Acute Toxicity Test of Zinc Chloride ($ZnCl_2$) in sobaity seabream (*Sparidebtex hasta)*

Elnaz Erfanifar[1, *], Vahide Jahanjo[2], Naghimeh Kasalkhe[1], and Elahe Erfanifar[3]

[1]Offshore Fisheries Research Center, Chabahar, Iran
[2]Azad University of Bandar Abbas, Iran
[3]Gorgan University of Agricultural Sciences and Natural Resource, Gorgan, Iran

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Abstract

The main objective of the present study was to investigate the sensitivity of marine fish sobaity seabream *(Sparidebtex hasta)* to Zinc Chloride in the toxicity test programs. In practice, experimental setup (Rang Finding Test) was constituted 25 fish (from 193 fish) to be placed in one replicates. All fishes were exposed to $ZnCl_2$ at several selected concentrations 0, 1, 5, 10, and 15 ppm (Rang Finding Test), then the fish were subjected to seven concentrations of $ZnCl_2$ (control, 10, 15, 20, 25, 30, 35, and 40 ppm). Each concentration contained seven fish with three replicate each. There was a simultaneous control group (n= 21; with no heavy metal additions) together with the heavy metal treatments, keeping all other conditions alike. Physicochemical parameters, such as dissolved oxygen, pH, and temperature of fiberglass tank as well as mortality rate of the fishes, were monitored daily. Number of mortality was registered after 24, 48, 72, and 96 hours. LC_{50} values were determined with probit analysis. The 96 hours LC_{50} value of $ZnCl_2$ to the fish was found to be 10.27 ppm. The mortality decreased with time, and most of the deaths occurred during the first 24 hours. In addition, behavioural changes increased with concentration enhancement.

Keywords: Zinc Chloride; Mortality; Acute toxicity; Marine fish; LC50.

1. Introduction

The contamination of natural aquatic resources, with heavy metals released from industrial, domestic, and other anthropogenic activities has become a matter of concern over the past few decades (Waqar *et al.*, 2013). Harmful effects of heavy metals on aquatic organisms can be detected by performing toxicity tests that allow establishing a dose-response relationship (Javed, 2013) which help us in predicting acute damage to aquatic fauna as well as in regulating toxic chemical discharges into the water bodies (Federation and APHA, 2005). Heavy metals are the most relevant of aquatic ecosystems considering the growing cases of environmental accidents in the last years in to the urban waters (Di Giulio and Hinton, 2008). Water pollution by industrial effluents containing organics and heavy metals pose a serious hazard to the aquatic biota and public health (Lawrence and Hemingway, 2003).

*Corresponding Author: elnaz_erfani@yahoo.com

Acute toxicity bioassays (LC_{50} and lethal concentration) were used to evaluate the toxicity of heavy metals and to assess the potentials of various fish species to the toxicity of metals (Abdullah *et al.*, 2007). Acute toxicity tests permit rapid assessment of the impacts of various toxicants on organisms. The criterion of lethal toxicity is mortality, the final response of an organism. However, in natural waters, long-term influence of low concentrations of pollutants or their mixtures mostly affects on the fish (Boudou and Ribeyre, 1997). The 96-h LC_{50} tests were conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metals pollution. Higher values of LC_{50} are less toxic because greater concentrations are required to produce 50% mortality in organisms. In addition, LC_{50} is the ambient aqueous chemical activity that causes 50% mortality in an exposed population. These calculations were based on two important assumptions explained in Di Giulio *et al.* (1993). The studies showed that fish is a good choice for studying the effects of heavy metals in aquatic ecosystems, because fish have high levels of food pyramid.

In this among, the Sobaity seabream (*Sparidebtex hasta*), the most appropriate target species, is in toxicology studies. The aims of the present study were to investigate acute effects of zinc chloride as potential dangerous additives to assess mortality effects of this heavy metal on a valuable cultured fish of Iran, *Sparidebtex hasta* (*S. hasta*). The *S. hasta*, locally known as sobaity in Kuwait, is native in Persian Gulf, western Indian Ocean, and coast of India (Yousif *et al.*, 2003). *S. hasta* is a silvery fish with tender flesh and a rich flavour. *S. hasta* is a shoreline surface fish, feeding in the wild by hunting small fishes (Kitto, 2004).

