

## Hepatic Responses of A Freshwater Fish Against Aquatic Pollution\*

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**Abstract.**- Freshwater fish, *Tor putitora* caught from polluted portion of River Kabul was studied for various hepatic biochemical parameters and was compared with control fish caught from non polluted Warsak Dam to know the possible toxic effects of pollution in the river. Fish liver was dissected out and analyzed for various biochemical parameters like total protein, soluble protein, total cholesterol, total lipid, glucose, free amino acid, DNA, RNA and enzymes such as amylase, GOT, GPT and LDH. The hepatic total proteins, soluble proteins, total cholesterol, total lipids, glucose content and free amino acids in fish sample 1 and sample 2 increased 29% and 16%, 6% and 15%, 75% and 68%, 41% and 65%, 47% and 26%, and 333% and 729%, respectively as compared to control sample. The DNA content decreased 14% and 20%, whereas the RNA decreased 41% and 32% in sample 1 and 2, respectively. The fish sample from site I and II showed 5% and 17% increased hepatic amylase activity, 69% and 72% decreased GOT activity in sample 1 and 2, respectively. The GPT activity decreased 45% in sample 1 and increased 28% in sample 2. The LDH activity was decreased 69% in sample 1, but showed 14% increase in sample 2 as compared to control sample. The increase and decrease in various biochemical parameters and enzymes in the liver of test fish samples in comparison with the control shows the adverse effect of aquatic pollution on the fish health. Aquatic pollution could be one of the major reasons of rapid decline in population of this endangered South Asian fresh water fish.

**Key words:** Fresh water fish, hepatic enzymes, DNA, RNA, hyperglycemia, hyperlipimia.

### INTRODUCTION

With exploding population and increasing industrialization and urbanization, water pollution by agricultural, municipal and industrial sources has become a major concern for the welfare of humanity. Water soluble toxicants from industrial and municipal wastes, leached soils and the atmosphere have rapidly transferred to natural bodies of water. While some of the pollutants decompose or volatilize, others form insoluble salts, which precipitate and get incorporated into the sediment (Bowen, 1979). Uptake of such toxicants by aquatic organisms like fish may be followed by metabolism of the toxicants into more toxic derivatives (Webb, 1975; Duffus, 1980). For example mercury from industrial effluents may be converted by microbial action into highly toxic methyl mercury which can then be taken up by fish. Many aquatic organisms have been known to concentrate toxic solutes from their habitat without any obvious damage to themselves. They thus act as

toxicant amplifiers, making the toxicants available to predators at dangerously high levels. Several cases of the adverse effects of environmental pollution on fish and fish consumers have been reported (Bowen, 1979; Dix, 1981).

Polluted waters have been reported for causing visible dermal and liver neoplasms. Polluted waters have also been reported of causing serious pathological liver conditions, particularly shrinkage of liver cells, which spread with the advancing age, also accompanied by the dissolution of the well-organized liver structure, blood clots and necrosis.

Liver is the main metabolic center where detoxification and drug metabolism take place which makes it greatly vulnerable to damage by toxic substances (Reddrop *et al.*, 1983).

Serious pathological liver conditions were diagnosed, in Flounder, *Platichthys flesus*, Ruffe, *Gymnocephalus cernua* and Smelt, *Osmerus eperlanus* caught in the River Elbe polluted waters. Particularly shrinkage of liver cells, which spread with advancing age and increasingly accompanied by other degenerative changes, such as dissolution of the well organized liver structure, blood clots and necroses were diagnosed. Neoplastic liver nodules were found in 32% of sexually mature Ruffe (Peters

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*et al.*, 1987). Winter flounder caught from polluted waters of Boston Harbor, Massachusetts, USA was found to have visible liver neoplasms (Huff *et al.*, 1991).

Since liver is the centre of metabolic activities and all toxic compounds are likely to be metabolized in this organ. The various hepatic enzymes are prone to toxic effects and can best be used as indicators of sublethal exposure of fish to toxic metals (Jackim *et al.*, 1970).

Lohner *et al.* (2001b) reported sunfish from a fly ash pond-receiving stream with high selenium levels from the receiving stream. Selenium levels were higher than toxic thresholds and were associated with adverse population effects and reproductive impairment. Tissue biochemistry was found to be indicative of metal exposure and effect, but varied widely. Liver glycogen and protein levels increased with increased liver metal levels. Lipid levels decreased with increasing metals.

