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Determination of the Lethal Concentration 50% (LC_{50}) of Hexavalent Chromium in Nile Tilapia (*Oreochromis niloticus*)

Debkanta Ghosh[1,2,*], Samir Kumar Saha[1]

[1]Department of Zoology, West Bengal State University, West Bengal, India
[2]Department of Zoology, Vidyasagar College for Women, Kolkata, India

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Abstract The contamination of heavy metals in water is a worldwide issue which is increasing day by day due to the different types of anthropogenic activities. These heavy metals pose a serious health risk on aquatic organisms. One such heavy metal is chromium. The present study is about to determine 24h, 48h, 72h and 96 hours LC_{50} value of hexavalent chromium for the Nile Tilapia, *Oreochromis niloticus*. In the present study, 24h, 48h, 72h and 96h of exposure of Nile Tilapia (*Oreochromis niloticus*) to potassium dichromate ($K_2Cr_2O_7$) was able to measure the hexavalent chromium lethal concentration 50 (LC_{50}) in Nile Tilapia (*Oreochromis niloticus*). A kind of computational and graphical technique can be applied to obtain an LC_{50} (median lethal concentration) from the response of concentration-mortality data produced by an acute mortality experiment. No process should be applied that does not evaluate both LC_{50} and its 95% confidence limits. The data were statistically analyzed by the application of SPSS software based on Finney's Probit Analysis Method and a 24h, 48h, 72h, and 96 h LC_{50} values for *Oreochromis niloticus* were found to be 121.06mg/l, 108.30mg/l, 99.31mg/l, and 93.49mg/l respectively. With the increase of the concentration of metal, the response of the fish mortality increased gradually. The mortality of the fish is directly proportional to the concentration of the exposed metals. The results indicate that hexavalent chromium revealed acute toxicity to fish when exposed for 96 hours and this could release the fact that exposure to hexavalent chromium may outcome in deleterious toxic effects to fish that influences the health of the aquatic environment.

Keywords Acute Toxicity, LC_{50}, Potassium Dichromate, Hexavalent Chromium, *Oreochromis niloticus*

1. Introduction

In ecotoxicology, tests mostly measured the response of toxic elements at various concentrations to the experimental organisms' forms for a particular time. The potency of the toxicant is measured by the median lethal concentration (LC_{50}) about the concentration-response [1,2]. Chromium is the most omnipresent heavy metal pollutant in the environment [3], reaching into the aquatic environment via the discharge of various industries. Different types of hexavalent forms of chromium are chromate (CrO_4^{-2}), hydrochlorate ($HCrO_4^{-1}$), and dichromate (CrO_7^{-2}) in the dissolved condition that fluctuates with pH value [4]. The stable forms of Chromium are trivalent and hexavalent forms that have biological importance. In the contaminated environments with low oxygen medium, the creation of hexavalent chromium, Cr (VI) is promoted by the oxidation of trivalent chromium (CrIII) [5]. Hexavalent chromium is more toxic than trivalent form because hexavalent chromium can easily enter the cell [6]. In the cell, the hexavalent chromium reduces to trivalent chromium which is combined with intracellular macromolecules [7]. The hexavalent chromium has the property of easy permeability and biotransformation which is responsible for its toxicity. Hexavalent chromium is the most stable form in the aquatic environment as it has an elevated bioaccumulation rate in the aquatic ecosystem and is reached to the food chain. In the standard LC_{50}-depended acute toxicity determination, the exposure time is set for 96 hours as it measures the median lethal concentration as less changeability than those at higher or lower centiles [8].

