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EFFECT OF CHROMIUM ON SCALE MORPHOLOGY IN SCALY CARP (*CYPRINUS CARPIO* L.)

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ABSTRACT

This study, it was aimed to be enabling to use of fish scale as a bio-indicator for heavy metal pollution that is gradually increase in natural waters. For this purpose scaled carps, *Cyprinus carpio* (Linnaeus, 1758) were exposed to 7.5, 15, 30 and 60 µg L^{-1} concentrations of trivalent chromium for 21 day and deformities appearing on the scales were examined under electron microscopy. It was found that scale deformations in scales were more in focus region and increased with increasing chromium concentration in water. Radius, circulars and lepidonts on scale damaged at various rates depending on chromium concentrations. In addition, more damage was observed in anterior region as compared with in posterior region of the scales. This study showed that scales were very affected from chromium accumulation. For these reasons scales have an important role as a bio-indicator to determine the pollution parameters, especially for heavy metal pollution.

Key words: Chromium toxicity, common carp, lepidont, scale morphology, radius.

INTRODUCTION

Water pollution causes negative effects on aquatic ecosystems, disruption of ecological balances and reduction or/and exhaustion of self-cleaning capability of all water in the world (Förstner and Wittmann, 1983). As heavy metals, especially cadmium, mercury, lead and chromium cannot be sent away from living organisms into which they enter with food chain by natural physiological mechanisms, they accumulate and cause toxic effect in cases where concentration exceed permissible levels (Anonymous, 1991; Nayak *et al*., 2005; Radhakrıshnan and Hemalatha, 2011).

Generally in fishes, the toxic effect of heavy metal influence physiological functions, individual growth and mortality. Fish can be used as an indicator of fresh water contamination by heavy metals because they occupy different trophic level in an aquatic ecosystem. High exposure concentration of heavy metals is known to be toxic for aquatic organisms but the low metal concentration also cause toxicity when they are introduced into the environment by a wide spectrum of natural and anthropogenic source. Heavy metals are non biodegradable and once they enter the aquatic environment their bio-accumulation may occur in fish tissue by means of metabolic activities and bioabsorption process.

Elemental chromium (Cr) is very stable, but is not usually found pure in nature. Chromium can exist in oxidation states ranging from -2 to +6, but is most frequently found in the environment in the trivalent (+3) and hexavalent (+6) oxidation states. The +3 and +6 forms are the most important because the +2, +4, and +5 forms are unstable and are rapidly converted to +3, which in turn is oxidized to +6 (Eisler, 1986). Because of its high biological activity and being more toxic, trivalent chromium (Cr^{3+}) was used in this study. Behaviour of Cr^{3+} in the living organisms depends on the oxidation stage, chemical features in the oxidation stage and physical structure (Mertz, 1987). Chromium is used as a pigment for the metal alloys, paints, cement, paper, rubber and other materials. Exposure to a lower level of chromium causes ulcer and irritation on the skin. Longer exposures can cause damages in kidneys, livers and blood circulation system and nerve tissues. Chromium increases in the body mostly by accumulating from the aquatic environments (WHO, 1996).

Demersal fish species reflect heavy metal concentration levels better than pelagic fish. In this study scale carp, *Cyprinus carpio* was used because of its easy adaptation to different conditions, growing and feeding conditions, being abundant in natural water, having high economic value and opportunity of feeding from the bottom.

One of the most important features of many fish noticed at first glance are their bodies being covered with scales. As being the outer structures of the fish body, scales are in a continuous and direct contact with pollutants present in the water. For these reasons, scales can play an important role to indicate pollution levels in water receiving pollutants. Any sudden change in the aquatic environment regarding metal pollution may cause changes and deformations in the shapes of the circuli and other structures in the scales of fishes (Johal and Dua, 1994). There have been many studies to investigate the chromium accumulation and its effects in the various organs of the fish so far. However, not enough information was found about the effect of chromium on the morphologic structure of scales. In this study how chromium affects the morphology of scales, especially the growth rings, was investigated.

MATERIALS AND METHODS

In this study, Chromium trioxide (anhydrous), well-known as chromium (VI) compound (Acharya *et al*., 2004), was obtained from Merck (Darmstadt, Germany) was used for preparation of the test medium.

