Effects of Zinc Chloride ($ZnCl_2$) sub-lethal concentrations on hepatic enzymes activity in Sobaity Seabream (Sparidebtex hasta)

Naghimeh Kasalkhe[1], Elnaz Erfanifar[1,*], Elahe Erfanifar[2], Mohammadreza Mirzaei[1], and Mahmmodreza Azini[1]

[1]Offshore Fisheries Research Center, Chabahar, Iran

[2]Gorgan University of Agricultural Sciences and Natural Resource, Gorgan, Iran

Received: 2016-12-21 *Accepted: 2017-01-28*

Abstract

Zinc, as one of the heavy metals and an environmental stressor, may alter many physiological processes like growth and serum parameters in fish. The main objective of this study was to determine the effects of Zinc Chloride sub-lethal concentrations (0.03 and 0.06 mg/L) on hepatic enzymes activity including enzymes, i.e. alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in Sobaity Seabream (Sparidebtex hasta). In this study, after determining the LC50, two sub-lethal treatments of Zinc chloride include 0.03, and 0.06 mg/L were considered that any treatment had three replications. Fish were exposed to different doses of sub-lethal for 1, 5, 10 and 15 days and samples of liver tissue were taken at the end of each period. The results showed that with increasing concentration of sub-lethal levels of AST, ALP, and ALT considerably increased. In addition, with prolonged exposure amount of enzymes AST, ALP, and ALT were increased significantly ($P<0.05$). This investigation suggested that those hepatic enzymes as stress indicators could be used as important and sensitive biomarkers in ecotoxicological studies, concerning the effects of metal contamination and fish health.

Keywords: Zinc Chloride; Hepatic enzymes; Sparidebtex hasta.

1. Introduction

Metal pollution of the sea is less visible and direct than other types of marine pollution but its effects on marine ecosystems and humans are very extensive. The presence of metals varies between fish species; depend on age, developmental stage and other physiological factors (Emami Khansari, 2005). Zinc metal is widely detected in water, although Zn compounds have relatively high solubility. Therefore, small amount of Zn in the water or in the diet is essential. The organisms will have internal

* Corresponding Author: elnaz_erfani@yahoo.com

mechanisms to transport Zn around the body in order to manufacture such vital enzymes. When the Zn in water rises to a level, the amount entering the organism through the gills exceeds the requirement for this metal. It was originally thought that the direct toxic action of Zn on fish was to precipitate the layer of mucus on the surface of the gill, causing suffocation (Andres *et al.*, 2000; Papagiannis *et al.*, 2004). However, excessive Zn in the aquatic environments is toxic (Huang *et al.*, 2010; Zheng *et al.*, 2011).

Actually, the fish toxicity by Zn has been well documented in various fish species (Dautremepuits *et al*., 2004; Giardina *et al.*, 2009). Fish readily absorb dissolved metals and may serve as indicators of the extent of pollution (Adham *et al.*, 2002; Farkas *et al*., 2002; Shukla *et al*., 2007). The silvery black porgy or blue finned seabream *S.hasta* (synonym *Acanthopagrus cuvieri*) locally known as Sobaity in Kuwait is native to Arabian Gulf, western Indian Ocean and coast of India (Yousif *et al.,* 2003).

Sobaity is a silvery fish with tender flesh and a rich flavor. It comes in habitats varying from shallow coastal waters to deep water. The fish feeds mostly on invertebrates and crustaceans (Bauchot and Smith, 1984; Kuronuma and Abe, 1986; Al-Abdessalaam, 1995). However, there is not enough information about the hepatic enzymes activity in *S.hasta* which is found in the Persian Gulf. This species has been introduced and cultured in Iran and other countries adjacent to the Persian Gulf (Jahantigh, 2015). Accordingly, the aim of this study is to improve current knowledge on the amount and distribution of hepatic enzymes activity in *S. hasta*.

2. Materials and methods

2.1. Fish holding conditions and acclimation

In this study, fish were fasting for a period of 24 hours before the sampling. Five fish per tank on the days of 1, 5, 10 and 15 was removed and clove extract anesthesia (1g/L). At the end of the weight and during individual fish were measured. *S. hasta* obtained from the Kolahi Culture Pond in Bandarabbas, Iran, were matched for the size (mean weight 4.8 g; mean length 6.26 cm), and then were transferred to the aquaculture research center of Offshore Fisheries Research Center Chabahr, Iran, in many containers equipped with an oxygen capsule and were acclimatized for a period of seven days at 30.5 ˚C under a constant 12:12 L:D photo period. Before toxicological tests, the fish were acclimated to laboratory conditions (PH 7.58, DO 8.85 mg O_2 L^{-1}, TDS=61.76 g/L, EC=37.95 µs/cm, and Salinity = 38 ppt) for a minimum of one week in a 300-L tank of seawater. Then, fish were fed at a feeding rate of 2% of body weight per day for 15 days. Acclimatized fish were fed daily with a formulated feed, and any fish that showed abnormal behavior were removed immediately from the tanks.

