of the entire region.

KEYWORDS: Trebinje, snakes fauna, Viperidae, Colubridae

REPRODUKTIVNE KARAKTERISTIKE DUNAVSKOG RAKA (*Pontastacus leptodactylus*) IZ RIJEKE MATURE

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Cilj ovog istraživanja je utvrditi reproduktivne karakteristike dunavskog raka, *Pontastacus leptodactylus* iz rijeke Mature u Srpcu. Rakovi su uzorkovani mjesečno pomoću LiNi vrša od maja do decembra 2019. godine. Uzorak su činila 54 raka (31 mužjak i 23 ženke). Od morfometrijskih karakteristika analizirane su: totalna dužina tijela (TBL), tjelesna težina (W), dužina kliješta (CLL), širina kliješta (CLW), dužina karapaksa (CPL), širina karapaksa (CPW), dužina abdomena (ABL) i širina abdomena (ABW). Maksimalna zabilježena dužina tijela iznosila je 133.7 mm kod mužjaka i 120.12 mm kod ženki, dok je maksimalna zabilježena težina tijela iznosila 55.6 g kod mužjaka i 49.8 g kod ženki. Najmanja fertilna ženka bila je dugačka 73.22 mm. Prosječan broj pleopodnih jaja (sa njihovim rasponom), bio je 259.32 (±SD 92.53; min. 74; max. 389). Prosječni prečnik i opseg jaja bio je 2.77 mm (±SD 0.111; min. 2.6 mm; max. 2.9 mm). Utvrđena je pozitivan korelacija (R^2 = 0.8774) između ukupne totalne dužine tijela ženki i broja pleopodnih jaja, a broj pleopodnih jaja prati povećanje dužine tijela. T - test pokazao je da postoje značane razlike između polova u CLW (p = 0.022), ABL (p = 0.006) i ABW (p = 0.027), što se objašnjava izraženim polnim dimorfizmom dunavskih rakova.

KLJUČNE RIJEČI: dunavski rak, *Pontastacus leptodactylus*, reproduktivne karakteristike, pleopodna jaja

REPRODUCTIVE CHARACTERISTICS OF DANUBE CRAYFISH (*Pontastacus leptodactylus*) FROM THE MATURA RIVER

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The aim of this study was to determine the reproductive characteristics of the danube crayfish, *Pontastacus leptodactylus* from the Matura River in Srbac. Crayfish were sampled monthly using LiNi traps from May to December 2019. A total of 54 crayfish were caught (31 males and 23 females). Some morphometric features were determined: body length (TBL), body weight (W), claw length (CLL), claw width (CLW), carapace length (CPL), carapace width (CPW), abdomen length (ABL) and abdomen width (ABW). The maximum recorded body length was 133.7 mm for males and 120.12 mm for females, while the maximum recorded weight was 55.6 g for males and 49.8 g for females. The smallest fertile female was 73.22 mm long. The average number of pleopodal eggs (with their range), was 259.32 (±SD 92.53; min. 74; max. 389). The average egg diameters and ranges were 2.77 mm (±SD 0.111; min. 2.6 mm; max. 2.9 mm). Positive correlation is significant ($R2 = 0.8774$) between total body size of females and the number of pleopodal eggs; the number of pleopodal eggs is followed by the increase of body length. The t-test showed significant differences between the sexes in CLW ($p = 0.022$), ABL ($p = 0.006$), ABW ($p = 0.027$) which are explained by the emphasized sexual dimorphism of the danube crayfish.

KEYWORDS: danube crayfish, *Pontastacus leptodactylus*, reproductive characteristics, pleopodal eggs

PRELIMINARNI PODACI O DISTRIBUCIJI GUŠTERA (REPTILIA: SQUAMATA, SAURIA) NA PODRUČJU NEVESINJSKOG POLJA I PODVELEŽJA - JUGOISTOČNA HERCEGOVINA

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Prema dostupnim literaturnim podacima, područje Nevesinjskog polja i platoa Podveležja su nedovoljno herpetološki istraženi. Ovaj prostor je biogeografski i klimatsko - ekološki prihvatljiv za nastanjenje većeg broja vrsta iz klase Reptilia. Osnovni cilj rada je utvrditi distribuciju guštera (Reptilia: Squamata, Sauria) na istraživanom području Nevesinjskog polja i platoa Podveležja. Istraživanje na području Nevesnjskog polja i Podveležja bilo je u periodu mjeseca Maja pa sve do Septembra 2018 god. Determinacijom herpetološkog materijala određeno je šest vrsta guštera: *Podarcis muralis* (Laurenti, 1768), *Lacerta viridis* (Laurenti, 1768), *Lacerta agilis* (Linnaeus, 17589), *Anguis fragilis* (Linnaeus, 1758), *Podarcis melisellensis* (Braun, 1877) i *Dalmatolacerta oxycephala* (Dumeril i Bibron, 1839). Vrste sa najvišim stepenom pojavnosti su: *Podarcis muralis* i *Lacerta viridis*, dok su manje česte *Dalmatolacerta oxycephala*, *Podarcis melisellensis* i *Lacerta agilis*. Rezultati rada ukazuju na karakterističnu pojavu za vrstu *Lacerta viridis* gdje se dominantnost ženki u periodu mjeseca Juna, Avgusta, Septembra 2018 god. jasno uočava, dok se za vrstu *Podarcis muralis* bilježi pojava krajem mjeseca Septembra 2018 god. Nakon dobivenih rezultata potvrđen je distributivni status i onih vrsta za koje u dosadašnjim literaturnim zapisima nije tačno naveden. Prisustvo vrste *Dalmatolacerta oxycephala* na osnovu dosadašnjih nalaza potvrđeno je u Avgustu 2018. godine na lokalitetu Podveležja (Srpača), dok je vrsta *Lacerta agilis* također potvrđena u Septembru 2019. godine na jezeru Alagovac.

KLJUČNE RIJEČI: bioraznolikost, Sauria, distribucija, Nevesinjsko polje, plato Podveležja

PRELIMINARY DATA ON THE DISTRIBUTION OF LIZARDS (REPTILIA: SQUAMATA, SAURIA) IN THE AREA OF THE NEVESINJE FIELDS AND PLATEAU OF PODVELEZJE - SOUTHEAST HERZEGOVINA

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According to the available literature data, the area of the Nevesinje field and the Podveležje plateau have been insufficiently herpetologically researched. This area is biogeographically and climatically - ecologically acceptable for the habitat of a large number of species from the class Reptilia. The main goal of this paper is to determine the distribution of lizards (Reptilia: Squamata, Sauria) in the investigated area of Nevesinje field and Podveležje plateau. The research in the area of Nevesnjsko polje and Podveležje was in the period of May until September 2018. Six species of lizards were determined by determining herpetological material: *Podarcis muralis* (Laurenti, 1768), *Lacerta viridis* (Laurenti, 1768), *Lacerta agilis* (Linnaeus, 17589), *Anguis fragilis* (Linnaeus, 1758), *Podarcis melisellensis* (Braunla, 1877) and *Dalmatolacerta oxycephala* (Dumeril and Bibron, 1839). The species with the highest incidence are: *Podarcis muralis* and *Lacerta viridis*, while less common are *Dalmatolacerta oxycephala*, *Podarcis melisellensis* and *Lacerta agilis*. The results indicate a characteristic phenomenon for the species *Lacerta viridis* where the dominance of females in the period of June, August, September 2018. clearly observed, while for the species *Podarcis muralis* the occurrence is recorded at the end of September 2018. After the obtained results, the distribution status of those species was confirmed, for which it has not been precisely stated in the previous literature records. The presence of the species *Dalmatolacerta oxycephala* based on previous findings was confirmed in August 2018 at the site Podveležja (Srpača), while the species *Lacerta agilis* was also confirmed in September 2019 on Lake Alagovac.

KEYWORDS: biodiversity, Sauria, distribution, Nevesinjsko polje, plateau of Podveležje

ERITROCITNI STATUS NEKIH AUTOHTONIH VRSTA RIBA

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Autohtone vrste riba predstavljaju značajnu komponentu ukupnog biodiverziteta. Njihovo proučavanje je od velikog značaja, jer je potpuno poznavanje preduslov za adekvatnu zaštitu i očuvanje. Cilj ovog istraživanja je bio ispitivanje eritrocitnog statusa nekih autohtonih vrsta riba slatkih voda Republike Srpske: dvoprugaste uklije - *Alburnoides bipunctatus* (Bloch, 1872), mrene - *Barbus barbus* (Linnaeus, 1758) i klena - *Squalius cephalus* (Linnaeus, 1758). Istraživanjem je obuhvaćeno 14 jedinki dvoprugaste uklije, 13 jedinki mrene i 11 jedinki klena. U dobijenim uzorcima krvi su standardnim metodama ustanovljeni sljedeći parametri eritrocitne loze: broj eritrocita, koncentracija hemoglobina (Hb), hematokrit (Hct) i vrijednosti hematoloških indeksa (MCV, MCH i MCHC). Pored navedenih parametara, istraživanjem su obuhvaćeni i osnovni morfometrijski parametri riba. Relevantnim statističkim metodama je izvršena obrada dobijenih rezultata i komparacija praćenih karakteristika. Na osnovu dobijenih podataka je zaključeno da rezultati odgovaraju podacima za navedene vrste sa drugih lokaliteta.

KLJUČNE RIJEČI: eritrociti, autohtone vrste riba, hematološki parametri

ERYTHROCYTE STATUS OF SOME INDIGENOUS FISH SPECIES

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Indigenous fish species represent a significant component of overall biodiversity. Study of these species is very important, because the complete knowledge is a prerequisite for adequate protection and preservation. The aim of this study was to examine the erythrocyte status of some indigenous fish species in fresh waters of the Republic of Srpska: *Alburnoides bipunctatus* (Bloch, 1872), *Barbus barbus* (Linnaeus, 1758) and *Squalius cephalus* (Linnaeus, 1758). The study included 14 individuals of *Alburnoides bipunctatus*, 13 individuals of *Barbus barbus* and 11 individuals of *Squalius cephalus*. Using standard methods the following erythrocyte parameters were determined in the obtained blood samples: red blood cell count, hemoglobin concentration (Hb), hematocrit (Hct) and hematological indices (MCV, MCH and MCHC). Beside these parameters, the basic morphometric parameters were also determined in the research. Relevant statistical methods were used to process the obtained results and compare the monitored characteristics. Based on the obtained data, it was concluded that the results correspond to the data for the mentioned species from other localities.

KEYWORDS: erythrocytes, indigenous fish species, hematological parameters

EFEKTI PRIMJENE SUPLEMENATA NA PARAMETRE ERITROCITNE LOZE WISTAR PACOVA

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Suplementi su dodaci ishrani koji mogu da pomognu, pojačaju ili asistiraju prirodnoj funkciji u organizmu. U svom sastavu najčešće sadrže različite minerale, vitamine, proteine, masne kiseline, probiotike ili druge metabolički aktivne supstance. Cilj ovog istraživanja je praćenje efekata primjene suplemenata na parametre eritrocitne loze Wistar pacova. Istraživanje je sprovedeno na ukupno 19 jedinki, koje su bile podijeljene u tri grupe. Prva eksperimentalna grupa je tretirana whey proteinom (protein surutke), druga eksperimentalna grupa je tretirana multivitaminom (u sastavu sadrži preko 20 vitamina i minerala), dok je treća grupa predstavljala kontrolnu grupu. Da bi se ispitao uticaj suplemenata na pojedine hematološke parametre u uzorcima krvi su pomoću hematološkog brojača određeni sljedeći parametri eritrocitne loze: broj eritrocita, hematokrit (Hct), srednja vrijednost zapremine eritrocita (MCV), srednja vrijednost količine hemoglobina u eritrocitu (MCH) i srednja vrijednost hemoglobina u litri eritrocita (MCHC). Koncentracija hemoglobina (Hb) je određena metodom po Drabkinu. Na osnovu dobijenih rezultata je uočeno da je srednja vrijednost broja eritrocita najniža u eksperimentalnoj grupi jedinki koje su tretirane whey proteinom. Vrijednost koncentracije hemoglobina u sve tri grupe je bila niža u odnosu na referentnu vrijednost. Srednja vrijednost hematokrita je pokazala odstupanja od referentnih vrijednosti u svim grupama. Za parametre MCH i MCHC je zabilježeno odstupanje u kontrolnoj i u grupi jedinki koje su tretirane multivitaminom. Na osnovu dobijenih rezultata se može zaključiti da pojedini suplementi mogu ostvariti uticaj na neke parametre eritrocitne loze Wistar pacova.

KLJUČNE RIJEČI: suplementi, Wistar pacovi, eritrociti, hematološki indeksi

EFFECTS OF SUPPLEMENT APPLICATION ON ERYTHROCYTE PARAMETERS OF WISTAR RATS

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Supplements are food addition that can help, enhance or assist natural body function. They usually contain minerals, vitamins, proteins, fatty acids, probiotics or some other metabolic active substances. The aim of this study was to investigate the effects of supplement application on erythrocyte parameters of Wistar rats. The research was conducted on 19 Wistar rats individuals. Individuals were randomly allocated into three groups. The first experimental group was treated with whey protein, the second experimental group was treated with a multivitamin (contains over 20 vitamins and minerals), while the third group represented the control group. In order to examine the influence of supplements on certain hematological parameters in collected blood samples the following erythrocyte parameters were determined using hematology analyzer: red blood cell count, hematocrite (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hemoglobin concentration (Hb) was determined using the Drabkin method. Results in our research showed decrease in red blood cells count in the experimental group of individuals treated with whey protein. The mean value of hemoglobin concentration was lower than the reference value in all three groups. The mean value for hematocrite showed deviations from the reference values in all groups. For MCH and MCHC parameters, deviations were observed in the control group and in the second experimental group. Based on the results, it can be concluded that certain supplements may have an effect on some erythrocyte parameters of Wistar rats.

KEYWORDS: supplements, Wistar rats, erythrocytes, hematological indices

HEAD CAPSULE SIZE AND SHAPE CHANGES DURING LATE POSTEMBRYOGENESIS IN MILLIPEDE *Megaphyllum unilineatum* (C. L. KOCH, 1838) (DIPLOPODA: JULIDA)

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Morphological variation of the head capsule in millipedes has been poorly investigated, at least by using geometric morphometrics. The aim of the present study is to explore head capsule size and shape differences between postembryonic stadia in *Megaphyllum unilineatum* (C. L. Koch, 1838) using aforementioned approach. Dorsal and ventral (gnathochilarium) head capsule sides of 91 individuals from the same locality, in different postembryonic stadia, were photographed using Carl Zeiss Axiocam MRc camera, and the scale was added to each picture. Landmarks were positioned in TpsDig program, whilst semi-landmarks were positioned in MakeFan program. Centroid size (CS) was calculated using CoordGen6 program, whilst CS differences were tested by ANOVA and *Post hoc* Tukey HSD for unequal N test in Statistica 7 program. Head capsule shape differences were obtained using Canonical Variate Analysis (CVA) in MorphoJ program. Gnathochilarium and dorsal side CS differed significantly among postembryonic stadia. Results of *Post hoc* test showed that differences in gnathochilarium and dorsal side CS differed significantly among all the stadia, with the exception stadia VI and VII, and stadia X and XI. Also, no significant differences in gnathochilarium CS were obtained between stadia IX and X. Results of CVA illustrated that gnathochilarium and dorsal side shape differed significantly between all postembryonic stadia, except between stadia X and XI. Additionally, no significant gnathochilarium shape differences were obtained between stadia VII and VIII. These results may indicate the presence of different developmental rates of gnathochilarium and dorsal head capsule side in sub-adults and adults of examined species.

KEYWORDS: millipedes: postembryonic stadia: geometric morphometrics

POSTEMBRYONIC DEVELOPMENT IN *Megaphyllum unilineatum* (C. L. KOCH, 1838) (DIPLOPODA: JULIDA)

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Millipedes (Diplopoda) are characterized by anamorphic development and three types of anamorphosis are recognized in these arthropods: euanamorphosis, hemianamorphosis and teloanamorphosis. Although postembryonic development of members of the order Julida is better known in comparison with the other diplopod groups, data on postembryogenesis in many julidans are still lacking. In this study, we examined postembryonic development of julidan millipede *Megaphyllum unilineatum* (C. L. Koch, 1838). Aims of this study were to determine the number of postembryonic stadia, to determine the first postembryonic stadium when animals can be sexed and to determine when the adulthood is reached in *M. unilineatum*. All individuals (400) examined in this study were sampled from one population in Belgrade (Serbia). In order to collect and to examine younger postembryonic stadia, animals were bred and reared in laboratory. Numbers of the following characters were used for stadia separation: total number of body rings, numbers of podous and apodous body rings, number of rows of ocelli and number of ocelli. In examined species, we have determined that postembryonic period of life cycle consists of eleven stadia and sexes can be distinguished from the stadium VI. Presence of mature eggs in ovaria in females and fully developed gonopods in males implies that adulthood is reached at the stadium IX. *Megaphyllum unilineatum* undergoes euanamorphosis during the postembryonic phase of life. Recorded patterns of changes in numbers of morphological characters used in this study offer reliable system for stadia delimitation in *M. unilineatum* and follow developmental pathways observed in Julida.

KEYWORDS: millipedes: anamorphosis: Julidae

THE EFFECTS OF ESTRADIOL THERAPY ON OXIDATIVE STRESS MARKERS IN ERYTHROCYTES AND BLOOD PRESSURE IN PREECLAMPSIA

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Preeclampsia is a specific syndrome in human pregnancy and a leading cause of maternal and fetal morbidity and mortality. The clinical manifestations of preeclampsia include the development of hypertension (≥ 140/90mmHg) and proteinuria (≥ 0.3 g/24 h) after the 20th gestation week. The only intervention that effectively reverses the syndrome of preeclampsia is delivery. The aim of this study was to determine effects of short-term estradiol therapy on oxidative stress markers in erythrocytes and blood pressure in preeclampsia. Two groups of women were recruited to the study: 22 healthy pregnant women and 20 pregnant women with preeclampsia. Intramuscular injections of 10 mg 17 β-estradiol were administrated to pregnant women during three days. Blood pressure was measured and venous blood was collected before and 24 h after each administration of estradiol injection. The following parameters of oxidative stress markers were determined in hemolysate: concentrations of superoxide anion - $O_2^{\cdot -}$, hydrogen peroxide - H_2O_2, nitrites - NO_2^-, peroxynitrite - $ONOO^-$ and lipid peroxide – LPO. The results of this study show that estradiol therapy significantly reduces mean arterial pressure and the concentrations of $O_2^{\cdot -}$, H_2O_2. NO_2^-, $ONOO^-$ and LPO in erythrocytes of preeclamptic women. The analysis of the relationship between decrease of the concentration of $O_2^{\cdot -}$, NO_2^-, $ONOO^-$ in erythrocytes and decrease of the mean arterial pressure in preeclampsia has shown a positive correlation. These results suggest that estradiol therapy leads to increased bioavailability of NO by reduction of oxidative stress parameters and contributes to erythrocytes mediated reduction of hypertension in preeclampsia.

KEYWORDS: preeclampsia, estradiol, oxidative stress markers

THE EFFECTS OF MATERNAL THROMBOPHILIA ON THE REDOX HOMEOSTASIS OF AMNIOTIC FLUID AND AMNIOTIC FLUID CELLS

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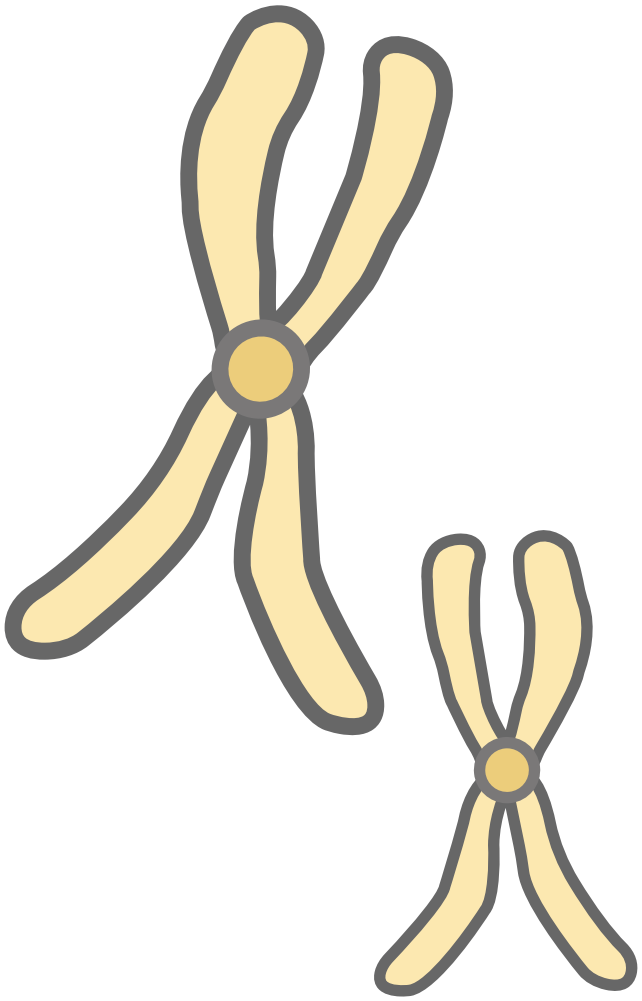
Thrombophilia in pregnancy is followed by a high prenatal morbidity and mortality. Considering that amniotic fluid and amniotic fluid cells reflect fetal health and status of metabolism, the determination of oxidative stress biomarkers in amniotic fluid and amniotic fluid cells can provide important information about health, development and mature of fetus during pregnancy. The aim of this study was to test the effects of maternal thrombophilia on the redox homeostasis of amniotic fluid and amniotic fluid cells in the first trimester of pregnancy. The study included two groups of pregnant women gestational age from 16 to 18 weeks: 32 healthy pregnant women and 23 pregnant women with hereditary thrombophilia. In amniotic fluid and amniotic fluid cells, concentrations of the following parameters of the oxidative stress markers were determined: superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxynitrite ($ONOO^-$), lipid peroxides (LPO), reduced glutathione (GSH) and oxidized glutathione (GSSG). The results of this study show that the concentrations of $O_2^{\cdot-}$, NO, $ONOO^-$ and LPO are significantly increased, while concentrations of H_2O_2, GSH and GSSG are significantly lower in amniotic fluid cells of pregnant women with thrombophilia. In the amniotic fluid of pregnant women with thrombophilia, an increased concentration of GSSG has been shown. Thrombophilia of the mother acts prooxidatively and induces increased of oxidative stress biomarkers and oxidative damage of lipids in the fetal cells.

KEYWORDS: thrombophilia, oxidative stress markers, amniotic fluid, amniotic fluid cells

Genetika i evolucija

Genetics and Evolution



MCM2, PCNA I EZH2 U LIMFOMAGENEZI

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Tumorske stanice nastaju nakupljanjem promjena u genomu normalnih stanica pa stoga zadržavaju dio karakteristika onih stanica iz kojih su i nastale. Iako više nisu pod kontrolom organizma, sustava ili tkiva u kojem se tumor razvija, tumorske stanice zadržavaju mogućnost komunikacije sa stanicama iz svog mikrookoliša i ta komunikacija može utjecati na promjene u njihovim osnovnim procesima poput replikacije i ponovne uspostave kromatina. Cilj ovog istraživanja bio je analizirati ekspresiju gena *MCM2*, *PCNA* i *EZH2* čiji produkti su ključni za regulaciju replikacije i ponovne uspostave kromatina i procijeniti prisutnost proteina koje ti geni kodiraju u normalnim i tumorskim B-stanicama. Korišteni su uzorci germinativnog centra tonzila (normalne B-stanice), folikularnog limfoma (indolentnog tumora porijeklom B-stanica) i dvije najčešće skupine difuznog B-velikostaničnog limfoma (agresivnog tumora B-stanica). Metode su uključivale lančanu reakciju polimerazom u stvarnome vremenu i imunohistokemijsko bojenje. Rezultati su pokazali da dolazi do značajnog smanjenja ekspresije proteina MCM2, PCNA i EZH2 u folikularnom limfomu u odnosu na normalne B-stanice, ali i do značajnog porasta njihove ekspresije u difuznom B-velikostaničnom limfomu u odnosu na folikularni limfom. Ovakvi rezultati sugeriraju da su za stupanj agresivnosti i razinu progresije limfoma značajne promjene osnovnih staničnih procesa, no da one ne pokazuju nužno i linearnost u odnosu na porast stupnja agresije tumora.

KLJUČNE RIJEČI: *MCM2*, *PCNA* i *EZH2*, B-limfomi

MCM2, PCNA AND EZH2 IN LYMPHOMAGENESIS

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Tumor cells develop by the accumulation of changes in the genome of normal cells and retain some of the characteristics of cells from which they originated. Although they are no longer under the control of the organism, system or tissue in which the tumor develops, tumor cells retain the ability to communicate with cells from their microenvironment and this communication can affect changes in their basic processes such as replication and chromatin reassembly. The aim of this study was to analyze the expression of *MCM2*, *PCNA* and *EZH2*, genes whose products are crucial for the regulation of replication and chromatin reassembly, and to assess the presence of proteins encoded by these genes in normal and tumor B-cell. Samples of the germinal center of the tonsils (normal B-cells), follicular lymphoma (indolent tumor of B-cell origin) and the two most common groups of diffuse large B-cell lymphoma (aggressive B-cell tumor) were used. Methods included real time-PCR analysis and immunohistochemical staining. The results showed that there is a significant decrease in the expression of MCM2, PCNA and EZH2 proteins in follicular lymphoma compared to normal B-cells, but also that there is a significant increase of their expression in diffuse B-cell lymphoma compared to follicular lymphoma. Such results suggest that changes in basic cellular processes are significant for the degree of aggression and the level of lymphoma progression, but that they do not necessarily show linearity when assessed in relation to the degree of tumor aggression.

KEYWORDS: *MCM2*, *PCNA* i *EZH2*, B lymphoma

PRISUSTVO HROMOZOMSKIH ABERACIJA KOD PAROVA SA STERILITETOM I HABITUALNIM POBAČAJEM

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Prema preporukama Svetske zdravstvene organizacije i Povelji međunarodnog prava ističe se da je osnovno ljudsko pravo pojedinca da slobodno i odgovorno, odlučuju o broju svoje dece kao i razmacima između porođaja. Neostvarivanje trudnoće nakon godinu dana redovnih polnih odnosa parove svrstava u grupu potencijalnih kandidata za lečenje od steriliteta. Od ukupnog broja pacijenata koji se leče od steriliteta u 7-12% uzrok je prisustvo hromozomskih aberacija u njihovim kariotipovima. Cilj ovog rada bio je da se ispita prisustvo i učestalost hromozomskih aberacija kod parova sa sterilitetom i habitualnim pobačajem u Univerzitetsko Kliničkom centru Republike Srpske (UKCRS). Habitualni pobačaj je spontani i ponovljeni pobačaj nepoznate etiologije. Analiza hromozoma podrazumeva uzimanje uzoraka krvi od pacijenata; kultivaciju ćelija te periferne krvi; preparaciju limfocita; bojenje hromozoma klasičnom tehnikom, tehnikom GTG i CBG traka i NOR; mikroskopsku analizu hromozoma i određivanje konstitucionog kariotipa pacijenata. Od 5.1.2009. do 31.12.2019. godine u Citogenetičkoj laboratoriji UKCRS urađeno je ukupno 3842 analiza kariotpa iz periferne krvi, pri čemu je 1956 pacijenata imalo uputnu dijagnozu habitualnog pobačaja ili sterilitet. Procenat pacijenata koji su imali aberacije u svojim kariotipovima a lečili su se od steriliteta i habitualnog pobačaja u UKCRS bio je 6,87%. .Najzastupljenije hromozomske aberacije u kariotipovima kako muških tako i ženskih pacijenata lečenih od steriliteta i habitualnog pobačaja bile su inverzije. Takođe, bilo je dva puta više pacijenata sa uputnom dijagnozom sterilitet u odnosu na one sa habitualnim pobačajem, a da su imali aberantne kariotipove. Važnost analize kariotipva pacijenata koji se leče od steriliteta i habitualnog pobačaja je velika.

KLJUČNE REČI: hromozomske aberacije, sterilitet, kariotip, habitualni pobačaj

PRESENCE OF CHROMOSOME ABERRATIONS IN PAIRS WITH STERILITY AND HABITUAL ABORTION

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According to recommendations of WHO and Charter of International Law basic human right of an individual is to decide freely on number of their children. Impossibility of conception after a year of regular sexual intercourse places couples in candidates for treatment of sterility. Of total number of patients treated for sterility in 7-12%, the cause is presence of chromosomal aberrations in their karyotypes. The aim of this study was to examine frequency of chromosomal aberrations in couples with sterility and habitual abortion at University Clinical Center of Republic of Srpska (UCCRS). Habitual abortion is spontaneous and repeated abortion of unknown etiology. Chromosome analysis involves taking blood samples from a patient; culturing cells and peripheral blood; lymphocyte preparation; chromosome staining by classical technique, GTG and CBG tape and NOR; microscopic analysis of chromosomes and determination of constitutional karyotype. From 5.1.2009. to 31.12.2019. total of 3842 analyzes of karyotypes from peripheral blood were performed in Cytogenetic Laboratory of UCCRS, and 1956 patients had diagnosis of habitual abortion or sterility. The percentage of patients who had aberrations in their karyotypes and were treated for sterility and habitual abortion at UCCRS was 6.87%. The most common chromosomal aberrations in the karyotypes of patients treated for sterility and habitual abortion were inversions. Also, there were twice as many patients with a referral diagnosis of sterility compared to those with habitual abortion, who had aberrant karyotypes. The importance of analysis of karyotypes of patients treated for sterility and habitual abortion is great.

KEYWORDS: chromosomal aberrations, sterility, karyotype, habitual abortion

ZNAČAJ PRENATALNE DIJAGNOSTIKE U OTKRIVANJU HROMOZOMSKIH ABERACIJA KOD PLODA

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Povećanje ukupne svesti budućih roditelja o značaju prenatalne dijagonostike u prevenciji hromozomskih abearcija kod novorođenčad veliki je zadatak i izazov. Hromozomske abracije u kariotipu ploda mogu da dovedu do velikih oštećenja u toku njegovog rasta i razvoja kao i otežanog života nakon rađanja. Cilj ovog rada bio je da se ispitaju prisustvo i učestalost hromozomskih aberacija u kariotipovima plodova nakon amniocenteze u Citogenetičkoj laboratoriji Univerzitetsko Kliničkog centra Republike Srpske (UKCRS) u periodu od sedam godina. Analiza hromozoma podrazumeva uzimanje uzoraka plodove vode; kultivaciju ćelija plodove vode; preparacija ćelija ploda; bojenje hromozoma klasičnom tehnikom, tehnikom GTG i CBG traka i NOR; mikroskopsku analizu hromozoma i određivanje konstitucionog kariotipa ploda. U periodu od 1.1.2009. do 31.12.2015. godine u Citogenetičkoj laboratoriji UKCRS urađeno je ukupno 4361 analiza kariotpa iz plodove vode, a njih 142 bili su aberantni kariotipovi. U radu je potvrđena korelacija između prisustva hromozomskih aberacija u kariotipovima plodova u odnosu na starost majke. Najčešća hromozomska aberacija u kariotipovima plodova bila je trizomija 21, zastupljena sa čak 32,98% od ukupnog broja aberantnih kariotipova. Rad pokazuje da je od ukupnog broja registrovanih novorođenčadi sa aberantnim kariotipovima, njih 68% dijagnostikovano još prenatalno. Visok procenat prenatalnih dijagnoza sa detekcijom aberantnih kariotipova potvrđuje veliki značaj amniocenteze u prevenciji rađanja novorođenčadi sa hromozomskim aberacijama.

