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Research Article

Acute Toxicity of Mercury Chloride (Hgcl2) and Cadmium Chloride (Cdcl2) on the behavior of freshwater fish, *Percocypris Pingi*

Abstract

The present study was performed to examine acute toxicity of two water-borne metals (Hg and Cd) by static bioassays. Fingerlings *Percocypris pingi* (*P. pingi*) were exposed to a range of concentrations of heavy metals and the mortality rate were investigated after 24, 48, 72 and 96h. The median lethal concentration (LC_{50}) was determined with Probit analysis. The LC_{50} values of mercury chloride for the *P. pingi* at 24, 48, 72 and 96h were 0.441, 0.347, 0.327 and 0.327 mg/L, respectively. But the LC_{50} values of cadmium chloride at 24, 48, 72 and 96h were 2.551, 2.341, 1.207 and 0.081mg/L, respectively. The toxicity ranking of the two heavy metals was Hg>Cd. The safe concentration of Hg and Cd were 0.0327 and 0.0081mg/L, respectively.

Abbreviations

CAT: Catalase; SOD: Superoxide Dismutase; GST: Glutathione S-Transferase; GPX: Glutathione Peroxidase; GR: Glutathione Reductase

Introduction

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 [1]. Environmental contamination by metals is a widespread phenomenon. Since heavy metals are not destroyed in living organisms through biological degradation, they have the ability to accumulate in various tissues and organs and even be biomagnified in the food chain [2]. When the amount of heavy metals in the medium reach to more than a certain limit it become toxic for those animals that live in that environment [3]. They pose serious hazards to freshwater fish resource for their toxicity and long persistence. They are also harmful to humans who are relay on aquatic products as food sources. This element has a long-biological half-life in humans and it gets accumulated in vital organs-especially in liver and kidney throughout their lives [4].

Toxicity tests are conducted to assess the effects of single or complex mixture of contaminants on the organisms. In freshwater, the existence or absence of fish has been extensively used as a biological indicator of the level of pollution [5]. *Percocypris pingi* (*P. pingi*), mainly in distributed in the upper reaches of the Yangtze River, was chosen for this study. However, the number of *P. pingi* is sharply reduced because of the destruction of environment by anthropic activities so that *P. pingi* becomes an endangered species. Acute toxicity tests experiments about *P. pingi* have not been reported. The study was to investigate the acute toxicity effects of two heavy metals as potential dangerous substances by assessing the mortality effects of mercury chloride (HgCl2) and cadmium chloride (CdCl2) pollutants on a freshwater fish, *P. pingi.* The results can provide theoretical support for other relevant researches and help aquaculture industry to monitor water quality safety for better economic benefits.

Materials and Methods

Experimental animals

The *P. pingi* were purchased from the Dadu river breeding station (Sichuan, China). *P. pingi* breeding base with average total length of 4±1cm and body weight of 0.7±0.3 g were obtained. The weight-matched fish were randomly distributed in seven tanks and numbered Group1 to Group 7 (n =9/group).

Reagents

Heavy metals: Mercury chloride and cadmium chloride were of analytical reagent grade and it purchased from

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Chengdu Kelong Chemical Reagent Factory (Sichuan, China). Mercury chloride and cadmium chloride were firstly dissolved in distilled water and then desired volume of the solution was mixed in tap water to obtain the corresponding mentioned toxicant concentrations [6].

Preparation of solutions: The above mentioned pure chloride compounds were dissolved in distilled water and stock solution was prepared. Ammonium sulfate standard stock solution can be prepared mixing 0.0472g in 100mL of distilled water and was diluted 10 times. Reagent A was prepared by containing 5g of phenol and 25mg of potassium nitroprusside in 100mL of distilled water. Reagent B was prepared by mixing 2mL of NaClO and 2.5g of NaOH in distilled water.

Acute toxicity test

Prior to the experiment the fish were being acclimatized for eight days to laboratory conditions under a simulated photoperiod of 12h light and 12h darkness in indoor tank (27.5×21×17 cm^3), with constantly aerated and filtered water at 17 before the experiments. Water was renewed every day and the fish were fed to satiety twice a day at 08:00 and 19:00 with commercial pellet diet (≥50% crude protein, ≥8% fat) (Shengsuo, Shandong, China). Dead fishes were immediately removed to avoid possible deterioration of the water quality [7]. Acclimated fishes were not fed for 1 day before the start of experiments until the end of the 96h experimental period. Thus, the volume of waste matter was minimized in order to not affect their living condition [8].

