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Effect of cadmium on Haematological changes in a freshwater catfish, *Heteropneustes fossilis*

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Abstract
Toxic pollutants such as heavy metals are particularly harmful to aquatic life. They can take up metals concentrated at different levels in their different body organs. Thus heavy metals acquired through the food chain as a result of pollution are potential chemical hazards and threatening consumers. The effects of exposure to any hazardous substance depend on the dose, exposure time, the mode of exposure, personal habits, traits, and presence of other chemicals. Therefore it is important to monitor heavy metals in aquatic environment, especially in fish. Increased loads of heavy metals in waste water may increase the risk of ground water contamination. The present work is designed to evaluate effect of cadmium on haematological parameters of *Heteropneustes fossilis.* The main haematological alteration resulting from exposure of *H. fossilis* to various concentrations of cadmium for 7, 14 and 21 days include significant decrease in haematocrit and haemoglobin concentration and in red blood cell counts. The white blood cell counts increased followed by a change in the composition as seen from the differential white blood cell counts. MCHC exhibited a significant decline when compared to control fishes. MCV and MCH values were found to exhibit a significant rise in treated fish than in control fish. The changes in the hematological parameters indicated that they can be used as indicators of cadmium related stress in fish on exposure to elevated levels in the water.

Keywords: *Heteropneustes fossilis*, cadmium, haematological parameters, in haematocrit and haemoglobin

1. Introduction
Fishes have been recognized as good accumulators of organic and inorganic pollutants (Eneji *et al.*, 2011) [11]. Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment. They can take up metals concentrated at different levels in their different body organs. Thus heavy metals acquired through the food chain as a result of pollution are potential chemical hazards and threatening consumers (Sani, 2011) [37]. At low levels, some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes. Other metals, such as cadmium lead are not known to have essential role in living organisms and are toxic even at low concentrations. The essential metals also become toxic at high concentrations. The consequence of heavy metal pollution can be hazardous to man through his food (Sani, 2011) [37].

Fish is the most susceptible of the aquatic animals to these metals. It is cheap, easy to get and is consumed in different forms such as boiling, frying in deep oil, smoking, sun drying amongst others. The physical and chemical environment in which the fish resides appears to influence the rate of bioaccumulation of trace elements in fish. Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid composition higher in cysteine than most other sources of protein. The effects of exposure to any hazardous substance depend on the dose, exposure time, the mode of exposure, personal habits, traits, and presence of other chemicals (Adekola *et al*., 2007) [1]. Therefore it is important to monitor heavy metals in aquatic environment, especially in fish. Increased loads of heavy metals in waste water may increase the risk of ground water contamination. The major anthropogenic sources of heavy metals include waste water run-off from roads, industrial wastes, from mining, manufacturing and metal finishing plants. The present work is designed to evaluate effect of cadmium on haematological parameters of *Heteropneustes fossilis.*

The freshwater catfish, *Heteropneustes fossilis*) is an important group of food fishes in India. This stinging catfish (*H. fossilis*) is commercially important and valuable food species also in many Asian countries (Akand *et al.*, 1991) [3]. *H. fossilis*, commonly known as Shing or Singhi is a popular catfish in India and found naturally in lakes, ponds, swamps and marshes, ditches, floodplains and in muddy rivers. It can survive at a reduced oxygen level (Stickney, 1979) [48]. It is characterized by an accessory respiratory organ (air breathing organ) which enables it to exist for hours when out of water or in indefinitely oxygen-poor water and even in moist mud (Akand *et al*., 1991) [3]. So, this species is very potential in seasonal water bodies of India.

2. Material and Methods
Collection of fishes
The freshwater catfish, *Heteropneustes fossilis* (Bloch) with a size range of 16-20 cm and, weighing 54 ±4 g irrespective of their sex, have been chosen as the test organism in the present study. The fishes were collected from the domestic fish market located at Guntur city ($16^0$20’ N $80^0$27’ E and 31 m elevation), Guntur district, Andhra Pradesh, India.

Acclimatization
Fishes were acclimatized to the laboratory conditions in large

fiber glass tanks with unchlorinated ground water for 3 to 4 weeks at a room temperature of 28 ± 2ºC. As these catfishes are benthic in nature, overcrowding was avoided by keeping small numbers of fishes in each tank. Water was changed on alternate days. Tanks were covered with fish netting to prevent the escape of fishes.

Selection of sub-lethal concentrations

In the present study 1/10th of the 96h LC_{50} value was taken as sub-lethal concentration (A). The two other doses, B & C, used were a reduction in concentration of the sub-lethal concentration (A) in a graded manner. The half concentration of the sub-lethal concentration A (50 % reduction) was used as the second dose (B) while the third dose (C) was 50 % reduction in concentration of the second dose B (Kayode *et al.*, 2016) [23].

Haematological studies

After determining 96 h LC_{50} value, 3 sub-lethal concentrations (A, B, C) of Cadmium chloride were taken and 10 fishes were introduced in each concentration. For each sub-lethal exposure, five replicates were maintained. The water was changed every day in the control and renewed in the treatment group, so that the concentration of cadmium chloride remained the same during the experimental period. *Heteropneustes fossilis* was exposed to sub-lethal concentration of Cadmium for 21 days. At the end of 7th, 14thand 21st day sampling was done. At the end of the exposure period, blood was taken by the following method. The fish were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry towel and the posterior half of its body was blotted with a clean coarse filter paper. Blood from the Control and Cadmium chloride treated fishes were obtained by severance of caudal peduncle and collected in Eppendorf tubes containing 1 % of Ethylene diamine tetra acetic acid (EDTA) as anticoagulant (Mgbenka *et al.*, 2003) [26]. Haematological parameters were estimated by standard methods as described by Hesser (1960) [18] and Blaxhall and Daisley (1973) [5].

Red Blood Corpuscular (RBC) Count

Red blood Corpuscles (RBC) count was done with a Neubauer chamber as described by Sohn and Henry (1969) [46]. The pipette with red glass bead was used for charging the counting chamber. All counts were done in triplicate.

