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Research Article

Investigation acute toxicity some of heavy metals at different water hardness

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ABSTRACT

All aquatic organisms are directly or indirectly affected by the physical characteristics of their environment, especially the chemical composition of the water. The high concentration levels, damage tissues and interfere with the normal growth and proliferation. The aim of this research was to the investigation acute toxicity of $ZnSO_4$ and $CuCl_2$ to *Gambusia holbrooki* by static bioassays at different water hardness. The acute toxicity of selected heavy metals to fish was determined in soft, hard and very hard water, respectively (25, 125 and 350 mg/L as $CaCO_3$). Results showed that water hardness had a significant effect in terms of Cu and Zn toxicity in fish. The toxicity of Cu and Zn increased with decreasing water hardness. Results indicated that an increase in water hardness (from 25 to 350 mg/L as a $CaCO_3$) substantially reduced the toxicity of Cu (up to 38-fold) and Zn (up to 264-fold) to fish at 96 hours exposure. The 96 hours LC_{50} values for fish were higher in the hard and very hard water compared to the soft water. Water hardness had a much smaller effect on the acute toxicity of Cu than the Zn in fish. Cu toxicity in all water types was higher than Zn toxicity for fish. In polluted areas, it is recommended to expand the aquaculture activities in the water resources that their hardness is higher than the average water hardness in the environment. The toxic effect of other heavy metals in more diverse aquatic animals should be investigated in the future research.

Keywords: Acute toxicity, Heavy metals, Static bioassay, Water hardness.

INTRODUCTION

Heavy metals are widely used in various industries and considered as common water pollutants. When the amount of heavy metals in a medium, reach to more than a certain limit, it becomes toxic for those animals that live in the environment (Alkarkhi *et al*., 2009). Low concentrations of some heavy metals are essential for aquatic animals. However, at high concentration levels, they accumulate in different organs, damage tissues and interfere with the normal growth and proliferation (Alkarkhi *et al*., 2009). That is why knowledge regarding the toxicity of heavy metals to aquatic organisms is of paramount importance (Rathore and Khangarot, 2003). Copper (Cu) and Zinc (Zn) that are often present in industrial wastewaters are hazardous to the aquatic ecosystem and pose possible human health risk effects. Besides their toxic and harmful effects to aquatic organisms, heavy metals accumulate throughout the food chain and may affect human well beings (Martins et al., 2004). All aquatic organisms are directly or indirectly affected by the physical characteristics of their environment, especially the chemical composition of the water (Gillis *et al.,* 2008). A number of investigators have reported that the toxic effect of heavy metals on freshwater organisms is affected by water hardness (Kim et al., 2001; Markich *et al.*, 2006). Studies have also shown that environmental factors play an important role in modifying the toxicity of metals (Vedamanikam and Shazilli, 2008). These reports show that increasing water hardness reduces the toxic effect of metals in aquatic organisms. The effects of metals on aquatic organisms have been the subject of numerous investigations (Martins et al., 2004; Kim *et al.,* 2001; Vedamanikam and Shazilli, 2008). Although many research have been conducted to assess the toxicity of heavy metals in algae, however, the number of studies dealing with the toxic effect of heavy metal on aquatic animals including fish are limited (Harmon *et al.*, 2005). The eastern mosquitofish, *Gambusia holbrooki*, is a small fish native to the south-eastern United States. In the past century, this species and its congener, western mosquitofish, *G. affinis*, have been stocked in both permanent and temporary water sources throughout the world for mosquito control (Leyse *et al.,* 2004; Ebrahimpour *et al*, 2010 a). In Iran, during 1922–1930, *Gambusia spp* were imported from Italy and introduced to Ghazian marshes on the Caspian littoral for the biological control of malaria (Ebrahimpour *et al*, 2010 a; Tabibzadeh *et al.*, 1970). In 1966, these fish were introduced to other parts of the country (Ebrahimpour *et al*, 2010 a; Tabibzadeh *et al.*, 1970). Due the importance of *Gambusia* fish in controlling malaria in local fresh water, it was introduced to many countries and now is considered as one of the major fresh water fish species all around the global (Garcia and Huffaker, 1979). The main objective of the present study was to investigate the acute toxicity of Cu, and Zn at different water hardness values to an exotic fish in Iran, *G. holbrooki.*

MATERIALS AND METHODS

A short acute toxicity assay (lethal concentration to 50% of the population) was designed to assess the toxicity effect of Cu and Zn on fish *Gambusia holbrooki* (Dural *et al.*, 2006). Fish were collected from the Ebn-e Hesam River in Khosf (in eastern Iran) over March and April 2009 and transported to the laboratory in polythene bags filled with river water. They were allowed to acclimate to the laboratory conditions for 8 days prior starting the experiments. The fish density in aquariums were kept about 1 gram per liter (TRC, 1984) to make the adaptation more convenient. There were 10 fish per aquaria and the fish were not fed during the study. The average wet weight (±SD) of the fish was 0.39 (±0.08) g. Aquariums held 50 litres of water and were fitted with artificial aeration to maintain oxygen levels (±SD) at an appropriate level (6.8±0.2) . The fish were exposed to Cu (as $CuCl_2$) and Zn (as $ZnSO_4$) in the aquarium systems. The exposure time of fish to Cu and Zn was 96 hours. The concentration ranges of tested heavy metals were determined through providing a serially diluted solutions (OECD, 1992). Preliminary tests were carried out to estimate the minimum nonlethal and maximum lethal concentrations of Cu and Zn. The concentrations of Cu or Zn added to each aquarium are presented in Table 1. Stock solutions (1000 mg/L) were prepared by dissolving analytical-grade of Cu and Zn (Merck Chemicals, Darmstadt, Germany) in distilled water.

