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The Acute and Chronic Toxicity of Copper on The Behavioral Responses and Hematological Parameters of Freshwater Fish, Common Carp (*Cyprinus carpio* L.)

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Abstract

The present study was conducted to examine toxicological effects of copper sulfate (Cu) in common carp fish (*Cyprinus carpio* L.). The LC_{50} (median lethal concentrations) of copper on *Cyprinus carpio* were 3.64, 3.36, 3.04, 2.65 mg/L respectively. In general, behavioral responses of the fishes exposed to copper included uncontrolled swimming, erratic movements, loss of balance, swam near the water surface with sudden jerky movements. Haematological parameters such, red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Packed cell volume (PCV), mean cell volume (MCV) mean cell haemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were studied. The obtained results indicated that the (RBC) and (WBC) have increased with increasing metal concentrations and exposure period. While, haemoglobin (Hb) had slightly increased compared with control fish. Other parameters like Packed cell volume (PCV), mean cell volume (MCV) mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) appeared likely being enhanced with increased exposure to studying heavy metals, and did not show any significant increase with different concentrations used in chronic exposure experiments.

Keywords: *Cyprinus carpio*, Copper, $LC_{50,}$ Behaviour, Hematological parameters.

السمية الحادة والمزمنة لمعدن النحاس على الاستجابة السلوكية والمحددات الدموية لأسماك المياه العذبة، الكارب الاعتيادي *Cyprinus carpio* L.

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الخلاصة

الدراسة الحالية أجريت لتقييم الآثار السمية لمعدن النحاس (Cu) على سمك الكارب الشائع بعد التعرض الحاد والمزمن. وكان التركيز المميت الوسطي LC_{50} على سمك الكارب 3.64، 3.36، 3.04، 2.65 ملغم/لتر على التوالي. بشكل عام، تضمنت الاستجابات السلوكية للأسماك المعرضة للنحاس سباحة غير منضبطة، وحركات غير منتظمة، وفقدان للتوازن، وسباحة بالقرب من سطح الماء مع حركات متشنجة مفاجئة. كما اظهرت نتائج دراسة مكونات الدم مثل ، خلايا الدم الحمراء (RBC) ، خلايا الدم البيضاء (WBC) ، الهيموغلوبين (Hb) ، حجم الخلية المرصوصة (PCV) ، متوسط حجم الخلايا (MCV) ، متوسط خلية الهيموغلوبين (MCH) و تركيز جسيمة الهيموغلوبين (MCHC) ، اشارات النتائج إلى أن خلايا الدم الحمراء (RBC) وخلايا الدم البيضاء (WBC) ازدادت مع زيادة تراكيز العناصر الثقيلة وفترات التعرض. في حين، سجل الهيموغلوبين (Hb) ارتفاعاً طفيفاً مقارنة مع اسماك السيطرة. اما في حالات حجم الخلية المرصوصة

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(PCV) ، متوسط حجم الخلايا (MCV) ، متوسط خلية الهيموغلوبين (MCH) و تركيز جسيمة الهيموغلوبين (MCHC) ، فقد ظهرت النتائج زيادة واضحة مع زيادة التعرض للمعادن الثقيلة ، الا انها لم تظهر اي زيادة هامة مع التراكيز المختلفة المستخدمة خلال تجارب التعرض المزمن.

Introduction

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. As a result of the pollutants transported from industrial areas in the environment and their chemical persistence and many freshwater ecosystems are faced with high levels of xenobiotic chemicals [1]. Copper (Cu), is one of the most important pollution-causing metals. It is considered to be an important xenobiotic, persistent and non-biodegradable chemical and also known to be the most common cumulative pollutants in terrestrial and aquatic ecosystem [2].

However, the higher concentration of Cu in an aquatic ecosystem would become toxic to organisms due to increased production of free radicals in the body, teratogenicity and chromosomal aberrations [3]. Furthermore, an excess of oral copper intake may cause intravascular hemolysis, liver cirrhosis, tachycardia and acute renal failure [4, 5].

Acute toxicity bioassays (LC_{50}) is conventional tools and very extensively used to assess the potency/toxicity of physiologically active heavy metals and also to evaluate the full potential of metal contamination on commercially and ecologically important species [6]. It is caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal's toxicity [7]. The exact causes of death due to metal poisoning are multiple and depend on time concentration [8]. Behavior change is obviously a very important individual's level response that is the result of molecular, physiological and ecological processes. The behavior allows an organism to adjust to external and internal stimuli in order to best meet the challenge of surviving in a changing environment. Thus, the behavior is a selective response that is constantly adapting through direct interaction with physical, chemical, social and physiological aspects of the environment [9]. Behavioral and morphological abnormalities as a result of sublethal toxicity can reduce an aquatic organism's health and fitness. Changes in behavior and morphology are thus proven to be more sensitive diagnostic endpoints than the mortality [10]. Behavior links physiological function with ecological processes, hence behavioral indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations. Importantly, studies are beginning to correlate physiological changes with behavioural disruption, thus providing ecological relevance to physiological measures of toxicity [11].

