*J. Int. Environmental Application & Science,* Vol. 13(2): 125-131 (2018)

*Research Paper*

# What can Multi-Biomarker Approach Tells Us about the Impact of Pollution on Freshwater Biota Health?#

Valbona Aliko[1,*], Valon Morina[2], Eldores Sula[3]

*[1*]Department of Biology, University of Tirana/Faculty of Natural Sciences, Boulevard "Zogu I", Tirana, Albania; [2]Prishtina University, Faculty of Geosciences and Technology, Mitrovica, Kosovo; [3] Department of Laboratory Technicians and Imaging, Aldent University, Tirana, Albania*

*Received April 04, 2018; Accepted June 29, 2018*

**Abstract:** The health of freshwater ecosystems and their biota exposed to continuous detrimental effects of environmental contaminants can be better assessed by integrating analytical chemical analysis with carefully selected biological endpoints measured in tissues of species of concern. These biological endpoints include molecular, biochemical and physiological markers (i.e. biomarkers) that when integrated, can clarify issues of contaminant bioavailability, bioaccumulation and ecological effects while enabling a better understanding of the effects of non-chemical stressors. Here, a battery of biomarkers, devised to measure cellular damage, antioxidant enzyme activity and physiological impairment, were combined with chemical analysis of water column, sediment and tissue, to determine exposure to and the effects of pollution at sites within Sitnica River (Kosovo). Carp fish, *Cyprinus carpio*, collected *in situ* through electrofishing were used as test species to determine the possible alterations in biochemical and physiological biomarkers. Our results confirmed a significant increase of hepatic alanine transaminase (ALT), aspartate transaminase (AST), glutathione-S-transferase (GST), catalase (CAT) and superoxide-dismutase (SOD) in blood. Both, blood glucose (GLU) and cortisol concentration were also significantly increased. Alteration in liver histological structure, increased in the frequency of micronuclei (MN) and nuclear abnormalities (ENA) in erythrocytes, were the most discriminating biomarkers among sites. This holistic approach to environmental assessment is encouraged as it helps to identify the integrated impact of chemical contamination on organisms and to provide a realistic measure of environmental quality.

***Key words**: Multi-biomarker approach, Freshwater biota health, Pollution*

## Introduction

Surface waters, such as rivers, receive large quantities of wastewater from industrial, agricultural, and domestic sources, including municipal sewage treatment plants. Water pollution is known to be detrimental to human health and aquatic ecosystems, since surface waters are used both as a source of drinking water and for agricultural, recreational and religious activities around the world (Ohe et al., 2004). The development of anthropogenic activities is the main factor leading to the increasing levels of contaminants in rivers environments. On the long-term effect, pollution of water column, river sediments affect freshwater biota health status due to bioaccumulation ability of different xenobiotics to animal tissues. That said, the combination of physicochemical and biological evaluation methods, is the best approach to assess the effect of xenobiotics to biota health. Because of, in natural environments, contaminants usually are present as very complex mixtures and there is no single biomarker that can give a complete diagnosis of environmental degradation. To overcome this difficulty, the use of a set of complementary biomarkers may be useful to evaluate the various responses to mixtures of pollutants in river organisms under stress (Lavado et al., 2006; TejedaVera et al., 2007; Frenzilli et al., 2008; Pandey et al., 2003; Tejeda-Vera et al., 2007; Linde-Arias et al., 2008a, b; Ruas et al., 2008; Falfushynska and Stolyar, 2009). Biomarkers complement and enhance the reliability of the chemical analysis data, offering more integral and biologically relevant information on the potential impact of toxic pollutants on the health of organisms (van der Oost et al., 2003; Hansen, 2003; Ferreira *et a*l., 2005; Parvez and Raisuddin, 2005; Parvez *et al*., 2006).

