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Research Article

# Evaluation of Toxic Stress of Copper Sulphate and Lead Nitrate on Hematological and Serum Biochemical Characteristics of Freshwater Cyprinid (*Labeo rohita*)

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***Abstract***

*L. rohita, the common freshwater cyprinid in Pakistan, is currently living in such habitats that are contaminated with heavy metals. Copper and lead metals are considered as the strong aquatic pollutants in this area. The aim of the study was to determine 96h $LC_{50}$ values of copper sulphate [$CuSO_4.5H_2O$] and lead nitrate [$Pb(NO_3)_2$] for L. rohita and also to demonstrate the effects of their acute concentrations on hematology and serum biochemistry of the candidate fish. The 96h $LC_{50}$ values for [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] were found to be 3.15 mg/L and 6.80 mg/L respectively. Based on $LC_{50}$ values, the experiments were conducted for 96h at acute concentrations of [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] and data revealed a significant decrease ($P<0.01$) in RBC count, Hb, WBC count, and PCV. In contrast, a significant increase ($P<0.01$) in mean corpuscular hemoglobin concentration (MCHC) values was observed for treated groups indicating the signs of anemia. Among biochemical indices, the significantly higher values ($P<0.01$) for plasma $NH_3$, LDH, TRIG, CHOL, LDL, VLDL, UA, CPK, and BRN were observed in experimental groups compared with the control. In contrast, lower value of AP, UR, HDL and ALB were observed in treated groups compared with control. The study revealed that copper sulphate and lead nitrate are toxic agents for L. rohita. The study also apprehends the fish consumers as a diet towards the similar hematological and biochemical consequences with the consumption of such metal accumulated fish meat.*

***Keywords:*** *Labeo rohita; Copper sulphate; Lead nitrate; Hematology; Serum biochemistry.*

## Introduction

The most common cause of water pollution in developing countries is domestic and industrial waste that is directly released into streams or ponds without treatment. These wastes mostly contain various types of pollutants such as heavy metals, radioactive substances, pesticides, herbicides and corrosive substances like acids and bases (Mhadhbi *et al.,* 2012). However in Pakistan, another prominent source of aquatic pollution is agriculture industry; where growers use pesticides to manage pests. These pesticides contain various heavy metals such as copper, lead, nickel, chromium, cadmium, zinc and manganese as active ingredients (Samanta *et al.,* 2005). Among these heavy metals, the copper and lead, being stable and persistent, are ubiquitous environmental pollutants and are recognized as strong toxic metals to living organisms, including fish and man. These pollutants in aquatic environment can pose adverse effects on growth, physiology, reproduction and survival risk of aquatic organisms especially on fish (Malik *et al.,* 2010).

Fish is the diverse class of aquatic animals (Hoar and Randall, 2001). Fish is an appropriate species among aquatic animals to act as a biological indicator of water pollution (Sudagar and Hajibeglou, 2010). Many ecological and physiological factors are known to affect fish hematology (Oshode *et al.,* 2008). As in veterinary medicine, the hematological, biochemical and serum parameters of fish are used as an index to appraise physiological responses and to identify structural and functional prestige of vigor during stress conditions in a number of fish species; including the influence of toxicity on ionic regulation and gill $Na^+/K^+$ - ATPase activity of teleost fish *etc.* can be used to monitor stress instigated by pollutants (Suvetha *et al.,* 2010). These hematological indices are expedient to afford evidence analogous to that given by human blood variables. It is studied by a number of researchers that piscine RBCs, however, are more sensitive to stressors and often demonstrate variation in morphology and negative efficiency as far as oxygen carriage is concerned (Rowan, 2007).