Procedure

The blood was taken in a vial containing Ethylenediamine tetra acetic acid (EDTA) as anticoagulant. Blood was drawn up to 0.5 mark in RBC pipette and immediately, the diluting fluid (Hayem's solution) was drawn up to the 101 mark (thus the dilution is 1:200). Pipette was shaken thoroughly and diluted blood was charged into the counting chamber, after discarding two drops. The solution was allowed to settle for few seconds and the number of RBCs was counted in five small squares of the RBC column under high power microscope and the number of RBCs per cubic mm was calculated.

$$\text{RBC (millions)} = \frac{\text{No. of cells X Dilution factor X Depth factor}}{\textit{Area counted}}$$

Estimation of Haemoglobin (Hb) content

Haemoglobin was determined by the Cyanmethemoglobin method (Dacie and Lewis, 1968)[9]. In this method, all types of Hb will be converted first to methemoglobin and then to cyanomethemoglobin which can be measured colorimetrically. Blood sample of 0.02 ml was pipetted into 5 ml of Drabkin's reagent (commercial name Aculute by Glaxo). It was shaken well and allowed to stand for 10 minutes. Sometimes a jelly like substance was seen in the solution formed by the ruptured cell walls of RBCs. It can be removed by centrifugation. Optical density was measured at 540 nm in a spectrophotometer against a reagent blank. Using a commercial cyanmethemoglobin standard, a standard graph was prepared from which the values of Hb can be read directly as g/dl.

Estimation of Packed Cell Volume (PCV)

PCV was estimated by employing the microhaematocrit method (Snieszko, 1960) [45]. Heparinized, non-clotted blood was collected in unheparinized even bored capillaries. It was allowed to run 1/2 to 3/4 lengths of capillary tube and the tubes were sealed with sealing wax on opposite sides. The tubes were then transferred to a high speed microhaematocrit centrifuge and were placed in the grooves of capillary head. They were centrifuged in the centrifuge at 12000 rpm for 5 minutes. PCV was measured directly on a microhaematocrit reader associated with the centrifuge as volume present.

White Blood Corpuscles (WBC) Count

WBCs were counted according to the method described by Donald Hunter and Bomford (1963) [10].

Procedure

Blood was collected in vials containing EDTA as anticoagulant. The blood was drawn up to 0.5 mark of WBC pipette and immediately diluted fluid, Turk's solution was drawn up to l 1 marks above the bulb. Solution was mixed thoroughly and was allowed to stand for 2 minutes. Solution was expelled and a drop of fluid was allowed to flow under the cover slip. It was allowed to stand for 2 minutes and the WBCs were counted in the 4 comer square millimeters. The number of WBCs per cubic millimeter was calculated accordingly.

$$\text{WBC (millions)} = \frac{\text{No. of cells X Dilution factor X Depth factor}}{\textit{Area counted}}$$

Calculation of RBC constants

Based on the results of the tests which measure total RBC, Hb and PCV several calculations have been derived which give quantitative information about the red blood corpuscles. These values are called RBC constants.

Mean Corpuscular Volume (MCV)

The mean corpuscular volume is the volume of the average cell or the average cell volume of all the RBCs.

$$MCV\ (\mu m3) = \frac{PCV\ \%}{RBC\ in\ million} X\ 10$$

Mean Corpuscular Haemoglobin (MCH)

MCH is the amount of Hb in the average RBC or average amount of Hb per cell in all the red cells.

$$MCH\ (pg) = \frac{Hb\ \left(\frac{g}{dl}\right)}{RBC\ in\ million} X\ 10$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is the portion of the average RBC containing haemoglobin or the concentration in the average cell.

$$MCHC\ (\%) = \frac{Hb\ \left(\frac{g}{dl}\right)}{PCV\ (\%)} X\ 100$$

3. Results and Discussion

Haematology deals with the study of blood and embraces various aspects of physiological, pathological as well as the biochemical characteristics of the blood. Fish haematology is a tool to assess the status of general health. A number of blood parameters such as total RBC count, total WBC count, hemoglobin (Hb) content, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) have been used as indicators of metal pollution in the aquatic environment. In the last three decades, studies on fish blood has gained increasing importance for fishery biologists and ichthyologists for regular monitoring of health of the fish stocks and to develop an information database about inter-specific and intra-specific variations in blood characteristics under exposure to heavy metals.

The results of the present investigation showed various anomalies in the blood of *H. fossilis* during prolonged exposure to cadmium. The mean±SD values of hematological parameters of control and treated fish under sub-lethal concentrations of Cadmium exposed for 7, 14 and 21 days are depicted in Table 1. Significant changes were not observed in the measured variables of fish maintained in uncontaminated water (control). The main haematological alteration resulting from exposure of *H. fossilis* to various concentrations of cadmium for 7, 14 and 21 days included significant decrease in haematocrit and haemoglobin concentration and in red blood cell counts and an increase in white blood cell counts (Table1). MCHC exhibited a significant decline when compared to control fishes. MCV and MCH values were found to exhibit a significant rise in treated fish than in control fish.

