Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 963 - 971

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CATALASE AND GLUTATHIONE-S-TRANSFERASE ACTIVITY IN DIFFERENT TISSUES OF FRESHWATER CATFISH C*LARIAS GARIEPINUS* ON EXPOSURE TO CADMIUM

SUMIT ROSE*[1], S.VINCENT[1] AND B.MEENA[2]

[1]Department of Zoology, Loyola College, Chennai-600 034, Tamil Nadu, India.
[2]Department of Zoology, Presidency College, Chennai-600 005, Tamil Nadu, India.

ABSTRACT

Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota. In vivo effects of Cadmium (Cd) levels of expression on antioxidant enzymes such as Catalase (CAT) and Glutathione-S-transferase (GST) were investigated in liver, kidney, gill and brain tissues of freshwater Catfish, *Clarias gariepinus.* CAT is a common antioxidant enzyme which breaks down hydrogen peroxide into oxygen and water and restricts the accumulation of carbon dioxide bubbles in the blood. is produced naturally in almost all living organisms. CAT levels were measured using spectrophotometrically at 570 nm. Activity of GST which plays an important role in the detoxification of xenobiotics was studied along with Cd. The GST levels were measured using spectrophotometrically at 340 nm. Hence CAT and GST enzymes can be considered as a sensitive bioindicator of the antioxidant defense system.

KEYWORDS: **Cadmium, Glutathione-S-transferase, Catalase, Antioxidant, *Clarias garipinus***

SUMIT ROSE
Department of Zoology, Loyola College, Chennai-600 034, Tamil Nadu, India.

INTRODUCTION

Cadmium is an ubiquitous heavy metal present in aquatic environments due to natural and anthropogenic sources and is usually present in trace amounts. Cd is naturally found on the earth's crust or occurring in combination with other elements such as Zn and Cu. Cd accumulates in drinking water and air then eventually accumulates in the body, causing a number of diseases such as Hypertension, Osteomalacia, gastric dysfunction, CNS dysfunction, and endocrine disorders in human[1,2]. Cd is primarily used for electroplating other metals and in nickeled batteries because of its relative resistance to corrosion and high electrical and thermal conductivity. These inputs may result in increased Cd levels of the aquatic ecosystems, which can be potentially toxic to organisms such as fishes. Fishes have been used as aquatic contamination indicators for many years[2]. Cd causes significant metabolic alterations and injuries to biological systems at different levels[3]. Cd after entering into the organism of fishes through the gills, binds to albumins and erythrocytes in the blood and then is transferred into tissues and organs where it is bound to proteins of low molecular weight producing metallothioneins by the induction of metallothionein mRNA synthesis[4]. About 75 % of the total accumulated Cd in the organism is deposited in the liver and kidney[5, 6], but it can also be deposited in the heart, gill and other tissues[7,8]. Fish tissues are endowed with antioxidant defense systems consisting of CAT, GST enzyme to protect them from oxidative stress caused by metals[9]. CAT, primary antioxidant defense component, eliminates hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O+O_2$) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes. CAT activity is considered as a sensitive biomarker of oxidative stress before hazardous effects could occur in fish[10, 11].

The majority of aquatic studies have been directed toward using GST as biomarkers of exposure to environmental chemicals[12]. One of the potential biomarkers is GST, which is a key to phase II detoxification enzymes. The phase II metabolism involves the conjugation of xenobiotics with endogenous substrate, thus facilitating their excretion[13]. In 1994, GST's have been recommended by the International Council for the Exploration of the Sea as biomarker which requires additional research before monitoring applications can be undertaken[14].Trace metals at the cellular level are often involved in oxidative stress, which results in the production of Reactive Oxygen Species (ROS). ROS include the superoxide radical (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical, all of which affects mainly lipids, proteins, carbohydrates, and nucleic acids[15]. The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging ROS[16]. Excessive ROS production in response to heavy metal pollution is the natural defense mechanism of biomolecules, leading to a cumulative damage[17]. When the antioxidant enzymes fail or insufficient, an increase of ROS production may originate oxidative damage[18]. Recent studies have suggested that certain GST isoenzymes may be involved in the regulation of stress-activated cell signaling pathways[19-21]. In present study, the effect of Cd on the enzyme CAT and GST activity in a freshwater catfish, *C. gariepinus* tissues were analyzed.

