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Acute Toxicity of Zinc Sulfate Heptahydrate ($ZnSO_4*7H_2O$) and Copper (II) Sulfate Pentahydrate ($CuSO_4*5H_2O$) on Freshwater Fish, *Percocypris pingi*

Ling Zeng, Lan Huang, Ming Zhao, Sheng Liu, Zhengjian He, Jupan Feng, Chuanjie Qin and Dengyue Yuan*

Department of Aquaculture, College of Life Sciences, Neijiang Normal University, Neijiang, Sichuan, 641100, PR China

***Corresponding author:** Dengyue Yuan, Department of Aquaculture, College of Life Sciences, Neijiang Normal University, Neijiang, Sichuan, 641100, PR China, Tel: +86(0832) 234-4599; E-mail: yuandengyue@163.com

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Abstract

This study was carried out to determine acute toxicity of two heavy metals (zinc and copper) to fingerlings *Percocypris pingi* by static bioassays. The experimental fish were subjected to a range of concentrations of heavy metals. At different exposure periods (24 h, 48 h, 72 h and 96 h), the mortality rate were separately investigated. The median lethal concentration (LC_{50}) was determined with Probit analysis. The LC_{50} values of zinc for the *Percocypris pingi* at 24 h, 48 h, 72 h and 96 h were 3.504 mg/L, 2.933 mg/L, 2.852 mg/L and 2.852 mg/L, respectively. But the LC_{50} values of copper at 24 h, 48 h, 72 h and 96 h were 1.730 mg/L, 1.389 mg/L, 1.340 mg/L and 1.340 mg/L, respectively. The heavy metals were ranked in order of toxicity: copper >zinc. The safe concentration of zinc and copper were 0.2852 mg/L and 0.1340 mg/L, respectively. Physicochemical parameters variables e.g. dissolve oxygen, total hardness, and ammonia nitrogen of aquaria were monitored under different concentrations of zinc (0, 1.60, 2.40, 3.20, 4.00, 4.80, 5.60) mg/L and copper (0, 0.60, 1.00, 1.40, 1.80, 2.20, 2.60) mg/L.

Keywords Acute toxicity; Heavy metals; LC_{50}; Safe concentration; *Percocypris pingi*

Introduction

Industrial development and chemical contamination threaten aquatic animal life [1]. Environmental pollutions with heavy metals were increased in the world. The natural aquatic systems may extensively be polluted with heavy metals released from domestic, industrial and other man-made activities [2]. Metals and metal compounds are transformed naturally such as by bacterial activity with formation of organic species that affect their mobility and accumulation in biotic as well as biotic systems [3].

Some heavy metals are basic elements of biochemistry in aquatic organisms, and they are normal parts of marine organisms and can be directly absorbed from water [4-6]. Heavy metals represent a crucial role in several enzymatic processes, including the functions of cell respiration, free radical defence, connective tissue synthesis, neurotransmitter function and so on [7,8]. But metals serve as an important component of the water contaminant that interferes with the integrity of biochemical and physiological mechanisms of aquatic organisms [9]. Zn and Cu are the two most common heavy metals. Additional, Zn and Cu are essential trace elements, required by all living organisms for several physiological functions and biochemical reactions. However, Zn and Cu are found to be poisonous to organisms when above the optimal concentration [3,10-12]. In particular, heavy metals such as Zn and Cu are accumulated and absorbed by the organism and these metals throughout the food chain can confer harmful effect on human health [13]. Therefore, toxicity tests had been performed on fishes to evaluate the effect of toxicants on various aquatic organisms under laboratory conditions [14]. There were studies reported that the LC_{50} 96 h was 32.24 ppm ± 1.41 ppm for Zn on Silver Dollar Fish (*Mtynnis fasciatus*) [15], the LC_{50} 96 h was 8.990 mg/L for Zn, 0.521 mg/L for Cu on *Modiolus philippinarm* and the LC_{50} 96 h was 2.337 mg/L for Zn, 0.023 mg/L for Cu on *Cerithidae cingulate* [16]. But acute toxicity test of Zn and Cu for *Percocypris pingi* hasn't been reported.

