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Research Article

Hepatopancreatic alteration in protein level in freshwater bivalve, *Lamellidens corrianus* (Lea) exposed to broad spectrum antibiotics

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Abstract

In India demand for freshwater pearls is day by day increasing due to a great fascination for pearls. To tied the knot between production and supply of pearls, freshwater bivalve culture sector is being actively involved for the good yield of pearls. In this practice to reduce the mortality rate of bivalves, antibiotic therapy is used to practice after insertion of beads in the bivalve shell or tissue. In the present study attempt was done to find out the impact of some antibiotics tetracycline, chloramphenicol and trimethoprim on the hepatopancreatic protein level. Hepatopancreas, the storage house of metabolic reserve and source of energy during physiological stress is greatly affected by the antibiotic exposure. After acute exposure of tetracycline (369.10PPM), chloramphenicol (470.37PPM) and trimethoprim (174.80PPM)) *Lamellidens corrianus* showed a most significant decrease in the protein level. The most depleted protein level was estimated to 41.03 % in case of chloramphenicol after 96 hours. The chronic concentration used of tetracycline 73.82 PPM, chloramphenicol 94.07 PPM and trimethoprim was 34.96 PPM resulted into a drastic effect in reduction of protein level upto 42.49 % in chloramphenicol exposed bivalves followed by trimethoprim and tetracycline exposed bivalves 38.49 % and 31.57% respectively.

Keywords: *Lamellidens corrianus,* Tetracycline, Chloramphenicol trimethoprim, Hepatopancreas, Protein.

Introduction

Although the traditional source of pearls has been saltwater mollusks, freshwater mussels, which live in ponds, lakes and rivers, can also produce pearls. China has harvested freshwater pearls in the form of mabe since the 13th century, and has leadership in freshwater pearl production. The United States was also a major source of natural freshwater pearls until over-harvesting and increasing pollution significantly reduced the number of available pearl-forming mussels in the US. Demand for freshwater pearls is day by day increasing due to fascination for pearls and to fulfill this demand many countries including India started freshwater pearl culture.

In this practice after a small surgery of bead implantation, treatment is used to give the bivalves by keeping them in the water mixed with antibiotics. The effect of these antibiotics on the physiology is not still studied. The biochemical evaluation of bivalve is thus needed to indicate the effect of these antibiotics on the condition of the cell and its content due to altered internal milieu of the organism. Effect of tetracycline, chloramphenicol and trimethoprim on the hepatopancreatic protein level is estimated. Hepatopancreas the storage house of metabolic reserve and source of energy during physiological stress is greatly affected by the antibiotic exposure stress.

Tetracyclines ($C_{22}H_{24}N_2O_8$) are effective against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. Tetracycline inhibits cell growth by inhibiting translation. It binds to the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome.

Chloramphenicol ($C_{11}H_{12}C_{l2}N_2O_5$) is also inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. It specifically binds to the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation.

Trimethoprim ($C_{14}H_{18}N_4O_3$) binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid for thymidine synthesis pathway and retard bacterial growth (Brogden, *et al.*, 1982) Proteins are the major biochemical component, which act as source of energy for various physiological functions including reproduction (Giese, 1969)

Materials and Methods

Freshwater bivalves *Lamellidens corrianus* were collected from Girna Dam Dist: Nasik (M.S.) situated at 20^o 28'58" N latitude and 74^o 43'13"E longitude. Freshly collected bivalves were brought to the laboratory, scrubbed to remove fouling matter. The animals were kept in 50 L aerated dechlorinated water for 4 days for acclimatization. Water temperature during the experimentation was 28.5^o C $\pm$ 2.0 ^{o}C and pH 7.3 $\pm$ 0.23.

During experimentation only those *L. corrianus* (65 to 80 mm length) showing movements and good health, were used for experimentation. The animals *L. corrianus* were divided into four batches, for acute and chronic exposures each of tetracycline, chloramphenicol and trimethoprim along with control. For each experiment 10 animals of approximately similar size were exposed to acute concentrations as 369.10 PPM, 470.37 PPM and 174.80 PPM and to chronic concentration as 73.82 PPM, 94.07 PPM and 34.96 PPM of tetracycline, chloramphenicol and trimethoprim respectively. *L. corrianus* were exposed to acute and chronic concentration up to 96 hrs and 21 days respectively.