MATERIALS AND METHODS

The fresh water catfish *C.gariepinus* was collected from Poondi fish farm, Thiruvallur District, Tamil Nadu, Southern India. The fishes were acclimatized in the laboratory in a stone tank (100L) at room temperature (30 $\pm 2^0$C) for 7 days. The $CdCl_2$ (Merck, Mumbai, India) solution was prepared in distilled water for acute toxicity studies. The various concentrations of $CdCl_2$ such as 5.0 ppm, 10.0 ppm and 15.0 ppm were prepared and fishes were exposed for a period of 24, 48, 72 and 96 hrs. Eighty fishes with similar size, length, weight about (approx. 30-35 g) were selected and divided into four groups. Each group has 20 fishes. One group was kept as control, the other three groups were transferred to stone

tanks (100 L) containing 5.0 mg L^{-1}, 10.0 mg L^{-1}, and 15.0 mg L^{-1} of $CdCl_2$, respectively. The test solutions were renewed daily to maintain the waterborne Cd concentration. In our biological experiments, GST and CAT enzyme levels were studied in the tissues of liver, kidney, gill and brain. The content of other heavy metals was also analyzed at the test water and found to be below detectable limit (BDL) to rule out their role.

CATALASE ENZYME ASSAY

Catalase levels in response to Cd treatments were evaluated by the method of Sinha *et al.,* 1972[22]. The tissues (50mg) were homogenized in 50 mM phosphate buffer, pH 7.0, and centrifuged at 16,000g for 45 min. The supernatant was used as the enzyme source. The reaction mixture contained 2 mL of phosphate buffer (pH 7.0) 0.45 mL H_2O_2, and 0.025 mL of enzyme source. The absorbance was read at 570 nm using spectrophotometrically and the enzyme activity was expressed as micromoles of H_2O_2 consumed/min/ mg protein.

GST ENZYME ASSAY

The GST levels in response to Cd treatments were analyzed in the tissues using the method of Habig *et al*., 1974[23]. Enzymatic assay was performed on *C.gariepinus* liver, kidney, gill and brain. The tissues (50mg) were homogenized in 50 mM Tris–HCl buffer, pH 7.4, and containing 0.2 M sucrose and centrifuged at 16,000g for 45 min at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 mL contained 2.4 mL of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 mL of 30 mM CDNB and 0.1 mL of 30 mM GSH, as enzyme source. The reaction was initiated by glutathione. The absorbance was read at 340 nm against a reagent blank. The results were expressed as µM/min/mg protein. The GST levels were measured using spectrophotometrically.

STATISTICAL ANALYSIS

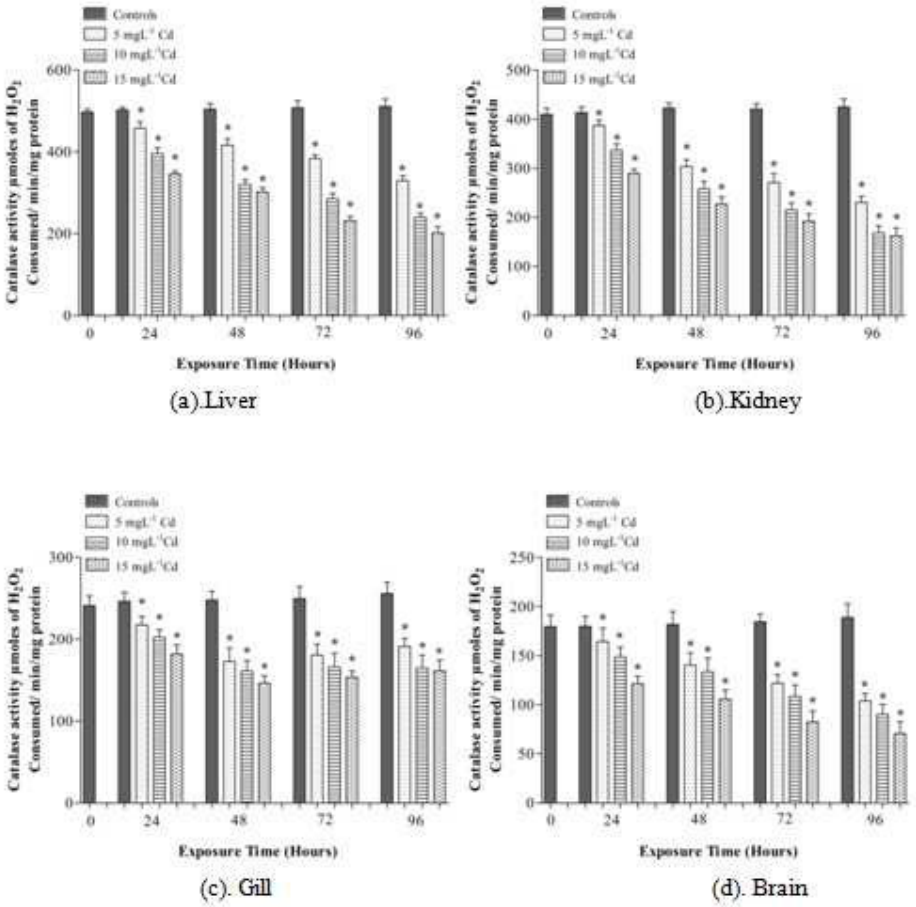
Statistical analysis of data was carried out using Graphpad Prism Version 5.0. The values are reported as mean ± SD. One-way analysis of variance was utilized to test the differences between the control, GST and CAT levels exposed for each sampling. The data of different hours of sampling were compared by ANOVA, Unifactorial analysis was used to test the differences between the control and treated groups.

