

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

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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  $\pm$  1.41 ppm for Zn

on Silver Dollar Fish (*Mtynniss 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 & Criteria Near 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  $\pm$  1 cm and body weight of 0.7 g  $\pm$  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 ( $27.5 \times 21 \times 17 \text{ cm}^3$ ) 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 ( $17^\circ\text{C}$  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 ( $\geq 50\%$  raw protein,  $\geq 8\%$  raw fat,  $\leq 3\%$  raw fiber,  $\leq 16.5\%$  raw ash,  $\leq 5\%$  calcium,  $\geq 1.0\%$  phosphorus,  $\leq 12\%$  moisture and  $\geq 2.0\%$  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  $50^\circ\text{C}$  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<sub>50</sub> 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 <sub>50</sub> (mg/L)	safe concentrations (mg/L)	Duration (h)	LC <sub>50</sub> (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<sub>50</sub> 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<sub>50</sub> 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).

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