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RESEARCH ARTICLE

TOXICITY STUDY OF LEAD NITRATE ON FRESHWATER FISH *CIRRHINA MRIGALA*.

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Abstract

Chemicals present in the environment tend to alter the physiology of flora and fauna. Heavy metals tend to accumulate in the biosphere by virtue of their non-biodegradable nature. It is known that accumulation of heavy metals in tissues and organs results in cellular and molecular damage in various species (Cestari et al., 2004; Flora et al., 2012). The present study demonstrates the morphological and biochemical effects associated with the accumulation of the heavy metal lead in adult fish, *Cirrhina mrigala* (Common Indian Carp). Adults of *Cirrhina mrigala* were exposed to increasing concentrations of lead nitrate for a span of 96 hours, post which anatomical and biochemical changes were monitored. External morphology of adult fish and that of the liver and intestine were also qualitatively examined. The liver of treated fish appeared enlarged and had more mass compared with the control, whereas the intestine showed discolouration indicating ingestion of lead. Enzymes indicating stress and cellular damage were assayed in liver extracts prepared from exposed fish. The enzymes studied were succinate dehydrogenase, alanine transaminase, aspartate transaminase, acid phosphatase and alkaline phosphatase. A significant increase was foundin the activity of all of the enzymes except succinate dehydrogenase probably indicating hepatotoxicity. Succinate dehydrogenase activity was seen to have decreased, possibly indicating oxidative stress. Analysis of the neural marker enzyme acetylcholinesterase in brain extracts from these fish also showed a significant decrease in its activity which could lead to a misregulation in neurotransmission. These results directly reflect on the toxicity caused by accumulation of lead in the adult fish.

Introduction:-

Contamination of aquatic ecosystems as a result of anthropogenic factors with heavy metals has been receiving increased worldwide attention due to its harmful effects on human health and other organisms in the environment. In such ecosystems, metals may precipitate or adsorb on the surface of solids, remain soluble or suspended or may be taken up by fauna and flora and accumulate in the tissues of organisms and exert toxic effects (Javed, 2012). One of the heavy metals – lead– is an important contaminant of the industrial age. The main sources of lead contamination are lead based paints, cars using unleaded gas, lead smelters, metal processing plants and incinerators. The main source of lead in drinking water is old lead piping and lead-combining solders. The amount of lead that may dissolve in water depends on acidity (pH), temperature, hardness and standing time of the water (Ruby et al., 1992).

Lead has no known biological benefit to humans. It is known to exert developmental and teratogenic effects in mammalian embryos (Flora et al., 2012). Lead intoxication may result in anaemia and disorders of the gastro-intestinal, renal and nervous systems (Gerber et al., 1980). It has been established that lead accelerates lipid peroxidation in hepatocytes, thereby resulting in hepatotoxicity and severe liver damage in rats (Sandhir and Gill, 1995).

The liver is the major site of elimination of toxins, and hence stress-induced alterations in intermediary metabolism are bound to affect the activity of oxidative enzymes such as succinate dehydrogenase (SDH; EC 1.3.99.1), alanine transaminase (ALT; EC: 2.6.1.2) and aspartate transaminase (AST; EC: 2.6.1.1). Cellular damage due to heavy metals results in the release of ALT and AST in the blood stream and the levels of these enzymes have the potential to indicate hepatocellular-toxicity. Alkaline phosphatase (ALP; EC: 3.1.3.1) and acid phosphatase (ACP; EC. 3.1.3.2) are present in almost all tissues primarily within the cell membrane and increase in activity during heavy metal toxicity. Acetylcholinesterase (AchE; EC: 3.1.3.7) regulates neurotransmission at synapses. Changes in the brain AchE activity help determine the impact of the xenobiotics on the nervous system and ascertain behavioural changes (Patra et al., 2001).

