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Stress Responses of Biomolecules and Dehydrogenase Activity in the Tissues of *Labeo rohita* against Pollutant Load in the Lakes of Bangalore

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Abstract: *The physico-chemical characteristics of the aqueous phase have direct influence on the metabolic activity of aquatic biota, in general and fish in particular. In the present investigation, water samples from selected water bodies, viz., Hebbal fish farm (control site), Vengaiah lake (receiving domestic sewage - Lake A) and Yellamallapa Chetty lake (receiving industrial effluents and drainage water - Lake B) were assessed for detection of their pollution status. Simultaneously, the muscle and gill tissue of fresh water fish, Labeo rohita reared in these water bodies, were excised and biomolecules (protein, glycogen and cholesterol) and dehydrogenases (succinic - , lactic- and malic –dehydrogenases) were subjected to analysis for their level and activity. The results obtained showed reduction in the level of protein and glycogen and a corresponding increase in cholesterol level in tissues of fish sampled from lake B when compared to those from lake A and control ones. A significant increase in lactic dehydrogenase activity with a marginal decrease in the activity of succinic and malic dehydrogenase was observed in both muscle and gill tissues of fish from lake B and an insignificant variation in those from lake A in contrast with control ones. Such changes in the biological systems can be correlated with the water parameters with the help of chemical processes. Stress response due to pollutant load in the mentioned water body in terms of fluctuations in biomolecule content and dehydrogenases actvity in the test fish resulted in variation in its metabolic pathways.*

Keywords: *Labeo rohita*, Biomolecules, Dehydrogenases, Pollutants, Metabolism.

Introduction

Water is a limited resource which cannot be produced as and when required by the technological means. The quality of water depends on the concentration of its physico-chemical parameters such as pH, DO, TDS, alkalinity, BOD, COD, temperature (natural), etc. With recent development of industries and sudden population growth, pollutants are constantly being discharged into fresh water bodies (lakes) which change the properties of water and adversely affect the flora and fauna of that particular water ecosystem. The degree of toxicity produced by the pollutants is dose independent upon environmental conditions such as temperature, pH, oxygen content and presence of residue molecules (Singh and Mishra, 2009). Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors (Arechon and Plump, 1990). Among the various pollutants, heavy metals, in particular, are widespread contaminants released into aquatic systems from numerous factories and industries. Some metals are known to be toxic even at low concentrations, including arsenic, cadmium, mercury and lead (Lee *et al.,* 2009). Others such as copper and cobalt, are known to be essential elements and play important roles in biological metabolism at very low concentrations (Lee *et al.,* 2009). The presence of heavy metals in fishes from the Coastal waters of Kapar

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and Mersing, Malaysia water was reported by Bashir *et al.* (2013). The indestructible nature and long term toxic effects of heavy metals including lead (Pb), nickel (Ni), manganese (Mn), zinc (Zn), cadmium (Cd) and chromium (Cr) to man as a result of consumption of organisms obtained from polluted rivers has raised scientific and environmental concerns (Kumar *et al.,* 2012). Heavy metals are among the most persistent pollutants in aquatic ecosystem because of their resistance to decomposition in natural conditions (Khan, 2011). The danger is that heavy metals even at low concentrations in fish and water have a particular significance in ecotoxicology and their toxic effects have been widely published for a number of water bodies (Ekeanyanwu *et al.,* 2011).

