Risk assessment of lead and cadmium on Juveniles of *Cyprinus carpio* in laboratory scale

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Abstract:

This paper assesses the risk of lead and cadmium heavy metals on *Cyprinus carpio* in laboratory conditions. The research determined the 96 hours LC50 value of lead nitrate and cadmium nitrate in the fish *Cyprinus carpio*. This study combined the ecological toxicology test with the ecological hazard description to determine the acute toxicity effects of lead and cadmium on *Cyprinus carpio*. Ecological toxicology took place in 96 hours with test concentration of 0.25; 0.5; 1.0; 1.5; 2.0 mg/l on lead and 0.5; 1.0; 1.5; 2.0; 3.0 mg/l on cadmium. Through probit analysis, the LC50 after 96 hours of lead and cadmium in *Cyprinus carpio* was found to be 0.987 mg/l and 1.171 mg/l, respectively. Through monitoring the biological behaviour of *Cyprinus carpio* when exposed to lead and cadmium, it was observed that the number of deaths is proportional to the concentration of chemical exposure time. The abnormal morphology and behaviour of the fish also increased with testing time and lead and cadmium concentration. *Cyprinus carpio* also absorbs lead and cadmium in its body; the cumulative content is similar as above.

Keywords: ***acute toxicity, cadmium nitrate, Cyprinus carpiro, lead nitrate, 96 hours LC50.***

Classification number: ***6.2***

Introduction

Toxic chemicals released into the environment, either from point sources such as industrial and municipal discharges or from non-point sources such as agricultural runoff and atmospheric deposition, are capable of contaminating surface waters and sediments [1]. Heavy metals are a group of toxic chemicals persistent in the environment, which are bio-accumulative and non-biodegradable in the food chain [2]. Heavy metals also disrupt and result in the contamination of ecosystems; they can be both carcinogenic and non-carcinogenic for human health. Heavy metals in the human body do not degrade, which accounts for their chronic toxicities. Air contaminated by heavy metals may pollute soil and water, resulting in contaminated crops and consumables. Erosion of natural deposits of rock minerals and atmospheric deposition of gaseous emissions from tailpipes of industrial engine allow the mobility of heavy metals into the aquatic environment. Heavy metals persist in the aquatic environment and, based on their available concentrations, bioaccumulate in the tissues of aquatic plants and animals.

Examples of heavy metals that have been released into the environment include cadmium (Cd), lead (Pb), nickel (Ni), arsenic (As), mercury (Hg) and chromium (Cr) among others; they are probable carcinogens in humans. Lead reduces and increases, while cadmium accumulates [3]. Lead and cadmium can cause damage to the nervous, cardiovascular, and human skeletal systems.

Fishes are organisms that survive mainly in water bodies. Fish is food to humans, as it remains a relatively cheap source of protein. The nutritional composition of fish encompasses both macro and trace nutrients beneficial to the human biological system. The major nutritional constituents of fish are water, proteins, lipids, minerals and vitamin B2 [4]. However, aquatic ecosystems polluted with cadmium and lead threatens the suitability of fish as an important food source for humans. Fish being the final chain in the aquatic food web is able to bioaccumulate heavy metals in the aquatic environment. The accumulated metals in fishes are transferable to humans through the food chain. Fish safety, just as food safety, is an important public health issue because humans can develop numerous diseases from the consumption of contaminated fish [5].

There have been many studies on the effects of toxins on the growth, development and reproduction of fish species [6]. According to EPA's Ecotoxicological Testing Guidelines, fishes are considered to be highly susceptible and can be easily observed during the test, so it is chosen as the ecotoxicological test organism [1]. LC50 is used to

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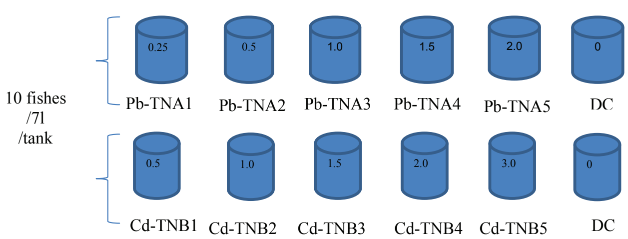
evaluate the effects of toxins on the test organisms through the lethal levels [6, 7].

