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Assessment of Zinc- and Lead-Induced Renal Toxicity in Freshwater Fish *Channa punctatus*

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ABSTRACT

In the present study, the renal toxicity caused by zinc and lead in the freshwater fish *Channa punctatus* was evaluated. Tests of acute toxicity of these metals showed that the 96-hour LC₅₀ values for zinc chloride between *Channa punctatus* fish was determined as 50.00 mg/L while it was 25.00 mg/L for lead acetate. This means that lead is more toxic than zinc. The sub lethal exposure of the fish to these metals resulted in biochemical changes over the period of 7, 15, 30, 45, and 60 days. There was an increase in the levels of urea, creatinine, uric acid, BUN, and potassium while sodium and chloride were reduced as the duration of exposure increased. These changes indicate the impairment of renal function such as filtration of toxins, excretion of nitrogenous waste, tubule functioning, and maintaining of the electrolyte balance. Pathological studies showing the renal degeneration such as tubular degeneration, vacuolization, congestion, and necrosis in the fish exposed to these metals with the effect being more intense in the lead-acetate exposed fish. This shows that both of these metals posed nephrotoxicity in *C. punctatus* but lead caused more renal damages as compared to zinc and demonstrates the environmental hazard caused by heavy metal pollution in the freshwater zone.

Introduction

The contamination of the aquatic ecosystems is caused due to the different factors like Industrialization, urbanization, agricultural operations, and disposal of untreated effluents. Heavy metals are the major pollutant amongst different pollutant categories as they have the ability to persist in nature, are non-biodegradable, can bio-accumulate, and create harmful physiological effects in the aquatic species. Both industrial effluents and sewage wastes introduce metals into rivers, lakes, ponds, and other water habitats and these metals accumulate in the aquatic species and sediments over time. Fish are very prone because of their regular contact with the water and the ability to accumulate metals through their gills, digestive system, and other body parts (Ashraj, 2005; Baharom & Ishak, 2015). Fish can also act as good bio-indicators for determining the level of aquatic pollution since changes in biochemistry, physiology, and histopathology could be observed after exposure to pollution. Different factors that influence the level of the metal accumulate in the fish include species, age, duration of exposure, physicochemical parameters of water, and the route of uptake. Among all other organs, kidneys serve as most vital organs in the body and hence renal tissues could become susceptible to heavy metal accumulation as these tissues play important role in excretion, osmoregulation, and electrolyte balance process. Zinc (Zn) serves as an essential trace mineral that plays a significant role in different biochemistry and physiology in organisms. However, when the concentration of zinc is high, it can have harmful effects on organisms and disturb their normal function. Bengari and Patil (1986) stated that zinc is an essential mineral that has both structural and functional importance, however, high concentration of zinc might affect the fish physiology negatively. Zinc can be absorbed by the fish through its gills and the digestive system and then it travels to different organs of the fish through blood (Spry *et al.*, 1988). Chronic exposure of fish to zinc has been shown to result in tissue accumulation in *Channa punctatus* and high level of zinc may

negatively affect ionic balance, acid-base equilibrium, gaseous exchange, and structural integrity of tissues (Agrahari and Gopal, 2007; Murugan *et al.*, 2008). On the contrary, lead (Pb) has no beneficial function in applying physiology of living organisms and it is an important aquatic toxicant, applied through industrial and human activities. Heavy metals, like lead can persist in aquatic environment and accumulate in fish, causing biochemical changes and modifying the structure of the fish. Exposure to lead can lead to changes in blood profile, affect metabolic functions in fish, and disrupt the function of some organs such as kidneys (Heath, 1987; Khare & Singh, 2002). The kidneys can react to metal-induced stress through disturbance in filtration, ionic regulation, nitrogenous waste excretion, and tissue structure. *Channa punctatus* (Bloch, 1793), also known as the spotted snakehead or spotted murrel, is a freshwater fish that has great economic importance and is widely distributed in South Asia. It can be found in ponds, rivers, lakes, ditches, etc., having a unique ability of survival in low-oxygen conditions. Due to wide distribution, adaptability to environment, and economic significance, this fish can be considered to be an appropriate candidate for toxicological studies. Thus, this research intends to analyze the harmful effects of Zn and Pb by assessing kidney parameters, including serum urea, creatinine, uric acid, blood urea nitrogen, sodium, potassium, and chloride, in order to analyze the nephrotoxic effects of metals on the kidneys of *Channa punctatus*.

Materials and Methods

Experimental Fish and Acclimatization- Healthy specimens of the freshwater fish *Channa punctatus* (Bloch, 1793) were collected from local freshwater bodies and transported carefully to the laboratory. The fishes were maintained in aquaria under controlled laboratory conditions at approximately 27 ± 1°C. During acclimatization, the fishes were provided with standard laboratory pellet feed and water *ad libitum*. Healthy and apparently disease-free individuals of relatively uniform size and weight were selected for the experimental study.



Heavy Metals and Preparation of Experimental Groups- Zinc (Zn) and lead (Pb) were selected to assess their nephrotoxic effects in *C. punctatus*. Zinc was administered in the form of zinc chloride ($ZnCl_2$), whereas lead was administered as lead acetate. Experimental fishes were divided into control and heavy-metal-treated groups. The control fishes were maintained under identical laboratory conditions without exposure to either Zn or Pb, while treated fishes were exposed to the respective heavy metal concentrations.

