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IMPACT OF ANTHROPOGENIC ACTIVITIES ON THE HAEMATOLOGICAL 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 biochemical parameters (lipid, protein and cholesterol) of investigated organ like liver of Labeo calbasu. The results showed that the biochemical changes induced in liver were mainly represented by depletion of protein, lipid and cholesterol contents in all studied fishes taken from different sites as compared to control fish sample. Haematological parameters like R.B.Cs, Hb, MCH, MCHC and PCV declined while WBCs and MCV increased as compared with the control fish.

KEYWORDS: Betwa River, Labeo calbasu, Haematological and 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. 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 elements. 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.

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 haematological parameters and biochemical parameters of investigated organ like liver of Labeo calbasu which will provide first hand information regarding their alterations and damages caused by anthropogenic activities.

AREA OF STUDY

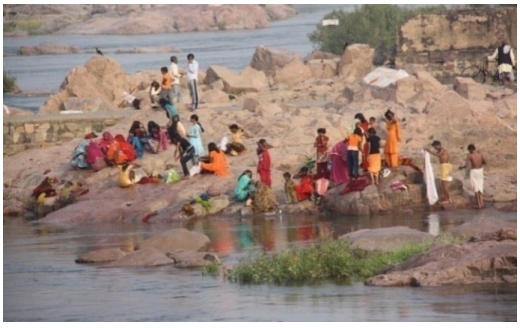
The Betwa or Vetravati is a river of great antiquity and immense mythological and religious values for the people of the Malva region of M.P for hundreds of years. This second largest river of the Malva region is not only important from the geo-ecological point of view but also has a significantly potent 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 23.2^0N). 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 25.55^0N). The river has a huge catchments area of around 46580 km.[2] During the course of its flow, Betwa receives 14 tributaries of which as many as 11 are located in the Madhya Pradesh. A site named as Ramghat on the Betwa River is a sacred ghat situated in the Vidisha district of M.P. It is a religious ghat and many temples are situated on both the banks of the river. About 1km length of this stretch is restricted for fishing and fishermen avoid to fish in this stretch.

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 (site1)



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 for various hematological and biochemical parameters to assess the effect of water pollution on the fish health with extrapolation to hazards to human health. The following techniques were adopted for quantifying the hepatic protein levels, total lipid and cholesterol. Protein was estimated following the method of **Lowry et al., (1951),**total lipid content as per the method of **Floch (1957)** and expressed as mg/g wet tissue weight. Cholesterol level was estimated by the method of **Karba and Kabra (1956)** and expressed as mg/100mg wet tissue weight.

For hematological studies, the fishes were anesthesized with 100 mg/L tricaine methane sulfonate (MS-222). The caudal peduncle was cut-off with a sharp razor blade and free flowing blood was collected for the hematological study. Haematological parameters were estimated by standard methods as described by **Blaxhall and Daisley (1973)**. The RBC and WBC cells count were made by Neubaur's hemocytometer. Hemoglobin percentage was determined by the method prescribed by **ICSH (1978).**

RESULTS

In the present investigation, the following observations were made in Labeo calbasu caught from Betwa River from different sites.

