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# The impact of the Sava river pollution on biomarkers response in the liver and gills of three cyprinid species

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Due to the presence of a large number of different pollutants, monitoring of the surface water quality based solely on the analysis of a limited number of xenobiotics, cannot be considered as reliable. Beside toxic, these agents can exert genotoxic effects, inducing damage in the DNA molecule, which, if not repaired, could lead to mutations and alterations in cells, tissues, organism, whole population and the ecosystem. The surface waters are under the pressure of both anthropogenic and natural sources of pollution. Additionally, extreme hydrological events, such as water scarcity and flooding, may further impair the state of freshwater bodies. Fish may be exposed to harmful substances through water, sediment and food. In ecogenotoxicity studies, gills are used as they represent the first organ in direct contact with water and waterborne pollutants, while the liver, as a key organ that controls many life functions is used as a major organ for metabolic breakdown of xenobiotics [1]. Common bream (*Abramis brama*), white bream (*Blicca bjoerkna*) and white-eye bream (*Ballerus sapa*) are three closely related, benthivorous cyprinids, native for the Sava River. The sampling site Duboko (23 rkm), on the Sava River, is chosen as it is exposed to the untreated wastewater from the town of Obrenovac (more than 70,000 inhabitants), intensive agricultural activity and close proximity to the largest thermal power plant in Serbia (TENTA) and belonging ash field. This study was conducted to assess the impact of multiple stressors during different seasons on different levels of biological organization, subcellular (genotoxic effect) and cellular/tissue level (histopathological effects), in the liver and gills of three bream species. As a biomarker of exposure DNA damage was measured by applying the alkaline comet assay, while histopathological alterations were monitored as a biomarker of effect. In parallel, concentration of metals and metalloids were assessed in gills, liver and muscle.

Basic physical (pH, temperature, oxygen concentration, electrical conductivity) and chemical ($NO_2$, $NO_3^-$, $NH_4^+$, $PO_4^{3-}$) parameters were measured on site. Microbiological indicators of faecal pollution, total coliforms (TC), *Escherichia coli* (EC) and Enterococci (EF) were assessed by using a most probable number approach (MPN). Presumptive *Clostridium perfringens* (CP) numbers were determined by using membrane filtration and incubation on TSC (Tryptose Sulphite Cycloserine) media. Sampling of fish tissue for comet assay and histopathological analyses was performed in 2014, during winter (January and February), spring (March and early June), and summer (late June, July, and August), once per month, for a total of 52 specimens. Analysis of metals and metalloids was performed only on fish sampled in February, early June and in August, in total 15 specimens. In the mid May extreme hydrological events led to extensive flooding in the studied area. Comet assay was conducted on the

liver and gill cells according to Kostić et al. [2]. Tail intensity, % of DNA in the comet tail (TI) was used to express the DNA damage level. Histopathological analyses included the use of conventional histological methods, staining of tissues sections using hematoxylin eosine differential staining, and examined under the microscope. The type and the extent of histological alterations were described by using a method developed by Bernet et al. [3]. Analysis of metals and metalloids was performed by inductively-coupled plasma optical emission spectrometry (ICP-OES), and included assessment of concentrations of 16 elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sr and Zn). To compare the total metal content in different tissues and through different seasons metal pollution index (MPI) was calculated according to equation MPI = (cf1 x cf2 x cf3 x…cfn) $^{1/n}$, where cfn = concentration of the metal n in the sample [4].

Statistical analysis of data from the individual months showed the highest DNA damage in gill cells during early June (spring). Gill histopathological index (IG) did not show significant seasonal variations, however it was the lowest during winter, the highest in spring, and slightly decreased in summer. A possible cause of this incidence could be a withdrawal of water which took place in June, after the flooding event that occurred in the middle of May. In liver the highest DNA damage was observed during August. Histopathological index of liver showed significantly higher values in summer in comparison to spring. This could be prescribed to a higher metabolic rate of fish liver during warm seasons and also could be a consequence of processing a large quantities of xenobiotics introduced into the water column due to withdrawal of water after floods and sediment disturbance. According to the MPI, gills were under the highest pressure of metal pollution during spring and summer. Liver was under the highest pressure of metal pollution during winter, while the muscle was the least affected tissue during all three seasons.

Overall, gills as the first organ in direct contact with water showed a higher response in terms of DNA damage (molecular level), while the liver as the major organ for processing of xenobiotics both from water and food showed a higher degree of histopathological alterations in comparison to gills (tissue/organ level). Increased response of both biomarkers during spring and summer indicates a joint effect of the flooding event and seasonal changes of climate and hydrological parameters.

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