Turkish Journal of Agriculture - Food Science and Technology, 7(9): 1424-1428, 2019
DOI: https://doi.org/10.24925/turjaf.v7i9.1424-1428.2727

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology

Congo Red Effects on Detoxification and Metabolic Enzymes in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792)

Durali Danabas[1,a,*], Nuran Cikcikoglu Yildirim[2,b], Numan Yildirim[3,c], Onder Aksu[1,d]

[1]*Munzur University, Fisheries Faculty, TR62000, Tunceli, Turkey*
[2]*Munzur University, Pertek Sakine Genç Vocational School, Department of Veterinary Medicine, TR62000, Tunceli, Turkey*
[3]*Munzur University, Tunceli Vocational School, Department of Plant and Animal Production, TR62000, Tunceli, Turkey*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 30/05/2019
Accepted : 05/08/2019

Keywords:
Congo Red
CYP1A1
GST
LDH
Rainbow trout

ABSTRACT

The aim of this study was to evaluate the activities of the biotransformation enzymes (Glutatyon S-Transferase (GST) and cytochrome P450 (CYP1A1)) and metabolic enzyme (Lactate dehydrogenase-LDH) in the liver of rainbow trout (*Oncorhynchus mykiss*) after 24 and 48 hour exposure to 0.5, 1.0 and, 2.0 mg l^{-1} of Congo red (CR). Enzyme activities were determined by using commercial kits with ELISA method. Congo red altered the activities of GST, CYP1A, and LDH enzymes in liver tissue of *O. mykiss* in a dose-dependent manner. The statistical differences in GST activities among the groups for 24 and 48 h were significant, but, LDH activities were significant for only 24 h. Exposure duration of CR didn't affect the biochemical response of rainbow trout. Thus, CR exposure changed the biotransformation and metabolic enzymes, and the changes of these enzymes activities may be used as a potential bioindicator of the CR exposure.

[a] *dalid07@gmail.com* *http://orcid.org/0000-0002-8947-3749*
[b] *nurancyildirim@gmail.com* *https://orcid.org/0000-0003-3975-6705*
[c] *numanyildirim44@gmail.com* *https://orcid.org/0000-0003-1109-8106*
[d] *onderaksu@munzur.edu.tr* *https://orcid.org/0000-0003-3735-6732*

Introduction

The environment is continuously loaded with chemicals released by industries (Van Der Oost et al., 2003). Synthetic dyes such as azo dyes are generally used in the different industries like food, textile, cosmetic, plastic, and pharmaceutical industries. A major part of these synthetic dyes have been shown to have the toxic effects for flora and fauna in the environment (Kagalkar et al., 2010; Lin et al., 2011). Quite a change of commercial dyes and pigments exists, and, annually exeeder amounts of 7×105 tons are produced in the world-around. However, between 10 and 15% of these amounts are released as waste maters during the dyeing processes (Gomez et al., 2007). Dye concentrations lower than 1.0 ppm may induce visible coloration and hence public complaints (Metivier-Pignon et al., 2007). Important biochemical responses occur after the organisms living in the aquatic environments have been exposed to different environmental organic/inorganic contaminants (Booth et al., 1988).

Biomarkers are some parameters measuring behavior, physiology, biochemistry, of organisms in response to pollution (Bottcher and Schroll, 2007; Dosnon-Olette et al., 2010). Metabolic and detoxification enzymes play an important role in maintaining cell homeostasis and their induction shows a specific response to pollutants. Thus, these responces were indicated as biochemical biomarkers of pollutant-based oxidative stress in several organisms living in aquatic environments (Regoli et al., 1998).

The biotranformation system can be categorized in to phase I and phase II enzymes, which protect cells against the deleterious effects of xenobiotics (Kaur and Kaur, 2015). The cytochrome (CYP) enzymes mediate phase I biotransformations, primarily acting as a monooxygenase, where an oxygen atom is incorporated into substrates. These reactions usually convert hydrophobic xenobiotics into polar forms, making them more water soluble and thus, more readily eliminated (Brown and Reisfeld, 2008).