The blood parameters have been used as a sensitive indicator of stress in fish exposed in different water pollutants and toxicants, such as metals, chemical, pesticides and industrial effluents etc. The evaluation of hematological characteristics of fish has become an important means of understanding normal and pathological processes and toxicological impacts [12]. In action, fish blood is greatly utilized in a toxicological investigation and environmental monitoring as a favorable indicator of physiological and pathological alterations. Hematological indices and blood parameters could be practical to the assessment of the effects of contaminants on fish [13]. Hematological indices, such as MCV, MCH and MCHC, hematological parameters, like Hb, PCV, RBC, and WBC amount, and biochemical parameters, like glucose, are greatly used to assess the toxic effect of environmental pollutants [14].

Common carp (*Cyprinus carpio*) represents an important farmed species. Common carp dominate wetlands and lakes because of their long lifespans and large size [15]. Generally, common carp considered is one of the major consumers as a food for Iraqi people and broadly used in the estimation of genotoxicity studies.

This aimed study investigates the toxic effects of copper sulphate on the standard test species, common carp (*Cyprinus carpio*), by determination of 96-hour LC_{50} values and evaluates behavioral disorders of the common carp exposed to different concentration of the toxicant. Also aimed to evaluate the hematological parameters of common carp (*Cyprinus carpio*) exposed to the toxicity of copper sulphate.

Material and Methods Experimental Fish

Fish *Cyprinus carpio* were obtained hatchery incubators (the city of Madaan, south of Baghdad) at a weight of 34.72±9.90 gm in the body plate-1.

The fish were being acclimated to the laboratory conditions for 10 days in glass aquaria (70×40×40 cm) before the initiation of the experiments. Water was renewed every day and a 16-8 h photoperiod was maintained during the acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during the acclimatization and test periods, but feeding was stopped one day prior to exposure for acute toxicity test only.

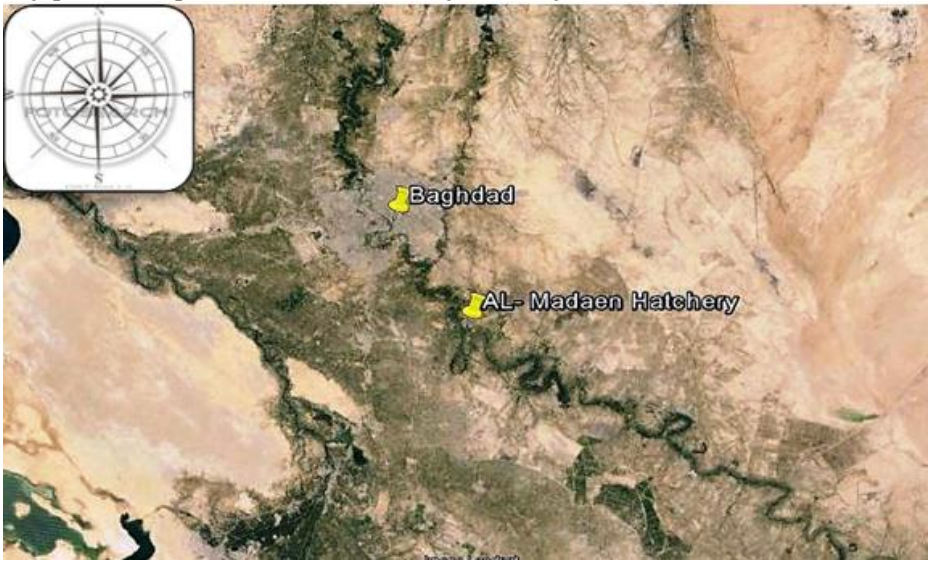

Plate 1- Al- Madaan Hatcheries in south of Baghdad (Google earth 2015).

