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IMPACT OF ANTHROPOGENIC ACTIVITIES ON THE HISTOLOGICAL AND BIOCHEMICAL PARAMETERS OF *LABEO CALBASU* CAUGHT FROM BETWA RIVER IN RAISEN DISTRICT (M.P).

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ABSTRACT:-

The present investigation was undertaken to assess the effect of human hazards on the histology of investigated organ, kidney of *Labeo calbasu* in which normal kidney showed large number of nephrons, each consisting of renal corpuscles, proximal and distal convoluted tubules. However affected kidney showed marked shrinkage and breakage of haemopietic tissues, degeneration of renal epithelium. Glomeruli showed shrinkage and breakage in Bowman's capsule, necrosis in renal tubules, hypertrophy of epithelial cells of renal tubules. Similarly, biochemical parameters (lipid, protein and cholesterol) of investigated organ, liver showed depletion in all selected samples in comparison with control.

Keywords: Betwa River, *Labeo calbasu*, Histological parameters, Biochemical parameters.

INTRODUCTION:

As all of us are aware about the fact that water is a primary driving force for major physical, chemical and biological changes all over the world. It is pertinent that oceans and seas contain approximately 97% where as fresh water resources contain 3% of the entire water reserve of the earth. It is impossible to sustain life more than few days without water **(WHO, 2005).** Water also played a pivotal role in the evolution of human civilization e.g. Rivers like Nile, Tigris, Indus and Ganges have been recognized as the life lines for ancient civilizations.

Until recent population explosion from few decades, that has caused an immense pressure on water resources, fresh water as a source has never been an issue of concern. However, due to intensive agricultural practices, extensive urbanization, rapid industrialization and burning of fossil fuels are amongst anthropogenic activities, which have increased rapidly and have been considered important for changing the natural conditions of an aquatic ecosystem. Thus degradation of natural ecosystems ultimately alters the structure and functions of aquatic biota due to the adverse effect of human activities. Not only in one part or in one area, but also throughout the world, fresh water resources are facing number of environmental problems largely associated with anthropogenic activities in their catchment areas. Especially in poor and developing countries, the pollution of fresh water resources is a matter of great concern because without considering environmental protective measures, pollutants are discharged directly or indirectly into rivers and streams.

There are various types of agents which are responsible for degrading the water quality e.g additives like fertilizers, pollutants and other soil improving agents which are used to increase the crop production, after heavy rain fall get dissolved and leach-down to ground water or moves to rivers/ streams with surface run off after soil saturation. Similarly, metals are also one of the important contaminant groups responsible for deterioration of surface water quality, which either originate naturally from parent rock material as a result of weathering or contributed from anthropogenic processes. Heavy metals like iron, copper, nickel, chromium and zinc are essential in living organisms because of their structural and functional roles in various physiological processes **(Wepener *et al.*, 2001),** where as non-metals have no known role in metabolic functions of the organisms and are toxic even in trace amounts. The absorbed heavy metals in organisms can bind with cellular components (i,e nucleic acids and proteins) and interfere with metabolic processes that lead to genotoxic, neurotoxic, mutagenic effects. So accumulation of heavy metals disrupts the physiology and histology of aquatic organisms that may lead to death.

For analysis of water pollution in a river, aquatic organisms are used because they are sensitive to any physical and chemical change in river water. Aquatic organisms such as algae, invertebrates, fishes and amphibians are important organisms for understanding the impacts of human activities in river ecosystem. Among aquatic organisms, fishes are good indicators of pollution stress and have wide range of tolerance. Fishes are sensitive to any type of human disturbance such as industrial effluents, municipal waste, river discharge and strongly influence the distribution, migration, colonization of fishes **(Plafkin *et al.*, 1989).**

So from few years importance of protection, restoration and management of aquatic resources has been realized all over the globe. Hence the present study was taken to study the effect of anthropogenic activities in Betwa river in Raisen district(M.P) on *Labeo calbasu* which will provide first hand information regarding their histological and biochemical damages caused by anthropogenic activities.

AREA OF STUDY:

The Betwa or Vetravati is a river of great antiquity and immense mythological and religious values for the people of the Malva region of M.P for hundreds of years. This second largest river of the Malva region is not only important from the geo-ecological point of view but also has a significantly potent socio-economic impact on the area through which it flows.

Betwa is an important tributary of the Yamuna, which in turn is a tributary of the river Ganga. This makes the Betwa as an important river of sacred gangetic river system. The river Betwa rises from the main Vindyan range in the extreme southwest of the Raisen district at jhirribarod village (Longitude $77^{0}24'$E & latitude 23.2^{0}N). It flows for estimated total length of 573 km of which 216km in M.P and 98 km in U.P and finally joins the river Yamuna at Hamirpur in U.P (Longitude 80.13^{0} and Latitude 25.55^{0}N). The river has a huge catchments area of around 46580 sq.kms. During the course of its flow, Betwa receives 14 tributaries of which as many as 11 are located in the Madhya Pradesh.