2.2. Exposure system

Prior to each trial, the tanks (60-L capacity) were filled with 30 Litters of dechlorinated tap water. Active groups of 15 fish were randomly transferred to 9 fiberglass tanks with continuous aeration. Stock solutions of $ZnCl_2$ were prepared by dissolving analytical grade Zinc Chloride ($ZnCl_2$ from Merck), in double distilled water. For the experiments, first 96 hours LC_{50} value of $ZnCl_2$ to the fish was found to be 10.27 ppm (Erfanifar *et al*., 2016). Thereafter, triplicate treatment groups (with control) were exposed to sub-lethal concentrations of 10% and 20% from the newly-determined LC_{50}-96h value for each metal according to a recent research on $Zncl_2$ for a period of 15 days (Sadeghi *et al*., 2015). In fact, the fish were exposed to control, 0.03 mg/L and 0.06 mg/L for 1, 5, 10, and 15 days.

2.3. Harvesting and Preparation of Fish hepatic

The days after 1, 5, 10 and 15, fish hepatic samples were collected. The preparation of Fish hepatic

Table 1. Change in serum enzyme of AST, ALT, and ALP of *S. hasta* exposed to sub-lethal concentration of $Zncl_2$ for short term (15 days)

Days	Treatment	AST	ALT	ALP
Day1	Control	172.33±1.20[a]	9.66±0.33[a]	52.00±0.57[a]
	T1	187.66±0.88[b]	22.00±0.57[b]	58.33±0.88[b]
	T2	193.33±0.88	33.00±0.88[c]	69.00±0.57[c]
Day5	Control	174.00±0.57[a]	12.00±0.57[a]	54.00±0.57[a]
	T1	186.66±0.88[b]	26.00±0.57[b]	66.00±0.57[b]
	T2	210.33±5.48[c]	37.33±0.88[c]	74.33±1.20[c]
Day10	Control	176.00±0.57[a]	12.33±0.33[a]	54.66±0.88[a]
	T1	198.00±0.57[b]	32.00±0.57[b]	71.33±0.88[b]
	T2	276.66±8.81[c]	41.33±1.85[c]	83.33±0.88[c]
Day15	Control	177.00±0.57[a]	13.00±0.57[a]	57.66±0.88[a]
	T1	251.00±10.81[b]	37.33±1.45[b]	74.66±1.45[b]
	T2	351.00±25.57[c]	46.00±2.08[c]	87.66±1.45[c]

a, b, c: Values in columns with different letters are significantly different

samples for measuring of alkaline phosphatase activity (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) was based on the method of in agreement with Cahu *et al.* (1999). The preparation method of physiologic serum was used for measuring the hepatic enzymes activity. In brief, the isolated fish hepatic were homogenized with a volume of phosphate buffered saline. Moreover, fish hepatic homogenates were centrifuged at individual conditions (10 minutes, 3000 g, 4 °C). The resultant supernatants were then collected and stored at -80 °C until analysis of enzyme activity. Furthermore, ALT, ALP and AST enzymes were determined using the Pars-Azmoon Diagnostics Infinity AST reagent Kit (Pars-Azmoon Co., Teheran, Iran) and a Sigma Diagnostics Infinity ALT Reagent Kit (Sigma, St. Louis, MO), respectively.

2.4. Statistical analysis

Initially, the raw data were checked for normality of distribution by Kolmogorov- Smirnov tests. All values were expressed as means ± standard error of means (SEM). Then, the analysis of differences between control and different sampling times in each exposure group was tested by one-way analysis of variance (ANOVA). Moreover, the post hoc Duncan's multiple range tests were used among treatment means with $SPSS_{22}$, which the significance was determined at $P< 0.05$ as described by Dytham (1999).

3. Results

Hepatic function was assessed by measuring the AST, ALT and ALP. Liver enzymatic parameters for *S. hasta* exposed to $Zncl_2$ for 15 days are shown in Table 1. As it is shown the mean values that have been labeled with the same superscript letters are significantly different ($P< 0.05$).