Animals sacrificed and hepatopancreas removed, dried out and ground into fine powdered form and Protein contents were estimated by (Lowry *et al.*,1951) method using Folin Phenol Reagent.

Protein estimation

10 mg of dry powder was homogenized in small amount of 10% TCA and the homogenate was diluted to 10 ml by 10% TCA, centrifuged at 3000 rpm for 15 minutes. The protein precipitate at the bottom of centrifuged tubes was dissolved in 10ml1.0 N NaOH solution. 0.1 ml of this solution of each powder was taken in test tubes containing 4.0 ml. freshly prepared Lowry's 'C'. After adding 0.5 ml. Folin's – phenol reagent, the test tubes were incubated in dark at 37^0 C for 30 minutes. The O. D. of blue colour developed was read at 530 nm along with blank. The protein content in different tissues was calculated using standard graph (using Bovine serum albumen) value in terms of mg protein/100 mg of dry tissue.

Results

After exposure to the acute and chrnic concentrations of antibiotics, all bivalves were seen with copious amount of mucus secretions to resist the environment created by water mixed with antibiotics. In the present study obtained results demonstrated that, after acute and chronic exposures to tetracyclines, chloramphenicol and trimethoprim a marked depletion in the protein contents in the hepatopancreas of freshwater bivalve *Lamellidens corrianus* was observed as compared to control. The obtained results are presented in the table nos.1 and 2. The results showed that, there was progressive decrease in the protein content with increase in exposure time.

After acute exposure *Lamellidens corrianus* of tetracycline, chloramphenicol and trimethoprim showed the most significant decrease in the protein level upto 41.03 % as in case of chloramphenicol after 96 hours. After chronic concentration the most significant depletion of protein level upto 42.49 % in chloramphenicol exposed bivalves followed by trimethoprim and tetracycline exposed bivalves 38.49 % and 31.57% respectively as shown in table 1 and 2.

Table 1: Impact of acute exposure of Tetracycline, Chloramphenicol and Trimethoprim on protein contents of hepatopancreas of *L. corrianus*

24 hrs				96 hrs			
Control	**Tetra**	**Chlor**	**Trimetho**	**Control**	**Tetra**	**Chlor**	**Trimetho**
53.703 ±2.175	52.098 ±1.6632 -4.999*	40.277 ±1.258 -24.999***	48.333 ±1.253 -10.000**	37.037 ±2.180	25.542 ±1.258 -31.034***	21.839 ±1.468 -41.034***	26.819 ±1.253 -27.586***

Values are expressed as mg/100mg dry weight of tissue.± indicates standard deviation of three independent replications.
+ or - indicates % variation over control. Significance: * P < 0.05; ** P < 0.01; *** P 0.001; NS = Non-significant.

Graph 1 showing Changes in protein level after acute exposure of Tetracycline, Chloramphenicol and Trimethoprim