Acute toxicity is often made up of morbidity or mortality from a quantitative standpoint which is determined as the LC_{50}. The LC_{50} stands for the dose of the substance that gives rise to mortality (LC_{50}) in 50% of the experimental organisms. The LC_{50} stands for the concentration of the substance to which the organisms were introduced that causes mortality (LC_{50}) in 50% of an exposed population [9]. A definite xenobiotic is hazardous for the aquatic ecosystem and the food chain can be constructed after acute toxicity of fish [10]. Heavy metal toxicities might be related to oxidative tissue damage because heavy metals function as a generator in the oxidative reaction of biological macromolecules. The vital problem in ecotoxicological-related experimental analysis is to manage the relative scarcity of toxicity-related literature influenced by environmental contamination that may induce both adaptive and adverse responses in organisms at different levels such as cells, tissues, and organs. The toxicity depends on several factors, such as concentration of toxicant, type of toxicant, duration of exposure, and susceptibility of the organisms [11]. For this reason, we analyzed LC_{50} of hexavalent chromium in different exposure periods (24h, 48h, 72h, and 96 h) and hexavalent chromium accumulation in different organs of *Oreochromis niloticus* in acute treatment. Toxicity studies of Hexavalent chromium in the fish models remain minimal and thus the current experiment was focused to evaluate the effects of acute toxicity of specific hexavalent chromium for 24h, 48h, 72h, and 96h in the freshwater fish, *Oreochromis niloticus.*

1.1. Test Animal

Nile tilapia, *Oreochromis niloticus* average weighing 90 gm and length 12 cm were collected from Amda beel, Ranaghat-I Block, West Bengal (23°11'N, 88°32'E). Fishes were acclimatized to the laboratory environment before tests with exposure to a continuous supply of water and a sufficient lighting arrangement. They were maintained in a healthy-oxygenated glass aquarium (60L volume), which was dechlorinated at the uniform break.

1.2. Preliminary Tests

The physicochemical property of the water was measured according to APHA instructions [10]. Temperature (26 ± 2 °C), pH (6.71 to 6.96), and TDS (821 to 852) of Water were recorded using the standardized protocol and were maintained throughout the test.

2. Materials and Methods

Test organisms, Nile Tilapia *(Oreochromis niloticus)* fishes were collected from Amanda Bell, Ranaghat, District: Nadia, West Bengal, India (23°11'N, 88°32'E) in October 2021. A total of twenty fishes (ranging between 10-12 cm in length and 90- 100 gm in weight and three months in age) were collected with the help of the fisherman using the gill net, retained in maintenance tanks, and instantly transported alive to the laboratory. The fishes were acclimated to the laboratory environment for a minimum of two weeks (14 days) before the test. The fishes were kept in glass aquaria (70L volume) filled with water (dechlorinated), constant oxygenation, and temperature of the water, 26± 2°C. The photoperiod was 14:10 (14 hours light/10-hour darkness) and fish were fed twice daily with available balanced fish food.

2.1. LC_{50} Determination

Potassium dichromate ($K_2Cr_2O_7$) was used for this experiment. Pure compound of $K_2Cr_2O_7$ was dissolved in deionized water and the stock solutions (1000 ppm) were prepared. The bioassay of acute toxicity for the chemical of this experiment, Potassium dichromate($K_2Cr_2O_7$) was carried out on the static system to determine the 24h, 48h, 72h, and 96-h LC_{50} in the experimental fish, *Oreochromis niloticus*. For the definitive test, the scale finding experiment was executed first to measure the concentration of the experimental solution. In the definitive test, ten fishes were used for every group. Groups 1, 2, 3, and 4 were exposed to rising concentrations of Potassium dichromate($K_2Cr_2O_7$) whereas the group1 was retained Potassium dichromate($K_2Cr_2O_7$) free water to serve as a control. The minimal concentrations of Potassium dichromate($K_2Cr_2O_7$) tested were: 25, 50, 80, 100, 120, 140 and 160 mg/l. Fishes were not supplemented for 24 hours before the tests. The duration of exposure for the experiment was 24h, 48h, 72h, and 96 h. The test was replicated three times and the number of dead fish was noted at 24h, 48h, 72h, and 96 h. The indicators for the death of fish were no activity, withdrawal of gill motion, and no response to soft stimulation, and dead fishes were withdrawn and thrown away after each monitoring. The 24h, 48h, 72h, and 96h LC50 determination in this experiment, and the toxicity value of Potassium dichromate($K_2Cr_2O_7$) upon the Nile Tilapia (Oreochromis niloticus) were measured using probit analysis method. For probit analysis with a confident limit of 5% level for LC_{50}, SPSS software was used [12]. LC_{50} values of mortality were determined for each exposure period was recorded.

