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An Assessment of Chromium (VI) induced Toxicity in Common Freshwater Fish *Anabas testudineus* (Bloch,1792)

Souvik Biswas[1], Pushmita Halder[1], Santanu Debnath[2*], Pratyush Ghosh[3] and Ashis Kumar Panigrahi[1]
[1]Eco-Toxicology, Fisheries and Aquaculture Extn. Laboratory, Department of Zoology, University of Kalyani, Kalyani, Nadia (West Bengal), India,
[2]Department of Zoology, Brahmananda Keshab Chandra College, Kolkata (West Bengal), India,
[3]Department of Zoology, Chandernagore College, Chandannagar, Hooghly (West Bengal), India,

(Corresponding author: Santanu Debnath)*
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ABSTRACT: Chromium, a heavy metal with potential toxicity, can be found in surface water and groundwater and may originate from both natural and anthropogenic sources. Industrial wastewater or effluents are the primary sources of the hazardous pollutant hexavalent chromium (Cr (VI)). This form of chromium pollution is a major environmental concern worldwide due to its persistence and extreme toxicity to living organisms. Chromium toxicity in fish can have a wide range of adverse effects, including physiological, and biochemical changes, and haematological alterations. Thus, this present study aims to assess the toxicity caused by potassium chromate and to observe different aspects of haematological, and behavioural alterations in common freshwater fish *Anabas testudineus*. Fish were subjected to increasing concentrations of potassium chromate ranging from 5-30 mg/L with different time exposure. The lethal and sub-lethal concentration of chromium (VI) was determined in *A. testudineus* through this study. This study revealed toxicity of chromium through altered morphology (losing of scale, shrinking of eyeballs, redness in eyes, etc.), and behavioural abnormalities (erratic swimming, hyperactivity, frequent surface visits, etc.). Moreover, notable haematological alterations, including changes in PCV, MCV, and MCHC, were observed. Based on these findings, it can be concluded that the heavy metal Chromium (VI) in the form of potassium chromate has adverse effects on *A. testudineus*.

Keywords: Chromium, Potassium chromate, *Anabas testudineus*, Haematological, Behavioral changes.

INTRODUCTION

Contamination free environment can produce food of high quality for human consumption. Heavy metals and other contaminants significantly negatively impact fish, which are economically very important. Metal contamination gradually poses a possible risk to aquatic organisms that are not the intended targets due to their bioaccumulative and non-biodegradable characteristics, particularly in fish.

The toxicity of a particular element is significantly reliant on its mobility since soluble species are more accessible than those absorbed by or adsorbed. The mobility is greatly influenced by the physicochemical environment; for example, metal species that are adsorbed to the mineral matrix at neutral pH are likely to desorb in the digestive tract in acidic pH conditions and be absorbed by an organism. Various indices are used to measure the degree of pollution and the hazards to the environment and health caused by the presence of metal enrichment of a specific environmental component in comparison to its natural concentration. Usman *et al.* (2017) found that the accumulation of heavy metals in common fresh water fish species *Labeo rohita* of river Kabul due to anthropogenic effect of industrial effluents.

Certain metals are classified as human carcinogens by the United States Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer (IARC) based on epidemiological and experimental investigations (Tchounwou *et al.,* 2012). Chromium (Cr), a metal, has emerged as a significant contaminant, which, because of its non-biodegradable nature and biochemical properties, has the potential to be hazardous to living things (Benjamin & Kutty 2019; Farag *et al.*, 2006; Velma *et al*., 2009). In India, Chromium pollution of the aquatic environment is frequently seen as a threat due to growing industry and a lack of accessible safe disposal methods. The situation is made worse by the fact that the drinking water system has been heavily contaminated by its obtainability in nature, either as dichromate in acidic zones or as chromate in alkaline conditions (Dutta *et al.*, 2016; Mitra *et al.*, 2020). The permissible levels of chromium for drinking water by the Indian drinking water Quality standard (IS: 10500:2012) is 50µgL, ingesting large doses of the highly carcinogenic metal hexavalent chromium (Cr^{6+}) may result in the death of both animals and humans (Zayed & Terry 2003).

