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RESEARCH ARTICLE

Biochemical studies in Muscle of fresh water fish, Labeo rohita (Ham.) exposed to heavy metals

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Abstract

The main aim of the present study is to know the effect of heavy metals in Tawa reservoir on Labeo rohita and their seasonal variations. During the study, Labeo rohita were exposed to pre monsoon, monsoon and post monsoon seasons at different sites in Tawa reservoir. The Protein quantity in muscle decreases when compared to the control (5.97±0.46 g/dl), pre monsoon (4.95±0.08 g/dl), monsoon (4.83±0.06 g/dl) and post monsoon (4.90±0.08 g/dl) periods. The Glucose quantity in muscle also decreases when compared with the controlled fish (91.47±1.17mg/dl), pre monsoon (88.61±0.28mg/dl) , monsoon (76.55±0.85mg/dl) and post monsoon seasons (86.58±0.59). The Lipid quantity of muscle also decreases during monsoon period (4.7±0.11mg/100ml) as compared to control (5.36±0.31mg/100ml), pre monsoon (4.93±0.12mg/100ml) and post monsoon seasons (4.76±0.08mg/100ml). The results are statistically significant at $p< 0.005$ level.

INTRODUCTION

Heavy metal accumulation in the tissue of fish is a general Phenomenon .The gills are the first target in metal accumulation because they are directly in contact with water. Heavy metals have been widely reported in aquatic animals. Heavy metals like Copper (Cu), cadmium (Cd), and Lead (Pb) are non essential, so even at low concentration they become toxic when ingested over a long period. Therefore presence of these metals in fish is regarded as hazardous to health.

Heavy metals are one of the major pollutants, which are present in small quantities in the earth's crust. Heavy metals constitute an important class of toxic substances, which are encountered in numerous occupational and environmental circumstances .The heavy metals are present at <0.1% level in earth's crust .But anthropogenic, geochemical factors are releasing a large number of toxic heavy metals into the aquatic ecosystem (Beddi and Engine, 2005). The heavy metals produce their toxicity by forming complexes or legends with organic compounds. These modified molecules lose their ability to function properly and thus result in malfunction or death of affected cells. When heavy metals bind to these groups they may inactivate important enzyme system or affect protein structure of an animal. Heavy metals are major pollutants of aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms (Maheswaran and Devepaul, 2008).

Heavy metals when reach the aquatic bodies deteriorate the life Sustaining quality of water and cause damage to both flora and fauna (Zyadah and Abdel –BakeY, 2000). The problem of heavy metal pollution increases many folds due to their long half life period and properties of non-biodegradability, bioaccumulation and biomagnifications (Burman and Lal., 1994).

The increasing importance of fish as a source of proteins, fats, minerals, vitamins and the interest in understanding the accumulation of heavy metals at the tropic level of food chain, extend the focus towards aquatic fish (Hinton and Lauren, 1990). Pollutants that are reaching from different sources into water bodies enter fish through five main routes, via food or non-food particles, gills, oral, consumption of water and the skin. On absorption, the pollutant is carried in blood stream to either a storage point or to the liver for transformation and for storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination of stored fat which is an extra hepatic tissue (Nussey et al., 2000).

In aquatic environment heavy metals in dissolved form are easily taken up by aquatic organisms where they strongly bind with sulphydril groups of proteins and accumulate in their tissues (Kargin, 1996). Because of accumulative characteristics in food chain, the level of heavy metals in tissue and organs of various fishes living in different aquatic environment have been investigated by many researchers (Ashraf et al., 1991). The accumulation of heavy metals in the tissue of organisms can result in chronic illness and cause potential damage to the population (Borlas, 1991).They also cause serious impairment in metabolic, physiological and structural systems when present in high concentration in the milieu (Tort el al., 1987).The Field of metal toxicology is one of the broadest area of toxicology.

Material and Methods:

For Bio-chemical estimations a part of muscle were dissected out quickly from each fish, weighed and stored at 10^{o}C. The spectrophotometric methods were used to quantify the protein levels (Lowry et al., 1951), total lipid (Floch, et al., 1957), glucose and glycogen (Kemp and Kite's 1954) and statistical significance of the data was assessed using the one way analysis of variance (ANOVA) test.