In the present investigation, *Labeo rohita,* a freshwater fish was sampled from the two polluted lakes, Vengaiah lake and Yellamallapa chetty lake (lake A and lake B respectively) and from control fish farm, for analyses of biochemical parameters and enzymatic activity of its muscle and gill tissues. The fish reared in lake A which received sewage and domestic waste from surrounding habitation and lake B which is adjacent to Cipla pharmaceutical company, were analysed. Fish from Hebbal fish farm were taken as control. Biomolecules as proteins, carbohydrates and lipids play a major role as precursors in fish under stress conditions. It is known that pollutants produce metabolic changes by way of influencing enzyme system. Decrease of protein level was reported by Dhapte *et al.* (2006) in fish *Nemacheilus botia* exposed to endosulfan. Decrease in glycogen content was also reported by Sobha *et al.* (2007) in the tissues of freshwater fish, *Catla catla* exposed to the heavy metal toxicant cadmium chloride. Cholesterol is required to build and maintain membranes. The industrial effluents from tannery, electroplating and textile mills caused marked depletion in biochemical composition (glycogen, protein and lipid) in tissues such as gill, liver, muscle and kidney of the fish, *Labeo rohita* (Muley *et al.,* 2007). Dehydrogenases are the redox enzymes. Succinic dehydrogenase (SDH) is a primary enzyme in the oxidative catabolism of sugars. Malate dehydrogenase (MDH) an enzyme in citric acid cycle that helps in the conversion of malate into oxaloacetate (using NAD+) as a catalyst and vice versa where as lactate dehydrogenase (LDH) converts pyruvate to lactate in absence or in short supply of oxygen. LDH is widely used as a biomarker of lesions in organ and tissue in toxicology. Ramakritinan *et al.* (2005) studied impact of distillery effluent on the activity of enzymes like LDH and SDH in the muscle, liver and brain tissues fresh water fish, *Cyprinus carpio.* The present study was aimed to compare and correlate the variations in biochemical constituents and enzymatic activity of the tissues of fish with the physico-chemical parameters of the selected water bodies to recognise changes in the metabolic pathways due to the presence of xenobiotic substances.

Materials and Methods

Sampling of water was done in acid washed (Nelson's water sampler) bottles from control site (Hebbal Fish farm), experimental sites of Lake (A) and (B) located in Bangalore city. Standard methods were used for determination of the physico-chemical parameters e.g., temperature, pH, BOD, COD, DO, TDS, phosphates, sulphates, nitrates and alkalinity etc., (APHA *et al.,* 2005). DO was fixed on site as per the standard method. Trace metals were analysed by using Atomic Absorption Spectrophotometer. The values of physico-chemical parameters were compared with the Indian standard values provided by BIS: 10500-1991 (Revised 2012). Test fish, *Labeo rohita* were sampled with the help of dragnet - brought to the lake bank alive from the three water bodies simultaneously along with water samples. Fish were anaesthetized using MS222 to retain the characteristics of biochemical constituents and enzymes of the tissues. The tissues such as muscle and gill were carefully excised out of the body of the fish at the site and transferred to buffer solution for analysis of biochemical constituents and dehydrogenases' activities. Proteins were estimated by Lowry's method (Lowry *et al.,* 1951), glycogen content by Anthrone reagent (Seifer *et al.,* 1950) by standard glucose solutions and cholesterol was estimated by Zlatki's, method (Zlatkis *et al.,* 1953). Succinic, malic and lactic dehydrogenases were estimated by continuous Spectrophotometric rate determination method (Bergmeyer and Bernt, 1974). One way ANOVA was applied to statistically evaluate the results and to find significant differences (Tukey's multiple comparison test) within the physico-chemical parameters of control fish farm, lake A and lake B, and correlation with the biochemical and dehydrogenases activity of tissues of fish.

Results and Discussion

Results: The physico-chemical characteristics of water samples from Hebbal fish farm, lake A and lake B were statistically analyzed and the data represented in Table- 1 showed that due to the presence of trace metals, low values of DO and pH and high values of all the remaining parameters, lake B (Yellamallappa chetty lake) was assessed to be industrially polluted when compared to the lake A (Vengaiah lake). Hebbal fish farm was taken as control site. All water parameters of control site were compared with lake A and lake B and inturn with the standard BIS: 10500-1991(Revised 2012). Physico-chemical parameters of lake A and B when compared to BIS standard revealed presence of high levels of temperature, total suspended solids, chemical oxygen demand, biological oxygen demand, conductivity, turbidity and alkalinity. Trace metals' content such as, aluminium, cadmium, copper, iron, lead and mercury in the present study showed relatively high level in water samples of lake B when compared to BIS values.

