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Investigating the 96h LC_{50} of Mercury and Cadmium on *Channa punctatus* (Bloch): A Comparative Acute Toxicity Bioassay

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Abstract

Acute and chronic exposure of heavy metals exerts detrimental effect at the cellular level and is a rising global concern. The pollutants once introduced in the water bodies, subsequently enters the food chain, and poses risks not only to the aquatic organisms, but to the human consumers as well. Therefore, understanding the toxicological level of these metals is vital for assessing the severity of potential risks. The study aimed to conduct a comparative analysis of the acute toxicity of mercury and cadmium on *Channa punctatus* through a 96-hour bioassay. It was carried out in a semi-static laboratory condition following the standard guidelines. The behavioural, and mortality response was recorded at the 24, 48, 72, and 96 h of exposure duration for both toxicants. The results demonstrated distinct differences in toxicity levels between mercury and cadmium. The 96h-LC_{50} value for cadmium was measured at 6.19 mg/l, while for mercury, it was significantly lower at 0.44 mg/l. It was revealed that even the trace concentration of metals can induce toxicity, if given for a prolonged period of time. Furthermore, the study observed heightened toxicity of mercury, exerting adverse effects at lower concentrations compared to cadmium within the same exposure duration.

Keywords: Acute Toxicity, Cadmium, *Channa punctatus*, Mercury, 96h-LC_{50}

1. Introduction

One of leading concern of heavy metal toxicity is its impact on fish, which are not only a key component of many ecosystems, but also a significant dietary source for various top consumers, including humans[1,2]. Rapid development in the industrial, agricultural, and domestic sectors, coupled with the imperative to meet the needs of a rapidly growing world population, has led to an increase in a variety of pollutants in recent decades[3,4]. Among these, the proliferation of carcinogenic heavy metals such as cadmium (Cd), lead (Pb), zinc (Zn), chromium (Cr), mercury (Hg), and others has become a rising concern. These heavy metals infiltrate ecosystems through diverse sources, including industrial effluents, mining activities, agricultural runoffs, and atmospheric deposition, posing a threat to environmental integrity and public health[5,6]. The relevance of this issue is exacerbated by the persistent, bio-accumulative, and biomagnifying behaviour of these heavy metals within the living world, particularly through the intricate web of the food chain. The accumulation of heavy metals in various tissues, manifests adverse effects, ranging from gill dysfunction, liver and kidney damage, ocular impairments to even carcinogenic outcomes in the organisms residing in contaminated waters[7,8].

Among these contaminants, cadmium and mercury stand out as notorious pollutants known for their detrimental effects on aquatic life[9-12]. Therefore, the study narrows its focus on cadmium, and mercury due to their well-documented detrimental impacts on fish, and human health. Cadmium, known for its nephrotoxicity, carcinogenic potential, and disruption of physiological

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processes, and mercury, particularly for its neurotoxic effects, bio accumulative nature, and severe impacts on the nervous system, stand as critical heavy metals necessitating thorough investigation[2,13-15]. Chaudhary *et al*.,[16] findings indicate that a sub-lethal exposure of cadmium (Cd) to *C. punctatus* over a 7-day period resulted in the generation of Reactive Oxygen Species (ROS) and inflammation, which was confirmed by the decreased expression of IL-10, and increased expression of NF-κB, iNOS, TNF-α, IL-1β, IL-6, IL-12. Similarly, the mercury induced genotoxic damage was showed by Gill *et al*.,[17] where comet assay and micronucleus assay validated the duration, and concentration dependent DNA damage.

