HRVATSKO MIKROSKOPIJSKO DRUŠTVO

POZIV NA 243. SASTANAK

Hrvatskog mikroskopijskog društva, koji će se održati u prostorijama **Fakulteta za kemijsko inženjerstvo i tehnologiju, Marulićev trg 20, mala predavaonica (I. kat)**, u

utorak, 29. siječnja 2019. u 16:00 sati
u organizaciji J. Macan i G. Kovačevića

uz sljedeći

D n e v n i r e d:

1. **Sjećanje na Marijana Tudju** (4. 8. 1942. – 24. 12. 2018.)

2. Izlaganja stipendista za IMC19 u Sydneyu:

Daniela Petrinec (PMF, BO): Visualisation of hunting nets formed by algae: a perfect hunting mechanism?

Petra Tramontana (PMF, BO): Free-living alga *Chlorella vulgaris* as freshwater ecosystem inhibitor?

Josip Barišić (IRB): The use of histopathological semi-quantitative scoring approach in zebrafish embryo toxicity tests

Vilko Mandić (FKIT): Regeneration performance of the nanostructured titania photocatalyst prepared by anodic growth

3. Razno

Tajnica:
Jelena Macan

Predsjednica:
Andreja Gajović

Visualisation of hunting nets formed by algae: a perfect hunting mechanism?

Petrinec, D.[1], Kovačević, G.[1], Tramontana, P.[1], Peharec Štefanić, P.[1] and Špoljar, M.[1]

[1] University of Zagreb, Faculty of Science , Department of Biology, Croatia

Green Hydra (*Hydra viridissima* Pallas, 1776) is a typical example of an endosymbiotic organism. In gastrodermal myoepithelial cells it contains unicellular photoautotrophic algae. Algae and especially microalgae take an important place as the primary producers and food resources in aquatic ecosystem sand are the basis of many food chains.Class Turbellaria is the most primitive group within the phylum Platyhelminthes. Turbellarians generally locomote by coordinated waves of cilia on a secreted mucus trail, though some species can swim by rhythmic muscle contractions.
In this experiment, the interaction between endosimbiotic algae *Desmodesmus subspicatus* (Chlorophyceae) (Chodat) Hegewald and Schmidt isolated from green hydra and predatory turbellarian species *Polycelis felina* (Dalyell, 1814) and *Dugesia gonocephala* (Duges, 1830) was observed at different light and temperature conditions: at 25 °C and exposed to daylight (photperiod 8 hs light, 16 hs dark) and at 13.5 °C in the dark, both separately with starved and fed animals.The results were recorded immediately after the experiment setup,and every 1, 8 and 24 hs after the beginning of the experiment, including the controls. Cladoceran *Daphnia magna* (Straus, 1820) was added to each glass dish as a prey (10 individuals). There were 5 replicas for each experiment, and the controls: altogether 220 dishes and 1540 animals. Hunting nets were visualized by adding the constant amount of algal suspension to each glass dish.For analysis of algae, light and cTEM microscopy were used. In this micro-ecosystem, turbellarians were characterized by phenomenon of mucus network emerging by the interaction with algae. The appearance of mucus and the formation of complex network occurred between 1 and 8 hs after the experiment setup.It was observed that the appearance of the nets was intensive due to the presence of algae this way changing the interactions within fresh water micro-ecosystem.The development of the nets was noticed even when only algae were present. But, in interaction with turbellarians especially with starved *P.felina* at the 13.5 °C this effect was much more intensive. Mostly after 8 hs *Daphnia* specimens were trapped within the nets but some remained alive and some of them managed to escape. After 24 hs within dishes where the nets were formed the highest density of dead *D.magna* was observed. There is a clear correlation between *D. Magna* density, algae and formation of the nets. The interaction of endosimbiotic algae and turbellarians created a mechanism that potentially gives more efficient hunting and stronger predatory role for turbellarians in the fresh water ecosystems. Further microscopic analyses could provide the insight into the complex formations of the hunting nets and food web interactions.

Free–living alga *Chlorella vulgaris* as freshwater ecosystem inhibitor?