Klopper-Sams *et al.* (1994) reported increased liver size and differences in serum chemistry in long nose suckers exposed to bleached-kraft effluent. Similarly, Lohner *et al.* (2001c) studied sunfish from a fly ash pond-receiving stream and an Ohive River, USA reference sites. Effects of elevated metal level on fish health were focused. Higher concentrations of selenium, copper, and arsenic were found in liver of effluent-exposed fish than in reference fish.

Occurrence of metal contaminants especially the heavy metals in excess of natural loads, has become a problem of increasing concern in aquatic ecosystems. This situation has arisen as a result of the rapid growth of population, industrial development and discharge of untreated industrial wastes, increased urbanization, expansion of natural resources, extension of irrigation and other modern agricultural practices as well as the lack of environmental regulations (Calvari and Naeve, 1994; Lester *et al.*, 1983; Bagatto and Ali Khan, 1987). It is a world wide problem and has created serious health concerns (Galindo *et al.*, 1986; Pastor *et al.*, 1988). Chromium, lead, mercury, zinc, copper and nickel are among the most harmful metallic pollutants. Bioaccumulation of these metals is known to adversely affect liver, muscle, kidney and other tissues of fish, disturb metabolism and hamper

development and growth of fish (Anadon *et al.*, 1984; Kadiiska *et al.*, 1985a,b; Birge *et al.*, 2000).

Fish collected from Boulder River and some of its tributaries receiving effluents from abandoned mines were analyzed for metal load. Data showed elevated concentrations of arsenic, cadmium, lead and zinc to varying degrees in biological tissues collected. Tissue damages in fish livers along with reduction in fish sizes and populations were also noted. It was suggested that exposure to metals might have resulted in a deterioration of fish health and a quantitative loss in fish populations. 100% mortality of fish placed in live containers in some sections of the basin creek was also observed (Farg *et al.*, 1999).

Pest control chemicals are poisons (Agnihotri *et al.*, 1976) that may accumulate in the food chain and cause widespread contamination of the environment (Kapoor *et al.*, 1980; Okondahoka *et al.*, 1984; Kaphalia *et al.*, 1986). The chlorinated hydrocarbon insecticides sprayed on crops ultimately enter the aquatic ecosystems i.e. rivers and finally oceans. This phenomenon remained a topic of lively debate in the recent past because of their toxic effects on the non-target organisms especially fish, birds and mammals (Javaid and Waiz, 1972; Kan and Tuinstra, 1975; Blus, 1978; Kan, 1978).

After ingestion through intestine the insecticide is taken into the liver through the portal blood where it is reported to induce number of changes (Kontek *et al.*, 1976; Morgan and Lin, 1978).

Insecticide residues pose a severe threat to our ecosystem because of their greater stability (Hamilton, 1985). In spite of the fact that majority of the American and European states have posed a ban on their use, these compounds are still quite extensively used in developing third world countries including Pakistan (Ali, 1989).

In order to avoid the use of such banned pesticides, it is essential to replace them with suitable pesticides that must be target-specific, non-toxic to other species, easily biodegradable and less persistent.

Tobacco, sugarcane, wheat and maize are the main crops of the area irrigated by River Kabul and its tributaries where substantial amounts of

insecticides per cropping season are applied. The widespread use of toxic insecticides has created a need for their monitoring in the River Kabul.

In the present study fish liver was studied for various biochemical parameters like total protein, soluble protein, total lipid, cholesterol, glucose, free amino acids, free fatty acids, and nucleic acids i.e. DNA and RNA. Among the enzymes GOT, GPT, LDH and amylase were studied to know the deleterious effect of pollution on these parameters.

### MATERIALS AND METHODS

Fish samples were collected from two sites (1 and 2) of the polluted portion of the River and were compared with control samples taken from the Warsak Dam reservoir (site 3).

Fishing was done during late night with the help of professional local fishermen. Gill nets (Patti) made locally of nylon were used for fishing as fish gear. Two fish samples were collected from the highly polluted belt of the Main River. One fish sample was collected from the Main River upstream Nowshera-Mardan Road Bridge (Site 1), while the second fish sample was taken downstream Nowshera-Mardan Road Bridge (Site 2) with a distance of 3 km. Both the above samples were considered fish samples from polluted water (test fish sample) and were compared with the third fish sample (control fish sample) collected from the non polluted Warsak Dam (Site 3) about 60 km upstream the polluted part of the River Kabul.