2.2. Statistical Analysis

All tests were conducted in triplicates for the perfection of the results. The number of fishes used in this study, the mortality rate, and the exposure concentrations in each test were fixed to a probit analysis using log10 concentration conversion using the SPSS statistical programming software.

3. Results

The LC_{50} values of 24h, 48h, 72h, and 96 hours of hexavalent chromium, Cr (VI) were 121.06mg/l, 108.30mg/l, 99.31mg/l, 93.49 mg/l respectively, and no death was recorded among the control group. The mean LC_{50} values of 24h, 48h, 72h, and 96 hours of potassium dichromate as Cr (VI) on *Oreochromis niloticus* individuals were found to be 121.06mg/l, 108.30mg/l, 99.31mg/l, 93.49 mg/l respectively, by the use of SPSS on Probit Analysis Method with a confident limit of 5% level for the four experiments periods. Figures 1, 2, 3 and 4 show the plot of probits and LC_{50} results. Tables 1, 2, 3, and 4 show the relationship between the mortality rate of Oreochromis niloticus and the concentration of potassium dichromate as Cr (VI) according to Finney's Probit Analysis method using SPSS software. The results acquired from static 24h, 48h, 72h, and 96h toxicity experiments of potassium dichromate as Cr (VI) for mature *Oreochromis niloticus* and estimated LC_{50} values and confidence limits are listed in Tables 5, 6, 7 and 8.

Table 1. The relation between the potassium dichromate concentration and the mortality rate of *Oreochromis niloticus (*24h)

Concentration (mg/l)	Number of exposed fish	Number ofdead fish	Observed Responses	Expected Responses	Residual	Probability
25	10	0	0	.000	.000	.000
50	10	0	0	.001	-.001	.000
80	10	1	1	.378	.622	.038
100	10	2	2	2.061	-.061	.206
120	10	3	3	4.848	-1.848	.485
140	10	7	7	7.334	-.334	.733
160	10	10	10	8.841	1.159	.884

Table 2. The relation between the potassium dichromate concentration and the mortality rate of *Oreochromis niloticus* (48h)

Concentration (mg/l)	Number of exposed fish	Number ofdead fish	Observed Responses	Expected Responses	Residual	Probability
25	10	0	0	.000	.000	.000
50	10	1	1	.158	.842	.016
80	10	1	1	1.998	-.998	.200
100	10	3	3	4.122	-1.122	.412
120	10	5	5	6.122	-1.122	.612
140	10	8	8	7.623	.377	.762
160	10	10	10	8.610	1.390	.861

Table 3. The relation between the potassium dichromate concentration and the mortality rate of *Oreochromis niloticus* (72h)

Concentration (mg/l)	Number of exposed fish	Number ofdead fish	Observed Responses	Expected Responses	Residual	Probability
25	10	0	0	.000	.000	.000
50	10	1	1	.176	.824	.018
80	10	2	2	2.535	-.535	.254
100	10	3	3	5.085	-2.085	.508
120	10	6	6	7.193	-1.193	.719
140	10	10	10	8.540	1.460	.854
160	10	10	10	9.283	.717	.928

Table 4. The relation between the potassium dichromate concentration and the mortality rate of *Oreochromis niloticus* (96h)

Concentration (mg/l)	Number of exposed fish	Number ofdead fish	Observed Responses	Expected Responses	Residual	Probability
25	10	0	0	.000	.000	.000
50	10	1	1	.190	.810	.019
80	10	2	2	3.028	-1.028	.303
100	10	4	4	5.882	-1.882	.588
120	10	8	8	7.959	.041	.796
140	10	10	10	9.095	.905	.910
160	10	10	10	9.625	.375	.962

