World Journal of Zoology 10 (3): 191-199, 2015
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2015.10.3.94169

Comparative Toxicity Study of Heavy Metals $HGCL_2$, AS_2O_3 & $CDCL_2$ to Freshwater Teleost Fish, *Amblypharyngodon mola*

A.D. Shelke and G.P. Wani

Department of Zoology, B.P. Arts, Science & Commerce College, Chalisgaon,
Dist. Jalgaon, (North Maharashtra University, Jalgaon) State- Maharashtra, India

Abstract: Fish constitutes are an important and cheap source of animal protein to human beings and a large number of people depend on fish and fishing activities for their livelihood. Increasing human influences through heavy metal pollution have however led to the depletion of our fish resources and substantial reduction in the nutritive values. The aim of this study was to determine the pollutants levels including the to evaluate toxicity of heavy metals in freshwater fish *Amblypharyngodon mola* exposed to Mercuric chloride, Arsenic trioxide and Cadmium chloride. Acute toxicity tests were carried out in the laboratory conditions. The LC_{10} values for 24 & 96 hours exposure to $HgCl_2$, As_2O_3 & $CdCl_2$ were calculated. Among all LC_{10} values of toxicants $HgCl_2$ is found to be more toxic than any other metal and *Amblypharyngodon mola*, showed more sensitivity to this metal. The LC_{50} values for 24 & 96 hours exposure to $HgCl_2$, As_2O_3 & $CdCl_2$ were calculated, LC_{10} and LC_{50} values for $HgCl_2$ are low when compared to As_2O_3 and $CdCl_2$.

Key words: $HgCl_2$ · As_2O_3 & $CdCl_2$, *Amblypharyngodon mola* · Acute toxicity test

INTRODUCTION

Heavy metals pollutants compare with other types of aquatic pollution, are less visible but its effects on the ecosystem and humans are intensive and very extensive due to their toxicity and their ability to accumulate in the aquatic organisms [1].

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [2, 3]. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants [4].

The danger of these heavy metals is their persistent nature as they remain in the biota for long period of time when they are released into the environment [5, 6]. As a result of these heavy metals pollution several endemic fish species have become threatened.

The acute toxicity test is used to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions [7]. Toxicity is a characteristic feature of an individual organism's response to a chemical at a particular concentration or dosage for a specific period. Comparative toxicity of mercury and chromium compounds was assay in the fish *Clarias batrachus* (Linn) by Rani *et al.* [7].

Majority of the studies concerning the effects of heavy metals on fish has been confined to the acute toxicity test with the death of fish as an end point. Hence, The aim of this study was to determine the pollutants levels including the to evaluate toxicity of heavy metals in freshwater fish, *Amblypharyngodon mola* exposed to Mercuric chloride, Arsenic trioxide and Cadmium chloride.

MATERIALS AND METHODS

For the present study the freshwater teleost fish, *Amblypharyngodon mola* were collected from Gadad and Girna rivers dams near Chalisgaon City, Maharashtra India (Latitude 20° 28' 58" N and longitude 74° 43'13"E) and brought to the laboratory. The fishes acclimatized to laboratory condition in 80L capacity glass aquarium for 10 days. During acclimatization green cabbage leaves were provided as a food. Medium sized

Corresponding Author: A.D. Shelke, Department of Zoology, B.P. Arts, Science & Commerce College, Chalisgaon, Dist. Jalgaon, North Maharashtra University, Jalgaon, State- Maharashtra, India. Tel: + 919765213047.

Amblypharyngodon mola of about 8 to 9 cm. in length and weighing 9-11 g was selected for experimental purpose. The quality of the water was determined according to APHA- 1985 and was as follows PH 7.1±0.25, Air temperature 32±2.0 C, water temperature 26.5±2.0, Dissolved oxygen 4.36±0.94, Bicarbonates 68±10, Total alkalinity 74.86 ± 10.50, Acidity 4.36±2.57 (except PH and temp.) all parameters are expressed in mg/l. The fishes were selected for evaluation of toxicity, lethal toxicity tests were conducted over 96 hours. Ten fishes were exposed to increasing concentrations of $HgCl_2$, As_2O_3 & $CdCl_2$. One aquarium was maintained as control along with each group. The water with respective dose of heavy metals was changed after every 24 hours. The no. of dead *Amblypharyngodon mola* were recorded and removed after 24 hours up to 96 hours. The same procedure was repeated till the constant mortality in respective doses of heavy metals.

Acute toxicity tests were carried out under static conditions up to 96 hours. The data collected was then analyzed statistically by means of the probit method on transforming the toxicity curve (% mortality versus conc.) in to regression line (Mortality in probit/log. conc.), Which allows the average medium lethal concentration of LC_{50} to be calculated for 24, 48, 72 and 96 hrs. The toxicity of three heavy metals $HgCl_2$, As_2O_3 and $CdCl_2$ to medium sized, freshwater teleost fish, *Amblypharyngodon mola* was assessed by classic method of probit analysis [8, 9].