The water quality in the stretch of the river Betwa extending from its origin near Mandideep industrial area up to Bhojpur remains poor because of the regular inflow of domestic wastes of the Bhopal city through the Kaliyasot river and industrial/domestic water from Mandideep. Owing to the above facts and public complaints from local agencies the overall quality of the river water in this area has been marked as poor. The quality of the Betwa river water improves after Bhojpur due to the confluence of some smaller rivers like Ricahan, Dawar etc. flowing from the forest area located in the central part of the district. The average quality in the north-western part of the district i.e. towards Vidisha District falls under a medium category with some patches of low quality attributed to the industrial/domestic contamination from isolated large industries and scattered settlements **(Lesser, 1978).** The present study was undertaken to assess “the impact of anthropogenic activities in the catchment area of Betwa River in Raisen district on *Labeo calbasu”* (Ham.)

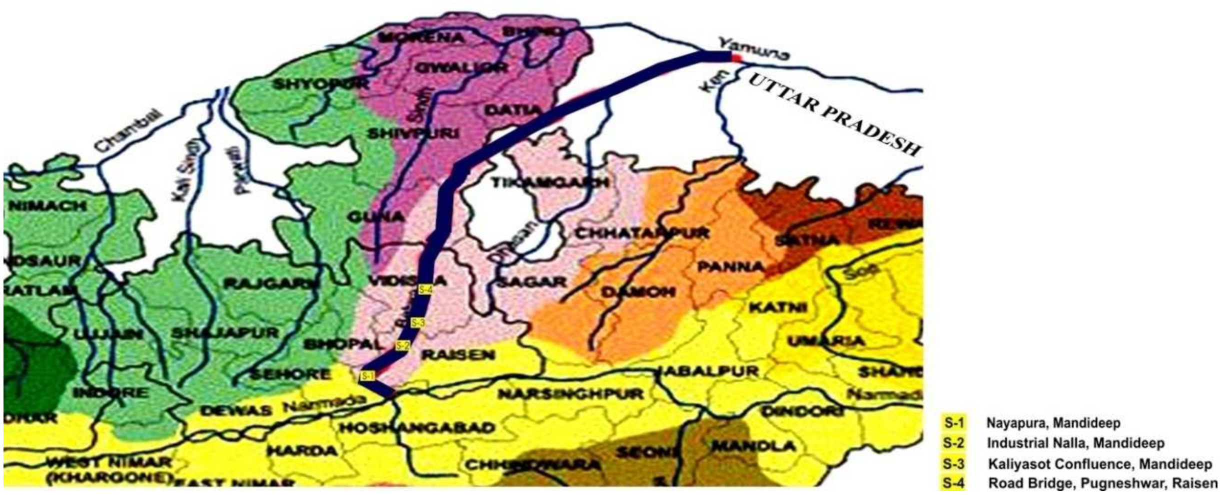
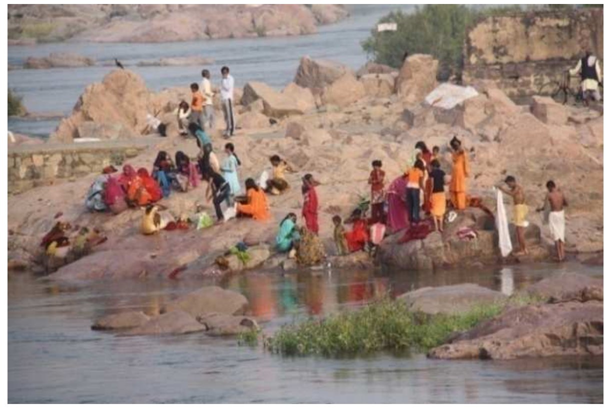


Fig: Map of Betwa River having catchment area of 216 Km² in M.P.



Nayapura, Mandideep (site 1)

Industrial nalla, Mandideep (site 2)

Kaliyasot confluence, Mandideep (site 3)

Road Bridge Pugneshwar,Raisen (site 4)

MATERIALS AND METHODS:

For evaluation of biological parameters four fish samples at different sites were collected from the highly polluted belt and less polluted belt of the Betwa River. One fish sample was collected from the area of Nayapura, Mandideep (site 1).The second fish sample was taken at Industrial Nalla, Mandideep(site 2) while as other two fish samples were collected from Kaliyasot confluence, Mandideep (site 3) and Road Bridge Pugneshwar, Raisen (site 4).All the above samples collected from sites1,2,3 and 4 of Betwa river were considered fish samples from polluted water (test fish samples) and were compared with the fish sample collected from Patra fish farm, Bhopal(Madhya Pradesh) which was considered as the control fish sample.

