ASSESSMENT OF POLLUTION INDUCED ANTIOXIDANT ENZYME ACTIVITY AND HISTOPATHOLOGICAL CHANGES AND IN A CAT FISH, HETEROPNEUSTES FOSSILIS

Dr. Reddy P. B.

Assoc. Professor, Department of Zoology, Govt. PG College, Ratlam, M.P. India

Abstract: To ensure the health of aquatic ecosystems and identification of species at risk from the harmful effects of water contaminants can be made possible by integrating biochemical and histopathological analysis of a selected species. The present investigation is aimed to evaluate the adverse effect of wastewater effluents on H.fossils, collected in two different zones (upstream and downstream) of Chambal River at Nagda, Ujjain, and M.P.India. Fish liver and kidneys from the upstream (US) and downstream (DS) were analyzed for oxidative stress responses and histopathology. Catalase and glutathione *S*-transferase activity was significantly higher at downstream ($p < 0.05$), signifying initiation of antioxidant defense mechanisms. The results also revealed that liver has a higher capacity and adaptability to neutralize ROS compared to kidney. Remarkable histopathological changes were observed in liver and kidney of the studied fish. The major changes include are cellular infiltration, haemorrhage, vacuolation of hepatocytes and necrosis in liver, and swollen with pyknotic nuclei in kidney. The remarkable increase of hepatic SOD, CAT and GST activities is related histological changes observed in liver compared to kidney, where pathological changes were not lessen by antioxidant defense system. These results pointed out that thermal hot spring effluent discharged into Chambal River at Nagda, induced the most significant health impact on the fish. The biomarker responses found in fish from the contaminated site were in accord with alteration of water quality parameters. On the whole, this study suggests that the stress enzymes along with histopathology can serve as very useful tool for monitoring *in situ* prolonged adverse effects of wastewater effluents on fish.

Keywords: H. Fossils, Histopathology, Stress Enzymes, Waste Water.

Introduction: Water is the most important natural resource and essential for survival to the human being and also for all living organisms. The input of manmade contaminants to aquatic ecosystems has the potential to affect entire aquatic ecosystem. Aquatic pollution is worldwide problem and rapid growth of industrialization and urbanization are major factors and responsible of aquatic pollution in the world. Fishes are one of the major and widely distributed organism in the water and easily susceptible to contaminants present in water [1], [2].Much concern has been raised in recent years that exposure to industrial and municipal wastewater treatment effluents containing xenobiotic chemicals can disrupt not only the endocrine function but also disrupt the overall the health of a fish or any other aquatic organisms [3]. Physiological and histological alterations in fish caused due to the exposure of contaminants are can be effectively used for environmental risk assessment [4], [1], [2], [5],[6]. For better management of aquatic ecosystem, it is essential to predict changes in ecosystem composition and function, as well as health status of organism by a known set of environmental parameters and contaminant concentrations. But, this is far more difficult than it seems, because of regular changes in the species composition and health of organisms in the field with tidal and seasonal cycles of ecosystem.

Liver is an important metabolic organ that executes many functions essential for survival. It detoxifies dangerous substances which come from both external and internal sources [7].While kidney extracts and flush out the waste from the blood, balance body fluids, form urine, and support in other important functions of the body. The malfunction or failure of the kidneys can lead to serious illness or even death [8].

The Chambal River at Nagda, Ujjain and (M.P. India) is highly polluted due to the dumping of both municipal and many industrial wastes. Therefore, we intended to study the pollution induced histopathological and biochemical changes in liver and kidney of a locally available cat fish, Heteropneustes fossilis.

Materials and Methods: ***Study Area***: The Chambal River flows at Nagda (Ujjain district of Madhya Pradesh, India) and is close to the tropic of cancer at 23‟27 N and 75‟25 and 517 meters above MSL. The river receives both treated and untreated municipal and industrial wastes continuously (Fig.1)

Sampling: The surface water samples from upstream and downstream were collected in summer (April, 2017) in glass bottles at about 10 cm below the surface and different parameters like pH, COD, BOD and DO, EC, TDS, TSS and hardness were analyzed according to APHA [9].