RESULTS

Catalase is an important enzyme in antioxidant defense system protecting animals from oxidative stress. The effect of 5, 10 and 15ppm of $CdCl_2$ solution on the four tissues such as liver, kidney, gill and brain is graphically represented in Graph 1. The highest level of CAT enzyme levels was shown in the values of 457.6 ± 16.07 (µmol H_2O_2 consumed/min/mg protein) in the liver during 24 hrs of Cd exposure with 5.0 mg L^{-1}, during 24 hours of Cd exposure, in the kidney CAT level is 386.6 ± 10.64 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. During 24 hours of Cd exposure, in gill, CAT level were showed 217.4 ± 10.28 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. During 24 hours of Cd exposure, the brain CAT level is 164.2 ± 13.77 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. The CAT levels of control tissues value were in the following order, liver 497.2 ± 7.98 (µmol H_2O_2 consumed/min/mg protein), in kidney 409.5± 12.11 (µmol H_2O_2 consumed/min/mg protein), in gill 241.7 ± 11.89 (µmol H_2O_2 consumed/min/mg protein) and brain 179.6 ± 11.78 (µmol H_2O_2 consumed/min/mg protein). CAT activities decreased on exposure to increase Cd concentration when compared to the control CAT activity. The CAT activity in the brain was found to be lower, when compared to all other tissues. The datas were subjected to statistical analysis of one way ANOVA and the values were found to be statistically significant at $P < 0.05$.

