Chromium (III and VI) induced Opercular alterations in an Indian Air-breathing fish, *Anabas testudineus* (Bloch)

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Abstract : The Indian Air-breathing fish, *Anabas tesudineus was* used as a model animal to investigate the responses to chromium sulphate and potassium dichromate in water bodies. The fish, *Anabas tesudineus* were exposed to sublethal concentration of 15.25mg/L of chromium sulphate and 1.95mg/L potassium dichromate for 24, 48, 72 and 96 hours as well as 15, 30 and 45 days to study alterations in opercular beats. The mean opercular movement of controlled *Anabas tesudineus* was found 48 (46-49)min^{-1}. In chromium sulphate treated fish, the opercular movement showed a mean initial significant increase from 49 to 56 min^{-1} during 24-72 hours duration and then it decreased significantly to 43 to 48 min^{-1} during 96 hours and long term treatment. In potassium dichromate treated fish, it showed a mean initial significant increase from 50 to 57 min^{-1} during 24-72 hours duration and then it decreased significantly to 41 to 46 min^{-1} during 96 hours and long term treatment. The experiment showed more adverse effect of potassium dichromate on opercular movement in comparison to chromium sulphate as the toxicant.

Changes in opercuiar beats are treated as the important diagnostic tools and indicators of stress due to pollutants and environmental fluctuations.

Key words: Chromium Toxicity, Opercular beats, *Anabas tesudineus*.

INTRODUCTION

Chromium is considered as one of the most common ubiquitous pollutants in the aquatic environment, but its pure metallic form is absent naturally. There are three oxidation states in case of Chromium *viz*., Cr (II), Cr (III) and Cr (VI). Among which Cr (II) is most unstable. Cr (III) and Cr (VI) are the stable oxidation state in the environment. Cr (VI) is generally considered 1,000 times more toxic than Cr(III) (Dayan and Paine 2001). Chromium is considered as potent toxicant to fish showing effects at physiological, histological, biochemical, enzymatic and genetic level (Heath 1987).

Anabas testudineus (Bloch) is a potamodromous freshwater fish belonging to the family Anabantidae of the order Perciformes. It plays a significant role in fisheries and aquaculture practices because of having a high market value as important food fish, not only in India (Kumar et al., 2013; Ray and Kumar, 2019) but also in Indonesia (Akbar et al., 2016). Respiration is one of the essential parameters on which depend many of the vital functions like growth, reproduction and metabolism of fish (Heath, 1987).

In view of this, toxicity tests will be performed on *Anabas testudineus* to elucidate the effects of chromium on the opercular movement of the fish.

The work may provide a future guideline for the scientific community and public officials involved in health risk assessment and management to ensure a better environmental condition for human health.

MATERIALS AND METHODS

Samples of *Anabas testudineus* (body weight: 10-70g; total length: 5-11cm) were obtained from local fish markets and ponds around Sasaram. They were maintained in large cement tanks and fed with chopped goat liver till the feed was acceptable.

The $Cr_2(SO_4)_3$ (MERCK, Mumbai, India; Purity 99%) as chromium (III) and $K_2Cr_2O_7$ (MERCK, Mumbai, India; Purity 99%) as chromium (VI) was used as the test chemicals in this experiment.

The water temperature remained at 26.0 ± 1.0^0C. Temperature the aquaria were not controlled and they enjoyed natural periodicities. After the acclimation of the fish in laboratory conditions for a fortnight, the fish were exposed to test concentrations of chromium sulphate and potassium dichromate. All fishes were fed daily with chopped goat liver. From time to time dissolved oxygen content was maintained in the aquarium with slow bubbling of air with an aerator, giving least interference to the fish.

Chromium sulphate and potassium dichromate treated as well as controlled specimens of fish were kept in glass jars and acclimatized for 10 minutes. After 10 minutes the number of opercular beat (min^{-1}) was enumerated for 10 minutes using a stop watch.

Data recorded on a Microsoft Excel spreadsheet. The results were expressed in mean and standard deviation for each group. Then data were tested for statistical significance by a two-way analysis of variance (ANOVA) using Graph Pad Prism 5.

RESULTS AND DISCUSSION

Toxicants are known to alter the oxygen uptake of fish. Jones (1964), Belding (1992) and Ranjeet et al, (2013) suggested that fish responds to toxic materials by increased or decreased opercular movements.

When exposed to different concentrations of chromium sulphate and potassium dichromate for 24 h, the fish showed a spurt in its opercular movements. Following prolonged exposure for 48, 72 and 96 hours as well as 15, 30 and 45 days to different concentrations of chromium sulphate and potassium dichromate, the fishes showed a reduction in its opercular movement in comparison to rise during 24 hours durations. However, a slight recovery after 48 hours of chromium sulphate and potassium dichromate exposure is seen in fishes exposed to different concentrations.

