HOW FLOODING MODIFIES GENOTOXIC RESPONSE IN FRESHWATER FISH?

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KAKO POPLAVE MODIFIKUJU GENOTOKSIČNI ODGOVOR SLATKOVODNIH RIBA?

Apstrakt

U ovom radu ispitivan je potencijal dve vrste roda *Abramis* (*Abramis bjoerkna* i A*bramis sapa*) kao bioindikatora genotoksičnog potencijala vode reke Save primenom komet testa. Izabrani lokalitet pripada oblasti intezivne poljoprivredne aktivnosti, izložen je otpadnim vodama grada Obrenovca i nalazi se u blizini najveće termoelektrane u Srbiji, "Nikola Tesla". Uzorkovanje je vršeno od januara do avgusta 2014. godine, obuhvatajući mesec maj kada je došlo do intezivnih poplava u slivu reke Save. Dobijeni rezultati ukazuju da se ispitivane vrste mogu koristiti za procenu genotoksičnog potencijala.

Ključne reči: poplave, reka Sava, genotoksikologija, komet test, Abramis sp.
Key words: flooding, Sava River, genotoxicology, Comet assay, Abramis sp.

INTRODUCTION

Chemical compounds from various sources significantly contribute to the contamination of both, the water column and sediments in rivers (Vargas et al., 2001). Many of these substances exhibit genotoxic potential causing formation of lesions in DNA molecule (Theodorakis, 2001). Due to their possible toxicity, genotoxicity and bioaccumulation potential,

heavy metals are receiving significant attention (Višnjić-Jeftić et al., 2010; Sunjog et al., 2012). Chronic exposure of fish to sublethal trace metal levels causes among others disturbed ion regulation, reduced swimming speed and reduced growth (Bervoets and Blust, 2003). Species of the genus *Abramis (A. bjoerkna* and *A. sapa)* are widely distributed in many European and Asian freshwater ecosystems (fishbase.org). These cyprinid fishes are closely associated with superficial sediment due to their benthic way of life (Breukelaar et al., 1994). Flood events account for 40% of all natural disasters which are affecting many countries worldwide. Since the sediment plays an important role as a reservoir of many pollutants (Yang et al., 2008) it is considered that remobilization of sediments under the impact of flooding introduce pollutants into the water column (Ockenfeld et al., 2005) increasing their availability to aquatic organisms. Knowing that various pollutants may exhibit genotoxic potential, Comet assay or single cell gel electrophoresis (SCGE), a rapid and sensitive method for measuring the level of DNA damage in individual cells, has found a wide application in genotoxicity studies (Tice et al., 2000). The aim of this study was to investigate application of comet assay for the assessment of DNA damage in blood cells of two *Abramis* species (*A. bjoerkna* and *A. sapa*) and their potential use as bioindicator organisms. Considering that the flooding occurred during our sampling period (from January to August 2014) a parallel examination of the flooding potential has been made to modify effects of pollutants already present in the environment on the freshwater fish.

MATERIAL AND METHODS

Sampling was performed on a monthly basis, from January to August 2014, with exception of April, due to unfavorable environmental conditions, on the site Duboko, approximately 23 km upstream of the Belgrade city center. This site was chosen due to the exposure to various pollutant pressures, intensive agricultural activity and untreated waste waters from the town of Obrenovac. Moreover, the largest Thermal Power plant in Serbia „Nikola Tesla" and associated ash fields are situated a few kilometers upstream from the sampling site. Physical and chemical parameters were measured on site. Present levels of metals micro constituents (Zn, Cu, Cr, Cd, Ni, As), total and dissolved, were obtained from the Agency for Environmental Protection (Serbia). For the analyses of microbiological water quality indicators of fecal pollution were monitored. Total coliforms (TC) and *E. coli* (EC) were determined by Most Probable Number (MPN) using Colilert 18 (IDEXX, Ludwigsburg, Germany). *Enteroocci* (EF) were determined by the MPN using MUD/SF microtiter plates (BIORAD, Vienna, Austria) according to ISO 7899-1:1998. For the isolation of presumptive *Clostridium perfringens* (CP) membrane filtration method and incubation on the tryptose-sulfite-cycloserine (TSC) media were applied, according to ISO 14189:2013. The study was carried out on the blood samples from the genus *Abramis* species *(A. bjoerkna*/*A. sapa*), total of 32 specimens ranging in length from 15-22.5 cm, and in weight 31-139 g. To obtain the baseline level of DNA damage we have implemented acclimation strategy, successfully applied in our previous studies (Kolarević et al., 2013; Vuković-Gačić et al., 2014). Cells viability was assessed with differential Acridin orange/Ethidium bromide staining. Under fluorescence viable cells appear uniformly stained in green, whereas cells with orange/red nuclei are considered dead (Squier and Cohen, 2001). The alkaline comet assay was performed according to the method of Singh et al. (1988) with slight modifications (Aborgiba et al., 2015). For statistical analysis data were analyzed using Statistica 6.0 Software (StatSoft,