Determination of LC_{50} - Acute toxicity tests were conducted to determine the 96-h median lethal concentration (LC_{50}) of Zn and Pb using a static-renewal bioassay. Experimental fishes were distributed among different aquaria containing 25 L of dechlorinated water and exposed to graded concentrations of zinc chloride or lead acetate. Survival and mortality were recorded after 24, 48, 72, and 96 h of exposure. The percentage mortality at 96 h was used for LC_{50} determination. The obtained mortality data were analyzed using the log-dose/probit regression method following Finney (1971).

Sublethal Exposure- Following determination of the 96-h LC_{50} values, sublethal concentrations of Zn and Pb were selected for assessment of renal toxicity. Control experiments were conducted simultaneously under comparable laboratory conditions. The source document describes exposure periods extending to 7, 15, 30, 45, and 60 days for biochemical and histopathological examination. The fishes were exposed to the respective heavy metals for 6 h per day during the experimental period. At the designated intervals, fishes from control and treated groups were sampled for further investigation.

Collection of Blood Samples- At the predetermined sampling intervals, experimental fishes were carefully handled and blood samples were collected from the cut caudal vein into clean, sterilized glass centrifuge tubes. The samples were allowed to clot for approximately one hour and subsequently centrifuged at 2500–3000 rpm for 30 min. The resulting supernatant serum was carefully separated using a fine pipette and transferred into clean sterilized glass vials for biochemical analysis.

Assessment of Kidney Function- Renal toxicity induced by Zn and Pb was evaluated through selected serum biochemical parameters associated with kidney function. The following parameters were assessed using appropriate kit methods: Serum urea, Serum creatinine, Serum uric acid, Blood urea nitrogen (BUN), Serum sodium (Na^+), Serum potassium (K^+), Serum chloride (Cl^-)

Alterations in these parameters were used to assess impairment of renal function following exposure to Zn and Pb. Values obtained from the treated groups were compared with those of the corresponding control group.

Collection of Kidney Tissue- At each scheduled sampling interval, fishes from the control and heavy-metal-treated groups were anesthetized and dissected. The kidneys were carefully removed and processed for histopathological examination. Particular care was taken during tissue collection to avoid mechanical damage that could interfere with microscopic interpretation.

Histopathological Examination of Kidney- Kidney tissues collected from control, Zn-exposed, and Pb-exposed fishes were washed with saline and fixed in Bouin's fluid. Following fixation, tissues were dehydrated through a graded series of ethanol and embedded in paraffin wax. Sections of approximately 5 μm thickness were prepared and stained with hematoxylin and eosin (H&E), following the procedure described in the source document with reference to Soliman *et al.* (2017). The stained sections were examined under a Biolux-NLCD digital microscope, and representative photomicrographs were obtained. Histopathological alterations in treated kidney tissues were compared with the normal renal architecture of control fishes.

Evaluation of Renal Toxicity- The nephrotoxic potential of Zn and Pb was evaluated by integrating changes in serum kidney-function biomarkers with histopathological alterations in renal tissues. Differences between control and metal-exposed fishes were used to characterize the extent of renal dysfunction and tissue injury associated with exposure to the two selected heavy metals.

Result

Experimental findings on the toxic nature of zinc chloride and lead acetate in *Channa punctatus*. These include acute, viz., 96 hour LC_{50} value, alteration of behaviour, liver function, kidney function and liver and kidney histopathological observations.

All the biochemical values are expressed as Mean $\pm$ SD. The data were statistically evaluated using One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT). Different superscript letters denote significant difference at $P < 0.05$.

1. Acute Toxicity Study

Table 1.1 Mortality Response of *Channa punctatus* Exposed to Zinc Chloride for 96 Hours

Concentration (mg/L)	No. of Fish Exposed	No. of Dead Fish	Mortality (%)	Survival (%)
Control	10	0	0	100
20	10	1	10	90
30	10	2	20	80
40	10	4	40	60
50	10	5	50	50
60	10	7	70	30
70	10	9	90	10
80	10	10	100	0

Calculated 96-h LC_{50} value of Zinc Chloride = 50.00 mg/L

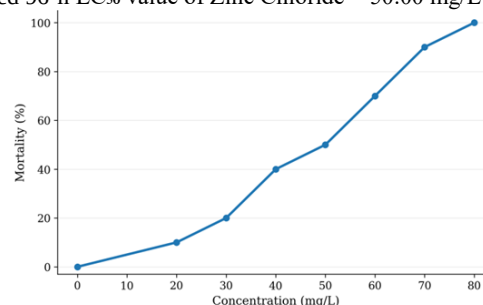


Figure 1.1. Concentration-mortality response of *Channa punctatus* exposed to zinc chloride for 96 hours.

The concentration-mortality response curve increased progressively for *Channa punctatus* with increasing concentration of zinc chloride at the end of 96-h exposure, with no mortality occurring in the controls, which affirmed that the laboratory conditions were appropriate and that the mortality was a result of the exposure to the toxicant. The mortality was mild at the lower concentrations and significantly marked among the Channa at the higher concentrations. The value for 50% mortality is at 50.00 mg/L of zinc chloride, and is viewed as the 96-h LC_{50} value. This indicated that zinc chloride acute toxicity, was exacerbated in *Channa punctatus* depending on the dosage of exposure.