The live specimens of *Labeo calbasu* measuring about 10-20 cms. and weighing approx. 80-125 gms were collected and brought to laboratory in polythene bags for studying the combined effect of agrochemicals and heavy metals due to anthropogenic activities. However, control fishes were transferred into the glass aquaria of 50 liters capacity containing well-aerated, unchlorinated ground water for 15 days acclimatization. The fishes were screened for any physical damage, disease and mortality. The immobilized, injured, abnormal and dead fishes were discarded immediately. Control fishes were fed every day twice with wheat flour pellets, boiled egg protein and grounded dried shrimps purchased from local fish market. Before stocking, the aquaria were washed with 0.1% $KMnO_4$ to free the walls from any possible fungal infections if any and acclimatization was judged satisfactory when the incidence of fish monolith was less than 10% of total fish during one week prior to the commencement of the experiment. The fishes were also treated with 0.1% $KMnO_4$ solutions to check any possible bacterial infections. The media in aquaria for control fish was renewed on alternate days to prevent accumulations of metabolites.

In addition, the individual fish in the control aquaria were considered dead when they failed to respond to touch stimulus. After the death of each fish, its body was removed immediately from the container along with the aquaria water allotted. After 15-day acclimatization of control fish, survived individuals in the container were dissected in the laboratory.

Both the test and control fish samples were compared to assess the effect of water pollution on the fish health with extrapolation to hazards to human health. The fishes were sacrificed by decaefication of the head and their kidney was dissected out quickly and was fixed in Bouin’s fixative, then paraffin sections were cut at 5-7 µm thick. Paraffin sections were stained with haematoxylin using eosin as the counter stain for normal histology. Similarly for biochemical estimations a part of liver samples were taken out from each group, weighed and stored at 10^{o}C. The spectrophotmetric methods were used to quantify the total lipids **(Folch, 1957)** protein levels **(Lowry *et .al.*, 1951)** and cholesterol contents by **Karba and Kabra (1956).**

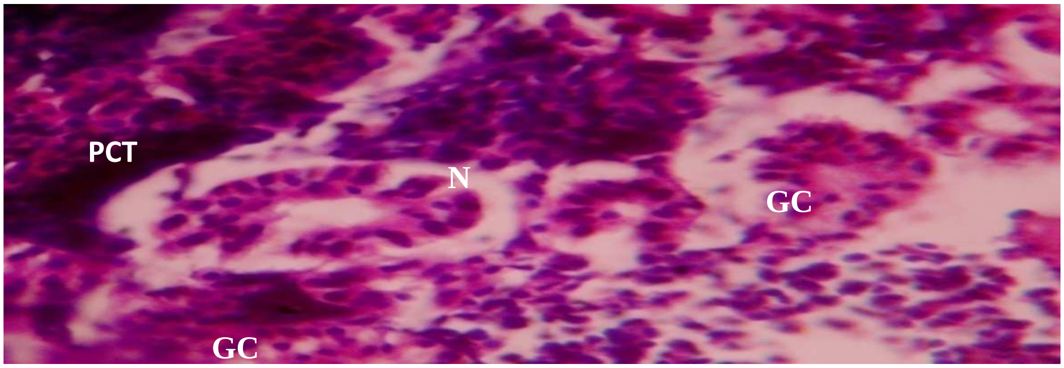
RESULTS AND DISCUSSION:

HISTOLOGICAL CHANGES:

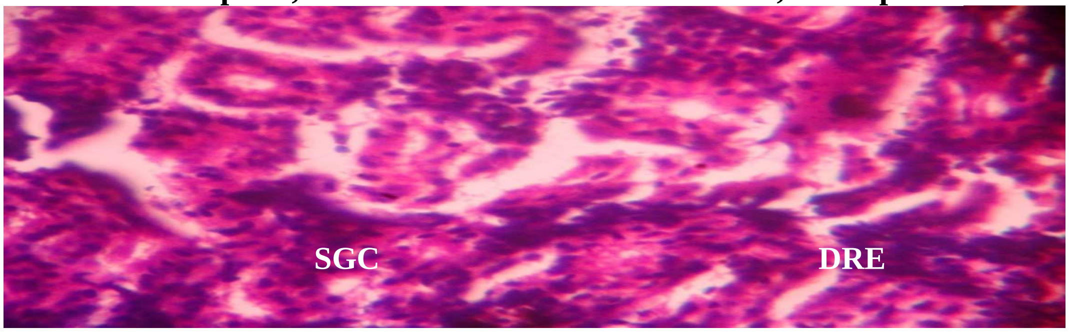
The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids and erythropoiesis. The kidney is one of the first organs to be affected by contaminants in the water. Thus, the present investigation will help in elucidating the normal histology of kidney of *Labeo calbasu* (Ham.) brought from Patra fish farm and effect of pollutants caused by anthropogenic activities on the histology of fishes collected from selected sites of Betwa River.