KLJUČNE REČI: amniocenteza, hromozomske aberacije, kariotip, prevencija

IMPORTANCE OF PRENATAL DIAGNOSIS IN DETECTION FOR FETAL CHROMOSOMAL ABERRATIONS

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Increasing overall awareness of future parents about the importance of prenatal diagnostics in prevention of chromosomal aberrations in newborns is a great task and challenge. Chromosomal abrasions in karyotype of fetus can lead to great damage during its growth and development, as well as difficult life after birth. The aim of this study was to examine frequency of chromosomal aberrations in fetal karyotypes after amniocentesis in Cytogenetic Laboratory of University Clinical Center of Republic of Srpska (UCCRS) over a period of seven years. Chromosome analysis involves taking samples of amniotic fluid; fetus water cell cultivation; fetus cell preparation; chromosome staining by classical technique, GTG and CBG tape technique and NOR; microscopic analysis of chromosomes and determination of the constitutional karyotype of the fetus. In the period from 1.1.2009. to 31.12.2015. year in the Cytogenetic Laboratory of UCCRS, a total of 4361 analyzes of karyotypes from amniotic fluid were performed, and 142 of them were aberrant karyotypes. The paper confirms the correlation between the presence of chromosomal aberrations in fetal karyotypes in relation to age of their mother. The most common chromosomal aberration in fetal karyotypes was trisomy 21, represented by as much as 32.98% of total number of aberrant karyotypes. The paper shows that out of total number of registered newborns with aberrant karyotypes, 68% of them were diagnosed prenatally. The high percentage of prenatal diagnoses with detection of aberrant karyotypes confirms great importance of amniocentesis in prevention of newborns with chromosomal aberrations.

KEYWORDS: amniocentesis, chromosomal aberrations, karyotype, prevention

POLIMORFIZAM rs1800795 U KANDIDATSKOM GENU ZA DUGOVJEČNOST *IL-6* U POPULACIJI ROMA HRVATSKE

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Trajanje životnog vijeka ovisi o međudjelovanju genetskih i okolišnih čimbenika te o stilu života. Jedna od teorija starenja istražuje ulogu imunološkog odgovora i bazira se na ideji da imunološki sustav s godinama gubi svoju učinkovitost, što dovodi do autoimunosti i smanjene sposobnosti reagiranja na infekcije. Ljudi koji se s ovim problemom bolje nose, aktiviranjem protuupalnog odgovora žive dulje. Budući da je interleukin-6 povezan s upalom, njegov je gen kandidat za istraživanja dugovječnosti. Romi su transnacionalna manjina prisutna u mnogim zemljama svijeta za koju se zna da ima kraći životni vijek od okolne populacije. Cilj ove studije bio je istražiti povezanost između polimorfizma rs1800795 u promotorskoj regiji *IL-6-174G/C* i dugovječnosti kod 315 ispitanika (171ž/144m, dobni raspon 18-72 godine, srednja dob 40,35 ± 13,71 godina), pripadnika socio-kulturno različitih i geografski udaljenih romskih skupina u Hrvatskoj; dvije skupine Vlaških Roma te jedna skupina Balkanskih Roma. DNK je ekstrahirana iz periferne krvi metodom isoljavanja, a genotipovi su određeni pomoću Kompetitivne alel-specifične PCR metode (KASP). Rezultati su pokazali da je G alel, u prethodnim istraživanjima povezanom s dugovječnošću, imao najmanju učestalost alela kod Međimurskih Roma (65,2%, $p<0,00001$), koji su ujedno imali i najnižu srednju dob (34,4±11,1 god, $p<0,0001$) u usporedbi s Baranjskim (73,0% i 45,0±14,2 god) i Balkanskim Romima (84,4% i 41,4±13,4 god). Analiza provedena odvojeno prema spolu pokazala je da je razlika bila značajna samo kod žena ($p<0,001$). Da zaključimo, *IL-6-174G* mogao bi doprinijeti trajanju životnog vijeka Roma u Hrvatskoj, a genetska različitost testiranih romskih skupina može biti posljedica njihove reproduktivne izolacije.

KLJUČNE RIJEČI: gen *IL-6*, rs1800795, dugovječnost, Romska populacija, Hrvatska

POLYMORPHISM rs1800795 IN LONGEVITY CANDIDATE GENE *IL-6* IN THE CROATIAN ROMA POPULATION

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Lifespan is determined by the interaction of genetic, environmental and lifestyle factors. One of the aging theories focuses on immune response, and argues that the immune system loses its effectiveness with age, leading to autoimmunity and a reduced ability to respond to infections. People who can better deal with this problem by activating the anti-inflammatory response live longer. Since interleukin-6 is related to inflammation, its gene is a candidate gene for longevity. Roma is a transnational minority present in many countries of the world, known to have shorter life expectancy than surrounding populations. Objective of this study was to investigate the association between rs1800795 in *IL-6-174G/C* promoter region and longevity in 315 subjects (171f/144m, age range 18-72 years, 40.35±13.71 yrs), members of socio-culturally different and geographically distant Roma groups in Croatia; two Vlax Roma groups, and one Balkan Roma group. DNA was extracted from peripheral blood using salting-out method and genotypes were determined using the competitive allele specific PCR method (KASP). Results showed that G allele, in previous research associated with longevity, had the lowest allele frequency in the Međimurje Roma (65.2%, $p<0.00001$), who also had the lowest mean age (34.4±11.1 yrs, $p<0.0001$) when compared to the Baranja (73.0% and 45.0±14.2 yrs) and the Balkan Roma (84.4% and 41.4±13.4 yrs). When analyzed separately by sex, this difference was significantly pronounced only in women ($p<0.001$). To conclude, *IL-6-174G* may be advantageous for lifespan in the Croatian Roma and genetic distinctiveness of tested Roma groups may be the result of reproductive isolation.

KEYWORDS: *IL-6* gene, rs1800795, longevity, Roma population, Croatia

ANALIZA EKSPRESIJE GENA *NOTCH* SIGNALNOG PUTA U ĆELIJAMA ORALNOG KARCINOMA TRETIRANIM EGZOZOMIMA MEZENHIMSKIH MATIČNIH ĆELIJA

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Kancerske matične ćelije (cancer stem cells-CSC), mala subpopulacija ćelija oralnog karcinoma, označene su kao nosioci sposobnosti inicijacije razvoja maligniteta. Smatraju se takođe glavnim uzročnikom recidiva i metastaza, kao i rezistencije na antineoplastičnu terapiju. *Notch* signalni put igra ključnu ulogu u promociji preživljavanja, proliferacije, samoobnavljanja i migracije CSC. U poslednjoj deceniji egzozomi su prepoznati kao posrednici u interćelijskoj komunikaciji. Predstavljaju nanovezikule koje sadrže signalne molekule (mikroRNK, proteine i dr), i učestvuju u regulaciji fizioloških i patoloških procesa, uključujući tumorigenezu. Cilj studije je bio ispitati efekat egzozoma iz mezenhimskih matičnih ćelija apikalne papile (stem cells from apical papilla-SCAP) na CSC.U studiji su korišćene sledeće ćelijske kulture: primarne, SCC-25 ćelijska linija kao i izdvojene dve ćelijske subpopulacije, CD44+ (CSC) i CD44-; sve četiri ćelijske kulture su tretirane egzozomima izolovanim iz SCAP. Procena vijabilnosti ćelija praćena je *Neutral Red* esejom i testom proliferacije (Tripan plavo). Metodom kvantitativnog PCR-a određen je nivo genske ekspresije molekula *Notch* signalnog puta. Tretman egzozomima izazvao je smanjenje vijabilnosti od 20% CD44+ ćelija. Pokazano je statistički značajno smanjenje ekspresije gena *Notch* signalnog puta (*Notch2*, *Notch3*), kao i liganda *Jagged 1*, što je posledično dovelo do smanjenja ekspresije gena *Hes* i *Hey* čiji produkti igraju ulogu u održavanju matičnosti, metastaziranju i rezistenciji na hemoterapiju. U subpopulaciji CSC došlo je do smanjenja ekspresije i transkripcionog faktora *Oct*-4, markera kancerske matičnosti. Egzozomi SCAP-a deluju umereno citotoksično na ćelije oralnog karcinoma. Svoje destvo ostvaruju preko *Notch* signalnog puta smanjujući ekspresiju gena čiji su produkti deo *Notch* signalne kaskade, kao i *Oct-4* gena.

KLJUČNE REČI: oralni karcinom, kancerske matične ćelije, dentalne matične ćelije, egzozomi, *Notch* signalni put

ANALYSIS OF NOTCH SIGNALING PATHWAY GENE EXPRESSION IN ORAL CANCER CELLS TREATED WITH EXOSOMES FROM MESENCHYMAL STEM CELLS

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Cancer stem cells (CSCs), a small subpopulation of oral cancer cells, have the capacity of tumor initiation. They are considered as the main cause of recurrence and metastasis, as well as resistance to antineoplastic treatments. The Notch signaling pathway plays a major role in promoting CSCs survival, proliferation, self-renewal and migration. In the last decade, exosomes have been recognized as mediators of intercellular communication. They represent nanovesicles that contain biological signaling molecules and participate in the regulation of physiological and pathological processes, including tumorigenesis. The aim of the present study was to examine the effects of exosomes originating from stem cells of apical papilla (SCAPs) on CSCs. Primary tumor cell cultures, SCC-25 cell line and two separated cell subpopulations (CD44+, CD44-) were used in the experiments and treated with exosomes isolated from SCAPs. Cell viability was assessed by Neutral Red and Trypan blue. Gene expression of Notch signaling pathway was determined by qPCR. Exosome treatment led to 20% decrease of CD44+ cells viability. A statistically significant down-regulation of expression of Notch pathway molecules (*Notch2, Notch3*) was shown. *Jagged1* was also down-regulated, which led to *Hes* and *Hey* (two genes involved in stemness, metastasis and drug resistance) expression decrease. Levels of *Oct-4*, another cancer cell stemness marker, were decreased as well. SCAP exosomes have a moderate cytotoxic effect on CSCs. They exert their effects via Notch signaling cascade as demonstrated by the reduced expression of all examined Notch pathway molecules. In addition, levels of *Oct-4* were also affected by exosome treatment.

KEYWORDS: oral cancer, cancer stem cells, dental stem cells, exosomes, Notch signaling pathway

POTENTIAL OF INDELS WITHIN ITS2 REGION FOR RESOLVING TAXONOMIC PROBLEMS IN *Merodon ruficornis* SPECIES GROUP (DIPTERA: *SYRPHIDAE)*

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Notwithstanding the conclusions of numerous studies that suggest reliability of including indels as an additional phylogenetic signal, most studies perform the phylogenies construction treating gaps as missing data. The inclusion of all available data is particularly relevant in the analysis of taxonomic boundaries within the monophyletic *Merodon ruficornis* species group, characterized by incongruence between morphological and molecular data. With the aim to examine the potential of the nuclear ribosomal internal transcribed spacer 2 (ITS2) in an integrative taxonomic approach, we investigated *M. loewi* and *M. armipes* — two species from the *ruficornis* group which were shown to share the same cytochrome c oxidase subunit 1 (COI) haplotype, despite the clear morphological differences between them. ITS2 region was sequenced in specimens belonging to both species and sequence analysis revealed presence of indel region(s) in the aligned dataset. In order to reveal the role of indels as a potentially valuable information for the species delineation within *Merodon*, gaps were treated in two ways: 1) by being excluded as missing data; and 2) by being included as a fifth character states under the maximum parsimony principle along with coding them as separate presence/absence characters in the maximum likelihood and Bayesian framework. Contrary to the treating gaps as missing data, their inclusion led to a high supported delineation between species as well as structuring at the intraspecific level observed in all phylogenetic trees. Indels within ITS2 region proved to be informative as an additional tool for resolving taxonomic problems within *Merodon*.

KEYWORDS: Syrphidae, *Merodon*, ITS2, indels, molecular taxonomy

POVEZANOST POLIMORFIZAMA C282Y I H63D GENA *HFE* SA NEPLODNOŠĆU KOD MUŠKARACA U SRBIJI

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Iako je ostvaren veliki napredak u pogledu dijagnostike i lečenja neplodnosti, još uvek nemamo rešenje za neplodnost kod velikog broja parova. Prema podacima iz literature, i dalje je otvoreno pitanje u kojoj meri mutacije gena *HFE* utiču na neplodnost muškaraca. Hereditarna hemohromatoza je bolest metabolizma koju karakteriše nakupljanje gvožđa usled njegove povećane apsorpcije. Za formu I ove bolesti, koja se nasleđuje autozomno recesivno, odgovoran je gen *HFE* koji se nalazi na kratkom kraku hromozoma 6. Opisano je više mutacija tog gena, ali sa hemohromatozom su najčešće povezane C282Y i H63D. Ciljevi našeg rada su bili utvrđivanje učestalosti genotipova *HFE* gena kod infertilnih muškaraca i korelacije *HFE* genotipa i fenotipa kod infertilnih muškaraca u Srbiji. Studija je obuhvatila 100 infertilnih muškarca, kao i kontrolnu grupu. Analiza polimorfizama C282Y i H63D u genu *HFE* urađena je amplifikacijama ciljnih regiona metodom PCR. Nakon provere amplifikacije, vršena je digestija PCR produkata restrikcionim enzimima *RsaI*(C282Y) i *BlcI*(H63D) i analiza dobijenih restrikcionih framenata RFLP metodom. Učestalost heterozigota kod neplodnih muškaraca bila je 9% za C282Y i 18% za H63D, i nije se značajno razlikovala od kontrolne grupe. Složeni heterozigot CY+HD utvrđen je kod 4% neplodnih muškaraca. Analiza asocijacija polimorfizma C282Y i nalaza spermograma je utvrdila prisustvo genotipa CC kod više od 90% muškaraca sa azoospermijom i oligospermijom. Analiza asocijacije serumske koncentracije FSH i polimorfizma C282Y kod neplodnih muškaraca je pokazala značajno veću učestalost heterozigota CY u grupi muškaraca sa povišenim vrednostima FSH u odnosu na grupu sa normalnim vrednostima FSH.

KLJUČNE REČI: *HFE* gen, neplodnost kod muškaraca, hereditarna hemohromatoza, polimorfizam C282Y, polimorfizam H63D

THE ASSOCIATION OF *HFE* GENE POLYMORPHISMS C282Y AND H63D WITH MALE INFERTILITY IN SERBIA

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Although great progress has been made in diagnosis and treatment of infertility, we still do not have solution for infertility in a large number of couples. According to literature, the question of how mutations in *HFE* gene affect male infertility remains open. Hereditary hemochromatosis is a metabolic disease characterized by iron accumulation due to its increased absorption. Type I of this disease is inherited recessively; it is due to mutations in *HFE* gene, located on the short arm of chromosome 6. Among the mutations described in this gene, C282Y and H63D are most commonly associated with hemochromatosis. The objectives of our study were to determine frequency of *HFE* genotypes and correlation of *HFE* genotype and phenotype in infertile men in Serbia. The study included 100 infertile men, as well as control group. Analysis of C282Y and H63D polymorphisms was performed by amplification of target regions by PCR. After checking the amplification, PCR products were digested with restriction enzymes *RsaI* (C282Y) and *BlcI* (H63D); the obtained restriction fragments were analyzed by RFLP. Heterozygote frequency in infertile men was 9% for C282Y and 18% for H63D, and did not differ significantly from control group. The complex heterozygote CY + HD was found in 4% of infertile men. Analysis of associations of C282Y polymorphism with spermogram results revealed the presence of CC genotype in more than 90% of men with azoospermia and oligospermia. Analysis of association of serum FSH concentration and C282Y polymorphism in infertile men showed significantly higher frequency of CY heterozygotes in group with elevated FSH values compared to group with normal FSH values.

KEYWORDS: *HFE* gene, male infertility, hereditary hemochromatosis, C282Y polymorphism, H63D polymorphism

SEQUENCE VARIABILITY IN THE MITOCHONDORIAL DNA CONTROL REGION OF THE EUROPEAN ROLLER (*Coracias garrulus*) FROM SERBIA

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The European Roller is a migratory species within the Coraciidae family and the only one occurring in Europe. During the last several decades, rollers were facing a drastic and rapid population decline. Starting from the 1950s, it was observed in Serbia as well, particularly in Vojvodina region, where some of the census data from the1990s indicated only around 15 breeding pairs. The placement of wooden nest-boxes since 2003 has had a positive effect on the population recovery, which today counts more than 160 breeding pairs. The aim of this study was to determine genetic variability of mitochondrial DNA control region sequences in rollers from Serbia. A partial fragment of mtDNA control region was amplified and sequenced in 55 individuals sampled across Serbia. Sequence analyses were performed using the SeqScape and BioEdit, while the molecular diversity parameters were calculated using DnaSP and Arlequin. A total of 22 haplotypes were revealed, with a high haplotype diversity (Hd) of 0.954, low nucleotide diversity (π) of 0.012, and an average number of pairwise differences (k) of 4.959. No signal of spatial structuring was observed, and neutrality tests (Tajima D and Fu´s Fs) values were negative indicating a signal of demographic expansion. In comparison to the central European populations, our preliminary results based on the mitochondrial DNA indicate higher genetic variability in rollers from Serbia, and a signal of population expansion. Further analyses based on microsatellites loci would be necessary in order to get more insight into the recent population size changes.

KEYWORDS: control region, European Roller, mtDNA, Serbia

Biohemija i molekularna biologija

Biochemistry and Molecular Biology

EML (EMSY-LIKE) PROTEINI SU ČITAČI HISTONSKIH MODIFIKACIJA KOJI UČESTVUJU U REGULACIJI RAZVIĆA SEMENA KOD *Arabidopsis thaliana* (L.) HEYNH.

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U XXI veku sve veću pažnju naučnika privlači oblast epigenetika koja se bavi otkrivanjem novih mehanizama koji učestvuju u regulaciji ekspresije gena. Epigenetički procesi uključuju složenu interakciju između metilacije DNK, modifikacija histona i nekodirajućih RNK. Međutim mnogi igrači su i dalje nepoznati, posebno proteini (tzv. histonski čitači), koji se vezuju za modifikacije histona koje čitaju i prevode u signal koji se potom prenosi do odgovarajućih regulatornih sistema u ćeliji. Dosadašnji podaci pokazuju da su čitači histonskih modifikacija biljaka uključeni u veliki broj različitih bioloških i razvojnih procesa u ćeliji, ali da je broj opisanih čitača relativno mali. Razviće semena kod skrivenosemenica nastaje kao rezultat procesa dvojnog oplođenja, u kome se jedna spermatična ćelija spaja sa jajnom ćelijom, dok se druga spermatična ćelija spaja sa centralnom ćelijom. Narušavanje ravnoteže u genomskom doprinosu roditelja često dovodi do brojnih poremećaja u razviću semena i embriona, a može dovesti i do potpunog prekida razvića semena. Jedan od glavnih mehanizama koji narušava ravnotežu odnosa roditeljskih genoma u semenu predstavlja represija sinteze auksina uz pomoć Polikomb represivnog kompleksa 2 (PRC2). Evolutivno konzervisana uloga PRC2 tokom razvića zasniva se na ulozi ovog kompleksa da postranslaciono modifikuje histon H3, a proteini koji prepoznaju posttranslacione modifikacije (PTM) histona, odnosno čitači histonskog koda, često su uključeni u regulaciju razvića u sprezi sa PRC2. Stoga čitači histonskog koda predstavljaju pogodne kandidate za dodatni uticaj na funkciju PRC2 kompleksa. U našem istraživanju je pokazano da su EML1 i EML3, proteini koji pripadaju EMSY-like Tudor/Agenet proteinskoj porodici, čitači H3K36me3 histonske modifikacije. Opsežnim analizama mutantnih fenotipova demonstrirano je da su EML1 i EML3 neophodni za sprečavanje razvića semena u slučaju izostanka oplođenja, kao i za regulaciju ravnoteže roditeljskih genoma nakon oplođenja. Pretpostavljeno je da EML1 i EML3 imaju ulogu u represiji ekspresije paternalnog alela regulisanjem

transporta aksina i transdukcije signala. Na osnovu dobijenih rezultata predložen je mehanizam dobijanja apomiktičnih semena kod *A. thaliana* kao i za prevazilaženje međuvrsnih seksualnih reproduktivnih barijera, zasnovan na finom podešavanju transporta auksina tokom razvića semena pomoću histonskih čitača EML1 i EML3.

KLJUČNE REČI: čitači histonskih modifikacija, apomiksija, razviće semena, auksin, *Arabidopsis thaliana*

MOLECULAR AND DEVELOPMENTAL ROLES OF EML (EMSY-LIKE) PROTEINS AS HISTONE MARK READERS IN SEED DEVELOPMENT OF *Arabidopsis thaliana* (L.) HEYNH.

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Epigenetics has gained significant attention in the past few years, as it has become evident that chromatin modifications play a major role in regulating gene expression. Among the epigenetic phenomena, many players and their function still remain unknown, especially the effector proteins (histone mark readers) that translate histone marks into an active or repressed chromatin state. So far a relatively few plant so-called histone readers have been identified to be instrumental for many developmental processes. Seed development in flowering plants is initiated by double fertilization of two female gametes by the two sperm cells, whereby fertilization of the haploid egg cell will generate the diploid embryo, while fertilization of the diploid central cell will generate the triploid endosperm. Any change in the genomic contribution of one parent often leads to severe defects including seed abortion. The repression of auxin synthesis by the Polycomb Repressive Complex 2 (PRC2) is a major mechanism contributing to sensing genome balance. Proteins involved in recognizing histone posttranslational modifications (PTMs) are often included in the regulation of plant development in conjunction with in PRC2. Results from our study show that EML1 and EML3, proteins that belong to the EMSY-Like Tudor/Agenet protein family, are H3K36me3 histone readers necessary to maintain parental genome balance in Arabidopsis. We furthermore show by analyzing the mutant phenotypes that both EML1 and EML3 are required to prevent seed development before fertilization, and to regulate the balance of parental contributions after fertilization. We hypothesize that EML1 and EML3 function to repress paternal gene expression by regulating auxin transport and signaling. Finally, we propose a mechanism of apomictic seed production in Arabidopsis, based on the fine-tuning of auxin flow during seed development, by the histone readers EML1 and EML3, which could be exploited for the engineering of asexual reproduction through seeds (apomixis), and for generating new interspecies hybrids.

KEYWORDS: Histone readers, apomixis, seed development, auxin, *Arabidopsis thaliana*

EFEKAT KRATKOROČNOG IZLAGANJA KADMIJUMU LARVI KUKURUZNOG PLAMENCA *Ostrinia nubilalis* (HBN.) NA AKTIVNOST ENZIMA SOD I GST

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Sve veća zagađenost životne sredine teškim metalima, kao što je kadmijum (Cd), predstavlja jedan od vodećih ekoloških problema u svetu. Od velike je važnosti razumeti kako povećana koncentracija ovog metala, u zemljištu i biljkama, utiče na žive organizme. Kao jedan od mehanizama štetnog delovanja Cd navodi se produkcija reaktivinih kiseoničnih vrsta (*engl.* reactive oxygen species, ROS), što dovodi do oksidativnog stresa. S druge strane, organizmi su tokom evolucije stekli mehanizme antioksidativne zaštite, poput enzima superoksid dismutaze (SOD) i glutation *S*-transferaze (GST), koji uklanjaju slobodne radikale iz ćelije. Cilj ovog rada je bio da se ispita u kojoj meri kratkoročno izlaganje kadmijumu larvi *Ostrinia nubilalis*, široko rasprostanjene i ekonomski značajne vrste insekta, utiče na aktivnost enzima SOD i GST. Larve poslednjeg stadijuma (peti instar, L5) izlagane su, tokom 48 sati, različitim koncentracijama kadmijuma. Formirane su četiri eksperimentalne grupe, kontrola (K), koja nije tretirana kadmijumom, i tri tretmana (Cd1, Cd2 i Cd3) tretirani sa 10, 50 i 100 mg Cd po kg podloge. Rezultati rada su pokazali da je aktivnost enzima SOD bila statistički značajno povećana u grupi Cd3, dok je aktivnost GST enzima bila značajno povećana u grupama Cd2 i Cd3. Dobijeni rezultati su u saglasnosti sa prethodno dokazanim mehanizmima delovanja Cd, gde je uočeno da velike koncentracije Cd utiču na porast atktivnosti ovih enzima, u cilju zaštite organizma od štetnog delovanja slobodnih radikala. Imajući u vidu da je *O.nubilalis* ekonomski značajna vrsta za poljoprivredu, smatramo da rezultati koji ukazuju na adaptivne mehanizme ove vrste imaju višestruki značaj.

KLJUČNE REČI: insekti, teški metali, toksičnost, oksidativni stres

EFFECT OF SHORT TERM CD EXPOSURE ON SOD AND GST ACTIVITY IN CORN PEST *Ostrinia nubilalis* (HBN.)

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Environmental pollution with heavy metals, such as cadmium (Cd), is one of the leading problems in the world. It is important to understand how the increased concentration of Cd in nature affects living organisms. The main mechanisms of Cd toxicity is the production of reactive oxygen species (ROS), which leads to oxidative stress. On the other hand, living organisms have acquired mechanisms of antioxidant protection, involving enzymes superoxide dismutase (SOD) and glutathione *S*-transferase (GST), which remove free radicals from the cell. The aim of this study was to examine how short - term exposure to cadmium in *Ostrinia nubilalis*, a widespread and economically important pest, affects the activity of the enzymes SOD and GST. Fully developed larvae (fifth instar, L5) were exposed for 48 hours to different concentrations of cadmium. Four experimental groups were formed, control (K) untreated group, and three treatments (Cd1, Cd2 and Cd3) treated with 10, 50 and 100 mg/kg^{-1} respectively. The results showed that the SOD activity was significantly increased in the Cd3 group, while the GST activity was significantly increased in the Cd2 and Cd3 groups. The obtained results are in accordance with the previously proven mechanisms of Cd action showing that high concentrations of Cd increase the activity of these enzymes, in order to protect the organism from the harmful effects of free radicals. Bearing in mind that *O. nubilalis* is an economically important species for agriculture, we believe that the results have multiple significance on the adaptive mechanisms of this species.

KEYWORDS: insects, heavy metals, toxicity, oxidative stress

ZNAČAJ BIOHEMIJSKIH PARAMETARA U PROCJENI RAZVOJA DIJABETIČKOG MAKULAROG EDEMA NAKON OPERACIJE KATARAKTE

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Dijabetički makularni edem je najčešći razlog gubitka vida osoba oboljelih od diabetes melitusa i njegova patogeneza je veoma kompleksna. Pored makularnog edema, razlog gubitka vida kod ovih pacijenata može biti i katarakta koja zahtijeva hiruršku intervenciju.Operacija katarakte može ubrzati nastanak makularnog edema postoperativno i to kod pacijenata kod kojih diabetes melitus traje duže od 10 godina,koji su na insulinskoj terapij,pacijenata sa lošom metaboličkom kontrolom koja se procjenjuje na osnovu povišenih vrijednosti biohemijskih parametara: glukoze u krvi (GUK) >10mmol/l, glikoziliranog hemoglobina (HbA_1c)>7%,lipoproteina niske gustine (LDL)>2,6 mmol/l i triglicerida>1,7mmol/l.Cilj ovog istraživanja je ispitati uticaj vrijednosti navedenih biohemijskih parametara na razvoj makularnog edema kod pacijenata oboljelih od diabetes melitusa nakon operacije katarakte, a koji se inače koriste kao markeri procjene stepena metaboličke kontrole.Studija presjeka je obuhvatila 30 pacijenta oboljelih od diabetes melitusa tip 2 kod kojih su pored standardne preoperativne pripreme, određeni i biohemijski parametri: vrijednosti HbA_1c,LDL i triglicerida.Vrijednosti GUKa određuju se standardno za svakog pacijenta.U studiji sprovedenoj na Klinici za očne bolesti tokom 2019.godine 7 ispitanika (22%) sa povišenim vrijednostima HbA_1c,LDL i triglicerida razvilo je makularni edem nakon operacije katarakte što za posljedicu ima smanjenje vidne oštrine operisanih pacijenata.Vrijednosti HbA_1c,LDL i triglicerida su bitni biohemijski parametri za procjenu mogućnosti razvoja dijabetičkog makularnog edema nakon operacije katarakte i biće uvršteni u preoperativni protokol pripreme pacijenata oboljelih od diabetes melitusa.

KLJUČNE RIJEČI: glikozilirani hemoglobin, sermski lipidi, dijabetički makularni edem, operacija katarakte

SIGNIFICANCE OF BIOCHEMICAL PARAMETERS IN ASSESSMENT OF POSTCATARACT DIABETIC MACULAR EDEMA DEVELOPMENT

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Diabetic macular edema is the most common cause of loss visual function in diabetic patients with complex pathogenesis. Other than macular edema, reason for loss of visual function in diabetic patients could be cataract which requires surgical intervention. Cataract surgery in dibetic patients can develop macular edema postopeatively,in patients with duration of diabetes more than 10 years,in patients with insulin treatment received,estimated metabolic control in relation of biochemical parameters, such as blood glucose (GUK) >10 mmol/l glycosylated hemoglobin (HbA_1c)> 7%,low-density lipoproteins (LDL)>2,6 mmol/l,triglycerids>1,7 mmol/l.Aim of this research is to examine the impact of values of biochemical parameters on macular edema development in diabetic patients following the cataract surgery, that are otherwise used as markers of metabolic control assessment.Cross-sectional study included 30 patients with type 2 diabetes mellitus who underwent certain laboratory parameters investigation such as HbA_1c,LDL and triglycerids beside standard preoperative procedure.In the study which took place at Eye Clinic during 2019. 7 participants (22%) with higher level of HbA_1c,LDL and triglycerids, developed macular edema what resulted in visual acuity decrease in operated patients.Level of HbA_1c,LDL and triglycerids are very important biochemical parameters in assessment of postcataract diabetic macular edema development. They will be put in preoperative preparation protocol for diabetic patients who need cataract surgery.