There are three subspecies which distributed in the upper reaches of the Yangtze River (*Percocypris pingi*) and its tributaries and from Yunnan Lancang River, in the Nampang Jiang and Lake Fuxian (*P. pingi regani*) and in the Upper Salween and in the Mekong (*P. pingi retrodorsis*), China. *Percocypris pingi* is a highly commercial fishery species, and also collected for aquaculture but the population of *Percocypris pingi* is declining as a result of anthropic activities and *Percocypris pingi* has "Near Threatened" status in "International Union for Conservation of Nature" (Red List Category & CriteriaNear Threatened ver 3.1). Therefore, the aim of this study was to investigate the acute effects of some heavy metals as potential dangerous substances by assessing the 50% lethal concentrations (LC_{50}) and the safe concentration of zinc sulfate and copper (II) sulfate pollutants on a freshwater fish, *Percocypris pingi.* The results of acute toxicity tests for zinc and copper will contribute to detect the toxicity of heavy metals to *Percocypris pingi*, and provide the environmental basis for the evaluation of environmental quality and the management of wastewater discharge.

Materials and Methods

Experimental fish

The fish seeds were purchased from the Dadu river breeding fish station (Sichuan, China). *Percocypris pingi* breeding base with average total length of 4 cm ± 1 cm and body weight of 0.7 g ± 0.3 g were obtained. Seven replicates each containing nine fish were exposed to two heavy metals, respectively.

Reagents

Heavy metals: Zinc sulfate heptahydrate and Copper (II) sulfate pentahydrate were of analytical reagent grade and it purchased from Chengdu Kelong Chemical Reagent Factory (Sichuan, China).

Preparation of solutions: The above mentioned metallic compounds were dissolved in deionized water and stock solution was prepared. Metal solutions were prepared by diluting of a stock solution with deionized water and then obtained the corresponding mentioned toxicant concentrations. Each concentration contained nine fish with one replicate each. Ammonium sulphate standard stock solution can be prepared mixing 0.0472 g in 100 ml of deionized water and was diluted 10 times. Reagent A was prepared by containing 5 g of phenol and 25 mg of potassium nitroprusside in 100 ml of deionized water. Reagent B was prepared by mixing 2 ml of NaClO and 2.5 g of NaOH in deionized water [17].

Acute toxicity test: Prior to toxicity testing, the fish were transferred to an indoor tank (27.5 × 21 × 17 cm^3) with constantly aerated and filtered water. In order to avoid the sudden stress of fish, all samples need adapt to laboratory conditions for one week (17°C with 12 h light and 12 h dark). Water was renewed daily and the fish were fed to satiety twice daily at 08:00 and 19:00 with commercial pellet diet (≥ 50% raw protein, ≥ 8% raw fat, ≤ 3% raw fiber, ≤ 16.5% raw ash, ≤ 5% calcium, ≥ 1.0% phosphorus, ≤ 12% moisture and ≥ 2.0% lysine), (Shengsuo, Shandong, China). During all the trials dead fish were immediately taken away to protect the water quality from possible deterioration [18]. Acclimated fish were kept unfed for 1 day before the start of experiments until the end of the 96h experimental period. Thus, the influence of waste matter was minimized in order to maintain their living condition [19].

Acute toxicity tests were done using different concentrations of zinc (0, 1.60, 2.40, 3.20, 4.00, 4.80, 5.60) mg/L and copper (0, 0.60, 1.00, 1.40, 1.80, 2.20, 2.60) mg/L throughout all experiments. Each concentration involved in nine fish. During acute toxicity experiments constant air was supplied to each aquarium. No food was provided to the specimens during the assay and the test media was not renewed [15]. The mortality rate was measured at different exposure periods (24 h, 48 h, 72 h and 96 h). The value of LC_{50} was the concentration of each heavy metal caused 50% mortality in fish at 96 h, calculated by Finney's Probit Analysis (SPSS Inc., Chicago, IL, USA). This study was performed in Conservation and Utilization of Fishes resources in the Upper Reaches of the Yangtze River Key Laboratory of Sichuan Province.

