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Cadmium Induced Toxicity and Antioxidant Activities in Labeo Rohita (Hamilton)

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Abstract The present study was carried out to observe the toxicity of cadmium at various sublethal concentrations in common edible fish, *Labeo rohita* (rohu) during various exposure periods. *Labeo rohita* was exposed to sublethal concentrations of cadmium chloride that is 1.0 mg l^{-1}, 1.5 mg l^{-1} and 2.0 mg l^{-1} along with control for 24hr, 48hr, 72hr and 96hr. The fishes were sacrificed and muscle was collected for the estimation of total proteins, carbohydrate, lipids and antioxidant enzymes i.e. superoxide dismutase (SOD), lipid peroxidation (LPO) and catalase (CAT) activities. The maximum decrease in protein, carbohydrate and lipid content were found to be 40.44%, 32.17% , 33.12% at 96hr of exposure at 2 mg l^{-1} of $CdCl_2$. Antioxidant enzyme activities were increased when fish exposed to sublethal concentration of cadmium chloride. The maximum increase in the level of catalase, lipid peroxidation, and superoxide dismutase was noted to be 141.2%, 94.6% and 71% respectively at 96hr exposure for 2.0 mg l^{-1} of $Cdcl_2$.The results revealed that cadmium is highly toxic in muscle of *Labeo rohita* as there was a drastic reduction of biochemical parameters (carbohydrate, protein, lipids) and changes in the level of antioxidant enzymes activities.

Keywords. Antioxidant, *Labeo rohita,* Heavy metal, CAT, LPO, SOD

1. Introduction

Rivers and lake shores are the areas primarily affected by diluted cadmium waste from industrial facilities in big cities [1]. Cadmium is a highly toxic heavy metal, since it causes deleterious effects in organisms at low levels of exposure [2]. Several studies showed that toxic agents may affect behavioral parameters [3,4,5,6,7]. Behavioral changes are good indicators of damage to the central nervous system, as a consequence of exposure to toxic agents [7]. Accumulation of cadmium in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly. By contrast, the excretion of cadmium from living organisms is a slow process. Various toxic effects of cadmium have been reported, as well as its accumulation in liver [8], kidney [9,10] and testes [11]. Cadmium can also replace essential metals such as copper and zinc in several metalloproteins, altering the protein conformation and affecting their activity because this element interacts ubiquitously with sulphydryl groups of amino acids, proteins and enzymes [12]. Thus, the toxic effects of cadmium are related to changes in natural physiological and biochemical processes in organisms. Fish promptly respond to cadmium and other contaminants with alterations in detoxification enzymes [13] and it is well known that these enzymatic responses are modulated by several factors, among them temperature, age, nutritional status [13] and oxygen availability [14].

The organisms developed a protective defense against the deleterious effects of essential and inessential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body [15,16]. A variety of contaminants including toxic heavy metals (cadmium, copper, mercury and zinc) are reported to be ubiquitously present in rivers, reservoirs and are disadvantageous for aquatic organisms [17]. In general, they are not biodegraded and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain [18,19,20,21,22,23].

2. Materials Methods

The test animals *Labeo rohita* (Rohu) having the length of 10-15 cm and weight of 10-12 g were collected from the nearest commercial fish breeding farm at Chiplima and they were acclimatized to the laboratory condition. The water used having DO 9.7 mg l^{-1}, temperature 18.9^{0}C, pH 7.23, conductivity 1.63 mmho/cm, TDS 0.685 mg l^{-1}. The water was aerated continuously to maintain the proper oxygen

concentration and fed with commercially available food. The water was renewed every 24hr.

The 96hr LC_{50} value of $CdCl_2$ was determined using probit analysis [24] and value was found to be 2.45 mg l^{-1} . Then sublethal concentrations i.e 2.0 mg l^{-1}, 1.5 mg l^{-1}, 1.0 mg l^{-1} of $CdCl_2$ was prepared along with control. Fish feeding was stopped before 24 hours of exposure to different sublethal concentrations of $CdCl_2$. Twenty numbers of fishes were exposed to each concentration for the study. Four individuals were taken out from each container (total 20) at 24, 48, 72 and 96 hour intervals and their length and weight were measured. Then these fishes were sacrificed and muscles was collected and stored in deep freezer to estimate biochemical constituents' subsequently.

