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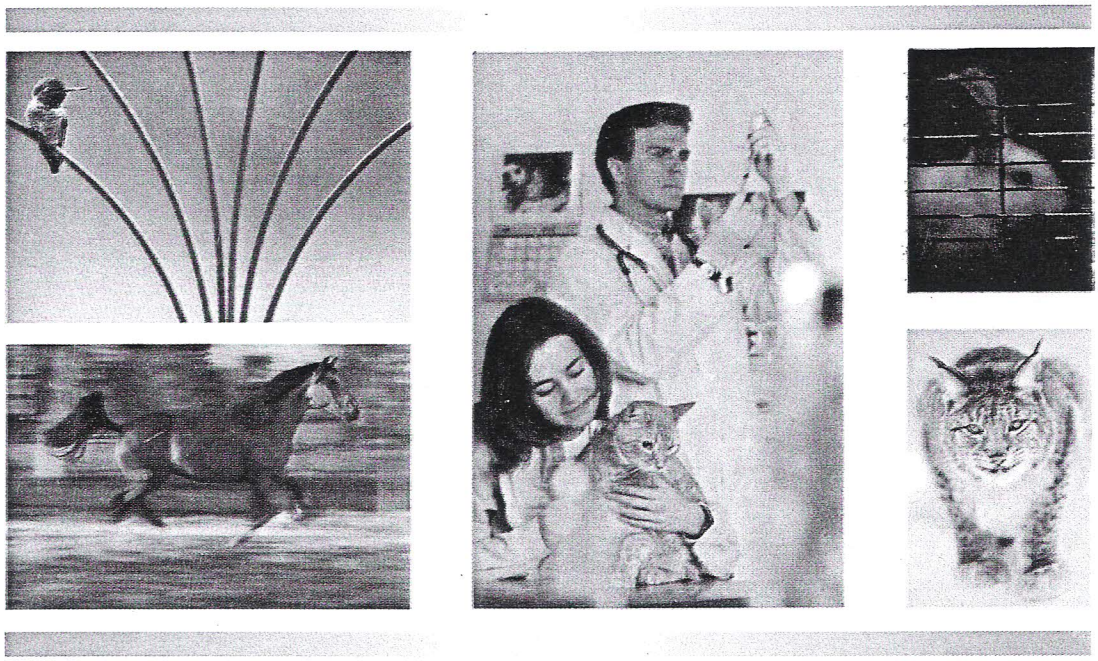

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Accumulation of Chromium in Liver, Gill and Muscle Tissues of *Orechromis niloticus*

[1]Nuray Ciftci, [1]Bedii Cicik, [2]Cahit Erdem, [1]Ozcan Ay and [1]Celal Gunalp
[1]Faculty of Aquaculture, Yenisehir Kampusu, University of Mersin,
C Blok, Kat:2, 33169 Mersin, Turkey
[2]Department of Biology, Faculty of Art and Sciences, University of Cukurova,
01330 Balcali Adana, Turkey

Abstract: The aim of the present study was to determine the levels of chromium in the liver, gill and muscle tissues of *Oreochromis niloticus* after exposing the animals to 1.0, 2.0 and 4.0 ppm chromium over 96 h. Metal levels in the tissues studied were determined using atomic absorbrtion techniques. Although, some behavioral disturbances were observed in the chromium exposed fish at the beginning of experiments, no mortalities were observed at the end of 96 h. Exposure to chromium increased tissue levels of the metal except the muscle tissue while tissue accumulation increased with increasing concentration of the metal in the medium. Maximum accumulation was observed in the gill tissue at the lowest concentration tested, whereas accumulation was higher in the liver tissue at 2.0 and 4.0 ppm Cr.

Key words: Chromium, tissue accumulation, *Oreochromis niloticus*, gill, muscle tissue, Turkey

INTRODUCTION

The levels of pollutants such as heavy metals in aquatic environments increased due to an increase in human population, industrial and technological development, widespread usage of chemical fertilizers and pesticides and discharge of domestic wastewater without refining which in turn become a global problem threatening all trophic levels. High levels of heavy metals result in mass deaths or habitat changes in aquatic organisms whereas at lower concentrations they mainly accumulate in metabolically active tissues interfering with metabolic and physiologic activities. Cr^{3+} was shown to play a role in protein, carbohydrate and lipid metabolism in mammals (Steven *et al*., 1976).