After dissection fish liver was taken out, washed with distilled water, and shifted to properly marked sterilized polythene bags and then stored in freezer (at  $-20^{\circ}\text{C}$ ) for further analyses.

#### *Processing of liver tissue*

Liver tissue after thawing were cut with razor, washed with distilled water and blotted with blotting paper. A weighed portion of liver was homogenized in 3 ml ice-cold saline (0.89% NaCl) solution for saline extract and 3 ml ethanol for ethanol extract in a motor driven teflon glass homogenizer. The homogenate was centrifuged at 4,000 rpm ( $3,500 \times g$ ) for 45 minutes at  $5^{\circ}\text{C}$  in a refrigerated centrifuge (DAMON/IEC DPR-6000) to get a clear saline supernatant and for 15 minutes at

$5^{\circ}\text{C}$  at the same speed for ethanol supernatant. Aqueous liver extract in ice-cold saline was used for the estimation of enzymes like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), amylase, lactate dehydrogenase (LDH) and other biochemical parameters like glucose, free amino acids (FAA) and protein (total and soluble) contents. For the estimation of cholesterol, total lipids and free fatty acids ethanol extract was used, which was extracted from separate, weighed liver tissue. Total protein content was estimated from the tissue processed for nucleic acid estimation. For this purpose the pellet obtained after extraction of DNA and RNA was crushed with 2.5 ml of 0.5 N NaOH to solublize the protein fraction for estimation.

#### *Biochemical analysis of fish liver*

Liver ethanol and saline extracts were used for the estimation of the following biochemical parameters to assess the effect of water pollution on the fish liver. Total and soluble proteins were determined by the method of Lowry *et al.* (1951), cholesterol according to the method of Liebermann and Burchardt described by Henry (1964), glucose contents were determined by O-toluidine method of Hartel *et al.* (1969), free amino acids by the method of Moore and Stein (1954), total lipid by the method of Zöllner and Kirsch as described by Henry and Henry (1974). Nucleic acids were extracted according to the method reported by Shakoori and Ahmad (1973). DNA and RNA contents were estimated according to the method mentioned by Schneider (1957).

Nucleic acid contents of liver tissue were extracted by the method described by Shakoori and Ahmed (1973). Weighed amount of liver tissue was crushed in boiling ethanol. 2-3 washings in methanol: ether (3:1 mixture) followed by three washings in ethanol. The crushed tissue was then desiccated over dry calcium chloride as a desiccant in the vacuum for 24 hours. RNA was extracted in 10% perchloric acid (PCA) after keeping at  $4^{\circ}\text{C}$  for 18 hours, while DNA was extracted after keeping in 10% PCA at  $65^{\circ}\text{C}$  for 30 minutes.

Among enzymes GOT and GPT were determined by the method of Reitman and Frankel (1957), LDH activity by the method based on the

method of Cabaud and Wroblewski (1958) and amylase activity according to procedure described by Wootton (1964).

## RESULTS

Table I and Figures 1 and 2 show the increase and decrease in various parameters in the liver of the test fish sample (1 and 2) in comparison with the control fish sample. Hepatic total protein, soluble protein, total cholesterol, total lipid, glucose and amino acids showed higher values in livers of fish from polluted water when compared with that of control water. Total proteins in liver of fish from site 1 and 2 showed 29.2% and 16.1% higher values, soluble protein showed 6% and 14.5%, total cholesterol showed 75% and 67.9%, total lipid showed 40.5% and 64.9%, glucose showed 46.9% and 25.92%, free amino acids showed 332.5% and 729% higher values, respectively, in fish sample 1 and 2 when compared with control fish sample caught from Warsak Dam. DNA content, however, showed non-significant decrease of 13.85 % in sample 1 and 19.85% in sample 2. The RNA content showed significant decrease of 40.75% in sample 1 and 31.46% in sample 2 when compared with the control samples.

Among liver enzymes, the fish from polluted sites 1 and 2 showed 5.1% and 17.2% higher amylase activity, respectively. The GOT activity showed 68.5% and 71.93%, GPT activity 44.5% and 27.68% decrease in sample 1 and sample 2 respectively, where as LDH activity was inhibited 68.72% in sample 1 and increased 139.57% in sample 2 when compared with control sample.

## DISCUSSION

Since liver is the main metabolic centre, where most of the xenobiotics are metabolized and detoxified. It is therefore, one of the forefront organs of the body, which faces the major onslaught of an unwanted chemical invasion. The liver function is therefore, likely to be disturbed under such circumstances (Balasubramanian *et al.*, 1977; Enan *et al.*, 1982; Reddy *et al.*, 1982a,b; Gopaldaswami and Aiyar, 1984; Abdullaev *et al.*, 1985).