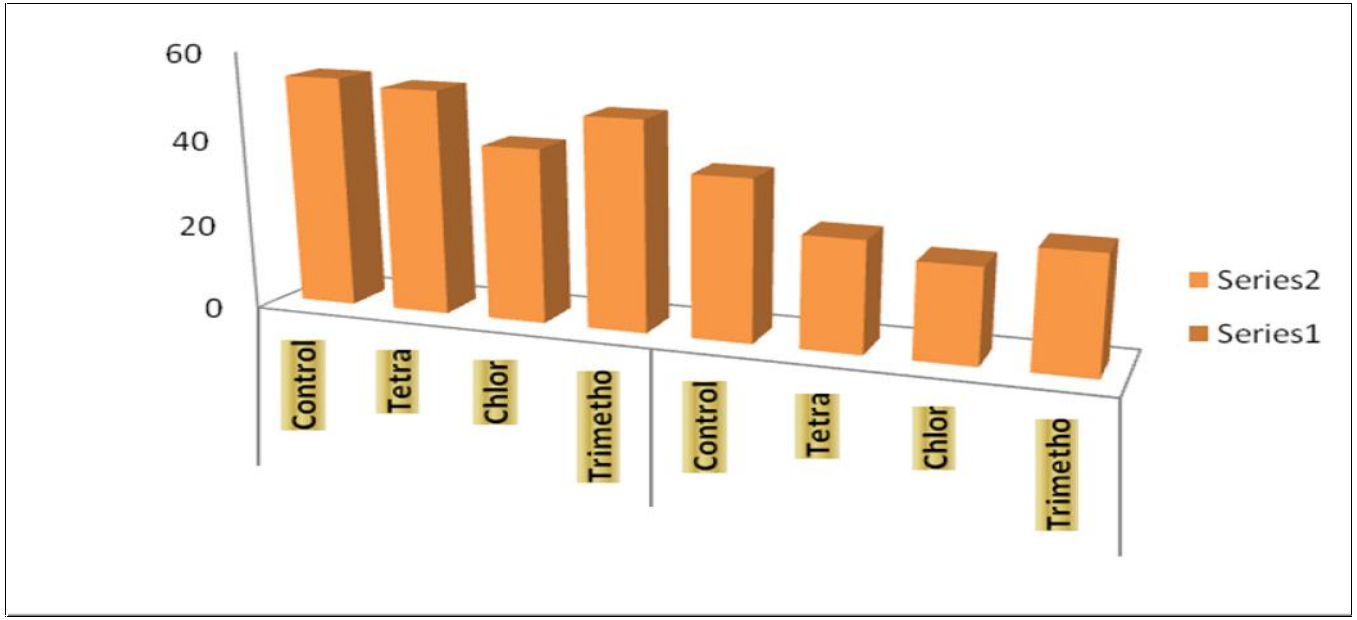
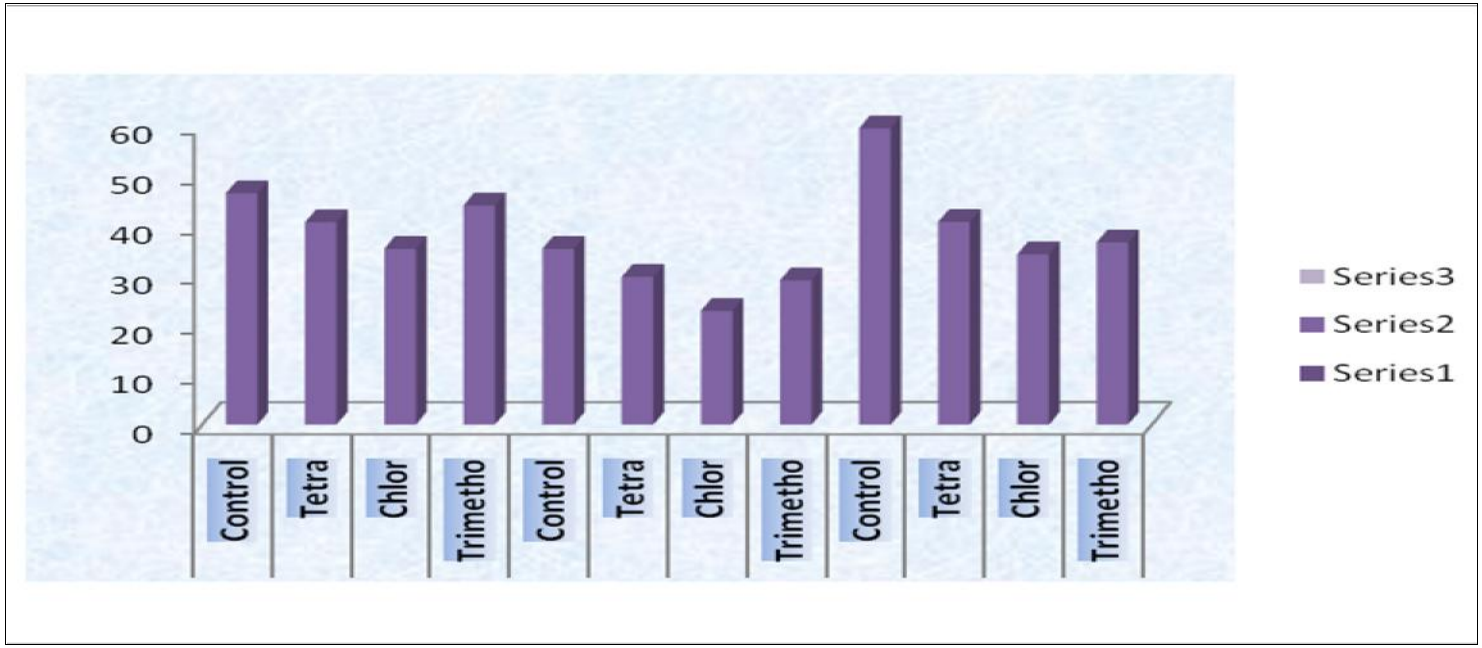