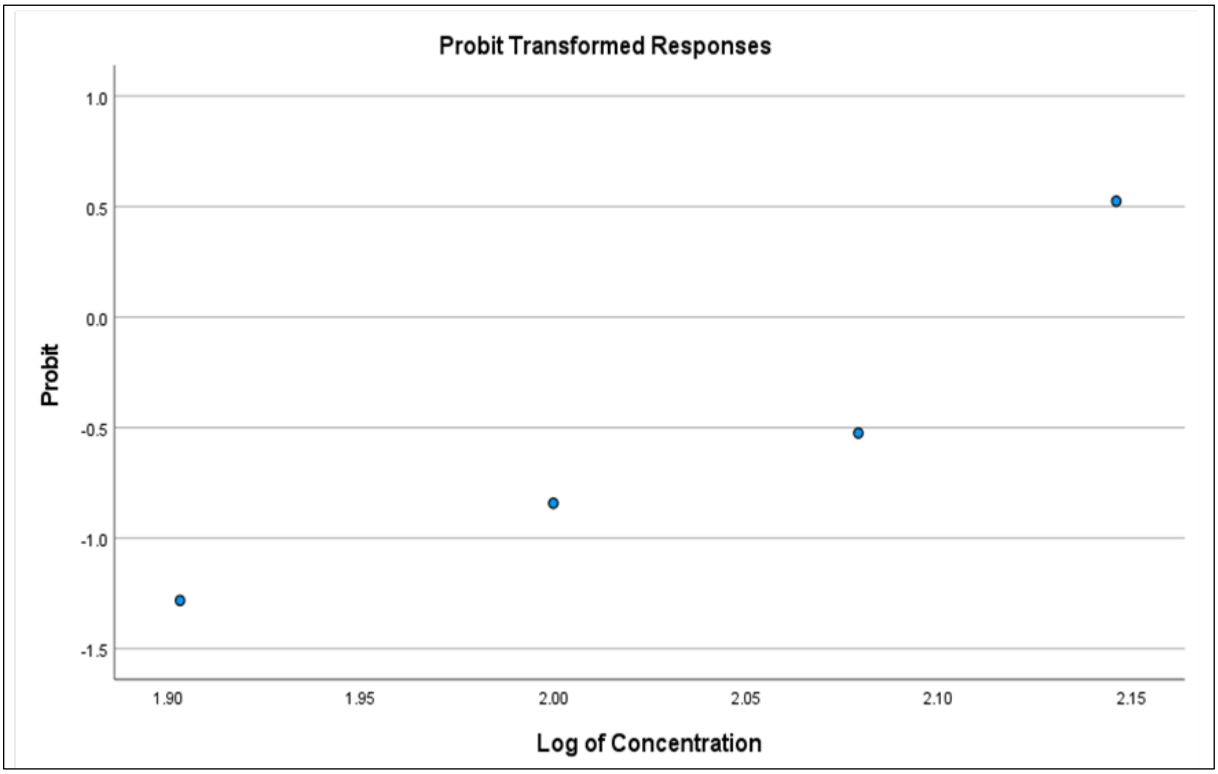

Figure 1. Plot of probits and predicted regression (24h)

