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Determination of Mercuric Chloride Toxicity on *Capoeta fusca* under Laboratory Conditions

Hamid Reza Pourkhabbaz[1*] and Ali Reza Pourkhabbaz[2,3]

1- Assistant Professor, Department of Environment, Faculty of Natural Resources, Behbahan Khatam Alanbia University of Technology.
2- Assistant Professor, Department of Environment, Faculty of Natural Resources, University of Birjand
3-Assistant Professor, Higher Educational Institute of Birjand Hormozan.

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Abstract

Acute toxicity testing of contaminants, continually released into the aquatic ecosystems from industrial and residential areas and representing a potential risk to the aquatic biota is important. Mercury is a highly toxic metal to which, due to its wide usage in agricultural, industrial, medical and other fields, exposure cannot be avoided. The purpose of this study was to examine the acute toxicity of mercuric chloride ($HgCl_2$) on a freshwater species of fish, *Capotea fusca,* according to the static test for calculating LC_{50} (lethality concentration for 50%). For this purpose, fish were exposed to mercuric chloride and were not fed for approximately 96 h by adding no food. Ten different concentrations in three replicates were chosen. For each treatment, 10 fish specimens were used. The solutions were prepared by dissolving mercuric chloride (Merck) in distilled water. Results represented that mortality decreased as exposure time increased, so that most of the mortality occurred during the first 24 h. Finally, the Conclusion showed that the LC_{50} value at 72 h and 96 h were 0.539 mg/L.

Keywords: *Capoeta fusca*, LC_{50}, Mercuric chloride, *Qanat*, Static bioassay.

تعیین اثر سمیت کلرید جیوه بر سیاه ماهی *Capoeta fusca* در شرایط آزمایشگاهی

حمیدرضا پورخباز[۱*] و علیرضا پورخباز[۲،۳]

۱- استادیار گروه محیط زیست، دانشکده منابع طبیعی، دانشگاه صنعتی خاتم الانبیاء بهبهان
۲- استادیار گروه محیط زیست، دانشکده منابع طبیعی، دانشگاه بیرجند
۳- استادیار، موسسه آموزش عالی هرمزان بیرجند

چکیده

آزمون سمیت حاد آلاینده‌ها، که بطور مداوم از نواحی صنعتی و مسکونی رها می شوند، نشان‌دهنده میزان خطر بالقوه این ترکیبات بر جانداران آبزی می‌باشد. جیوه یک فلز بسیار سمی است که به دلیل کاربرد وسیع آن در کشاورزی، پزشکی و سایر فعالیت‌ها، نمی‌توان از درمعرض قرار گرفتن آن اجتناب شود. هدف این مطالعه، آزمایش سمیت حاد کلرید جیوه ($HgCl_2$) بر ماهی آب شیرین، *Capotea fusca*، بر اساس آزمون پایایی جهت محاسبه LC_{50} می‌باشد. بدین منظور، این ماهی بدون افزودن مواد غذایی حدود ۹۶ ساعت در معرض کلرید جیوه قرار گرفت. ۱۰ غلظت مختلف در سه تکرار انتخاب شدند و برای هر تیمار ۱۰ نمونه ماهی استفاده گردید. محلول‌ها با حل کلرید جیوه در آب مقطر آماده شدند. نتایج مشخص کرد که مرگ و میر با گذشت زمان کاهش می‌یابد، به‌طوری‌که بیشتر مرگ ومیرها در طی ۲۴ ساعت اولیه اتفاق افتاد. سرانجام، نتیجه‌گیری نهایی نشان داد که مقدار LC_{50} در طی ۷۲ و ۹۶ ساعت ۰/۵۳۹ میلی گرم در لیتر بود.

کلمات کلیدی: *Capoeta fusca*، LC_{50}، کلرید جیوه، قنات، آزمون آماری زیستی.

* Corresponding author. *E-mail Address*: pourkhabbaz@yahoo.com

Introduction

A *qanat* is a traditional water management system in Iran used to provide a reliable supply of water to human settlements or for irrigation in arid and semi-arid climates; this technology is known to have developed in ancient Persia and then spread to other cultures. *Qanats* are up to 3000 year-old artificial sub-horizontal underground water channels of 5 to 80 km long (Stiros, 2006). The term *qanat*, deriving from an ancient Semitic word meaning "to dig", describes an underground water channel, consisting of vertical shafts connected at the bottom to a sub-horizontal tunnel (Stiros, 2006).