Nowadays industrial wastes are one of the main reasons for water, soil and air pollution. All over the world, this is a matter of concern because this pollution brings changes in the environment, which in turn adversely affect living organisms and play a role in changing their genetic material. Potassium dichromate is one of the key components used in many industries and is responsible for the alternation of the water quality, which produces health risks. Many reports point to excessive juvenile fish mortality and decreased adult breeding potential after chronic exposure to heavy metals (Bhatkar, 2011; McLeay, 1975).

Generally, in different laboratories and industries, potassium chromate a common inorganic salt is used as an oxidizing reagent (Sanyal *et al*., 2017, 2017). The toxicity of Chromium (Cr) depends on its solubility and various oxidative state (Dutta *et al*., 2016; Abhijit Mitra, 2013; Mitra *et al*., 2020). As Cr exists in various oxidative states, the most common states are divalent [Cr (II)], trivalent [Cr (III)], and hexavalent [Cr (VI)](Nisha *et al.,* 2016). Among those oxidative states, Cr (VI) has more toxic effects as it possesses mutagenic and teratogenic properties (Velma *et al*., 2009). Another reason for Cr (VI) toxicity is that it can easily pass through the cell membrane (D'Agostini *et al.,* 2000; De Flora *et al.,* 1989; De Flora *et al*., 2018; De Flora *et al*., 1997).

Cr (VI) is one of the heavy metals, which affects fish and aquatic species even present in a very low concentration and also affects its morphology, enzymatic functions, physiology and biochemical balances (Heath, 2018). Many researchers reported that various fishes are adversely affected by Cr (VI) toxicity, which in turn disbalances their biochemical reactions, and specific and non-specific immunity (Lohner *et al.,* 2001.; Prabakaran *et al*., 2007; Sastry & Sunita 1983). Glutathione-S-transferase (GST), Aminotransferase (ALT), and aspartate Aminotransferase (AST) are the common enzymes, which are affected by the Cr (VI) toxicity (Dey *et al*., 2019).

We looked into the toxicity and pattern of Cr bioaccumulation in fish for two reasons: (i) the metal has been found in moderate to high concentrations in fish from rivers (Sanyal *et al.,* 2017) and sewage-fed wetlands (Adhikari *et al.,* 2004; Raychaudhuri *et al*., 2008) in the Indian state of West Bengal (W.B.), and (ii) studies on Cr bioaccumulation in Indian edible fish are lacking. Higher trophic levels of the marine food web do not appear to collect Cr (Quarcoo *et al*., 2015). Cr has been found in algae, aquatic plants, crustaceans, and fish in freshwater habitats (Dwivedi *et al.,* 2010; Marchese *et al.,* 2008; Sanyal *et al.,* 2015).

Anabas testudineus is a common freshwater fish of the Anabantidae family that is endemic to Asia and can thrive in low-quality or polluted water. *A. testudineus* is a robust fish that can survive out of water for 6 to 10 hours. However, these industrial toxins have severely harmed this fish population and its natural habitat. So, overall it offers the characteristics to consider as a model species. Therefore, this present study aimed to investigate the toxic effect of Cr(VI) on *Anabas testudineus* fish and evaluate its haematological and behavioural alterations.

MATERIALS AND METHODS

Healthy *A. testudineus* specimens (average length of 5.87±0.74 cm, the average weight of 20.75±2.48 g) were collected from a local fish farm in Naihati. The fish specimens were acclimatized in the laboratory for 15 days after being collected. The fish were fed a pelleted commercial fish feed twice a day during this time. The garbage was siphoned off daily. The potassium chromate (K_2CrO_4) used in this study was obtained from the local market.

Physico-chemical Parameters. A piece of portable equipment was used to test the temperature, pH, and total dissolved solids (TDS). Standard (APHA, 2005) procedures were used to calculate dissolved oxygen (DO), ammonia, alkalinity, and hardness.