Scale common carps (*Cyprinus carpio*) were supplied from State Hydraulic Works, 9th Region Management, Keban Fisheries Department Management (Elazığ/Turkey). The scale carp used in the controlled laboratory conditions were placed in two tanks 100x120x60 cm (in 500 litters fibreglass tanks) and left for adaptation. Before metal exposure, fish were acclimatized until the death rate fell under 1%. The average weight of fishes was 100 ± 5 g. After acclimation, the fishes were placed in glass aquaria measuring 30x100x40 cm, with 22-25 °C water temperature, 8-8.5 mg L^{-1} dissolved oxygen and 8-9 pH value. Water temperature and dissolved oxygen were determined with YSI 52 portable oxygen meter and pH was determined with Thermo Scientific Orion 3 Star portable pH meter. All aquaria were aerated using an aeration pump. During adaptation and study period, all fish were fed with commercial fish feed to an equivalent of 2 % body weight twice daily. Uneaten food and the feces were removed at 30 minutes after feeding from all tanks daily. Chromium level in feed and test water were not in detectable limit.

Nearly 15-20 fish were put into each test aquarium (in duplicate) with 7.5, 15, 30 and 60 µg L^{-1} chromium concentrations for 21 days. Test medium were renewed twice a week 3/4 ratio using renewal test technique (APHA, 1985). Actual concentration of chromium level in test solution was found close to prepared concentration. At the end of exposure, scales from each fish were taken, washed and dried. For examination, dorsal area of scales was gold plated at thickness of 100 ^{0}A (Watt, 1985) at Inonu University Central Research Laboratories and then examined using JEOL JSM-7001F Scanning Electron Microscope (SEM) at Firat University Electron Microscopy Laboratory. Their photos were taken. The use of fish and the experimental protocol were approved by the Animal Experimentation Ethics Committee of the Firat University (FUAEEC) (Elazig, Turkey).

RESULTS AND DISCUSSION

In this study, no deformations were observed on the scales of the control group (Figure 1a). Normal scale structure has circuli (C) present all over. These are the signs of calcium deposition or the growth rings. In the anterior region, maximum number of circuli was seen showing bifurcation at different levels. The fully formed circuli have row of lepidonts (L) (Figure 1b). Lepidonts are tooth shaped structures on the outer surface of the circuli of scales. These structures help scale to remain fixed in the body of the fish and scales start detaching from the body when these structures are damaged (Dua and Gupta, 2005; Chernova, 2010).

It was found that focus (F) parts of all the exposed scales were deformed considerably. These deformations were observed in the scale of fish exposed to 30 and 60 µg L^{-1} chromium in comparison with control (Figure 2a-d). It was observed that there were dissections in the radii (R) and circuli. Deformations on the scales of fish exposed to 7.5 and 15 µg L^{-1} of chromium concentration were found to be relatively smaller, but damages on the scales of fish exposed to 30 and 60 µg L^{-1} of chromium concentration were larger and especially these concentrations greatly damaged the radii and circuli shape (Figure 3a-d). Tandon and Johal (1993) reported that the feeding conditions of the fish, changes in the water quality and especially heavy metal pollution altered the annulus formation in the scales. In their study on *Tor putitora*, they reported that maximum Ca accumulation and minimum Fe accumulation caused changes on annulus formation and the aluminium accumulation also caused brittleness in the margin of scales., Rishi and Jain (1998) reported that fish group (*Cyprinus carpio*) which was exposed to different level (14.5, 29, 43.5 and 58 mg L^{-1} test solution) of cadmium concentration, lepidont and circulus were damaged in both anterior and posterior parts of the scales. These deformaties increased with the increase in concentration level and reached up to choromatophores and radii, especially in the anterior part of scales. They also observed that all the calcareous parts were deformed. Lin-Sun *et al*. (2009) determined malformation, thickening on the scales and tears in the edges of the scales of *Oreochromis* spp. which were obtained from rivers in which heavy metal level was high. Heavy metals accumulate and affect more on scales in comparison with other organs, due to they were exposed to pollution and environmental conditions at first and directly. This accumulations and effects change according to the time period and concentration level. Therefore scales could be used as bio-indicator for heavy metal pollution, which is one of the pollution factors (Johal and Sawhney, 1997; Rashed, 2001; Darafsh *et al*., 2008).

Experimental groups, deformations were observed in the lepidonts of scales of fish as parallel with increasing of chromium concentration. Only small erosions were observed in the edges of the lepidonts in the scale of fish kept in 7.5 and 15 $\mu g\ L^{-1}$ of chromium concentrations. In the fish kept in 30 and 60 $\mu g\ L^{-1}$ chromium concentrations, lepidont deformations were higher level and detached from the bases of the most lepidonts especially in the 60 $\mu g\ L^{-1}$ of concentration group (Figure 4a-d). Rishi and Jain (1998) reported that lepidonts were the first parts to be affected from the exposure to heavy metals, and these damages increased in a parallel way with the increase of concentration. Similar findings were reported on *Channa punctatus* by Dua and Johal (2000) with Johal (2001). Dua and Gupta (2005) reported that demage to anteior part of lepidonts were observed at low concentration of mercury, but the higher concentrations imparted damage to the extent that the whole rows of lepidonts were sloughed off. Khanna *et al*. (2007) reported that there was fractured or damaged level of lepidont damages on the scales of the fish which were caught from the areas where human activity was intensive parts of Ganga River.

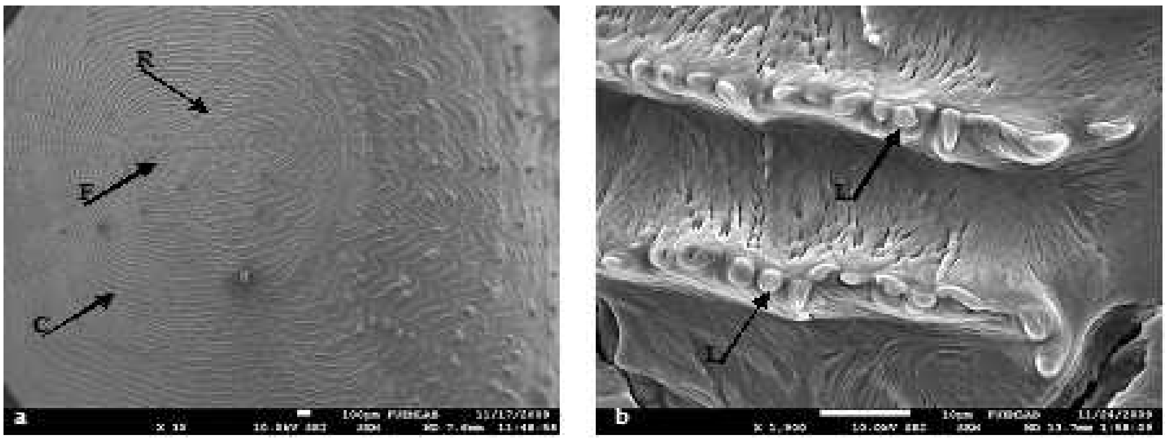

Figure 1: Scale's micrograph of *C. carpio* from control group by SEM (a: general view, b: lepidont view).

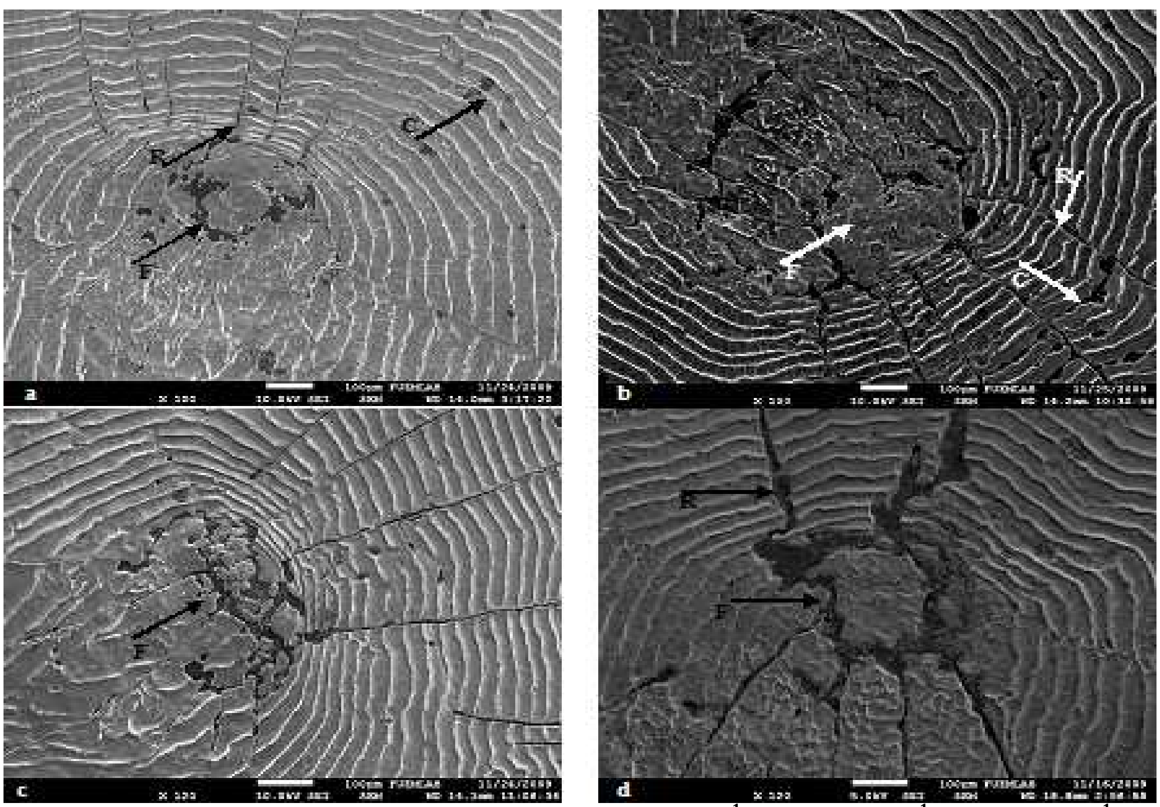

Figure 2: Focus micrograph of *C. carpio* scales by SEM. (a: 7.5 μgL^{-1} Cr; b: 15 μgL^{-1} Cr; c: 30 μgL^{-1} Cr; d: 60 μgL^{-1} Cr).