The histology of the kidney from control group of *Labeo calbasu* is made up of a large number of nephrons, each consisting of renal corpuscles, proximal and distal convulated tubules. The inter tubular space is full of lymphoid tissue which is uneventually distributed. Showing well vasculorised glomerulus capsule and renal tubules. The kidney histology of *Labeo calbasu* caught from site 1 (Nayapura, mandideep), showed marked glomeruli shrinkage and breakage of the outer wall of haemopoietic tissues and also exhibited degeneration and flattening of renal epithelium. Histology of the kidney of *Labeo calbasu* caught from site 2 (Industrial nalla, Mandideep), also showed glomeruli shrinkage and breakage leaving a large space in Bowman's capsule. Haemopoietic tissues also showed degeneration. Necrosis was observed in renal tubules. The epithelial cells of the proximal convoluted tubule were degenerated and their nuclei were found enlarged.

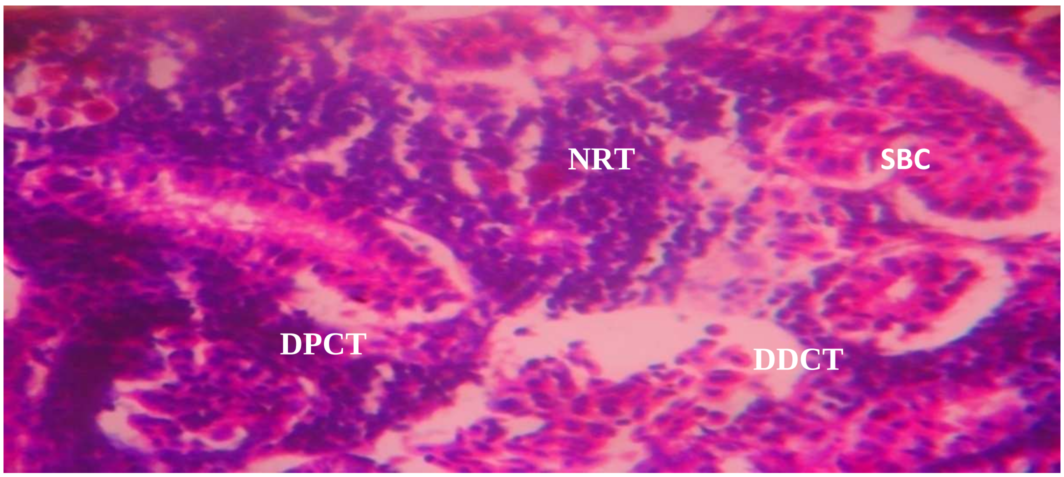
Histology of the kidney sections of *Labeo calbasu* caught from site 3(Kaliyasot, Mandideep), showed disintegration of convoluted tubules with large intra- cytoplasmic vacuoles in the epithelial cells. Shrinkage and degeneration of the glomeruli, increase of space within the Bowman's capsule was also seen. Histology of the Kidney of *Labeo calbasu* caught from site 4 (Road Brigde, Pugneshwar, Raisen), showed necrosis of tubular epithelium, degeneration of the epithelial cells of the renal tubules, degeneration of the glomeruli, hypertrophy of the epithelial cells of the renal tubules, narrowing of the tubular lumen and glomerular contraction in the Bowman's capsule.



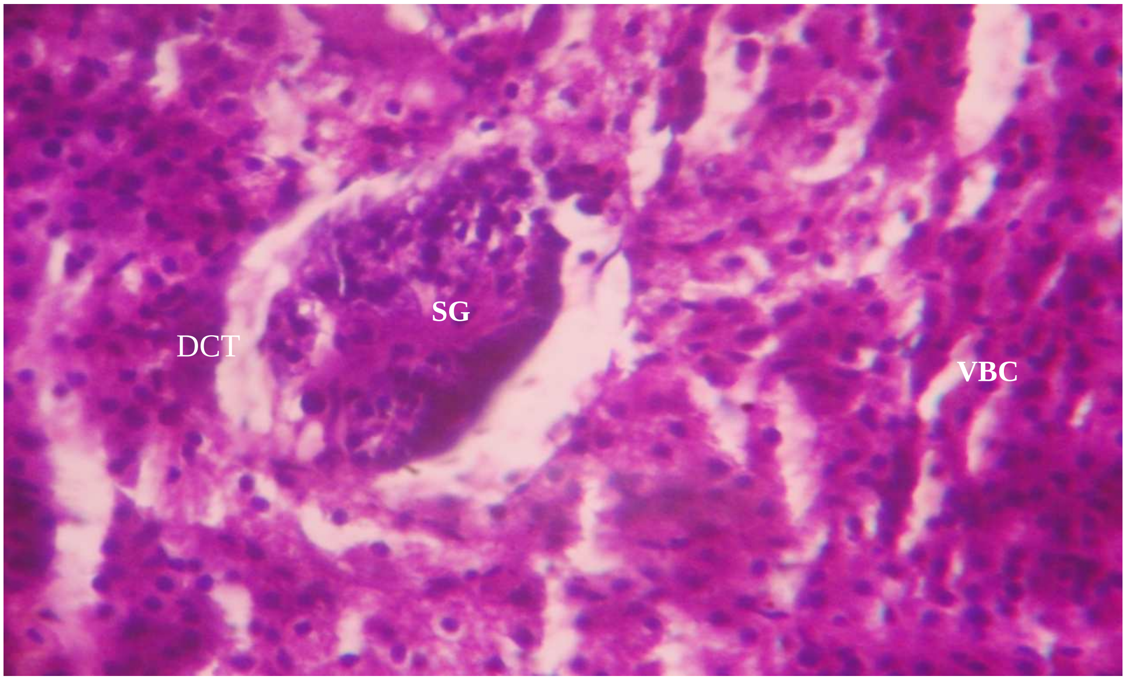
GC=Glomeruli capsule, PCT= Proximal convoluted tubule, N= Nephron