Water properties

Physico-chemical characteristics were analyzed as follows temperature, pH, dissolved oxygen and electrical conductivity (EC) using a portable pH/ EC meters. Salinity (S) was calculated depending on the value of electrical conductivity by using the following equation as recommended. Dissolved oxygen (DO) was measured by Winkler method [16].

$$S\ (\%) = EC\ (\mu S/cm) \times 0.00064$$

Acute Toxicity Test

Acute toxicity tests were carried out for a period of 96 h. The mortality rate was determined at the end of 24, 48, 72 and 96 hours and dead fish were removed when observed. During the experimental period the control and copper exposed fishes were kept under constant observation to study the behavioral changes. Acute toxic effect of metal copper on the fishes was determined by the use of Finney Probit Analysis [17].

Behavioral studies

During the acute exposure period, the behavioral changes and morphological abnormalities of the healthy/control fish and the fish exposed to various concentrations of copper were regularly monitored and evaluated for behavioral changes. Behavioral observations were recorded for air gulps, startle response, mode of swimming, schooling, equilibrium and general activity of fish during the experiment. Data was also collected for morphological studies that included the effects on fish coloration, fish scales, presence/absence of hemorrhages and any other abnormality in the structure such as abnormal lateral flexure and posturing of pectoral fins.

Sub-chronic experiments

Safe concentration (Sc) of copper was determined following the formula given by Hart *et al.*, [18]:

$$Sc = LC_{50}\ (24hr.) - 0.3/[(LC_{50}\ (48hr.) - LC_{50}(24hr.)]x$$

Where, safety factor equal 2 or 3

Chronic toxicity test

Three groups of fishes were used in each aquarium and subjected to 0.5, 0.9 and 1.2 mg Cu /L. and left for 3 and 6 weeks. Water was refreshed every 48 hr. to remove any wastes and provide oxygen. Fishes were fed once per day [19].

Blood Sampling

Blood samples were used to measure red blood cells count (RBCs), white blood cells (WBCs), hemoglobin (Hb) concentration and packed cell volume (PCV), which was done. PCV was determined by spinning the blood sample contained in heparinized capillary tubes in a micro-hematocrit centrifuge. The Hb concentration was determined using the cyanomethemoglobin method and RBCs and WBCs count was carried out in a modified Neubauer chamber after saline (0.9% NaCl solution) dilution of the blood differential white cell counts were done on blood films stained with Giemsa. The blood indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were then calculated using the blood measurements above [20].

Statistical analysis

All experiments were repeated three replicates. Data were analyzed with SPSS program software (Version 13.0). The LC_{50} values were calculated using probit analysis statistical method. The Microsoft Excel 2007 was used to find regression equation (Y= mortality percentage; X=log of concentrations), the LC_{50} was derived from the best-fit line obtained. The data were subjected to use SPSS and statistical tests for the significant differences were used (T test).

Results and Discussion

Water properties

Table 1- shows the results of the physicochemical properties of the laboratory. Some physical and chemical properties were measured in the laboratory such as Temperature, pH, EC, DO and Salinity.

Physico-chemical properties	Mean and Standard Deviation
Temperature (c°)	26±1.4
Hydrogen ion concentration(pH)	7.5±0.8
Electrical conductivity (µs/cm)	1170.5±31.15
Dissolved oxygen (D.O) (mg/l)	6.9±0.8
Salinity (ppt)	0.684±0.101

Acute Toxicity Test

The exposure value for the element copper recorded during a period of 24, 48 and 72 and 96 hours were 0.5, 1, 2, 3, 4, 5 and 6 mg /L, While, recorded values for median lethal concentrations 3.644, 3.365, 3.044 and 2.655 mg/L during a period of 24, 48 and 72 and 96 hours, respectively (Table-2 and Figures -1, -2, -3 and -4). Compared with other fishes, these results agreed with the results of a recent study [21] where they found the 96h LC_{50} values for [$CuSO_4.5H_2O$] to be 3.15 mg/L. Mariappan and Karuppasamy [22] found that the median lethal concentration (LC_{50}) of copper to *Cyprinus carpio* L. was 38.36 mg/L.

Table 2- LC_{50} values of copper (mg/L)