KEYWORDS: glycosylated hemoglobin, serum lipids, diabetic macular edema, cataract surgery

KLONIRANJE SEKVENCI ZA miR-7 I miR-34A I NJIHOVA EKSPRESIJA U GLIOBLASTOMSKOJ STANIČNOJ LINIJI

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Male nekodirajuće RNA mikroRNA (miRNA, miR) reguliraju proteinsku ekspresiju i sudjeluju u brojnim staničnim procesima. miR-7 i miR-34a se smatraju tumorskim supresorima, budući da njihova ekspresija inhibira staničnu proliferaciju, potiče apoptozu i povećava osjetljivost na kemoterapeutike. Cilj ovog rada je istraživanje utjecaja miRNA na proliferaciju i preživljenje stanične linije glioblastoma A1235. Metode su obuhvatile konstrukciju ekspresijskih plazmida sa sekvencama pri-miRNA-7 i pri-miRNA-34a, uzgoj staničnih klonova transfekcijom plazmida sa sekvencama miR, te detekciju zrelih miR procesom koji je uključivao reverznu transkripciju i umnažanje metodom kvantitativnog PCR u stvarnom vremenu. Otpornost na kemoterapeutik istražena je praćenjem proliferacije sojeva s povećanom ekspresijom miR-7 i miR-34a u prisustvu alkilirajućeg agensa. Rezultati su pokazali povećanje ekspresije zrelih miR-7 i miR-34a u stanicama nakon unosa plazmida s njihovim sekvencama. Međutim, nije bilo morfoloških niti bitnih razlika u brzini proliferacije stanica i otpornosti na alkilirajući agens kod stanica s povećanom ekspresijom miRNA u odnosu na kontrolne stanice. Rezultati ukazuju na moguće stanično-specifične uloge miR-7 i miR-34a ovisne o unutarstaničnoj signalnoj mreži.

KLJUČNE RIJEČI: miRNA-7, miRNA-34a, ekspresija, glioblastomska stanična linija

miR-7 AND miR-34 SEQUENCE CLONING AND EXPRESSION IN A GLIOBLASTOMA CELL LINE

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Small non-coding RNAs, microRNAs (miRNA, miR) regulate the protein expression and take part in different processes in the cell. miR-7 and miR-34a are known as tumour suppressors as their expression can influence cell proliferation, apoptosis and resistance on chemotherapeutics. The aim of this work was the analysis of the effect of these miRs on the proliferation and survival in the glioblastoma cell line A1235. Methods comprised expression plasmid construction containing pri-miRNA sequences for miR-7 and miR-34a, cell transfection with plasmid constructs and detection of mature miRs by reverse transcription and real time quantitative PCR reaction in the clones. Resistance on chemotherapeutic was investigated by cell growth follow up in strains with increased miR-7 and miR-34a expression. Results showed increase in mir-7 and miR-34a expression after transfection with corresponding plasmids. Obtained cell strains had the same morphology as their parental cells and the cell proliferation and resistance on alkylating agent was the same as in control cells. These results indicate the existence of cell-specific roles of miR-7 and miR-34a dependent on the intracellular milieu.

KEYWORDS: miR-7, miR-34a, expression, glioblastoma cell line

HIV-1 SUBTYPE DISTRIBUTION IN NEWLY DIAGNOSED PATIENTS IN 2019 IN CROATIA

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Croatia is a South-Eastern European country with a low prevalence of HIV-1 infection and centralised system of clinical care. In the period 1985-2019, a total of 1748 persons have been diagnosted with HIV-infection. Molecular epidemiology of HIV-1 infection in Croatia is characterised by the high prevalence of subtype B infection, particularly among men who have sex with men (MSM) with a small proportion of patients (mainly heterosexuals) infected with non-B subtypes and circulating recombinant forms (CRF). The aim of this study was to analyse recent trends in HIV-1 molecular epidemiology in Croatia. The study included 85 HIV-infected persons diagnosed at the University Hospital for Infectious Diseases, Zagreb during 2019 (83% national coverage). The mean age was 35.7 years (SD 18-72) and males accounted for 95.3% of the study population (81/85). The most common route of transmission was sex between men (88.2%, 75/85), followed by heterosexual transmission (11.8%, 10/85). Partial *pol* gene sequences were generated from 83/85 samples and analysed by using REGA HIV-1 subtyping tool version 3.0. Subtype B was detected in 75/83 samples (90.4%), followed by subtype A (4.8%, n= 4), CRF02_AG (1.2%, n= 1), CRF06_CPX (1.2%, n=1), CRF01_AE (1.2%, n=1) and CRF12_BF (1.2%, n=1). The majority of subtype B-infected patients were MSM (84.3% 70/83) with only 7 patients belonging to the heterosexual transmission group. In a non-B subtype group, 6/8 patients were males. The results of this study confirm that subtype B epidemics of subtype B among MSM remains the main driving force of HIV-1 epidemics in Croatia.

KEYWORDS: HIV-1, MSM, subtype B, molecular epidemiology

REGULACIJSKI LIMFOCITI T U LIMFOMAGENEZI

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B-stanični limfomi su heterogena skupina tumora koji čine 85% svih ne-Hodgkinovih limfoma (NHL, eng. *non-Hodgkin lymphoma*), tumora porijekla stanica limfoidne loze. B-limfomi nastaju iz različitih razvojnih stadija limfocita B. Osim većine ne-Hodgkinovih limfoma, klasični Hodgkinov limfom (cHL, eng. *classical Hodgkin's lymphoma*) također nastaje iz limfocita B. Jedna od komponenti tumorskog mikrookoliša su pomoćnički limfociti T koji eksprimiraju transkripcijski faktor FOXP3 (CD4+ FOXP3+), a još se nazivaju i regulacijski limfociti T (T_{reg}). Regulacijski limfociti T odgovorni su za održavanje imunosne tolerancije i homeostaze imunosnog sustava. Njihov je utjecaj na ponašanje tumora i njegov odgovor na terapiju opažen je u različitim vrstama tumora. Cilj ovog istraživana bio je evaluirati značaj stanica T_{reg} u najčešćim skupinama NHL-a (folikularni limfom, limfom stanica plaštene zone, difuzni B-velikostanični limfom) i u klasičnom Hodgkinovom limfomu. Metodom imunohistokemijskog bojenja detektirani su markeri poput CD20 kojim je određeno porijeklo tumorskih stanica iz limfocita B u uzorcima tumorskog tkiva fiksiranog formalinom i uklopljenim u parafin. Istom su metodom tipizirane stanice tumorskog mikrookoliša na temelju markera CD68, CD3, CD4, CD8, PD-1, FOXP3, CD14, CD21. Utvrđena je statistički značajna korelacija između broja stanica T_{reg} i broja makrofaga u mikrookolišu tumora u MCL. Inverzna korelacija između broja stanica T_{reg} i broja stanica koje eksprimiraju marker PD-1 opažena je u cHL. Statistički značajna korelacija između broja stanica T_{reg} i preživljenja pacijenata nije utvrđena u analiziranim skupinama limfoma B-porijekla. Ovakvi rezultati sugeriraju da CD4+ FOXP3+ limfociti T utječu na sastav tumorskog mikrookoliša u pojedinim tipovima limfoma B-porijekla.

KLJUČNE RIJEČI: B-limfomi, transkripcijski faktor FOXP3, tumorski mikrookoliš

REGULATORY T-LYMPHOCYTES IN LYMPHOMAGENESIS

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B-cell lymphomas are a heterogenous group of tumours that account for 85% of all non-Hodgkin lymphomas (NHL), tumours derived from cells of lymphoid lineage. B-lymphomas originate from various developmental stages of B lymphocytes. Classical Hodgkin's lymphoma (cHL) also originates from B lymphocytes. Helper T lymphocytes expressing the transcription factor FOXP3 (CD4+ FOXP3+), also known as regulatory T lymphocytes (Tregs), are a component of tumour microenvironment (TME). FOXP3 regulates expression of various genes in Tregs responsible for the maintaining of immune tolerance and homeostasis of the immune system. It has been shown that Tregs influence the behaviour of tumour cells. The aim of this study was to evaluate the importance of Tregs in the most common groups of B-lymphomas (follicular lymphoma (FL), mantle cell lymphoma (MCL), diffuse large B cell lymphoma (DLBCL)) and in classical Hodgkin's lymphoma. Origin of the tumour cells was determined by the detection of markers like CD20 by immunohistochemical staining in formalin-fixed, paraffin-embedded tissue samples. The same method was applied to evaluate the contents of TME based on markers CD68, CD3, CD4, CD8, PD-1, FOXP3, CD14, CD21. Statistically significant correlation between the numbers of Tregs and macrophages in TME was observed in MCL. Inverse correlation between the numbers of Tregs and cells expressing PD-1 was observed in cHL. No statistically significant correlation between the number of Tregs and the survival of the patients was observed in studied types of B-cell originated lymphoma. These results suggest that Tregs affect the contents of TME in B-cell originated lymphoma.

KEYWORDS: B lymphoma, transcriptional factor FOXP3, tumour microenvironment

BIOCHEMICAL RESPONDS OF PURE AND MIXED FUNGAL CULTURES TO THE PRESENCE OF SODIUM TRIPOLIPHOSPHATES

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Sodium tripolyphosphate (STPP) has a wide broad application in different industrial processes but mainly as builder in household cleaning products. The use of STPP has been associated with the environmental problem named as eutrophication. From the environmental point of view, it is important to identify microorganisms which have ability to reduce STPP from environment. Theaim of this research was to investigate and enhance the knowledge of the chemical and biochemical responds of pure cultures *Trichoderma viride* and *Geotrichum candidum* and their co-culture to the presence STPP added in growth liquid medium at concentration 0.5%, during cultivation process of 8 days. For this purpose, the changes in the pH value, redox potential, proteolytic activity of liquid growth media and total dry weight biomass (DWB) of all cultures were measured. The addition of STPP in growth medium had influence on increase in initial pH values and decrease in redox potential values compared to control. The STPP caused inhibitory effect on protease activity of all fungal cultures in following order: *T. viride* (3.60%), mixed culture (9.77%) and *G. candidum* (60.33%). After 8 days cultivation, DWB of *T. viride* was slightly inhibited (1.06%) whereas the DWB of *G. candidum* (0.58%) and mixed culture (9.53%) were slightly and middle stimulated by addition of 0.5% STPP. The results obtained in this study is interesting for further examination of the potential role of mixed culture in polyphosphates removal from industrial and waste water treatment plants and their potential application in the biotechnological processes.

KEYWORDS: sodium tripolyphosphate, *Trichoderma viride, Geotrichum candidum*

ANALIZA EKSPRESIJE CD31 U TKIVU HUMANE POSTELJICE

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Posteljica je privremeni organ za rast i razvoj fetusa koji obezbjeđuje dotok kiseonika i hranjivih materija iz majčinog organizma. CD31 je protein koji se nalazi na površini monocita, neutrofila, trombocita, makrofaga, limfocita i u međućelijskim spojevima endotelnih ćelija kapilara i krvnih sudova posteljice. CD31 imunoreaktivnost nije nađena u trofoblastu humane posteljice. Cilj rada je da se utvrdi ekspresija CD31 i vaskularni parametri u tkivu posteljice. Imunohistohemijska analiza urađena je na deset uzoraka humane posteljice koji su uzeti na srednjoj udaljenosti između središta i margine posteljice. Nakon standardne histološke obrade tkiva, rezovi su bojeni hematoksilin-eosin bojenjem i imunohistohemijskom metodom sa anti-CD31 antitijelom. Analizirana je CD31 ekspresija i pomoću LAS V4.3 softvera su utvrđeni vaskularni parametri CD31 pozitivnih krvnih sudova: *capillary area density* (CAD) *i capillary number density* (CND). U ispitivanim uzorcima posteljice CD31 imunoreaktivne endotelne ćelije visokog stepena imunoreaktivnosti identifikovane su u krvnim sudovima horionske ploče, krvnim sudovima stable resica i intermedijarnih resica i kapilarima terminalnih resica. Imunoreaktivnost nije utvrđena u sinciciotrofoblastu i citotrofoblastu posteljice. Prosječna vrijednost CAD u terminalnim resicama iznosila je 41,56%, a CND 7,84 kapilara/1000µm^2. U tkivu humane posteljice pozitivna CD31 ekspresija je prisutna u endotelu svih krvnih sudova horionske ploče i resičnog stabla. Pozitivna CD31 ekspresija nije utvrđena u sinciciotrofoblastu i citotrofoblastu. U terminalnim resicama humane posteljice CAD iznosi 41,56%, a CND 7,84 kapilara/1000µm^2 .

KLJUČNE RIJEČI: placenta, CD31, imunohistohemija

ANALYSIS OF CD 31 EXPRESSION IN HUMAN PLACENTA TISSUE

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The placenta is a temporary organ for the growth and development of the fetus. CD31 is a protein found on the surface of monocytes, neutrophils, platelets, macrophages, lymphocytes, and in the intercellular junctions of endothelial cells capillaries and placental blood vessels. CD31 immunoreactivity was not found in the human placental trophoblast. The aim of this study was to determine the expression of CD31 and vascular parameters in placental tissue. Immunohistochemical analysis was performed on ten human placental samples that were taken at a medium distance between the center and the margin. After standard histological tissue processing, sections were stained with hematoxylin-eosin staining and immunohistochemical method with anti-CD31 antibody. CD31 expression and vascular parameters of CD31 positive blood vessels: capillary area density (CAD) and capillary number density (CND) were analyzed by using LAS V4.3 software. In the examined placenta samples, CD31 immunoreactive endothelial cells of a high degree of immunoreactivity were identified in the blood vessels of the chorionic plate, the blood vessels of the stem, and intermediate villi, and the capillaries of the terminal villi. The average value of CAD in terminal villi was 41,56%, and CND was 7,84 capillary/1000μm^2. In human placental tissue, positive CD31 expression is present in the endothelium of all blood vessels of the chorionic plate and the villous trees. Positive CD31 expression wasn't detected in syncytiotrophoblast and cytotrophoblast. In the terminal villi of the human placenta, CAD is 41.56% and CND is 7.84 capillaries/1000μm^2 .

KEYWORDS: placenta, CD31, immunohistochemistry

EFFECT OF ACRYLAMIDE TREATMENT ON THE ACTIVITY AND EXPRESSION OF ANTIOXIDANT ENZYMES IN RAT HEPATOCYTES

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Acrylamide (AA) is carcinogen, mutagen and neurotoxic substance present in fried, roasted and baked starch-based goods. AA is formed in Maillard reaction from asparagine and carbonyl sources, such as reducing sugars, during thermal food processing at temperatures between 120 and 180°C. The aim of our study was to determine whether acrylamide treatment affects the expression and activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in hepatocytes. Rat hepatoma cell line H4IIE was treated with 4 mM (IC_{20}) and 4.5 mM (IC_{50}) of AA for 24 h. Relative mRNA expression for SOD1, SOD2 and CAT was quantified using real-time RT-PCR. Total SOD activity was measured by the method based on inhibition of superoxide radical production in the xanthine–xanthine oxidase reaction at 550 nm. CAT activity was measured as the rate of hydrogen peroxide decomposition at 240 nm. After AA treatments, mRNA level for SOD2 and CAT significantly increased in a *concentration-dependent manner in* H4IIE cells. *Treatment with higher AA concentration (4.5 mM) significantly increased transcription of SOD1.* Total SOD activity significantly decreased in a concentration-dependent manner, while there was no significant difference in CAT activity between control and AA-treated cells. Our results indicate that acrylamide exerts toxic effects on hepatocytes by altering expression of SOD and CAT, and activity of SOD enzyme.

KEYWORDS: acrylamide, hepatocytes, superoxide dismutase, catalase

EFFECT OF SUBCHRONIC ACRYLAMIDE EXPOSURE ON CYTOCHROME P450 2E1 EXPRESSION IN RAT LIVER

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Acrylamide (AA) is toxic chemical that can be formed as a food contaminant during the high-temperature cooking of many common foods such as potato products, breads and coffee. AA is metabolized by enzyme cytochrome P450 2E1 (CYP2E1) to glycidamide in liver. The objective of our study was to determine whether acrylamide treatment disturbs CYP2E1 expression in rat liver. Adult male Wistar rats were subchronicly (three weeks) treated with 25 mg/kg or 50 mg/kg body weight (bw) of AA. Formalin-fixed paraffin-embedded liver tissue was cut into 5 μm thin sections and immunostained with anti-CYP2E1 antibody. The amount of CYP2E1 in immunostained sections was determined using Windows based ImageJ program (ImageJ, Version 1.50f). We measured the optical density (OD) of immunolabeled CYP2E1, since OD is proportional to the concentration of the stain. In immunostained liver sections of all animal groups *intralobular gradient* in the intensity of CYP2E1 *immunoreactivity was detected.* CYP2E1 positive hepatocytes were dominantly distributed in centrilobular regions. Treatment with AA at a dose of 25 mg/kg bw led to decrease in the *intensity of immunostaining.* Detected decrease was confirmed by significantly reduced percentage contribution of positive, high positive and total positive cells, as well as, OD of immunolabeled CYP2E1. Contribution percentage of low positive, positive, high positive and total positive cells, and OD of immunostained CYP2E1 in group treated with 50 mg/kg bw was also decreased compared to the control, but without statistical significance. Our results indicate that AA application stimulate hepatocytes to downregulate CYP2E1 expression.

KEYWORDS: acrylamide, liver, cytochrome P450 2E1, rat

PREVALENCE OF HUMAN LEUKOCYTE ANTIGEN HLA-B*57:01 IN HIV-1 INFECTED PATIENTS IN CROATIA

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The presence of human leukocyte antigen HLA-B*57:01 is associated with abacavir hypersensitivity. Despite its efficacy, approximately 5% of individuals who receive abacavir develop an immune-mediated hypersensitivity reaction (HSR). Therefore it is important to determine the carrier frequency prior to choosing optimal therapy for HIV-1 infected patients, since B*57:01 prevalence varies among different populations. Screening for HLA-B*57:01 allele was introduced in 2009 as a part of routine care for all patients; newly-diagnosed patients entering clinical care, treatment naive patients diagnosed with HIV-infection prior to January 2009 and treatment- experienced patients. The aim of this study was to determine the prevalence of the HLA-B*57:01 allele in the HIV- positive patients receiving clinical care in Reference center in Croatia, from January 2017 until September 2020. Blood samples from 374 HIV-infected patients (353 males, 20 females, 1 transgender, median age 37 years, range 18-85 years) in Croatia were analyzed. Presence of HLA-B*57:01 allele was determined by Real-Time amplification test for the detection of HLA-B using the HLA B*57:01 Real-Time kit (Sacace Biotechnologies, Como, Italy). Fifteen of 374 HIV-infected patients (4%) were positive for the presence of HLA-B*57:01 allele in the period from January 2017 until September 2020. In conclusion, the prevalence of HLA-B*57:01 allele in Croatian HIV-infected patients is slightly lower than the prevalence in Caucasian HIV-infected patients worldwide (5.6%).

KEYWORDS: HLA-B*57:01 status, abacavir, HIV-infected patients

OPTIMIZATION OF A FLUORESCENT CELL ASSAY IN YEAST FOR IDENTIFICATION OF GLUCOCORTICOID RECEPTOR LIGANDS

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Glucocorticoids are steroid hormones released from the adrenal cortex. They regulate different physiological processes, including immune function, and their action is mediated through glucocorticoid receptors (GRs). Glucocorticoid drugs are used to treat various inflammatory and autoimmune diseases (asthma, allergy, rheumatoid arthritis, *etc.*) but their long-term use is associated with undesirable side effects. Therefore, current research is focused on development of novel selective modulators of GR activity. The aim of the present study was to optimize a fluorescence cell assay in yeast for identification of GR ligands, because there is a lack of non-transcriptional and non-radioactive GR binding assays in the literature. We expressed the ligand-binding domain of GR fused with yellow fluorescent protein (YFP) in *Saccharomyces cerevisiae*. For yeast transformations, a standard lithium acetate/polyethylene glycol method was used. Binding of ligand to the expressed receptor leads to increased fluorescence intensity in yeast cells due to receptor dimerization and fluorescence resonance energy transfer (FRET) between two YFP molecules. Relative binding affinity was measured by fluorescence microscopy and fluorimetry. Using this hormone-sensitive fluorescent biosensor, fluorescence intensity was found to correlate with ligand binding affinity, which was expressed as fold fluorescence relative to controls. Prednisolone showed strong, dose-dependent binding for GR. In the presence of negative control estradiol, fluorescence was less intense and localized to organelles, while in recombinant cells treated with high-affinity ligand fluorescence was spread throughout the cytoplasm. This optimized yeast-based assay may be used as biosensor for testing libraries of new synthetic or natural compounds with potential GR-mediated anti-inflammatory activity.

KEYWORDS: glucocorticoid receptor, FRET, prednisolone, biosensor

MOLECULAR DYNAMICS OF HUMAN 3α-HYDROXYSTEROID DEXYDROGENASE COMPLEXED TO NADP$^+$ AND NOVEL BILE ACID DERIVATIVE INHIBITORS

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Human type III 3α-hydroxysteroid dehydrogenase (3α-HSD-III) is a member of the aldo-keto reductase (AKR) superfamily, and is involved in the development and progression of many types of cancers, including lung and prostate cancer. AKR enzymes are involved in xenobiotic degradation. Human type III 3α-HSD deactivates widely used chemotherapeutic drugs such as cisplatin, but also acts by reduction of steroid hormones from their more potent to less potent forms. Bile acids have been shown to be strong and selective inhibitors of 3α-HSD-III. In this work we conducted molecular docking of novel bile acid derivatives against the crystal structure of human type III 3α-HSD complexed to ursodeoxyholic acid and NADP$^+$ (PDB ID: 1IHI). Molecular docking results were validated by enzymatic assays using recombinant enzyme expressed and purified from BL21 *E. Coli*. Enzymatic activity was measured in the presence of inhibitors. The most promising compounds with the largest estimated binding affinity and *in vitro* inhibition potential inhibitors were further studied by molecular dynamics. Simulations were performed with periodic boundary conditions, for 20 ns, using the NAMD platform. *In vitro* enzyme activity assays confirmed inhibitors with estimated IC50 in the sub-micromolar range. Molecular docking predicted that these compounds could bind to the enzyme active site. Molecular dynamics simulations confirmed the stability of the enzyme-coenzyme-inhibitor complex. RMSD (root mean square deviation) values for C-alpha protein atoms were ~1.5 Å. RMSD. Analysis of ligand binding revealed three conformational clusters during the simulation. Results indicate 4-bromo-3-oxo derivatives could bind to 3α-HSD-III active site and inhibit enzymatic activity.

KEYWORDS: 3α-HSD-3, bile acids, enzyme inhibition, molecular dynamics

ACKNOWLEDGEMENT: This work was supported by Ministry of Science, Education and Technological Development of the Republic of Serbia (Grant No. 172021).

SADRŽAJ FENOLA I ANTIOKSIDATIVNI KAPACITET LISTOVA VRSTE *Inula helenium* L. (ASTERACEAE) TOKOM CVJETANJA I PLODONOŠENJA

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Evroazijska vrsta *Inula helenium* L. pripada tribusu *Inulae* familije Asteraceae. *I. helenium* široko se upotrebljava u konvencionalnoj i tradicionalnoj medicini kao antitusik, dijafortetik i antiseptik. Cilj istraživanja je bio utvrđivanje sadržaja sekundarnih metabolita i antioksidativnog kapaciteta u etanolnom ekstraktu listova vrste *I. helenium* tokom cvjetanja i plodonošenja. Uzorci korišteni u našem istraživanju su sakupljeni na lokalitetu Mliništa (1150 m n.v.) u Republici Srpskoj tokom jula (uzorak I – faza cvjetanja) i avgusta (uzorak II – faza plodonošenja) 2020. godine. Koncentracija ukupnih fenola u uzorku I bila je 4,80±0,116 mg/mL, a u uzorku II 4,49±0,102 mg/mL u odnosu na standard galne kiseline, dok su koncentracije flavonoida bile 0,429±0,012 mg/mL u uzorku I i 0,397± 0,037 mg/mL u uzorku II, u odnosu na kvercetin kao standard. Tečnom hromatografijom pod visokim pritiskom detektovan je katehin u koncentracijama od 0,95 mg/mL u uzorku I i 0,71 mg/mL u uzorku II. Antioksidativni kapacitet listova vrste *I. helenium* određen je na osnovu sposobnosti etanolnog ekstrakta da uklanja ABTS radikal i H_2O_2. U uzorku I, IC_{50} za sposobnost uklanjanja ABTS radikala bila je pri koncentraciji ukupnih fenola od 0,078 mg/mL, dok je u uzorku II, IC_{50} bila pri koncentarciji ukupnih fenola od 0,120 mg/mL. Za razliku od ABTS radikala znatno veće koncentracije ukupnih fenola vrste *I. helenium* dovele su do uklanjanja 50% H_2O_2: 1,356 mg/mL u uzorku I i 1,513 mg/mL u uzorku II. Dobijeni rezultati ukazuju da listovi vrste *I. helenium* tokom cvjetanja imaju veći sadržaj sekundarnih metabolita i veći antioksidativni kapacitet u odnosu na fazu plodonošenja.

KLJUČNE RIJEČI: *Inula helenium*, sadržaj flavonoida, katehin, ABTS test, uklanjanje H_2O_2

Jovana Govedar and Anđela Košpić jednako su doprinijele radu.

PHENOL CONTENT AND ANTIOXIDANT CAPACITY OF *Inula helenium* L. (ASTERACEAE) LEAVES DURING THE FLOWERING AND FRUITING

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Eurasian species *Inula helenium* L. belongs to the tribus *Inulae* of the family Asteraceae. *I. helenium* is widely used in conventional and traditional medicine as an antitussive, diaphoretic and antiseptic agents. The aim of the study was to determine the content of secondary metabolites and antioxidant capacity in ethanol leaf extract of *I. helenium* during flowering and fruiting. Plant material used in our research was collected at the locality Mlinište (1150 m a.s.l.) in the Republic of Srpska during July (sample I – flowering phase) and August (sample II – fruiting phase) in 2020. The total phenols concentration in samples I and II was 4.80±0.116 and 4.49±0.102 mg/mL, respectively, relative to the gallic acid standard. In contrast, the flavonoid concentrations were 0.429±0.012mg/mL (sample I) and 0.397±0.037 mg/mL (sample II), relative to quercetin as standard. Catechin at concentrations of 0.95 mg/mL and 0.71 mg/mL in samples I and II, respectively, were detected by high-pressure liquid chromatography. The antioxidant capacity of *I. helenium* leaves was determined based on the ability of the ethanol extract to remove the ABTS radical and H_2O_2. In sample I, the IC_{50} for the ability to remove ABTS radicals of samples I and II were found to be 0.078 and 0.120 mg/mL, respectively. In contrast to ABTS radicals, significantly higher concentrations of total phenols in *I. helenium* led to the removal of 50% H_2O_2: 1.356 mg/mL in sample I and 1.513 mg/mL in sample II. The obtained results indicate that the leaves of *I. helenium* in the flowering have a higher secondary metabolite content and a higher antioxidant capacity in relation to the fruiting phase.

KEYWORDS: *Inula helenium*, flavonoid content, catechin, ABTS test, H_2O_2 removal

Jovana Govedar and Anđela Košpić have contributed equally to this work.

ODREĐIVANJE KOMPONENTI ANTIOKSIDATIVNOG SISTEMA I MARKERA TOKSIČNOSTI U TKIVU TRITONA (*Lissotriton vulgaris greacus*)

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Organizmi su konstantno izloženi uticajima različitih antropogenih i abiotičkih faktora iz spoljašnje sredine koji ih dovode u stanje oksidativnog stresa. Genetske razlike, kao i razlike u fazi razvoja organizma, mogu biti uzrok različitog odgovora organizma na oksidativni stres. Cilj rada je bio ispitivanje aktivnosti glutation-S-transferaze (GST; EC 2.5.1.18) i koncentracije glutationa (GSH) kao komponenti antioksidativnog sistema (AOS) i aktivnosti acetilholinesteraze (AChE; EC 3.1.1.7) kao markera toksičnosti u tkivu repatog vodozemca tritona (*Lissotriton vulgaris greacus*) sa tri lokaliteta na području Republike Srpske koji se razlikuju po stepenu zagađenosti (Bijela gora, Ubla i Gacko). Pored uticaja lokaliteta, ispitivan je i uticaj polova. Nakon pripreme uzoraka, aktivnost GST, koncentracija GSH i aktivnost AChE su određene spektrofotometrijski. Najmanje vrijednosti ispitivanih parametara su izmjerene kod uzoraka tritona na lokalitetu Bijela gora (aktivnost GST: 1,0 mmol/min/mg prot., koncentracija GSH: 3,2 mmol/mg prot., aktivnost AChE: 0,8 nmol/min/mg prot.) koji je zaštićeno područje i bez izraženog antropogenog uticaja. S druge strane, najveće izmjerene vrijednosti svih parametara su bile na lokalitetu Gacko (aktivnost GST: 2,4 mmol/min/mg prot., koncentracija GSH: 6,6 mmol/mg prot., aktivnost AChE: 2,6 nmol/min/mg prot.) gdje postoji više izvora zagađenja, kao i najizraženiji antropogeni uticaj. Dobijeni rezultati su pokazali da na vrijednosti komponenti AOS i AChE kod uzoraka ženki tritona vjerovatno utiče i njihov reproduktivni status. Aktivnost AChE je najosjetljivija na stepen zagađenosti lokaliteta, te se može koristiti kao pouzdan biomarker zagađenja spoljašnje sredine.

KLJUČNE RIJEČI: antioksidativni sistem, triton, glutation-S-transferaza, glutation, acetilholinesteraza

DETERMINATION OF ANTIOXIDANT SYSTEM COMPONENTS AND TOXICITY MARKERS IN TRITON TISSUE (*Lissotriton vulgaris greacus*)

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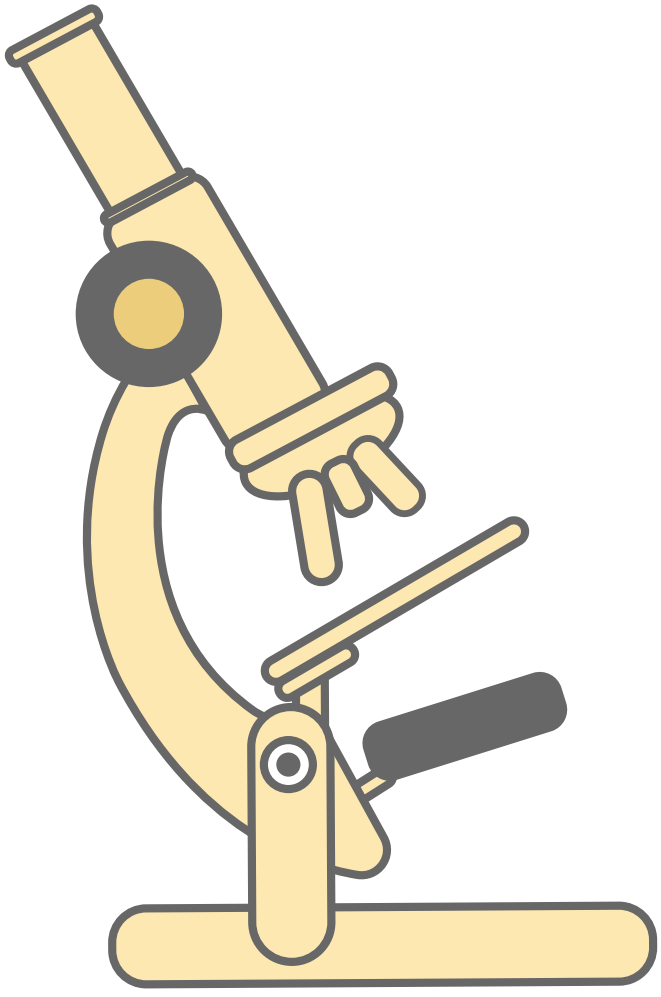
Organisms are exposed to the influences of anthropogenic and abiotic factors from the external environment that brings them into a state of oxidative stress. Genetic differences as well as differences in the stage of development can be the cause of the different responses to oxidative stress. The paper aimed to examine the activity of glutathione-S-transferase (GST; EC 2.5.1.18) and the concentration of glutathione (GSH) as components of the antioxidant system (AOS) and the activity of acetylcholinesterase (AChE; EC 3.1.1.7) as marker of toxicity in amphibian triton tissue (*Lissotriton vulgaris greacus*) from three localities in the territory of Republic of Srpska that differ in the degree of pollution (Bijela gora, Ubla and Gacko). Alongside the influence of the locality, the influence of the sexes was also examined. After sample preparation, parameters were determined spectrophotometrically. The lowest values of the examined parameters were measured in triton samples at the Bijela gora site (GST activity: 1,0 mmol/min/mg prot., GSH concentration: 3,2 mmol/mg prot., AChE activity: 0,8 nmol/min/mg prot.), which is a protected area without anthropogenic impact. The highest measured values were at the Gacko site (GST activity: 2,4 mmol/min/mg prot., GSH concentration: 6,6 mmol/mg prot., AChE activity: 2,6 nmol/min/mg prot.) with several sources of pollution and most pronounced anthropogenic impact. The results showed that the values of parameters in samples of triton females are influenced by their reproductive status. AChE activity is most sensitive to the degree of contamination of the site and can be used as a reliable biomarker of environmental pollution.

