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Comparison of Lethal Concentrations (LC_{50}-96 H) of $Cdcl_2$, $Crcl_3$, and Pb $(NO_3)_2$ in Common Carp (*Cyprinus carpio*) and Sutchi Catfish (*Pangasius Hypophthalmus*)

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ABSTRACT

Background: The present study compared lethal concentrations (LC50-96 h) of $CdCl_2$, $CrCl_3$, and Pb $(NO_3)_2$ between two scaled and scaleless freshwater fish species: *Cyprinus carpio* (Cyprinidae) and *Pangasius hypophthalmus* (Pangasiidae).

Methods: The experimental fishes were obtained from fish markets/ponds with average lengths and weights of approximately 11.68 ±1.92 and 9.8±1.9 cm, and 25.92±6.3 and 18.61±3.22 g for *C. carpio* and *P. hypophthalmus*, respectively. The fishes were exposed to different concentrations of cadmium (Cd) (0, 10, 20, 40, 80, 100, 120, 200 mg L^{-1}) lead (Pb) (0, 20, 40, 50, 60, 90, 150 mg L^{-1}), and chromium (Cr) (0, 5, 10, 20, 30, 40 mg L^{-1}) for 96 h. Physicochemical parameters, such as dissolved oxygen, pH, and temperature of aquaria as well as mortality rate of the fishes, were monitored daily.

Results: The 50% lethal concentrations (LC_{50}-96 h) of $CdCl_2$, $CrCl_3$, and Pb $(NO_3)_2$ for *P. hypophthalmus* were found at 64.89, 7.46, and 48.06 mg L^{-1}, and those of $CdCl_2$, $CrCl_3$, Pb $(NO_3)_2$ for *C. carpio* were detected at 84.8, 17.05, and 77.33 mg L^{-1}. The ratios of heavy metal toxicity factors (TF) were greater for common carp compared to those for the catfish.

Conclusion: Our preliminary findings suggest that common carp *C. carpio* with higher LC_{50} (and LC_{100}) values appears to be more tolerant to heavy metals exposure than the catfish (*P. hypophthalmus*). This may be due to the resistance to the heavy metals through protection from the carp's scaled body versus scaleless body of the catfish.

Keywords: Cadmium, Chromium, Lead, Fish Scales, 50% Lethality.

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INTRODUCTION

Heavy metals pollution of the aquatic environment has been marked as a serious health concern, which its introduction into these ecosystems happens through various routes such as industrial effluents and wastes, agricultural pesticide run-off, domestic garbage dumps, and mining activities (1). A potential threat for aquatic organisms is contamination arisen from being exposed to significant amounts of heavy metals, which at high concentrations can cause harmful effects on metabolic, physiological, and biochemical systems of fishes (2-5) together with long-term eco-toxicological effects (6). Thus, these metals are a matter of environmental and health concern because of their toxic

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potential to be accumulated in food chain (7).

Toxicity testing has been widely used as a tool to identify suitable organisms as a bio-indicator and to derive water quality standards for chemicals. It is also considered an essential tool for assessing the effects and fate of toxicants in aquatic ecosystems (8). Because toxicity studies quantify an organism's response to a biologically active material (9) and are useful in determining water quality, it is, therefore, crucial to restore and resolve metal pollution through environmental monitoring. Fish absorb dissolved or available metals and can, therefore, serve as a reliable indication of metal pollution in an aquatic ecosystem (10).

Common carp (*Cyprinus carpio*) accounts for an important farmed species. Hence the optimum culture conditions and caveat on severe aftermath of environmental pollutants including such heavy metals as Cd, Cr, and Pb can effectively help improve cultivation of this valuable species. The scaleless fish, *Pangasius hypophthalmus* (sutchi or striped catfish), is native to the Mekong River drainage, which emerged as a major species for aquaculture purposes research focus, outside of tropical regions of South East Asia, and can be successfully cultured in the western tropics. Briefly, both species are of aquaculture interest, which have achieved commodity status on world seafood markets. Furthermore, development of this catfish culture industry has faced difficulties partly related to the limited knowledge of biology, ecology, and physiology reported in some cultivated stocks (11,12).

Both lethal and sub-lethal concentrations of heavy metals determine the sensitivity of individual organisms across species. Such changes differ from metal to metal, from species to species, and from one experimental condition to another. The exact causes of death due to metal poisoning are multiple and depend on time-concentration (13). The susceptibility of fish to a particular heavy metal is also a very important factor for LC_{50} values (14). Conducting 96-h LC_{50} tests, therefore, makes it possible to measure the susceptibility and survival potential of organisms to particular toxic heavy metals. Because the 96-h LC_{50} values of fish vary from species to species and from metal to metal (15-17), it would be appealing to scrutinize variations in metal accumulation and toxicity in catfish with that in a species like carp from similar environment for various purposes (18). Therefore, the purpose of this study was to determine and compare the LC_{50} of $CdCl_2$, $CrCl_3$, and $Pb(NO_3)_2$ in two scaled *C. carpio* and scaleless *P. hypophthalmus* fish species, and to compare the level of sensitivity in each species from exposure to different Cd, Cr, and Pb concentrations.

