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Research Article

Aquatic Toxicology

Effect of Copper Sulfate On Hematological and Histological Parameters of Freshwater Fish *Oreochromis niloticus* at Various Sublethal Concentration

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Abstract: Heavy metals pollution is a great environmental problem in recent years throughout the world. Various activities such as domestic, anthropogenic activities, volcanic eruptions and industrial activities which increase their level of heavy metals concentration into the environment. Copper sulfate is used as an algaecide and fungicide in the fields of agriculture and aquaculture being discharged into water bodies and it can affect the aquatic organisms, especially fishes. The present study deals with the effect of copper sulfate on various hematological parameters of the experimental fish *Oreochromis niloticus. Oreochromis niloticus* is an important freshwater fish in commercial aquaculture. Fish is a significant food component of human food. Fish are the suitable bio-indicators of heavy metal contamination. Heavy metals are oxidative stress inducers in fish and can reflect aquatic contamination. The Objective of the present study was to analyze the hematological effects of heavy metal CuSO4 on the freshwater fish *O.niloticus*. The LC50 value of copper sulfate found to be 39.2 mg/L. The fish *Oreochromis niloticus* were experimented for the different sub lethal concentrations of 1/10, 1/20 and 1/30 of copper sulfate for the period of 30 days to get hematological changes. At the end of the experiment the result shows that the Red Blood Cell (RBC) count and the percentage of hemoglobin (Hb) were significantly decreased as the sub-lethal concentration of heavy metal Copper sulfate increased, while the level of WBC, MCV and MCH significantly increased. The effect of copper sulfate was observed by histopathological investigation of gill and liver of treated fish. The aim of present study is to investigate the hematological and histopathological effects of copper sulfate for the period of 30 days at various sublethal concentration in various tissues of the freshwater fish *O. niloticus.*Dietary data were collected in detail through face-to-face interview on tilapia *O.niloticus* consumption. Intake of fish (g) was computed. Statistical analyses were conducted using SPSS software for Windows and all tests were considered significant at p<0:05.

Keywords: Copper Sulfate, *Oreochromis niloticus*, Hematology, RBC, WBC, MCV, MCH and Hb, Histopathology.

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1. INTRODUCTION

Pollution in the Environmental has been the main cause of the toxic contamination of aquatic media all over the world.[1]This waste contains mainly heavy metals, pesticides, radioactive substances and herbicides.[2]Heavy metal pollution is the major common toxic pollutants in aquatic media.[3]The transformation of heavy metals can cause bioaccumulation in the tissues of organism and disrupting the biological chain.[4]Heavy metals waste discharged into aquatic media that can pollute the water bodies.[5]Aquatic fish are the reflect metal contamination in the aquatic media.[6]Heavy metals CuSO4 accumulate in various organs like muscle, gill, kidney and liver of the fish body.[7]Among various heavy metal, the copper is being recognized as toxic metals to aquatic living organisms. Pollutants in aquatic media can create adverse consequences on reproduction and growth of aquatic organisms mainly on fish.[8] Copper sulfate is used in reservoirs, ponds, lakes to control the algae populations.[9]Fish are good food sources for humans. Fish are suitable bio indicators of heavy metal contamination. In the present study, the blood parameters and histopathology of the fish were used as indicators of their physiological state. Blood parameters in fish have been frequently used as valuable indicators of environmental monitoring in the presence of toxicants.[10]The hematological parameters are important to evaluate the effects of metal pollutants.[11]Hematological parameters are used to detect physicochemical changes in a fish species and to assess functional and structural health during various stress conditions.[12]Fish is sensitive to pollution-induced stress, and changes to the hematological parameters, such as RBC, WBC and hemoglobin content, can be used to monitor toxic stress caused by heavy metals.[13]Studying various blood parameters in aquatic fish has become a significant health measure.[14]The present study was an attempt to find out the various effect ofcopper sulfate on hematological parameters like RBC, WBC, Hb, MCH and MCV levels of *Oreochromis niloticus*. The study of the hematological parameter is an important tool and this can monitor pathological changes in fishes.[15]In recent years fish hematology has become an important tool of fisheries biologists and researchers.[16] Pathological observations have beensuggested as a relevant methodology for the evaluation of aquatic contaminations.[17] Gill histopathological alterations like hyperplasia have been observed in fish submitted to CuSO4.[18]Heavy metal accumulation in various organ is the important route cause through which a metal pollutant can enter across the food chain of human being resulting in health risks.[19]The present experiment provides the effect of CuSO4 at different sublethal concentration of 96 h LC50 using freshwater fish *Oreochromis niloticus*. Aim of this study was to investigate hematological and histopathological effects of CuSO4 for the period of 30 days in liver and gill of freshwater fish tilapia, *Oreochromis niloticus*, exposed to different sublethal concentrations of CuSO4 (1/10, 1/20 and 1/30).