RESULTS

From the results of LC_{10} and LC_{50} values, it is quite clear that the *Amblypharyngodon mola* is more sensitive to the tested heavy metal mercuric chloride. According to the toxicity of these heavy metals to the fishes, they can be arranged as $HgCl_2 > As_2O_3 > CdCl_2$.

Table 1: Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal $HgCl_2$ (24 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 24 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	2	0.3010	10	1	10	3.7184	3.71	0.3358	3.3589	3.719	1.0110	12.4917	0.3043	46.4566	3.7600	3.6936
2.	3	0.4771	10	3	30	4.4756	4.50	0.5809	5.8099	4.476	2.7719	26.0051	1.3224	116.3988	12.4070	4.4852
3.	4	0.6020	10	5	50	5.0000	5.10	0.6343	6.3431	5.000	3.8185	31.7155	2.2987	158.5775	19.0927	5.0466
4.	5	0.6989	10	7	70	5.5244	5.50	0.5809	5.8099	5.524	4.0605	32.0938	2.8379	177.2861	22.4304	5.4821
5.	6	0.7781	10	8	80	5.8416	5.90	0.4714	4.7144	5.840	3.6682	27.5320	2.8542	160.7868	21.42275	5.8381
Total									Sw= 26.0362		Swx= 15.3301	Swy = 129.8381	Swx^2= 9.6175	Swy^2= 659.5058	Swxy = 79.1128	

$$\bar{X}=\frac{Swx}{Sw}=\frac{15.3301}{26.0362}=0.5887$$

$$\bar{Y}=\frac{Swy}{Sw}=\frac{129.8381}{26.0362}=4.9868$$

$$b=\frac{Swxy-\bar{X}\,[Swy]}{Swx^2-\bar{X}\,[Swx]}=\frac{79.1128-0.5887 \times 129.8381}{9.6175-0.5887 \times 15.3301}=\frac{79.1128-76.4315}{9.6175-0.5927}\ \frac{2.6763}{0.5954}=4.4946$$

Regeression Equation - $Y = \bar{Y} + b\,(X-\bar{x})$

$Y = 2.3407 + 4.4949x$

Table 2: Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal $HgCl_2$ (96 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 96 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	0.3	0.4771	10	3	30	4.4756	4.45	0.5578	5.5788	4.477	2.6616	24.9762	1.2698	111.8188	11.9159	4.3673
2.	0.5	0.6989	10	5	50	5.0000	5.30	0.6160	6.1609	4.991	4.3058	30.7490	3.0093	153.4685	21.4902	5.1294
3.	0.7	0.8450	10	7	70	5.5244	5.85	0.5026	5.0260	5.496	4.2469	27.6228	3.5886	151.8154	23.3409	5.6314
4.	0.9	0.9542	10	9	90	6.2816	6.30	0.3358	3.3589	6.281	3.2050	21.0972	3.0582	132.5118	20.3409	6.0066
5.	1.1	1.0413	10	10	100	-	-	-	-	-	-	-	-	-	-	-
1 added in Log value						Total			Sw= 20.1246		Swx= 14.4193	Swy = 104.4452	Swx^2= 10.9259	Swy^2= 549.6145	Swxy = 76.8776	

$$\bar{X}=\frac{Swx}{Sw}=\frac{14.4193}{20.1246}=0.7165$$

$$\bar{Y}=\frac{Swy}{Sw}=\frac{104.4452}{20.1246}=5.1899$$

$$b=\frac{Swxy-\bar{X}\,[Swy]}{Swx^2-\bar{X}\,[Swx]}=\frac{76.8776-0.7165 \times 104.44}{10.9259-0.7165 \times 14.4193}=\frac{76.8776-74.8349}{10.9259-10.3314}\ \frac{2.0427}{3.4359}=3.4356$$

Regeression Equation - $Y = \bar{Y} + b\,(X-\bar{x})$

$Y = 2.7281 + 3.4359x$

Table 3: Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal AS_2O_3 (24 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 24 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	3	0.4771	10	3	30	4.4756	4.45	0.5578	5.5788	4.477	2.6616	24.9762	1.2698	111.8188	11.9159	4.3673
2.	5	0.6989	10	5	50	5.0000	5.30	0.6160	6.1609	4.991	4.3058	30.7490	3.0093	153.4685	21.4902	5.1294
3.	7	0.8450	10	7	70	5.5244	5.85	0.5026	5.0260	5.496	4.2469	27.6228	3.5886	151.8154	23.3409	5.6314
4.	9	0.9542	10	9	90	6.2816	6.30	0.3358	3.3589	6.281	3.2050	21.0972	3.0582	132.5118	20.3409	6.0066
5.	11	1.0413	10	10	100	-	-	-	-	-	-	-	-	-	-	-
					Total				Sw= 20.1246		Swx= 14.4193	Swy = 104.4452	Swx^2= 10.9259	Swy^2= 549.6145	Swxy = 76.8776	

$$\overline{X}=\frac{Swx}{Sw}=\frac{14.4193}{20.1246}=0.7165$$

$$\overline{Y}=\frac{Swy}{Sw}=\frac{104.4452}{20.1246}=5.1899$$

$$b=\frac{Swxy-\overline{X}\,[Swy]}{Swx^2-\overline{X}\,[Swx]}=\frac{76.8776-0.7165 \times 104.4452}{10.9259-0.7165 \times 14.4193}=\frac{76.8776-74.4315}{10.9259-10.3314}\frac{2.0427}{0.5945}=3.4356$$