MATERIALS AND METHODS

P.hypophthalmus was purchased from local aquarium shops and *C. carpio* was obtained from Nasr Fish Culture pond (Sari, Iran) with approximate total length of 8.0-14.0 and 7.0-12 cm, respectively. The fishes were transferred to aquarium room located at SANRU. Prior to toxicity testing, the fishes were acclimatized for one week under laboratory conditions (25 ± 1^{o} C with 12h light: 12h dark). Water quality parameters (TDS = 600 mg L^{-1}, pH = 6.75, EC = 1 ds/m, DO = 5-8 mg L^{-1}) were measured during the experiment. The experimental aquaria were aerated through air stones. Cd, Cr, and Pb heavy metals in the forms of cadmium chloride ($CdCl_2.H_2O$, BDH), chromium chloride ($CrCl_3.6\ H_2O$, APLICAM), and lead (II) nitrate ($Pb\ (NO_3)_2$, MERK) were used as toxicants.

Acute cadmium, lead, and chromium toxicity experiments were performed using different concentrations of Cd (0, 10, 20, 40, 80, 100, 120, and 200 mg L^{-1}), Pb (0, 20, 40, 50, 60, 90, 150 mg L^{-1}), and Cr (0, 5, 10, 20, 30, 40 mg L^{-1}) during 96 h. Metal solutions were prepared by diluting of a stock solution with well water. Each concentration contained six fish with one replicate each. There was a simultaneous control group (n= 10; with no heavy metal additions) together with the heavy metal treatments, keeping all other conditions alike. The concentration of each heavy metal caused 50% mortality in fish for 96 h was taken as the LC$_{50}$ value, calculated by Finney's Probit Analysis (in SPSS, V. 16). During the toxicity test, the fishes were not fed. The numbers of dead fish were counted daily and removed immediately from the aquaria. The degree of fish susceptibility to each heavy metal was determined as toxicity factor (TF). This factor is calculated by the ratio of LC$_{50}$ of a metal in one fish species to that in the other, i.e.: LC$_{50}$ *C. carpio*/ LC$_{50}$ *P. hypophthalmus*, at different intervals tested. Then the LC$_{50}$ of the two species was statistically compared through linear regression and evaluated by one-sample t-test (e.g. 19). To do this, first, the TF ratios were obtained by estimating LC$_{50}$ of each examined metal for each species at different times. Next, one-sample t-test was employed using SPSS software.

RESULTS

The 50% lethal concentrations (LC$_{50}$-96h) of $CdCl_2$, $CrCl_3$, Pb $(NO_3)_2$ for *Pangasius hypophthalmus* were 64.89, 7.46, and 48.06 mg L^{-1}, and those for *Cyprinus carpio* were 84.8, 17.05, and 77.33 mg L^{-1}, respectively.

Table 1 (A and B) shows lethal concentrations (LC$_{50}$- 96 h) of Cd, Cr, and Pb in common carp and sutchi (striped) catfish, respectively, at four durations. It is evident that the required concentrations of all the examined heavy metals to reach lethal doses are far greater in common carp compared with those required in the catfish.

Time-response mortality of the two fish species resulting from different concentrations of individual heavy metals (Cd, Cr, and Pb) are presented in Tables 2 to 4. The Cd-response of the sutchi catfish (*P. hypophthalmus*) began at 40 mg L^{-1} (48 and 72 h), which displayed higher mortalities with rising levels of cadmium especially at 24 and 48 h. The common carp (*C. carpio*) initially responded to a Cd concentration of 80 mg L^{-1} (96 h), which exhibited rather elevated mortalities with increasing levels of cadmium, especially at 120 and 200 mg L^{-1}. Both species were equal in zero mortality in the control groups.

Table 2 also indicates that LC$_{100}$ values for the common carp (*C. carpio*) and sutchi catfish (*P. hypophthalmus*) were at 120 and 100 mg L^{-1} of Cd (after 48 h), respectively.