Table 1: Cadmium induced changes in Hematological parameters of *H. fossilis*

Treatments	Exposure period	Experiment						
		RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (µm^3)	MCH (pg)	MCHC (%)
Control	7th Day	3.10±0.112	10.52±0.16	38.94±1.16	3.72±0.160	119.80±3.26	33.93±1.22	27.01±0.34
	14th Day	3.11±0.342	10.64±0.68	38.98±1.18	3.78±0.226	121.15±4.12	34.21±1.02	27.29±0.64
	21th Day	3.11±0.645	10.72±0.12	38.98±0.96	3.78±0.468	121.92±2.62	34.46±1.46	27.50±0.46
A	7th Day	2.08±0.342	8.28±0.34	37.18±0.84	3.98±0.234	199.42±2.88	39.80±1.20	22.27±0.48
	14th Day	1.66±0.112	7.02±0.48	35.06±0.82	4.09±0.146	260.60±3.12	42.28±1.16	20.02±0.74
	21th Day	1.22±0.126	5.72±0.34	32.88±0.68	4.24±0.248	367.86±3.78	46.88±1.28	17.39±0.66
B	7th Day	2.42±0.246	9.64±0.28	38.18±0.66	3.90±0.106	164.38±4.12	39.83±0.86	25.27±0.78
	14th Day	1.98±0.242	8.16±0.46	36.68±0.96	3.98±0.426	210.50±3.46	41.21±0.96	22.24±0.84
	21th Day	1.78±0.422	7.20±0.68	35.26±0.88	4.08±0.346	240.78±2.86	42.69±1.08	20.41±0.56
C	7th Day	2.76±0.116	9.82±0.24	38.85±0.86	3.82±0.420	140.76±2.68	35.57±1.28	25.27±0.86
	14th Day	2.48±0.112	9.10±0.26	37.86±0.68	3.88±0.268	160.72±2.78	36.69±1.02	24.03 ±0.66
	21th Day	2.04±0.246	8.46±0.42	36.24±0.84	3.96±0.262	202.15±3.26	41.47±0.98	23.34±0.82

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

A = Sub-lethal conc. (2.068 ppm)**; B** = 50% SL of A (1.034 ppm)**; C** = 50% SL of B (0.517 ppm)

Red Blood corpuscles (RBC)

The present study reveals that there is a significant reduction in RBC of fish after 7, 14 and 21 days of exposure to sub-lethal concentrations of cadmium than in the control fish. The number and percent change in RBC of *H. fossilis* exposed to sub-lethal concentrations of cadmium for 7, 14 and 21 days are given in Table 2 and Figure 1. The maximum percent change of total RBC count was found in treatment- A for 21days (60.77%) and minimum in treatment-C for 7 days (10.96%). The total RBC count was found to decrease with an increase in dosage and duration.

The reduction of RBC at sub-lethal level of cadmium might be due to the destruction of mature RBCs and inhibition of erythrocyte production due to the reduction of haemo synthesis affected by pollutants (Hussein *et al.*, 2011). Gill and Epple (I993) [15] found a significant reduction in RBC in American eel (*Anguilla rostrata*) after exposure to 150 µg Cd/L. Karuppaswamy *et al.* (2005) [26] found a significant decrease in total erythrocyte count in air breathing fish, *Channa punctatus* after exposure to sub lethal dose of Cadmium. The decrease in RBC count may be attributed to haematopathology or acute haemolytic crisis that result in severe anemia in most vertebrates including fish species exposed to different environmental pollutants.

Annune *et al.* (1994) [4] reported a non-significant decrease and swelling of red blood cells in *Oreochromis niloticus*. Flos *et al.* (1987) [12] and Soundararajan *et al.* (2014) [47] stated that the swelling of red blood cells (erythrocytes) may be due to an increase in protein and carbon dioxide in the blood. The decrease in the total erythrocyte count (TEC) may be due to the cytotoxic effect of heavy metal compounds on the erythropoietic tissue. Such a disturbance in bone marrow leads to alteration of cell cycle and reduction in erythropoeisis (Tariq *et al.*, 2006) [49].

Table 2: Changes in RBC and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : RBC(millions)						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	3.10±0.112	2.08±0.342	32.90	2.42±0.246	21.93	2.76±0.116	10.96
2	14th Day	3.11±0.342	1.66±0.112	46.62	1.98±0.242	36.33	2.48±0.112	20.25
3	21th Day	3.11±0.645	1.22±0.126	60.77	1.78±0.422	42.76	2.04±0.246	34.40

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

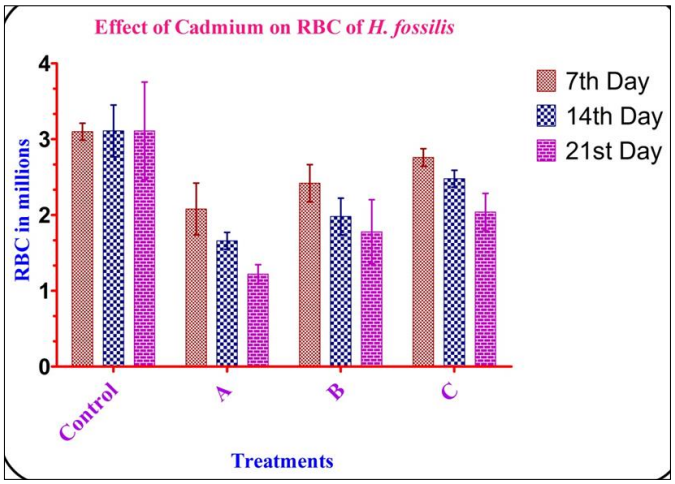

Fig 1: Changes in RBC in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

Haemoglobin (Hb) content

In this study, Hb content significantly decreased in fish exposed to sub-lethal concentration of cadmium for 7, 14 and 21 days than that of control fish. The Hb content and percent changes of effected fish Hb are tabulated in Table 3 and Figure 2. The maximum percent change of Hb content was found in treatment- A for 21days (46.64%) and minimum in treatment-C for 7 days (6.65%). The total Hb concentration was found to decrease with an increase in dosage and duration. These results may be due to cumulative response of cadmium toxicity towards excessive red cell destruction there by lead to anemic to protect the fish against infections under cadmium stress (Kaoud *et al.*, 2011) [20].

According to the results obtained from the present study, Hb seems to be the best blood indicator of environmental stress. Khalesi *et al.* (2014) [4] and Cazenave *et al.* (2005) [8] suggested that the increase in Hb concentration could be a reliable first indicator of an adaptational improvement in the oxygen transporting capacity of the blood. In addition to behavioral and morphological adjustments, fish could respond to low oxygen levels by adjusting several physiological and biochemical parameters (Val *et al.*, 1998) [51]. Reduction in haemoglobin concentration may probably be due to production of reactive oxygen species under the influence of heavy metal cadmium which results in destruction of the red blood cell membrane and its function (Tariq, 2006) [49]. The observed depletion in the haemoglobin values in the fish could also be attributed to the lysing of erythrocytes. The significant reduction in these parameters is an indication of severe anemia caused by exposure of the experimental fish to cadmium in the water (Kaoud *et al.*, 2011) [20].