1. Catalase enzyme Activity in C.gariepinus on exposure to Cd

Graph 1



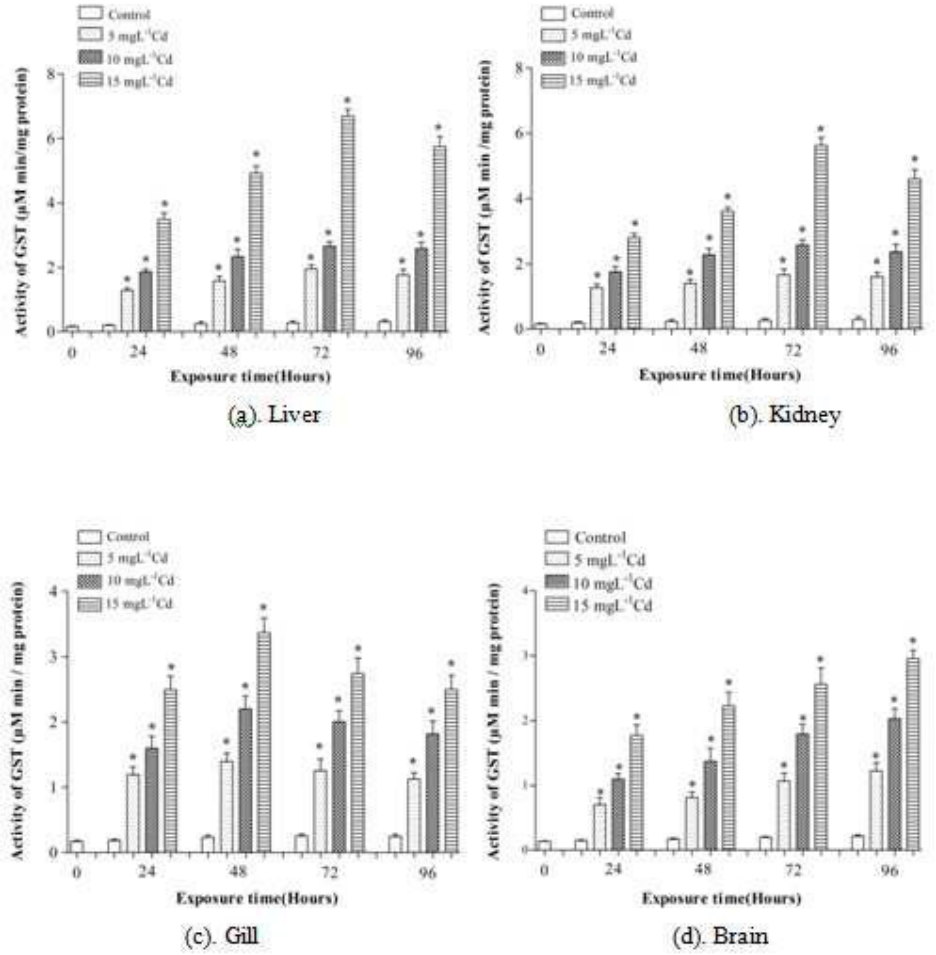
*Graph 1. (a,b,c and d) activity of CAT (μmol H_2O_2 consumed/min/mg protein) in C.gariepinus on various concentrations of $CdCl_2$ (Control, 5.0 ppm, 10.0 ppm and 15.0 ppm) for a period of 24, 48, 72 and 96 hrs. The results were represented as Mean ± SD. Statistical comparisons were made against Control fish on each sampling day. (*Statistically significant at P < 0.05)*

ACTIVITY OF GST ENZYME

GST activities in response to Cd treatments is analyzed in the liver, kidney, gill and brain of *C.gariepinus* for a period of 96 hrs. These data were graphically represented in Graph 2. The highest level of GST enzyme activity shows values of 6.695± 0.213 (μM min/mg protein) in the liver during 72 hrs of Cd exposure with 15.0 mg L^{-1}, during 72 hours of Cd exposure in the kidney, GST levels shows 5.631 ± 0.0.237 (μM min/mg protein) when treated with 15.0 mg L^{-1} of $CdCl_2$. During 48 hours of Cd exposure in the gills, GST level shows 3.366 ± 0.224 (μM min/mg protein) when treated with 15.0 mg L^{-1} of $CdCl_2$. During 96 hours of Cd exposure in the brain, GST level shows 2.958 ± 0.126 (μM min/mg protein) when treated with 15.0 mg L^{-1} of $CdCl_2$. The GST levels in various control tissues showed the following pattern as, liver (0.173 ± 0.0017), kidney (0.1609 ± 0.0171), gill (0.170 ± 0.017) and brain (0.134 ± 0.006) μM min/mg protein. In the liver and kidney, GST enzyme levels gradually increased rapidly to reach a peak during 72 hours of exposure and then declined gradually during 96 hours of exposure. In gill, GST enzyme levels decreased after 48 hours. The brain showed the lower GST levels than all other tissues were studied. In the brain, GST enzyme levels gradually increased during 96 hours of exposure. The datas were subjected to statistical analysis of one way ANOVA and the values were found to be statistically significant at $P < 0.05$.

2. GST enzyme Activity in C.gariepinus on exposure to Cd

Graph 2



Graph 2. (a,b,c and d) activity of GST (µM/mn/mg of protein) in C.gariepinus on various concentrations of $CdCl_2$ (Control, 5.0 ppm, 10.0 ppm and 15.0 ppm) exposed for a period of 24, 48, 72 and 96 hrs. The results were represented as Mean ± SD. Statistical comparisons were made against Control fish on each sampling day. (*Statistically significant at P < 0.05)