Table1. Lethal concentrations (LC$_{50}$-96 h, mg L^{-1}) of Cd, Cr, and Pb for common carp (*C. carpio*) (A) and sutchi catfish (*P. hypophthalmus*) (B)

A)

Duration (h)	Cd	Cr	Pb
24	287.47	67.84	480.64
48	137.45	33.67	228.7
72	131.38	21.24	96.19
96	84.8	17.05	77.32

B)

Duration (h)	Cd	Cr	Pb
24	128.24	27.43	156.4
48	83.63	17.57	86.57
72	69.74	9.8	62.22
96	64.89	7.46	48.06

Table 2. Mortalities of *P. hypophthalmus* and *C. carpio* recorded at different concentrations (mg L^{-1}) of Cd during 96 h

Cd concentration (mg L^{-1}) **Duration (h)**	**Control**	**10**	**20**	**40**	**80**	**100**	**120**	**200**
***P. hypophthalmus* (sutchi catfish)**								
24	0	0	0	0	2	4	4	3
48	0	0	0	1	1	1	2	2
72	0	0	0	1	0	1	0	1
96	0	0	0	0	0	0	0	0
***C. carpio* (common carp)**								
24	0	0	0	0	0	2	3	0
48	0	0	0	0	0	1	3	3
72	0	0	0	0	0	1	0	0
96	0	0	0	0	3	0	0	3

Table 3. Mortalities of *P. hypophthalmus* and *C. carpio* recorded at different concentrations (mg L^{-1}) of Cr during 96 h

Cr concentration (mg L^{-1}) **Duration (h)**	**Control**	**5**	**10**	**20**	**30**	**40**
***P. hypophthalmus* (sutchi catfish)**						
24	0	2	1	3	0	0
48	0	1	3	1	0	1
72	0	0	2	2	3	3
96	0	0	0	0	2	0
***C. carpio* (common carp)**						
24	0	0	1	1	3	0
48	0	0	0	1	3	1
72	0	0	0	1	1	4
96	0	0	1	0	0	1

Table 4. Mortalities of *P. hypophthalmus* and *C. carpio* recorded at different concentrations (mg L^{-1}) of Pb during 96 h

Pb concentration (mg L^{-1}) **Duration (h)**	**Control**	**20**	**40**	**50**	**60**	**90**	**150**
***P. hypophthalmus* (sutchi catfish)**							
24	0	0	0	1	1	0	3
48	0	0	2	0	2	3	2
72	0	0	0	0	2	1	1
96	0	0	0	2	0	2	0
***C. carpio* (common carp)**							
24	0	0	0	0	1	1	0
48	0	0	0	0	2	0	1
72	0	0	0	0	0	2	4
96	0	0	0	1	0	2	0

The mortality of *P. hypophthalmus* initiated at a Cr concentration of 5 mg L^{-1}, which showed greater fish death as the level of chromium was raised. In the common carp, on the other hand, a Cr density of 10 mg L^{-1} started fish demise and higher Cr levels caused elevated mortalities. No fish death was noticed in the control groups for both species. Table 3 indicates that LC_{100} values for the carp and catfish were at 30 and 10 mg L^{-1} of Cr (after 72 h), respectively.

Concerning lead, the initial response of *P. hypophthalmus* was recorded at 40 mg L^{-1} of Pb (48 h; Table 4). Further concentrations of Pb produced more mortality in the fish. Comparably, *C. carpio* showed primary death at 50 mg L^{-1} (96 h) of Pb and continued dying with additional levels of Pb. Table 4 also indicates that the carp and catfish showed equal LC_{100} values at 90 mg L^{-1} of Pb (after 96 h).

TOXICITY FACTOR (TF)

The tested heavy metals were significantly different in TF ratios at different times ($P< 0.05$, Table 5) indicating that *C. carpio* required higher levels of Cd, Pb, and Cr than *P. hypophthalmus* to reach LC_{50} values. The descending trends of Cd, Pb, and Cr LC_{50}s at different intervals were not significant in *C. carpio* ($P>0.05$). In *P. hypophthalmus*, Cd and Pb LC_{50}s did not significantly reduce with time ($P>0.05$) while the decrease in Cr LC_{50} from 24 to 96 h was significant ($P<0.05$).

Table 5. Toxicity factors (TFs) in *C. carpio* and *P. hypophthalmus* subjected to the heavy metals at different times

Duration (h)	Cd	Cr	Pb
24	2.31	2.47	3.07
48	1.64	1.91	2.64
72	1.88	2.16	1.54
96	1.3	2.28	1.6