Soundararajan *et al.* (2014) [47] reported the reduction in Hb content of *Heteropenustes fossilis* exposed to zinc. Similarly the decreased amount of Hb could be corroborated with previous investigations in *Oreochromis mossambicus* exposed to copper and zinc (Senthamilselvan, 2015) [40]. Pamila *et al.* (1991) [32] have reported that the reduction in Hb content might be due to the inhibitory effect of toxic substance on the enzyme systems involved in the synthesis of hemoglobin. Joshi *et al.* (2002) [19] have reported that heavy metal exposure decreased Hb due to impaired intestinal absorption of iron. Karuppasamy *et al.* (2005) [22] observed a significant decrease in Hb in *Channa punctatus* exposed to cadmium. The changes in Hb may be due to immunological reactions to produce antibodies to cope up with the stress induced by cadmium (Vijay Ramdas, 2013) [52].

Table 3: Changes in Hb and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : Hb g/dl						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	10.52±0.16	8.28±0.34	21.29	9.64±0.28	8.36	9.82±0.24	6.65
2	14th Day	10.64±0.68	7.02±0.48	34.02	8.16±0.46	23.31	9.10±0.26	14.47
3	21th Day	10.72±0.12	5.72±0.34	46.64	7.20±0.68	32.84	8.46±0.42	21.08

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

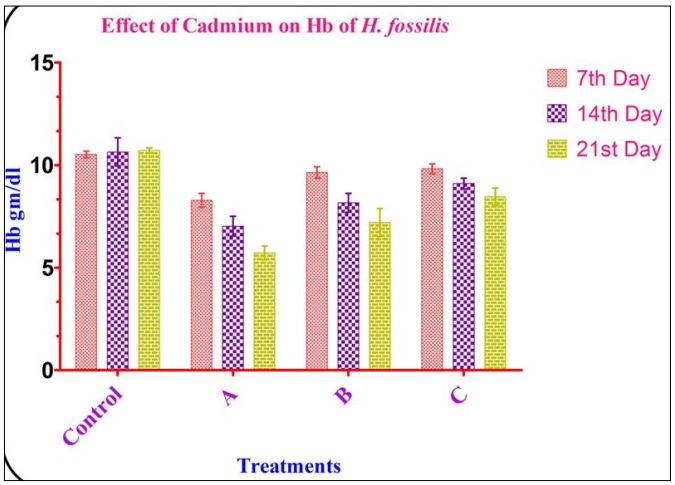

Fig 2: Changes in Hb in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

Packed Cell Volume (PCV)

The present study reveals that the fish exposed to cadmium showed significant decline in PCV. Calculated values of PCV in control and treated fish along with percent change over control are given in Table 4 and Figure 3. The total PCV percentage was found to decrease with increase in dosage and duration. The maximum percent change of packed cell volume was found in treatment-A for 21days (15.64 %) and minimum in treatment-C for 7 days (0.23 %).

The decrease in PCV may be due to the disturbances that occurred in both metabolic and hemopoietic activities of the fish exposed to sub-lethal concentrations of cadmium (Santhakumara *et al.*, 2000) [38]. Mazon *et al.* (2002)[25], Kuruppasamy *et al.* (2005) [22], Gupta *et al.* (2009) [16] and Sharma and Langer (2014) [12] reported that hemopoietic organs get impaired by toxicity resulting in slower erythropoiesis and thereby lead to reduction in PCV.

The present results are in agreement with the earlier works reported (Kuruppasamy, 2000; Tyagi and Srivastava, 2005; Gupta, 2008; Olanike *et al.*, 2008, Witeska *et al.*, 2010 and Kaoud *et al.*, 2011) [21, 50, 17, 29, 55, 20]. Joshi *et al.* (2002) [19] and Sandeep *et al.* (2013) [36] suggested that decrease in PCV is due to hemodilution mechanism because of gill damage or impaired osmoregulation and impaired intestinal absorption of iron. However, Witeska (2005) [52] in *Cyprinus carpio* and Caravalho and Fernandes (2006) [7] in *Prochilodus lineatus* observed copper induced blood alternations, characterized by an increase in PCV. They suggested that heavy meals induced a hypoxic condition in fish that stimulates the spleen for RBC production and release of stored erythrocytes into the circulation. Similar result has also been reported earlier by Ghazaly (1992) [13], Palackova *et al.* (1994) [31] and Wilson and Taylor (1993) [53].

Table 4: Changes in PCV and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : PCV %						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	38.94±1.16	37.18±0.84	4.51	38.18±0.66	1.95	38.85±0.86	0.23
2	14th Day	38.98±1.18	35.06±0.82	10.05	36.68±0.96	5.90	37.86±0.68	2.87
3	21th Day	38.98±0.96	32.88±0.68	15.64	35.26±0.88	9.54	36.24±0.84	7.03

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

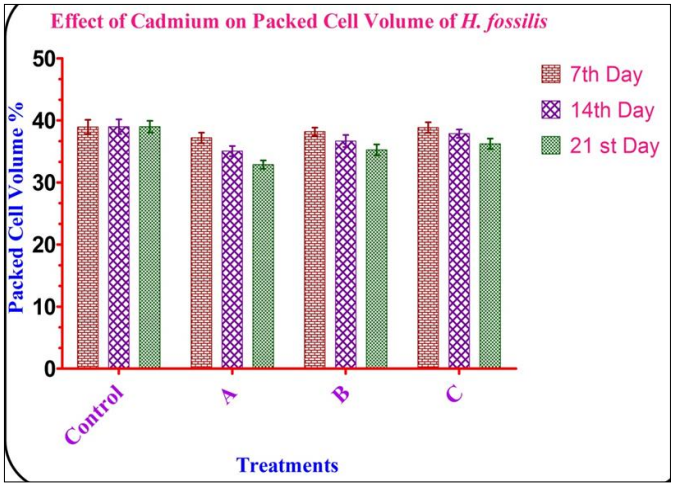

Fig 3: Changes in PCV in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

The Packed cell volume (PCV) appears to be positively correlated with erythrocyte count. Fall in the number of red blood cells followed by PCV confirms anemia in *Heteropneustes fossilis.* The probable mechanisms for developing anemia in *Heteropneustes fossilis* could be due to the loss of erythrocytes as compensatory erythropoiesis could not be observed, which was reflected in the absence of immature erythrocytes in the peripheral blood.

