INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-2, April-June-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490
Received: 5th Jan-2016 *Revised: 9th Feb 2016* *Accepted: 10th Feb 2016*
Research article

ACUTE EFFECT OF CHROMIUM TOXICITY ON THE BEHAVIORAL RESPONSE OF ZEBRA FISH *DANIO RERIO*

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ABSTRACT: In aquatic ecosystems, domestic wastes as well as industrial effluents of heavy metal contaminants stand for predominant chemical form of the metal. Chromium is highly toxic to both aquatic and soil environment. Aim of the present study was to determine acute effect of chromium trivalent (Cr- III) and hexavalent (Cr-VI) toxicity on the behavioral response of zebra fish *Danio rerio*. The 96 hours LC_{50} of chromium trivalent and hexavalent was determined to be 105.279 mg/l and 26.03 mg/l by experimental fishes were exposed in different concentrations. Jerky movements, secretion of mucus, opening mouth for gasping, normal color change, erratic swimming, color turns were observed. Consequently fish moved to the corners of the experimental trough then loss of equilibrium followed by hanging vertically in water became death. The behavior showed that acute chromium toxicity is severely affects the fundamental organs and behavior which is harmful to fishes in aquatic ecosystem. There is need to control the usage of chromium especially Cr-VI because of its observed toxicity level.
Key words: Pollution, Chromium, Acute toxicity, Zebra fish

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INTRODUCTION

The exponential increase of human population and concurrent industrial development, the environmental pollution problem mounts a serious threat to natural ecosystems, human and other living organisms. Tannery industries produce great amount of effluents from different nature, most of them are highly pollutants. When toxic substances accumulate in the environment and in food chains, they can greatly interrupt biological processes [1]. Non essential heavy metals are usually potent toxins and their bioaccumulation in living tissues leads to intoxication, decreased fertility, cellular and tissue damage, cell death and dysfunction of variety of organs [2]. The presence of toxic and polluting heavy metals from industrial effluents, discharges from mines and their removal have received much attention in recent years. The amount of heavy metals in the industrial waste waters often contains considerable and would endanger the public health and the environment if discharged without adequate treatment. Chromium, a potential pollutant, is well known for its mutagenic [3] and carcinogenic [4] effects in humans, animals and plants. Extensive use of chromium in tanning industries had resulted in chromium contaminated soil and ground water at production sites which pose a serious threat to human health, fish and other aquatic biodiversity [5]. Heavy metal contamination severely interferes with ecological balances of an ecosystem and anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation [6].

Acute effects of Cr metal toxicity at various functional levels across fish species have been reported [7-9]. The use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms is well documented by number of studies [10-13]. Lethal concentration (LC_{50}) is the particular concentration of a chemical in the experimental water at which just 50% of the text organisms are able to survive for a specific period of exposure [14,15] (APHA, 1960; Haniffa and Porchellvi, 1984). Zebra fish has been suggested as a model organism for environmental and human risk assessment of Endocrine Disrupting Chemicals (EDC's), to determine endocrine activities of chemicals, and to study mechanisms of endocrine disruption [16] and for toxicological studies based upon the International Organization for Standardization and the Organization for Economic Co-operation and Development [17]. The present study was undertaken to determine the behavioral response on the acute toxicity of the metal Cr-III and Cr-VI on the zebra fish, *Danio rerio*.

METERIALS AND METHODS

The experimental animals, zebra fish, *Danio rerio* (5 ± 0.25 gm; 2 ± 0.25 inches) were collected from an aquarium, brought to the laboratory and maintained in 50 liter capacity glass tanks at the rate of 100 fish /tank. The tank was provided good aeration, light /dark photoperiod and the fishes were acclimatized in laboratory condition before starting the toxicological studies.

The acute toxicity of two different heavy metals Cr -III (CrO_3) and Cr- VI ($CrCl_3$.6H2O) were studied at different concentrations (80, 90, 95, 100,105,110,115, 120,125 mg/l and 10, 20, 22, 24, 28, 30, 32 mg/l) of both metals separately following Finney (1971) [18] and the effects of mortality were recorded after every 24 hrs up to 96 hours. Three replicates were performed for each concentration. The regression method of probit analysis[18] and IBM SPSS statistics 21 were used to calculate the LC_{50} values.

