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The Comparison of Acute Toxicity (96h) of Copper ($CuSO_4$) in *Cyprinus Carpio and Rutilus Rutilus*

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Abstract

The experiments were conducted to study of the acute toxicity of *Cyprinus carpio and Rutilus rutilus* by added copper salt in basins. 13 fishes with average weight of 2±0.5g to *Rutilus rutilus* and 20±2.2g *Cyprinus carpio* were exposed to different concentrations of copper salt respectively.

The experiments were done by Static Method during 96 hours. A group of fish was considered as control samples. The different concentrations of copper such as 0, 0.1, and 0.2, 0.3, 0.4, 0.45 and 5mgL^{-1} were used to Rutilus *rutilus* and *Cyprinus carpio* fishes. Under stable condition (T^{OC} and pH), the lethal concentration of copper was measured of 0.4mgL^{-1} and 0.45mgL^{-1} to *Rutilus rutilus* and *Cyprinus carpio* respectively during of 96 hours. The results indicated the significant differences were observed between treatments of fish with each other and also with the control samples.

With increasing of copper in each treatment, the mortality rate of fish significantly was increased. Histopathological findings showed that major lesions were hemorrhage, hyperemia, hyperplasia and epithelial cells necrosis in total fish. Also in the lesion fish were observed degenerated tubules of their kidney, expansion of Bowman's capsule and hepatocytes necrosis.

Keywords: acute toxicity, copper, static method, *Rutilus rutilus, Cyprinus carpio*

1. Introduction

Fish production capacity can increase by identifying environmental factors and providing an appropriate environment for the fish (Imanpoor, Ahmadi & Kabir, 2011). It has been established that heavy metals have wide spectrum of negative Influence on fish organisms, disrupting endocrine system, and inducing decrease of quantity and quality of offspring (Popek, Kleczar, Nowak & Epler, 2009).

Heavy metal enters the aquatic environment naturally through weathering of the earth crust. In addition to geological weathering, human activities have also introduced large quantities of metals to localized area (Thirumavalavan, 2010). Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Gharedaashi et al., 2013).

Heavy metal contamination usually causes depletion in feed utilization in fish and such disturbance may result in reduced fish metabolic rate and hence causing reduction in their growth (Gharedaashi et al., 2013). Growth is a sensitive and reliable endpoint in chronic toxicological investigations. However, Copper (Cu) is an essential metal for all organisms including fish. It has an important role in metabolism and its concentration is well regulated. However, Cu is one of the most toxic metals to fish and affects various blood parameters, growth, behavior, enzyme activity, and reproduction (Gharedaashi, Nekoubin, Imanpoor & Taghizadeh, 2013). Copper toxicity occurs when a specific amount of metal binds to physiologically active biological membranes, generally outcompeting cations injuring the physiological mechanism.

This threshold level depends on animal species and life stage. Water chemistry largely influences copper toxicity. It is known that copper bioavailability and toxicity occur when the amount of copper in water exceeds the combined capability of dissolved organic matter (DOC) to bind metal, cations to outcompete the metal for binding to the biotic ligand, water chemistry to transform the metal to non-toxic species, particulate organic carbon (POC) to absorb the metal, and mineral particles to incorporate the metal into their matrix (Martinsi &

Bianchini, 2008). Fish is an indicator to measure the freshwater contamination by heavy metals because they occupy different trophic levels in an aquatic ecosystem (Gharedaashi et al., 2013, Imanpour et al., 2011). LC50 is the biological index of 50% mortality in an ex- posed population. The 96-hour LC50 tests are conducted to measure the susceptibility and mortality potential of biota to particular toxic substances Higher LC50 values are less toxic because greater concentrations are required to produce 50% mortality in exposed animals (Gharedaashi et al., 2013).

Rutilus rutilus and *Cyprinus carpio* is a fish of the family Cyprinidae from brackish water habitats of the Caspian Sea and from its freshwater tributaries. It is typically a medium sized fish. The population seems to have collapsed due to over exploitation and marine pollution. Its flesh and roe is enjoyed as food, and highly prized in Guilan and Mazandaran provinces in Iran. However, ecotoxicological studies involving fresh water and marine species are needed for a better understanding of toxic effects induced by copper contamination in seawater animals. Therefore, the main goals of the present study were to determine the acute copper toxicity and to describe the kinetics of copper accumulation in gills, kidney and liver of *Cyprinus carpio and Rutilus rutilus*. In this respect, this study can help in the formulation of new concentration limits for copper, to improve environmental legislation (Campagna et al., 2008).

