

Effect of Various Doses of Cr (VI) on Survival and Growth of *Cyprinus carpio*

Tayybah Shaheen¹ and Farhat Jabeen*²

¹Government College for women Karkhana Bazar Faisalabad, Pakistan

²Department of Zoology, Government College University, Faisalabad, Pakistan

Abstract.- This study assessed the impact of different doses of hexavalent chromium [(Cr (VI)] on survival and growth of *Cyprinus carpio*. It was investigated that Cr (VI) adversely affected the survival and growth of *Cyprinus carpio* in temporal dose pattern through ambient water. Fish refused to accept the feed immediately after the exposure but after 6 to 7 hours it began to take feed. In this study it was revealed that Cr (VI) exposure significantly altered several physicochemical variables of the water: Cr (VI) increased BOD, COD, CO₂, alkalinity, calcium, hardness, chlorides, TDS and turbidity whereas, pH and DO were decreased on Cr (VI) exposure. Growth decreased significantly with the increase in Cr (VI) concentration and the rate of decline in length (24%) was less than weight (51%). Mortality was 40% in exposed fish. Actual chromium concentration in tanks varied from 36 to 118µg/ml exposed with 25 to 150mg/l of Cr (VI). Exposure of fish to different chromium concentrations in water significantly (P<0.05) reduced their feed consumption and specific growth rate as compared to control. This decrease was linearly correlated with the increase in chromium concentration.

Key words: Physicochemical parameters survival growth mortality ambient water.

INTRODUCTION

Fish is an important source of animal protein. Its nutritional value is very high not only for normal human requirements but also for prevention of many metabolic ailments and cardiac troubles (Lemos *et al.*, 2005). However the fish habitats are being contaminated alarmingly through a number of aquatic pollutants (Charles *et al.*, 2006; Oner *et al.*, 2009). These pollutants have not only depleted the fish stock in a number of water bodies but also have threatened the human health by incorporating into food chain. Among these pollutants heavy metals are most injurious for fish life. Metal pollution has curtailed fish production and resultantly the human population is facing a great shortage of aquatic animal protein (Lu *et al.*, 2015). Therefore, it is necessary to control the use of heavy metals because they are posing hazardous impacts to aquatic fauna which ultimately leads to serious human health concern (Ambreen *et al.*, 2015).

Biological interest in chromium (Cr) has risen due to its prominent role in industrial pollution and toxicity. Cr exists primarily in Cr (III) and Cr (VI)

oxidation states; the later, hexavalent species, being considered as more toxic in the environment due to its higher solubility and mobility. These species are known to be associated with a spectrum of DNA lesions occurring during Cr (VI) exposure (Reynolds *et al.*, 2004). Though in small traces it played an important role in growth (Tylor, 1999) and metabolism yet at higher concentration (250-300 mg/l for freshwater fish), Cr is highly toxic, mutagenic (Jim *et al.*, 2006; Laura *et al.*, 2006), carcinogenic (Singh *et al.*, 2005; Prabakaran *et al.*, 2006), teratogenic (Krishnani *et al.*, 2006; Mokhtar *et al.*, 2006), and highly mobile and incorporating metal (food chain) (Vinodhini and Narayanan, 2009).

The effect of Cr (VI) on survival of fish in term of LC₅₀ has been investigated from 142-250 mg/l for freshwater fish. This variation in LC₅₀ depends on size, species, source of chromium (VI) and physical conditions i.e. temperature, pH, and DO etc. (Mishra and Mohanti, 2009). Cr has detrimental effect on fish growth and survival (Shaheen *et al.*, 2012). It has not only damaged fish fertility but also produced defects in progeny. Exposure of breeders before spawning induced cumulative effect on gonads which in turn produced weak, meager and retarded offsprings due to malfunctioning of trophic hormones (Kobayashi and Okamura, 2005; Imai and Nakamura, 2006).

