



Hrvatsko biološko društvo
SOCIETAS BIOLOGORUM CROATICA
Croatian Biological Society

14. HRVATSKI BIOLOŠKI KONGRES
s međunarodnim sudjelovanjem

14th CROATIAN BIOLOGICAL CONGRESS
with International Participation

Pula, 12 - 16. 10. 2022.



14. *Hrvatski
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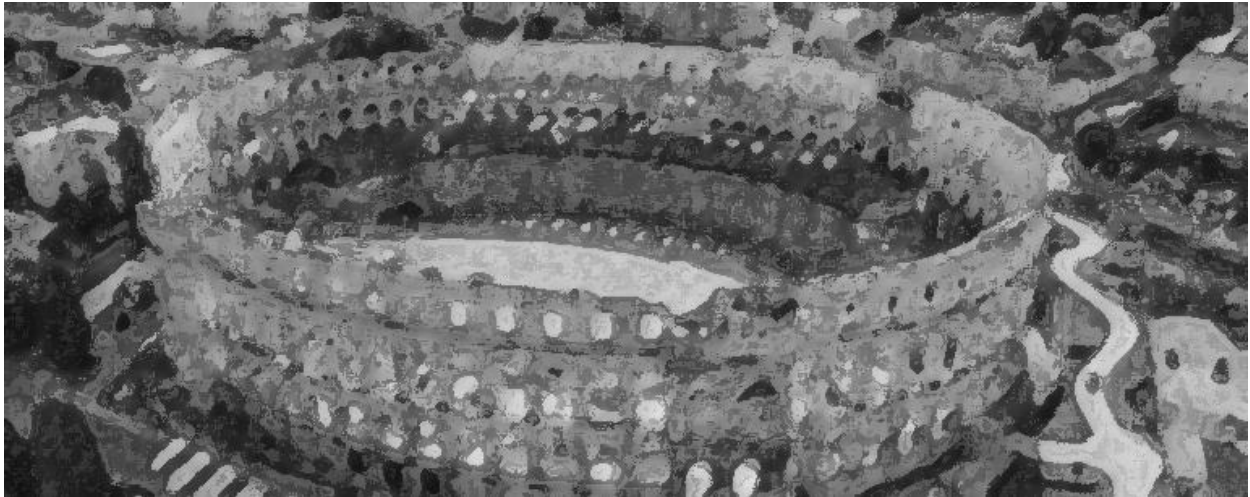
ZBORNİK SAŽETAKA
BOOK OF ABSTRACTS



**14th Croatian Biological
Congress**
Pula, October 12 -16, 2022



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SOCIETAS BIOLOGORUM CROATICA
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12 - 16. listopada 2022.

Pula, Hrvatska

14th CROATIAN BIOLOGICAL CONGRESS

With International Participation

12nd - 16th October 2022

Pula, Croatia

ZBORNİK SAŽETAKA BOOK OF ABSTRACTS

Zagreb, 2022.

**ZBORNİK SAŽETAKA
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OF THE 14th CROATIAN BIOLOGICAL CONGRESS**

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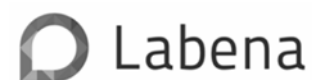
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Orašnici (TOR) i Butišnici (TBU). Prosjek specifičnog rasta algi i postotak inhibicije izračunat je prema normi ISO 8692:2012 nakon 24, 48 i 72 h inkubacije mjerenjem optičke gustoće spektrofotometrom na 670 nm. Konačni rezultat testa predstavlja najviša koncentracija pri kojoj nije uočena inhibicija (<20 %), odnosno LIDA-vrijednost (eng. Lowest Ineffective Dilution). Rezultati testa ukazuju na potencijalnu toksičnost otpadne vode IWW (LIDA = 32, 72 h). Za ostale postaje LIDA iznosi: KRS i TBU = 1, TOR = 2 i MWW = 4, odnosno porast toksičnosti u otpadnih voda: KRS = TBU < TOR < MWW < IWW. Provedeni testovi potvrdili su alge kao osjetljive bioindikatore te ukazali na toksični utjecaj industrijskih otpadnih voda na rijeku Krku i na važnost pravilnog pročišćavanja otpadnih voda prije ispuštanja u okoliš. Stoga je potrebno provoditi daljnji monitoring kako bi se izbjegle ozbiljne posljedice na biotu i Nacionalni park Krka.

