Acute Toxicity of Mercuric Chloride ($HgCl_2$), Lead Chloride ($PbCl_2$) and Zinc Sulfate ($ZnSO_4$) on Silver Dollar Fish (*Metynnis fasciatus*)

Ali Sadeghi[*1] *and Mohammad Reza Imanpoor* [2]

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ABSTRACT

Background: Since heavy metals can accumulate in the tissues of aquatic organisms, they can cause a range of hazardous effects which can become harmful to humans. The aim of this study was to investigate the acute effects of some heavy metals as potential dangerous substances by assessing the mortality effects of Mercuric Chloride (HgCl2), Lead Chloride (PbCl2) and Zinc Sulfate (ZnSO4) pollutants on a freshwater fish, silver dollar (*Metynnis fasciatus*).

Methods: Fish samples were exposed to different concentrations of mercuric chloride ($HgCl_2$), lead chloride ($Pbcl_2$) and zinc sulfate ($ZnSO_4$) for 96h and their cumulative mortality was calculated in 24h intervals. Results were analyzed by SPSS 16 to obtain number of cumulative mortality and lethal concentrations (LC_{10-99}).

Results: LC_{50}-96h was 0.94±0.41ppm, 86.84±1.04ppm and 32.24±1.41ppm for mercuric chloride, lead chloride and zinc sulfate, respectively. So, mercury had the highest toxicity to silver dollar fish.

Conclusion: Mercuric chloride and lead chloride has the lowest and highest rate of mortality among these tree metals on silver dollar fish; however, the mortality rate was increased with increasing concentrations of toxins with time.

Keywords: Fishes; Lead Chloride; Lethal Dose 50; Mercuric Chloride; Water Pollution, Chemical; Zinc Sulfate.

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INTRODUCTION

Various chemicals derived from agricultural procedures and industrial effluents, e.g. heavy metals, may contaminate a variety of different water environments. Since metals are not degradable and potentially toxic when bound to endogenous compounds in living organisms [1], they can produce a range of hazardous effects in aquatic organisms; from alterations in single cells to changes in whole populations [2].

The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in ecosystem make these chemicals harmful to aquatic ecosystems and, consequently, to humans who are dependent on aquatic products as food sources [3, 4]. The concentration of heavy metals in aquatic animals is related to several parameters, e.g. food habits and foraging behavior of the fish [3], tropic status, source of particular metals, distance of the animal from the pollutant source, the presence of other ions in the ecosystem [5], temperature, transport of metal across membranes, the metabolic rate of the animal [6] and seasonal variation in the taxonomic composition of different tropic levels affecting the level and accumulation of heavy metal in the fish tissue [7].

Median lethal dose (LC_{50}) tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in animals [8]. Heavy metals such as mercury, cadmium and lead are toxic to aquatic animals at very low concentrations and have no beneficial effect to living beings [9].

1. PhD Student, Department of Fishery, Payame Noor University (PNU), Iran.
2. Department of Fishery, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran.
* Corresponding Author: E-mail: sadeghi.a_shilat@yahoo.com

The aim of this study was to investigate the acute effects of some heavy metals as potential dangerous substances by assessing the mortality effects of Mercuric Chloride ($HgCl_2$), Lead Chloride ($PbCl_2$) and Zinc Sulfate ($ZnSO_4$) pollutants on a freshwater fish, silver dollar (*Metynnis fasciatus*).

MATERIALS AND METHODS

This is an experimental study that was carried out in Gorgan University of Agricultural Sciences & Natural Resources in December 2014. The selected fish species was Silver dollar (*Metynnis fasciatus*); 21 for each concentration. In sum there were 105 fishes in 5 groups for each heavy metal. Test chambers were 120-litre glass aquaria. All fishes were acclimated for a week in these aquaria before assays with continuous aeration and water temperature was regulated at 25°C by aquarium heaters. The fishes were fed twice daily with formulated feed and dead fishes were immediately removed to avoid possible water deterioration [10].

Mercury test concentrations were 0, 0.02, 0.5, 1 and 2ppm of $HgCl_2$; lead chloride concentrations were 0, 40, 80, 120 and 160ppm of $PbCl_2$; and Zinc concentrations were 0, 15, 30, 60 and 90ppm of $ZnSo_4$. Groups of 21 fishes were exposed for 96h in glass aquaria with 120l of the test medium. During acute toxicity experiment, the water in each aquarium was aerated and the temperature was kept at 25°C. No food was provided to the specimens during the assay and the test media was not renewed.

Mortality rates were recorded at 0, 24, 48, 72 and 96h. Acute toxicity tests were carried out [11]. The nominal concentrations of mercuric chloride, lead chloride and zinc sulfate, estimated to result in 50% mortality of silver dollar fish within 24h, 48h, 72h, and 96h were attained by probit analysis by Finney's method and using the maximum-likelihood procedure in SPSS 16 (SPSS Inc.; Chicago, USA) [12].

The LC_{50} value was obtained by fitting a regression equation arithmetically and also by graphical interpolation using logarithms of mercuric chloride, leads chloride and zinc sulfate concentrations versus probity value of percentage of mortality. After the acute toxicity test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each measured endpoint [13].

RESULTS

No fishes died during the acclimation period before exposure, and no control fishes died during acute toxicity tests.

The mortality of silver dollar fish increased significantly with increasing concentrations from 0.5ppm to higher concentrations for mercuric chloride and 40ppm to higher concentrations for lead chloride and 15ppm to higher concentrations for zinc sulfate (Table 1).

Table 1. Cumulative mortality of silver dollar fish (n=21 for each concentration) exposed to acute Mercuric Chloride ($HgCl_2$), Lead Chloride ($PdCl_2$), and Zinc Sulfate ($ZnSO_4$) based on time (24-96h).

Parameters	24h	48h	72h	96h
Mercuric Chloride Concentration (ppm)				
Control	0	0	0	0
0.02	0	0	0	0
0.50	0	0	0	0
1.00	2	7	10	13
2.00	5	12	18	21
Lead Chloride Concentration (ppm)				
Control	0	0	0	0
40	0	0	0	0
80	2	4	5	8
120	8	12	15	20
160	21	21	21	21
Zinc Sulfate Concentration (ppm)				
Control	0	0	0	0
15	0	0	0	0
30	2	3	4	7
60	5	10	17	21
90	21	21	21	21

Lethal concentrations (LC_{10-99}) of each variable were presented in Table 2. The LT_{50} (median lethal survival time) could be estimated for each concentration.

Acute toxicity testing statistical endpoints of mercuric chloride, lead chloride and zinc sulfate for silver dollar fish during different times after exposure was shown in Figure 1.

DISCUSSION

It is evident from the results that the tested metals concentration had direct effect on the LC_{50} values of the respective fishes. LC_{50} values indicated that mercury is more toxic than the other two metals. Obtained LC_{50}s in the present study correspond to values that have been published in the literature for other species of fishes. The differences in acute toxicity may be due to differences in water quality and test species [14]. The susceptibility of fish species to a particular heavy metal is a very important factor for LC_{50} levels. Fishes that are highly susceptible to the toxicity of one metal may be less or even not susceptible to the toxicity of another metal at the same level in the ecosystem. Conversely, a metal which is highly toxic to a fish species at low concentrations may be less or even non-toxic to other species at the same or even higher concentrations [15]. The fishes exposed to lead chloride and zinc sulfate can compensate for these pollutants. After full compensation 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 subsequent effects on fitness [14].

Because of the lack of available data on the effects of lead chloride and zinc sulfate on the respective LC_{50} values of the studied species, the results of the present study were not compared with those of other studies. However, some justifications have been provided by a number of studies. Many aquatic species show a vast range of LC_{50} for mercury chloride, which for saltwater fish can vary from 36μg/l (juvenile spot) to 1678μg/l (flounder) and for saltwater invertebrates from as low as 3.5μg/l (mysid shrimp) to 400μg/l (soft clam) [16, 17].

Table 2. Lethal concentrations (LC_{10-99}) of Mercuric Chloride ($HgCl_2$), Lead Chloride ($PdCl_2$), and Zinc Sulfate ($ZnSO_4$) based on time (24-96h) for silver dollar fish.