Acute Toxicity Bioassay. Chromium's median lethal concentration (LC_{50}) was estimated using a four-day static acute toxicity test. A rough range-finding test was done before calculating the actual concentration of the test solution for the conclusive test of 12.02mg/L for 96 hours. In the typical day/light conditions, a group of 10 randomly selected fish specimens was treated to varied concentrations of the test chemical (0, 5, 10, 15, 20, 25, 30mg/L) in the definitive test. Frequent monitoring was performed to observe fish mortality. At the end of 24, 48, 72, and 96 hours of exposure, the number of dead fish was also reported. The dead specimens were removed as quickly as possible.

Behavioral Observations. The behaviour of the control and chromium (as potassium chromate) exposed groups was critically watched and documented (qualitative analysis) during the toxicity test. This study took into account fish behaviour such as swimming pattern (normal/erratic), activity pattern (hypoactive/normal/hyperactive), the extent of mucous secretion (no mucous secretion/low mucous secretion/heavy mucous secretion), frequency of surface visit, opercular movement (low/normal/high), and so on.

Observations on haematological parameters. Upon completion of the acute toxicity testing, the haematological effects of Chromium (as potassium chromate) were evaluated using two sublethal concentrations of 12.02 mg/L – 10% (1.202mg/L) and 30% (3.606mg/L) (of the 96-hour LC_{50} value, respectively). A control group was also created in which no chromium was used. Water was swapped every 24 hours in the treatment sets to maintain the chromium concentration constant during the exposure period. Two sets were prepared, one for up to seven days and the other for fourteen days. The chromium-exposed groups and the control group were provided with a pelleted fish meal once daily. During the experimentation phases, no deaths were documented. Fish from each group were sedated on the 7th and 14th days before their blood was taken. The caudal puncture was used to collect blood using 3ml sterile plastic syringes washed with an anticoagulant (1% EDTA). TLC (Total Leukocyte Count) was determined using

Neubauer's Haemocytometer and RBC dilution fluid (Agarwal *et al.*, 2006). The haemoglobin (Hb) was calculated using the acid-hematin technique (Sood, 2010). Packed Cell Volume (PCV) was calculated using the microhematocrit method (May *et al.*, 1975; Schalm *et al.*, 1975). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated using conventional formulas (Dacie & Lewis 1991).

Statistical Analysis. Finney's Probit Analysis method was used to calculate the LC_{50} value for 96 hours with 95% confidence limits, slope, and intercept values (Lewis & Finney 1972). The obtained values for haematological examinations are shown as mean and SD. All statistical analyses were carried out using the SPSS 21.0 statistics package.

RESULTS

Table 1: Shows the mean and standard deviation (SD) of the water quality metrics.

Parameters	Value
Temperature (°C)	27.2±0.3
pH	7.29±0.04
TDS (mg/L)	673.87±16.42
DO (mg/L)	6.43±0.35
Alkalinity (mg/L)	112.47±2.45

An acute toxicity test demonstrated that *A. testudineus* mortality is proportional to toxicant concentration. During the experiment, no one died in the control group. The proportion of *A. testudineus* deaths caused by the toxicity of chromium.

Table 2: Percentage mortality of *A. testudineus* after 96 h of exposure to Potassium chromate.

Concentrations of Potassium chromate (mg/L)	Number of fish used for the experiment	Mortality rate (%)
0	10	0
5	10	20
10	10	30
15	10	50
20	10	80
25	10	90
30	10	100

Table 3: LC_{50} Values of the Potassium chromate as chromium after 96 hours Exposure Period (with 95% confidence limit).

Exposure Period (hr)	LC_{50} (mg/L)	95% confidence limit		Intercept
		Upper	Lower	
96hr.	12.02	8.076	16.533	-3.266±0.979

Table 4: Behavioural responses of Koi (*Anabas testudineus*).

