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Histological Changes of Electric Yellow Cichlid (*Labidochromis caeruleus*) Exposed to Acute and Chronic Cadmium Concentrations

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ABSTRACT

In this study, electric yellow cichlid were (*Labidochromis caeruleus*) exposed to acute cadmium (Cd) concentrations (0, 50, 60, 70, 80 mg l^{-1}) for 72 h, and chronic Cd concentrations (0.00, 0.25, 0.50, 1.00, 2.00 mg l^{-1}) for 20 d to examine histological alternation. In addition to the recovery was detected after 10 d. After chronic trial, rest of the fish were exposed to just water not containing cadmium for 10 day for recovery. Gills, liver, muscle, and spleen samples were collected from randomly selected fish. The tissue samples prepared with standard techniques for haematoxylin and eosin (H&E). Cadmium depending on concentrations increase caused severe disorders on fish. After acute exposure, several changes were detected such as edema, hyperplasia, breakdown in epithelial tissues of gills; vacuolation and necrosis in liver; increase of hemosterin clustering and necrosis in spleen. After chronic exposure, some symptoms were also found such as edema, aneurysm, degermation of secondary lamellae in gills; degeneration and necrosis in liver; degeneration and granulomas in spleen. Additionally, after recovery of chronic Cd exposure some of the same symptoms were observed such as edema, hyperplasia and aneurysm in gills; vacuolar degeneration and necrosis in liver; granulomas and degeneration in spleen.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi 7(8): 1216-1221, 2019

Akut ve Kronik Kadmiyum Konsantrasyonlarına Maruz Bırakılmış Sarı Prenses Balıklarının Histolojik Değişiklikleri

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ÖZ

Bu çalışmada, histolojik değişimleri incelemek için sarı prenses balıkları (Labidochromis caeruleus) akut cadmiyum konsantrasyonlarına (0, 50, 60, 70, 80 mg l^{-1}) 72 saat süreyle ve kronik kadmiyum konsantrasyonlarına (0,00; 0,25; 0,50; 1,00; 2,00 mg l^{-1}) 20 gün süreyle maruz bırakılmıştır. Ayrıca kronik deneme sonunda arta kalan balıklar 10 gün süreyle kadmiyum içermeyen temiz suya maruz bırakılmıştır. Bu iki denemeden sonra seçilmiş balıklardan solungaç, karaciğer ve dalak örnekleri toplanmıştır. Doku örnekleri standart hematosilen-eozin tekniğiyle hazırlanmıştır. Kadmiyum artan konsantrasyonlara bağlı olarak balıklarda önemli bozukluklara neden olmuştur. Akut maruziyet sonrası solungaçlarda ödem, hiperplazi, epitel dokuda bozukluklar; karaciğerde vakuoleşme ve nekroz; dalakta hemosterin kümeciklerinde artış ve nekroz gibi çeşitli bozukluklar saptanmıştır. Kronik maruziyet sonrasında solungaçlarda ödem, anörizm, sekonder lamellerde dejenerasyon; karaciğerde dejenerasyon ve nekroz; dalakta dejenerasyon ve granuloma gibi bazı semptomlar bulunmuştur. Ayrıca kronik kadmiyum maruziyetinden sonraki iyileşme evresinde, solungaçlarda ödem, hiperplazi ve anörizm; karaciğerde vakular dejenerasyon ve nekroz; dalakta granüloma ve dejenersyon gibi bazı benzer semptomlar gözlenmiştir.

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Introduction

Nowadays, water pollution is an important problem in the world. There are many pollutants present in the environment. Heavy metals are more vital than others. They are generally found trace amount in the environment. After industrial, agricultural and mining activities, huge amount of waste discharged to seas, lakes and rivers without purification (Marcussen et al., 2007). It has been reported that heavy metals accumulated in aquatic animals (Abdel-Warith et al., 2011; Jalaludeen et al., 2012; Youis et al., 2013). In such manner, they participate to food chain and increased from one level to another. At the end, they cause a dangerous situation for humans, in the upper level of food chain (Özkan et al., 2018).

