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ORIGINAL RESEARCH ARTICLE

Studies on Biochemical Changes in the Fish *Ctenopharyngodon idella* in Relation to Arsenic Toxicity

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ABSTRACT

Arsenic is a pollutant widely distributed in nature and released into the environment through industrial processes and agricultural practices. Arsenic after reaching the aquatic bodies deteriorate the life, sustaining the quality of water and cause damages to both flora and fauna. Millions of people world-wide are chronically exposed to inorganic forms of the environmental toxicant arsenic in drinking water. Protein is one of the most important nutrients in human diets and fish has become an increasingly important source of protein in most of the developing countries. Among the various fish tissues is the major contributor of proteins and the whole mass of the body. The goal of this study is to assess the changes in the biochemical compositions in, gill, liver, kidney and brain of *Ctenopharyngodon idella* due to arsenic intoxication. The fish were exposed to sub-lethal concentration of arsenic trioxide of the 96 hrs LC_{50} values 89 mg/L for the periods of 7, 14 21 and 28 days. The results of the present study indicate that arsenic intoxication induces in decrease level protein content in, gill, liver, kidney and brain of Grass carp *Ctenopharyngodon idella.* The significant alterations showed toxic effects of heavy metal arsenic trioxide at biochemical levels.

Key words: Arsenic trioxide, Protein, Freshwater fish and *Ctenopharyngodon idella*.

1. INTRODUCTION

Industrial, agricultural and domestic effluents generally contain a wide variety of organic and inorganic pollutants such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids which are invariably discharged into rivers, canals and streams without scientific treatment. Such contaminants usually change water quality and may cause many diseases, structural alterations and functional changes in the organs of the animals [1]. Arsenic is an element which is present at low concentrations in air, soil and water. Arsenic, a non-essential trace element, a potent toxin, mutagen and xenobiotic metalloid has recently appeared as a major pollutant of drinking water in several districts of West Bengal, Tamilnadu and Andhra Pradesh. At present, one of the most worldwide environmental problems is that the drinking water has been polluted by arsenic. Arsenic poisoning from underground drinking water in Bangladesh was first identified in 1993 in the Nawabgonj District [2]. The detection of arsenic in milk and meat is a new finding [3].

Organic arsenic exposure can also occur by eating food. Organic arsenic is less harmful than inorganic arsenic. Inorganic arsenic trioxide is a component of geologic formation which may be washed out into the ground water. Arsenic poisoning can be related to human activities such as mining and ore smelting but it is more often associated with dissolved solids naturally endemic in the aquifer environment. Chronic arsenic toxicity due to drinking of arsenic contaminated ground water is a major environmental health hazard throughout the world [4]. Chronic arsenic poisoning results from drinking contaminated well water over a long period of time. Acute arsenic exposure may promote immediate gastrointestinal tract infection whereas chronic effects may exert degenerative, inflammatory and neoplastic changes of respiratory, haematopoietic, cardiovascular and nervous system [5].

Fish is a major and easily available source of food in nature for mankind. It provides a significant amount of animal protein intake in the diet of a large population. It has been advised that fish

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should be consumed two or three times weekly because of the pharmaceutical effects of omega 3 polyunsaturated fatty acids which exist abundantly in fish oil. But, wide ranges of contaminants are continuously introduced into the aquatic environments and fish from polluted waters seriously threaten human health due to the bioaccumulation of metals in muscle and other tissues. Fish, as a living bio indicator organism, play an increasingly important role in monitoring of water pollution since they respond with great sensitivity to changes in the aquatic environment [6].

However, all the metals taken up by fish are not accumulated because fishes have the ability to regulate their body metal concentration to some extent [7] showed that excretion of metals could occur through the gills, bile (*via* faeces), kidney and skin. There are five potential routes for a pollutant to enter a fish. These routes are through the food, non-food particles, gills, oral consumption of water and the skin. Once the pollutants are absorbed, they are transported by the blood to either a storage point (that is, bone) or to the liver for transformation and storage [8]. If the pollutants are transformed by the liver, they may be stored there or excreted in the bile or passed back into the blood for possible excretion by the gills or kidneys, or stored in fat, which is an extra hepatic tissue [9].