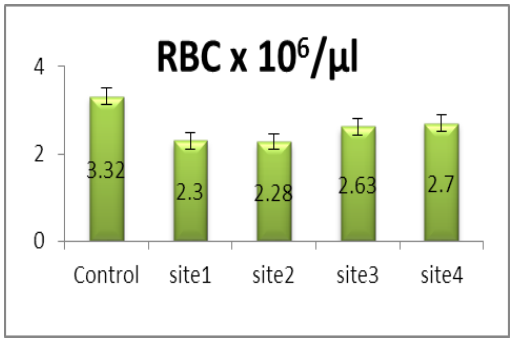
HAEMATOLOGICAL CHANGES

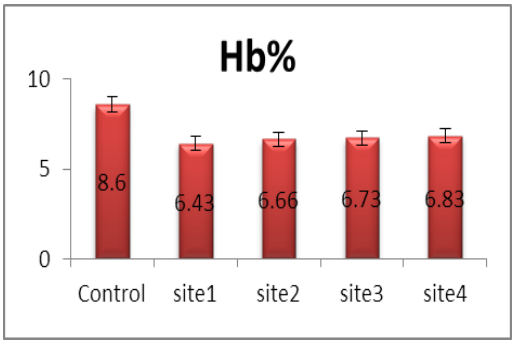
Among hematological indices, the RBC count in Labeo calbasu lowered down in polluted samples mostly in site 1 and site 2 fish samples than site 3 and site 4 samples, as compared with that of control fish. RBC count was $3.32\pm0.23x10^6$/µL in control fish sample,(highest), $2.3\pm0.27x10^6$/µL (site1fishsample),$2.28\pm0.26x10^6$/ µL(site2) (least), $2.63\pm0.21x10^6$/µL(site3) and $2.7\pm0.26x10^6$/µL(site 4). Similarly, hemoglobin percentage also declined in all samples, mostly in fishes taken from site1 as compared to control sample. In control hemoglobin percentage was observed to be 8.6±0.51%, 6.43±0.19% (site 1), 6.66±0.17%(site 2),6.73±0.18% (site 3) and 6.83±0.16% (site 4). Hematocrit (PCV) value also decreased in all the

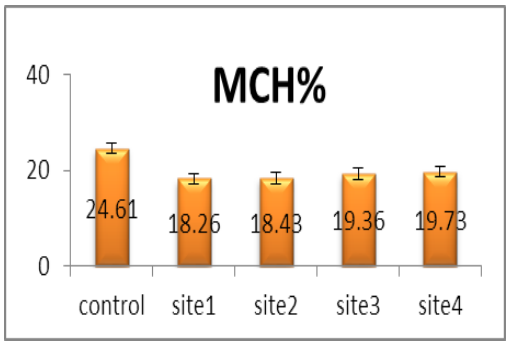
samples as compared to control sample but the most profound decline was observed in fish collected from site 2.In control fish PCV percentage was 26.96±0.45% and 20.03±0.14% at site 2 fish (least Value of PCV),20.76±0.04% in site 1fish,21.8±0.28% in site 3 fish sample and 21.16±0.29% in site 4 fish sample. WBC count on the other hand increased in all fish samples caught from selected sites of Betwa River as compared to the fish considered as control collected from Patra fish farm. However, in fish, collected from site 1 shows highest value of WBC as compared to other sites and least value of WBC was observed in control fish. Range of WBC in control was $4.53\pm0.28x10^3/\mu L$,$6.66\pm0.04x10^3/\mu L$ at site1, $6.46\pm0.25x10^3/\mu L$ (site2), $6.1\pm0.03x10^3/\mu L$(site3) and $5.63\pm0.06x10^3/\mu L$(site 4). Like above blood parameters i,e RBC, PCV, Hb% in contrast of WBCs, MCH and MCHC parameters also showed decline in their contents from control.MCH content in control fish, Labeo calbasu was 24.61±0.24% while in site 1 least value i,e 18.26±0.57% of MCH was observed. Fishes caught from site 2,site 3 and site 4 have MCH value as 18.43±0.56%,19.36±0.58% and 19.73±0.55%.MCHC also follows the same procedure as Hb%, RBC, MCH and PCV i,e in control MCHC content was 27.00±0.39% (Highest) while in site1fish sample, MCHC value was 23.1±1.40%(Least),23.86±1.56%(site 2),22.3±1.30%(site 3) and 22.53±1.32% (site 4).Like WBC,MCV also showed increase in all selected samples in contrast of control.MCV in control group was found to be 84.99±0.45% (Least)while at site 1 the value of MCV was 91.2±0.29%,92.11±0.07% at site 2(Highest) 89.76±0.28% at site 3 and 87.56±0.26% at site 4 caught fishes, as shown in table I.