Rivers are the primary recipients of chemical, industrial and agricultural waste in India. The main species of fish found in the Indian rivers are the common edible carps. *Cirrhina mrigala* (Common Indian carp) is endemic to the Indo-Gangetic riverine system (Jayaram, 1981) and is one of three main freshwater carp species of Southeast Asia. To determine the extent of damage to the adult carps due to heavy metal pollution of the rivers, *Cirrhina mrigala* was exposed to lead for 96 hours. Post exposure, the adult fish were assessed for morphological defects, biochemical changes and oxidative stress.

Materials and methods:-

Collection and maintenance of *Cirrhina mrigala*:-

Cirrhina mrigala were procured from the Department of Fisheries based in Goregaon, Mumbai, India. Approximately 200 fish were purchased at a time. The fish used for the study were then acclimatized in groups of eight in 2 ft. tanks, for a span of seven days. Two batches of fish were used for conducting the experiment. All the tanks were supplied with adequate aeration with aerators. The tanks were filled with dechlorinated water to a predetermined level of 12 litres. The fish were fed freeze-dried *Daphnia* twice a day and one-fifth of the tank water was changed every day.

Treatment of adult fish with lead nitrate:-

Fish in the control group were maintained on the same diet and in dechlorinated water without treatment with lead nitrate. Other batches of fish were exposed to 60ppm, 75ppm and 90ppm of lead nitrate for 96 hours. The 96 hour LC_{50} for lead nitrate in *Cirrhina mrigala* is 300ppm (Senthilkumaar et al., 2012).

Morphometric analysis of fish:-

Fish from the control batch and the 60ppm, 75ppm and 90ppm lead nitrate-treated fish were killed by cold necrosis post 96 hours of exposure and were then weighed and the length of the fish measured. Lead treated and untreated fish were dissected and changes in internal morphology were qualitatively observed.

Protein estimation by Folin-Lowry method:-

Liver homogenates of fish were prepared in phosphate buffer and were used to estimate the amount of protein by the method devised by Lowry et al. (Lowry et al., 1951).

Enzyme assays:-

Post 96 hours of treatment with lead nitrate, liver and brain homogenates were prepared from adult *Cirrhina mrigala*for enzyme assays. Liver homogenates were used to assay for AST, ALT, ACP, ALP and SDH, whereas brain homogenates were used to assay for AchE. A 1% liver homogenate was prepared in 0.1M phosphate buffer (pH 7.7) in an ice bath. The homogenate was centrifuged at 4000 rpm for 10 minutes at 4^{o}C. The supernatant was used for the assays. A 1% brain homogenate was similarly prepared in phosphate buffer (pH 8.0).

Succinate dehydrogenase assay:-

The activity of SDH (an enzyme found in the inner mitochondrial membrane) was estimated by a method devised by Nachlas et al. (Nachlas et al., 1960). Activity of SDH was expressed as nmol/minute/mg of protein.

Acid phosphatase and alkaline phosphatase assays:-

The activity of ACP (a lysosomal enzyme) and ALP (a plasma membrane-tethered enzyme) was estimated by Bessay's method (Bessay et al., 1946). Activity of ACP and ALP was expressed as nmol/minute/mg of protein.

Aspartate transaminase and alanine transaminase assays:-
The activity of AST and ALT (both enzymes found in the mitochondria as well as the cytosol) was estimated by Reitman and Frankel's method (Reitmen and Frankel, 1957). Activity of AST and ALT was expressed as nmol/minute/mg of protein.

Acetylcholinesterase assay:-
The activity of AchE (a post-synaptic membrane bound enzyme) was estimated by Ellman's method (Ellman et al., 1961). Activity of AchE was expressed as umol/minute/mg of protein.

Results:-

Morphological changes: The fish in the control batch and the 60ppm, 75ppm and 90ppm lead nitrate treated batches were starved during the 96 hour exposure period. Length measurements showed no significant difference in the fish exposed to lead nitrate versus the control. On dissecting the fish, marked differences in the colour of the intestine of the control and lead-treated fish were visible. The fish in the control set displayed dark colouration in the intestine (Fig. 1A), which was not observed in fish treated with 60ppm (Fig. 1B), 75ppm (Fig. 1C) and 90ppm (Fig. 1D) lead. This change in colour could be attributed to the accumulation of lead nitrate in the intestine of the fish.

