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Lead Nitrate Induced Acute Toxicity in the Freshwater Fishes Channa Punctatus and Heteropneustes Fossilis

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Abstract Heavy metal pollution in aquatic bodies has become a matter of great concern. This study examined the acute toxicity of lead nitrate to the freshwater fishes Channa punctatus and Heteropneustes fossilis. They comprise an important link in the food chain, and defilement by heavy metal produces an imbalance in the aquatic environment and other fauna. The fishes were exposed to different concentrations of lead nitrate, and % mortality was recorded during 24, 48, 72, and 96 hours. The lethal concentration (LC50) value of lead nitrate for 96 hours against C. punctatus and H. fossilis were found 158.171 and 280.074 mg/l, respectively, using the Probit analysis statistical method. The mortality of the fish was directly proportional to the concentration. The results indicated that a lower concentration of lead was highly toxic to C. punctatus than H. fossilis. Assessment of LC50 also revealed that the amount of lead present in freshwater might be lethal to all the aquatic fauna. Exposure to lead affected human health, which accounted for the death of 1.06 million people and loss of healthy life, amounting to nearly 24.4 million. Hence, strict regulations should be imposed to dispose of heavy metals in aquatic bodies to conserve valuable biological diversity.

Keywords: *lead nitrate, Channa punctatus, Heteropneustes fossilis, LC_{50}, probit*

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1. Introduction

At present, the impact of heavy metals on aquatic fauna is attracting widespread attention, especially in studies linked to industrial contamination [1]. Due to massive discharges of trash and leaching, the quality of both surface and groundwater has been heavily affected [2]. These heavy metals are significant pollutants. Their continuous persistence leads to bioaccumulation and biomagnification, influencing various physiological, cellular, and biochemical functions [3,4]. Due to lead toxicity, USEPA and EPA do not relax in lead contamination in drinking water [5,6]. An extremely high concentration of lead is reported in the sediment of Gomati (~90mg/l) [7] and Kali River (81mg/l) [8]. Fishes live close to their surroundings which contains different heavy metals, bacteria, remnant pharmaceuticals, coliform, microplastics, pesticides, etc. Therefore, cumulative impacts of these toxicants may cause harmful effects on aquatic flora, fauna, and other living beings of the food web [9,10]. Fishes, being dominant dwellers of the underwater world, are regarded as good indicators for heavy metal pollution [11,12].

Among the heavy metals, lead (Pb) is a major aquatic contaminant in various parts of the world. It is also considered an acutely toxic and non-biodegradable element [13,14]. Pb is graded number second on the top 20 list of ATSDR's and known as a puissant environmental contaminator [15]. The accumulation of Pb in the environment is primarily through the exhaust pipes of various automobiles in gasoline and has wide usage in paint [16]. The industrial process contributing to lead are the plumbing of pipes, lead ammunition, accumulator batteries, pewterware, toys, and faucets, etc. [17,18]. In freshwater, Pb predominantly exists as a divalent cation (Pb^{2+}) under the acidic state and formulates lead carbonate ($PbCO_3$) and hydroxide (Pb $(OH)_2$) under alkaline conditions. Generally, Pb^{2+} also exists in free ionic form, and in this form, it is bioavailable and most toxic. As a result, Pb becomes one of the dangerous environmental poisons encountered in everyday life, which gets into the environment and eventually enters the human and animal bodies [19].

The acute toxicity test is used to evaluate the concentration of test material or the amount of toxicant that induces a pernicious effect on a group of test organisms in a short-term exposure under monitored conditions [20]. Toxicity is attributed to an individual organism's response to a chemical at a specific concentration or dosage for a particular period [21]. In the present study, attempts were made to determine the

comparative research on the acute toxicological effects of exposure of lead nitrate [$Pb(NO_3)_2$] on edible and freshwater fishes, C. punctatus, and H. fossilis.

2. Materials and Methods

2.1. Test Fishes and Their Collection

Healthy and disease-free fishes, *C. punctatus* 'Bajaria' (weight 110-125 gm, length 16-18 cm, n= 40) and *H. fossilis* 'Singhi' (weight 50-60 gm, length 14-16 cm, n= 40) were collected from an individual group with the help of fisherman from local fish markets of Rithora and Dohra, Bareilly (U.P) respectively.

