



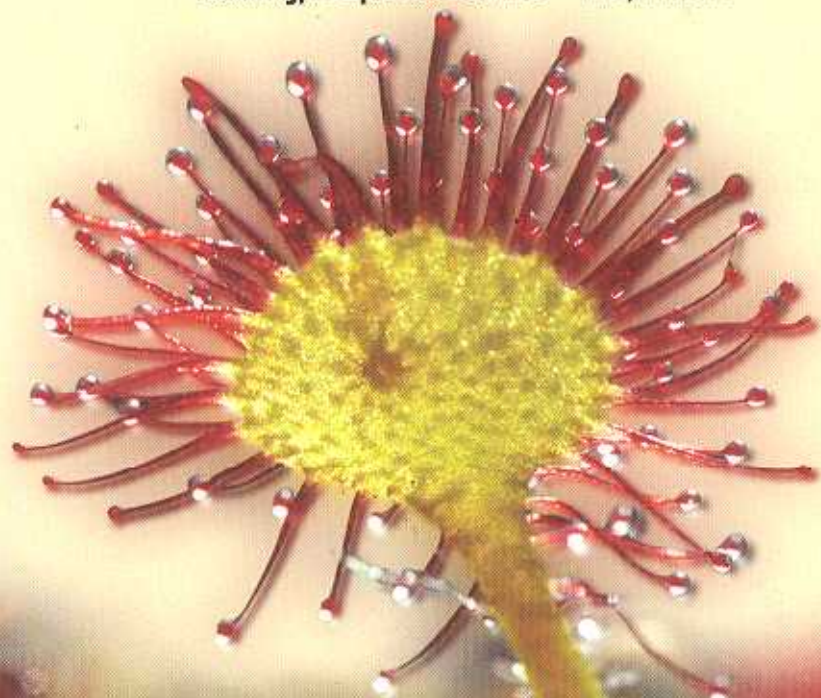
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ZBORNİK SAŽETAKA
PROCEEDING OF ABSTRACTS

Uvodna tematska predavanja
Introductory Lectures

IL-18

EKOTOKSIKOLOŠKI POKAZATELJI ONEČIŠĆENJA U KLENU
(*Leuciscus cephalus* L.) – ISTRAŽIVANJA U OKVIRU EU FP6 PROJEKTA
“SARIB”

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Glavni cilj EU FP6 projekta *SARIB* (*Sava River Basin: Use, Management and Protection of Resources*) je utvrđivanje razine onečišćenja rijeke Save. Uz korištenje suvremenih analitičko-kemijskih metoda za određivanje prisustva organskih zagađivala i teških metala, u vodi, sedimentu i tkivu riba mjerene su i odgovor niza ekotoksikoloških biomarkera. Aktivnost 7-etoksirezorufin deetilaze (EROD), bioaktivacijski potencijal i metaboliti policikličkih aromatskih amina mjereni su u jetri, odnosno žuči klena *Leuciscus cephalus*, vrste rasprostranjene i često korištene u monitorinzima zagađenja europskih rijeka. Klenovi su izlovljavani na pet postaja: Otok Samoborski (referentna postaja), Sava Jarun (uzvodno od glavnih ispusta grada Zagreba), te Oborovo, Lukavec Posavski i Jasenovac (opterećeni komunalnim i industrijskim otpadnim vodama Zagreba i Siska). U jetri klenova izlovljenih u rujnu 2005. g. zabilježena je značajna indukcija aktivnosti EROD: 3.2 puta na postaji Sava Jarun, 3.9 puta na Oborovu, 4.8 puta na Lukavcu Posavskom, te 7.2 puta na postaji Jasenovac u odnosu na kontrolnu nezagađenu postaju Otok Samoborski. Najviša vrijednost (20.7 pmol/min/mg_{protein}) dobivena na postaji Jasenovac predstavlja ~50% maksimalne EROD aktivnosti koja je izmjerena u riba laboratorijski tretiranih modelnim EROD inducerom α -naftoflavonom (25 mg/kg). Količina CYP1A proteina mjerena ELISA metodom pokazala je identičan trend kao i EROD aktivnost. Značajno povećan bioaktivacijski potencijal jetre klena izmjerene je na svim lokacijama u odnosu na Otok Samoborski. Metaboliti policikličkih aromatskih ugljikovodika u žuči nisu pratili obrazac EROD aktivnosti i bioaktivacijskog potencijala. U izlaganju ćemo dati i usporedbu s vrijednostima istih biomarkera izmjerenih u drugim europskim rijekama i/ili monitoring programima.