RESULTS

The toxicity tolerance values varied in different size, weight and age groups of the fish in a stable environmental condition. In parameter estimates the variables of both Cr-III and Cr-VI concentrations are statistically significant (Table 1 and 5). In Cr-III, the logistic LC_{50} concentration of expected response is 4.915 and the residual value is .085 shown in the (Table. 3). In Cr-VI, the expected response is 4.951 and the residual value is .049 shown in the (Table.7). The observed percentage mortality of *Danio rerio* for Cr- III and Cr-VI in static tests and LC_{50} values of Cr- III and Cr-VI with 95% confidential limits in static tests from 24 hours up 96 hours .Control mortality value was zero in the experimental fish. The estimated 96h LC values for Cr- III is 105.27 mg/l and that of Cr-VI is 26.03 mg/l and its SE (standard error) 3.858 (Table 4 and 8). The graphs were depicted in probit values for mortality rate and toxicant concentration of Cr- III and Cr-VI were shown in Fig. 1 and 2. The mortality was increased, as the concentration of the toxicant was increased.

Table 1. Parameter estimates for the probit analysis in Cr-III

Parameter		Estimate	Std error	Z	Sig.	95% Confidence Interval	
						Lower bound	Upper bound
PROBIT[a]	Conc	18.566	3.858	4.813	.000	11.005	26.127
	Intercept	-37.546	7.820	-4.801	.000	-45.366	-29.727
a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Table 2. Chi-Square tests of Cr-III

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.588	6	.953[a]
a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.				
b. Statistics based on individual cases differ from statistics based on aggregated cases.				

Table 3. Log concentration, observed responses in Cr- III exposed zebra fish, *D.rerio*

Number		Conc	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.954	10	1	1.031	-.031	.103
	2	1.978	10	2	2.037	-.037	.204
	3	2.000	10	4	3.392	.608	.339
	4	2.021	10	5	4.915	.085	.491
	5	2.041	10	6	6.382	-.382	.638
	6	2.061	10	7	7.618	-.618	.762
	7	2.079	10	8	8.544	-.544	.854
	8	2.097	10	10	9.169	.831	.917

Table 4. Acute toxicity of Cr-III on zebra fish, *Danio rerio* during 96h

Point	96h LC_1 (mg/l)	96h LC_5(mg/l)	96h LC_{10}(mg/l)	96h LC_{50}mg/l	96h LC_{99}(mg/l)	S.E
	78.893	85.851	89.808	105.279	140.489	3.858

Table 5. Parameter estimates for probit analysis in Cr-VI

	Parameter	Estimate	Std error	Z	Sig.	95% Confidence Interval	
						Lower bound	Upper bound
PROBIT[a]	Conc	21.362	4.222	5.060	.000	13.088	29.637
	Intercept	-30.240	5.989	-5.049	.000	-36.228	-24.251

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Table 6. Chi-Square tests of Cr-VI

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.835	5	.975[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

a. Statistics based on individual cases differ from statistics based on aggregated cases.

Table 7. Log concentration, observed responses in Cr-VI induced zebra fish, *Danio rerio*

	Number	Conc	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.301	10	0	.072	-.072	.007
	2	1.342	10	1	.591	.409	.059
	3	1.380	10	2	2.252	-.252	.225
	4	1.415	10	5	4.951	.049	.495
	5	1.447	10	7	7.503	-.503	.750
	6	1.477	10	9	9.058	-.058	.906
	7	1.505	10	10	9.722	.278	.972

Table 8. Acute toxicity of Cr-VI on zebra fish, *Danio rerio* during 96h

Point	96h LC_1 (mg/l)	96h LC_5(mg/l)	96h LC_{10}(mg/l)	96h LC_{50}mg/l	96h LC_{99}(mg/l)	S.E
	20.260	21.805	22.675	26.034	33.454	3.858