Results and Discussion:

Protein:

Under present investigation it has been observed, that during monsoon period the protein level decreases maximum (4.83±0.06g/dl) as compared to pre monsoon (4.95±0.08g/dl) and post monsoon (4.9±0.08g/dl) in muscle. Studies on biochemical constituents of blood also help to understand the physiological status of fish with changing ecophysiological conditions. In present study change in protein content in stress response to heavy metals in the reservoir lead to irreversible and reversible disturbances of integrated functions such as physiological behavior and reproduction, which are also supported by Collivin (1984) in Perca , fluviatilis exposed to copper and M. vittatus to Chromium and Mercury (Sivakami et al., 1994; Kasthuri et al., 1997). Decrease in protein content during monsoon seasons also affects the other biochemical parameter. Depletion in tissue protein in Labeo rohita due to mixture of heavy metals present in the reservoir, toxicity may be attributed to either rapid utilization of body protein or poor intake of dietary protein by fish, which support to view of Severson (1981) who opined that the heavy metals in general interfere with protein synthesis. Further under stress conditions the dietary protein consumed by the fish is not utilized by the body tissue as also suggested by Bhaskaran and Palanichamy (1991) hence the treated fish meet out their extra energy demands from protein metabolism, which is mobilized to produce glucose, the instant energy, which is made available for fish by the process of gluconeogenesis, also, reported by (Vasanthi et al., 1987).

The reduced level of protein content in present study may be attributed to metabolic utilization of keto acids to gluconeogenesis pathway for the synthesis of glucose or for the maintenance of osmoregulation and ionic balance. Similar results were also noticed by (Srivastava et al., 1996). The decrease in protein content also suggested an increase in proteolytic activity and possible utilization of its products for metabolic purpose. Depletion of tissue protein in fishes exposed to various toxicants have already been reported by Ram and Sathyanesan (1984) and James et al., (1995). Umminger (1970) suggested that protein also being energy source is to spare during chronic period of stress. Smet and Blust (2001) observed the change in protein metabolism in cyprinus carpio and found the stress respond change due to cadmium exposure.

Dubale and Shah (1981) recorded hypoproteinaemia in Channa punctatus exposed to two very low concentrations to malathion for 14 to 21 days, corroborate undoubtedly to our study. They also suggested that the sharp rise of acid phosphates the synthesis of RNA and protein is impaired resulting in a depleted level of protein. In fact the quantity of protein depends on the synthesis of RNA, which plays an important role in protein synthesis. Decrease in protein content of Muscle after exposure to concentration of heavy metals(As , Cu) is also attributed to active transdeamination of amino acid to keto acids which may be transferred to TCA cycle through amino transferase probably to subsidize extra energy with increased energy demand and a tissue repair due to stress environment with the stress condition caused by heavy metal exposure Syverson (1977) and Ram lingam et al., (2000). Which corroborate with findings of (Sreedevi et al., 1992). The reduced protein level in present study may also suggest increased proteolysis and it is also possible that utilization of degraded products for metabolic processes like increased rate of homeostasis and tissue repair under metal stress a similar view with Neff (1985). A similar opinion with Pradhan and Iota (1993), the decrease level of protein in muscle after 30 day exposure to copper and mercury may be attributed to lack of protein biosynthesis or inhibition of translation or may also be due to poor rate of absorption of aminoacid glycine due to damage of intestinal villi induced by these metals. The effect of exposure to sub lethal concentration of the organophosphate on muscle of Labeo rohita was studied after 15, 30 and 45 days. At the end of the period, the muscle protein and RNA level decreases Das and Mukherjee (2000). Haggi and Adhanmi (1977) also discussed that an insecticide may affect the protein synthesis either by inhibiting the RNA synthesis resulting in a low RNA thus low protein level or uptake of amino acid is hampered into the polypeptide chain. Insecticide may also alter the protein concentration through impairing the synthesis and metabolism of protein, DNA, RNA and by altering the activity of lysosomal enzyme as autolysis. The most probable reason of low level of protein in different tissue in this study may be parallel to above findings. Fish to survive in adverse environmental condition need more energy leading to breakdown of protein to fulfill extra alternate precursor for extra energy requirement. The observation is in conformity with earlier finding of Somnath (1991).

