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IMPACT OF ANTHROPOGENIC ACTIVITIES ON THE HAEMATOLOGICAL 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 various blood parameters of the fish, *Labeo calbasu* caught from Betwa river in Raisen district of M.P. 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 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 *Labeo calbasu* that will provide first hand information regarding their haematological 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 sq. kms. 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 river Betwa plays a significant role in the human life of the villages located in Mandideep, Bhojpur, and Raisen areas. It has become polluted at some places of Mandideep due to industrial activities and the confluence of sewage, domestic wastes and industrial effluents of many big and small enterprises with various types of organic compounds and heavy metals detrimental to human health and aquatic organisms.

Urban areas, farms, factories and individual households- all contribute to the contamination of this river. Moreover, water of Betwa River is widely used for drinking, agricultural and power generation purposes.

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.)

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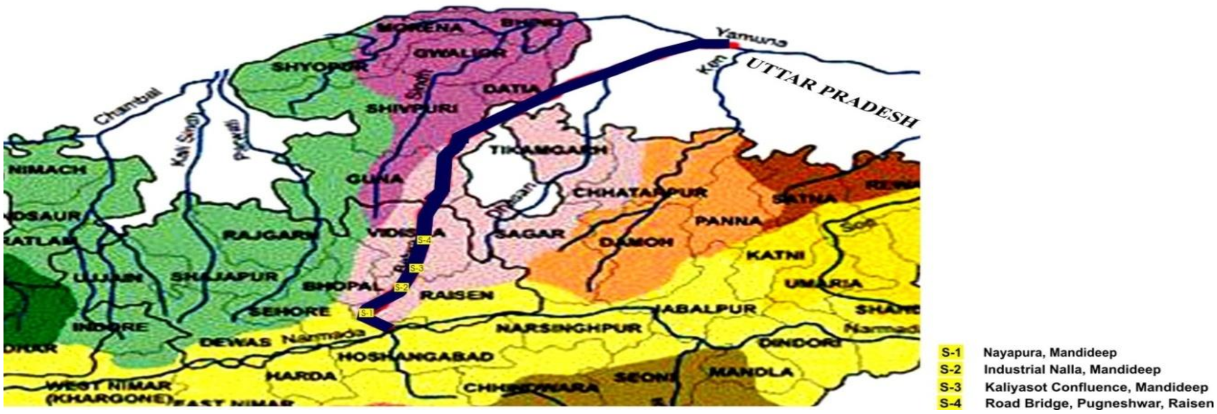

Figure 1: Map of Betwa River having catchment area of 216 Km² in M.P.

Nayapura, Mandideep (site 1)

Industrial nalla, Mandideep (site 2)

Kaliyasot confluence (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 sites 1, 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 parameters to assess the effect of water pollution on the fish health with extrapolation to hazards to human health.

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).

It has been observed that profound effect of various kinds of pollutants was found at Nayapuara site and Industrial nalla site caught fishes as compared to Kaliyasot site and Road Bridge, Pugneshwar site caught fishes.

RESULTS AND DISCUSSION

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

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 $3.32\pm0.23x10^6/\mu L$ in control fish sample, (highest), $2.3\pm0.27x10^6/\mu L$ (site 1 fish sample), $2.28\pm0.26x10^6/ \mu L$ (site 2) (least), $2.63\pm0.21x10^6/\mu L$ (site 3) and $2.7\pm0.26x10^6/\mu L$ (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 $4.53 \pm 0.28 x10^3/\mu L$, $6.66 \pm 0.04 x10^3/\mu L$ at site1, $6.46 \pm 0.25 x10^3/\mu L$ (site2), $6.1 \pm 0.03 x10^3/\mu L$ (site3) and $5.63 \pm 0.06 x10^3/\mu L$ (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 site1 fish 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.

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. Both leucopenia and conversely leukocytosis (increased number of WBC) have also been reported in fish populations exposed to heavy metals (Mishra and Srivastava, 1980; Gill and Pant, 1987).