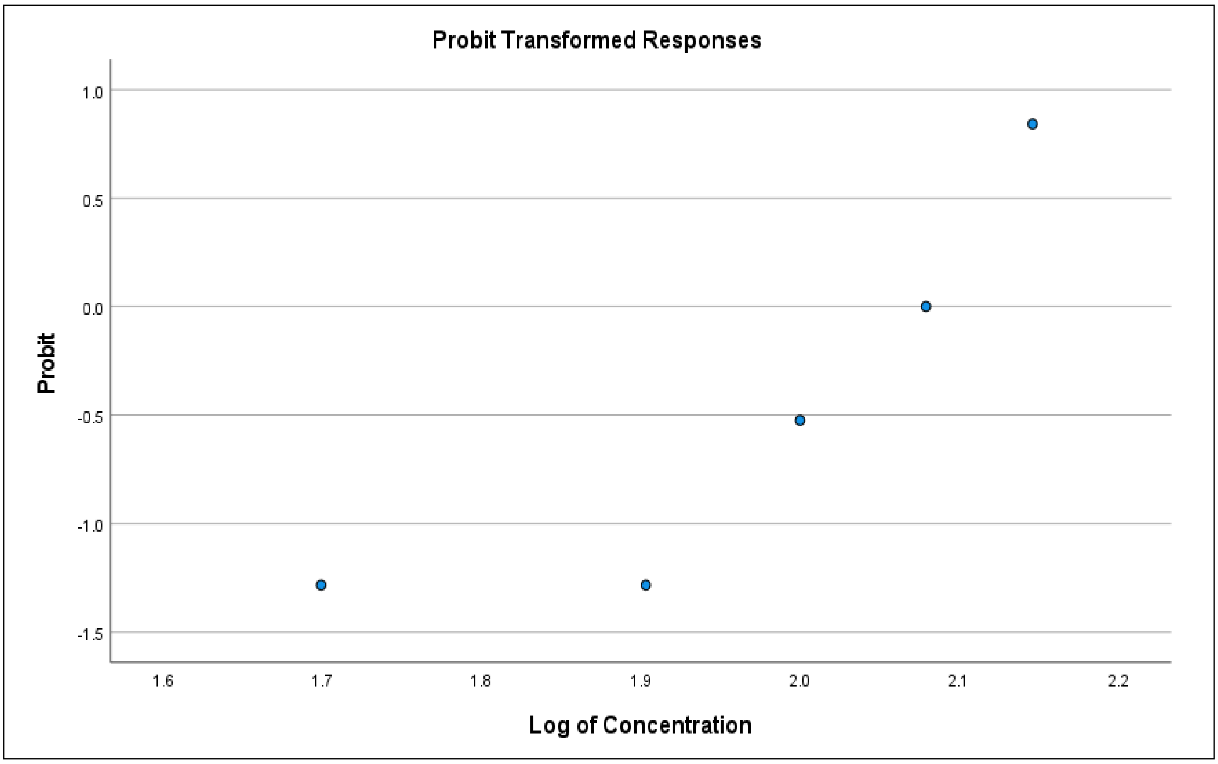

Figure 2. Plot of probits and predicted regression (48h)