* Corresponding author: farjabeen2004@yahoo.co.in
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Per acre fish yield in Pakistan has been curtailed from 1000 kg to 200 kg in the last 15 years (Javed, 2005; Rauf *et al.*, 2009) due to introduction of chromium rich metallic effluent from textile, electroplating, sugar, leather tanning, paper and pulp industries of Kasaur, Multan, Faisalabad, Wazirabad, Mial (Punjab), Hyderabad, Maleer, Karachi east (Sindh), Dikari, and Nasirabad (Balochistan), Attock (NWFP), (EPA, 1999). It is one of the factors behind the depletion of aquatic life from freshwater bodies (like Deg, Rohi, Aik, Ravi, Soan, Chenab, Jehlem and Sutlug River) of Pakistan (EPA, 1999; Zia and Ashraf, 2006; Saima *et al.*, 2008). Considering the degree of damage and to learn the causes and effect of metallic exposure a lot of research has been carried yet, it needs more studies to eliminate the damaging effect of metallic toxicity. Since metal toxicity has direct effect on per acre fish yield the present study was designed to check the effect of Cr VI on fish growth and physicochemical parameters of ambient water. This will not only determine the abnormalities in fish due to Cr VI but will also explain which sublethal concentration is relatively safer and which is relatively fatal for fish health.

MATERIALS AND METHODS

Cyprinus carpio breeders (W=500±9.5g; L=25.60±2.6cm) were acclimatized for a period of one month in cemented tanks. These were fed with fish feed (30% protein and 8% carbohydrates) @7% of wet body spread over two feedings (Jhingran, 1995) through out the year adjusting on weight and mortality. Aeration was provided with automatic compressor. The water was removed on every alternate day to siphon off the unconsumed food. 252 *C. carpio* breeders were divided at random in to control (n=36) and treated (n=216) @12 breeders/tank. Control were kept in normal water while experimental group were separately exposed to various sub lethal Cr (VI) concentrations (25, 50, 75, 100, 125 and 150mg/l) up to one year in triplicate for each concentration (Mckim, 2002). Actual chromium in water is determined/month on atomic absorbent through out the year. Temperature, dissolved oxygen and pH were monitored daily (using YSI-Model-57 and Lutron-pH meter) while

free carbon dioxide (CO₂), alkalinity (as CaCO₃), total hardness (as CaCO₃) chloride, total dissolved solids (TDS), Biological Oxygen Demand (BOD), Chemical oxygen demand (COD), carbonates and bicarbonates were monitored after every two weeks following APHA (1995). Water samples were collected from surface, column and bottom of each tank from each concentration separately using Kemmerer and Van Dorn Bottle sampler. Two sub-stations were fixed in the east and west corner of each tank for each concentration. Water samples were mixed to have a composite sample in each tank/concentration. These water samples were stored in sterilized borosil bottles of one-liter capacity and were carried to the laboratory in an insulated box at 4°C and stored in refrigerator before analysis, which was conducted within 24 hour of sample collection. Mortality was determined daily while weight (on digital balance: Varion M 1275) and length (using graduated meter rod) on monthly basis. Weight limit was kept nearest to 0.01g where as length limit was nearest to 0.1 cm. For determining weight every month 5 fishes were selected randomly from each concentration/ tank, immersed in MS222 (0.5gm/l solution: Sandoz) for 45 seconds to 1 minute before weighing and marked with permanent marker after weight. Feed conversion ratio was calculated by gram feed dry matter consumed per gram weight gain while specific growth rate was measured as percentage of body weight gain per day (100 x Final weight (g) - Initial weight (g)/ time in days). Data was statistically analyzed using ANOVA with Turkey's on average mean data of 12 months for water and growth using SPSS, Pearson correlation matrix and condition factor $K = W \times 100/L^3$ (Weatherly and Gill, 1987).

RESULTS AND DISCUSSION

Cr (VI) exposure significantly altered the physicochemical characteristics of the tank water having fish. The level of tolerance in fish depends on a number of factors such as alkalinity, hardness, pH, DO, species and concentration of metal in the medium. In present investigation water temperature varied from a maximum of 46°C in May to minimum of 12°C in January. Temperature

Table I. Effect of Cr (VI) on physicochemical parameters of ambient water containing *Cyprinus carpio* breeders for one year.