Arsenic exerts its toxic effects through an impairment of cellular respiration by inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation. Arsenic toxicity results from its ability to interact with sulfahydryl groups of proteins and enzymes, and to substitute phosphorus in a variety of biochemical reactions [10]. Proteins are basic molecules to any living system. In cells, they function as enzymes, structural materials, lubricants and carrier molecules. The toxicity tests are needed to evaluate the nature and degree of adverse effects of toxic compounds on the organisms. The toxicity of the trivalent arsenic lies in its ability to bind to the sulphur groups of essential cysteine's in proteins. These groups can be important for the 3 D structure of the proteins and enzyme substrate inter-actions. Because arsenicals in trivalency form binds to two sulphur groups, they can cross link proteins, distorting their overall shape and impeding their function [11]. Keeping in view of the harmful effects of arsenic in fish, the effects of sub-lethal concentrations of arsenic trioxide in *Grass carp* was investigated in the present study.

2. MATERIALS AND METHODS

The freshwater fish, Grass carp *Ctenopharyngodon idella* having weight 14 - 16 g and length 12 - 14 cm were collected from the fish farm located on PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1 % $KMNO_4$ solution and then kept in plastic pools for acclimatization for a period of 9 days. They were fed on rice bran and oil cake daily. The arsenic trioxide was used in this study and stock solutions were prepared. Arsenic trioxide LC_{50} was found out for 96 hrs 89 mg/L [12] respectively taken as sub-lethal concentration of this study. Fifty fish were selected and divided into 5 groups of 10 each. The first group was maintained in free from arsenic trioxide and served as the control. The other 4 groups were exposed to sub-lethal concentration of arsenic trioxide in 10 litre capacity aquaria. The 2nd, 3rd, 4th and 5th groups were exposed to arsenic trioxide for 7, 14, 21 and 28 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein estimation. The protein content of the tissues were estimated by the method of [13, 14] respectively.

Statistical Analysis

The data were analyzed by applying Analysis of Variance two way ANOVA to test the level of significance. Results were presented as means ± SE. P values < 0.05 were regarded as % changes over the control to student 't' test the level of significance [15].

3. RESULTS AND DISCUSSION

The protein levels in gill, liver, kidney and brain of Grass carp *Ctenopharyngodon idella* exposed to sub-lethal concentration of heavy metal arsenic trioxide showed significant decrease when compared to control fish. The decrease in gill, liver, kidney and brain of *Ctenopharyngodon idella* protein levels were more pronounced at 28 days of exposure periods (**Table 1**).

Table 1: The level of Protein in the selected tissues of freshwater fish Grass carp *Ctenopharyngodon idella* exposed with sub-lethal concentration of arsenic trioxide

Organs	Control	Exposure period in days			
		7 days	14 days	21 days	28 days
Gill %COC	68.08± 1.23	64.15± 1.10 -5.77	61.34±1.65 -10.97	57.88± 1.97 -14.98	55.31± 2.07 -18.75
Liver %COC	105.66± 2.14	93.00± 2.32 -12.89	86.28± 2.35 -18.34	79.92± 2.46 -24.36	71.11± 2.67 -32.69
Kidney %COC	72.35± 1.46	63.27± 1.84 -11.56	59.66± 2.29 -17.52	55.87± 2.37 -22.78	50.96±2.41 -29.56
Brain %COC	81.23± 1.52	77.26± 1.65 -4.88	73.09±1.83 -10.02	69.58± 2.08 -13.34	67.26±2.22 -17.19

The values are mean ± S.E six individual observations. (Values are expressed as mg/g wet wt. of tissue).
*significant (P<0.05) of % COC – Per cent change over the control.

Increased industrialization, heavy population and urbanization growth created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among the aquatic pollutants, heavy metals are of greatest concern. Heavy metals after reaching the aquatic bodies deteriorate the life, sustaining the quality of water and cause damages to both flora and fauna. The problem increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications [9, 16].

The effect of arsenic in fish arsenic is graded as one of the most toxic elements to fish. Acute exposure can result in immediate death because of arsenic induced increases in mucus production, causing suffocation, or direct detrimental effects on gill epithelium. Gill surfaces are the first target of water-borne metals [17]. The micro-environment of the gill surface consists of an epithelial membrane which primarily contains phospholipids covered by a mucous layer. According to the gill surface is negatively charged and thus provides a potential site for gill-metal interaction sites for positively charged metals [18]. Liver is the main organ responsible for detoxification of harmful substances which reach it through circulation. Thus the liver is most susceptible to toxicants entering the body of animals. The high degree of liver damage in acute treatment than the chronic exposure with mercury on *Channa punctatus* [19]. Liver plays an important role in detoxification of toxins by breaking down substances and metabolic product as a result of which hepatic cells exhibit more damage than cells from any other organs when an animal is exposed to a toxicant. These heavy metal toxicants are accumulated in the fish through general body surface which affect severally their life support system at molecular biochemical levels. Once these toxic substance enters into body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders [20]. Further, liver plays an important role in the synthesis of proteins. The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination [21].