**Table I.- Biochemical components of liver of *Tor putitora* caught from Warsak Dam (control) and two sites (site 1 and site 2) from polluted portion of River Kabul.**

Parameters (mg/g)	Control (n=6)	Site 1 (n=5)	Site 2 (n=5)
Total protein	302.75± 9.89a	391.20± 6.19***	351.4± 13.63*
Soluble protein	158.11± 11.23	167.59± 1.71	181.02± 16.68
Total cholesterol	5.28± 0.19	9.24± 0.81***	8.88± 0.43***
Total lipid	46.75± 8.72	65.97± 13.04	77.02± 8.25*
Glucose	18.58± 7.68	27.29± 9.86	23.39± 1.94
Free amino acid	3.89± 0.74	16.86± 2.85***	32.32± 1.82***
DNA	1.35± 0.12	1.16± 0.072	1.08± 0.14
RNA	14.95± 0.33	8.86± 1.83**	10.25± 1.84*
Amylase (IU/g)	61.57± 1.71a	64.69± 3.02	72.15± 3.60*
GOT (mIU/mg)	474.68± 27.89	149.50± 12.44***	133.23± 10.81***
GPT (mIU/mg)	511.40± 39.06	283.73± 73.26*	652.94± 36.12*
LDH (IU/g)	3533± 1116.8	1105± 301.92	8464± 240.4**

Mean±SEM; Student's 't' test; \*P<0.05, \*\*P<0.01; \*\*\*P<0.001 For statistical significance enzymes in muscle of test fish samples has been compared with control.

Abbreviations used: : DNA, deoxy ribonucleic acids; RNA, ribonucleic acids; LDH, lactate dehydrogenase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; CPK, creatine phosphokinase. Enzyme units: IU (international unit), transformation of one micromole of substrate in one minute under the conditions of the test.

Control, fish sample from non polluted Warsak Dam; Site 1, polluted portion of River Kabul upstream Nowshera-Mardan Road Bridge; Site 2, downstream to Site 1 where Nowshera city sewage also joins the main river.

Liver homogenates were studied for the evaluation of various biochemical parameters including some enzymes. Liver biochemical parameters included total protein, soluble protein, total cholesterol, total lipid, glucose, free amino acids DNA and RNA. Total protein, soluble protein, cholesterol, lipid, glucose, free amino acids and DNA were elevated, while RNA was inhibited when compared with control. This increase or decrease of

various biochemical parameters in comparison to control fish exhibits the ongoing toxicity of various toxicants present in the ambient habitat, where fish under investigation resided.

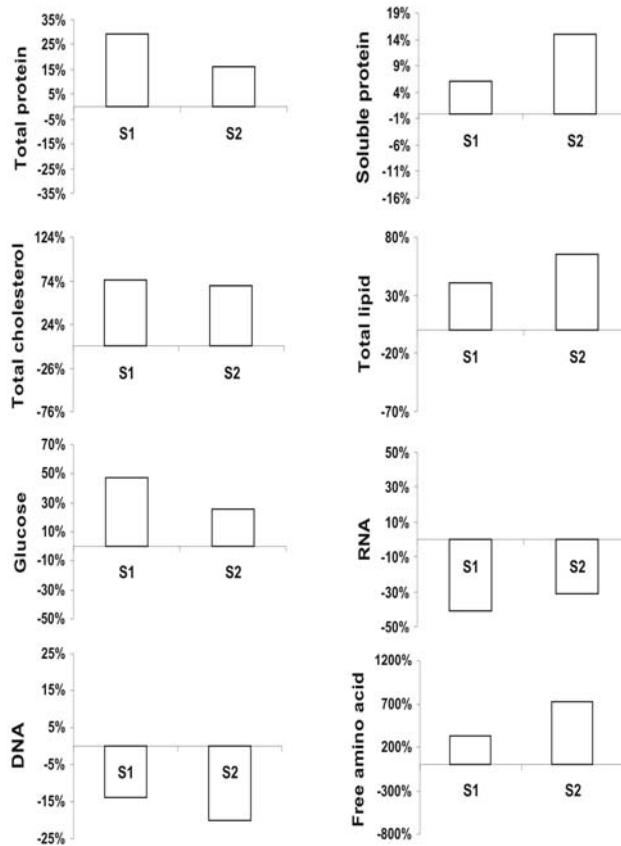


Fig. 1. Biochemical responses in the liver of *Tor putitora* showing % increase (+) or decrease (-) captured from two polluted sites (S1 and S2) of River Kabul receiving industrial effluents.

