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Influence of Water Hardness and Ph on Acute Toxicity of Hg on Fresh Water Fish *Capoeta fusca*

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Abstract: The objective of this study was to utilize static test for examining the acute toxicity of Hg to a native fish, *Capoeta fusca*, by static bioassay. The acute toxicity of Hg to *C. fusca* was determined in the three different water hardness (soft, hard and very hard water) and two different pH (5 and 9). The 96 h LC_{50} for Hg at the soft, hard and very hard was found to be 0.118, 0.154 and 0.180 mg/L, respectively, while the 96 h LC_{50} Hg at the pH 5 and pH 9 was found to be 0.032 and 0.396 mg/L, respectively. Results showed that toxicity of Hg reduced by increasing water hardness, also by increasing water pH toxicity of Hg decreased. So, Hg is more toxic in the soft water than in the hard water as well as pH 5 than pH 9.

Key words: Soft water % LC_{50} % Static bioassay % Lethality % *Capoeta fusca*

INTRODUCTION

Qanat is a water-management system used to provide a reliable supply of water to settlements or for irrigation in hot, arid and semi-arid climates; the technology is known to have developed in ancient Persia and then spread to other cultures [1]. Qanats unique environment for fishes and comprises an adit which taps the groundwater and provides a permanent flow. In many districts of the plateau of Iran, fishes are only observed in Qanats, some of them have flowed for hundreds of years [2]. Fishes in Qanats, all over Iran constitute 25 species in Coad’s research, e.i. 40% of the plateau fauna. One of the most significant fishes in Qanats of the east of Iran is the *Capoeta fusca* belonging to the family cypriniod [3].

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades. Among the various toxic pollutants, heavy metals are particularly severe in their action due to persistence is biological amplification through the food chain [4, 5]. Heavy metals have long been recognized as serious pollutants of the aquatic ecosystem. The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to living beings are mercury, cadmium and lead [6]. 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 [7]. This element is classified as one of the most toxic metals, which are introduced into the natural environment by human interferences [8]. Adequate management of our environment requires the correct tools which will allow us to accurately predict the fate and effects of contaminants within the environment.

The purpose of fish acute toxicity is for decision whether a certain xenobiotic is dangerous for the aquatic environment. Acute toxicity tests are short-term tests designed to measure the effects of toxic agents on aquatic species during a short period of their life span [9]. Bioassays allow study, under controlled conditions, of some parameters such as mortality, behavior alterations, or damage in tissues or cells and can help predicting some effects of mercury in natural aquatic eco-systems [10].

The purpose of this research is to determine the acute toxicity of Hg as $HgCl_2$ to a native fish, *Capoeta fusca*, which has been reported only from the east of Iran, at two different pH and three hardness levels of water. These species of fish have been recognized as great important ones from the genetic conservative point of view.

MATERIALS AND METHODS

Birjand is the center of province of South Khorasan in the east of Iran. There are no permanent rivers in the province. However, there are valuable sources of native

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Table 1: Physiochemical properties of test water

	Test water				
Parameter	pH= 5	pH= 9	Soft water	Hard water	Very hard water
Total hardness (as CaCO3, mg/l)	285±5	275±5	50	150	275
pH	5	9	7.5±0.3	7.9±0.3	8.1±0.3
Temperature (°C)	19.1±0.1	18.3±0.1	19.2±0.2	19.2±0.3	19.2±0.2
Dissolved oxygen (mg/l)	6.2±0.1	6.3±0.05	6.4±0.2	6.4±0.05	6.5±0.1
Mg (mg/l)	37.3±1.5	37±1	42.3±1.5	44±1	48.3±2
Nitrite (mg/l)	0.41	0.01	0.03	0.5	0.07
Nitrate (mg/l)	0.04	0.06	0.02	0.01	0.03
Ammonia (mg/l)	0.12	0.11	0.13	0.19	0.14

Table 2: Concentrations of the Hg used in this investigation using *Capoeta fusca*