Kidney is an important organ of excretion and osmoregulation and it is highly susceptible to toxic substances because of its high blood supply. Kidney plays a major role in the elimination of most of the toxicant and considered as a major target organ for toxicity impact [22]. The kidney and liver have been proposed as the major target organs for environmental contaminants such as heavy metals and they are important organs for metabolic waste excretion and heavy metals elimination in fish [23]. Fish is an important source of protein rich food for humans and has become a popular component of human diet during the lost few decades in entire Indian subcontinent.

Proteins are the building units of the body and are also the most abundant macromolecules in the cells constituting half of their dry weight [24]. They regulate and integrate various physiological and metabolic processes [25] in the body. Further, the protein content of the fish body mainly determines the quality and food value of it flesh. The maximum concentration of protein in the liver clearly shows the importance of liver in the body metabolism as well as it the major storehouse of various metabolites [26]. Further, a higher content of the protein in the liver may be attributed to the fact that liver synthesizes most of the plasma protein [27].

Proteins are one of the most important and complete group of biological materials comprising of the nitrogenous constituents of the body and performing different biological functions. Metallothioneins (MT) are a group of cytoplasmic proteins involved in metal regulation [28]. In fish, metallothioneins have been found in high concentrations in most of the vital organs such as liver, kidney, gill, and intestine [28]. They belong to a class of low-molecular-weight cysteine-rich metal-binding proteins found in a large number of prokaryotes and eukaryotes. It is a small protein easily induced by heavy metals, hormones, and a variety of chemicals even at acute stress [29].

The decrease in protein level observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes. According to [30] proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. The depletion of protein fraction may have been due to their degradation and possible utilization for metabolic purposes. Enhanced protease activity and decreased protein level have resulted in marked elevation of free amino acid content in the fish tissues [11]. [31] have reported that arsenic

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induced toxic effects is related to its high ability of reacting with protein and non-protein thiol groups resulting in an alteration of critical cellular pathways.

The fish products have attracted a source of high amounts of significant nutritional components and considerable source of protein in the human diet [32-34]. Plasma proteins were decreased significantly with exposure period of chromium. This could be attributed to renal excretion or impaired protein synthesis or due to liver disorder [35]. [36] have reported that there is an appreciable decline in different biochemical constituents in various tissues in fresh water fish *Labeo rohita* under chromium stress. The continuous reduction of gill protein content was reported in *Anabas testudineus* when exposed to sub-lethal concentration of Cuman L. [22].

The results of the present study showed that the sub-lethal concentrations of heavy metal arsenic trioxide significantly altered the protein levels in gill, liver, kidney and brain of Grass carp *Ctenopharyngodon idella* after 7, 14, 21 and 28 days exposure. The decrease in gill, liver, kidney and brain of *Ctenopharyngodon idella* protein levels were more pronounced at 28 days of exposure periods. It is toxic even at low concentrations and has no known function in biochemical processes [37]. It is known to inhibit active transport mechanisms, involving ATP, to depress cellular oxidation reduction reactions and to inhibit protein synthesis [38]. Exposure to the arsenic salt did not cause significant alteration in the protein level of the brain prior to 30 days of treatment. Although the toxicity of arsenic on the protein content of the brain manifested late, its concentration fell to about 55% of the control value after 60 days [39]. This might perhaps be due to the capacity of arsenic to cross the blood brain barrier and accumulate in the brain causing degenerative changes [40]. [41] also noticed decreased amount of proteins in the brain of *Catla catla* exposed to 0.1 ppm of arsenic trioxide.