DISCUSSION

CATALASE

CAT being a primary antioxidant defense component, eliminates hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O+O_2$) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes, is considered as a sensitive biomarker of oxidative stress before hazardous effects could occur in fish[10, 11]. CAT is an important enzyme in antioxidant defense system protecting animals from oxidative stress. The highest CAT activity was determined in liver tissue compared to other tissues, which are in agreement with other literatures[10, 24]. These data are in accordance with those reported in other fish species where CAT activity is distributed in a decreasing order, as follows: liver > kidney > heart> brain > muscle[24]. In the present study, Cd decreased the CAT activity in the liver. The reduction may be associated with the direct binding of metal to –SH groups in the enzyme molecule. Liver CAT activity was found to be inhibited following both *in vivo* and *in vitro* exposure to dissolved Cd^{2+} at a concentration greater than 1 mg/L in the killifish, *Fundulus heteroclitus* and the authors suggested a direct effect of Cd^{2+} on high molecular weight compounds like CAT[25]. The liver was found to be stronger in the face of oxidative stress than the other tissues and a

uniform organ with the highest antioxidant enzyme activities (CAT). This could be related to the fact that the liver is the site of multiple oxidative reactions and maximal free radical generation[26]. The highest inhibition was observed in the kidney, this can be associated with the effective antioxidant system in this tissue where there is higher metal bioaccumulation and related to metal binding protein synthesis and non-enzymatic antioxidant mechanisms as shown by Dautremepuits *et al.,*2004[27]. Moreover, this can be attributed to the possible induction of stress proteins and non-enzymatic antioxidant formation[9]. Gill is the first affected organ when fish is exposed to metals. It was determined that there was no significant change in CAT activity in the liver and gill and this could be associated with a higher activity of GPX, which acts as a defense against the formation of H_2O_2 or effective antioxidant responses due to a higher renovation of gill epithelium. The lowest activity of CAT was measured in the gill tissue; this can be explained by the increased generation of H_2O_2, which led to a decreased CAT activity[28]. The brain is susceptible to oxidative damage through the free radicals as it contains high amounts of unsaturated lipids and utilizes about 20% of total oxygen demand of the body[29]. The specific activity of brain CAT was found to be lower, which may be related to the direct binding of metal ions to –SH groups in the enzyme molecule, increased hydrogen peroxide and superoxide radical due to oxidative stress. It was indicated that rapid inactivation of CAT at high hydrogen peroxide concentration was due to the conversion of active enzyme compound to inactive compounds[30]. In general, inhibition of CAT activity in all tissues of *C. gariepinus* might have resulted due to the direct effect of metals.

GST ENZYME

GST is a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism. GST was shown to catalyze essential steps in the biosynthesis of prostaglandins and leukotrienes[31]. GST plays a critical role in mitigating oxidative stress in all life forms and GST activity also has been widely used as a biomarker to detect stress. As an antioxidant enzyme, a GST activity has either a significant increase or decrease with different patterns according to the exposed elements or exposure conditions. GST activity varied in different tissues and organs of aquatic animals[32]. The GST activity levels of *C. gariepinus* were in the following order liver > kidney > gill > brain. These results are in accordance with the works of whitefish tissues[33]. The concentration of Cd levels in liver and kidney is much higher than gill and muscle in fishes because the liver and kidney are the major targets for Cd distribution to detoxify them by binding with MT. The gills are the major entry site of heavy metals and act as a transient store for accumulation of metals[34].

There are various modes of Cd uptake in aquatic organism, were it is most readily absorbed by organisms directly from the water in its free ionic form Cd (II), Metal ions are usually absorbed through passive diffusion or carrier mediated transport by the gills while metals associated with organic materials are ingested and absorbed by endocytosis through the intestine. It has been suggested that Cd ions enter the chloride cells in the gills through calcium channels[35]. Cd heavy metal after entering into the organism of fishes through the gills, binds to albumins and erythrocytes in the blood and is then transferred into tissues and organs where it is bound to proteins of low molecular mass producing metallothioneins by the induction of metallothionein mRNA synthesis[4]. The present study has also shown higher concentrations of GST activity in the liver than the gills. About 75 % of the total accumulated Cd in an organism deposits in the liver and kidneys[5, 6], but it can also be deposited in the heart, gills and other tissues[7, 8].

The role of the liver in antioxidant enzyme response as a result of its higher sensitivity to metals comparing to the kidney has been studied by various investigations as the liver has to overcome the oxidative stress than the other tissues because of the high antioxidant enzyme activities[36]. Liver of vertebrates exhibits a high metabolism and oxygen consumption and it is the main organ of xenobiotic detoxification. It is a particularly rich source of GST[37]. Gills uptake the heavy metals from the site and directly interact with the toxic medium[38]. But however their GST enzyme

levels are low, compared to liver and kidney. The GST activity increases steadily during 24 hrs and 48 hrs of exposure and then decline slowly during 72 hrs and 96 hrs of exposure. This variation might due to the time taken for the metal to be transported to other detoxifying organs.