2. Materials and Methods

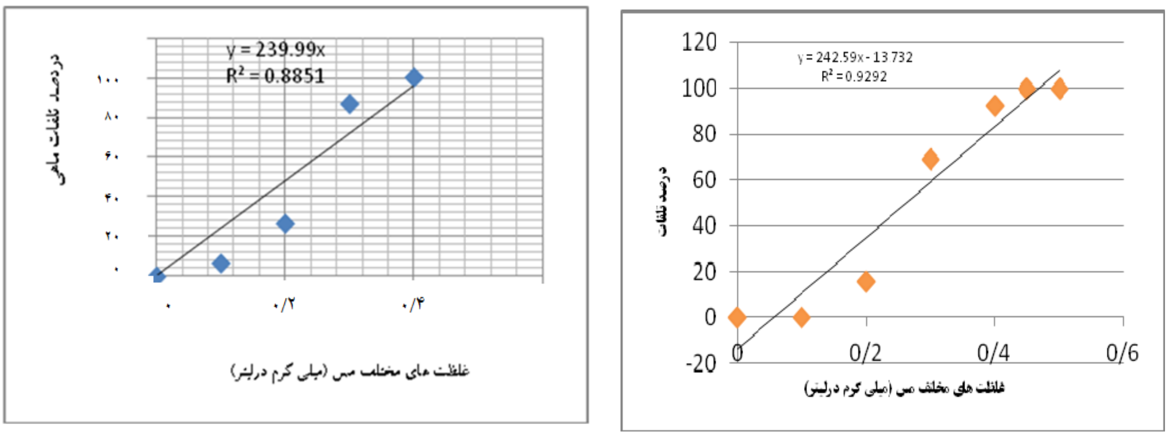
The experiments were performed in laboratory of Gonbad Kavous University, which located in eastern north of Iran, Golestan Province. The fresh water fish were collected from the fish farm located in Golestan Province. This fishes were brought to the laboratory and transferred to the fiber glass tanks of 500 liters capacity containing chlorine free aerated well water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions. Temperature in all stages was maintained to 25-26ºC and pH was equivalent 7.8-8.2. The experiments were done by Water Static Method (APHA, 1998; Peyghan, 1999) during 96hours (4 days). Fishes were divided into two equal groups each comprising of 13 fishes. Each group was kept in separate plastic tanks. The first group was kept as control; the fishes were maintained in water containing normal water without any treatment. Different amounts 0, 0.1, 0.2, 0.3, 0.4, 0.45 and 0.5 mgL^{-1} of CuSo4 salt was used. All fishes were placed in 21 basins (with 3-5 liters capacity) and in each basin, 13 fishes with average weight 20±3 and 2±0.5 g respectively to *Cyprinus carpio and Rutilus rutilus* were placed. The treatments of fish were recorded for each 12h by observation of their behavior and mortality. Stock solutions of copper sulfate were prepared by dissolving analytical grade copper sulfate (CuSO4.5H2O from Merck) in double distilled water. Air-stone has been used in treatment as an aerator. In duration time of the experiments till to last test, at each 12 hours, the amount of Cu in the water basin was measured and also all nominal behaviors of toxicity on fishes were observed. The number of dead fish was counted every 12 h and removed immediately from the aquaria. The mortality rate was determined at the end of 24, 48, 72 and 96 h. During the toxicity test, the fishes were not fed. The samples were taken from gill, kidney and liver of fishes and histopathological sections were prepared. Eventually, Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) Duncan (1957).

3. Results and Discussion

Acute toxicity of copper sulfate showed that mortality is directly proportional to the concentration of the copper sulfate while the percentage of mortality is virtually absent in control (Table 1). Based on the results in preliminary stage, highest mortality rate were observed in concentration of 0.3mgL^{-1} ($p<0.05$). The lethal concentrations of Cu were equivalent to 0.45 and 0.4 mgL^{-1} of that in *Cyprinus carpio and Rutilus rutilus* respectively for a period of 96 hours. The rates of mortality for first duration of the experiments were high. Mortality percentages of fishes were compared by exposing of the basins in different concentration of copper (0, 0.1, 0.2, 0.3, 0.4, 0.45 and 0.5 mgL^{-1}) after 96 hours as showed in Tab 1. All fish were survived in control at the duration time. The results have revealed that the mortality percentage was increased significantly with increasing of copper concentration ($p<0.05$). The LC_{50} (median lethal concentration) was obtained 0.262 and 0.208 mgL^{-1} to *Cyprinus carpio and Rutilus rutilus* respectively that resulted from a linear regression and relationship between the mortality percentage and different used concentrations of Copper (figure. 1). In this experiment the behavior of fish remarkably changed due to the treatment of copper sulfate when compared to the control. The various locomotary responses exhibited by fish due to sublethal concentrations of copper sulfate during initial stage of exposure included restlessness, erratic and fast swimming, abrupt change in position and direction, jumping and overall hyperactivity were noticed. The fish showed surfacing tendency throughout the experimental period. At the beginning of the test, some behavioral symptoms such as gasping, swallowing water, the curvature of muscles, hit the basin sides, lack of balance and severe reaction to external factors were observed in total fish especially to *Rutilus rutilus*. At duration of the experiments the copper poisoned fish sat on the floor of the basins and finally died. The most absorption of copper was recorded at the first 12 hours after starting of the test. So, the results have revealed that the mortality percentage in the lethal concentration of copper was 38.46, 69.23, 92.32, 100 in 24, 48, 72 and 96 hours to *Cyprinus carpio* while the mortality percentage in the lethal concentration of copper was 53.84, 84.61, 84.61, 100 in 24, 48, 72 and 96 hours to *Rutilus rutilus* (figure. 2).

Table 1. Mortality percentages of fishes in different concentration of copper

Treatment	**Concentration of Copper**	**Mortality rate**		**Mortality %**	
		Cyprinus sp.	*Rutilus sp.*	*Cyprinus sp.*	*Rutilus sp.*
1	Control	0	0	0	0
2	0.1	0	1	0	7.69
3	0.2	2	4	15.38	30.77
4	0.3	9	11	69.23	84.62
5	0.4	12	13	92.31	100
6	0.45	13	13	100	100
7	0.5	13	13	100	100



B. A.

Figure 1. A linear regression of fish mortality in different concentration of copper

a: *Cyprinus carpio* b: *Rutilus rutilus*.

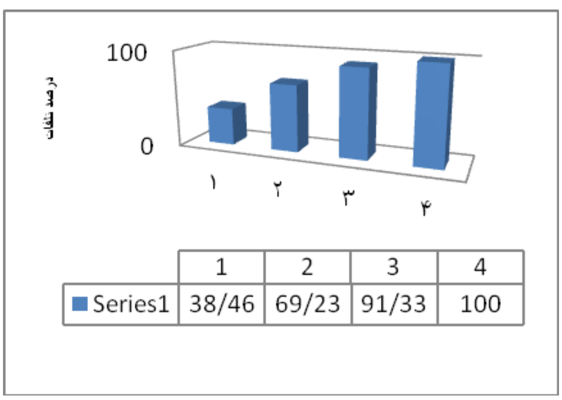


Figure 2. Mortality rate of fishes in different treatments in main test during 96h

The study of histological samples shows, the common lesions of fish gill which exposed on lethal concentration of copper were observed in different cases such as hyperplasia, edema, hyperemia, hemorrhage, expansion of secondary lamella, epithelial cells necrosis of gill and inflammation (Figure. 3, 4, 5, 6).The major lesions on kidney of fishes were detected such as expansion of Bowman's capsule, hemorrhage, hyperemia, degenerated tubules of kidney, epithelial cells necrosis of kidney and a lot of monocellular. In kidney of the control fish samples was observed any lesions (Figure. 7, 8). The liver lesions in the samples were contained of hyperemia, hemorrhage; inflammatory cells infiltration and hepatocytes necrosis (Figure. 9, 10). But in the control samples were not observed lesions. However, effects of toxicity were different in fishes.

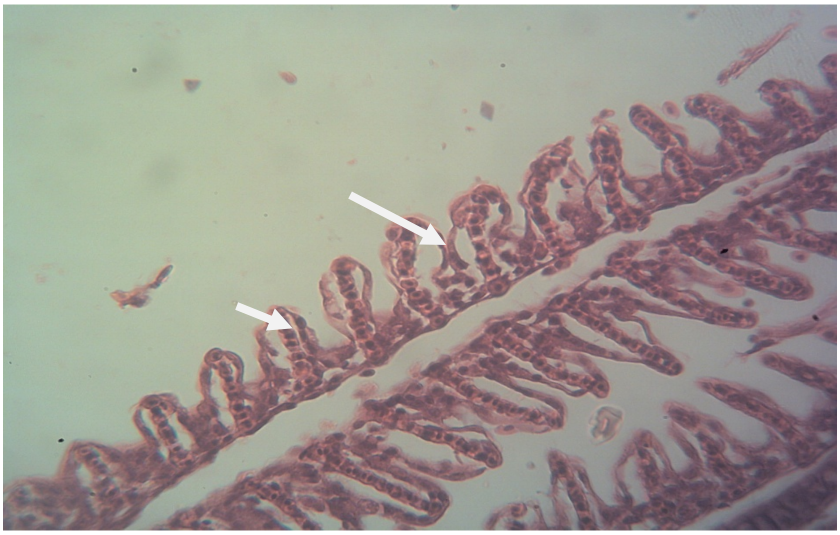


Figure 3. Arrows show edema of fish gills that exposed on lethal toxicity of copper in *Rutilus rutilus* (*100)

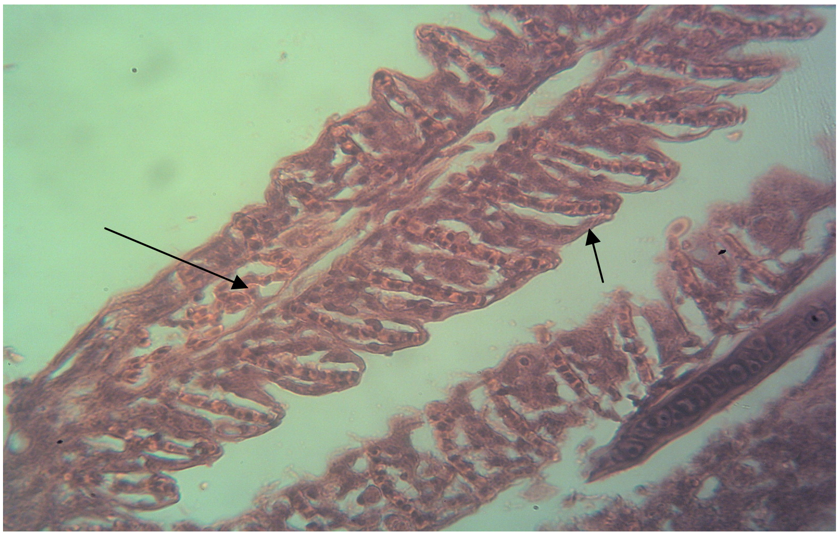


Figure 4. Arrows show edema and hyperemia of fish gills that exposed on lethal toxicity of copper in *Cyprinus carpio* (*100)

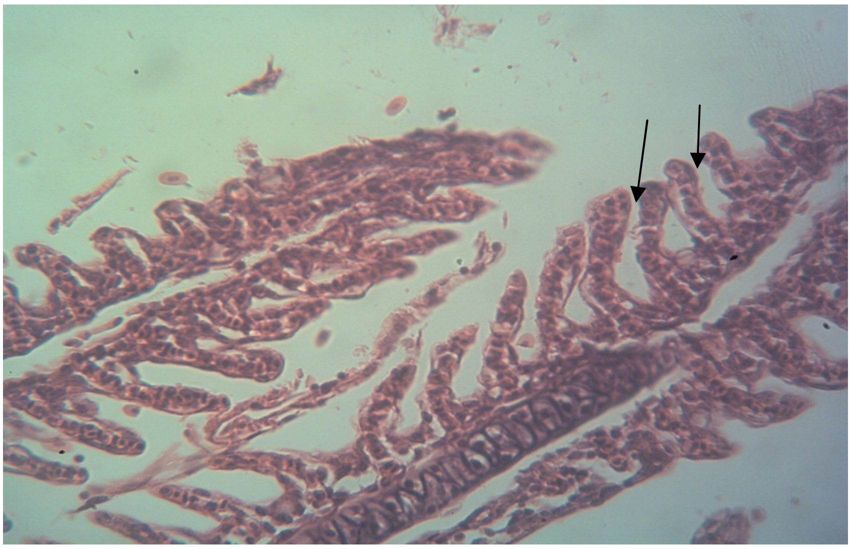


Figure 5. Arrows show clubbing of fish gills that exposed on lethal toxicity of copper in *Cyprinus carpio* (*100)

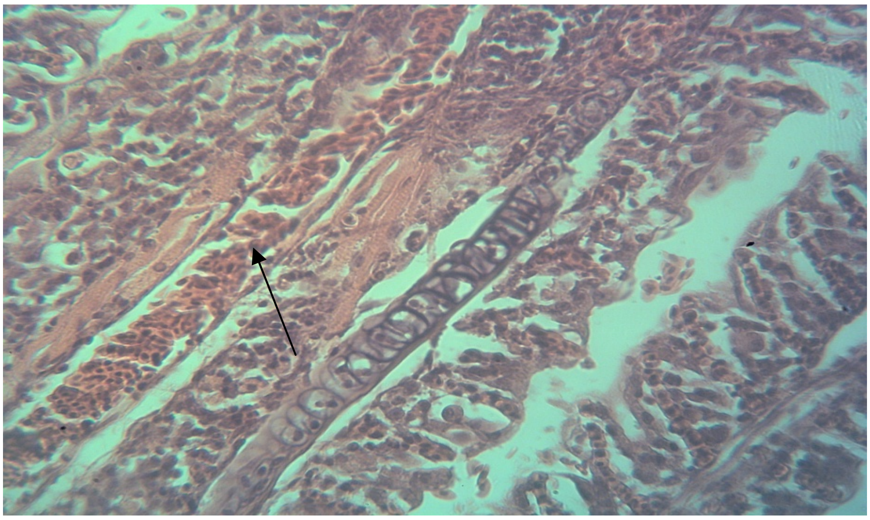


Figure 6. Arrows show hyperemia of fish gills that exposed on lethal toxicity of copper in *Rutilus rutilus* (*100)

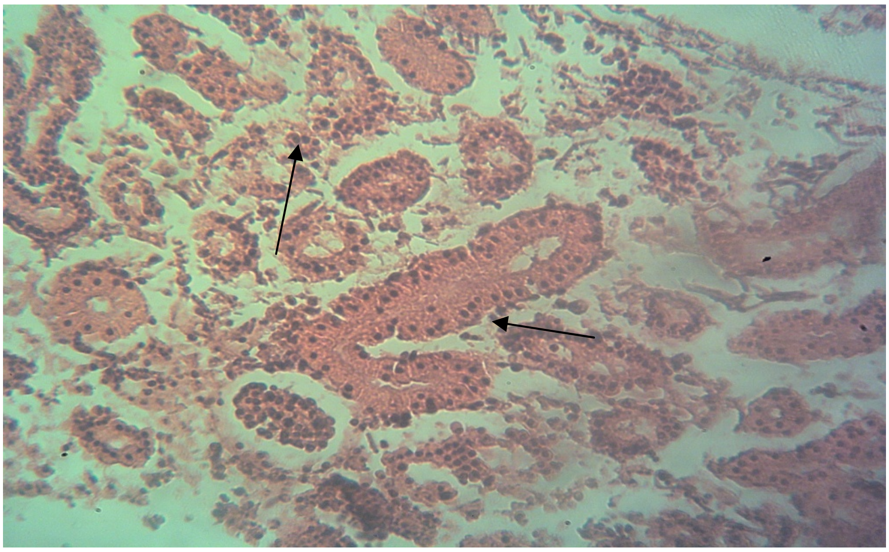


Figure 7. Arrows show degenerated tubules of kidny histological section that exposure to lethal copper toxicity in *Rutilus rutilus*. (* 400)

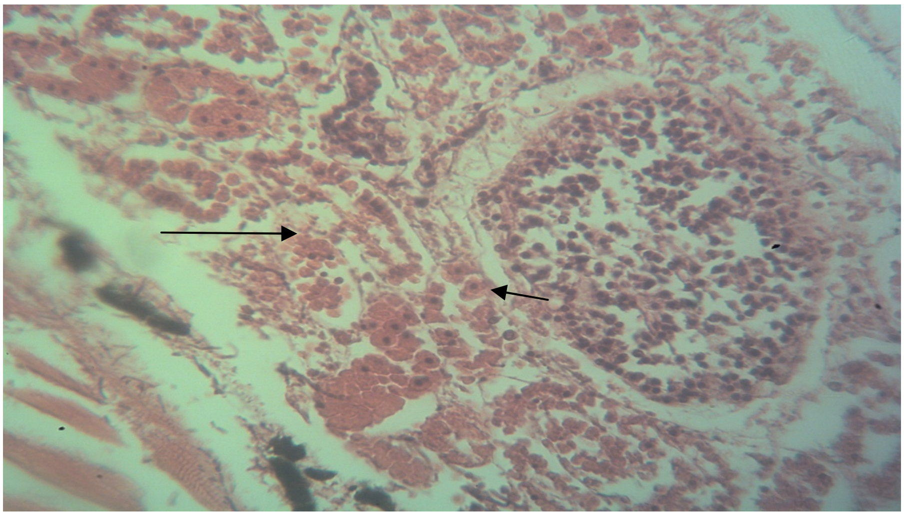


Figure 8. Arrows show hemorrhage and degenerated tubules of kidney histological section that exposure to lethal copper toxicity in *Cyprinus carpio* (* 400)

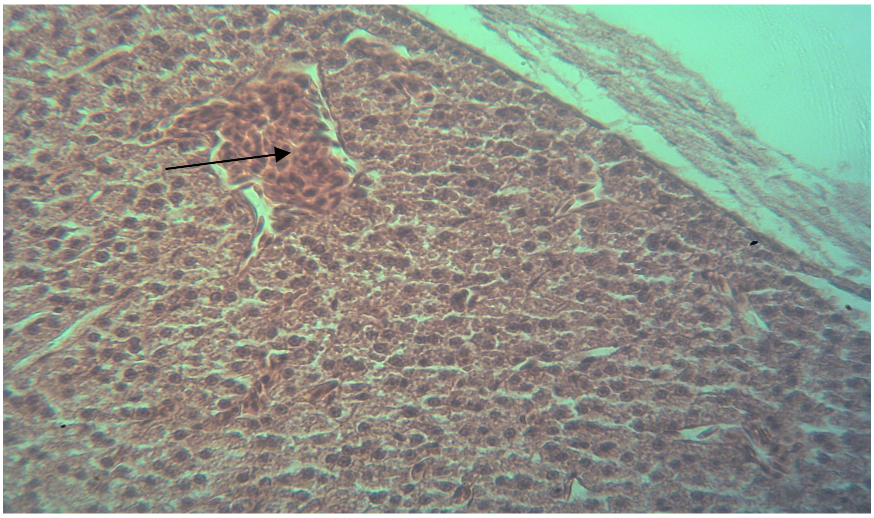


Figure 9. Arrows show hemorrhage into liver cells histological section that exposure to lethal copper toxicity in *Cyprinus carpio* (* 100)

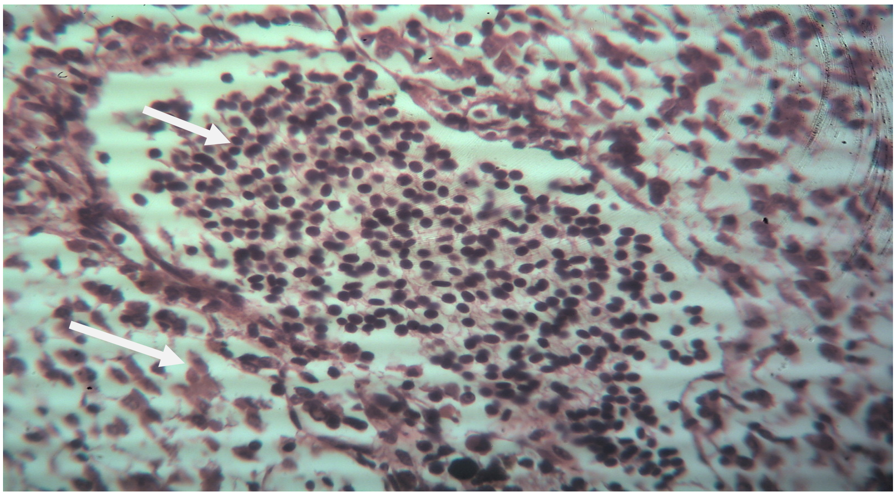


Figure 10. Arrows show inflammatory cells infiltration and hepatocytes necrosis histological section that exposure to lethal copper toxicity in *Rutilus rutilus* (* 100)

4. Discussions

Determination of the chemistry of the experimental media used to perform toxicity tests is of great important for a correct prediction of copper speciation, bioavailability, and its subsequent toxicity. In fact, several physical and chemical water parameters (e.g., pH, alkalinity, hardness, and ion concentration) have shown to influence copper toxicity (Martinsi & Bianchini, 2008). Toxic metals are recognized as one of the most hazardous environmental pollutants and are toxic to many living organisms. Copper sulphate is known for their action on biological tissue (Thirumavalavan, 2010). Fish are often used as sentinel organisms for ecotoxicological studies because they play a number of roles in the trophic web, accumulate toxic substances and respond to low concentrations of mutagens. Therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems (Khoshnood et al., 2014). Despite the many studies of the toxicity of copper, there have been few reports of the c toxicity of copper to *Cyprinus carpio and Rutilus rutilus* to assess these effects (Martinsi & Bianchini, 2008; Campagna et al. 2008; Thirumavalavan, 2010; Gharedaashi et al., 2013). Therefore, the objective of this study is to evaluate the toxicity of the copper ion on the behavior and histopathological of *Cyprinus carpio and Rutilus rutilus* exposed to lethal concentrations of copper. Based on the results, MATC (maximum acceptable toxicant concentration) for *Cyprinus carpio* that was below of 0.1 mgL^{-1} of copper. So, there was no mortality rate of fish in this concentration. However, there was no significant difference in histopathological sing in this concentration; therefore it can be effect on the growth during the period of culture. For example, the threshold levels of effect of copper on growth for rainbow trout 0.130mgL^{-1} were reported by Muir (1982). Bertoletti (2000), in evaluating the chronic toxic effects of various chemical compounds at different initial phases of the life cycle of *Danio. rerio* (Cypriniformes, Cyprinidae), under the same experimental conditions as this study, found a NOEC of 92.0 µg.Cu.L–1 for survival, i.e., a concentration roughly several times less than the NOEC calculated in this study. On the hand other, the variability of the toxicity can be attributed to several factors, such as: the sensitivity of the test organisms, which is reflected in their tolerance to toxic substances; the size of the organisms used in each bioassay and intrinsic factors of each individual (Campagna et al. 2008; Jahanbakhshi , Hedayati & Pirbeigi, 2015). So, there were the most reactions of toxicity for *Rutilus rutilus* which had least weight in this experiment.

Lloyd (1961) reported for the rainbow trout *(O.mykiss* Walbaum, 1792) the toxicity ratio by dissolved compounds such as ammonia and salts of zinc salts, lead and copper, and also phenols derivatives, were raised as markedly below 60% oxygen saturated. The studies have shown that the intensity of the lesions depends on the type of pollutant, concentration and time of exposure.

These changes can impair the exchange of gases necessary to metabolism, ionic regulation and acid-base equilibrium (Mazon, Cerqueira & Fernandez, 2002). A response was generally observed in the mortality rate which increases with increased concentration of copper (Table 1). In this research showed, that mortality rate is directly proportional to the concentration of the copper sulfate while the percentage of mortality is virtually absent in control (Table 1). Despite copper's importance as a micronutrient in the metabolism of aquatic organisms; the work showed that even at very low concentrations this metal can compromise the survival of fishes. Similar results have been reported by Gharedaashi et al., (2013). In this study the lethal concentration of copper was equal to 0.45 and 0.4 mgL^{-1} to *Cyprinus carpio and Rutilus rutilus* respectively. The mortality levels found in the acute toxicity bioassays were difference to those reported by Bertoletti (2000). Richey & Roseboom (1978). Richey & Roseboom (1978) illustrated that the 48 h-LC50 of Cu to rainbow trout (*Oncorhynchus mykiss*) in size of a yearling and alkalinity of 250, was 0.75 mg/l. (Jahanbakhshi et al., 2015).

This fact may be related to the exposure period, which was longer in the present study, and also to the different sensitivity of the species used. For example, *Rutilus rutilus* was sensitive than Cyprinus carpio in this research. In the natural environment, toxic substances are normally found at sublethal concentrations, and for this reason it is essential for effects that are not immediately visible (growth, morphological and physiological changes, etc.) to be evaluated in toxicity tests. Organisms submitted to chronic concentrations have reduced resistance, and instead of using energy for growth and reproduction, will divert it to detoxification of the pollutant. This can cause reduced size, reproduction and survival of fish, and consequently increase the vulnerability of the species itself.

The LC_{50} (median lethal concentration) was obtained 0.262 and 0.208 mgL^{-1} to *Cyprinus carpio and Rutilus rutilus* respectively that resulted from a linear regression and relationship between the mortality percentage and different used concentrations of Copper (figure 1). According to Gharedaashi et al., (2013) that median lethal concentration (LC50) of copper sulfate to Caspian Sea kutum *(Rutilus frisii kutum)* for 96 h of exposure was 2.310 ppm. However, the median lethal concentration 96 h (LC50) value of copper sulfate in other aquatic organisms were such as: the LC50 for *R. sumatrana*, for 24, 48, 72 and 96 hours for Cu were 54.2, 30.3, 18.9 and 5.6 µg^{-1}L and For *P. reticulata*, LC50 for 24, 48, 72 and 96 hours for Cu were 348.9, 145.4, 61.3 and 37.9 µg^{-1}L respectively (Shuhaimi-Othman et al., 2010), the 24 h- LC50 of Cu was reported as 1.17 mg/L for *P. reticulate* (Park & Heo, 2009) which were lower than present study. Gomes, Chippari-Gomes & Gomes (2009) reported that with juvenile Brazilian indigenous fishes, curimata *Prochilodus vimboides* and piaucu *Leporinus macrocephalus,* 96 h- LC 50 of copper were 0.047 and 0.090 mg/L, for *curimata* and *piauçu*, respectively, which were lower than present study. This indicates that different organisms have different sensitivity to toxic substance. The toxicity reported by other studies differs from this study probably due to different species used, aged, size of the organism, test methods and water quality such as water hardness, as this could affect toxicity.

The most mortality has happened at earlier hours of the tests same the results were obtained by others (Mokarami & Emadi 2007; Jahanbakhshi et al., 2015). Fish exposed to copper were stressed progressively with time before death. The respiratory impairment due to the toxic effect of copper on the gills of *R. rutilus* was similar to *C. carpio*; however, effectively rate of copper was higher for *R. rutilus* to *C. carpio*. This result was similar to the reports of Khoshnood et al., (2014) and Peyghan et al. (2012). According to Thirumavalavan (2010), the level of glucose and lactic acid were increased in the blood of fresh water fish *Catla catla* exposed with sublethal concentration of copper sulphate. It can be as a useful indicator of stress in fish and it is found to be elevated during the toxic stress. They revealed a decrease in aerobic and an increase in anaerobic respiration is for this reason.

Because of the vital functions of the gills (respiration, osmorregulation and excretion), they are in direct contact with the external environment, which facilitates interaction with toxic substances in the water. For this reason, they are considered excellent indicators of environmental quality (Campagna et al., 2008). Death could have, therefore, occurred either by direct poisoning or indirectly by making the medium unconducive for the fish or even by both. The abnormal behavior observed during the exposure period like restlessness and surface to bottom movement were similar to the observations of Peyghan et al. (2012); and Naeemi (2013). Many studies showed the effects of toxic metals on gills of the fish species, including increase of epidermal thickness and lamellar width, fusion of secondary lamellae, hyperplasia, club-shaped cartilaginous tissue, aneurysm, edema and necrosis in epithelium region (Peyghan, 1999; Mokarami & Emadi 2007; Farhangi & Hajimoradloo, 2008; Peyghan et al., 2012; Jahanbakhshi et al., 2015). Previous studies suggested edematous changes in the gill were most probably due to the increase in capillary permeability. Hyperplasia was considered as a protective mechanism from environmental irritant by decreasing the respiratory surface and increasing the toxicant–blood diffusion distance and its intensification could result in the thickness of epithelial layers, which could be supported by the increases of epithermal thickness and lamellar width. Thus, all the lesions found in the present study would probably inhibit the respiratory, secretory and excretory functions in the gill of fish (Khoshnood et al, 2104). However, various damages may result in fish mortality, such as degenerated tubules of kidney and glumeroles, necrosis of hepatocytes of liver. Toxicity cause disorganizing kidney and liver function. Similar results were reported by Peyghan (1999) and Farhangi & Hajimoradloo (2008).

5. Conclusion

According to Table 1 (determination of toxicity in different concentration), copper for *C.carpio* and R. rutilus are medium toxicity. Due to the vicinity of these two species, further studies should be conducted on acceptable level of this toxic substance. However, toxicity of copper was rather for *R. rutilus* to *C. carpio*. The results of these studies may provide guidance to selection of acute toxicity to be considered in the field of biomonitoring efforts designed to detect the bioavailability of copper sulfate and early warning indicators of this heavy metal toxicity in Caspian Sea fishes.

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References

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed.). New York, American Public Health Association. In: Gharedaashi *et al.,* 2013.

Bertoletti, E. (2000). Estimativa de efeitos tóxicos crônicos com Danio rerio (Pisces, Cyprinidae). São Paulo: Faculdade de Saúde Pública, Universidade de São Paulo – USP, Master thesis in Public health, in: Campagna et al., 2008.

Campagna, A. F., Fracácio, R., Rodrigues, B. K., Eler, M. N., Fenerich-Verani, N., & Espíndola, E. L. G. (2008). Effects of the copper in the survival, growth and gill morphology of *Danio rerio* (Cypriniformes, Cyprinidae). *Acta Limnol Brasilia, 20*(3), 253-259. https://doi.org/10.5132/jbse.2008.01.003

Duncan, B. D. (1957). Duncan's multiple range tests for correlated and hetenoscedastic mean. Biometrics. 13: 359-364 in Thirumavalavan, R. 2010. https://doi.org/10.2307/2527799

Farhangi, M., & Hajimoradloo, A. M. (2008). Effect of Clinoptilolite Zeolite to prevent of mortality in rainbow trout *(Oncorhynchus mykiss)* by total ammonia (NH4-N) concentration. 1ST International congress on aquatic animal health management and disease, January 27-28, Tehran, Iran. pp:35-38.

Gharedaashi, E., Nekoubin, H., Imanpoor, M. R., & Taghizadeh, V. (2013). Effect of copper sulfate on the survival and growth performance of Caspian Sea kutum, *Rutilus frisii kutum*. Springer Plus, 2:498 (5P). https://doi.org/10.1186/2193-1801-2-498

Gomes, L. C., Chippari-Gomes, A., & Gomes, T. (2009). Acute toxicity of copper and cadmium for piauçu, *Leporinus macrocephalus*, and curimatã. *Biology of Sciences, 31*(3), 313–315. https://doi.org/10.4025/actascibiolsci.v31i3.5069

Imanpoor, M. R., Ahmadi, A. R., & Kabir, M. (2011). Effects of sub lethal concentration of Chloramines T on growth, survival, haematocrit and some blood biochemical parameters in common carp fry (*Cyprinus carpio*). *ACCL Bio flux, 4*(3), 280-291.

Jahanbakhshi, A., Hedayati, A., & Pirbeigi, A. (2015). Determination of acute toxicity and the effects of sub-acute concentrations of CuO nanoparticles on blood parameters in *Rutilus rutilus*. *Nanomed Journal, 2*(3), 195-202.

Khoshnood, Z., Jamili, Sh., Khodabandeh, S., Mashinchian Moradi, A., & Motallebi Moghanjoghi, A. A. (2014). Histopathological effects and toxicity of atrazine herbicide in Caspian Kutum, *Rutilus frisii kutum*, fry. *Iranian Journal of Fisheries Sciences, 13*(3), 702-718. https://doi.org/10.3354/dao02850

Loyed, R. (1961). Effect of dissolved oxygen concentration on the toxicity of several poisons to rainbow trout *(salmo gairdneri Richardson)*. *J.Exp.Biol, 38*, 447-455.

Martinsi, S. E., & Bianchini, A. (2008). Copper accumulation and toxicity in the Plata pompano *Trachinotus marginatus* Cuvier 1832 (Teleostei, Carangidae). *Pan-American Journal of Aquatic Sciences, 3*(3), 384-390.

Mazon, A. F., Cerqueira, C. C. C., & Fernandez, M. N. (2002). Gill cellular changes induced by copper exposure in the South American tropical fish *Prochilodus scrofa*. *Environment Research. Section., 88*(1), 52-63. https://doi.org/10.1006/enrs.2001.4315

Mokarami, Gh., & Emadi, H. (2007). Study of application of zeolite (Clinoptilolite) in salt water by absorption of ammonia and its effects on growth and survival *penaeus indicus. Iran journal of fisheries sciences, 2*, 127-138.

Muir, J. F. (1982). Recalculated water system in aquaculture. In: Effects of ammonia on growth and molting of penaeus japonicus juveniles. Chen J.C., pp. 249-260, Aquaculture. 104. https://doi.org/10.1016/0044-8486(92)90207-2

Naeemi, A., Jamili, S., Shabanipour, N., Mashinchian, A., & Shariati Feizabadi, S. (2013). Histopathological changes of gill, liver and kidney in Caspian kutum exposed to Linear Alkylbenzene Sulfonate. *Iranian Journal of Fisheries Sciences, 12*(4), 887-897.

Park, K., & Heo, G. J. (2009). Acute and sub-acute toxicity of copper sulfate pentahydrate (CuSO4.5H2O) in the guppy *(Poecilia reticulata)* (toxicology). *J. veter. Med. Sci., 71*(3), 333. https://doi.org/10.1292/jvms.71.333

Peyghan, R. (1999). Study of experiential of acute toxicity by ammonia in common carp (*Cyprinus carpio*) based on histopathological changes and serum enzymes and possibility of prevent it by zeolite. Dissertation of Aquatic Animal Health Management and Diseases. Tehran University. 104p.

Peyghan, R. T., Dadar, M., & Rezaei, R. (2012). Histopathological study of gill lesion of rainbow trout in cold water fish farm of Kohgiluyeh Boyer Ahmad province. *Journal of Aquaculture Sciences, 1*(1), 14-24.

Popek, W., Kleczar, K., Nowak, M., & Epler, P. (2009). Heavy metals concentration in the tissues of perch (*Perca fluviatilis*) and bleak (*Alburnus alburnus*) from Czarna Orawa River, Poland. *AACL Bioflux., 2*(2), 205-208.

Richey, D., & Roseboom, D. (1978). Acute toxicity of copper to some fishes in high alkalinity water. Illinois state water survey, Urbana, Circular. 131: 24. In: Jahanbakhshi *et al*., 2015. https://doi.org/10.5962/bhl.title.119542

Shuhaimi-Othman, M., Nadzifah, Y., & Hassan, S. (2010). Toxicity of copper and cadmium to freshwater fishes. *World Acad. Scie. Engin. Tech., 65*, 869–871.

Thirumavalavan, R. (2010). Effect of copper on carbohydrate metabolism fresh water, *Catla catla. Asian Journal of Science and Technology, 5*, 095-099.