Regeression Equation - $Y = \overline{Y} + b\,(X-\overline{x})$

$Y = 2.7281 + 3.4359x$

Table 4: Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal AS_2O_3 (96 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 96 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	1.2	0.0791	10	2	20	4.1584	4.15	0.47144	4.7144	4.160	0.3729	19.6119	0.0294	81.5855	1.5512	3.9813
2.	2.7	0.4313	10	4	40	4.7467	5.00	0.63662	6.3662	4.749	2.7457	30.2330	1.1842	143.5769	13.0393	4.9131
3.	4.2	0.6232	10	6	60	5.2533	5.50	0.58099	5.8099	5.240	3.6207	30.4438	2.2564	159.5259	18.9724	5.4208
4.	5.7	0.7558	10	8	80	5.8416	5.85	0.50260	5.0260	5.841	3.7986	29.3568	2.8710	171.4734	22.1876	5.7716
5.	7.2	0.8573	10	9	90	6.2816	6.10	0.40474	4.0474	6.264	3.4698	25.3529	2.9746	158.8106	21.7348	6.0401
					Total				Sw= 25.9639		Swx= 14.0077	Swy = 134.9984	Swx^2= 9.3156	Swy^2= 714.9723	Swxy = 77.4853	

$$\overline{X}=\frac{Swx}{Sw}=\frac{14.0077}{25.9639}=0.5395$$

$$\overline{Y}=\frac{Swy}{Sw}=\frac{134.9984}{25.2639}=5.1994$$

$$b=\frac{Swxy-\overline{X}\,[Swy]}{Swx^2-\overline{X}\,[Swx]}=\frac{77.4853-0.5395 \times 134.9984}{9.3156-0.5395 \times 14.0077}=\frac{77.4853-72.8316}{9.315-67.5571}\frac{4.6537}{1.7585}=2.6464$$

Regeression Equation - $Y = \overline{Y} + b\,(X-\overline{x})$

$Y = 3.7721 + 2.6456x$

Table 5: Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal $CdCl_2$ (24 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 24 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	5	0.6989	10	1	10	3.7184	3.70	0.3358	3.3589	3.719	2.3475	12.4917	1.6406	46.4568	8.7303	3.6202
2.	7	0.8450	10	3	30	4.4756	4.45	0.5578	5.5788	4.477	4.7140	24.9762	3.9834	111.8188	21.1045	4.4712
3.	9	0.9542	10	5	50	5.0000	5.05	0.6366	6.3662	5.000	6.0746	31.8310	5.7964	159.1550	30.3730	5.1073
4.	11	1.0413	10	7	70	5.5244	5.50	0.5809	5.8099	5.524	6.0498	32.0938	6.2997	177.2866	33.4190	5.6146
5.	13	1.1139	10	9	90	6.2816	5.90	0.4714	4.7144	6.216	5.2313	29.3047	5.8494	182.1580	32.6420	6.0375
					Total				Sw= 25.8282		Swx= 24.4372	Swy = 130.6974	Swx^2= 23.5696	Swy^2= 676.8752	Swxy = 126.2688	

$$\overline{X}=\frac{Swx}{Sw}=\frac{24.4377}{25.82821}=0.9461$$

$$\overline{Y}=\frac{Swy}{Sw}=\frac{130.6974}{25.8282}=5.0602$$

$$b=\frac{Swxy-\overline{X}\,[Swy]}{Swx^2-\overline{X}\,[Swx]}=\frac{126.2688-0.8461 \times 130.6974}{23.5696-0.9461 \times 24.4377}=\frac{126.2688-123.6528}{23.5696-23.1205}\frac{2.616}{0.4491}=5.8249$$

Regeression Equation - $Y = \overline{Y} + b\,(X-\overline{x})$

$Y = 0.4508+ 5.8249x$

The results obtained after toxicity evaluation of *Amblypharyngodon mola* are cited in Tables 1 to 6 and Figs. 1 to 6.

The LC_{10} and LC_{50} values for heavy metal pollutants are summarized in Table 7 and Figs. 7 and 8. In the present study The LC_{10} values for 24 & 96 hours were calculated, $HgCl_2$ reported 2.0253 & 0.1941 ppm values respectively. The LC_{10} values for As_2O_3 were 1.9418 & 0.9543 ppm respectively, the LC_{10} values for $CdCl_2$ was 5.1970 & 3.1109 ppm respectively.