Goran Kovačević, Petra Tramontana, Daniela Petrinec, Petra Peharec Štefanić, Maria Špoljar

Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia

Free-living unicellular algal species *Chlorella vulgaris* Beij. [K&H, 1992] strain SAG 211 - 11b is often found in freshwater habitats and overall shows high adaptability. Turbellaria are one of the traditional sub-divisions of the phylum Platyhelminthes (flatworms). As predators they inhabit freshwater or moist terrestrial environments. They move by cilia on the ventral dermis, allowing them to glide along on a film of mucus.
In this experiment were used free-living unicellular photoautotrophic alga *C. vulgaris* and turbellarians *Dugesia gonocephala* (Duges, 1830) and *Polycelis felina* (Dalyell, 1814). The influence of algae on the behavior of turbellarians was assessed.

Algae were cultured on a sterile deep stock agar and were growing in test tubes of 16 cm in length and 15 mm in diameter in air chamber under sterile conditions at 24ºC and constant light. By standardizing the method of maintaining algal culture, a constant amount of clonal cultures suitable for performing the experiment was obtained. Turbellarians were isolated from their natural habitat, cultivated at 13.5 ºC and fed with aquatic crustaceans *Artemia salina*. The experiment was conducted under different temperature and light conditions: at 25 ºC and exposed to daylight (photoperiod 8 hs light, 16 hs dark) and at 13.5 ºC in the dark. Different number of turbellarians was added in experimental dishes (60 ml): 5 dishes with one specimen in each (first setup with *P. felina* and second with *D. gonocephala*) and 5 dishes with 5 specimens in each (first setup with *P. felina* and second with *D. gonocephala*) at both temperatures, including both fed and starved animals separately, and in both sets with constant concentrations of *C. vulgaris* added to each experimental dish. The results were recorded immediately after the experiment setup and every 1, 8 and 24 hs after the beginning of the experiment. There were 5 replicas for each experiment, including controls. Altogether 170 experimental dishes, 240 *P. felina* and 240 *D. gonocephala* individuals were used. For analysis of algae, light and cTEM microscopy were used.

After one hour noticed was the sediment of algae formed at the bottom of each experimental dish as well as the inhibition of turbellarians *D. gonocephala* by *Chlorella*, mitigating their movement and coordination. In the suspension of algae, *D. gonocephala* exhibited extremely numb behaviour. Individuals that were sporadically moving had extremely slow and uncoordinated behaviour and this was specifially expressed only on the walls of the glass dish where no algal sediment was present. *D. gonocephala* that rested, mostly rested in the algal sediment at the bottom of the dish. This phenomenon of inhibition of motion and coordination was also present in *P. felina*, but it was extremely expressed in *D. gonocephala*.

The use of histopathological semi-quantitative scoring approach in zebrafish embryo toxicity tests

Barisic, J.[1], Babic, S.[1], Klobucar, G.I.V.[2], Topic Popovic, N.[1], Sauerborn-Klobucar, R.[3], Strunjak-Perovic, I.[1] and Coz-Rakovac, R.[1]

[1] Ruder Boskovic Institute, Croatia, [2] Faculty of Science, Croatia, [3] Pathcon Laboratories, Croatia

Zebrafish (*Danio rerio*) has proven to be a highly useful model organism in many toxicological studies, and is the ideal experimental organism in ecotoxicology and pharmacology. Nowadays, due to the strict regulations in animal experiments ethical considerations and implementation of 3R principles, the early life-stage test using the zebrafish embryo, up to 120 hours post fertilisation, become one of the most widely used tools for understanding of how chemicals in our environment affect aquatic organisms. Despite the increasing use of novel molecular techniques in pathology, histopathology has initially been and is still used as standard diagnostic tool in human and veterinary medicine. However, multi-parametric and semi-quantitative scoring of a lesion magnitude has become a common approach to handle histopathologic information in biomedical research in order to obtain more relevant and statistically significant results.

