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Asim Ullah
Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences Lahore, Pakistan

Hameed Ur Rehman
Department of Chemistry, Kohat University of Science and Technology, Kohat-26000, KPK, Pakistan

Alam Zeb Khan
Department of Zoology, Kohat University of Science and Technology, Kohat-26000, KPK, Pakistan

Ziafat Rehman
Department of Microbiology, Abbottabad University of Science and Technology, Pakistan

Saeed Ahmad
Department of Microbiology, Abbottabad University of Science and Technology, Pakistan

Asima Bakht
Department of Pharmacy, Kohat University of Science and Technology, Kohat-26000, KPK, Pakistan

Mujaddad Ur Rehman
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Zain UlAbdin
Department of Zoology, Shaheed Benazir Bhutto University Sheringal Dir upper, KPK, Pakistan

Zona Amjad
Faculty of Fisheries and Wildlife, University of veterinary and animal sciences Lahore, Pakistan

Muhammad Fahim Qureshi
Department of Zoology, Kohat University of Science and Technology, Kohat-26000, KPK, Pakistan

Correspondence
Hameed Ur Rehman
Department of Chemistry, Kohat University of Science and Technology, Kohat-26000, KPK, Pakistan

Determination of 96-Hr LC_{50} of Lead Nitrate for a Fish, *Oreochromis niloticus*

Asim Ullah, Hameed Ur Rehman, Alam Zeb Khan, Ziafat Rehman, Saeed Ahmad, Mujaddad Ur Rehman, Asima Bakht, Zain Ul Abdin, Zona Amjad, Muhammad Fahim Qureshi, Javid Khan

Abstract
Metals are of special concern because of their diversified effects and the range of concentrations that could cause toxic ill-effects to the fish. Generally, heavy metals exert their toxic effects in organisms by generating reactive oxygen species, causing oxidative stress. This paper emphasizes on the determination of 96-hr LC_{50} value of Lead Nitrate for the fish, *Oreochromis niloticus.* The fish species were acclimatized in laboratory condition for 20 days. The stock solution of lead nitrate was prepared and fish were treated with different gradually increasing concentration of metals for 96 hours. The result showed that LC_{50} of Lead Nitrate for the fish, *Oreochromis niloticus* 44 mg/l. the mortality of the fish is directly proportional to the concentration of the expose metals.

Keywords: 96-Hr LC_{50}, Lead Nitrate, *Oreochromis niloticus.*

Introduction
Fishery resources are an important source of both macro- and micro-nutrients for humans. Internationally fish accounts for about 17 percent of animal protein ingestion show that fish is the most important animal-source food in the diets of more than one billion people [1].
Due to feeding and living in the aquatic environments fish are particularly uncovered and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants [2, 3]. Fish, in contrast with invertebrates, are more sensitive to many toxicants and are a difficult test subject for suggestion of ecosystem health [4, 5].
Heavy metals are produced from a variety of natural and anthropogenic sources [6]. In aquatic environments, heavy metal pollution results from nonstop atmospheric evidence, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, and also via wastewater treatment plants [7, 8].
The many sources of water pollution cause difficult consequences to aquatic life. Fish and aquatic life those are at the top of the aquatic food chain are exposed to higher levels of toxins directly from the polluted water and by feeding on other fishes who are already exposed to high levels of toxins in water [9, 10].
Water pollution with heavy metals affects various physiological processes in fishes including breeding and development. The effects of waterborne metals on fishes are related to their uptake and accumulation by the organism, resulting in metal-induced disturbances in the structure and function of various tissues and organs [11].
Lead as a heavy metal is known to cause lethal effects on aquatic organisms. *Oreochromis niloticus* is a commercial fish and widely preferred as edible fish in Pakistan. It is very important to evaluate edible organisms like *Oreochromis niloticus* from toxicity point of view as health of human being is directly associated with it. During the present course of investigation acute toxicity tests of Lead Nitrate to determine 96-hr LC_{50} have been conducted on *Oreochromis niloticus*, a fish having high nutritional value as well as a it serves as a good pollution indicator.

Material and Methods
The present investigations were conducted at fish seed hatchery, Department of Fisheries and aquaculture, University of Veterinary and Animal Sciences, Ravi Campus. Individuals of *Oreochromis niloticus* were obtained from grow out ponds kept in holding tanks, supplied with flow through aerated water and acclimatized for 2 weeks, in the laboratory before

conducting acute toxicity tests. Laboratory tests were conducted to determine the 96-hr LC_{50} and lethal concentrations of lead (Pb) for *Oreochromis niloticus* at constant temperature (30 ºC) and pH (7.5) of water.