Channa punctatus, the spotted snakehead, emerges as an exemplary model organism for this study. Their sensitivity to heavy metals, relatively simple maintenance, and relevance in aquatic ecosystems make them an ideal organism for toxicity assays aimed at understanding the effects of Cd and Hg exposure[18,19]. The 96-hour acute toxicity bioassay emerges as a vital tool for assessing short-term adverse effects of pollutants on aquatic organisms, providing crucial insights into their tolerance levels and potential impacts on the ecosystem. Several studies have been reported the 96-h acute toxicity value on Channa punctatus for cadmium[7,8,10,20], and for mercury[21-24]. But limited literatures have done a comparative study in the recent years taking cadmium, and mercury as the exposure toxicant[13]. Therefore, the primary objective of this paper is to conduct a comparative toxicity assay of cadmium and mercury on Channa punctatus, aiming to contribute valuable data to the existing body of knowledge. This study holds substantial significance in elucidating the differential effects of these heavy metals on aquatic organisms, thus contributing to informed environmental policies, and strategies for safeguarding both ecosystem health and human well-being.

2. Materials and Methods

2.1 Test Organism

The test organism is *Channa punctatus* (Order: Perciformes; Family: Channidae), commonly known as the spotted snakehead is often used as a test organism in toxicity assays due to its sensitivity to various pollutants and chemicals. Their relatively simple maintenance, rapid growth, and sensitivity to environmental changes make them valuable indicator species for toxicity testing. The species usually grow in low lying, stagnant water bodies[25], with optimal temperature range between 22-28 °C[26], pH between 6.5-8.5, and can live at low oxygen level of 4-5 mg/l[27].

Class: Actinopterygii
Order: Perciformes
Family: Chanidae
Genus: *Channa*
Species: *punctatus*
Vernacular Name: Goroi (Bihar)
Habitat: Freshwater bottom dwelling
Feeding Habit: Carnivore

Salient identifying characteristics:

- Elongated body with fairly rounded caudal fin.
- Large and irregular shape scales on the head.
- Large mouth with 3-6 canine teeth.
- Straight lateral line with a slight curve over the fourth anal ray.
- Body mostly dark with light gray or dull white colour on the abdomen.
- Dark bands or irregular spots passes from the dorsum of the body to the middle of the body.

Figure 1. *Channa punctatus* and its identifying key characteristics.

2.2 Preparation of Test Chemicals

The 1000 ppm stock solutions were prepared using 0.1719g of $CdCl_2.H_2O$ [Fisher Scientific, CAS No: 35658-65-2], and 0.1354 g of $HgCl_2$ [Sisco Research Laboratory, CAT No: 7487-94-7] in 100 ml of deionised water[28]. Thereafter, test solutions for the range finding test, and the LC_{50} toxicity assay was prepared by diluting stock solution to the desired concentrations of toxicant. Prior to the stock, and test solution preparation all the glassware were thoroughly acid washed following the standard procedure.

2.3 Toxicity Bioassay

The 96-hour toxicity bioassay of cadmium and mercury with *Channa punctatus* was carried out in the Environmental Biology Laboratory in a semi static condition following the standard guidelines of APHA[28], and OECD[29]. Healthy juvenile specimens of *Channa Punctatus* were brought to the laboratory and were given profile attic treatment of 0.05% of potassium permanganate to avoid any dermal infections. Six individuals of the species were randomly selected and were sacrificed for the estimation of Cd and Hg in the muscle tissue of the fishes. Further, the experimental fishes were acclimatised for 10 days in a 500-litre glass aquarium. During the acclimatisation period, water has been changed at every 24-hour intervals. and were given proper oxygenation through aerators. Moreover, selected water quality parameters have been monitored at regular intervals less than 2% mortality was recorded during the acclimatisation phase.

After the 10 days of acclimatisation healthy active fishes were selected for the exploratory range finding test for the selected toxicants. Then after a set of ten healthy juvenile individuals of *Channa punctatus* of mean length 9.66 ± 0.377 cm, and mean weight 5.7 ± 0.244 g were transferred to a 50-litre glass aquarium. Fishers were exposed to a concentration of 0.2, 0.4, 0.6, 0.8, 1.0 mg/l of $HgCl_2$, and 2, 4, 6, 8, 10 mg/l of $CdCl_2.H_2O$. Meanwhile, a control tank was maintained at the similar laboratory conditions. The behavioural changes, and mortality was carefully recorded at every 24 h, 48 h, 72 h and 96 h of the exposure durations. The dead fishes were removed immediately. The data obtained were then subjected for the estimation of 96 h LC_{50} value for the heavy metal of interest, following probit analysis method suggested by Finney[30] on Microsoft Excel (2021). The same has been carried out on SPSS version 22.0 to check any calculation ambiguity determined for the 96-hour LC_{50} value of mercury and cadmium.