Table 1.2 Mortality Response of *Channa punctatus* Exposed to Lead Acetate for 96 Hours

Concentration (mg/L)	No. of Fish Exposed	No. of Dead Fish	Mortality (%)	Survival (%)
Control	10	0	0	100
10	10	1	10	90
15	10	2	20	80
20	10	3	30	70
25	10	5	50	50
30	10	7	70	30
35	10	8	80	20
40	10	10	100	0

Calculated 96-h LC_{50} value of Lead Acetate = 25.00 mg/L

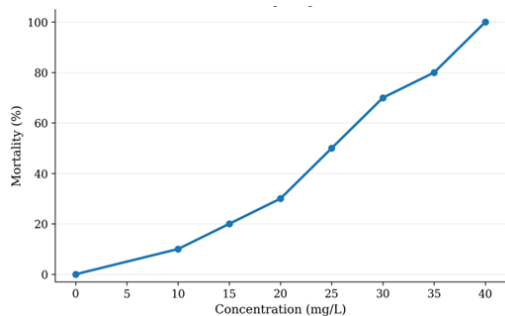


Figure 1.2. Concentration-mortality response of *Channa punctatus* exposed to lead acetate for 96 hours.

The mortality response of *Channa punctatus* on exposure to increasing concentrations of lead acetate increased stepwise with increasing concentration. Death was not recorded from unexposed control fishes. Death in exposed fishes occurred within a concentration range in 96 hr. The LC₅₀ value was recorded at 25.00 mg/L (half of the series of 50% mortality). Compared with zinc chloride, mortality occurred at lower concentration with lead acetate indicating higher acute toxicity. The steep gradient curve at higher concentration suggest severe physiological distress and impairment of normal mechanisms of rest and regulation.

Table 1.3 Probit Analysis for Determination of 96-h LC₅₀ of Zinc Chloride

Concentration (mg/L)	Log Concentration	Mortality (%)	Probit Value
20	1.301	10	3.72
30	1.477	20	4.16
40	1.602	40	4.75
50	1.699	50	5.00
60	1.778	70	5.52
70	1.845	90	6.28

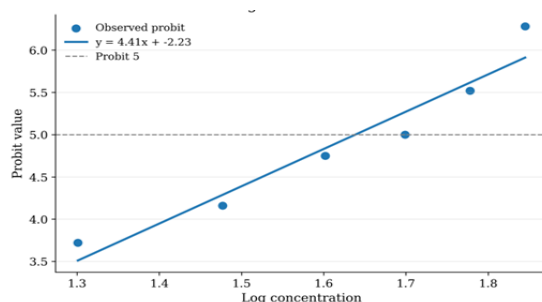


Figure 1.3. Probit regression graph for determination of 96-hour LC₅₀ value of zinc chloride in *Channa punctatus*.

The probit regression graph for zinc chloride showed a positive relationship between log concentration and probit mortality response. As the concentration of zinc chloride increased, the probit value also increased, confirming a concentration-dependent toxic response. The regression line crossed probit value 5 at approximately 50.00 mg/L, indicating the 96-hour LC₅₀ value or the concentration of zinc chloride required to kill 50% of exposed fishes was 50.00 mg/L. The probit analysis confirms the reliability of acute toxicity assessment and supports selection of sub-lethal concentration for chronic exposures in studies.

Table 1.4 Probit Analysis for Determination of 96-h LC₅₀ of Lead Acetate

Concentration (mg/L)	Log Concentration	Mortality (%)	Probit Value
10	1.000	10	3.72
15	1.176	20	4.16
20	1.301	30	4.48
25	1.398	50	5.00
30	1.477	70	5.52
35	1.544	80	5.84

Table 1.5 Summary of LC₅₀ and Selected Sub-lethal Concentrations

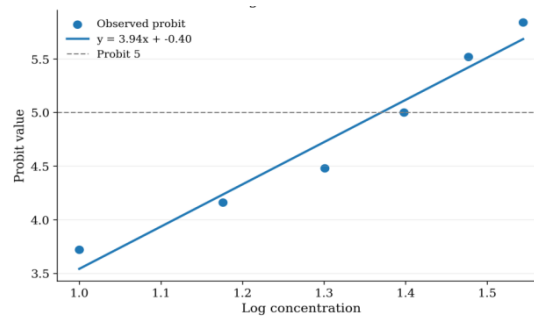


Figure 1.4. Probit regression graph for determination of 96-hour LC₅₀ value of lead acetate in *Channa punctatus*.

The probit regression graph of lead acetate showed a clear increase in probit values with increasing log concentration. The LC₅₀ value was determined where the regression line crossed probit 5, corresponding to 25.00 mg/L. This lower LC₅₀ value indicates greater toxicity of lead acetate compared with zinc chloride. The linear trend in probit response confirms that mortality was directly related to toxicant concentration. The result suggests that lead acetate strongly affects survival of *Channa punctatus* even at comparatively lower concentrations, making it a more potent aquatic toxicant.

Toxicant	96-h LC ₅₀ Value	1/10th of LC ₅₀	Selected Sub-lethal Concentration
Zinc Chloride	50.00 mg/L	5.00 mg/L	5.00 mg/L
Lead Acetate	25.00 mg/L	2.50 mg/L	2.50 mg/L

LC₅₀ values were calculated by Finney's probit method after 96-hour acute toxicity exposure.