Stress is an energy-arduous metabolic process and the fish have to try to mobilize energy substrates to handle with the stress state (Vijayan *et al.,* 1996 a). Thus, hematological indices such as RBCs count, PCV and Hb concentration have been used as signs for weighing fish

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health (De Pedro *et al.,* 2005). Thus, hematology unlocks the door for a number of investigators of worldwide to emphasize their attention in studying the variables, upsetting the living organisms including both fish and human being. Therefore, the hematological variables in fish are considered as useful biosensors for aquatic pollution. Recent studies have suggested that any type of alteration in fish blood cell characteristics (number, shape, components, etc.) as well as in plasma enzymes profile could be diagnostic indicators of environmental stress (Llorente *et al.,* 2002). Although the perturbation induced by [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] in fish blood parameters is well documented, but the variability of the reported results are largely dependent on the intensity of pollution, fish species, age, sex and water quality characteristics. Therefore, hematological studies have been frequently used as biosensor veritable tools of evaluating the state health of fish in ichthyologic research (Palacious and Risbourg, 2006). Joshi *et al*. (2002) suggested that heavy metal exposure is the root cause of diminution of the RBCs, Hb and PCV due to reduced intestinal captivation of iron. The elevations in the levels of serum uric acid of fish exposed to metals or any pollutant is the reflection of reduced renal function or kidney damage (Okonkwo and Ejike, 2011).

In Pakistan, copper ($Cu^{+2}$) levels in fresh water bodies such as rivers, streams, ponds *etc* range between 0.07 to 5.14 mg/L while that of lead ($Pb^{+2}$) ranges from 1.12 to 5.65 mg/L which are significantly greater than the scientifically accepted (<1.0 mg/L) level (Nergis *et al.,* 2009).

*L. rohita*, the common freshwater cyprinid, is chiefly cultivated in rivers, ponds, lakes *etc* for public consumption as a rich protein source in Pakistan. At present, the natural habitats of this fish are declining because of pollution with heavy metals. Based on current status of our natural aquatic environments and the frequent use of pesticides and their penetrating impacts on these environments, the present study was conducted to sort out the after effects of the dissolved elements in water bodies on the fish. The trials in the experiments were conducted to investigate 96h $LC_{50}$ values of [$CuSO_4.5H_2O$] and that of [$Pb(NO_3)_2$], the active ingredients of most pesticides frequently used by growers, with emphasis to investigate the effect of acute concentrations of [$CuSO_4.5H_2O$], [$Pb(NO_3)_2$] and their mixture on hematology and plasma biochemistry of economically important freshwater cyprinid fish, (*L. rohita*). To the best of our knowledge, it is the first study in Pakistan pertaining to hematological and biochemical consequences in *L. rohita* due to heavy metal toxicity.

## Materials and Methods

### *Experimental animals*

Juveniles of *L. rohita*, of both sex, with total body length ranging between 7.6-11.7 cm and body weight between 5.15-11.40 g were used.

### *$LC_{50}$ determination*

To determine the 96h $LC_{50}$ values, 15 juveniles of *L. rohita* were exposed to each of the seven concentrations {0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 7.0 mg/L of [$CuSO_4.5H_2O$]}. In another experiment, 15 juveniles were exposed to one of the seven concentrations {1.5, 3.0, 4.5, 6.0, 9.0, 12.0, 18.0 mg/L of [$Pb(NO_3)_2$]}. The fish mortality was recorded after 24, 48, 72, 96, 120, 144 and 168 hours. The 96h $LC_{50}$ values for [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] and their 95% confidence limit was determined using probit analysis (Latif, *et al.,* 2013). The control mortality was corrected using Abbott (1925) formula, where necessary. The 96h $LC_{50}$ value of [$CuSO_4.5H_2O$] for *L. rohita* was found to be 3.15 mg/L and for [$Pb(NO_3)_2$] it was 6.80 mg/L.

### *Experimental design*

Four groups of fish juveniles, 19 individuals in each group, in four separate well aerated concrete tanks were exposed for 96h. i). Control group (C), exposed to toxicant free lab water, ii). Experimental Group 1 (EG-1), exposed to 3.15 mg/L of [$CuSO_4.5H_2O$], iii). Experimental Group 2 (EG-2), exposed to 6.80 mg/L of [$Pb(NO_3)_2$] and iv). Experimental Group 3 (EG-3), exposed to 1.575 mg/L of [$CuSO_4.5H_2O$] + 3.40 mg/L of [$Pb(NO_3)_2$]. The physicochemical characteristics of the laboratory were: Capacity of each tank = 300 L; temperature= 25.0±1.0°C, pH= 6.5-6.8. All experiments were carried out in triplicate in semi-static systems with renewal of water after every 12h interval, with the regular addition of fresh solution of toxicant with same concentration to sustain the nominal concentrations of [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$]. Cares were taken during handling of fish to avoid stress. Temperature, pH and oxygen concentration of water were maintained throughout the experiments as previously described by Latif, *et al.,* (2013). Blood samples for hematological and biochemical tests were collected from both the experimental and control fish juveniles that survived the 96h toxicant exposure period, following the international criteria for aquatic life protocol used by Ololade and Oginni, (2010); Rey Vazquez and Guerrero, (2007). All the experimental procedures and fish handling protocols were approved by Ethical Committee of Zoology Department, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

### *Blood sample collection and measurement of hematological indices*

At the end of each experimental period, the fish was anesthetized by using tricaine methanesulfonate as anesthetics and 1-2 ml of blood sample was collected from the *v. caudalis* using heparinized syringe (Heparin inj.) from both experimentally treated fish and control group. 0.5 ml of the blood sample was directly used to measure the hematological parameters while the remaining volume of the blood sample was preserved in vials containing 400µl of 0.5M EDTA for the determination of biochemical indices. The hematocrit (PVC) was determined using the microhematocrit method of Snieszko (1960), while the hemoglobin (Hb) was measured using

**Table 1** Hematological profile of *L. rohita* in EG-1, EG-2 and EG-3, when compared with the control

| **Parameters** | (C) (N=19) (Mean ± S.D). | EG-1 (Cu treated) (N=19) (Mean ± S.D). | EG-2 (Pb treated) (N=19) (Mean ± S.D). | EG-3 (Cu+ Pb treated) (N=19) (Mean ±S.D). |
|---|---|---|---|---|
| RBC Count($x10^6$ $\mu l^{-1}$) | 1.220±0.0184 | 1.1795±0.0181 [a] | 1.1874±0.0156 [a] | 1.0184±0.0248 [a] |
| Hemoglobin (g/dl) | 4.473±0.331 | 4.142±0.208 [a] | 4.204±0.243 [a] | 4.023±0.430 [a] |
| PCV (% ) | 13.416±0.993 | 12.432±0.631 [a] | 12.626±0.723 [a] | 12.06±1.29 [a] |
| MCV(Fl) | 109.96±8.40 | 105.14±6.14 [b] | 106.04±6.28 [b] | 118.5±13.30 [a] |
| MCH(Pg) | 36.65±2.80 | 35.05±2.05 [b] | 35.35±2.09 [b] | 39.50±4.43 [a] |
| MCHC(g/dl) | 26.69±2.62 | 29.92±2.92 [a] | 30.52±5.38 [a] | 32.40±7.82 [a] |
| WBC Count (x $10^3$ $\mu l^{-1}$) | 8.19±1.06 | 6.79±1.043 [a] | 7.52±1.13 [b] | 6.50±1.97 [a] |

The hematological values are expressed as the mean ± S.E. Superscript "a" statistically highly significantly different (a = 0.001) from control according to Duncan's New Multiple Range Test. Superscript "b" indicates non-significant (b = 0.05)

**Table 2** Serum biochemical profile *L. rohita* in EG-1, EG-2 and EG-3, when compared with the control

| **Parameters** | **(C)** (N=19) (Mean ± S.D). | **EG-1 (Cu treated)** (N=19) (Mean ± S.D). | **EG-2 (Pb treated)** (N=19) (Mean ± S.D). | **EG-3 (Cu+ Pb treated)** (N=19) (Mean ± S.D). |
|---|---|---|---|---|
| Triglycerides (mg/dl) | 57.77±2.78 | 67.27±3.74 [a] | 62.39±5.24 | 70.97±4.03 [a] |
| Cholesterol (mg/dl) | 106.02±5.35 | 125.02±6.16 [a] | 112.16±7.93 | 139.1±18.20 [a] |
| H.D.L(mg/dl) | 21.63±1.94 | 16.17±1.77 [a] | 18.34±1.98 [a] | 14.15±1.90 [a] |
| L.D.L(mg/dl) | 56.92±3.25 | 76.41±4.76 [a] | 64.75±4.41 [a] | 89.0±12.30 [a] |
| V.L.D.L(mg/dl) | 11.553±0.557 | 13.455±0.748 [a] | 12.48±1.05 [b] | 14.194±0.807 [a] |
| LACT(µg/dl) | 1.522±0.0821 | 1.744±0.382 [b] | 1.843±0.401 [b] | 1.858±0.426 [b] |
| AST(U/L) | 33.09±1.83 | 35.23±3.40 [b] | 36.56±4.31 [b] | 39.26±6.66 [a] |
| ALT(U/L) | 32.40±1.89 | 36.66±4.82 [b] | 34.09±3.85 [b] | 38.39±8.25 [b] |
| L.D.H(U/L) | 59.54±2.38 | 83.04±7.53 [a] | 70.30±14.70 [b] | 97.80±17.00 [a] |
| GGT(U/L) | 15.076±0.969 | 17.49±1.24 [a] | 16.00±2.88 [b] | 19.48±1.51 [a] |
| ALP(U/L) | 3.569±0.307 | 3.921±0.502 [b] | 4.387±0.829 [a] | 4.262±0.613 [a] |
| Glucose(mg/dl) | 7.363±0.578 | 8.47±1.70 [b] | 8.81±1.95 [b] | 9.81±2.62 [a] |
| TP(g/dl) | 4.406±0.242 | 3.928±0.338 [a] | 3.973±0.549 | 3.485±0.406 [a] |
| ALB(g/dl) | 1.345±0.131 | 1.002±0.133 [a] | 1.152±0.169 [a] | 0.9958±0.0997 [a] |
| GLOB(g/dl) | 3.066±0.405 | 2.927±0.547 [b] | 2.824±0.490 [b] | 2.886±0.271 [b] |
| Acid Phosphatase (U/L) | 1.2637±0.073 | 1.227±0.168 [b] | 1.1184±0.050 [a] | 1.094±0.124 [a] |
| Uric Acid(mg/dl) | 0.843±0.125 | 1.298±0.210 [a] | 1.162±0.218 [a] | 1.561±0.244 [a] |
| CPK(U/L) | 102.96±1.70 | 107.45±6.12 [b] | 108.90±7.29 [b] | 156.20±29.00 [a] |
| Bilirubin(mg/dl) | 0.1110±0.0098 | 0.1754±0.0145 [a] | 0.1665±0.0101 [a] | 0.1826±0.0283 [a] |

The serum biochemical values are expressed as the mean ± S.E. Superscript "a" statistically highly significantly different (a = 0.001) from control according to Duncan's New Multiple Range Test. Superscript "b" indicates non-significant (b = 0.05)

**Table 3** Serum gas and ion profile of *L. rohita* in EG-1, EG-2 and EG-3, when compared with the control

| **Parameters** | **(C)** (N=19) (Mean ± S.D). | **EG-1 (Cu treated)** (N=19) (Mean ± S.D). | **EG-2 (Pb treated)** (N=19) (Mean ± S.D). | **EG-3 (Cu+ Pb treated)** (N=19) (Mean ± S.D). |
|---|---|---|---|---|
| Sodium (m.mol/l) | 1.668±0.187 | 1.488±0.147 [b] | 1.523±0.150 [b] | 1.285±0.130 [a] |
| Potassium (m.mol/l) | 1.429±0.166 | 1.234±0.160 [a] | 1.255±0.166 | 1.008±0.137 [a] |
| $Ca^{+2}$(m.mol/l) | 2.353±0.271 | 1.864±0.193 [a] | 1.976±0.171 [a] | 1.587±0.225 [a] |
| $Mg^{+2}$ (m.mol/l) | 1.291±0.111 | 1.413±0.187 | 1.451±0.159 [a] | 1.547±0.251 [a] |
| pH | 7.368±0.155 | 6.646±0.749 [a] | 6.849±0.248 [a] | 6.759±0.204 [a] |
| Ammonia (m.mol/l) | 707.04±5.30 | 852.00±11.50 [a] | 799.4±81.5 [a] | 875.4±83.8 [a] |
| $PO_2$ mm Hg | 58.49±2.72 | 47.63±2.97 [a] | 52.34±1.63 [a] | 46.34±1.82 [a] |
| $O_2$ Sat (%) | 95.21±1.84 | 89.84±2.14 [a] | 91.89±2.02 [a] | 89.52±6.97 [b] |
| $PCO_2$(mm Hg) | 25.12±1.34 | 33.24±2.70 [a] | 32.01±2.56 [a] | 40.26±4.31 [a] |

The serum gas and ion values are expressed as the mean ± S.E. Superscript "a" statistically highly significantly different (a = 0.001) from control according to Duncan's New Multiple Range Test. Superscript "b" indicates non-significant (b = 0.05)

Hb test kit where it was converted into red cyanomethemoglobin under the effect of potassium ferricyanide and potassium cyanide, following the protocol used by Ahmed Hamid *et al.,* (2013). RBC count was carried out using haemocytometers and WBC counts were measured using light microscope with an improved Neubauer hemocytometer, following the protocol used by Shah and Altindag, (2005). The other derived hematological indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae as described by Blaxhall and Daisny (1973).