Table 1. The statistical summary of analysed physico chemical parameters during the toxicity assay

	Average	Standard Deviation
pH	7.99	0.13
Electrical conductivity	0.68	0.07
Dissolved Oxygen	6.06	1.04
Turbidity	7.09	2.86
Total Alkalinity	238.5	2.62
Total Hardness	210.75	2.48

Note: Unit in mg/L, Except EC (mS/cm), Turbidity (NTU) and pH.

3. Results

The physico-chemical attributes of the test aquarium water have not shown any significant variations during the acclimatization, and the exposure phases Table 1. The pH remains in the optimal range (6.5-8.5), with a mean value of 7.99 ± 0.13. The water temperature varies from 26 °C to 27.5 °C, and has not exceeded the fluctuation beyond 2°C[29]. The Dissolved Oxygen level was maintained above 5 mg/l, throughout the experiment, which has not gone below 5.25 mg/l. The chemical variables such as total hardness, and total alkalinity that were marked within the guideline value[29] (250 mg/l), to perform the toxicity assay. The concentration of total hardness ranged from 208 to 216 mg/l, while the alkalinity has ranged between 234 to 242 mg/l. However, less than 2% mortality was recorded during the acclimatization phases, and no mortality was recorded in the control tank during the LC_{50} experiment.

The level of cadmium, and mercury prior to the experiment was found BDL (Below Detection Limit) for cadmium (<0.002 mg/l) and for mercury (<0.0002 mg/l) in the muscle tissue of *Channa punctatus*. The result of the percentage cumulative mortality at different doses of Cd, and Hg, at the different time intervals has been given in Table 2, and Table 3 respectively. After the 24 h of exposure, only one individual of the species died at the highest dose of Cd (10 mg/l), whereas, 10% mortality was recorded at 0.6 mg/l, and 20% has died in each of the tanks exposed to 0.8, and 1.0 mg/l of Hg. The result marked the death of all the individuals of *Channa punctatus* exposed

Table 2. Percentage cumulative mortality at different exposure level of $CdCl_2.H_2O$ during the bioassay

Concentration of $CdCl_2.H_2O$		**% cumulative mortality after time (h)**			
in mg/l	Log Conc.	24	48	72	96
0.0 (Control)		0	0	0	0
2	0.301	0	0	0	0
4	0.602	0	0	0	10
6	0.778	0	0	10	40
8	0.903	0	20	50	80
10	1	10	60	80	100

Table 3. Percentage cumulative mortality at different exposure level of $HgCl_2$ during the bioassay

Concentration of $HgCl_2$		**% cumulative mortality after time (h)**			
in mg/l	Log Conc.	24	48	72	96
0.0 (Control)		0	0	0	0
0.2	-0.6989	0	0	10	20
0.4	-0.3979	0	10	20	30
0.6	-0.2218	10	20	40	50
0.8	-0.0969	20	30	50	70
1	0	20	50	70	90

to 10 mg/l of cadmium, at the 96 h of toxicity assay. In the case of mercury, highest mortality was recorded after 48 h (3 individuals) at the dose 1.0 mg/l, which follows a couple of death at 72 h and 96 h at the similar dose. The result clearly revealed the toxic behaviour of the metals, as the mortality increases with the increase in the concentration of the metals.

Consequently, Table 4 summarises the LC$_{50}$ value of cadmium, and mercury at different exposure durations. It was observed that the concentration at which mercury has caused 50% mortality in just 24 h is 1.19 mg/l, which is noted 89.12 mg/l for cadmium. As the exposure duration is increased to 48 h, 72 h and 96 h, the LC$_{50}$ value of mercury has decreased to 0.87 mg/l, 0.74 mg/l, and 0.44 mg/l mg/l respectively. A similar scenario was observed in case of cadmium. However, the 96 h LC$_{50}$ value of mercury is 63.02% lower than the 24 h LC$_{50}$ value, while it has experienced a decline of 93.05% in cadmium. This potentially suggests the delayed response of cadmium in inducing toxicity among the exposed fishes.