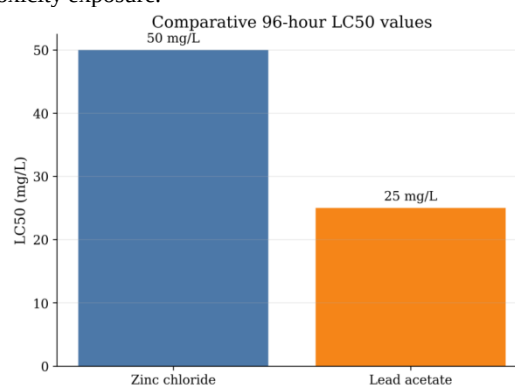


Figure 1.5. Comparative 96-hour LC₅₀ values of zinc chloride and lead acetate in *Channa punctatus*.

The comparative LC₅₀ indicated that the value for zinc chloride is 50.00 mg/L on 96 hour LC₅₀ as against 25.00mg/L for lead acetate. The graph indicates that lead acetate is more toxic than Zinc chloride for *Channa punctatus*. Since less lead acetate is needed to produce mortality as compared to zinc concentration, this makes lead acetate a more potent toxicant acutely.

1.3 Behavioural Alterations During Acute Toxicity Exposure

Table 1.6 Behavioural Changes Observed in *Channa punctatus* During 96-h Exposure to Zinc Chloride and Lead Acetate

Behavioural Response	Control Group	Zinc Chloride Exposure	Lead Acetate Exposure
Swimming activity	Normal	Mild to moderate erratic swimming	Marked erratic swimming
Opercular movement	Normal	Increased	Highly increased
Surface gulping	Absent	Mild	Moderate to severe
Mucus secretion	Normal	Increased	Highly increased
Body balance	Normal	Slight loss of balance	Frequent loss of balance
Response to stimulus	Active	Reduced	Highly reduced
Restlessness	Absent	Moderate	Severe

Bottom settling	Occasional	Frequent at higher concentration	Very frequent
Loss of equilibrium	Absent	Observed at high dose	Prominent at high dose
Mortality	Nil	Concentration-dependent	Concentration-dependent

Control fishes behaved normally, displayed active and proper response to external stimuli; exhibited normal opercular movement and showed no signs of stress. Fishes in progressively acute stage of poisoning experiment occasionally displayed increased opercular movement, erratic swimming, excessive mucus secretion, exhibited surface gulping and show less response to stimulus; all these changed to varied extent depending on dose and duration of exposure.

Fishes exposed to lead acetate showed more severe disturbance of behaviour; markedly restless, rapid opercular movement, frequently exhibited surface gulping due to loss of equilibrium and tended to settle at bottom at higher concentration. The greater severity of the disturbance of behaviour showed that stress level of toxic action was greater in *Channa punctatus* during acute exposure by lead acetate.

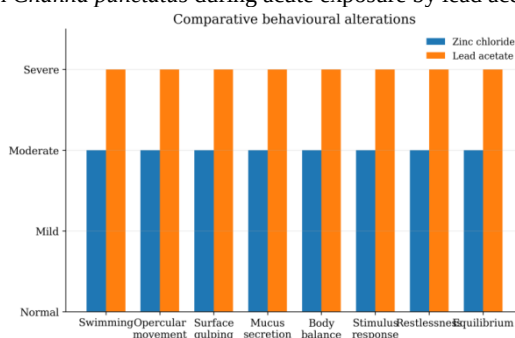


Figure 1.6. Comparative behavioural alterations observed in *Channa punctatus* during acute exposure to zinc chloride and lead acetate.

Behavioural changes were noted in fishes that were exposed to zinc chloride and lead acetate. Control fishes exhibited normal swimming, reactivity, and normal opercular movements. Opercular movements were increased; erratic swimming, surface gulping, mucus secretion, and restlessness were observed. Fishes showed decreased response to stimulus and loss of equilibrium. Observed characteristics increased with the time of toxicant contact. Lead acetate induced greater departure from normal behaviour than did zinc chloride. These indicate that fishes were subject to respiratory distress, impairment of nervous functions, disorder of osmoregulation, and physiological distress. Behavioural changes (departure from normal) indicated early signs of heavy metal toxicity.

1.4 Summary of Acute Toxicity Results

Table 1.7 Comparative Acute Toxicity of Zinc Chloride and Lead Acetate in *Channa punctatus*

Toxicant	96-h LC ₅₀ Value	Toxicity Level	Behavioural Severity	Relative Toxicity
Zinc Chloride	50.00 mg/L	Moderate	Moderate	Less toxic
Lead Acetate	25.00 mg/L	High	Severe	More toxic

The 96-h LC₅₀ value of zinc chloride was recorded as 50.00 mg/L, whereas the LC₅₀ value of lead acetate was 25.00 mg/L. Since a lower LC₅₀ value indicates higher toxicity, lead acetate was found to be more toxic than zinc chloride in *Channa punctatus*. The behavioural observations also supported this result, as lead-exposed fishes showed more severe signs of physiological stress.