The present study on biochemical profile and enzymatic activity in muscle and gill tissue of fish were analysed statistically as shown in Table 2 and 3. An appreciable reduction was recorded in the level of protein and glycogen content in the two tissues of fish from lake B (Protein - 13.10 ± 0.89 and 8.26 ± 0.42 and glycogen - 2.20 ± 0.18 and 1.63 ± 0.08 respectively) when compared to those of lake A (Protein - 17.03 ± 0.57 and 16.22 ± 0.45 and glycogen - 3.45 ± 0.14 and 2.03 ± 0.12 respectively) and control ones (Protein - 18.33 ± 0.82 and 17.83 ± 1.17 and glycogen - 4.08 ± 0.25, 2.24 ± 0.10 respectively). Cholesterol content in the muscle and gill tissue (3.2 ± 0.22 and 2.4 ± 0.14) of fish from lake B showed a significant increase when compared to those of lake A (2.2 ± 0.09 and 1.6 ± 0.09) and control ones (2.0 ± 0.09 and 1.4 ± 0.14).

With similar lines as biochemical profile the succinic dehydrogenase activity in the muscle and gill of control fish showed a higher level (43.33 ± 0.50 and 20.58 ± 0.55 respectively) when compared to those of lake A (41.52 ± 0.91and 18.9 ± 0.4 respectively) and Lake B (33.38 ± 0.78 and 12.8 ± 0.72 respectively). Inspite of the differences in the physico-chemical parameters of two lakes and control site, the muscle tissue showed a higher SDH activity than gill tissue in general but a reducing trend was observed from the fishes sampled from control site to polluted lake A and B. A marked reduction was also recorded in malate dehydrogenase activity of muscle and gill tissue sampled from lake B (35.83 ± 2.14 and 16.32 ± 0.81) and also lake A when compared to those of control ones (47.83 ± 1.17 and 22.75 ± 1.17) respectively. But the activity of lactic dehydrogenase of the muscle tissue of lake B showed a significant increase of 383.67 ± 4.84 when compared to control fish and of those observed in the tissues from lake A (326.33 ± 4.41). An increase of LDH activity found in fish from lake A and B was significant in the muscle tissues when compared to those in the gill tissue. Thus, out of the three dehydrogenases, the activity of succinic and malic dehydrogenase was lower but of lactic dehydrogenase was higher in the muscle and gill tissue of the fish sampled from lake B. An insignificant variation in biochemical levels and dehydrogenase activity in both the tissues from Lake A were observed when compared to those of control ones.

Table 1 Physico-Chemical parameters of fish farm (Control), Vengaiah lake (A) and Yelemallappa Chetty lake (B).

Sl. no	Parameters	Standards BIS: 10500-1991 (Revised 2012)	Control (farm)	Lake A	Lake B
1.	Temperature (C^0)	22-28	26 ± 0.63	26 ± 0.63	28 ± 1.26
2.	pH Value	06.50 - 08.50	7.87 ± 0.08	7.65 ± 0.16	6.85 ± 0.74
3.	Color (Pt-Co scale)	5 - 25	3.1 ± 0.63	4.3 ± 0.63	6.1 ± 0.49
4.	Odor	UOB	UOB	UOB	Fishy
5.	Turbidity, NTU	05 - 20	7.8 ± 0.15	21 ± 0.89	34.2 ± 2.14[b]
6.	Conductivity mmho /cm	300	483 ± 9.54	837 ± 42.80[a]	1207 ± 35.15[a]
7.	Total Alkalinity as $CaCO_3$, mg/l	200 - 600	202 ± 1.38	290 ± 1.60[a]	544 ± 11.07[a]
8.	Total Dissolved solids, mg/l	500 - 2000	420 ± 7.69	750 ± 1.41[a]	985 ± 2.93[a]
9.	Total Suspended solids, mg/l	100	92 ± 0.82	150 ± 0.82[a]	260 ± 4.73[a]
10.	D.O, mg/l	4.0 - 6.0	3.7 ± 0.05	3.7 ± 0.05	1.2 ± 0.08
11.	B.O.D, mg/l	2 - 6	6 ± 0.76	24 ± 1.21	113 ± 1.33[a]
12.	C.O.D, mg/l	200	76 ± 1.72	126 ± 2.25[a]	374.7 ± 2.88[a]
13.	Total Phosphorus, mg/l	-	0.35 ± 0.01	1.02 ± 0.01	2.42 ± 0.01
14.	Nitrates as NO_3,mg/l	45 - 100	2.13 ± 0.28	2.25 ± 0.20	3.87 ± 0.10
15.	Sulphates as SO_4, mg/l	200 - 400	62 ± 0.52	103 ± 1.21[a]	210 ± 0.52[a]
16.	Aluminium as mg/l	0.03- 0.2	0	0.067 ± 0.002	3.7 ± 0.089
17.	Arsenic as mg/l	0.05	0	0	0.003 ± 0.001
18.	Cadmium as mg/l	0.01	0.001	0.04 ± 0.01	0.124
19.	Copper as mg/l	0.05 – 1.5	0.013	0.03	0.32 ± 0.01
20.	Iron as mg/l	0.3 - 1	0.04 ± 0.008	0.13 ± 0.022	3.68 ± 0.004
21.	Lead mg/l	0.05	0.004 ± 0.001	0.04 ± 0.012	0.37 ± 0.020
22.	Zinc as mg/l	5 - 15	0.54 ± 0.02	1.88 ± 0.01	2.68 ± 0.01
23.	Mercury, mg/l	0.001	0	0	0.028