**Corresponding: E-Mail: valbona.aliko@fshn.edu.al; Tel: +377 (044) 151 321; Fax: + 381 (028) 530 446*

*#This paper has been presented in ISESER-2018, Konya, Turkey*

The aim of this study is to show the usefulness of multi-biomarker approach in measuring the impact of pollution on freshwater biota health of Sitnica River, Kosovo. Sitnica River is the longest river that flows completely within Kosovo's borders. It is heavily polluted with heavy metals (Zn, Pb, Cd, As, Cu, Ni, Mn), solid and urban wastes (sulphates, nitrates), Cl, phenol, and ash from lignite burning (Berisha et.al, 2008: Jusufi et. al., 2004, Gashi et.al 2011), and aromatic polycyclic hydrocarbons as biphenyl polychlorures -PCB (V.Čalić et al., 2005). Along its stream from Ferizaj to Mitrovica, it represents the most polluted river in Kosovo (KAPA 2010). Regarding the physical parameters such as suspended substances are present and exceed the maximum allowed values. This comes from the fact that in this river, are flowed one branch of the Nerodime River as well as rivers: Graqanka, Pristina, Drenica, Llapi, Trepca and other smaller water streams, where all these rivers converge Sitnica River and contain urban and industrial wastewater discharged. The measured chemical parameters such as COD and BOD5 indicate also for the permanent river pollution. The concentration level of phenols and heavy metal concentration in exceeded allowed values of the fourth category of the quality of surfaced waters. Sitnica River is classified as a "dead" river, since its diversity of species is very poor (Dakonta, 2009), especially at Vragoli and Plemetin sampling sites, due to high levels of water and sediment pollution.

As presented in the present article, numerous biomarkers used in eco-physiological study of effects of Sitnica River pollutants on carp fish gave us successfully evidence on the effects of contamination on the exposed biota. This holistic approach to environmental assessment is encouraged as it helps to identify the integrated impact of chemical contamination on organisms and to provide a realistic measure of environmental quality.

## Materials And Methods

### Sampling sites

Three sampling sites (S1-Ferizaj, S2-Vragoli, and S3-Plemetin) alongside Sitnica River characterized by highly industrial activity and heavily urbanization, were selected, Figure 1. Ferizaj is situated nearby river source, so this site is relatively clean and it was used as reference site. The other two sampling sites are heavy polluted by industrial and urban discharges, (Berisha et.al, 2008; Jusufi et. al., 2004; Gashi et.al 2011). Animals specimens of *Cyprinus carpio* (n= 42) at approximately the same size, were collected with electro fishing method (Hans Grassl GmbH).


**Figure 1**. Map of sampling sites alongside Sitnica River

The animals were transported to the laboratory for further biomarker analysis, keeping in the containers of river water with constant aeration. Water column and sediment analysis were conducted in all sites. Heavy metal concentration in liver tissue was measured according to AOAC Official Method 999.11 (Atomic Absorber -Varian).

### Blood and tissue sample preparation

Blood was collected from anesthetized animals using ether. The blood was taken by caudal vein using 2 ml sterile plastic disposable syringes fitted with 0.8 x 38-mm hypodermic needles. Further on, 1 ml blood was taken using EDTA as anticoagulant. Blood serum was separated by centrifugation (5000 rpm /10 min) and then were used to determinate the biochemical parameters (plasmatic glucose, total

protein), hepatic enzymes (AST and ALT), and oxidative stress enzymes (SOD, CAT, GST). Two blood smears for each fish were immediately prepared with fresh blood drops and allowed to air dry. Slides were fixed with ethylic alcohol (5 minutes) and stained with 10% Giemsa-Romanowsky stain (Sigma-Aldrich). All slides were prepared and stained by the same individual. Slides were examined using a Zeiss oil-immersion light microscope with 1000X magnification. In total 58 800 erythrocytes from 42 fish individuals were evaluated. Liver tissue were conserved in formalin 10%, prepared for microscopy, and observed and photographed.