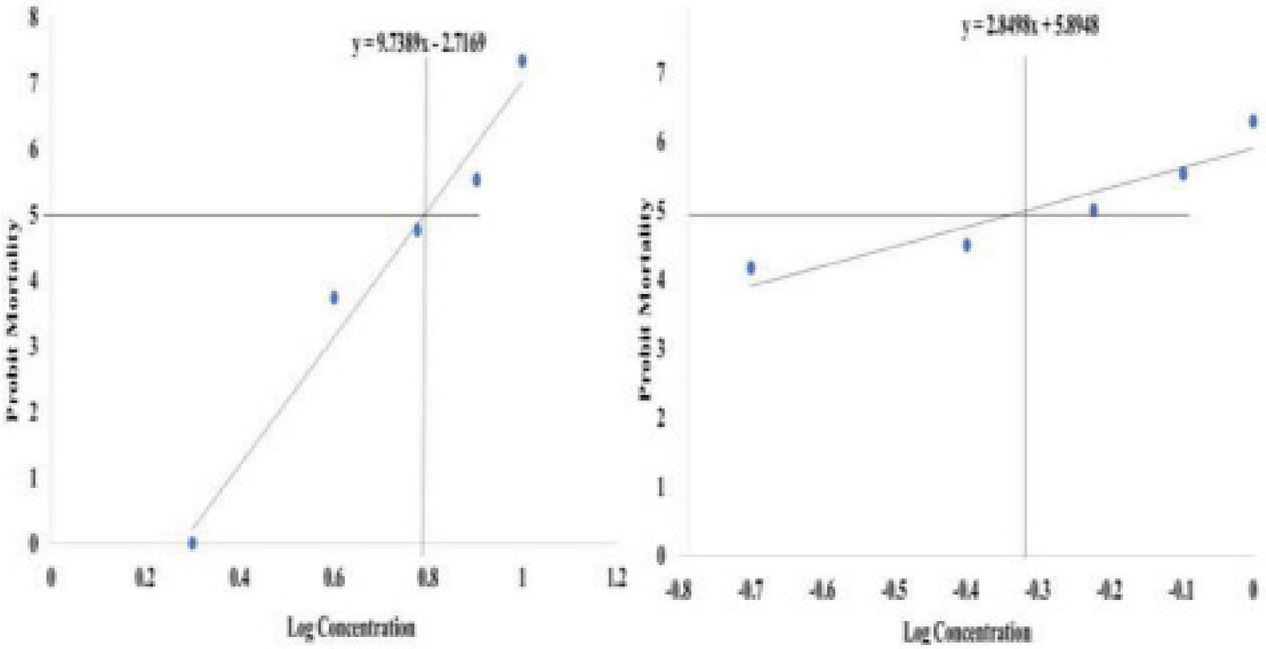
Table 4. The LC$_{50}$ value of $HgCl_2$, and $CdCl_2.H_2O$ at different exposure durations

	LC$_{50}$ (in mg/l)	
Exposure duration (in hour)	**Hg**	**Cd**
24	1.19	89.12
48	0.87	14.35
72	0.74	9.48
96	0.44	6.19

The Finney method of probit analysis has revealed the 96 h LC$_{50}$ value of Cd and Hg as 6.19, and 0.44 mg/l (Figure 2). Figure 3 depicts the dose-response relationship curve for the 96-h acute toxicity assay of the studied metals. The x-axis represents the increasing doses of respective metals, whereas % mortality has been taken as a response on the y-axis to investigate the relationship. The results profoundly showed, that under the given set of environmental conditions, mercury can be more detrimental than cadmium. A small spike in the concentration of Hg from 0 to 0.2 mg/l has caused death to 20% of the individuals, whereas at similar the similar scale no death was recorded for cadmium. Therefore, the 96 h Lowest Observable Adverse Effect Level (LOAEL) for mercury was noted to be 0.2 mg/l, and for cadmium it was marked at 4 mg/l.