There are many studies about heavy metal accumulation in fish (Kalay and Canlı, 2000; Olvisk et al., 2001; Mol et al., 2010; Begum et al., 2013; Bashir et al., 2013). They showed that heavy metals accumulated in viseral organs and muscle tissue of fish. Heavy metals participate in many biochemical activities in fish (Olsson, 1998). As a result of that, they cause many advers effects on vital organs (gills, liver, kidney and spleen) of fish.

Some studies on histological alterations of different species of fish exposed to cadmium have been presented. Thophon et al. (2003) exposed white sea bass (*Lates calcarifer*) to acute cadmium concentrations (0.0, 5.6, 10.0, 18.0, 25.0, 32.0 mg l^{-1}) for 96 h and chronic cadmium concentration (0.8 mg l^{-1}) for 3 months. Van Dyk et al. (2007) exposed *Oreochromis mossambicus* to 0.018 mg l^{-1} of cadmium + 0.16 mg l^{-1} of zinc and 0.03 mg l^{-1} of cadmium + 0.3 mg l^{-1} of zinc for short period (96 h) and long period (672 h). Liu et al. (2011) exposed *Synechogobius hasta* to acute cadmium concentrations (0.0, 0.1, 0.2, 0.4, 0.8, 1.6 mg l^{-1}) for 96 h and chronic cadmium concentrations (0.00, 0.10, 0.17, 0.29 mg l^{-1}) for 15 d. Yılmaz et al. (2011) exposed *Leuciscus cephalus* to 0, 1, 2 mg l^{-1} of cadmium concentrations for 10 d. Bais and Lokhande (2012) exposed *Ophicephalus striatus* to 0.63 mg l^{-1} of cadmium concentration for 96 h. Omer et al. (2012) exposed Nil tilapia (*O. niloticus*) to 5, 10 mg l^{-1} of cadmium concentrations for 7 d. Selvanatan et al. (2013) exposed *Clarias batrachus* to 0.00, 0.12, 0.17, 0.30, 0.66 mg l^{-1} of cadmium concentrations for 30 d. Younis et al. (2015) exposed *O. niloticus* to 1.68, 3.36, 5.04 mg l^{-1} of cadmium concentrations for 20 d.

The electric yellow cichlid, *Labidochromis caeruleus* is one of the most popularaquarium fish species. It is found in the phylum *Chordata*, class *Actinopterygii*, order *Perciformes* and familiy *Cichlidae*. They have beautiful yellow color and called as yellow lab cichlid, electric yellow cichlid, yellow labido, lemon yellow lab, yellow prince. They are endemic species in Lake Malawi of Africa. They reach 8-10 cm of maximal length and 6-10 years old in their life spaces. Minimal tank volume should be 114 L. They prefer 23.9-26.1°C water temperature, pH 7.8-8.9 and 10-20°dH (dGH or German degree or deutsche Härte) hardness. It is enough to rechange about 10-20% of tank water in a week. They want rocks and caves on the sandy ground in aquarium. They are mount breeder fish. Egg incubation takes 3-4 weeks (Anonymous, 2019).

There is no information about histological changes of electric yellow cichlid exposed to Cd in literature. Because of that, this species was prefered to study on this research. Additionally, higher concentrations of cadmium were used in this study. Since LC_{50} values of cadmium (50% mortality for test animals) were preferred to use in acute Cd toxicity. Some studies (Thophon et al., 2003; Yılmaz et al., 2004; Benjamin and Thatheyus, 2012) were used to detect the Cd concentrations that studied in this research. The treshhold values for acute Cd toxicity were found in white sea bass *(L. calcarifer*) for 96-h LC_{50} 20.12 mg l^{-1} (Thophon et al., 2003), guppy (*Poecilia reticulata*) for 96-h LC_{50} 30.4 mg l^{-1} (Yılmaz et al., 2004). and tilapia (*O. niloticus*) for 72-h LC_{50} 106.88 mg l^{-1} (Benjamin and Thatheyus, 2012).

The objectives of this study were to examine the effects on viseral organs and muscle of electric yellow cichlid, *Labidochromis caeruleus*, exposed to acute, and chronic Cd concentrations.