Abbreviations used: S1, *Tor putitora* sampled from polluted site 1 of River Kabul upstream to Nowshera-Mardan Road Bridge; S2, *Tor putitora* sampled from polluted site 2 of River Kabul downstream to Nowshera-Mardan Road Bridge.

DNA, deoxy ribonucleic acids; RNA, ribonucleic acids.

In the present study total protein in both the fish samples from polluted waters were elevated significantly. Probably the phenomenon of protein synthesis was enhanced to provide immunity against

the ambient toxicants. In a previous study liver protein levels were found to increase significantly in fish from the ash pond discharges and these levels were positively correlated with liver metal levels (Lohner *et al.*, 2001b). It is well known that metals can be sequestered in tissues and be partially bound to metal-binding proteins such as metallothionein (MT) in the liver (Benson and Birge, 1985). MT has already been isolated from fish liver (Carpene and Vařák, 1989) and is low molecular-weight cytosolic protein. MT and other metalloproteins are also known to be involved in the metabolism and detoxification of certain toxicants including trace metals in aquatic organisms (Roesijadi, 1994).

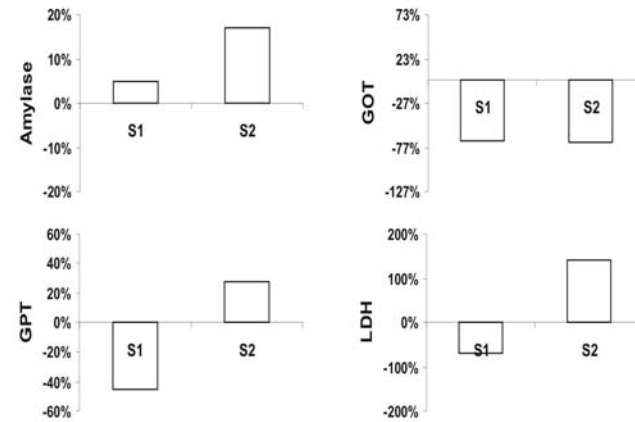


Fig. 2. Biochemical responses in liver of *Tor putitora* showing % increase (+) or decrease (-) captured from two polluted sites (S1 and S2) of River Kabul receiving industrial effluents.

Abbreviations used: S1, *Tor putitora* sampled from polluted site 1 of River Kabul upstream to Nowshera-Mardan Road Bridge; S2, *Tor putitora* sampled from polluted site 2 of River Kabul downstream to Nowshera-Mardan Road Bridge.

GPT, glutamate pyruvate transaminase; GOT, glutamate oxaloacetate transaminase; LDH, lactate dehydrogenase.

Most probably accumulated proteins in the liver in the present study could be metalloproteins, which were limiting the toxicity of metals in *Tor putitora* from River Kabul and makes their existence possible; it would seem likely that these same proteins would provide protection to other fish

populations that are similarly exposed. MT has been found in all types of organisms including mammals (Probst *et al.*, 1977), fish (Benson and Birge, 1985), molluscs (Roesijadi, 1994), and blue-green algae (Maclean *et al.*, 1972); however, fish in Belews Lake and Lake Martin were apparently susceptible to the ash pond metals and did not benefit from the action of such proteins (Sorenson *et al.*, 1982, 1984). Although such proteins may have been present in these susceptible fish populations, not all circumstances will allow them to be protective. Natural populations of fish can develop elevated tolerances to certain metals, but exposure must be at tolerable concentrations of metals in order for this phenomenon to be triggered. In addition, the tolerance is lost when exposure to the metals is persistent (Benson and Birge, 1985). Hilmy *et al.* (1987) observed increases in liver total protein levels in catfish (*Clarias lazera*) and bolti (*Tilapia zilli*) exposed to zinc.

Free amino acids like total protein also increased significantly in both the test samples. Most probably proteins under the influence of toxicants while providing immunity to the body were also splitting into free amino acids in the liver; secondly the high free amino acids may also be due to more and rapid absorption of these contents from the intestine. In another study in response to sublethal toxicity of sodium arsenite on protein metabolism in teleost fish, *Tilapia mossambica*, an increase in total protein and amino acids was found. It was suggested that the fish was able to respond to the stressful situations by gearing up the metabolic activity as revealed by the elevated protein and amino acid content (Shobha *et al.*, 2001).