2.2. Acclimatization and Feeding

The fishes were firstly treated with a solution of 0.02% $KMnO_4$ for 2 minutes to remove any dermal ailments. They were acclimatized for 10 days in the laboratory in glass aquaria (75×38×30 cm) having 20-liter non-chlorinated tap water and were fed on pelleted diet. Maintenance of aquaria was done using aerated tubed motorized pump to avoid hypoxic environment. For covering of aquaria, monofilament netting was used to obstruct the fishes from jumping out of the testing experiment. Feeding was ceased 24 hours before the initiation of the experiment. Water in the aquaria was changed every 24 hours, leaving no defecation, residual food, or dead fish, and well kept in a 12-12-hour photoperiod during acclimatization and testing periods.

2.3. Preliminary Tests

The physicochemical properties of the tap water were examined by following precautionary measures and procedures as given by APHA [22]. Tap water used for maintaining fish in aquaria had a pH 6.9-7.2, temperature ranged from 26-28 °C, dissolved oxygen 9.5 to 10.0 mg/l, and total hardness 255-260 mg/l as $CaCO_3$.

2.4. Test Chemical

Analytical grade lead nitrate $Pb(NO_3)_2$ in white powdered form, (Purity 98.5 %, molecular weight 331.21 g/mol) Merck, India (Ltd.) was prepared in water and the test concentration was prepared by dilution.

2.5. Acute Toxicity Test

Short-term acute toxicity tests were conducted by adopting the renewal bioassay method followed by [23,24,25] guidelines throughout 96h, using different concentrations of toxicants.

The experiment was performed in a static system in glass aquaria and the acclimatized fishes were grouped into four experimental groups each consisting of ten fishes. Group I fishes were maintained as control without any treatment, groups II, III, and IV fishes were treated to different concentrations in mg/l concentrations of toxicants for 96 hours mentioned in Table 1.

The sub-lethal concentration of test chemicals was freshly prepared in distilled water before mix up in aquaria water. The concentration of each test media was increased in a gradual manner and the amount of metal concentration was maintained to as per mortality rate from 0 % to 100%. Few pilot tests were conducted for the selection of lethal concentration and the different range of concentrations was selected to examine the rate of mortality. The mortalities were observed at the intervals of 24, 48, 72, and 96 hours of the experiment, and dead fishes were removed instantaneously from the test medium to prevent any organic deterioration and oxygen deduction.

Each experiment was repeated thrice to attain constant findings. The categorization of experimental groups based on the LC_{50} value and from the literature survey of the highest level of Pb contamination of natural freshwater bodies.

2.6. Behavioral Responses

During the acute toxicity bioassay, behavioral responses of fish such as convulsions, equilibrium status, fin movement, hyperactivity, and swimming rate in exposed as well as the control group was noted as suggested by Rand [26].

2.7. Evaluation of Median Lethal Concentration (LC_{50})

The concentration of the toxicant at which 50 percent of the test species dies during a particular period or the concentration lethal to one-half of the test population is referred to as median lethal concentration (LC_{50}) or median tolerance limit.

The LC_{50} values of toxicants were estimated by using the following methods:

i. Direct interpolation method: On transforming toxicity curve plotted between % mortality vs. concentration for 96 hours.

ii. Finney's Probit method: It is the standard method to examine the dose-response data. The Probit values of % mortality was obtained from Finney's table [27] given below (Figure 1). The curve was plotted between the log concentrations and Probit kill values by drawing a line corresponding to the 50% mortality at 96 hours.

iii. By SPSS Probit analysis: The actual LC_{50} data was analyzed using Probit analysis with SPSS 21 statistical analysis software. In the parametric procedure and long history of statistical applications, Probit analysis is based on linear regression technique following transformation of toxicity data. The LC_{50} value (with 95% confidence limits), the correlation between mortality (Probit) against the $\log_{10}$ concentrations and the regression line equation were also obtained through best-fit line.