Materials and methods

Each Cd concentration was replicated in three different aquaria. Water was renewed daily with the same Cd concentrations for each concentration. Laboratory temperature was hold constantly at 24°C. Water parameters were measured once a day with multi parameter (WTW Multi 3420 set G, Weilheim, Germany). Hardness, alkalinity, ammonia and nitrite were measured once at the beginning of experiment. Fish were fed *ab libitum* once a day. For each aquarium, additional aeration was supplied during the trial. Mortality was recorded every day for each aquarium.

Acute Exposure of Cd Concentration

This study consist of two trials: acute and chronic exposures. In the acute trial, laboratory cultured fish were used (1.95±0.18 g and 51.79±1.60 mm, n= 10) and ten fish were stocked per 5 L (35x16x25 mm). They were starved for 24 h before experiment and they were exposed to 0, 50, 60, 70, 80 mg l^{-1} of cadmium chloride ($CdCl_2.H_2O$, Fluka catalog no: 20899-25G-F) for 72 h. After 24, 48 and 72 h, four fish were randomly selected from each aquarium to collect their gills, liver, spleen and muscle tissues.

Chronic Exposure of Cd Concentration and Recovery

In the chronic trial also, laboratory cultured fish were used (6.43±1.32 g and 71.41±4.8 mm, n= 15), and fifteen fish were stocked per 15 L (50×20×30 mm). They were exposed to 0.00, 0.25, 0.50, 1.00, 2.00 mg l^{-1} of cadmium chloride ($CdCl_2.H_2O$, Fluka catalog no: 20899-25G-F) for 20 d. After 10 and 20 d, four fish were randomly selected from each aquarium to collect their gills, liver, and spleen and muscle tissues.

In the recovery part of chronic trial, the rest of the alive fish from chronic trial were hold in clean water for 10 d to get ride of Cd accumulation from their bodies. They were stocked in 5 L of clean water (35x16x25 mm). After 10 d, four fish were randonly selected from each aquarium to collect their gills, liver, spleen and muscle tissues.

Histological Analysis

Fish were anaesthetized with 400-500 mg l^{-1} tricaine methansulphonate (MS-222) after each trial (Topic Popovic et al., 2012). Gill, liver, and spleen tissue samples were collected from randomly selected fish and fixed in the 10% of formalin for at least 24 h. Then, they were dehydrated in water and graded ethanol concentrations (distelled water, 70, 80, 96, 100%) and xylene (100%) (Roberts, 2012), embedded in paraffin wax and cut in a rotary microtome (Leica RM 2125 RTS, Nussloch, Germany) about 5 µ. After that, those sections mounted on glass slides and deparaffinized in xylen and stained with hematoxylin and eosin (H&E) method (Culling et al., 1985). Tissue preparations were examined by a light microscope (Euromex Novex B series, Arnhem, Netherlands). Symptoms were recorded by taking microphotographs by microcamera (Novex Cmex DC 5000, Arnhem, Netherlands).

Results

For two trials, water quality parameters are given in Table 1. In the acute trial, at 50-80 mg l^{-1} of Cd concentrations, several symptoms were seen such as edema, hyperplasia in the gill cells (Figure 1); degeneration, vacuolation (Figure 2B and 2C), and necrosis (Figure 2D) in the liver cells; increase of homesiderin clustering and necrosis in spleen cells (Figure 3). No symptom was detected in muscle tissues. The survival rate was 100% during 72 h, at acute Cd exposure.