UOB: Unobjectionable, BDL: Below Detectable Limits

The superscripts a and b indicate statistical mean differences at $p < 0.001$ and 0.01 respectively.

Discussion: Physico-chemical and toxicological bioassay is the basic tool for evaluation of a complex industrial waste (Webner *et al.,* 1989). Conductivity, alkalinity, total dissolved solids, total suspended solids, nitrates, sulphates, biological oxygen demand and chemical oxygen demand of lake B showed significant variation in its level when compared to those of control site resulting in its algal growth, eutrophic and anaerobic condition. Murdoch *et al.* (2001) also reported that phosphate and nitrate are responsible nutrients for increase in algae growth and other toxic blooms which leads to eutrophication of lakes causing decrease in dissolved oxygen and a simultaneous increase in BOD. High levels of TDS, TSS, total phosphorus etc and trace

Table 2 Levels of protein, glycogen and cholesterol in the different tissues of *L. rohita* from fish farm (Control), Vengaiah lake (A) and Yelemallapa Chetty lake (B).

Biochemical constituents	Tissue	Control	Lake A	Lake B
Protein	Muscle	18.33 ± 0.82	17.03 ± 0.57	13.10 ± 0.89
	Gill	17.83 ± 1.17	16.22 ± 0.45	8.26 ± 0.42
Glycogen	Muscle	4.08 ± 0.25	3.45 ± 0.14	2.20 ± 0.18
	Gill	2.24 ± 0.10	2.03 ± 0.12	1.63 ± 0.08
Cholesterol	Muscle	2.0 ± 0.09	2.2 ± 0.09	3.2 ± 0.22
	Gill	1.4 ± 0.14	1.6 ± 0.09	2.4 ± 0.14

Values expressed mg/g wet weight of tissues. Values are expressed as Mean ± S.D; sample size (n) = 6

Statistically significant mean difference at $p < 0.0001$.

Table 3: Succinic-, Malic- and Lactic dehydrogenase activity in muscle and gill tissues of L. rohita from fish- farm (Control), Vengaiah lake (A) and Yelemallappa Chetty lake (B).

Dehydrogenases'activity	Tissue	Control	Lake A	Lake B
Succinic dehydrogenase (SDH)	Muscle	43.33 ± 0.50	41.52 ± 0.91	33.38 ± 0.78
	Gill	20.58 ± 0.55	18.9 ± 0.4	12.8 ± 0.72
Malic dehydrogenase (MDH)	Muscle	47.83 ± 1.17	43.83 ± 1.47	35.83 ± 2.14
	Gill	22.75 ± 1.17	20.53 ± 0.86	16.32 ± 0.81
Lactic dehydrogenase (LDH)	Muscle	300.83 ± 4.45	326.33 ± 4.41	383.67 ± 4.84
	Gill	124.67 ± 4.50	152.33 ± 4.18	212.67 ± 5.16

Values expressed as U/ml enzyme. Values are expressed as Mean ± S.D; sample size (n) = 6

Statistically significant mean difference at $p < 0.0001$.

metals like cadmium, lead and mercury more than the permissible BIS standard values caused degradation of water quality of lake B. Similar results were recorded by Adeyemo (2003) in his work on consequences of pollution and degradation on Nigerian aquatic environment on fisheries resources and Murdoch *et al.* (2001) during his work on watershed inventory and stream monitoring methods. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to contamination of lake water with domestic, industrial and agricultural wastes causing greatest threat to the health of aquatic ecosystem (Joshi *et al.,* 2002). This problem of environmental pollution and its deleterious effects on aquatic biota, including fish is receiving focus during the last few decades (Jagadeesan *et al.,* 2001). Almost all the trace metals that contaminate the system get readily absorbed by plants and then animals reported to be relatively toxic at levels slightly above than those required for maintaining normal metabolic activities of the body (Chakraborthy *et al.,* 2004).

