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Behavioural Responses and Changes in some Haematological Parameters of the Cichlid Fish, *Oreochromis niloticus*, Exposed to Trivalent Chromium

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ABSTRACT. Specimens of *Oreochromis niloticus* were exposed to different concentrations (17.5-27.5 mg/l) of trivalent chromium in the form of $CrCl_3$. Lc. 50 for 96 h was computed as 23.7 mg/l. The fishes exposed to sublethal concentrations (3.0 and 6.0 mg/l) showed remarkable changes in behaviour like cough, yawn, fin flickering, jerking movement, nudge and nip. A positive dose-response relationship was demonstrated for these changes in behaviour. Frequency of occurrence of such behavioural changes was more pronounced in the beginning of treatment and decreased gradually by prolongation of time, but remained higher than the control. Changes in haematological parameters such as total leucocyte and erythrocyte counts, haemoglobin and haematocrit were apparent in the fish exposed to trivalent chromium. The response of fish to chromium was dose dependent. MCH and MCHC values were significantly affected ($P < 0.001$).

Heavy metals entering aquatic ecosystem through industrial wastes, mining, agricultural development, fossil fuel combustion can have a wide range of adverse effects on aquatic communities[1,2]. Survey of literature reveals that heavy metals like cadmium, copper and mercury are studied extensively but very little information are available regarding the effect of chromium to the aquatic communities[3]. Most of the work done on the chromium toxicity is based on acutely lethal concentrations[4-12]. Accumulation of chromium by the fish from the metal polluted environment is reported by Onwumere and Oladimiji[13] and Tariq *et al*[14]. Changes in haematological parameters in fish after the exposure to chromium are available in the work of Gautam and Gupta[15]. It is reported that chromium affects the chromatids exchange, DNA synthesis, respiratory organs and lower parts of the intestine[16-18].

Chromium, in its salts, is usually present in travelent or hexavalent states, both of which are water soluble. Undre alkaline pH, the trivalent form changes into the hexavalent[10].

Trivalent chromium, in the form of $CrCl_3$, was chosen for use in the present investigation. Available literature reveals that trivalent chromium, due to its low absorptivity, is less toxic to animals compared to hexavalent chromium[19,20]. Thus, the author has made an attempt to study its toxicity to the freshwater fish, *Oreochromis niloticus,* by registering mortality, changes in behaviour and some haematological parameters such as total leucocytes and erythrocytes counts, haemoglobin, haematocrit and some erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC).

Material and Methods

Live and healthy specimens of *Oreochromis niloticus* were obtained from a fish farm located at Deerab about 80 km southwest of Riyadh. The average length and weight of the test specimens were 13.78 ± 1.2 cm and 41.8 ± 2.3 gm, respectively. In laboratory, the fish were kept in glass aquarium and left for 4 weeks to acclimatize to the laboratory conditions. During the period of acclimation, the fish were fed a commercial fish food to satiety twice daily. The fish were unfed during the period of exposure. The laboratory water was analysed for dissolved oxygen (7.5 ppm) hardness (273 ppm as $CaCo_3$), temperature (22°C) and pH (7.5).

When the acclimation period ended, which was Judged by normal activity and feeding of the fish, ten fishes, randomly selected from the stock, were transferred to each aquarium containing 30 liters of water. Required concentration of chromium 17.5, 20.0, 22.5, 25.0 and 27.5 mg/l were prepared by adding a known volume of stock solution of trivalent chromium in the form of $CrCl_3$. A control set was run with same number of fish and same volume of water. Water of all the aquaria, including control, was aerated with mechanical air pumps. The medium of experimental tanks was renewed every 24 hours. The fish were exposed for 96 hours and their mortality was registered separately for each concentration. Lc-50 for 96 h was computed from a graph made from $\log_{10}$ concentrations of $CrCl_3$ and probit of kill according to the method of Finney.[21]

In another set of experiments, five fish were exposed to each of the sublethal concentrations, 3.0 and 6.0 mg/l, for 4 days. A control set , having the same number of fish and same quantity of water, was run simultaneously for comparison. Changes in behavioural pattern of fish due to chromium were observed and counted as they were easily identifiable and countable. These behaviours are described briefly in Table 1. The observations were made for 30 minutes for each tank at 24 h, 48 h, 72 h and 96 h of exposure. Time of observation was rotated from morning to evening to avoid the diurnal fluctuations in behaviour.