Determination of plasmatic glucose concentration was done quantitatively by glucose oxidase enzymatic method. Total protein concentration (TP) was determined in a spectrophotometer at 700 nm according to the method of Lowry et al. (1951) using a standard curve of bovine serum albumin (BSA). Aspartate aminotransferase activity (AST) and alanine aminotransferase (ALT) were done using enzymatic-colorimetric methods by means of commercial kits (Roche) using the analyzer model Cobas Integra 400 –Roche. The activity of glutathione S-transferase (GST) was determined by monitoring the complexation of reduced glutathione with the substrate 1-chloro-2, 4-dinitrobenzene (CNDB) in a spectrophotometer at 340 nm (Titertek Multiskan R spectrophotometer MCC/340). The catalase activity (CAT) was determined from the rate of decomposition of $H_2O_2$ by the enzyme, based on the decrease in absorbance at 240 nm. The concentration of superoxide dismutase (SOD) was determined by colorimetric test (Biovision) and expressed in U/ml.

Hematological parameters like red blood cells (RBC) and white blood cells (WBC) were determined using Bürker hemocytometer (Ochei & Kolhatkar, 2005), while packed cell volume (PCV) was measured by micro method (Golden and Farb, 1971). Size and shape factor of RBC and their nuclei, were also calculated according to the formulae: ES= [(A x B x π)/4] Ns= [(a x b x π)/4]. Erythron Profile was determined by calculating the percentage of different kind of erythrocytes (normal, in division, enucleated, degenerated, micro-nucleated). In total, 2000 RBC per individual were evaluated. To evaluate genotoxicity, the micronucleus test (MN) was performed with fish erythrocytes according to the technique described by Heddle (1973) and Schmid (1975) and the occurrence of erythrocytic nuclear abnormalities (ENA) was analyzed according to Carrasco et al. (1990). The ENA were classified according to Monteiro et al (2011). A total of 1000 erythrocytes/fish, were examined microscopically. The mean frequency of micronuclei (MN) and erythrocytic nuclear abnormalities (ENA) of each site was calculated and expressed per 1000 cells (‰).

**Statistical analysis**

All data were expressed as mean value ± SE. One sample T-test ($p<0.05$) was used to compare means of erythrocyte deformities among different sampling sites.

**Results and Discussions**

The most dominant fish specimens found in Sitnica River were cyprinid fish, represented mostly by *Cyprinus carpio*, which is characteristic for low quality freshwater bodies. This is directly related with high level of multifactorial pollution of Sitnica River. The results of heavy metals concentration in liver of carp fish collected at polluted sites alongside of Sitnica River, are shown in Table 1. These results were in strong correlation with physicochemical analysis and heavy metal concentrations found in river water column and sediments.

**Table 1**. Concentrations of heavy metals in fish liver

| | **Ferizaj (S1)** | **Vragoli (S2)** | **Plemetin (S3)** |
|---|---|---|---|
| **Heavy Metals** | Concentrations (mg/L) | | |
| Zinc (Zn) | 0.1585 | 0.3525 | 0.359 |
| Copper (Cu) | 0.022 | 0.061 | 0.0575 |
| Cadmium (Cd) | 0.014 | 0.014 | 0.014 |
| Lead (Pb) | 0.048 | 0.048 | 0.048 |

There were no marked differences in evaluated heavy metal concentrations measured at three sampling sites. These findings are in accordance with water column pollution found by previous studies (Gashi et al. 2011).

Different biomarker levels measured in fish sampled from three sampling sites are shown in the Table 2. The data obtained from measurements of biochemical parameters showed that the levels of glucose in blood plasma of carp fish from Sitnica River were significantly higher ($p<0.05$) compared with the control group.