Compliance with Ethical Standards: Disclosure of potential conflicts of interest: Authors declare that they have no conflicts of interest.

Ethical Statement: Authors declare that the study does not involve any work on humans. Ethical clearance was obtained for the present work.

Table 1. Experimental design for the evaluation of LC_{50} of $Pb(NO_3)_2$ in *C. punctatus* and *H. fossilis*

Toxicant	Fish Species	Sub Lethal Doses (mgl^{-1})			
		Group I	Group II	Group III	Group IV
Lead nitrate ($Pb(NO_3)_2$)	*C. punctatus*	Control	50	100	200
	H. fossilis		200	250	300

TRANSFORMATION OF PERCENTAGE MORTALITIES TO PROBITS

(Finney. 1971)

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
%	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Figure 1. Finney's table showing transformation of Percentage Mortalities to Probits

3. Results

3.1. Behavioral Changes

During the current study, it was noted that the *C.punctatus* and *H.fossilis* exposed to different concentrations of $Pb(NO_3)_2$ at acute exposure induces behavioral changes. Specifically, at a high concentration of toxicant after 2-3 days, fishes showed darting movement and swimming disturbances. As the concentration of toxicant increases, fishes appeared to induce mucus secretion over the gills causing rapid movement of opercula to make great efforts for movement. However, the control group of fish maintained in normal water were found to be active throughout the experiment. After 3-4 days of toxicant exposure opercula beat counts were found lower than that of the control group. Thus, toxicant entered their body, their mouth remained open with the eventual death of the fishes. The results showed adverse effects of Pb on fishes.

3.2. Acute Toxicity

Lethal toxicities of $Pb(NO_3)_2$ compound were assessed among two freshwater fish *C. punctatus* and *H .fossilis*. The mortality of the fishes was directly proportional to the concentration. No mortality was recorded among the control groups of all the experiments. However, it was also proved that an increase in concentration of test chemical was required as a lethal dose for these freshwater fishes. Total mortality of fish was recorded after exposure of 96 hours.

3.2.1. Acute Toxicity in *C. punctatus* Exposed to $Pb(NO_3)_2$

The concentrations tested for the lethal toxicity of $Pb(NO_3)_2$ ranged from 100 mg/l to 300 mg/l and the 96 h LC_{50} value was worked out by direct interpolation method and Probit SPSS 21 software and estimated LC_{50} value against *C. punctatus* were found 200 and 158.171 mg/l respectively.

Table 2. Relation between concentration of $Pb(NO_3)_2$ and the % mortality of the *C. punctatus*

S.No	Dose ($Pb(NO_3)_2$)mg/l	Log conc.	No. of fish taken	% Mortality after exposure to $Pb(NO_3)_2$				Probit kill Mortality at			
				24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	0	0.00	10	0	0	0	0	0.00	0.00	0.00	0.00
2	100	2.00	10	0	10	20	30	0.00	3.72	4.16	4.48
3	200	2.30	10	0	20	40	50	0.00	4.16	4.75	5.00
4	300	2.47	10	10	30	50	90	3.72	4.48	5.00	6.28