Lipid:

It has been observed that during monsoon period the lipid level decreases maximum(4.7±0.11mg/100ml) as compared to pre monsoon(4.93±0.12mg/100ml) and post monsoon (4.76±0.08mg/100ml) in muscle of Labeo rohita. The decreased level of lipid in muscle during monsoon seasons may be attributed due to damage of intestinal villi induced by these heavy metals. The Lipid level during the Pre moonson season was less reduced while the higher concentration of heavy metals during monsoon season showed there is tremendous fall of lipid level as compared to the control group, the highest percentage of lipid was found in control group while the lowest value was recorded during monsoon season, similar results were also observed by Virk and Sharma (1999).

GLUCOSE:

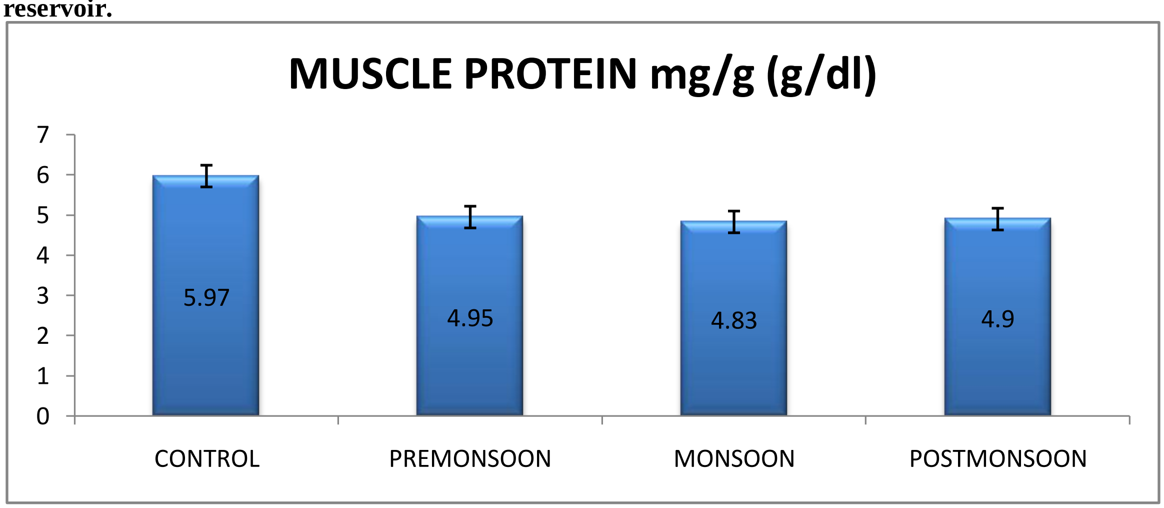
Under present investigation it has been observed that during monsoon period the glucose level decreases maximum (76.55±0.85mg/dl) as compared to pre monsoon(86.61±0.28mg/dl) and post monsoon (86.58±0.59mg/dl) in muscle of Labeo rohita. The decreased level of glucose in muscle tissues during monsoon seasons may be attributed due to damage of intestinal villi induced by these heavy metals. The data on the effect of heavy heavy metal mixture in the reservoir (Cadmium chloride, Lead chloride and zinc chloride and copper chloride) on glucose content of the muscle tissues of Indian Major Carp Labeo rohita are represented in Table(1) .Glycogen levels are found to be the highest in liver tissue of fishes, as it is chief organ of carbohydrate metabolism in animals, followed by muscle tissues. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. The glycogen contents in muscle tissues of fish Labeo rohita, under the heavy metals stress, present in the Tawa reservoir during different monsoon seasons were decreased as compared to that of the fish under control. The maximum decrease was observed during monsoon period as compared to pre monsoon and post monsoon seasons in muscle tissue exposed under heavy metal intoxication . A steady decrease in the tissue glucose content clearly indicates that effects of heavy metals are persistent under prolonged exposure periods. This disruption of biological oxidation process agrees with earlier report of (Reddy et al., 1991). This change suggesting that glycogen utilization by anaerobic glycolysis perhaps to meet the energy warranted by intoxicated environment. Decrease in glucose level in the muscle of Labeo rohita reflect the hypoglycemic condition which most probably could be because of renal failure to reabsorb 100% glucose and its redistribution to the tissue cells via the blood capillaries. The presence of hypoglycemia in the muscle confirms the Kidney failure. Possibly the renal impairment could be due to the toxic effects of heavy metals present in the Tawa reservoir. Sastry and Shukla (1990) reported that the muscle Glucose content was decreased due to Cd toxicity in murrel fish.. The average glycogen content was observed to decrease significantly in the muscle of Parreysia favidens under $HgCl_2$ intoxication (Bhamre et al., 2001). Glycogen depletion is more prevalent under hypoxic conditions that may stimulate phosphorylase activity bringing about a drop in glycogen level. In general, when the animal undergone to stress and strain, then the glycogen reserves were used as substitution of metabolic requirement to meet the energy demands through glycolysis or Hexose Monophosphate shunt pathway. It is assumed that decrease in Glucose content may be due to the inhibition of hormones which contribute to glycogen synthesis. Decrease in liver and muscle Glucose levels is corroborated with the earlier reports of many workers .The diminution of glycogen would result in the disruption of enzymes associated with carbohydrate metabolism.