Exposure Period and Concentration																
	24 hrs				48 hrs				72 hrs				96 hrs			
Behavioral Responses of Koi (*Anabas testeudineus*)																
	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Erratic Swimming	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
Gulping air at surface	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
Opercular movements	A	F	F	F	A	N	N	N	A	N	N	S	A	N	S	S
Loss of equilibrium	A	A	A	A	A	A	A	A	A	A	A	P	A	A	A	P
Hitting against wall	A	A	A	P	A	A	A	A	A	A	P	P	A	A	A	P
Restlessness	A	A	P	P	A	A	P	P	A	A	A	A	A	A	A	A
Sluggishness	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
Fish lied at surface	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P	P
C =Control, T_1=1.202mg/L, T_2 =3.606mg/L, T_3 =12.02mg/L Less= L, More= M, Fast = F, Normal = N, Slow = S, Present = P, Absent= A																

Table 5: Morphological responses of Koi (*Anabas testudineus*).

Exposure Period and Concentration																
	24 hrs				48 hrs				72 hrs				96 hrs			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Morphological Changes of Koi (*Anabas testudineus*)																
Loosening of scales	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
Shrinking of eye balls	A	A	A	A	A	A	A	A	A	P	P	P	A	P	P	P
Redness in eyes	A	A	A	A	A	A	A	A	A	A	A	P	A	P	P	P
Profuse mucous secretion	A	P	P	P	A	P	P	P	A	P	P	P	A	P	P	P
Bleeding from gills	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P	P
Hemorrhages at mouth	A	A	A	A	A	A	A	A	A	A	A	P	A	A	P	P
C =Control, T_1=1.202mg/L, T_2=3.606mg/L, T_3 =12.02mg/L Less= L, More= M, Fast= F, Normal= N, Slow= S, Present= P, Absent= A																

Table 6: Hematological parameters of *A. testudineus* exposed in two (1.202mg/L & 3.606mg/L) sublethal concentrations of 12.02mg/L.

Parameter	Day of Exposure	Control	Concentration	
			1.202mg/L	3.606mg/L
PCV (%)	7	31.78±0.76	28.77±0.16*	25.52±0.24**
	14	31.81±0.72	27.07±0.15**	24.33±0.28***
TLC ($10^3 mm^{-3}$)	7	17.25±0.12	20.72±0.09***	20.97±0.07***
	14	17.64±0.30	20.7±0.23*	21.95±0.06***
MCV (10^{-4}fL)	7	150.67±0.12	143.69±0.30***	129.07±0.20***
	14	150.44±0.133	141.12±0.42***	129.07±0.12***
MCH (10^5pg)	7	38.52±0.44	36.95±0.09*	35.46±0.51*
	14	38.28±0.531	36.25±0.29*	35.09±0.11**
MCHC (g%)	7	33.85±0.10	35.61±0.27**	37.36±0.22***
	14	33.80±0.177	37.3±0.66**	38.49±0.13***
Hb (gm/dL)	7	12.1±0.36	10.53±0.28*	9.59±0.20**
	14	12.17±0.17	10.48±0.11**	9.78±0.12***
TEC ($x10^6 mm^{-3}$)	7	3.57±0.23	2.70±0.19*	2.18±0.18*
	14	3.43±0.25	2.35±0.12*	1.95±0.03**
*denote P<0.05 level of statistical significance. **denote P<0.01 level of statistical significance & ***denote P<0.001 level of statistical significance.				

DISCUSSIONS

Control fishes. The fishes were very active and showed well-synchronized movement. They mostly settled at the base of the experimental tub, while sometimes came on the surface of the water and actively responded to slight disturbances. No mortality was recorded in the control during the experimental period of 96 hours (Tables 4 & 5).

Treated fishes. Treated fishes showed erratic and rapid swimming in one Lethal concentration of 12.02mg/L and the two sub-lethal concentrations of 1.202mg/L (10% of Lethal concentration) and 3.606mg/L (30% of Lethal concentration). The effect was more pronounced at 24h while less at 96h. In 12.02mg/L it was 20 min at 24h, 15min at 48h, 15min at 72h and 10min at 96h, in 3.606mg/Lit was 12min at 24h, 10min at 48h and 10min at 72h and 96h and in 1.202mg/L it was 9min at 24h and 6min for 48h, 72h and 96h. Gulping air at the surface or jumping out of the water was also observed, it was maximum in 12.02mg/L and 3.606mg/L concentrations and increased with the duration of exposure (24hrs, 48hrs, 72hrs and 96hrs) while it was minimum in 1.202mg/L for at all exposure periods.