Exposure the protein contents of skin and gills decreased substantially. The main reason for protein decrease in these boundary tissues might be due to excessive synthesis followed by sloughing of the slime (made up of glycoprotein) induced by the arsenic stress for inducing increased secretion of slime in the skin and gills the concentration of RNA also increases [42]. The other probable reason for the loss of proteins might be due to rejection of damaged cellular components of the skin and gills rendered by the contact stress of different toxicants [42-44], including an arsenic salt [45]. The concentration of malathion, thiodon and ekalux significantly reduced the total protein in liver of *Oreochromis mossambicus* [46]. Similar observations were noted when the fish were exposed to pollutants [39]. The protein contents in the liver of *Catla catla* depleted under the sub-lethal stress of chromium [47]. [46] have reported a decrease in muscle and liver protein in *Channa punctatus* exposed to mercury, cadmium and lead for periods of 30 days. [48] have reported the decrease in protein content of liver, muscle and kidney in *Channa punctatus* when exposed to sub-lethal concentration of sugar mill waste.

The rapid depletion in total protein content due to active degradation of proteins under stress is dependent on the development of resistance towards the pollutant stress. The decrease of total protein might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthetic machinery [49, 50]. The decreased protein level was observed in the kidney tissue of *Catla catla* at sub-lethal concentration of chromium [47]. The protein content of the freshwater fish decreased with increasing concentration of textile mill effluent [51]. Many investigators have also recorded such a reduction in protein content in different tissues when the fish were exposed to different pollutants [52-54]. It is evident that proteins are degraded to meet the energy requirements during arsenic trioxide exposure.

4. CONCLUSION

In conclusion, this study showed that arsenic trioxide altered the protein metabolism in the freshwater fish *Ctenopharyngodon idella* Grass carp by affecting the levels of protein in gill, liver, kidney and brain due to impairments in energy requiring vital processes.

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REFERENCES

1. Sharma R.K. and Agrawal M. (2005): Biologicaleffects of heavy metals: An overview. J. *Environ. Biol.,* 26 (2), 301-313.
2. Smith AH, Ligas EO and Rahman M (2000). Contamination of drinking water of arsenic in Bangladesh. A public health

emergency. *Bulletin of World Health Organization,* 78: 1093-1103.

3. Awal MA (2007). Detection of arsenic in the food chains and animal samples and study of the preventive measure using the best cost-effective agricultural products based spirulin against arseniasis in man and livestock. Annual Research Report (2006-2007), USDA-Bangladesh collaborative research.
4. Horacio O, Gonazalez, Roling JA, Baldwin WS. Bain L. J (2006). Physiological changes and differential gene expression in mummichogs (*Fundulus heteroclitus*) exposed to arsenic. *Aquat. Toxicol*. 77: 43-52.
5. Neiger RD and Osweiler GD (1989). Effect of subacute low level dietary sodium arsenite on dogs. *Fundamental and Applied Toxicology*. 13: 439-451.
6. Santhananm R, Sukumaran N and Natarajan PA. (1987). Manual of fresh water aquaculture. Oxford and FBH publishing co PVT Ltd, New Delhi-2.
7. Romanenko VD, Malyzheva TD,YU Yevtushenko N (1986). The role of various organs in regulating zinc metabolism in fish. *Hydrobiol. J* 21(3) 7-12.
8. Heath AG (1987). Water Pollution and Physiology. CRC press Inc., Boca Raton.145 pp.
9. Heath A. (1995): Water pollution and fish physiology. 2nd edn, Lewis Publishers, Boca Raton. pp. 125- 140.
10. Samuel, S., R. Kathirvel, T. Jayavelu & P. Chinnakkannu. (2005). Protein oxidative damage in arsenic induced rat brain: Influence of DL-α-lipoic acid. *Toxicology Letters*, 55: 27-34.
11. Seshagiri R, Moorthy KS, Reddy BK, Swamy KS, Chethy CS (1987). Effect of benthiocarb on protein metabolism of teleost, *Sarotherodon mossambica*. *Indian J. Environ. Health*, 29: 440-450.
12. Sprague, J. B., (1971). Measurement of pollutant toxicity to fish, III sublethal effects and 'safe' concentrations. Water Res. 5: 245-266.
13. Lowry, O.H., N.J. Rosenbrough, A.L. Far and R.J. Randall, (1951). Protein measurements with Folin- Phenol reagent. *J.Biol. Chem*. 193: 265-275.
14. Moore, S and H.H. Stein, (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem*. 22: 907.
15. Duncan, B.D., (1957). Multiple range tests for correlated and heteroscedastic means. *Biometrics,* 13: 359-364.
16. Verma R.S., Khan M.A., Tripathi R., Shukla S. and Sharma U.D. (2005): Heavy metal toxicity to fresh water prawn, Macrobrachium dayanum (Crustacea-Decapoda). *Aquacult., 6(1), 57-62.*
17. Spicer, J.I. and Weber, R.E., (1991). Respiratory impairment in crustaceans and molluscs due to exposure to heavy metals. *Comp. Biochem. Physiol*., 100C: 339-342.
18. Reid, S.D. and Mcdonald, D.G., (1991). Metal binding activity of the gills of the rainbow trout (*Onchorhynchus mykiss*). *Can. J. Fish. aquat. Sci*., 48: 1061-1068.
19. Gallagher, E.P. and R.T. Di Giulio, (1992). A comparison of glutathione dependent enzymes in liver, gills and posterior kidney of channel catfish *(Ictalurus punctarus). Comp. Biochem. Physiol.*, 102: 543.
20. Braunbeck, T. and Segnor, H. (1992). Pre-exposure, temperature, acclimation and diet as modifying factors for the tolerance of Golden ide *(leuciscus idus melanotus*) to short time exposure to 4-chloroaniline. *Eco toxology and enveionmental safety,* 24.72,-94.
21. Petrivalsky, M., M. Machala, K. .Nezveda, V. Piacka, Z. Syobodova and P. Drabek, (1997). Glutathione depentdent detoxifying enzymes in *rainbow trout* liver: search for specific biochemical markers of chemical stress. *Environ. Toxicol. Chem*. 16: 1417-1421.
22. Eva, C., (1990). Toxicity and impact of carbonate fungicide cumin L. on respiratory and protein metabolism of an air breathing fish *Anabas testudineus* (Bloch). M.Phil. Thesis, Annamalai University.
23. Laurent, P., Dunel, S., (1980). Morphology of gill epithelia in fish. Am. *J. Physiol*. 238R, 147-159.
24. Jaleel, C. A., Gopi, R., Kishorekumar, A., Mathivanan, P., Sankar , B and Pannerselvam, R., (2008). Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Cathranthus roseus. Acta Physiologiae Plantarum,* 30 : 287- 292.