The biochemical constituents viz. total Proteins, carbohydrate ,Lipids and antioxidant enzyme activities i.e. Lipid peroxidation, Catalase, and Superoxide dismutase (SOD) were estimated by standard methods [25,26,27,28,29,30]. In the mean time the fishes were observed regularly for the study of behavioural and morphological changes.

3. Results

3.1. Behavioural and Morphological Changes

Following treatment with sublethal concentrations of Cadmium chloride the fishes showed exciting aggressive behavior and symptoms of restlessness. Erratic swimming pattern was also observed. The fishes frequently dashed against the wall of the containers. Sometimes they swam with their head downward, jabbing, scarping the bottom of the containers. Erratic opercula movement was also noticed which may be due to difficulties in normal respiration. The movement was jerky. Ultimately the fish became quiescent.

Large scale pigmented disturbance leading to colour change was observed usually pale and dull. There was excessive mucous secretion, which formed a thick coating over the body of the fishes even at lower concentrations.

3.2. Effect of $Cdcl_2$ in Protein, Lipid and Carbohydrate content of *Labeo rohita*

When the muscles were subjected for estimation of total proteins (mg gm^{-1} muscle tissue) at various sublethal concentrations for different exposure periods a gradual decrease was noticed . From the observation the protein content of *Labeo rohita* at zero conc. was found to be 36.5, 36.2, 36.1, 36.1 for 24, 48, 72, and 96 hour of observation. At 1.0, 1.5, 2.0 mg l^{-1} the percent decrease was recorded as 4.63%, 9.72%, 15.09%, respectively for 24 hour of exposure. For the same concentrations after 48 hour of exposure the decrease was noticed as 7.8%, 12.45%, 24.83%. Similarly the decrease was found to be 13.79%, 18.28%, 32.96% after 72 hour of exposure period and after 96 hour the percent decrease was 19.39%, 24.65%, 40.44% respectively (Table 1).

By analyzing the data statistically (Two-way ANOVA) they are found to be significant at $p \leq 0.05$ level of significance (F1=9.488, F2 = 40.04745).

When the muscles were subjected for estimation of carbohydrate (mg gm^{-1} muscle tissue) at various sublethal concentrations for different exposure period a gradual decrease was noticed. At zero concentration the carbohydrate content was found to be 26.7, 26.6, 25.9, 25.8 for 24 ,48 ,72, and 96 hour of observation. The percent decrease of carbohydrate was estimated as 7.8%,17.9%, 29.5%, at 1.0 mg l^{-1} ,1.5 mg l^{-1} and 2.0 mg l^{-1} espectively during 24 hour of exposure. For the same concentrations after 48 hour of exposure the decrease was noticed as 10.1%, 18.42%, 31.2%. Similarly the decrease was found to be 12.35%, 23.5%, 30.8% after 72 hour of exposure period and after 96 hour the percent decrease was 11.24%, 28.68%, 32.17% respectively.(Table 1)

Two way ANOVA test showed a significant difference between the exposure duration and different concentrations of cadmium at $p \leq 0.05$ level (F1=25.23358, F2=605.5937)

When the muscle were subjected for estimation of lipid (mg gm^{-1} muscle tissue) at various sublethal concentrations for different exposure period a gradual decrease was noticed. It was found that the lipid content in muscles of *Labeo rohita* at zero concentration was 65.8, 65.5, 65.3, 65.2 for 24, 48, 72, and 96 hour of observation. The percent declined was recorded as 3.95%, 9.57%, 15.6%, respectively at the chosen concentrations for 24 hour of exposure. For the same concentrations after 48 hour of exposure the decrease was noticed as 5.19%, 11.75%, 20.6%.Similarly the decrease was found to be 8.2%, 16.38%, 25.42% after 72 hour of exposure period and after 96 hour the percent decrease was 12.8%, 22.2%, 33.12% respectively (Table 1).