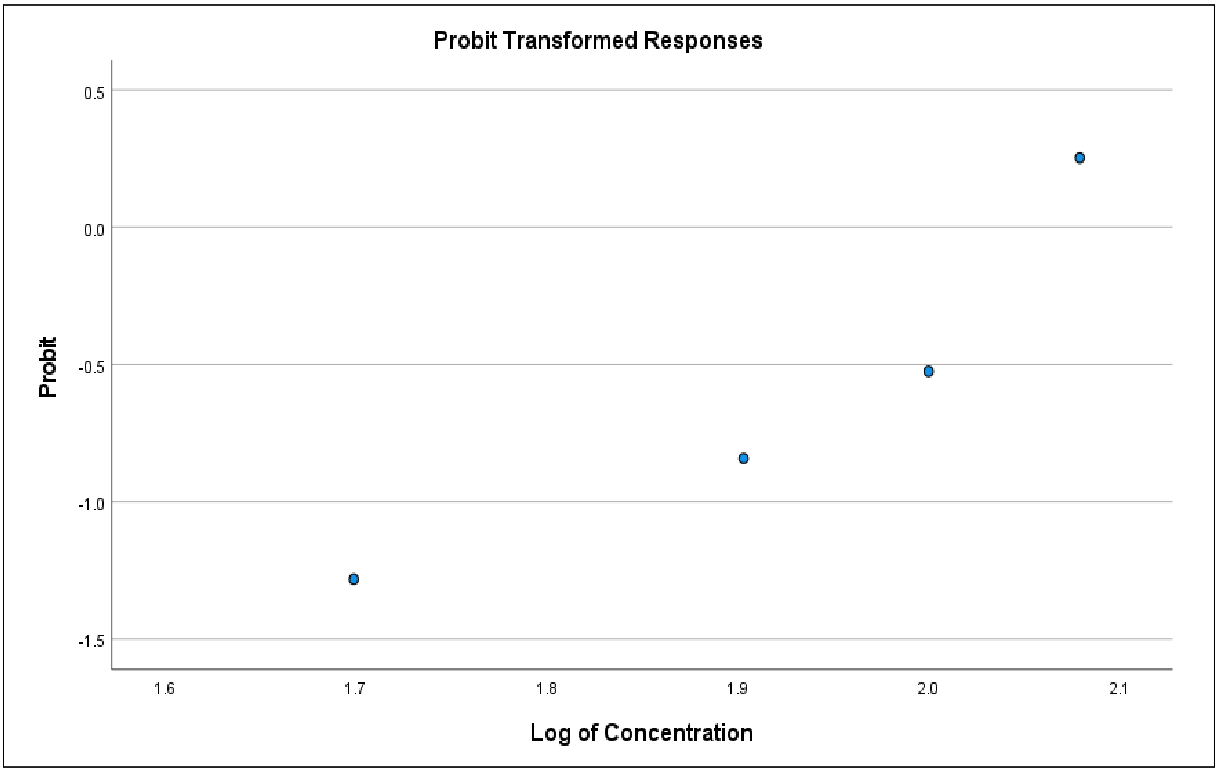

Figure 3. Plot of probits and predicted regression (72h)

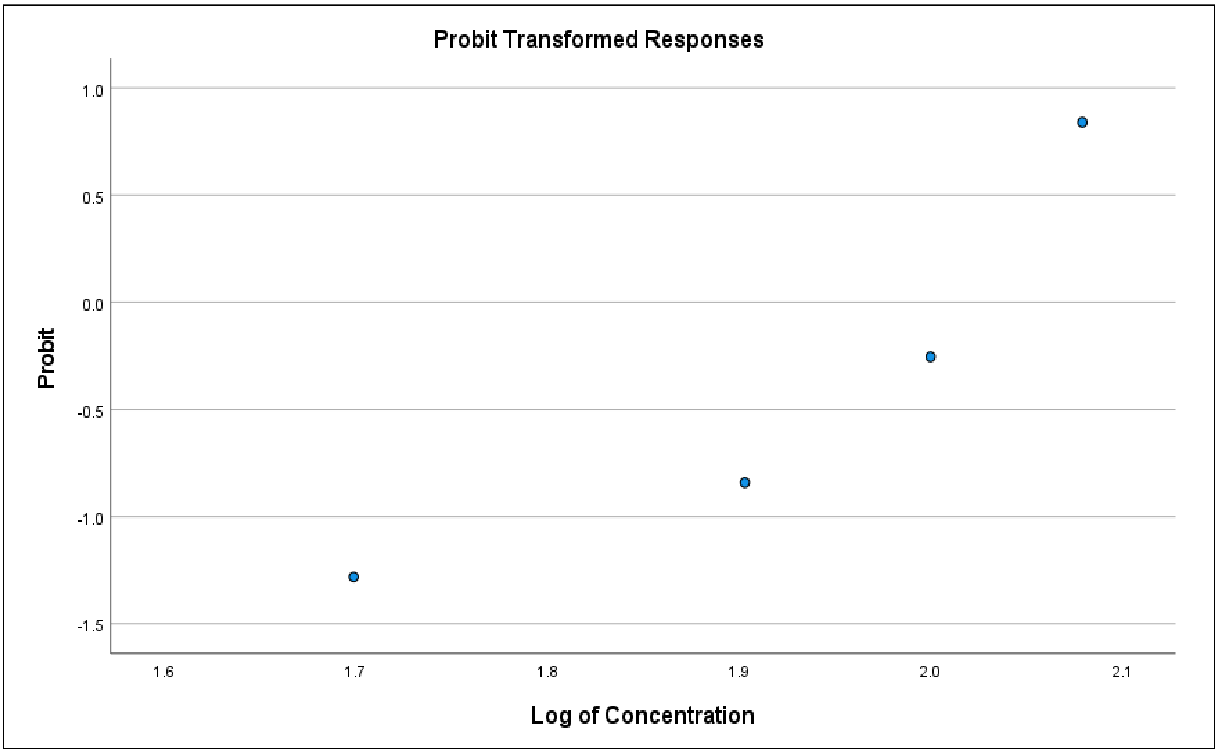

Figure 4. Plot of probits and predicted regression (96h)

Table 5. Probit(Finney DJ.) analysis of 95% confidence limits for different concentrations of potassium dichromate in *Oreochromis niloticus* (24h)