KEYWORDS: antioxidant system, triton, glutathione-S-transferase, glutathione, acetylcholinesterase

Mikrobiologija

Microbiology



PROMENA ULTRASTRUKTURNE GRAĐE BETA ĆELIJA PANKREASA PACOVA USLED UTICAJA VISOKOFREKVENTNIH ELEKTROMAGNETNIH POLJA

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Tehnologija proizvodnje visokofrekventnih elektromagnetnih polja u svetu danas velikom brzinom napreduje, zato je neophodno neprestano testiranje njihovog uticaja na žive sisteme i okolinu. Cilj rada bio je da se dokaže uticaj visokofrekventnih elektromagnetnih polja na promenu ultrastrukturnih morfoloških i stereoloških parametara beta ćelija pankreasa pacova. Pacovi su izlagani viskofrekventnim elektromagnetnim poljima frekvencije od 3 GHz mesec dana. Uporedo sa zračenom bila je i kontrolna grupa pacova koji nisu bili izlagani elektromagnetnim poljima spomenute frekvencije. Promene su verifikovane na elektronmikrografijama beta ćelija sa transmisionog elektronskog mikroskopa, Zeiss EM902, pri uvećanju od 12000-29000 puta. Parametri stereološke nepristrasne analize organela određivani su po Kavalierijevom principu uz uoptrebu P2 mnogonameske mrežice. Korišćeni parametri ultrastrukturne morfološke analize beta ćelija bili su: oblik, veličina, broj i gustina organela. Dok su parametri ultrastrukturne stereološke analize istih ćelija bili: volumenska i numerička gustina organela. Vrednosti analiziranih parametara obrađene su u SPSS progamu i stepeni značajnosti njihovih promena testirani su Fisherovim i t-testom. Svi analizirani ultrastrukturni morfološki i stereološki parametri beta ćelija pankreasa pacova izlaganih visokofrekventnim elektromagnetnim poljima bili su izmenjeni u odnosu na iste parametre kod kontrolne grupe pacova. Zaključak rada je da visokofrekventna elektromagnetna polja frekvencije 3GHz imaju uticaj na ultrastrukturu beta ćelija pankreasa pacova.

KLJUČNE REČI: elektronski mikroskop, organele, stereologija, morfologija, elektromagnetna polja

ULTRASTRUCTURAL CHANGES IN RAT'S PANCREATIC BETA CELLS AFTER HIGH FREQUENCY ELECTROMAGNETIC FIELDS EXPOSURE

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The technology of production of high frequency electromagnetic fields is advancing at a rapid pace today, so it is necessary to constantly test its impact on living systems and the environment. The aim of this study was to prove the exposure of high frequency electromagnetic fields on the change of ultrastructural morphology and stereology parameters of rat pancreatic beta cells. Rats were exposed to electromagnetic fields at a frequency of 3 GHz for one month. There was also a control group of rats that were not exposed with electromagnetic fields. The changes were verified on beta cell electron micrographs from a transmission electron microscope, Zeiss EM902, at a magnification of 12000-29000X. The parameters of stereological analysis organelle's were determined to the Cavalieri's principle with P2 multipurpose grid. The parameters of ultrastructural morphological analysis of beta cells used were: shape, size, number and density of organelles, and stereological analysis of the same cells were: volume and numerical density of organelles. The values of the analyzed parameters were processed in the SPSS program and the degree of significance of their changes was tested by Fisher and t-test. All analyzed ultrastructural morphology and stereology parameters of rat pancreatic beta cells exposed to high frequency electromagnetic fields were changed in relation to the same parameters in the control group of rats. The conclusion of the paper is that high frequency electromagnetic fields of frequency 3GHz have an influence on the ultrastructure of beta cells of the pancreas of rats.

KEYWORDS: electron microscope, organelles, stereology, morphology, electromagnetic fields

PROMENA GRAĐE TIMUSA PACOVA NAKON IZLAGANJA VISOKOFREKVENTNIM ELEKTROMAGNETNIM POLJIMA

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Doprinos timusa u održavanju imuniteta orgnizama daje mu ulogu testnog organa u *in vivo* eksperimentima o uticaju elektromagnetnih polja na žive sistema. Nivoi frekvencija elektromagnetnih polja koja koriste uređaji za svoj rad svakodnevno rastu i menjaju se. Međutim, eksperimenti i modeli koji testiraju uticaje ovih polja na biljni i životinjski svet ne mogu da prate njihov brz razvoj. Cilj rada bio je da se pokaže uticaj visokofrekventnih elektromagnetnih polja na promene histoloških i stereoloških parametara tkiva timusa pacova. Eksperimenitalne životinje, pacovi, izlagani su viskofrekventnim elektromagnetnim poljima frekvencije od 3 GHz mesec dana. Uporedo sa zračenom bila je i kontrolna grupa pacova koji nisu bili izlagani elektromagnetnim poljima spomenute frekvencije. Promene su verifikovane na presecima tkiva timusa na svetlosnom mikroskopu Leica DM8000 M, na uvećanju od 200 puta, sa MEGA VIEW kamerom i pratećim softverskim sistemom. Preseci tkiva timusa tretirani su hematoksilin-eozinom i toluidin bojama radi bolje vizuelizacije timocita i mastocita. Parametri stereološke analize ćelija timusa određivani su po Kavalierijevom principu uz uoptrebu P2 mnogonameske mrežice. Korišćeni parametri histološke analize preseka tkiva timusa bili su: broj, oblik i veličina svih tipova ćelija timusa; nukleocitoplazmatični odnos i mitotski indeks timocita i mastocita. Dok su parametri sterološke analize preseka timusa bili: volumenska i numerička gustina ćelija timusa. Svi parametri tkiva timusa pacova izlaganih eksperimnetalnim poljima bili su izmenjeni u odnosu na iste parametre kontrolne grupe pacova. Zaključak rada je da elektromagnetna polja frekvencije 3GHz imaju uticaj na histološku građu timusa pacova, a samim tim i na njegovu funkciju.

KLJUČNE REČI: histologija, timociti, mastociti, elektromagnetna polja, imuni sistem

CHANGE IN STRUCTURE OF RATS THYMUS AFTER EXPOSURE TO HIGH FREQUENCY ELECTROMAGNETIC FIELDS

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Thymus has role like test organ in *in vivo* experiments. In our case experiment testing exposure of electromagnetic fields on living systems, and their work are growing and changing every day. However, experiments and models that test the effects of these fields on plants and animals can't follow their development. The aim of study was to show changes of histological and stereological parameters in rat thymus which were exposed of electromagnetic fields. Experimental animals, rats, were exposed to electromagnetic fields at a frequency of 3 GHz for one month. Control group of rats that were not exposed to mentioned frequency. Changes were verified on sections of thymus tissue on a Leica DM8000 M light microscope, at magnification of 200X, with MEGA VIEW camera and accompanying software system. Thymus tissue sections were treated with hematoxylin-eosin and toluidine colors for better visualization of thymocytes and mastocytes. Parameters of stereological analysis of thymus cells were determined according to the Cavalieri's principle with use of P2 multipurpose grid. Parameters of histological analysis of thymus section of tissue were: number, shape and size of all types of thymus cells; nucleocytoplasmic ratio and mitotic index of thymocytes and mastocytes. Parameters of sterological analysis of thymus section were: volume and numerical density thymus cells. All analyzed parameters of rat thymus tissue exposed to fields were changed in relation in control group. The conclusion of work is that fields of frequency 3GHz have an influence on histological structure of rat's thymus and thus on their function.

KEYWORDS: histology, thymocytes, mast cells, electromagnetic fields, immune system

ZNAČAJ DETEKCIJE MUTACIJA GENA KOD PACIJENATA SA MELANOMOM

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Melanomi su grupa najagresivnijih i za današnje metode lečenja najrezistentniji zloćudni tumori kod ljudi. U odabiru terapije za melanome koristi se i molekularna metoda analize *BRAF V600E* mutacija u uzorcima tkiva koža obolelih pacijenata u Univerzitetsko Kliničkom Centru Republike Srpske (UKCRS). Cilja rada bio je da se pokaže značaj i distribucija detekcije *BRAF V600E* mutacija u genomima pacijenata obolelih od melanoma. Studija u radu obuhvata 393 testirana uzorka kože sa melanomima pacijenata u periodu od pet godina. Uzorci kože su prošli kroz standardnu proceduru za mikroskopsku analizu tkiva, a nakon nje je iz histoloških preseka određivano prisustvo/odsustvo *V600E* mutacije gena *BRAF* na PCR (Polymerase Chain Reaction - reakcija lančanog umnožavanja DNK molekula) COBAS® 4800 sistemu. Parametri analize uzoraka u radu bili su: koncentracija izolovane DNK iz uzoraka obolele kože od melanoma; uspešnost detekcije *BRAF V600E* mutacija u uzorcima kože obolelih pacijenata od melanoma; kao i polna i starosna kategorija obolelih od metastatskog melanoma u korelaciji sa prisutnom/odsutnom detekcijom *BRAF V600E* mutacije u njihovim genomima. Srednja vrednost godina starosti svih pacijenata obolelih od melanoma u UKCRS bila je 58,62. Detektovane su mutacije *BRAF V600E* u 56,26% uzoraka kože u odnosu na ukupan broj pacijenata. Detekcija ove mutacije bila je najčešća kod muškaraca starosne dobi od 45-55 godina, dok je najređa bila kod dečaka i devojčica do 15 godina starosti. Testiranje uzoraka i detekcija *BRAF V600E* mutacije na genomu obolelih od melanoma pokazuju izuzetan značaj u cilju odabira adekvatne terapije i daljeg toka lečenja pacijenta.

KLJUČNE RIJEČI: *BRAF V600E*, PCR, koža, genom, polna distribucija

THE IMPORTANCE OF DETECTION GENE MUTATION IN PATIENTS WITH MELANOMA

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Melanomas are group of most aggressive and most resistant on treatment malignant tumors in humans. Molecular method of analysis of *BRAF V600E* mutations in samples of diseased skin tissue of patients at the University Clinical Center of Republic of Srpska (UCCRS) is used for treatment of melanoma. The aim of this study was to show importance of detecting *BRAF V600E* mutations in genomes of patients with melanoma. Study includes 393 tested skin samples with human melanoma over a period of five years. Skin samples underwent a standard procedure for microscopic tissue analysis, followed by histological sections to determine the presence/absence of the *V600E* mutation in the *BRAF* gene on the PCR (Polymerase Chain Reaction) in COBAS® 4800 system. The parameters of the analysis in the paper were: concentration of isolated DNA from samples of melanoma skin; success of detection of *BRAF V600E* mutations in skin samples of patients with melanoma; gender and age of patients in correlation with presence/absence of detection of *BRAF V600E* mutation in their genomes. The mean age of patients with melanoma in UCCRS is 58.62 years. *BRAF V600E* mutations were detected in 56.26% of skin samples from melanoma patients. Detection of mutation was most common in men aged 45-55 years, while it was least common in boys and girls up to 15 years of age. Testing of samples and detection of *BRAF V600E* mutation on genome of melanoma are in order to select the appropriate therapy and further course of treatment of the patient.

KEYWORDS: *BRAF V600E*, PCR, skin, genome, gender distribution

ADHEZIVNA SPOSOBNOST VRSTA IZ RODA *Klebsiella* I NJIHOVA KOAGREGACIJA SA *Enterococcus faecalis*

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Bakterije mlečne kiseline i enterobakterije su normalni činioci mikroflore mlečnih proizvoda proizvedenih na tradicionalan način zbog čega često stupaju u međusobne interakcije. U ovom radu ispitivani su hidrofobnost i sposobnost adhezije za epitel svinjskog creva, kao i ko-agregacija između *Klebsiella* spp. i *Enterococcus faecalis* KGPMF 49. Bakterije korišćene u ovom istraživanju su izolovane iz autohtonog, tradicionalno proizvedenog sira iz Sokobanje (jugoistočna Srbija). Procenat adhezije u prisustvu različitih rastvarača je meren spektrofotometrijskom metodom, a sposobnost adhezije bakterija za epitel svinjskog creva je detektovana fluorescentnim mikroskopom. Najveći procenat je izmeren u prisustvu hloroforma, a najmanji u prisustvu ksilena (hloroform < etil acetat < ksilen). *Klebsiella pneumoniae* KGPMF 13 i *Klebsiella ornithinolytica* KGPMF 9 su pokazale sposobnost adhezije za epitel tankog creva svinje. Vrste iz roda *Klebsiella* pokazuju različit stepen koagregacije sa *E. faecalis* KGPMF 49. *Klebsiella ornithinolytica* KGPMF 8 pokazala je najveći procenat ko-agregacije (32,3%). Kao kontrola, korišćen je standardni soj *Klebsiella pneumoniae* ATCC 70063. Na osnovu rezultata može se zaključiti da sir iz Sokobanje sadrži enterobakterije koje poseduju sposobnost adhezije i koje stupaju u interakciju sa drugim vrstama. Ovaj rad doprinosu znanju o enterobakterijama izolovanih iz sireva proizvedenih na tracionalan način.

KLJUČNE RIJEČI: *Klebsiella* spp., autohtoni sir, adhezivna svojstva, *Enterococcus faecalis*

ADHESIVE ABILITY OF THE SPECIES FROM GENUS *Klebsiella* AND THEIR CO-AGGREGATION WITH *Enterococcus faecalis*

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Lactic acid bacteria and enterobacteria are normal members of the microflora in the dairy products produced in the traditional way, which is why they often interact with each other. In this work, the hydrophobicity and the adhesion ability at porcine intestinal epithelium, as well as co-aggregation between *Klebsiella* spp. and *Enterococcus faecalis* KGPMF 49 were investigated. Bacteria used in this study were isolated from indigenous, traditionally produced cheese from Sokobanja (southeastern Serbia). The percentage of adhesion in the presence of different solvents was measured by spectrophotometric method while the ability of bacteria to adhere to the porcine intestine epithelium, was detected by a fluorescent microscope. The highest percentage was measured in the presence of chloroform, and the lowest in the presence of xylene (chloroform < ethyl acetate < xylene). *Klebsiella pneumoniae* KGPMF 13 and *Klebsiella ornithinolytica* KGPMF 9 showed the adhesion ability to porcine intestine epithelium. Species of the genus *Klebsiella* showed a different degree of co-aggregation with *E. faecalis* KGPMF 49. *Klebsiella ornithinolytica* KGPMF 8 showed the highest percentage of co-aggregation (32.3%). A standard strain, *Klebsiella pneumoniae* ATCC 70063, was used as a control. Based on the results, it can be concluded that the cheese from Sokobanja contains enterobacteria that could adhere and interact with other species. This paper contributes to the knowledge of enterobacteria isolated from cheeses produced in a traditional way.

KEYWORDS: *Klebsiella* spp., autochthonous cheese, adhesion properties, *Enterococcus faecali*

MIKROBIOLOŠKA ISPRAVNOST I IDENTIFIKACIJA DOMINANTNE MIKROBIOTE DUVAN ČVARAKA

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Među mnogim tradicionalnim mesnim proizvodima poreklom sa Balkanskog poluostrva, tradicionalna „grickalica“ pod nazivom „čvarci“ veoma je popularna za konzumaciju i trgovinu. U Srbiji se čvarci prave od svinjskog mesa i masti, topljenjem i prženjem u rastopljenoj masti. Postoje tri različite vrste čvaraka, a njihova razlika potiče u veličini komada. Čvarci mogu biti napravljeni od velikih komada, sitnih komada ili komadića poput sićušnih niti, koji se u Srbiji nazivaju „duvan čvarci“. Ovaj proizvod mora da ima prirodnu boju, ne sme da sadrži delove koji su zagoreli ili raspadnuti kao ni otpad (npr. dlačice sa kože); mora biti hrskav i imati karakterističan miris i ukus. Ovaj istraživački projekat zasnovan je na istraživanju mikrobiološke ispravnosti i identifikaciji dominantne mikrobiote duvan čvaraka proizvedenih u seoskim domaćinstavima. Analiza mikrobiološke ispravnosti pokazala je da u proizvodu nije detektovano prisustvo enterobakterija, koagulaza-pozitivnih stafilokoka, kvasca i plesni, kao ni *Salmonella* spp. i *Shigella* spp. Korišćenjem biohemijskih testova i MALDI-TOF masene spektrofotometrije, identifikovana je dominantna mikrobiota proizvoda. U okviru bakterija mlečne kiseline, identifikovani su *Lactobacillus sakei* i *Lactobacillus curvatus*, dok su u okviru koagulaza-negativnih stafilokoka identifikovani *Staphylococcus xylosus* i *Staphylococcus equorum*. Rezultati ovog istraživanja su pokazali da su ispitivani uzorci duvan čvaraka mikrobiološki ispravan proizvod i po prvi put dali saznanja o dominantnim članovima mikrobiote.

KLJUČNE RIJEČI: *duvan čvarci*, mikrobiološka ispravnost, dominantna mikrobiota, *Lactobacillus* spp., *Staphylococcus* spp.

MICROBIOLOGICAL SAFETY AND IDENTIFICATION OF DOMINANT MIKRIOBIOTA FROM "*DUVAN ČVARCI*"

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Among many traditional meat products from Balkan peninsula, the traditional pork meat snack called "*čvarci*" (in Serbian: *чварци*) is very popular for consumption. In Serbia, *čvarci* are made from pork meat and fat, by melting and frying in the melted fat. There are mainly three different types of *čvarci*, and their difference originated from the size of every piece. It could be a large piece, small pieces, or pieces like tiny threads, which are called in Serbia the "*duvan čvarci*". This product must have a natural color, no detection of over burnt and rancidity, no contaminants (e.g. hairs), must be crispy and have a distinctive odor and taste. This research project is based on the investigation of the microbiological safety and identification of dominant microbiota of "*duvan čvarci*" originated from local households. Microbiological safety analysis indicated that enterobacteria, coagulase-positive staphylococci, yeast, and molds as well as *Salmonella* spp. and *Shigella* spp. were not detected. By using a biochemical test and MALDI-TOF mass spectrophotometry, the dominant microbiota was identified. Among lactic acid bacteria, it was identified *Lactobacillus sakei* and *Lactobacillus curvatus*, while among coagulase-negative staphylococci, it was identified *Staphylococcus xylosus* and *Staphylococcus equorum*. The results from this study indicated that examined samples of *duvan čvarci* are a microbiologically safe product and, for the first time, gives evidence about the dominant members of the microbiota.

KEYWORDS: *duvan čvarci*, microbiological safety, dominant microbiota, *Lactobacillus* spp., *Staphylococcus* spp.

ANTIMIKROBNA I ANTIOKSIDATIVNA AKTIVNOST ACETONSKIH EKSTRAKATA DVE JESTIVE, POLIPORNE VRSTE GLJIVA TRULEŽNICA: *Laetiporus sulphureus* I *Meripilus giganteus*

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Poliporne vrste gljiva truležnica predstavljaju veliku i široko rasprostranjenu grupu gljiva iz razdela Basidiomycota. Pored njihovog dobro poznatog značaja u prirodnim ekosistemima, mnoge poliporne gljive truležnice poseduju izražena bioaktivna svojstva. U ovom istraživanju prikazana su antimikrobna i antioksidativna svojstva acetonskih ekstrakata dve jestive vrste *Laetiporus sulphureus* (Bull.) Murrill i *Meripilus giganteus* (Pers.) P. Karst., koje su uzorkovane u Batočini, u centralnoj Srbiji. Antimikrobna aktivnost ekstrakata testirana je na 10 vrsta gljiva i pet vrsta bakterija mikrodilucionom metodom (resazurin je korišćen kao indikator bakterijskog rasta). Antioksidativna aktivnost ekstrakata testirana je uz pomoć 2,2-difenil-1-pikrilhidrazil (DPPH) metode i određivanjem redukcionog kapaciteta. Minimalne inhibitorne koncentracije (MIC) za vrstu *L. sulphureus* bile su u opsegu 0,62-20 mg/ml, a za *M. giganteus* 0,31-20 mg/ml. IC_{50} vrednost u DPPH metodi za *L. sulphureus* bila je 730,84 μg/ml, dok je za *M. giganteus* bila 712,31 μg/ml. Apsorbanca za redukcioni kapacitet za *L. sulphureus* bila je u opsegu 0,010-0,032, dok je za *M. giganteus* iznosila 0,042-0,076. Obe vrste su pokazale umereno jaku antimikrobnu i antioksidativnu aktivnost. U svim testovima vrsta *M. giganteus* je pokazala bolje rezultate od vrste *L. sulphureus*. Rezultati sprovedenog istraživanja upućuju na to da obe vrste poseduju lekoviti potencijal i da stoga obe mogu biti korišćene kao funkcionalna hrana.

KLJUČNE REČI: Bioaktivnost, gljive, funkcionalna hrana, *Laetiporus sulphureus*, *Meripilus giganteus*

ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITY OF ACETONIC EXTRACTS OF TWO EDIBLE, WOOD-DECAYING POLYPORES: *Laetiporus sulphureus* AND *Meripilus giganteus*

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Wood-decaying polypore mushrooms represent a large and widespread group of fungi of the phylum Basidiomycota. Apart from their well-known importance in natural ecosystems, many wood-decaying polypores have potent bioactive properties. This study shows the antimicrobial and antioxidative properties of acetonic extracts of two edible species *Laetiporus sulphureus* (Bull.) Murrill and *Meripilus giganteus* (Pers.) P. Karst., collected in Batočina, central Serbia. The antimicrobial activity of extracts was tested on 10 species of fungi and five species of bacteria by the microdilution assay (resazurin was the indicator of bacterial growth). The antioxidative activity of extracts was tested by the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay and reducing power assay. MIC values in microdilution assay for *L. sulphureus* were in range 0.62-20 mg/ml and for *M. giganteus* 0.31-20 mg/ml. The value of IC_{50} in DPPH assay for *L. sulphureus* was 730.84 μg/ml and for *M. giganteus* 712.31 μg/ml. The absorbance in reducing power assay for *L. sulphureus* was in range 0.010-0.032 and for *M. giganteus* 0.042-0.076. Both species displayed moderately strong antimicrobial and antioxidative activities. In all assays *M. giganteus* expressed better activity than *L. sulphureus*. The results indicate that both species have therapeutic potentials and can be used as functional foods.

KEYWORDS: Bioactivity, functional food, *Laetiporus sulphureus*, *Meripilus giganteus*, mushrooms

UČESTALOST BAKTERIJA *Escherichia coli* I *Shigella spp*. NA POVRTLARSKIM KULTURAMA NA PODRUČJU SEMBERIJE

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U poslednje vrijeme sve češće se javljaju epidemije koje su nastale kao rezultat prenošenja patogenih mikroorganizama konzumiranjem kontaminiranog svježeg povrća. Povrće ne predstavlja prirodno stanište za bakterije koje izazivaju oboljenja kod čovjeka. Dok neki autori smatraju da do kontaminacije dolazi u procesu prerade i pakovanja, odnosno na putu od proizvođača do krajnjeg potrošača, drugi pretpostavljaju da su đubrenje i navodnjavanje neprečišćenom vodom neki od mogućih puteva prenosa patogenih bakterija na poljoprivredne kulture. Cilj provedenog istraživanja je bio da se ukaže na eventualno prisustvo potencijalno patogenih sojeva bakterija na pojedinim povrtlarskim kulturama (krompir, zelena salata, špinat, paradajz, paprika, mrkva, peršun, crveni luk) na području Semberije. Ekstrakcija bakterija iz uzoraka je vršena potapanjem maceriranog biljnog tkiva u rastvoru za ekstrakciju sa sterilnom peptonskom vodom. Za izolaciju potencijalnih patogena korištene su indirektne odgajivačke metode na visoko selektivnim hromogenim podlogama. Nakon poređenja dobijenih izolata sa pozitivnim kontrolama izdvojene su suspektne kolonije i izvršena je potvrda sojeva ekstrakcijom DNK i AmpliTest PCR metodom sa specifičnim prajmerima. Prisustvo bakterije *Escherichia coli* je potvrđeno na krtolama krompira, plodovima paradajza i paprike babure i na lukovici crvenog luka, dok je *Shigella spp.* izolovana na lišću zelene salate, na plodovima paradajza, na krtolama krompira, kao i na korjenu mrkve i peršuna. Prisustvo potencijalnih patogena na svježem povrću, posebno onom koje se konzumira bez prethodne termičke obrade, je alarmantno. Kako bi se sprečile negativne posljedice neophodno je precizirati puteve njihove transmisije kao i ispitati njihovu vijabilnost u za njih nespecifičnim ekološkim nišama kao što su poljoprivredne kulture.

KLJUČNE RIJEČI: povrće, *Escherichia coli*, *Shigella spp*.

FREQUENCY OF BACTERIA *Escherichia coli* AND *Shigella spp*. IN VEGETABLE IN THE AREA OF SEMBERIJA

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Recently, epidemics that have occurred as result of transmission of pathogenic microorganisms by consuming contaminated fresh vegetables have become more frequent. Vegetables don't represent natural habitat for bacteria that cause diseases in humans. While some authors believe that contamination occurs in process of processing and packaging, on way from the producer to final consumer, others assume that fertilization and irrigation with untreated water are some of the possible ways of transmitting pathogenic bacteria to the crops. The aim of research was to indicate possible presence of potentially pathogenic strains of bacteria on certain vegetables (potato, lettuce, spinach, tomato, paper, carrot, parsley, red onion) in the area of Semberija. Extraction of bacteria from the samples was performed by immersing the macerated plant tissue in extraction solution with sterile pepton water. Indirect culture methods on highly selective chromogenic media were used to isolate certain potential pathogens. After comparing obtained isolates with positive controls, suspicious colonies were isolated and strains were confirmed by DNA extraction and AmpliTestPCR method with specific primers. The presence of *Echerichia coli* was confirmed on potato tubers, tomato and peppers fruits and on red onion bulbs, while *Shigella spp.* was isolated on lettuce leaves, tomato fruits, potato tubers and carrot and parsley roots. The presence of potential pathogens on fresh vegetables, especially those consumed without prior heat treatment, is alarming. In order to prevent negative consequences, it is necessary to specify routes of their transmission as well as to examine their viability in non-specific ecological niches such as crops.

KEYWORDS: vegetables, *Escherichia coli*, *Shigella spp*.

CHLAMYDIA AND GONORRHEA PREVALENCE AMONG MEN WHO HAVE SEX WITH MEN IN COMMUNITY BASED CENTRE IN ZAGREB, CROATIA

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Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) infections are among the most common bacterial sexually transmitted infections and disproportionately affect men who have sex with men (MSM). Large proportion of chlamydia and gonorrhea infections in men are asymptomatic, and therefore often undiagnosed and untreated which increases chances for complications and risk of transmissions. The aim of this study was to determine prevalence of chlamydia and gonorrhea infection among community-venue attending MSM in Zagreb, Croatia, between November 2019 and February 2020. Testing sites for each participant were determined based on self-reported sexual risk behavior. Urine, rectal and/or pharyngeal sample were pooled and real time PCR detection of CT and/or NG DNA was performed using Xpert CT/NG assay on the Cepheid GeneXpert Instrument Systems. The median age of 89 participants was 32 (standard deviation 8.39, age range 19-53 years). Most of the respondents (59.55%) had at least some university education, were employed (71.91%) and lived in an urban area (87.64%). Infection with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* was detected in 14 (15.73%) participants, with 3 cases of coinfection with both bacteria. A total of 11 participants (12.36%) tested positive for chlamydial infection and 6 participants (6.74%) for gonococcal infection. The prevalence of CT and NG in MSM in our study is expectedly higher compared with Croatian general population and consistent with studies conducted in similar settings. However, well-educated men were reached disproportionately and different recruitment approach may be needed in order to include a more diverse spectrum of MSM.

KEYWORDS: sexually transmitted infections, chlamydia, gonorrhea, men who have sex with men, community testing

BEZBEDNOST HRANE I ZDRAVLJE POTROŠAČA

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Trovanje hranom je oboljenje nastalo konzumiranjem kontaminirane hrane. Najčešći uzročnik trovanja hranom su mikroorganizmi (bakterije, plesni, virusi i paraziti) ili njihovi toksini koji mogu kontaminirati hranu u bilo kom trenutku prerade ili proizvodnje. Kontaminacija takođe može nastati i u domaćinstvu ukoliko se hrana nepravilno čuva ili priprema. Primeri nebezbedne hrane uključuju sveže meso, piletinu, nepasterizovano mleko, voće i povrće i sirove školjke. Simptomi trovanja hranom, koji mogu započeti nekoliko časova nakon konzumiranja kontaminirane hrane, često podrazumevaju mučninu, povraćanje ili dijareju. Trovanje hranom je najčešće blago i rešava se bez lečenja, ali u nekim slučajevima je neophodna bolnička nega. Svaki pokušaj da se proceni učestalost bolesti koje se prenose hranom u Srbiji veoma je težak, delom zbog toga što takve bolesti nisu jasno označene kao prehrambene, a delom zbog toga što pacijenti ne traže uvek lekarsku pomoć. Cilj ovog rada bio je da predstavi najznačajnije uzročnike trovanja hranom u Srbiji, prema javno dostupnim podacima o zaraznim bolestima, Instituta za javno zdravlje Srbije. Najčešći uzročnici ovih bolesti su bakterije kao što su *Salmonella sp*. *Campilobacter jejuni, Escerichia coli*, *Staphylococcus aureus, Shigella sp*., kao i neke vrste virusa, plesni i parazita. Obzirom da su crevne zarazne bolesti na drugom mestu po zastupljenosti i da se njihova učestalost povećava poslednjih godina, neophodno je kontinuirano edukovati stanovništvo o načinima prenošenja ovih bolesti i merama prevencije kako bi se smanjio rizik od izloženosti potrošača infekcijama prenetim hranom.