Acute toxicity from single compounds is continually released into aquatic ecosystems from industrial and residential areas and represents a potential risk to the aquatic biota. Mercury is a highly toxic metal, resulting in a variety of adverse results on health, affecting the neurological, renal, respiratory, immune, dermatologic, and reproductive systems (Risher and Almer, 2005; Sharma *et al.,* 2007). The wide use of mercury in agriculture, industry, medicine, and other fields makes exposure to it unavoidable and, consequently, it is of global concern because of its high toxicity at low concentrations and its bioavailability (Gray *et al.,* 2002; Khangarot, 2003; Vázquez-Núñez *et al.,* 2007). The toxicity of mercury depends greatly on the forms of the mercury compounds (inorganic and organic). Both inorganic and organic mercury in waters pose considerable risk to aquatic biota since mercury in both forms is cumulatively toxic (Skubal and Eshkov, 2002). Inorganic mercury presented in the environment is a well-established toxicant to human health (Sharma *et al.,* 2007). It forms salts in two ionic states, namely mercury (I) and mercury (II). Mercury (II), or mercuric salts, is much more commonly found in the environment than mercury (I), or mercurous salts (Boening, 2000). Mercury chloride ($HgCl_2$) is a highly toxic environmental contaminant and it presents a serious hazard to the public health and a threat to most life forms.

According to Coad (1998), distribution of *Capoeta fusca* is reported only in the western region of Asia and eastern Iran; distribution of this species is reported in Afghanistan, mostly orginating from Iranian drainage of the Namakzar (Coad, 1981). This species is very important because of its genetic values. The aim of this investigation was to examine the acute toxicity of mercuric chloride ($HgCl_2$) to a native freshwater fish, *Capoeta fusca,* according to static tests.

Materials and Methods

Birjand is the capital of South Khorassan Province in theE of Iran. This province has a dry climate with a significant difference between day- and night-time temperatures and an annual rainfall of 172 mm; the basin area received a total rainfall of 76 mm. Birjand is an important regional centre of some agriculture and pasturage.

During April 2012, *Capoeta fusca* (Nikolskii, 1897) belonging to the family Cyprinidae were collected from three *qanats* in Birjand. The live fish were transported to a laboratory in polythene bags filled with *qanat* water. Prior to the experiment the fish were acclimatized for six days to laboratory conditions in six pre-cleaned glass aquaria with tap water. The fish were exposed to mercuric chloride ($HgCl_2$) in the aquarium systems and were not fed during the experiment. The aquaria were fitted with artificial aerators to maintain oxygen levels. The exposure time of fish to mercuric chloride was 96 h, without adding any food. Sets of 10 fish specimens were exposed randomly to 50 litres water. The average wet weight (±SD) of fish used in experiments was 5.3 (±1.4) g.

The exploratory range of concentration of test chemicals was determined by a series of range finding experiments (OECD, 1992). Preliminary tests were carried out to estimate the minimum lethal and maximum non-lethal concentrations (the minimum concentration that calculated to kill 100% of fish at 96 h and the maximum concentration that calculated not to kill any fish at 96 h) of mercuric chloride. The initial concentration of mercuric chloride added to each aquarium was accurately calculated. The concentration range of mercuric chloride used was 1 to 0.125 (mg/l). Thereafter, upon reaching final concentrations, ten different concentrations in decreasing amounts (1, 0.902, 0.804, 0.707, 0.610, 0.512, 0.416, 0.319, 0.222 and 0.125 (mg/l) in three-replicates were chosen. For each treatment, 10 fish specimens were used. The solutions were prepared by dissolving analytical-grade mercuric chloride (Merck) in distilled water. A control was used for the test with three replicates. The experiment and control water used in the investigation consisted of natural tap water. No mortality was observed in the controls during the experimental period. Dissolved oxygen (mg/l), temperature (ºC) and pH were recorded individually in each test container at exposure times of 24, 48, 72 and 96 h. Water quality of the experimental tank was determined according to standard procedures (Weiner, 2000). Total hardness, Phosphate, magnesium, nitrite, nitrate and ammonia (mg/l) were determined before starting the experiments using a photometer (Palintest, 8000). Mortalities were recorded at 24, 48, 72, and 96 h of exposure, and the dead fish were regularly removed from the test solution.