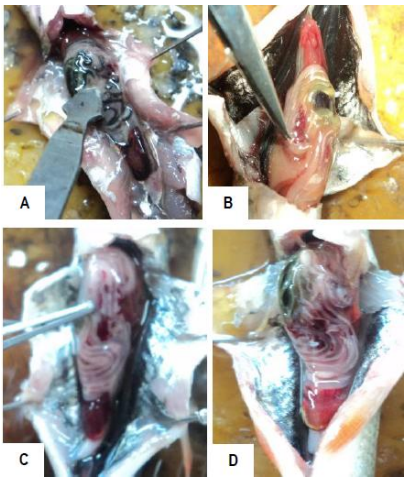

Fig. 1:- Accumulation of lead nitrate in the intestines of *Cirrhina mrigala*. (A) Untreated fish [control] displaying dark colouration in the intestines; (B) Fish treated with 60ppm lead displaying loss of colouration in the intestine; (C) Fish treated with 75ppm lead displaying loss of colouration in the intestine; (D) Fish treated with 90ppm lead displaying loss of colouration in the intestine.

Fish exposed to 60ppmand 75ppm lead displayed a definite visible enlargement in the size of the liver compared to that of the untreated fish (Fig. 2A) and showed a well defined structure (Fig. 2B and 2C). The 90ppm lead treated fish, however, showed an enlargement of the liver and a distorted structure (Fig. 2D). These results indicate that lead probably causes necrosis of the liver. Microscopy could be used in future to assess cellular damage in the liver due to lead.

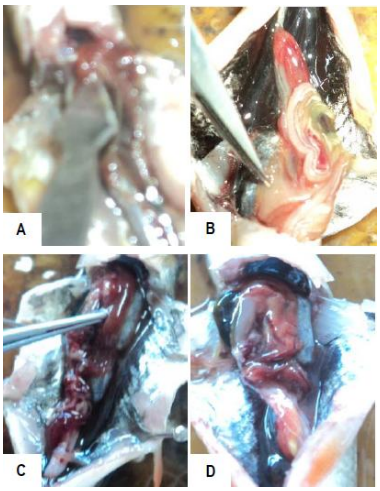

Fig. 2: Visible changes in liver morphology of Cirrhina mirgala post lead treatment. (A) Liver of untreated fish displaying normal morphology; (B, C) Liver of fish treated with 60ppm and 75ppm lead visibly enlarged; (D) Liver of fish treated with 90ppm lead enlarged with an irregular structure.

Protein estimation by Folin-Lowry Method:-

The total protein content of the liver and the brain did not vary significantly between the control and the treated fish. The liver and brain extracts were then further used for enzyme assays.

Biochemical enzyme markers:-

The biochemical marker enzymes assayed in the study were SDH, ACP, ALP, AST, ALT and AchE. Dose dependent changes were observed in the activity of each of the enzymes in the lead-treated and untreated fish (Fig. 3). A 50% decrease in the activity of SDH was seen in fish exposed to 75 ppm and 90 ppm lead (Fig. 3A). In contrast, about two-fold increase was noted in the activity of ACP, ALP and ALT in fish exposed to 75ppm lead (Fig. 3B, 3C and 3E). Large increase in AST activity (eight-fold) was observed in fish treated with 75ppm lead (Fig. 3D). An increase in ACP, ALP, AST and ALT activities are indicative of cellular damage and hence, the enlargement of the liver. There was a significant 40% decrease in the activity of AchE in the brain extracts of fish exposed to 90 ppm lead (Fig. 3F). This may lead to disturbances in neural activity, affecting movement. These findings are summarized in Table 1.

Table 1:Activities of enzymes before and after treatment with increasing concentrations of lead nitrate.