Physicochemical Parameters	Dose (mg/l)						
	Control	25	50	75	100	125	150
Temperature (°C)	24.42 ^a	27.00 ^a	27.38 ^a	28.65 ^a	30.50 ^a	30.50 ^a	32.66 ^a
pH	9.53 ^a	8.41 ^b	7.38 ^c	7.36 ^c	6.87 ^{cd}	6.31 ^{de}	5.99 ^e
DO	8.99 ^a	5.94 ^b	5.43 ^{bc}	5.06 ^{bc}	4.46 ^{cd}	3.49 ^d	2.33 ^e
BOD (mg/l)	8.78 ^a	55.46 ^b	78.83 ^{bc}	101.00 ^{cd}	136.58 ^d	194.66 ^e	283.66 ^f
COD (mg/l)	46.46 ^a	134.50 ^{0b}	190.96 ^c	198.75 ^c	250.96 ^d	339.83 ^e	413.00 ^f
Free CO ₂ (mg/l)	5.66 ^a	18.75 ^b	20.75 ^b	24.33 ^b	36.58 ^c	51.58 ^d	61.58 ^d
Alkalinity (mg/l)	453.66 ^a	465.66 ^a	482.25 ^{ab}	491.25 ^{ab}	516.66 ^b	525.16 ^{bc}	569.00 ^c
Calcium (mg/l)	97.75 ^a	114.33 ^{ab}	148.66 ^{bc}	171.83 ^{cd}	199.25 ^d	287.50 ^e	307.46 ^e
Hardness (mg/l)	250.33 ^a	304.66 ^b	319.83 ^{bc}	348.00 ^c	357.66 ^c	408.46 ^d	467.58 ^e
Chlorides (mg/l)	42.66 ^a	56.75 ^{ab}	83.50 ^{bc}	101.00 ^c	156.00 ^d	163.83 ^d	249.96 ^e
Turbidity (NTU)	15.50 ^a	37.83 ^b	53.75 ^c	86.08 ^d	97.46 ^d	128.16 ^e	145.83 ^f
TDS	469.46 ^a	512.33 ^{ab}	530.58 ^{bc}	556.58 ^{bc}	577.33 ^{cd}	622.66 ^d	828.66 ^e

Values in columns having no common letters are significantly different; P<0.05

remained fluctuated with non significant increase (Table I). Deleterious effect of Cr (VI) increased with the increase in temperature, which in turn stress the fish and increases its metabolic rate beyond normal level and hence affected the pace of fish growth (Mayor and Rao, 2004; Arshad *et al.*, 2007).

DO decrease from 9mg/l of control to 2.1mg/l at 150mg/l Cr (VI) concentration. DO significantly decrease at 25, 125 and 150mg/l Cr (VI) concentration (Table I). BOD significantly increase (P<0.01) from 8mg/l of control to 283 mg/l and COD from 46 of control to 413mg/l at 150mg/l Cr (VI) concentration (Table I) in temporal dose pattern. Temperature BOD and COD are positively correlated with each other while DO is negatively correlated with these parameters. Presently with the increase in temperature toxicity of Cr (VI) also increased which in turn reduced DO level. With the decrease in DO level the amount of BOD and COD increased which showed that when DO levels fell below saturation (9.7 to 1.6 mg/l), anaerobic bacteria took over the further breakdown of fish feed. Such anaerobic conditions persisted at 125 and 150 mg/l Cr (VI) concentration for an extended period, that's why water become septic, *i.e.* blackish brown colored, in few months (May- July) with foul smelling which is in accordance with (Chukwu and Nwankwo, 2005; Nussey *et al.*, 2006; Xiaoli *et al.*, 2007).

Changes in Physicochemical parameters of

ambient water were compared with their respective control ones. The statistical significance has been determined by Tukey's test and its probability is represented by superscript. (DO, dissolved oxygen; BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand; TDS, Total dissolved solids).

pH decreased from 9.5 to 6 while CO₂ increased (P<0.001) from 6 to 61mg/l and alkalinity from 454mg/l of control to 569mg/l at 150mg/l Cr (VI) concentration in 12 months except at 50mg/l (Table I). Level of significance varied within the concentrations. Changes in pH of ambient water produced subtle changes in the functional and structural affinity of aquatic organisms (Arvind, 2003; Ogundiran and Afolabi, 2008).