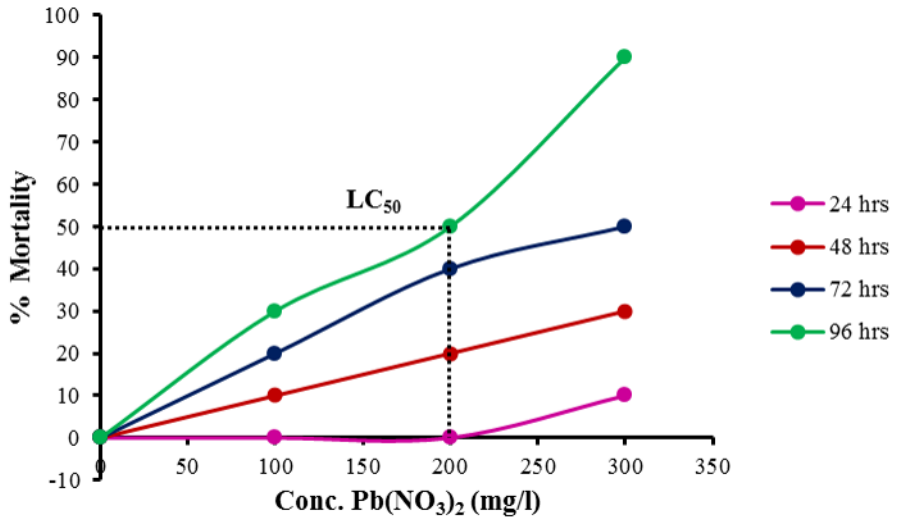

Figure 2. Dose-response curves for $Pb(NO_3)_2$ to the fish *C. punctatus*

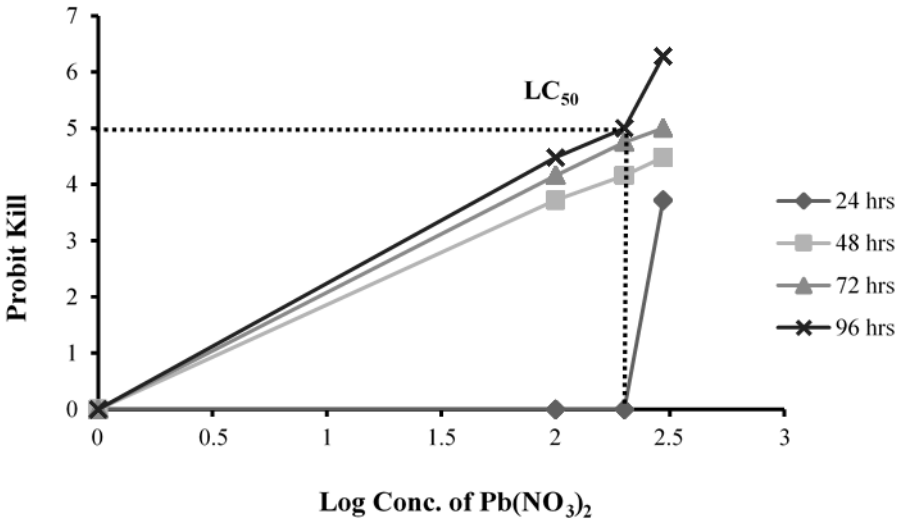

Figure 3. Plot of log Conc. of $Pb(NO_3)_2$ versus Probit kill of *C. punctatus* showing LC_{50} at 96 h

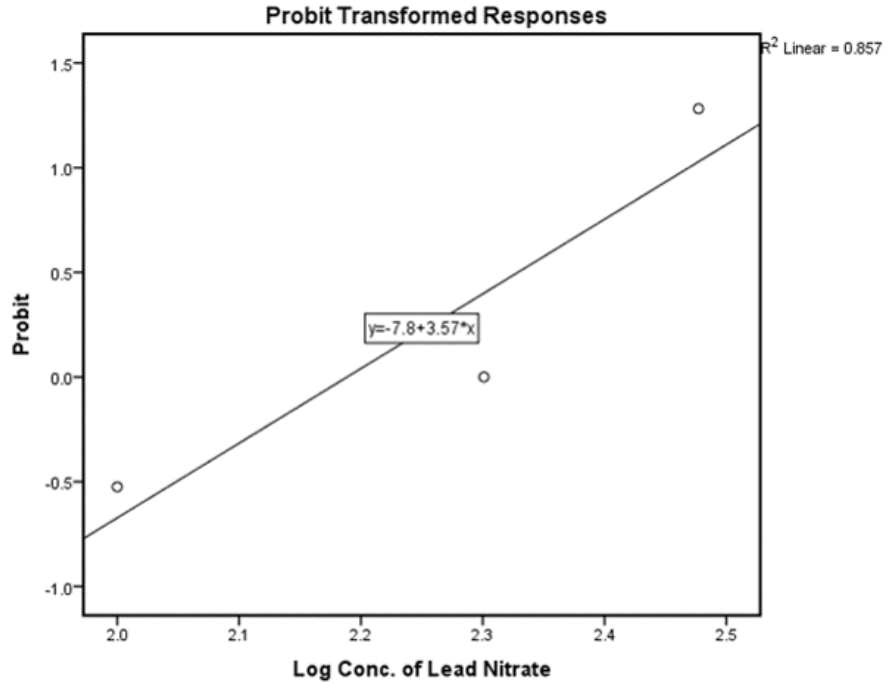

Figure 4. Regression line between the Probit of *C. punctatus* and log conc.of $Pb(NO_3)_2$