2. Kidney Function Parameters

Table 2.1 Comparative Effect of Zinc Chloride and Lead Acetate on Blood Urea Level in *Channa punctatus*

Exposure Period	Control (mg/d L)	Zinc (mg/d L)	Lead (mg/d L)	Zinc vs Control (%)	Lead vs Control (%)	Significance
7 Days	18.64 ± 0.82	22.38 ± 1.04	24.16 ± 1.12	+20.06	+29.61	b

15 Days	18.71 ± 0.79	27.46 ± 1.26	30.84 ± 1.38	+46.77	+64.83	c
30 Days	18.58 ± 0.86	34.72 ± 1.54	39.68 ± 1.72	+86.87	+113.56	d
45 Days	18.69 ± 0.81	41.86 ± 1.83	48.42 ± 2.06	+123.97	+159.07	e
60 Days	18.62 ± 0.84	49.74 ± 2.18	59.36 ± 2.42	+167.13	+218.80	f

Blood urea level increased significantly in both zinc chloride and lead acetate exposed groups. The elevation was exposure-duration dependent, with the highest value recorded after 60 days. Lead acetate produced a greater increase than zinc chloride, indicating more severe renal dysfunction and impaired nitrogenous waste excretion.

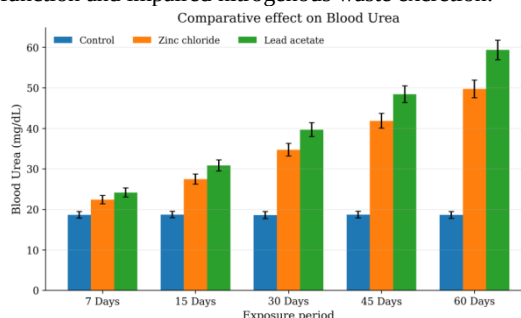


Figure 2.1. Comparative effect of zinc chloride and lead acetate on blood urea level in *Channa punctatus*.

Blood urea level increased significantly in both zinc chloride and lead acetate exposed fishes. Urea is a nitrogenous waste product, and its elevation indicates of renal excretion or altered protein catabolism. The increase was time - dependent and the highest level was recorded after 60 days. More increase with lead acetate than zinc chloride denotes greater nephrotoxicity. Increased urea indicates reduced efficiency of kidneys in eliminating by-products of metabolism. This result clearly indicates that chronic heavy metal condition disturbed renal function and nitrogen metabolism in *Channa punctatus*.

Table 2.2 Comparative Effect of Zinc Chloride and Lead Acetate on Serum Creatinine Level in *Channa punctatus*

Exposure Period	Control (mg/d L)	Zinc (mg/d L)	Lead (mg/d L)	Zinc vs Control (%)	Lead vs Control (%)	Significance
7 Days	0.62 ± 0.04	0.78 ± 0.05	0.84 ± 0.05	+25.81	+35.48	b
15 Days	0.63 ± 0.03	0.96 ± 0.06	1.08 ± 0.07	+52.38	+71.43	c
30 Days	0.61 ± 0.04	1.21 ± 0.08	1.39 ± 0.09	+98.36	+127.87	d
45 Days	0.62 ± 0.03	1.48 ± 0.10	1.76 ± 0.11	+138.71	+183.87	e
60 Days	0.63 ± 0.04	1.82 ± 0.12	2.24 ± 0.14	+188.89	+255.56	f

Serum creatinine concentration increased progressively in treated fishes. The highest creatinine value was observed in lead acetate exposed fishes after 60 days. Increased creatinine level indicates impaired renal filtration, reduced excretory efficiency, and progressive nephrotoxicity.

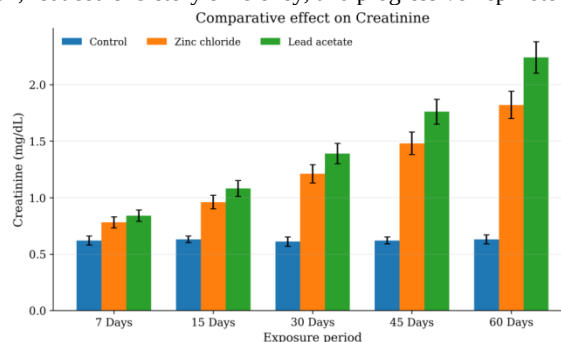


Figure 2.2. Comparative effect of zinc chloride and lead acetate on

serum creatinine level in *Channa punctatus*.

Serum creatinine level was higher in treated fishes compared to control fishes. Creatinine, an important indicator of the kidney's filtering ability, was elevated in the treated fishes indicating diminished glomerular filtration, impaired function, and resultant build-up of metabolic waste. Increase in serum creatinine was gradual in those fishes treated with zinc chloride while fishes on lead acetate had high serum creatinine level. Maximum value (day 60) indicates chronic renal affliction. Since amorphous crytalline creatinine in serum was excreted mainly through the kidneys as urine - raised levels in serum confirm nephrotoxic damage. This result establishes further that lead acetate produced more renal toxicity in *Channa punctatus* than zinc chloride.