High concentration of free CO₂ may be due to the decomposition of Cr (VI) at the bottom of cemented tanks, DO depletion or because of large amount of organic matter in tanks as unconsumed food, which fish did not consume because of Cr (VI) stress, which disturbed its metabolism. Increased CO₂ enhance the concentrations of carbonates and bicarbonates which in turn increased alkalinity. Similar results were described by Olumukoro and Egborge (2004). pH is negatively correlated with temperature, BOD, COD, alkalinity, CO₂, TDS, turbidity, chlorides, and calcium. Calcium increased significantly from 98 of control to 307mg/l, hardness from 250 of control to 467, chlorides from 43 of control to 250mg/l, TDS from 469 of control

to 828mg/l at 150mg/l and turbidity from 15 of control to 145 NTU at 150mg/l Cr (VI) concentration (Table I) in 12 months. Present results indicated calcium/chloride rich water above 25mg/l Cr (VI). It has been observed that the amount of total hardness increased with increase in concentration of Cr (VI). Abnormalities due to hardness caused slow growth, anxiety and eventual fish death (Boyd, 1995). Maximum value of TDS was noted above 100 mg/l Cr (VI) tanks with frequent fish kill. This may be due to high amount of dissolved solids present in tanks and DO depletion. Similar results were studied by Xiaoli *et al.* (2007). They observed that the large amount of total solids might result in high osmotic pressure, which in turn caused imbalance osmotic regulation and led to suffocation of aquatic animals particularly fishes present in tanks. Maximum turbidity values were observed at 75,100, 125, and 150 mg/l while turbidity level remained more or less same at 25 and 50 mg/l throughout the experiment. Low level of water during summer and high level of water in winter may be the proper reasons for changes in the turbidity. These observations are in conformity with those of Wayne and Ming (2004), Mathivan *et al.* (2006) and Oygard *et al.* (2007).

Sublethal concentrations of Cr (VI) produced deleterious effects on growth and fish development. The feed offered was not taken up by the fish when immediately exposed to different chromium concentrations as compared with control but after 5 to 6 hrs they started feeding. Weight gain and SGR of *C. carpio* significantly decrease with the increase in Cr (VI) concentrations. This decrease was linearly correlated with the increase in chromium concentration. Result significantly differ between weight (df=6, 77; F=203.6; P<0.001) and length (DF=6, 77; F=23.5; P<0.05) at various Cr (VI) concentrations. Weight increase from initial 500±9.5 to 856±216g in control while it decreased to 495±63, 473± 31, 436±49, 424±49, 405±59g at 50, 75, 100, 125 and 150mg/l except at 25mg/l Cr (VI) concentration that had non significant increase in weight (517±60) (Fig. 1). SGR decrease from 97.2% of control to 4.38, -1.3, -7.3, -17.5, and -20.7 and -26% at 25, 50, 75, 100, 125 and 150mg/l Cr (VI) concentration. Length significantly (P<0.001) decreased from 39± 2.5 cm of control to 32±0.8,

31±0.8, 31±0.41, 30±0.4, 29±0.5 and 28±0.909 at 25, 50, 75, 100, 125 and 150mg/l Cr (VI) concentration (Fig.2). Exposure of fish to different chromium concentrations in water significantly reduced feed consumption. FCR significantly (P<0.001) decreased from 3.5 of control to 2.71, 2.56, 2.44, 1.98, 1.82 and 1.41 at 25, 50, 75, 100, 125 and 150mg/l Cr (VI) concentration. The decrease in weight gain, SGR, feed consumption and FCR with increase in chromium concentration was noted throughout the experiment and did not return to normal until the end of experiment, even when the fish appeared to acclimatize to chromium exposure. Reduced growth in terms of SGR, FCR, weight and length could be because of (i) less feed intake due to Cr (VI) stress, (ii) decreased protein synthesis, (iii) general slow down of normal

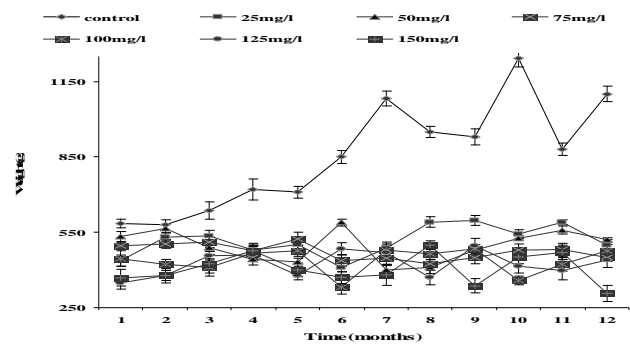


Fig. 1: Body weight of *Cyprinus carpio* after one year of chronic-active exposure to Cr (VI); each dose had 3 replicates of 12 fish.

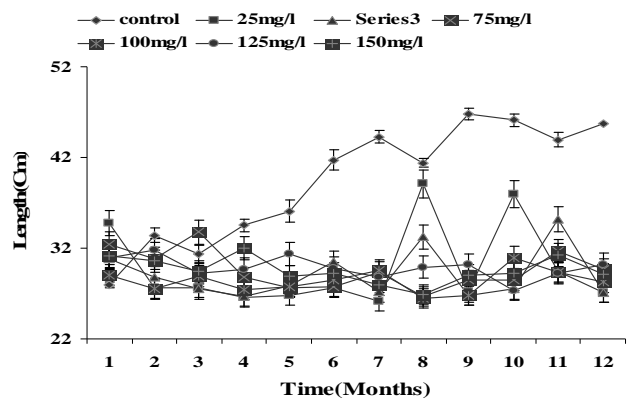


Fig. 2: Body Length of *Cyprinus carpio* after one year of chronic-active exposure to Cr (VI); each dose had 3 replicates of 12 fish.

metabolism, or (iv) combined action of all these factors (Robert and Oris, 2004; Pamela *et al.*, 2007).

In Pakistan per ace fish yield decreased due to Cr (VI) which produced suffocation in fish by increasing BOD/COD. Secondly due to disturbance in fish metabolic system its efficacy to take feed and feed conversion ratio is also affected. Chromium stress may induce energy depletion in fish which decrease liver and muscle glycogen content that ultimately retarded fish growth and yield (Hayat *et al.*, 2007). In Indian major carps weight significantly decrease (8% of wet body weight) on exposure to various doses of manganese and nickel while control gain (6% of wet body weight) after 30 days (Javed, 2005). These support present findings where control gain 84% weight while exposed lost 43% weight at 150mg/l Cr (VI) concentration. The main reason behind this rapid lose is that Cr(VI) not only cumulated in tissues during aquatic organisms growth (bioaccumulation) but also biomagnify up the food chain and thus fish faced both metallic stress as well as starvation. Value of condition factor (K) varied from 1.4 to 1.9 with the increase in chromium concentration. Fish with a high value of "K" are lighter, while fish with a low "K" value are heavier (Weatherley and Gill, 1987; Wooton, 1998). Present decreasing trend of condition factor in control and increasing trend in exposed fish suggest that increase in weight here is less than cube of its length.

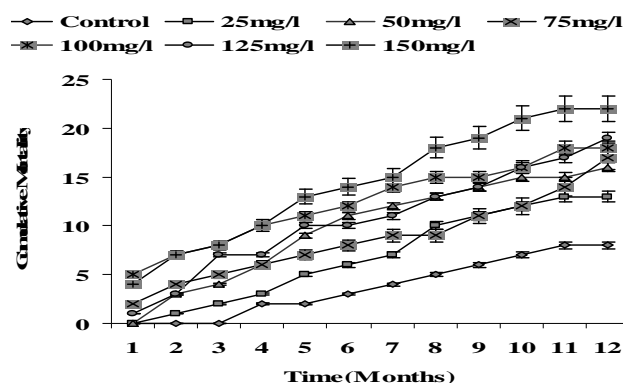


Fig. 3. Cumulative mortality in *Cyprinus carpio* under the influence of sublethal Cr (VI) doses for one year; each dose had 3 replicates of 12 fish.

Similar results were interpreted by Sirashanthini *et al.* (2009), Charles *et al.* (2006) and Patton *et al.* (2007). Mortality increased with increase in time and concentration (Fig. 3). Mortality increased from 21% of control to 40, 43, 45, 50, 53 and 61% at 25, 50, 75, 100, 125 and 150mg/l Cr (VI) concentration. Fish survival increased from 39% in exposed to 79% in control with overall 40% mortality. Experiment conducted on survival and growth, showed temporal dose pattern. The period from one to five months is characterized by very low mortality, which is apparently related with the resistance of breeders to metallic toxicity. During the late months the mortality tends to increase, suggesting accumulative effect of Cr (VI) that reduced feed uptake and deteriorated metabolic rate of fish. Decreased DO Level and increased temperature in summer may also contribute to fish death? Elliot and Hemingway (2002) and Dinesh *et al.* (2005) also observed similar results. Restlessness at higher Cr (VI) concentration throughout the year caused maximum mortality.

CONCLUSIONS

Cr (VI) not only deteriorates water quality of ambient water beyond normal range but also affected fish growth and survival in concentration dependent manner.

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