Time (hours)	LC_{50} (mg/L)
24 hr	3.644
48 hr	3.365
72 hr	3.044
96 hr	2.655

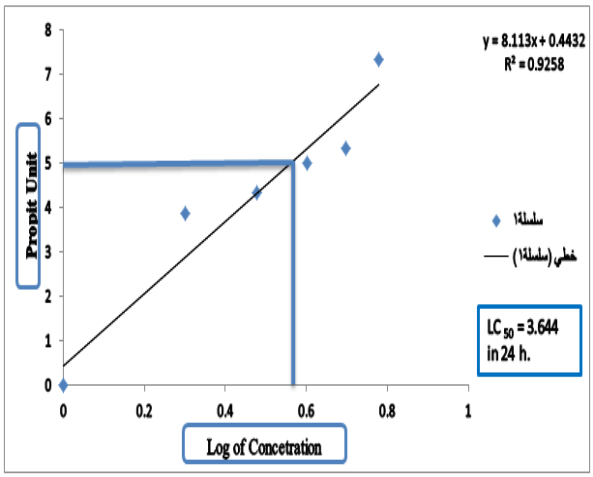

Figure 1-Toxicity curve of the copper after 24hr. of exposure

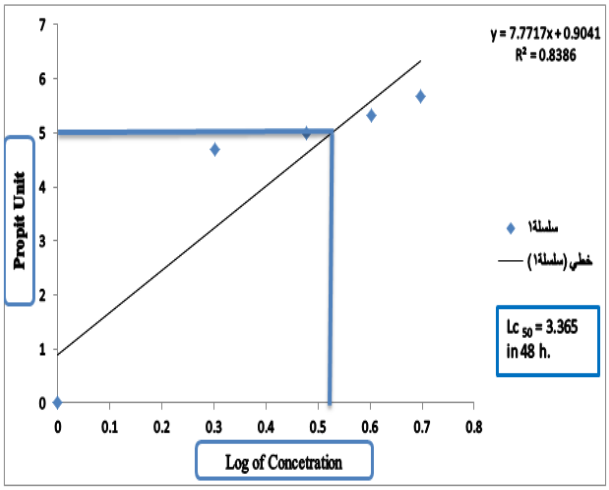

Figure 2- Toxicity curve of the copper after 48hr. of exposure

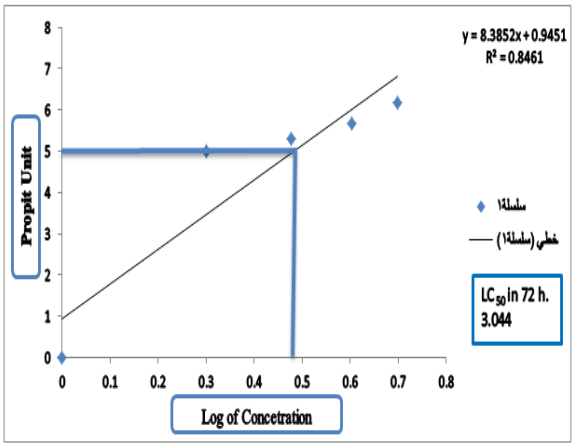

Figure 3- Toxicity curve of the copper after 72 hr. of exposure

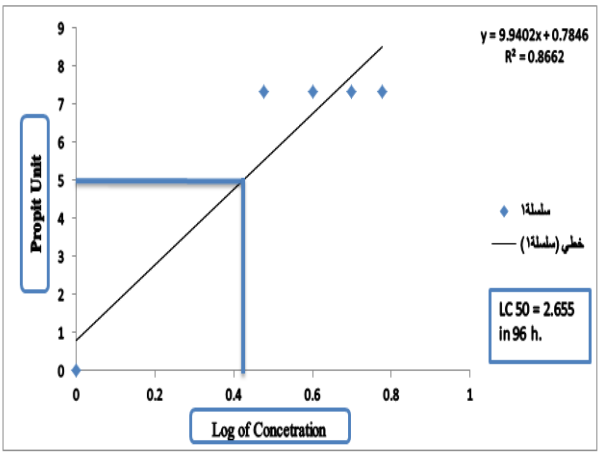

Figure 4- Toxicity curve of the copper after 96 hr. of exposure

Behavioral responses

The behavior and condition of the fishes in both control and testing solution were noted every 24 h up to 96 h. The fishes showed a marked change in their behavior when exposed to different copper concentrations. The behavioral changes observed in fish are as follows in the table-3. The fish showed some abnormal activities like erratic swimming, equilibrium loss and enhanced surfacing behavior. The metals concentrations showed the direct relationship with fish hyperactivity and convulsions rate. The intensity of toxicity of copper chloride concentrations were most obvious than cadmium chloride in the first hour of exposure [23]. Hiatt *et al.*, [24] stated that respiratory poisons induce symptoms of gulping, swimming at the surface, and depressed activity.

Table 3- The behavioral changes in common carp (*Cyprinus carpio)* for copper

Concentration (mg/L)	Behavioral changes
Control	There was no change in the behavior and the swimming patterns and no mortality in the control group through the whole testing period.
0.5	There were no mortality and natural movements of fish were recorded.
1	Natural movement and no changes in behavior were seen.
2	Abnormal swimming and the fish tended together at the surface, fast movement with the presence of mortality.
3	Seen an imbalance in the movements of fishes.
4	The increase in opercula movement due to faster breathing.
5	Fish did not the response to external effect.
6	Clear behavioral changes after exposure to copper such as fast swimming, lack of balance and respond to external stimuli, finally all fish died.

