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Effect of Environmental Stresses on Lipid and and Haematological Profiles of the Air Breathing Catfish *Clarias batrachus* (Linn.)

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ABSTRACT

Fishes adopt themselves to some extent when natural waters have high BOD, heavy metals and ozone contamination. The present work studied the effects of hypoxia, anoxia, ozone, copper sulphate and a combination of copper sulphate + ozone on the lipid and hematological profiles of *Clarias batrachus*. In case of lipid profile, total cholesterol level (195.5 ± 1.5) was highest in normal condition, triglyceride (204 ± 3.162), HDL (38 ± 1.224) and VLDL (40.75 ± 1.478) in 2 hour hypoxia, and LDL (129 ± 2.236) in copper sulphate + ozone treated condition. Analysis of blood samples showed elevation Hemoglobin level (9.7 ± 0.070) was highest in 4 hour hypoxia condition, RBC count (3 ± .070) and glucose level (186.75 ± 1.478) in 2 hour hypoxia , PCV percentage (28 ± 0.707) in anoxia , and total protein in normal (6 ± 0.070) and 2 hour hypoxia (6 ± 0.141) conditions.

Keywords: Hypoxia, Anoxia, Ozone, Copper Sulphate, Lipid profile, RBC, Heamoglobin, *Clarias batrachus*.

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INTRODUCTION

Fishes when faced with high BOD evolve special behaviours to survive[1]. In hypoxic habitats fishes may have more hemoglobin RBCs in their blood, and thus increasing capacity to transport oxygen. Their body tissues may contain more myoglobin, a molecule that act as oxygen store[2]. When oxygen is rare and metabolic demand is low, as in a cold water fish for example, anaerobic metabolism can contribute to survival for days, weeks, or even months[3]. Heavy metals at high concentrations cause harmful effects on metabolic, physiological, and biochemical systems of fishes. Fish have been largely used as bio-indicators for environmental pollutants due to the sensitivity of their biochemical and hematological parameters[4]. Geological weathering and human activities have introduced large quantities of metals to localized areas leading to bioaccumulation and transfer to man through food web [5, 6]. Copper is a trace element responsible for the function of specific enzymes. But this metal becomes toxic at higher concentrations. Use of Copper sulphate ($CuSO_4$) as a fungicide in agricultural practices and for control of algae and pathogens in fish culture ponds have increased the copper concentrations in aquatic systems. Copper sulphate toxicity to fish varies with the species and the physical and chemical characteristics of the water. Adverse effect of copper to some fresh water fishes like *Lepidocephalichthys thermalis*, the rainbow trout have been well documented[7]. Ozone is 3000 times faster than chlorine as a disinfectant removes colour and odour of water. Overdose of ozone can burn fish gills, and also oxidizes some of the biochemical compounds present in living organisms, including amino acids, pyrimidine nucleotides, fatty acids, flavins, and proteins containing sulfhydryl groups [8]. The Walking catfish, *Clarias batrachus* are mostly encountered in muddy or swampy water of high turbidity[9]. Its intolerance to cold temperature is range limiting[10]. Behavioral avoidance of environmental extremes during cold/dry seasons involves burrowing into pond and river banks to enter dormancy. Deep water may also serve as a thermal "Refugio" during cold snaps[11]. The species thrives in estuarine waters up to 18 ppt salinity[9]. Several physiological adaptations like greatly reduced gas bladder and gills becoming highly vascularized arborescent (tree-like) organs acting as accessory breathing structures. Due to these special characteristics, this fish was chosen for the present work to observe the changes under severe environmental hypoxia, the effect of ozone and the toxic heavy metal copper on them.

MATERIALS AND METHODS

Adult catfishes (*Clarias batrachus*) which were off their reproductive period were procured locally. Fish were fed fish pellets once a day and kept in aerated water tanks for two weeks for acclimatization.