Physicochemical parameters of test mediums during acute toxicity test of fish [17].

Ammonia nitrogen: In 10ml of water sample, 10 ml of stock solution, 1.25 ml of reagent A and 1.25 ml of reagent B were mixed and heated in 50°C water bath for 20 minutes. After cooling to room temperature, concentrations were measured with spectrophotometer (T6, PERSEE, China). Standards curve was run at 625 nm. Ammonia nitrogen was made at 12 h intervals during 96 h concentration of LC_{50}.

Dissolve oxygen: Dissolve oxygen of the test medium was measured by Dissolved oxygen meter (METTLER TOLEDO, USA).

Total hardness: Total Hardness was tested by Water Quality Rapid-Test Kits (Mingde, Beijing, China).

Behavioural studies: All samples were exposed to heavy metals (zinc and copper) respectively, and the behavioural changes and morphological abnormalities were studied under constant observations. Then the phenomena about behavioural observations were recorded for startle response, mode of swimming, equilibrium and general activity of fish during acute toxicity trials. Data was also collected for morphological studies that included the effects on fish coloration, appearance and any other abnormality in the structure such as abnormal lateral flexure and posturing of pectoral fins [20].

The fish were cared for and the experimental procedures were described in this study in accordance with the guidelines approved by Neijiang normal university (Sichuan, China)

Results

Acute toxicity tests

No mortality was observed during the acclimation period before exposure, and no mortality was observed among control fish. This study tested the mortality of *Percocypris pingi* for zinc and copper at different concentrations during the exposure times at 24 h, 48 h, 72 h and 96 h (Table 1). Acute toxicity of zinc and copper showed that mortality is not directly proportional to concentration of two heavy metals during the exposure times at 24 h, 48 h, 72 h and 96 h. Different degrees of poisoning symptoms were recorded in this study under two heavy metals with different concentrations.

Parameters	No. of mortality			
	24 h	48 h	72 h	96 h
Zn^{2+} Concentration (mg/L)				
Control	0	0	0	0
1.6	1	1	1	1
2.4	2	4	4	4
3.2	5	5	5	5
4	6	6	7	7
4.8	6	9	9	9
5.6	8	9	9	9
Cu^{2+} Concentration (mg/L)				
Control	0	0	0	0
0.6	0	0	0	0
1	2	2	2	2
1.4	5	6	6	6
1.8	5	7	7	7
2.2	7	8	9	9
2.6	6	9	9	9

Table 1: Mortality of *Percocypris pingi* (n=9 for each concentration) exposed to acute Zinc sulfate heptahydrate and Copper (II) sulfate pentahydrate.

Most of them swam slowly or stayed still in the water and the bottom of glass aquaria at low concentrations of heavy metals. With the concentrations increased, the samples produced startle response and moved fast. *Percocypris pingi* gradually became weakened and then died in the bottom of glass aquaria.

Effects of a heavy metal zinc: There was 100% mortality at concentration for zinc 4.80 mg/L and 5.60 mg/L after 48 h (Table 1). The values of LC_{50} for *Percocypris pingi* obtained for zinc at different periods of exposure were summarized in Table 2. The safe concentration of zinc is 0.2852 mg/L for *Percocypris pingi*, Chinese freshwater aquaculture water quality standard in the maximum allowable concentration of the specified value is 0.1 mg/L (Table 3), and so the tolerance of *Percocypris pingi* for zinc in water is higher than the state water quality standards.

A)			B)		
Duration (h)	LC_{50} (mg/L)	safe concentrations (mg/L)	Duration (h)	LC_{50} (mg/L)	Safe concentrations (mg/L)
24	3.504	0.2852	24	1.73	0.134
48	2.933		48	1.389	
72	2.852		72	1.34	
96	2.852		96	1.34	

Table 2: The 50% lethal concentrations (LC_{50} 96 h, mg/L) and safe concentrations of Zn A) and Cu B) for *Percocypris pingi*.

Heavy metal	Standard value
Zn	≤ 0.1mg/L
Cu	≤ 0.01mg/L

Table 3: Chinese freshwater aquaculture water quality standard.