Sub-chronic exposure

Three series of exposure were conducted after we calculated safe concentrations for long-term exposure to copper Table-4. In series 1, fish specimens exposed to 0.5 mg/L of copper, while in other series experiments studied fish placed in 0.9 and 1.2 mg/L respectively. In this exposure, no mortality was observed in all periods of exposure for each tested concentrations.

Table 4- Safe concentrations values of exposure to copper

Element	Sc, when x=2	Sc, when x=3
Copper	0.93	0.86

Blood parameters

Hematological indices are very important parameters for the evaluation of fish physiological status under metallic stress. Table-5 shows mean ± standard deviation of fish blood contents after being exposed to different copper concentrations for three and six weeks.

Table 5-Mean ± standard deviation of several blood contents in fishes subjected to different copper concentrations for three and six weeks.

Variables	Control	Copper					
		3 weeks			6 weeks		
		0.5	0.9	1.2	0.5	0.9	1.2
RBC 10^6 /µL	0.72±0.42	1.26±0.233	1.31±0.124	0.943±0.184	0.96±0.026	0.92±0.042	0.747±0.113
WBC 10^3/µL	93.17±31.6	119.87±4.25	120.1±4.23	125.2±3.544	128. 3±0.282	124.23±1.59	130.57±3.00
Hb g/dL	7.07±0.45	7. 43±1.438	7.5±0.748	5.4±0.864	5.83±0.31	5.8±0.294	4.8±0.535
PCV %	22.9±1.73	26.1 ± 6.534	28.77±2.613	19.9±2.222	20.97±1.04	22.13±0.974	17.07±2.22
MCV Nm^3	221.1±4.55	205.77±27.9	219.8±6.231	214.17±17.2 8	217.6±8.58	240.6±1.499	229.6±9.50
MCH pg	71.43±0.12	59.1±0.804	57.2±0.589	57.6±1.840	60.53±2.88	63.03±0.918	64.77±3.47
MCHC g/dL	32.23±0.74	29.3±4.348	26.03±0.579	26.97±1.406	27.93±2.41	26.23±0.543	28.23±0.531

Figures -5,-6,-7,-8,-9,-10 and -11 Reveal the changes in the blood of fish *Cyprinus carpio* exposed to sublethal concentrations of copper exposed fish for 3 and 6 weeks. The RBC count was increased throughout the study period when compared to the control. Similar work carried out by Thangam *et al.*, [25] reported that RBC count was decreased throughout the study period when compared to the control showing a minimum percent decrease of -2.22 at the end of 7th day and a maximum percent decrease of 18.36 at the end of 35th day. The white blood cell count was increased throughout the study period when compared to the control. The exposure times had significantly ($P \leq 0.01$) affected mean WBC values but These data gave no significant effects of different copper concentrations ($P<0.05$) on mean WBC value. The value of LSD (8.726 103/µL) had confirmed such effects. [21] has evaluated that revealed a significant decrease ($P<0.01$) in RBC count, Hb, WBC count, and PCV compared to control, where the decrease in WBC count was non-significant. Represent the data on changes in the hemoglobin and PCV content of the fish *Cyprinus carpio* exposed to sublethal concentrations of copper for 35 days. The hemoglobin content in the copper exposed fish was found to be decreased when compared to the control.[26] Showed that copper exposed group with alkaline water had a decreased in RBC, PCV and Hb than those kept in copper exposed group in acidic water. In the case of *C. carpio*, blood changes to be affected by the water pH, decrease of the PCV, RBCs, Hb concentrations in copper exposure in water pH 5.0 compared to in copper exposure in water pH 9.0. Other parameters like mean cell volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) appeared likely being enhanced with increased exposure to studying heavy metals, and did not show any significant increase with different concentrations used in chronic exposure experiments. Abdul Latif *et al.*, [21] found a significant increase ($P \leq 0.01$) in mean corpuscular hemoglobin concentration (MCHC) values treated groups indicating the signs of anemia. Singh *et al.*,[27] have noticed that the impact of copper on haematological profile of freshwater fish and that represent with that mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) values and revealed that significant increase during 15 and 30 days of exposures, and both the values were found significantly increased at the end of the experimental period.