Chromium is a trace element which can be present in various chemical forms and is active biologically at 3^{+} and 6^{+} valences (Towil *et al*., 1978; Langard and Norseth, 1979). In addition to its usage in metal coating and leather tanning industries it is also used widely in stainless steel, ferrochromium, phosphate fertilizers and pigment production as a raw material (Langard and Norseth, 1979).

Chromium was shown to accumulate mainly in metabolically active organs such as liver, gill and kidneys at high concentrations. It was shown that fish go under some behavioral alterations such as suspending feeding, irregular swimming and accelerated operculum movement when first encountered with chromium (Svecevieius, 2007, 2009). Chromium also caused structural changes such as hypertrophy and hyperplasia at gill epithelium, degeneration in fin rays and weakening of immune system (Synder and Valle, 1991; Bennani *et al*., 1996; Arunkumar *et al*., 2000).

O. niloticus shows a wide range of distribution in tropical climates it is easy to cultivate, resistant species against diseases and pollutants and is economically important since it is widely consumed as a protein source.

It is important to determine the levels of pollutants in tissues of fish species that are consumed as protein sources, since these pollutants are passed cumulatively along the food chain. Present study was undertaken to determine the levels of chromium in liver, gill and muscle tissues of *O. niloticus* after exposing animals to 1.0, 2.0 and 4.0 ppm concentrations of the metal over 96 h.

MATERIALS AND METHODS

O. niloticus having 22.11±1.27 g weight and 11.75±0.21 cm in total length were obtained from the farming tools of Cukurova University, Faculty of Aquaculture were used in the present study. Since the accumulation and toxicity of metals are dependent upon the physical and chemical characteristics of water, experiments were run under controlled laboratory conditions at Mersin University, Aquaculture Faculty laboratories (Table 1).

Corresponding Author: Cahit Erdem, Department of Biology, Faculty of Art and Sciences, University of Cukurova, 01330 Balcali Adana, Turkey

Table 1: Some physical and chemical properties of the experimental media

Temperature (°C)	24±1
Illumination regime (light/dark)(h)	12/12
Total Hardness (ppm $CaCO_3$)	246.4±3.28
Total Alkalinity (ppm $CaCO_3$)	409±0.63
pH	8.02±0.06
Dissolved O_2 (mg L^{-1})	7.42±0.47

Fish were adapted to laboratory conditions for 1 month in 8 glass aquaria 40×120×40 cm in height. The sized four aquaria were used in the experiments taking the chromium concentrations tested. The first three aquaria were filled with 120 L of 1.0, 2.0 and 4.0 ppm chromium solutions and the fourth one filled with the same amount of chromium free tap water and used as control. Experiments were run in triplicate being two fish in each replicate hence six fish were placed in each aquarium. Fish were fed once a day with readymade fish feed (Pinar, pellet No. 2) at amounts of 2% of the total biomass. Aquaria were aerated with a central aeration system.

$K_2Cr_2O_7$ (Merck) was used in the preparation of experimental solutions. Experimental solutions were replaced daily with freshly prepared 1000 ppm Cr stock solution by making necessary serial dilutions in order to prevent concentration changes due to precipitation, adsorption to glass and evaporation.

At the end of the experimental period fish were removed from the aquaria, washed with tap water and dried with Whatman filter paper. Liver, gill and muscle tissues were dissected from each specimen separately and were brought to constant weight at 150°C for 72 h. Dried tissues were weighted and digested in nitric acid (HNO_3, 65%, O.A.: 1.40, Merck)and perchloric acid ($HClO_4$, 60%, O.A.:1.53, Merck) mixture (2:1; v/v)at 120°C for 4 h (Muramoto, 1983). The digested tissue samples were then transferred into polyethylene tubes and their volumes were made up to 10 mL with distilled water.

Tissue levels of chromium were determined using Perkin-Elmer 2380 Atomic Absorbtion Spectrophotometer. Statistical analysis of the experimental data was carried out using variance analysis and Student Newman Keuls Procedure.

RESULTS AND DISCUSSION

The effects of heavy metals depend on the chemical form of the metal, its concentration and exposure period, interaction with other metals, the species in concern and to its developmental stage and also to the chemical and physical properties of water. No mortality was observed in *Channa punctatus* exposed to 2.6 ppm Cr over 30 days of exposure while caused 100% mortality after exposure to the same concentration over 120 days (Sastry and Sunita, 1984). There was no mortality in *O. niloticus* exposed to 1.0, 2.0 and 4.0 ppm Cr over 96 h. This can be explained by the prevention of metal to cause its toxic effects by excretion, storage and detoxification mechanisms of this species.

Table 2: Accumulation of chromium in liver, gill and muscle tissues of *O. niloticus* (μg Cr g^{-1} d.w.)

	Tissues ($\bar{X}\pm S\bar{x}$ *)		
Concentration (ppm Cr VI)	Liver	Gill	Muscle
0.0	0.60 ± 0.61^{ax}	0.46 ± 0.13^{ax}	0.53 ± 0.35^{ax}
1.0	3.55 ± 0.33^{bx}	4.06 ± 0.17^{by}	0.87 ± 0.08^{az}
2.0	$7.34\pm.05^{cx}$	4.87 ± 0.19^{cy}	0.72 ± 0.08^{az}
4.0	43.42 ± 2.44^{dx}	9.66 ± 0.28^{dy}	0.61 ± 0.12^{az}

*SNK = Letters a, b, c and d show differences concentrations, x, y and z among tissues Data shown with different letters are significantly different at the $p<0.05$ level. $\bar{X}\pm S\bar{x}$: Mean±standart error

Toxic materials, probably due to their effects on gill tissue, cause some behavioral alterations such as movement toward surface, increase in operculum movement, disorders in swimming coordination and rejection to take of feed. These discrepancies in behavior were observed in *Cyprinus carpio* exposed to cadmium (Karaytug *et al*., 2007), *Lobeo rohita* exposed to copper (Venkataramana and Radhakrishnaiah, 2001), *Anguilla anguilla* exposed to lead (Ciftci *et al*., 2008), *Tilapia zilli* and *Clarias lazera* exposed to zinc (Hilmy *et al*., 1987) and *O. niloticus* exposed to chromium in the present study which returned to normal with the prolongation of exposure period. Fish first exposed to waterborne metals undergoes into a stress condition which is a possible consequence of disturbance of ion transport due to necrosis and apostosis of chloride cells and respiratory cells at gill tissue (Lauren and McDonald, 1987). However, on prolonged exposure periods, acclimation occurs by the replacement of damaged cells and restoration of ion transport (Li *et al*., 1998; Dang *et al*., 1999).

Metal a ccumulation in liver and gill tissues of *O. niloticus* increased with increasing concentrations of chromium in the media while no statistical difference was found in muscle accumulation compared with the control fish. Accumulation of Cr was highest in gill tissue at 0.1 ppm Cr while the highest levels of chromium were measured in the liver tissue at 2.0 and 4.0 ppm Cr (Table 2).

Although, tissue accumulation of heavy metals depends on various factors, it primarily a function of the metabolic was highest in kidney tissue in *Scardinius erythrophta* activity of the organ concerned. Tissue accumulation *Lamus* exposed to 20 ppm Cr over 24 h (Van Hoof and Van San, 1981) where as highest chromium levels were found in liver tisues of both *C. carpio* and *Tilapia nilotica* exposed to 1 ppm Cr over 16 days (Canli and Kargin, 1995). Chromium accumulation was found to be higher in gill tissue of *O. niloticus* when exposed to 1.0 ppm Cr over 96 h where the levels of the metal in liver tissue exceeded the gill levels in the

following two higher concentrations of the present study. Chromium seems to accumulate in gill tissue when animals exposed to low levels of the metal since gills are direct contact with the surrounding water. The carrying capacity of the gill tissue, however is exceeded at prolonged exposure periods and the metal is being transferred to the liver tissue for detoxification and to the kidney tissue for being excreted.

No statistical difference was found between the chromium exposed and control animals at the concentrations tested, suggesting muscle is not active tissue in metal accumulation.

CONCLUSION

Chromium accumulated in metabolically active tissues such as gill and liver of *O. niloticus* exposed to 1.0, 2.0 and 4.0 ppm Cr concentrations of over 96 h whereas muscle accumulation of the metal was not statistically different compared with the control fish.

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