Table 3. Lethal concentration of $Pb(NO_3)_2$ on *C. punctatus* depending on time of exposure

Point	Conc.(mg/l)
LC_{10}	65.410
LC_{20}	88.570
LC_{30}	110.207
LC_{40}	132.837
LC_{50}	158.171
LC_{60}	188.338
LC_{70}	227.011
LC_{80}	282.468
LC_{90}	382.483

The mortality of *C. punctatus* at different concentrations of $Pb(NO_3)_2$ along with the Probit kill values are summarized in Table 2 and the dose-response curves are plotted between concentration vs. % mortality occurred at 24, 48, 72, and 96 hours respectively is shown in Figure 2. At 96 h, the mortality of fishes was observed in all groups ranged from 30% to 90% is shown in Figure 3 with Probit kill values and was directly proportional to the concentration with the regression line equation is shown in Figure 4. Exploratory test also showed 100% mortality at above 300 mg/l within 96 hours of exposure. The lethal concentration required to cause 10% mortality in the fish exposed to the toxicant was 65.410 mg/l. This gradually increased from this point until it reached 158.171 mg/l concentration that resulted in 50% mortality of the fish. As the amount of bioaccumulation continued with the increase in exposure period, the concentration required to trigger 90% mortality was 382.483 mg/l. The lethal points are shown in Table 3.

3.2.2. Acute Toxicity in *H. fossilis* Exposed to $Pb(NO_3)_2$

The concentrations tested for the lethal toxicity of $Pb(NO_3)_2$ ranged from 250 mg/l to 350 mg/l and the 96 h LC_{50} value was worked out by direct interpolation method and Probit SPSS 21 software and estimated LC_{50} value against *H. fossilis* were found 300 and 280.074 mg/l respectively.

The mortality of *H. fossilis* at different concentrations of $Pb(NO_3)_2$ along with the Probit kill values are summarized in Table 4 and the dose-response curves are plotted between concentration vs. % mortality occurred at 24, 48, 72, and 96 hours respectively is shown in Figure 5. At 96 h, the mortality of fishes was observed in all groups ranged from 40% to 80% is shown in Figure 6 with Probit kill values and was directly proportional to the concentration with the regression line equation is shown in Figure 7. Exploratory test also showed 100% mortality at above 350 mg/l within 96 hours of exposure. The lethal concentration required to cause 10% mortality in the fish exposed to the toxicant was 185.726 mg/l. This gradually increased from this point until it reached 280.074 mg/l concentration that resulted in 50% mortality of the fish. As the bioaccumulation continued with the increase in exposure period, the concentration required to trigger 90% mortality was 422.351 mg/l. The lethal points are shown in Table 5.

Table 4. Relation between concentration of $Pb(NO_3)_2$ and the % mortality of the *H. fossilis*

S.No	Dose ($Pb(NO_3)_2$) mg/l	Log conc.	No. of fish taken	% Mortality after exposure to $Pb(NO_3)_2$				Probit kill Mortality at			
				24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	0	0.00	10	0	0	0	0	0.00	0.00	0.00	0.00
2	250	2.39	10	0	10	20	40	0.00	3.72	4.16	4.75
3	300	2.47	10	0	10	30	50	0.00	3.72	4.48	5.00
4	350	2.54	10	10	40	70	80	3.72	4.75	5.52	5.84