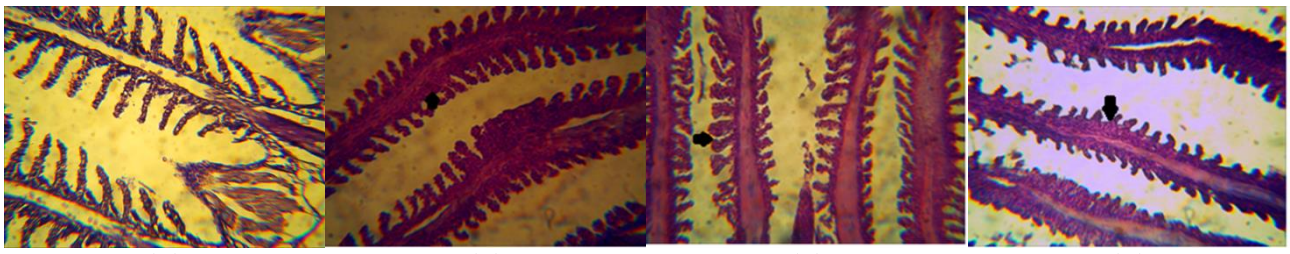
After chronic trial, Cd exposure caused some clinical symptoms that could be seen by eyes such as hemorrhagic liver at 1.00-2.00 mg $l^{-1}$of Cd, hydropic spleen and anemic gills at 2.00 mg l^{-1} of Cd. After chronic Cd exposures were fulfilled, some symptoms were detected by microscope examinations. Edema, aneurysm, degeneration of secondary lamellae of gills at 0.25-2.00 mg l^{-1} of Cd (Figure 4); vacuolar degeneration of hepatocyte cells and necrosis in liver (Figure 5); granulomas and degeneration were examined in spleen at 0.25 mg l^{-1} of Cd (Figure 6). Their severities increased as Cd concentration and duration increased. The survival rate was between 53.33-66.67% for 20 d (Table 2). After recovery from chronic Cd toxicity, several symptoms were observed such as aneurysm and edema at 0.25-1.00 mg l^{-1} of Cd; hyperplasia, and edema at 2.00 mg l^{-1} of Cd in gills (Figure 7); vacuolar degeneration and necrosis in liver at 0.25-2.00 mg l^{-1} of Cd (Figure 8); granulomas and degeneration in spleen at 0.25-2.00 mg l^{-1} of Cd (Figure 9). The survival rate was between 30.0% and 60.0% (Table 2).

Table 1 Water quality parameters of water source during the acute, and chronic trials, and recovery

Parameter	Acute Trial	Chronic Trial	
		Trial Part	Recovery Part
Temperature	24.1±0.44	23.2±0.68	22.8±0.23
pH	8.85±0.16	8.61±0.23	8.97±0.08
Oxygen	8.1±0.04	7.90±0.56	7.83±0.07
Hardness	738.0	738.0	738.0
Alkalinity	609.0	609.0	609.0
Ammonia	0.22	0.22	0.22
Nitrite	0.0069	0.0069	0.0069

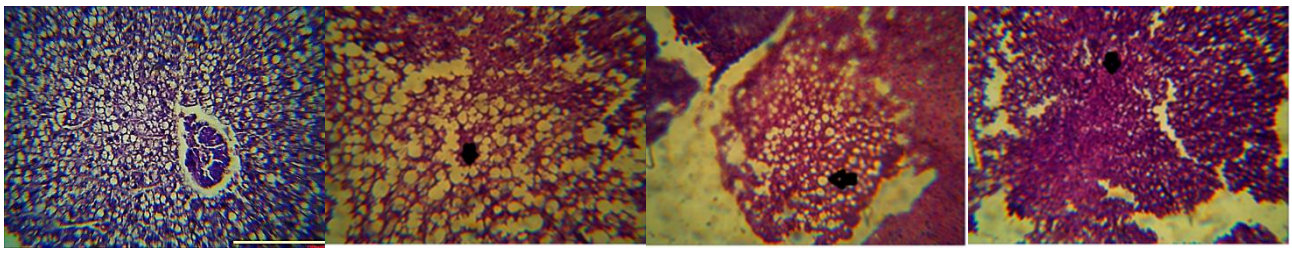
Table 2 Survival in the chronic and recovery trials

Cd Exposure	Chronic Trial	Recovery Trial
0.00 mg l^{-1}	100.00±0.00	100.00±0.00
0.25 mg l^{-1}	66.67±0.00	40.00±28.28
0.50 mg l^{-1}	53.33±9.43	50.00±0.00
1.00 mg l^{-1}	60.00±0.00	30.00±14.14
2.00 mg l^{-1}	66.67±0.00	60.00±28.28