Activity of enzymes expressed as nmol/minute/mg of protein						
Group / Enzyme	**SDH**	**ACP**	**ALP**	**AST**	**ALT**	**AchE (µmol/minute/ mg of protein)**
Control	2.560 ± 0.144	0.340 ± 0.001	0.027 ± 0.0004	0.800 ± 0.048	5.07 ± 0.067	14.950 ± 0.078
60ppm lead	2.099 ± 0.040	0.512 ± 0.007	0.063 ± 0.003	4.042 ± 0.367	4.911 ± 0.096	12.211 ± 0.258
75ppm lead	1.241 ± 0.040	0.699 ± 0.013	0.043 ± 0.002	6.387 ± 0.450	5.891 ± 0.135	13.261 ± 0.363
90ppm lead	1.664 ± 0.056	0.527 ± 0.004	0.020 ± 0.001	1.365 ± 0.122	4.986 ± 0.135	8.395 ± 0.375

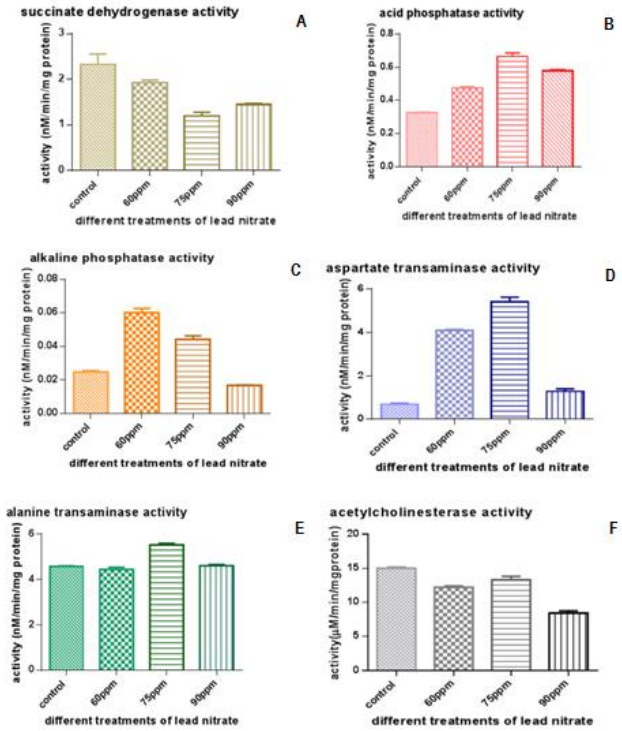

Fig. 3: Changes in the activity of stress biomarker enzymes in liver (SDH, ACP, ALP, AST and ALT) and brain extracts (AchE) post treatment with 60, 75, and 90 ppm lead nitrate. (A) SDH, (B)ACP, (C) ALP, (D) AST, (E) ALT, and (F) AchE. Control fish were not exposed to lead nitrate.

Discussion:-

The accumulation of lead nitrate in the intestine of adult fish, as opposed to that of the control fish, was clearly visible. The unexposed fish showed a black colouration in the intestine, indicative of normal morphology. The fish treated with increasing concentrations of lead nitrate showed varying accumulation of lead as was evident by the whitening of the intestine. Discolouration of the intestine has also been observed in fresh water fish *Leuciscus cephalus* exposed to cadmium (Sures and Siddall, 1999). However, to determine the extent of accumulation of lead nitrate in the body of the adult fish, estimation of lead in dry weight ash needs to be carried out.

It has been established that succinate dehydrogenase is sensitive to many toxicants, including heavy metals (Sastry and Sharma, 1980; Natarajan, 1984). Studies have revealed a steady decrease in succinate dehydrogenase activity in the liver of freshwater fish on exposure to copper, cadmium and other heavy metals (Sastry and Subhadra, 1982; James et al., 1992; Radhakrishnaiah et al., 1992). In the present study the activity of succinate dehydrogenase decreased in liver extracts of adult *Cirrhina mrigala*exposed to sublethal concentrations of lead suggesting that inhibition of mitochondrial oxidation of succinate may result in a decreased energy production. The suppression of succinate dehydrogenase activity indicates impairment of the oxidative metabolic cycle and hence an increase in anaerobic glycolysis to meet the energy demands of the organism (Rajeswari et al., 1989).

