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[Research]

Effect of Copper sulphate on behavioral and histopathological changes in roach, *Rutilus rutilus caspicus*

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ABSTRACT
The research was accomplished in order to study behavioral and histopathological effects of Copper on *Rutilus rutilus caspicus* under experimental condition. The study was performed using Water Static Method during 96 hours. 15 fish with averaged weight 2±0.5 g in weight were encountered to different concentrations (0, 0.1, 0.2, 0.3, 0.4 mg/l) of Copper. A group of fish was considered as control. Under stable condition and aeration, the lethal concentration was 0.4 mg/l. Results indicated that there was significant differences between treatments when copper concentration increased ($p<0.05$). it was found that in the high concentration of Copper nominal signs of toxicity such as convulsion, air gulping and flared operculum were observed. Histopathological sings were hyperplasia, edema, hyperemia, hemorrhage, expansion of Bowman's capsule and hepatocytes necrosis. In control group no lesion was observed.

Key word: *Behavioral, Histopathological, LC50, Toxicity, Copper Sulphate, Rutilus rutilus caspius.*

INTRODUCTION

Heavy metals are the most important toxic pollutants which threaten the environment (Rahbar Hashemi, 2013). Copper is one of the most toxic metals to aquatic organisms and ecosystem. Because copper is an algicide, and algae are at the base of food chains, so the amount of algae present in an aquatic ecosystem will affect the amount of food available for aquatic animals including zooplankton, insect, shellfish, fish and aquatic mammals. Copper is highly toxic in aquatic environments and has some effects on fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity (Horne & Dunson 1995). Copper accumulates in many different organs of fish and mollusks.

Studies carried out on different fish species have revealed that toxic metals can produce toxic effects on fish by prevent of growth, physiological, biochemical, reproduction activities, and mortality (Imanpour Namin, 2011). However, the concentration of these elements above tolerable levels is a disturbance factor for species survival and ecosystem stability. 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 (Lemus & Hung 1999). The lethal concentration in varieties of fishes is different and depends on species, age and ambient factors in water (Lemus & Hung

1999). Therefore, it is important to determine copper accumulation in different tissues of the exmined species to identify possible biotic ligands (toxicity sites) (Witeska & Jezierska 2003). However, the goals of the present study were to determine sublethal concentration of copper and to study of behavioral and histopathological changes in roach, *R. r. caspius)* under experimental condition.

MATERIALS AND METHODS

Preparing Fish

This study was carried out in Laboratory of Natural Resources Department in Gonbad Kavous University. *R. r. caspius* averaged 2±0.5 g in weight was obtained from Sijaval Fish Culture Center. Fish were acclimated to laboratory conditions for one week. They were reared in fiber glass tanks filled with 300 L of fresh water (under constant aeration, temperature 26°C and pH 8.7, Hardness = 390 mgL^{-1}). The experiment was performed by Water Static Method during 96h.

Determination of LC50

Five flasks with 3 litters volume were used with different concentrations such as 0, 0.1, 0.2, 0.3 and 0.4 mg/l of Cu (prepared with $CuSO_4.5H_2O$). Each flask contained 15 fish, with their relative duplicates, for a 96 hour exposure period. The amount of copper sulphate to be added in each aquarium was calculated according to the volume of each aquarium. Observations were made at 24, 48, 72 and 96 hours post-exposure. Fish were not fed during this period. The mortality rate was recorded thereafter dead fish were removed every 24 hours.

Behavioral and Histopathological Examinations

The behavioral changes of the healthy fish and the fish exposed to various doses of copper sulphate were recorded. Samples were randomly taken from gill, kidney and liver of fishes exposed to lethal concentration of copper and histopathological sections were prepared.

Statistical Analysis

Comparisons of mean values were performed using One-Way ANOVA followed by the Duncan's test. In all cases, the significance level adopted was 5%.

RESULTS

After the 96-h exposure, there were 0, 8.88, 33.33, 79.99 and 100% mortality at the 0, 0.1, 2.0, 3.0 and 4.0 mg/ l copper respectively (Table 1). Survival rate of control was 100%. Fig 1 shows the relation between the Copper sulphate concentration and survival rate of *R. r. caspius.* The results obtained from 96-h toxicity experiment revealed that there was significant difference between treatments when copper concentration increased ($p<0.05$) (Fig 1). 96-hour LC_{50} was measured 0.228 mg/l using of probit software (1.5).

Table 1. The relation between the copper sulphate concentration and the mortality rate of *R. r. caspius.*

Concentration of Copper	N	Percent of mortality Subset for alpha = 0.05			
		1	2	3	4
0	3	.0000			
0.1	3	8.8833			
0.2	3		33.3300		
0.3	3			79.9933	
0.4	3				1.0000E2
Sig.		.570	1.000	1.000	1.000

Means for groups in homogeneous subsets are displied.

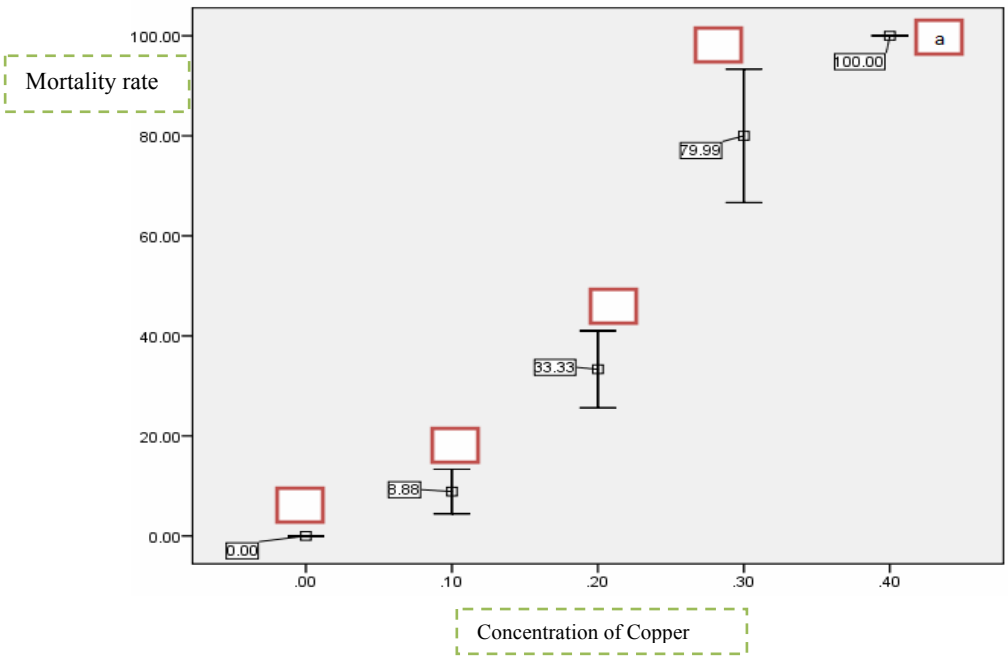

Fig. 1. *R. r. caspius* mortality in different concentrations of Copper

The behavioral changes of *R. r. caspius* exposed to various concentrations of copper are as follows:

In control group, there were no behavioral changes and no mortality throughout the experiment. In groups exposed to capper, there were vertical and downward swimming patterns and sudden movements. The fish became hyperactive and showed imbalance in posture and capsizing in water. Finally the fish sank down to the bottom and became motionless.

The common lesions of fish gill exposed to copper lethal concentration were hyperplasia, edema, hyperemia, hemorrhage and expansion of secondary lamellae (Fig. 2- 3). The major lesions in kidney were expansion of Bowman's capsule, hemorrhage, hyperemia, degeneration in tubules (Fig. 4-5). The lesions in liver were hyperemia, hemorrhage, inflammatory cells infiltration and hepatocytes necrosis (Fig. 6- 7). In control group no lesion was observed.

Fig. 2. Gill histological section that exposure to lethal Concentration of Copper. Arrow shows edema (× 400).

Fig. 3. Gill histological section that exposure to lethal Concentration of Copper. Arrow shows hemorrhage (× 400).

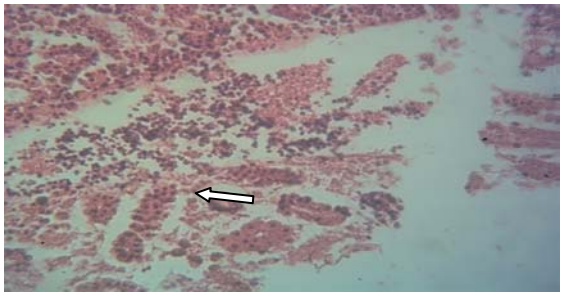

Fig. 4. kidney histological section exposed to lethal Concentration of copper. Arrow shows inflammation (× 400).

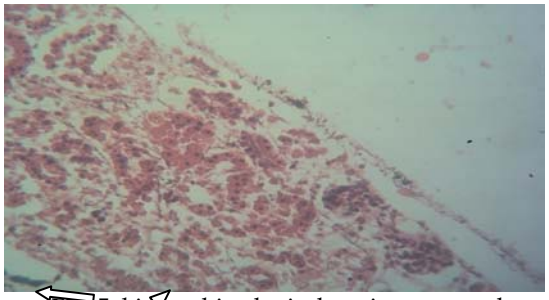

Fig. 5. kidney histological section exposed to lethal Concentration of copper. Arrows show degenerate tubules of kidney (× 400).

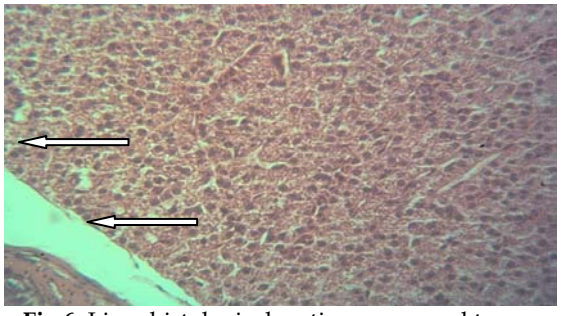

Fig 6. Liver histological sections expos ed to lethal concentration of copper. Arrow shows necrosis(× 200).

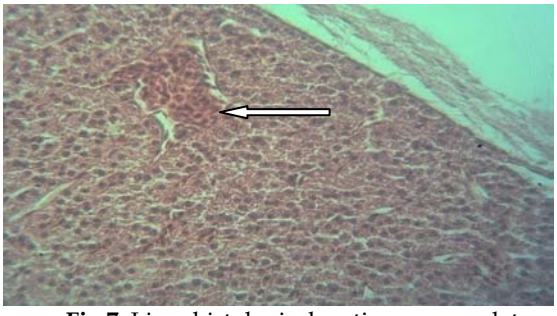

Fig 7. Liver histological sections exposed to lethal concentration of copper. Arrow shows hemorrhagic area around hepatocytes (× 200).

DISCUSSION

Copper has been used as a chemical tool in freshwater farm ponds and aquaculture. It is both an effective algicide and an anti parasite disinfectant. Copper has low marginal safety. However, Copper can be used to control algae in ponds. Within the aquatic environment, at high concentrations, it exerts adverse effects by accrue of structural damage, which affects the growth, development and survival of fish (Tuurala & Soivio 1982). A response was generally observed in the mortality rate which increases with increased concentration of copper (Table 1). The lethal concentration in varieties of fish is different. It depends on species, age and environmental factors such as temperature, pH and hardness (Witeska & Jezierska 2003; Giguere *et al.* 2004; Wong *et al.* 1977, Lemus & Hung 1999). Lemus and Hung (1999) reported that temperature is a determining factor in the survival rate of the species. They reported that copper was more toxic in *Petenia kraussii* juveniles exposed to higher temperatures. Ololade and Oginni (2010) demonstrated that water pH and hardness have significant direct relationship with 96 h LC_{50} of copper in fish. The most mortality was happened at the first hours of exposure. Similar behavioral pattern has been reported about other toxicants such as ammonia on *Oncorhynchus mykiss* (Farhangi & Hajimoradloo 2008), Zinc on *Clarias gariepinus* (Ololade & Oginni 2010) and Zinc on *Poecilia reticulate* (Gul *et al.* 2009). All these observations were more pronounced with increasing concentrations of toxicant. Consequently, the percentage and number of survived fish decreased with increasing concentrations of the toxicant in water (Table 1). The differences between the mortality and survival rate in the control and experimental treatments were statistically significant ($p < 0.05$) particularly at higher concentrations. Similar results have been reported by Farhangi and Hajimoradloo (2008); Farhangi (2010) and Gul *et al.* (2009). No mortality was observed in Concentration 0.05 mg/l of copper. The fish that is highly susceptible to the toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of

that metal (Abdullah & Muhammad 2006). Salmonids are generally sensitive to the toxin metals than the other fish species (Abdullah & Muhammad 2006). For example, reported lethal concentration to copper in *Oncorhynchus mykiss* was 0.125 mg/l. (Bagdonas & Vosylienė 2006). However, in the present study, the 96-h LC50 of copper for *R. r. caspius* was 0.228 mg/l (Table 1). Sprague (1969) observed variability in acute toxicity even in a single species and single toxicant depending on fish size, age and condition of examined species along with experimental factors. Abdullah and Muhammad (2006) indicated from their experimental study on *Catla catla* that 30 days old fish were more sensitive to metal toxicity than 60 and 90 days old fish respectively. Therefore, *R. r. caspius* fry were used in the present study. Neurological impairment has been observed in factory workers exposed to copper dust (ATSDR 1990). Behavioral changes in fish are an adequate biomarker for pollution monitoring and management of aquatic environment, employment of fish species in different aquatic environments is recommended to determine their differential sensitivity and applicability in aquatic eco-toxicology and pollution management (Ezeonyejiaku *et al*. 2011). In this study, the fish exposed to copper were observed to be highly irritable and displayed frenzied swimming when they were manipulated; their bodies were covered with thick mucus and finally died with mouth opened. These observations were similar to those of Olaifa *et al.* (2004) who worked on *Clarias gariepinus* to copper and Farhangi (2010) who worked on *Cyprinus carpio* exposed to zinc. Khunyakari *et al.* (2001) indicated increasing mucus secretion in *Poecilia reticulata*, exposed to increasing concentration of heavy metals (Nickle, Pb, Zn). The primary effect of copper in fish occurs on gills which are structurally damaged (Kirk & Lewis 1993). This damage includes collapse of lamellae, lifting of lamellar epithelium away from pillar cells and swelling of epithelial cells (Ezeeonyejiaku *et al*. 2011).

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 2010. However, $CuSo_4$ is a choice disinfectant for many fish treatments, despite its toxicity and needs to be used with caution.

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اثرات رفتاری و آسیب شناسی سولفات مس در ماهی سفید

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چکیده:

مطالعات بمنظور بررسی اثرات آسیب شناسی و تغییرات رفتاری مسمومیت با مس درماهی سفید تحیت شرایط آزمایشی صورت گرفت. مطالعات به روش آب ساکن در مدت 96 ساعت اجرا شد. 15 قطعه ماهی با وزن 0/5 ± 2 در معرض غلظت های مختلفی از سولفات مس (0/1،0، 0/2، 0/3، 0/4 میلی گرم در لیتر) قرار گرفت. یک گروه از ماهیها بعنوان شاهد در نظرگرفته شد. تحت شرایط ثابت و هوادهی، غلظت کشنده مس برابر 0/4 میلی گرم درلیتر بدست آمد. نتایج نشان داد با افزایش غلظت مس اختلاف معنی داری درنرخ تلفات ماهی بدست آمد($p<0.05$). آزمایشات نشان داد در غلظتهای بالای مس علائم ظاهری مسمومیت همچون تشنجات عصبی، بلعیدن هوا از سطح آب و حرکات ناموزون سرپوشهای آبششی دیده شد. مشاهدات آسیب شناسی شامل هیپرپلازی، ادم ، پرخونی، خونریزی، اتساع کپسول بومن و نکروز سلولهای کبدی بود. در گروه شاهد ضایعه ای مشاهده نشد.

* مولف مسئول