Two way ANOVA test showed a significant difference between the exposure duration and different concentrations of cadmium at $p \leq 0.05$ level (F1=8.446672, F2=41.39044).

3.3. Effect of $Cdcl_2$ in antioxidant enzyme activities Catalase activity

From this experiment ,it was observed that there was increase the level of catalase activity (nMoles $mg^{-1}sec^{-1}$) in muscle, when the test species were exposed to different sublethal concentrations of cadmium in different time courses. The catalase activity at control were 5.6,5.9,6.1,6.3 during 24,48,72 and 96 hour of observation. The catalase activity in chemically exposed test organism increase over zero concentration was 16%, 44%, and 105.3% respectively for 24 hour of exposure. After 48 hour of observation the enzyme activity was increased up to 27.1%, 57.6%, 110% and at 72 hour the increase was recorded as 36%, 85.2%, 137.7%. After 96 hour of exposure the increase was estimated as 82%, 130.15%,141.2% respectively.(Figure 1).

In order to know the difference between the two variable ,exposure duration and concentration when data were subjected to two -way ANOVA found to be significant at $p \leq 05$ level of significance (F_1=,11.022 F_2=32.307).

Table 1. Showing percent decrease over zero concentration in protein, carbohydrate, lipid content (mg/gm muscle tissue), of Labeo rohita exposed to different sublethal concentrations of $CdCl_2$ in different exposure periods.

Duration 0f exposure(hr)	Different Con. (ppm)	Protein	Carbohydrate	Lipid
24hr	0	36.5±0.108	26.7±0.213	65.8±0.325
	1.0	34.81±0.262 (4.63%)	24.6±0.247 (7.8%)	63.2±0.231 (3.95%)
	1.5	32.95±0.238 (9.72%)	21.9±0.168 (17.9%)	59.5±0.143 (9.57%)
	2.0	29.3±0.231 (15.09%)	18.8±0.403 (29.5%)	55.5±0.195 (15.6%)
48hr	0	36.2±0.170	26.6±0.301	65.5±0.265
	1.0	33.35±0.182 (7.8%)	23.9±0.218 (10.1%)	62.1±0.341 (5.19%)
	1.5	31.69±0.175 (12.45%)	21.7±0.231 (18.42%)	57.8±0.321 (11.75%)
	2.0	27.2±0.312 (24.83%)	18.3±0.275 (31.2%)	52±0.276 (20.6%)
72hr	0	36.1±0.253	25.9±0.219	65.3±0.146
	1.0	31.12±0.231 (13.79%)	22.7±0.210 (12.35%)	59.9±0.293 (8.2%)
	1.5	29.5±0.182 (18.28%)	19.8±0.231 (23.5%)	54.6±0.234 (16.38%)
	2.0	24.2±0.145 (32.96%)	17.9±0.164 (30.8%)	48.7±0.341 (25.42%)
96hr	0	36.1±0.175	25.8±0.154	65.2±0.234
	1.0	29.1±0.323 (19.39%)	22.9±0.212 (11.24%)	56.8±0.149 (12.8%)
	1.5	27.2±0.243 (24.65%)	18.4±0.324 (28.68%)	50.7±0.128 (22.2%)
	2.0	21.5±0.305 (40.44%)	17.5±0.243 (32.17%)	43.6±0.138 (33.12%)