KLJUČNE REČI: trovanje hranom, bakterije, gljive, javno zdravlje

FOOD SAFETY AND CONSUMERS HEALTH

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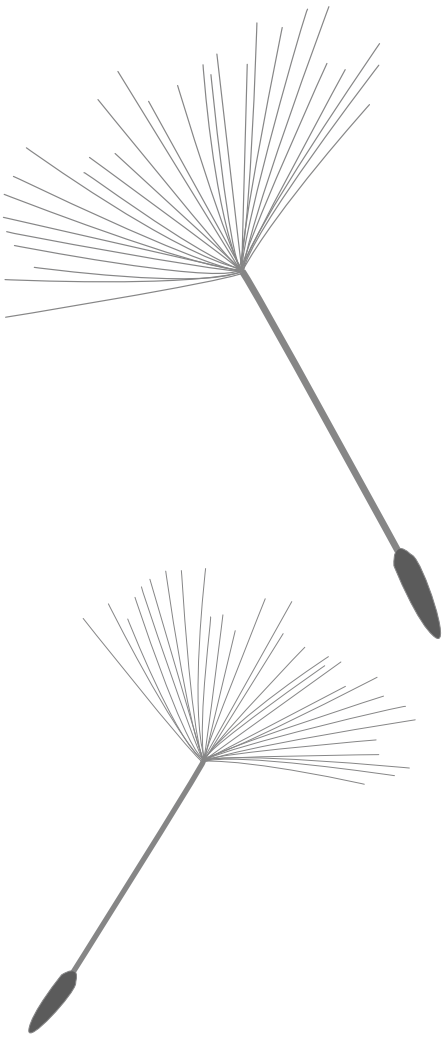
Food poisoning is illness caused by eating contaminated food. The most common causes of food poisoning are microorganisms (bacteria, molds, viruses and parasites) or their toxins which can contaminate food at any point of processing or production. Contamination can also occur at home if food is incorrectly handled or cooked. Examples of unsafe food include uncooked meats, poultry, unpasteurized milk, fruits and vegetables, and raw shellfish. Food poisoning symptoms, which can start within hours of eating contaminated food, often include nausea, vomiting or diarrhea. Most often, food poisoning is mild and resolves without treatment, but in some case are necessary a hospital care. Any attempt to evaluate the incidence of food – borne diseases in Serbia is very difficult, partly because such diseases are not clearly designated as food-borne, and partly because patients do not always seek a medical attention. The aim of this paper was to present the most significant causes of food poisoning in Serbia, according to publicly available data on infectious diseases of the Institute of Public Health of Serbia. The most common causes of these illnesses are bacteria such as *Salmonella sp*. *Campilobacter jejuni, Escerichia coli*, *Staphylococcus aureus, Shigella sp*., as well as some types of viruses, molds and parasites. Given that intestinal infectious diseases are the second most common and their frequency has increased in recent years, it is necessary to continuously educate the population about ways of transmitting these diseases and prevention measures to reduce the risk of consumer exposure to foodborne infections.

KLJUČNE REČI: food poisoning, bacteria, molds, public health

Ekologija biljaka

Plant Ecology



ECOLOGICAL – PHYTOGEOGRAPHICAL CHARACTERISTICS OF THE WEED FLORA OF VEGETABLES CROPS IN THE SURROUNDING OF ČENTA VILLAGE (SOUTH BANAT, SERBIA)

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There is a recognized need for research on weed flora of Čenta (Southern Banat, Serbia) due to its multifold scientific and practical significance. In this paper, the weed flora of garden vegetable crops at the studied locality is presented from floristic, ecological and phytogeographic standpoints. Plant material was collected in the field from April 2017 to October 2019 in plots of garden vegetable crops (potatoes, peas, beans, onion, garlic, cabbage, Savoy cabbage, cauliflower, tomato, bell pepper, carrot, parsley and celeriac). There were 35 recorded weed species from 16 families and 31 genera. The analysis of representation of plant life forms in the weed flora of garden vegetable crops at Čenta has shown predominance of therophytes (65.71%), followed by geophytes and hemicryptophytes at 17.4% each. The analysis of ecological indices for five main ecological factors (humidity, soil acidity, soil fertility, light and temperature) has shown dominance of plants preferring moderately humid habitats, in soils with neutral to weakly acidic reaction and medium amount of mineral matter, semi-open to open regarding the light regime, and mesophilic to thermophilic regarding the temperature regime. The phytogeographic analysis of weed flora has shown presence of 10 different floristic elements, grouped into five main area types. The results of this research will be used to expand the network of studied urban units in Serbia, which are used in various comparative analyses, and also as a good foundation for selecting adequate measures of weed suppression.

KEYWORDS: Čenta village, south Banat, ecological indices, life forms, phytogeographycal characteristics, weed flora

CORRELATION PATTERNS IN THREE *Lamium* SPECIES GROWN IN TWO LIGHT AND THREE DENSITY TREATMENTS

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Phenotypic integration is a correlation structure between the traits of an organism. It could be seen both as an adaptation to a set of environmental conditions, and as a constrain to evolution. Plastic responses of different traits and strength and patterns of their integration change throughout the lifespan of an organism and should be higher under stress. That is why there is a need for monitoring trait responses at different ontogenetic stages, as well as under different environmental treatments. In this study we examined between trait correlations in three *Lamium* species – annual *Lamium purpureum*, and perennial *Lamium album* and *Lamium maculatum*, grown in two light treatments (low and high) and three planting densities within light treatments (1, 3 and 5 plants per pot). The goal was to examine how pattern and strength of phenotypic correlations and the level of phenotypic integration changed in different light and density treatments, ontogenetic stages and among three *Lamium* species. *L. purpurem* showed greater integration in comparison to the other two perennial species before applying environmental treatments, and greater variability in phenotypic correlations in response to light and density at the earlier ontogenetic stage. Variability in phenotypic response intensified over time in perennials, *L. album* and *L. maculatum*, when they become more similar in their reaction to annual *L. purpureum*. Leaf width and leaf length, as functionally and developmentally related traits, were the most integrated in all three species in comparison to other measured traits.

KEYWORDS: *Lamium album* (White Dead Nettle), *Lamium maculatum* (Spotted Dead Nettle), *Lamium purpureum* (Red Dead Nettle), phenotypic integration

SEASONAL VARIABILITY IN CORRELATION PATTERNS AMONG *Iris variegata* L. GENOTYPES GROWING IN CONTRASTING LIGHT CONDITIONS

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Higher plants have developed protection mechanisms in order to diminish the effects caused by the presence of free radicals generated during high irradiance, and one of the detoxification mechanisms is the synthesis of secondary metabolites. In this study we investigated the amount and pattern of individual phenotypic responses (represented through targeted phenolics content in 68 genotypes of *Iris variegata*) to seasonal changes in environmental conditions under two experimental light regimes. The goal was to examine how environmental stressors mold the interrelationships between metabolite traits. Genotypes of *I. variegata* were collected in Deliblato sands Special Nature Reserve in Serbia, from two types of natural habitats (exposed and shade) and transferred into the experimental field conditions providing either 1. high light intensity and higher red / far red light ratio and 2. low light intensity and lower red / far red light ratio. After period of acclimatization leaves were collected during spring, summer and fall of one experimental year and subjected to UHPLC/qqqMS quantification of 10 selected phenolic compounds belonging to the groups of phenolic acids, flavonoids and xanthones. The correlations between all studied compounds within two light treatments and three seasons were in almost all cases significant and positive. Number of statistically significant correlations markedly decreased through the vegetative period in both light treatments. The experiment was conducted on a large number of genotypes of *I. variegata* and points to the need of involving a number of factors in future ecological and evolutionary researches.

KEYWORDS: correlation patterns, phenolic compounds, light treatments, within season variability, *Iris variegata*

VARIJABILNOST SESKVITERPENA U ETARSKOM ULJU VRSTE *Teucrium montanum* L. (LAMIACEAE) SA SERPENTINITSKIH I KREČNJAČKIH STANIŠTA

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Seskviterpenska jedinjenja pokazuju širok spektar bioloških aktivnosti, koja mogu doprineti adaptaciji biljaka na određene tipove staništa. Cilj prikazanog istraživanja je određivanje kvalitativne varijabilnosti i relativne zastupljenosti seskviterpena u uzorcima vrste *Teucrium montanum* L. u zavisnosti od tipa podloge. Dvadeset uzorka etarskih ulja dobijeno je metodom hidrodestilacije po Clevenger-u od nadzemnih biljnih delova. Deset uzoraka etarskog ulja biljaka sa serpentinita i deset sa krečnjaka su ispitani sveobuhvatnom GCxGC-MS metodom. Rezultati su pokazali da populacije sa serpentinitskih staništa sintetišu 2 puta više seskviterpena nego sa krečnjačkih staništa. Na osnovu prosečnih vrednosti relativne zastupljenosti u uzorcima ulja *Teucrium montanum* sa oba tipa staništa pokazano je da su seskviterpeni zastupljeni sa 23,28%. U uzorcima sa serpentinita izdvaja se seskviterpenski ugljovodonik *β*-guaien (0,92%), dok je od oksidovanih seskviterpena najzastupljeniji 5-isopropenil-2-metil-2-cikloheksen-1-il pivalat (4,81%). Takođe, u uzorcima sa krečnjaka izdvaja se seskviterpenski ugljovodonik *β*-guaien (0,54%), dok je od oksidovanih seskviterpena najzastupljeniji farnezil aceton (5,9,13-pentadekatrien-2-on, 6,10,14-trimetil, (E,E)) – 0,94%. Seskviterpenski ugljovodonik 6-epi-*β*-kubeben je jedino detektovan u populacijama sa krečnjaka. Rezultati predstavljeni u ovom istraživanju pokazali su da edafske karakteristike zemljišnog supstrata utiču na kvalitativni sastav i relativnu zastupljenost seskviterpena u etarskom ulju vrste *Teucrium montanum* i doprinose diferencijaciji specifičnih hemotipova.

KLJUČNE REČI: eterična ulja, serpentinit, krečnjak, *Teucrium monatnum*, varijabilnost, GCxGC-MS

VARIABILITY OF SESQUITERPENES IN ESSENTIAL OIL OF *Teucrium montanum* L. (LAMIACEAE) FROM SERPENTINITE AND CALCAREOUS HABITATS

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Sesquiterpene compounds show a wide range of biological activities, which could contribute to the adaptation of plants to certain habitat types. This study aimed to determine the qualitative variability and relative percentage of sesquiterpenes in samples of *Teucrium montanum* L. in relation to the type of substrate. Twenty essential oil samples were obtained by the Clevenger hydrodistillation method from aboveground plant parts. Ten samples of essential oil from serpentinite and 10 from calcareous habitats were tested by the comprehensive GCxGC-MS method. Based on the average values of the relative percentage in the samples of *Teucrium montanum* essential oil from both types of habitats, it was shown that the sesquiterpenes were represented by 23.28%. By comparing the values of sesquiterpenes from examined habitats, it was shown that the plants from serpentinite habitats synthesize 2 times more sesquiterpenes than plants from calcareous habitats. Among the most abundant compounds in the serpentinite samples for hydrocarbon sesquiterpenes was *β*-guaiene (0.92%), and for oxygenated sesquiterpenes, it was 5-isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate (4.81%). Among the most abundant compounds in calcareous samples from the group hydrocarbon sesquiterpenes was *β*-guaiene (0.54%), and from oxygenated sesquiterpenes is farnesyl acetone (5,9,13-pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)) – 0.94%. The sesquiterpene hydrocarbon 6-epi-*β*-cubeben was only detected for calcareous populations. The results presented in this study showed the significant influence of the substrate on the qualitative composition and relative percentage of sesquiterpenes in *Teucrium montanum* essential oil. The edaphic characteristics of substrate could affect the essential oil biosynthesis, which affects the differentiation of specific chemotypes.

KEYWORDS: *Teucrium montanum*, essential oil, serpentinite, calcareous, variability, GCxGC-MS

VARIJABILNOST ANTIOKSIDATIVNE AKTIVNOSTI VRSTE *Mentha pulegium* (LAMIACEAE) SA RAZLIČITIH STANIŠTA

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Mentha pulegium pored nezaslanjenih može da naseljava zaslanjena staništa. Cilj istraživanja je uporedna analiza sekundarnih metabolita (fenolna jedinjenja i flavonoidi) i antioksidativne aktivnosti *M. pulegium* sa dve slatine (Lalinačka i Oblačinska) i jednog nezaslanjenog staništa (Zasavica) u etanolnim i etil acetatnim ekstraktima nadzemnih biljnih delova. Količina fenolnih jedinjenja *M. pulegium* sa Oblačinske i Lalinačke slatine izražena kao ekvivalent galne kiseline bila je 140,94 mg GA/g i 92,91 mg GA/g za etanolni, kao i 65,49 mg GA/g i 116,64 mg GA/g za etil acetatni ekstrakt. Vrednosti *M. pulegium* sa Zasavice iznosile su 67,02 mg GA/g za etanolni i 57,05 mg GA/g za etil acetatni ekstrakt. Koncentracija flavonoida *M. pulegium* sa Oblačinske i Lalinačke slatine prikazana kao ekvivalent rutina iznosila je 87,13 mg Ru/g i 87,46 mg Ru/g za etanolni, kao i 208,07 mg Ru/g i 129,52 mg Ru/g za etil acetatni ekstrakt. *M. pulegium* sa Zasavice imala je vrednosti 162,64 mg Ru/g za etanolni i 219,62 mg Ru/g za etil acetatni ekstrakt. Antioksidativna aktivnost *M. pulegium* sa Oblačinske i Lalinačke slatine imala je IC_{50} vrednosti 325,42 μg/mL i 458,85 μg/mL za etanolni, kao i 100,26 μg/mL i 405,85 μg/mL za etil acetatni ekstrakt. Vrednosti *M. pulegium* sa Zasavice su iznosile 508,85 μg/mL za etanolni i 435,66 μg/mL za etil acetatni ekstrakt. *M. pulegium* sa slatina je imala veću količinu ukupnih fenolnih jedinjenja i veću antioksidativnu aktivnost, dok je sa nezaslanjenog staništa imala veću količinu flavonoida. Zaslanjenost zemljišta utiče na povećanu sintezu sekundarnih metabolita koji biljkama omogućavaju adaptaciju na stresne uslove staništa.

KLJUČNE REČI: sekundarni metaboliti, antioksidativna aktivnost, *Mentha pulegium*, zaslanjena staništa

VARIABILITY OF ANTIOXIDATIVE ACTIVITY OF *Mentha pulegium* (LAMIACEAE) FROM DIFFERENT HABITATS

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In addition to nonsaline, *Mentha pulegium* can also inhabit saline habitats. The aim of this study was analysis of phenolic compounds and flavonoids, as well as antioxidant activity of *M. pulegium* from two saline (Lalinačka and Oblačinska slatina) and nonsaline habitat (Zasavica) in ethanolic and ethyl acetate extracts. The total phenolics of *M. pulegium* from Oblačinska and Lalinačka slatina expressed as gallic acid equivalent were 140.94 and 92.91 mg GA/g for ethanolic, as well as 65.49 and 116.64 mg GA/g for ethyl acetate extract. In the samples from Zasavica were 67.02 mg GA/g for ethanolic and 57.05 mg GA/g for ethyl acetate extract. The concentration of flavonoids in *M. pulegium* from Oblačinska and Lalinačka slatina shown as the rutine equivalent were 87.13 and 87.46 mg Ru/g for ethanolic, as well as 208.07 and 129.52 mg Ru/g for ethyl acetate extract. Samples from Zasavica had values of 162.64 mg Ru/g for ethanolic and 219.62 mg Ru/g for ethyl acetate extract. The antioxidant activity (IC_{50}) of *M. pulegium* from Oblačinska and Lalinačka slatina was 325.42 and 458.85 μg/mL for ethanolic, as well as 100.26 and 405.85 μg/mL for ethyl acetate extract. The values of samples from Zasavica were 508.85 μg/mL for ethanolic and 435.66 μg/mL for ethyl acetate extract. *M. pulegium* from saline habitats had higher amount of total phenolic compounds and higher antioxidant activity, while from nonsaline habitat had more flavonoids. Soil salinity affects the increased synthesis of secondary metabolites that allow plants to adapt to stressful habitat conditions.

KEYWORDS: secondary metabolites, antioxidant activity, *Mentha pulegium*, saline habitats

FANEROFITE U FLORI BILEĆE

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U radu su prikazani rezultati istraživanja dendroflore područja opštine Bileća. Floristička istraživanja su vršena tokom vegetacijske sezone, 2017. i 2018. godine. Potvrđeno je prisustvo 114 vrsta i podvrsta biljaka, grupisanih u 73 roda i 35 porodica. U pogledu životnih formi dominiraju fanerofite iz grupe listopadnog drveća. Identifikacija biljnih taksona je vršena na osnovu florističke literature. Taksonomski status, pripadnost familijama i nomenklatura prikazani su prema Euro+Med PlantBase. Florni elementi su dati prema Oberdorfer-u. Indikatorske vrijednosti biljnih vrsta za osnovne ekološke faktore su određene po Kojiću i saradnicima. Životne forme su određivane prema Ellenberg-u i Mueller-Dambois-u, bazirano na principima Raunkiaer-a, kao što je prikazano u Flori Srbije. Prema biljnogeografskoj analizi najčešće su vrste iz submediteranske, evroazijske i subatlanske areal grupe. Analizom ekoloških indeksa za osnovne ekološke faktore utvrđena je dominacija submezofita. Većina vrsta preferira neutrofilnu do bazofilnu reakciju zemljišta koje je siromašno hranljivim materijama. U odnosu na svjetlost najbrojnije su poluskiofite, odnosno biljke polusjene, dok u odnosu na temperaturu dominira grupa smještena na prelazu, između mezotermnih i termofilnih vrsta biljaka.

KLJUČNE RIJEČI: dendroflora, životne forme, florni elementi, ekološki indeksi, Bileća

PHANEROPHYTE IN THE FLORA OF THE MUNICIPALITY OF "BILEĆA"

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The paper presents the results of research on the dendroflora of the municipality of Bileća. Floristic investigation was conducted in the vegetation season, 2017. and 2018. The presence of 114 species and subspecies of plants was confirmed, grouped into 73 genera and 35 families. In terms of life forms, dominated phanerophytes from the group of deciduous trees. Identification of plant taxa was determined on the basis of floristic literature. Taxonomic position, family affiliation and nomenclature are given according to Euro+Med PlantBase. The floristic elements are given according to Oberdorfer. Indicator values of plant species for basic ecological factors were determined by Kojić and associates. Life forms were determined according to Ellenberg and Mueller-Dambois, based on Raunkiaer's principles, as shown in the Flora of Serbia Phytogeographical analysis showed that species are most often from sub-Mediterranean, Eurasian and sub-Atlantic areal groups. The analysis of ecological indices for basic ecological factors determined the dominance of submezophytes. Most species prefer a neutrophilic to basophilic soil reaction. In relation to light, the most numerous species are hemisciophyte, half-shaded plants, while in relation to temperature, dominated the group located at the transition between mesothermal and thermophilic plant species.

KEYWORDS: dendroflora, life forms, floristic elements, ecological indices, Bileća

Ekologija životinja

Animal Ecology

ZAJEDNICE MAKROINVERTEBRATA U EKSTREMNIM STANIŠTIMA

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Slatkovodna staništa skromnih dimenzija mogu biti veoma značajna sa aspekta biodiverziteta, ne zbog toga što podržavaju veliko bogatstvo vrsta već zbog toga što mogu da sadrže endemične vrste. Neki od ovih prostorno ograničenih habitata mogu predstavljati ekstremno okruženje zato što fizicko-hemijski parametri u njima imaju vrednosti izvan opsega 'normanih' odnosno očekivanih za takve ekosisteme. Ovakvi uslovi zahtevaju da organizmi koji opstaju u njima imaju specifične adaptacije. Sa druge strane, ti uslovi jako doprinose jedinstvenosti zajednicama koje naseljavaju ovakva staništa. Postoji čitav spektar ekstremnih vodenih staništa u pogledu temperature, hemijskih karakteristika, varijabilnosti hidroloških uslova ili insolacije i sl. Ovo izlaganje biće fokusirano na posebnost zajednica makroinvertebrata koje naseljavaju termalne izvore, slatine i vodopade. Osim što se ovi ekosistemi odlikuju karakteristicnim taksonomskim sastavom i strukturom zajednica, ove zajednice su specifično funkcionalno oblikovane. Biće dat literaturni pregled dosadasnjih istrazivanja makroinvertebratskih zajednica extremnih stanista u Srbiji i okruženju. Takodje će biti prodiskutovani rezultati sopstvenih istraživanja koja su kategorisana prema tipu ekstremnih staništa na: istraživanja makroinvertebratske zajednice toplih izvora; istraživanja zajednica dva tipa slatina; istraživanja makroinvertebratske zajednice vodopada. Obzirom da su istraživanja ovakvih stanista i njihovih zajednica, a jos vise njihovog funkcionisanja, sporadična, ovo predavanje predstavlja i pokušaj podizanja svesti o važnosti ovih habitata koji su među najugroženijim u Evropi.

KLJUČNE RIJEČI: makroinvertebrate, ekstremna staništa, termalni izvori, slatine, vodopadi

MACROINVERTEBRATE COMMUNITIES IN EXTREME ENVIRONMENTS

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Freshwater environments that often occur at small spatial scales may be highly important from biodiversity standpoint, not because of high species richness but because they may include populations of endemic species. Some of these spatially limited habitats may represent extreme environments, as their physicochemical parameters show values outside of "normal" limits expected for such ecosystems. These conditions demand specific adaptations from the surviving organisms while at the same time they also strongly contribute to uniqueness of communities inhabiting these habitats. There is a whole spectrum of extreme aquatic habitats in terms of temperature, chemical composition, variability of hydrological condition or insolation etc. This discussion will focus on unique characteristics of macroinvertebrate communities inhabiting thermal springs, salt marshes and waterfalls. In addition to characteristic taxonomic composition and structure, the communities in these ecosystems are specifically functionally outlined. This paper will include an overview of previous research on macroinvertebrate communities of extreme habitats in Serbia and its surroundings, as well as a discussion of our original research, categorized according to type of extreme habitats into: research of macroinvertebrate community of thermal springs; research of communities in two types of salt marshes; research of macroinvertebrate community of waterfalls. As studies of these habitat types and their communities, and especially regarding their ways of functioning, are sporadic, this presentation is also an attempt to raise awareness on importance of these habitats, which are often among the most vulnerable aquatic habitats in Europe.

KEYWORDS: macroinvertebrates, extreme habitats, thermal springs, salt marshes, waterfalls

KVALITETA ARHIVSKOG MATERIJALA ZA DNA-BARKODIRANJE

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Makrozoobentos se već desetljećima determinira na morfološkoj razini. Razvojem molekularnih metoda unatrag desetak godina postalo je moguće učiniti taksonomsku identifikaciju i korištenjem DNA-barkodiranja. Dodatno, postojanje arhivskih zbirki uzoraka makrozoobentosa omogućava istraživanja kroz vrijeme ukoliko je materijal u njima pohranjen tako da su očuvane makromolekule. Cilj ovog istraživanja bio je analizirati očuvanost molekula DNA u arhivskim uzorcima ovisno o fiksativima korištenima za pohranu. Korištene su zbirke uzoraka makrozoobentosa sakupljene 1992., 2008., 2016. i 2020. godine pohranjene u različitim fiksativima. Odabrane vrste iz pojedine zbirke obrađene su na dva načina: 1) jedinke su uklopljene u parafinske kocke čiji su rezovi korišteni za izolaciju molekula DNA i procjenu kvalitete tkiva, 2) DNA je izolirana iz originalnih, neuklopljenih uzoraka. Metode su uključivale izolaciju DNA iz oba tipa uzoraka setom kemikalija Zymo Research Quick-DNA/RNA kit, a lančanom reakcijom polimerazom umnožen je fragment gena *COI* koji je služio kao barkod. Umnoženi fragmenti sekvencirani su Sangerovom metodom. Očuvanost morfologije tkiva procijenjena je korištenjem rezova tkiva jedinki obojenih hemalaumom i eozinom. Rezultati su pokazali da je iz arhivskih uzoraka moguće izolirati DNA zadovoljavajuće koncentracije i čistoće bez obzira na starost zbirke i fiksativ. Ograničavajući faktor kod uzoraka fiksiranih formalinom bila je fragmentacija DNA dok su uzorci fiksirani u etanolu imali DNA zadovoljavajuće kvalitete. Očuvanost tkiva za morfološke analize bila je zadovoljavajuća samo kod uzoraka pohranjenih u formalinu. Na temelju ovih rezultata može se zaključiti da je zbirke makrozoobentosa potrebno pohranjivati u alkoholu za molekularne analize i paralelno u formalinu za morfološka testiranja.

KLJUČNE RIJEČI: fiksativi, izolacija DNA, DNA-barkodiranje, očuvanost tkiva

QUALITY OF ARCHIVED MATERIAL FOR DNA BARCODING

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Macrozoobenthos has been determined at the morphological level for decades. With the development of molecular methods, it became possible to make taxonomic identification using DNA-barcoding. Additionally, with the existence of archival collections it became possible to research macrozoobenthos samples over time if they are stored in fixatives that preserve macromolecules. The aim of this study was to analyze the preservation of DNA molecules in archived samples depending on the fixatives used for storage. Samples collected in 1992, 2008, 2016 and 2020, stored in various fixatives were used. The samples were analyzed using two approaches: 1) selected individuals were embedded in paraffin blocks and their sections were used for DNA isolation and tissue quality assessment, 2) DNA was isolated from the original, non-embedded samples. Methods used in this study included DNA isolation with Zymo Research Quick-DNA/RNA kit, and polymerase chain reaction for amplification of barcode (fragment of *COI* gene). The amplified fragments were sequenced by the Sanger method. Preservation of tissue morphology was evaluated using tissue sections stained with hemalum and eosin. The results showed that DNA isolates from archived samples have adequate concentration and purity regardless of the fixative or the age. Samples stored in formaldehyde solution had fragmented DNA molecules while samples stored in ethanol had adequate quality. Tissue morphology was well preserved only in samples stored in formaldehyde solution. Based on obtained data it can be concluded that macrozoobenthos archives should be stored in ethanol for molecular analyses and, in parallel, in formaldehyde solution for morphological testing.

KEY WORDS: fixatives, DNA isolation, DNA barcoding, tissue preservation

STANJE TAKSONA ZOOBENTOSA KAPTIRANIH IZVORA NACIONALNOG PARKA KOZARA

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Nacionalni park Kozara se nalazi na središnjem dijelu planine Kozara (sjeverozapadni dio Republike Srpske, Bosna i Hercegovina). Iako je ovo područje poznato po gustoj hidrografskoj mreži sa mnogobrojnim izvorima i potocima, nedostaju znanja o naselju beskičmenjaka koji žive na dnu ovih akvatičnih ekosistema. Cilj ovog rada je utvrđivanje stanja taksona zoobentosa 10 kaptiranih izvora na području Nacionalnog parka Kozara. Semikvantitativni uzorci zoobentosa su sakupljani ručnom mrežom (prečnik okaca 250 μm) tokom 2019. godine. Pronađeni su predstavnici 44 taksona beskičmenjaka (srednja vrijednost ± standardna devijacija: 15,60 ± 6,43). Zglavkari (Arthropoda) su predstavljeni najvećim brojem taksona. Predstavnici familije Chironomidae su imali najveću abundanciju u većini izvora koji su obuhvaćeni ovim istraživanjem. Pronađeni su u svim kaptiranim izvorima zajedno sa još dva taksona, Nematoda i Oligochaeta. Predstavljeni preliminarni rezultati obuhvataju taksone različitih nivoa klasifikacije, pa se očekuje da će nastavak ovog istraživanja doprinijeti kvalitetnijem sagledavanju taksonomskog spektra zoobentosa kaptiranih izvora Nacionalnog parka Kozara.

KLJUČNE RIJEČI: zoobentos, kaptirani izvori, Nacionalni park Kozara

STATE OF ZOOBENTHOS TAXA IN CAPTURED SPRINGS OF KOZARA NATIONAL PARK

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Kozara National Park is located in the central part of Kozara Mountain (the northwestern part of the Republic of Srpska, Bosnia and Herzegovina). Although this area is known for a high-density hydrographic network with numerous springs and streams, there is a lack of knowledge about the invertebrate assemblages that inhabit the bottoms of these aquatic ecosystems. The aim of this study is to determine the state of zoobenthos taxa of 10 captured springs within the area of Kozara National Park. Semi-quantitative samples of zoobenthos were collected by hand net (250 μm mesh apertures) in 2019. Representatives of 44 invertebrate taxa were recorded (mean ± standard deviation: 15.60 ± 6.43). Arthropods were represented by the highest number of taxa. In most springs covered by this study the representatives of the family Chironomidae had the highest abundance. They were found in all captured springs, together with two more taxa, Nematoda and Oligochaeta. Presented preliminary results include taxa of different classification levels, so it is expected that the continuation of this investigation will contribute to a better understanding of zoobenthos taxonomical spectrum in captured springs of Kozara National Park.

KEYWORDS: zoobenthos, captured springs, Kozara National Park

PRVI NALAZI *Cyzicus* sp. (CRUSTACEA, SPINICAUDATA) U BOSNI I HERCEGOVINI

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Rod *Cyzicus* pripada grupi rakova velikih branhiopoda (klasa Branchiopoda, red Spinicaudata). Ovaj rod je karakterističan za područje Laurazije, sa geografskim rasprostranjenjem koje obuhvata Evroaziju, Afriku i Sjevernu Ameriku. U toku 2016. godine je zabilježen u Bosni i Hercegovini u poplavnim područjima na desnoj obali rijeke Save, u blizini naselja Gaj i Bajinci na području Republike Srpske. Na svim pomenutim lokalitetima pronađene su biseksualne populacije sastavljene od velikog broja jedinki u barama sa plitkom, blago alkalnom vodom, bogatom makrofitama. Do sada nije bilo podataka o pojavi roda *Cyzicus* u Bosni i Hercegovini. Ovo je prvi nalaz tog taksona na teritoriji ove zemlje. Naši nalazi ukazuju da bi i druga močvarna područja u Bosni i Hercegovini mogla sadržavati populacije spinikaudata, čemu bi u budućnosti trebalo posvetiti više pažnje.

KLJUČNE RIJEČI: *Cyzicus*, prvi nalazi, Bosna i Hercegovina

FIRST RECORDS OF *Cyzicus* sp. (CRUSTACEA, SPINICAUDATA) IN BOSNIA AND HERZEGOVINA

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Genus *Cyzicus* belongs to large branchiopod crustaceans (Class Branchiopoda, Order Spinicaudata). The genus appears to be Laurasian, with geographical range encompassing Eurasia, Africa, and North America. In 2016 it was recorded in Bosnia and Herzegovina in the stagnant inundated areas at the right bank of Sava River, near settlements Gaj and Bajinci at the territory of Republic of Srpska. Bisexual populations with high abundance were recorded at all study sites, in ponds with shallow, slightly alkaline water rich in submerged macrophytes. Until now there were no records of the occurrence of genus *Cyzicus* in Bosnia and Herzegovina. This is the first time that this taxon was reported in the territory of this country. Our findings also indicate that other wetland areas in Bosnia and Herzegovina may contain spinicaudatan populations as well, and should be paid more attention in future.