Waste water from many industrial production activities in Vietnam, especially waste water from the recycling of metals, contains lead and cadmium. When theses concentration exceeds the allowed standard, they affect humans through biological amplification in the food chain. *Cyprinus carpio* is a common food source in Vietnam so, if it is living in a polluted environment, it poses a high risk to health.

Methods

Juveniles of *Cyprinus carpio* with a mean body weight 8-10 g and standard length of 8-9 cm were used for the study. They were collected from the fish research laboratory of the Research Institute for Aquaculture No 1, Vietnam. The fishes were acclimatised in the laboratory for 5 days in plastic tanks of 20 litres capacity before the experiment. During the acclimatisation, the fishes were fed daily with regular feed stock, i.e., Durhante fish pellet, which was equivalent to 5% mean body weight of a fish. Natural groundwater was used to feed the fishes. Water samples were taken at three different sampling times and analysed for cadmium and lead. The acceptable feeding water must be free of heavy metals. Commercial lead nitrate and cadmium nitrate were used as Cd and Pb sources, respectively. Only individuals who are still healthy will be used for ecological toxicology.

Each experiment was conducted with 5 different concentrations of lead and cadmium and a control sample. After conducting two exploratory experiments, the experimental concentration range was determined as follows:



The concentrations of Pb and Cd in the fishes were determined after 6h, 12h, 24h… 96h of the experiment. The meat of the fishes was homogenised and added with nitric acid-peroxide. Then, the samples were digested using a microwave digester (MWS-2). The completely digested samples were allowed to cool to room temperature; then, they were filtered (glass wool) and made up to 50 ml. All the digested samples were analysed using an atomic absorption spectrophotometer (Thermo Scientific) and an air-acetylene flame.

Homogenised samples were spiked with three different concentrations of heavy metals for determination recovery. Each running in triplicate and blanks were carried through the whole procedure described above. The recovery of result analysis ranged from 70.7% to 118.5%.

Ecotoxicity testing was performed by using the APHA method and EPA guidelines. During the experiment, oxygen was continually pumped to maintain the required DO level for the fish, and the temperature and pH parameters were measured daily.

All research results were evaluated and analysed in Excel. The LC50 of lead and cadmium in *Cyprinus carpio* was determined through probit analysis [8].

Results and discussion

Physical-chemical characteristics of water samples

The physical-chemical characteristics of sample waters were analysed. Its main parameters were investigated, including temperature (28-29°C), pH (7.1-7.6), dissolved oxygen (6.7-8.4 mg/l), hardness (43.1-52.5 mg $CaCO_3$/l) and total alkalinity (112-120 mg $CaCO_3$/l). Cadmium and lead were not detected in the samples. The results were compared with the Food and Agriculture Organisation's (FAO) [9a, 9b] guidelines for fish pond water quality, as shown in Table 1.

Table 1. Physical-chemical characteristics of water samples.

Parameter	Range	FAO guideline
Temperature (°C)	28-29	25-30
pH	7.1-7.6	6.5-8.5
Dissolved Oxygen (mg/l)	6.7-8.4	>3
Hardness (mg $CaCO_3$/l)	43.1-52.5	>25*
Total alkalinity (mg $CaCO_3$/l)	112-120	>25*
Cadmium (mg/l)	ND**	
Lead (mg/l)	ND**	

Source: FAO [9a]; *FAO [9b], ** not detected.

Effects of lead on Cyprinus carpio

Through confirmed experiments and results in 96 hours, the following was observed: The higher the dose and longer the exposure time, the greater the impact of chemicals. Specifically, at the lowest Pb^{2+} concentration of 0.25 mg/l, the number of dead fish was the lowest, and at the highest concentration of 2.0 mg/l, the number of dead fish was the highest. During the first 3 hours, the fishes were not

Table 2. Probit for 96 hours exposure to lead.

Concentration (ppm)	Log_{10} Concentration	Total no of test fish	Replicate 1			Replicate 2			Replicate 3		
			No of death	*% mortality*	*probit*	*No of death*	*% mortality*	*probit*	*No of death*	*% mortality*	*probit*
0	0	10	-	-	-	-	-	-	-	-	-
0.25	-0.602	10	2	20.00	4.16	2	20.00	4.16	2	20.00	4.16
0.5	-0.301	10	4	40.00	4.75	4	40.00	4.75	3	30.00	4.48
1.0	0	10	4	40.00	4.75	4	40.00	4. 75	4	40.00	4.75
1.5	0.176	10	6	60.00	5.25	6	60.00	5.25	7	70.00	5.52
2	0.301	10	7	70.00	5.52	7	70.00	5.52	7	70.00	5.52

affected but, after 96 hours, the lowest and highest number of dead fish increased. It is evident that at the same time, the mortality variation was very large for the selected concentration range.