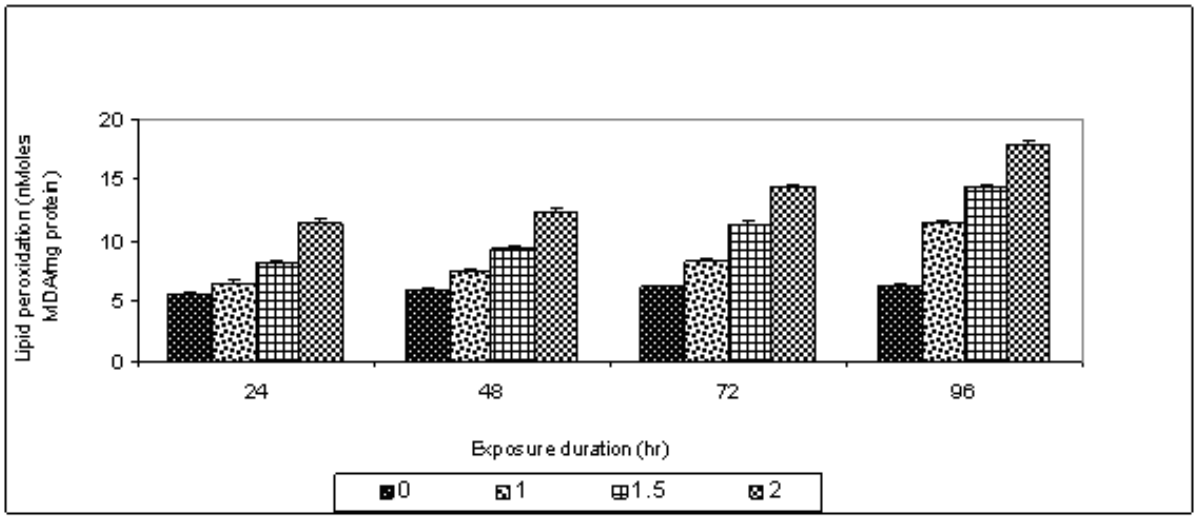

Figure 1. Catalase activity (nMoles/mg/sec muscle tissue) in *Labeo rohita* exposed to different sublethal concentrations of cadmium chloride in different exposure periods

3.4. Lipid Peroxidation Activity

Figure 2 showed that the level of LPO activity (nMoles MDA $^{-1}$ mg^{-1} protein) in muscle tissue of *Labeo rohita* was increase upon exposed to different sublethal concentrations of cadmium in different time courses. The LPO activity at control was 8.3, 8.9, 9.1, 9.3 during 24, 48, 72 and 96 hour of observation. Upon exposed to the chosen sublethal concentrations the percent increase over zero concentration was 9.6%,38.5%,74.6% for 24 hour of exposure. After 48 hour of observation the enzyme activity was increased up to 11.2%, 41.5%, 78.6% and at 72 hour the increase was recorded as 24.1%, 63.7%,90%. During 96 hour of exposure the increase was found as 48.3%, 47.39%,94.62% respectively.

In order to know the difference between the two variables ,exposure duration and concentration when data were subjected to two –way ANOVA found to be significant at $p \leq 0.05$ level of significance (F_1=9.37227, F_2=31.7615).

3.5. Superoxide Dismutase Activity

Further, it was observed that there was increase in the level of superoxide dismutase activity (U/mg^{-1} protein) in muscle, when Labeo rohita were exposed to different sublethal concentrations of cadmium in different time courses. The SOD activity at control were 15.6,16.1,16.3,16.9, during 24,48,72 and 96 hour of observation. The activity in chemically exposed organism at different sublethal concentrations increased over zero concentration was 12.17%,27.56%,43.5% for 24 hour of exposure. After 48 hour of observation the enzyme activity was increased up to 12.4%, 27.32%, 52.1% and at 72 hour the increase was recorded as 15.9%, 32.5%, 58.8%. After 96 hour of exposure the increase was found to be 14.20%, 38.4% and 71.0% respectively. (Figure 3)

In order to know the difference between the two variable ,exposure duration and concentration when data were subjected to two -way ANOVA found to be significant at $p \leq 0.05$ level of significance (F_1=,7.759 F_2=63.297).