It is evident from the Tables 1 and 2 that there was rapid increase in opercular beats in chromium sulphate and potassium dichromate treated fish for 24 hours. A progressive inhibition of opercular movement in comparison to 24 hours duration was observed.

The rate of variation in opercular movement was recorded from 54 (+14.89%) to 46 (-2.12%) per minute in short term exposure of 15.25mg/L of chromium sulphate when compared to controlled value of 48. Statistical analysis indicated that there was a significant difference in the opercular beat under controlled and chromium sulphate ($F=71.18$; $p<0.001$ and 3.00 $p<0.05$) (Table 1). But, the variation in opercular movement counted from 48 (0 to -2.04%) to 43 (-8.51%) per minute in long term exposure of chromium sulphate when compared to controlled value of 48. Statistical analysis indicated that there was a significant difference in the opercular beat under controlled and chromium sulphate ($F=92.50$; $p<0.001$ and 45.63 $p<0.001$) (Table 1). F value indicated more pronounced change in opercular movement of *Anabas testudineus* was found during long term exposure when compared to corresponding short term exposure of chromium sulphate (Table 1).

The rate of variation in opercular movement was recorded from 56 (+19.13%) to 44 (-6.39%) per minute in short term exposure of 1.95mg/L of potassium dichromate when compared to controlled value of 48. Statistical analysis indicated that there was a significant difference in the opercular beat under controlled and potassium dichromate ($F=82.66$; $p<0.001$ and 0.6496 $p>0.05$) (Table 2). But, the variation in opercular movement counted from 46 (-4.16 to -6.12%) to 41 (-12.78%) per minute in long term exposure of potassium dichromate when compared to controlled value of 48. Statistical analysis indicated that there was a significant difference in the opercular beat under controlled and potassium dichromate ($F=95.00$; $p<0.001$ and 36.00 $p<0.001$) (Table 2). The observations indicated more pronounced change in opercular movement of *Anabas testudineus* was found during long term exposure when compared to corresponding short term exposure of potassium dichromate (Table 2).

The initial increase in opercular movements in early hour indicates that the fish adaptively increased its opercular movement to increase its oxygen uptake to meet the toxicant stress. Progressive decline in opercular movement of fish exposed to chromium sulphate and potassium dichromate concentrations up to 45days and in frequency of surfacing indicates the severe inhibitory effect of toxicants on the respiratory movement of the fish.

In *Channa punctatus,* it has been reported tendency of convulsion and increased opercular movement altered than control when exposed in 20 mg/l and 40 mg/l concentration of potassium dichromate (Mishra and Mohanty, 2008).

Pandey et al, (1976) have reported that Malathion cause a significant decrease in opercular beating in *Channa punctatus*. Choudhary (1981) in *Heteropneustes fossilis* found a decrease in opercular beating after the treatment of Malathion. Some other workers also found a marked decrease in opercular breathing of *Tilapia mossambica* when exposed to sublethal concentration of Malathion and Methyl Parathion. Prakash et al, (1990) in *Tilapia mossambica* found a decrease in opercular beating after ambient ammonia stress. However, Baskaran and Palanichamy (1990) found a marked increase in opercular beating of *Oreochromis mossambicus* when exposed to sublethal concentration of ammonium chloride.

Cirrhinus mrigala exposed to cobalt chloride at selected periods showed a decrease in their ventilation rate up to 27.91% in lethal concentration at 240hr of exposure, while, in sublethal concentrations initially increased up to 23.95 and 27.91% at 96 and 240hr of exposure followed by a decline up to 24.70 and 12.94% at 960hr of exposure to 39.45 and 13.10 mg/l concentration respectively (Kumari and Shahi, 2014).

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Table-1: Effect of 15.25mg/L of chromium sulphate on opercular movement of *Anabas testudineus*.