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. Qayyum and Shami, (1983) have reported that insecticide aldrin treatment to fish produced a decline in erythrocyte count, Hb concentration and PCV values. In present study, PCV decreased in all studied samples which correlates with the finding of Lohner *et al.*,(2001) who reported significantly altered hematological parameters in sunfish inhabiting selenium-laden coal ash effluents and concluded leucopenia (decrease in WBC), reduced PCV and hemoglobin. Reduced packed cell volume and hemoglobin was correlated with the presence of anemia. Reduced PCV or hematocrit (volume of RBCs per unit volume of whole blood; hematocrit is synonymous with PCV, although a small portion of PCV includes WBC and Platelets) in fishes exposed to higher selenium and metal contaminated effluents have been reported in number of other studies (Sorenson *et al.*, 1982).

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.*

Gupta *et al.*, (1995) studied the effects of sublethal concentrations of chlordane and malathion (organophosphate) on hematological parameters of *Notopterus notopterus* and examined the exposed fish exhibited higher values of prothrombin time (PT), white blood cells (WBC) and packed cell volume (PCV). Gupta *et al.*, (2009) studied the effect of lindane on hematological indices of minor carp *Labeo boga* and reported increase in percentage of WBC count with the increase concentration of lindane.

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.

From the present study, it was clear that the water quality of River Betwa has been degraded qualitatively, as the concentration of some constituents has passed the permissible limits recommended by WHO, and this could pose a great threat to all kinds of life directly or indirectly.

Table 1: 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

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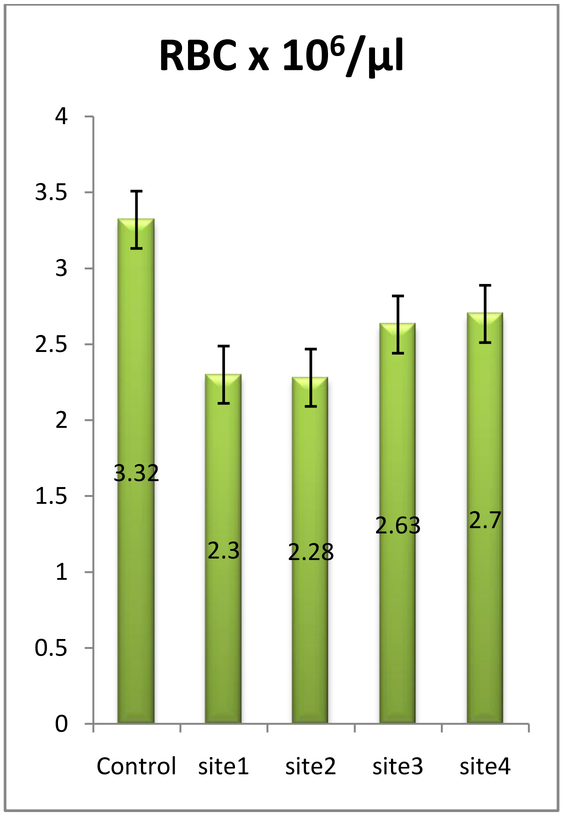