**Table 2**. Different biomarker levels measured in fish from Ferizaj, Plemetin and Vragoli sampling sites. Significant values are accepted for $p<0.05$, Paired samples T-test.

| **Biomarkers** | **S1 (Ferizaj)** | **S2 (Plemetin)** | **S3 (Vragoli)** |
|---|---|---|---|
| Glu (mg/dL) | 102±9.5 | 120±7.2 | 203±13.1 |
| AST (IU/L) | 420±0.3 | 532±23.3 | 615±31.5 |
| ALT (IU/L) | 37±0.12 | 86±1.3 | 532±11.24 |
| TP (g/dL) | 3.9±1.3 | 2.42±0.6 | 1.96±0.21 |
| SOD (U/ml) | 3.2±0.01 | 4.1±0.3 | 5.02±0.3 |
| CAT (nmol/ml) | 83.57±3.3 | 105.9±2.3 | 329±4.1 |
| GST (nmol/ml) | 0.5±0.03 | 0.7±0.01 | 0.81±0.1 |
| RBC | 127±0.7 | 0.12±118 | 0.4±121 |
| WBC | 7277.7±618 | 5430±0.78 | 4.983±1.3 |
| PCV | 39.7±2.3 | 6.32±4.3 | 6.062±5.3 |
| MN | 0.2±0.3 | 0.84±0.3 | 1.75±0.3 |
| ENA | 0.2±0.3 | 0.84±0.3 | 1.75±0.3 |

All biochemical and physiological biomarkers have been altered due to the presence of heavy metals found in river and animal tissues. Glucose and hepatic transaminases are significantly increased in all three sampling sites, while total protein values are significantly decreased compared with control. Elevated levels of glucose are normal in the animals under stress and it is related with secretion of catecholamine and corticosteroid hormones from surreal glands. Stress is an energy demanding process since the animal under stress conditions use energy to oppose the stress effects. Our present data support very well these facts. Glucose levels can be a very sensitive parameter of water stress in carp fish (Aliko et al 2011, Sula and Aliko, 2017a).

Our results show than the activity of AST and ALT were increased significantly ($P<0.05$) (Table 2). AST exhibited a higher increase than ALT. ALT is a key metabolic enzyme released on the damage of hepatocytes. Increased level of ALT indicates an adaptive response to its leakage into the blood stream due to the presence of water toxicity. It also has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress induced toxicity in liver. This result is in accordance with the results of previous researchers on fresh water fish (Aliko *et al* 2011; Morina et al. 2013; Sula & Aliko, 2017a).

Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures to metals present in industrial effluent like may lead these metals to accumulate in the liver and cause pathological alterations. Moreover, cell injury of certain organs like liver leads to the release of tissue specific enzymes into the bloodstream. Significant increase in transaminases (AST and ALT) activity in fish of polluted areas could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver (Reddy & Baghel, 2012).

Antioxidant enzyme activities of CAT, SOD and GST showed a significant increase ($p<0.001$) in the liver of fish from three sampling sites, most notably in sites of Vragoli and Plemetin ($S_2$ and $S_3$), Table 2. Among three enzymes, the activity of blood catalase CAT activity was the mos remarkably increased in all sites. All values were significant for $p<0.05$.

Hematological parameters were all altered with a significant decrease of red blood cell number and packed cell volume, and a significant increase of white blood cell number, Table 2. The presence of heavy metals in river water had caused the changes of size and form factor of erythrocytes, as well as of their nuclei. Micronucleus assay (MN) and erythrocytic nuclear abnormalities (ENA) revealed a significant genotoxicity and cytotoxicity related with heavy metals and other pollutants present in Sitnica River. The most frequent among the erythrocyte nuclear abnormalities observed in fish from

polluted sampling sites of Vragoli and Plemetin, comparing to reference site, were cell and nuclear deformity and degenerating cells (swollen and hemolyzed cells)., Figure 2. According to our opinion, based on results taken from similar studies done in amphibians and carp fish (Aliko *et al*. 2008, 2011; Morina et al. 2013; Sula and Aliko, 2017a), the presence of degenerated erythrocytes is an indicator of areas with chronic pollution. This finding is supported also by the increase of neutrophils and decrease in leukocytic cells found in fish taken from polluted sites. The histological analysis of fish liver tissue confirmed permanent damages with nontumoral lesions, heterogenous parenchyma, cytoplasmic vacuolization and necrotic foci, as the most frequent abnormalities, Fig.2. The liver of the fish from river source (site1-Ferizaj) exhibited a normal architecture and there were no pathological abnormalities, with hepatocytes presenting a homogenous cytoplasm, and a large central or sub central spherical nucleus.