In the present study, similar pattern of reduction in RBC, Hb and PCV indicates anaemic stage of experimental fish caused due to decreased erythropoietic activity or increased destruction of blood cells. The reduction in red blood cell count and haemoglobin percentage indicates the occurrence of acute anaemia (Prasanta Nanda, 1997) [33].

White Blood Corpuscles (WBC)

The results of the present study i.e. calculated values for total WBC in control and exposed fish along with percent change over control are given in Table 5 and Figure 4. The total WBC was found to increase with increase in dosage and duration. The maximum percent change of WBC was found in treatment-A for 21days (12.16%) and minimum in treatment-C for 7 days (2.65%).

Leucocytes or WBC are cells of immune system which play a key role in both non-specific and specific immune responses in protecting the body against foreign substances. Review of literature shows two opposite lines of response of leucocytes

to different heavy metals. One group of researchers reported increase in WBC in fish in response to heavy metal toxicity (Buthelezi, 2000; Singh and Tandon, 2009; Raina and Sachar, 2014 and Sharma and Langer, 2014) [6, 43, 34, 42]. Increase in WBC count can be attributed to a stimulation of the immune system in response to tissue damage caused by heavy metals. Moraes (2007) [27] stated that one of the most elementary ways to assess the immune system is to explore changes in the WBC count.

The other group researchers advocated decrease in WBC in response to heavy metals in fish (Witeska, 2005; Oliveira *et al.*, 2006; Olanike, 2007; Olanike *et al.*, 2008; Safahieh *et al.*, 2010 and Witeska *et al.*, 2010) [54, 30, 28, 29, 35, 55]. The decline in WBC count is due to release of epinephrine during stress because of heavy metal toxicity and weakening of the immune system (Olanike, 2007) [27]. The results of the present study are in accordance with first group of researchers who have observed increase in WBC of fish on exposure to cadmium.

Table 5: Changes in WBC and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : WBC (millions)						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	3.72±0.160	3.98±0.234	6.98	3.90±0.106	4.84	3.82±0.420	2.65
2	14th Day	3.78±0.226	4.09±0.146	8.02	3.98±0.426	5.29	3.88±0.268	2.69
3	21th Day	3.78±0.468	4.24±0.248	12.16	4.08±0.346	7.94	3.96±0.262	4.76

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

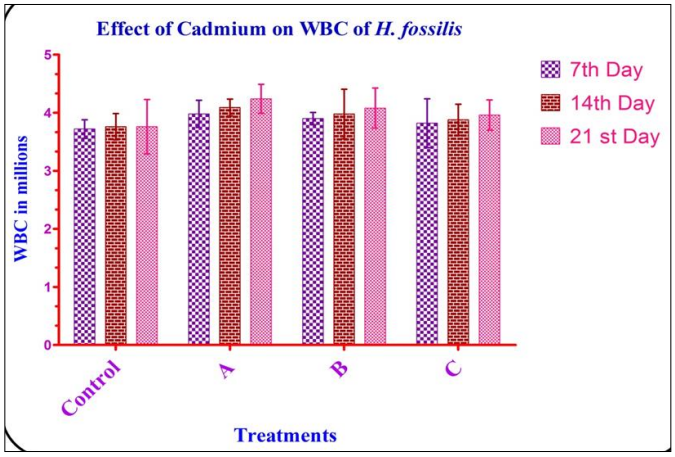

Fig 4: Changes in WBC in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

Mean Corpuscular Volume (MCV)

MCV (µm3) values were found to exhibit significant rise in treated fish exposed to various sub-lethal concentrations of cadmium for 7, 14 and 21 days than in control fish. The MCVs and their percent changes are represented in Table 6 and Figure 5. The total MCV was found to increase with increase in dosage and duration. The maximum percent change of MCV was found in treatment-A for 21days (201.72%) and minimum in treatment-C for 7 days (17.50%).

Mean corpuscular volume (MCV) is one of the important blood parameters which gives an indication of the status of size of RBC. In the present study a marked increase in MCV was observed which can be attributed to reduction in total erythrocyte count because MCV and total erythrocyte count exhibit inverse relationship with each other. This is in agreement with the works of Shah (2006) [41] in *Tinca tinca*, Olanike (2007) [29] in *Clarias gariepinus*, Afaq (2009) [2] in *Cirrhinus mrigala*, Gupta *et al.* (2009) [16] in *Labeo boga*, Sandeep *et al.* (2013) [36] in *Labeo rohita*, and Sharma and Langer (2014) [42] in *Garra gotyala*. The increase in MCV may be due to the enlargement of RBCs as a result of hypoxic condition or osmotic disturbances and uptake of electrolytes and water into the cells accompanied by acidification of cytoplasm of RBC or macrocytic anemia in fishes exposed to metal pollution (Sinha *et al.*, 2000) [44].