2. MATERIALS AND METHODS

2.1 Collection of Fish

Fish tilapia *Oreochromis niloticus*is one of the commercial species. *Oreochromis niloticus*of mixed sexes and fairly uniform size were obtained from Tilapia Research Center, Barur in Krishnagiri district, Tamilnadu, India and authentication of Dr.Somu Sundar Lingam, Assistant Professor, Incharge of TRC Barur. The fish collected were transported in water filled polythene bags. The fishes brought to the aquaculture laboratory in the Department of Zoology, Govt. Arts College for Men, Krishnagiri were acclimatized in cemented fish tank before they were used for the experiment. The fish disinfected with potassium permanganate (0.1%) solution were maintained in a well-aerated tap water tank for the period three weeks. Experimental fishes were screened for physical damage and mortality. The test solution renewed every 24 hours to maintain O2 level.

2.2 Experimental Design

In the present experiment, the LC50 value of CuSO4 was calculated according to Finney,1971[20] and it was found to be 39.2 mg/L. The average body weight of 23g to 25g (15-18cm in length) fish were collected from the acclimatized tank and divided into 4 groups, contain 10 fishes. Three of these groups were exposed to sub-lethal concentration for 1/10, 1/ 20 and 1/30 of the LC50 of Cuso4 for 30 days. The fourth, unexposed group served as a control group. The water was renewed every day to maintain oxygen level. Dissolved O2 was added with a biological filter. Fish were fed twice a day. Temperature was maintained at 26 ± 1°C. Test fish Mortalities within each group were recorded every day. (Figure.1,2,3)

Figure 1 illustrates that the morphological changes of fish O.niloticus experimented with sublethal concentrations of CuSO4 for the period of 30 days.

Fig.1.Toxic effect of CuSO4 on fish *Oreochromis niloticus*

Figure 2,3 illustrates that the laboratory work of researches and experimental setup in aquaculture laboratory at where the work was carried out.

Fig.2,3. Experimental setup in aquaculture laboratory

2.3 Water Quality

Chlorine free water was used in all the tests (Table.1)

2.3.1 Characteristics of the Experimental water

Table.1. Water Quality

S.No	Parameters	Units	Value
1	**pH**	-	7.3 to 7.5
2	**Temperature**	^{o}C	26 ± 1
3	**Alkalinity as CaCO3**	mg/L	255 to 260
4	**Dissolved Oxygen**	mg/L	7.2 to 7.4
5	**Total hardness as CaCO3**	mg/L	300 to 320
6	**Salinity**	mg/L	0.42 to 0.49

Water quality maintained for the study (Committee on Water Quality Criteria, 1972)[21]

2.4 Hematological Studies

2.4.1 Collection of Blood Sampling

At the end of 30 days of exposure periods, the blood sample was collected from both CuSO4 treated (1/10, 1/20, 1/30) fish and control fish. The fish were caught with the least disturbance. The blood sample was collected from the experimented fish using the technique of cardinal vein puncture by using a syringe containing 0.1ml of 0.2% EDTA. The blood was mixed with the heparin anticoagulant solution (1:10 physiological saline). The blood cells counted manually using Neubauer chamber and microscope after making a special type of wet mount. To estimate various parameters in the blood (RBC, WBC and Hb), the following procedures were adapted.