Table 2.3 Comparative Effect of Zinc Chloride and Lead Acetate on Serum Uric Acid Level in *Channa punctatus*

Expo sure Perio d	Control (mg/dL)	Zinc (mg/d L)	Lead (mg/d L)	Zinc vs Contr ol (%)	Lead vs Contr ol (%)	Signi fican ce
7 Days	2.18 ± 0.11	2.64 ± 0.13	2.82 ± 0.14	+21.1 0	+29.3 6	b
15 Days	2.21 ± 0.10	3.16 ± 0.15	3.48 ± 0.17	+42.9 9	+57.4 7	c
30 Days	2.19 ± 0.12	3.84 ± 0.18	4.36 ± 0.21	+75.3 4	+99.0 9	d
45 Days	2.20 ± 0.11	4.58 ± 0.22	5.34 ± 0.25	+108. 18	+142. 73	e
60 Days	2.18 ± 0.10	5.42 ± 0.26	6.48 ± 0.31	+148. 62	+197. 25	f

Serum uric acid level increased significantly in both heavy metal exposed groups. The rise was more marked in lead acetate exposure. Elevated uric acid suggests impaired renal clearance, altered purine metabolism, and increased metabolic stress under chronic heavy metal toxicity.

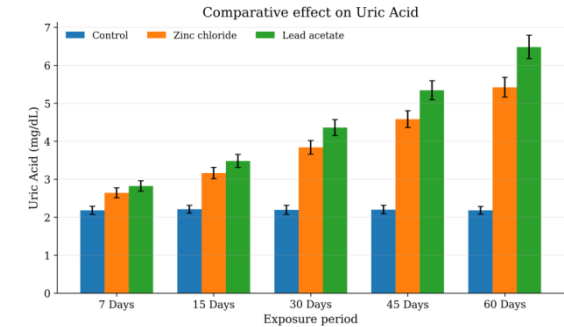


Figure 2.3. Comparative effect of zinc chloride and lead acetate on serum uric acid level in *Channa punctatus*.

Serum uric acid level increased significantly in zinc chloride and lead acetate exposed groups. Elevated uric acid indicates impaired renal clearance, altered purine metabolism, and increased metabolic stress. The increase was progressive from 7 to 60 days, showing duration-dependent nephrotoxicity. Lead acetate caused a greater rise than zinc chloride, suggesting stronger renal damage. Accumulation of uric acid reflects reduced excretory ability of kidney tissues under heavy metal stress. This result confirms that chronic exposure disturbed nitrogenous waste metabolism and renal function in *Channa punctatus*.

Table 2.4 Comparative Effect of Zinc Chloride and Lead Acetate on Blood Urea Nitrogen (BUN) Level in *Channa punctatus*

Expos ure Period	Contr ol (mg/d L)	Zinc (mg/d L)	Lead (mg/d L)	Zinc vs Contr ol (%)	Lead vs Contr ol (%)	Signi fica nce
7 Days	8.71 ± 0.38	10.46 ± 0.49	11.29 ± 0.52	+20.0 9	+29.6 2	b
15 Days	8.74 ± 0.37	12.83 ± 0.59	14.41 ± 0.64	+46.8 0	+64.8 7	c
30 Days	8.68 ± 0.40	16.23 ± 0.72	18.54 ± 0.80	+86.9 8	+113. 59	d
45 Days	8.73 ±	19.56	22.63	+124.	+159.	e

Days	0.38	± 0.86	± 0.96	05	22	
60 Days	8.70 ± 0.39	23.24 ± 1.02	27.74 ± 1.13	+167. 13	+218. 85	f

BUN level showed a significant and progressive increase in zinc chloride and lead acetate exposed fishes. The maximum rise was observed after 60 days, particularly in the lead acetate group. Increased BUN confirms renal impairment and reduced elimination of nitrogenous metabolic waste.

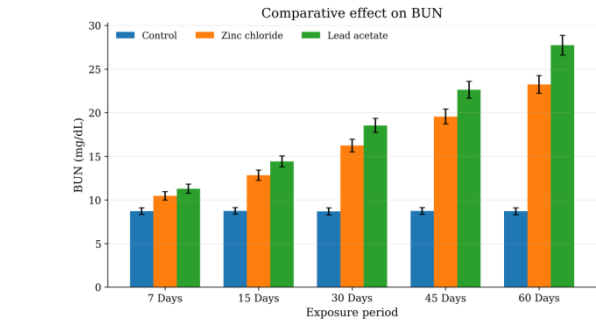


Figure 2.4. Comparative effect of zinc chloride and lead acetate on blood urea nitrogen (BUN) level in *Channa punctatus*.

Blood urea nitrogen level increased continuously in both treated groups. BUN is directly related to urea metabolism and renal excretory performance. Increased BUN indicates reduced elimination of nitrogenous wastes and impaired kidney function. The rise was more pronounced in lead acetate-treated fishes, especially at 45 and 60 days. This suggests that lead acetate produced more severe nephrotoxic effects than zinc chloride. The progressive elevation of BUN confirms duration-dependent renal impairment. Thus, BUN alteration provides strong biochemical evidence of heavy metal-induced nephrotoxicity in *Channa punctatus*.

Table 2.5 Comparative Effect of Zinc Chloride and Lead Acetate on Serum Sodium Level in *Channa punctatus*

Exp osu re Perio d	Contr ol (mmo l/L)	Zinc (mmol /L)	Lead (mmol /L)	Zinc vs Contr ol (%)	Lead vs Contr ol (%)	Significa nce
7 Day s	142.3 6 ± 3.18	136.42 ± 2.86	133.7 4 ± 2.72	-4.17	-6.05	b
15 Day s	142.1 8 ± 3.05	130.86 ± 2.64	126.5 8 ± 2.48	-7.96	-10.97	c
30 Day s	142.5 2 ± 3.22	124.74 ± 2.36	118.9 2 ± 2.21	-12.48	-16.56	d
45 Day s	142.2 9 ± 3.14	118.36 ± 2.18	111.4 8 ± 2.05	-16.82	-21.65	e
60 Day s	142.4 4 ± 3.26	112.68 ± 2.04	104.8 6 ± 1.92	-20.89	-26.38	f

Serum sodium level decreased progressively in zinc chloride and lead acetate exposed fishes. The reduction was more pronounced in lead acetate treatment, especially at 45 and 60 days. Decreased sodium concentration indicates disturbance in osmoregulation, impaired renal tubular reabsorption, and electrolyte imbalance caused by chronic heavy metal exposure.

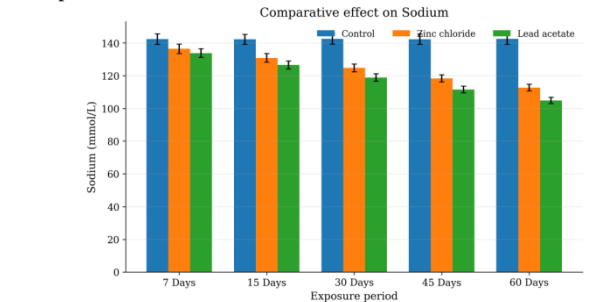


Figure 2.5. Comparative effect of zinc chloride and lead acetate on serum sodium level in *Channa punctatus*.

Serum sodium level decreased progressively in zinc chloride and lead acetate exposed fishes. Sodium is essential for osmoregulation, nerve function, and maintenance of extracellular fluid balance. Reduced sodium concentration indicates impaired renal tubular reabsorption and disturbed ionic regulation. The decline was greater in lead acetate exposure, showing stronger renal and osmoregulatory stress. The maximum decrease after 60 days suggests chronic disruption of electrolyte balance. This result indicates that heavy metal exposure affected kidney-mediated ion regulation and disturbed physiological homeostasis in *Channa punctatus*.

Table 1.2 Comparative Effect of Zinc Chloride and Lead Acetate on Serum Potassium Level in *Channa punctatus*

Exposure Period	Control (mmol/L)	Zinc (mmol/L)	Lead (mmol/L)	Zinc vs Control (%)	Lead vs Control (%)	Significance
7 Days	4.62 ± 0.18	5.18 ± 0.22	5.36 ± 0.24	+12.12	+16.02	b
15 Days	4.59 ± 0.17	5.74 ± 0.26	6.12 ± 0.29	+25.05	+33.33	c
30 Days	4.64 ± 0.19	6.46 ± 0.31	7.08 ± 0.34	+39.22	+52.59	d
45 Days	4.61 ± 0.18	7.24 ± 0.36	8.16 ± 0.41	+57.05	+77.01	e
60 Days	4.63 ± 0.20	8.06 ± 0.42	9.42 ± 0.48	+74.08	+103.46	f

Serum potassium concentration increased significantly in treated fishes compared with control. The rise was exposure-duration dependent and was highest in the lead acetate group after 60 days. Increased potassium may be associated with renal tubular dysfunction, cellular injury, leakage of intracellular potassium, and impaired ionic regulation.

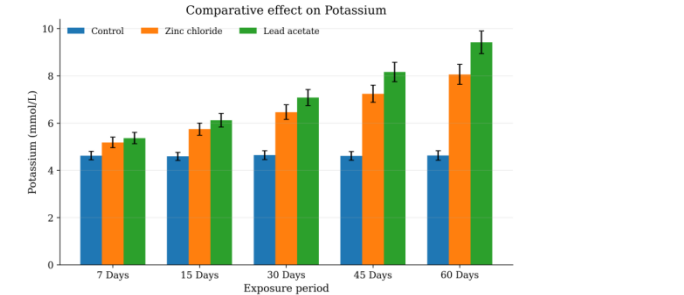


Figure 2.6. Comparative effect of zinc chloride and lead acetate on serum potassium level in *Channa punctatus*.

Serum potassium concentration increased significantly in both metal-exposed groups. Potassium is mainly an intracellular ion, and its elevation in serum may result from cellular injury, membrane damage, and impaired renal excretion. Zinc chloride caused a moderate increase, while lead acetate produced a stronger rise. The increase became more pronounced with prolonged exposure, especially after 60 days. Elevated potassium indicates renal tubular dysfunction and disturbance in cellular ion transport. This result confirms that heavy metal exposure caused serious electrolyte imbalance and nephrotoxic stress in *Channa punctatus*.

Table 2.7 Comparative Effect of Zinc Chloride and Lead Acetate on Serum Chloride Level in *Channa punctatus*

Exposure Period	Control (mmol/L)	Zinc (mmol/L)	Lead (mmol/L)	Zinc vs Control (%)	Lead vs Control (%)	Significance
7 Days	106.48 ± 2.34	101.62 ± 2.18	99.84 ± 2.06	-4.56	-6.24	b
15 Days	106.31 ± 2.26	96.74 ± 1.96	93.58 ± 1.84	-9.00	-11.97	c
30 Days	106.56 ± 2.41	91.36 ± 1.82	86.72 ± 1.68	-14.2	-18.62	d

				6		
45 Days	106.42 ± 2.29	85.48 ± 1.64	79.36 ± 1.52	-19.68	-25.43	e
60 Days	106.67 ± 2.38	79.24 ± 1.48	71.86 ± 1.36	-25.72	-32.63	f

Serum chloride level declined significantly in zinc and lead exposed fishes. The reduction was more severe in lead acetate exposure. Decreased chloride level indicates altered acid-base balance, renal tubular disturbance, and osmoregulatory stress under chronic heavy metal toxicity.