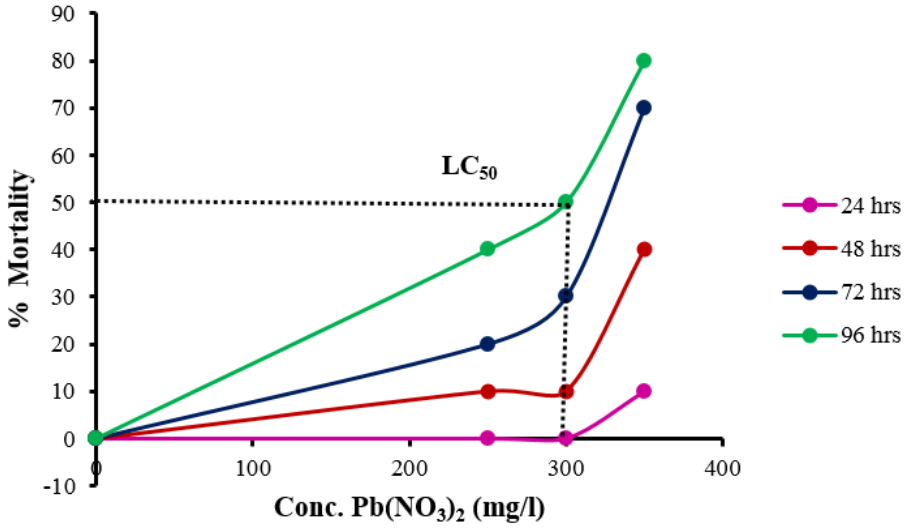

Figure 5. Dose-response curves for $Pb(NO_3)_2$ to the fish *H. fossilis*

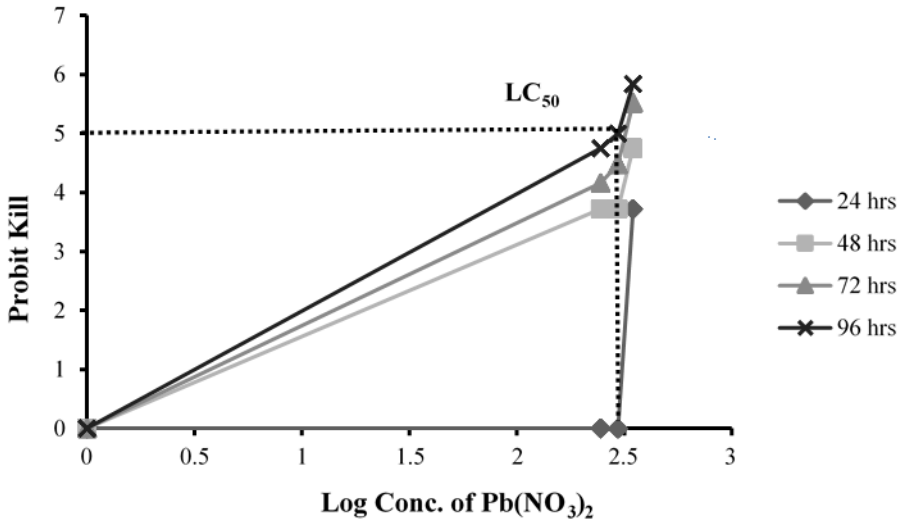

Figure 6. Plot of log Conc. of Pb $(NO_3)_2$ versus Probit kill of *H. fossilis* showing LC_{50} at 96 h