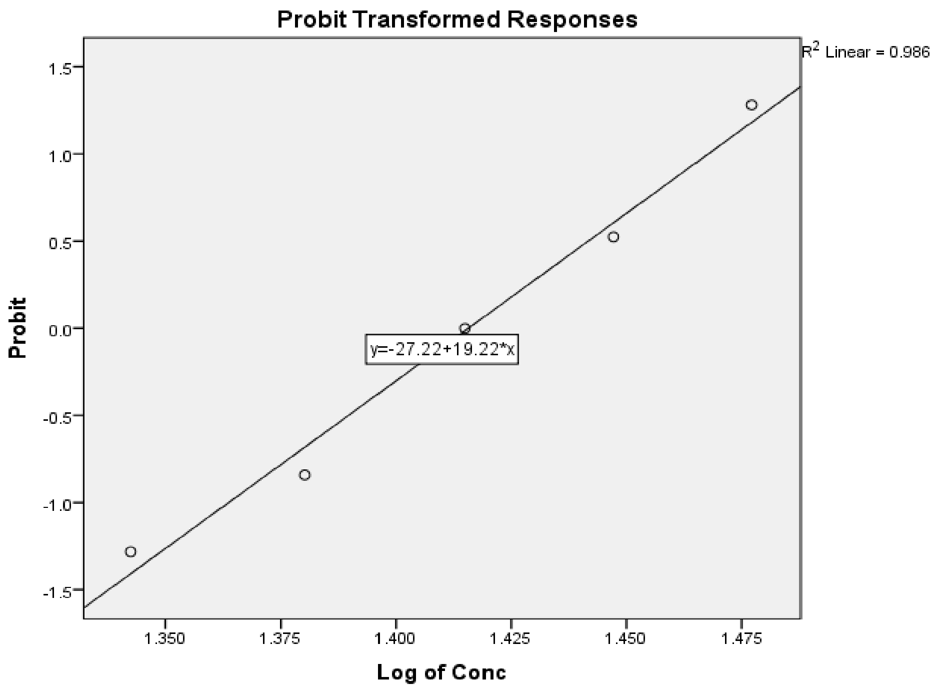

Fig. 1. Cr-III

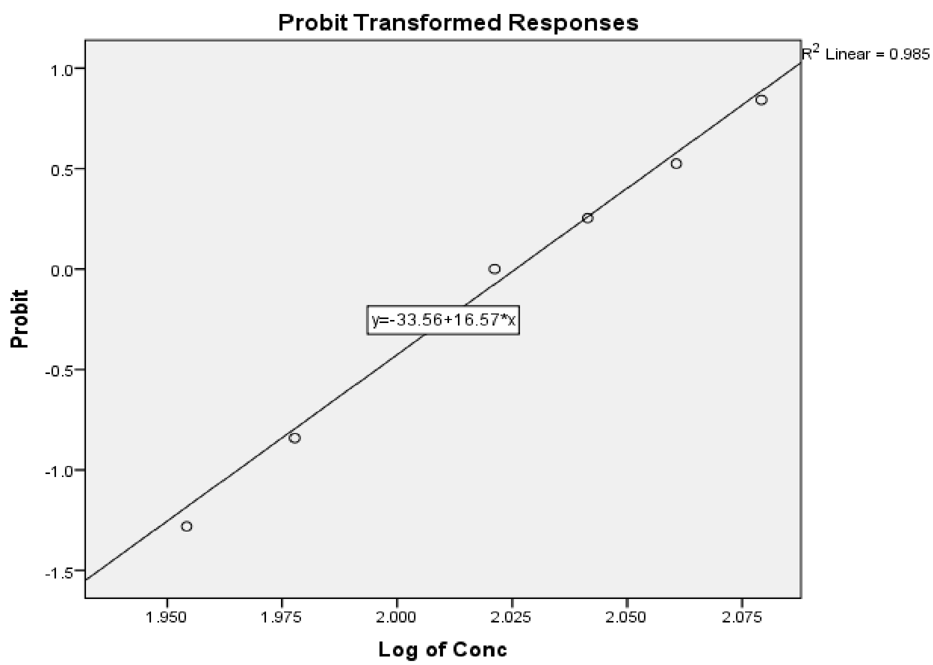

Fig. 2. Cr-VI

Fig. 1 & 2 The linear graph represents probit mortality of experimental fish against log concentration in Cr-III and Cr -VI exposed zebra fish, *Danio rerio*