Table 1. Concentrations of two heavy metals used in this study

Element	Soft water	Hard water	Very hard water
$CuCl_2$	0.005,0.01,0.02,0.04 and 0.08 mg/L	0.016,0.031,0.062, 0.125, 0.25,5 and 1 mg/L	0.16,0.31, 0.62, 1.25 and 2.5 mg/L
$ZnSO_4$	0.16, 0.31, 0.62, 1.25 and 2.5 mg/L	30, 40, 50, 60, 70 and 80 mg/L	80, 100, 120, 140 and 160 mg/L

A control was used for the tests with 3 replicates of each treatment. No mortality was observed over the experiment in controls. The water used over the experiment consisted of three different hardness levels (soft water, hard water and very hard water; with 25, 125 and 350 mg/L $CaCO_3$, respectively). Dissolved oxygen (mg/L), temperature (ºC) and pH were recorded individually in each test container at exposure times of 24, 48, 72 and 96 hours. Water quality indices of the experimental tanks were determined using standard protocols EPA (2010) Total hardness, magnesium, nitrite and ammonia (mg/L) were determined prior starting the experiments by photometer, Palintest, 8000. Mortalities were recorded at 24, 48, 72 and 96 hours of fish exposure to heavy metals and the dead fish were removed regularly from the test solutions. LC_{50} values were calculated through the data collected in the acute toxicity bioassays using the U.S Environmental Protection Agency (EPA) method "Probit analysis program (version 1.5)"(EPA, 2009).

RESULTS AND DISCUSSION

The physiochemical properties of the test and river waters are shown in Table 2. The physiochemical parameters analyzed during the bioassays showed that the water hardness concentrations in the river water (>500 mg/L as $CaCO_3$) were much higher than the test waters (350, 125 and 25 mg/L as $CaCO_3$). Lethal concentration to 50% of the population of Cu and Zn are shown in Table 3.

Table 2. Physiochemical properties of the river water and test water (M±SD)

Parameter	River water	Test water: very hard water	hard water	soft water
Total hardness ($CaCO_3$, mg/L)	>500	350 ± 4.2	125± 3	25 ± 1
pH	7.8 ± 0.1	7.75 ± 0.2	7.75 ± 0.2	7.75 ± 0.2
Temperature (ºC)	13.3	13. 6 ± 0.2	13.6 ± 0.2	13.6 ± 0.2
Dissolved oxygen (mg/L)	6.7 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	6. 8 ± 0.2
Mg (mg/L)	16 2 ± 4	45 ± 2	24 ± 2	2.1± 0.3
Nitrite (mg/L N)	0.0 5	0. 007	0. 006	-
Ammonia (mg/L N)	0.5	0.02	0.01	-

Table 3. LC_{50} of Cu and Zn (mg/L) for *Gambusia holbrooki*

Water hardness	LC_{50} values and 95% confidence limits: 24 hours	48 hours	72 hours	96 hours
Copper				
Soft	0.05	0.026	0.019	0.017
	(0.037 – 0.076)	(0.020 – 0.032)	(0.015 – 0.023)	0.014 -0.0 20
Hard	1.34	0.48	0.25	0.17
	(0.68-5.30)	(0.31-0.94)	(0.18-0.37)	(0.09-0.35)
Very hard	2.54	1.13	0.84	0.65
	(.89-4.59)	(0.89-1.52)	(0.67-1.06)	(0.54-0.79)
Zinc				
Soft	1.58	0.67	0.52	0.46
	(1.17-2.48)	(0.54- 0.85)	(0.41- 0.64)	(0.38 -0.56)
Hard	66.05	58.38	52.3	48.1

	(61.1-72.9)	(53.5-64.2)	(41.4-64.2)	(32.4- 61.5)
Very hard	135.8	126.4	122.9	121.6
	(128.6-136.7)	(120.3-132.9)	(116.9-129.1)	(115.8-127.4)