KEYWORDS: *Cyzicus*, first records, Bosnia and Herzegovina

IHTIOFAUNA DONJEG DIJELA TOKA RIJEKE VRBANJE (REPUBLIKA SRPSKA, BiH)

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Diverzitet slatkovodne ihtiofaune Bosne i Hercegovine (BiH) broji oko 118 vrsta riba i čini oko 20% evropske slatkovodne ihtiofaune. Sliv rijeke Vrbanje nalazi se u središnjem dijelu BiH i jedan je od autohtonih slivova u Republici Srpskoj. Vrbanja je jedna od najvećih desnih pritoka rijeke Vrbas, pri čemu se donji dio toka pruža na oko 28 km. Ihtiološka istraživanja donjeg dijela toka rijeke Vrbanje provedena su tokom 2013. godine, na tri lokaliteta, a uzorkovanje je vršeno elektroagregatom za lov ribe ELT 62 II GI, 3 kW. Konstatovan je kvalitativno/kvantitativni sastav ihtiofaune kao i određeni indeksi diverziteta (Simpsonov indeks, Šenon-Viverov indeks, indeks dominantnosti i Sorensenov indeks sličnosti). Ustanovljeno je da donji dio toka rijeke Vrbanje naseljava 10 vrsta riba iz tri familije (Cyprinidae, Cobitidae i Percidae), pri čemu je familija Cyprinidae pokazivala najveće bogatstvo vrsta. Vrste koje su dominirale kako sa brojem jedinki, tako i biomasom bile su *Barbus balcanicus* i *Squalius cephalus,* što se u mnogome razlikuje od ihtiofaune koja je naseljavala isto područje sredinom 20. vijeka. Ovakav sastav ihtiofaune donjeg dijela toka rijeke Vrbanje dovodi se u vezu kako sa faktorima životne sredine tako i sa uticajem određenih ljudskih aktivnosti.

KLJUČNE RIJEČI: ihtiofauna, diverzitet, ciprinidi, rijeka Vrbanja

ICHTHYOFAUNA OF LOWER COURSE OF VRBANJA RIVER (REPUBLIC OF SRPSKA, B&H)

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The diversity of freshwater ichthyofauna of Bosnia and Herzegovina (B&H) numbers about 118 fish species and makes up about 20% of European freshwater ichthyofauna. The Vrbanja River Basin is located in the central part of B&H and is one of the indigenous basins in the Republic of Srpska. Vrbanja is one of the largest right tributaries of the Vrbas River, with the lower part of the course stretching for about 28 km. Ichthyological research of the lower course of the Vrbanja River was carried out during 2013, at three localities, and sampling was performed with an *electric fishing device* ELT 62 II GI, 3 kW. The qualitative and quantitative composition of ichthyofauna as well as some diversity indices (Simpson index, Shannon-Weaver index, Dominance index and Sørensen similarity index) was determinated. It was found that the lower course of the Vrbanja River is inhabited by 10 fish species belonging to three families (Cyprinidae, Cobitidae and Percidae). Family Cyprinidae has the greatest richness of species. Species dominant with number of individuals and biomass were *Barbus balcanicus* and *Squalius cephalus* which differs greatly from the ichthyofauna that inhabited the same area in the mid-20th century. This composition of ichthyofauna of the lower course of the Vrbanja River is related to the environmental factors and impact of certain human activities.

KEYWORDS: ichthyofauna, diversity, cyprinids, Vrbanja River

REPRODUKTIVNI CIKLUS MEDITERANSKE DAGNJE (*Mytilus galloprovincialis* LAMARCK, 1819) U BOKOKOTORSKOM ZALIVU (CRNA GORA, JUGOISTOČNI JADRAN)

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Mediteranska dagnja (*Mytilus galloprovincialis* Lamarck, 1819) je glavna uzgojna vrsta u akvatorijumu Bokokotorskog zaliva, kao i u oblasti cijelog Jadranskog mora. Glavni cilj ovoga rada je bio ispitati godišnji reproduktivni ciklus kod mediteranske dagnje na osnovu kvantitativnih i kvalitativnih analiza. Reproduktivni ciklus kod ove vrste je praćen od februara 2015 do januara 2016. godine na dva uzgajališta u Bokokotorskom zalivu. Anaziran je stadijum razvoja gonada, srednji gonadni indeks (SGI) i veličina oocita na ukupno 180 jedinki kao i odnos polova na ukupno 1260 jedinki. Odnos polova je bio 1:1 (χ^2=3.12; p=0.07). Gametska aktivnost se može opisati kao kontinuirana tokom godine, dok se tokom avgusta javlja neaktivni period kada je više od 50% ispitivanih jedinki bilo u stadijumu mirovanja. Najintenzivniji mrijest je bio tokom zime i tokom proljeća. SGI je bio najniži tokom avgusta 2015. godine, a najvisočiji tokom oktobra, novembra i decembra 2015. godine kada je i najveći broj jedinki bio u zrelom stadijumu. Kvantitativna analiza (broj i dijametar oocita) se poklapa sa kvalitativnom analizom. Dijametar oocita se kretao od minimalne vrijednosti 13.8 µm u martu 2015. godine do maksimalne vrijednosti 132.63 µm u decembru 2015. godine. Najveći broj oocita je izmjeren u novembru 2015. godine (1305 oocita), dok je najmanji broj izmjeren u junu 2015. godine (30 oocita). Najveće srednje vrijednosti dijametra oocita su bile tokom novembra i decembra 2015. godine (66.31±13.08 µm; 66.58±12.09 µm). Dobijeni rezultati ukazuju na kontinuiranu godišnju gametsku aktivnost mediteranske dagnje i da je najintenzivniji mrijest tokom januara, februara, marta i aprila. Podaci o godišnjem reproduktivnom ciklusu uzgajanih školjki su od velikog značaja za uzgajivače, jer na osnovu ovih podataka, kao i na osnovu podataka o kondicionom indeksu, uzgajivači mogu da utvrde najbolje vrijeme za berbu uzgajanih školjki.

KLJUČNE RIJEČI: *Mytilus galloprovincialis*, mrijest, oogeneza, gonadni indeks, Bokokotorski zaliv

REPRODUCTIVE CYCLE OF THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis* LAMARCK, 1819) IN BOKA KOTORSKA BAY (MONTENEGRO, SOUTH-EAST ADRIATIC)

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Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) is one of the most important aquaculture species in Boka Kotorska Bay, as well as in the Adriatic Sea. The main goal of this study was to investigate annual reproductive cycle of Mediterranean mussel by quantitative and qualitative analysis. Reproductive cycle of this species is monitored between February 2015 and January 2016 on two farms in Boka Kotorska Bay. Gonad development stage, mean gonad index (MGI) and oocytes morphometry were analyzed on total 180 individuals, as well as sex ration on total 1260 individuals. Sex ratio was 1:1 (χ^2=3.12; p=0.07). Gametogenic activity can be described as continuous, while during August 2015 inactive period appeared, when more than 50% of investigated individuals were in resting stage. The most intense spawning appeared during winter and spring. MGI showed minimal value in August 2015, while maximum values were during October, November and December 2015, when the highest number of individuals were ripe. Quantitative analysis (oocytes number and diameter) was in accordance with qualitative analysis. Oocytes diameter ranged from minimal 13.8 μm during March 2015 up to maximal value 132.63 μm during December 2015. The highest number of oocytes were measured during November 2015 (1305 oocytes), while the lowest number were measured during June 2015 (30 oocytes). The highest mean oocytes diameter was during November and December 2015 (66.31±13.08 μm; 66.58±12.09 μm, respectively). Obtained results indicate on continuous gamete activity during the year in Mediterranean mussel, and that the most intense spawning appeared during January, February, March and April. Data about annual reproductive cycle of farming bivalves are of great importance to farmers, because based on those data, as well as data about condition index, farmers can determine the best time for bivalves harvesting.

KEYWORDS: *Mytilus galloprovincialis*, spawning, oogenesis, gonad index, Boka Kotorska Bay

NEW RECORDS OF ENDEMIC SPECIES OF EARTHWORM THE *Allolobophora mayeri* (MRŠIĆ, 1990) (LUMBRICIDAE) IN BOSNIA AND HERZEGOVINA

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The earthworm fauna of Bosnia and Herzegovina has been sparsely researched and the knowledge on earthworm fauna is largely based on old literature. Identifying the earthworm material recently collected from of Bosnia and Hercegovina (Cincar Mountain), resulted in a new record of the endemic species *Allolobophora mayeri*. The Cincar Mt. is part of the Dinaric mountain range located in the southwestern part of Bosnia and Herzegovina. *Allolobophora mayeri* is a Bosnian endemic species, so far been found at only one locality Čajniče near Goražde (narrow range of Balkan endemism) in eastern Bosnia and Herzegovina. After almost thirty years, our research indicated that *All. mayeri* is still present in the in Bosnia and Herzegovina. Nevertheless, taking into account the new locality, it is evident an extension of the distribution of this species to the west. Further, the new localities from Cincar Mt. represent the westernmost limit of the species' natural range for now. Our research has shown that this species belongs to a broad range of Balkan endemism. This paper reports the new record of species *All. mayeri* in Bosnia and Herzegovina, thus improving the knowledge of its distribution area.

KEYWORDS: earthworm, new record, *Allolobophora mayeri*, Bosnia and Herzegovina, Cincar Mountain

NOVI NALAZI VRSTE *Ancilus recurvus* MARTENS, 1873 U BOSNI I HERCEGOVINI

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Godine 2016. nađena je vrsta *Ancilus recurvus* Martens, 1873 kao nova vrsta za Bosnu i Hercegovinu. Nekoliko primjeraka nađeno je u potočiću koji otiče iz kaptiranog vrela ispod brda Lisac pored puta kroz dolinu Idbar. U zadnje vrijeme su intenzivirana istraživanja vodenih puževa na područjima kraških polja istočne i zapadne Hercegovine, što je potaklo i istraživanja izvorskih puževa u drugim dijelovima BiH. Cilj ovih istraživanja je, sa jedne strane oživljavanje malakoloških istraživanja kod nas, a sa druge utvrđivanje potpunije biogeografije za pojedine vrste. Tako je u zadnjih nekoliko mjeseci vrsta Ancilus recurvus nađena na još četiri lokaliteta. Ukupno je sakupljeno 14 živih primjeraka iz vrela kod restorana "Studenac" i kod Karamehmedovića harema (Trebinje), te u vrelu Husremovac, kod sela Ledići (Trnovo). Suhe kućice su izolovane iz pijeska u pećini Vrilo (vrelo Ričine, Buško blato) u vrijeme kad je pećina bila suha. Glavna karakteristika svih staništa je da se radi o izvorima čiste vode (nisu zabilježena nikakva zagađenja), a prva dva lokaliteta su u vezi sa pećinom Vruljak. Novi nalazi ove vrste u različitim dijelovima Bosne i Hercegovine pokazuju da ova vrsta ima široko rasprostranjenje i da su razlozi njenog kasnog otkrića prije svega u nedovoljnom kapacitetu istraživača malakologa kod nas.

KLJUČNE RIJEČI: vodeni puževi, Planorbidae, *Ancilus recurvus*, Bosna i Hercegovina

NEW FINDINGS OF *Ancilus recurvus* MARTENS, 1873 IN BOSNIA AND HERZEGOVINA

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In 2016, the species *Ancilus recurvus* Martens, 1873 was found as a new species for Bosnia and Herzegovina. Several specimens were found in a stream that flows from a captured spring below the Lisac hill along the road through the Idbar valley. Recently, research on water snails in the karst fields of eastern and western Herzegovina has been intensified, which has encouraged research on spring snails in other parts of B&H. The goal of these researches is, on the one hand, the revival of malacological research in our country, and on the other hand, the determination of a more complete biogeography for certain species. Thus, in the last few months, the species Ancilus recurvus has been found in four more localities. A total of 14 live specimens were collected from the spring near the restaurant "Studenac" and near the Karamehmedovića harem (Trebinje), and in the spring Husremovac, near the village of Ledići (Trnovo). Dry houses were isolated from the sand in the Vrilo cave (Ričina spring, Buško blato) at a time when the cave was dry. The main characteristic of all habitats is that they are sources of clean water (no pollution was recorded), and the first two sites are related to the Vruljak cave. New findings of this species in different parts of Bosnia and Herzegovina show that this species is widespread and that the reasons for its late discovery are primarily in the insufficient capacity of malacologists in our country.

KEYWORDS: water snails, Planorbidae, *Ancilus recurvus*, Bosnia and Herzegovina

PRILOG POZNAVANJU SLIJEPIH MIŠEVA BRDA MOŽURA

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Poznavanje slijepih miševa Crne Gore je nedovoljno radi čega je pokrenuto višegodišnje istraživanje za potreba mreže NATURA 2000. Bolje je istražena mediteranska biogeografska regija od alpske, ali i ona dosta fragmentarno zbog nepostojanja istraživačkih kapaciteta. U zaleđu Bara i Ulcinja jedini lokalitet koji je istraživan je Pećina na Veljoj Gorani. Tokom ljeta i jeseni 2019. godine provedeno je istraživanje slijepih miševa brda Možura koje se nalazi na području opština Ulcinj i Bar. Reljef brda karakterišu vrtače, škrape, skrašćene depresije i krečnjački greben. Korištene su standardne tehnike istraživanja (vizuelno opažanje, hvatanje nevidljivim mrežama, snimanje ručnim i automatskim ultrazvučnim detektorima (Pettersson D240X, EchoMeter Touch 2 za android mobilni telefom i Pettersson D500X). Za analizu snimaka korišten je softver Bat Sound 4.03 (Pettersson Elektronik). Ukupno je registrovano šest vrsta slijepih miševa: *Hypsugo savii, Miniopterus schreibersii, Myotis oxygnathus, Nyctalus noctula, Pipistrellus pipistrellus* i *Tadarida teniotis*. Registrovana je i fonetska grupa *Pipistrellus kuhlii/nathusii.* Obzirom da se na brdu Možura nalazi vjetropark sa 23 vjetroturbine postoji potencijalna opasnost od stradanja za vrste *Hypsugo savii, Nyctalus noctula, Pipistrellus pipistrellus* i *Pipistrellus kuhlii* koje lete i do visina elisa vjetroturbine.

KLJUČNE RIJEČI: slijepi miševi, vjetroturbine, ugroženost, brdo Možura, Crna Gora

CONTRIBUTION TO THE KNOWLEDGE OF BATS OF THE MOŽURA HILL

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Knowledge of the bats of Montenegro is insufficient, which is why a multi-year research was launched for the needs of the NATURA 2000 network. In the hinterland of Bar and Ulcinj, the only locality that has been explored is the Pećina na Veljoj Gorani cave. During the summer and autumn of 2019, a survey of bats of the Možura hill was conducted. Mozura hill, belongs to the territory of Municipalities Bar and Ulcinj. The relief is characterized by numerous sinkholes, limestone pavement, karstified depression and limestone ridges. Standard research techniques were used (visual observation, capture by mist net, recording by manual and automatic ultrasonic detectors (Pettersson D240X, EchoMeter Touch 2 for android mobile phone and Pettersson D500X). Bat Sound 4.03 (Pettersson Electronics) software was used to analyze the recordings. A total of six species were registered: *Hypsugo savii, Miniopterus schreibersii, Myotis oxygnathus, Nyctalus noctula, Pipistrellus pipistrellus* and *Tadarida teniotis*. The phonetic group *Pipistrellus kuhlii / nathusii* were also registered. Since there is a wind farm with 23 wind turbines on Možura Hill, there is a potential of collision risk and bat mortality for the species *Hypsugo savii, Nyctalus noctula, Pipistrellus pipistrellus* and *Pipistrellus kuhlii*, which fly up to the height of the wind turbine blades.

KEYWORDS: bats, wind turbines, vulnerability, Možura hill, Montenegro

MOLECULAR IDENTIFICATION OF GREEN FROGS (ANURA: RANIDAE: *Pelophylax*) OF WESTERN BALKANS (BOSNIA & HERZEGOVINA AND MONTENEGRO)

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The genus *Pelophylax*, known as green frogs, compromise highly aquatic, sun-loving frogs which can be found in a wide variety of generally well-vegetated and sunny water bodies. They are notoriously difficult to distinguish since identification based on the analysis of morphological traits leads to frequent mistakes. Here, we analyzed 127 samples of genus *Pelophylax* within 18 different localities in Bosnia and Herzegovina and two in Montenegro. Determination of species of the genus *Pelophylax* was performed based on the size polymorphisms of PCR amplicons of serum albumin intron-1 (SAI-1) detected using agarose gel electrophoresis. Hybrids were recognized by the presence of fragments characteristic for both parent species (dual bands on gel). Based on the fragment sizes, we identified five different species: (1) *Pelophylax kurtmuelleri* (30.7%), (2) *P.ridibundus* (26.8%), (3) *P. shqipericus* (0.8%), (4) *P. lessonae* (0.8%), and (5) *P.* kl. *esculentus* (0.8%), while population allotment of hybrids was high (40.1%). Our results suggest that two new species of green frogs establish a viable population in Bosnia and Herzegovina: *P. kurtmuelleri* and *P. shqipericus*. We discuss their new autochthonous and allochthonous distribution borders, so as their invasive character.

KEYWORDS: *Pelophyax*, electrophoresis, species identification, western Balkans

GREEN FROGS (*Pelophylax* spp.) SCREENINGS OF AMPHIBIAN EMERGING PATHOGENS *Batrachochytrium dendrobatidis* AND *Ranavirus*

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One of the most important driver of amphibians' continuing global declining trend are emerging infectious diseases which cause amphibian mass mortality worldwide. Here we present screening results on genus *Pelophylax* (Amphibia, Anura) of two deadly amphibian pathogens *Batrachochytrium dendrobatidis* (Bd) and *Ranavirus* (Rv). A total of 15 different sites in four different bioregions (mediterranean, submediterranean, continental and pannonian) of Bosnia and Herzegovina (western Balkans) were researched between April and July of 2019. Total sample size was 137 with 2-30 specimens of *Pelophylax* spp. per site. Quantitative PCR has detected Bd on 51 (37%) individuals and in seven sites, while Rn was not ascertained. Presence of Bd in B&H further confirms its active spreading and closing its distribution circle in Balkan Peninsula, so as prevalence of fungus in *Pelophylax* spp. which remains genus significance as source of infection. Since no mortality nor clinical signs of Bd disease was recorded during fieldwork we can attribute genus *Pelophyax* as „Troyan horse" for deadly fungus.

KEYWORDS: pathogens, *Batrachochytrium, dendrobatidis, Ranavirus*, Balkan

SUBBIOCODE – RAZVOJ NOVIH ALATA ZA BRZU PROCJENU PODZEMNOG BIODIVERZITETA U BOSNI I HERCEGOVINI

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Podzemna staništa razlikuju se od nadzemnih u tome, da imaju potpunu tamu, stabilne klimatske uslove i nedostatak primarnih producenta. Tu žive vrste, specializirane za život u podzemlju, a njihova brojnost je globalno najveća baš u Dinaridima. Međutim, podzemna fauna u regiji je veoma ugrožena, posebno u područjima u kojima se podudaraju ambiciozni ekonomsko-razvojni planovi i manjak znanja o bogatstvu podzemnih vrsta. U 2019. godini pokrenut je projekt SubBIOCODE - Razvoj novih alata za brzu procjenu podzemnog biodiverziteta u Bosni i Hercegovini u širem slivu rijeke Trebišnjice kojeg finansira „Critical Ecosystem Partnership Fund“. Cilj projekta je poboljšati znanje o raširenosti vrsta, razviti protokole za brzu procjenu podzemnog biodiverziteta, utvrditi prioritetne lokacije za očuvanje podzemnih staništa i uključiti lokalne sudionike u provođenje terenskih studija i obavljanje aktivnosti na zaštiti podzemnih staništa. Ciljeve ćemo dostići kroz edukaciju lokalnih sudionika, pripremom baze podataka o raširenosti podzemnih vrsta, listom ugroženosti izabranih vrsta i izborom najbogatijih lokaliteta. U projektu smo postavili internet stranicu www.subbiocode.net, započeli sa analizama biodiverziteta koristeći molekularne alate, s pripremom podataka za bazu i sa uključivanjem lokalnih studenata u projektne radionice i druge aktivnosti. Zbog korona virusa, veće terenske aktivnosti su odgođene, a neke se odvijaju u manjem obimu. Edukacija i sastanci partnera iz BiH i Slovenije se vrše preko video veza.

KLJUČNE RIJEČI: zaštita podzemnog biodiverziteta, zaštita prirode, edukacija, pećine i izvori, BIH

SUBBIOCODE – DEVELOPING NEW TOOLS FOR RAPID ASSESSMENT OF SUBTERRANEAN BIODIVERSITY IN BOSNIA AND HERZEGOVINA

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Differently from surface habitats subterranean habitats are characterized by constant darkness, stable climate and lack of primary producers. They are inhabited by species specialized for life in subterranean realm. Dinarides are renown as a global hotspot in richness of subterranean species. Yet, subterranean fauna of the region is highly endangered, especially in areas where ambitious economic-developmental plans and incomplete knowledge on subterranean species richness coincide. In 2019, a project SubBIOCODE - Developing new tools for rapid assessment of subterranean biodiversity in Bosnia and Herzegovina, financed by the "Critical Ecosystem Partnership Fund", was launched in the Trebišnjica River Basin. Main goals of the project are to improve the knowledge on species distributions in the region, develop protocols for rapid subterranean biodiversity assessment, identify conservation priorities in subterranean habitats and involve local stakeholders in conducting field studies and conservation activities. The goals will be achieved by educating the local stakeholders, preparing a database on subterranean species distributions, assessing the endangerment status of the selected species and identifying the species richest localities. In the project, we launched an internet page www.subbiocode.net, started the analyses of subterranean biodiversity using molecular tools, formation of the database and inclusion of local students in project workshops and other activities. Due to the corona virus, extensive field work activities had to be postponed, while some are executed in a smaller extent. Education and meetings of partners from BIH and Slovenia continue using the online video conference tools.

KEYWORDS: subterranean biodiversity conservation, nature protection, education, caves and springs, BIH

Zaštita biodiverziteta

Biodiversity Conservation

ULOGA NAUKE U ZAŠTITI BIODIVERZITETA

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Nučnici širom sveta se već dugi niz godina bave istraživanjima vezanim za zaštitu biodiverziteta, tako da danas raspolažemo ne samo dobro utemeljenim teorijskim postavkama za očuvanje svih vidova biodiverziteta (ekosistemskog i specijskog diverziteta kao i zaštitom genetičkih resursa biljnih i životinjskih vrsta i mikroorganizama), već i brojnim empirijskim studijama, u kojima su obrađeni jedan ili više taksona sa različitih aspekata relevantnih za njihovo očuvanje. Naime, moramo znati čime raspolažemo da bismo mogli da preduzmemo adekvatne mere zaštite. U tom smislu, naročito su značajna empirijska istraživanja botaničara, zoologa, populacionih genetičara i evolucionih biologa, koji u svojim naučnim publikacijama, na osnovu rezultata istraživanja na morfološkom i/ili molekularnom nivou, na nivou genoma, itd., daju preporuke za očuvanje taksona kojima se bave. Istraživanja ovog tipa su naročito intenzivirana poslednjih decenija, i to najviše zbog globalnog otopljavanja klime koje već dovodi do redistribucije areala rasprostranjenja biljnih i životinjskih vrsta, i koje je već rezultiralo izumiranjem nekoliko vrsta vodozemaca. Rezultati naučnih istraživanja se koriste kao osnova za formulisanje globalnih programa i strategija za zaštitu biodiverziteta od strane međunarodnih organizacija, poput IUCN, WWF, FAO, itd., koji se kroz zakone i druge propise implementiraju u državama širom sveta. Danas je zaštita biodiverziteta jedan od strateških ciljeva većine zemalja u celom svetu. Međutim, u zemljama na području Balkana, evidentna je slaba povezanost nauke i državnih institucija koje imaju zajednički cilj – zaštitu biodiverziteta, a u prevazilaženju ovog raskola, značajnu ulogu može imati nevladin sektor, putem savetodavne uloge. Na primeru Pančićeve omorike (*Picea omorika* (Panč.) Purk.), vinove loze (*Vitis vinifera* L.) kao i domaćeg magarca (*Equus asinus L.*) demonstriram ulogu nauke u zaštiti genetičkih resursa biljnih i životinjskih vrsta u Srbiji, kao i moguće načine povezivanja nauke, zakonodavstva i prakse.

KLJUČNE RIJEČI: očuvanje genetičkih resursa biljnih i životinjskih vrsta, teorijska i empirijska istraživanja, vladin i nevladin sektor, povezivanje nauke i prakse

THE ROLE OF SCIENCE IN BIODIVERSITY CONSERVATION

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Scientists around the world have been researching biodiversity for many years, so today we have not only well-founded theoretical assumptions for the conservation of all types of biodiversity (ecosystem and species diversity as well as the protection of genetic resources of plant and animal species and microorganisms), but also numerous empirical studies, in which one or more taxa have been processed from different aspects relevant to their conservation. Namely, we must know what we have at our disposal in order to be able to take adequate protection measures. In this sense, empirical research by botanists, zoologists, population geneticists and evolutionary biologists is especially important, who in their scientific publications, based on the results of research at the morphological and/or molecular level, at the genome level, etc., give recommendations for conservation of the taxa in question. Research of this type has been particularly intensified in recent decades, mainly due to global warming, which is already leading to the redistribution of plant and animal species distribution areas, and which has already resulted in the extinction of several amphibian species. The results of scientific research are used as a basis for the formulation of global programs and strategies for biodiversity protection by international organizations, such as IUCN, WWF, FAO, etc., which are subsequently implemented in countries around the world through laws and other regulations. Today, the protection of biodiversity is one of the strategic goals of most countries around the world. However, in the Balkan countries, there is a weak connection between science and state institutions that have a common goal - the protection of biodiversity, and in overcoming this divide, the non-governmental sector can play a significant role, through an advisory role. On the example of Serbian spruce (*Picea omorika* (Panč.) Purk.), grapevine (*Vitis vinifera* L.) and domestic donkey (*Equus asinus* L.), I demonstrate the role of science in the protection of genetic resources of plant and animal species in Serbia, as well as possible ways of connecting science, legislation and practice.

KEYWORDS: conservation of genetic resources of plant and animal species, theoretical and empirical research, governmental and non-governmental sector, linking science and practice

ŽIVOTINJE VLAŽNIH I EFEMERNIH VODENIH STANIŠTA KAO INDIKATORI GLOBALNIH KLIMATSKIH PROMENA

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Vlažna staništa se definišu kao područja sa tranzicionim ekosistemima, između terestričnih i akvatičnih. Voda se u njima može zadržavati tokom čitave godine ili samo tokom dela sezone. Javljaju se u regionima sa različitim klimatskim, geološkim i orogeografskim uslovima i odlikuju širokim spektrom fizičkih, hemijskih i bioloških karakteristika i procesa. Stoga su ova staništa pogodna za razvoj velikog broja živih organizama. Za zokruživanje životnog ciklusa i dugoročno preživljavanje akvatičnih vrsta je neophodno integralno korišćenje vlažnog područja i njegovih resursa. Ove vrste su dobri pokazatelji stanja životne sredine i poznate su kao vodeće (flagship) vrste. Jedan od tipičnih primera su rakovi, koji čitav svoj životni ciklus zaokružuju u okviru istog vodenog basena. Druga grupa životinja provodi samo deo svog životnog ciklusa u vlažnim staništima ili su u određenoj fazi životnog ciklusa zavisne od drugih organizama u vodenom basenu. Primer su vodozemci, čiji opstanak larvenog stadijuma u velikoj meri zavisi od vodenih basena, pogotovu onih bez predatora. Veliki broj insekata takođe zavisi od vlažnih staništa u pogledu razvića larvi. Vodene ptice se hrane i gnezde na ovim staništima, ili ih koriste kao odmorišta. U poslednje vreme pretnja globalnim zagrevanjem budi interesovanje i zabrinutost o uticaju klimatskih promena na biološku raznovrsnost. Prema nedavnom istraživanju, degradacija biodiverziteta vodenih ekosistema odvija se na planetarnoj skali, bez znakova skorijeg oporavka. Neke vrste su osetljivije na ove pretnje i reaguju brže na promene. One imaju uske granice tolerancije na fizičko-hemijske karakteristike i sezonsko trajanje staništa, često su lokalno distribuirane i endemične za određeno područje. Sve navedeno ukazuje na značaj praćenja ovih vrsta u cilju procene integriteta vlažnih i efemernih vodenih staništa, a mere preduzete za njihov opstanak mogu doneti korist ekosistemu u celini. Prema ovom konceptu, monitoring i upravljanje vlažnim staništima se fokusira na jednu ili nekoliko „krovnih vrsta“, koje predstavljaju potrebe većine ostalih vrsta u tom staništu. Nadgledanje njihovih reakcija (morfoloških, fizioloških i bihejvioralnih) štiti sve druge vrste i alarmira na negativne promene staništa, mnogo pre nego što one postanu očigledne i nepovratne.

KLJUČNE REČI: vlažna staništa, klimatske promene, flagship vrste

WETLAND AND EPHEMERAL AQUATIC ANIMALS AS INDICATORS OF GLOBAL CLIMATE CHANGE

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Wetlands are defined as areas with transitional ecosystems, between the terrestrial and aquatic. Water can be retained in them throughout the year or only during part of the season. They occur in regions with different climatic, geological and oro-geographical conditions and are characterized by a wide range of physical, chemical and biological characteristics and processes. Therefore, these habitats are suitable for development of a large number of living beings. Integral use of the wetlands and its resources is necessary to complete life cycle and long-term survival of aquatic species. These species are good indicators of the state of the environment and are known as leading (flagship) species. One of the typical examples are shrimps, which complete their entire life cycle within the same water basin. The second group of animals spends only part of their life cycle in wet habitats, or depends on other aquatic organisms at a certain stage of their life cycle. An example is amphibians, whose survival of the larval stage largely depends on water basins, especially those without predators. A large number of insects also depend on wetlands in terms of larval development. Waterfowl feed and nest in these habitats, or use them as resting places. Recently, the threat of global warming has aroused interest and concern about the impact of climate change on biodiversity. According to a recent study, the degradation of the biodiversity of aquatic ecosystems takes place on a planetary scale, with no signs of imminent recovery. Some species are more sensitive to these threats and react faster to changes. They have narrow tolerance limits for physico-chemical characteristics and seasonal habitat duration and are often locally distributed and endemic to a particular area. All of the above points the importance of monitoring these species, in order to assess the integrity of wetland and ephemeral aquatic habitats. The measures taken for their survival can benefit the ecosystem as a whole. According to this concept, wetland monitoring and management focuses on one or more “umbrella species”, which represent the needs of most other species in that habitat. Monitoring their responses (morphological, physiological and behavioral) protects all other species and alarms negative habitat changes, long before they become so obvious and irreversible.