Opercular movements were fast at 24h, normal at 48h and 72h and slow at 96h in all three sub-lethal concentrations. Loss of equilibrium in fishes was rarely seen. It was shown only in 12.02mg/L after 72h and 96h of exposure periods while absent in other concentrations and exposure duration. Fishes were hitting against the wall at 12.02mg/L for all-time intervals, in 3.606mg/L only at 72h and it was absent in 1.202mg/L.

Fishes showed restlessness in 3.606mg/L and 12.02mg/L concentrations at 24h and 48h of exposure periods. Fishes became sluggish after 72h and 96h time intervals in 3.606mg/L and 12.02mg/L concentrations.

After 96h intervals, all fishes died which were kept at 12.02mg/L (Lethal) concentration and 2 fishes died at 3.606mg/L (Sub lethal) concentration. Before death, they jumped out of the water, gulped air and then laid down at the surface of the water with jerky movements. Lastly, dead fishes laid down on the surface of the water with bellies upward and open mouths.

Several investigators have established to detection of fish physiology and pathology using the normal ranges for several blood values in fish, and the examination of blood indices provides accurate details on the status of the toxicant-exposed animal's chronic stress, deficits, and metabolic abnormalities. Thus, the antagonistic effects of toxicants on the blood constituents of an organism can be assessed by haematological parameters. Blood comprises a liquefied part termed plasma, which leads to the formation and function of three different cells namely erythrocytes, leukocytes, and thrombocytes. Erythrocytes are red blood corpuscles (RBCs) that are in better amounts in the bloodstream, and fish erythrocytes are nucleated where they carry oxygen and carbon dioxide gas. Leukocytes are white blood corpuscles (WBC), which are the defensive cells used widely to evaluate the immune system whereas the role of thrombocytes in blood clotting, organic defence, and phagocytic function (Hrubec *et al.*, 2000).

The packed cell volume (PCV) level was also reduced by 10% and 30% condition after 7 days (Table 6) and 14 days (Table 6) correspondingly as compared to the control condition. In this case, a distorted RBC shape or a decrease in RBC production brought on a decreased PCV.

The average size or volume of a red blood cell is determined by the term "mean corpuscular volume" (MCV). Accordingly, low MCV indicates microcytic, the size is smaller than the average size, normal MCV indicates normocytic with the normal average size of RBC and high MCV indicates macrocytic representing larger than the average size of RBC. Under the potassium chromate exposure to 7 days and 14 days, conditions also decreased respectively as compared to the control condition. Low MCV in the blood of the potassium chromate-exposed fish was consistent with microcytic anaemia as same in Benjamin & Kutty (2019).

The amount of haemoglobin that typically resides inside a single red blood cell is known as mean corpuscular haemoglobin (MCH). Potassium chromate exposure significantly (P<0.05) decreased the mean corpuscular haemoglobin in the failure of blood

osmoregulation, aberrant haemoglobin production, and abnormal plasma osmolarity were found in all treated groups (Stookey *et al*., 2007). Sharma & Langer (2014) proved that MCH rises due to increased RBC lysis and a decrease in cellular blood iron, which represents a decrease in Hb concentration due to metal toxicity.

The average amount of haemoglobin found inside a single red blood cell is calculated as the mean corpuscular haemoglobin concentration (MCHC). Exposure to Potassium chromate at sublethal concentration decreased the mean corpuscular haemoglobin concentration after 7 days and 14 days of treatment than that of the control group. The results suggest that exposure to sublethal concentration of Potassium chromate drastically affected the total population of red cells in *Anabas testudineus* in a time-dependent manner. Red blood cell size is represented by MCV, whereas mathematical measurements of haemoglobin concentration and blood oxygen carrying capacity are made using MCH and MCHC (Groff & Zinkl 1999). The reduction in the levels of MCH and MCHC in chromate-exposed groups indicates diminished oxygenation and insufficient production of red cells with insufficient haemoglobin.