Effects of a heavy metal copper: There was 100% mortality at concentration for copper 2.20 mg/L after 72 h and 2.60 mg/L after 48 h (Table 1). The LC_{50} values of copper for the *Percocypris pingi* at 24 h, 48 h, 72 h and 96 h were 1.730 mg/L, 1.389 mg/L, 1.340 mg/L and 1.340 mg/L, respectively (Table 2). For *Percocypris pingi*, the safe concentration of copper is 0.1340 mg/L. This is higher than the Chinese freshwater aquaculture water quality standard(0.01 mg/L) (Table 3).

Physicochemical variables studied during acute toxicity tests

In the study, dissolve oxygen, total hardness, ammonia nitrogen contents were measured (Table 4). The results showed significant differences under different concentrations of heavy metals for *Percocypris pingi*.

Metal concentration (mg/L)	Dissolve Oxygen (mg/L)	Total Hardness (mg/L)	Ammonia Nitrogen (mg/L)
Zn Concentration (mg/L)			
1.6	8.60 ± 0.53	8.80 ± 0.44	0.32 ± 0.25
2.4	8.17 ± 1.07	8.65 ± 0.29	0.49 ± 0.25
3.2	9.28 ± 0.21	8.95 ± 0.29	0.38 ± 0.19
4	9.58 ± 0.11	8.95 ± 0.29	0.62 ± 0.40
4.8	9.34 ± 0.19	9.24 ± 0.44	0.63 ± 0.41
5.6	9.09 ± 0.15	8.95 ± 0.29	0.47 ± 0.22
Cu Concentration (mg/L)			
0.6	8.26 ± 0.07	8.8 ± 1.32	0.31 ± 0.10
1	8.23 ± 0.27	8.8 ± 1.32	0.47 ± 0.19
1.4	8.23 ± 0.14	9.68 ± 0.44	0.43 ± 0.14
1.8	8.28 ± 0.15	10.12 ± 0.88	0.44 ± 0.10
2.2	8.19 ± 0.05	8.80 ± 0.88	0.50 ± 0.16
2.6	8.23 ± 0.17	9.68 ± 0.44	0.40 ± 0.04

Table 4: Mean of physicochemical properties of the test medium at different concentrations of Zn and Cu for *Percocypris pingi*.

Discussion

Zinc is a ubiquitous metal existed in environment, and is an essential trace metal for all living organisms, which is crucial to over 300 enzymes and other proteins [21]. However, zinc is potentially toxic at elevated concentration. Some studies had reported that the mechanism of its toxicity was disrupting calcium homeostasis through the induction of hypocalcaemia and disturbing acid-base balance [22]. In this study, the LC_{50} values of zinc at 24 h, 48 h, 72 h and 96 h were 3.504 mg/L, 2.933 mg/L, 2.852 mg/L and 2.852 mg/L, respectively. Obtained LC_{50}s not correspond to values that have been published in the literature for other species of fishes. Abdulali Taweel and M. Shuhaimi-Othman noted that the LC_{50} 24 h, 48 h, 72 h and 96 h of zinc for tilapia fish were 64.897 mg/L, 37.306 mg/L, 22.700 mg/L, 16.177 mg/L, respectively [23]. With the concentrations of zinc increased, the mortality of *Percocypris pingi* was increased. The LC_{50} values of zinc for *Percocypris pingi* were 2.852 mg/L at 72 h and 96 h, probably because fish can produce metal-binding metallothioneins which play an important role in metal homeostasis and in protection against heavy metal toxicity in vertebrates and invertebrates [24]. It had reported that the concentration of zinc increased in livers might promote the synthesis of various proteins and other molecules which have high affinities for metal-forming complexes [25].