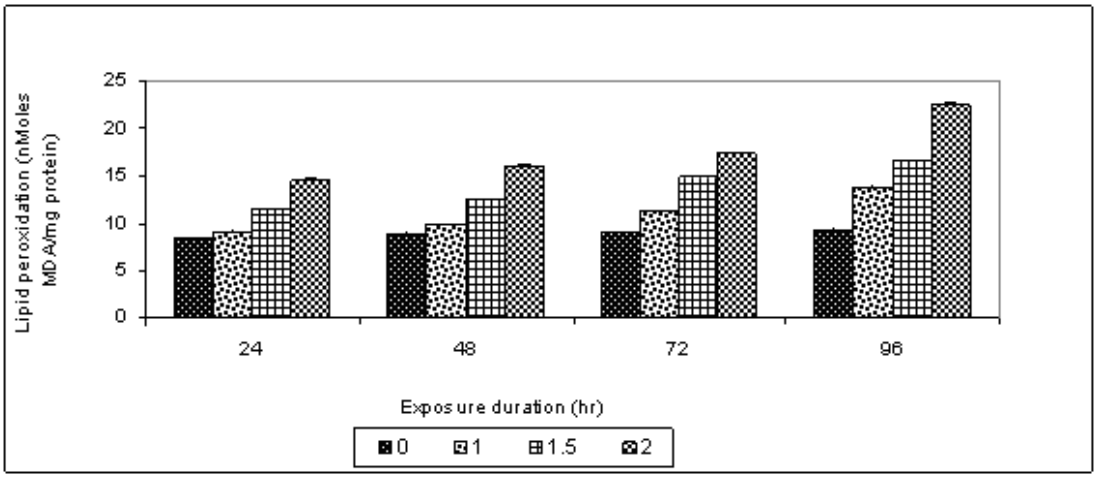

Figure 2. Lipid peroxidation activity (nMoles/ MDA/ mg protein muscle tissue) in *Labeo rohita* exposed to different sublethal concentrations of cadmium chloride in different exposure periods

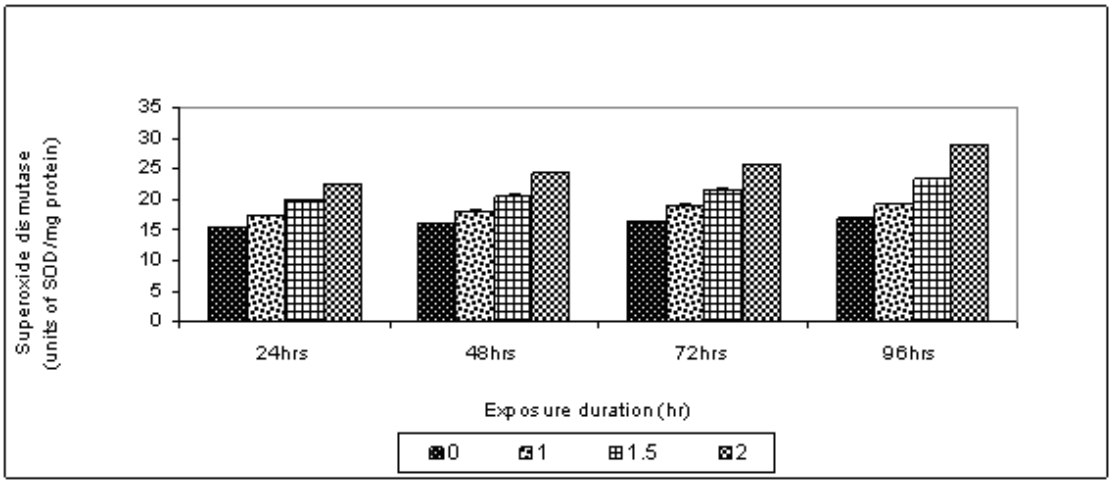

Figure 3. Superoxide dismutase activity (units of SOD/mg protein muscle tissue) in *Labeo rohita* exposed to different sublethal concentrations of cadmium chloride in different exposure periods

4. Discussion

4.1. Behavioural and Morphological Changes

Behavioural and morphological alterations as noted in the course of investigation were also noticed by many workers under heavy metals exposure [31]. Toxic effects of cadmium in the brain and nervous system may be associated with aggressive behavior in fish species [32]. The rate of opercula movement and rate of heart beat are highly conspicuous in fishes reference to toxic substances [33]. The excess secretion of mucous and body dispigmentation are due to change in number and area of mucous gland and chromatophores caused by the dysfunctions of the endocrine gland [34].