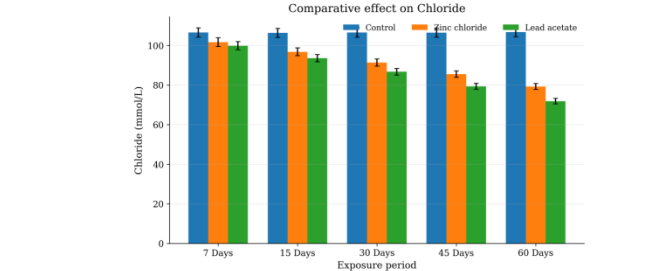


Figure 2.7. Comparative effect of zinc chloride and lead acetate on serum chloride level in *Channa punctatus*.

Serum chloride level decreased gradually in zinc chloride and lead acetate exposed fishes. Chloride is important for osmotic pressure, acid-base balance, and electrical neutrality of body fluids. Its reduction indicates impaired renal tubular function, disturbed acid-base regulation, and osmoregulatory imbalance. The decline was more severe in lead acetate-treated fishes, showing greater toxicity. The lowest chloride level after 60 days reflects chronic disruption of ionic homeostasis. This result supports the conclusion that heavy metals produced renal dysfunction and electrolyte imbalance in *Channa punctatus*.

3. Histological Examination

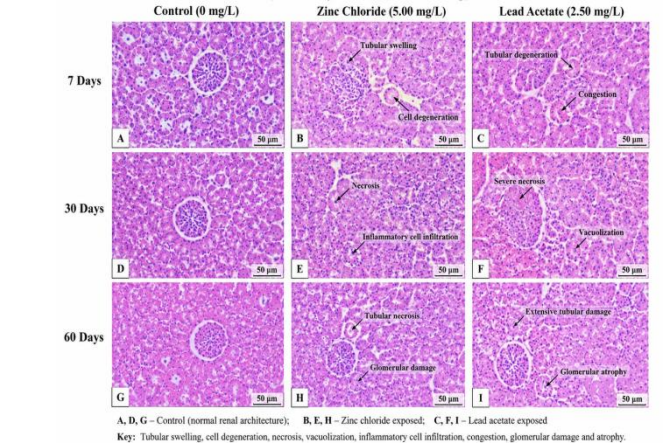


Figure 3.1 Histopathological alterations in kidney of *Channa punctatus* following zinc and lead exposure.

Discussion

The study showed that significant toxicity affecting the kidneys was noted in the species *Channa punctatus* resulting from the use of zinc chloride and lead acetate. In this case, the lead compound was reportedly around what can be described as 25.00 mg/L or lower than zinc chloride of 50.00 mg/L which means that the level of toxicity for lead exceeds the use of zinc chloride. On top of that, some behavioral changes, namely erratic swimming, increased amount of opercular movement and surface gulping without movement against the current of water and excessive production of mucus were noted after prolonged exposure to zinc chloride and lead acetate. Also, in case of exposure to lead acetate for 60 days levels of urea increased by 218.80%, levels of creatinine increased by 255.56%, levels of uric acid improved by 197.25% and the levels of BUN increased by 218.85% relative to the level of the compound administered. Changes in the levels of nitrogenous wastes indicate that kidney could no longer perform its excretory functions effectively as far as animals used in the study were concerned. Therefore, it is quite apparent why heavy metals accumulate in fish and how their distribution is affected by various parameters having to do with water composition as well as the conditions of exposure and tissue composition. As a matter of fact, heavy metals, including zinc accumulate in fish tissues over time leading to toxic effects due to the increased concentration of these heavy metals

(Murugan *et al.*, 2008). Also, there are some electrolyte changes in the serum of fish caused by use of both zinc chloride and lead acetate. The level of sodium was reduced while the amount of potassium increased in the serum of fish throughout the experiment.

Conclusion

It has been shown in the present experiment that zinc chloride and lead acetate have important nephrotoxic effects, affecting the freshwater fish, *Channa punctatus*. The concentration and exposure time affected the nephrotoxicity of both heavy metals; however, the nephrotoxic effect of lead acetate was relatively more acute because of the low 96-h LC₅₀ value of lead acetate. The increase in serum urea, serum creatinine, uric acid, blood urea nitrogen, and serum potassium, with a decrease in sodium and chloride, will further confirm the kidney damage as due to lack of renal filtration and also loss of electrolyte and osmoregulatory balance caused by nephrotoxicity of heavy metals. Histopathology also confirmed the nephrotoxicity of heavy metals, as indicated by the various forms of damage to tubular epithelium following heavy metal poisoning. The combined results indicate that Zn and Pb have important nephrotoxic effects in freshwater fish, *Channa punctatus* due to prolonged exposure to these heavy metals.

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