*Determination of serum biochemical parameters*

For blood biochemistry, the blood samples were subjected to centrifuge at a rate of 10,000 rpm for 5-8 minutes in TG20-WS Tabletop High Speed Laboratory Centrifuge Machine, serum was separated out and the various metabolites, ions, gases and enzymes were analyzed from the serum. Serum biochemical indices which were analyzed by using Hitachi 902 automatic analyzer (Japan) are glucose (GLU), Cholesterol (Chol), total protein (TP), albumin (ALB), total globulins (GLOB), $PO_2$, $PCO_2$, $O_2$ saturation , triglycerides (TRG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), creatine phosphokinase (CPK), alkaline phosphatase (ALP), lactate (LACT), gamma glutamyltransferase (GGT), acid phosphatase, uric acid, bilirubin (BRN), sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) (Kengkoom *et al*. 2012). The test was performed as stated in service manual for model 902 automatic analyzer Hitachi, Ltd. 1997.

*Statistical analysis*

All the data are expressed as mean and standard error of mean. The statistical package, Minitab-14 was used for the data analysis. The statistical differences were determined by using one way analysis of variance (ANOVA) and the level of significance was set at $P \leq 0.05$, following the statistical protocol, introduced by Zar (1996).

## Results

*Hematological profile*

In exposed fish, RBC count, hemoglobin, PCV and WBC counts decreased significantly ($P<0.01$) for EG-1, EG-2 and EG-3, compared to control (Table 1). MCV and MCH for EG-1 and EG-2 were not different to control, but for EG-3, these were least significantly higher than control (Table 1). Maximum variations in hematological profile were observed for EG-3, where *L. rohita* was exposed to 0.1575 mg/L of [$CuSO_4.5H_2O$] and 0.340 mg/L of [$Pb(NO_3)_2$].

*Serum biochemical profile*

Among lipid profile indices in exposed fish, the plasma ammonia, TRIG, Cholesterol, LDL, VLDL, uric acid, CPK and bilirubin showed significant higher values ($P<0.01$) for EG-1, EG-2 and EG-3 compared with control (Table 2 & 3). Liver function and health parameters such as glucose, AST, ALT, LDH, GGT, except GLOB were significantly higher ($P<0.01$) for EG-1 and EG-3 than control (Table 2). Marked two folds increase was observed for LDH levels for EG-1, EG-2 and EG-3 compared with control. In contrast, acid phosphatase was the only enzyme showed significantly lower values ($P<0.01$) for EG-2 and EG-3, but was not significantly different for EG-1 compared with the control (Table 2).

*Serum pH, arterial blood gases and ionic profile*

Serum pH values were decreased for EG-1, EG-2 and EG-3 compared with the control (Table 3). The concentrations of $Na^+$ were not significantly different for EG-2 than the control. However the sample for EG-1 and EG-3 showed a significant decrease in $Na^+$ levels than the control. Similarly, the concentrations of $K^+$ were significantly lower ($P<0.01$) for EG-1, EG-2 and EG-3 than the control (Table 3). The samples for EG-1, EG-2 and EG-3 showed significant increase in the concentrations of $Mg^{+2}$compared with control (Table 3). In contrast to $Mg^{+2}$, the concentrations of $Ca^{+2}$ were significantly lower ($P<0.01$) for EG-1, EG-2 and EG-3 than the control (Table 3). A decrease in oxygen saturation (%) and $PO_2$ in serum but an increase in $CO_2$ concentration was observed for EG-1, EG-2 and EG-3 compared with the control (Table 3).