Fish: The cat fish (H.fossils) of more or less of same size and body weight (irrespective of the sex) were randomly collected from both the study stations. After morphometry, the liver and kidney of the fish were carefully removed and weighed and processed for the study of oxidative stress (OS) markers and histopathological studies. Both the tissues were homogenized in 4 volumes of homogenizing buffer (50 mM Tris - HCl mixed with 1.15% KCl and pH adjusted to 7.4), using Teflon Homogenizer (Remi,India). The resulting homogenate was centrifuged at 10,000g for 20 min in a Remi (India) centrifuge at 0–4°C. The supernatant was transferred and stored –20°C until analysis.

Histopathology: Routine histology was used to produce tissue sections for histological analyses. Both the tissues (Liver and Kidney) were preserved in 4% buffered formalin for 24-72 hours. Then after, the samples were transferred to 50% ethanol for indefinite time. For embedding, tissues were dehydrated further through 70%, 95% and absolute ethanol and cleared in xylene, and soaked in paraffin wax with melting Point: 58-60°**C** (K.K. Chemicals, Mumbai). Sections were cut at 7 µm using a rotary microtome (Atico Medical Pvt Ltd, Ambala). All the sections were and dewaxed overnight in an oven at 60°C. Dried sections were stained with routine haematoxylin and eosin stains. After staining, sections were dehydrated in increasing graded concentrations of ethanol (30-100%), cleared in xylene, and mounted in DPX. Tissue sections were examined microscopically using Olympus light binocular microscope (Olympus, Japan) and microphotographs were taken using digital camera DSX510(Olympus, Japan) connected to microscope.

Oxidative Stress Markers: The stress enzymes were assayed spectrophotometrically by using a kit provided by OxiSelect™ (BIOGENUIX MEDSYSTEMS PVT. LTD. New Delhi). The activity of superoxide dismutase (SOD) at 550 nm, (one unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation [10]. Catalase (CAT) was assayed according to Mohanty J.G [11] at 240 nm and Glutathione *S*-transferase (GST) activity was determined by the method of Habig et al [12] using 1 chloro 2,4 dinitrobenzene as substrate at 340 nm. Glutathione (GSH) was determined according to Jollow et al. [13].
All values were expressed as mean ± standard error. Data have been presented as mean values ± standard error (S.E.).

The significance of difference between experimental and control data was statistically analyzed using student (T) test.

Results: The entire mean data were examined for differences between polluted and reference sites**.** Morphological abnormalities like shedding of scales, discoloration, injury of skin, cracks and necrosis of fins, eye deformities,, lower lip extension and mucus secretion all over the body were observed in fish exposed to waste water at downstream.

Water Quality Parameters: Results of surface water quality parameters of Chambal River at Nagda are summarized in Table.1. Results indicate that almost all water parameters studied were higher than the approved limit by the Central Pollution Control Agency, i.e. [14] (CPCB, 1995). On the whole, the water quality of downstream of Chambal River at Nagda was comparatively poor and not suitable for agriculture and domestic uses. The level of contamination at downstream of the river is mainly due to indiscriminate discharges of municipal and industrial wastes [1], [12].

Table 1: Values of Water Quality Parameters of Chambal River at Nagda during Summer 2017

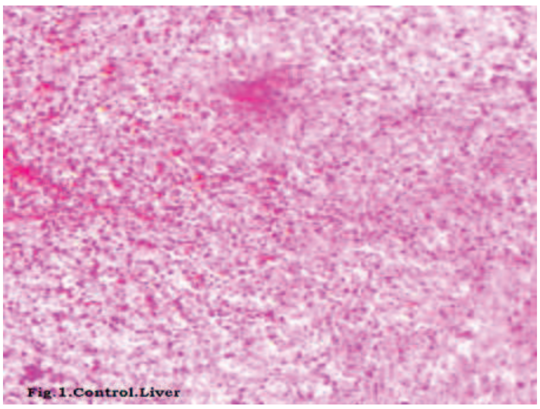
Parameter	Station I	Station II
Colour	Turbid	Grayish
Odor	No specific odour	No specific odour
BOD, mg/L	10.0±0.01	51.2±4.8
COD mg/L	12.0± 1.4	32.0±1,8
DO mg/L	7.5±0.1	8.9±**0.07
EC,umho/cm	85±2.3	419±13.2**
pH	7.1±0.09	8.8±0.9
TDS mg/L	110.10±5.3	392.1±3.2
Temperature 'C	22.4±0.33	22.8±0.67
Total hardness	200±9.67	1380±34.3
TSS mg/L	26.1±2.2	140.2±8.76

Oxidative Stress Markers: The results of oxidative stress markers in liver and kidney of H.fossils are presented in Table 2. Results confirm that three hepatic oxidative enzyme activities (GST, GSH and SOD) were significantly (P> 0.01) elevated in fish collected from Downstream (Juna Nagda) in comparison with reference site. Catalase is a major antioxidant enzyme found in almost all aerobic organisms. The activity of the enzyme differs significantly in different tissues, and elevated level in organs with high oxidative stress [3]. The activities of catalase (CAT) enzyme in the present investigation were significantly reduced both in the liver and kidney.

