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Statistical Analysis of Acute Toxicity and the Effects of Lead Nitrate on the Behavior of Heteropneustes Fossilis(Bloach)

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Abstract: Heavy metals are continually released in to the aquatic environment from natural processes such as volcanic activity, weathering of rocks and industrial processes [24]. Today, aquatic animals are exposed to different concentrations of metals in aquatic environments. These metals tend to accumulate in their bodies which could influence all aspects of organisms' life [5] 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 H.fossilis were found 157.08 mg/l using the Profit analysis statistical method. The mortality of the fish was directly proportional to the concentration. 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: Transportation Problems, Existing Solution Methods, Trapezoidal Fuzzy Number, Ranking of Median Method, Optimal Solution, Decision Making.

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. Due to lead toxicity, USEPA and EPA do not relax in lead contamination in drinking water [3,4]. 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 [5,6]. Pb is graded number second on the top 20 list of ATSDR's and known as a puissant environmental contaminator [7]. The accumulation of Pb in the environment is primarily through the exhaust pipes of various automobiles in gasoline and has wide usage in paint [8]. The industrial process contributing to lead are the plumbing of pipes, lead ammunition, accumulator batteries, pewterware, toys, and faucets, etc. [9,10]. 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 bio available 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 [11]. 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 [12]. Toxicity is attributed to an individual organism's response to a chemical at a specific concentration or dosage for a particular period [13]. In the present study ,attempt were made to determine the acute toxicological effects of exposure of lead nitrate [$Pb(NO_3)_2$] on edible and freshwater fishes, H. fossilis.

Behavioral changes in animals are indicative of internal disturbances of the body functions.(MOREIRA 2008) Moreover, the interaction of fish behavior related to the ecology can be easily observed .Fish behavior, can be defined as an adaptation of fish to the different physical and chemical stimuli associated with any kind of toxicants. The information on fish behavior helps in sustainable harvest and research. Behavioral changes in fish can help in determining and understanding the change in environment.(Hellou,2011) An infected fish shows altered behavior towards toxicants, especially heavy metals.

2. MATERIALS AND METHODS

2.1 Test Finishes and their Collections

Irrespective of the sex, healthy specimens of H.fossilis of 80-82 g of body weight and 27-29 cm length belonging to a single population were collected locally at Chidambaram, Tamil - Nadu, India.

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 with minced goat liver. 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 [14]. Tap water used for maintaining fish in aquaria had a pH 6.9-7.4, temperature ranged from 28-30 °C, dissolved oxygen 9.5 to 10.0 mg/l, and total hardness 40.44 mg/l .

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

Short-term acute toxicity tests were conducted by adopting the renewal bioassay method followed by [15,16,17] 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.

Cumulative Mortality

Concentration ppm	No. of died fish			
	24 h	48 h	72 h	96 h
140	0	0	0	0
150	0	2	6	10
160	0	3	7	12
170	0	5	9	15
180	2	10	12	18

Point	24 h	48 h	72 h	96 h
LC01	168.32	136.85 (110.26-147.30)	121.97 (91.74-134.86)	125.59 (109.93-134.16)
LC10	187.99	155.24 (141.68-161.64)	142.10 (123.26-150.24)	138.87 (128.03-144.92)
LC30	203.67	170.08 (163.66-179.51)	158.74 (150.09-165.28)	149.35 (142.42-153.85)
LC50	215.29	181.19 (173.54-207.18)	171.39 (164.66-184.46)	157.08 (152.23-161.51)
LC70	227.57	193.02 (181.94-227.99)	185.05 (175.30-212.15)	165.20 (160.71-171.67)
LC80	235.34	200.55 (186.90-246.34)	193.83 (181.34-231.80)	170.32 (165.21-179.04)
LC90	246.55	211.48 (193.86-274.49)	206.71 (189.75-262.54)	177.68 (171.12-190.40)
LC99	275.37	239.88 (211.06-355.51)	240.82 (210.72-353.87)	196.45 (185.01-221.54)

Evaluation of Median Lethal Concentration (Lc50)
The concentration of the toxicant at which 50 percent ofthe 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:

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

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

Acute toxicity test

According to results obtain from range finding test, Acute toxicity tests were carried out in order to calculate the 96h-LC for Lead nitrate. Concentration for Acute toxicity tests were [control, 140,150,160,170,180 and 190 ppm]. Mortality was recorded after 24, 48, 72 and 96h and LC_{50} values and its confidence limits [95%] were calculated by Boudou and Ribeyre [9]. Percentages of fish mortality were calculated for each Cupper Sulphat concentration at 24, 48, 72 and 96 h of exposure.

Also LC_{50} values were calculated from the obtained data in acute toxicity bioassays, by Finney's method of ''probit analysis'' and with SPSS computer statistical software. In Finney's method, the LC_{50} value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis [10].

The LC1,10,30,50,70,80,90,99 values in Table – 2 were derived using simple substitution probit of 1,10,30,50,70,80,90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. Lead nitrate. The 95% confidence limits for LC_{50} were estimated by using the formula LC_{50} [95% CL] = LC_{50} ±1.96 [SE [LC_{50}].

3. RESULTS

The results from the acute toxicity test as for the $Pb(NO_3)_2$ on Heteropneustes Fossilis are shown in Table =2, and there was no mortality in the control group throughout the experiment are shown in Table -1. Fish mortality was increased significantly when the concentrations and the time of exposure were increased.

During the current study, it was noted that the Heteropneustes *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 fects of $Pb(NO_3)_2$ on fishes. The 96 hrs LC_{50} value of lead nitrate were found to be 157.08 mg/l.

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 [19,20]. In polluted regions, exposure of fish to heavy metals resulted in the transmission of chemicals into the biological system and induced biochemical disturbances [21,22,23]. The amount of Pb present in fresh-

water bodies varies from 18 to 1,559 µg/L reported by ILA [24] and Church [25].
Alterations in behavior resulting from stress are a very sensitive indicator of potential toxic impacts [26.27.28]. Fish exposed to different doses of toxicants exhibited marked behavioral changes [29]. 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. [12] in *Lates calcarifer* exposed to chromium and mercury metal. Kumar et al. [30] 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[31,32]. 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 [33,34]. It is evident from the results thatthe concentration of Pb metal has a direct effect on the LC_{50} values. Samuel et al. [44] also d one the acute toxicity assessment on *Clarias gariepinus* when exposed to Pb anddemonstrated 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 concentration of lead nitrate increased in the freshwater fish *H.fossilis*. These findings were supported with the observation by many researchers in different species exposed to $Pb(NO_3)_2$.

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