At the end of exposure, all the fishes from each concentration were sacrificed and their blood was collected in heparinized vials by cutting the caudal peduncle. Samples of clotted blood were discarded. Experiments with lethal and sublethal concentrations were run in duplicates. Erythrocyte and leucocyte counts were made with Neubaur haemocytometer. Haemoglobin was estimated by the method of Blaxhall and Daisley[22]. The blood samples were centrifuged at 6000 rpm for 10 minutes in haematocrit tubes for the estimation of haematocrit value. The erythrocytic indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the method of Ghai[23]. Data were subjected to statistical analysis (student "t" test) to find out significance of differences between the treated fish and control ones.

Results

Freshwater fish, *Oreochromis niloticus,* was exposed to different lethal concentrations (17.5, 20.0, 22.5, 25.0, 27.5 mg/l) of trivalent chromium in the form of chromium chloride ($CrCl_3$). The 96 hours Lc-50 was found to be 23.7 mg/l (Fig. 1). Fish exposed to these concentrations initially showed hyperexcitation, restlessness, abnormal swimming and secretion of mucus which was followed by the loss of balance and succumbing of the fish.

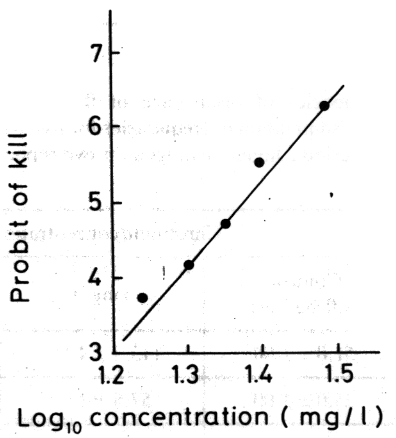

FIG. 1. Relationship between probit of kill and $\log_{10}$ concentrations (mg/l) of trivalent chromium.

Observations made on the fish exposed to sublethal concentrations of chromium indicated a remarkable change in the behaviour. The most significant effect of chromium was on respiration as indicated by increased frequency of coughs and yawns ($P < 0.01$). The increase was more pronounced in the initial stage of exposure (24 h) as compared with other periods of exposure.

Throughout the whole period of exposure to trivalent chromium, the frequency of aggressive responses like nudge and nip and also some other responses such as fin flickering, partial and S-jerk and burst swimming increased significantly ($P < .01$) as compared with the control (Table 1). The frequency of the first three responses was higher during the 24 h period of exposure than that in other periods of exposure (Fig. 2). The frequency of all the aforementioned responses, except that of the S-jerk response, was significantly high in the higher concentration as compared with that of the lower one (Table 2). The trivalent chromium-intoxicated fish secreted a large quantity of mucus.

TABLE 1. Behaviour of *Oreochromis niloticus* monitored in the present study.

Behaviour	Description
Cough	Rapid, repeated opening and closing of mouth and opercular covering with partial extension of fins.
Yawn	Wide opening of mouth and hyperextension of fins.
S-jerk	Movement of body sequentially from head to tail.
Partial-jerk	Movement of head or tail only.
Fin-flickering	Repeated extension and contraction of dorsal fin.
Burst swimming	Sudden and rapid movements (forward).
Nudge	Movement of the fish towards another fish.
Nip	Biting.

These terms were described by Henery and Atchison[2].

TABLE 2. Changes in frequencies of occurrence of the behaviour of *Oreochromis niloticus*. Data are summation of frequencies for five fish per tank for four observations (total period 2 hours) averaged for two replications.