According to the results of the enzymes activity estimation, the level of AST increased considerably from 172.33 IU/L on the first day to 351 IU/L on day 15 (Figure 1). There are significant differences between the values ($P<0.05$) which are indicated in Table 1 and Figure 1 with difference letters.

The Figure 1 illustrates that the Serum AST increased after 15 days, when the fish were exposed to the both concentrations of $Zncl_2$. This elevation continued during the time and increased in $zncl_2$

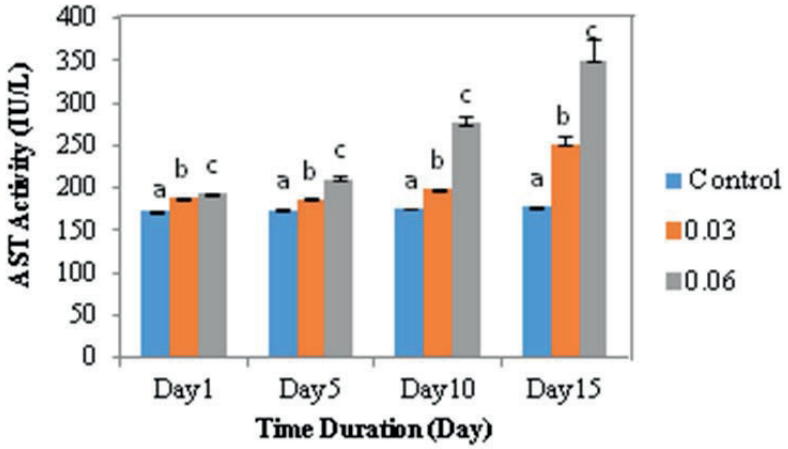

Figure 1. The effects of different sub-lethal $Zncl_2$ concentrations on serum enzymes activities

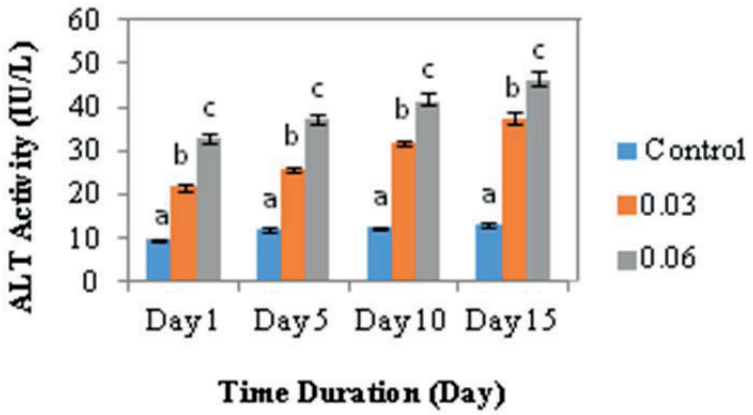

Figure 2. Effects of different sub-lethal $Zncl_2$ concentrations on serum enzymes activities

level so that in the 15th day, the AST activity was greatest in the group exposed to high $zncl_2$ compared to the control. Data are expressed as mean ± standard error (SE). Means that are shown with different letters (a, b, and c) are significantly different from each other ($P<0.05$).

In addition, based on the results of the enzymes activity estimation, the level of ALT increased notably from 9.66 IU/L on the first day to 46 IU/L on day 15 (Figure 2). These differentiations are shown with different letters (a, b, c) in the figure.

The Figure 2 shows that the Serum ALT increased after 15 days, when the fish were exposed to the both concentrations of $Zncl_2$. This elevation continued and increased in $zncl_2$ level with the intention that the ALT activity in the 15th day was the maximum in the group exposed to high $zncl_2$ in comparison with the control. The data are stated as mean ± SE. Furthermore, the means with different letters are significantly different from each other ($P<0.05$).

Furthermore, according to the results of the enzymes activity estimation, the level of ALP increased significantly from 52 IU/L on the first day to 87.66 IU/L on day 15 (Figure 3).

The Figure 3 indicates that Serum ALP increased after 15 days, when the fish were represented by the both concentrations of $Zncl_2$. This elevation continued and increased in $zncl_2$ level, therefore, the ALP activity in the 15th day was the most in the group subjected to high $zncl_2$ in respect to the control. The data is again expressed as mean ± SE. Moreover, the means values that are shown with different letters (a, b, and c), are significantly different from each other ($P<0.05$).