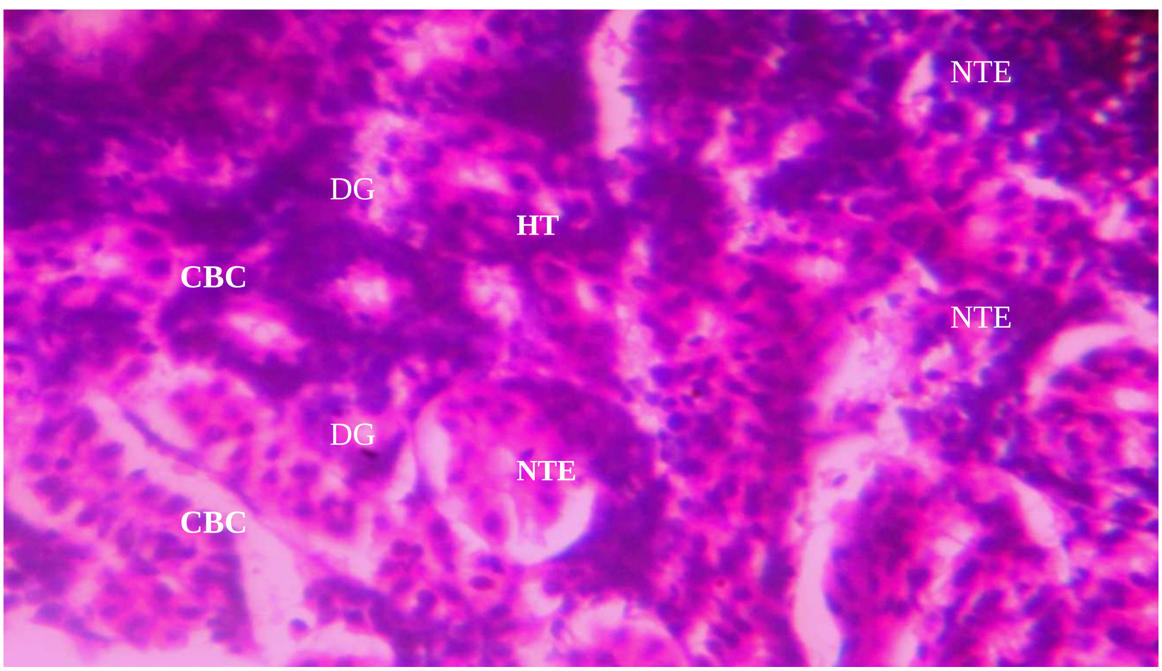
SGC= Shrinkage of glomeruli capsule, DRE= Degeneration of renal epithelium



SBC= Shrinkage of Bowman's capsule, NRT= Necrosis of renal tubules, DDCT= Degenerated distal convoluted tubule, DPCT= Degenerated proximal convoluted tubule.



DCT= Degenerated convoluted tubules, SG= Shrinkage of glomeruli, VBC= Vacuolation of Bowman's capsule



NTE= Necrosis of tubular epithelium, DG= Degeneration of glomeruli,

HT=Hypertrophy, CBC=Contraction of Bowman's capsule.

Similar histopathological lesions were recorded previously in kidney tissues of treated fishes with different pollutants. They include necrosis and degeneration of hemopoietic tissue and vacuolation of renal tubular cells in winter pounder (*Pseudopleuronectes americanus*) poisoned by copper **(Baker, 1969). Sastray and Sharma,(1979)** observed a number of striking changes in the histological structure of the kidney of *Channa punctatus* exposed to sublethal concentration of 0.01ppm of endrin for a span of 30 days and found that the shrinkage of glomerulus was the visible sign of intoxicatication. Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman's space **(Takashima and Hibiya, 1995).**

The present study is in proximity with the results observed by **Tilak *et al.*, (2001)** who noticed severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm and vacuolization in kidney tissues of *Ctenopharyngodon idella* after exposure to fenvalerate. **Cengiz, (2006)** observed degeneration in the epithelial cells of renal tubules, pycnotic nuclei in the hematopietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intercytoplasmic vacuoles in the epithelial cells with hypertrophied cells and narrowing of the tubular lumen was observed in the kidney tissues of fish exposed to detamethrin. Our results are in agreement with the changes in the kidney of Zebra fish, *Danio rerio* exposed to sublethal concentrations of chlropyriphos **(Scheil *et al.*, 2009).**

BIOCHEMICAL CHANGES:

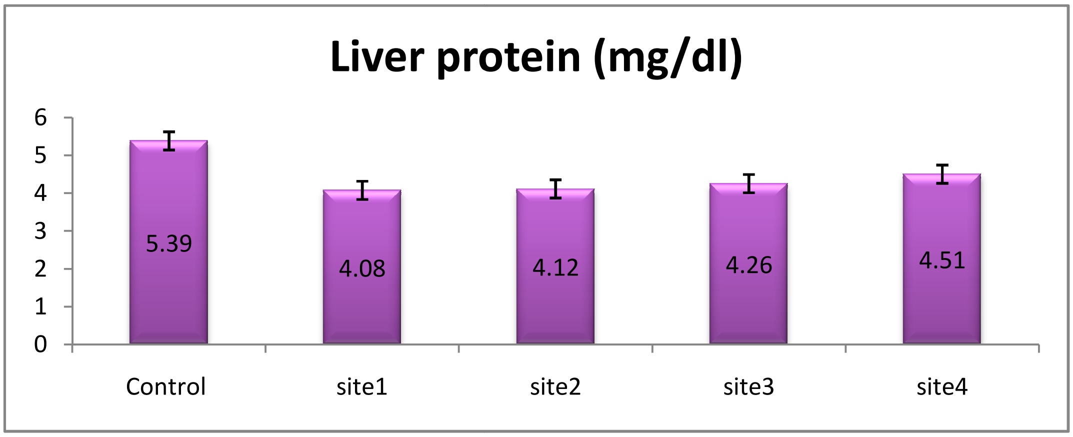
Liver homogenates were studied for the evaluation of various biochemical parameters including total protein, total lipid and total cholesterol. During experimentation all biochemical parameters i,e total protein, total lipid and total cholesterol decreased when compared with control. This decrease of 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.

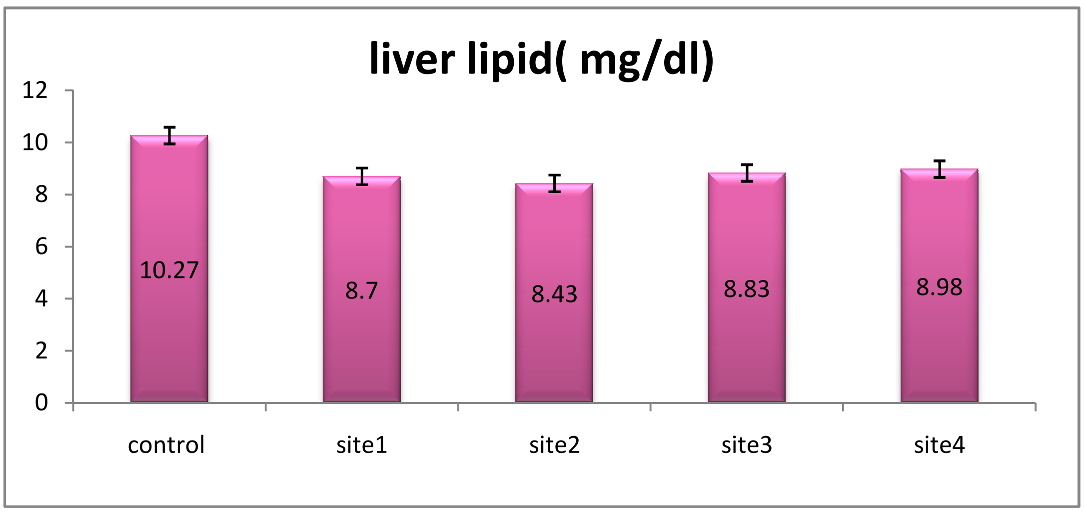
In response to aquatic pollutants caused by anthropogenic activities, the total protein in all the four selected fish samples was significantly decreased. Total protein decreased most in the sample fish caught from site 1, which valued 4.08±0.20 mg/dl followed by fish sample caught from site 2, which valued 4.12±0.01 mg/dl. However, sample fish caught from site 3 and site 4 also showed depletion in total protein contents but not so much as was observed in first two fish samples. The value of total protein in the fish caught from site 3 valued 4.26±0.18 mg/dl and 4.51±0.10 mg/dl in site 4 fish sample as compared to control, which showed highest protein content of 5.39±0.09 mg/dl. Likewise, total lipid also decreased in all fish samples from polluted waters. The total lipid depletion was most profound in fish sample caught from site 2, which valued 8.43±0.09 mg/dl followed by sample fish caught from site 1 which valued 8.7±0.16 mg/dl. The fish samples from other two sites i,e site 3 and site 4 also showed depletion in their total lipid contents but the depletion was not as much as in site 1 and site 2 fish samples. The values of total lipid contents in sample fish from site 3 and site 4 was found to be 8.83±0.04 mg/dl and 8.98±0.10 mg/dl while in control sample, total lipid content showed highest value i,e 10.27±0.25 mg/dl. Similarly, total cholesterol also decreased in all selected samples as compared to control, however the most significant depletion was found in the sample fish caught from site 2, which valued 60.11±0.58 mg/100mg followed by the sample caught from site 1 which valued 62.83±0.41 mg/100mg. Likewise, total cholesterol in other two sample fishes from site 3 and site 4 also showed a marked depletion which valued,63.53±0.33mg/100 mg and 63.83±0.44 mg/100mg.