Table: 6 Calculations of regression equation for LC_{10} and LC_{50} of *Amblypharyngodon mola* exposed to heavy metal $CdCl_2$ (96 hours)

Sr. No	Conc. in ppm.	Log of Conc. x	No. of animals exposed n	Mortality for 96 hours r	% Morality P=100 r/n	Empirical probit	Expected probit y	Weighing coefficient w	Weight w = nw	Working probit y	W x	W y	W x^2	W y^2	W x y	Improved Expected probit y'
1.	4.0	0.6020	10	2	20	4.1584	4.15	0.4714	4.7144	4.160	2.8380	19.6119	1.7085	81.5855	11.8060	4.1767
2.	5.5	0.7403	10	4	40	4.7467	7.75	0.6160	6.1609	4.747	4.5609	29.2457	3.3764	138.8293	21.6505	4.7579
3.	7.0	0.8450	10	6	60	5.2533	5.20	0.6274	6.2742	5.253	5.3016	32.9583	4.4799	173.1299	27.8493	5.1979
4.	8.5	0.9294	10	7	70	5.5244	5.50	0.5809	5.8099	5.524	5.3997	32.0938	5.0185	177.2861	29.8279	5.4609
5.	10.0	1.0000	10	8	80	5.8416	5.85	0.5026	5.0260	5.841	5.026	29.3568	5.026	171.4730	29.3568	5.7576
					Total				Sw= 27.9854		Swx= 23.1262	Swy = 143.2665	Swx^2= 19.6093	Swy^2= 742.3038	Swxy = 120.4905	

$$\overline{X}=\frac{Swx}{Sw}=\frac{23.1262}{27.9854}=0.8263$$

$$\overline{Y}=\frac{Swy}{Sw}=\frac{143.2665}{27.9854}=5.1193$$

$$b=\frac{Swxy-\overline{X}\,[Swy]}{Swx^2-\overline{X}\,[Swx]}=\frac{120.4905-0.8263x\ 143.2665}{19.6093-0.8263\ x\ 23.1262}=\frac{120.4905-118.3811}{19.6093-19.1091}\ \frac{2.1097}{1.7585}=4.2025$$

Regeression Equation - $Y = \overline{Y} + b\ (X-\overline{x})$

$Y = 1.6468+ 4.2025x$

Table 7: Comparative study of LC_{10} and LC_{50} values of $HgCl_2$, As_2O_3 & $CdCl_2$ to *Amblypharyngodon mola.*

		LC_{10} values in ppm. (in hrs.)		LC_{50} values in ppm. (in hrs.)	
Sr. No.	Name of the pollutant	24 hrs.	96 hrs.	24 hrs.	96 hrs.
1	$HgCl_2$	2.0253	0.1941	3.9048	0.4583
2	As_2O_3	1.9418	0.9543	4.5837	2.9115
3	$CdCl_2$	5.1970	3.1109	8.6253	6.2791

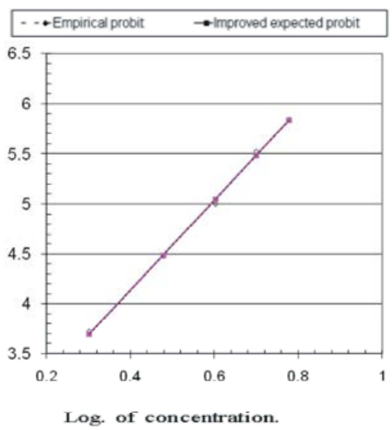

Fig. 1: Provisional and Regression line for *A. mola* exposed to Mercuric Chloride for 24 hrs

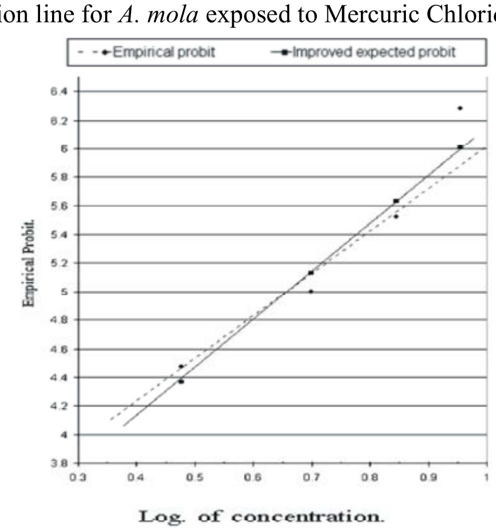

Fig. 2: Provisional and Regression line for *A. mola* exposed to Mercuric Chloride for 96 hrs