4. Discussions

The induction of toxicity in an organism is a complex interplay between the extrinsic environmental factors, and the degree to which an organism is responding towards those factors. The extrinsic factors such as pH, temperature, dissolved oxygen, and certain anions govern the chemical forms of the metals, whereas, the presence of suspended particles, organic matter, nutrients, etc drives the bioavailability of various contaminants in the aquatic environment[31]. In addition, it is very well documented that the exposure duration, age, sex, size, uptake pathway are some intrinsic factors that influence the toxicity at the intra and inter specie level of a metal exposure[32-34]. An aquatic environment with an acidic pH = 5.6, can lead to 50% mortality in the *Channa punctatus*[35]. The study reported acidic pH has led to the dissociation of branchial epithelium, decrease in interlamellar spaces, and increased opening of mucous gland in the species. Singh *et al.*[26] reported a fluctuation from the optimum



(a) (b)

Figure 2. **(a)** Graph determining LC$_{50}$ value of cadmium. **(b)** Graph determining LC$_{50}$ value of Mercury.

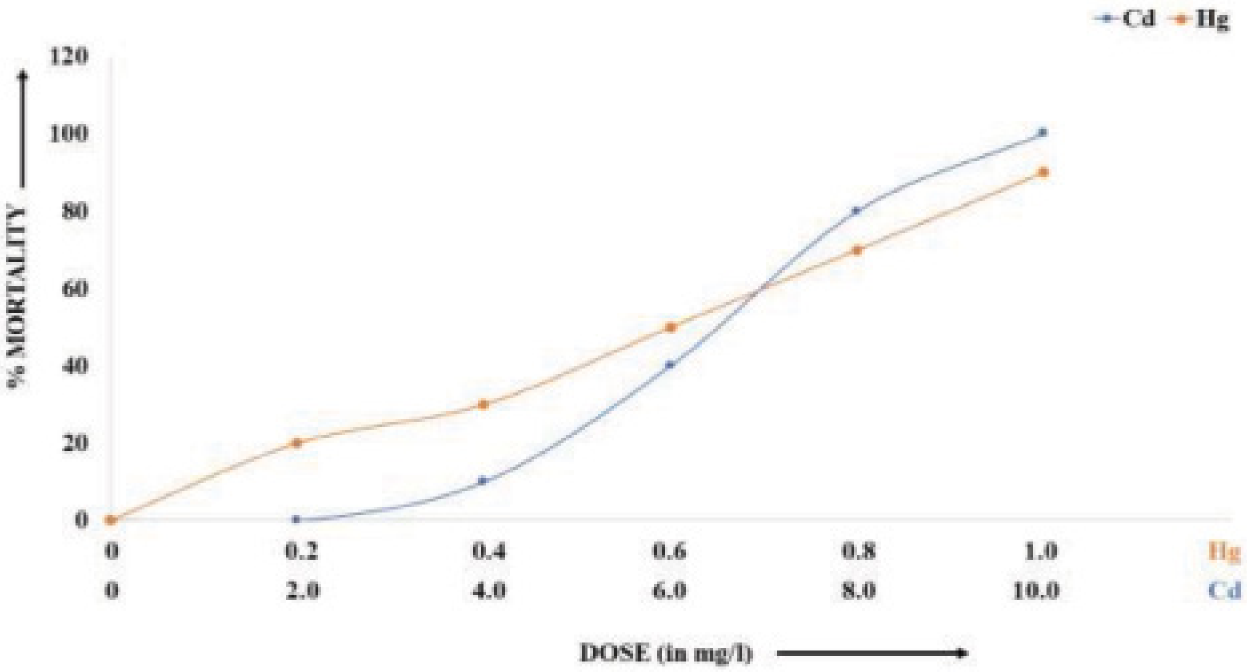


Figure 3. Dose-response curve of exposed fish % mortality and the increasing concentration of toxicants.