2.4.2 *Estimation Of Total* RBC , WBC, Hb, MCV, MCH

The counting of RBC and WBC is done by manually using a microscope after making a special type of wet mount. Mukherjee (1988).[22]The method used to count the RBC cell is popularly known as hemocytometry. Number of RBC count per sq.mm calculated by using the following formula,

$$\text{Total RBC} = \frac{\text{No. of Cells x Dilution factor x Depth factor}}{\text{Total No. of Small Squares}} = \text{cubic mm}$$

To enumerates the level of WBC the following formula is used,

$$\text{Total WBC} = \frac{\text{No. of Cells counted x Volume of the Square x Dilution factor}}{\text{No. of Squares (4)}} = \text{cubic mm}$$

Acid haematin method adapted for the estimation of Hemoglobin (Sahli, 1962).[23]The blood indices like MCV, MCH were then calculated using the method of Lee et al., (1998).[24]The following formula was used for calculating MCV, MCHin each sample of blood.

$$MCV = \frac{hematocrit\ (\%)}{RBC\ \ count\ (millions/mm^3 blood)} \times 10$$

$$MCH = \frac{Hb\ (in\ g/d\ L)}{RBC\ (\ in\ millions/\mu L)} \times 10$$

2.5 Analysis Of Histology

To analyze the tissue damage caused by heavy metal CuSO4, the Liver and Gill of the treated and control tissues were fixed in fluid of Bouin's. The standard histological technique was adapted after 24 hr, by the method of Gurr(1959)[25] to observe the histological variations.

2.6 Dietary survey on Human health risk

A dietary survey was conducted based on a questionnaire method. In the present experiment a questionnaire survey was conducted on average of 1000 healthy adults randomly from the general population at different fish shops nearby KRP Dam at Krishnagiri ,Krishnagiri district, Tamil Nā du. Dietary fish intake data were collected in detail through face-to-face questionnaire-based interviews. Data survey lasted 35 days, until the end of 5 weeks. The questionnaire included quantities and types of fish consumption. Only fish tilapia was included in this survey. Intake was computed in gram (g). Statistical analyses were conducted.

3. STATISTICAL ANALYSIS

Observed data were statistically analyzed and performed using SPSS 20.0 software by ANOVA along with DMRT (Duncan, 1957)[26] which was applied to find significant variations between different control means and experimental means for the collected data. The observed data were presented as mean ± SE and significance were declared at ($P < 0.05$).

4. RESULT

3.1 *Hematological Studies*

The blood parameters of fish *O. niloticus* treated with various sub-lethal concentrations of CuSO4 for the period of 30 days showed decreased levels of RBC, Hb and increased level of WBC, MCV, MCH at the end of all experiments when compared with the control group. Significant variations were observed in the level of Hb in the treatment groups. The Hb level in the control group was 5.3±0.07, whereas fish *O. niloticus* in the CuSO4 treatment groups showed decreased level of Hb (4.6±0.02(1/10), 4.1±0.09(1/20), 3.8±0.02(1/30) (g/dl). The level of RBC in control was 2.82±0.14(×10^6/µl) and fish treated with CuSO4 showed decreased level of RBC count (1.62±0.11(1/10), 2.09±0.17(1/20), 2.31±0.18 (1/30)(×10^6/µl)). The results of the present experiment showed that MCV and MCH levels significantly raised as the concentration of CuSO4 increased. MCV value observed for the control group was115.8 ± 1.16 while the values observed for fish *O. niloticus* exposed to CuSO4 increased respectively, 140.6±1.10, 132.7±1.07, and 121.3±1.02 . MCH value noted for the control group was 34.2 ± 2.09 while the values observed for fish *O. niloticus* exposed to CuSO4 increased respectively, 50.0±1.07, 41.3±1.06, and 36.8±1.02. However, the WBC results in this experiment indicated that the level of WBC increased as the CuSO4 concentration increased . The WBC count of control group was 76.0±0.05(×10^3), whereas WBC count in fish *O. niloticus* in the treatment groups to 103.0±0.01(1/10), 92.4±0.05(1/20), 83.2±0.03(1/30) (×10^3) increased and sublethal concentration of CuSO4 increased while compare with control group. Maximum declined levels ofHb, RBCand increased levels of WBC, MCV, MCH were observed in the period of 30 days. (Table.2).