Table.2. Comparison of Oxidative Stress Markers of H.fossils from Chambal River (n = 8)

Oxidative markers	Liver		Kidney	
	Upstream	Downstream	Upstream	Downstream
CAT U/mg protein	179.6 ± 14.1	119.4 ± 13.1	153.4 ± 11.8	113.4 ± 19.8
SOD U/mg protein	7.8 ± 0.8	14.2 ± 0.3	5.7 ± 0.3	9.7 ± 0.4
GST U/mg protein	1287.0 ± 104.7	1492.0 ± 121.9	1164.0 ± 104.5	1464.0 ± 98.4
GSH (nmol/g tissue	0.294 ± 0.0028	1.294 ± 0.0021	0.192 ± 0.0018	1.5621± 0.0021

Histopathology: The histo pathological alterations of liver and kidney are shown in plate I and II**.** The control fish (reference site) showed clearly the homogeneous structure of the parenchyma in both liver and kidney. The major alterations in the liver include irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation and the presence of eosinophilic granules in the cytoplasm (Fig. 4b Cytoplasmic and nuclear degeneration was found very common. The tissue was slightly to moderately damaged. The alterations found in the kidney are shown in Plate II. The kidney of H.fossils from downstream also exhibited marked pathological changes. The major changes include glomerular expansion, resulting in reduction of Bowman's space, severe necrosis, swelling in renal tubules, cellular hypertrophy and granular cytoplasm. The epithelial cells of the distal convoluted tubule (DCT) were decreased in size. (Plate. II).



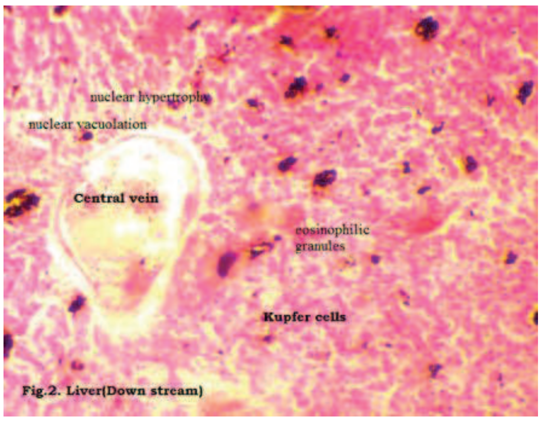
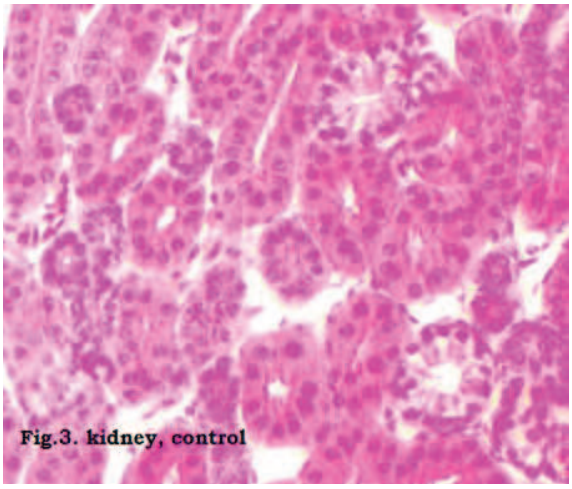


Plate.1. Variation in Histopathology of Liver of Heteropneustus Fossilis in Two Different Stations of River Chambal

Fig.1. Control Fish Showing Normal Structure. Fig.2. Liver of Fish from Station from Downstream Showing Nuclear Hypertrophy, Nuclear Vacuolation and the Presence of Eosinophilic Granules