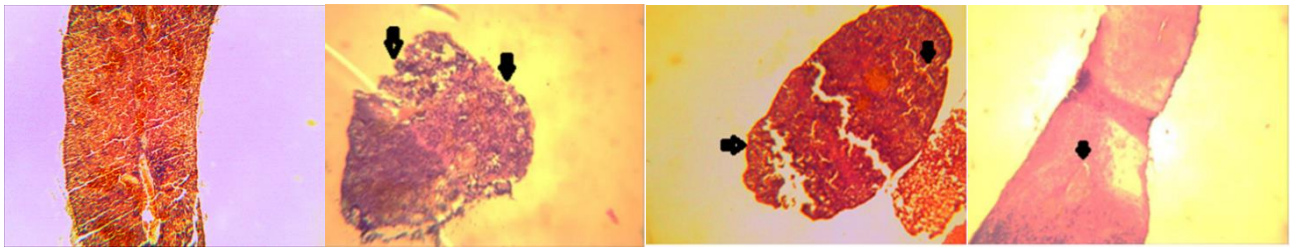
(A) (B) (C) (D)

Figure 1 Gills in the acute trial for 72 h (40× H&E), A: Control, B: hyperplasia in the 50 mg/L Cd exposure, C: hyperplasia in the 60 mg/L Cd exposure, D: hiperplasia and degeneration in the 80 mg/L of Cd exposure



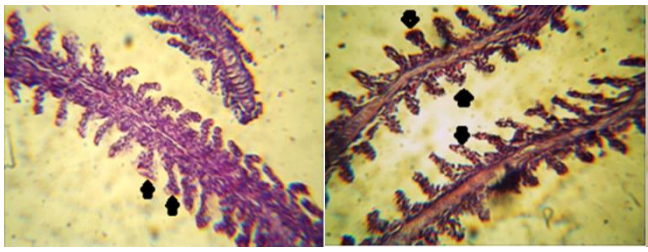
(A) (B) (C) (D)

Figure 2 Liver in the acute trial for 72 h (40× H&E), A: Control, B: vacuolations in the 50 mg/L of Cd exposure, C: vacuolations in the 60 mg/L of Cd exposure, D: necrosis in the 80 mg/L of Cd exposure (arrow)



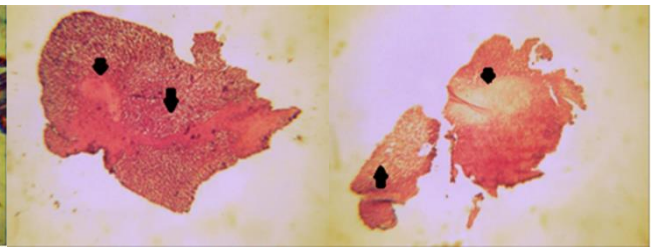
(A) (B) (C) (D)

Figure 3 Spleen in the acute trial for 72 h (10× H&E), A: Control, B: necrosis in the 50 mg/L of Cd exposure, C: necrosis in the 60 mg/L of Cd exposure, D: homesterin clustering in the 80 mg/L Cd exposure (arrow)



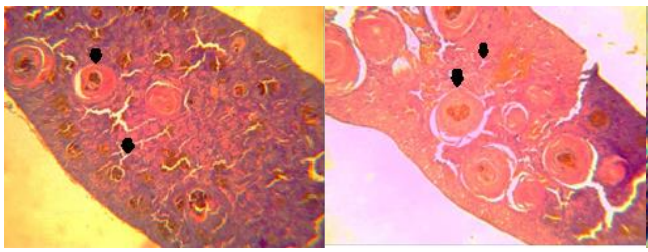
(A) (B)

Figure 4 Gills in the chronic trial for 20 d (40× H&E), A: edema, aneurysm in the 0.25 mg l^{-1} of Cd exposure, B: degeneration, edeme, and aneurysm in the 2 mg l^{-1} of Cd exposure (arrow)