Table. 2 Changes in hematological parameters of *Oreochromis niloticus* experimented to different sub lethal concentration of copper sulfate for the period of 30 days

Hematological parameters	Control	Experiment 1 (1/10)	Experiment 2 (1/20)	Experiment 3 (1/30)
RBC (count×10^6)	2.82±0.14	1.62±0.11	2.09±0.17	2.31±0.18
WBC (count×10^3)	76.0±0.05	103.0±0.01	92.4±0.05	83.2±0.03
HB (g/dl)	5.3±0.07	4.6±0.02	4.1±0.09	3.8±0.02
MCV(fl)	115.8±1.16	140.6±1.10	132.7±1.07	121.3±1.02
MCH (pg)	34.2±2.09	50.0±1.07	41.3±1.06	36.8±1.02

All the values mean ± SD of six observations Values which are not sharing common superscript differ significantly at 5% (p < 0.05) Duncan multiple range test (DMRT)

Table I illustrates that the fish *O.niloticus* treated with various sublethal concentrations of CuSO4 for the period of 30 days showed various changes in their hematological parameters. The level of RBC, Hb were decreased and the level of WBC, MCV, MCH were increased at the end of 30 daysexperiment. Heavy metal CuSO4 affect the hematological study in fish.

4.1 Histological studies

The present study is to investigate the histopathological effects of copper sulfate on the freshwater fish *Oreochromis niloticus* for the period of 30 days of exposure. End of the exposure period, fish from the control and treated group were sacrificed, and the tissues of gill and liver were removed from the experimental group as well as from the control group for histopathological examination.

4.1 Histology and pathological examination in the liver tissues

The liver of freshwater fish, *Oreochromis niloticus*(control group) comprises polygonal structural hepatocytes with distinct vesicular nucleus (VN). Hepatopancreatic cells (HS), blood sinusoids (BS) and cytoplasm with vacuolation (CV) were also noted among the hepatocytes (Plate.1). The Liver of experimental fish, *Oreochromis niloticus* exposed to different sublethal concentrations of CuSO4 for the period of 30 days showed significant histopathological alterations like space formation (SF), rupture of hepatocytes (RH), vacuolization (V), enlargement of hepatocytes (EH). Severe structural damages were observed in the liver of experimental fish *O. niloticus* (1/10 of sublethal concentration) at the end of 30th days of exposure period.

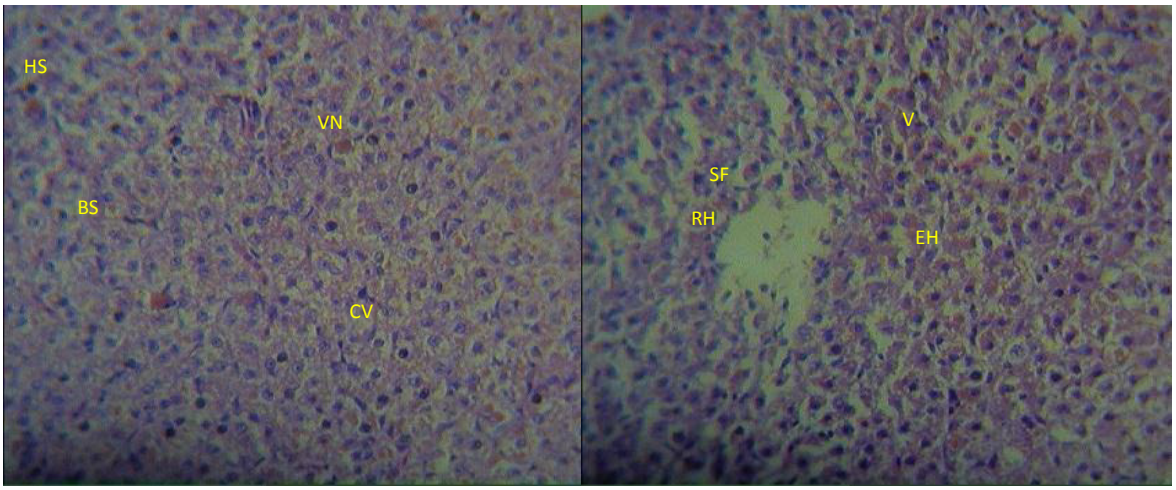

PLATE – A **PLATE – B**

Figure.4. Histology and Histopathology OfLiver (PLATE-A and B)