Behavioural response of zebra fish due to toxicity:

In this study adult fish showed normal behavior in non exposed control group but in toxic medium chromium exposed fishes had jerky movements, secretion of mucus, opening mouth for gasping, normal color change, erratic swimming, color turns were observed. Consequently fish moved to the corners of the experimental trough then loss of equilibrium followed by hanging vertically in water. Lastly the high concentration of chromium exposed fishes was found dead at the bottom of the tank. These symptoms were showed that the normal behavior has changed due to toxicity.

DISCUSSION

During the present study, the rate of mortality increased with increasing exposure of duration and concentrations of the chromium. These results have important implications for ecological risk assessments; mainly focus on the toxicity of individual chemicals as the basis for estimating impacts to imperiled aquatic species [18]. The importance of multiple stressors is widely recognized in aquatic ecotoxicology [19]. Bioassay is a necessity to determine the concentration of a toxicant, which could be allowed in waters without adverse effects on the living organisms [20]. Further, it is an important test to determine the concentration of a toxicant which may be allowed in receiving waters without adverse effects on living resources [21]. Acute toxicity test was conducted to determine LC_{50} values of Cr-III and Cr-VI to the adult and embryo of zebra fish, *Danio rerio*. LC_{50} values of Cr-VI have been reported for a limited number of species [22-31]. Available data on the acute toxicity of Cr-III and Cr-VI to fish species is scarce. Tolerance of an organism is often measured in terms and of the level of lethal factor and length of time the animal survives [32]. In the present study, the survival time of *Danio rerio* was reduced, the concentration of Cr-III and Cr-VI was increased which indicating that there is a direct proportional relationship between survival and concentration of Cr-III and Cr-VI. Similar results have been previously reported by Gutierrez and collaborators [33].

Metal ions and their complex exhibit widening toxicity to the organism that ranges from sublethal to lethal depending upon the time of exposure and the prevailing conditions in the ambient water [34]. LC_{50} values differ from species to species for the same toxicant due to the mode of action and responses of the animals [35]. The toxicity tests have also been influenced by the size, age [36] sex [37] and the nutrient supply [38-40]. The toxic effects may include both lethal and sublethal concentration which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behavior [41]. Determination of lethal concentration (LC_{50}) of *Clarias batrachus* for cadmium chloride and mercuric chloride were 8.51 ppm and 1.81 ppm, respectively [32]. Acute toxicity of chlorantraniliprole to fresh water fish C*tenopharingodon idella* (Valenciennes, 1844) resulted the estimated 96h LC_{50} value by using probit analysis as 11.008mg/L[19]. Ashish [42] described the acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch), reported that the 96 h LC_{50} of chromium salt, potassium dichromate for *C. punctatus*, 41.75 mg/L, Josefina [43] reported that lethal concentration LC_{50} of Cr-VI for *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) after 96 h treatment was 21.4 mg/l. Tabinda [44] reported acute toxicity of chromium for three species of fishes *Ctenopharyngodon idella*, *Cyprinus carpio* and *Tilapia nilotica* were 122.61, 180.08, 238.90 mg/L respectively. Azmat [45] also determined 96 hr LC_{50} concentrations (77.01, 114.61, 98.29 mg/l) of chromium for three fish species *Catla catla*, *Labeo rohita* and C*irrhina mrigala* has taken the data published earlier for other fish species; the SMAV (Species Mean Acute Value) of Cr-VI in fish reported to be ranging from 30.0 mg/l to 139.9 mg/L (US EPA, 1985). In the present study, 96hr LC values of zebra fish species *Danio rerio* found to be Cr- III is 105.27 mg/L and that of Cr-VI is 26.03 mg/l. The variation of LC values is due to its dependence upon various factors such as toxicant sensitivity, concentration and duration of exposure.