Table 6: Changes in MCV and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : MCV(µ m^3)						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	119.80±3.26	199.42±2.88	66.46	164.38±4.12	36.89	140.76±2.68	17.50
2	14th Day	121.15±4.12	260.60±3.12	115.10	210.50±3.46	73.75	160.72±2.78	32.66
3	21th Day	121.92±2.62	367.86±3.78	201.72	240.78±2.86	97.49	202.15±3.26	65.80

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

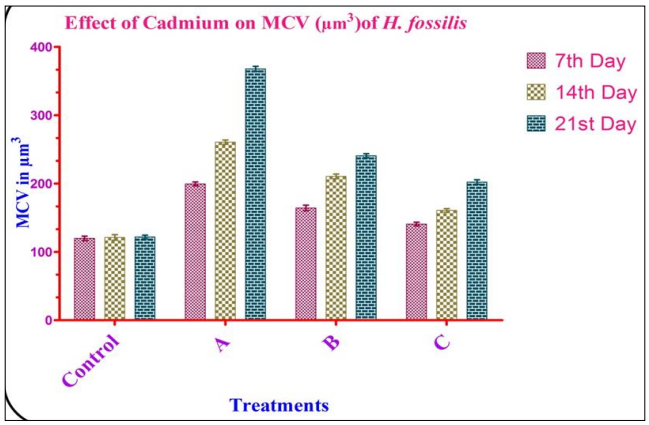

Fig 5: Changes in MCV in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

Mean Corpuscular Haemoglobin (MCH)

The present study reveals that the fish exposed to cadmium showed significant increase in MCH. Calculated values for MCH in control and exposed fish along with percent change over control are given in Table 7 and Figure 6. The total MCH was found to increase with increase in dosage and duration. The maximum percent change of WBC was found in treatment-A for 21days (36.04%) and minimum in treatment-C for 7 days (4.83%).

MCH represents the average weight of haemoglobin in RBC. MCH exhibited an increasing trend in all exposure periods of sub-lethal concentrations. These results are in line with the findings of Sandeep *et al*. (2013) [36], Raina and Sachar (2014) [34] and Sharma and Langer (2014) [42]. Such changes in MCH may either be due to the increased lysis of RBCs or reduction in cellular blood iron thereby resulting in reduced Hb synthesis (Sharma and Langer, 2014). In contrast to the above results, no significant effects of metal toxicity on MCH have been observed by Oliveira *et al*. (2006) [30], Carvalho and Fernandes (2009) [7] and Safahieh *et al*. (2010) [35] in fish.

Table 7: Changes in MCH and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : MCH (pg)						
		Treatments						
		Control	A	% Change	B	% Change	C	% Change
1	7th Day	33.93±1.22	39.80±1.20	17.30	39.83±0.86	17.39	35.57±1.28	4.83
2	14th Day	34.21±1.02	42.28±1.16	23.59	41.21±0.96	20.46	36.69±1.02	7.25
3	21th Day	34.46±1.46	46.88±1.28	36.04	42.69±1.08	23.88	41.47±0.98	20.34

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

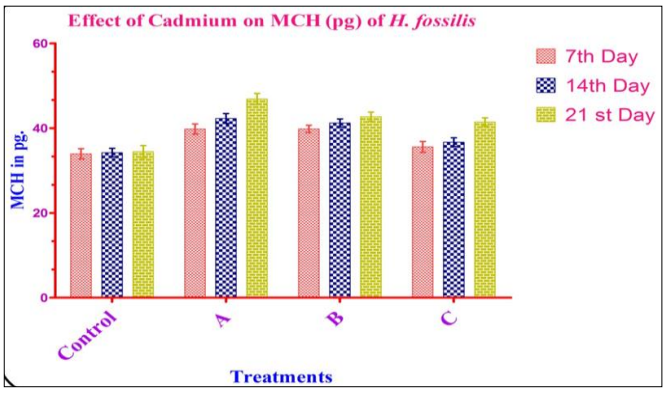

Fig 6: Changes in MCH in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

Mean corpuscular Haemoglobin Concentration (MCHC)

In the present investigation, significant decrease in MCHC was noticed in fish exposed to cadmium than in control. The MCHC values and percent changes are presented in Table 8 and Figure 7. The total MCHC was found to decrease with increase in dosage and duration. The maximum percent change of MCHC was found in treatment-A for 21days (36.76%) and minimum in treatment-C for 7 days (6.44%).

MCHC is the indication of average concentration of haemoglobin in RBC cells, calculated based on Hb and PCV. Decrease in Hb and PCV coupled with increase in MCV might be the reason for the decreased MCHC.

Table 8: Changes in MCHC and percent change over the control in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

S. No	Exposure Period	Parameter : MCHC %						
		Control	Treatments					
			A	% Change	B	% Change	C	% Change
1	7th Day	27.01±0.34	22.27±0.48	17.55	25.27±0.78	6.44	25.27±0.86	6.44
2	14th Day	27.29±0.64	20.02±0.74	26.64	22.24±0.84	18.50	24.03±0.66	11.95
3	21th Day	27.50±0.46	17.39±0.66	36.76	20.41±0.56	25.78	23.34±0.82	15.13

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test)

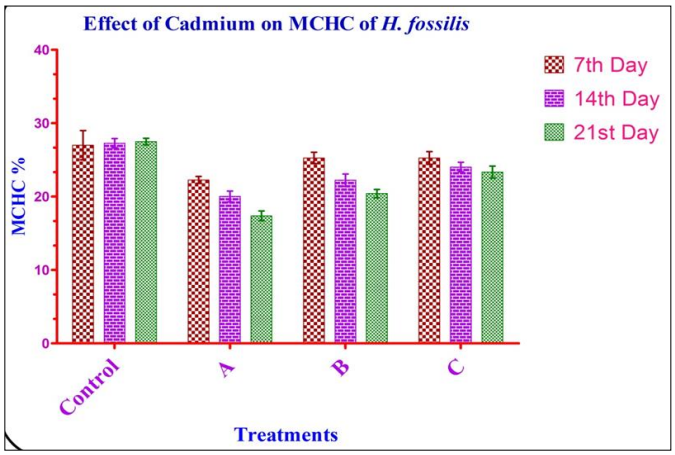

Fig 7: Changes in MCHC in the blood of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7, 14 and 21 days

The present results are in line with the findings of Sandeep *et al.* (2013) [36], Raina and Sachar (2014) [34] and Sharma and Langer (2014) [42]. Such changes in MCHC may be either due to the increased lysis of RBCs or reduction in cellular blood iron thereby resulting in reduced Hb synthesis (Sharma and Langer, 2014) [42]. The low concentration of MCHC during the treatment might have resulted from decrease in Hb synthesis due to toxic action or swelling of erythrocytes by which blood oxygen transport capacity is increased when fish were subjected to less effective gas exchange (Saravanan *et al.*, 2011) [39]. Alterations in MCV, MCH and MCHC clearly indicated that the fish are under chemical stress, which lead to pathological condition in the tissues. Gill *et al.* (1991) [14] have also studied the haematology of different fish in response to different chemical stresses and the present observations corroborate with their findings.