In the soft water with concentrations of 0.08 mg/L Cu, the mortality at 96 hours of exposure was 100%, while in the hard water the mortality at 96 hours in 0.08 mg/L Cu was 30%, and in the very hard water at 96 hours of exposure no mortality were observed at 0.16 mg/L Cu. In the hard water with concentrations of 1 mg/L Cu, the mortality at 96 hours of exposure was 100%, while in the very hard water the mortality at 96 hours in 1 mg/L Cu was 75%. In the soft water, the LC_{50} values for Cu at 24, 48 and 72 hours of exposure were 0.05, 0.026 and 0.019 mg/L, respectively, while in hard water it was 1.34, 0.48 and 0.25 mg/L, respectively. However, in very hard water with the same period of exposure, the LC_{50} values were 2.45, 1.13 and 0.84 mg/L, respectively. In the soft water with concentrations of 2.5 mg/L Zn, the mortality at 96 hours of exposure was 100%, while in the hard and very hard waters at 96 hours of exposure no mortality were observed at 30 and 80 mg/L Zn, respectively. In the hard water with concentrations of 80 mg/L Zn, the mortality at 96 hours of exposure was 100%, while in the very hard water at 96 hours no mortality was recorded at 80 mg/L Zn. In the soft water, the LC_{50} values for Zn at 24, 48 and 72 hours of exposure were 1.58, 0.67 and 0.52 mg/L, respectively, while in hard water it was 66.05, 58.38 and 52.3 mg/L, respectively. However, in the very hard water with the same period of exposure, the LC_{50} values were 135.8, 126.4 and 122.9 mg/L, respectively. The acute toxicity of Cu and Zn to the *Gambusia holbrooki* was evaluated at three different water hardness by static bioassays for calculating the LC_{50}. What is more important in assessing the chemical toxicity of a compound is a threshold concentration and the concentration gradient curve (Kamrin, 2000). Comparing the effects of the two metals at three different water hardness on fish, it was observed that Cu at the soft water was more toxic with the LC_{50} value of 0.017 mg/L, while Zn at the very hard water was less toxic with the LC_{50} value of 121.6 mg/L. Results of the present study indicated that a 12-fold increase in water hardness (from 25 to 350 mg/L as a $CaCO_3$) substantially reduced the toxicity of Cu (up to 38-fold) and Zn (up to 264-fold) to fish at 96 hours exposure. Similarly, Ebrahimpour et al (2010 b) reported that a 9.5-fold increase in water hardness (from 40 to 380 mg/L as CaCO3) largely reduced the toxicity of Cu (up to 6.5-fold) and Zn (up to 7.5-fold) to *Capoeta fusca* at 96 hours exposure. They also showed that in the hard water (150 mg/L as $CaCO_3$), the LC_{50} values for Cu at 96 hours exposure was 6.45 mg/L, respectively, while in the very hard water (380 mg/L as $CaCO_3$), the LC_{50} values for Zn at 96 hours exposure was 87.2 mg/L, respectively. Strauss (2003) showed that a 7 fold increase in water hardness (range 16 to 112 mg/L $CaCO_3$), considerably reduced the Cu toxicity (up to 239 fold) at 96 hours in the long blue nails tilapia fish (*Oreochromis aureus*). It seems the kind of fish species is more important than the sensitivity of fish to a toxic element. The rate of the toxicity is affected by kind and the concentration of heavy metals, species under the test and the water type.

Results of this study indicated that *G. holbrooki* is sensitive to Cu and Zn toxicity in soft water. The less the water hardness, the fish were more sensitive to Cu and Zn toxicity. Exposure of fish to Cu and Zn at three different water hardness led to various LC_{50} values. Heavy metals are more toxic in the soft water than in the hard water because they are more soluble in the soft waters. It is known that the dissolved forms of heavy metals act as active toxic agents(Rathore and Khangarot, 2003). Perschbacher and Wurts (1999) showed that as water hardness increased, the catfish *(Ictalurus punctatus)* survival rate was significantly improved from 10% in soft water (10 mg/L as CaCO3) compared to 95% in very hard water (400 mg/L as CaCO3). In fresh water as water hardness increases, heavy metal toxicity decreases due to the competition between heavy metal ions and calcium and magnesium ions for the uptake sites of organisms (Kim et al., 2001; Pyle *et al.*, 2002; Ebrahimpour, 2010 b; Javid et al., 2007). In the natural environment, calcium and magnesium are present at much higher concentrations than the heavy metals. Therefore, by competing with heavy metals and blocking their access to aquatic organisms, calcium and magnesium levels are important considerations with respect to the toxic effects of heavy metals upon biota in aquatic systems (Kim et al., 2001; Garcia and Huffaker, 1979). According to Penttinen et al (1998) the uptake of calcium and magnesium ions by the cell membrane leads to the stabilization of calcium and magnesium, and this reduces the permeability of the cell membranes to metal ions. Water hardness reduces metals toxicity by saturating the gill surface binding sites with Ca^{2+} (Pyle *et al.*, 2002).

CONCLUSIONS

Fish were more sensitive to Cu and Zn toxicity when the water hardness decreased. Results indicated that an increase in water hardness (from 25 to 350 mg/L as a $CaCO_3$) substantially reduced the toxicity of Cu (up to 38-fold) and Zn (up to 264-fold) to fish at 96 hours exposure. In polluted areas, it is recommended to expand the aquaculture activities in the water resources that their hardness is higher than the average water hardness in the environment. The toxicity effect of other heavy metals on more diverse aquatic animals should be investigated in the future research.

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