4.2. Biochemical Changes

In the present investigation, the biochemical parameters like total proteins, total lipids and total carbohydrate content of muscles tissue in *Labeo rohita* showed decline following the exposure to various sublethal concentrations.The reduced protein content indicated proteolysis in tissue which forms aminoacids and used in TCA cycle for energy production during stress condition.The protease activity increased in tissue and caused decrease in tissue protein.The other reason is to meet energy demands during pollution stress mobilization of proteins might have taken place. The decreased protein content in white muscles by cadmium exposure, could be related to decreased specific growth rate (SGR) [35]. Following cadmium chloride treatment (20 mg g^{-1}) the plasma protein level decreased in *Symbaranchus bengalensis* and *Anabas testudineus* [36]. Significant fall in total protein content of tissues in *Channa punctatus* exposed to Hg, Pb ,Cu, Cd and Cr was also reported [37]. The decrease in carbohydrate content may indicate an immediate utilization to meet the excess demand of energy. This is perhaps achieved by rapid glycogenolysis through activation of glycogen transferase respectively [38]. Loss of lipid from gonadal tissue may be due to mobilization of a tissue lipid into the metabolism and that activated lipases may have depleted total lipid [39].

In this study, LPO, SOD and CAT activities in muscle tissues showed differences in enzyme activity parallel to metal bioaccumulation. Accumulation of Cd and its effect on SOD and LPO in the catfish *Clarias gariepinus* is dependent on concentration, tissue and time [40]. The most obvious effect of cadmium exposure was the increased hepatic lipid peroxidation. It was reported that lipid peroxidation is a consequence of cadmium exposure in both fish and mammals [41, 42, 10]. Tissue and cell membrane lipoperoxidation caused by ROS have been considered to be proportional to antioxidant content [43]. SOD catalyzes the destruction of O_2- by dismutation and H_2O_2 formation. Catalase catalyze the conversion of hydrogen peroxide to water. some researchers have reported that antioxidant enzymes are often elevated as a direct response to the ROS challenge and actually represent an attempt to detoxify. For example, SOD and CAT were elevated in the erythrocytes of *Carassius auratus* treated with cadmium for 15 days [44]. Cadmium-exposed fish showed an enzymatic response profile with high activity for SOD and CAT.

5. Conclusion

In the present investigation cadmium was found to be highly toxic *in Labeo rohita* as there was a drastic change in behavioural and morphological characteristics and reduction in biochemical lparameter.

The findings of the present study substantiate earlier findings that antioxidant enzymes such as SOD, CAT and lipid peroxidation in fish can be effectively used as biomarkers of cadmium toxicity.

The present study also emphasizes that fish can serve as valuable bioindicators and can serve as useful alternative models for understanding cadmium toxicity.

REFERENCES

[1] Randi, A.S., J.M. Monserrat, E.M. Rodriquez and L.A. Romano, 1996. Histopathological effects of cadmium on the gills of the freshwater fish, Macropsobrycon uruguayanae Eigenmann 1915 (Pisces, Atherinidae). J. of Fish Diseases, 19: 311-322.

[2] Cope, W. G., J. Wiener, M. T. Steingraeber & G. J. Atchison. 1994. Cadmium, metal-binding proteins, and growth in bluegill (Lepomis macrochirus) exposed to contaminated sediments from the upper Mississippi River basin. Canadian Journal of Fish and Aquatic Science, 51: 1356-1367.

[3] Abdul Farah, M., B. Ateeq, M. Niamar Ali, R. Sabir & W. Ahmad.2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere, 55: 257-265.

[4] Lefebvre, K.A., V. L.Trainer & N. L. Scholz. 2004. Morphologicalabnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin. Aquatic Toxicology, 66: 159-170.

[5] Nakayama, K, Y. Oshima, T. Yamaguchi, Y. Tsuruda, I. J. Kang, M. Kobayashi, N. Imada & T. Honjo. 2004. Fertilization success and sexual behavior in male medaka Oryzias latipes, exposed to tributyltin. Chemosphere, 55: 1331-1337.

