

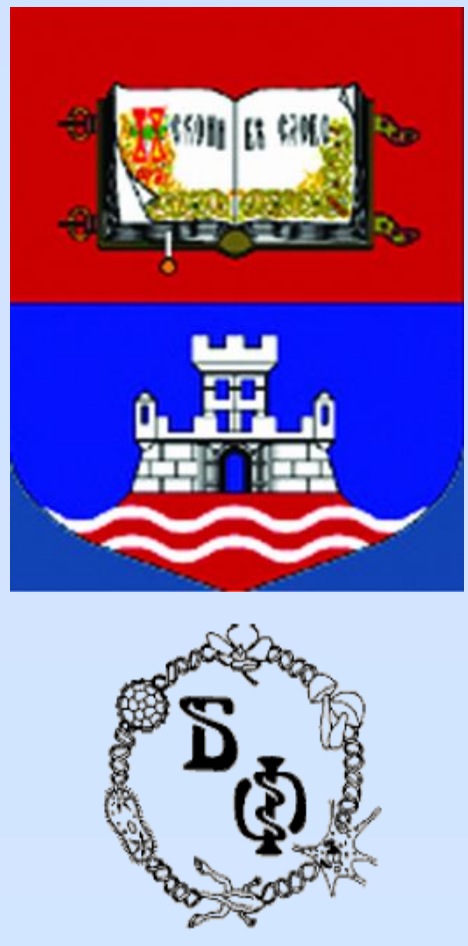
FRESHWATER MUSSELS IN THE ECOGENOTOXICOLOGICAL STUDIES-APPLICATION OF COMET ASSAY

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"This project received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n. 2652

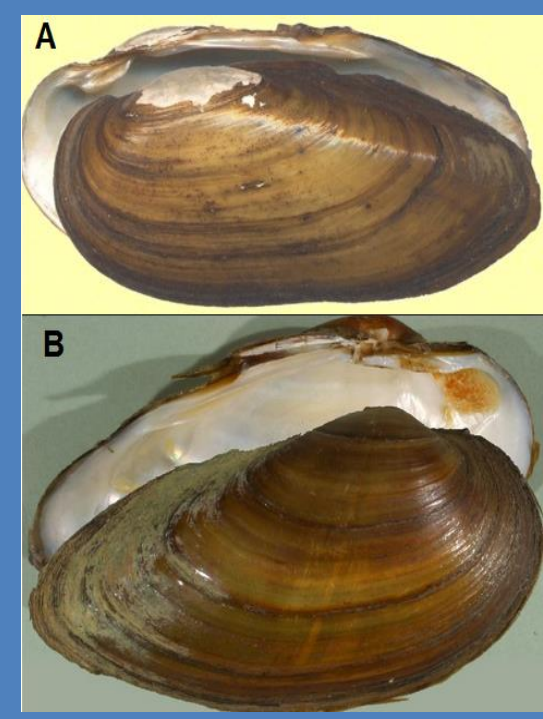


Introduction

Presence of pollutants in aquatic ecosystems can be detected by a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status and DNA integrity. The integrity of cellular DNA is continuously attacked by various agents resulting in DNA lesions. Unrepaired DNA lesions may block replication and transcription, potentially leading to cell death or generating mutations. The main objectives of ecogenotoxicology studies are detection of the origin of genotoxic pollution and their effect on individuals and populations.

Freshwater mussels

Mussels are commonly employed in the ecogenotoxicological studies. They have several characteristics, such as wide distribution, filter feeding, a sessile life form and an ability to accumulate pollutants, which makes them favorable organisms for estimating the environmental pollution level and the bioavailability of various types of pollutants. Different approaches are used for assessing the conditions of ecosystems, the passive (collection of mussels from selected locations) and the active biomonitoring (subsequent exposure of mussels from unstressed populations at polluted sites). Moreover, in *ex situ* assessment, the genotoxic effect of different compounds can be detected in laboratory conditions.