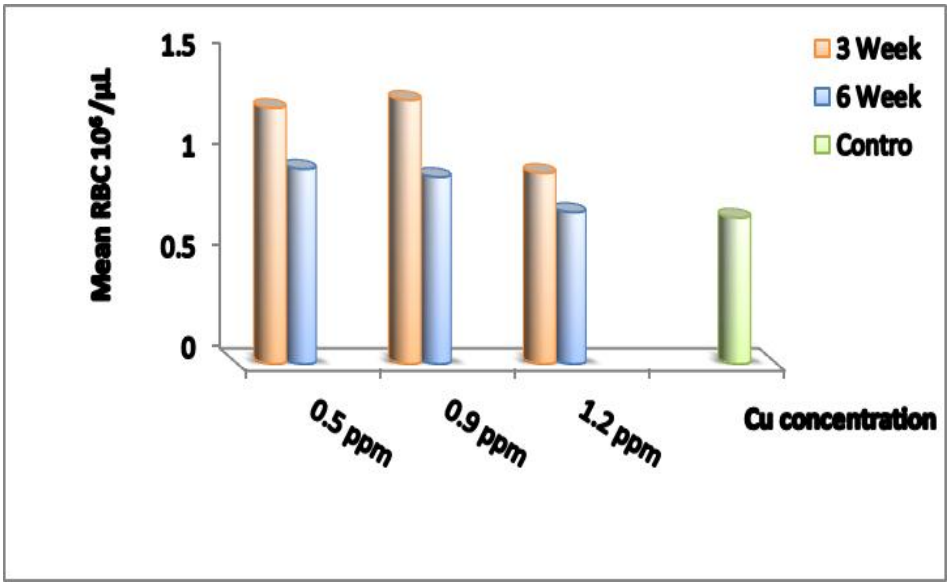

Figure 5- Mean RBC in the blood of fishes exposed to Copper for 3 and 6 weeks and a control sample.

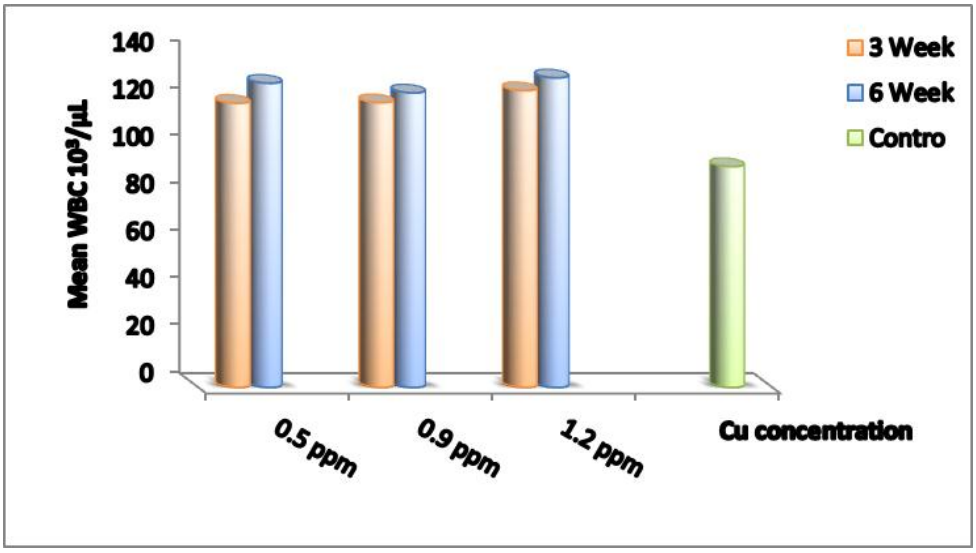

Figure 6- Mean WBC (10^3/µL) in the blood fishes exposed to Cu for 3 and 6 weeks and a control sample.