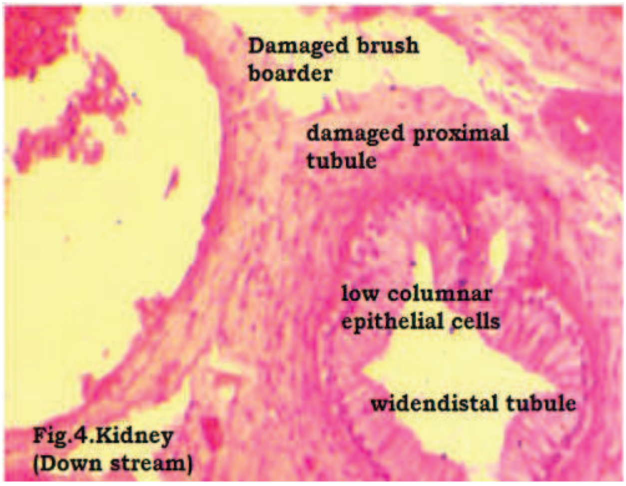


Plate.II. Plate.1. Variation in Histopathology of Kidney of Heteropneustus Fossilis in Two Different Stations of River Chambal.
Fig.3. Control Fish Showing Normal Structure. Fig.4. Kidney of Heteropneustus Fossilis from Station from Downstream Showing Glomerular Expansion, Resulting in Reduction of Bowman's Space, Severe Necrosis, Swelling in Renal Tubules, Cellular Hypertrophy and Granular Cytoplasm

Discussion: The aim of this study was to assess the extent of pollution load at Chambal River on oxidative stress biomarkers and histopathological studies in the liver and kidney of H.fossils. Modern agricultural practices, Industrial effluents, and Municipal wastes are known as major sources of anthropogenic pollution [1], [2], [15], [16], and [17]. Many Rivers and lakes in India too are known to be polluted due to discharge of untreated municipal sewage [1], [2], [15], [18], agriculture runoff and discharge of industrial effluents [19], [20]. A lot of contaminants can induce oxidative stress at some extent and can damage the cell membranes by generating free radicals (ROS) and/or changing the activity of antioxidant enzymes [21].

The physico chemical analysis of surface waters like DO, BOD, EC, TDS and hardness of Rivers and lakes has long been used to evaluate the quality of water. In the present study, all the studied parameters like DO, DO, BOD, EC, TDS were much higher in the downstream of the River than reference site and exceeded standards of WHO and CPCB. The increased BOD and depletion in DO in the downstream directly are reflecting the presence of high levels of organic and inorganic pollutants at this station. The preferred concentration of DO for the commercial fisheries in India is 4 mg/l [14]. But in our study at Juna Nagda the DO concentration was found to be less than 5 mg/l (4.4). The depletion in oxygen and increase in BOD may cause aquatic hypoxia and affect the growth in aquatic organisms [22], Dissolved oxygen (DO) plays an important role in survival, physiological and normal metabolic functions of aquatic organisms. It is rationally suggested that increased O_2 (hyperoxia) levels increase ROS generation but living beings have certain specific adaptive mechanisms to prevent negative consequences of high ROS [23]. The highest values of conductivity were also found at downstream that has been associated with impaired water bodies which may reflect a decrease in water quality [24]. At Juna Nagda (downstream) we detected an increased loading of nutrients which indicates the discharge of effluents into the river at Juna Nagda.

The histopathological lesions observed in this study might cause storage of bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes and thus caused metabolic disorders [25]. It implies that that the bile is getting accumulated not being released from the liver to cause possible damage to the hepatic metabolism [26].

The histopathological alterations seen in the kidney and liver in the present study point out that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress [27]. This outcome thus confirms that histopathology is able to evaluate the early effects and such studies are good biomarkers for field assessment, especially in tropical areas which are naturally subject to a diversity of ecological variations. In conclusion, the present study showed that histopathology is a useful biomarker for the assessment of aquatic contamination.