Parameters	24h	48h	72h	96h
Mercuric Chloride ($HgCl_2$)				
LC_{10}	1.37±0.54	0.75±0.37	0.60±0.39	0.73±0.41
LC_{20}	1.80±0.54	1.86±0.37	0.84±0.39	0.80±0.41
LC_{30}	2.10±0.54	1.32±0.37	1.00±0.39	0.85±0.41
LC_{40}	2.37±0.54	1.53±0.37	1.14±0.39	0.90±0.41
LC_{50}	**2.62±0.54**	**1.72±0.37**	**1.28±0.39**	**0.94±0.41**
LC_{60}	2.86±0.54	1.91±0.37	1.41±0.39	0.98±0.41
LC_{70}	3.13±0.54	2.11±0.37	1.55±0.39	1.02±0.41
LC_{80}	3.44±0.54	2.35±0.37	1.72±0.39	1.08±0.41
LC_{90}	3.87±0.54	2.68±0.37	1.95±0.39	1.15±0.41
Lead Chloride ($PdCl_2$)				
LC_{10}	89.06±0.98	74.71±0.78	69.10±0.78	62.69±1.04
LC_{20}	99.54±0.98	86.45±0.78	80.33±0.78	70.98±1.04
LC_{30}	107.11±0.98	94.92±0.78	88.42±0.78	76.96±1.04
LC_{40}	113.57±0.98	102.16±0.78	95.34±0.78	82.07±1.04
LC_{50}	**119.61±0.98**	**108.92±0.78**	**101.80±0.78**	**86.84±1.04**
LC_{60}	125.65±0.98	115.68±0.78	108.27±0.78	91.62±1.04
LC_{70}	132.11±0.98	122.92±0.78	115.18±0.78	96.72±1.04
LC_{80}	139.67±0.98	131.39±0.78	123.28±0.78	102.70±1.04
LC_{90}	150.16±0.98	143.13±0.78	134.50±0.78	111.00±1.04
Zinc Sulfate ($ZnSO_4$)				
LC_{10}	40.97±0.64	32.58±0.52	26.69±0.54	25.17±1.41
LC_{20}	48.76±0.64	40.67±0.52	33.24±0.54	27.59±1.41
LC_{30}	54.38±0.64	46.50±0.52	37.96±0.54	29.34±1.41
LC_{40}	59.18±0.64	51.48±0.52	42.00±0.54	30.84±1.41
LC_{50}	**63.66±0.64**	**56.14±0.52**	**45.77±0.54**	**32.24±1.41**
LC_{60}	68.15±0.64	60.80±0.52	49.54±0.54	33.63±1.41
LC_{70}	72.95±0.64	65.78±0.52	53.58±0.54	35.13±1.41
LC_{80}	78.57±0.64	71.61±0.52	58.30±0.54	36.88±1.41
LC_{90}	86.36±0.64	79.70±0.52	64.85±0.54	39.30±1.41

The 96h LC_{50} value for catfish exposed to Hg^{2+} under static test conditions has determined to be 570µg/l [18]. The 96h LC_{50} value of mercury chloride for chub is found to be 205µg/l and 96h LC_{50} for trout is 814µg/l [19]. For the estuarine fish (*Pomatoschistus microps*), LC_{50} of copper and mercury at 96h were 568µg/l and 62µg/l, respectively [16].

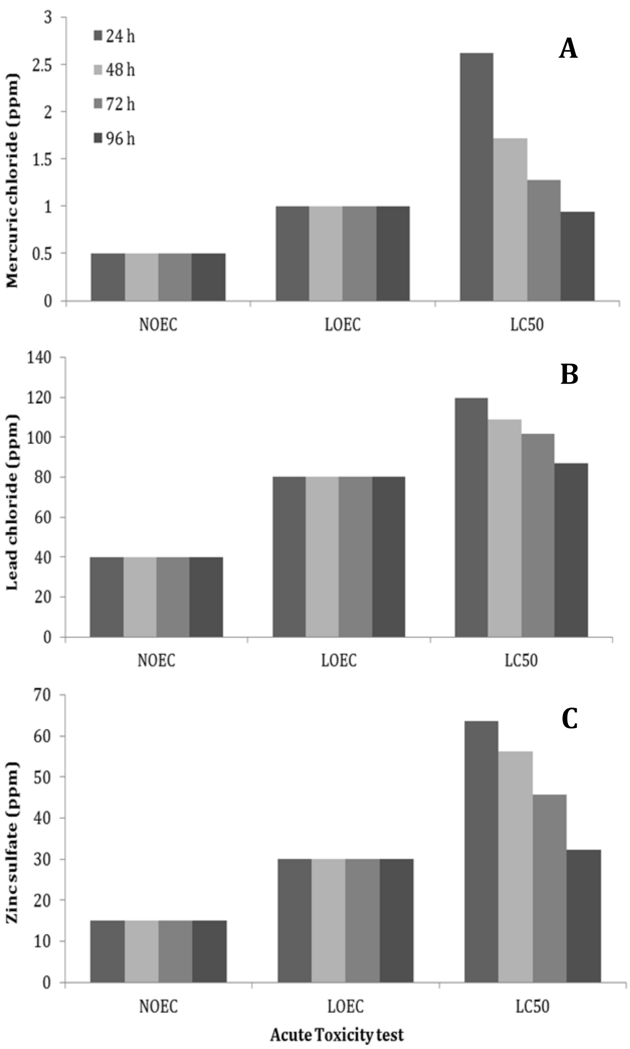

Figure 1. Acute toxicity testing statistical endpoints in silver dollar fish exposed to Mercuric Chloride (A); Lead Chloride (B); and Zinc Sulfate (C) in different times (24h, 48h, 72h and 96h).

Other studies show different results. For example, FAO/UNEP [20] has reported that the 96h LC_{50} values of mercury chloride is 350µg/l for catfish, 220µg/l for rainbow trout, 90µg/l for striped bass and 75µg/l for brook trout. Eisler & Gardener have reported the 96h LC_{50} values of mercury chloride as 37µg/l for fathead minnow, 160µg/l for bluegill, 903µg/l for sunfish and 200µg/l for rainbow trout [21]. In a study by Agarwal, the 96h LC_{50} values of mercury is 75µg/l for the catfish (*Sarothrodon mossambicus*), 33µg/l for rainbow trout (*Salmo gairdneri*), 110µg/l for the banded killifish (*Fundulus diaphanous*) and 90µg/l for the striped bass (*Roccus saxatilis*) [22]. Thus, it can be concluded from the present study that silver dollar fishes are not highly sensitive to $HgCl_2$ and, therefore, can be considered as suitable toxicological models. The LC_{50} value reported in the present study for $HgCl_2$ was lower than the values reported by Agarwal for the *Channa punctatus* (Bloch) at 48h (3.512µg/l), 72h (2.951µg/l), and 96h (3.113µg/l) but higher than those reported by Rathore & Khangarot for the *Channa marulius* (0.432µg/l for 72h and 0.314µg/l for 96h) [23].

Chronic toxicity values are much lower than acute values that highlights the cumulative effects of relatively low concentrations of mercury in water (i.e., <1µg/l). In aquatic toxicology, if LC_{50} concentration is less than 1000µg/l, the chemical is considered low toxic, and with LC_{50} concentrations of 1000-10000µg/l it is described as moderately toxic [24]. Therefore, we concluded that mercury chloride was highly toxic to silver dollar fish, while zinc sulfate and lead chloride had low toxicity. A safe level of mercury in aquaculture is only 1µg/l with LC_{50} range of 10-40µg/l, whereas LC_{50} values for other heavy metals are higher than mercury (cadmium 80-420, copper 20-100, zinc1000-10000, and lead 1000-40000µg/l) [10]. LC_{50} values vary for each species and the accumulation of heavy metals in the body of fish depends upon several factors. It is evident that concentrations of lead chloride and zinc sulfate and the physiological responses, affect the LC_{50} values of the fish. Silver dollar fish might have developed increased resistance to lead and zinc through acclimation. During acclimation, various proteins are released in the body of fish which may detoxify the metal ions. This may cause higher levels of heavy metals being required to cause effects, resulting in higher LC_{50} levels [25].

The selection of heavy metals may be an important tool for assessment of the effects of pollutants in aquatic ecosystems. The three metals used in our experiment demonstrated their potential for use in bioassays. By comparing the sensitivity of these metals to common reference toxicants, we conclude that silver dollar fish can be used as a suitable model for toxicity testing in ecotoxicological studies. Further studies should examine the effects of other contaminants as well as complex mixtures of pollutants, on this species to assess their suitability for detecting pollution, in order to develop aquatic ecosystem monitoring programs.

CONCLUSION

Mercuric chloride and lead chloride has the lowest and highest rate of mortality among these tree metals on silver dollar fish; however, the mortality rate was increased with increasing concentrations of toxins with time.

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