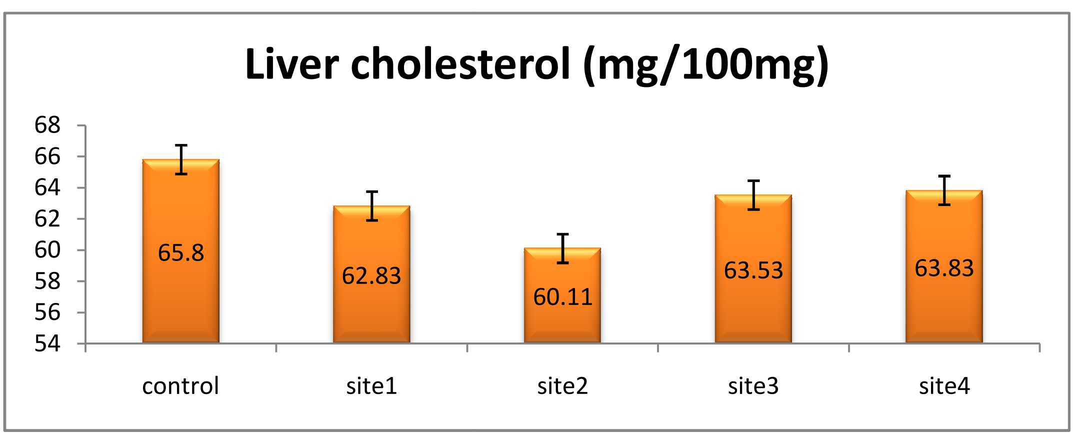
Table showing changes in protein, lipid and cholesterol content in the liver of *Labeo calbasu* during Jan.2011- Dec. 2012.

S. no.	Organ investigated	Parameters studied	control	SD	Site 1	SD	Site 2	SD	Site 3	SD	Site 4	SD
1.	Liver	Protein(mg/dl)	5.39±0.09	0.31	4.08±0.20	0.68	4.12±0.01	0.06	4.26±0.18	0.60	4.51±0.10	0.33
2.		Lipid(mg/dl)	10.27±0.25	0.86	8.7±0.16	0.55	8.43±0.09	0.03	8.83±0.04	0.15	8.98±0.10	0.35
3.		Cholesterol (mg/100mg)	65.8±0.31	1.05	62.83±0.41	1.33	60.11±0.58	1.92	63.53±0.33	1.10	63.83±0.44	1.47

Fig. Biochemical responses in the liver parameters of *Labeo calbasu* showing increase (+) or decrease (-) in their contents, caught from different sites of Betwa river with relation to control.







In the present investigation, depletion in the total protein was observed in liver of *Labeo calbasu* sampled from four selected sites of Betwa river. The variation in protein distribution suggests a gradual difference in metabolic calibers of various tissues and it is a physiological strategy adopted by the animal to adjust itself to the changing metabolic system. The decrease in the protein content as observed in the present study over the control could be due to metabolic utilization of the ketoacids to gluco-neogenesis pathway for the synthesis of glucose, as also reported by **Somnath, (1991)** in tissues of fish *Labeo rohita* exposed to acute and sublethal concentrations of tannic acid. When an animal is under toxic stress, diversification of energy occurs to accomplish the impeding energy demands and hence the protein level is depleted **(Neff, 1985)** which justifies our findings in the fish *Labeo calbasu* caught from Betwa river.

