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CRYOPRESERVATION OF FISH BLOOD – USEFUL TOOL FOR ASSESSING GENOTOXIC POTENTIAL OF AQUATIC ECOSYSTEMS

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One of the major limitations in performing ecogenotoxicological studies is the distance between research field and the laboratory. As some of the methods used in ecogenotoxicology require fresh biological material with intact cell viability, transfer of samples to the laboratory within a few hours after sampling is usually required. To overcome this issue, we have introduced cryopreservation in our research as a possible solution. Cryopreservation is a method which includes preservation of intact, living cells at low temperature for a long time. In natural conditions freezing, forming of ice crystals and dehydration could destroy cell structures. To avoid this consequence, specific compounds were introduced, cryoprotective agents, in the method of cryopreservation. The main characteristic of these compounds is their ability to reduce ice crystal formation in cells at any temperature.

We have applied cryopreservation in the evaluation of genotoxic potential along different river streams (the Adige River, the Sava River and the Velika Morava River basin). For this purpose, we focused on the level of DNA damage of cryopreserved fish blood cells (Salmo cenerinus, Salmo marmoratus, Alburnus alburnus) by using the comet assay.

To test whether cryopreservation has the impact on cell viability, or that it induces additional DNA damage, we employed preliminary experiments in 4 Abramis brama and 8 A. alburnus specimens. Namely, from every specimen two blood samples were taken: one for analyzing cells viability and the level of DNA damage of fresh blood, and another for observing cell viability and DNA damage of cryopreserved samples. The viability of cell blood was determined by using acridine orange/ethidium bromide differential staining. For analyzing the level of DNA damage alkaline comet assay was used. Obtained results indicated that cryopreserved blood cells had approximately the same viability and the level of DNA damage as nonpreserved blood samples.

According to our results, cryopreservation is a very useful method in genotoxicology and could have many benefits: blood samples should not be analyzed immediately after sampling; samples could be transported in liquid nitrogen without concern about additional DNA damage.