## Discussion

Fish is the heterogeneous group of aquatic animals. Since the fish are ectothermic aquatic vertebrates, having economic values as natural resources of proteins and other fish product(s), their physiologic, pathologic and toxicologic studies are emerging fields at the present days. Such fields pertaining to fish physiology, pathology and toxicology are further worth attention due to the significance of fish in human nutrition, as people of almost every nation agree to include fish in their normal diet as a rich source of quality protein (Saksena, 1999). It is informed that important elements responsible for normal physiology of animals and their intact structure of various organs for physiological life sustaining processes are hematopieotic tissue, hematological parameters, blood serum biochemistry, lipid profile and circulating blood (Davis, 1997). The toxic substances cause fluctuations in hematological parameters, either by enhancing their number or concentration by promoting their biosynthetic activities or their fall in number or concentration by suppressing their biosynthetic sites (Scott and Sloman, 2004). In clinical and veterinary medical sciences, the hematological parameters are considered as good health indicators of animals as well as for environment (Schuett *et al.,* 1997). The quality and quantity of blood cells (RBCs and WBCs), which are prime important blood parameters are used in evaluating the health of the

animals. Any type of fluctuation in these parameters is the reflection of stress, caused by some contaminant in the environment (Tierney *et al.,* 2004). Sahan *et al.,* (2007) gave preference to hematological parameters to evaluate the effect of water pollution stress in fish. The study showed that [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] significantly caused alterations in hematological and biochemical parameters in *L. rohita*. In view of the results the [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] were included in the lists of compounds strongly toxic to fish. In our study, for *L. rohita* 96h $LC_{50}$ values for [$CuSO_4.5H_2O$] was found to be 3.15 mg/L and for [$Pb(NO_3)_2$] 6.50 mg/L. These values showed nonconformity with 96h $LC_{50}$ values of 0.56 mg/L for [$CuSO_4.5H_2O$] and 27.2 mg/L for [$Pb(NO_3)_2$] for *L. rohita* stated by Adhikari (2003) and Abdullah *et al.,* (2007) respectively. Several aspects including composition of toxicant, experimental situations and sex or age of fish may affect the sensitivity of fish to heavy metal exposure.

The exposure of *L. rohita* to acute concentrations of [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$] and their combination caused a significant decrease ($P<0.01$) in RBCs count for EG-1, EG-2 and EG-3, that is the sign of hemolysis, leading to anemic situation for exposed groups. The another concrete evidence of anemic situation for exposed groups was the decrease in PCV, hemoglobin and a decrease in TP, all these signs of prevalence of anemia were also observed for EG-1, EG-2 and EG-3. The decrease in RBCs count may perhaps be from the inhibition of DNA synthesis in red blood cell production, or impaired intestinal absorption of iron or possible disruption of hematopoiesis and hypoxia, induced by exposure to the selected toxicants (Shah, 2006). Similar diminutions in RBCs count have been stated by Musa and Omoregie, (1999) when the fish was exposed to polluted environment under laboratory conditions. Thus, the significant decrease in these hematological parameters is an obvious clue of prevalence of severe anemia induced by exposure of the experimental fish to copper and lead in the water, as reported by Maheswaran *et al.,* (2008). These alterations were attributed to direct or feedback responses of structural damage to RBC membranes, blood cell injury resulting in hemolysis, as is evident from hypercholesterolemia in treated groups of fish, because cholesterol, the prime important component of almost all types of eukaryotic cell membrane, indicating impairment in cholesterol contribution in RBC membrane maintaining structural integrity, thus resulting in hemoglobinemia and hemoglobinuria. The decrease of RBC count may be due to hypocalcemia, as calcium is the main regulator of permeability of cell membrane to water and inorganic ions. Its low levels causes hemolysis due to endosmosis of water, causing severe anemia (Saiki *et al.,* 1995). The results of hypocalcemia in the present study match with the data presented by other authors (Tulasi *et al.,* 1990). In the study, the MCV values showed an induction for EG-3 compared with control, but the increase for EG-1 and EG-2 is non-significant, indicating the incidence of macrocytosis, consequently anemia for EG-3, where fish was administered in combination of copper and lead metals. Increase in MCH values for EG-3 in the study was another concrete evidence of anemia for EG-3. Increased MCHC values (hyperchromia) were observed for exposed groups. Such conditions were found where the hemoglobin is abnormally concentrated inside the red blood cells. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes, blood cell injury resulting in hemolysis (Gross, 1997), as is evident from hypercholesterolemia.