KEYWORDS: wetlands, climate change, flagship species

UTJECAJ VIŠESTRUKIH STRESORA NA ZAVIČAJNE ZAJEDNICE SLATKOVODNE IHTIOFAUNE

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Kopnene vode pokrivaju samo 0,8% zemljine površine, od toga dostupno je oko 0,3% slatke vode u kojima živi čak 50 % poznatih ribljih vrsta. Unatoč toj bogatoj raznolikosti, raznolikost slatkovodnih riba u svijetu opada i sada se smatra da je slatkovodna riba najugroženija skupina kralježnjaka. Opadanje ihtiodiverziteta u kopnenim vodama uglavnom je izazvano djelovanjem čovjeka što uključuje eutrofikaciju, gubitak i uništavanje staništa, izolaciju ribljih populacija uslijed gradnje brana, izmjene vodenih tokova, invazije egzotičnih vrsta, prelov i klimatske promjene. Cilj ove rada je utvrditi različite stresore koji su tijekom proteklog vremena uzrokovali poremećaje u sastavu zajednica slatkovodnih riba u slivu rijeke Save te njihov potencijalni utjecaj. Rijeka Sava je oduvijek bila važna za komercijalni i rekreacijski ribolov lokalnog stanovništva, a tako je i danas. Početkom 20. stoljeća na rijeku Savu u Hrvatskoj još nisu utjecale glavne ljudske aktivnosti i bila je bogata je ihtiofaunom. Međutim, u Sloveniji je osnovana rudarska industrija dvadesetih godina koja je emitirala ugljikovu prašinu u rijeku Savu. Istodobno je u njenom srednjem dijelu započela izgradnja nasipa za ublažavanje poplava kao i gradnja hidroelektrana u gornjem toku. Nadalje, 1980-ih godina u Sloveniji je izgrađena nuklearna elektrana Krško (NE), a 2010. godine hidroelektrana Krško (HE). Slijedom toga, zbog tih aktivnosti izgubljena su mnoga poplavna područja nizvodno i promijenjeno je stanište. Istodobno su i mnoge strane vrste riba ušle u Savu (namjernim unošenjem i slučajno) te su zabilježene njihove samoodržive populacije. Osim toga, uočeno je da se sastav zavičajnih ribljih vrsta u uzvodno i nizvodno promijenio te da je migratornim vrstama opstanak upitan. Temeljem navedenog može se zaključiti da je u rijeci Savi identificirana većina stresora zabilježenih u literaturi (zagađenje, nasipi, brane, gubitak staništa, strane vrste), a koji značajno utječu na sastav ribljih zajednica i na gubitak ihtioraznolikosti.

KLJUČNE RIJEČI: stresori, kopnene vode, zavičajne ribe, rijeka Sava, raznolikost

EFFECT OF MULTIPLE STRESSORS ON NATIVE FRESHWATER FISH COMMUNITIES

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Fresh waters covering only 0.8% of the Earth's surface area, of which about 0.3% is available, but support around 50% of the total fish species. Despite rich diversity, ihtiodiversity is in decline worldwide and now freshwater fish are thought to be the most threatened group of vertebrates. Globally, the drivers of the declines and pressures on fresh waters are almost completely human and include eutrophication, habitat loss and population isolation through loss of access from the damming of rivers, flow alteration, habitat destruction, exotic species invasion, overharvesting and climate change. The aim of this study is to identify the different stressors that have caused disturbances in the composition of freshwater fish communities in the Sava River Basin over time and their potential impact. The Sava River was important for commercial and recreational fishing for the local population, which still remains today. At the beginning of the 20th century, the Sava River in Croatia was unaffected by major human activities and rich in ichthyofauna. However, the 1920s mining industry was established in Slovenia, which emitted carbon dust into the Sava River. At the same time, the construction of embankments to mitigate flooding started in the middle section. Furthermore, in the 1980s, the Krško nuclear power plant (NPP), and in the 2010s, the Krško hydropower plant (HPP) were built in Slovenia. Consequently, due those activities many flooding areas was lost and habitat has been changed. At the same time many exotic fishes entered to the Sava (by stocking for purpose and by accident) and their self-sustaining populations was recorded. Also, it was observed that the composition of native fish species upstream and downstream has been changed and that the survival of migratory species become questionable. It can be concluded that the most of the stressors recorded in the literature (pollution, embankments, dams, habitat loss, alien species) have been identified in the Sava River, which significantly affect the composition of fish communities and cause the loss of ichthyodiversity.

KEYWORDS: stressors, freshwaters, indigenous fish, Sava River, diversity

BENTOSKE ZAJEDNICE RIJEKE NERETVE

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U radu je prikazano istraživanje bentoskih zajednica rijeke Neretve, u svrhu procjene kvalitete i funkcije ekosustava tekućica, a sukladno odredbama Okvirne direktive o vodama - ODV (2000/60/EZ). Istraživanja su provedena tijekom 2018. godine, a obuhvatila su ukupno 22 postaje, među kojima šest postaja duž longitudinalnog profila Neretve i 16 postaja na pritokama ovih rijeka. Za procjenu ekološkog stanja tekućica, ODV definira zajednice fitobentosa, makrofitske vegetacije i makroskopskih beskralješnjaka kao ključne sastavnice bioloških elemenata procjene, uz ihtiofaunu te podržavajuće fizikalno-kemijske i hidro-morfološke elemente. Biološki elementi kvaliteta voda su uzorkovani slijedeći propisane protokole: europski standardi EN13946:2014 za fitobentos i EN14184:2014 za makrofitsku vegetaciju, AQUEM metodologija i multi-habitat shema uzorkovanja za makroskopske beskralješnjake, te standardne metode uzorkovanja ihtiofaune pomoću istosmjerne struje (DC) polja (Starfish protokol), dok su fizikalno-kemijski pokazatelji izmjereni na terenu (WTW kombinirane elektrode) i u laboratoriju standardnim metodama analize (APHA, ISO). Za procjenu raznolikosti korišteni su: Pantle-Buck indeks saprobnosti (S), prošireni biotički indeks (EBI), Trent biotički indeks (TBI) te Shannon-Weaverov indeks raznolikosti. Na svim postajama zabilježen je visoki stupanj raznolikosti i brojnost svojti. Za prirodne tekućice, visoko ekološko stanje utvrđeno je za ukupno osam postaja, dok je njih devet imalo dobro ekološko stanje, sukladno tipologiji tekućice. Izmijenjena vodna tijela, njih ukupno pet, bila su u granicama maksimalnog i dobrog ekološkog potencijala. Za sva istraživana vodna tijela utvrđeno je odgovarajuće (najmanje dobro) stanje voda, propisano ODV-om i sukladno važećoj zakonskoj regulativi (Odluka o karakterizaciji površinskih i podzemnih voda, referentnim uvjetima i parametrima za ocjenu stanja voda i monitoringu voda, „Službene novine F BiH“ br. 1/14).

KLJUČNE RIJEČI: bentoske zajednice, rijeka Neretva, ekološki status, ekološki potencijal

BENTHIC ASSEMBLAGES OF THE NERETVA RIVER BASIN

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The paper presents a survey of benthic communities of the Neretva River, for the purpose of assessing the quality and function of running waters ecosystems, in accordance with the provisions of the Water Framework Directive - WFD (2000/60 / EC). The research was conducted during 2018, and included a total of 22 stations, including six stations along the longitudinal profile of the Neretva and 16 stations on the tributaries of these rivers. To assess the ecological status of streams, the WFD defines communities of phytobenthos, macrophytes vegetation and macrozoobenthos as key components of the biological assessment elements, in addition to ichthyofauna and supporting physicochemical and hydro-morphological elements. Biological elements of water quality were sampled according to the prescribed protocols: European standards EN13946: 2014 for phytobenthos and EN14184: 2014 for macrophytes vegetation, AQUEM methodology and multi-habitat sampling schemes for macrozoobenthos, standard methods of sampling ichthyofauna using direct current (DC) fields (Starfish protocol), while physico-chemical parameters were field measured (WTW combined electrodes) and in the laboratory by standard methods of analysis (APHA, ISO). The following indices were used to assess diversity: the Pantle-Buck Saprobity Index (S), the Extended Biotic Index (EBI), the Trent Biotic Index (TBI), and the Shannon-Weaver Diversity Index. A high degree of diversity and abundance of taxa was recorded at all stations. For natural streams, a high ecological status was determined for a total of eight stations, while nine of them had a good ecological status, according to the typology of the stream. The modified water bodies, five in total, were within the limits of maximum and good ecological potential. For all investigated water bodies, the appropriate (least good) water status has been determined, prescribed by the WFD and current legislation (Decision on characterization of surface and groundwater, reference conditions and parameters for water status assessment and water monitoring, "Official Gazette of F BiH" No. 1/14).

KEYWORDS: benthic assemblages, the Neretva River, ecological status, ecological potential

KATEGORIJE UGROŽENOSTI I STATUSI ZAŠTITE POJEDINIH SLATKOVODNIH VRSTA NA PODRUČJU BOSNE I HERCEGOVINE

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U ovom radu su analizirane kategorije ugroženosti i statusi zaštite vrsta koje se nalaze u Crvenoj listi zaštićenih vrsta flore i faune Republike Srpske (*Cyprinus carpio, Lampetra planeri, Leucaspius delineatus, Romanogobio kesslerii, Romanogobio uranoscopus, Squalius svallize, Telestes metohiensis* i *Zingel streber*) sa ciljem analize i usklađivanja statusa zaštite i kategorija ugroženosti u Bosni i Hercegovini. Razmatrani su statusi zaštite i kategorije ugroženosti pomenutih vrsta na globalnom i evropskom nivou, kao i u zemljama regiona (Srbija i Hrvatska). U Srbiji je zaštićena jedino vrsta *Romanogobio kesslerii*, dok su u Hrvatskoj sve vrste zaštićene i nalaze se unutar Crvene knjige slatkovodnih riba Hrvatske, izuzev *Telestes metohiensis* koja je u Hrvatskoj potpuno iščezla. Prema podacima iz Crvene liste faune Federacije Bosne i Hercegovine samo *Cyprinus carpio* ne spada u ugrožene vrste i smatra se da je izrazito brojan, što se kosi sa podacima za ostale evropske zemlje gdje spada u osjetljivu grupu. U Uredbi o strogo zaštićenim i zaštićenim vrstama Republike Srpske nalaze se sve vrste osim *Lampetra planeri*, čije prisustvo je upitno za prostor BiH. Za opstanak ugroženih vrsta potrebno je utvrditi postojeće stanje populacija, izraditi i primijeniti akcijske planove, obezbijediti adekvatnu zaštitu, pratiti stanje populacija i revidirati podatke.

KLJUČNE RIJEČI: slatkovodne vrste, kategorije ugroženosti i zaštite, Crvena lista, Crvena knjiga

THREAT CATEGORIES AND PROTECTION STATUS OF CERTAIN FRESHWATER SPECIES IN THE TERRITORY OF BOSNIA AND HERZEGOVINA

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This paper analyzes the categories of endangerment and protection status of species that are in the Red List of protected species of flora and fauna of the Republic of Srpska (*Cyprinus carpio, Lampetra planeri, Leucaspius delineatus, Romanogobio kesslerii, Romanogobio uranoscopus, Squalius svallize, Telestes metohiensis* and *Zingel streber*) with the aim of analyzing and harmonizing the protection status and vulnerability categories in Bosnia and Herzegovina. The protection statuses and endangered categories of the mentioned species at the global and European level, as well as in the countries of the region (Serbia and Croatia) were discussed. In Serbia, only the species *Romanogobio kesslerii* is protected, while in Croatia all species are protected and are included in the Red Book of Freshwater Fish of Croatia, except for *Telestes metohiensis*, which has completely disappeared in Croatia. According to the data from the Red List of Fauna of the Federation of Bosnia and Herzegovina, only *Cyprinus carpio* does not belong to the endangered species and is considered to be extremely numerous, which contradicts the data for other European countries where they belong to the vulnerable category. The Decree on Strictly Protected and Protected Species of the Republika Srpska contains all species except *Lampetra planeri,* whose presence is questionable for the territory of Bosnia and Herzegovina. For the survival of endangered species, it is necessary to determine the current state of populations, develop and implement action plans, provide adequate protection, monitor the state of populations and revise data.

KEYWORDS: freshwater species, endangered and protected categories, Red List, Red Book

PRIJEDLOG MJERA ZA REVITALIZACIJU ZAŠTIĆENOG STANIŠTA TIŠINA

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Zaštićeno stanište Tišina nalazi se na teritoriji opštine Šamac na sjeveru Republike Srpske i zauzima površinu od 196,49 ha od čega 58,84 ha ili 30% čine vodena i vlažna staništa sa tipičnom makrofitskom vegetacijom i vegetacijom šuma mekih lišćara. Ovo područje predstavlja kompleks različitih ekosistema koji se sastoje od močvare i bare Mala Tišina, Velika Tišina, bare Odmut i povremenog vodotoka Žandrak. Područje je zaštićeno prema Zakonu o zaštiti prirode RS i trenutno nema ni jedan međunarodni status zaštite, ali je predloženo za područje mreže Natura 2000. U sklopu močvarno- barskog kompleksa Tišina i Odmut, do danas je registrovano 175 vrsta biljaka, 125 vrsta ptica, 8 vrsta vodozemaca, 4 vrste gmizavaca i 21 vrsta riba. Područje se nalazi pod dejstvom antropogenih pritisaka kao što su poljoprivredne djelatnosti, prisustvo invazivnih vrsta biljaka i riba, ispuštanje neprečišćenih otpadnih voda u bare, ilegalna sječa šume i krivolov. U radu su prikazane neke konvencionalne mjere za revitalizaciju zaštićenog staništa i ekoremedijacijske mjere koje podrazumjevaju postavku ekosistemskih procesora kao mjera smanjenja zagađujućih materija. Ekosistemski procesori se mogu izgraditi u domaćinstvima koja ne posjeduju septičke jame, za prečišćavanje otpadnih voda domaćinstava, i u melioracijskim kanalima u svrhu prečišćavanja otpadnih voda sa poljoprivrednih površina. Vrste biljaka koje se najčešće koriste u te svrhe su *Typha latifolia, Carex* sp*., Iris pseudacorus, Salix* spp*., Alnus glutinosa, Quercus robur* i *Phragmites australis* koje su već prisutne na teritoriji močvarno- barskog kompleksa. Predložene mjere ne zahtjevaju velika finansijska ulaganja i u praksi daju dobre rezultate.

KLJUČNE RIJEČI: zaštićeno stanište Tišina, Natura 2000 stanište, antropogeni pritisci, mjere revitalizacije, ekosistemski procesori

PROPOSAL OF MEASURES FOR REVITALIZATION OF PROTECTED HABITAT TIŠINA

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The protected habitat "Tišina" is located on the territory of the municipality of "Šamac" in the north of the Republic of Srpska and covers an area of 196.49 ha, of which 58.84 ha or 30% are aquatic and wet habitats with typical macrophyte vegetation and soft deciduous forest vegetation. This area is a complex of different ecosystems consisting of the wetland and pond "Mala Tišina", "Velika Tišina", pond Odmut and the watercourse "Žandrak". The area is protected under the RS Law on Nature Protection and currently doesn't have any international protection status but is proposed for the Natura 2000 network. Within the wetland-marsh complex, up to this point are registered 175 species of plants, 125 species of birds, 8 species of amphibians, 4 species of reptiles and 21 species of fish. The area is under the anthropogenic pressures such as agricultural activities, invasive species of plants and fish, the discharge of untreated wastewater, illegal logging and poaching. The paper presents some conventional measures for the revitalization of protected habitats and ecoremediation measures that include ecosystem processors as a measure to reduce pollutants. Ecosystem processors can be built in households for the treatment of household wastewater, and in reclamation canals for the agricultural wastewater treatment. The most used plant species are *Typha latifolia*, *Carex* sp., *Iris pseudacorus*, *Salix* spp., *Alnus glutinosa*, *Quercus robur* and *Phragmites australis*, which are already present in the wetland complex. The proposed measures do not require large financial investments and give good results in practice.

KEYWORDS: protected habitat "Tišina", Natura 2000 habitat, anthropogenic pressures, revitalization measures, ecosystem processors

ALGAE DIVERSITY IN MOUNTAIN STREAMS IN THE AREA OF VRANICA MOUNTAIN (BOSNIA AND HERZEGOVINA)

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Mountain streams as well as other aquatic ecosystems in high mountain areas of Bosnia-Herzegovina represent important habitats for a large number of rare and endangered algae species. The area of Vranica mountain is in particular characterized by the diversity of these habitats. The aim of this paper is to determine the diversity of algae in the mountain streams in the area of Vranica mountain. Due to the specific geological foundation, aquatic ecosystems on the mountain are stable throughout the year. Field investigation and sampling has been conducted at 27 sites during summer and autumn seasons in 2018. Aside from sampling of algae of phytobenthos, the basic physical and chemical parameters of water were measured (temperature, pH, electrical conductivity, oxygen concentration, turbidity and amount of formed organic matter). A total of 78 taxa from six classes of algae were found, as follows: *Cyanophyceae* (1), *Bangiophyceae* (1), *Chrysophyceae* (1), *Xanthophyceae* (1), *Bacillariophyceae* (61) and *Conjugatophyceae* (9). The most numerous algal species were diatoms as follows: *Odontidium mesodon, Achnanthidium minutissimum, Cocconeis lineata, Cocconeis placentula, Meridion circulare, Gomphonema minusculum, Tetracyclus rupestris, Diatoma ehrenbergii, Encyonema minutum, Ulnaria ulna, Encyonema silesiacum, Hannea arcus*. In addition, representatives of other classes of algae as *Bangia atropurpurea, Tribonema vulgare, Hydrurus foetidus, Pleurotaenium trabecula* were identified. Based on obtained results, it can be concluded that mountain streams in the area of Vranica mountain provide favourable habitat for diverse species of algae.

KEYWORDS: algae, mountain streams, biodiversity, conservation, biomonitoring

DIVERZITET MEDONOSNIH BILJAKA U UZORCIMA MEDA IZ BOSNE I HERCEGOVINE

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Bosna i Hercegovina (BiH) se odlikuje visokim specijskim diverzitetom biljaka, ali o njihovoj zastupljenositi u medu još uvijek nema potpunih podataka. Melisopalinološkom analizom uzoraka meda može se utvrditi tačan botanički sastav uzorka kao i broj polenovih zrna svake identifikovane biljne vrste. Ovo istraživanje je imalo za cilj utvrditi stepen raznolikosti medonosnih biljaka u uzorcima meda porijeklom iz BiH. Za potrebe istraživanja, tokom dvije vegetacijske sezone, prikupljeno je 50 uzorka meda. Melisopalinološka metoda koja je primjenjena u izradi i analizi preparata je u skladu sa Pravilnikom o metodama za kontrolu meda i drugih pčelinjih proizvoda Bosne i Hercegovine. Analiza preparata obuhvatala je identifikaciju medonosnih biljaka na osnovu mikromorfoloških osobenosti polenovih zrna kao i tačan broj polenovih zrna svake identifikovane biljke. Za precizno utvrđivanje stepena raznolikosti medonosnih biljaka u uzorcima korišten je Shannon-Weaver indeks diverzita (H'), a za potpunu interpretaciju korišten je indeks ujednačenosti ili Eveness indeks (E). U istraživanju ukupno je identifikovana 51 različita medonosna biljka i 14118 polenovih zrna. Vrijednost Shannon-Weaver indeksa bila je u intervalu od 1.11 do 2.52, dok je vrijednost Eveness indeksa varirarala od 0.63 do 1. Uzimajući u obzir vrijednosti indeksa možemo zaključiti da se analizirani uzorci odlikuju visokom raznolikošću medonosnih biljaka.Visoke vrijednosti oba tipa indeksa direktan su odraz florističke raznolikosti autohtone apiflore i ekološke jedinstvenosti prostora BiH.

KLJUČNE RIJEČI: melisopalinologija, biodiverzitet, polen, medonosne biljke

DIVERSITY OF HONEY PLANTS IN HONEY SAMPLES ORIGINATING FROM BOSNIA AND HERZEGOVINA

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Bosnia and Herzegovina (B&H) is characterized by high diversity of plants at species level, but there is still no complete data on their presence in honey. Melissopalynological analysis of honey samples can determine the exact botanical composition of the sample as well as the number of pollen grains of each identified plant species. This research aimed to determine the degree of diversity of honey plants in honey samples originating from B&H. For the research purposes, during two vegetation seasons, we have collected 50 honey samples. The microscopic slides for the applied melissopalynological method were prepared and analised according to the *Rule Book on methods for control of honey and other bee products in Bosnia and Herzegovina*. The analysis of microscopic slides included the identification of honey plants based on the micromorphological features of pollen grains, as well as the exact count number for each identified plants. The Shannon-Weaver Diversity Index (H ') was used to accurately determine the degree of diversity of honey plants in the samples, and the uniformity index or Eveness index (E) was used for complete interpretation. In the research were identified 51 honey plants and 14 118 pollen grains. The Shannon-Weaver value ranged between 1.11 and 2.52, while the Eveness index varied from 0.63 to 1. Taking into account the values of the index, we can conclude that the analyzed samples are characterized by a high diversity of honey plants. The high values of both types of indices are a direct reflection of the floristic diversity of the autochthonous apiflora and the ecological uniqueness of the BiH area.

KEYWORDS: melissopalynology, biodiversity, pollen, honey plants

PREGLED OBJAVLJENIH RADOVA O ZAJEDNICI MAKROINVERTEBRATA U MALIM VODENIM TELIMA NA TERITORIJI BALKANSKOG POLUOSTRVA

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Termin mala vodena tela obuhvata male stajaće (lentičke) i tekuće (lotičke) slatkovodne ekosisteme. U ovaj tip ekosistema spadaju: gornji tokovi reka, kanali, mala jezera, bare, močvare, ritovi, plavna područja, ribnjaci i sl. Veliki deo ovih ekosistema predstavlja ekotone. Poznato je da ekotoni imaju veliki specijski diverzitet i to čini mala vodena tela vrednim proučavanja i zaštite. Cilj ovog rada je da predstavi i analizira publikovane naučne radove u kojima je sa različitog aspekta proučavana zajednica makroinvertebrata u malim vodenim telima. Izvori nisu razmatrani kao posebna celina već samo kao deo gornjih tokova reka. Ograničili smo se na radove koji obuhvataju informacije vezane za područje Balkanskog poluostrva (područje Srbije, Crne Gore, Severne Makedonije, Bosne i Hercegovine, Albanije, deo Slovenije, Hrvatske, Grčke, Bugarske, Rumunije). Najstariji rad koji smo pronašli datira iz 1994. a najskoriji iz 2020. godine, zbog čega smo se odlučili za period izmedju tih godina. Analizirali smo 63 publikovanih radova na ovu temu. Najviše radova je objavljeno 2014. godine. Najveći broj publikovanih radova se odnosi na lokalitete u Hrvatskoj (24 radova). Sa druge strane, našli smo po jedan rad vezan za lokalitete u Albaniji, Sloveniji, Crnoj Gori i Bosni i Hercegovini. U 18 radova je analizirana cela zajednica makroinvertebrata a u 16 samo asambleja Diptera. Zaključujemo da najveću pažnju privlače mala jezera (21 publikacija) a najmanju ribnjaci (5 publikacija). Najveće napore treba koncentrisati na istraživanje ribnjaka zbog najmanjeg interesovanja. Iako po dekadama raste interesovanje u naučnim krugovima, nedovoljno pažnje se poklanja proučavanju i očuvanju ovog tipa ekosistema.

KLJUČNE REČI: makroinvertebrate, Balkansko poluostrvo, mala vodena tela

OVERVIEW OF PUBLISHED PAPERS ON MACROINVERTEBRATE ASSEMBLAGE IN SMALL WATER BODIES IN BALKAN PENINSULA

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The term of small water bodies includes small standing (lentic) and flowing (lotic) freshwater ecosystems. This type of ecosystem includes: upper parts of river, ditches, small lakes, ponds, swamps, marshes, flood zones, fishponds etc. Most of these ecosystems are ecotones. The ecotones are known for their high species diversity and therefore small water bodies are worthy of studying and protection. The goal of this paper is to analyze scientific papers connected with macroinvertebrate community of small water bodies. Springs were not discussed as a separate unit but only as the uppermost part of river catchments. We decided to include only the papers presenting information from Balkan Peninsula (Serbia, Montenegro, Northern Macedonia, Bosnia-Herzegovina, Albania, part of Slovenia, Croatia, Greece, Bulgaria and Romania). Period from 1994-2020 was chosen for this overview. Our analysis included 63 papers published on this topic. Most papers have been published 2014. The greatest number of published papers pertains to localities in Croatia (24 papers). We found only single papers related to the localities in Albania, Slovenia, Montenegro and Bosnia-Herzegovina. There were 18 papers with analyses of entire macroinvertebrate communities and 16 papers with just the assemblage of Diptera. Small lakes brought most attention to researchers (21 publications) while fishponds were least studied (5 publications). The greatest efforts should be concentrated on the research of fishponds due to the least interest. Although the interest of scientific circles has been increasing in recent decades, research and conservation of this type of ecosystem are still paid insufficient attention.

KEYWORDS: macroinvertebrates, Balkan Peninsula, small water bodies

Zaštita životne sredine

Environmental Protection

POREĐENJE IMTA I UZGOJA MEDITERANSKE DAGNJE (*Mytilus galloprovincialis* L.) U MONOKULTURI U BOKOKOTORSKOM ZALIVU

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Integralna multi-trofička akvakultura (IMTA) je oblik akvakulture gdje se produkti metabolizma uzgajanih riba kao i ostaci nepojedene hrane, koji predstavljaju opterećenje za vodeni medijum, mogu iskoristiti kao hrana organizmima nižeg trofičkog nivoa kao što su školjke i na taj način dovesti do povećanja produktivnosti cijelog sistema i smanjiti negativan uticaj intenzivnog uzgoja riba na vodeni ekosistem. Prirast i kondicioni indeks (KI) kod mediteranske dagnje (*Mytilus galloprovincialis* Lamarck, 1819) su praćeni od januara 2015 do januara 2016 godine na tri pozicije: NBL – pozicija pored uzgajališta ribe, NUD – pozicija udaljena 100 m od uzgajališta sa ribom i SVN – monokultura. Najintenzivniji prirast na sve tri pozicije je bio tokom proljećnog perioda, a najmanje intenzivan tijekom ljetnjeg perioda. Po završetku eksperimenta sve jedinke sa sve tri pozicije su dostigle komercijalnu dužinu od 50 mm. Rezultati ANOVA testa su pokazali da postoje statistički značajne razlike u prirastu između pozicija ($F=36.32$; $p<0.001$), a Turkey *post hoc* test je pokazao da je prirast na NUD i SVN poziciji statistički značajno veći u poređenju sa NBL pozicijom. Vrijednosti KI na poziciji NUD su bile veće od vrijednosti na pozicijama SVN i NBL, osim u razdoblju od oktobra do decembra kada je KI bilo sličan na pozicijama NBL i NUD i statistički značajno visočiji u poređenju sa SVN pozicijom (ANOVA sa Turkey *post hoc* testom), što ukazuje na to da se tokom hladnijeg perioda godine dagnje vjerovatno hrane nutrijentima koji vode porijeklo sa uzgajališta riba. Visok mortalitet zabilježen je na poziciji NBL, što je najvjerojatnije rezultat velike količine obraštajnih organizama. Dobijeni rezultati ukazuju da su neophodna dodatna istraživanja i uključivanje većeg broja biomarkera kao npr. analize stabilnih izotopa C i N, sa ciljem utvrđivanja benefita IMTA uzgoja.

KLJUČNE RIJEČI: IMTA, dagnje, prirast, kondicioni indeks, Bokokotorski zaliv

IMTA vs. MONOCULTURE FARMING OF MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis* L.) IN BOKA KOTORSKA BAY

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Integral multi-trophic aquaculture (IMTA) is a form of aquaculture where metabolic products of fish and uneaten food, which presents negative pressure for ecosystem, can be used as food in lower trophic organisms as bivalves, leading to the higher productivity of the entire system as well as reducing of negative fish farming impact. *The growth rate and condition index (CI) were monitored* in Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) since January 2015 up to January 2016 on three different positions: *NBL – position near fish cages, NUD – position 100 m removed from fish cages and SVN – monoculture. The most intense growth in mussels was recorded in spring, and the least intense during summer. On the end of experiment, all monitored individuals from all three positions achieved commercial size, 50 mm. Results of ANOVA showed that there are statistically significant differences in growth rate among positions* ($F=36.32$; $p<0.001$), while Turkey *post hoc* test showed that growth rates on NUD and SVN position are similar and significant higher compare to NBL. During most of investigated period, CI was higher on NUD compare to other two positions, except in period October to December, when CI was similar among NBL and NUD position and statistically significant higher compare to SVN (ANOVA with Turkey *post hoc* test), indicating on fact that during cold period mussels probably feed on the nutrients from fish farm origins. The highest mortality was recorded at NBL position, what is probably due to the effect of fouling organisms. Obtained results indicate that additional and more detailed studies are needed as well as monitoring of other biomarkers as C and N stable isotopes, with the aim of determining the benefits of IMTA.

KEYWORDS: IMTA, mussels, growth, condition index, Boka Kotorska Bay

IMPACTS OF SMALL HYDRO POWER PLANT AND FISH POND ON DIATOM COMMUNITY (PRIŠTAVICA RIVER, SERBIA)

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The mountain streams are under increasing pressure due to the construction of small hydro power plants (SHPs) and fish ponds (FPs). About 90 SHPs have been built in Serbia so far, and a total of 850 are planned. Objective of this study was to investigate the influence of SHP and FP on the spatial and temporal distribution of diatom assemblages in the Prištavica River. Epilithic samples were collected in July and October 2019 from 4 sites. SHP is located between the sites 3 and 4 and FP between 1 and 2. Permanent slides were made, and then qualitative and quantitative analysis of diatoms was performed. The results suggest that construction of SHP and FP have significant impact on physical and chemical water parameters which varied among the sampling sites. A total of 78 diatom taxa belonging to 28 genera were identified. Impact of SHP on diatom species diversity was noticed in summer, when observed species diversity was significantly lower at site 4 compared to others. Sites directly after the FP and SHP (sites 2 and 4) were distinguished by the same dominant species. *Achnanthidium minutissimum* and *Cocconeis euglypta* were dominant taxa at these sites in summer and *Nitzschia amphibia* in autumn. This indicates that both constructions built on the Prištavica River have a similar impact on the diatom community structure and thus probably on the water quality. Further research is necessary to improve management strategies which will enable sustainability and diversity of diatoms community as well as other aquatic organisms.