EKOTOXICOLOGICAL INDICATORS OF POLLUTION IN THE
EUROPEAN CHUB (*Leuciscus cephalus* L.) – RESEARCH WITHIN THE

EU FP6 "SARIB" PROJECT

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The main goal of the FP6 SARIB project (*Sava River Basin: Use, Management and Protection of Resources*) is to evaluate the extent of pollution of the Sava river (Croatia). Besides the advanced analytical-chemical methods for PAHs, PCBs and heavy metal presence in the water, sediment and tissues of a common cyprinid freshwater fish European chub (*Leuciscus cephalus*), various ecotoxicological biomarkers (EROD activity, bioactivation potential, PAH bile metabolites) have been measured. The sampling survey involved 5 stations: Otok Samoborski (reference site), Sava Jarun (upstream from the main Zagreb city outlet), and Oborovo, Lukavec Posavski and Jasenovac (inputs of domestic and industrial effluents from the Zagreb and the Sisak city area). Significant induction of EROD activity was observed in chub caught in September 2005. In comparison to the Otok Samoborski the EROD activity was higher 3.2 fold at Sava Jarun location, 3.9 fold at Oborovo, 4.8 fold at Lukavec Posavski and 7.2 fold at Jasenovac. The highest value (20.7 pmol/min/mg_{PROT}) obtained for Jasenovac was ~50% of maximal EROD activity measured in fish laboratory treated with model EROD inducer α -naphthoflavone (25 mg/kg). The same trend as for EROD activity was observed for the amount of CYP1A protein. Significantly higher bioactivation potential was measured at all locations in comparison to control site. PAH bile metabolites did not follow the pattern observed for the EROD activities and bioactivation potential. In addition, the results described will be compared with data available for the same set of biomarkers measured in other European rivers and/or monitoring programs.

IL-19

UČINAK KOLEBANJA ČIMBENIKA OKOLIŠA NA BIOLOŠKE
 POKAZATELJE STRESA U DAGNJI *M. galloprovincialis*

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ESTROGENICITY OF WATER SAMPLES FROM ADRIATIC COAST AND FROM MUNICIPAL SEWAGE TREATMENT PLANTS IN CROATIA USING YEAST ESTROGEN SCREEN BIOASSAY

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In the nineties the endocrine disruption was observed in wild populations of male fish, by measuring vitellogenin induction and feminization of reproductive organs. This generated growing concern that hormonally active agents (i.e. endocrine disruptors) may cause harmful endocrine effects in aquatic organisms. Chemical analysis has revealed the presence of various substances that possess estrogenic activity, like natural and synthetic estrogens, phytoestrogens and industrial chemicals «xenoestrogens» (alkylphenols and their metabolites, pesticides, UV-filters) in effluents of sewage treatment plants (STP). In this survey we used Yeast estrogen screen (YES) bioassay to determine for the first time xenoestrogen potential of environmental samples from Croatia. This assay uses recombinant yeast *Saccharomyces cerevisiae* transfected with hER (human estrogen receptor) to measure agonists and antagonists of binding on ER. Estrogenic activity for each sample was determined by comparing dose response curves of samples with reference dose response curve of 17 β -estradiol (E2). Estrogenic potential of samples were finally expressed in E2 equivalents (EEQ). Results showed that maximal determined concentration of EEQ in STP effluents did not exceed 0.25 ng/L (1 pM E2) what is very low when compared with other similar surveys of waste water samples in Europe. In the second study xenoestrogen potential of coastal water samples collected seasonally from 24 locations along the Adriatic coast was determined using YES bioassay. Results revealed seasonal variation in EEQ concentrations of xenoestrogens with average concentration of 5.3 ng/L EEQ in March, 46.2 ng/L EEQ in June and 40.7 ng/L of EEQ in August, and the highest 80.7 ng/L of EEQ determined in October.