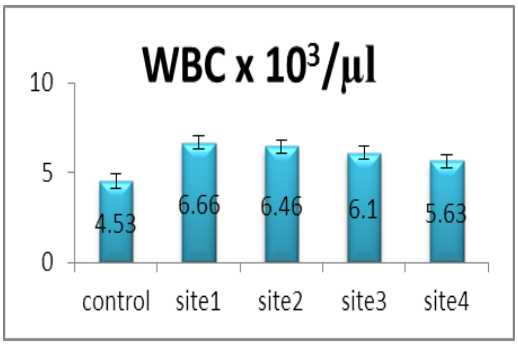
Table I: showing changes in haematological parameters of Labeo calbasu in relation to control caught during Jan.2011- Dec.2012.

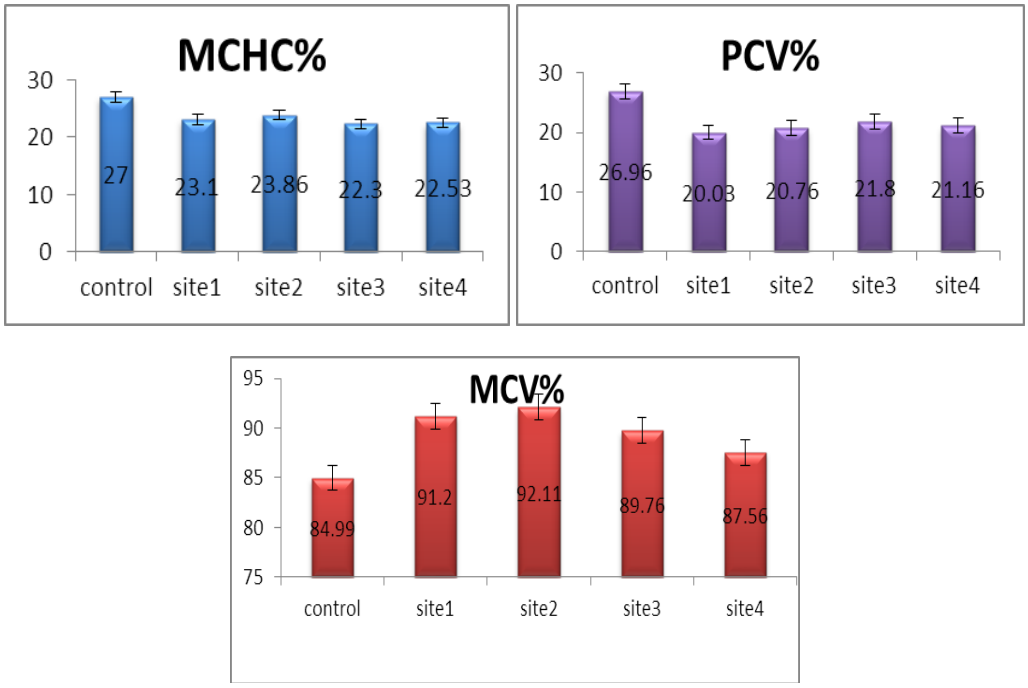
Blood Parameters	Control		Site 1		Site 2		Site 3		Site 4	
	Mean± SE	SD	Mean± SE	SD	Mean± SE	SD	Mean± SE	SD	Mean± SE	SD
RBC x $10^6/mm^3$	3.32±0.23	0.77	2.3±0.27	0.91	2.28±0.26	0.87	2.63±0.21	0.70	2.7±0.26	0.87
Hb %	8.6±0.51	1.70	6.43±0.19	0.6	6.66±0.17	0.56	6.73±0.18	0.60	6.83±0.16	0.55
WBC x $10^3/mm^3$	4.53±0.28	0.95	6.66±0.04	0.15	6.46±0.07	0.25	6.1±0.03	0.1	5.63±0.06	0.20
MCH%	24.61±0.24	0.82	18.26±0.57	1.92	18.43±0.56	1.87	19.36±0.58	1.95	19.73±0.55	1.85
MCHC%	27.00±0.39	1.31	23.1±1.40	4.6	23.86±1.56	5.2	22.3±1.30	4.34	22.53±1.32	4.39
PCV%	26.96±0.45	1.50	20.03±0.14	0.47	20.76±0.04	0.15	21.8±0.28	0.95	21.16±0.29	0.98
MCV%	84.99±0.45	1.52	91.2±0.29	0.96	92.11±0.07	0.25	89.76±0.28	0.95	87.56±0.26	0.87









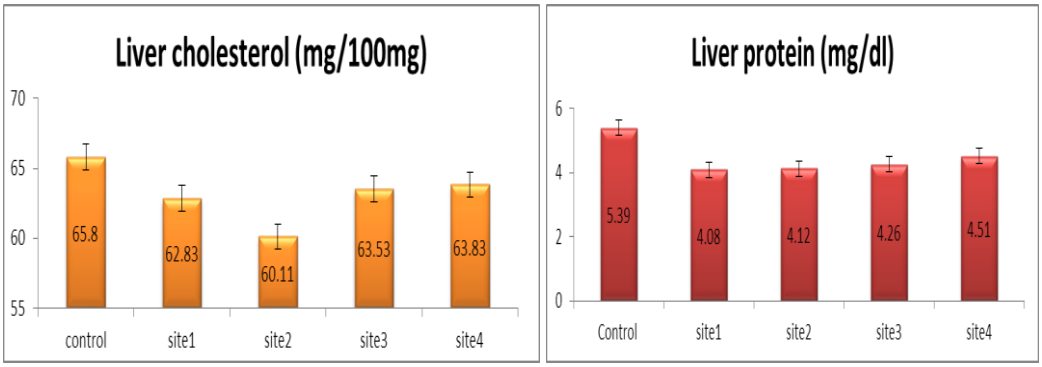


Haematological changes in Labeo calbasu caught from different sites of Betwa river during Jan. 2011- Dec.2012.

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 as shown in table II.



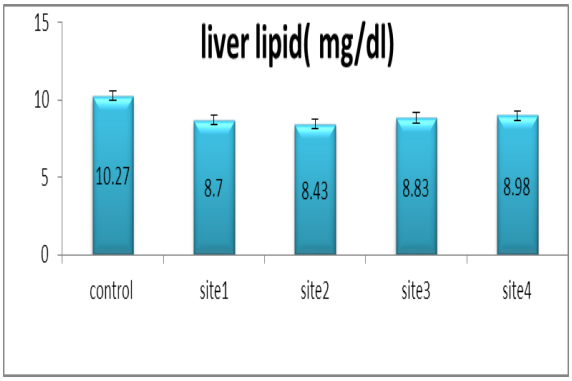


Fig. II. Biochemical responses in the liver parameters of Labeo calbasu showing increase (+) or decrease (-) in their contents, caught from different sites of Betwa river with relation to control.

RESULTS AND DISCUSSION

Haematological Parameters

The decreased Hemoglobin observed in the present investigation on Labeo calbasu was apparently due to decreased RBC count, which in turn led to decrease PCV. The decreased RBC count may be due to inhibited RBC production and or due to hemoglobin synthesis. Ambient toxicants might have caused disintegration of RBC cells, which in turn have caused reduction of hemoglobin and hematocrit count. This confirms the presence of anemia, solely due to the presence of pollutants in the water body and inside of Labeo calbasu caught from Betwa River at different sampling stations. Anemia of this type characterized by reduced RBC count, Hematocrit and hemoglobin contents have also been reported by several workers after insecticide feeding **(Mandal et al., 1986).**