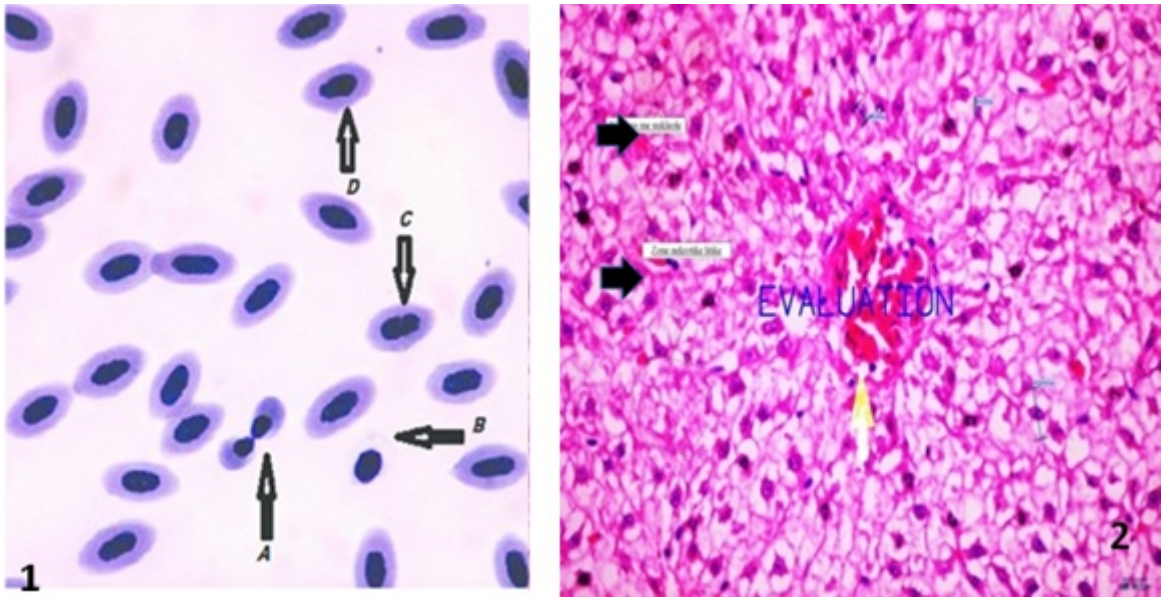


**Figure 2.** Erythrocyte cellular and nuclear anomalies and histological pathologies of *C. carpio* liver

Our results indicate that the fish are under a highly stressful condition due to the presence of industrial effluents. These findings are in agreement with those of Stehr et al, 1998; Chovanec et al., 2003; Ebrahimi and Taherianfard, 2011; Dhevakrishnan and Zaman, 2012; and Sula and Aliko, 2017b, which had reported that risk for cellular and nuclear vacuolization increased with the presence of aromatic and chlorinated hydrocarbons in sediments. Our findings are supported by the fact that Vragoli and Plemetin sampling sites were reported to have the highest level of heavy metals.

## Conclusions

In summary, our results show that the combination of biomarkers of response at different levels of sentinel organism's organization is the most valuable approach, because it can give a more balanced view on organism response to water pollution, comparing to single biomarkers. This holistic *in situ* approach to environmental assessment is encouraged as it helps to identify the integrated impact of chemical contamination on organisms and to provide a realistic measure of environmental quality.