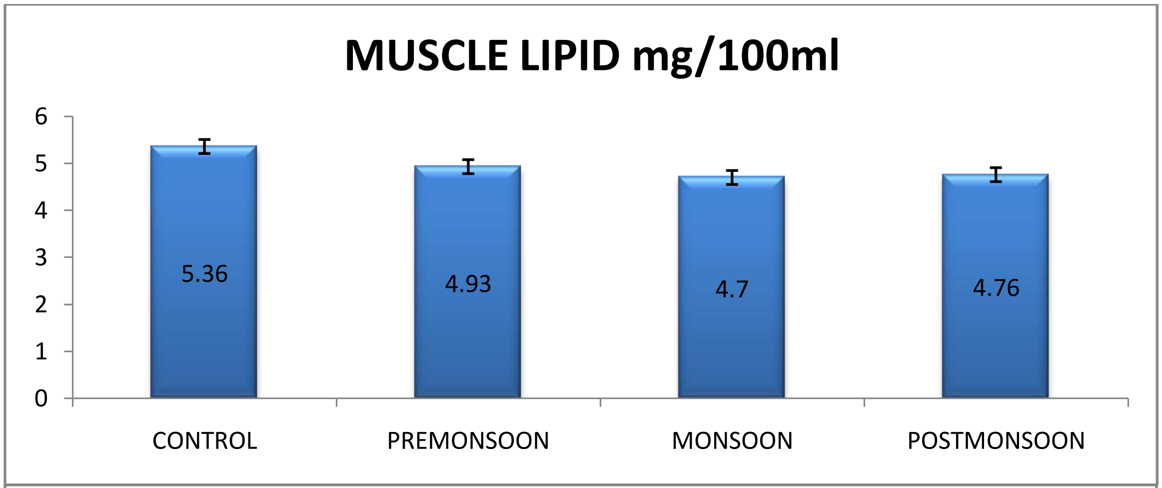
BIOCHEMICAL ANOVA TABLE 1: Biochemical parameters of Labeo rohita, caught from Tawa reservoir during pre monsoon, monsoon and post monsoon seasons:

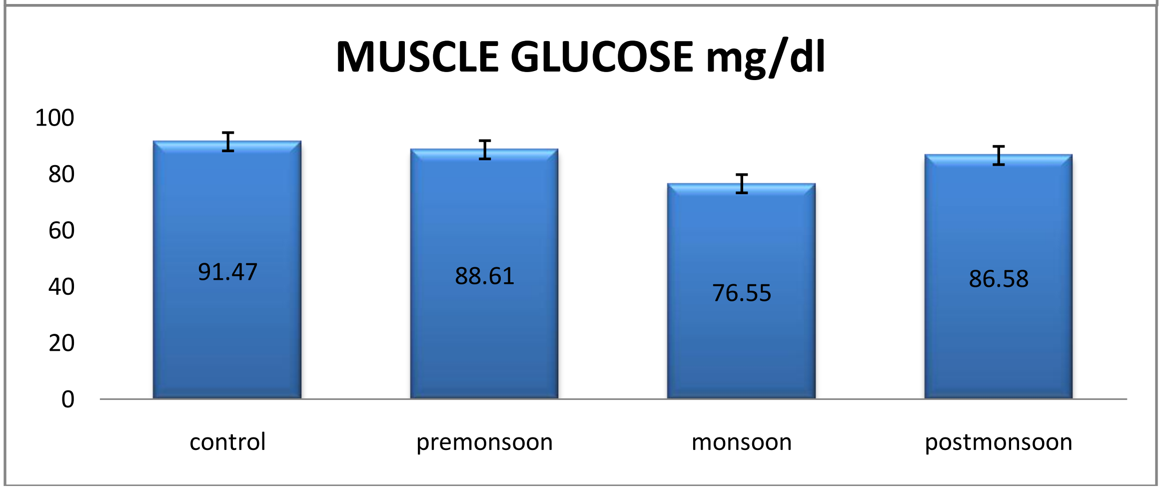
Serial. No	ORGANS	Control	Pre monsoon	Monsoon	Post monsoon	Significance
1	Muscle protein g/dl	5.97±0.46	4.95±0.08*	4.83±0.06*	4.90±0.08*	P <0.05
2	Muscle glucose mg/dl	91.47±1.17	88.61±0.28	76.55±0.85	86.58±0.59	P>0.05
3	Muscle lipid mg/100ml	5.36±0.31	4.93±0.12	4.7±0.11	4.76±0.08	P>0.05