Acid phosphatase is a marker enzyme of lysosomes and exists in a latent form. Stimulation or inhibition of this enzyme can result in disturbances in metabolism. Change in the activity of acid phosphatase, due to oxidative stress, is a characteristic of tissue damage (Biber et al., 1981). In the present study, an increase in acid phosphatase activity was observed. This finding is in agreement with various studies carried out on heavy metal toxicity on freshwater fish in the liver and kidneys (Sastry and Subhadra, 1985; Sastry and Gupta, 1979). The rise in the activity of acid phosphatase due to lead toxicity suggests putative hepatocellular damage in the organism (Sharma, 1999). It has been suggested that hyperactivity of acid phosphatase indicates proliferation of lysosomes in an attempt to sequester the toxic xenobiotic (Gill et al., 1992).

Increased activity of alkaline phosphatase has been found in pathological processes such as liver impairment, kidney dysfunction and bone diseases, and has been attributed to significant liver damage (Kendall et al., 1970). Alkaline

phosphatase is a membrane-bound enzyme found at the bile pole of hepatoctyes (Garnero and Delmas, 1993). Various studies have established changes in the activity of alkaline phosphatase as an important indicator of oxidative stress. Lead nitrate is considered as an activator of alkaline phosphatase as is also evident in the present study. Toxicity studies performed on freshwater fish using heavy metals such as hexavalent chromium and mercuric chloride, and organic chemicals such as diethyl phthalate and endosulfan, have revealed an increase in the activity of alkaline phosphatase in the treated fish (Ghorpade et al., 2002; Agarwal and Sastry, 1979; Gill and Pant, 1981). A similar increase in ALP was observed in lead exposed *Cirrhina mrigala* in the present study. Thus, it can be concluded that short term, acute exposure to lead results in significant hepatocellular damage in *Cirrhina mrigala*.

Aspartate transaminase and alanine transaminase link amino acids to intermediates of pathways involved in energy generation, particularly the tricarboxylic acid cycle. It is, hence, expected that increased energy demand, as a result of oxidative stress, will result in mobilization of existing energy sources, including amino acids, resulting in the activation of enzymes of amino acid metabolism (Masola et al., 2008). Aspartate transaminase and alanine transaminase are frequently used in diagnosis of tissue damage, as markers of necrosis caused by pollutants, in various tissues (de la Torre et al., 2000). In the present study, the activity of aspartate transaminase and alanine transaminase increased in *Cirrhina mrigala* exposed to sublethal concentrations of lead for 96 hours as seen in the levels in liver extracts. This may be due to necrosis of hepatocytes leading to increased permeability of the cell membrane resulting in damage to tissues. This suggests an increased participation of amino acids in energy metabolism in response to an increased energy demand to cope with the metabolic stress caused by lead nitrate.

Acetylcholinesterase is primarily localized at pre-synaptic axonal membranes within synapses in the central nervous system, while in synapses of the peripheral nervous system and at neuromuscular junctions, it is localized on the post-synaptic dendrites. On excitation of the post-synaptic neuron by acetylcholine, acetylcholinesterase hydrolyzes acetylcholine into acetate and choline, thereby regulating the critical window for neurotransmission (Sussman et al., 1991). In the present study, a significant decrease in acetylcholinesterase activity was observed in brain extracts of the lead-treated adult fish as compared with those of untreated fish. Decrease in activity of acetylcholinesterase maybe indicative of damage to the nervous system. This decrease in activity maybe due to inhibition of acetylcholinesterase by direct binding of lead with the enzyme (Passowet al., 1961) or the toxic effects produced by them on tissues (Blackwood et al., 1961) leading to decreased synthesis of enzymes (Hirth, 1964). Significant decreases in acetylcholinesterase activity have been reported in various studies conducted using a number of xenobiotics thus indicating that decrease in the activity of acetylcholinesterase can be considered as a biological indicator of stress in freshwater fish.

In conclusion, the heavy metal lead seems to accumulate in the body of the fish and causes changes in the metabolic enzyme activities in the liver and the brain. This study reveals the hazards associated with the accumulation of heavy metals in the environment and their modus operandi for toxigenesis within fauna.