Results of various enzyme activities are summarized in Table.2. The liver is the most frequently targeted organ in terms of xenobiotics induced toxicity. It is the most active organ in antioxidant defence, playing also an important role in redox metabolism [3]. The SOD–CAT system provides the first line defense against reactive oxygen species (ROS). Typically a synchronized stimulation response in the activities of SOD and CAT is observed when exposed to pollutants [28]. In the present study hepatic SOD, GST and GPx activities were elevated in both tissues of H.fossils of downstream while there was a significant decrease in the main antioxidant enzyme catalase in the CAT was found in second (CAT). Our results are in agreement with earlier studies of Stanic B et al [29], in Acipenser ruthenus and Valavanidis, A et al [30] in C. carpio, brown trout (S. trutta) collected in two different sites of polluted area. The increased SOD and GST activity in the liver and kidney of the cat fish, H.fossils may be clarified as a reparation mechanism against various contaminants present in water [31], [32]. However, the antioxidant defense status varies from tissue to tissue depending on habitat, age, sex, thermoregulatory capacity, metabolic rate and nutritional status of the organism [33]. Besides, the level of oxidative stress or injury depends on duration and concentration of the active toxic compound(s) present in polluted site and also depends on the ability of the organism to recover [7].

Conclusions: The overall renal and hepatotoxic effect of pollutants at Chambal River is probably related to a production of free radicals (ROS), which alters the antioxidant status and membrane stability. Water chemistry analysis at two sites indicated that the downstream at Juna Nagda is highly polluted with higher biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH and low dissolved oxygen (DO) when compared to the upstream (Methwasa). Strong industrial activity coupled with intensive use of chemicals in agricultural practices and discharges of untreated municipal effluent directly into the River are the major sources of the pollution. The study therefore provides a balanced use of biomarkers of oxidative stress in biomonitoring of aquatic pollution. The sampling site is the source of municipal, industrial and dyeing effluent. This could be a reason for higher pH, BOD and COD levels in this site [15]. Therefore, much more experiments are needed on fish populations to resolve the issue of pollution impact in Chambal River at Nagda.

References:

1. Reddy, P.B., 2012.[a] Evaluation of potential biomarkers for effluent induced hepatotoxicity. *International Journal on Applied Bioengineering*, *6*(2).
2. Reddy, P.B., 2012.[b]. Histopathogical studies as potential and direct biomarkers of pollution. *Trends in Life sciences*, *1*(1), pp.27-31.
3. Anil Patil and Reddy, P.B.2017. Endosulfan induced oxidative stress in Tilapia mossambica. Life Sciences International Research Journal: Volume 4 Issue 1,209-214.
4. Van der Oost, R., Beyer, J. and Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology*, *13*(2), pp.57-149.
5. Reddy, P.B. and Kusum, W., 2013. Using histopathology of fish as a protocol in the assessment of aquatic pollution. *Journal of Environmental Research and Development*, *8*(2), p.371.
6. Reddy, P.B. and Rawat, S.S., 2013. Assessment of aquatic pollution using histopathology in fish as a protocol. *International Research Journal of Environment Sciences*, *2*(8), pp.79-82.
7. Reddy, P.B., 2016. European Journal of Biomedical and Pharmaceutical sciences. *European Journal of Biomedical*, *3*(12), pp.595-600.
8. Bellomo, R., Ronco, C., Kellum, J.A., Mehta, R.L. and Palevsky, P., 2004. Acute renal failure–definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Critical care*, *8*(4), p.R204.
9. APHA. 1998. In Standard methods for the examination of water and waste lands.1998; 20th edition, Washington, D.C. American Public Health Association.
10. Lepock JR, Arnold LD, Torrie, BH, Andrews B, Kruuv J. Structural analyses of various Cu2+, Zn2+-superoxide dismutases by differential scanning calorimetry and Raman spectroscopy. *Archives of biochemistry and biophysics*, 1985; *241*(1): 243-251.
11. Mohanty JG, Jaffe JS, Schulman, ES, Raible DG. A highly sensitive fluorescent micro-assay of H 2 O 2 release from activated human leukocytes using a dihydroxyphenoxazine derivative. Journal of immunological methods, 1997; 202(2): 133-141.
12. Habig, WH; Pabst, MJ; Jacoby, WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem* **1974**, *249*, 7130–7139.