Values are Mean ± SEM, ANOVA test, n =3, * = Significant at p <0.05, NS = Not Significant. For statistical significance biochemical parameters in the muscle of test fish samples has been compared with control.

Fig.1-3: Biochemical responses in the muscle of Labeo rohita showing % increase (+) or decrease (-) captured during pre monsoon, monsoon and post monsoon seasons in Tawa reservoir.







Conclusion:

Considerable changes were observed in Biochemical parameters of Labeo rohita during pre monsoon, monsoon and postmonsoon seasons due to accumulation of mixture of various Heavy metals. These variations represent disturbances in the metabolic processes of the organism. Measurement of these Biochemical parameters indicates the effects of stress and abnormality in Labeo rohita which confirms the increasing concentration of heavy metals and threat to the aquatic biota.

References:

Ashraf (1991). The level of heavy metals in tissue and organs of various fishes living in different aquatic environment have been investigated by many researchers. Pak. J. Bot., 42(1): 141-154, 2010.

Beddi and Engine (2005): The heavy metals are present at 0.1% level in earth's crust. But anthropogenic, geochemical factors are releasing a large no. of toxic heavy metals into the aquatic ecosystem.2005; Volume 435 Number 7046pp 1137-1286.

Bhamre (2001).Effect of mercuric chloride on glycogen content of fresh water mussel. Parreysia fevidens. Poll.Res.,20(1):13-15.

Bhaskaran and Palanichamy (1991). Use of Biochemical parametes in Biomonitoring of pesticide pollution in some freshwater fishes. J. Excotoxical, Environ. Monit., 2: 103 -109.

Borlas (1991). The accumulation of heavy metals in the tissue of organisms can result in chronic illness and cause potential damage to the population. Environmental Toxicology, 14:367-373.

Burman and Lal (1994): Problem of heavy metal pollution increases many folds due to their long half life Period properties of non-biodegradability, bioaccumulation and biomagnifications .Our Nature, 8:180-184.

Collivin (1984). Change in protein content in stress response to heavy metals in the reservoir lead to irreversible and reversible disturbances of integrated functions such as physiological behavior and reproduction. Indian J. Comp. Anim. Physiol., 1(2): 37-39.

Das and Mukherjee (2000). Das, B. K. 1998. Studies on the effect of some pesticides and commonly used chemicals on Indian major carps and their ecosystem. Ph.D. Orissa University of Agriculture and Technology. Bhubaneswar. Orissa.

Dubale and Shah (1981). Toxic effects of cadmium nitrate on liver of Channa punctatus. Experntia. 35**:** 643.

Floch(1957).A simple method for the isolation and purification of total lipids from animal tissues.The Journal of Biological chemistry, 266:497-509.

Haggi and Adhanmi (1977).Insecticide may affect the protein synthesis either by inhibiting the RNA synthesis resulting in a low RNA thus low protein level or uptake of amino acid is hampered into the polypeptide chain.

Hinton and Lauren (1990). The increasing importance of fish as a source of proteins, fats, minerals and vitamins. Mar. Ecol. Prog. Ser.vol. 91:127-133, 1992.