Decrease in protein content in the present finding may be due to the impairment of protein synthesis or increase in the rate of its degradation to amino acids. This may be fed to TCA cycle through amino-transferase probably to cope with high energy demands in order to meet the stress condition. The decrease in proteins might be due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in cytoplasm**.**

The reduction in the protein content in the present investigation in the liver of *Labeo calbasu* suggests that the tissue protein undergoes proteolysis, which results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. **Schmidt, (1975)** also postulated that the decreased trend of the protein content in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing free amino acids for the synthesis of glucose, or due to directing free amino acids for the synthesis of necessary proteins or for the maintenance of osmotic and ionic regulation. It is evident that the proteins are degraded to meet the energy requirements during pollutants exposure. **Hymavathi,(2001)** reported a decrease in the biochemical profile (total proteins, carbohydrates and lipids) of *Channa orientalis* from a habitat polluted by slaughterhouse wastes when compared to an unpolluted habitat of Mudasarlova stream of Visakhapatnam. This is in agreement with the present investigation on *Labeo calbasu.*

Lipid plays an important role in metabolic activities of animals because they are source of energy and are involved in the building of cellular components .They are stored in the form of metabolites and provide energy when an organism faces adverse conditions**.** It is used as energy reservoir, stored and transported in the form of glycerol esters **(Patole *et al.*, 2008).** Lipid plays an important fuel reserve of the fish during stress situation so glycogenolysis, proteolysis and hydrolysis of lipids have been reported to generate more energy through gluconeogenesis in order to cope with the increased energy demands occurring due to metal toxicity in fish **(Gunstone, 1960).**

The decreased value of lipid in the present investigation indicated that there were major changes like accumulation of lipid contents and alteration in the lipid metabolism due to pollutant stress, which showed hazardous effect on the fresh water fish, *Labeo calbasu.* A decrease in the total lipid content in the liver of *Labeo calbasu* caught from various sites exposed to pollutants caused by anthropogenic activities suggests that lipid might have been channeled for energy production for other metabolic functions in which these products play a vital role during stress condition. Similarly, **Ravathi *et al.*, (2005)** also observed the decreased level of lipid in the fish *Gambusia affinis* when exposed to tannery effluent. Depletion of lipid content may be due to lypolysis or the mitochondrial injury, which impaired the function of TCA cycle and the fatty acid oxidation mechanism. Significant depletion of lipid in liver of the studied fish may be associated with the inhibition of lipid synthesis by pollutants drifted from various industries located in the nearby areas of Betwa River and agricultural run-offs washed away by small streams or by rain in rainy seasons or comparatively more utilization of stored lipids as an immediate source of energy to with stand stress.

Cholesterol is the major sterol in animal tissue. Cholesterol and its esters with long chain fatty acids are important components of plasma lipoprotein and of the outer cell membrane. It is the major precursor of steroid hormones such as sex hormones and adrenal cortical hormones, which effect cellular activities by influencing gene expression. It stimulates utilization of fatty acids and ketogenesis. Cholesterol is also important for the synthesis of bile acids, which facilitate the emulsification, and digestion of lipids in the small intestines.

The results shown in the below table show a significant decrease in cholesterol content in the studied tissues of *Labeo calbasu* which signals the hypocholesterolemic conditions due to metal intoxication. The decrease was found to be high in sample caught from site 2 and less in control brought from Patra fish farm. Decrease in total cholesterol level in all the samples can also be correlated with the inhibition of protein metabolism and switching on the energy production source to some other metabolite. The hypo-cholesterolemia could be attributed to the less amount of cholesterol produced by liver or more excretion of cholesterol to the bile duct as a result of stress. In response to stress situation, hypothalamus secretes corticotrophin-releasing hormone, which stimulates the anterior pituitary to release corticotrophin by which adrenal cortex is stimulated to secrete corticoid hormones. These hormones influence the anti-inflammatory and anti-allergic actions along with carbohydrate metabolism **(Lehinger, 1982).**That's why probably, the biosynthesis of steroids along with inhibition of cholesterol synthesis is another cause of reduction in cholesterol content in present findings. The reduced cholesterol may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced reabsorption of dietry cholesterol as reported by **Jayantha Rao *et al.*,(1984)** . Significant decrease in cholesterol level may also be due to decreased synthesis, which in turn may be due to the lack of cholesterol starting material (acetyl co-enzyme A) partly in the glucose deficient environment and partly due to extra needs of energy for the body to detoxify the toxic compounds, as also stated by **Ali, (1989). Srivastava *et al.*, (2002)** have attributed the lowering of cholesterol in the liver of *Channa punctatus* exposed to zinc, to an increase in lipid utilization for meeting additional energy requirements under stress condition**.**

From the above findings, it was evident that Betwa river has definitely metal stress and which is related to increasing industrial 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, herbicides and hydrocarbons in the waters of Betwa river, because river Betwa and its tributaries are the ultimate source of irrigation and sink of agricultural wastes for the whole district of Raisen.

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