Many studies have suggested CYP1A mRNA level and CYP-dependent enzymatic activity in fish liver in response to the presence of pollutants in the aquatic environment (Moore et al., 2003; Binelli et al., 2006; Santos and Martincez, 2012).

Glutatyon S-Transferase is a phase II family, detoxifying enzymes with a determinant function in the biotransformation processes (Thom et al., 2001) by catalyzing the conjugation of different electrophilic compounds with glutathione; the resulting conjugates being more water soluble and easily can be able to extract (Cheung et al., 2001). It has been reported that an increasing in the GST activity of liver in various fish species, results from exposure to PCBs, PAHs, OCPs, metals, and pesticides (Lopes et al., 1999).

Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in especially all major organ systems. The extracellular LDH appearance is used to detect cell damage or cell death (Glick, 1969; Moss and Henderson, 1986; Lott and Nemensanszky, 1987).

Azo dyes compounds characterized by the presence of one or more azo groups (—N═N—) constitute the most class of dyes in the textile industry and have a serious environmental impact (Demirci and Hamamcı, 2013). The immediate toxic impact of dyes and degraded products on different aquatic organisms being has been demonstrated (Copaciu et al., 2013).

The aims of the present study were to measure detoxification enzymes (GST and CYP1A1) and a metabolic enzyme (LDH) in liver of rainbow trout (*O. mykiss*) exposing to CR and determine the biochemical response of CR in rainbow trout liver simultaneously.

Materials and Methods

Experimental Model Animal

Rainbow trout, weighing 139.0±25.0 g (mean±SE), were obtained from a local commercial fish farm (Tunceli, Turkey). They were held in a fish tank (2.0×2.0×1.0 m). The temperature and photoperiod were 18.0±0.5°C and 12:12 light-dark cycle, respectively. For 2 days before the experiment, fish were acclimatized.

Experimental Design

Congo Red (CR, product code C6277) was purchased from Sigma-aldrich. Experiment was performed in four aquariums (90.0 l), each stocked with 10 fish, i.e. three aquariums with 0.5 mg l^{-1} (Group A), 1.0 mg l^{-1} (Group B), and 2.0 mg l^{-1} (Group C) of CR and one control aquarium with no containing CR. The CR concentrations in exposure tank spectrofotometrically confirmed. In 24 h, half of the experimental fish was sampled and other half in 48 h. However, 5 fish were sampled in the beginning of the experiment for 0 h.

Biochemical Analysis in Model Animal

Fish were anaesthetized to deep sedation with 0.7 g l^{-1} benzocaine dissolved in ethyl alcohol (Sardella et al., 2004) and anesthesia of fish being observed as deep sedation, loss of swimming actions and partial loss of equilibrium (Altun and Danabas, 2006), and then, dissected for obtaining of liver tissue. The blood in the tissue samples was removed. Then, the samples have been weighed and homogenized. The homogenized samples have been centrifuged; the supernatants were obtained and kept at -70°C until their analyses. The animal experiments in this study were performed in accordance with the rules in guidelines for animal research and health from the National Institute of Health.

Glutatyon S-transferase: The activity of GST was determined by using The Cayman Chemical Glutathione S-Transferase Assay kit for fish (catalog No. GST: 703302). This assay measures total GST activity by measuring the conjugation of 1-chloro-2.4-dinitrobenzene (CDNB) with reduced glutathione.

Cytochrome P450: The activity of CYP1A1 was determined by using the cusabio fish CYP1A1 ELISA kit (catalog No: CSB-EL006395FI). This assay employs the competitive inhibition enzyme immune assay technique.

Lactate dehydrogenase activity: The activity of LDH was determined by using fish LDH ELISA Kit produced by My BioSource Chemical Company (catalog No: LDH: MBS-013278). The ELISA analytical biochemical technique of the MBS013278 kit is based on LDH antibody-LDH antigen interactions (immunosorbency) and an HRP colorimetric detection system to detect LDH antigen targets in samples.

Statistical Analysis

Among the all experimental groups, to determine the statistical differences in all analysed parameters, One-Way ANOVA and the Duncan Multiple Range Test were used ($P<0.05$).

Results

Glutatyon S-Transferase, CYP1A1 and LDH activities in liver tissue of *O. mykiss* exposed to CR textile dye in different concentrations was investigated and the results are shown in Table 1.