From the results, our study is in conformity with the postulation of **Hamilton et al., (1978)** who concluded that insecticides like dieldrin become immunogenic and provoked a chemical immune-haemolytic anemia. Existing data indicates that pollutants like insecticides after entering into the blood stream have produced a number of abnormalities in the blood itself and in other blood formatting tissues **(Christopher, 1969).** Increase in MCV and decrease in MCH and MCHC was evident in the present investigation. This data confirmed that anemia produced was macrocytic hypochromatic type. The MCHC is not influenced by the blood volume or by the number of cells in the blood but can be interpreted incorrectly only when new cells, with a different hemoglobin concentration, are released into blood circulation **(Soivo and Nkinmaa, 1981).** Previous reports also ascertain that prolonged reduction in hemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants. In another study a significant decline in MCH and MCHC have also been reported by **Khattak and Hafeez, (1996)** in fish, Cyprinion watsoni exposed to extensively used agriculture pesticide malathion. WBC count increased reflecting the occurrence of leucocytosis (WBC increase) in all fish samples caught from Betwa river. This was perhaps, a typical defensive response of the fish against a toxic invasion and second most common probability may be leukemia or blood cancer during which the number of WBCs increases.

The insecticide treatment has repeatedly been found to cause a decrease in hemoglobin content and RBC count **(Shakoori et al., 1988).**The decreased synthesis of hemoglobin and slower activity of hemopoietic tissue under the influence of aldrin may explain the low values of hemoglobin and breakdown of RBC. In a study of sunfish from a lake, which received selenium-laden effluent from a power plant in Texas, **Sorenson and Bauer, (1983)** noted a reduction in the number of erythroblasts or immature erythrocytes. A reduction in the number of immune erythrocytes of immature erythrocytes could be indicative of a reduced rate of erythropoiesis, which could lead to reduced oxygen availability to tissues. This erythrocytic fragility or hemolytic action could inhibit the synthesis of new hemoglobin or erythrocytes, thus explaining the low PCV detected in our study on Labeo calbasu.

Thus from the above observation, it was evident that the hematology of the studied fish, Labeo calbasu was highly influenced by the pollutants present in the vicinity of Betwa river. However, the most drastic changes in blood parameters were observed in the fish samples caught from first two sites than that of other two sites, which ascertains that more the pollution in the water body, more variations will be in the hematological parameters.

Biochemical Parameters

Liver

Liver is the primary organ of detoxification and hence it is expected that toxicant could reach there for detoxification and disposal. This results in structural changes in the liver, in the arrangement of hepatic cords leading to the alteration of liver metabolism and its biochemical content. The pollutants act as one kind of stress and organism respond by developing necessary potential occurring in body give first indication of stress. During stress an organism needs sufficient energy which is supplied from reserve food material i,e proteins, lipids, cholesterol etc. 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).**

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. **Srinivas, (1991)** who reported decreased lipid content in Tilapia mossambica exposed to atrazine found similar results. 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**.**