Element	Soft water	Hard water	Very hard water
	0.032, 0.062, 0.125, 0.25 and 0.5 mg/L	0.062, 0.125, 0.25 and 0.5 mg/L	0.062, 0.125, 0.25, 0.5 and 1 mg/L
$HgCl_2$	pH= 5	pH= 9	
	0.07, 0.015, 0.031, 0.62 and 0.125 mg/L	0.062, 0.125, 0.25, 0.5 and 0.75 mg/L	

fish population in its Qanats. During September and October 2010, *C. fusca* [11] belonging to the family cyprinidae, with average weight ± SD of 3.49 ± 0.69 g and average length of 7.50 ± 0.40 cm were obtained from a Qanat in Birjand. The fish were transported to the laboratory in polyethylene bags by water of Qanat. Fishes was acclimatized for one week to the laboratory conditions in pre-cleaned glass aquariums with tap water. Thereafter, sets of 8 fish specimens (in triplicate) were exposed randomly to 40 litres of water in the aquarium system. The exposure time to Hg (as $HgCl_2$) was 96 hours, without adding any food. For determination the mortality limits of Hg as well as survival experiment, treatments and replication were considered based upon OECD [12].

Stock solutions (1000 mg/L) were prepared by dissolving analytical-grade Hg (as $HgCl_2$) in distilled water. Preliminary tests were carried out to estimate the minimum lethal and maximum nonlethal concentrations of Hg (as $HgCl_2$). The initial concentration of Hg (as $HgCl_2$) added to each aquarium were accurately calculated (Table 2). The experimental water use in the investigation consisted of three types of water (soft, hard water and very hard water; nominal levels of 50, 150 and 275 mg/L as $CaCO_3$, respectively) and two different pH level (pH 5 and pH 9). Dissolved oxygen (mg/L), temperature (°C) and pH were recorded individually in each testing aquarium at exposure times. Quality of water in the experimental tank was determined according to standard procedures. Total hardness, magnesium, nitrite, nitrate and ammonia (mg/L) were determined before starting the experiments by a photometer, Palin test 8000 (Table 1). Lethal concentration for 50% (LC_{50}) values were calculated from the data obtained in acute toxicity bioassays, by using the EPA computer probit analysis program (Version 1.5).

RESULTS AND DISCUSSION

Hg levels can be alternated with different conditions. Factors influencing Hg levels includes exogenous (characteristics of the water as oxygen concentration, pH, hardness, temperature, etc) and endogenous (characteristic of the individuals or species as habitat, food preferences, metabolic rate, age, etc), which may change the state of toxicity of chemical as Hg [9, 13]. Hypoxic conditions, temperature, enhance and acidifications generally render the fish more sensitive to toxication whereas increases in mineral contents (as hardness) decrease metal toxicity [14, 15].

The percentage mortality of *C. fusca* for the three water hardness tests at 96 hours exposure to various concentrations of Hg is presented in figure 1. In the soft and hard water with concentration of 0.5 mg/L, the mortality at 96 hours exposure was 100% (Figure 1a and b), while in the very hard water at 96 hours exposure no mortality were observed (Figure 1c). In the very hard water with concentration of 1 mg/L, the mortality at 96 hours exposure was 100%. Also, the parentage mortality of *C. fusca* for the two pH testing water at 96 hours exposure to various concentration of Hg is presented in figure 2. In the pH 5 with concentration of 0.125 mg/L, the mortality at 96 hours exposure was 100% (Figure 2a), while in the pH 9 at 96 hours exposure no mortality were observed (Figure 2b). In the pH 9 with concentration of 0.75 mg/L, the mortality at 96 hours exposure was 100.