Glutatyon S-Transferase Activities

In the control group, GST activity decreased, when compared with T_0 during 24 and 48 h, but this was not statistically significant ($P>0.05$). In the Group A (0.5 mg l^{-1}) and B (1.0 mg l^{-1}), there was an increase during 24 and 48 h when compared to T_0, but this increase was statistically insignificant ($P>0.05$). The activity of GST decreased slightly in 48 h according to 24 h. Group C (2.0 mg l^{-1}) showed the highest increase during 24 h, but there was a decrease for 48 h. This statistical difference was significant ($P<0.05$)

Glutatyon S-Transferase activities were increased in all the groups exposed to different doses of dye when compared to the control group during 24 and 48 h. The GST activities in 48 h were lower than 24 h. The differences among the groups for 24 and 48 h were statistically found as significant ($P<0.05$).

Cytochrome P450 Activities

The CYP1A1 activities were increased in the control group during 24 h compared to 0 h, but decreased during 48 h ($P>0.05$). The CYP1A1 activity in Group A decreased during 24 and 48 h when compared to 0 h ($P>0.05$). The CYP1A1 activity in Groups B and C increased during 24 and 48 h ($P> 0.05$).

Table 1 Glutathione S-transferase (nmol/min/ml), and Cytochrome P450 1A1 (pg ml^{-1}) and Lactate dehydrogenase (U l^{-1}) activities in liver of rainbow trout (*O. mykiss*) exposed to different doses of CR

Parameters	T_0	Groups	Exposure duration	
			T_{24}	T_{48}
GST (nmol/min/ml)	$149.76\pm6.14_q$	Control	101.88 ± 23.82^a_q	77.53 ± 33.51^a_q
	$149.76\pm6.14_q$	A(0.5 mg l^{-1})	$188.86\pm28.12^{ab}_q$	170.82 ± 31.45^b_q
	$149.76\pm6.14_q$	B(1.0 mg l^{-1})	$198.14\pm54.03^{ab}_q$	177.43 ± 15.78^b_q
	$149.76\pm6.14_q$	C(2.0 mg l^{-1})	27882 ± 16.93^b_p	$121.27\pm0.12^{ab}_q$
CYP1A1-P450 (pg ml^{-1})	$205.82\pm38.72_q$	Control	252.10 ± 44.10^a_q	$198.63\pm40.34^{ab}_q$
	$205.82\pm38.72_q$	A(0.5 mg l^{-})	160.17 ± 3.17^a_q	172.96 ± 37.41^a_q
	$205.82\pm38.72_q$	B(1.0 mg l^{-1})	235.00 ± 15.39^a_q	$233.69\pm3.84^{ab}_q$
	$205.82\pm38.72_q$	C(2.0 mg l^{-1})	212.96 ± 28.80^a_q	280.86 ± 3.56^b_q
LDH (U l^{-1})	$628.03\pm112.29_q$	Control	$500.18\pm13.45^{ab}_q$	582.94 ± 80.46^a_q
	$628.03\pm112.29_q$	A(0.5 mg l^{-})	718.16 ± 53.16^b_q	636.50 ± 22.24^a_q
	$628.03\pm112.29_q$	B(1.0 mg l^{-1})	$534.60\pm40.66^{ab}_q$	663.53 ± 66.34^a_q
	$628.03\pm112.29_q$	C(2.0 mg l^{-1})	492.09 ± 109.25^a_q	474.02 ± 24.22^a_q

Different letters (a and b) show statistical differences of Duncan multiple range test among control and all applications of groups in same hour for same parameter. Different letters (q and p) show statistical differences of Duncan multiple range tests among exposure durations (T_0, T_{24} and T_{48} hours) in same group

When CYP1A1 activity compared to the control group, a decrease in the all experimental groups was observed for 24 h ($P>0.05$). There was a decrease in the Group A, and an increase in the Groups B and C ($P<0.05$), when compared to the control group for 48 h.