LC_{50} values were calculated from the data obtained in acute toxicity bioassays, using EPA method "Probit analysis program (version 1.5)" and with Minitab statistical software (version 14).

Result

The extrapolation of laboratory data to the field is not always meaningful and, so, it is difficult to decide on an acceptable concentration based on the laboratory experiments that may be considered 'safe' in the field (Pandey *et al.,* 2005). An acute effect normally occurs shortly after exposure with a single concentration of hazardous chemicals. The magnitude of the effect depends on the innate toxicity of the substance, the duration of exposure and the type of organisms. The acute toxicity of mercuric chloride ($HgCl_2$) to this freshwater fish, *Capotea fusca*, was evaluated using static bioassays for calculating the LC_{50} (lethality concentration for 50%). The physiochemical properties of the test water and *qanat* water are given in Table 1. The physical and chemical parameters analyzed during the bioassays showed no differences among the ranges of 10 concentrations, neither in test water nor *qanat* water, while hardness concentrations were higher in *qanat* water than in test water.

The LC_{50} value for mercuric chloride, calculated by the EPA method and Minitab statistical software at 24, 48, 72 and 96 h of exposure, is shown in Table 2.

Figure (1) shows that fish mortality increased as the concentration of mercuric chloride increased, which indicates a direct proportional relationship between mortality and the concentration of mercuric chloride.

Table 1: Physiochemical properties of the *qanat* water and test water.

Parameters	Value (*qanat* water)	Value (test water)
pH	8.4 ± 0.2	8.3 ± 0.1
Dissolved oxygen (mg/l)	5.8 ± 0.2	6.1 ± 0.2
Temperature (ºC)	19.5 ± 0.5	21 ± 0.6
Total hardness (as $CaCO_3$, mg/l)	430 ± 6.3	305 ± 5.2
Magnesium (mg/l)	51 ± 4	48 ± 3
Nitrite (NO_3- N, mg/l)	0.002	0.003
Nitrate (NO_3- N, mg/l)	0.43 ± 0.2	0.45 ± 0.1
Ammonia (NO_3- N, mg/l)	0.02	0.01

Table 2: Lethal concentration (LC_{50}) with a 95% confidence limit (in parentheses) of mercuric chloride (in mg/l), estimated by Minitab and the EPA method.

Time	LC_{50} calculated by Minitab	LC_{50} calculated by EPA
24	0.575 (0.542 – 0.609)	0.576 (0.509 – 0.638)
48	0.545 (0.513 – 0.578)	0.549 (0.515 – 0.582)
72	0.539 (0.508 – 0.571)	0.539 (0.505 – 0.572)
96	0.539 (0.508 – 0.571)	0.539 (0.505 – 0.572)

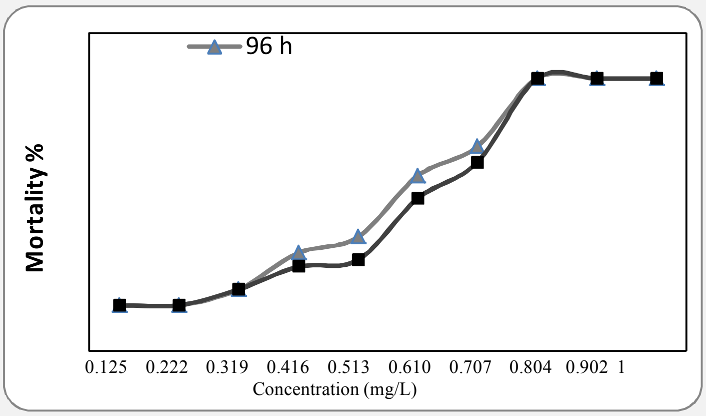

Figure 1: Percentage mortality of *Capoeta fusca* after 24 h and 96 h exposure to different concentrations of mercuric chloride.