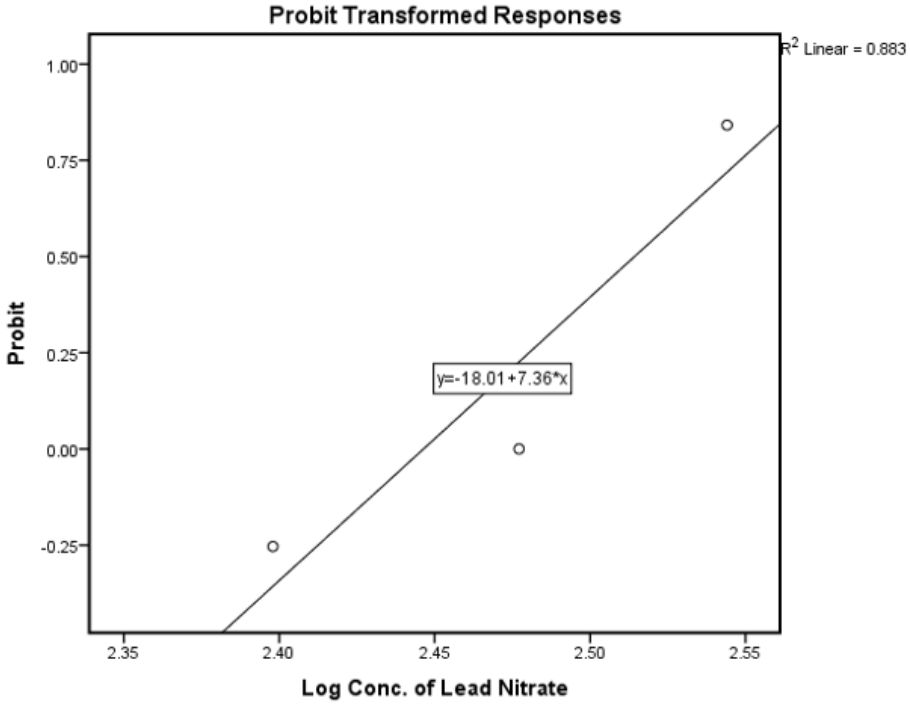

Figure 7. Regression line between the Probit of *H. fossilis* and log conc.of $Pb(NO_3)_2$

Table 5. Lethal concentration of $Pb(NO_3)_2$ on *H. fossilis* depending on time of exposure

Point	Conc.(mg/l)
LC_{10}	185.726
LC_{20}	213.852
LC_{30}	236.741
LC_{40}	258.229
LC_{50}	280.074
LC_{60}	303.767
LC_{70}	331.339
LC_{80}	366.803
LC_{90}	422.351

3.2.3. Relative Toxicity of $Pb(NO_3)_2$ against the Freshwater Fish, *C. punctatus*, and *H. fossilis* at 96 h

LC_{50s} obtained in the present study were compared with each other and findings showed that at 96 h, a lower concentration of Pb is found to be highly toxic to *C. punctatus* as compared to *H. fossilis.* The computed statistical of logistic LC_{50} concentration with 95% confidence limits, regression equation, R-value, standard error, and chi-square are summarized in Table 6.

Table 6. Comparative statistical LC_{50} analysis of Pb $(NO_3)_2$ against *C. punctatus* and *H. fossilis* with SPSS Probit method

Toxicant	Fish Species	LC_{50} Value (mg/l)	Regression equation $Y=y^{-}+(X-x^{-})$	R- Value	S.E	Chi-squa-re	95% Confidence limits	
							Lower Bound	Upper Bound
Lead Nitrate ($Pb(NO_3)_2$)	*C. punctatus*	158.171	-7.8+3.57*x	0.857	.011	1.28	.780	5.904
	H. fossilis	280.074	-18.01+7.36*x	0.883	.077	.463	-.771	15.138

4. Discussion

A bioassay is an essential tool for evaluating the impact and the fate of toxicants in the aquatic environment. Heavy metals from anthropogenic origins, such as Pb, have been admitted as significant contaminants in the aquatic environment [28,29]. In polluted regions, exposure of fish to heavy metals resulted in the transmission of chemicals into the biological system and induced biochemical disturbances [30,31,32]. The amount of Pb present in freshwater bodies varies from 18 to 1,559 µg/L reported by ILA [33] and Church [34].

Alterations in behavior resulting from stress are a very sensitive indicator of potential toxic impacts [35,36,37]. Fish exposed to different doses of toxicants exhibited marked behavioral changes [38]. In the present analysis, darting movement and attempting to escape from toxic water were observed in fishes. Fishes appeared to induce mucus secretion over the gills causing rapid movement of opercula to make great efforts for movement. Similar symptoms were also observed by Senthamilselvan et al. [20] in *Lates calcarifer* exposed to chromium and mercury metal. Kumar et al. [39] observed the initial increase of mucus secretion, loss of buoyancy, and balance with change in body coloration in *Clarias batrachus* after exposure to copper sulfate.

