EVALUATION OF RIVER WATER GENOTOXICITY WITH COMET ASSAY IN DIFFERENT TISSUES OF EUROPEAN CHUB

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PROCENA GENOTOKSIČNOSTI KORIŠĆENJEM KOMETA TESTA U RAZLIČITIM TKIVIMA KLENA IZ REČNE VODE

Apstrakt

Cilj ovog rada je bila analiza oštećenja molekula DNK primenom komet testa (engl. *Single Cell Gel Electrophoresis,* SCGE) na klenu (*Squalius cephalus* L.) kao potencijalnom model organizmu za procenu genotoksičnosti vode Kolubarskog basena. Kolubarski basen je bogat nalazištima lignita pa kao takav trpi veliki pritisak rudarskog postrojenja, "Kolubara", pored već postojećeg uticaja komunalnih voda kao i spiranja sa obradivih površina. Uzorkovanja su vršena mesečno tokom sezone 2011/2012 god. Jedinke klena sa Zlatarskog jezera, Uvac, su korišćene kao prirodna kontrolna populacija.

Komet test je osetljiva i relativno jednostavna metoda primenljiva na velikom broju različitih organizama i tkiva. Za detekciju oštećenja DNK molekula u ovom radu korišćena su tri tkiva: krv, jetra i škrge.

Ključne reči: *ekogenotoksikologija, komet test, klen, reke*
Keywords: ecogenotoxicology, comet assay, European chub, river

INTRODUCTION

The European chub, *Squalius cephalus*, an ubiquitous fish species displaying a wide ecological niche, feeding on a low trophic level and widely distributed all over Europe, offers possibilities to investigate various polluted contexts (Durand et al., 1999). The use of chub as bioindicator has already been reported in field studies assessing the environmental quality of inland waters differently impacted by anthropogenic activities (Winter et al., 2005; Randak et al., 2006; Krca et al., 2007; Pavlica et al., 2011). Several biomarkers have also been characterized in this species in response to chemical pollutants, including levels of DNA strand breaks (SB), ethoxyresorufin *O*-deethylase (EROD) activity, polycyclic aromatic hydrocarbon (PAH) metabolites in bile, DNA adducts, erythrocyte micronucleation, inhibition of acetylcholinesterase (AChE), activities of glutathione *S*-transferases (GST) and vitellogenin gene induction (Devaux et al.,1998; Flammarion et al., 2000; Machala et al., 2001; Winter et al., 2005; Randak et al., 2006; Krca et al.,2007, Frenzilli et al., 2008).

The comet assay, also referred to as the single cell gel electrophoresis assay (SCGE), is arapid, visual, and quantitative technique for measuring DNA damage in eukaryotic cells (Singh et al., 1988).

The aim of the present research was to evaluate the application of the comet assay on different tissues of *Squalius cephalus* as a model organism for monitoring the pollution of Kolubara basin.

MATERIAL AND METHODS

Field sampling was conducted at two sites (N 44◦26'05.26'' E 20◦15'22.95'', N 44◦29'40.67'' E 20◦17'07.88'') at Kolubara basin. Kolubara basin has rich deposits of lignite and hence the whole area is under intensive mining activity. Total of 65 specimens of chub were caught by electrofishing device ELEMAX SHX 2000 (SAWAFUJI). Average body length and weight were: 22.75±7.22 cm and 163.27±164.92 g.

Uvac River "Zlatar" reservoir was used as a reference site. This is a protected natural area of great importance with very low anthropogenic impact. Total of 11 specimens were used for analysis. Average body length and weight were: 23.89 ± 6.97 cm and 194.7 ± 217.87 g, respectively. The alkaline comet assay procedure was performed in accordance with the method described by Singh et al. (1988) but with some modifications (Sunjog et al., 2014). Results of the comet assay were compared by analysis of variance, one-way ANOVA (Stat Soft, , 2007). Results that yield $p < 0.05$ are considered border-line statistically significant.

RESULTS AND DISCUSION

Results presented in Fig. 1 represents DNA damage expressed with TI (tail intensity or % the DNA in tail), and shows the difference in level of DNA damage in three tissues (blood, liver and gills) at Kolubara basin compared with reference site, Uvac. All tissues were significantly different compared to reference site. Gills showed the best response as compared to other tissues. Gills may be more prone to injury than other tissues, due to a high respiratory blood flow and permanent contact with the water environment. Blood was less sensitive in comparison to other tissues. This might be due to circulation of blood cells in the bloodstream, which indicates that blood could be used as a biomarker only for acute

contaminations. It was determined that the turnover rate of fish erythrocytes is approximately 100 days (Devaux et al., 1998; Fischer et al., 1998; Buschini et al., 2004).

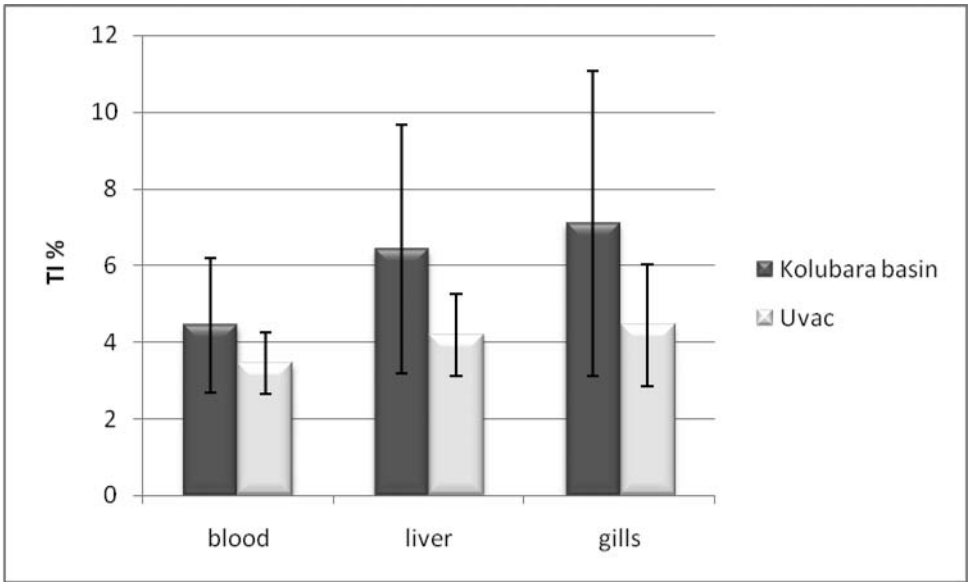

Figure 1. DNA damage of blood, liver and gills at Kolubara basin compared with reference site

Tissue specific responses are expected because of variations in alkali-labile sites and cell types with different background DNA single-strand break levels, due to variations in excision repair activity, metabolic activity, antioxidant concentrations, or other factors (Lee & Steinert, 2003). However, although the blood gave the lowest response to DNA damage compared to other tissues, it was still possible to observe the significant difference compared to Uvac.

CONCLUSION

Overall the results obtained in this study confirmed that *S. cephalus* can be used for the assessment of river basin genotoxicity based on comet assay. All tested biomarkers are sensitive and suitable for this type of research.

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