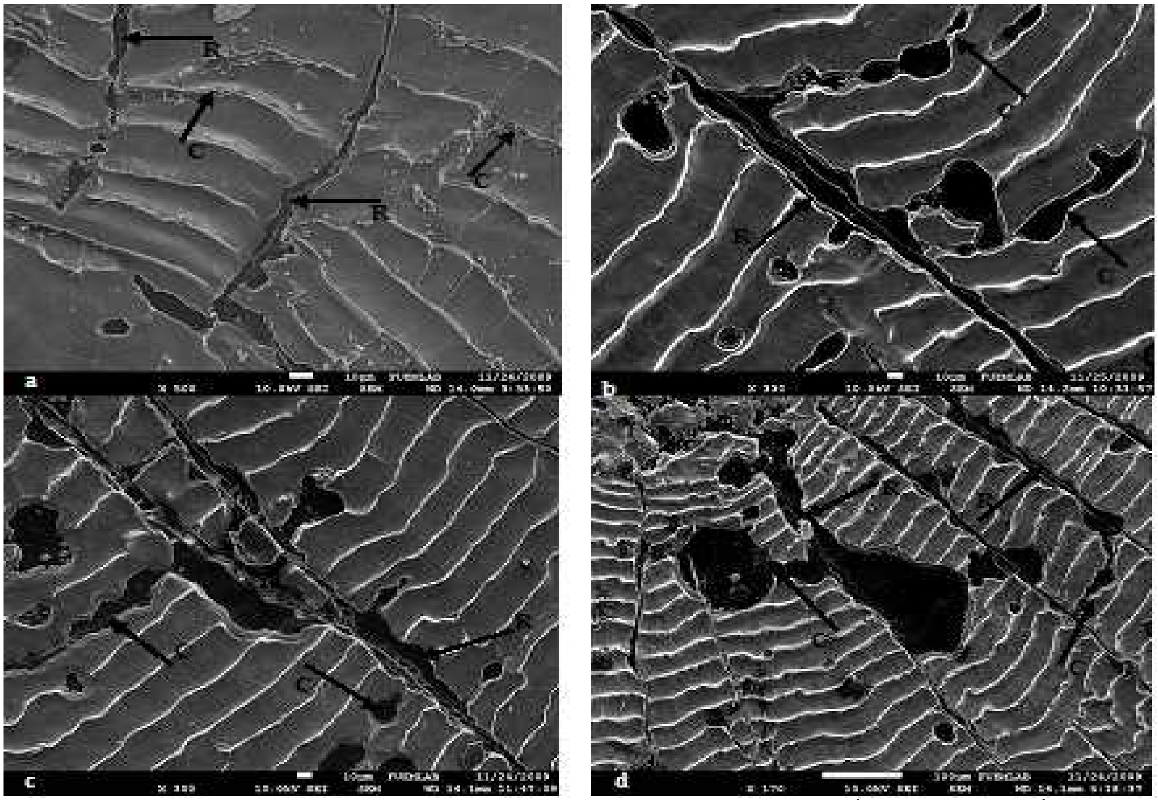

Figure 3: Radii and circuli micrograph of *C. carpio* scales by SEM. (a: 7.5 μgL^{-1} Cr; b: 15 μgL^{-1} Cr; c: 30 μgL^{-1} Cr; d: 60 μgL^{-1} Cr).

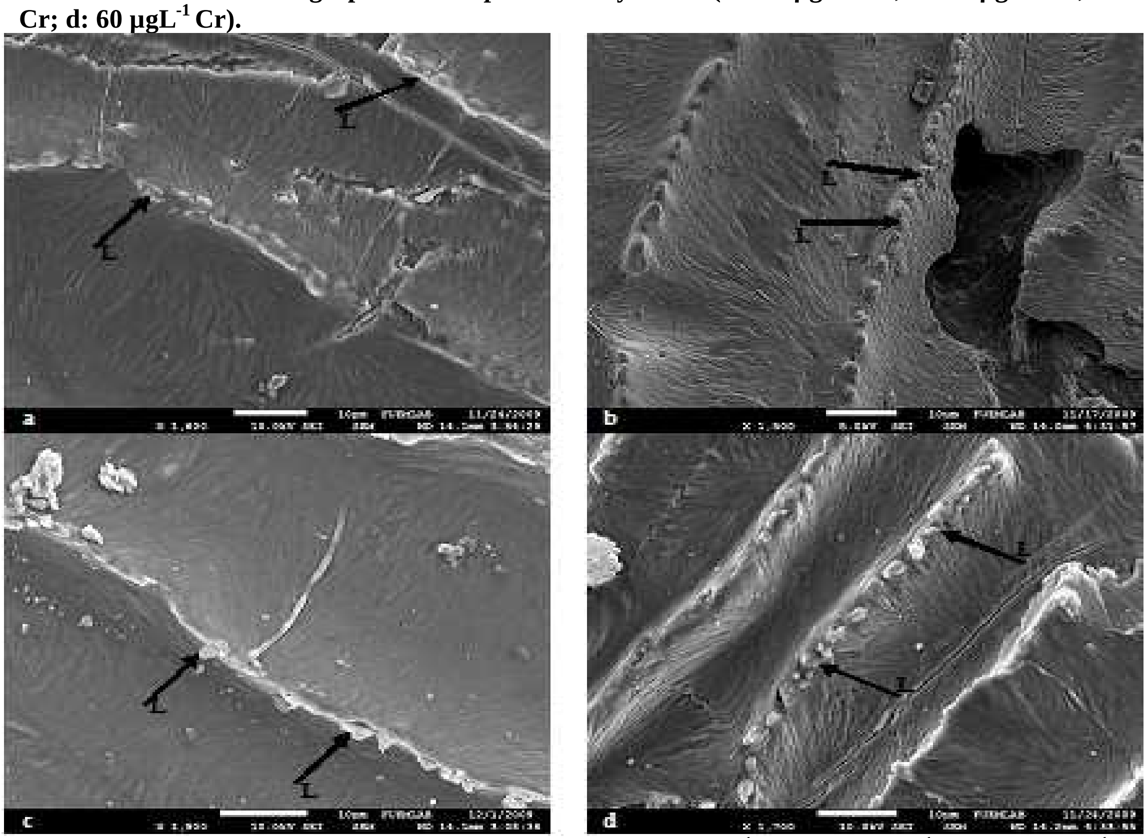

Figure 4: Lepidonts micrograph of *C. carpio* scales by SEM (a: 7.5 μgL^{-1} Cr; b: 15 μgL^{-1} Cr; c: 30 μgL^{-1} Cr; d: 60 μgL^{-1} Cr).

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