CONCLUSION

In conclusion, our results indicate that antioxidant enzyme assay can be used as bioindicator for acute exposure to Cd in fresh water fish catfish *C. gariepinus* and in other fishes can be used as a bioindicator for acute exposure to Cd. This metal stimulated rapidly the antioxidant system as evidenced by an increase in GST activities in the detoxifying organs such as liver and kidney. GST is one of the intensely investigated conjugation enzymes, and is in the second stage of xenobiotic detoxification. It is very often used as a biochemical marker of aquatic environment contamination with exogenous substances. The response of CAT activity in different tissues of *C.gariepinus* exposed to sublethal concentrations of $CdCl_2$ solution was found to be variable depending on the tissues and duration of exposure periods. Hence the CAT and GST activity can be considered as a sensitive biomarker for biomonitoring the aquatic environment contaminated with chemicals and this may provide a useful data for future investigations.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Park TS, Taurine: its physiological roles and nutritional significance. Korean J Nutr, 34:597-607, (2001).
2. Ahn CS, Effects of taurine supplementation on serum lipidperoxide levels in middle-aged women. Korean J Food Nutr, 20: 440-449, (2007).
3. Pratap H, BONGA SEW: Effects of water-borne cadmium of plasma cortisol and glucose in the cichlid fish *Oreochromis mosambicus*. Comp Biochem Physiol C, 95: 313-317, (1990).
4. George SG, Todd K, Wright J, Regulation of metallothionein in teleosts: induction of MT mRNA and protein by cadmium in hepatic and extra hepatic tissues of a marine flatfish, the turbot (*Scophthalmus maximus*). Comp Biochem Physiol C, 113: 109-115, (1996).
5. Marafante E, Binding of mercury and zinc to cadmium-binding protein in liver and kidney of goldfish (*Carassius auratus* L.). Experientia, 32: 149-150, (1976).
6. Kraal MH, Kraak MHS, De Groot CJ, Davids C, Uptake and tissue distribution of dietary and aquaeous cadmium by carp (*Cyprinus carpio*). Ecotoxicol Environ Saf, 31:179-183, (1995).
7. Vigh P, Mastala Z, Balogh KV, Comparison of heavy metal concentration of grass carp (*Ctenopharyngodon idella*) in a shallow eutrophic lake and a fish pond (possible effects of food contamination). Chemosphere, 32: 691-701, (1996).
8. Melgar MJ, Perez M, Garcia MA, Alonso J, Miguez B, The toxic and accumulative effects of short-term exposure to cadmium in rainbow trout (*Oncorhynckus mykiss*). Vet Hum Toxicol, 39:79-83, (1997).
9. Basha PS, Rani AU, 2003. Cd-induced antioxidant defense mechanism in freshwater teleost Oreochromis mossambicus (Tilapia). Ecotoxicol Environ Saf, 56:218–221, (2003).
10. Gul S, Belge-Kurutas E, Yıldız E, Sahan A, Doran F, Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey Environ Int, 30:605-609, (2004).
11. Sanchez W, Palluel O, Meunier L , Coquery M, Porcher JM, Ait-Aissa S, Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. Environ Toxicol Pharmacol, 19:177–183, (2005).
12. Schlenk D, Celander M, Gallagher E, George S, James M, Kullman S, Van den hurk P, Willett K, Biotransformation in Fishes. In: Di Giulio RT, Hinton DE,