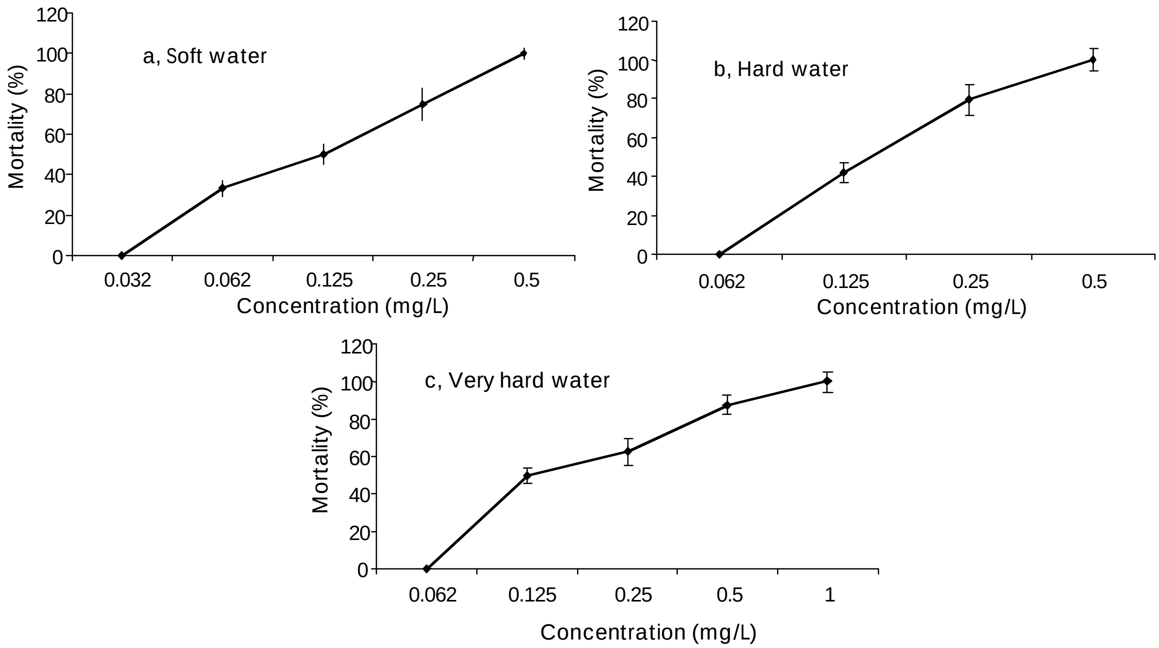

Fig. 1: Percentage mortality of *Capoeta fusca* after 96 hours exposure to different concentrations of Hg at three different water hardness.

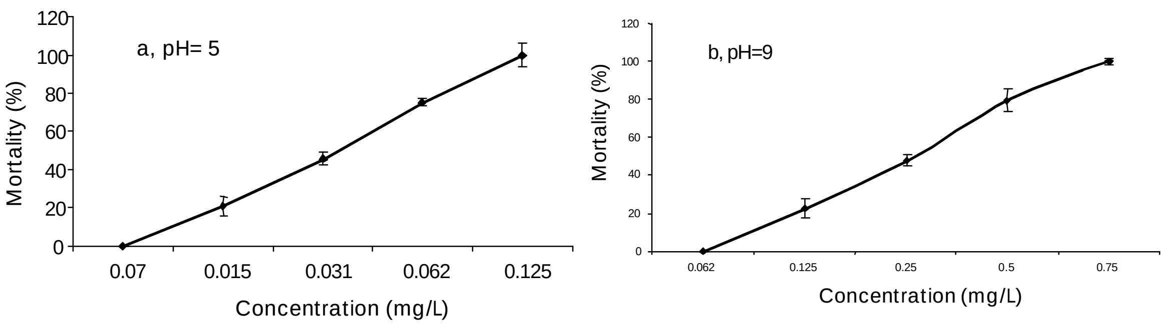

Fig. 2: Percentage mortality of *Capoeta fusca* after 96 hours exposure to different concentrations of Hg at two different pH.

The LC_{50} values of Hg to the *C. fusca* were generated from the mortality data. Comparing the effects of the Hg at three different water hardness and two pH on the *C. fusca*, it were observed that Hg at the soft water and pH 5 was more toxic to the fish with LC_{50} value of 0.118 and 0.032 mg/L at 96 hours, respectively (Table 3). The results of the present study indicated that a 5.5-fold increase in water hardness (from 50 to 275 mg/L as $CaCO_3$) substantially reduced the toxicity of Hg only a 1.5-fold to *C. fusca* at 96 hours exposure. Also, the result of the present study indicated that increasing pH (from pH=5 to 9) substantially reduced the toxicity of Hg up to 12-fold to *C. fusca* at 96 hours exposure. Kumar and Gupta [16] pointed that a 1.08-fold increase in hardness (from 270 to 560 mg/L as a $CaCO_3$) substantially reduced the toxicity of Hg to 1.3-fold, 1.2-fold and 1.01-fold to fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mirigala*, respectively, at 96 hour exposure. Also, Pyle *et al.* [17] showed that a 7-fold increasing water hardness (20 to 140 mg/L as $CaCO_3$) mainly reduced the toxicity of Ni (up to 5-fold) to larval fathead minnows (*Pimephales promelas*) at 96-hours exposure. The studies of Rathore and Khangarot [15] showed that by increasing hardness water from 12 to 300, Hg acute toxicity on *Tubifex muller* has been reduced. A report by Khangarot *et al*. [18] showed that with increasing pH 5.5-8.5 the acute toxicity to fry of common carp (*Cyprinus carpio*) lowered. This reduction of metal accumulation at high pH also resulted in a concurrent reduction of toxicity.