Table 2: Impact of chronic exposure of Tetracycline, Chloramphenicol and Trimethoprim on protein contents of hepatopancreas of *L. corrianus*

7 days				14 days				21 days			
Contro	**Tetra**	**Chlor**	**Trimetho**	**Contro**	**Tetra**	**Chlor**	**Trimetho**	**Contro**	**Tetra**	**Chlor**	**Trimetho**
46.296 ±2.512	40.509 ±4.085 -12.499*	35.185 ±1.6632 -28.999***	43.859 ±2.357 -5.263**	35.185 ±1.253	29.555 ±2.175 -15.999**	22.777 ±2.180 -35.263***	28.851 ±2.512 -18.000**	59.259 ±3.287	40.545 ±2.517 -31.578***	34.074 ±1.6632 -42.499***	36.444 ±1.253 -38.499***

Graph 2 showing Changes in protein level after chronic exposure of Tetracycline, Chloramphenicol and Trimethoprim



Discussion

Pesticides, particularly organophosphate compounds, are used in some areas of the world to regulate pests such as shrimps in fish ponds as well as ectoparasitic infestations. Most of these chemicals are toxic to aquatic life at lower concentrations than those used to treat fish (Barg, 1992, Beveridge and Phillips, 1990).
There is larger risks of serious disease outbreaks in aquaculture practices threatening other farmed and wild populations. Intensive systems demand treatment of water by chemicals, and drugs for disease prophylaxis and treatment. The overuse of these chemicals and drugs creates pollution as well as develops resistant strains of pathogens (Pullin, 1989; GESAMP, 1991; Barg, 1992).

The hepatopancreas in bivalve mollusc serves as a site for the storage of metabolic reserves which provides a source of energy utilized during gametogenesis and during the periods of physiological stress (Bayne, 1976; Muley 1988)**.** At high pollution stress however, protein synthesis can be suppressed indicating disturbance of normal metabolic processes (Pottinger *et al.,* 2002). To cope up stressful conditions there is extra energy expenditure of the animal. It is carried out through different ingredients of food as carbohydrates, fats and ultimately through proteins. The depletion of protein content suggests an increased rate of proteolysis to build energy to overcome problems of different pollutants. They may be mobilized in to TCA cycle through aminotransferase system (Jadhav *et al.,* 1995) and maintained energy level at the expense of proteins (Muley and Mane, 1995). The fall in protein level during pollutant exposure may be due to increased catabolism and decrease in protein synthesis. (Vincent *et al.,* 1995; Waykar and Lomte, 2001)

The higher depletion of protein in the hepatopancreas might be due to high metabolic potency and efficiency of the gland under pollutant stress .The digestive gland is the main site of degradation and detoxification of toxicants and hence resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein is the tool to access the extent of toxicity (Singraju *et al.,* 1991; Mule & Lomte 1995; Jadhav, 1993).

Several authors have reported depletion in proteins in fishes exposed to various toxicants affecting nutritive value of edible organisms (Meenakshi, 1998). Adverse effect on protein metabolism due to heavy metal studied by Kristen (2007) Nickel effect in fresh water bivalve, *Lammellidens marginalis* by Andhale, (2011). Moore (1991) stated that pathological reactions of the lysosomal system in hepatopancreatic cells of bivalve molluscs have proven to be sensitive bioindicators of Mussel. In the present study, the destruction of the basement membrane of the hepatic tubules and cell surface of the digestive cells after cadmium exposure probably caused disfunction of the surface receptors resulting in the disturbance of lysosomal system functioning. The results recorded in the present study are in harmony with the results of previous investigators (Lomte *et al.,* 2000; Mahajan and Zambare, 2005; Gulbhile, 2006; Satyaparameshwar *et al.,* 2006; Pardeshi and Gapat, 2012).