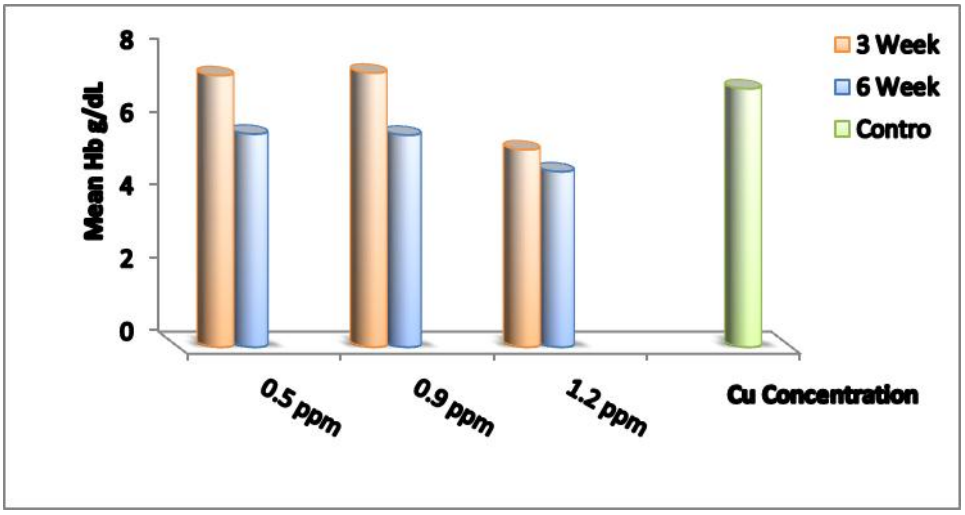

Figure 7- Mean Hb (g/dL) in the blood of fishes exposed to Cu for 3 and 6 weeks and a control sample.

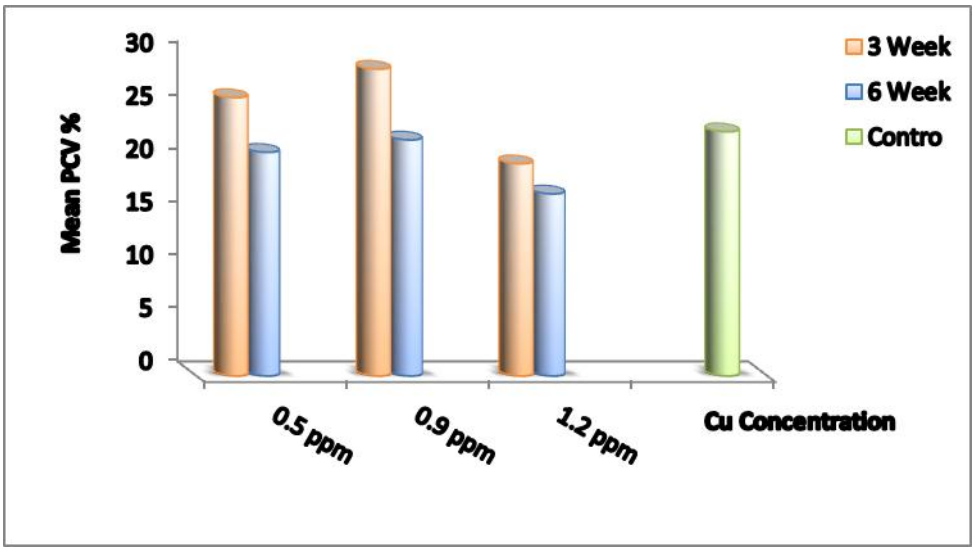

Figure 8- Mean PCV (%) in the blood of fishes exposed to Cu for 3 and 6 weeks and a control sample.

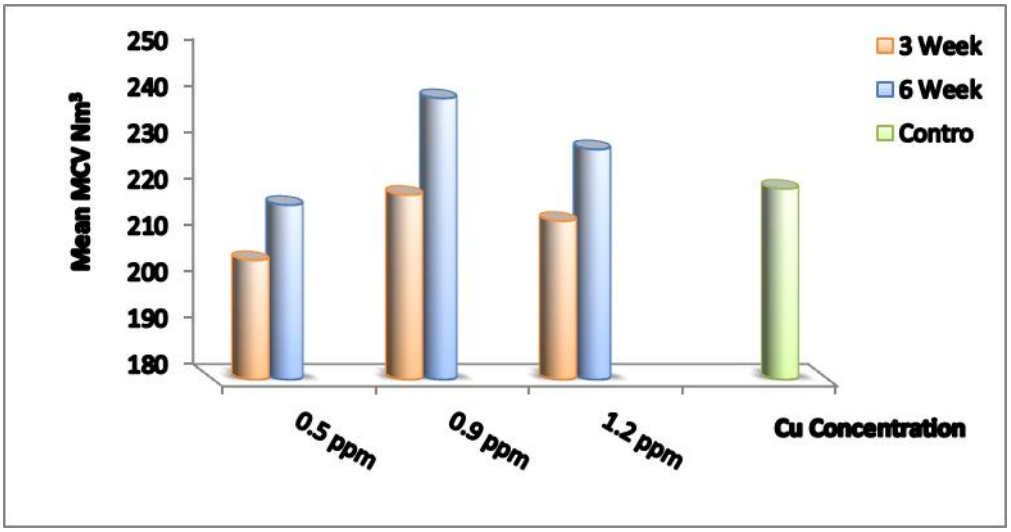

Figure 9- Mean MCV (Nm^3) in the blood of fishes exposed to Cu for 3 and 6 weeks and a control sample.