Inc.). Kolmogorov-Smirnov test for normality of distribution was used for the OTM values. Non-parametric Man-Whitney U test was applied with significance level $p < 0.05$ for differences between samples and corresponding negative control (acclimation). Correlations between the level of DNA damage and concentrations of heavy metals in water were investigated using Spearman correlation test ($p < 0.05$).

RESULTS

The highest water level was recorded in May, when flooding occurred in the Sava River Basin (Fig. 1-a). As soon as the flooding occurred, the majority of inhabitants from the town of Obrenovac were evacuated. During the most critical months of flooding (May and June) the highest concentrations of heavy metals in water (Mn, Cd, Pb, Co, As and B) were recorded. High positive correlation was shown between the water level and concentrations of Ni ($r = 0.81$), Cd ($r = 0.71$), Co ($r = 0.65$), Mn ($r = 0.63$) and Pb ($r = 0.61$). In the month with the highest water level (May) the highest concentrations of TC were observed, while during this and following month there has been a drastic decline in the concentrations of fecal pollution indicator bacteria - EC, EF and CP (Fig. 1-c). Additionally, significant negative correlation was observed between the water level and EC concentrations ($r = -0.86$). Viability of blood cells ranged from 87-100%. Compared to the baseline level of DNA damage (OTM = 2.2 ± 0.2), obtained after 10 days of acclimation, the highest level of DNA damage was recorded in June, while March was the month with the lowest level of DNA damage (Fig. 1-b). Significant positive correlation was observed between the DNA damage level and concentration of dissolved arsenic ($r = 0.76$) and high for total arsenic concentrations ($r = 0.71$). DNA damage level was in negative correlation with Zn levels ($r = -0.79$), with the level of $NH4^+$ ($r = -0.77$), EF ($r = -0.79$) and CP ($r = -0.79$).

DISCUSSION

In this study we have assessed the level of DNA damage in blood cells of two *Abramis* species (*A. bjoerkna* and *A. sapa*) and their potential use in genotoxicity monitoring of the Sava River. Extremely unfavorable hydrological conditions led to the occurrence of flooding in May 2014. This caused the evacuation of the entire town of Obrenovac thus excluding the impact of domestic waste water on the studied site. Due to this sudden decrease in concentrations of main fecal indicator bacteria was observed. This is evidenced with the observed negative correlation between fecal indicator bacteria concentrations and the water level.

On the other hand the impact of the Thermal Power Plant "Nikola Tesla" and the ash fields increased. It is well established that flooding leads to sediment movement and perturbation, causing the release of many hazardous substances already present in the environment, such are heavy metals and metalloids (Wölz et al., 2009). When the flood wave reached its peak, during May and June, the highest concentrations of Cd, Co, Mn, Pb, As and B were recorded. The study of Kostić et al. (2012) is dealing with the presence of majority of these elements in the fly ash disposal field of power plant "Nikola Tesla". Thus, it could be assumed that they were displaced when flooding occurred which elevated their concentrations in water. This is supported with the observed positive correlation between concentrations of Ni, Cd, Co, Mn, Pb and water level. The level of DNA damage in blood cells significantly