Probability	95% Confidence Limits for Concentration		
	Estimate	Lower limit	Upper limit
.010	70.383	43.164	84.921
.020	75.002	48.555	88.878
.030	78.088	52.303	91.514
.040	80.493	55.300	93.568
.050	82.504	57.854	95.288
.060	84.255	60.113	96.790
.070	85.821	62.159	98.137
.080	87.248	64.044	99.371
.090	88.566	65.800	100.515
.100	89.797	67.454	101.590
.150	95.079	74.666	106.287
.200	99.497	80.797	110.376
.250	103.451	86.293	114.224
.300	107.135	91.360	118.036
.350	110.666	96.102	121.958
.400	114.125	100.576	126.116
.450	117.573	104.818	130.627
.500	121.069	108.860	135.608
.550	124.669	112.739	141.175
.600	128.436	116.508	147.464
.650	132.449	120.236	154.642
.700	136.815	124.015	162.951
.750	141.688	127.967	172.770
.800	147.318	132.270	184.745
.850	154.164	137.223	200.112
.900	163.231	143.448	221.693
.910	165.500	144.959	227.299
.920	168.000	146.607	233.569
.930	170.793	148.428	240.684
.940	173.968	150.473	248.912
.950	177.660	152.822	258.669
.960	182.099	155.608	270.659
.970	187.708	159.075	286.213
.980	195.432	163.765	308.355
.990	208.256	171.363	346.937

Table 6. Probit(Finney DJ.) analysis of 95% confidence limits for different concentrations of potassium dichromate in *Oreochromis niloticus* (48h)

Probability	95% Confidence Limits for Concentration		
	Estimate	Lower limit	Upper limit
.010	46.918	22.717	62.476
.020	51.750	27.113	67.024
.030	55.071	30.321	70.108
.040	57.709	32.974	72.540
.050	59.948	35.295	74.594
.060	61.922	37.393	76.401
.070	63.706	39.329	78.033
.080	65.346	41.143	79.534
.090	66.875	42.859	80.933
.100	68.315	44.497	82.252
.150	74.610	51.901	88.069
.200	80.024	58.523	93.192
.250	84.981	64.724	98.052
.300	89.694	70.667	102.898
.350	94.294	76.435	107.919
.400	98.877	82.064	113.293
.450	103.523	87.568	119.203
.500	108.309	92.953	125.849
.550	113.315	98.237	133.451
.600	118.640	103.462	142.264
.650	124.406	108.706	152.612
.700	130.786	114.092	164.950
.750	138.039	119.800	179.991
.800	146.590	126.107	198.967
.850	157.228	133.494	224.269
.900	171.717	142.981	261.500
.910	175.412	145.320	271.481
.920	179.516	147.885	282.791
.930	184.140	150.737	295.813
.940	189.445	153.964	311.113
.950	195.683	157.703	329.586
.960	203.273	162.179	352.767
.970	213.010	167.813	383.609
.980	226.680	175.545	428.973
.990	250.027	188.340	511.938

Table 7. Probit (Finney DJ.)analysis of 95% confidence limits for different concentrations of potassium dichromate in *Oreochromis niloticus* (72h)

Probability	95% Confidence Limits for Concentration		
	Estimate	Lower limit	Upper limit
.010	46.531	24.682	60.669
.020	50.854	28.782	64.724
.030	53.803	31.720	67.458
.040	56.133	34.118	69.606
.050	58.103	36.196	71.415
.060	59.834	38.059	73.002
.070	61.394	39.767	74.430
.080	62.825	41.357	75.741
.090	64.156	42.854	76.960
.100	65.406	44.276	78.106
.150	70.845	50.629	83.126
.200	75.488	56.230	87.488
.250	79.714	61.424	91.564
.300	83.710	66.379	95.555
.350	87.591	71.186	99.605
.400	91.441	75.898	103.838
.450	95.326	80.548	108.382
.500	99.311	85.156	113.374
.550	103.462	89.742	118.975
.600	107.858	94.333	125.377
.650	112.598	98.975	132.821
.700	117.819	103.752	141.640
.750	123.725	108.795	152.333
.800	130.651	114.324	165.735
.850	139.215	120.731	183.447
.900	150.791	128.862	209.165
.910	153.729	130.851	215.990
.920	156.985	133.027	223.692
.930	160.645	135.439	232.514
.940	164.834	138.160	242.823
.950	169.744	141.302	255.191
.960	175.701	145.051	270.592
.970	183.311	149.751	290.893
.980	193.940	156.170	320.390
.990	211.957	166.725	373.352