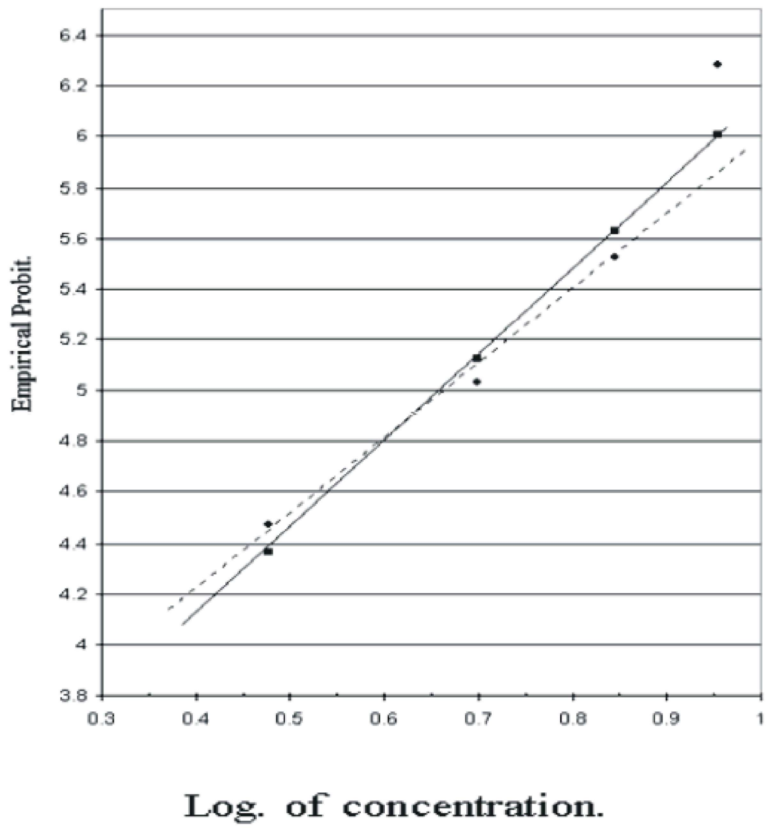

Fig. 3: Provisional and Regression line for *A. mola* exposed to Arsenic Trioxide for 24 hrs

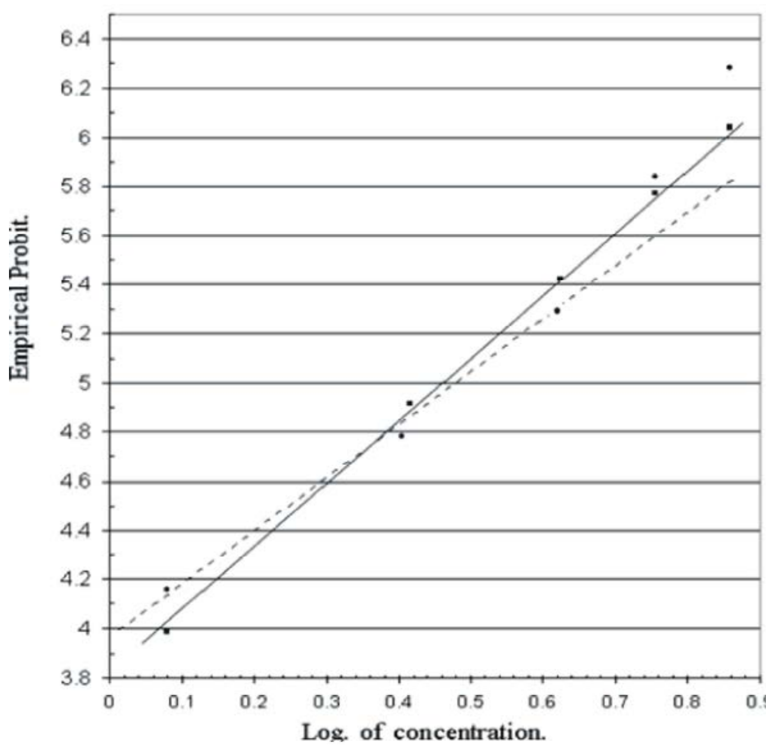

Fig. 4: Provisional and Regression line for *A. mola* exposed to Arsenic Trioxide for 96 hrs