Experimental Design

The fish were separated into two groups for exposure to hypoxia, anoxia, ozone and sub lethal concentration of heavy metal, copper ($CuSO_4.5H_2O$), and ozone along with copper sulphate. The first group were again separated into four experimental groups I, II, III, and IV with 4 fish in each tank. Group I served as control and kept under normoxia. Groups II and III were subjected to hypoxia, the former for 2 hour and the latter for 4 hour, where as group IV fishes were subjected to anoxia. Hypoxia was induced by disconnection of the aeration system and avoiding the fish to reach the surface by an iron net fitted one-inch below the water surface to prevent bimodal respiration. Anoxia was induced by disconnection of the aeration system and by fitting a glass slab one-inch below the water surface to completely cut off oxygen supply. After the period of exposure to normoxia, hypoxia and anoxia, the fish were quickly removed from the tanks and blood samples were collected by using the heart puncture technique for taking of lipid profile and haematological profile[12]. The second group fish were again separated into three experimental groups. The first group was exposed to sub lethal concentration of copper sulphate (1 mg/l) added in the water for a period of 96 hour, the second group was supplied with ozone along with sub lethal concentration of copper sulphate for 96 hour and the third group was supplied with ozone alone. A parallel control was also maintained in tank containing normal water without any treatment. At the end of the exposure period, blood was collected from the fish for determination of lipid and hematological profiles.

Lipid Profile Determination

Lipid profile was determined according to the methods of Trinder (1969), Allain *et al* (1974), Flegg (1972), Grillo *et al* (1981) and Demacker *et al* (1980)[13, 14, 15, 16, 17].

Haematological parameter Determination

Red blood cells (RBC) were determined by methods of Ochei and Kolhatkar (2005), total haemoglobin (Hb) was determined based on its complete conversion into Cyanmetahaemoglobin read at 540 nm, and hematocrit (Ht) or packed cell volume (PCV) was read after centrifugation as usual[12]. The blood samples were centrifuged at 12,000 g for 3 min at room temperature to harvest the plasma. The harvested plasma was utilized for multi parameter studies, as many metabolites remain stable in their concentration in plasma if stored properly (Allian *et al*, 1974)[14]. Total protein and blood glucose was determined using analytical kit.

Statistical Analysis

All the data were collected from 4 animals for each set of experiment and were statistically analysed for student "t" test at 0.05% probability.

RESULTS AND DISCUSSION

Lipid profile of blood sample showed that total cholesterol level was highest in normal condition and lowest in ozone treated condition (Figure 1). Triglyceride level was highest in 2 hr hypoxia condition and lowest in ozone treated condition (Figure 2). HDL (High density lipid) level was highest in 2 hr hypoxia condition and lowest in copper sulphate + ozone treated condition (Figure 3). LDL (Low density lipid) level was highest in copper sulphate + ozone treated condition and lowest in 2 hr hypoxia condition (Figure 4). VLDL level was highest in 2 hr hypoxia condition and lowest in ozone treated condition. VLDL (Very low density lipid) level was highest in 2 hr hypoxia condition and lowest in ozone treated condition (Table 1).

Table-1 Lipid Profile: VLDL (mg/ml) in different Conditions

Sl. No.	Treatment Parameter	VLDL(mg/dl)
1	Normal	35.35
2.	2 hr Hypoxia	40.75
3	4 hr Hypoxia	32.50
4	Ozone	23.87
5	$CuSO_4$	35.5
6	$CuSO_4$ + Ozone	32.75