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References:-

1. **Agrawal, M. K. and Sastry, K. V. (1979).** $HgCl_2$ induced enzymological changes in kidney and ovary of a teleost fish Channa punctatus. Bull. Environ. Contam. Toxicol. 22(1-2): 38-43.
2. **Bessay, O.A., Lowky, O.H. and Brock, M.J. (1946).** A method for the rapid determination of alkaline phosphatase with five cubic millileters of serum. J. Biol. Chem. 164: 312-319.
3. **Biber, J., Stieger, B., Hasse, W. and Murer, H. (1981).** A high yield preparation for rat kidney brush border membranes: different behaviour of lysosomal markers. Biochim. Biophys. Acta. 647(2): 169-176.
4. **Blackwood, C. and Mandl, I. (1961).** An improved test for the quantitative determination of trypsin, trypsin-like enzymes, and enzyme inhibitors. Anal. Biochem. 2(4): 370-379.
5. **Cestari, M.M., Lemos, P.M.M., Ribeiro, C.A.O., Costa, J.R.M.A., Pelletier, E., Ferraro, M.V.M., Mantovani, M.S. and Fenocchio A.S. (2004).** Genetic damage induced by trophic doses of lead in the neotropical fish Hopliasmalabaricus (Characiformes, Erythrinidae) as revealed by the comet assay and chromosomal aberrations. Genet. Mol. Biol. 27(2): 270-274.
6. **de la Torre, F.R., Salibian, A. and Ferrari, L. (2000).** Biomarker assessment in juvenile Cyprinuscarpioexposed to waterborne cadmium. Environ. Pollut. 109: 277-282.
7. **Ellman, G.L., Courtney, K.D., Andres, V. And Featherstone, R.M. (1961).** A new and rapid determination of acetylcholinesterase activity. Biochem. Pharmacol. 7(2): 88-95.
8. **Flora, G., Gupta, D. and Tiwari, A. (2012).** Toxicity of lead: A review with recent updates. Interdiscip. Toxicol. 5(2): 47-58.
9. **Garnero, P. and Delmas, P.D. (1993).** Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. J. Clin. Endocrin. Metab. 77(4): 1046-1053.
10. **Gerber, G.B., Leonard, A. and Jacquet, P. (1980).** Toxicity, mutagenicity and teratogenicity of lead. Mut. Res./Rev. Genet. Toxicol. 76(2): 115-141.
11. **Ghorpade, N., Mehta, V., Khare, M., Sinkar. P., Krishnan, S. and Rao, C.V. (2002).**Toxicity study of diethyl phthalate on freshwater fish Cirrhinamrigala. Ecotoxicol. Environ. Saf. 53(2): 255-258.
12. **Gill, T. S. and Pant, J. C. (1981).**Effect of sublethal concentration of mercury in teleost Puntius conchonius: Biochemical haematological responses. Ind. J. Exp. Biol. 19: 571-573.
13. **Gill, T. S., Tewari, H. and Pande, J. (1992).** Short and long-term effects of copper on the rosy carb Puntius conchonius. Ecotoxicol. Environ. Saf. 23: 294-297.
14. **Hirth, L. (1964).** Enzyme damage due to heavy metal poisoning. Munch. Med. Wochenschr. 106: 985-988.
15. **James, R., Sampath, K. and Ponmani, K.P. (1992).** Effect of metal mixture on activity of enzymes and their recovery in Orechromismossambicus. Ind. J. Exp. Biol.30: 496-499.
16. **Javed, M. (2012).** Effects of zinc and lead toxicity on the growth and their bioaccumulation in fish. Pak. Vet. J. 32(3): 357-362.
17. **Jayaram, K. (1981).** The Freshwater Fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka. Calcutta: Zool. Surv. Ind. 2: 475.
18. **Kendall, M.J., Cockel, R., Becker, J. And Hawkins, C.F. (1970).** Raised serum alkaline phosphatase in rheumatoid disease. An index of liver dysfunction?. Annals Rheum. Dis. 29(5): 537-540.
19. **Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951).** Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1): 265-275.
20. **Masola, M., Chibi, M., Kandare, E., Naik, Y.S. and Zaranyika, M.F. (2008).** Potential marker enzyme and metal- metal interactions in Helisomaduryiand Lymnaeanatalensis exposed to cadmium. Ecol. Environ. Saf. 70: 79-87.
21. **Nachlas, M.M., Margulies, S.I. and Seligman, A.M. (1960).** A colorimetric method for the estimation of succinate dehydrogenase activity. J. Biol. Chem. 235(2): 499-503.
22. **Natarajan, G.M. (1984).** Effect of lethal (LC 50/48 hr) concentration of metasystox on some selected enzyme systems in the air breathing fish, Channastriatus(Bleeker). Comp. Physiol. Ecol. 9: 29-32.
23. **Patra, R.C., Swarup, D. And Dwivedi S.K. (2001).** Antioxidant effects of α-tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology. 162(2): 81-88.
24. **Passow, H., Rothstein, A. and Clarkson, T. W. (1961).** The general pharmacology of the heavy metals. Pharmacol. Rev. 13: 185-224.
25. **Radhakrishnaiah, K., Venkataramana, P., Suresh, A. and Sivaramakrishna, B. (1992).** Effect of lethal and sub lethal concentration of copper on the glycogen in liver and muscle of fresh water teleost, Labeorohita(Ham). J. Environ. Biol. 139(1): 63-68.
26. **Rajeswari, K. and Reddy, J. (1989).** Impact of thiodon on the metabolic pathway of the fish Tilapia