Behaviour	Chromium concentrations		
	Control (0.0 mg/ml)	3 mg/l	6 mg/l
Cough	51.0 ± 1.00	113 ± 4.50*	168.5 ± 1.50*
Yawn	25.0 ± 1.00	57.5 ± 1.50*	79.5 ± 5.50*
Fin flickering	429.0 ± 5.00	588.5 ± 2.50*	616.5 ± 6.50*
Nudge	172.0 ± 1.00	235.0 ± 4.00*	253.0 ± 3.00*
Nip	197.0 ± 1.00	266.5 ± 6.36*	280.0 ± 2.00*
Partial-jerk	30.5 ± 1.05	37.5 ± 2.5*	40.0 ± 2.00*
S-jerk	20.0 ± 2.00	37.5 ± 1.50*	34.5 ± 2.50*
Burst swimming	12.5 ± 0.05	28.5 ± 1.50*	31.5 ± 0.05*
Chafe	18.5 ± 2.50	20.0 ± 2.00*	18.5 ± 1.50*

*Significant difference between the experimental and control means ($P < .01$).

Haematological observations embodied in Table 3 indicate that the trivalent chromium-exposed fish revealed a significant increase in the erythrocyte count and haemoglobin and haematocrit concentrations. The leucocyte count was significantly reduced. Values of MCHC and MCH increased significantly in the trivalent chromium concentrations considered. However, the increase in MCV value was only significant in the 3 mg/l concentration.

TABLE 3. Variations in the blood parameters of *Oreochromis niloticus* due to chromium intoxication.

Blood parameters	Chromium concentrations		
	Control	3 mg/l	6 mg/l
RBC (million/mm^3)	1.65 ± 0.018	1.73 ± 0.021*	1.68 ± 0.030*
WBC (cell × 1000/mm^3)	35.05 ± 0.297	33.55 ± 0.184**	33.41 ± 0.310*
Haemoglobin	6.55 ± 0.013	7.734 ± 0.009**	8.15 ± 0.075**
Haematocrit (%)	32.676 ± 0.165	35.792 ± 0.069**	37.214 ± 0.135**
MCV (fl)	19.80 ± 0.203	20.83 ± 0.260*	19.98 ± 0.290
MCH (Pg)	39.74 ± 0.438	45.02 ± 0.556**	43.77 ± 0.920*
MCHC (%)	20.067 ± 0.107	21.608 ± 0.037**	21.900 ± 0.240**

* Significant difference between treatment and control means ($P < .01$).
** Significant difference between treatment and control means ($P < 0.001$).

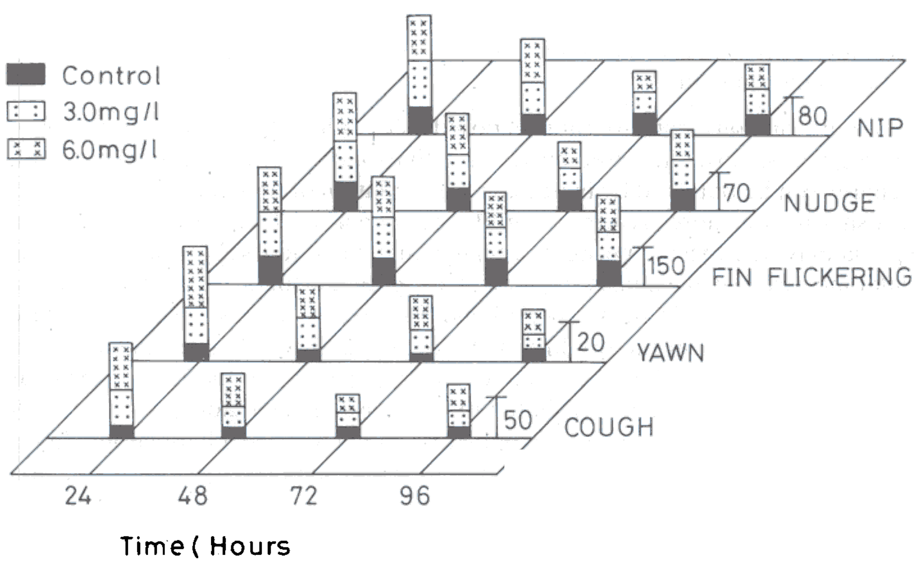

FIG. 2. Frequencies of occurrence of the different behaviour of control and chromium exposed *Oreochromis niloticus* at different time intervals.

Discussion

Results of the present investigation revealed that trivalent chromium exerts toxic effect on the fish but, comparative to other heavy metals (cadmium, copper, zinc, ... etc.), it is less toxic. The 96 hours Lc-50 value (23.7 mg/l) is quite high when compared with that of cadmium (5.2 mg/l) reported from the same laboratory for the same species[24]. Some investigations made on different species of fish showed a differential toxic nature of chromium. The 96 hours Lc-50 values obtained for different species of fish varied from 4.4 mg/l to 97.9 mg/l[8]. The value reported here fits in this range.