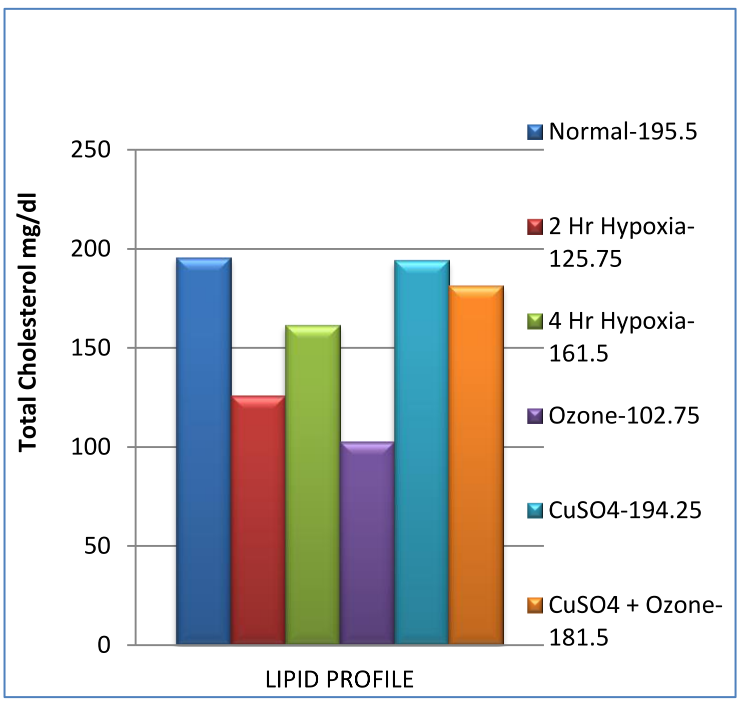

Figure 1. Total Cholesterol(mg/dl) in different coditions

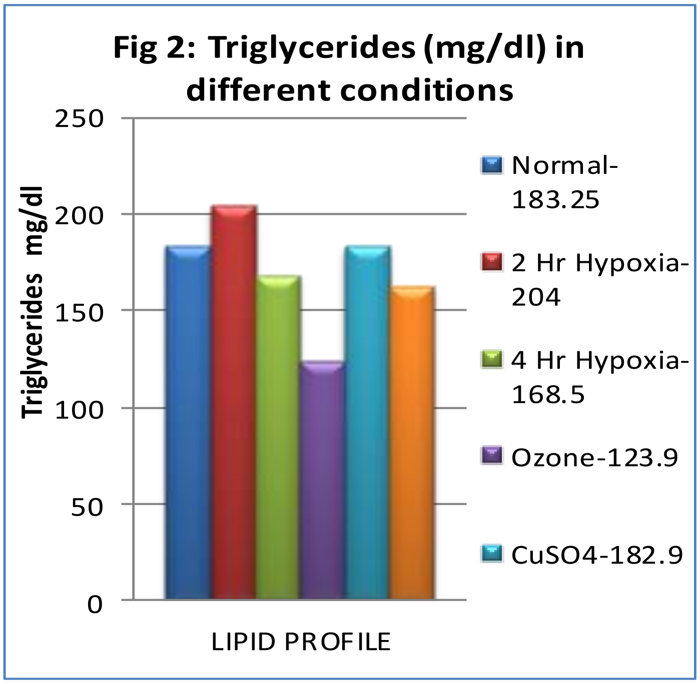

Figure 2. Triglycerides (mg/dl) in different conditions

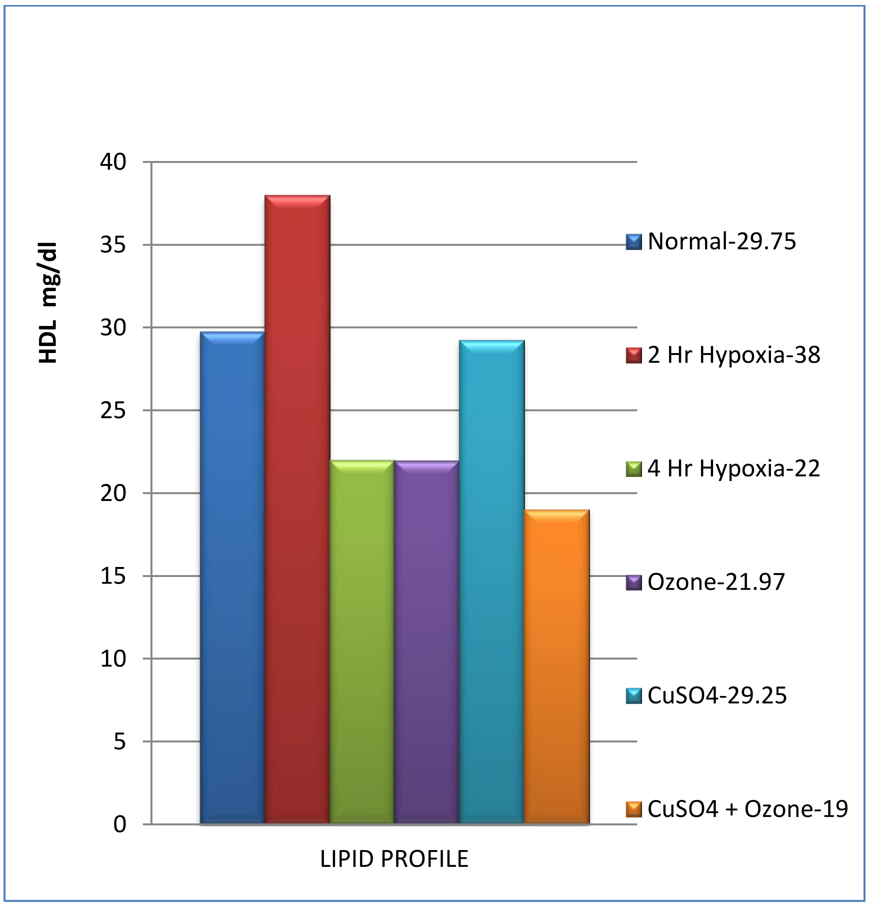

Figure 3. HDL(mg/ml) in different conditions

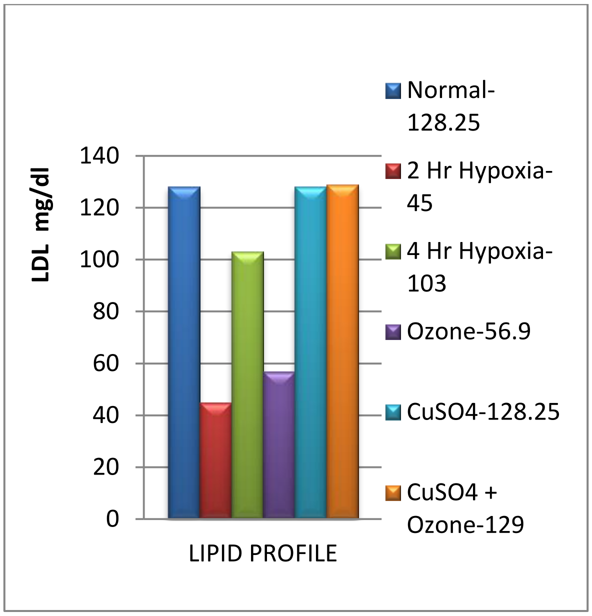

Figure 4. LDL(mg/dl) in different conditions

Analysis of Blood Sample

Examination of blood sample of *C. batrachus* showed that the hemoglobin content was highest in 4 hour hypoxia condition and lowest in ozone treated condition (Figure 5). Highest number of RBC was present in 2 hour hypoxia condition and lowest in ozone treated condition (Figure 6). PCV (Packed Cell Volume) percentage was highest in anoxia condition and least in ozone treated condition (Figure 7). Blood glucose was highest in 2 hour hypoxia condition and lowest in 4 hour hypoxia condition (Figure 8). Normal and 2 hour hypoxia condition contained highest amount of protein and ozone treated condition the lowest (Table 2).