OP-55

KONCENTRACIJA METALOTIONEINA I METALA (Zn, Cu, Cd) U CITOSOLU JETRE KLENA (*Leuciscus cephalus* L.) IZ RIJEKE SAVE

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Na postajama rijeke Save od Otoka Samoborskog do Jasenovca (rujan, 2005.) određeni su biometrijski parametri (dužina, masa klena i masa jetre kao glavnog detoksifikacijskog organa), starost i spol klena (*Leuciscus cephalus* L.), odabranog kao bioindikatorskog organizma. Također, u citosolu jetre provedena su ispitivanja razine metala (Zn, Cu, Cd) i metalotioneina (MT) kao biomarkera izloženosti metalima. Ustanovljena je visoka pozitivna korelacija biometrijskih parametara ($p < 0,01$). U citosolskoj frakciji izoliranoj centrifugiranjem homogenata tkiva na 50000xg, određena je koncentracija metala (atomska apsorpcijska spektrometrija) i MT (diferencijalna pulsna voltametrij). Usporedbom koncentracija metala u citosolu jetre klena na odabranim postajama uočeno je da su uzvodno od Zagreba povećane razine Zn i Cu, dok se razine Cd povećavaju nizvodno od Zagreba, s najvišim vrijednostima na postaji Jasenovac. Koncentracije MT ne razlikuju se značajno među postajama, ali su više vrijednosti zabilježene na Jasenovcu gdje je povećana i razina Cd. Statistička analiza rezultata za svaku postaju ukazuje na pozitivnu korelaciju MT i esencijalnih metala (Zn, Cu). Samo u jedinkama s postaje Oborovo ustanovljena je statistički značajna korelacija svih promatranih parametara (MT, esencijalnih metala Zn i Cu i biometrije). Za pravilnu interpretaciju razine MT i izloženosti klena metalima, neophodno je sustavno praćenje i prikupljanje većeg broja podataka radi definiranja osnovne razine tog biomarkera.

METALLOTHIONEIN AND METAL (Cu, Zn, Cd) CONCENTRATIONS IN LIVER CYTOSOL OF CHUB (*Leuciscus cephalus* L.) FROM SAVA RIVER

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Biometric parameters (length, mass of chub and liver as main detoxification organ), age and sex were determined in chub (*Leuciscus cephalus* L.), selected as bioindicator organism in Sava river, from Otok Samoborski to Jasenovac (September, 2005). Also, metal (Zn, Cu, Cd) and metallothionein (MT) levels as biomarker of metal exposure were measured in cytosolic fraction of liver. Biometric parameters have positive and significant correlation ($p < 0,01$). In the cytosolic fraction of liver, prepared by centrifugation of tissue homogenate at 50000xg, metals (atomic absorption spectrometry) and MT (differential pulse voltammetry) were measured. Comparison of metal levels in liver cytosol from selected locations indicated to higher values of Zn and Cu upstream of

Zagreb, while Cd levels increased downstream of Zagreb, with the highest value at Jasenovac. MT levels at different locations were comparable, except at Jasenovac where the highest values were also recorded with higher Cd. Statistical analysis of results at each sampling site indicate to the correlations between MT and essential metals (Zn, Cu). Statistically significant correlations between all parameters (MT, essential metals Zn and Cu and biometry) were detected on Oborovo location. More data need to be collected for correct interpretation of MT levels and chub exposure to metals, in order to define constitutive level of that biomarker.