Changes in the behaviour of *Oreochromis niloticus* exposed to chromium chloride may be due to the manifestations of the disturbances in the psysiological mechanism which is supposed to initiate, maintain and terminate the behaviour[25]. The hyperexcitation, Jerking movement of the metal-exposed fish may be due to the toxic and irritating effect of toxicant and fish struggled in an attempt to get relieved from the stressful environment.

The elevated muscular activity of fish undoubtedly require more supply of oxygen for the oxidation of fuel molecules, on the other hand, mucus deposition on the grills reduces the gaseous diffusion causing less supply of oxygen[26]. Therefore, the increased demand of oxygen and reduced supply of it may be compensated by increasing the frequencies of coughs and yawns. Both responses have a clearing effect on gills[27,28] and thus the gaseous exchange through the gills is increased. Hexavalent chromium exposure increases the frequencies of cough and ventilation rate of fish, *Salvelinus fontinalis;*[5] and give considerable support to the present investigation. Reduction in the frequency of behavioural responses considered in the present investigation after prolonged exposure can be ascribed to the adaptation of fish to such stressfull environment.

It is quite obvious that chromium exposed fish would require considerable amount of energy to perform increased muscular activity induced by toxicant. That amount of energy can be obtained by increasing the oxidation of fuel molecules. Therefore, the fish will use more oxygen to fulfill the increased demand of energy. Tissue hypoxia may be expected because of high demand and low supply of oxygen due to hinderance caused by mucus deposition on gills and damage of gill lamillae by chromium[18]. Hence, in such hypoxic condition there is a stress mediated release of new erythrocytes from the erythropoietic organs and synthesis of haemoglobin in older cells to improve the oxygen carrying capacity of blood[29-33]. Similarly, Majewski and Giles[34] have attributed the increased haemoglobin in the metal exposed fish to the toxicant induced hyperactivity and impaired gill function. High values of MCH and MCHC registered in the chromium exposed fish may be due to the fact that the fish would synthesize more haemoglobin under stress to increase the oxygen carrying capacity of blood. The high value of MCV of the toxicant exposed fish can be ascribed to the swelling of the red blood cells[33].

Reduction in the WBC count of the chromium exposed fish may be a consequence of a sharp decline in number of lymphocytes and thrombocytes. This change may be due to the enhanced secretion of adrenocorticotrophic hormone (ACTH) which results in the higher blood-titers of corticosteroids which bring about the lysis of lymphocytes and thrumbocytes. It is reported that qualitative response of the pituitary and interrenals, that is production of the ACTH and corticosteroids, in different stimuli is identical but quantitative response depends upon the nature of stress[35,36]. Previous studies[33,37,38] lend a considerable support to the present line of reasoning and leave no scepticism about the above mentioned process.

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الاستجابات السلوكيـة والتغـيرات في بعض مكونات دم أسمـاك البـلطي نتيجـة لتعريضـها للكـروم الثـلاثي

حمود بن فارس القحم
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المستخلص . لقد تم في هذه الدراسة غمر البلطي النيلي في عدة تراكيز (١٧,٥-٢٧,٥ ملجرام/لتر) من الكروم الثلاثي ، حيث وجد أن التركيز الحرج (Lc50) يساوي ٢٣,٧ ملجرام/لتر . ولقد لوحظ على الأسماك المغمورة تغيرات في السلوك (مثل التثاؤب ، الحركة الدودية ، اضطراب في الزعانف ، السعال ، زيادة في السلوك العدائي ، زيادة في عدد كرات الدم الحمر ، نقص في كرات الدم البيض ، زيادة في الهيموجلوبين) وتغيرات إيجابية في MCH و MCHC ($P < 0.001$) .

إزدادت التغيرات السلوكية في البداية ولكنها بدأت في التناقص التدريجي مع زيادة الفترة الزمنية ، لكنها بقيت بمعدل أعلى من سلوك الأسماك التي في حوض المراقبة (الكنترول) .