2. Materials and Methods

2.1. Fish and condition

For this experiment, 193 healthy specimens *S. hasta* (mean weight 4.8 g; mean length 6.26 cm) were captured from the coastal water of Chabahar. The fishes were transported to aquaculture research center of Offshore Fisheries Research Center, in a many containers equipped with an oxygen capsule and were acclimatized for a period of seven days at 30 °C under a constant 12:12 L:D photo period. Before toxicological tests, the fish were acclimated to laboratory conditions (*p*H 7.58; DO 8.25 mg O_2 L-1) for a minimum of one week in a 300-L tank with seawater. Acclimatized fish were fed daily with a formulated feed. Firstly, using deionized water, all fiberglass tanks in this experiment were washed, and thoroughly rinsed. Prior to each trial, the tanks (60 L capacity) were filled with 50 L of dechlorinated tap water. A number of 24 fiberglass tanks, each of which stocked with seven fishes, were used in our experiments for each metal. Stock solutions of $ZnCl_2$ was prepared by dissolving analytical grade Zinc Chloride ($ZnCl_2$ from Merck), in double distilled water. The 21 fish were used per concentration of each heavy metal. Separate groups of 21 fish each served as controls for Zinc Chloride. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of water quality.

2.2. Rang Finding Test

All fishes were exposed to $ZnCl_2$ at several selected concentrations (0, 1, 5, 10, and 15 ppm) to determine $ZnCl_2$ dose in acute toxicity test. Groups of 25 fish (each stocked with 5 fishes) were exposed to different concentrations of $ZnCl_2$ for 96 hours in fiberglass tanks. Medium test was not renewed during the assay and no food was provided to the animals. Values of mortality were measured at times of 0, 24, 48, 72 and 96 hours.

2.3. Acute toxicity tests

Concentrations for acute toxicity tests were (control, 10, 15, 20, 25, 30, 35, and 40 ppm). Boudou and Ribeyre recorded mortality after 24, 48,72 , and

96 hours, and LC_{50} values, and its confidence limits (95%) (Hotos and Vlahos, 1998). Furthermore, they calculated percentages of fish mortality for each of Zinc Chloride concentration at 24, 48, 72 and 96 hours of exposure.

Furthermore, LC_{50} values were calculated from the obtained data in acute toxicity bioassays, by Finney's method of "probit analysis" and with SPSS statistical software. In Finney's method, the LC_{50} value derived by fitting a regression equation arithmetically and a graphical interpolation by taking logarithms of the chemical concentration test on the X-axis and the probit value of percentage mortality on the Y-axis (Finney, 1971).

The $LC_{1,\ 10,\ 30,\ 50,\ 70,\ 80,\ 90,\ 99}$ values were derived using simple substitution probit of 1, 10, 30, 50, 70, 80, 90, and 99, respectively, for probit of mortality in the regression equations of probit of mortality vs. Zinc Chloride. The SE of LC_{50} is calculated from the following formula:

$$\mathrm{SE\ (LC50)} = \frac{1}{b\sqrt{\mathrm{pnw}}}$$

which b is the slope of the Zinc Chloride/probit response (regression) line; p is the number of Zinc Chloride used, n is the number of animals in each group, and w is the average weight of the observation. The 95% confidence limits for LC_{50} were estimated using the formula LC_{50} (95% CL) = LC_{50} ±1.96 [SE (LC_{50})] (Hotos and Vlahos, 1998).

2.4. Behavior observation

Physiological responses like rapid operculum movement and frequent gulping of air were observed during the initial stages of exposure after it became occasional.

3. Results

The results from acute toxicity test as for the Zinc Chloride on *S. hasta* are shown in Table 2. There was no mortality in the control group throughout the experiment Table 1. Fish mortality increased significantly when the concentrations and the time of exposure enhanced.

As it expected, the 96-h LC_{50} values decreased with prolonged exposure time. This denotes on increasing in toxicity with exposure duration. Before death, fish showed rapid gill movement, nervous movements, erratic swimming, imbalance and inability to remain upright. The 96 hours LC_{50} value of Zinc Chloride to the fish was 10.27 ppm.