Table 3: Lethal concentration (LC_{50}) whit 95% confidence limit (in parentheses) of Hg estimated by EPA method

	LC_{50} values (mg/L) and 95% confidence limits			
Hg	24 hours	48 hours	72 hours	96 hours
Water hardness				
Soft	0.195 (0.157-0.245)	0.122 (0.035-0.426)	0.128 (0.102-0.161)	0.118 (0.093-0.149)
Hard	0.226 (0.190-0.269)	0.186 (0.155-0.225)	0.154 (0.129-0.184)	0.154 (0.129-0.185)
Very hard	0.366 (0.138-1.317)	0.256 (0.207-0.339)	0.198 (0.032-0.715)	0.180 (0.19-0.22)
pH				
pH=5	0.032 (0.026-0.041)	0.032 (0.026-0.041)	0.032 (0.026-0.041)	0.032 (0.026-0.041)
pH=9	0.453 (0.385-0.535)	0.396 (0.332-0.473)	0.396 (0.332-0.473)	0.396 (0.332-0.473)

Water hardness is well known to attenuate metal toxicity and is major factor, which influences the toxic effects of heavy metals on fish [9, 17]. Toxicity of the metal is usually concerned with impairment of active Ca^{2+} transport in fish by the competitive blockade of epithelial Ca^{2+} channels in the gill epithelium [19]. Fish are near to their ionoregulatory threshold and gill structural integrity is jeopardized due to the lack of available Ca^{2+} in very soft water. Ambient metals change the place of Ca^{2+} from the negatively charged gill surface owing to structural damage and a reduction in osmotic wholeness [17]. In soft water, this change of gill tissue is clear by the observed high toxicity of metals [20]. The results of the study showed that *C. fusca* is so to Hg toxicity at soft water. Metals were more toxic at low hardness, so lowering water hardness, increases sensitivity of *C. fusca* to toxicity of Hg. In the soft water, the LC_{50} values for Hg at 24, 48 and 72 hours of exposure were 0.195, 0.122 and 0.128 mg/L, respectively, while in the hard water were 0.226, 0.186 and 0.154 mg/L, respectively. In the very hard water with the same period of exposure, the LC_{50} values were 0.366, 0.256 and 0.198 mg/L, respectively. In the hard water, the LC_{50} value at 96 h is the same as 72 h, showing no mortality occurrence after 72 h.

One of the most important problems with the correct measurement of the toxicity of metals to significant testing species is the controlling of pH through the ecotoxicity assay. The pH changes can also happen when using natural surface waters as test medium, through the ecotoxicity assay on transportation (because of the loss of chemical equilibrium when sampling). In the other hand, as the pH of the environment can affect mucus secretion or production reduction in the pH may change the mucus, because of reduction of metal uptake [21]. At low pH, metals usually found in their most bioavailable form as divalent cations. In this way ameliorating effect of low pH was attributed to H^+ competition with metal ions at gill surfaces [17]. Also, the results showed that *C. fusca* react the same to Hg toxicity at in low-pH water (pH 5) than in high-pH water (pH 9), that may be because of its own acid toxicity inducing bicarbonate loss in the body fluid [22]. In the pH 5, the LC_{50} values for Hg at 24, 48 and 72 hours of exposure were 0.032, 0.032 and 0.032 mg/L, respectively, while in the pH 9 were 0.453, 0.396 and 0.396 mg/L, respectively. In the pH 5, the LC_{50} value at 72 and 48 h is the same as 24 h, showing no mortality occurrence after 24 h. This is due to 100% mortality in the first 24 hours occurred. Also, in the pH 9, the LC_{50} values for Hg at 48 h is the same as 72 h, showing no mortality occurrence after 48 h. The major cause of mortality might be due to respiratory epithelium damage by oxygen culmination during the formation of a mucus film over the gills of fishes [7].

CONCLUSION

The *C. fusca* (black fish) is a native fish of eastern Iran. In conclusion, we can conclude that the Hg is more toxic in the soft water than in the hard water as well as pH=5 than pH=9 on the *C. fusca*.

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