A – Section of liver taken from control fish O. niloticus showing normal histoarchitectural pattern (Xca 200)
B – Section of liver taken from high sublethal concentration(1/10) of CuSO4 treated fish O. niloticus for the period of 30 days (XCa 200)

Plate A and Plate B (Figure,4) illustrates that the fish *O.niloticus* treated with various sublethal concentrations of CuSO4 for the period of 30 days showed various Histopathological changes in their Liver architecture. Space formation, Rupture of hepatocytes, Vacuolization are the common histopathological changes were observed at the end of 30 days experiment.

4.2 Histology, Histopathological examination of gills

The gill of freshwater fish *O.niloticus*(control group) showed four-gill arches. Primary gill lamellae (PGL) of control fish arranged alternately on both sides of the interbrancheal septum. Secondary gill lamellae (SGL) of control fish arranged on both sides of the Primary gill lamellae. Primary gill lamellae comprises blood vessels (BV), lining epithelial cells. Secondary gill lamellae consisted of blood spaces (BS), contractile pillar cell system. At the base of gill filament mucous cells (MC) were observed (Plate-C).The Gill of freshwater fish, *O. niloticus* exposed to different sublethal concentrations of CuSO4 for the period of 30 days showed histopathological alterations like fusion in secondary gill lamellae (FSL), swelling of secondary gill lamellae (SSL), curling of secondary gill lamellae (CSGL), Rupturing of secondary gill lamellae (RSGL), cytoplasmic vacuolization (CV), damaged RBC cells (DRBC) (Plate-D). Significant histopathological variations were observed at 1/10 sublethal concentration of CuSO4.

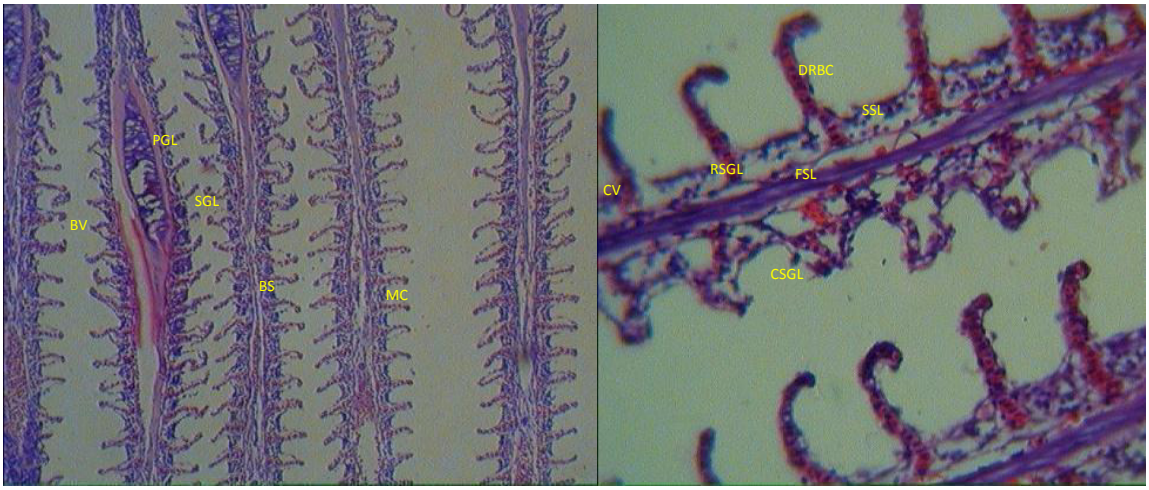

PLATE – C **PLATE – D**

C – Section of Gill taken from control fish O. niloticus showing normal histoarchitectural pattern (Xca 200)
D – Section of Gill taken from high sublethal concentration(1/10) of CuSO4 treated fish O. niloticus for the period of 30 days (XCa 200)

Fig.6,7 Histology and Histopathology of Gill (PLATE-C and D)

Plate C and Plate D (figure.6,7) illustrates that the freshwater fish *O.niloticus* experimented with different sublethal concentrations of CuSO4 for the period of 30 days showed different changes in their Gill architecture. Swelling of secondary gill lamellae, curling of secondary gill lamellae, rupturing of secondary gill lamellae are the common histopathological changes observed at the end of 30 days experiment.