Fishes are sensitive to contamination and the pollutants may disrupt some physiological and biochemical processes when they enter the organs of fishes (Tulasi *et al.,* 1992) as also observed in the present study on *Labeo rohita* from lake B. Fish at a higher level of food chain accumulate a significant amount of pollutants and this accumulation depends on the intake and elimination from the body (Karadede *et al.,* 2004). Exposure of aquatic organisms to metal pollution is a major concern today. The present study showed alteration in the physiological condition and metabolic activities of test fish, *Labeo. rohita* sampled from lake B when compared to those observed in lake A and control site. This can be attributed to the exposure of fish to the significant variation in levels of physico-chemical parameters including trace metals. Since metals act as mutagenic/genotoxic compounds, interfering with xenbiotic metabolic pathways affecting glycolysis, the Krebs cycle, oxidative phosphorylation, protein, amino acid metabolism as well as carbohydrate and lipid metabolism as reported by Drastichova *et al.* (2005) in his cytochemical studies of carp neutrophil after acute exposure to cadmium.

Fish are an important source of protein to man; muscles and gills are the vital organs which have direct contact with the medium through which pollutants enter into their body. In the present study, since high levels of nutrient and trace metals were recorded from water of lake B and subsequently variation in the levels of biomolecules and dehydrogenase activity in the muscle and gill tissue of test fish from the same lake was observed. Therefore, enzymes, could be used (as biomarkers) to identify possible environmental contaminations before the health of aquatic organisms is seriously affected (Barnhoorn, 1996) and to develop water quality indices (Mekkawy *et al.,* 2009). Biochemical approaches can be used to provide an early warning of potentially damaging changes in stressed fish. Reduction in protein and glycogen content in both the tissues of fish in the present study proves that protein has undergone hydrolysis and oxidation whereas glycogen has been utilised rapidly to meet the increasing demand for energy for the survival of fish which is stressed due to the degraded water quality of lake B. De Smet and Blust (2001) also supported this observation by stating that exposure to cadmium caused an increase in the role of proteins for the energy production to combat stress and also due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm may be used to replace the loss of proteins during physiological stress (Patil *et al.,* 2011).

According to Cicik and Engin (2005), cadmium stress caused alteration in the glycogen content through glycolysis or hexose monophosphate pathway in muscle and liver tissue of *Cyprinous carpio* and by Paraquat in African Catfish (Kori- Siakpere *et al.,* 2007). These results are in agreement with the present work on muscle and gill tissue of *Labeo rohita* from lake B which was contaminated with significantly high level of trace metals (Al, Cd, Fe, Pb, Zn and traces of Hg). Depletion in glycogen content may be due to rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Jha and Jha,1995).The decline in glycogen might be partly due to its utilization in the formation of glycoprotein and glycolipids, which are the essential constituents of various cells and other membranes (Vutukuru, 2005).

Stimulation of cholesterol observed in both the tissues of fish may be due to their exposure to pollutants, lipophilic in nature present in lake B. Meenakumari *et al*. (2010) reported that cholesterogenesis increases on the interference of xenobiotics with feedback mechanism. In similar lines with the present study, Muley *et al.* (2007) reported that an increase in cholesterol level can be attributed to the inhibition of steroidogenesis. Under stress conditions, liver undergoes metabolic changes which disturb the excretory mechanism leading to an increase in cholesterol content (Goksyr *et al.,* 1994). The exposure of common carp to heavy metals significantly elevated the concentrations of red blood cells, blood glucose and total cholesterol level (Vinodhini and Narayanan, 2009). A decreasing trend in all the biochemical constituents (total proteins, carbohydrates and lipids) in the tissues of *Channa orientalis* was reported by Hymavathi and Rao (2001) from the habitat polluted by slaughterhouse wastes when compared to the fishes of unpolluted habitat of Mudasarlova stream of Visakhapatnam. The changes exhibited by the biochemical constituents in muscle and gill tissues in the present study due to degradation in the water quality of the lakes can be attributed to proteolysis, glycogenolysis with activation of glycogen phosphorylase and non utilisation of cholesterol to overcome metabolic stress for their survival in non conducive environment of lake B. Degraded condition of the water quality of lake B required surplus oxygen intake by the fish causing an increase in respiratory activity. This resulted in excess utilisation of glycogen and protein in gill when compared to muscle tissue.