The mortality Probit of LC_{50} values was calculated for the lethal concentration of toxicants $Pb(NO_3)_2$ during acute exposure periods. Results of the current study have showed that the LC_{50} values of *C. punctatus* and *H. fossilis* were found 158.171 mg/l and 280.074 mg/l respectively. From the Probit analysis, the points at which the toxicants began to cause lethal effects on the fish varied significantly [40,41]. The lethal concentration required to provoke 10% mortality in the fish exposed to the toxicants until 50% mortality of fish were also assessed and proved that as the level of bioaccumulation continued may cause lethality to the organisms [42,43]. It is evident from the results that the concentration of Pb metal has a direct effect on the LC_{50} values. Samuel et al. [44] also done the acute toxicity assessment on *Clarias gariepinus* when exposed to Pb and demonstrated the lethal points from LC_{10} to LC_{95}.

Different researchers have observed similar results on LC_{50} in different fish species in response to various toxicants. Hence, our current findings are in agreement with many workers [29,45,46,47,48,49]. Pandit et al. [50] also reported the LC_{50} of $Pb(NO_3)_2$ in two air-breathing fish, *Channa punctatus* (177.8 mg/l) and *Clarias batrachus* (346.6 mg/l). The toxic effects of Pb on common carp *Cyprinus carpio* was reported by Paul et al. [51] and evaluated LC_{50} were 328 mg/l for 96 hours. Al-Balawi et al. [52] registered the LC_{50} value for the African catfish, *Clarias gariepinus* as 122 mg/l when exposed to different concentrations of lead acetate and further observed the sub-lethal effects on the growth and reproduction of fishes. Exposure of Pb at all the concentrations showed a reduced growth rate and hence, inversely proportional to growth.

Results of the current study showed that the mortality was increased as the concentrations of $Pb(NO_3)_2$ increased in both the freshwater fish *C. punctatus* and *H.fossilis*. These findings were supported with the observation by many researchers in different species exposed to $Pb(NO_3)_2$ toxicant [50,53-59]. Hence, our current findings demonstrated that both the fishes *C. punctatus* and *H. fossilis* are sensitive to $Pb(NO_3)_2$ decreasing their survival capacity when increasing the exposure time. Manjeet [60] explained in his study that there are differences in the value of LC_{50} found in the same fish species for the same heavy metal. Sometimes it is observed that some fishes are very sensitive towards the toxicity caused by one heavy metal and show less sensitivity towards another equally toxic heavy metal at the same concentration. This is attributed to the fact that the accumulation of metals in living organisms depends on the concentration of metal taken up by the organism from their surroundings and mechanism of metal's distribution in their body organs and the inherent ability of fish to concentrate metal [61,62]. Krewski et al. [63] described the toxicity tolerance values varied in different toxicants against different size and test species in a stable environmental condition. Pb has also been shown to inhibit immune phagocytosis and compromised immune activity [64].

The result of the current study showed that the metal Pb was more toxic to *C. punctatus* as compared to *H.fossilis* and induced acute toxic effects in the form of behavioral alterations in both the fishes. Therefore, this study will assist in formulating the use of Pb metal and should be rigorously controlled and regulated by creating suitable legislation to impede its bioaccumulation in the environment that mitigates the negative impact on aquatic creatures.

5. Conclusion

Impacts of heavy metals on fish are much more intricate to illustrate due to the dynamical nature of the aquatic ecosystem. Results of the present study conclusively showed that the amount of Pb in water impair feral populations of freshwater fish by altering their physiological behavior and abilities which ultimately affect their diversity and environmental permanence. These alterations might be potentially disruptive to the survivability of the fish in their natural environment and the findings have important implications for ecological risk assessments. Hence, there is a consistent need to determine the toxicity of heavy metals because the consumption of heavy metals in the form of food may impair the health of human beings via the food chain.

Highlights

i) Lead is highly toxic metal.

ii) Lower concentration of lead was highly toxic to *C. punctatus* as compared to *H. fossilis*. iii) Results of LC_{50} revealed that the amount of lead present in freshwater may be lethal to all the aquatic fauna.

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Authors Contribution

SN executed experimental work and prepared manuscript; NG planned, supervised and edited manuscript; VG edited the manuscript; AT executed experimental work.

Conflict of Interest

None

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