Acute mercury and cadmium toxicity experiments were performed using different concentrations of Hg (0, 0.18, 0.26, 0.34, 0.42, 0.50 and 0.58 mg/L) and Cd (0, 0.20, 0.60, 1.00, 1.40, 1.80 and 2.20mg/L) during 96h. Each concentration contained nine fishes. During acute toxicity experiment, the water in each aquarium was aerated. No food was provided to the specimens during the assay and the test media was not renewed [9]. The mortality rate was determined at the end of 24, 48, 72 and 96 h, and dead fishes were removed when observed. The concentration of each heavy metal caused 50% mortality in fish for 96h was taken as the LC_{50} value, calculated by Finney's Probit Analysis (SPSS Inc., Chicago, IL, USA). This study was carried out in Conservation and Utilization of Fishes resources in the Upper Reaches of the Yangtze River Key Laboratory of Sichuan Province.

Determination of physico-chemical parameters

Ammonia nitrogen: In 10mL of water sample, 10mL of stock solution, 1.25mL of reagent A and 1.25mL of reagent were mixed and heated in 50 water bath for 20 minutes. After cooling to room temperature, concentrations were measured with spectrophotometer (T6, PERSEE, china). Standards curve was run at 625nm. Ammonia nitrogen were 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).

Behavioral studies

During the experimental period the control and Hg and Cd exposed fishes were kept under constant observation to study the behavioral changes and morphological abnormalities. Behavioral observations were recorded for startle response, mode of swimming, equilibrium and general activity of fish during the experiment. 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 [10].

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

Results

Acute toxicity tests

No fishes died during the acclimation period before exposure. The mortality of studied fish for Hg and Cd at different concentrations were examined during the exposure times at 24, 48, 72 and 96 h (Table 1). *P. pingi* exposed during the period of 24, 48, 72 and 96 h had significantly increased number of dead individual with increasing concentration.

Acute toxicity of a heavy metal Hg: There was 100% mortality at concentration for Hg 0.58mg/L (Table 1). We study the lethal concentrations (LC_{50}-96 h) of heavy metals in *P. pingi* respectively with at four durations (Table 2). For *P. pingi* LC_{50} for 24, 48, 72 and 96h for Hg were 0.441, 0.347, 0.327 and 0.327mg/L, respectively. The safe concentration of

Table 1: Mortality of *P. pingi* (n=9 for each concentration) exposed to acute mercury chloride (HgCl2) and cadmium chloride (CdCl2)

Parameters	No. of mortality			
	24h	48h	72h	96h
Mercury Chloride Concentration (mg/L)				
Control	0	0	0	0
0.18	0	1	1	1
0.26	1	4	4	4
0.34	3	6	7	7
0.42	1	2	3	3
0.50	6	8	8	8
0.58	9	9	9	9
Cadmium Chloride Concentration (mg/L)				
Control	0	0	0	0
0.20	0	0	0	4
0.60	0	0	6	7
1.00	0	0	5	7
1.40	0	0	6	7
1.80	1	2	3	7
2.20	2	3	7	9

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Hg is 0.0327 mg/L for *P. pingi* (Table 2), Chinese freshwater aquaculture water quality standard in the maximum allowable concentration of the specified value is 0.0005mg/L (Table 3), and so the tolerance of *P. pingi* for Hg in water is higher than the state water quality standards.

Acute toxicity of a heavy metal Cd: There was 100% mortality at concentration for Cd 2.2mg/L after 96h (Table 1). For *P. pingi* LC_{50} for 24, 48, 72 and 96h for Cd were 2.551, 2.341, 1.207 and 0.081mg/L, respectively. The safe concentration of Cd on *P. pingi* is 0.0081mg/L. This is lower than the Chinese freshwater aquaculture water quality standard (0.005mg/L) (Table 3).

Physico-chemical variables studied during acute toxicity tests

In the study, ammonia nitrogen, dissolve oxygen and total hardness contents showed significant vary differences under different concentrations of heavy metals for *P. pingi* (Table 4).

Table 2: Lethal concentrations (LC_{50}-96 h, mg/L) and safe concentrations of Hg A) and Cd B) for *P. pingi*

A			B		
Duration (h)	LC_{50} (mg/L)	safe concentrations (mg/L)	Duration (h)	LC_{50} (mg/L)	safe concentrations (mg/L)
24	0.441		24	2.551	
48	0.347		48	2.341	
72	0.327	0.0327	72	1.207	0.0081
96	0.327		96	0.081	

Table 3: Chinese freshwater aquaculture water quality standard.

Heavy metal	Standard value
Hg	≤0.0005 mg/L
Cd	≤0.005 mg/L

Table 4: Mean of physico-chemical properties of the test medium at different concentrations of Hg and Cd for *P. pingi*

Metal concentration (mg/L)	Ammonia Nitrogen (mg/L)	Dissolve Oxygen (mg/L)	Total Hardness (mg/L)
Mercury Chloride Concentration (mg/L)			
0.18	0.33±0.17	8.51	9.09
0.26	0.53±0.35	8.88	9.09
0.34	0.35±0.20	8.87	9.24
0.42	0.32±0.24	9.07	9.39
0.50	0.48±0.41	9.01	9.24
0.58	0.33±0.09	9.02	8.95
Cadmium Chloride Concentration (mg/L)			
0.20	0.32±0.12	9.01	9.09
0.60	0.66±0.46	9.18	9.24
1.00	0.55±0.40	9.13	9.09
1.40	0.64±0.43	9.18	9.24
1.80	0.71±0.53	9.27	9.09
2.20	0.72±0.54	9.34	9.39