## References

Aliko V, (2008) Comparative blood morphology in the peripheral blood films from different vertebrate classes with an adaptation and evolutionary approach. *Proceedings of Int. Confer.Biolo. & Environ. Sci., pp:*19-27.

Aliko V, Sula E, (2011) Study of fish stress physiology through evaluation of physiological and biochemical biomarkers in blood. *Master Thesis*. 50 pg.

Berisha L, Arbneshi, T, Rugova, M, (2008) The level concentration of lead, cadmium, copper, zinc and phenols in water of Sitnica River, *J. Int. Balwois. Ohrid*, Republic of Macedonia.

Čalić V, Miosic N, Cencic K, Zdenka P, Mladen P, Nena P, Rugova A, Arbneshi T, (2005) Pollution of river sediments in Bosnia and Herzegovina and Kosovo with polychlorinated biphenyls. *25th International Symposium on Halogenated Environmental Organic Pollutants and Persistent Organic Pollutants (POPs)-DIOXIN*.

Carrasco KR, Tilbury KL, Myers MS, (1990) Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. *Can Aquat Sci*.; **47**:2123–2136.

Chovanec, A., Hofer, R., Schiemer, F., 2003, Fish as bioindicator. In: Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), *Bioindicators and Biomonitors. Elsevier Science Ltd, Oxford* (Chapter 18).

Dakonta, (2009) Consulting services for environmental assessment and remedial action plan for Mitrovica Industrial Park, Kosovo, UNDP.

Dhevakrishnan, R. and Zaman M. H., 2012, Effect of Cauvery River pollutants on histopathological alterations in gill and liver tissues of freshwater fish *Labio rohita*, *Environmental Toxicology Int.J.Cur. Tr. Res*.1/2:65-71.

Ebrahimi, M., Taherianfard, M., 2011, The effects of heavy metals exposure on reproductive system of cyprinid fish from Kor River, *Iranian Journal of Fisheries Sciences*, 10/1:13-21.

Falfushynska, H.I., Stolyar, O.B., 2009. Responses of biochemical markers in carp *Cyprinus carpio* from two field sites in Western Ukraine. *Ecotoxicology and Environmental Safety*, 72, 729–736.

Ferreira, M., Moradas-Ferreira, P., Reis-Henriques, M.A., 2005. Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and founder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic Toxicology*, 71, 39–48.

Frenzilli, G., Falleni, A., Scarcelli, V., Del Barga, I., Pellegrini, S., Savarino, G., Mariotti, V., Benedetti, M. Fattorini, D., Regoli, F., Nigro, M., 2008, Cellular responses in the cyprinid *Leuciscus cephalus* from a contaminated freshwater ecosystem. *Aquatic Toxicology*, 89, 188–196.

Gashi, F., Franciskovic-Bilinski, S., Bilinski, H., Troni, N., Bacaj, M., Jusufi, F., 2011, Establishing monitoring network on Kosovo rivers: preliminary measurements on the four main rivers (Drini i Bardhe, Morava e Binqes, Lepenc and Sitnica). *Environ. Monitoring & Assessment*, 175, 1-4:279-289.

Goldenfarb, P.B., Bowyer, F.P., Hall, E. and Brosious, E., 1971, Reproducibility in the hematology laboratory: the microhematocrit determination. *American Journal of Clinical Pathology*, 56 (1), 35-39. PMid:5556212

Hansen, P.D., 2003, Biomarkers. In: Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), Bioindicators and Biomonitors. *Elsevier Science Ltd, Oxford* (Chapter 6).

Jusufi, S., Arbneshi, T., Shehdula, M., Bacaj, M., 2004, Phenol Concentration in Sitnica River. Proceedings of the II Congress of Ecologist of the Republic of Macedonia, with *J. Int. Environmental Application & Science*, 3 (2): 66-73 Special issues of Macedonian Ecological Society Vol 6, pp 568 – 571. Published by Macedonian Ecological Society. Skopje.