Pure compound of Lead Pb $(No_3)_2$ was dissolved in deionized water and stock solutions (1000 ppm) were prepared.

Acute toxicity tests were performed in glass aquaria attached with aeration system. Glass aquaria were filled with 80-liter metal free tap water and stock solution was added on metallic ion basis to get the desired concentration of metal. To avoid stress to the fish, the desired metal concentration in each aquarium was attained within 7 hours of the start of the experiment.

Twenty individuals of experimental fish were tested against concentration of metal for the determination of both 96-hr LC_{50} and lethal concentrations. During acute toxicity trials, the fish were subjected to 12-hr photoperiod. The fish were not fed during acute toxicity trials. The concentration of Lead was started from zero up to the onset of 96-hr LC_{50} and lethal concentrations. The aquarium was examined after every 2 hours for fish mortality. The dead fish were weighed individually after being lightly blotted dry at the time of mortality observations and were dissected and their body organs viz. gills, liver, skin and muscle for the determination of their respective exposure metals by following the methods of S.M.E.W.W. (1989).

For acute toxicity tests, the mortality data were statistically analyzed by using Probit Static Bioassay test system. The 96-hr LC_{50} and lethal concentrations were determined along with 95% confidence intervals. The data obtained from present investigation were statistically analyzed by using SPSS 13.0 computer program.

Results and Discussion

Twenty individuals of *Oreochromis niloticus* were tested against lead concentration for the determination of both 96-hr LC_{50} and lethal concentrations at constant temperature (30 ºC) and pH (7.50) of water. During the present investigation the 96-hr LC_{50} of Lead Nitrate

for the fish, *Oreochromis niloticus* was found to be 44 mg/l (table.1). Similar results were also found by other researchers with different heavy metals for the same fish. The value of LC_{50} may vary in the same fish for the same heavy metals determined by some scientists. This is attributed to the fact that several factors including differences in the test species, age, feeding habit, sex, composition of toxicant and also the experimental conditions under which the tests are performed [12, 13].

The high level of total dissolved solids in the water resultantly causes hardness. This high hardness play to reduce the availability of Lead Nitrate to the fish [14]. The susceptibility of *Oreochromis niloticus* to the toxic effect of Lead Nitrate is directly proportional to the concentration and duration of dose. If the dose increases the rate of mortality will also increase hence, confirms the observations made in *Oreochromis niloticus* by the effect of lead nitrate.

Table 1: Relation between concentration of Lead Nitrate and the percentage mortality of the fish

Dose mg/l	Log of concentration	Total individuals	Mortality	Mortality %	Probit kill
0	-	20	0	0	0
4	0.6020	20	0	0	0.03
8	0.9031	20	0	0	0.06
12	1.0792	20	1	5	0.41
16	1.2041	20	2	10	1.18
20	1.3010	20	3	15	2.32
24	1.3802	20	4	20	3.71
28	1.4471	20	5	25	5.22
32	1.5051	20	6	30	6.74
36	1.5563	20	7	35	8.21
40	1.6020	20	8	40	9.58
44	1.6434	20	10	50	10.83
48	1.6812	20	11	55	11.96
52	1.7160	20	12	60	12.96
56	1.7482	20	13	65	13.84
60	1.7781	20	14	70	14.62
64	1.8062	20	15	75	15.29
68	1.8325	20	16	80	15.88
72	1.8573	20	18	90	16.40
76	1.8808	20	20	100	16.85

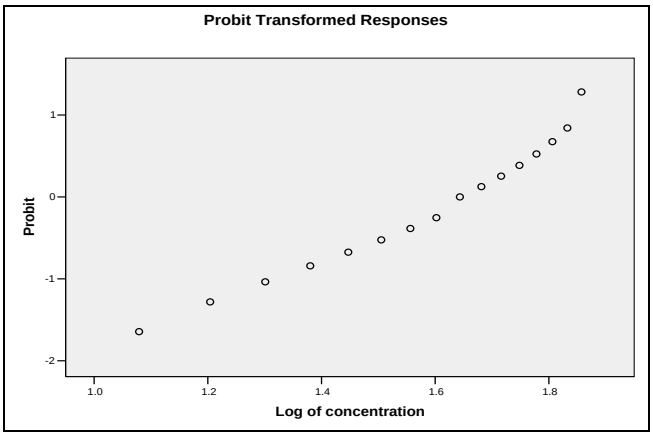

Fig 1: Regression line between the probit kill of *Oreochromis niloticus* and log concentration of Lead Nitrate

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