Table 2.Total Proteins (gm/dl) in different Conditions

Sl. No.	Treatment Parameter	Total Protein(g/mdl)
1	Normal	6
2	2 hr Hypoxia	6
3	4 hr Hypoxia	5.2
4	Ozone	4.95
5	$CuSO_4$	5.8
6	$CuSO_4$ + Ozone	5.75

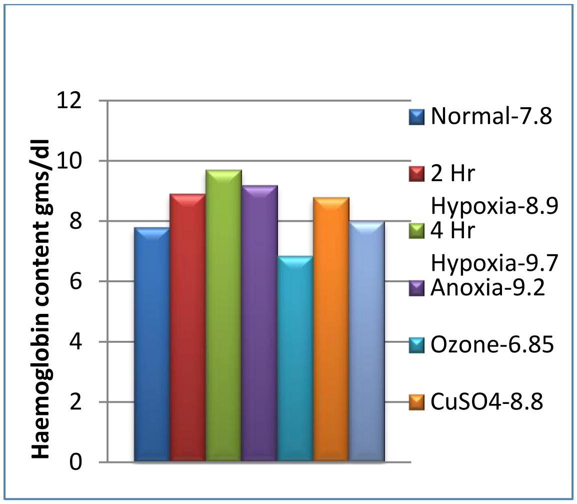

Figure 5. Haemoglobin content(gm/dl) in different conditions

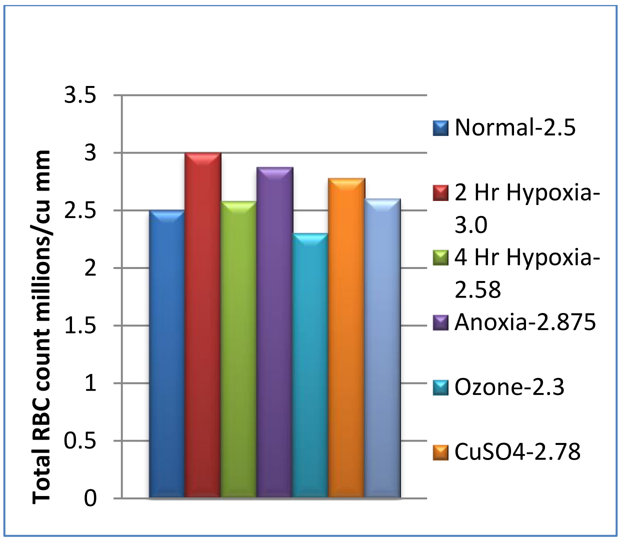

Figure 6. Total RBS count (millions/cu mm) in Different conditions

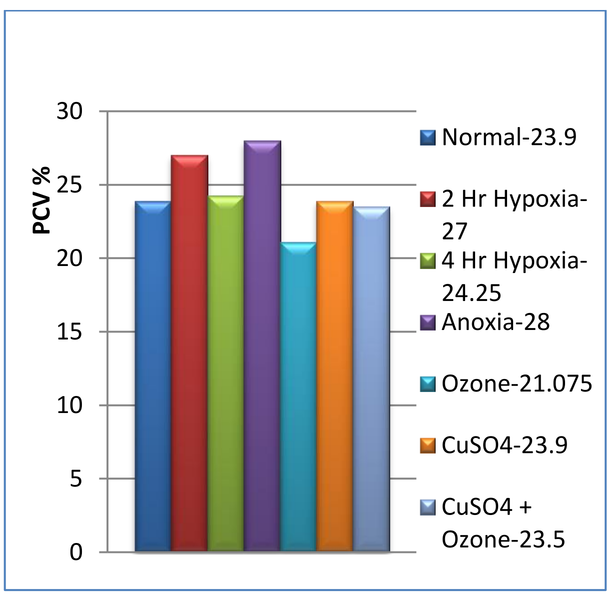

Figure 7. PCV(%) in different conditions

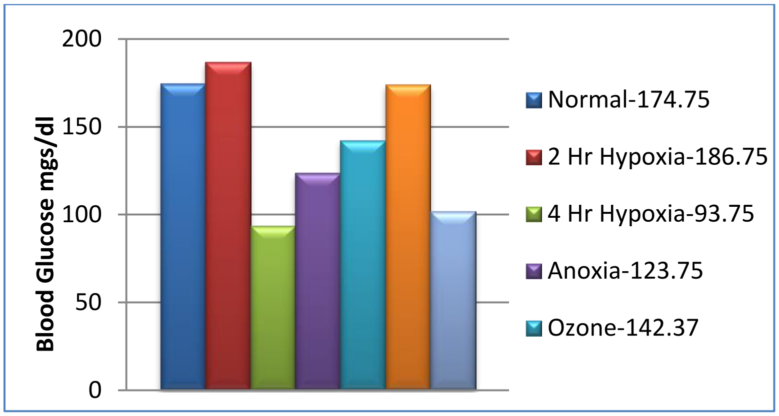

Figure 8. Blood glucose (mg/dl) in different conditions

Determination of lipid profile is important study with reference to *Clarias batrachus* and due to nutritional value as they play very important, physiological role in human nutrition[17]. In case of hypoxia, total cholesterol level and LDL level decreased in both 2 hour and 4 hour hypoxia. Triglyceride level was elevated in 2 hour hypoxia but reduced in 4 hour hypoxia condition. HDL and VLDL level showed similar trend. This may be due to increase or decrease in enzyme activity to break down the lipids to meet the energy or nutritional demands. Lipid blocks the metabolism of hepatic triglycerides due to the defective synthesis of very low density lipoproteins which are involved in the transport and mobilisation of triglycerides to extra hepatic tissues. On exposure of *C. batrachus* to sublethal concentration of heavy metal copper (copper sulphate) total cholesterol, triglyceride, HDL level slightly decreased, while VLDL level increased slightly. When the fishes were exposed to copper sulphate + ozone, all the parameters decreased in comparison to the fishes exposed alone to copper sulphate except VLDL level, which may be due to the oxidizing effect of ozone. When the fishes were exposed to ozone alone all the parameters were reduced in comparison to the ones exposed to sublethal concentration of copper sulphate and normal environmental condition. Several species besides *Clarias batrachus* have been reported as responsive to environmental oxygen concentration, particularly in terms of blood