[6] Yilmaz, M., A. & Gül, E. Karaköse. 2004. Investigation of acute toxicity and the effect of cadmium chloride (CdCl2.H2O) metal salt on behavior of the guppy (Poecilia reticulatus). Chemosphere, 56: 375-380.

[7] Sloman, K. A., G. R. Scott, Z. Diao, C. Rouleau, C. M. Wood & D. G. McDonald. 2003. Cadmium affects the social behaviour of rainbow trout, Oncorhynchs mykiss. Aquatic Toxicology, 65: 180-185.

[8] Novelli, E. L. B., E. P. Vieira, N. L. Rodrigues & B. O. Ribas. 1998. Risk assessment of cadmium toxicity on hepatic and renal tissues of rats. Environmental Research, 79: 102-105.

[9] Klaassen, C. D. & J. Liu. 1997. Role of metallothionein in cadmiuminduced hepatotoxicity and nephrotoxicity. Drugs and Metabolism Review, 29: 79-102.

[10] Novelli, E. L. B., A. M. Lopes, A. S. E. Rodrigues, J. L. V. B. Novelli Filho & B. O. Ribas. 1999. Superoxide radical and nephrotoxic effect of cadmium exposure. International Journal of Environment and Health Research, 9: 109-116.

[11] Shen, H. M. & S. Sangiah. 1995. Na+,K+-ATPase, glutathione and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice. Archives of Environmental Contamination and Toxicology, 29: 174-179.

[12] Serafim, A. and Bebianno, M.J. 2007. Kinetic model of cadmium accumulation and elimination and metalothionein response in Ruditapes decussates. Environmental Toxicology and Chemistry, 26: 960-969.

[13] Jimenez, B. D. & J. J. Stegeman. 1990. Detoxication enzymes as indicators of environmental stress on fish. In: Adams, S. M. (Eds). American Fisheries Symposium 8, Maryland Pp. 67-79.

[14] Wilhelm Filho, D. 1996. Fish antioxidant defenses: a comparative approach. Brazilian Journal of Medical and Biological Research, 29: 1735-1742.

[15] Abou EL-Naga, E.H., K.M. EL-Moselhy, M.A. Hamed, 2005. Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish Mugil seheli. Egyptian. J. Aquat. Res., 31(2): 60-71.

[16] Filipovic, V., B. Raspor, 2003. Metallothionein and metal levels in cytosol of liver, kidney and brain in relation to growth parameters of Mullus surmuletus and Liza aurata. From the eastern Adriatic Sea. Water Res., 37(13): 3253-3262.

[17] Olsson, P.E., 1998. Disorders associated with heavy metal pollution. In: Fish Diseases and Disorders Volume 2 (Non-infectious Disorders). (Eds. Leatherland, J.E. and Woo, P.T.K.), CABI International, U.K. pp: 105-131.

[18] Das, S. and A. Kaviraj, 2000. Cadmium accumulation in different tissues of common carp, Cyprinus carpio treated with activated charcoal, EDTA and single superphosphate. Geobios., 27: 69-72.

[19] Laxi, R., 2005. Cadmium contamination in common Indian food items, Himalayan J. Environ. Zool., 19-23

[20] Jayakumar, P. and V.I. Paul, 2006. Patterns of cadmium accumulation of the catfish Clarias batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. Veterinarshki Archiv., 76: 167-177.

[21] Kumar, P., Y. Prasad, A.K. Patra and D. Swarup, 2007. Levels of Cadmium and Lead in Tissues of Freshwater Fish (Clarias batrachus L.) and Chicken in Western UP (India). Bull. Environ. Contamin. and Toxicol., 79: 396-400.

[22] Kumar, P., Y. Prasad, R. Ranjan, D. Swarup, A.K. Pattanaik and R.C. Patra, 2008. Accumulation Pattern of Cadmium in Tissues of Indian Catfish Clarias batrachus. Animal Nutrition. and Feed Technol., 8(1): 115- 119.