Increase in lipid, soluble protein, cholesterol and glucose in the liver could also be related to the abundance of available food to the fish. This indicates that the fish is not under nutritional stress, but the stress is rather toxicological. However, in a similar study lipid levels in fish from both Stingy Run sampling efforts were significantly lower than those in fish from the Ohio River indicating possible nutritional stress (Lohner *et al.*, 2001b). The tissue-specific lipid content of fish can also be influenced by a variety of factors, including diet (Fletcher, 1984), reproductive condition (Love, 1970), and water temperature (Hansen and Abraham, 1983).

River Kabul has definitely heavy metals stress (Shakoori and Yousafzai, 2006, 2007, 2008a,b) which is evident from the present results, and which has been related to increasing mining and anthropogenic activities occurring in the vicinity of the river. The second stress which has also a strong possibility may be the presence of a variety of pesticides, insecticides and herbicides in the waters of River Kabul which has not been included in this study, but could be strongly believed, because River Kabul and its tributaries are the ultimate source of irrigation and sink of agricultural wastes for the whole valley of Peshawar. The presence of both the inorganic and organic pollutants in the river waters (Shakoori and Yousafzai, 2008a,) can cause a variety of diseases and discomfort in the inhabiting fish population, which is evident from our results too. Significant increase in total protein in the liver of both the treated samples indicates the induction of hyperproteinaemia.

Highly significant increase in total cholesterol in both the test samples reflects the presence of a marked hypercholesterolaemia. Excess of hepatic lipids in both the treated samples signals the presence of hyperlipaemia, while the increase in glucose in both the samples favors the induction of hyperglycemia. In another study, Gill *et al.* (1991) have also reported the induction of hepatic hyperproteinaemia, hyperlipaemia, and an increase in glucose, free fatty acids and cholesterol levels in the liver of *Barbus conchoni* after feeding endosulfan to the fish.

Increase in glucose reflects the conversion of glycogen into glucose due to glycogenolysis for energy use. Glucose might be rapidly converting into energy rich adenosine triphosphate bonds (ATP) for sequestering the effect of variety of toxicants in the aquatic habitat. Moreover, locally *Tor putitora* is coined the name Mahaseer also for being stronger and powerful fish, and some times it carry away the bait as well, while trying for escape. This favors the increase in the amount of glucose and its burning for high energy production. In a same study aspects of carbohydrate metabolism were analyzed in liver of the freshwater fish, *Labeo rohita*, exposed to a lethal and sublethal concentration of cypermethrin. All exposed fish exhibited a hyperglycemic condition (Philip *et al.*,

1995).

Heavy metals like chromium produces conspicuous changes in blood and tissue metabolite levels with acute and chronic poisoning and it causes hyperglycemia, glycogenolysis in the brain and liver (Abbasi and Soni, 1984; Ginter *et al.*, 1989). Cholesterol level in blood, liver, kidneys, testes and ovaries increases after acute chromium intoxication in mammals (Gauglhofer, 1984). Chronic exposure, however, leads to hypercholestermia and elevated levels of tissue cholesterol (Muller *et al.*, 1979).

As reported by Ulmer and Vallee (1969) lead produced number of metabolic disorders through its effect on mitochondria, which resulted in impaired respiration and phosphorylative capacity. It is assumed that increase in glucose content of the liver may be related to decreased glucose after impairment of mitochondrial function. The increase in cholesterol in the present result may be due to the increased synthesis through diverting glucose towards lipid metabolism or due to kidney retention disease by decrease in excretion of lipid including cholesterol. Rana *et al.* (1980) have reported increase in lipid contents of kidney after lead administration.

Both the DNA and RNA contents were decreased in the present investigation. The decrease of both the nucleic acids shows the hepatic cells damage, which was clearly visible during sample collection, as the liver tissue was damaged and showing the signs of tissue necrosis. Dissolution of liver started soon after fish sample collection. In a similar study Iqbal (1988) reported a decrease in hepatic DNA and increase in RNA after 1 week feeding of mercuric chloride, an increase in DNA and decrease in RNA after 2 weeks feeding and a decrease in both the DNA and RNA contents after 3 and 4 weeks feeding to *Ctenopharyngodon idella*. The increase and decrease in DNA and RNA seems to be the aging phenomenon. Most probably for short term exposures fish have responded the toxicant by developing immunity, but prolong exposure have failed the body defence mechanism and tissue degeneration started. This seems to be happened in the present study also. Fish might have resisted the ambient toxicants for some time after which tissue necrosis have started and resulted in

the decrease of both DNA and RNA contents.