13. Jollow, DJ; Michell, JR; Zampaglione, N; Gillete, JR. Bromobenzene induced liver Necrosis: Protective role of GSH and evidence for 3, 4 -Bromobenzene oxide as the Hepatotoxic metabolite. *Pharmacology* **1974**, *11*, 151–169.
14. CPCB. 1996. Statistics, U., Central Pollution Control Board. New Delhi, October, 1996.
15. Reddy, P.B. and Baghel, B.S., 2012. Impact of Industrial waste water on the Chambal River and Biomarker responses in fish due to pollution at Nagda. MP India. *DAV Int. J. Sci*, *1*(1), pp.86-91.
16. Rajput, R.S., Pandey, S. and Bhadauria, S., 2017. Status of water pollution in relation to industrialization in Rajasthan. *Reviews on Environmental Health*. Rev Environ Health. 2017 Sep 26;32(3):245-252.
17. Hamilton, P.B., Rolshausen, G., Webster, T.M.U. and Tyler, C.R., 2017. Adaptive capabilities and fitness consequences associated with pollution exposure in fish. *Phil. Trans. R. Soc. B*, *372*(1712), p.20160042.
18. Swain, A.K., Jena, M., Das, B. and Swain, R., 2017. CONCERN OVER POLLUTION IN TWO MAJOR RIVERS OF ODISHA, INDIA: A CASE STUDY OF MAHANADI AND BRAHMANI RIVERS. *With the blessings of*, *29*, p.1.
19. Rajaram, T. and Das, A., 2008. Water pollution by industrial effluents in India: discharge scenarios and case for participatory ecosystem specific local regulation. *Futures*, *40*(1), pp.56-69.
20. Voulvoulis, N. and Georges, K., 2016. Industrial and Agricultural Sources and Pathways of Aquatic Pollution. In *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 29-54). IGI Global.
21. Sachdev, S. and Davies, K.J., 2008. Production, detection, and adaptive responses to free radicals in exercise. *Free Radical Biology and Medicine*, *44*(2), pp.215-223.
22. Mallya, Y.J., 2007. The effects of dissolved oxygen on fish growth in aquaculture. *The United Nations University fisheries training programmer, Final project, pp30*.
23. Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. *Aquatic toxicology*, *101*(1), pp.13-30.
24. Olsen, J., Melbye, M., Olsen, S.F., Sørensen, T.I., Aaby, P., Nybo Andersen, A.M., Taxbøl, D., Hansen, K.D., Juhl, M., Schow, T.B. and Sørensen, H.T., 2001. The Danish National Birth Cohort-its background, structure and aim. *Scandinavian Journal of Social Medicine*, *29*(4), pp.300-307.
25. Ruan, P., Yang, C., Su, J., Cao, J., Ou, C., Luo, C., Tang, Y., Wang, Q., Yang, F., Shi, J. and Lu, X., 2013. Histopathological changes in the liver of tree shrew (Tupaia belangeri chinensis) persistently infected with hepatitis B virus. *Virology journal*, *10*(1), p.333.
26. Neuschwander-Tetri, B.A., 2010. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steato hepatitis: the central role of non triglyceride fatty acid metabolites. *Hepatology*, *52*(2), pp.774-788.
27. Camargo, M.M. and Martinez, C.B., 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical ichthyology*, *5*(3), pp.327-336.
28. Stoliar OB, Lushchak VI. Environmental pollution and oxidative stress in fish. INTECH Open Access Publisher. 2012.
29. Stanic B, Andric N, Zoric S, Grubor-Lajsic G, Kovacevic R. Assessing pollution in the Danube River near Novi Sad (Serbia) using several biomarkers in sterlet (Acipenser ruthenus L.). Ecotoxicology and Environmental Safety, 2006; 65(3): 395-402.
30. Valavanidis, A, Vlahogianni, Th, Dassenakis, M, Scoullos, M. Molecular Biomarkers of Oxidative Stress in Aquatic Organisms in Relation to Toxic Environmental Pollutants. Ecotoxicology and Environmental Safety, 2006; 64(2): 178–189.
31. Dimitrova, M.S.T., Tsinova, V., Velcheva, V.: Combined effects of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (Cyprinus carpio). Comp. Biochem. Physiol. Part C., 1994; 108: 43-46.
32. Vinodhini, R. and Narayanan, M., 2009. Biochemical changes of antioxidant enzymes in common carp (Cyprinus carpio L.) after heavy metal exposure. *Turkish Journal of Veterinary and Animal Sciences*, *33*(4), pp.273-278.
33. Hansen, B.H., Rømma, S., Garmo, Ø.A., Olsvik, P.A. and Andersen, R.A., 2006. Antioxidative stress proteins and their gene expression in brown trout (Salmo trutta) from three rivers with different heavy metal levels. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *143*(3), pp.263-274.

***