Copper mainly comes from factory effluents, which are involved in manufacturing of electronic goods, fungicides, fertilizers [26]. In this study, the LC_{50} values of copper at 24 h, 48 h, 72 h and 96 h were 1.730 mg/L, 1.389 mg/L, 1.340 mg/L and 1.340 mg/L, respectively. And the LC_{50} values of copper for *Percocypris pingi* were both 1.340 mg/L at 72 h and 96 h. It probably because the trophic status, metabolic rate of *Percocypris pingi* and physicochemical properties of water also influenced mortality [14]. Generally, increasing concentration of the copper was in keeping with the increase of mortality for *Percocypris pingi*, which was also time-dependent and suggested that the concentration of copper had direct effect on the LC_{50} values for the *Percocypris pingi*. It was reported that metals can increase or decrease the activities of hepatic enzyme and cause histopathological hepatic alterations [27]. Meanwhile, some trace metals as co-substrates that are a vital part of many biological enzyme systems and they can catalyse oxidation or reduction reactions [12]. So we assumed that the death of *Percocypris pingi* was related to pathological injury in various tissues such as liver and kidneys caused by copper [28-32]. In addition, some investigations indicated that copper can lead to dysfunction of organism by binding histidine, cysteine and methionine containing proteins [33]. Some studies about other species of fishes showed different results. Abdulali Taweel and M. Shuhaimi-Othman noted that the LC_{50} 24 h, 48 h, 72 h and 96 h of copper for tilapia fish were 3.286 mg/L, 1.860 mg/L, 1.368 mg/L, 1.093 mg/L, respectively [23]. Ansari [2] had reported that the LC_{50} values of copper for zebrafish at 24 h, 48 h, 72 h and 96 h were 285.12 mg/L, 196.06 mg/L, 99.75 mg/L and 78.17 mg/L, respectively [3]. Thus *Percocypris pingi* was very sensitive to copper, which suggested that *Percocypris pingi* was useful to monitor the water quality as an early warning tool.

When the *Percocypris pingi* were acutely exposed to heavy metals, severe mortality was observed in this study. With the increased of concentration and time duration of metal exposure, the survival rate of *Percocypris pingi* decreased. It probably because heavy metals exposure can result in the increase of reactive oxygen species (ROS) e.g. Hydrogen peroxide, superoxide radicals and hydroxyl radicals, leading to impairment of normal oxidative metabolism and finally to oxidative stress in aquatic organisms [34]. In our previous study, we had reported that acute toxicity of mercury and cadmium on *Percocypris pingi*. The LC_{50} 96 h values of mercury chloride for the *Percocypris pingi* was 0.327 mg/L. But the LC_{50} 96 h value of cadmium chloride was 0.081 mg/L [17]. And in this study, the LC_{50} 96 h values of zinc for the *Percocypris pingi* was 2.852 mg/L. But the LC_{50} 96 h values of copper were 1.340 mg/L. The results showed that the toxicity ranking of four heavy metals were Cd>Hg>Cu>Zn.

Other studies reported that the heavy metals have a complex effect on the aquatic environment and this effect relies on the physicochemical characteristics of water and fish species [35,36]. And metal toxicity may be modified by physicochemical parameters for *Percocypris pingi* [17]. So we detected the physicochemical characteristics (e.g. Dissolve oxygen, total hardness, ammonia nitrogen) of water in different concentration of heavy metals (Table 4). The results showed that the physicochemical of water were related to the concentration of heavy metals, but they were disproportional. Therefore, we assumed that there were other factors which were working on and need to be further studied.

Conclusion

This study was done to assess the acute toxicity of heavy metals about zinc and copper for *Percocypris pingi*. The results of the present study strongly indicate that *Percocypris pingi* was very sensitive to heavy metals in the aquaria and the toxicity ranking of the two heavy metals was copper>zinc. About further studies, in order to illustrate the effects of heavy metals we plan to study the chronic toxicity of zinc and copper during the development stage of *Percocypris pingi* and we plan to explore the relationship between the growth rate of *Percocypris pingi* and the toxicity of the mixture of heavy metals (zinc and copper).