In another study by Iqbal (1988) the amount of both DNA and RNA decreased after 24 hours and 48 hours feeding of mercuric chloride to *Ctenopharyngodon idella*, however, after 6 and 12 hours feeding the DNA content decreased while RNA increased as compared to control. Joshi and Desai (1988) after feeding monocrotophos to freshwater fish, *Tilapia mossambica* have reported a significant decrease in RNA content, while DNA and protein also decreased but this decrease was non significant.

The enzymes (ATPase, dUTPase, AP, and glycosylase) are commonly used as markers of cellular alterations. The activity of these types of enzymes is used as a biomarker, due to their involvement in the adaptive cellular response to the potential cytotoxicity or genotoxicity of pollutants such as heavy metals. Many metals are genotoxic and have the capacity to cause various types of DNA damage. Metals can also inhibit DNA repair. For example, mercury inhibits dUTPase, which prevents the incorporation of dUTP into DNA by DNA polymerases (Williams, 1986). The cellular metabolism of genotoxic chemicals can be relatively complex and often results in structural alterations to the DNA molecule. Cellular responses can lead to repair of the damage or death of the cells containing the damaged DNA. Tumors may result from those cellular lesions that are not repaired. Studies of mammalian genotoxicity have utilized enzymatic and histochemical markers to detect hepatocellular alterations and tumors and similar studies have been done with fish (Lohner *et al.*, 2001a,b).

It has been reported that chromium also produces DNA breakage and inactivates polymerases which affect DNA replication in mammals (Snow and Xu, 1988, Douglas *et al.*, 1980). Chromium also forms complexes with nucleotides, preventing the binding of DNA molecules (Petrilli *et al.*, 1985; Wolf and Ottenwaelder, 1987, Wolf *et al.*, 1989). As reported by DeFlora (1981) and Petrilli and DeFlora (1982), hexavalent chromium causes DNA damage. It also induces misincorporation of nucleotides in *in vitro* DNA transcription assays (IARC, 1980; Levis and Bianchi, 1982). Potassium dichromate induced dominant lethal mutations in mice treated *in vivo*

(Paschin *et al.*, 1982) and chromosomal aberrations in mammalian cells *in vitro* (Levis and Bianchi, 1982; Leonard and Lauwerys, 1980). The decrease in DNA content could be related to hepatic necrosis and or to inhibited DNA replication. The reduced RNA content may be due to decreased RNA synthesis due to heavy metal toxicity.

Liver function tests are usually recognized as the reliable indicator of liver metabolism (Tseng *et al.*, 1988). The raised enzymatic activity in the liver may be because of induction of enzyme synthesis (Street, 1969; Kimbrough *et al.*, 1971; Krample and Hladka, 1975), while their low levels could either be due to enzymatic inhibition (Hendrickson and Bowden, 1976; Meany and Pocker, 1979) or due to liver damage without any regeneration.

Among liver enzymes, amylase, GOT, GPT and LDH were elevated in the sample 1 showing acute liver damage (hepatitis), while in sample 2 all these enzymes were inhibited showing hypocondition or dysenzymia. The main hepatic cellular component to be affected by the ambient toxicants seems to be the cell membrane. Aquatic toxicants either have increased the membrane permeability causing enhanced leaching out of the enzymes, or reduced the permeability forcing the enzymes to accumulate in the cells. Cellular damage is another reason for decreased synthesis of enzymes in living organisms.

Alteration in plasma enzymes is direct measure of histopathological effects in the liver. The raised enzymatic activity in the blood is attributed to liver damage, while their low levels show the regenerative power of liver to minimize cell membrane injury. High level of enzymes in the cells reflects the enhanced biosynthesis of enzymes or disturbance in the cell membrane integrity, which does not allow the enzymes to move freely (Anjum, 1991; Ali *et al.*, 1992). The increased enzymatic activities in the liver might be due to increased enzyme synthesis to counter the damage caused by toxicants. The decreased activities of enzymes may be attributed to decreased enzyme synthesis or it may also be due to changes in permeability of hepatic cells as a result of which some soluble enzymes leave the cells resulting in lower enzyme activities in the cells. Cell membranes being selective in passage of different molecules in

different circumstances favor the passage of one sort of molecule and stop the other. This could explain the induction and inhibition of different enzymes at a time or at different times and circumstances. Both the possibilities seem to be activated in different samples under the influence of different toxicant levels.