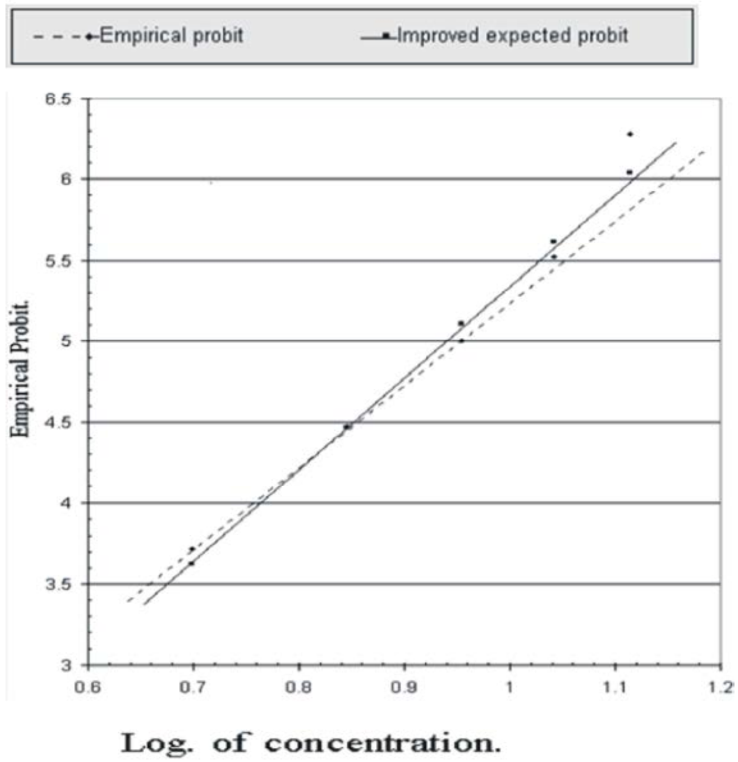

Fig. 5: Provisional and Regression line for *A. mola* exposed to Cadmium Chloride for 24 hrs

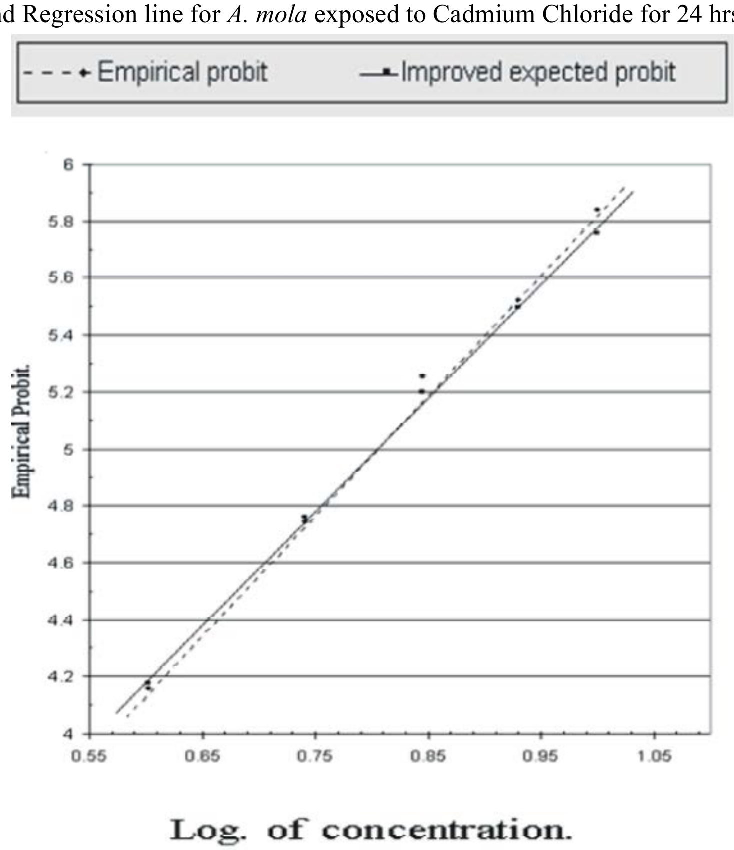

Fig. 6: Provisional and Regression line for *A. mola* exposed to Cadmium Chloride for 96 hrs

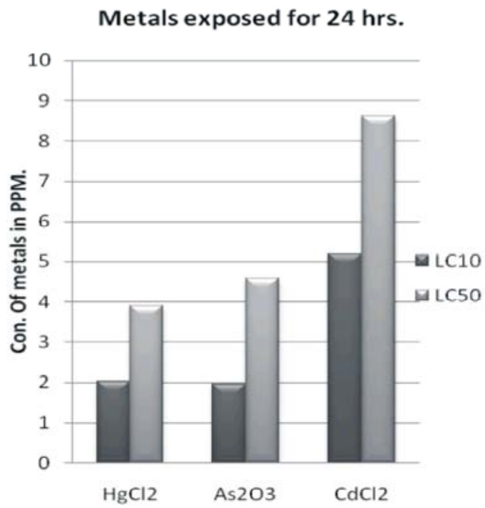

Fig. 7: Comparison of 24 hrs. LC_{10} and LC_{50} Values of $HgCl_2$ As_2O_3 & $CdCl_2$

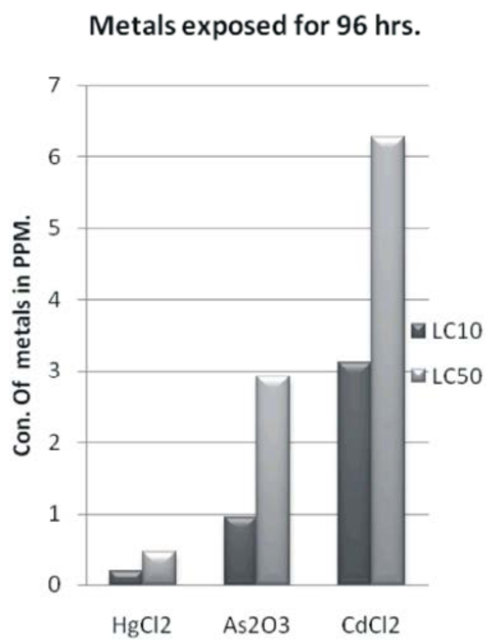

Fig. 8: Comparison of 96 hrs. LC_{10} and LC_{50} Values of $HgCl_2$, As_2O_3 & $CdCl_2$

Among all LC_{10} values of toxicants $HgCl_2$ is found to be more toxic than any other metal and *Amblypharyngodon mola*, showed more sensitivity to this metal.

The LC_{50} values for 24 and 96 hours were calculated, $HgCl_2$ showed 3.9048 & 0.4583 ppm values respectively. The LC_{50} values for As_2O_3 were 4.5837 & 2.9115 ppm respectively, the LC_{50} for $CdCl_2$ was 8.6253 and 6.2791 ppm respectively. LC_{50} values for $HgCl_2$ are low when compared to As_2O_3 and $CdCl_2$.

DISCUSSION

In the present study, LC_{10} and LC_{50} values indicated that mercury is more toxic to *A. mola* and may be very harmful to this fish. However, the study showed the LC_{10} and LC_{50} values vary for each species and the accumulation of heavy metals in the body of fish depends upon several factors. It is evident that concentrations of As_2O_3 and $CdCl_2$ and physiological response affect the LC_{10} and LC_{50} values of the fish. It may be due to the increased resistance of *A. mola to* As_2O_3 and $CdCl_2$ through acclimation. During acclimation, various proteins are released in the body of fish which may detoxify the metal ions. This may cause higher levels of heavy metals being required to cause effects, resulting in higher LC_{50} amounts [10].

Many aquatic species show a vast range of LC_{50} for mercury chloride. The 96 h LC_{50} value for catfish exposed to Hg^{2+} under static test was determined to be 570 μg/l [11]. The 96 h LC_{50} value of mercury chloride for chub was found to be 205 μg/l and 96 h LC_{50} for trout 814 μg/l [12]. A safe level of mercury in aquaculture is only 1 μg/l with LC_{50} range of 10-40 μg/l of, whereas LC_{10} and LC_{50} values for other heavy metals are higher than mercury (cadmium 80-420, cooper 20-100, zinc1000-10000, lead 1000-40000 μg/l) [13].

This work was supported by Ebrahimpour *et al*. [14] which he reports that the concentration of $HgCl_2$ increased, fish mortality also increased, indicating that there is a direct proportional relationship between mortality and concentration of $HgCl_2$. Hedayati, *et al.* [15] reported that the mortality of fish *Cyprinus carpio* exposed to mercury chloride might be due to respiratory epithelium damage by oxygen culmination during the formation of a mucus film over the gills of fish. In the present study also, the decrease survival of fish in mercury treatment may be due to respiratory epithelium damage by oxygen culmination.

In the present study the Arsenic trioxide and Cadmium chloride level was increased, the survival time of fish. *A. mola* was reduced. The above observation was supported by Azmat and Javed [16] who reports that the disruption of the gill epithelium, production of mucus on gills, inability to osmoregulate of the chromium of the blood have all been found to be associated with harmful chromium levels and a pronounced accumulation of mucus on the gills and a sloughing off of the gill epithelial tissue may severely impair bronchial O_2 diffusion. This combined with marked reduction in blood O_2 carrying capacity due to heavy metals, results in eventual cellular anoxia. In the present study also a similar mechanism may be operating in fish, when it was exposed to Arsenic trioxide and Cadmium. Hence, the present study clearly explains when Arsenic trioxide and Cadmium chloride levels are increased, the mortality rate of fish increased significantly.

In the present acute study, Mercuric chloride, Arsenic trioxide and Cadmium chloride level was increased; the survival time of *A. mola* was reduced. Similar work was carried out by Hedayati *et al*. [15] in *Cyprinus carpio* exposed to combined metals, mercury chloride and lead chloride and zinc sulphate. Fish that are highly susceptible to the toxicity of one metal may be less or even not susceptible to the toxicity of another metal at the same level of that metal in the ecosystem. The gills are considered the main site of entry for the dissolved metals. Thus, they represent the target for the toxic action of metals [17]. However, the variety of cell-types of the gills (chloride cells, mucus cell, pillar cells and undifferentiated cells) makes it difficult to interpret the possible mechanisms of metal accumulation [18]. These responses resulted in decreased oxygen tension in the blood or might be connected to a disorder in osmoregulation. It may lead dead of the fish. In the present study also a similar mechanism may be operating in fish, when it was exposed to Mercuric chloride, Arsenic trioxide and Cadmium chloride.