Earlier several other workers also studied the effect of different contaminants on the embryo and juveniles of zebrafish [46-48]. Ansari and Ansari [49] described that the embryos of zebra fish were less sensitive to heavy metals as compared to larvae, due to the presence of chorion and reported the same. Li *et al.*, [50] experimented on zebra fish and its result clearly indicates that sodium arsenite exposed embryos exhibited delayed hatching. Likewise, Scheil *et al.*, [51] reported the effect of 3, 4-dichloroaniline and diazinon on zebrafish embryos and larvae and found that they causes impairment in development, locomotor activity and mortality. For that reason, it may be attributed that the hatching was exaggerated due to the inhibition of some hatching enzymes. As the former experiment, it is clear from the present study of Cr- VI exposed zebra fish, *Danio rerio* embryos also showed delayed hatching when compared to Cr-III exposed embryos and it was also depend upon higher concentrations. The estimated 96hr LC values for Cr- III is 77.72 mg/L and that of Cr- VI is 13.43 mg/l. Xu *et al.*,[52] reported that the exposure to sub-lethal doses of metals might have caused acetylcholinesterase inhibition, which could drastically affect growth, hatchability, survival, feeding and reproductive success of fishes. Therefore heavy metals should be treated before discharging into aquatic systems to prevent serious problems.

The behavioral patterns are the most perceptive indicator of possible toxic effects. Behavioural responses are important to survival because they are necessary to perform essential life functions [53]. In the present study, Cr-III and Cr-VI exposed fishes had jerky movements, secretion of mucus, opening mouth for gasping, normal color change, erratic swimming were observed. The above symptoms are due to inhibition of AChE activity to accumulation of acetylcholine in cholinergic synapses following hyper stimulation [54]. The faster opercular movement in fish has been reported to increase significantly following the exposure of toxins [55]. Karuppasamy [56] has observed the characteristics of restlessness, increased activity and opercular movements of fish put into hypoxic conditions [57-58]. Lateral swimming and loss of equilibrium were possibly due to the damage of nervous system [59] which is controlling all the vital activities. In the present study, fish moved to the corners of the experimental trough then loss of equilibrium followed by hanging vertically in water. As mentioned already, these symptoms are also due to the inhibition of AChE activity and destruction of neural system. Related behavior changes were observed by Timchalk *et al*. [60] and Rathnamma and Nagaraju [19]. The impacts of pollution on behavior have been reviewed with primary reference to aquatic animals by Atchison *et al.*[61]. The changes in behavior might be due to hyperactivity of the neural mechanism, as well as, for hyper secretion of stress hormones secretary from endocrine glands. Studies on the lethal concentration limits usually provide measures of relative toxicity of different chemicals and also the sensitivity of fish species, but not throw right on the maximum concentration to toxic materials which would not affect the metabolism of the fish. Behavioral study is necessary to monitoring that relates to the organisms behavior in the field in order to develop a more accurate assessment of the hazards that a contaminant may cause in natural systems. Therefore this acute study suggested that adequate care must be taken to avoid the release of Cr-III and Cr- VI metal toxicity to aquatic environment.

CONCLUSION

Aquatic pollution becoming a huge threat to the human population and the ecosystems. During the present study, the survival time of *Danio rerio* was reduced, in proportion to the increased exposure concentration of Cr-III and Cr- VI; which indicating that there is a direct correlation between survival and concentration of Cr-III and Cr-VI. The acute toxicity effects on the behavior of the experimental fishes. Present study alarming the need for monitoring the industrial effluents for Cr concentration level.

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Lazarus, Chairman, Institute for Environmental Research and Social Education, Nagercoil, India for his support to complete this work. We also thankful to the HOD, Dept. of Zoology, S.T. Hindu College, Nagercoil, India for the facilities extended to carry out this research.

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International Journal of
Plant, Animal and Environmental Sciences

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences