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Acute Toxicity Test of Mercuric Chloride ($Hgcl_2$), Lead Chloride ($Pbcl_2$) and Zinc Sulphate ($Znso_4$) in Common Carp (*Cyprinus carpio*)

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Abstract

The main objective of the present study was to investigate the sensitivity of freshwater fish *Cyprinus carpio* to some heavy metals in the toxicity test programs. Fish were exposed to the wide range of mercury chloride, lead chloride and zinc sulphate with expose of metal salts in water. The acute toxicity of mercury chloride (Hg), lead chloride (Pb) and zinc sulphate were evaluated by static bioassays and calculation of the LC_{50} (lethality concentration for 50%). The LC_{50} of mercury chloride was found to be higher than other species, while lead chloride had the lowest one. The 96h LC_{50} were 0.93 ± 0.71, 58.0 ± 0.28 and 41.1 ± 0.20 ppm for the Hg, Pb and Zn respectively.

Keywords: Freshwater fish; Heavy metal; LC_{50}; Toxicity test

Introduction

(Various) chemicals (derived) from agricultural procedures and industrial effluents, such as heavy metals, (may contaminate) a variety of different water environments. Since metals are not degradable and potentially toxic when bound to endogenous compounds after they enter the body [1], they can produce a range of hazard effects in aquatic organisms, ranging from alterations in a single cell to changes in whole populations [2].

The concentration of heavy metals in aquatic animals is related to several parameters, such as the food habits and foraging behavior of fish [3], tropic status, source of a particular metal, distance of the animal from the pollutant source and the presence of other ions in the ecosystem [4], temperature, transport of metal across the membrane and the metabolic rate of the animal [5] and the seasonal variation in the taxonomic composition of different tropic levels affecting the level and accumulation of heavy metal in the fish tissue [3].

The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the ecosystem make these harmful chemicals to the aquatic ecosystem and, consequently, to humans who depend on aquatic products as sources of food. Since heavy metals can accumulate in the tissues of aquatic organisms, these tissue concentrations of heavy metals can be of public health concern to both organisms and humans [6,7].

Lethal Concentration of 50% (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 are never beneficial to living beings [9], Thus, the aim of the present study was to investigate the acute effects of some heavy metals as potential dangerous additives by assessing the mortality effects of these pollutants on valuable freshwater fish, common carp (*Cyprinus carpio*).

Material and Methods

Water-only toxicity tests were carried out on cultured common carp (approximately 18 g & 12 cm) using three metal salts ($HgCl_2$, $PbCl_2$ and $ZnSo_4$). Criteria for selecting healthy, disease-free fish that were not previously exposed to any pollution agents or toxicants one was based on their activity and external appearance. Once selected, the fish were maintained alive on board in a fiberglass tank. Samples were transferred to a 400-L aerated tank equipped with aeration.

All samples were acclimated in a 15 fiberglass tank for 7 days at 25°C under a constant 12:12 light: Dark photoperiod the fish were fed with commercial formulated plate daily with a commercial feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality [10].

Mercury test concentrations were 0, 0.05, 0.2, 0.5 and 1 ppm of $HgCl_{2;}$ lead chloride test concentrations were 0, 3, 15, 60 and 1 ppm of $PbCl_2$; and Zinc tested concentrations were 0, 5, 10, 20, 40 and 60 ppm of $ZnSo_4$. Groups of 21 fish were exposed for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortalities were measured at time 0, 24, 48, 72 and 96 h [8].

Acute toxicity tests were carried out in order to calculate the 96h-LC_{50} for metals, based on Hotos and Vlahos [11]. Mortality was recorded after 24, 48, 72 and 96 h and LC_{50} values and its confidence levels (95%) were calculated. Percentages of fish mortality were calculated for each metal concentration at 24, 48, 72 and 96 h of exposure. LC_{50} values were calculated from the data obtained in acute toxicity bioassays, by Finney's [12] method of "probit analysis" and with SPSS computer statistical software. In Finney's method, the LC_{50} value is derived by fitting a regression equation arithmetically and graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis [12].

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Concentration (ppm)	No. of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.05	0	0	0	0
0.20	0	0	0	0
0.50	0	0	0	0
1.00	1	6	10	15

Table 1: Cumulative mortality of common carp during acute exposure to $HgCl_2$ (n=21, each concentration).

Concentration (ppm)	No. of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
3	0	0	0	0
15	0	0	0	0
60	0	1	6	13
120	3	10	19	21

Table 2: Cumulative mortality of common carp during acute exposure to $PbCl_2$ (n=21, each concentration).

Concentration (ppm)	No. of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
5	0	0	0	0
10	0	0	0	0
20	0	0	0	0
40	0	3	5	10
60	4	9	16	21

Table 3: Cumulative mortality of common carp during acute exposure to $ZnSo_4$ (n=21, each concentration).

The 95% confidence levels of the LC_{50} values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan [13]. Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. it is derived from the Normal Equivalent Deviate (NED) approach developed by Tort, et al. who proposed measuring the probability of responses (i.e., proportion dying) on a transformed scale based in terms of percentage of population or the standard deviations from the mean of the normal curve [7].

The $LC_{1,10,30,50,70,90,99}$ values were derived using simple substitution probit of 1, 10, 30, 50, 70, 90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. metals. The 95% confidence limits for LC_{50} were estimated by using the formula LC_{50} (95% CL) = LC_{50} ± 1.96 [SE (LC_{50})]. The standard error SE of LC_{50} is calculated from the formula:

$$SE(LC_{50}) = 1 / b\sqrt{pnw}$$

Where: b=the slope of metals/probit response (regression) line; p=the number of metals used, n=the number of animals in each group, w=the average weight of the observations [11]. At the end of acute test, the Lowes Observed Effect Concentrations LOEC and No Observed Effect Concentration NOEC were determined for each endpoint measured. In addition, the Maximum Acceptable Toxicant Concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC [8].

Results

All controls resulted in low mortalities (less than 5%), which indicated the acceptability of the experiments. The mortality of common carp for mercury chloride, lead chloride and zinc chloride during the exposure times at 24, 48, 72 and 96 h respectively are indicated in Tables 1-3.

Exposure of fish during the period 24-96 h resulted in significantly increased number of dead fish l with increasing concentration. There were significant differences in number of dead fish between the duration 24-96.

Considering the mercury bioassay, the lowest concentration causing 100% of fish mortality was 1 mg/l at 96 h, while the highest concentration causing no fish mortality was 0.5 mg/l at 96 h. There was 100% mortality at concentrations as 120 ppm for lead and 60 ppm for zinc within the 96 h after dosing, and no mortality at 15 and 20 ppm within the exposure times for the plumb and zinc respectively.

Median lethal concentrations for 1%, 10%, 30%, 50%, 70%, 90% and 99% tests are shown in Tables 4-6.

For the 96 h experiment, Hg was particularly (toxic?) with LC_{50} values of 0.93 mg/l. Because mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure times (24, 48, 72, or 96 hrs). The straight line of best fit is then drawn through the points. These were time–mortality lines. As there can found LC_{50} of plumb was higher than other metals, however mercury had the lowest one.

Point	Concentration (ppm) (95 % of confidence limits)			
	24 h	48 h	72 h	96 h
LC_1	-	0.73 ± 0.83	0.62 ± 0.84	0.64 ± 0.71
LC_{10}	-	0.89 ± 0.83	0.80 ± 0.84	0.77 ± 0.71
LC_{30}	-	1.00 ± 0.83	0.93 ± 0.84	0.86 ± 0.71
LC_{50}	-	**1.08** ± 0.83	**1.03** ± 0.84	**0.93** ± 0.71
LC_{70}	-	1.16 ± 0.83	1.12 ± 0.84	0.99 ± 0.71
LC_{90}	-	1.27 ± 0.83	1.25 ± 0.84	1.08 ± 0.71
LC_{99}	-	1.43 ± 0.83	1.44 ± 0.84	1.21 ± 0.71

Table 4: Lethal Concentrations (LC_{1-99}) of $HgCl_2$ (mean ± Standard Error) depending on time (24-96h) for common carp.