correlated with dissolved arsenic in water, and highly correlated with total arsenic level. The study of Ramírez and García (2005) reported that arsenic induced increase in micronucleus frequencies in gill cells of zebra fish (*Danio rerio*). Significant negative correlation between DNA damage level and zinc levels in water is not surprising, since according to Galaris and Evangelou (2002) zinc appears to prevent and reduce oxidative stress. Negative correlation between the concentrations of fecal indicator bacteria EF, CP and $NH4^+$ concentration in water points out that observed genotoxic effects in fish should not be linked to wastewaters of the town Obrenovac.

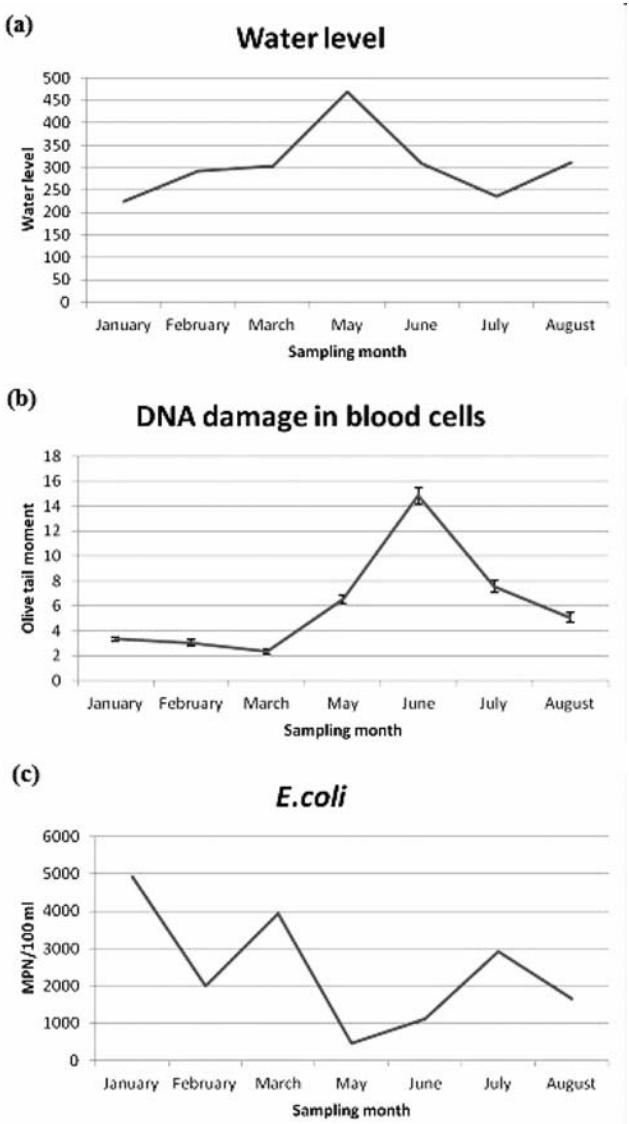

Figure 1. Comparative view in variation pattern of water level (a), DNA damage in blood cells (b) and *E.coli* concentration (c), during sampling period.

CONCLUSIONS

Obtained results show that examined species of the genus *Abramis* may be useful organisms for the assessment of genotoxic potential by the comet assay. Additionally, we showed the potential of flooding to modify water quality by remobilisation of pollutants already present in the environment which is reflected on the DNA damage in studied organisms.

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REFERENCES

Aborgiba, M., Kostić, J., Kolarević, S., Kračun-Kolarević, M., Elbahi, S., Knežević-Vukčević, J., Lenhardt, M., Paunović, M., Gačić, Z., Vuković-Gačić, B. (2015): Flooding modifies the genotoxic effects of pollution on a worm, a mussel and two fish species from the Sava River. Science of The Total Environment, In press, doi:10.1016/j.scitotenv.2015.03.120

Bervoets, L., Blust, R. (2003): Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. Environmental pollution, 126(1): 9-19.