temperature range (22-28°C) for *Channa punctatus*, affects the metabolic functions. A lower temperature affects the food consumption rate, and other enzymatic intermediates, whereas, temperature >35°C has been invariably linked with the high Hsp70 level in the fish. Not only in *Channa punctatus*, thermal stress leading to reduced food consumption rate, and digestive activities were also reported in *Catla catla*, and *Clarias batrachus*[36,37]. Similarly, effect of salinity is demonstrated by the work of Dubey *et al.*,[38] who found severe impact of salinity in the fishes exposed to 18 to 20 g/l of salinity. In this regard the study has maintained the water parameters and have tried to acclimatise fishes in a static environmental condition to all possible extent. As the water parameter was within the acceptable guidelines of OECD[29], and the mortality recorded were less than 2% it ensures a suitable condition to perform toxicity assay. The average value of water temperature, pH, DO, EC, Turbidity were respectively. The optimal conditions with minimal variations in water quality parameters might have negligible impact on the mortality of fishes, which is validated with the 0% death recorded in control aquarium during the toxicity bioassay.

Clearly, the difference in the 96-hour LC_{50} value of Hg, and Cd explains the differential toxic behaviour of various contaminants. Table 5. summarises the 96-h LC_{50} value of mercury and cadmium, that showed result similar to the present study on *Channa punctatus*, and some of the other aquatic experimental organisms observed by various studies. Previous researchers reported the 96-h LC_{50} value of mercury in *Channa punctatus* lies between 0.64 to 2.11 mg/l, whereas, it has marked as low as 0.5 mg/l in *Cyprinus carpio*. In contrary, the 96-h LC_{50} value of cadmium showed variations by the several research. The results of the present study mainly corroborate with the findings of Gupta and Rajbanshi[10], and Chandra and Verma 7. A study by Ramesh and Ramachandra[39] reported the toxicity value of cadmium in *Channa punctatus* was 0.559 mg/l, whereas a remarkably high value of was 30 mg/l and 80.62 mg/l observed by Amin *et al.*,[40] and Singh and Saxena[8] respectively. Akter *et al.*,[41] suggests these variations in the acute toxicity bioassay depends on the difference in species, and environmental factors. Moreover, the age, size, sex of similar test organism, and the selection, and preparation of stock standard of chemical toxicant might also lead variations in the results. The observed severity of fishes towards mercury than cadmium, agrees to the 96-h comparative toxicity assay of Leblond and Hontela[42], and Raj *et al*[13].

Table 5. Showing the 96h-LC_{50} value of mercury and cadmium observed by studies conducted on by various researchers

Metal	**Species**	**96 h LC_{50} value**	**References**
Mercury	*Channa punctatus*	1.8	Sastry *et al.*[21]
	Channa punctatus	2.11	Agarwal[22]
	Channa punctatus	1.21	Pandey *et al.*[15]
	Channa punctatus	0.81	Yadav and Trivedi[23]
	Channa punctatus	1.38	Gill *et al.*[17]
	Channa punctatus	1.12	Kumar *et al.*[1]
	Channa punctatus	0.64	Ramesh *et al.*[24]
	Channa punctatus	0.78	Trivedi *et al.*[2]
	Cirrhinus mrigala	1.11	Gupta and Kumar[53]
	Rosbora daniconius	0.8	Gupta and Rajbanshi[54]
	Cyprinus carpio	0.5	Sivaramakrishnan and Radhakrishnan[55]
	Cyprinus carpio	0.105	Dhara *et al*[56]
	Channa gachua	0.188	Dhara *et al.*[56]
	Clarias batrachus	0.89	Kumari and Chand[9]
Cadmium	*Channa punctatus*	7.4	Gupta and Rajbanshi[10]
	Channa punctatus	6.81	Gupta and Rajbanshi[10]
	Channa punctatus	11.2	Sastry and Shukla[11]
	Channa punctatus	11.8	Shukla *et al.*[20]
	Channa punctatus	14.95	Tiwari *et al.*[57]
	Channa punctatus	9.908	Chandra and Verma[7]
	Channa punctatus	80.62	Singh and Saxena[8]
	Channa punctatus	30	Amin *et al.*[40]
	Catla catla	4.53	Sobha *et al.*[58]
	Cyprinus carpio	4.5	Ramesha *et al.*[59]
	Clarias batrachus	103	Ahmad *et al*[60]
	Trichogaster fasciata	49.5	Roy *et al.*[61]
	Mystitus viltatus	17.94	Rao and Manjula[62]
	Wallago attu	32.96	Batool *et al.*[12]
	Clarias batrachus	82.66	Dhara *et al.*[63]
	Channa marulius	75.7	Batool *et al.*[12]