Kharat *et al.,* 2009 studied depletion in protein content in the ovary, hepatopancreas, gill and muscles of *Macrobrachium kistnensis* exposed to different concentrations of tributyltin chloride stress on protein metabolism similar results were obtained by (Cochrane *et al.,* 1991; Lundebye *et al.,* 1997; Sole and Porte, 2000)They stated that digestive gland was the most affected organ followed by gonad and gill. The decrease in protein contents may be due to altered size of pores in membrane (Abel, 1974) or diminished protein synthesis (Reddy, 1979). To elevate the level of repair enzymes, the proteolytic action increases (Kabeer *et al.,* 1977). The depletion in protein content caused rise in the amino acid pool (Omata *et al.,* 1978) due to toxicant stress. Inhibition in the protein synthesis was reported to be possible due to non-selective blocking of phosphorylation process in the central nervous system and tissues (Kuznetsov *et al.,* 1984; Kawamata *et al.,* 1987).

References

Abel, P.D. (1974) Toxicity of synthetic detergents to fish and aquatic vertebrates. *J. Fish Biol*., 6:279-298.

Andhale, A.V. (2011). Effect of nickel induced biochemical alterations in fresh water bivalve, *Lamellidens marginalis*. J. Ecobiotechnol., 3, 18-24.

Barg, U.C, (1992), Guidelines for the promotion of environmental management of coastal aquaculture development. FAO Fisheries Technical Paper No 328.

Bayne, B.L., 1976. Marine Mussels, their Ecology and Physiology, Cambridge University Press, London, pp: 495.

Beveridge, M.C.M. and M.J. Phillips. (1990). Environmental impact of tropical inland aquaculture. Conference on environment and third world aquaculture development, Rockefeller Foundation, Bellagio, Italy, 17–22 September.

Cochrane, B.J., Irby, R.B. and Snell, T.W.(1991). Effects of copper and tributyltin on stress protein abundance in the rotifer *Brachionus plicatilis*. Comp. Biochem. Physiol. 98C, 385–90.

Corbicula straitella. Ph.D. Thesis. Marathwada University, Aurangabad (M.S.) India.

GESAMP. (1991), (IMO/FAO/Unesco/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution), Reducing environmental impacts of coastal aquaculture. Rep. Stud. GESAMP, (47):35 p.

Gulbhile, S. D. (2006): Caffeine (1,3,7 trimethylxanthine) supplementation: possible recovery in mercury and arsenic induced alterations in the freshwater bivalve, *Lamellidens corrianus*. Ph.D. Thesis submitted to Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Jadhav, S.M. 1993. Impact of pollutants on some physiological aspects of the fresh water bivalve, *Corbicula straitella*. Ph.D. Thesis. Marathwada University, Aurangabad (M.S.) India.

Jadhav, S., Y.B. Sontakke and V.S. Lomte, (1995). Effect of carbaryl on protein content of freshwater bivalve Corbcula striatella. Indian J. Comp. Ani. Physiol., 13, 33-35.

Kabeer, A.I., Siviah, S. and Raman Rao, K.V. (1977) On the possible significance of changes in organic constituents in selected tissues of malathion exposed snail, Pila globosa (Wainson). Comp. Physiol. 94: 81-82.

Kawamata, O., Rasotoa, B., Omata, S.and Sugano H. (1987) Decrease in protein phosphorylation in central and peripheral nervous tissue of methyl mercury treated rats, *Arch. Toxicol*., 59 (5): 346-352.

Kharat. P.S., Laxmi B. Ghoble, K.B. Shejule, R.S. Kale and B.C. Ghoble, (2009). Impact of TBTCl on Total Protein Content in Freshwater Prawn, *Macrobrachium kistnensis*, Middle-East Journal of Scientific Research 4 (3): 180-184.

Kristen, H. (2007) Effect of some heavy metals on protein and collagen biosynthesis in rabbit dental pulp in-vitro. Europian J. Oral Sci., 85, 266-271.

Kuznetsov, D.A., Zivijalov, N.V., Govorkov, A.V.and Sibileba, M.T. (1984) Methyl mercury induced non-selective blocking of phosphorylation process as a possible causes of protein synthesis inhibition *in vitro* and *in vivo*. *Toxicol*. *Lett*., 36(2): 153-160.