References

1. Kumar M, Raj A, Kumar R (2017) Toxic Effects Of Cu(So4) on Gill And Liver Tissues Of Fresh Water Catfish Clarias Batrachus (Linn.). International Journal of Advanced Research 5: 801-806.
2. Velez D, Montoro R (1998) Arsenic speciation in Manufactured Seafood Products. Journal of Food Protection 61: 1240-1245.
3. Ansari SB (2014) Acute Toxicity of Copper, Cadmium and Arsenic to Zebrafish, Danio rerio (Cyprinidae). Trends in Biosciences 7: 2357-2360.
4. Grosell M, Wood CM, Walsh PJ (2003) Copper homeostasis and toxicity in the elasmobranch Raja erinacea and the teleost Myoxocephalus octodecemspinosus during exposure to elevated water-borne copper. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 135: 179-190.
5. Erickson RJ, Benoit DA, Mattson VR, Nelson Jr. HP, Leonard EN (1996) The effects of water chemistry on the toxicity of copper to fathead minnows. Environmental Toxicology & Chemistry 15: 181-193.
6. Ober AG, Gonzalez M, Maria IS (1987) Heavy metals in molluscan crustacean and other commercially important Chilean marine coastal water species. Bulletin of Environmental Contamination & Toxicology 38: 534-539.
7. Baker JTP (1969) Histological and Electron Microscopical Observations on Copper Poisoning in the Winter Flounder (Pseudopleuronectes americanus). Journal of the Fisheries Research Board of Canada 26: 2785-2793.
8. Bury NR, Jie L, Flik G, Lock RAC, Bonga SEW (1998) Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro. Aquatic Toxicology 40: 193-202.
9. Alireza P, Borhan M, Sinkakarimi MH, Ghasem R, Robabeh V (2016) Acute Toxicity Bioassay of the Mercury Chloride and Copper Sulphate to Rutilus rutilus caspicus and Rutilus frisii kutum. International Journal of Aquatic Biology 4: 25-30.