Lactate Dehydrogenase Activities

The LDH activities were reduced when compared to 0 h during 24 and 48 h in the control group and Group C ($P>0.05$). In Group A, there was an increase during 24 and 48 h compared to 0 h ($P>0.05$). A decrease in the Group B was observed during 24 h, although an increase was observed for 48 h compared to 0 h ($P>0.05$).

The LDH activities were increased in Groups A and B, but decreased in Group C ($P<0.05$) compared to the control group during 24 and 48 h. The differences among the experimental groups were statistically significant for only 24 h ($P<0.05$).

Discussion

The discharge of waste matters into the aquatic environments including the dye-containing effluents is undesirable, because of not only their colours, but also numerous of these dyes released some breakdown products. They have some harmful effects (e.g.; carcinogenic, toxic or mutagenic, etc.) to the aquatic organisms (Suteu et al., 2009; Zaharia et al., 2009) depending on the dose and exposure duration (Yusuff and Sonibare, 2004). Without advisable treatment process, if these dyes were leave din to the aquatic environments, they can remain in there for long times (Hao et al., 2000).

As the experimental species, the rainbow trout (*O. mykiss*), one of the most important fish models in the aquatic toxicology researches and of commercially important in our country, was exposed to CR as dye-kinds to determine its acute effects on biochemical and physiological responses of fish in the present research. This was done by analysing detoxification enzymes (GST and CYP1A1) and a metabolic enzyme (LDH) in liver tissue.

Glutathione S-transferases (GSTs) are a large family of enzymes catalyse the conjugation of (reduced) glutathione to a range of endogenous and xenobiotic substrates. One of their important roles is their contribution to phase II in biotransformation of exogenous compounds; typically, conjugation to glutathione makes them more soluble, simplifying their elimination from the organisms (Hajime et al., 2005). The GST activities in *Xenopus laevis* tadpoles were clearly increased after a 24 h exposure to Astrazon Red FBL, Remazol Red RR, and Remazol Turquoise Blue G-A compared to control (Güngördü et al., 2013). Similar GST induction were determined in hepatopancreas tissue of crayfish (*Astacus leptodactylus*) exposed to some ones of untreated textile wastewaters (Aksu et al., 2015). In a dose dependent manner, increasing of GST activities over control was determined in kidney, gills and brain of *Labeo rohita*, while a decreasing was noticed in the liver and muscle tissues, after 96 h exposurance to acid black dye (Kaur and Kaur, 2015). Sun et al. (2006; 2007) found that GST inductions in goldfish (*Carassius auratus*) that were exposed to HC Orange No. 1 dye. An increase in the activities of GST, in fish induced by azo dye, has been observed by Peng et al. (2010) and Yin et al. (2007). Aksu et al. (2017) determined the effects of two different textile dyes (Remazol Brillant Blue R (RBBR) and CR) on the enzymatic activities of GST, CYP1A1 and LDH, in the hepatopancreas tissue of crayfish, *A. leptodactylus*. They stated that GST and LDH activities increased and the CYP1A1 activities decreased after the exposurance to the CR; and suggested that these detoxification and metabolic enzymes were useful markers for further evaluating the physiological effects of CR and RBBR on crayfish, densely and continuously exposure the stress effects. In present study, the activities of GST increased in all the groups exposed to different doses of dye when compared to the control group during 24 h and 48 h. ($P<0.05$). The increased activities of GST support that GST enzyme would play an important role in detoxification of CR in *O. mykiss*. The increased GST activities were probably a metabolic adaptation to the exposure to CR and were defence against oxidative damage.

In fish, many xenobiotics act as CYP1A inducers, and CYP1A has been affirmed as a biomarker for the evaluation of pollution in the aquatic environments and organisms. Additionally, increment of CYP1A is closely related to detrimental effects such as apoptosis and

embryonic mortality in exposed fish (Dong et al., 2009). Thus, the interaction of environmental contaminants with CYP1A1 enzyme is likely to have an important toxicological relevance in fish. Environmental contaminants can induce CYP1A1 and the induction response can be monitored by measuring the change in the CYP1A1 activities (Cousinou et al., 2000; Rees and Li, 2004). In present study, an increase was observed in the Groups B (1.0 mg l^{-1}) and C (2.0 mg l^{-1}), when compared to the control group during 24 h and 48 h. An increase in CYP1A1 activity clearly indicates that the CR dye induced ROS generation in the tested fish model which was in turn responsible for an increase in the enzyme level of fish liver tissue.