Experimental setup included 48 hours exposure of zebrafish embryos to wastewaters collected within and downstream of the WWTP, and evaluation of toxic effects using biomarkers on tissue and the whole organism level during an early onto-genesis. In this study, we focus on the proposal of a new approach concerning the application of histopathology scoring system using light microscopy imaging. First, histopathological alterations of zebrafish embryo eye, brain, trunk and tail were described in detail. Second, to all observed histopathological alterations a reaction pattern was given (1 - 3) according to the pathological severity (Table 1). Next, each alteration was assessed and quantified using a scoring system ranging from 0 to 6, depending on the degree and extent of the alteration as follows: (0) none; (1 - 2) lower incidence; (3 - 4) medium incidence and (5 - 6) high incidence. Intermediate values were also considered. Scored index for each alteration was obtained by multiplying histopathological alteration index with severity, and a body part score was derived from the sum of scored indexes.

Using this approach, we were able to observe statistically significant differences between embryos exposed to different samples including the degree of alterations between different parts of the embryo. This procedure resulted in a much more sensitive semi-quantitative histopathological assessment of waste water induced changes in zebrafish embryo tissues. Obtained results showed that using this novel approach on zebrafish embryos can be used as a valuable tool for potential toxic evaluation of waste waters. Therefore, we encourage other researchers to use this scoring system for ecotoxicological assessment using zebrafish embryos.

Table1. Reaction pattern and scoring system for semi-quantitative histopathological alteration assessment

Scoring system	**Reaction pattern index**		
	1	**2**	**3**
Brain	Irregular cell compactness	Cloudy swelling of brain tissue	Reduced brain tissue mass
	Dilated brain ventricles	Nuclear alteration of brain tissue	Defective organization in the forebrain/midbrain/hindbrain
Eye	Poorly organized retina	Undeveloped eye cup	Eye deformity
	Undeveloped lens	Lens dysplasia	Necrosis
Trunk	Tissue blood accumulation	Lifting of muscle fibres from basal membrane	Yolk sac oedema
	Disrupted myofibril architecture/structure	Notochord deterioration	Necrosis
Tail	Blood accumulation	Lifting of muscle fibres from basal membrane	Curving of tail tip
	Disrupted myofibril architecture/structure	Notochord deterioration	Tail tip necrosis

Physical Sciences (PS3 - Thin films, coatings and surfaces)

Regeneration performance of the nanostructured titania photocatalyst prepared by anodic growth

Mandic, V.[1], Panzic, I.[2], Plodinec, M.[2] and Brnardić, I.

[1] Faculty of Chemical Engineering and Technology, Marulićev trg 19, HR-10000 Zagreb, Croatia

[2] Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia

[3] Faculty of Metallurgy, Aleja narodnih heroja 3, HR-44000 Sisak Croatia

Previously we observed the possibility to modify the titania nanotube anodization process to yield highly effective photocatalysts with reduced level of order, i.e. increased microstructural inhomogeneity at horizontal level and chemical inhomogeneity at vertical scale. Basically the defects induced on behalf of such modification favour high fotocatalytic activity. Here we investigate the durability and reusability of the as-prepared photocatalyst samples. The photodegradation of acetylsalicylic acid was repeated several times using the same selected samples. Results were modelled and photocatalyst lifetime was discussed. Vibration spectroscopies and depth sensitive diffraction techniques were used to monitor the samples before and after the extensive use in order to evaluate the contribution of the as-formed different areas in the final photocatalytic efficiency. Samples retain photocatalytic properties for reasonably long time. Here we test a novel concept of photocatalyst regeneration where the used samples were electrochemically re-synthesized in order to enable new activation in degradation efficiency. The reactivated photocatalyst was subjected to degradation tests to show remarkable photocatalytic activity regeneration. For the first time the cross-section HR electron microscopy of cycled and then regenerated samples offered the insight in the complex microstructural and chemical evolution on behalf of photodegradation and anodic re-growth. This novel concept may be considered as ground-breaking in terms of facile, cheap and environmental photocatalytic micro-pollutants removal.

The financial support of the Croatian Microscopy Society and CSF project No 9419 are gratefully acknowledged.

In memoriam na Dr. Sci. Marijana Tudju
(O. Milat, na sprovodu 31. 12. 2018.)