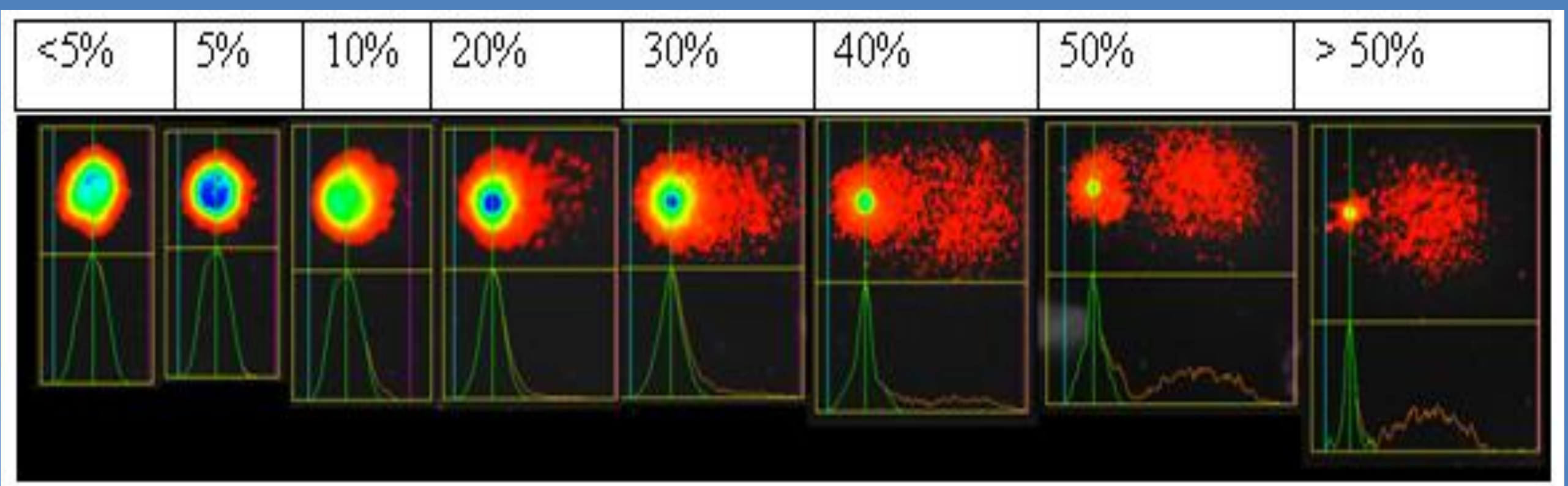


Unio pictorum (A) and *Unio tumidus* (B)

Comet assay

The comet assay (single cell gel electrophoresis, SCGE), is a sensitive and rapid technique for detection of DNA damage in individual cells based on the migration of denatured DNA during electrophoresis (damaged nuclei form comet-like shapes). It has been accepted as one of the major tools for assessing pollution related genotoxicity in aquatic organisms. In our experiments we performed alkaline version of comet assay described by Singh et al. (1988).

Images of 250 nuclei per each concentration of test substance were analyzed with a fluorescence microscope and scored using analysis software (Comet Assay IV Image analysis system, PI, UK). Tail intensity and Olive tail moment are chosen as relevant measure of DNA damage.



Micrographs of the scored nucleoids of haemocytes. Above micrographs approximate values of TI% are shown. The higher damage it is the higher TI% will be.