Behavioral response on *P. pingi*

There are different degrees of poisoning symptoms in two different concentrations. Most of them swam slowly or stayed still in the water and the bottom of glass aquaria. The behaviors of samples with low concentrations were same as the control group. During eight hours, it seems nothing changed. But with the concentrations of heavy metals increased, the behaviors of fish change obviously. *P. pingi* moved fast and performed upset in high concentrations group. As time goes on, the activity of *P. pingi* gradually weakened and the mortality rate was increasing. *P. pingi* swam slowly and died in the end.

In conclusion, the study clearly indicates that mercury chloride and cadmium chloride concentration caused mortality to the *P. pingi* and this could be one of the reasons for population decline of *P. pingi.*

Discussion

This study was done to assess the toxicity of heavy metals and the lethal median concentration (LC_{50}) of *P. pingi.* The results showed that the toxicity of two heavy metals had different effects on *P. pingi.* The LC_{50} values of mercury chloride (HgCl2) at 24, 48, 72 and 96 h were 0.441, 0.347, 0.327 and 0.327mg/L, respectively. The LC_{50} values of cadmium chloride (CdCl2) were 2.551, 2.341, 1.207 and 0.081mg/L, respectively. The results of Zeynab Abedi and Mohammad kazem Khalesi were different from our study. In the Common Carp (*Cyprinus carpio*), the LC_{50}-24, 48, 72 and 96h of CdCl2 were 2.31, 1.64, 1.88 and 1.3mg/L, respectively [11]. Thus *P. pingi* is sensitive to Cd. The widespread presence of metals in the environment is causative of a series of deleterious imbalances in exposed organisms, which can result in toxic insult [12]. At elevated concentrations, heavy metals can be absorbed through biological membranes of cell, system, organ or organism [13]. Furthermore, at higher levels of biological organization (tissue, organ and whole organism) heavy metals induce changes in metabolism, biochemistry, physiology, histology and inhibit synthesis of proteins and nucleic acid [14,15]. High concentration of metals can cause oxidative stress that damages the fish tissues specifically the liver and kidney which offer an antioxidant defense systems.

Hg pollution in water mainly comes from industrial and agricultural waste gas, waste water and waste residue [16]. In this study, the result showed that LC_{50} values of mercury chloride for *P. pingi* were 0.327 mg/L at 72 and 96h. It probably because the enzymes activities of fish increased and the inactivation of reactive molecules formed during oxidative stress, which could provide an additional protection against the oxidative damage induced by mercury [17]. There are several antioxidant enzymes in the liver and kidney, such as catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR). They are known to be sensitive to metals [18]. So we assume that the death of *P. pingi* is related to oxidative stress caused by metals. Other investigation indicated that the sub-acute and chronic mercury concentrations may cause several changes in the biochemical and hormone parameters of the studied fish and we can use these changes as biomarkers for

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mercury detection [19]. Thus we may can detect the mercury pollution according to the death of fish.

Cadmium is a toxic and fast heavy metal for fish. It is important for us to prevent and control the pollution of cadmium in terms of environmental quality or water quality standard. Cadmium levels have been reported in the range of 0.01~4.16μg/g dry weight in fish species from Iskenderun Bay [20], 0.45~0.90μg/g dry weight in fish species from Aegean Seas (Turkey) [21], and 0.09~0.48μg/g dry weight in fish species from the middle Black Sea (Turkey) [22]. It suggested that the concentration of Cd was significantly affected by the sampling site. In the study, the concentration of heavy metals by *P. pingi* may affect the mortality of fish. Previous studies suggested that the high levels of SOD, xanthine oxidase and GPX can increase the levels of GST and CAT, which shows a possible shift towards a detoxification mechanism under long-term exposure cadmium [18].

Therefore, we also think the physico-chemical characteristics of the test medium along with biotic factors are key to know the mechanisms of affecting LC_{50} concentrations of fish in toxicity tests. Some reports indicated that the carbon dioxide contents of the test mediums also increased significantly at higher concentrations of metals to the fish (*Labeo rohita*) [23]. High levels of metallic ions can induce oxidative stress in fish and prompt carbon dioxide liberation in water through respiration reaction, so carbon dioxide concentrations of the test medium increased significantly. We suppose that environmental conditions such as ammonia nitrogen, oxygen concentration and total hardness may affect the metal toxicity to *P. pingi.* So we measured the contents of three physico-chemical parameters. According to the results, we think that physico-chemical parameters may modify metal toxicity for *P. pingi*. About further studies, we plan to measure other physico-chemical characteristics such as pH, temperature, salinity and presence of other metals to prove our suspects and help aquaculture industry to monitor water quality safety for better economic benefits.

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