Kosovo Environmental Protection Agency (KEPA). Report: The state of water in Kosovo. Retrieved, November 3, 2012: www.ammkrks.net/repository/docs/raporti_ujerave_2010-angl.pdf.

Lavado, R., Urena, R., Martin-Skilton, R., Torreblanca, A., del Ramo, J., Raldu´ a, D., Porte, C., 2006, The combined use of chemical and biochemical markers to assess water quality along the Ebro River. *Environmental Pollution*, 139, 330–339.

Linde-Arias, A.R., Ina´ cio, A.F., Novo, L.A., Alburquerque, C., Moreira, J.C., 2008b, Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul. *Environmental Pollution*, 156, 974– 979.

Linde-Arias, A.R., Inacio, A.F., Alburquerque, C., Freire, M.M., Moreira, J.C., 2008a, Biomarkers in an invasive fish species, *Oreochromis niloticus*, to assess the effects of pollution in a highly degraded Brazilian River. *Science of the Total Environment*, 399, 186–192.

Monteiro, V., Cavalcante, G.S.M., Vilela, M.B.F.A., Sofia, S.H., Martinez, C.B.R., 2011, In vivo and in vitro exposures for the evaluation of the genotoxic effects of lead on the neotropical freshwater fish *Prochilodus lineatus*. *Aquatic Toxicology*, 104: 291-298.

Morina, V., Aliko, V., Sula, E., Gavazaj, F., Kastrati, Dh., Cakaj, F., 2013, Histopathologic biomarkers of fish liver as good bioindicator of water pollution in Sitnica River, Kosovo. *Global Journal of Science Frontier Research Agriculture and Veterinary*: 13/5:1-5.

Ochei, J., Kolhatkar, A., 2005, Medical Laboratory Science: Theory and Practice. Tata McGraw – Hill Publishing Company, New Delhi, pp. 281–283.

Ohe, T., Watanabe, T. and Wakabayashi, K., 2004, Mutagens in surface waters: a review. *Mutation Research*, vol. 567, p. 109-149.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S., 2003, Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallagoattu (Bl. & Schn.) *Science of the Total Environment*, 309, 105–115.

Parvez, S., Pandey, S., Ali, M., Raisuddin, S., 2006, Biomarkers of oxidative stress in Wallagoattu (Bl. and Sch.) during and after a fish-kill episode at Panipat, India. *Science of the Total Environment*, 368, 627–636.

Parvez, S., Raisuddin, S., (2005) Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). *Environmental Toxicology and Pharmacology*, 20, 112–117.

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 M.P. India. ISSN: 2277-5536 (Print version).

Ruas, C.B.G., Carvalho, C.S., Araujo, H.S.S., Espındola, E.L.G., Fernandes, M.N., (2008) Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. *Ecotoxicology and Environmental Safety*, 71, 86–93.

Schmid, W., 1975) The micronucleus test. *Mutat. Res*. 31: 9-15.

Stehr, C.M., Johnson, LL., Myers, M.S., 1998, Hydropic vacuolation in the liver of three species of fish from the US West Coast: lesion description and risk assessment associated with contaminant exposure. PMID: 9676252.

Sula, E., Aliko V, (2017a) Effects of stressors on hematological and immunological response in the fresh water crucian carp fish, *Carassius carassius. Albanian J. Agric. Sci.* (Special edition): 575 – 581.

Sula, E., Aliko, V., (2017b) Pathological changes in liver morphology of Crucian carp (*Carassius carassius*) from Seferani Lake in Dumrea region. *Albanian J. Agric. Sci.* (Special edition): 583 – 590.

Tejeda-Vera R, Lo´pez-Lo´pez E, Seden˜o-Dı´az JE, (2007) Biomarkers and bioindicators of the health condition of *Am. Splen.* & *Goodea Atripinnis* (Pisces: *Goodeaidae*) in the Ameca River, Mexico. *Environ. Int.*, **33**, 521–531.

Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003, Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxi. & Pharm.*, **13**, 57–149.