Probit analysis was applied to the mean results calculated in Table 2 to determine the LC50 of the carp.

Effect of cadmium on Cyprinus carpio

Through confirmed experiments and monitoring results in 96 hours, we see that the effect of toxicity is greater when the dose is higher and exposure time is longer. Specifically, at the lowest Cd^{2+} concentration of 0.5 mg/l, the lowest number of dead fish was recorded, and at the highest concentration of 3.0 mg/l, the highest number of dead fish was recorded. During the first 3 hours, the fish was not affected but, after 96 hours, the lowest and highest number of dead fish increased. It is evident that at the same time, the mortality variation was very large for the selected concentration range.

The 96-hour 50% lethal concentration (LC50-96 hours) of both cadmium and lead was calculated using the regression method. The number of deaths of test fish observed at each concentration after 96 hours of exposure for each of the three replicates is shown in the probit Tables 2 and 3. LC50-96 hours of cadmium and lead for *Cyprinus carpio* is shown in Table 4. Similar to the result presented in Table 1, the number of dead fishes was proportional to the lead test concentration in the three replicates.

Table 3. Probit for 96 hours exposure to cadmium.

Concentration (ppm)	Log_{10} Concentration	Total no of test fish	Replicate 1			Replicate 2			Replicate 3		
			No of death	*% mortality*	*Probit*	*No of death*	*% mortality*	*probit*	*No of death*	*% mortality*	*Probit*
0	0	10	-	-	-	-	-	-	-	-	-
0.5	-0,301	10	2	20.00	4.16	3	30.00	4.48	2	20.00	4.16
1.0	0	10	4	40.00	4.75	4	40.00	4.75	4	40.00	4.75
1.5	0.176	10	6	60.00	5.25	5	50.00	5.00	5	50.00	5.00
2.0	0.301	10	8	80.00	5.84	7	70.00	5.52	8	80.00	5.84
3.0	0.477	10	8	80.00	5.84	8	80.00	5.84	8	80.00	5.84

Table 4. LC50-96 hours of cadmium (Cd) and lead (Pb) for *Cyprinus carpio.*

Heavy metal	Replicate 1 LC50 (mg/l)	Replicate 2 LC50 (mg/l)	Replicate 3 LC50 (mg/l)	Mean LC50 (mg/l)
Cadmium	1.147	1.161	1.202	1.171
Lead	0.996	0.996	0.968	0.987

LC50-96 hours results show that there was no significant difference in the three replicates for both Cd and Pb. Although no death was recorded in the control groups, the mortality percentage of the test organism increased by increasing the test concentration. The increase in mortality with increase in toxicant concentration may be due to the increase of toxicant solubility and species' susceptibility. It accompanies the high toxicant concentration in the aquatic medium (LC50) of lead in the three replicates, which was significantly lower than those of cadmium. This result suggests that lead is more toxic to *Cyprinus carpio* than cadmium. Higher LC50 connotes less toxicity. Higher concentration is required to achieve a 50% mortality of test organisms.

Some authors in the world have also conducted toxicological studies to determine the LC50-96 hours in some organisms and obtained different results. Specifically, according to the result of Brraich Onkar Singh and Kaur Manjeet, the concentration of lead nitrate (LC50-96 hours) in Labeo rohita is 34.20 mg/l [10]. Zeynab Abedi1, et al. identified the LC50-96 hours of $CdCl_2$, $CrCl_3$ and Pb $(NO_3)_2$ for P. hypophthalmus as 64.89, 7.46 and 48.06 mg/l, respectively [11]. This suggests that the toxicological effects of lead and cadmium on different species and in different experimental conditions will yield different results.