Discussion

The LC_{50} values at 72 h and 96 h were 0.539 mg/l by Minitab and EPA. The LC_{50} value at 48 h was 0.545 and 0.549 mg/l by Minitab and EPA, respectively, while the LC_{50} value at 24 h was 0.575 and 0.576 mg/l, respectively; these are extremely close. In this study, the LC_{50} value for mercuric chloride in fish (*Channa punctatus)* at 96 h was lower than the value (1.20 mg/l) reported by Pandey *et al.,* (2005). Khangarot (1981) also reported a comparatively lower LC_{50} value (0.314 mg/l) at 96 h in *Channa marulius*. The following LC_{50} values at 96 h for mercuric chloride have been reported: for catfish *(Heteropneustes fossilis)*, 0.35 mg/l; rainbow trout (*Oncorhynchus mykiss)*, 0.22 mg/l; striped bass (*Roccus saxatilis),* 0.09 mg/l; and brook trout (*Salvelinus fontinalis)*, 0.075 mg/l (Pandey *et al.,* 2005); banded killifsh *(Fundulus diaphanous),* 0.11 mg/l; pumpkinseed *(Lepomis gibbosus),* 0.3 mg/l; white perch *(Roccus americanus),* 0.22 mg/l; carp *(Cyprinus carpio)*, 0.18 mg/l and american eel *(Anguilla rostrata),* 0.14 mg/l (Boening, 2000). The LC_{50} for mercuric chloride at 96 h was determined in this study to be 0.539 mg/l. Comparing our data with those available in the literature may not be meaningful because various factors may influence bioassay techniques, such as differences in fish (e.g. species, weight, size) and other environmental factors (Pandey *et al.,* 2005). Several important factors are known to make heavy metals biologically less active and therefore less toxic. Water hardness has a significant effect on heavy metal toxicity. In general, a higher level of hardness is beneficial because it reduces metal toxicity to fish (Weiner, 2000). Rathore and Khangarot (2003) found that the toxicity of mercuric chloride decreased with increasing water hardness. The LC_{50} value for mercuric chloride was lower in the present study than the values available in the literature; the reason for this might be due to high water hardness (> 300 mg/l). In the present study, the pH of the test water was higher than 7. Khangarot *et al.* (1985) reported that the acute toxicity to fry of common carp *(Cyprinus carpio)* decreased with an increasing pH from 5.5 to 8.5. It was found that at low pH (<7), mercury was more toxic compared with a high pH (>7); this might be due to acid toxicity itself causing bicarbonate loss in the body fluid (Das and Sahu, 2005).

Results of the bioassay are still fragmentary and highly variable as the change in species, chemical, biological and environmental factors influence the toxicity (Das and Sahu, 2005). It seems that two factors, namely water hardness and pH levels, could affect the acute toxicity of mercuric chloride on the *Capoeta fusca.* Figure 1 indicated the relationship between the concentration of mercuric chloride and percentage mortality of *Capoeta fusca* after 24 h and 96 h exposure to different concentrations of mercuric chloride. It clearly shows that mortality decreases over time, so that most of the mortality occurred during at the first 24 h. The reason might be due to prior intoxication during the proceeding hours, which enhanced over the subsequent hours' increase in time (Das and Sahu, 2005). Also, mortality depends on the retention time of mercuric chloride in water. At the end of the first 24-h period, most of the mercuric chloride in the water had been taken up by the fish, and its concentration in the water decreased.

Abnormality in test animal behaviour varied according to the test solution concentration (Das and Sahu, 2005). Fish exposed to different concentrations of mercuric chloride (especially at high concentrations '1, 0.902 and 0.808 mg/l') showed abnormal behaviour. They tried to avoid the toxic water by swimming fast and jumping, and they showed jerky movements. Finally, they settled on the bottom of aquarium and, after some time, they turned belly-up and died. The major cause of mortality might be due to respiratory epithelium damage by oxygen culminating in the formation of a mucus film over the gills of the fish (Das and Sahu, 2005).

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