Dragi Marijane,
U ovom trenutku po Tvojoj smrti, obraćajući se Tebi zapravo se obraćam svima nama ovdje prisutnima, Tvojima najbližima, Tvojim prijateljima, i suradnicima.

Govorim u ime Hrvatskoga mikroskopijskog društva želeći Ti izraziti zahvalnost što si bio naš član. Izraziti našu zahvalnost Tebi, a istovremeno iskazati naš ponos i zadovoljstvo nas u Društvu što smo imali čast i sreću družiti se s Tobom kao znanstvenikom, stučnjakom i čovjekom.
Nesumljivo Ti pripada čast što si davne 1984. godine bio u grupi od desetak nas osnivača tadašnje Sekcije za elektronsku mikroskopiju Hrvatskoga prirodoslovnog društva, a koja je tijekom 90-tih prerasla u sadašnje Hrvatsko mikroskopijsko društvo. Oduvijek smo se s veseljem družili na brojnim mjesečnim sastancima, i više smo puta s radoznalošću odslušali Tvoja predavanja.
Odajemo Ti zahvalnost što si od početka radio u Organizacijskom odboru tadašnjega "5. Jugoslavenskoga simpozija iz elektronske mikroskopije" na Plitvicama 1986.g. Značajno si doprinijeo uspješnosti toga događaja kako prezentiranjem svoga znanstvenog rada tako i popratnim druženjima u opuštenoj i prijateljskoj atmosferi.
Sudjelovao si, predstavljajući naše Hrvatsko mikroskopijsko društvo, i u nizu kasnijih domaćih i međunarodnih znanstvenih i stručnih skupova, posebno u Drugom Hrvatskomu simpoziju 1996.g., u Prvom kongresu Hrvatskoga društva za EM 1999.g. u Zagrebu, i u Drugom u Topuskom 2006.g.
Sudjelovao si u seriji Multinacionalnih kongresa od Stare Lesne u Slovačkoj 1995., i Portoroža u Sloveniji 1997., preko Veszprema do Pule 2003.g., a o svom si radu izlagao i na drugim konferencijama izvan mikroskopijskog društva.

Ali, nije sada ovdje trenutak za detaljno nabrajanje svih Tvojih stručnih i organizacijskih aktivnosti, objavljenih radova i Tvog CV-a; to ćemo zasigurno napraviti drugdje, na prvom slijedećem mjesečnom sastanku našega mikroskopijskog društva.
Mi koji Te znamo još od rada u Željezari u Sisku, preko Chromosa, do Plive, često smo koristili Tvoje znanje, Tvoju vještinu i Tvoju stručnost. Jer, od tih osamdesetih i devedesetih godina bio si prvi i najbolji među nama u Hrvatskoj u istraživanjima materijala tehnikama takozvane "Scanning elektronske mikroskopije" i mikroanalize. Svima koji su od Tebe tražili stručnu pomoć pomagao si, ne samo svojim znanjem i umijećem, već i svojom otvorenom spremnošću, ljubaznosću i dobrotom.
Nisi bio od onih koji bi se nametali, uguravali, i svojatali rezultate i postignuća drugih, nego upravo suprotno; svoje si rezultate velikodušno prinosio u zajednička stručna znanja kako u laboratorijima i firmama u kojima si radio, tako i k nama, u naše mikroskopijsko društvo.
Hvala Tebi i Tvojima najbližima što si bio takav znanstvenik i čovjek da smo iz poznanstva i druženja s Tobom i mi imali važnu životnu dobit; obogatili smo svoju stručnost, i uživali smo kao ljudi.

Ovdje je sada trenutak kad mogu reći da ćemo uspomenu na Tebe čuvati u našem sjećanju; da ćemo to svoje sjećanja prenijeti na mlađe suradnike i članove našega društva; da ćeš u našem sjećanju trajno biti

i ostati ne samo kao izvrstan znanstvenik, stručnjak, i znalac svoga posla, nego ćemo Te još više pamtiti kao ljubaznog i susretljivog suradnika, i velikodušnog čovjeka. Hvala Ti!