4.2 Survey On Fish Consuming Human Population

Fish tilapia consumption during the intervention period was observed every week with a dietary recall questionnaire. From the present data survey, it was observed that tilapia consuming population increased at different fish shops nearby KRP Dam at Krishnagiri ,Krishnagiri district, Tamilnadu. The average fish consumption by the consumers at different fish shops nearby KRP Dam, Krishnagiri district plotted in the table. Shop-1 (215.8%), Shop-2 (214.0%), Shop-3 (151.0%), shop-4 (166.4%), - shop-5 (235.6%)). Average of 982.8 % fish (*O. niloticus*) consumers were enrolled in the 5 weeks study (Table.3). The number of fish consuming people were increased in various shops nearby KRP Dam areas at Krishnagiri. At the end , heavy metal intake through fish also increased in the particular area. Consumption of heavy metal exposed fish may affect the health of Humans.

Table. 3. Survey of fish *O. niloticus*consumer in different fish Shops in near KRP Dam, Krishnagiri district

Places	1st week of survey	2nd week of survey	3rd week of survey	4th week of survey	5th week of survey	Average
Shop-1	210	218	191	242	218	215.8
Shop-2	200	216	173	211	270	214.0
Shop-3	174	139	145	147	150	151.0
Shop-4	145	187	138	200	162	166.4
Shop-4	280	253	205	190	250	235.6
						982.8

Survey on Tilapia consuming population at various shops near KRP Dam, Krishnagiri

Table 3 illustrates that the consumer of fish *O.niloticus* in various fish shops near KRP Dam at Krishnagiri district were increased. The consumer ratio in all shops (Shop-1, Shop-2, Shop-3, Shop-4 and Shop-5) were randomly high. Average of 982.8 % fish consumers were observed in the 5 weeks study. Heavy metal CuSO4 intake through fish also increased in the area of KRP Dam at Krishnagiri district. Consumption of CuSO4 exposed fish may affect the health of Humans.

4.3 Effect

Heavy metal CuSO4 is considered as an oxidative stress inducer in fish and humans. The present experiment on freshwater fish *O.niloticus* studied to CuSO4, showed histopathological alterations and variations in the blood parameters. The humans affected by toxic fish consumption may also get variations in their blood parameters. The present study advises consumers to monitor the heavy metal

mixture in the water bodies of particular areas along with health care advice.