Table 8. Probit (Finney DJ.) analysis of 95% confidence limits for different concentrations of potassium dichromate in *Oreochromis niloticus* (96h)

Probability	95% Confidence Limits for Concentration		
	Estimate	Lower limit	Upper limit
.010	46.331	25.747	59.613
.020	50.303	29.612	63.323
.030	52.999	32.351	65.815
.040	55.121	34.570	67.768
.050	56.910	36.481	69.409
.060	58.478	38.186	70.845
.070	59.889	39.743	72.136
.080	61.181	41.187	73.318
.090	62.380	42.543	74.416
.100	63.504	43.827	75.447
.150	68.380	49.522	79.943
.200	72.521	54.497	83.821
.250	76.272	59.083	87.415
.300	79.806	63.439	90.901
.350	83.227	67.658	94.401
.400	86.609	71.797	98.015
.450	90.011	75.894	101.841
.500	93.490	79.976	105.989
.550	97.104	84.066	110.583
.600	100.919	88.191	115.777
.650	105.019	92.388	121.767
.700	109.521	96.721	128.824
.750	114.596	101.294	137.346
.800	120.524	106.287	147.991
.850	127.822	112.035	161.999
.900	137.636	119.260	182.208
.910	140.117	121.017	187.544
.920	142.863	122.935	193.550
.930	145.945	125.055	200.413
.940	149.465	127.442	208.408
.950	153.584	130.189	217.966
.960	158.569	133.457	229.818
.970	164.919	137.541	245.359
.980	173.755	143.096	267.783
.990	188.654	152.180	307.620

4. Discussion

Results of this study showed that the LC_{50} values of 24h, 48h, 72h, and 96 h of hexavalent chromium exposure in Nile tilapia, *Oreochromis niloticus* were 121.06mg/l, 108.30mg/l, 99.31mg/l, 93.49 mg/l respectively, and no death was recorded among the control group.

Heavy metals are the most hazardous pollutant in the aquatic ecosystems that are released from different industries' effluent [13]. In the heavy metals polluted areas, exposure of fish to the heavy metal leads to reactions between biological organizations and chemicals resulting in biochemical interferences. Oxidative stress is a deleterious reaction resulting from the exposure of heavy metals, cells, or tissues to excess values of free radical toxicants, especially reactive oxygen species (ROS) [14,15]. It is induced by the presence of molecules having unpaired electrons, usually obtained from oxygen and its various reactive intermediates. Aquatic animals have developed antioxidant defense systems that interrupt Reactive oxygen species (ROS) for oxidized components [16]. It has been well documented that the LC_{50} value of hexavalent chromium toxicity depends on various factors such as species, age, weight, sex, temperature, pH, animal susceptibility, etc. [17]. The use of the LC_{50} test has become widespread as a general measure of chemical toxicity. It is useful to consider the repeat determination of the LC_{50} before conducting laboratory experiments. The present experiment was focused on evaluating the acute toxicity of hexavalent chromium in the Nile tilapia, *Oreochromis niloticus.*

5. Conclusions

It is concluded that aquatic organisms become sensitive to high concentrations of some heavy metal in the aquatic ecosystem, which causes harmful effects on them. It helps us to determine the permissible limit of a toxicant in an ecosystem. Acute toxicity test reveals the health of given aquatic ecosystem. Evaluation of median lethal concentrations (LC_{50}-96 h) of hexavalent chromium as potassium dichromate by probit analysis was 93.49 mg/l in the freshwater fish, *Oreochromis niloticus.* Hexavalent chromium has been recognized as serious toxicant of the aquatic environment. It caused serious impairment in physiological, metabolic and structural system, when present in high concentration.

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