Haemoglobin is a conjugated protein that contains the prosthetic group heme and the protein component apoprotein globin (Zhiteneva *et al.,* 1989) Haemoglobin is the primary intracellular protein of the RBCs that transport oxygen and carbon dioxide to tissues during respiration. Potassium chromate when exposed to fish at a sublethal concentration significantly ($P<0.05$) decreased the concentration of haemoglobin in all treatment groups in a time-dependent manner when compared to the corresponding control group. Anaemia in fish may result from blood cell damage, which may be the cause of the decrease in haemoglobin concentration following chromate exposure. The production of haemoglobinas well as oxygen binding capacity was significantly reduced in the present study (Mini, 2015; Benjamin & Kutty 2019).

The TEC, haemoglobin (%), and mean cell haemoglobin (MCH) of *A. testudineus* exposed to chromium were all significantly lower, indicating that the fish was anaemic, which could be related to iron deficiency and its subsequent decreased usage for haemoglobin synthesis. Anaemia in fish is an early sign of chromium toxicity, both acute and chronic. Furthermore, a significant decrease in TEC, haemoglobin (%), MCH, and hematocrit was observed in *Channa punctatus* treated with both copper and chromium, with the decrease being more evident in chromium-exposed fish, indicating that the metal causes acute anaemia under hazardous conditions (Singh, 1995; Velma *et al*., 2009; Vutukuru, 2005).

Leucocytes, also known as white blood cells, are immune system cells that play an important part in both non-specific and specific immunological responses in protecting the body from foreign substances. They are also one of the most basic ways to assess the immune system (Moraes *et al*., 2007). In the present study, there was a significant increase in TLC as observed in Cr-treated fish. With exposure to several heavy metals, similar findings were obtained in different fishes *Oreochromis mossambicus* (Buthelezi *et al.,* 2000); *Labeo boga* (Mitra *et al.,* 2020)**;** *Wallago attu* (Sharma & Langer 2014; Singh & Tandon 2009); *Cyprinus carpio* Koi (Ali & Khan 2018). Although previously it was reported that Cd exposure reduced the concentration of leucocytes (WBC) in lesser-spotted dogfish (*Scyliorhinus canicula*) (Tort & Torres, 1988); an increase in WBC count can be attributed to immune system stimulation in response to tissue damage induced by heavy metals. The incorporation of a foreign harmful salt in the bloodstream also caused an increase in TLC (Mitra *et al.,* 2020).

CONCLUSIONS

The present study is an ex-situ analysis which shows the toxic effect of Cr(VI). Cr(VI) is a toxic heavy metal which enters into the aquatic ecosystem via the effluents discharged from numerous industries. Fishes are highly susceptible to this heavy metal because this metal is assimilated through ingestion or gill uptake. Accumulation of this heavy metal in the hepatic and renal tissues impacts various organisms such as the gill, kidney, ovary and liver which inturn adversely affect its behaviour, biochemical activity, physiological activities and metabolic functions. This present study shows the adverse effect of potassium chromate in common freshwater fish *Anabas testudineus* and its exposure to the LC_{50} resulted in a significant alteration in haematology.

FUTURE SCOPE

The present study has shown that *A. testudineus* is a reliable bio-indicator for metallic pollution in freshwater ecosystems. Further research can be conducted to explore the long-term effects of metallic pollution on *A. testudineus*, including its behaviour, physiology, and biochemistry. While *A. testudineus* is a reliable bio-indicator for metallic pollution, comparative studies can be conducted with other fish species to evaluate their effectiveness as bio-indicators. This could provide a better understanding of the impact of metallic pollution on different fish species and their ecosystems.

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Conflict of Interest. None.

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