Point	Concentration (ppm) (95% of confidence limits)			
	24 h	48 h	72 h	96 h
LC_1	93.04 ± 0.21	82.11 ± 0.13	11.7 ± 0.19	32.1 ± 0.28
LC_{10}	115.4 ± 0.21	100.5 ± 0.13	43.9 ± 0.19	43.7 ± 0.28
LC_{30}	113.6 ± 0.21	113.9 ± 0.13	67.2 ± 0.19	52.1 ± 0.28
LC_{50}	**142.8 ± 0.21**	**123.1 ± 0.13**	**83.3 ± 0.19**	**58.0 ± 0.28**
LC_{70}	154.1 ± 0.21	132.4 ± 0.13	99.5 ± 0.19	63.8 ± 0.28
LC_{90}	170.3 ± 0.21	145.7± 0.13	122. ± 0.19	72.2 ± 0.28
LC_{99}	192.7 ± 0.21	164.2 ± 0.13	155. ± 0.19	83.8 ± 0.28

Table 5: Lethal Concentrations (LC_{1-99}) of $PbCl_2$ (mean ± Standard Error) depending on time (24-96h) for common carp.

Point	Concentration (ppm) (95 % of confidence limits)			
	24 h	48 h	72 h	96 h
LC_1	48.9 ± 0.26	20.8 ± 0.02	17.7 ± 0.02	27.6 ± 0.20
LC_{10}	58.1 ± 0.26	39.3 ± 0.02	32.4 ± 0.02	33.7 ± 0.20
LC_{30}	64.7 ± 0.26	52.8 ± 0.02	43.2 ± 0.02	38.1 ± 0.20
LC_{50}	**69.3 ± 0.26**	**62.1 ± 0.02**	**50.6 ± 0.02**	**41.1** ± 0.20
LC_{70}	73.9 ± 0.26	77.0 ± 0.02	58.0 ± 0.02	44.1 ± 0.20
LC_{90}	80.6 ± 0.26	84.8 ± 0.02	68.7 ± 0.02	48.5 ± 0.20
LC_{99}	89.8 ± 0.26	103.4 ± 0.02	83.5 ± 0.02	54.6 ± 0.20

Table 6: Lethal Concentrations (LC_{1-99}) of ZnSo4 (mean ± Standard Error) depending on time (24-96h) for Common carp.

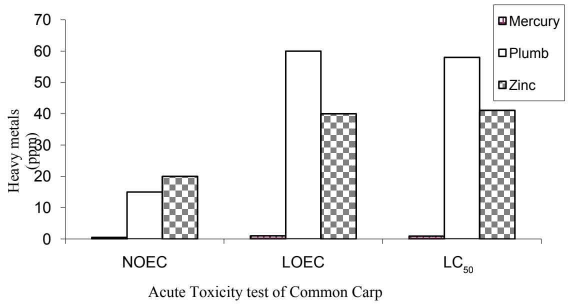

Figure 1: Acute toxicity testing statistical endpoints of mercury, plumb and zinc in common carp.

Toxicity Testing Statistical Endpoints are shown in Figure 1. LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values were the same for all studied metals. However LC_{50} (the median Lethal Concentration) values between metal showed significant differences. The Maximum Acceptable Toxicant Concentration (MATC) for mercury, plumb and zinc were 0.09, 5.8 and 4.1 ppm respectively.

Discussion

It is evident from the results that the heavy metal concentration has a direct effect on the LC_{50} values of the respective fish. LC_{50} values indicated that mercury is more toxic to than other (metals). LC_{50}s obtained in the present study correspond to values that have been published in the literature for other species of fish.