The changes in WBC count can be manifest with the form of leucocytosis, as lymphopenia and heterophilia are common characteristic responses of the animals under stress (Joshi, 2002). Since fish in contrast to mammals have no lymph nodes and their bones are without medullary cavity, hematopoietic tissue is limited to the stroma of the spleen and the interstitium of their kidney. To a lesser extent hematopoietic tissue is also confined to periportal areas of the liver, the intestinal submucosa and the specialized lymphoid organ, the thymus (Mumford *et al.,* 2007). In the leukocyte series, WBCs count were found to exhibit a highly significant decrease ($P<0.01$) for EG-1 and EG-3, compared to control except for EG-2, where the decrease in WBC count was non-significant. The significant decrease of neutrophils, thrombocytes and lymphocytes count for EG-1 and EG-3 may be due to increased concentration of toxicants in various tissues of fish (Ololade and Oginni, 2010). It is reported that when the animals are in stress the cortisol secretion is induced resulting in shortening the life expectancy of lymphocytes and induce their apoptosis (Verburg *et al.,* 1999) and inhibit their proliferation (Espelid *et al.,* 1996). The observed reduction in WBC count for toxicant exposed groups agrees with the reports that fish is in stress and the release of cortisol during stress causes a decrease of leukocyte count, which shows the weakening of the immune system (Olanike *et al.,* 2008). These changes in WBC count could be manifest with the form of leucocytosis, as lymphopenia and heterophilia are common characteristic responses of the animals under stress (Joshi *et al.,* 2002). This indicated a low concentration of these cell types and increased MCV values ($P<0.01$) for EG-3, an indication of immunity loss in toxicant exposed fish and the values of leucocytes found in the study were in agreement with the data reported by others (Velisek *et al.,* 2009), but our results showed contradiction to those of Velisek *et al.,* (2009), where an increase in leukocytes was observed in trout exposed by [$CuSO_4.5H_2O$]. In our data, the decrease in serum ALB was also observed, further a supportive indication of depressive immune response caused by metal toxicity for treated groups of fish. AST and ALT are bio-sensitive indicators of liver damage or injury from different types of diseases (Vinodhini and Muthuswamy, 2008). The results for EG-1 and EG-3 indicate an induction of both transaminases (ALT and AST), suggesting amplified transamination processes due to amino acid input into the TCA cycle in order to cope with the energy crisis during heavy metals based stress (Satyaparameshwar *et al.,* 2006). As the fish gill epithelium is the primary site for exchange of respiratory gases (Evans *et al.,* 2005). The results of any abnormality in plasma levels of $CO_2$ and $O_2$ in fish is a clue of either in damage of gills or hemoglobin abnormality. In the study, an elevation in $CO_2$ and a

depression in $PO_2$ and $O_2$ saturation (%) were observed for EG-1, EG-2 and EG-3. These results could be correlated with the levels of hemoglobin, as the hemoglobin levels were found to have decreased values for EG-1, EG-2 and EG-3 compared with the control.

## Conclusions

The study suggested that the hematological and biochemical indices of *L. rohita* are target parameters for [$CuSO_4.5H_2O$] and [$Pb(NO_3)_2$]. This data verifies that the vicissitudes in hematological and biochemical indices may be used as sensitive biomarkers for animal health evaluation, especially in regions that are naturally affected by heavy metals, causing stress in fish on exposure to elevated levels in the water. Exposure of *L. rohita* and other allied fish species to higher concentrations of copper and lead demonstrated a toxic poisoning. The study may also conclude that higher fish mortality is expected under a stationary bioassay method. The fish mortality at massive level is almost an observable situation in most of the aquatic environments particularly during drought when there is no or little flow of the river system, because of an increased concentrations of these metals. Secondly, the bioaccumulation of these metals in edible parts of fish i.e muscles is reported by a various authors (Khadiga et al., 2002). The consumption of fish as a diet from such metal polluted areas is directly toxic threat to human blood characteristics. Thus sincere attentions should be devoted to minimize the risk of copper and lead pollution in the ambient environment to save living organism including human population from adverse effects of these pollutants.

## Conflict of interest

Authors declared no conflict of interest with any one.

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