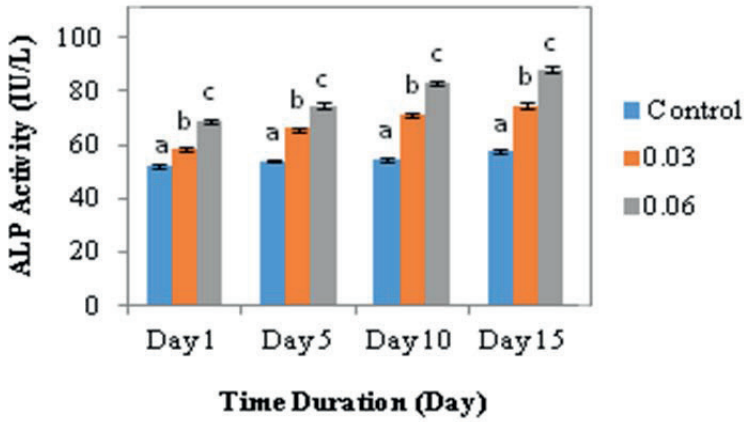

Figure 3. Effects of different sub-lethal $Zncl_2$ concentrations on serum enzymes activities

4. Discussion and Conclusion

Although the toxicity of zinc to several fish species has been documented, the toxicity of this metal is not well-known for all aquatic organisms (Küçükoğlu *et al.*, 2013). The heavy metal may cause injury to the organisms and the damaged tissues subsequently malfunction (Thirumavalavan, 2010). In fish, the liver acts as an important organ for uptake, accumulation, biotransformation, and excretion of toxicant during sub-lethal treatment (Maher *et al.*, 1999; Pedlar and Klaverkamp, 2002). Some results obtained from this study were in contrast with other relevant studies which may be to the differences in factors that influence toxicity (e.g., species, life history stage, water quality, exposure duration, zinc concentration). However, there are similarities between our findings and those from other studies investigating zinc toxicity. In the present study, the level of ALP, AST, and ALT increased over a 15-day period and the higher concentration caused more significant effects on fish. The results showed the effects of Zinc Chloride ($ZnCl_2$) sub-lethal concentrations on the hepatic enzymes activity in *S. hasta*. These results were in agreement with the findings of Younis *et al*., (2012) who stated that there was significant increase in the level of AST and ALT of zinc trioxide treated Nile tilapia *(Oreochromis niloticus)* compared to the control group. Based on the Younis *et al.,* (2012), the significant increase in AST and ALT levels in zinc treated fish with short and long term sub-lethal exposure indicated that hepatic damage due to zinc accumulation which in turn released these enzymes into the bloodstream.

In addition, Roy and Bhattacharya (2005) noted significant changes in serum AST and ALT in Channa punctatus exposed to As_2O_3, and indicated that the changes may be due to histopathological lesions in liver. Therefore, Nile tilapia exposed to zinc cause histopathological injury in the liver which is in agreement with the Abdel-Warith *et al.*, (2011). These results were in agreement with the findings of in another investigation (Heydarnejad *et al.*, 2013), which both AST and ALT activity changed significantly by Cu exposure so that both enzymes increased in a 15-day period by Cu in *Oncorhynchus mykiss.*

The represented effects of heavy metals on serum or liver enzyme activities including ALP, AST and ALT activities of several teleost species that are in agreement with those results by Vaglio and Landriscina (1999), Torre *et al.* (2000), and Kim and Kang (2004). Furthermore, these results stated the overall changes in concentrations and enzymes of these plasma/serum enzymes of a number of fish activities following heavy metal exposure occur either species were highly increased in the fish treated due to leakage of these enzymes from hepatic cells and with heavy metals including cadmium and zinc.

The authors suggest that the liver is rich in AST and ALT; therefore its damage can result in the liberation of large quantities of these enzymes into the blood. Therefore, increases in AST and ALT

activities in the serum of heavy metal in treated fish are assumed to be a result of liver damage by the heavy metals. The results in the current study showed that the activity of AST and ALT increased with the increase of zinc chloride concentration for both short and long term exposure of fish. Thus it is suggested that enzyme bioassay can be used as a valuable tool to assess a change or damage caused to an organism due to administration of heavy metals. These findings are in agreement with Harper research (Harper, 1978). Overall, the changes in concentrations and enzymes activities following heavy metal exposure occur either due to (i) leakage of these enzymes from hepatic cells and thus raising levels in blood, (ii) increasing synthesis and (iii) enzyme induction of these enzymes according to Shakoori, 1990. This investigation recommends that those hepatic enzymes as stress indicator could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health.

Acknowledgements

We are grateful to the Offshore Fisheries Research Center Chabahar for providing technical and financial facilities.

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