The differences in acute toxicity may be due to changes 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. Fish 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 of that metal 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 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 [14].

Because of the lack of available data on the effects of plumb and zinc on the respective LC_{50} values of all studied species, the results of the present study have not been compared with those of other studies and discussed accordingly. However, some justifications have been provided following various studies. Many aquatic species show a vast range of LC_{50} for mercury chloride, which for saltwater fish can vary from as high as 36 µg/l (juvenile spot) to 1678 µg/l (flounder), to as low as 3.5 µg/l (mysid shrimp) to 400 µg/l (soft clam) for saltwater invertebrates [16,17]. The 96 h LC_{50} value for catfish exposed to Hg^{2+} under static test was determined to be 570 µg/l [18]. The 96 h LC_{50} value of mercury chloride for chub was found to be 205 µg/l and 96 h LC_{50} for trout 814 µg/l [19]. For the estuarine fish *Pomatoschistus microps,* LC_{50} of copper and mercury at 96 h were 568 µg/l and 62 µg/l, respectively [16].

Other studies show different results. For example, FAO/UNEP [20] find that the 96 h LC_{50} values of mercury chloride for cat fish are 350 µg/l, rainbow trout 220 µg/l, striped bass 90 µg/l and brook trout 75 µg/l. The 96-h LC_{50} values of mercury chloride 37 µg/l for fathead minnow, 160 µg/l for bluegill sunfish, 903 µg/l for rainbow trout, 200 µg/l for rainbow trout [21]. For mercury, 96 h LC_{50} values for the catfish (*Sarothrodon mossambicus*) are 75 µg/l), 33 µg/l for the rainbow trout (*Salmo gairdneri*), 110 µg/l for the banded killifish (*Fundulus diaphanous*) and 90 µg/l for the striped bass (*Roccus saxatilis*) [22]. Thus, it can be concluded from the present study that common carp are not highly sensitive to $HgCl_2$ and, therefore, can be considered as a suitable toxicological model.

The LC_{50} values reported in the present study for $HgCl_2$ were lower than the values reported by [22] for the *Channa punctatus* (Bloch) at 48, 72, and 96 h and reported LC_{50} values of 2.512, 2.291, and 2.113 mg/L, respectively, at 48, 72, and 96 h. however, the present values were higher than those of 0.432 and 0.314 mg/L, respectively, at 72 and 96h in *Channa marulius* [23].

Chronic toxicity values are much lower than acute values and highlight the adverse effects of relatively low concentrations of mercury in water (i.e., <1 µg/L). In aquatic toxicology, if LC_{50} concentration is smaller than 1000 µg/l, the chemical is highly toxic, and considered moderately toxic between 1000-10000 µg/l [24] (Louis et al., 1996). Therefore we conclude that mercury chloride is highly toxic to common carp, which zinc and plumb are not toxic.

A safe level of mercury in aquaculture is only 1 µg/l with LC_{50} range of 10-40 µg/l of, whereas LC_{50} values for other heavy metals are higher than mercury (cadmium 80-420, cooper 20-100, zinc1000-10000, lead 1000-40000 µg/l) [10]. Thus, in the present study, LC_{50} values indicated that mercury is more toxic to *C. carpio* and may be very harmful to this fish. However, the study showed the 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 plumb and zinc and physiological response affect the LC_{50} values of the fish. It may be due to the increased resistance of carp to plumb and zinc through acclimation. During acclimatiion, 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} amounts [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 demonstrate their potential for use in bioassays. By comparing the sensitivity of these metals to common reference toxicants, we conclude that common carp can be used as a suitable model for toxicity determinations in ecotoxicological studies. Further studies should examine other contaminants of this species to assess their suitability for detecting toxicity, as well as complex mixtures of pollutants, in order to develop aquatic ecosystem monitoring programs.

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