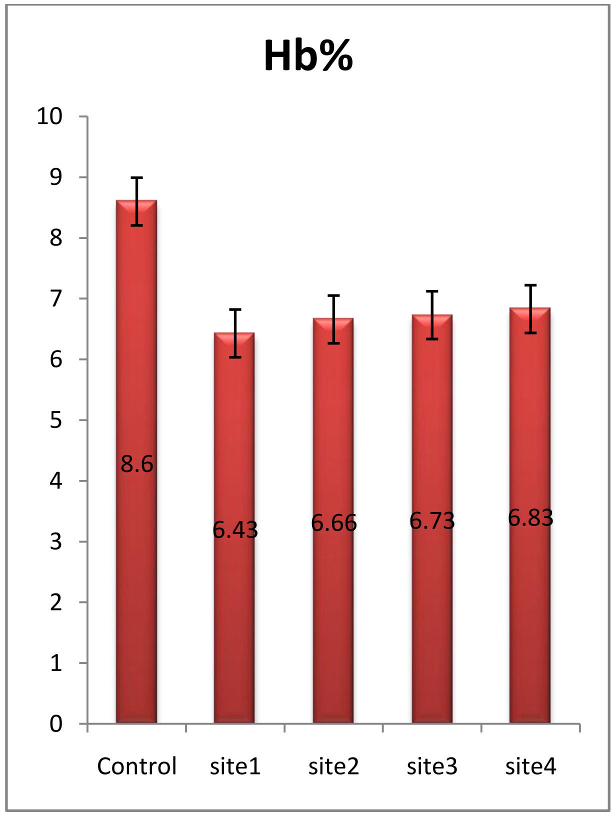

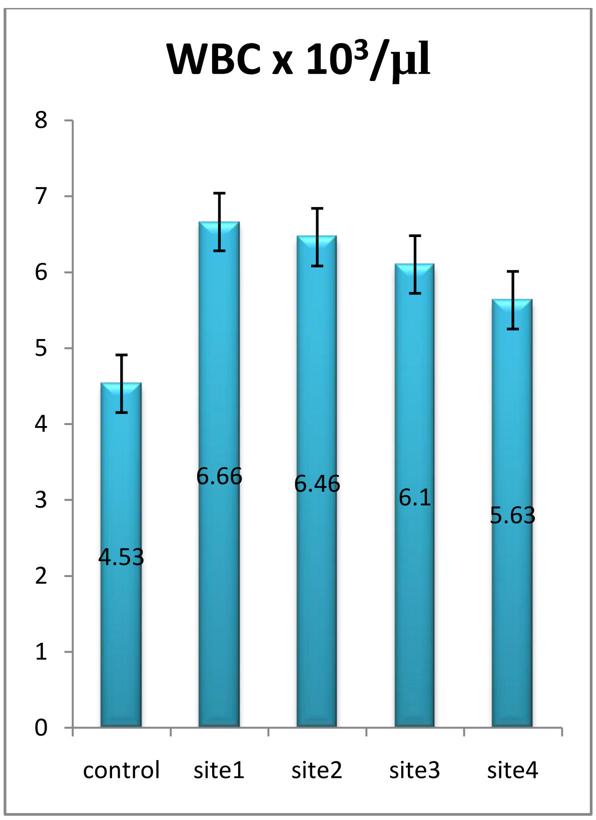

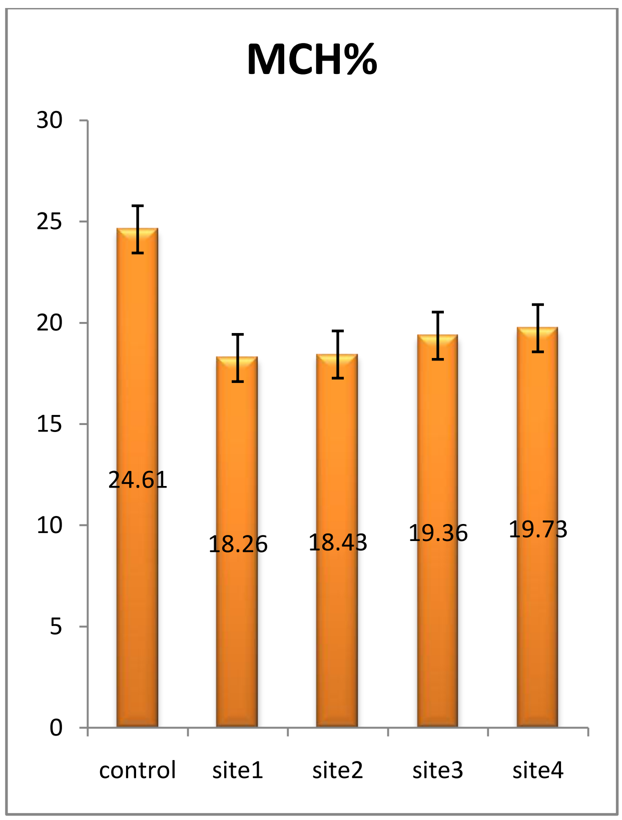

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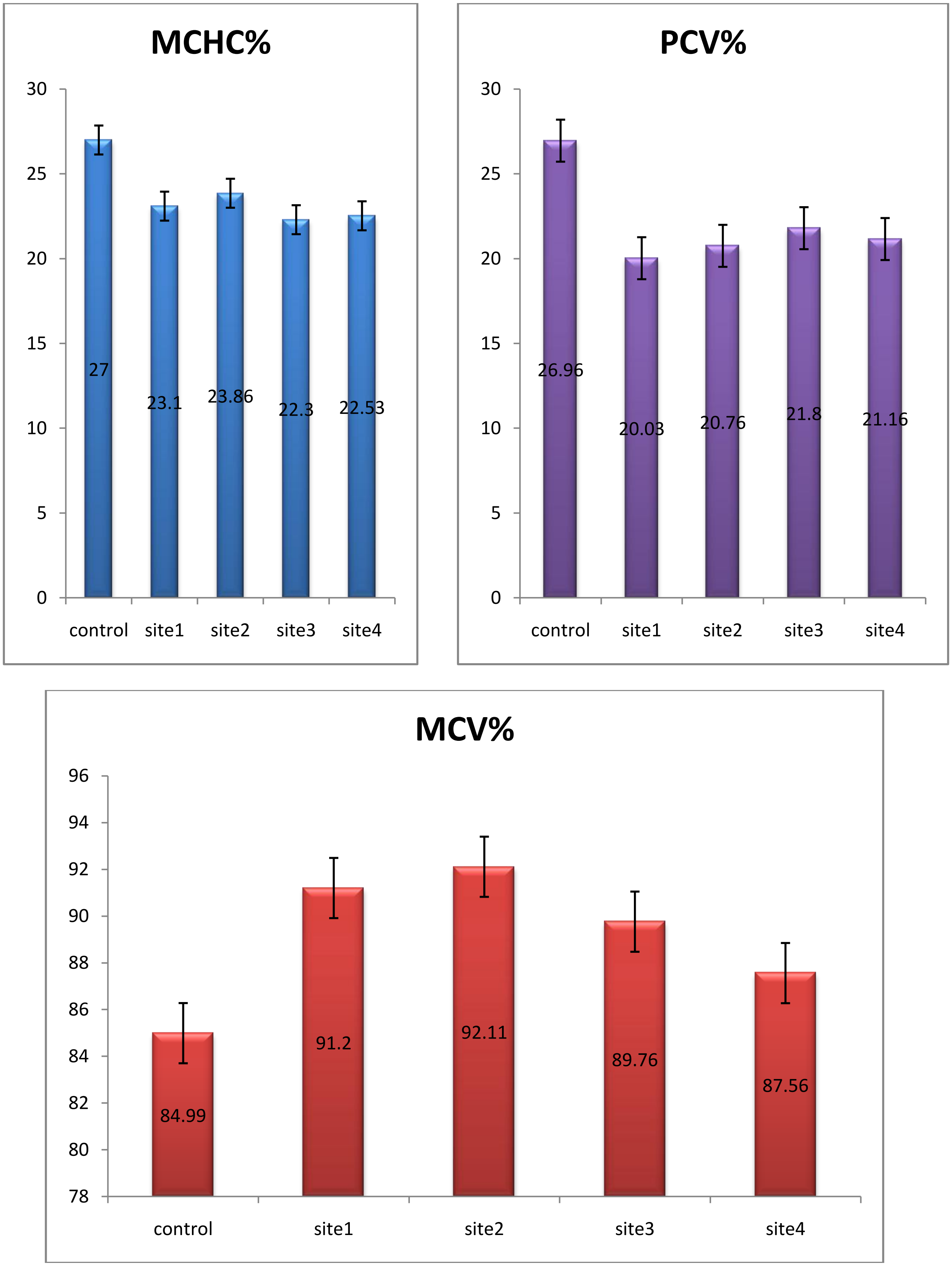

Figure 2: Haematological changes in *Labeo calbasu* caught from different sites of Betwa river during Jan. 2011- Dec. 2012.

The study confirms the altered water quality of Betwa River may be because of discharging industrial, agricultural and sewage effluents, which affect directly on water quality and fish health and human health indirectly. Furthermore, the uses of haematological parameters as biomarkers are very realistic approaches. The results ascertain that the selected fish, *Labeo calbasu* (Ham.) was highly influenced due to the impact of anthropogenic activities on Betwa River as it is highly devastated by various kinds of industries, agricultural practices and many other activities which play a pivotal role in degrading the water quality of Betwa River directly and the aquatic organisms indirectly.

Even though at present, the condition of Betwa overall is not so bad but if the same continues in future, the Betwa river would be completely polluted and becomes unfit for portability and other purposes. So it is time to preserve and protect this valuable river as well as the aquatic fauna, especially *Labeo calbasu* which is very sensitive to pollutants than other fishes as its body is very delicate and usually used to live in natural conditions. For this, various measures have to be taken which will control the contamination from different sources. These include proper management of wastes, industrial effluents etc. and above all the public awareness is must for the conservation of this sacred river and the aquatic organisms especially the studied fish.

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