Table 1. Cumulative mortality of gray mullet during acute exposure to Zinc Chloride (n=21, effective dose)

Concentration (ppm)	No. of died fishes			
	24h	48h	72h	96h
Control	0	0	0	0
10	0	6	9	12
15	18	18	18	18
20	18	18	18	18
25	18	19	21	21
30	21	21	21	21
35	21	21	21	21
40	21	21	21	21

Table 2. Lethal Concentrations (LC) of $_{1-99}$ of Zinc Chloride depending on time (24-96h) for gray mullet

	Concentration (ppm) (95 % of confidence limits)			
point	24h	48h	72h	96h
LC_1	1.252 (-47.22-8.7)	-2.72 (-1088-1.77)	-1.75 (-9.83-2.39)	-3.71
LC_{10}	7.45 (-22.75-12.61)	4.12 (-1.19-7.21)	4.08 (-1.18-6.94)	2.5
LC_{30}	11.75 (-5.64-16.1)	9.09 (5.61-11.37)	8.31 (4.8-10.44)	7.11
LC_{50}	14.73 (4.93-19.7)	12.53 (10.03-14.55)	11.25 (8.82-13.14)	10.27
LC_{70}	17.71 (12.36-26.55)	15.97 (13.93-18.25)	14.18 (12.26-16.33)	13.42
LC_{80}	19.51 (15.03-32.48)	18.05 (16-20.78)	15.95 (14.05-18.55)	15.33
LC_{90}	22.01 (17.58-41.87)	20.94 (18.61-24.55)	18.41 (16.27-21.89)	1797
LC_{99}	27.94 (21.92-65.88)	27.79 (24.25-34.03)	24.25 (21.02-30.34)	14.25

4. Discussion

The present research aim is to investigate acute toxicity test of Zinc Chloride ($ZnCl_2$) in *S. hasta*. Aquatic toxicity tests may provide insights to the relative sensitivity of *S. hasta* to Zinc Chloride, which may also provide suitable data on the impact of Zinc Chloride on water environment, as these species hold in important positions in aquatic ecosystems. Toxicity even within a species and with the same toxicant depends on the size, age, and condition of the tested organism as well as on experimental factors. The differences in acute toxicity could even be due to the changes in water quality and test species (Rathore and Khangarot, 2002).

In the present study, LC_{50} values indicated that Zinc Chloride ($ZnCl_2$) was toxic to the studied fish. The 96 hours LC_{50} value of $ZnCl_2$ to the fish was found to be 10.27 ppm. A significant increase in mortality was observed in *S. hasta* exposed to 10 ppm of Zinc Chloride. LC_{50} values indicated that Zinc Chloride is a toxic metal for *S. hasta*. LC_{50} obtained in the present study correspond to values published in the literature for other species of fish. Many aquatic species show a vast range of LC_{50} for Zinc Chloride, which for saltwater fish can vary from as high as 1500 µg/liter for flagfish (*Jordanella floridae*), 815 µg Zn/liter for Chinook Salmon (Spehar, 2011). While the observed acute toxicity (LC_{50}) value for 96-h is very low for Zinc Chloride compared to other heavy metals such as mercury.

In addition, acute toxicity of metals varied significantly among fish species. The fish that is highly susceptible to a metal may show higher resistance to another metal at the same concentration (Biuki *et al.*, 2010). The fish exposed to plumb and zinc can compensate for the pollutant. If it cannot successfully compensate for contaminant effects, an altered physiological stage may be reached in, which the fish species continues to function and in extreme cases, the acclimation response may be exhausted with a subsequent effect on fitness (Hedayati and Safahieh, 2011).

All the observations indicated the impact of Zinc Chloride toxicity and caused behavioral alterations, such as those observed in this study may result in severe physiological problems, ultimately leading to the death of fish. Therefore, the present investigation demonstrated a relation among survival and mortality rates. Thus, this study could be used as a tool for creating awareness among the local farmers and industrial, so that the use of the highly toxic heavy metals could be minimized.

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