25. Jaleel, C. A., Gopi, R., and Pannerselvam, R., (2007). Alterations in the lipid peroxidation, electrolyte leakage, and proline metabolism in *Cathranthus roseus* Under treatment with triadimefon, a systemic fungicide, *Comptes Rendus Biologies*, 330 : 905-912.
26. Guyton, C.A and Hall, E.J., (1996). Text Book of Medical Physiology Winth, in: W.B. Saunders Company, USA, p. 1148.
27. Lehininger, P A.L., (1983). Biochemistry, Kalyani Publishers, New Delhi, p. 1-1104.
28. Roesijadi G (1992) Metallothionein in metal regulation and toxicity in aquatic animals. *Aquatic Toxicol* 22:81–114.
29. Kagi JHR (1993) Evolution, structure and chemical activity of class I metallothioneins: an overview. In: Suzuki KT, Imura N, Kimura M (eds) Metallothionein III: biological roles and medical implications. Birkhauser Verlag, Berlin, pp 29–56.
30. Nelson, D.L. and M.M. Cox, (2005). Lehininger Principles of Biochemistry. 3rd Edn., Macmillan Worth Publishers, New York.
31. Habib GM, Shi Z, Lieberman MW (2007). Glutathione protects cells against arsenite-induced toxicity. *Free Radic Biol Med*. 42: 191-201
32. Ozogul, Y., Ozyurt, G., Ozogul, F., Kuley, E. and Polat, A., (2005). Freshness assessment of European eel (*Anguilla anguilla*) by sensory chemical and microbiological methods. *Food Chemistry*, 92, 745–751.
33. Duran, A. and Talas, Z. S., (2009). Biochemical changes and sensory assessment on tissues of carp *(Cyprinus carpio, Linnaeus 1758)* during sale conditions. *Fish Physiology and Biochemistry*, 35, 709–714.
34. Yousefian, M., Sharifrohani,M., Hosseinzadeh- Sahafi, H., Laloei, F. and Makhdoomi, C., (2011). Heritability estimation for growth-related traits in juvenile wild common carp (*Cyprinus carpio* L.) in the south of Caspian Sea. *Iranian Journal of Fisheries Sciences*, 10**(4),** 740- 748.
35. Kori-Siakpere O, Ubogu EO (2008). Sub-lethal haematological effects of Zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). *Afr. J. Biotechnol*. 7(12): 2068-2073.
36. Vutukuru, S.S. (2005). Acute effect of Hexavalent chromium on survival, oxygen consumption, haematological parameter and some biochemical profiles of the Indian Major carp, *Labeo rohita. Int. J. Environ. Res. Public Health*; 2 (3)456-462.
37. Burden, V. M.; Sandheinrich, M. B. and Caldwell, C. A. (1998). Effects of lead on the growth and α aminolvulinic acid dehydratase activity of juvenile rainbow trout, *Oncorhynchus mykiss. Environ. Poll*., 101: 285- 289.
38. Waldron, H. A. and Stofen, S. (1974): Subclinical Lead Poisoning. Academic Press, New York, 1- 224.
39. Randhir Kumar and Tarun K. Banerjee., (2012). Study of sodium arsenite induced biochemical changes on certain biomolecules of the freshwater catfish *Clarias batrachus*. *Neotropical Ichthyology*, 10(2): 451-459,
40. Rodriguez, V. M., L. Carrizales, M. S. Mendoza, O. R. Fajardo & M. Giordano. (2002). Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicology and Teratology, 24: 743-750.
41. Thirumavalavan, R. and S. Samipillai. (2010). Arsenic induced biochemical changes in the brain tissue of fresh water fish, *Catla catla*. *International Journal of Current Research*, 1: 30-34.
42. Banerjee, T. K. (2007). Histopathology of respiratory organs of certain air-breathing fishes of India. *Fish Physiology and Biochemistry*, 33: 441-454.
43. Parashar, R. S. and T. K. Banerjee. (2002). Toxic impact of lethal concentration of lead nitrate on the gills of air breathing catfish, *Heteropneustes fossilis* (Bloch). *Vet Arhiv*, 72: 167-183.
44. Hemalatha, S. and T. K. Banerjee. (1997). Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the air-breathing catfish (*Heteropneustes fossilis, Bloch). Biological Research*, 30: 11-21.
45. Singh, A. K. and T. K. Banerjee. (2008). Toxic impact of sodium arsenate (Na2HAsO4. 7H2O) on skin epidermis of the air-breathing catfish *Clarias batrachus (Linn.). Veterinarski Arhiv*, 78: 73-88.