[23] Kumar, P., Y. Prasad, A.K. Patra, R. Ranjan, R.C. Patra, D. Swarup and S.P. Singh, 2009. Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidativee stress in freshwater catfish (Clarias batrachus). The Sci. Total Environ., 407: 5024-5030.

[24] Finny, D.J. 1971. Probit Analysis. 3rd edition, Cambridge University Press, Cambridge; 333.

[25] Samseifter, S., Dayton Novic, B. and Edward, M. N. 1949.The estimation of glycogen with the anthrone reagent. Federation Proc; 8: 249.

[26] Lowry O H, Rosebrough N J, Farr A L & Randall R J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem; 193:265,

[27] Folch, J., M. Lees, and G. H. Sloane-Stanely. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem; 226:497–507.

[28] Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95:351–8.

[29] Aebi, H. 1974.Catalase. In: Bergmeyer HV, editor. Methods in enzymatic analysis. Vol 2, New York: Acadamic press; 674 - 84.

[30] Kazari Das, Samanta L and Chainy GBN. 2000. A modified spectrophotometric assay for superoxide dismutase using nitrite formation by superoxide radicals. IJBB; 37:201-04.

[31] Bengtsson B.E..1974. Effect of Zinc on growth of the minnow Phoxinus phoxinus. Oikos. 25:370-373.

[32] Provias, J. P., C. A. Ackerley & L. E. Becker. 1994. Cadmium encephalopathy: a report with elemental analysis and pathological findings. Acta Neuropathology, 88: 583-586.

[33] Thiede, W. 1965 uber die hormonale basis der adaptation on extreme temperature bein sdchweltlraer XI phosphomishelleni,Heckel (peecilidae) pisces.

[34] Pandey. S., Parvez, S., Sayeed. I., Haque, R., Bin- Hafeez, B. and Raisuddin, S. 2003. Biomarker of oxidativestress: a comparative study of river Yamuna fish Wallago attu. Science of the total Environment. 309: 105- 115

[35] Almeida, J. A., E. L. B. Novelli, M. Dal Pai Silva & R. Alves Jr. 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, Oreochromis niloticus. Environmental Pollution, 114: 169-175.

[36] Ashram, Roshan Ara, Manjula Yadav, Dillep Kumar and B.N. Yadav. (2003). The toxicity effects of Cadmium chloride on some biochemical parameters of Symbaranchus bengalensis and Ananbas testudineus. Bio-Science. Res. Bull. 19(1):75-78.

[37] Jana,,S. and Bandyophyay N. 1987 .Effect of heavy metals on some biochemical parameters in the freshwater fish,Channa punctatus .Environ.Ecol.5:488-493.

[38] Revathi, K., Gulati, S. and Sharief, D. 2005. Tannary effluent induced biochemichal changes in the larvivorous fish, Gambusia affinis.Poll.Res. 24(4):815-818.

[39] Jha, M.M. 1992. studied on the effects of nickel chloride to some organs of the climbing perch, Anabas testudineus

(Bloch): Ph. D. Thesis. L.N. Mithila university,Darbhanga.

[40] Asagba, S. O., Eriyamremu, G. E., Igberaese, M. E. 2008. Bioaccumulation of cadmium and its biochemical effect on selected tissues of the catfish (Clarias gariepinus). Fish. Physiol. Biochem. 34, 61-69.

[41] Manca, D., Ricard, A. C., Trottier, B., and Chevalier, G. 1991. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology 67, 303-323.

[42] Oner G, Izgut-Uysal, VN., Senturk UK. 1994. Role of lipid Peroxidation in cadmium-induced impairment of the gastric mucosal barrier. Food Chem Toxicol. Sep;32(9):799-804.

[43] Novelli Filho, J. L. V. B., E. L. B. Novelli & B. O. Ribas. 2000. Effect of alpha tocopherol on superoxide radical and toxicity of cadmium exposure. International Journal of Environmental Health Research, 10: 125-134.

[44] Zikic RV, Stajn AS, Pavlovic SZ, Ognjanovic BI, Saicic ZS. 2001. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of Goldfish, (Carassis auratus gibelio) exposed to cadmium. Physiol Res 50:105–111.