Activity of dehydrogenases can be considered as marker of mitochondrial abundance indicating state of fish health and its physiological condition. Inhibition of mitochondrial oxidation of succinate may lead to drop in energy production resulting in suppression of SDH activity which indicates impairment of oxidative metabolic cycle. In similar lines MDH is also dependent on the TCA products and inhibition in its activity is a result of reduction in oxidative metabolism. In the present study, the pollutants in lake B induced a marked decrease in SDH and MDH activity in fishes causing disturbances in oxidative process in the muscle and gill tissue of fish of the lake. These observations tally with reports by Almeida *et al.* (2002) in muscle of *Oreochromis mossambicus* exposed to cadmium and by Rajamannar and Manohar (2000) in different tissues (gill, liver, muscle and brain) of adult or fingerling of *L. rohita* exposed to lethal or sublethal concentrations of lead and copper or organochlorine (DDT and BHC) and organophosphorous (Dichlorvos and Monocrotophos) compounds resulting in a gradual decrease in SDH activity. The significant elevation of LDH in the present study in both muscle and gill tissue regardless of the water bodies suggested that pyruvate; the end product of glycolysis is not routed to Kreb's cycle but to the lactic acid cycle. This is in agreement with the reports of cadmium stress on the metabolic response of Nile Tilapia by Almeida *et al.* (2001). Decline in SDH and MDH activity and concurrent increase in LDH activity in the tissues of fish from lake B due to the degradation of water quality indicated that fishes were unable to bear the pollutant stress which resulted in fall of oxidative metabolism in TCA cycle and which inturn resulted in shift towards anaerobiosis at organ level. Similar results were reported by Ramkritinan *et al.* (2005). The statistical correlation between water parameters and dehydrogenases activity as well as biomolecule constituents revealed that fish is surviving in hypoxic condition with high levels of BOD and COD and variation in the nutrient level and other parameters in lake B. The changes in enzymes may be induced secondarily as a result of damaged cells or these effects may be due to accumulation of trace metals and fluctuation in metabolism of biomolecules in muscle and gill tissues.

The above results indicate that the respiratory activity of fish under stress was affected and it tried to bear the toxic effects by undertaking an anaerobic metabolism due to adverse environmental conditions. Thus, under anaerobic (stressful) conditions the elevated activity of LDH and reduced activity of SDH and MDH reflects the metabolic capacity of tissues after long term exposure to pollutants in water bodies. The effects of these enzymes probably are only a part of general metabolic response to stressful conditions due to significant variation in physico chemical parameters of lakes and may not be specific for trace metals. Fish flesh provides an excellent source of nutrition for human diet and it has relatively high digestibility, biological and growth promoting value but the nutritive value of the fish in question has reduced due to its exposure to various pollutants and is assumed to be unhealthy for human consumption. Therefore, management, conservation and periodic monitoring of these lakes are suggested for the survival of its flora and fauna.

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References

Adeyemo, O. K. (2003) Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources. *The Environmentalist.,* **23,** 297–306.

Almeida, J. A., Diniz, Y.S., Marques, S. F., Faine, L. A., Ribas, B. O., Burneiko, R. R. and Novell, E. I. (2002) The use of oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. *Environ. Int.,* **27**, 673–679.

Almeida, J. A., Novelli, E. L., Dal Pai Silva, M. and Junior, R. A. (2001) Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus. Environ. Pollut*., **114**, 169–175.

APHA, AWWA and WPCF. (2005) In Standard methods for examination of water and wastewater. (21st Eds: Andrew D. Eaton; Clesceri L.S.; Rice E. W.; Greenberg A.E.), The Edition AM. Publ. Health Association, Washington, D .C.

Arechon, N. and Plump, J. A. (1990) Sublethal effects of Malathion on channel cat fish, *Ictalaurus punctatus. Bull.Environ. Contam. Toxicol*., **44**, 435–442.