5. DISCUSSION

The use of heavy metals has been causing environmental problems and their deposition in aquatic biota influencing functional, structural and activities of the aquatic ecosystem. In the present experiment significant reduction of RBC was observed in fish *O. niloticus* treated with different sublethal concentrations of CuSO4 (1/10, 1/20,1/30) for the period of 30 days of exposure periods. Similar results of reduction of Hb% and RBC count in fish exposed to various heavy metals have been observed by Goel and Sharma (1987)[27] and Goel et al. (1985).[28]Disrupted hemoglobin synthesis and blood cell injury were reported due to copper induced stress (Mckim et al., 1970).[29] Increased number of WBC observed in CuSO4 treated fish *O. niloticus* at different sublethal concentrations (1/10, 1/20 and 1/30) for the period of 30 days of exposure periods. Increasing WBC count has been considered to be an adaptation method of fish to meet stressful conditions. Under stress conditions the toxic substances activate and promote the lymphocytes, which may lead to increase the number of WBC (El-Sayed *et al*., 2007).[30]Mishra and Srivastava (1980)[31] also observed increased levels of WBC count when freshwater fish were exposed to heavy metals. Atamanalp and Yanik (2003)[32], Atamanalp et al. (2002a)[33] found a significant variation in the Hb, MCH, MCHC count and erythrocyte sedimentation rate in rainbow trout (*Oncorhynchus mykiss*) following cypermethrin acute exposure. In the present study, fish *O. niloticus* when exposed to various sublethal concentrations of CuSO4 for 30 days showed various histopathological alterations in the gills such as excessive secretion of mucous in the Intercellular spaces, fusion of secondary gill lamellae, damaged RBC cells, curling of secondary gill lamellae, cytoplasmic vacuolization, rupturing of secondary gill lamellae. Saphia Ali Aitte (2020)[34] observed histopathological changes like necrosis in the secondary epithelial gill lamella, excessive hematoma and appearance vacuolation. Gill histological changes have been also noted by several authors in freshwater fish submitted to copper (Karan et al.,1998).[35]Enlargement of hepatocytes, vacuolization of the tissue, rupture of hepatocytes and their nuclei, necrosis were the important abnormalities studied in the liver of *O. niloticus* exposed to different (1/10, 1/20, 1/30) sublethal concentrations of CuSO4. The result of the present study is similar with the observation of Sarkar *et al.*, (2005)[36] such as formation of rupture in blood vessels, vacuoles, degenerations of cytoplasm in hepatocytes in the liver of freshwater fish *Oreochromis niloticus* exposed to cypermethrin for the period of 10 days. Cell proliferation and thickening of gill filament is a histological alteration found in fishes exposed to the copper by Arellano et al., (1999).[37]Alternations in the tissues of liver could be explained by Cu-induced oxidative stress (Hoyle et al.,2007).[38]Heavy metal may cause adverse health effects like nephrotoxicity.[39] It may causes various health effects such as constipation, anemia and vomiting.[40]Similar results were also reported by El- Sadaawy, 2013.[41] Fishes are more sensitive to contaminants and water pollutants that may severely damage the organs of the aquatic fish.[42] In the present experiment, liver tissues lost their architecture on exposure to CuSO4. Similar copper induced histopathological changes reported in liver and gills of freshwater fish *O.niloticus* by Abdel Tawwab et al., (2001).[43] The present study states that hematological and histological variations are observed in the different organs of freshwater fish *Oreochromis niloticus*by heavy metal CuSO4 depending upon the toxicity and consumption of freshwater fish affected by heavy metal toxicity increase the health risk of the people.

6. CONCLUSIONS

It is concluded that the present experiment is to analyze the various effects of heavy metal CuSO4 at various sub lethal concentrations in *O. niloticus*for the period of 30 days. Significant changes observed in hematological parameters like RBC, WBC, Hb, MCV and MCH and histopathological changes in Gill, liver of *O. niloticus*would be due to the toxicological effect of heavy metal CuSO4. This might be related to the adaptive response by freshwater fish towards heavy metal toxicity in an aquatic environment. From this study it is clear that aquatic pollution due to CuSO4 has a deleterious influence on aquatic organisms like fish and humans. Therefore, the presence of heavy metal CuSO4 in aquatic media should be a more concern for the potential risk on the freshwater fishes and human health. Results obtained from this experiment can be useful for further research and analyze the safe level usage of heavy metals like CuSO4 in the aquatic environment. The present study hereby concluded that the heavy metal CuSO4 toxicity analysis, histopathological and Hematological examination of fish serve as a bio-indicator of the freshwater habitats. The consumption of freshwater fish like *O.niloticus* as a diet from such heavy metal polluted areas is a toxic threat to humans . Attention should be devoted to reducing the risk of heavy metal CuSO4 pollution in the surrounding environment to protect living organisms including the human population from the effects of these heavy metal pollutants.

3. ACKNOWLEDGEMENT

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7. ATHOR CONTRIBUTION STATEMENT

Mr. Sasikumar.S conceptualized and gathered the data with regard to this work. Dr.Prakash Sahaya Leon. J analyzed these data and necessary data were given to designing of the manuscript. All authors discussed the results and contributed to the final manuscript.

8. ABREVIATIONS

CuSO4-Copper sulfate

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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