Lactic dehydrogenase is a hydrogen transferring enzyme that catalyses the oxidation of L-lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor (Kurutaş et al., 2006). In this study, LDH activities were increased in Groups A and B, when compared to control during 24 and 48 h. After exposurance to the CR, the increased LDH activity in the fish tissues observed in the present study, may show the increased rate of conversion of lactate to pyruvate and then to glucose. This result is in agreement with those suggested for other aquatic species exposed to azo dye (Güngördü et al., 2013). The liver glycogen of fish was decreased, when they exposed to different xenobiotics. The an attenuation in the glycogen stores may possibly due to that the hepatic synthesis of detoxifying enzymes requires high energy levels (Begum and Vijayaraghavan, 1995; Hori et al., 2006). Some fish exposed to dyes, show an enhancing in the opercular rate, which reflects high oxygen demand. This may be a causative factor to induce the anaerobic oxidation to release energy by enhancing of the activities of LDH (Nassr-Allah and Abdel-Hameid, 2007).

The tested dye affected the biomarker enzymes of aquatic organism (*O. mykiss*) in different levels and concentrations. The alteration of GST, CYP1A1, and LDH activity uncovered metabolic changes after CR exposure and CR could be metabolized, which can cause a high level of oxidative stress. The possible toxicity mechanism of rainbow trout can be explained with related oxidative stress mechanism, in which tested dye were transformed by GST and oxidative stress injury to the aerobic respiration.

Conclusion

The present study indicates important biochemical effects of CR in rainbow trout liver, suggests that exposure to CR may cause marked changes in fish. The experimental results demonstrated that the CYP1A1, GST and LDH can be used as biomarkers in evaluating the impact of CR exposure on the *O. mykiss* could be used as a useful tool in the research of the toxicology of CR. Detoxification enzymes (GST and CYP1A1) and a metabolic enzyme (LDH) could provide some different useful parameters for evaluating the physiological effects of azo dyes like CR in the rainbow trout, but a more detailed laboratory study will be carried out before these findings are applied to monitor the residual CR in aquatic organisms and environment. However, it is well known that acute toxicity in fish is unlikely to occur at the lower measured environmental concentrations; therefore, more long-term experiments at lower CR concentrations are needed to validate these parameters as biomarkers in large-scale environmental monitoring programmes.

Acknowledgments

This work was supported by Munzur University, Coordinatorship Unit of Scientific Research Projects (Tunceli, TURKEY) (MUNIBAP) (project no: MFTUB015-21).

Authors' Contributions

All responsibilities and contributions of every author are equal.

Conflict of Interest Disclosure

There is no conflict of interest among authors of this manuscript.

References

Aksu O, Yildirim NC, Yildirim N, Danabas D, Danabas S. 2015. Biochemical response of crayfish *Astacus leptodactylus* exposed to textile wastewater treated by indigenous white rot fungus *Coriolus versicolor*. Environmental Science and Pollution Research, 22: 2987–2993.

Aksu O, Yildirim NC, Danabas D, Yildirim N. 2017. Biochemical impacts of the textile dyes Remazol Brillant Blue R and Congo Red on the crayfish *Astacus leptodactylus* (Decapoda, Astacidae). Crustaceana, 90(13): 1563-1574.

Altun T, Danabas D. 2006. Effects of short and long exposure to the anesthetic 2-phenoxyethanol mixed with ethyl alcohol on common carp (*Cyprinus carpio* L., 1758) fingerlings. Isr. J. Aquac. Bamidgeh, 58: 1-5.

Begum G, Vijayaraghavan S. 1995. Carbohydrate metabolism in hepatic tissue of freshwater *Clarius batracus* L. during dimethoate exposure. Fd. Chem. Toxic, 33: 23-426.

Binelli A, Ricciardi F, Riva C, Provini A. 2006. New evidences for old biomarkers: Effects of several xenobiotics on EROD and AChE activities in Zebra mussel (*Dreissena polymorpha*). Chemosphere, 62: 510–519.