Lomte, V. S., Chuadhari, R. T. and Masarrat, S. (2000): Changes in protein content of fresh water bivalve, Parreysia cylindrica on exposure to nickel chloride and lead chloride. Ind. J. Environ. Ecoplan., 3: 63-65.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Rendel (1951). Protein estimation with folin-phenol reagent. J. Biol. Chem., 193, 265-278.

Lundebye, A.K; Langston W.J; Depledge M.H 1997. Stress proteins and condition index as biomarkers of tributyltin exposure and effect in mussels. Journ. of Ecotoxico.Vol -6. Pages 127-136.

Mahajan, A. Y. and S. P. Zambare (2005): Effect if Lead chloride on oxygen consumption of the freshwater bivalve *Corbicula stritella* J. Aqua. Biol. 230(2): 184-186.

Meenakshi, V. and K. Pugazhendhi (1998a) Toxicity of mercuric chloride to the fingerlings of freshwater fish Cyprinus carpio (Linn.). Uttar Pradesh J. Zool., 18, 145-148 (1998a).

Moore, M.N., (1991). Lysosomal changes in the response of molluscal hepatopancreatic cells to extra-cellular signals, Histochem. J., 23: 495-500.

Muley, S.D., 1988. Ph.D. Thesis, Marathwada University, Aurangabad, M.S, pp: 292.

Mule, M.B. and Lomte, V.S. (1995). Copper sulphate induced alterations of protein in freshwater gastropod, Thiara tuberculata. J. Ecobiol.7(3): 177-180.

Muley, D.V. and U.H. Mane (1995). Endosulfan toxicity to freshwater mussel *Lamellidens marginalis* and pH induced changes- A biochemical approach. Ind. J. Comp. Ani. Physiol., 13, 21-26.

Omata, S., Sakimura, K., Tnubaki, H. and Sugano, H. (1978) *In vivo* effect of methyl mercury on protein synthesis in brain and liver of rat. *Acta Pharmacol. Toxicol*., 44:367-378.

Pardeshi, A. and Gapat, M. (2012) Ascorbate effect on protein content during nickel intoxication in the freshwater bivalve, Lamellidens corrianus. Bioscience Discovery, 3(2); 270-274.

Pottinger, T. G., Carrick, T. R. and Yeomans, W. E. (2002): The three-spined stickleback as an environmental sentinel: effects of stressors on whole-body physiological indices. J. Fish Biol., 61; 207–219.

Pullin, R.S.V. 1989. Third-world aquaculture and the environment. Naga, The ICLARM Quarterly, January 1989, Vol. 12, No. 1, pp 10–13.

Reddy, S. (1979) Biochemical and physiological effects of certain insecticides on cockroach, *Periplaneta Americana*. Ph.D. thesis, Kakatiya University, Warangal, India.

Satyaparmeshwar, K., Ravinder Reddy, T. and Vijaykumar, N. (2006): Effect of chromium on protein metabolism of freshwater mussel, *Lamellidens marginalis*. Journal of Envin. Biol., 27 (2); 401 – 403.

Singaraju, R., Subrmanian, M.A. and Varadaraj, G. 1991. Sublethal effects of malathion on protein metabolism in the freshwater field crab, *Paratelphusa hydrodromos*. J. Ecotoxicol. Environ. Monit. I (1): 41-44.

Sole M. Morcillw Y. and Porte C. 2000. Stress-Protein Response in Tributyltin–Exposed clams. Journ. of Bulletin of Environmental Contamination and toxicology.Vol. 64. Pages 852-858.

Vincent, S., T. Ambhore, L. C. A. Kumar and M. Selvanayagam (1995): Biochemical response of the Indian major carp, *Catla catla* (Ham) to chromium toxicity. Indian J. Environ. Health, 37(3); 190-196.

Waykar Bhalchandra, Lomte, V. S. and Zambare, S. P. (2001): Effect of cypermethrin on the ascorbic acid content in the mantle, foot, gill, digestive gland and whole body tissues of fresh water bivalve, *Parreysia cylindrica*. J. Aqua. Biol., 16(2); 57-61