DISCUSSION

This study was done to assess the sensitivity of the scaled *Cyprinus carpio* and the scaleless *Pangasius hypophthalmus* fish species to cadmium, chromium, and lead through determination of acute 96-h LC_{50} values induced from exposure to different concentrations of the introduced heavy metals. As shown in Table 1 (A and B), *C. carpio* and *P. hypophthalmus* displayed contrasting tolerances to the applied densities of Cd, Cr, and Pb. The data clearly indicate that considerably more densities of the heavy metals are needed to induce dose-response mortality in the common carp than those required in the catfish. This implies that *C. carpio* should be more tolerant of the toxicant burden in comparison with *P. hypophthalmus*. This characteristic, in addition to species-specificity of responses (e.g.15), might have arisen from the fact that because a function of fish skin is to provide an effective protective barrier against environmental chemicals, and as common carp (and scaled fish in general: 20) have thicker epidermis covered by scales, they can effectively shield the animal against extra permeation of toxicants. Also, it is generally accepted that factors such as skin thickness and scale coverage are the determinants in the percutaneous (via skin) uptake rate of toxicants in fish (21). In brown bullhead (*Ameiurus nebulosus*), an important percentage of mercury uptake from the water occurred because of its scaleless permeable skin (22). Further evidence presented suggests protection against heavy metals toxicity by scales in three freshwater species making them tolerant to lethal concentrations of lead or mercury (23). The scales buffered the pH of lead nitrate solution and removed lead (and mercury) from water. The same authors also studied (24) the reduction of lead nitrate toxicity in water due to the presence of scales. They additionally reported reduced toxicity of 27 metals following filtration through scales and concluded that the keratin in scales may be the most important ectodermal secretion in absorbing metals and in providing protection against their toxic levels. Likewise, the sorption and removal of heavy metals by fish scales was investigated (25, 26, 27); metals accumulation in fish scales is increased during exposure (28). On the other hand, a general conclusion (reviewed by 21) deduced that scaled and scaleless fish showed the same general pattern of response (deterioration and death) to sub-lethal chronic metal concentrations. Similarly, there was not a significant difference between field samples of *Cyprinus carpio* and *Clarias lazera* (scaleless) in Cd concentrations of their comparable tissues (29). The above discrepancies might have occurred because toxicity and accumulation of heavy metals in fish are mainly dependent upon metals concentration and exposure period, although such other factors as water salinity, pH, hardness and temperature, ecological needs, size and age, life cycle, capture season, and feeding habits of fish also play significant roles (4).

Comparison of the numbers of dead fishes at different levels of the heavy metals indicate that lethality of the metals lies in order of Cr > Pb > Cd. Accordingly, chromium was more toxic than cadmium and lead to both fishes and *P. hypophthalmus* showed more sensitivity than *C. carpio* to all the metals. This corroborates the review of (14) stating that the acute toxicity of chromium to fish reveals the differences in the 96h-LC_{50} values between fish species, which can be attributed to the complicated metal-induced changes in the physiology and survival of aquatic organisms under metallic stress.

Lead has been reported as the most toxic element to aquatic invertebrates, algae and fish at the lowest concentration (30, 31), the toxicity of which is strongly influenced by lower water hardness (32). Moreover, the concentration of heavy metals in fish is related to several factors such as physicochemical properties of the water (e.g. water hardness: 33) and the presence of other ions in the environment (34, 35). The high total water hardness (TDS: ca. 600 mg L^{-1}) in this study, therefore, could have probably reduced Pb toxicity rendering it an intermediate lethality. Similar order of toxicities and bioaccumulations (Cr > Pb > Cd) have been reported in two freshwater and five coastal fish species (36, 37).

Cadmium belongs to the most toxic water contaminants. Lethal values (96 h LC_{50}) for fish range from 0.5 μg dm^{-3} (38) to 21.1 mg dm^{-3} (39) depending both on intrinsic factors such as fish species or age, and on environmental conditions (40). Exposure of *C. carpio* to 0.560 mg L^{-1} cadmium [Cd $(NO_3)_2$] killed all fish in 8 days (41) whereas 1.5 – 6 mg L^{-1} of lead ($PbNO_4$) for two months caused no mortality in this species (42). In catfish *Heteropneustes fossilis*, LC_{50} of cadmium chloride was found to be 50.41 mg L^{-1} (4), which tends to proximate that (>60 mg L^{-1}) detected in this study. Reported LC_{100} of Cd as 86.32 mg L^{-1} (43) is almost near that (100 mg L^{-1}) found in this study (43).

The ratios of heavy metal toxicity factor (TF) calculated in here were greater for common carp compared to those for the catfish. Conversely, relatively higher copper TF ratio was found for the catfish *Clarias gariepinus* (TF= 1.19) as opposed to that estimated in tilapia *Oreochromis niloticus* (TF= 1.0) (20). This disparity might be due to the physicochemical characteristics of the test media (39, 44), species and ages, and their sensitivity rates to the tested heavy metals.

CONCLUSION

The development and use of toxicity tests provide types of data regarding toxic responses of different fish species, which could be more effectively used in predictive toxicology and risk assessment. The common carp (*C. carpio*) with higher

LC_{50} (and LC_{100}) values appears to be more tolerant to heavy metals exposure than sutchi catfish (*P. hypophthalmus*). This attribute may be due to the increased resistance to the heavy metals through protection from the carp's scaled body versus scaleless body of the catfish. However, further studies are recommended to confirm it because various factors influence toxicant responses in natural and controlled ecosystems.

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