James (1995). Effect of metal mixtures on activity of two respiratory enzymes and their recovery in Oreochromis mossambicus. Indian J. Exp. Biol., **30:** 496-499.

Kargin (1996). In aquatic environment heavy metals in dissolved form are easily taken up by aquatic organisms. Pakistan Vet. J., 29(1): 24-26.

Kasthuri et al., 1997). Sub lethal effect of lead on feeding energetics,growth performance, biochemical composition and accumulation ofthe estuarine catfish, Mystus gulio (Hamilton). J. Environ. Biol., 18,95-101 (1997).

Kemp and Kite's (1954). A calorimetric micro method for the determination of glycogen J. Biochem., 1954, 56, 640-648.

Lowry (1951). Protein measurement with Folin Phenol Reagent. J. Biol. Chem. 1951, 193, 265-275.

Maheswaran and Devepaul (2008): Heavy metals are major pollutants of aquatic environment. International Journal of Integrative Biology ISSN0973-8363.

Neff (1985). Use of biochemical measurement to detect pollutant medicated damage to fish. ASTM spec Tech. publ., 854: 154–183.

Nussey (2000). Bioaccumulation ofchromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae) from Witbank Dam,Mpumalanga. Water SA, 26: 269-284.

Pradhan and Iota (1993). The decreased level of protein in different tissue like liver, intestine and muscle. WorldWideScience.org.

Ram and Sathyanesan (1984). Organophosphate induced biochemical changes in the brain, liver and ovary of the fish Channa punctatus .Proc. Ind. Nath. Sci. Acad., 51 (2): 537-542.

Ramlingam (1988).Effect of DDT Malathion and mercury onthe liver histomorphology of the fish Sartherodon mossambicus. Environ.Ecol.6(3):761-762.

Reddy(1991). Methylparathion induced alterations in the tissue carbohydrate catabolism of marine prawn, Metapenaeus monoceros. Bull. Environ. Contam. Toxicol., **47:** 925-932.

Sastry and Shukla (1990). Effects of paper mill effluent on fish life. Indan J. Environ. Prot., **8**(1): 31 – 38.

Severson (1981). Who opined that the heavy metals in general interfere with protein synthesis. Our Nature 8:180-18.

Sivakami (1994). Perca fluviatilis exposed to copper and M. vittatus to Chromium and Mercury.Environ. Ecol.12 (2):259-266.

Smet and Blust (2001). Change in protein metabolism in cyprinus carpio and found the stress respond change due to cadmium exposure. Punjab Univ. J. Zool., Vol. 20 (2), pp. 209-214.

Somnath (1991). Effect of acute and sub lethal concentration of tannic acid on the protein, carbohydrate and lipid levels in the tissue of the fish Labeo rohita. J. Environ. Biol., 12(2): 107-112.

Sreedevi et al., 1992). Decrease in protein content of Liver, Gills, and Muscle after exposure to concentration of heavy metals.Environ.pollut.76,355.

Srivastava (1996). Gluconeogenesis pathway for the synthesis of glucose or for the maintenance of osmoregulation and ionic balance. J.Adv. Zool.17, 38-43.

Syverson (1977). Decrease in protein content of Liver, Gills, and Muscle after exposure to concentration of heavy metals (As ,Cu).Neuropathol. Appl.Neurobiol:3,225-236.

Tort (1987). Heavy metals cause serious impairment in metabolic, physiological and structural systems when present in high concentration in the milieu. Camp. Biochem. Physiol. 87-C, 349-354.

Umminger (1970). Physiological studies on super-cold killi-fish Fundulus heteroclitus. III. Carbohydrate metabolism and survival at subzero temperature. J. Exp. Zool., **173:** 159 – 174.

Vasanthi (1987). A shift in metabolic pathway of Sarotherodon mossmbicus (Peters) exposed to Thiodon (Endosulfan), Proc, Indian, Acad., Sci (Arim. Sci). 96**:** 56 – 66.).

Virk and Sharma (1999). Biochemical changes induced by Nickel and Chromium in the liver of Cyprinus carpio. L. Poll. Res. 18 (3): 217-222.
Zyadah, M.A and Abdelbaky, T.E. (2000): Toxicity and bioaccumulation of copper, zinc and cadmium in some aquatic organisms. Bull. Env.Contam. Toxical. 64, 64: 740-747.