Barnhoorn, I. (1996) Effects of manganese on the haematology of the *Oreochromis mossambicus* and the bioaccumulation of metals in *Labeo umbratus*. Rand Afrikaans University, South Africa.

Bashir, F. H., Othman, M. S., Mazlan, A. G., Rahim, S. M. and Simon, K. D. (2013) Heavy metal concentration in fishes from the coastal waters of Kapar and Mersing, Malaysia. *Turk. J. Fish. Aquat. Sci.,* **13**, 375–382.

Bergmeyer, H.U. and Bernt, E. (1974) In: Methods of enzymatic analysis. (Bergmeyer H.U. ed.)Volume 2, 574-578, Academic Press, New York, NY and Bergmeyer H.U.:1974, Methods of Enzymatic Analysis, Vol. I, 2nd ed, pp. 485–486.

Chakraborthy, R., Sudip, D., Dhar, S. P., Tiabah, R.C., Myrboh, B., Ghosh, D. and Sharma, D. K. (2004) Determination of few heavy metals in some vegetables from north eastern region of india in relation to human health. *Poll. Res.,* **23**, 537–547.

Cicik, B. and Engin, K. (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). *Turk. J. Vet. Anim. Sci*., **29**, 113–117.

De Smet, H. and Blust, R. (2001) Stress responses and changes in protein metabolism in carp *Cyprinus Carpio* during cadmium exposure. *Ecotoxicol Environ. Saf.,* **48**, 255–262.

Dhapte, A. S., Wash, S. B., Gulbhile, S. D. and Mule, M. B. (2006) Effect of endosulfan on protein content of *Nemacheilus Botia*. *J. Aquat. Biol*., **22**, 198–202.

Drastichova, J. E., Švestkova, V. L. and Svobodova, Z. (2005) Cytochemical study of carp neutrophil granulocytes after acute exposure to cadmium. *J. Appl. Ichthyol*., **21**, 215–219.

Ekeanyanwu, C. R., Ogbuinyi, C. A. and Etienajirhevwe, O. F. (2011) Trace metal distribution in fish tissues, bottom sediments and water from Okumeshi River in Delta State, Nigeria. *Environ. Res. J.*, **5**, 6–10.

Goksyr, A., Beyer, J., Husy, A. M., Larsen, H. E., Westrheim, K., Wilhelmsen, S. and Klungsyr, J. (1994) Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus Morhua*) caged in a polluted fjord (S rfjorden, Norway). *Aquat. Toxicol*., **29**, 21–35.

Hymavathi, V. and Rao, L. M. (2001) Effect of slaughter house pollution on the biochemical composition of *Channa orientalis*. *J. Environ. Biol*., **22**, 209–212.

Jagadeesan, G., Jebanesan, A. and Mathivanan, A. (2001) In Vivo recovery of organic constituents in gill tissue of *Labeo rohita* after exposure to sub lethal concentrations of mercury. *J. Exp. Indelleriia.,* **3**, 22–29.

Jha, B. S. and Jha, A. K. (1995) Biochemical effect of nickel chloride on the liver and gonads of the freshwater climbing perch *Anabus testiduneus* (Bloch). *Proc. Nat. Acad. Sci. India.,* **65**, 39–46.

Joshi, P. K., Bose, M. and Harish, D. (2002) Haematological changes in the blood of *Clarias Batrachus* exposed to mercuric chloride. *Ecotoxiocol. Environ. Monit.,* **12**, 119–122.

Karadede, H., Kalai, M. and Unlu, E. (2004) Concentrations of some heavy metals in water, sediment and fish species from the Ataturk Dam Lake (Euphrates), Turkey. *Chemosphere.,* **41**, 1371–1376.

Khan, A. T. (2011) Trace element in the drinking water and their possible health effect in Aligarh city. *Ind. J. Wat. Res. Proct*., **3**, 522–530.

Kori- Siakpere., Ovie., Adamu., Kabir., Achakpokri and Aghogho, J. (2007) Sublethal effects of paraquat on plasma glucose levels and glycogen reserves in liver and muscle tissues of African catfish (*Clarias Gariepinus*) under laboratory condition. *J. Fish. Aquat. Sci*., **2**, 243–247.