mossambica. Environ. Ecol., 7(4): 863–866.
27. **Reitman, S. and Frankel. S. (1957).** A colorimetric method for the determination of serum glutamic oxalacetatic and serum glutamic pyruvic transaminases. Am. J. Clin. Path. 28(1): 56-63.
28. **Ruby, M.V., Davis, A., Kempton, J.H., Drexler, J.W. and Bergstrom, P.D. (1992).** Lead bioavailability – Dissolution kinetics under simulated gastric conditions. Environ. Sci. Technol. 26(6): 1242-1248.
29. **Sandhir, R. and Gill, K.D. (1995).** Effect of lead on lipid peroxidation in liver of rats. Biol. Trace Elem. Res. 48(1): 91-97.
30. **Sastry, K.V. and Gupta, P.K. (1979).** The effect of cadmium on the digestive system of the Teleost fish Heteropneustesfossilis. Environ. Res. 19(2): 221-230.
31. **Sastry, K. V. and Sharma, K. (1980).** Mercury induced haematological and Biochemical anomalies in Ophiocephalus (Channa) punctatus. Toxicol. Lett. 5: 245-249.
32. **Sastry, K.V. and Subhadra, K. (1982).** Effect of cadmium on some aspects of carbohydrate metabolism in a freshwater catfish Heteropneutesfossilis. Toxicol. Lett. 14: 45–55.
33. **Sastry, K. V. and Subhadra, K. (1985).** In vivo effect of cadmium on some enzyme activities in tissue of the fresh water cat fish, Heteropneustesfossilis. Environ. Res. 36(1): 32-45.
34. **Senthilkumaar, P., Eswari, S., Velmurugan, K. and Sarojini, S. (2012).** Independent toxic effects of metallic compounds in Indian major Carps. Ind. J. Sci. Tech. 5(8): 3142-3149.
35. **Sharma, B. (1999).** Effect of carbaryl on some biochemical constituents of the blood and liver of Clariasbatrachus, a fresh- water teleost. J. Toxicol. Sci. 24(3): 157-164.
36. **Sures, B. and Siddall R. (1999).** Pomphorhynchuslaevis: The Intestinal Acanthocephalan as a Lead Sink for its Fish Host, Chub (Leuciscuscephalus). Exp. Parasit. 93(2): 66-72.
37. **Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. and Silman, I. (1991).** Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science. 253(5022): 872-879.