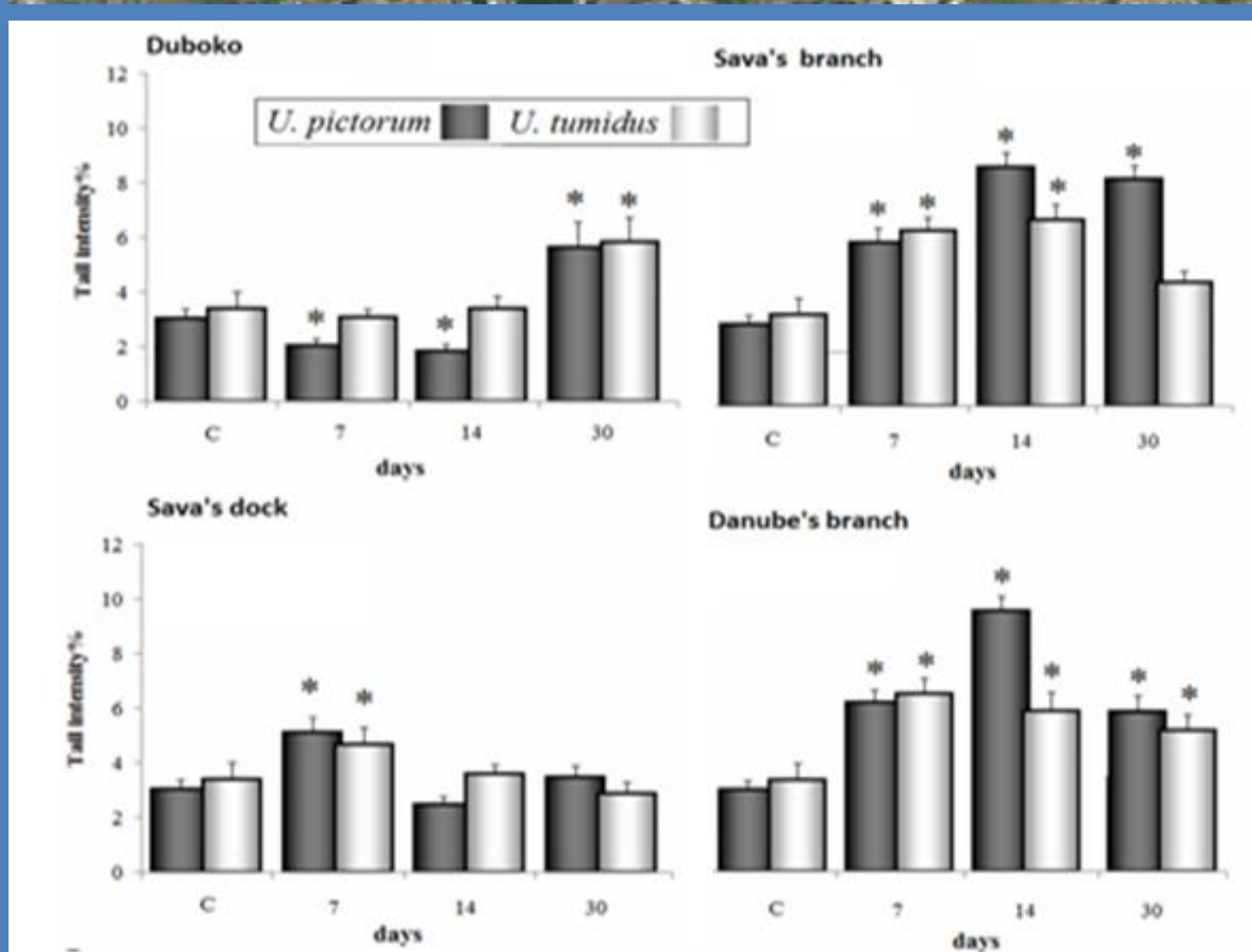
Application of mussels in biomonitoring

In situ

Ex situ

Active biomonitoring:

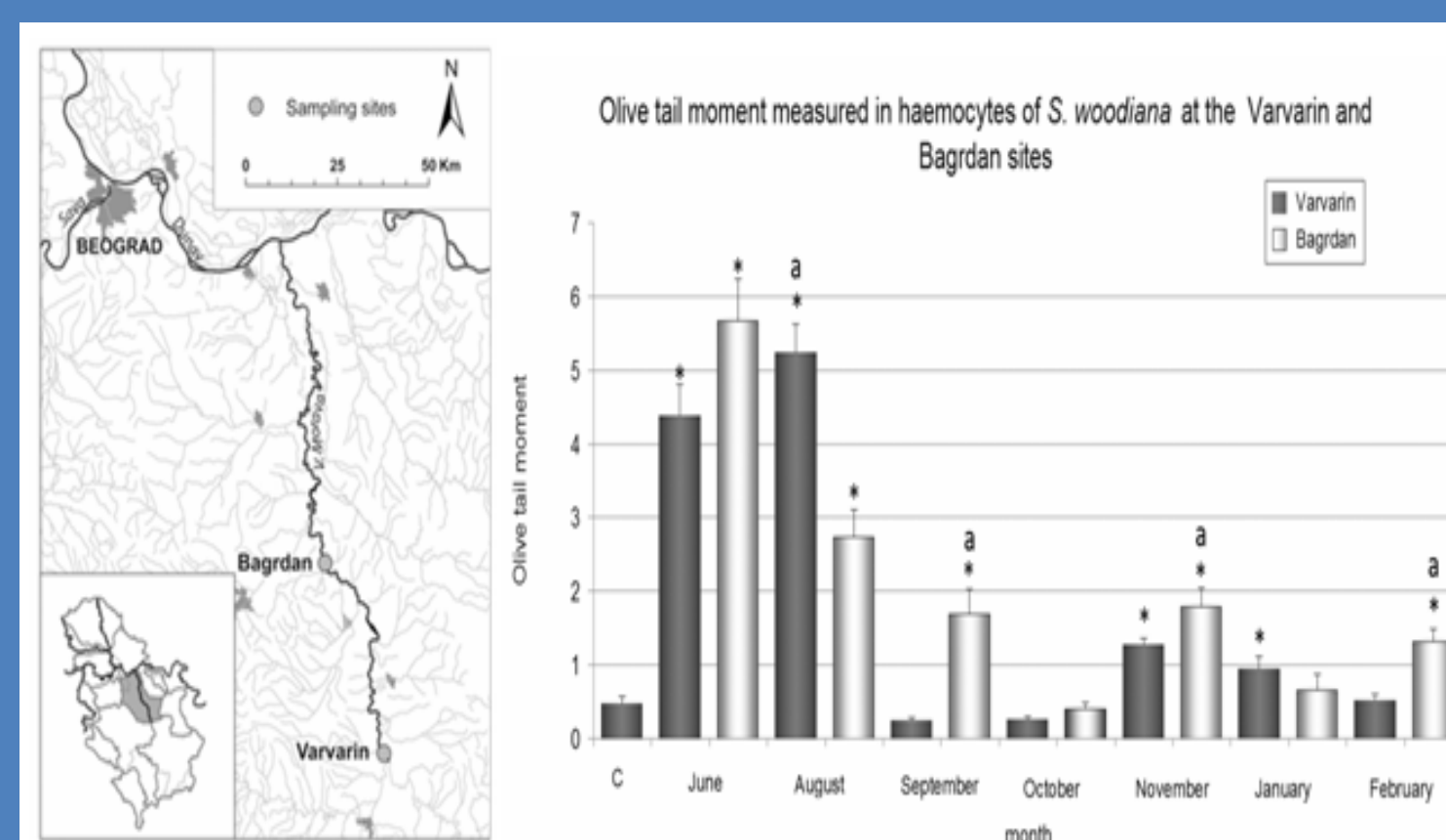
- Active biomonitoring implies translocation of specimens from unstressed populations to selected sites of interest.
- In active approach issues related to availability of specimens and effects of age, gender and physiological condition are avoided.
- This approach enables research on highly polluted sites.
- Specimens of *Unio* sp. were exposed at selected sites (Figure below) at the Sava and Danube rivers which are under the impact of untreated wastewaters of Belgrade city. Haemolymph was sampled after 7, 14 and 30 days of exposure (Results presented on figure below).



Vuković-Gačić et al. (2013) Comparative study of genotoxic response of freshwater mussels *Unio tumidus* and *Unio pictorum* to environmental stress. Hydrobiologia DOI 10.1007/s10750-013-1513-x

Passive biomonitoring:

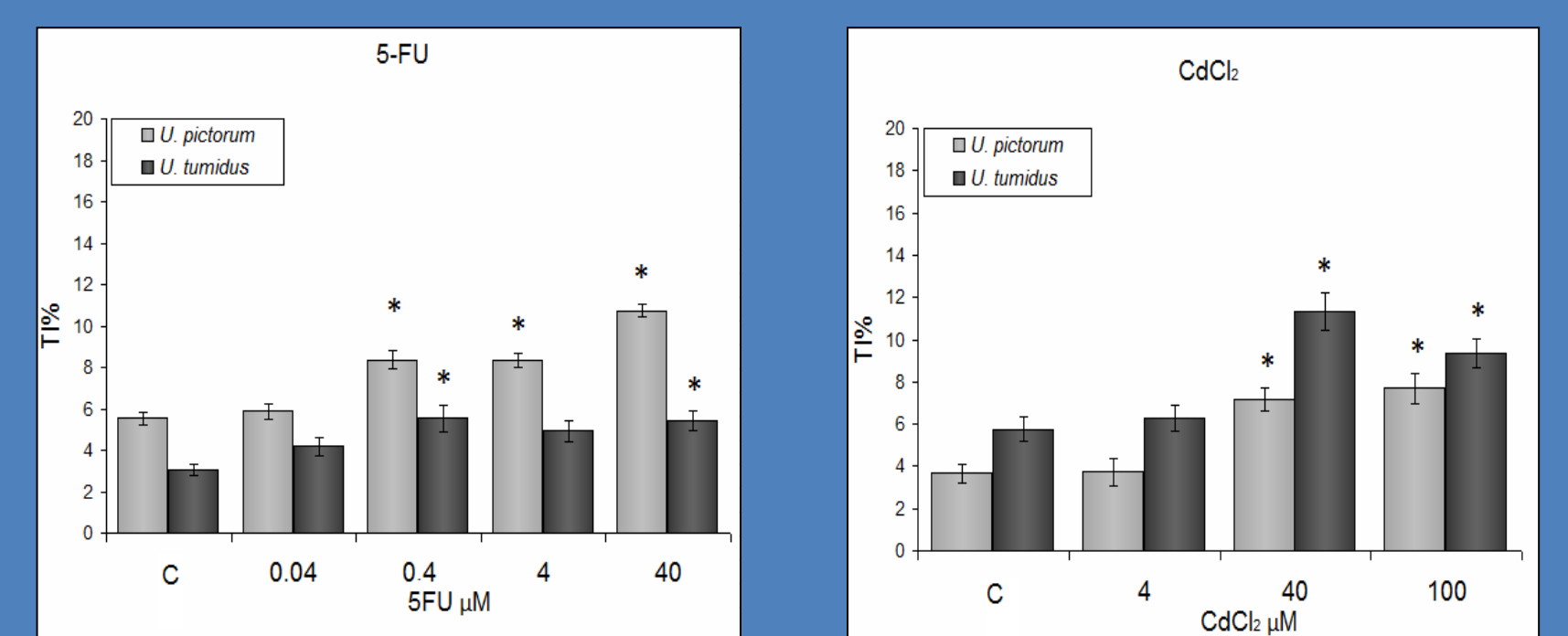
- Passive biomonitoring implies collection of specimens which inhabit selected sites of interest.
- Easily performed, suitable when research area is situated far from the laboratory.
- Study was performed on the Velika Morava River, significant Danubes tributary.
- Specimens of *Sinanodonta woodiana* collected monthly on two sites with different pollution pressure.
- Significant correlation was detected between the level of DNA damage in haemocytes and concentration of heavy metals in water.
- Seasonal variation in the level of DNA damage was observed, results are shown on the figure below.



Kolarević et al. (2013) Monitoring of DNA damage in haemocytes of freshwater mussel *Sinanodonta woodiana* sampled from the Velika Morava River in Serbia with the comet assay. Chemosphere 93, 243-251.

Cytostatics as emerging pollutants

- Studies performed *ex situ* are significant research tool for prediction of the effect of single compounds in environment.
- Obtained data are useful for risk assessment.
- Among emerging pollutants, cytostatics represent hot topic as serious threat to non-target organisms due to their cytotoxicity, genotoxicity and mutagenicity.
- As part of research for Cytotreat project (EU Commission, FP7) we are investigating impact of short term exposure to mostly used drugs: 5-Fluorouracil, Cisplatin, Etoposide Vincristine and Imatinib mesylate with Cadmium chloride as positive control (selected results represented on figure below).
- Data obtained so far indicate that cytostatics can induce increase in DNA damage in concentrations similar to ones measured in wastewater.



Gačić et al. (2014). The impact of *in vivo* and *in vitro* exposure to base analogue 5-FU on the level of DNA damage in haemocytes of freshwater mussels *Unio pictorum* and *Unio tumidus*. Environmental Pollution 191, 145-150.

Conclusions

Our studies indicate that freshwater mussels can be used as reliable bioindicators. Research performed *in situ* showed that selected species can detect genotoxic pollution present in environment. Results of the research performed *ex situ* showed harmful effects of environmentally relevant concentrations of cytostatics. Studies performed *ex situ* on mussels could be significant research tool for prediction of the effect of single compounds in environment.