Amylase activity in the present investigation increased in both the treated samples. Amylase is secreted by the exocrine region of pancreas. The increased activity may be due to pancreatitis or due to the damage of the amylase secretary cells. There is also the possibility that greater amounts of amylase were secreted into the intestine, which consequently enhanced starch digestion and transferred itself and the degradation products into portal blood and then into liver and hepatic cells through assimilation, which may also be accounted for hyperglycemic response in the present results. In a similar study *Cirrhina mrigala* exposed to lead acetate, for one week (short term) and eight weeks (long term) periods were found with increased hepatic amylase activity (Mujeeb, 1985).

The aquatic toxicants seems to have apparently hindered protein metabolism as is evident from high total protein, soluble proteins and free amino acids levels, but on the other hand have compensated by accelerating the enzyme (amylase) of carbohydrate metabolism and hence provides extra source of energy for the cells. Conversely the glucose, cholesterol and lipid levels increased as an alternate source of energy.

In a previous study treatment with copper sulphate ( $\text{CuSO}_4$ ), paraquat (PQ) and methidathion (MD) caused tissue damage and stress effects in carp, indicated by the increased liver LDH and GOT enzymes activities (Asztalos *et al.*, 1990). The effect of exposure to sub-lethal concentrations of the organophosphate pesticide, quinalphos (1.12, 0.22 mg/l) on enzyme activities in liver of the Indian major carp, *Labeo rohita* was studied after 15, 30 and 45 days. Under the influence of pesticide LDH levels in liver was elevated (Das and Mukherjee, 2000) as happened in treated sample 2 in the present study. In another study GOT and GPT were investigated after exposure to sub-lethal concentrations of  $\text{NH}_3\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  to the freshwater fish, *Catla catla* (Hamilton), *Labeo*



*rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). Depletion in the enzyme activities was observed in all the three exposed fish species (Tilak *et al.*, 2002). Activities of both the transaminases, GOT and GPT are also inhibited in sample 1. Gertig and Nowaczyk (1975) have reported decreased hepatic GPT, GOT and LDH activities like fish sample 1 of the present study.

Physiological and biochemical perturbations in the liver of *Carassius auratus* were investigated *in vivo* following 40 days of exposure to ytterbium solutions of different concentration. GPT activity in goldfish liver was stimulated at 0.05 mg/L Yb<sup>3+</sup> and inhibited at higher Yb<sup>3+</sup> concentrations (Hongyan *et al.*, 2002).

LDH is an active enzyme of glycolysis which catalyses the conversion of pyruvic acid to lactic acid, thus switching on the anaerobic respiration from the aerobic respiration. High activity of LDH suggests that pollutants like chromium have caused the depletion of oxygen in tissues, forcing the cells to get energy through anaerobic process. Another factor is also possible for explaining that high activity is the enhanced synthesis of LDH in the cells. In the present study LDH decreased in sample 1 and increased in sample 2.

In another study of similar nature the effect of exposure to sub-lethal concentrations of cypermethrin (a synthetic pyrethroid pesticide) on blood, muscle and liver of the Indian major carp, *Labeo rohita* was studied. LDH activity in brain and liver was elevated, but inhibited in kidney (Das and Mukherjee, 2003). Anjum (1991) reported increased LDH activity in liver cells after chromium administration to rabbits. Chromium is a powerful mutagen, which causes chromosomal aberrations in mammalian cells (Levis and Bianchi, 1982) and cell transformation in number of systems (Leonard and Lauwerys, 1980). Under chromium effects cellular structures like lysosomes, microsomes, mitochondria and cytosol soluble enzymes are disrupted and undergo enzymatic disorganization (Merkureva *et al.*, 1982; Pant and Gill, 1984; Srivasta *et al.*, 1985). Shakoori and Yousafzai (2008a) have reported heavy metal load including chromium in River Kabul which well explain the raised enzymatic activities in the present investigation.

## CONCLUSION

The presence of disenzymia, hyperglycemia, hyperlipemia, hypercholesterolemia, hyperproteinemia in the liver of fish under investigation explains the disturbance of biochemical processes occurring in the fish body caused by the ambient pollutants, thus disrupting the fish health, growth and obviously the fish population in the river.

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