parameter variations[14, 15, 16, 17]. Blood responses are considered fundamental to internal adjustments to cope with a number of stresses like hypoxia, anoxia condition etc. Among these, the increase of hematocrit is usually observed as the result of erythrocyte swelling, decrease of plasma volume, increase of red blood cell number or a combination of such factors[14]. *C. batrachus* presented the usual blood response to hypoxia, i.e., increase in hematocrit (PCV). Hemoglobin concentration also increased, along with increase in RBC count, which may be due to enhanced erythropoiesis. Blood glucose level increased in 2 hour hypoxia, may be due to its mobilization from liver to meet the increase in energy demand, but decreased in 4 hour hypoxia. Protein showed a different trend than glucose, in 2 hour hypoxia condition there was no change in protein level compared to normal condition, but decreased in 4 hour hypoxia condition suggesting stress induced proteolysis. The toxic effects of copper are related to its capacity for catalyzing oxidative reactions that leads to the production of Reactive Oxygen Species (ROS) [18, 19, 20]. These highly reactive compounds may also induce tissue alterations and change some physiologic responses of fish, thus leading to oxidative stress[18, 21, 22, 23]. Elevated levels of copper while acute, may lead to death whereas chronic effects could be reduced growth, shorter life span, reproductive problems, reduced fertility and behavioral changes[18, 24]. Furthermore, copper can affect metabolic activity at the biochemical levels[18, 25].

CONCLUSIONS

From the above discussion, it is concluded that stressful environmental condition especially hypoxia affect the normal metabolic activities in the air breathing catfish. Determination of lipid profile is an important study since different environmental conditions affect the lipid profile, thus affecting its nutritional quality. Animals can adapt themselves to a certain limit in changed environmental conditions, but after that the animal cannot adapt and die

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