46. Palanichamy, S. and P. Baskaran. (1995). Selected biochemical and physiological responses of the fish Channa striatus as biomonitor to assess heavy metal pollution in freshwater environment. *Journal of Ecotoxicology and environmental Monitoring*, 5: 131-138.
47. Vincent, S., T. Ambrose, L.Cyril Arun Kumar and M.Selvanaygam, (1995). Biochemical response of the Indian major carp,. *Catla catla (Ham.) to* chromium toxicity. *Indian, J.Environ. Hlth,* 37(3): 192-196.
48. Karupasamy, S., (1990). Toxicity impact of sugar mill effluent on freshwater fish *Channa punctatus (Bloch*). M.Phil. Thesis, Annamalai university, Annamalai nagar, India.
49. Premdas, F.H. and J.M. Anderson, (1963). The uptake and detoxification of C^{14} labelled DDT in Atlantic Salmon *Salmo salar. J.Fish. Res.Bd.Can.,* 30:837.
50. Arora, H.C., V.P. Sharma, S.N. Chattopathya and L.P. Sinha, (1972). Bioassay studies of some commercial organic insecticide. Part III. Trials of *Cirrhinus mrigala* with 6 insecticides. *Indian J. Environ. Hitch*, 14: 353-359.
51. Nish and N.P.Shukla, (1986). Effect of texitile liquid effluent on the freshwater fish. Indian *J.Environ. Protc.*, 6: 189- 192.
52. Ram , RN. and A.G. Sathyanesan, (1984). Mercuric chloride induced changes in protein, lipid and cholesterol levels of the liver, kidney and ovary of the fish, *Channa punctatus. Environ.ECOL.,* 113-117.
53. Palanichamy, S., P. Baskaran and M. P. Balasubramanian. (1986). Sublethal effects of malathion, thiodon and ekalux on protein, carbohydrate and lipid contents of muscle and liver of *Oreochromis mossambisus. Proc. Sym. Pest. Resid . env. Pollu.,* 97-102.
54. Jeyachandran, K.P.S and S.Chockalingam, (1987). Effect of tannery effluent on the respiratory parameters and biochemical contents in an air breathing fish, *Channa punctatus (Bloch). Comp. Physiol. Ecol.,* 12: 197-200.