KEYWORDS: small hydro power plant, fish pond, diatom community

ZAGAĐENJE VAZDUHA PRIZEMNIM OZONOM I NJEGOV UTICAJ NA ZDRAVLJE LJUDI I VEGETACIJU

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Prizemni ozon je jedna od značajnijih zagađujućih materija u vazduhu. Povećane koncentracije ozona u vazduhu mogu negativno da utiču na zdravlje ljudi i vegetaciju. Cilj rada je poređenje maksimalne dnevne osmočasovne srednje vrijednosti koncentracija prizemnog ozona u periodu od tri godine (2017-2019) za grad Prijedor sa ciljnim vrijednostima i dugoročnim ciljevima za zaštitu zdravlja ljudi koje su propisane direktivom 2008/50/EC. Radi utvrđivanja uticaja ozona na vegetaciju i šume izračunati su AOT40 indeksi. Dobijene vrijednosti su poređene sa pomenutom direktivom i domaćim zakonodavstvom. Referentna metoda za mjerenje koncentracija prizemnog ozona opisana je u standardu BAS EN 14 625, Kvalitet vazduha ambijenta - Standardna metoda za određivanje koncentracija ozona ultraljubičastom fotometrijom. Mjerni uređaj za prizemni ozon na automatskoj stanici za monitoring kvaliteta vazduha u Prijedoru je API Teledyne – 400. Rezultati su pokazali da maksimalna dnevna osmočasovna srednja vrijednost za period od tri godine iznosi 148,2 $\mu g/m^3$, i prekoračena je u odnosu na ciljnu vrijednost. Srednja vrijednost AOT40 za tri godine iznosi 19557,6 $\mu g/m^3 \cdot h$ za zaštitu vegetacije i prekoračena je u odnosu na ciljnu vrijednost. Vrijednost AOT40 je 35354,4 $\mu g/m^3 \cdot h$ za zaštitu šuma.

KLJUČNE RIJEČI: prizemni ozon, vegetacija, zdravlje ljudi, AOT40

GROUND LEVEL OZONE AIR POLLUTION AND ITS IMPACT ON HUMAN HEALTH AND VEGETATION

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Ground-level ozone is one of the most significant pollutant in the air, and increased concentrations of ozone in the air can negatively affect human health and vegetation. The aim of this paper is to compare the maximum daily average eight-hour value over a period of three years (2017-2019) for the city of Prijedor with the target values and long-term goals for the protection of human health from Directive 2008/50/EC. Also, in order to determine the impact of ozone on vegetation and forests, the AOT40 index was calculated and the obtained values were compared with the mentioned directive and domestic legislation. The reference method for measuring ground-level ozone concentrations is described in BAS EN 14 625, Ambient air quality - Standard method for the determination of ozone concentrations by ultraviolet photometry. The measuring instrument for ground-level ozone at the automatic air quality monitoring station in Prijedor is the API Teledyne-400. The results indicate that the maximum daily mean eight-hour value for a period of three years is 148.2 $\mu g/m^3$ and is exceeded in relation to the target value. The average value of AOT40 for three years is 19557.6 $\mu g/m^3 \cdot h$ for vegetation protection and is exceeded in relation to the target value. The value of AOT40 is 35354.4 $\mu g/m^3 \cdot h$ for forest protection. The paper explains in more detail the obtained results.

KEYWORDS: ground-level ozone, vegetation, human health, AOT40

KVALITET VODE REKA BANJE I POCIBRAVE

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Reka predstavlja prirodni vodotok, i kao takva pogodna je za mnoge ljudske aktivnosti kao i za zadovoljavanje životnih potreba stanovništva, samim tim reke su izložene antropogenim faktorima zagađenja. Kako bi se antropogeni faktori zagađenja kontrolisali, a kasnije i njihov uticaj smanjio, potrebno je nad svim vodnim telima vršiti redovan monitoring, odnosno, pratiti stanje kvaliteta vode. Cilj ovog istraživanja jeste određivanje kvaliteta vode reka Banje i Pocibrave i za tu svrhu određen je SWQ indeks (*Serbian Water Quality).* SWQ indeks je prilagođen WQ (Water Quality) indeksu i zasniva se na analizi bakterioloških i fizičko-hemijskih parametara kvaliteta vode. Uzorci za analizu uzeti su na 3 lokalitetita, po jedan na srednjem delu toka reka Banje i Pocibrave i jedan na ušću samih reka. Za SWQ indeks, merenja su izvršena za sledeće fizičko-hemijske parametre: suspendovane materije, pH vrednost, temperaturu, amonijum jone i fosfate. Dobijeni rezultati fizičko-hemijskih analiza, kao i podaci o brojnosti koliformnih bakterija su obrađeni u SWQI softveru. Dobijene vrednosti SWQI pokazuju da reku Pocibravu odlikuje dobar status (75), reku Banju odličan (92), dok se lokalitet koji se nalazi na ušću Pocibrave u Banju takođe odlikuje odličnim statusom (91). Iz navedenih rezultata možemo zaključiti da su prema SWQ indeksu obe reke u dobrom stanju.

KLJUČNE RIJEČI: kvalitet vode, Banja, Pocibrava, fizičko-hemijski i bakteriološki parametri

ZAHVALNICA: Ovaj rad je urađen u okviru boravka u IS „Petnica“ i delom je finansiran Ugovorom o realizaciji i finansiranju naučno-istraživačkog rada NIO u 2020. godini (Evidencioni broj: 451-03-68/2020-14/ 200032).

WATER QUALITY OF RIVERS BANJA AND POCIBRAVA

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The river is a natural watercourse, and as such is suitable for many human activities as well as for meeting the living needs of the population, thus the rivers are exposed to anthropogenic pollution factors. In order to control anthropogenic pollution factors, and later to reduce their impact, it is necessary to perform regular monitoring of all water bodies, ie to monitor the state of water quality. The aim of this research is to determine the water quality of the rivers Banja and Pocibrava and for this purpose the SWQ index (Serbian Water Quality) was chosen. The SWQ index is adjusted the WQ (Water Quality) index and is based on the analysis of bacteriological, physical and chemical parameters of water quality. Samples for analysis were taken at 3 localities, one at the middle part of the Banja and Pocibrava rivers and one at the mouth of the rivers themselves. For the SWQ index, measurements were performed for the following physical and chemical parameters: suspended solids, pH value, temperature, ammonium ions and phosphates. The obtained results of physical and chemical analyzes, as well as data on the number of coliform bacteria were processed in the SWQI software. The obtained SWQI values show that the river Pocibrava has a good status (75), the river Banja has an excellent status (92), while the locality located at the mouth of the Pocibrava in Banja also has a excellent status (91). From the above results we can conclude that according to the SWQ index both rivers are in good condition.

KEYWORDS: water quality, river Banja, river Pocibrava, physical, chemical and bacteriological parameters

MICROPLASTICS IN WATER AND ITS IMPACT ON THE ENVIRONMENT

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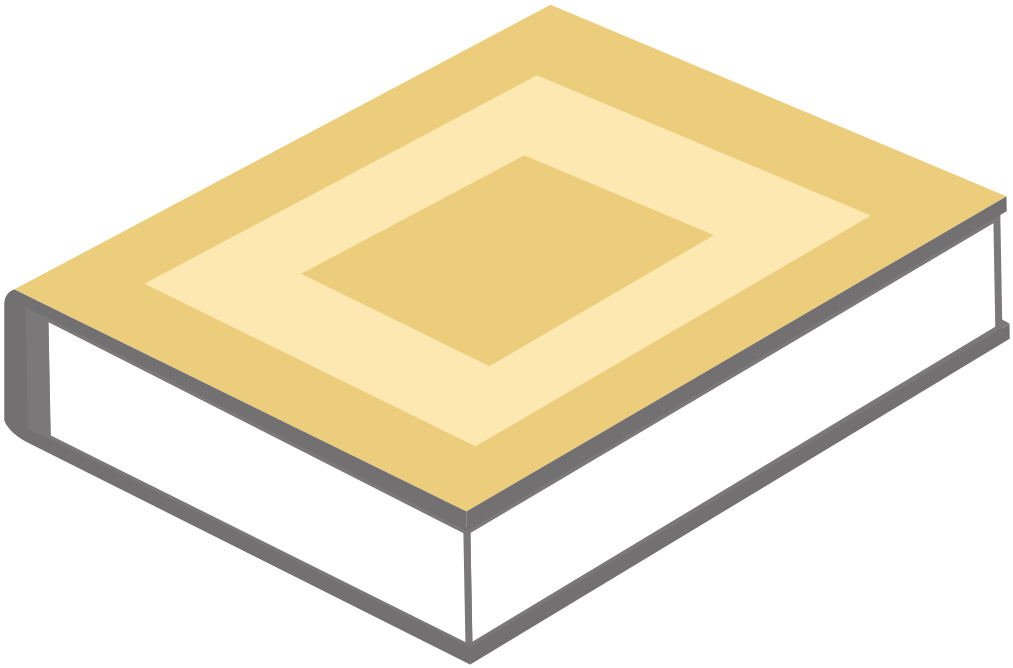
Microplastics (MP) pollution is a global problem of environmental protection, representing a great concern of both scientists and the general public. MP are found in all ecosystems, while the presence of MP in aquatic ecosystems attracts special attention. Dimensions of MP do not exceed 5 mm, hence are not easily relatable to the source of pollution, neither easy to remove from the environment. The aim of this research is to review the methods of sampling and detection of MP in water. By reviewing the literature, an overview of the source of MP in water and its transfer is summarized and also an analysis of the impact of MP on the health of living organisms. Identification and quantification of MP is performed by visual inspection accompanied by chemical characterization. Chemical characterization is usually performed using Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy. Specialized nets are used for sampling MP in water. There is no standard net/pore size, therefore is difficult to compare and match results. Due to the lack of uniformity in MP sampling methods, little is known about their time and geographical distribution. Although the number of studies related to MP has increased significantly in recent years, it is important to examine the interactions between water, air, soil and MP and to identify deficiencies and directions in research. New research is needed for a development strategy to reduce the amount of MP in the environment, to establish management and remediation measures, and to reduce the risk of MP pollution.

KEY WORDS: microplastics, environment, water

ACKNOWLEDGEMENTS: This work is financed by the Agreement on the realization and financing of scientific research work of NIO in 2020 of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Registration number: 451-03-68 / 2020-14 / 200032; 451-03-68 / 2020-14 / 200134).

Biologija i ekologija: položaj u školama

Biology and Ecology: Position in Schools



NASTAVNI PROGRAMI BIOLOGIJE U OSNOVNOJ ŠKOLI U SRBIJI I REPUBLICI SRPSKOJ

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Biologija je prirodna nauka koja se intenzivno razvija, a njeni sadržaji, uključujući sve njene discipline, su veoma značajni za obrazovanje učenika osnovnih škola. Prema Nastavnom planu za osnovnu školu biologija je kao nastavni predmet u Republici Srpskoj zastupljena od 6. do 9. razreda, a u Republici Srbiji od 5. do 8. razreda osnovne škole. U radu su analizirani nastavni programi biologije u osnovnoj školi u Srbiji i Republici Srpskoj. U istraživanju je primenjena metoda teorijke analize. Važeći programi biologije u Republici Srpskoj i prethodni program biologije u Republici Srbiji su bili veoma slični. U 5. razredu u Srbiji i 6. razredu u Republici Srpskoj proučavani su sadržaji iz botanike (osnove citologije, morfologije, anatomije fiziologije i sistematike biljaka). U 6. razredu u Srbiji i u 7. razredu u Republici Srpskoj proučavani su sadržaji iz zoologije (osnove morfologije, anatomije, fiziologije i sistematike životinja i osnove evolucije). U 7. razredu u Srbiji i u 9. razredu u Republici Srpskoj proučavani su sadržaji iz nauke o čoveku (osnove morfologije, anatomije, fiziologije, genetike i evolucije čoveka). U 8. razredu u oba programa prouavani su sadržaji iz ekologije i zaštite životne sredine. Raspored obrazovno-vaspitnih sadražaja u oba programa biologije bio je linijski. Biološki sadržaji nadovezivali su se u logičnom sledu, po biološkim disciplinama. Njihov najveći nedostatak je bila njihova preopširnost i težak akademski prikaz u važećim udžbenicima. Pored toga sadržaji su međusobno nedovoljno povezivani, ali ni sa programima drugih prirodnih nauka. Zbog toga je za učenike osnovne škole bilogija bila među težim nastavnim predmetima. Programi biologije u osnovnoj školi u Republici Srbiji su u međuvremenu značajno promenjeni. Raspored sadržaja u novim programima biologije je spiralan. U svim razredima proučava se pet istih nastavnih tema: Jedinstvo građe i funkcije kao osnova života, Život u ekosistemu, Nasleđivanje i evolucija, Poreklo i raznovrsnost života i Čovek i zdravlje, a njihovi sadržaji se u svakom sledećem razredu proširuju i produbljuju. Novi Programi nastave i učenja biologije za osnovnu školu u Srbiji usmereni se na ishode učenja i kompetencije koje učenici treba da steknu posle obrade svake nastavne teme u svakom razredu i u programu u celini. U prethodnom programu biologije u Srbiji sadržaji iz ekologije i zaštite životne sredine

proučavani su u 8. razredu, a proučavaju se u 8. razredu i u važećem programu biologije u Republici Srpskoj. U novom programu Nastave i učenja biologije za osnovnu školu u Srbiji saržaji iz ekologije i zaštite životne sredine proučavaju se u svim razredima u nastavnoj temi Život u ekosistemu. Promene u programima biologije u Republici Srpskoj su svakako neophodne i nalaze se u fazi pripreme za sledeću školsku godinu. One treba da prate savremena dostignuća u biologiji kao nauci, kao i u metodici nastave biologije. Promene treba da budu studiozno pripremljene, na bazi iskustava primene ranijih programa biologije i otklanjanja njihovih nedostataka. Promene u programima treba takođe da budu primerene uzrastu učenika i njihovim saznajnim sposobnostima.

KLJUČNE REČI: osnovna škola, biologija, programi biologije, Republika Srbija, Republika Srpska

BIOLOGY SYLLABI IN PRIMARY SCHOOLS IN SERBIA AND THE REPUBLIC OF SRPSKA

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Biology is a natural science that is developing intensively and its contents, covering all biological disciplines, are very important for primary school education. According to the Primary Education Curriculum, biology is studied from the sixth to the ninth grade in the Republic of Srpska, and from the fifth to the eighth grade of primary school in the Republic of Serbia. The paper analyzec the Syllabi of Biology in primary School in Serbia and the Republic of Srpska. The method of theoretical analysis is applied in the research. The current biology syllabi in the Republic of Srpska and the former biology syllabi in the Republic of Serbia were very similar. In the fifth grade in Serbia, i.e. the sixth grade in the Republic of Srpska, botany (fundamentals of cytology, morphology, anatomy, physiology and plant systematics) were studied. In the sixth grade in Serbia, i.e. the seventh grade in the Republic of Srpska, zoology topics (fundamentals of morphology, anatomy, physiology, animal systematics and evolution) were covered. The seventh grade in Serbia and the ninth in the Republic of Srpska included anthropology contents (fundamentals of morphology, anatomy, physiology, genetics and human evolution). The eighth grade of primary school in both curricula covered the topics related to ecology and environmental protection. The organization of the contents in both curricula was linear and all biology topics developed in a logical order, following the included disciplines. The greatest shortcoming of such an organization is seen in a very large and too detailed coverage of topics and a high level of academic writing style in the textbooks. The contents were neither interrelated nor connected to other science subjects and because of this biology was considered one of the hardest school subjects. In the meantime, biology syllabi in the Republic of Serbia have undergone considerable changes and the organizational pattern of the contents is spiral. In all grades the following five topics are covered: Unity of Form and Function as the Basis for Life; Life in an Ecosystem; Inheritance and Evolution; The Origin and Diversity of Life; Humans and their Health. The contents of these topics are given a more in-depth insight in each consecutive grade. The new syllabi are focused on the learning outcomes and the competencies that students are expected to acquire after each presented topic in each of the four grades. As for ecology and environmental protection, the former curriculum in Serbia

included these topics in the eighth grade and the same holds true for the eighth grade in the Republic of Srpska. The new syllabi in Serbia include ecology and environmental protection topics in all four grades under the general topic Life in an Ecosystem. Changes in the biology syllabi in the Republic of Srpska are certainly necessary and they are currently in the preparation phase for the new school-year. The changes should include the latest advances in biological disciplines and teaching methodology and need to be systematically prepared, based on the experience with previous syllabi and their detected shortcomings. The changes should be in line with the students' age and their cognitive abilities.

KEYWORDS: primary school, biology, biology syllabi, the Republic of Serbia, the Republic of Srpska

POTENCIJALI NASTAVE BIOLOGIJE KOJA SE REALIZUJE NA DALJINU PUTEM GOOGLE PLATFORME - PREDNOSTI I NEDOSTACI

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Napretkom informaciono komunikacionih tehnologija stvorili su se uslovi za uvođenje inovacija u nastavni proces i njegovo osavremenjivanje. Brojne su platforme koje nude mogućnost realizovanja nastave na daljinu, pri kojoj učesnici nastavnog procesa mogu da komuniciraju sinhrono, kao da su u učionici. Ove platforme se najčešće koriste u okviru predmeta računarstvo i informatika, tehničko i informatičko obrazovanje i drugih srodnih predmeta i to često samo kao podrška neposrednoj nastavi koja se odvija u učionici. Privremenim zatvaranjem škola zbog proglašenja pandemije koronavirusa početkom 2020. godine, platforme koje omogućuju nastavu na daljinu dobijaju na značaju i počinju masovno da se koriste u okviru svih nastavnih predmeta, pa i biologije. Jedna od online platformi koja se koristi za realizaciju nastave na daljinu je Google Classroom u koju je integrisan Google Meet. Ova platforma nudi nekoliko mogućnosti za prevazilaženje problema otežane komunikacije između učesnika usled novih okolnosti koje su trenutno prisutne u obrazovno-vaspitnom radu. Primena Google Classroom platforme ima brojne prednosti, ali i neke nedostatke. Pored toga što omogućuje sinhronu komunikaciju između nastavnika i učenika koji od kuće prate nastavu, postavljanje nastavnih materijala u virtuelno okruženje i mogućnosti da nastavnik koristi određene alate za pravljenje kvizova, testova i drugih sličnih sadržaja, ovakav tip nastave zahteva od nastavnika dodatna angažovanja i posebnu pripremu za čas. Cilj ovog rada je da prikaže prednosti i nedostatke Google Classroom platforme u nastavi biologije, kao i mogućnosti koje nudi ova platforma.

KLJUČNE REČI: nastava biologije, nastava na daljinu, google classroom, google meet

POTENTIALS OF BIOLOGY TEACHING THAT IS CARRIED OUT REMOTELY VIA GOOGLE PLATFORM - ADVANTAGES AND DISADVANTAGES

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Advances in information and communication technologies have created the conditions for the introduction of innovations in the teaching process and its modernization. There are numerous platforms that offer the possibility of realizing distance learning, in which participants in the teaching process can communicate synchronously, as if they were in a classroom. These platforms are most often used in the subjects such as computer science and informatics, technical and information education and other related subjects, and often only to support the direct teaching that takes place in the classroom. With the temporary closure of schools because of a coronavirus pandemic in early 2020, platforms that enable distance learning are gaining in importance and are widely used in all subjects, including biology. One of the online platforms used for distance learning is Google Classroom, which integrates Google Meet. This platform offers several possibilities for overcoming the problem of difficult communication between participants due to the new circumstances that are currently present in the educational work. The Google Classroom platform has a number of advantages, but also some disadvantages. In addition to enabling synchronous communication between teachers and students who follow classes from home, placing teaching materials in a virtual environment and the possibility for the teacher to use certain tools to create quizzes, tests and other similar content, this type of teaching requires additional engagement and special preparation for lectures. The aim of this paper is to show the advantages and disadvantages of the Google Classroom platform in teaching biology, as well as the possibilities offered by this platform.

KEYWORDS: biology teaching, distance learning, google classroom, google meet

ZNAČAJ I PRIMENA PROBLEMSKI ORIJENTISANE NASTAVE BIOLOGIJE U FUNKCIONALNOM OSNOVNOM OBRAZOVANJU ODRASLIH

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Brojna empirijska istraživanja u funkcionalnom osnovnom obrazovanju odraslih (FOOO) su potvrdila značaj primene aktivnih metoda učenja u sticanju novih znanja, povezujući ih sa prethodnim znanjem i životnim iskustvom. Jedan od nastavnih pristupa koji stavlja odraslog učenika u središte nastavnog procesa i podstiče ga na samostalno definisanje i istraživanje životnih problema je problemska nastava/učenje. Savremeni didaktičari ističu značaj dominantne primene problemskog učenja u nastavi biologije i drugih prirodnih nauka u FOOO, jer ona razvija jednu od ključnih kompetencija odraslih − sposobnost rešavanja problema. U problemskoj organizaciji nastave obrazovno-vaspitni rad počinje definisanjem problema, a nastavlja se analizom podataka, traženjem i nalaženjem strategija koje vode rešenju postavljenog problema. Ovakav tip nastave zahteva od nastavnika posebnu pripremu za čas, fleksibilno vreme trajanja časa, pripremu materijala/sredstava za rad na času, organizovanje timskog rada u rešavanju problemskih zadataka i/ili mogućnost izvođenja terenskog rada u neposrednoj okolini škole. Pretražujući scenarije za sprovođenje problemske nastave u realizaciji bioloških sadržaja u FOOO, može se uočiti da su oni malobrojni i samim tim nedovoljni za njenu uspešnu primenu u realizaciji Nastavnog programa biologije u FOOO. U cilju prevazilaženja ovog problema i unapređenja kvaliteta života odraslih, u radu su date teorijske osnove obrazovanja odraslih u Republici Srbiji, a zatim teorijski i praktični aspekti primene problemske nastave u realizaciji bioloških sadržaja u FOOO. U radu je prikazan i scenario za čas koji treba da doprinese profesionalnom osnaživanju nastavnika za primenu problemske nastave biologije u FOOO i obezbedi joj adekvatno mesto u ovom vidu obrazovanja.

KLJUČNE REČI: obrazovanje odraslih, nastava biologije, problemska nastava, scenario za čas

SIGNIFICANCE AND APPLICATION OF PROBLEM-BASED BIOLOGY LEARNING IN FUNCTIONAL BASIC EDUCATION OF ADULTS

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Numerous empirical studies related to functional basic education of adults (FBEA) have comfirmed the significance of active learning in gaining new knowledge, by linking it to backround knowledge and life experience. An approach that puts the adult student in the centre of learning and stimulates their independent defining and researching of real-world problems is the problem-based teaching and learning. Contemporary didactics experts emphasise the significance of dominant application of problem-based teaching and learning of biology and other sciences in FBEA, as the approach develops one of the key competencies of adults – problem-solving. Problem-based teaching and learning starts with a problem defining and continues with data analysis and searching and finding the strategies that lead to the problem solution. It requires a detailed lesson planning, flexible lesson duration, preparation of the necessary material and organisation of team work in solving problem-based tasks and/or conducting field work in the school vicinity. The search for problem-based biology lesson scenarios in FBEA shows that they are scarce and therefore insufficient for successful application in the biology syllabi. In order to overcome this problem and encance the life quality of adults, the paper gives a theoretical overview of the education of adults in the Republic of Serbia and then focuses on theoretical and practical aspects of applying problem-based learning of biology content in FBEA. The paper offers a lesson scenario as a support to biology teachers to apply problem-based learning and thus provide an adequate position of this approach in adult education.

KEYWORDS: adult education, biology teaching, problem-based learning, lesson scenario

TEŽNJA KA ZDRAVIM NAVIKAMA U OSNOVNOŠKOLSKOM OBRAZOVANJU U REPUBLICI SRBIJI

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U okviru programa biologije u osnovnoj školi uči se i o značaju zdrave ishrane i fizičke aktivnosti za zdravlje ljudi. Cilj rada bio je da u odeljenjima od 5. do 8. razreda osnovne škole istražimo zdravstvene navike učenika. Intervjuisali smo 415 učenika (uzrasta od 11 do 14 godina). Radeći zadatke alternativnog i višestrukog izbora, učenici su mogli da se opredele za određeni odgovor u vezi toga šta jedu za doručak, koliko vode piju u toku dana i da li i koliko praktikuju fizičke vežbe. Da bismo utvrdili da li postoje razlike u ispitivanim navikama između dečaka i devojčica, odnosno među uzrastima koristili smo Hi-kvadrat test (χ^2). Koeficijent kontingencije smo koristili za merenje stepena povezanosti pola i odgovora učenika na određena pitanja. U vezi sa ishranom, uočili smo u svim razredima značajnu razliku između dečaka i devojčica u tome šta jedu za doručak. Takođe smo uočili značajnu razliku između uzrasta: kod devojčica je postojala snažna korelacija između odgovora na određeno pitanje o ishrani i starosti, dok kod dečaka nismo ustanovili takvu povezanost. U navikama vezanim za uzimanje vode nije bilo značajne razlike između dečaka i devojčica. Međutim, uočili smo značajnu razliku u konzumiranju vode među uzrastima. Razlika u bavljenju fizičkim aktivnostima između dečaka i devojčica javila se jedino u 7. razredu. Samo kod devojčica primetili smo značajnu razliku između uzrasta, ali korelacija između odgovora i starosti učenica je bila mala. Rezultati ovog istraživanja pružaju značajne informacije pri planiranju strategija promocije zdravih stilova života među decom školskog uzrasta.

KLJUČNE REČI: Osnovna škola, ishrana učenika, unos vode, fizičke aktivnosti

STRIVING FOR HEALTHY HABITS IN PRIMARY EDUCATION IN THE REPUBLIC OF SERBIA

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The Biology curriculum in primary school addresses various topics, including healthy diet, a school-based and out of school physical activities. The aim of the study is to investigate health habits of students from 5th to 8th grade of primary school. We interviewed 415 students (ages 11 to 14). Doing an alternative choice and the multiple choice tests, they could opt for a particular answer about their breakfast diet, drinking of water habits, and their physical activity behaviors. We used Chi-square test (χ^2) to determine if there are differences in examined habits between boys and girls, and among ages, respectively. The Coefficient of contingency we used to measure the degree of association between the ages and student's answer to specific questions. Related to diet, we observed a significant difference between boys and girls in what they eat for breakfast, in all grades. We also noted significant difference between ages: in girls, there was a strong correlation between answers to a certain diet question and the age, while in boys we have not established such association. In habits related to water consumption, there was no significant difference between boys and girls. However, we noticed a significant difference in water consumption between ages. Difference in doing physical activities between boys and girls exists only in the 7th grade. Only in girls, we noticed significant difference between ages, but the correlation between response and age was weak. Results of this research provide important information in implementation of strategies for promotion healthy lifestyles among school-age children.

KEYWORDS: Primary School, diet, water intake, physical activities

RAZVOJ EKOLOŠKE SVIJESTI KROZ EKOLOŠKO OBRAZOVANJE I VASPITANJE UČENIKA OSNOVNE ŠKOLE

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Razvoj savremenog društva je usko povezan sa brzim industrijskim i tehnološkim napretkom, a time i sve većim uticajem čovjekovih aktivnosti na životnu sredinu. Tokom čitavog života čovjek je u direktnom kontaktu sa svojom okolinom, crpi iz nje brojne resurse i narušava prirodnu ravnotežu, što dovodi do mnogih globalnih, regionalnih i lokalnih ekoloških problema. Zato je veoma važno da stvaranje i razvoj ekološke svijesti i odgovornog odnosa prema životnoj sredini počnu još od ranog uzrasta, pri čemu obrazovanje ima najvažniju ulogu. Ekološko obrazovanje i vaspitanje su prisutni na svim nivoima savremenog školovanja, od predškolskih ustanova do fakulteta, konstantno ukazujući na opasnosti koje su rezultat narušavanja životne sredine i potrebu očuvanja osjetljive ekološke ravnoteže. Zadaci ekološkog obrazovanja i vaspitanja obuhvataju razvoj ekološke svijesti i ekološke kulture, pravilan odnos prema životnoj sredini i razvoj ekološkog ponašanja, stavova i djelovanja. Iz tih razloga, ciljevi istraživanja predstavljeni u ovom radu obuhvataju procjenu nivoa razvijenosti ekološke svijesti učenika Osnovne škole „Branko Ćopić" u Banjoj Luci. Istraživanje je provedeno na uzorku od 150 učenika osmog i devetog razreda. Podaci su prikupljeni metodom ankete i statistički obrađeni, da bi se utvrdio nivo razvijenosti ekološke svijesti u odnosu na razred i pol. Iz rezultata istraživanja može se zaključiti da anketirani učenici imaju veoma razvijenu ekološku svijest, što je rezultat ekološkog obrazovanja i vaspitanja. Može se zaključiti da se ekološka svijest može i dalje razvijati kroz različite praktične aktivnosti i sticanje ličnih iskustava vezanih za ekologiju.

KLJUČNE RIJEČI: ekološko obrazovanje, razvoj ekološke svijesti, osnovna škola, anketa za učenike

DEVELOPMENT OF ECOLOGICAL AWARENESS THROUGH ECOLOGICAL EDUCATION AND UPBRINGING OF PRIMARY SCHOOL STUDENTS

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The development of modern society is closely linked to rapid industrial and technological progress, and growing impact of human activities on the environment. Throughout life, man is in direct contact with his environment, draws numerous resources from it and disturbs natural balance, which leads to many global, regional and local environmental problems. It is very important that the origination and development of environmental awareness and responsible attitude towards the environment start from an early age, with education playing the most important role. Environmental education and upbringing are present at all levels of modern schooling, from preschools to faculty, constantly pointing out the perils result from environmental damage and the need to preserve a sensitive ecological balance. The tasks of ecological education and upbringing include the development of ecological awareness and culture, proper attitude towards environment and development of ecological behavior, stances and actions. Objectives of research presented in this paper include an assessment of the level of development of environmental awareness of pupils in the Elementary School "Branko Ćopić" in Banja Luka. The research included 150 eighth and ninth grade pupils. Data were collected by survey and statistically processed, to determine the level of development of environmental awareness in respect to grade and gender. From the results it can be concluded that surveyed pupils have a very developed environmental awareness, which is result of environmental education and upbringing. It can be concluded that environmental awareness can be further developed through various practical activities and gaining personal experiences related to ecology.

KEYWORDS: environmental education, development of environmental awareness, elementary school, pupil survey

INDEKS AUTORA/*AUTHOR INDEX*

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CIP - Каталогизација у публикацији
Народна и универзитетска библиотека
Републике Српске, Бања Лука

57(048.3)(0.034.2)
502/504(048.3)(0.034.2)

СИМПОЗИЈУМ биолога и еколога Републике Српске (4 ; 2020 ; Бања Лука)
Zbornik sažetaka / IV simpozijum biologa i ekologa Republike Srpske - SBERS2020 Prirodno-matematički fakultet, Univerzitet u Banjoj Luci, 12-14. novembar 2020. = Book of abstracts / IV symposium of biologists and ecologists of Republic of Srpska with international participation - SBERS2020 Faculty of Natural Sciences and Mathematics, University of Banja Luka 12-14 November 2020. ; [urednik Duško Jojić]. - Бања Лука : Природно-математички факултет, 2020. - 199 стр.

Način pristupa (URL): https://pmf.unibl.org/wp-content/uploads/2020/11/zbornik_SBERS2020.pdf. - Текст на срп. и енгл. језику.

ISBN 978-99955-21-86-8

COBISS.RS-ID 129895425