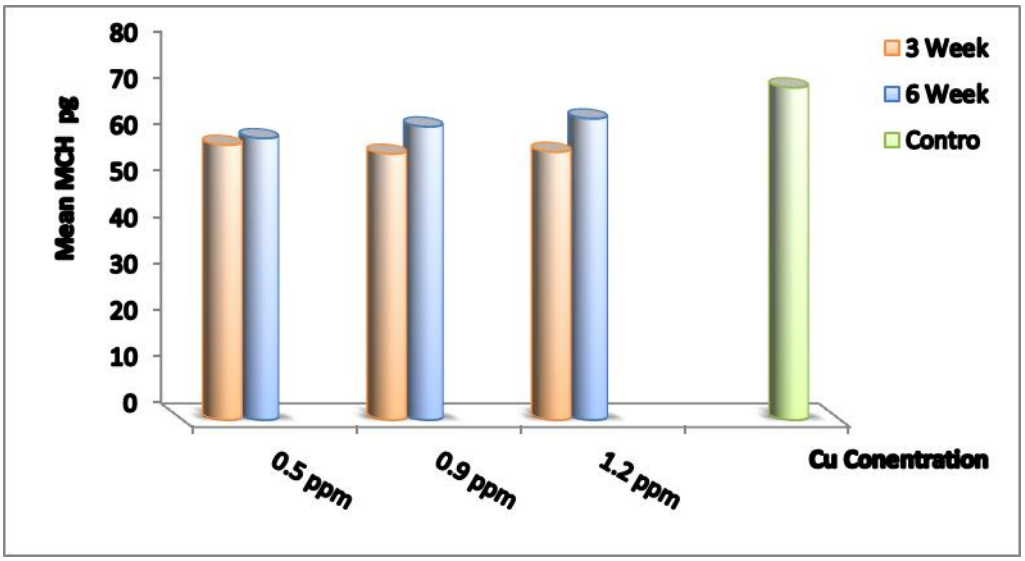

Figure 10- Mean MCH (pg) in the blood of fishes exposed to Cu for 3 and 6 weeks and a control sample.

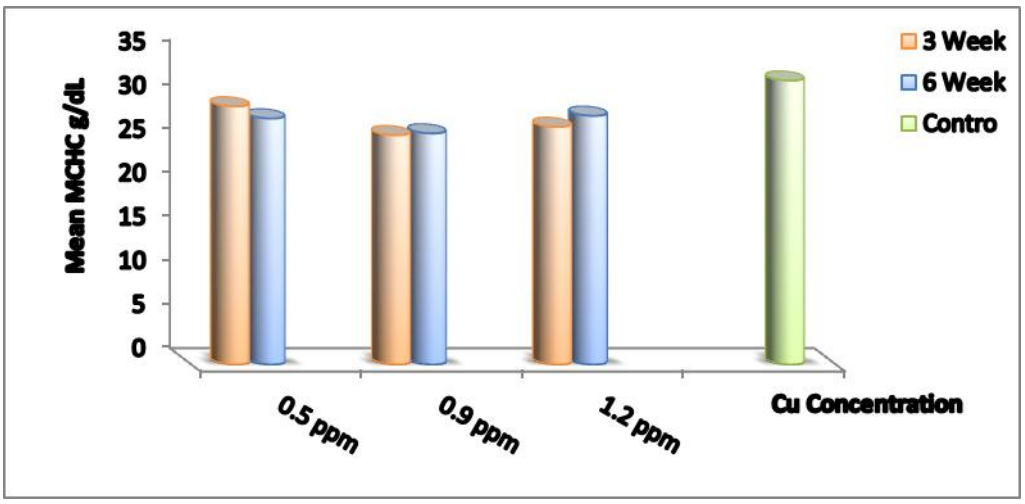

Figure 11- Mean MCHC (g/dL) in the blood of fishes exposed to Cu for 3 and 6 weeks and a control sample.

Conclusions

According to the results of this experiment, the LC_{50} values decreased with time, and about 50% of all mortalities occurred at the first 24 h. It was found that there was a positive relationship between the mortality and concentration levels; when the concentration level increased, the mortality rate increased as well. In the present study, the toxicity of copper and its harmful effect revealed various prominent abnormal behaviour such as erratic swimming, convulsion, loss of balance and difficulty in breathing in the exposed animal. The current study showed that the examined copper has profound effects of *Cyprinus carpio* represented by noticeable changes in hematological parameters. The blood parameters of *C. carpio* exposed to copper sulfate showed ion regulatory interference, but also compensatory responses to allow fish to endure and showed that a changes significantly affected hematology of *C. carpio.*

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