All the values are expressed in mg/l.

The result revealed prolonged exposure of a trace concentration of a toxicant increases its potential to cause detrimental impact on an organism[7]. Similar conclusions are made by various researchers working on *Channa punctatus*[43], *Oncorhynchus mykiss*[44], *Cirrhinus mrigala*[45], *Cyprinus carpio*[46], *Heteropneustes fossilis*[47] and many. In the present study the LC_{50} value has gradually decreased from 1.19 mg/l, and 89.12 mg/l in the 24th hour to 0.44 mg/l, and 6.19 mg/l in the 96th hour for mercury, and cadmium. This evidently suggest either the higher exposure level of toxicants, or the higher exposure duration of small concentration of same toxicant can exert detrimental impact. Pandey *et al.*,[15] has discussed the altered behaviour leading to subsequent mortality is associated with disturbed nervous/cellular enzyme system emerges with the onset of toxicant exposure. In addition, gills, being the primary site for adsorption of heavy metals undergoes progressive physical retaliation, which led to suffocation in an aquatic organism. Maruthanayangam *et al.*,[48] also noted that the primary cause of cadmium compound toxicity to fish *Channa punctatus* is gill injury, which hindered the fish to absorb oxygen from the water and resulted in anoxia. The longer the toxicant exposure period, the more clumping of the gills occurs. The two afore mentioned reasons could possibly explain higher mortality at a higher dose over a short exposure duration. However, the acute toxicity developed even at a small dose at the 96 hours is might be because of the accumulation of metals in various tissues, obstructing metabolic pathways. Reddy *et al.*,[49] and Sastry and Sharma[50] have shown that acute exposure of mercury causes significant reduction in the Ca-ATPase pump and Na-K-ATPase pump respectively. In a study conducted by Hasan *et al*.,[51] in Bangladesh he showed that the individuals of *Channa punctatus* that are exposed to high level of mercury, were reported with dead liver cells. Damage in the renal, and hepatic tissue due to the toxic behaviour of mercury chloride were also reported by[19,52]. A severe anaemia, and decrease in many haematological profile Haemoglobin (Hb), Red Blood Cells (RBC), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), and Platelet count (PLT) was noticed to be associated with the acute exposure of cadmium chloride[39].

5. Conclusion

In conclusion, the 96h LC_{50} relative toxicity levels of mercury, and cadmium on *Channa punctatus* has documented the heightened toxicity of mercury compared to cadmium. The findings also noted inconsistencies in toxicity patterns across studies. Interestingly, while mercury showed consistency in its potential toxicity in different studies, the variability in reported LC_{50} values of cadmium on *Channa punctatus* is varying. The LC_{50} value of cadmium ranged between 5 to 15 mg/l across different researchers, underscores the inconsistency surrounding cadmium's impact on species. Despite the established knowledge of higher toxicity in mercury, the remarkable fluctuations observed in cadmium toxicity levels among studies, emphasize the need for more standardized methodologies, and comprehensive investigations to ascertain a more precise understanding of cadmium's effects. The study further recommends exploration into the factors influencing cadmium toxicity, potentially including environmental variables, and specimen characteristics to establish a more reliable framework for future research, and regulatory considerations in aquatic life and human health.

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