editors. The Toxicology of Fishes. CRC press, New York, 153-234, (2008).
13. Van der Oost R, Beyer J, Vermeulan NPE, Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology &Pharmacology, 13(2): 57-149, (2003).
14. ICES (International Council for the Exploration of the Sea) Report of the working group on the biological effects of contaminants. CM 1994/ENV: 3 Ref. general secretary, Pleadgade, Copenhagen, 1261; 2-4, (1994).
15. Monferran MV, Pesce SF, Cazenave J, Wunderlin DA, Detoxification and antioxidant responses in diverse organs of *Jenynsia multidentata* experimentally exposed to 1, 2-and 1, 4-dichlorobenzene. Environ Toxicol, 23:184-192, (2008).
16. Vieira LR, Gravato C, Soares AMVM, Morgado F, Guilhermino L, Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: Linking biomarkers to behavior. Chemos, 76: 1416-1427, (2009).
17. Runnalls TJ, Hala DN, Sumpter JP, Preliminary studies into the effects of the human pharmaceutical Clofibric acid on sperm parameters in adult Fathead minnow. Aquat Toxicol, 84: 111-118, (2007).
18. Faria M, Lopez MA, Sanjuan MF, Lacorte S, Barata C, Comparative toxicity of single and combined mixtures of selected pollutants among larval stages of the native freshwater mussels (*Unio elongatulus*) and the invasive zebra mussel (*Dreissena polymorpha*), Sci Tot Environ, 408: 2452-2458, (2010).
19. Cho SG, Lee, YH, Park HS, Ryoo K, Kang KW, Park J, Eom SJ, Kim MJ, Chang TS, Choi SY, Glutathione S-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase1. Jou Biol Chem, 276:12749–12755, (2001).
20. Wang T, Arifoglu P, Ronai Z, Tew KD, Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. Jou Biol Chem, 276:20999–21003, (2001).
21. Townsend DM, Tew KD, The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene, 22:7369–7375, (2003).
22. Sinha AK. Colorimetric assay of Catalase. Analytical Biochemistry, 47:2:389-394, (1972).
23. Habig WH. Glutathione-S-transferase. The fist enzymatic step in mercapturic acid formation. Jou Biol Chem, 249:7130-7139, (1974).
24. Hidalgo MC, Exposito A, Palma JM, Higuera M, Oxidative stress generated by dietary Zn-deficiency: studies in rainbow trout (Oncorhynchus mykiss). Int Jou Biochem and Cell Biol, 34:183-193, (2002).
25. Pruell RJ, Engelhardt FR, Liver Cd uptake, catalase inhibition and Cd thionein production in the killifish (Fundulus heteroclitus) induced by experimental Cd exposure. Mar Environ Res, 3: 101–111, (1980).
26. Avci A, Kaçmaz M, Durak I, Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicol Environ Saf, 6: 101–105, (2005).
27. Dautremepuits C, Paris-Palacios S, Betoulle S, VernetG, Modulation in hepatic and head kidney parameters of carp (Cyprinus carpio L.) induced by copper and chitosan. Comp Biochem Physiol C, 137: 325-333, (2004).
28. Radi AAR, Matkovics B, Effects of metal ions on the antioxidant enzyme activities, protein contents and lipid peroxidation of carp tissues. Comp Biochem Physiol C, 90: 69–72, (1998).
29. Jena BS, Nayak SB, Patnaik BK, Age-related changes in catalase activity and its inhibition by manganase (II) chloride in the brain of two species of poikilothermic vertebrates. Arch Gerontol Geriatr, 26:119-129, (1998).
30. Wong DWS, Whitaker JR, Catalase. Handbook of Food Enzymology. New York, USA, p. 389, (2002).
31. Skipsey M., Andrews CJ, Townson JK, Jepson I, Edwards R, Substrate and thiol specificity of a stressinducible glutathione transferase from soybean. FEBS Lett, 409: 370-374, (1997).

32. Farombi EO, Adelowo OA, Ajimoko YR, Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. Environ Res Public Hlth, 4(2):158-165, (2007).
33. Mannervik B, The isozymes of Glutatione Transferase. Adv Enzymol Relat Areas Mol Biol, 57, (1985).
34. Soegianto A, Charmantier DM, Trilles J, Charmantier G, Impact of Cd on the structure of gills and epipodites of the shrimp Penaeus japonicus (Crustacea: Decapoda), Resources Aquatic Living, 12:57–70, (1999).
35. OLSSON PE, KILLE P, Functional comparison of the metal-regulated transcriptional control regions of metallothionein genes from cadmium-sensitive and tolerant fish species. Biochim Biophys Acta, 1350: 325-334, (1997).
36. Anushia C, Sampath Kumar P, Karthikeyan P, Biochemical changes induced in T*ilapia mossambicus* during copper and cadmium acute intoxication. International Journal of Institutional Pharmacy and Life Sciences, 2: 38-52, (2012).
37. Nimmo IA, The glutathione S-transferase of fish. Fish Physiol Biochem*,* 3(4): 163-172, (1987).
38. Mohamed S, Kheireddine O, Wyllia HM, Roquia R, Aicha D, Mourad B, Proportioning of biomarkers (GSH, GST, AchE, Catalase) indicator of pollution at Gambusia affinis (Teleostei Fish) exposed to cadmium. Environ Res J, 2: 177-181 (2008).