Effect of lead and cadmium on Cyprinus carpio

This experiment was conducted according to the concentrations selected in the previous exploratory experiment. The confirmed experiment was repeated twice. The results have been averaged as follows:

Average number of dead fish = (1^{st} death + 2^{nd} death+ 3^{rd} death)/3

The results are shown in Fig. 1 and Fig. 2 below:

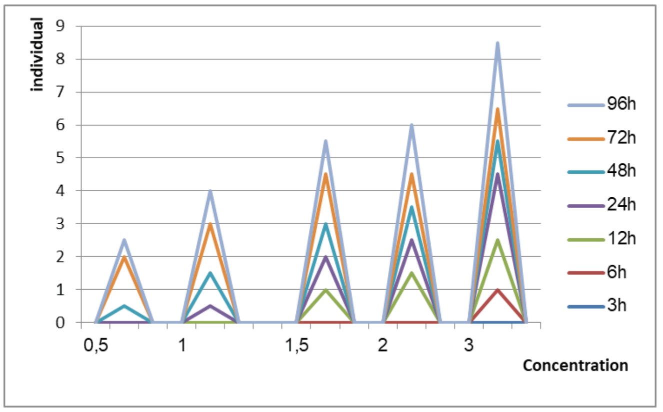


Fig. 1. Effect of lead on *Cyprinus carpio.*

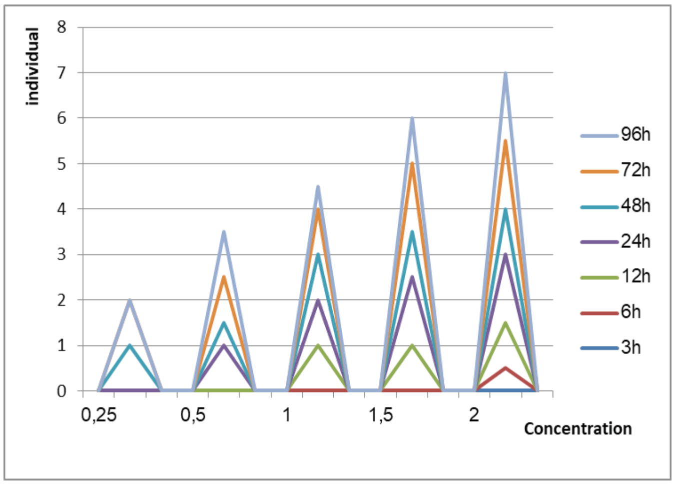


Fig. 2. Effect of cadmium on *Cyprinus carpio.*

The confirmed and monitored results after 96 hours (Fig. 3) showed that the effect of chemicals was greater when the dose was higher and exposure time was longer. Specifically, when Pb^{2+}, Cd^{2+} concentrations were the lowest at 0.25 ppm and 0.5 ppm, respectively, the number of dead fishes was minimum; at the highest concentration of lead at 2.0 ppm and cadmium at 3.0 ppm, the number of dead fishes was the highest. During the first 3 hours, the tested fishes were not affected but, after 96 hours, any amount of concentration increased the number of dead fish. At the same time, variation in lethal effects was significant for the range of concentrations chosen.

Effect of concentration and exposure time of lead and cadmium to Cyprinus carpio

The effect of lead and cadmium exposure on the *Cyprinus carpio's* bioassay is shown in Table 5.

Table 5. Expression of *Cyprinus carpio* in the experiments with lead.

Expression	Normal	Impacted	Poisoned
Swimming at the tank bottom	x		
Normal breathing	x		
Fast swimming		x	
Losing the swimming direction, rushing into the tank		x	x
Change eye colour (light yellow to grey brown)		x	
Scabbing, red marks on the body		x	x
Bleeding bring		x	x
Mucus secretion		x	x
Breathing by oral and bring		x	
Loss of balance, sluggishness, abdominal swimming		x	x
Die			x

The impacts of lead and cadmium exposure on *Cyprinus carpio* are shown in Figs. 3 and 4. The number of fishes is correlated to the level of concentration as well as exposure time. The percentage of normal fish diminishes. From the starting point up to 48 hours of observation, the percentage of affected fishes increases slightly due to prolonged exposure. After 48 hours, the adversely affected fishes increase suddenly with the exposure time being prolonged to 72 hours and 96 hours.

The test concentration of cadmium is two-fold with that of lead, but the effects appear almost simultaneously. It is suggested that the toxic levels of lead are two times greater than that of cadmium.