Body weight of fish (g)	Opercular movement in controlled fish (min^{-1})	Type of Exposure						
		Acute (hours)				Chronic (days)		
		24	48	72	96	15	30	45
15.4±0.81	47.0±2.0	53.0±2.0 (+12.76%)	52.0±2.0 (+10.64%)	49.0±2.0 (+4.26%)	46.0±2.0 (-2.12%)	45.0±2.0 (-4.26%)	44.0±2.0 (-6.38%)	43.0±2.0 (-8.51%)
23.2±0.90	48.0±2.0	53.0±2.0 (+10.42%)	52.0±2.0 (+8.33%)	49.0±2.0 (+2.08%)	48.0±2.0 -	47.0±2.0 (-2.08%)	46.0±2.0 (-4.17%)	44.0±2.0 (-8.33%)
35.1±0.71	47.0±1.0	53.0±2.0 (+12.77%)	52.0±2.0 (+10.64%)	48.0±2.0 (+2.13%)	48.0±2.0 (+2.13%)	47.0±2.0 -	46.0±2.0 (-2.13%)	44.0±2.0 (-6.38%)
43.1±0.51	48.0±3.0	54.0±2.0 (+12.50%)	53.0±2.0 (+10.42%)	50.0±2.0 (+4.17%)	49.0±2.0 (+2.08%)	48.0±2.0 -	47.0±2.0 (-2.08%)	46.0±2.0 (-4.17%)
58.1±0.62	49.0±2.0	53.0±2.0 (+8.16%)	52.0±2.0 (+6.12%)	50.0±2.0 (+2.04%)	49.0±2.0 -	48.0±2.0 (-2.04%)	47.0±2.0 (-4.08%)	46.0±2.0 (-6.12%)
62.1±1.12	47.0±2.0	54.0±2.0 (+14.89%)	51.0±2.0 (+8.51%)	47.0±2.0 -	47.0±2.0 -	46.0±2.0 (-2.13%)	45.0±2.0 (-4.26%)	44.0±2.0 (-6.38%)
39.5	**48**	**53 (+10.42%)**	**52 (+8.33%)**	**49 (+2.08%)**	**48 -**	**47 (-2.08%)**	**46 (-4.15%)**	**45 (-6.25%)**
		F = 71.18 (p<0.001) and 3.00 (p<0.05) (F at df: 3 and 15 at 5% = 3.3, 1% = 5.4% and 0.1% = 9.3) (F at df: 5 and 15 at 5% = 2.9, 1% = 4.6% and 0.1% = 7.6)				F = 92.50 (p<0.001) and 45.63 (p<0.001) (F at df: 2 and 10 at 5% = 4.1, 1% = 7.6% and 0.1% = 14.9) (F at df: 5 and 10 at 5% = 3.3, 1% = 5.6% and 0.1% = 10.5)		

Figures in parentheses indicate percent increase/decrease in opercular movement in comparison to controlled condition.

Table-2: Effect of 1.95mg/L of potassium chromate on opercular movement of *Anabas testudineus*.

Body weight of fish (g)	Opercular movement in controlled fish (min^{-1})	Type of Exposure						
		Acute (hours)				Chronic (days)		
		24	48	72	96	15	30	45
15.0±0.81	47.0±2.0	56.0±2.0 (+19.13%)	55.0±2.0 (+17.04%)	49.0±2.0 (+4.26%)	44.0±2.0 (-6.39%)	43.0±2.0 (-8.52%)	42.0±2.0 (-10.65%)	41.0±2.0 (-12.78%)
23.1±0.90	48.0±2.0	55.0±2.0 (+14.58%)	54.0±2.0 (+12.50%)	51.0±2.0 (+6.25%)	46.0±2.0 (-4.16%)	45.0±2.0 (-6.25%)	44.0±2.0 (-8.33%)	42.0±2.0 (-12.50%)
35.6±0.71	48.0±1.0	54.0±2.0 (+12.50%)	53.0±2.0 (+10.42%)	50.0±2.0 (+4.17%)	46.0±2.0 (-4.16%)	45.0±2.0 (-6.25%)	44.0±2.0 (-8.33%)	42.0±2.0 (-12.50%)
43.4±0.51	48.0±3.0	54.0±2.0 (+12.50%)	54.0±2.0 (+12.50%)	52.0±2.0 (+8.33%)	47.0±2.0 (-2.08%)	46.0±2.0 (-4.16%)	45.0±2.0 (-6.25%)	43.0±2.0 (-10.42%)
58.8±0.62	49.0±2.0	54.0±2.0 (+10.20%)	53.0±2.0 (+8.16%)	52.0±2.0 (+6.12%)	47.0±2.0 (-4.08%)	46.0±2.0 (-6.12%)	45.0±2.0 (-8.16%)	44.0±2.0 (-10.20%)
62.1±1.12	48.0±2.0	55.0±2.0 (+14.58%)	53.0±2.0 (+10.42%)	50.0±2.0 (+4.17%)	45.0±2.0 (-6.25%)	44.0±2.0 (-8.33%)	43.0±2.0 (-10.41%)	42.0±2.0 (-12.50%)
39.67	**48**	**55 (+14.58%)**	**54 (+12.50%)**	**50 (+4.17%)**	**46 (-4.16%)**	**45 (-6.25%)**	**44 (-8.33%)**	**42 (-12.50%)**
		F = 82.66 (p<0.001) and 0.6496 (p>0.05) (F at df: 3 and 15 at 5% = 3.3, 1% = 5.4% and 0.1% = 9.3) (F at df: 5 and 15 at 5% = 2.9, 1% = 4.6% and 0.1% = 7.6)				F = 95.00 (p<0.001) and 36.00 (p>0.05) (F at df: 2 and 10 at 5% = 4.1, 1% = 7.6% and 0.1% = 14.9) (F at df: 5 and 10 at 5% = 3.3, 1% = 5.6% and 0.1% = 10.5)		

Figures in parentheses indicate percent increase/decrease in opercular movement in comparison to controlled condition.