10. Wang H, Liang Y, Li S, Chang J (2013) Acute toxicity, respiratory reaction, and sensitivity of three cyprinid fish species caused by exposure to four heavy metals. Plos One 8: e65282.
11. Abdullah S, Javed M, Javed A (2007) Studies on Acute Toxicity of Metals to the Fish (Labeo rohita). International Journal of Agriculture & Biology 9: 333-337.
12. Kousar S, Javed M (2012) Evaluation of acute toxicity of copper to four fresh water fish species. International Journal of Agriculture & Biology 14: 801-804.
13. Ahmed M, Ahmad T, Liaquat M, Abbasi KS, Farid IBA, et al. (2016) Tissue specific metal characterization of selected fish species in Pakistan. Environ Monit Assess 188: 212.
14. Ali Gul (2016) Investigation of acute toxicity and LC50 value of Cu for a fish Oreochromis niloticus. Journal of Entomology and Zoology Studies 4: 605-607.
15. Sadeghi A, Imanpoor MR (2015) Acute Toxicity of Mercuric Chloride (HgCl2), Lead Chloride (PbCl2) and Zinc Sulfate (ZnSO4) on Silver Dollar Fish (Metynnis fasciatus). Iranian Journal of Toxicology 9: 1301-1306.
16. Ramakritinan CM, Chandurvelan R, Kumaraguru AK (2012) Acute Toxicity of Metals: Cu, Pb, Cd, Hg and Zn on Marine Molluscs, Cerithedia cingulata G, and Modiolus philippinarum H. Indian Journal of Geo-Marine Sciences 41: 141-145.
17. Yuan D (2017) Acute Toxicity of Mercury Chloride (Hgcl2) and Cadmium Chloride (Cdcl2) on the behavior of freshwater fish, Percocypris Pingi. International Journal of Aquaculture and Fishery Sciences 3: 66-70.
18. Johnston EL, Keough MJ, Qian PY (2002) Maintenance of species dominance through pulse disturbances to a sessile marine invertebrate assemblage in Port Shelter, Hong Kong. Marine Ecology Progress Series 226: 103-114.
19. Zarei I, Pourkhabbaz A, Alipour H, Khazaei SH (2013) Acute Toxicity and the Effects of Copper Sulphate (CuSO4.5H2O) on the Behavior of the Black Fish (Capoeta Fusca). Iranian Journal of Toxicology 6: 771-778.
20. Abedi Z, Khalesi M, Eskandari SK, Rahmani H (2012) Comparison of Lethal Concentrations (LC50-96 H) of Cdcl2, Crcl3, and Pb (NO3)2 in Common Carp (Cyprinus carpio) and Sutchi Catfish (Pangasius Hypophthalmus). Iranian Journal of Toxicology 6: 672-680.
21. Muralisankar T, Bhavan PS, Radhakrishnan S, Seenivasan C, Srinivasan V, et al. (2015) Effects of dietary zinc on the growth, digestive enzyme activities, muscle biochemical compositions, and antioxidant status of the giant freshwater prawn Macrobrachium rosenbergii. Aquaculture 448: 98-104.
22. Niyogi S, Wood CM (2006) Interaction between dietary calcium supplementation and chronic waterborne zinc exposure in juvenile rainbow trout, Oncorhynchus mykiss. Comp Biochem Physiol C Toxicol Pharmacol 143: 94-102.
23. Taweel A, Shuhaimi-O M, Ahmad AK (2013) In vivo Acute Toxicity Tests of Some Heavy Metals to Tilapia Fish (Oreochromis niloticus). Journal of Biological Sciences 13: 365-371.
24. Alipour H, Pourkhabbaz A, Hassanpour M (2016) Determination of metals (As, Cu, Fe, and Zn) in two fish species from the Miankaleh wetland. Archives of Polish Fisheries 24: 2.
25. Bharti S, Banerjee TK (2011) Bioaccumulation of metals in the edible catfish Heteropneustes fossilis (Bloch) exposed to coal mine effluent generated at Northern Coalfield Limited, Singrauli, India. Bull Environ Contam Toxicol 87: 393-398.
26. Mazon AF, Cerqueira CC, Fernandes MN (2002) Gill cellular changes induced by copper exposure in the South American tropical freshwater fish Prochilodus scrofa. Environ Res 88: 52-63.
27. Paris-Palacios S B-RS, Vernet G (2000) Biochemical and (ultra)structural hepatic perturbations of Brachydanio rerio, (Teleostei, Cyprinidae) exposed to two sublethal concentrations of copper sulfate. Aquatic Toxicology 50: 109-124.
28. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, et al. (2007) Histopathological changes in liver and gill epithelium of Nile tilapia, Oreochromis niloticus, exposed to waterborne copper. Pesquisa Veterinaria Brasileira 27: 103-109.
29. Nawaz M, Manzl C, Krumschnabel G (2005) In vitro toxicity of copper, cadmium, and chromium to isolated hepatocytes from carp, Cyprinus carpio L. Bull Environ Contam Toxicol 75: 652-661.
30. Varanka Z, Rojik I, Varanka I, Nemcsok J, Abraham M (2001) Biochemical and morphological changes in carp (Cyprinus carpio L.) liver following exposure to copper sulfate and tannic acid. Comparative Biochemistry & Physiology Toxicology & Pharmacology 128: 467-468.
31. Al-Bairuty GA, Shaw BJ, Handy RD, Henry TB (2013) Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 126: 104-115.
32. Jiraungkoorskul W, Sahaphong S, Kangwanrangsan N (2007) Toxicity of copper in butterfish (Poronotus triacanthus): tissues accumulation and ultrastructural changes. Environ Toxicol 22: 92-100.
33. Grosell M, Wood CM (2002) Copper uptake across rainbow trout gillsmechanisms of apical entry. Journal of Experimental Biology 205: 1179-1188.
34. Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101: 13-30.
35. Takasusuki J, Araujo MRR, Fernandes MN (2004) Effect of Water pH on Copper Toxicity in the Neotropical Fish, Prochilodus scrofa (Prochilodondidae). Bulletin of Environmental Contamination and Toxicology 72: 1075-1082.
36. Lauren DJ, Mcdonald DG (1986) Influence of Water Hardness, pH and Alkalinity on the Mechanisms of Copper Toxicity in uvenile Rainbow Trout, Salmo gairdneri. Can J Fish Aquat Sci 43: 1488-1496.