In the present study, decreased survival rate of fish in the concentration of metals such as Mercuric chloride, Arsenic trioxide and Cadmium chloride toxicity can cause all forms of physiological changes. Fish exposed to different doses of pollutants displayed marked behavioural changes. Those symptoms were hyper-activity and attempts to jump out due to skin irritation, restlessness, respiratory distress, loss of balance, gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement, back stroke, excessive accumulation of mucus, all these ending in death. The heavy metals may be an important tool for assessment of the effects of pollutants in aquatic ecosystems. The Three metals used in our experiment to demonstrate their potential for use in bioassays.

REFERENCES

1. Edem, C.A., B. Akpan and M.I. Dosunmu, 2008. A comparative assessment of heavy metals and hydrocarbon accumulation in *Sphyrena afra, Orechromis niloticus* and *lops lacerta* from Anantigha Beach market in Calabar-Nigeria. Afr. J. Environ. Pollut. & Health, 6: 61-64.
2. Farombi, E.O., O. A. Adelowo and Y.R. Ajimoko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish *Clarias gariepinus* from Nigeria ogun river. Int. J. Environ. Res. Public Health, 4(2): 158-165.
3. Vosyliene, M.Z. and A. Jankaite, 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija, 4: 12-17.
4. Olaifa, F.G., A.K. Olaifa and T.E. Onwude, 2004. Lethal and sublethal effects of copper to the African Cat fish *Clarias gariepnus*. Afr. J. Biomed. Res., 7: 65-70.
5. Bolormaa, O., J. Baasansuren, W.M. Kawasaki and T. Hattori, 2006. Analysis of heavy metals in water samples from a mining area in Mongolia. J. of Nuclear Instr. and Methods in Physics Res., 243: 161-166.
6. Yoon, Seokjoo, Sang-Seop Han and S.V.S. Rana 2008. Molecular markers of heavy metal toxicity – A new paradigm for health risk assessment. J. Environ. Biol., 29: 1-14.
7. Rani, M.J., M.M.C. John, M. Uthiralingam and R. Azhaguraj. 2011. Acute Toxicity of Mercury and Chromium to *Clarias batrachus (Linn)* Bioresearch Bulletin., 5: 368-372.
8. Finney, D.J., 1971. Probit analysis 3rd ed, 444pp Cambrige University Press., London.
9. Busvine, J.R., 1971. Critical review of the techniques for testing insecticides common wealth Agricultural Bureau, England, pp: 267-282.
10. Deb, S.C. and T. Fukushima, 1999. Metals in aquatic ecosystems: Mechanism of uptake, accumulation and release. Int. J. Environ Stud., 56: 385-33.
11. Elia, A.C., R. Galarini, M.I. Taticchi, A.J. Dorr and L. Mantilacci, 2003. Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. Ecotoxicol. Environ. Saf., 55: 162-167.
12. Verep, B., E.S. Beayli, I. Altinok and C. Mutlu, 2007. Assessment of mercuric chloride toxicity on rainbow trouts *Oncorhynchus mykiss* and chubs *Alburnoides bipunctatus*. Pak J. Biol Sci., 10: 1098-1102.
13. Johnston, E.L., M.J. Keough and P.Y. Qian, 2002. Maintenance of species dominance through pulse disturbances to a sessile marine invertebrate assemblage in Port Shelter Hong Kong. Marine Ecology Progress Series, 226: 103-114.

14. Ebrahimpour, M., M. Mosavisefat and R. Mohabbati, 2010. Acute toxicity bioassay of mercuric chloride: An alien fish from a river. Toxicological and Environmental Chemistry, 92: 169-173.
15. Hedayati, A, A. Jahanbakhshi, F. Shaluei and S.M. Kolbadinezhad, 2013. Acute toxicity test of mercuric chloride ($HgCl_2$), lead chloride ($PbCl_2$) and zinc sulphate ($ZnSo_4$) in Common Carp *Cyprinus carpio*. Journal of Clinical Toxicology, 3: 1-4.
16. Azmat, H. and M. Javed, 2011. Acute toxicity of chromium to *Catla catla, Labeo rohita* and *Cirrhina mrigala* under laboratory conditions. International Journal of Agriculture Biology, 13: 961-965.
17. Olsson, P.E., P. Kling and C. Hogstrand, 1998. Mechanisms of heavy metal accumulation and toxicity in fish. In: W.J. Langston and M.J. Bebianno, (Eds.,). Metal metabolism in Aquatic Environments, Chapman and Hall, London, pp: 321-350.
18. Alvarado, N.E., K.I. Quesada, L. Hylland and M. Marigomez, Soto, 2006. Quantitative changes in metallothionein expression in target cell-types in the gills of turbot, *Scophthalmus maximus* exposed to Cd, Cu and Zn and after a depuration treatment. Aquatic Toxicology, 77: 64-77.