Ključne riječi: rijeka Krka, otpadne vode, testovi toksičnosti, zelena alga

ASSESSMENT OF THE WASTEWATER TOXICITY IN THE KARST AREA USING ALGAL GROWTH INHIBITION TEST

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Toxicity assessment is performed by acute tests that indicate potential toxic effect on aquatic organisms. In our research, toxicity testing was performed in the upstream flow of the Krka River, directly impacted by inappropriately treated industrial and municipal wastewaters, using green algae (*Pseudokirchneriella subcapitata* (Korshikov) F. Hindák, 1990). Toxicity of water in karst area was tested at five sites, Krka River source (KRS, reference site), near municipal (MWW) and industrial wastewater outlets (IWW) and in tributaries Orašnica (TOR) and Butišnica (TBU). The average algal specific growth and the percentage of inhibition was calculated according to ISO 8692:2012 after 24, 48 and 72 h incubation by measuring the optical density on a spectrophotometer at 670 nm. The final result of the test is presented by the highest concentration at which no inhibition was observed (<20%), called LID_A value (Lowest Ineffective Dilution). Results indicated the potential toxicity of IWW (LID_A = 32, 72 h). For other sites LID_A was: KRS and TBU = 1, TOR = 2 and MWW = 4, therefore indicating increase of toxicity in wastewaters: KRS = TBU < TOR < MWW < IWW. Toxicity testing confirmed algae as sensitive bioindicators and pointed to the toxic impact of industrial wastewater on the Krka River and the importance of proper wastewater treatment before discharge into the environment. Therefore, further monitoring is needed to avoid serious consequences for biota and the Krka National Park.

Keywords: Krka River, wastewaters, toxicity testing, green algae

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EFFECT OF ACRYLAMIDE TREATMENT ON THE ACTIVITY AND EXPRESSION OF GLUTATHIONE-S-TRANSFERASE 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°C and 180°C. The aim of our study was to determine whether acrylamide treatment affects the expression and activity of glutathione-S-transferase (GST) in hepatocyte. Viability of rat hepatoma cell line - H4IIE upon AA treatment was assessed by the MTT viability assay. Cells were cultivated in a 96-well plate and exposed to increasing concentrations of AA (2.5–5.5 mM) for 24 h. AA concentration of 4 mM induced death of 17.62% H4IIE cells, while 4.5 mM AA caused death of 48.45% cells. Therefore, AA concentrations of 4 mM and 4.5 mM were taken as IC20 and IC50, respectively. H4IIE cells were treated with IC20 and IC50 of AA for 24 h. Relative mRNA expression for GSTA2 and GSTP1 was quantified using real-time RT-PCR. Total GST activity was determined spectrophotometrically at 340 nm using DTNB as the substrate. After AA treatments, mRNA level for GSTA2 and GSTP1 and total GST activity increased in a concentration-dependent manner in H4IIE cells. Only treatment with higher AA concentration (4.5 mM) proved to be statistically significant for all three examined parameters. Our results indicate that by altering expression and activity of GST enzyme acrylamide exerts toxic effects on hepatocytes.

Keywords: Acrylamide, GST activity, GST expression, MTT viability assay, H4IIE cell line

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UČINAK CaCl₂ I CaBr₂ NA RAZMNOŽAVANJE VRSTE *Daphnia magna* STRAUS

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Koncentrirane otopine soli CaCl₂ i CaBr₂ gustoće do 2.3 kg/L redovito se koriste tijekom specijalnih operacija u istraživanjima i proizvodnji prirodnog plina i sirove nafte. Različite koncentracija soli visoke gustoće često se ostavljaju u isplačnim jamama uz bušotine kao otpad te tako zagađuju površinske i podzemne vode. U ovom radu istraživali smo učinke CaCl₂ i CaBr₂ na vodenbuhu *Daphnia magna* u 21-dnevnom testu reprodukcije. Tri pokusne koncentracije (1925 mg L⁻¹, 481 mg L⁻¹ i 240 mg L⁻¹) prouzročile su značajno smanjenje razmnožavanja s povećanjem doze (p<0.001) i povećanje smrtnosti. Prosječan broj neonata po leglu smanjio se u sve tri CaCl₂ koncentracije i prosječan broj legala značajno se smanjio u najtoksičnijoj otopini (1925 mg L⁻¹). Obje ispitane koncentracije CaBr₂ su bile vrlo toksične za odrasle (50 and 60 % smrtnost) i embrije tako da su u CaBr₂ 1066 mg L⁻¹ i 533 mg L⁻¹ bila proizvedena samo abortirana jaja što je pokazalo embriotoksičnost te supstance. Uspoređujući dobivene rezultate s onima dobivenim u prethodnom istraživanju na slatkovodnom pužu *Planorbarius corneus* L. može se zaključiti da je *Daphnia magna* pokazala veću osjetljivost na ove kemikalije od puža. Rezultati