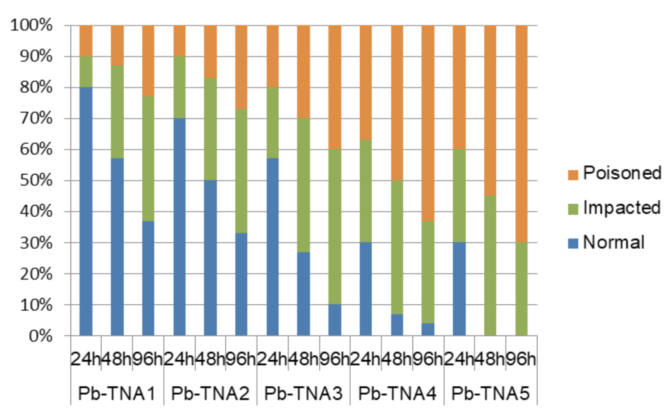


Fig. 3. Indication of the effect of lead on *Cyprinus carpio.*

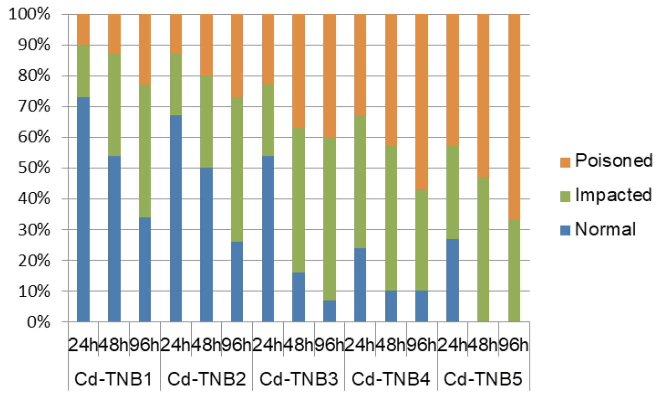


Fig. 4. Indication of the effect of cadmium on *Cyprinus carpio.*

Concentration of lead and cadmium in Cyprinus carpio

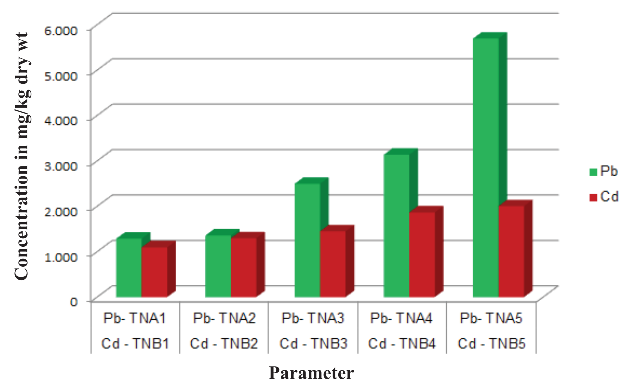


Fig. 5. Concentration of lead and cadmium in *Cyprinus carpio.*

Figure 5 shows that the concentration of lead and cadmium in *Cyprinus carpio* increases with the experimental concentration range. The lowest cumulative concentrations are found in formula Pb-TNA1, Cd-TNB1 and the highest in Pb-TNA5, Cd-TNB5. *Cyprinus carpio* accumulates lead content higher than cadmium.

The result shows that the concentration of Pb and Cd causes a significant effect on the *Cyprinus carpio* in 96 hours. It directly affects the physiological health, and it indirectly affects populations and ecosystems, which may affect human health if humans consume fishes from the affected ecosystem.

Conclusions

The effect of chemicals is greater when the dose is higher and exposure time is longer. The result showed that the LC50 of Cd and Pb were 1.171 mg/l and 0.987 mg/l, respectively. Our study provides good information about LC50 of two heavy metals on the juveniles of *Cyprinus* *carpio*, which is a useful basis for risk assessment.

Fish abnormalities (effects, poisoning) increase with an increase in the concentration of lead and cadmium and longer exposure time to them. The test concentration of cadmium is two-fold with that of lead, but the effects appear almost simultaneously. It has been suggested that the toxic levels of lead are two times greater than that of cadmium.

The concentration of lead and cadmium in *Cyprinus carpio* increases with the experimental concentration range. *Cyprinus carpio* accumulates lead higher than cadmium.

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