REFERENCES

1. Wepener, V., Van-Vuren., JHJ., Du-Preez, HH. The effect of iron and manganese at an acidic PH on the hematology of the banded Tilapia (Tilapia sparrmanii, Smith). Bull.Environ.Contam.Toxicol, 2001; 49(4): 613-619.
2. Lesser, H. Medium scale geomorphological mapping and landscape ecology // Guide to medium scale geomorphological mapping IG.U commission of Geomorphological survey and mapping/ Department of Geography. Basel, 1978; 149-151.
3. Lowry, HO., Rosebrough, NJ., Farr, AL., Randall, RJ. Protein measurement with the Folin fenol reagent. The Journal of Biological Chemistry, 1951; 193:265-275.
4. Folch, J., Less, M., Sloane-Stanley, CH. A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 1957; 266: 497-509.
5. Blaxhall, PC and Daisley, KW. Routine hematological methods for use with fish blood.J.Fish-Biol., 1973; 5(6): 771-781.
6. International Committee For Standardization in Heamatology, Recommmendations for reference method for hemoglobinometry in human blood (ICSH Standard EP 6/2: 1977) and specifications for international hemiglobincyanide reference preparation (ICSH standard EP 6/3:1977).J.Clin.Pathol, 1978; 31: 139.
7. Mandal, PK and Kulshrestha, AK. Histo-pathological changes induced by DDT in the live kidney and intestine of certain fishes. Experimentia, 1986; 18: 506-509.
8. Hamilton, HE., Morgan, DP and Simmons, A. A pesticide (dieldrin) induced immune-heamolytic anemia.Environ.Res., 1978; 17: 155-164.
9. Christophers, AJ. Heamatological effects of pesticides. Ann. N.Y. Acad. Sci., 1969; 160: 325-355.
10. Soivio, A and Nikinmea, M. The swelling of erythrocytes in relation to the oxygen affinity of the blood of the rainbow trout, Salmo gairdeneri Richardson.-In: Pickering.A.D.(ed.), Stress and fish 103-119.Academic Press, London. 1981.
11. Khattak, IUD, Hafeez, MA. Effect of malathion on blood parameters of the fish, Cyprinion watsoni. Pak. J. Zool, 1996; 28: 45-49.
12. Shakoori, AR., Ali, S.S and Saleem, MA. Effect of six months feeding of cypermethrin on the blood and liver of albino rats.J.Biochem.Toxicol. 1988; 3: 59-71.
13. Soreson, EMB., Cumbia, PM., Baur,TL., Bell, HS. and Harlan, CW., Selenium accumulation and cytotoxicity in teleosts following chronic environmental exposure.Bull.Environ.Contam. Toxicol., 1983; 13: 153-162.
14. Balasubramanian, A., Murthy, NVK and Ramakrishnan., S. Additive effect of acetyl salicyclic acid on some toxic chemicals. A study of liver function tests, Indian J. med. Res., 1977; 66: 155-159.
15. Somnath, B. Effect of acute and sublethal concentration of tannic acid on the protein, carbohydrate and lipid levels in the tissues of the fish Labeo rohita. J. Environ. Biol., 1991; 12(2): 107-112.
16. Neff, J M. Use of biochemical measurement to detect pollutant medicated damage to fish. ASTM spec Tech. publ., 1985; 854: 154–183.
17. Schmidt-Nielson, B Comparative physiology cellularion and vol.regulation.J.Exp. zool, 1975; 194: 107-220.
18. Hymavathi, V and Rao, L M. Effect of slaughterhouse pollution on the biochemical composition of Channa orientalis. J.Environ. Biol., 2001; 22(3): 209-212.
19. Patole, SS., Patil, M and Mahajan, RT. Studies on metabolic changes during intoxication by phytotoxicants. in fish, Nemacheilus evizardy (Day) Int. J. Pharmacol. Biol. Sci., 2002; 2: 63-68.
20. Gunstone, FF (1960) An Introduction to the Chemistry of Fats and Fatty Acids. Asian Publishing House, Bombay, Kolkata and Madras.
21. Srinivas, T., Prasad, TA., V.Rafi, Ghulam, Md. and Reddy., DC Effect of atrazine on some aspects of lipid metabolism in fresh water fish, Tilapia mossambica. Biochemistry international, 1991; 23(3): 603-609.

22. Revethi, K and Suman Gulathi, Dawood Sharief, Tannery effluent induced biochemical changes in the larvivorous fish Gambusia affinis. Poll. Res., 2005; 24(4): 815-818.
23. Schmidt- Nielson, B (1975) Comparative physiology cellularion and vol.regulation. J.Exp. zool, 194: 107-220.
24. Lehninger, AL (1982).Principles of Biochemistry. Worth Pub., New York, 1st edition.
25. Jayantha Rao, K., Azhar Baig, MD and Ramamurthy, K. Effect of systemic pesticide phosphamidon on some aspects of fresh water fish Tilapia mossambica: Indian .J. Environ.1-Hit, 1984; 26: 60-64.
26. Ali, SS (1989) Morphological and biochemical hazards caused by some organochlorinated insecticides on blood and liver of rat. Ph.D. thesis, Department of Zoology, University of the Punjab, Lahore.
27. Srivastava, N., N Kaushik and P. Gupta, Zinc induced changes in the liver and muscle of fish, Channa punctatus (Bloch). J. Ecophysiol. Occup. Hlth, 2002; 2: 197-204.