4. Conclusion

The circulatory system of fish is in close association with the external environment and with every tissue. It is sensitive to foreign stimuli and reflects the homeostasis of the animal. Thus haematological studies helped to check the systemic responses during stress conditions due to cadmium. The main haematological alteration resulting from exposure of *H. fossilis* to various concentrations of cadmium for 7, 14 and 21 days include significant decrease in haematocrit and haemoglobin concentration and in red blood cell counts. The white blood cell counts increased followed by a change in the composition as seen from the differential white blood cell counts. MCHC exhibited a significant decline when compared to control fishes. MCV and MCH values were found to exhibit a significant rise in treated fish than in control fish. The changes in the hematological parameters indicated that they can be used as indicators of cadmium related stress in fish on exposure to elevated levels in the water.

5. References

1. Adekola FA, Eletta OAA. A study of heavy metal pollution of Asa River, Ilorin. Nigeria; trace metal monitoring and geochemistry. Environmental Monitoring and Assessment. 2007; 125(1-3):157-163.
2. Afaq S. Toxicological effects of Leather dyes on total Leucocyte count of fresh water teleost, *Cirrhunus mrigala* (Ham). Biology and Medicine. 2009; 1(2):134-138.
3. Akand AM, Hasan MR, Habib MAB. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, *Heteropneustes fossilis*. *In*: S. S. De Silva (ed.). Fish nutrition research in Asia. Proc. of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines. 1991; 93-100.
4. Annune PA, Ebele SO, Oladimeji AA. Acute toxicity of cadmium to juveniles of *Clarias gariepinus (Teugels*) and *Oreochromis niloticus* (Trewavas). Journal of Environmental Science and Health Part A. 1994; 29(7):1357-1365.
5. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. J Fish. Biol. 1973; 5:771-781.
6. Buthelezi PP, Wepener V, Cyrus DP. The sub-lethal effect of zinc at different water temperatures on selected haematological variables in *Oreochromis mossambicus*. American Journal of Aquatic Sciences. 2000; 25:146-151.
7. Carvalho CS, Fernandes MN. Effect of temperature on copper toxicity and haematological responses in the neotropical fish *Prochilodus scofa* at low and high pH. Aquaculture. 2009; 251:109-117.
8. Cazenave J, Wunderlin DA, Hued AC, de los Angeles Bistoni, M. Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842), (*Pisces, Callichthyidae*) captured from pristine and polluted water. Hydrobiologia. 2005; 537(1-3):25-33.
9. Dacie JV, Lewis K. Practical Haematology. Churchill, London, 1968.
10. Donald Hunter, Bomford. A Dangerous Misprint. British Medical Journal. 1963; 1:1024.
11. Eneji IS, Sha'Ato R, Annune PA. Bioaccumulation of heavy metals in fish (*Tillapia zilli and Clarias gariepinus*) organs from river Benue, North Central. Pakistan Journal of Analytical/ Envt. 2011; 12(1&2):25-31.
12. Flos R, Tort L, Balasch J. Effects of zinc sulphate on haematological parameters in the dogfish *Scyliorhinus canicula* and influences of MS 222. Marine environmental research. 1987; 21(4):289-298.
13. Ghazaly KS. Sub-lethal effects of nickel on carbohydrate metabolism, blood and mineral contents of *Tilapia nilotica*. Water, Air, Soil Pollution. 1992; 64:525.
14. Gill S, Tewari H, Pande J. Effects of waterborne copper and lead on the peripheral blood in the Rosy Barb, Barbus (*Conchonius* Hamilton). Environ. Contam. Toxicol. 1991; 40:606-612.
15. Gill TS, Epple A. Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata*). Ecotoxicology and environmental safety. 1993; 25(2):227-235.
16. Gupta A, Rai DK, Pandey RS, Sharma B. Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environ Monito Assess. 2009; 157:449-458.
17. Gupta K. Copper induced toxicity on haematological and haemopoietic profile of *Puntius sophore* (Ham.). M.phil. Dissertation, University of Jammu, India, 2008.