Kumar, B., Sajwan, K. S. and Mukherjee, D. P. (2012) Distribution of heavy metals in valuable coastal fishes from north east coast of India. *Turk. J. Fish. Aquat. Sci.,* **12**: 81–88.

Lee, Q. D., Shirai, K., Nguyen, D. C., Miyazaki, N. and Arai, T. (2009) Heavy metals in a tropical eel *Anguilla marmorata* from the central part of Vietnam. *Water Air Soil pollut.,* **204**, 69–78.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, B. J. (1951) Protein measurement with folin-phenol reagent. *J. Biol. Chem*., **193**, 265–275.

Meenakumari, S., Parrey, S. A. and Saravanan, T. S. (2010) Ambient copper induced alterations in hematology and biochemistry of the major carp *Labeo rohita* (Hamilton). *IJIB.,* **10**, 119–123.

Mekkawy, I. A. A., Mahmoud, U. M., Osman, A. G. and Sayed, A. E.-D. H. (2009) Effects of ultraviolet a on the activity of two metabolic enzymes, DNA damage and lipid peroxidation during early developmental stages of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Fish Physiol. Biochem.*, **36**, 605–626.

Muley, D. V., Karanjkar, D. M. and Maske, S. V. (2007) Impact of industrial effluents on the biochemical composition of fresh water fish *Labeo rohita*. *J. Environ. Biol.,* **28**, 245–249.

Murdoch, T., Cheo, M. and O'Laughlin, K. (2001) Streamkeeper's field guide: Watershed inventory and stream monitoring methods. Adopt –A- Stream Foundation, Everett, WA, pp. 297.

Patil, H. Z., Mahale, P. N. and Kulkarni, A. B. (2011) Alterations in the biochemical contents in foot of freshwater snail, *Indoplanorbis exustus* exposed to heavy metals. *J. Exp. Sci.,* **2**, 12–14.

Rajamannar, K. and Manohar, L. (2000) Reduced respiratory enzyme activity under pesticide toxic stress in the fish, *Labeo rohita*. *J. Ecobiol.*, **12**, 135–144.

Ramakritinan, C. M., Kumaraguru, A. K. and Balasubramanian, M. P. (2005) Impact of distillery effluent on carbohydrate metabolism of freshwater fish, *Cyprinus carpio. Ecotoxicology.,* **14,** 693–707.

Seifer, S., Sayton, S., Navie, B. and Murthy, G. R. (1950) The estimation of glycogen with the anthrone reagent arch. *Biochem. Biophys*., **25**, 191–200.

Singh, S. and Mishra, R. N. (2009) Occurrence of organochlorine pesticides residues in Kuano River of eastern Uttar Pradesh. *J. Environ. Biol*., **30**, 467–468.

Sobha, K., Poornima, A., Harini, P. and Veeraiah, K. (2007) A study on biochemical changes in the fresh water fish, *Catla Catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu university: J. Sci. Engg. Technol.*, **3**, 1–11, DOI: 10.3126/kuset.v3i2.2890

Tulasi, S. J., Reddy, P. U. and Ramana Rao, J. V. (1992**)** Accumulation of lead and effects on total lipid derivatives in the freshwater fish *Anabus testudineus* (Bloch). *Ecotoxicol. Environ. Saf.,* **23**, 33–38.

Vinodhini, R. and Narayanan, M. (2009) The impact of toxic heavy metals on the haematological parameters in common carp (*Cyprinus carpio* l.). *Iran. J. Environ. Hlth. Sci. Eng.*, **6**, 23–28.

Vutukuru, S. S. (2005) Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the indian major carp, *Labeo rohita*. *Inter. J. Environ. Res. Public Hlth.*, **2**, 456–462.

Webner, C. I., Pelteir, W. H., Norberg-King, T. J., Homing, W. B., Kessler, F., Menkedick, J., Eiheisel, T. W. P., Lewis, A., Klemm, D. J., Pickering, O. H., Robinson, E. L., Lazorchak, J., Wymer, L. and Fryberg, R. (1989) Short term methods for estimating the chronic toxicity of effluents and receiving waters of fresh water organisms. 2nd, ed. *EPA-600/4-89-001 and supplement EPA-600/4-89-001 A*, Cincinnati, Ohio.

Zlatkis, A., Zak, B. and Boyle, A. J. (1953) A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med*., **41**, 486–492.