Booth CE, McDonald DG, Simons BP, Wood CM. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the Brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences, 45: 1563–1574.

Bottcher T, Schroll R. 2007. The fate of isoproturon in a fresh water microcosm with *Lemna minor* as a model organism. Chemosphere 66: 684–689.

Brown CM, Reisfeld B. 2008. Cytochromes P450: A structure-based summary of biotransformations using representative substrates. Drug Metabolism Reviews, 40: 1–100.

Cheung C, Zheng G, Li A, Richardson B, Lam P. 2001. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. Aquat. Toxicol. 52: 189–203.

Copaciu F, Opris O, Coman V, Ristoiu D, Niinemets Ü, Copolovici L. 2013. Diffuse water pollution by anthraquinone and azo dyes in environment importantly alters foliage volatiles, carotenoids and physiology in wheat (*Triticum aestivum*). Water Air Soil Pollut. 224: http://dx.doi.org/10.1007/s11270-013-1478-4.

Cousinou M, Nilsen B, Lopez-Barea J, Dorado G. 2000. New methods to use fish cytochrome P4501A to assess marine organic pollutants. Sci. Total Environ. 247: 213–225.

Demirci O, Hamamcı DA. 2013. Antioxidant responses in *Phanerochaete chrysosporium* exposed to Astrazone Red FBL textile dye. Cell Biochemistry and Function, 31(1): 89-90.

Dong XL, Zhu LS, Wang JH, Wang J, Xie H, Hou XX, Jia WT. 2009. Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). Chemosphere. 77: 404–412.

Dosnon-Olette R, Trotel-Aziz A, Couderchet M, Eullaffroy P. 2010. Fungicides and herbicide removal in Scenedesmus cell suspensions. Chemosphere, 79: 117–123.

Glick JH. 1969. Serum lactate dehydrogenase isoenzyme and total lactate dehydrogenase values in health and disease, and clinical evaluation of these test by means of discriminant analysis. Am. J. Clin. Pathol., 52: 320–328.

Gomez V, Larrechi MS, Callao MP. 2007. Kinetic and adsorption study of acid dye removalusing activated carbon. Chemosphere, 69: 1151–1158.

Güngördü A, Birhanli A, Ozmen M. 2013. Biochemical response to exposure to six textile dyes in early developmental stages of *Xenopus laevis.* Environ. Sci. Pollut. Res., 20: 452–460.

Hajime O, Ozaki K, Yoshikawa H. 2005. Identification of cytochrome P450 and glutathione-S-transferase genes preferential lyexpressed in chemosensory organs of the swallow tail butterfly, *Papilio xuthus* L. Insect Biochem. Mol. Biol. 8: 837–846.

Hao OJ, Kim H, Chang PC. 2000. Decolorization of wastewater. Critical Reviews in Environmental Science and Technology. 30: 449-505.

Hori TSF, Avilez IM, Inoue LK, Moraes G. 2006. Metabolical changes induced by chronic phenol exposure in matrinxã *Brycon cephalus* (teleostei: characidae) juveniles. Comp. Biochem. Physiol., 143(1): 67-72.

Kagalkar AN, Jagtap UB, Jadhav JP, Govindwar SP, Bapat VA. 2010. Studies on hytoremediation potentiality of *Typhonium flagelliforme* for the degradation of Brilliant Blue. Planta, 232(1): 271–285.

Kaur S, Kaur A. 2015. Variability in antioxidant/detoxification enzymes of *Labeo rohita* exposed to an azo dye, acid black (AB). Comparative Biochemistry and Physiology, Part C, 167: 108–116.

Kurutaş EB, Doran F, Çıralık H. 2006. The effect of endosulfan on lactic dehydrogenase enzyme system in liver of *Mus musculus*: A histochemical study. Eur J Gen Med. 3(4): 148-151.

Lin YH, He XB, Han GM, Tian QJ, Hu WY. 2011. Removal of Crystal Violet from aqueous solution using powdered mycelial biomass of *Ceriporia lacerata* P2. Journal of Environmental Sciences, 23(12): 2055–2062.