Breukelaar, A. W., Lammens, E. H., Breteler, J. G. K., Tatrai, I. (1994): Effects of benthivorous bream (*Abramis brama*) and carp (*Cyprinus carpio*) on sediment resuspension and concentrations of nutrients and chlorophyll a. Freshwater Biology, 32(1): 113-121.

Galaris, D., Evangelou, A. (2002): The role of oxidative stress in mechanisms of metal-induced carcinogenesis. Critical reviews in oncology/hematology, 42(1): 93-103.

http://www.fishbase.org/

Kolarević, S., Knežević-Vukčević, J., Paunović, M., Kračun, M., Vasiljević, B., Tomović, J., Vuković-Gačić, B., Gačić, Z. (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(2): 243-251.

Kostić, O., Mitrović, M., Knežević, M., Jarić, S., Gajić, G., Đurđević, L., Pavlović, P. (2012): The potential of four woody species for the revegetation of fly ash deposits from the 'Nikola Tesla-a' thermoelectric plant (Obrenovac, Serbia). Archives of Biological Sciences, 64(1): 145-158.

Ockenfeld, K., Böhme, M., Knöchel, A., Geller, W. (2005): Displacement of pollutants during the River Elbe Flood in August 2002. Acta hydrochimica et hydrobiologica, 33(5): 391-394.

Ramírez, O. A. B., García, F. P. (2005): Genotoxic damage in zebra fish (*Danio rerio*) by arsenic in waters from Zimapan, Hidalgo, Mexico. Mutagenesis, 20(4): 291-295.

Singh, N. P., McCoy, M. T., Tice, R. R., Schneider, E. L. (1988): A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental cell research, 175(1): 184-191.

Squier, M.K., Cohen, J.J. (2001): Standard quantitative assays for apoptosis. Molecular biotechnology, 19: 305-312.

Sunjog, K., Gačić, Z., Kolarević, S., Višnjić-Jeftić, Ž., Jarić, I., Knežević-Vukčević, J., Gačić-Vuković, B., Lenhardt, M. (2012): Heavy metal accumulation and the genotoxicity in barbel (*Barbus barbus*) as indicators of the Danube River pollution. The Scientific World Journal, 2012.

Theodorakis, C. W. (2001). Integration of genotoxic and population genetic endpoints in biomonitoring and risk assessment. Ecotoxicology, 10(4): 245-256.

Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.-C., Sasaki, Y. F. (2000): Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environmental and molecular mutagenesis, 35(3): 206-221.

Vargas, V. M. F., Migliavacca, S. B., de Melo, A. C., Horn, R. C., Guidobono, R. R., de Sá Ferreira, I. C. F., Pestana, M. H. D. (2001): Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 490(2): 141-158.

Višnjić-Jeftić, Ž., Jarić, I., Jovanović, L., Skorić, S., Smederevac-Lalić, M., Nikčević, M., Lenhardt, M. (2010): Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (*Alosa immaculata* Bennet 1835) from the Danube River (Serbia). Microchemical journal, 95(2): 341-344.

Vuković-Gačić, B., Kolarević, S., Sunjog, K., Tomović, J., Knežević-Vukčević, J., Paunović, M., Gačić, Z. (2014): Comparative study of the genotoxic response of freshwater mussels *Unio tumidus* and *Unio pictorum* to environmental stress. Hydrobiologia, 735(1): 221-231.

Wölz, J., Cofalla, C., Hudjetz, S., Roger, S., Brinkmann, M., Schmidt, B., Schäffer, A., Kammann, U., Lennartz, G., Hecker, M., Schüttrumpf, H., Hollert, H. (2009): In search for the ecological and toxicological relevance of sediment re-mobilisation and transport during flood events. Journal of Soils and Sediments, 9(1): 1-5.

Yang, Y., Ligouis, B., Pies, C., Grathwohl, P., Hofmann, T. (2008): Occurrence of coal and coal-derived particle-bound polycyclic aromatic hydrocarbons (PAHs) in a river floodplain soil. Environmental Pollution, 151(1): 121-129.