18. Hesser EF. Methods for routine fish hematology. The Progressive Fish Culturist. 1960; 22(4):164-171.
19. Joshi PK, Bose M, Harish D. Haematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. Ecotoxicol. Environ. Monit. 2002; 12:119-122.
20. Kaoud HA, Zaki MM, El-Dahshan AR, Saeid S, El Zorba HY. Amelioration the toxic effects of cadmium-exposure in nile tilapia (*Oreochromis niloticus*) by using *Lemna gibba* L. Life Science Journal. 2011; 8(1):185-195.
21. Karuppasamy R. Impact of pheny mercuric acetate (PMA) on the biomodel respiration in an airbreathing fish *Channa punctatus* (Bloch). Journal of Environmental Pollution. 2000; 7:287-293.
22. Karuppasamy R, Subathra S, Puvaneswari S. Haematological responses to exposure to sublethal concentration of cadmium in air-breathing fish *Channa punctatus* (Bloch). Journal of Environmental Biology. 2005; 26(1):123-128.
23. Kayode JS, Adelusi AO, Nawawi MNM, Bawallah M, Olowolafe TS. Geoelectrical investigation of near surface conductive structures suitable for groundwater accumulation in a resistive crystalline basement environment: a case study of Isuada, southwestern Nigeria. J Afr. Earth Sci. 2016; 119:289-302.
24. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure Novelty and Significance. Hypertension. 2014; 64(4):897-903.
25. Mazon AF, Monteiro EAS, Pinheiro GHD, Fcrnandes MN. Haematological and physiological changes induced by short-term exposure to copper in the fresh water fish, *Prochilodus scrofa*. Brazilian Journal of Biology. 2002; 62(4A):621-631.
26. Mgbenka BO, Oluah NS, Umeike I. Effect of Gammalin 20 (lindane) on differential white blood cell counts of the African catfish, *Clarias albopunctatus.* Bulletin of Environmental Contamination and Toxicology. 2003; 71(2):248-254.
27. Moraes FR. Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. Veterinay Clinical Pathology, Madison. 2007; 36:49-54.
28. Olanike KA. Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead. Turkish Journal of Fisheries and Aquatic Sciences. 2007; 7:163-169.
29. Olanike K, Funmilola A, Olufemi B, Olajide O. Sub lethal concentrations toxicity and blood profile of adult *Clarias gariepinus* exposed to lead nitrate. Int. J Hematol. 2008; 4:2-10.
30. Oliveira Ribeiro CA, Filipak Neto F. Haematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methyl mercury, inorganic lead and tributyltin chloride. Environmental Research. 2006; 101:74-80.
31. Palackova J, Pravda D, Fasiac K, Celechovska O. Sublethal effects of cadmium on carp (Cyprinus carpio) fingerlings. Sublethal and Chronic effects of pollutants on Freshwater fish (R. MuKller and R. Lloyd, Eds.). Arnette Blackwell SA, France. 1994; 53-61.
32. Pamila D, Subbaiyan PS, Ramaswamy M. Toxic effects of chromium and cobalt on *Sarotherodan mossambicus* (Peters). Indian. J Environ. Health. 1991; 33:218-225.
33. Prasanta Nanda. Haematological changes in the common Indian catfish *Heteropneustes fossilis* under nickel stress. J Ecobiol. 1997; 9(4):243-246.
34. Raina S, Sachar A. Effect of heavy metal zinc and carbamate pesticide sevin on haematological parameters of fish, *Labeo boga*. International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3(5):12636-12644.
35. Safahieh A. Hedyati A, Savari A, Movahedinia A. Experimental approaches of hematotoxic and immunotoxic effects of mercury chloride on yellowfin sea bream (*Acanthopagrus latus*). American-Eurasian Journal of Toxicological Sciences. 2010; 2(3):169-176.
36. Sandeep V, Praveena M, Kavitha N, Jayantha Rao K. Impact of Tannery Effluent, Chromium on Hematological Parameters in a Fresh water Fish, *Labeo rohita* (Hamilton). Res. J of Animal, Veterinary and Fishery Sciences. 2013; 1(6):1-5.
37. Sani U. Determination of some heavy metals concentration in the tissues of Tilapia and Catfishes. Biokemistri. 2011; 23(2):73-80.
38. Santhakumara M, Balaji M, Amudu K. Adaptive changes in respiratory movements of an air breathing fish Anabus testudineus exposed to organophosphate pesticide monocroptophos. Eco. Env. Conserv. 2000; 6(1):67-69.
39. Saravanan M, Karthika S, Malarvizhi A, Ramesh M. Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: Hematological, biochemical, ionoregulatory and enzymological responses. J Haz. Mater. 2011; 195:188-194.
40. Senthamilselvan Chezhian A, Suresh E. Acute toxicity of chromium and mercury to Lates calcarifer under laboratory condition. Int. J of Fisheries and Aquatic Studies. 2015; 2(4):54-57.
41. Shah SL. Hematological parameters in tench *Tinca tinca* after short term exposure to lead. J Appl. Toxicol. 2006; 26:223-228.
42. Sharma J, Langer S. Effect of Manganese on haematological parameters of fish, *Garra gotyla gotyla*. Journal of Entomology and Zoology Studies. 2014; 2(3):77-81.
43. Singh BP, Tandon PK. Effect of river pollution on haematological parameters of fish *Wallago attu*. *Res.* Environ. Life Sci. 2009; 2(4):211-214.
44. Sinha AK, Sinha MK, Adhikari S. Effect of the copper toxicity on haematological profile of Indian major carp *Labeo rohita*. Hand book Industry Environment and Pollution. 2000, 166-172.
45. Snieszko SF. Microliacmatocrit as a tool in fisheries management. Special Scientific Reports-Fisheries. No.3l4. U. S. Dept. Inter. Fish and Fisheries WildLife Special Sci-Report. 1960, 15.
46. Sohn ID, Henry IB. *In*: Todd-Sunford clinica diagnosis by Laboratatory methods, 14th edn., W. B. Saunders company, Philadelphia, London, Toronto. 1969; 139-143.
47. Soundararajan M, Veeraiyan G. Effect of heavy metal

arsenic on haematological parameters of fresh water fish *Tilapia mossambica*. Int. J Modn. Res. Rev. 2014; 2(3):132-135.
48. Stickney RR. Principles of Warm Water Aquaculture. New York: John Wiley and Sons, 1979.
49. Tariq J, Ashraf M, Jaffar M, Afzal M. Pollution status of the Indus river, Pakistan, through heavy metal and macronutrient contents of fish, sediment and water. Water Research. 1996; 30(6):1337-1344.
50. Tyagi A, Srivastava N. Haematological response of fish *Channa punctatus* (Bloch) to chronic zinc exposure. J Env. Biol. 2005; 26(2):429-432.
51. Val AL, Silva MNP, Almeida-Val VMF. Hypoxia adaptation in fish of the Amazon: a never ending task. S Afri J Zool. 1998; 33(2):107-114.
52. Vijay Ramdas B. Protective role of ascorbic acid on the cadmium induced changes in hematology of the freshwater fish, *Channa orientalis* (Schneider). Adv Appl Sci Res. 2013; 4:305-308.
53. Wilson RW, Taylor EW. The physiological responses of fresh water rainbow trout, *Oncorhynchus myskiss*, during acutely lethal copper exposure. Comparative Physiologv. 1993; 163:38-47.
54. Witeska M. Stress in fish. Hematological and Immunological effects of heavy metals, Electronic Journal of Ichthyology. 2005; 1:35-41.
55. Witeska M, Kondera E, Szymanska M, Ostrysz M. Hematological changes in common carp (*Cyprinus carpio* L.) after short-term Lead (Pb) exposure. Polish J of Environ. Stud. 2010; 19(4):825-831.