Lopes PA, Pinheiro T, Santos MC, Mathias ML, Collares-Pereira MJ, Viegas-Crespo AM. 1999. Response of antioxidant enzyme in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Sci Total Environ. 280: 153–163.

Lott JA, Nemensanszky E. 1987. *Lactate dehydrogenase.* In: Lott JA, Wolf PL, eds. Clinical Enzymology, a Caseoriented Approach. p. 213–244.

Metivier-Pignon H, Faur C, Cloirec PL. 2007. Adsorption of dyes onto activated carbon cloth: Using QSPRs as tools to approach adsorption mechanisms. Chemosphere, 66: 887–889.

Moore MJ, Mitrofanov IV, Vlentini SS, Volkov VV, Kurbskyi AV, Zhimbey EN, Eglinton LB, Stegeman JJ. 2003. Cytochrome P450 1A expression, chemical contaminants and histopathology in roach, goby and sturgeon and chemical contaminants in sediments from the Caspian Sea, Lake Balkhash and the fly River Delta, Kazakhstan. Mar. Pollut. Bull. 46: 107–119.

Moss DW, Henderson AR. 1986. *Enzymes.* In: Burtis CA, Ashwood ER eds. Tietz Textbook of Clinical Chemistry. 2nd edn. Philadelphia, Saunders Co., p. 735–896.

Nassr-Allah H, Abdel-Hameid NAH. 2007. Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. Turkish Journal of Fisheries and Aquatic Sciences, 7: 131-138.

Peng S, Xing Y, Rui L, Jian-ping C. 2010. Effects of nitrobenzene on liver antioxidant defence system of *Carassius auratus*. Chem. Res. Chin. Univ., 26(2): 204–209.

Rees CB, Li W. 2004. Development and application of a real-time quantitative PCR assay for determining CYP1A transcripts in three genera of salmonids. Aquat. Toxicol., 66: 357–368.

Regoli F, Nigro M, Orlando E. 1998. Lysosomal and antioxidant responses to metals in the Antarctic scallop *Adamussium colbecki*. Aquat. Toxicol., 40: 375–392.

Santos TG, Martincez CB. 2012. Atrazine promotes biochemical changes and DNA damage in a neotropical fish species. Chemosphere, 89: 1118–1125

Sardella BA, Matey V, Cooper J, Gonzalez RJ, Brauner CJ. 2004. Physiological, biochemical and morphological indicators of osmoregulatory stress in `California' mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to hypersaline water. Journal of Experimental Biology, 207: 1399-1413.

Sun Y, Yu H, Zhang J, Yin Y, Shen H, Liu H, Wang X. 2006. Bioaccumulation and antioxidant responses in goldfish *Carassius auratus* under HC orange no. 1 exposure. Ecotoxicol. Environ. Saf., 63: 430–437.

Sun Y, Yin Y, Zhang J, Yu H, Wang X. 2007. Bioaccumulation and ROS generation in liver of freshwater fish, goldfish *Carassius auratus* under HC orange no. 1 exposure. Environ. Toxicol., 22: 256–263.

Suteu D, Zaharia C, Muresan A, Muresan R, Popescu A. 2009. Using of industrial waste materials for textile wastewater treatment. Environmental Engineering and Management Journal, 8(5): 1097-1102.

Thom R, Dixon DP, Edwards R, Cole DL, Lapthorn AJ. 2001. The structure of azeta classglutathione S-transferase from *Arabidopsis thaliana*: Characterisation of a GST with novel active-site tyrosine catabolism. J. Mol. Biol. 308: 949–962.

Van der Oost R, Beyer J, Vermeulen NPE. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol., 13: 57–149.

Yin Y, Jia H, Sun Y, Yu H, Wang X, Wu J, Xue Y. 2007. Bioaccumulation and ROS generation in liver of *Carassius auratus*, exposed to phenanthrene. Comp. Biochem. Physiol. C, 145: 288–293.

Yusuff RO, Sonibare JA. 2004. Characterization of textile industries effluents in Kaduna, Nigeria and pollution implications. Global Nest: Int. J., 3: 212-221.

Zaharia C, Suteu D, Muresan A, Muresan R, Popescu A. 2009. Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. Environmental Engineering and Management Journal, 8: 1359-1369.