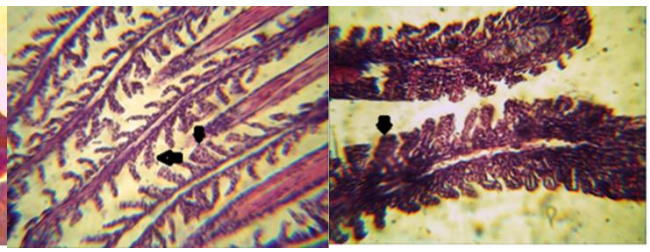
(A) (B)

Figure 5 Liver in the chronic trial for 20 d (10× H&E), vacuolations and degeneration (arrow) A: 0.25 mg l^{-1} of Cd exposure and B: 2 mg l^{-1} of Cd exposure (arrow)



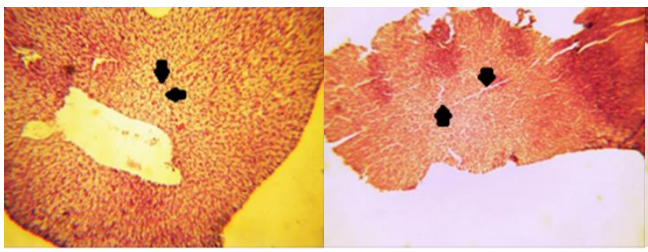
(A) (B)

Figure 6 Spleen in the chronic trial for 20 d (10x H&E), granulomas and degeneration A: 0.25 mg l^{-1} of Cd exposure and B: 2 mg l^{-1} of Cd exposure (arrow)



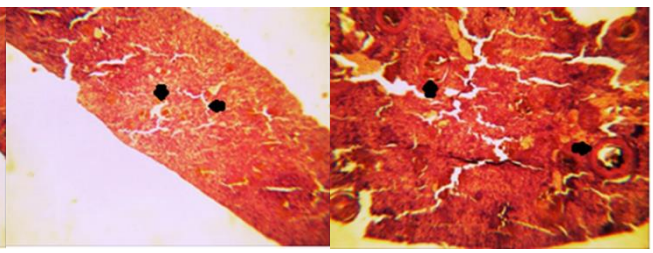
(A) (B)

Figure 7 Gills in the recovery trial for 10 d (40x H&E), A: edeme and aneurysm in the 0.25 mg l^{-1} of Cd exposure, B: hyperplasia and edema in the 2 mg l^{-1} of Cd exposure (arrow)



(A) (B)

Figure 8 Liver in the recovery trial for 10 d (10x H&E), vacuolations and degeneration A: 0.25 mg l^{-1} of Cd exposure and B: 2 mg l^{-1} of Cd exposure (arrow)



(A) (B)

Figure 9 Spleen in the recovery trial for 10 d (10x H&E), degeneration and granuloma A: 0.25 mg l^{-1} of Cd exposure and B: 2 mg l^{-1} of Cd exposure (arrow)

Discussion

Cd exposure causes histological changes in electric yellow cichlid. Because it is reported by Olsson (1998) that decrease of Ca^{+2}, Na^{-}, Cl^{-} and K^{+} levels, increase of hyperglycemia and hypermagnesemia in the plasma, increase of mucus cells in intestine and gills, increase of number of chloride cell in the epithelium of gills, and deformations in vertebrate.

Histological effects of Cd on various species have been investigated in some studies. There are many similar results between our study and previous ones. In this study, edema, aneurysm, hyperplasia, breakdown in epithelial tissues, degeneration of secondary lamellae was identified in gills. Vacuolar degeneration and necrosis in hepatocyte cells of liver, and degeneration and granulomas in spleen were investigated. Thophon et al. (2003) found edema, degeneration of pillar cells, aneurysm, and hyperplasia in gills; and blood congestion of sinusoids, hydropic swelling, vacuolation, and black granulation in liver of *L. calcarifer*. Van Dyk et al. (2007) detected hyalisation, vacuolation and blood congestion in liver of *O. mossambicus*. Liu et al. (2011) reported degeneration of seconder lamellea, aneurysm, edema in gill; blood congestion and vaculation in liver and spleen of *S. hasta*. Yılmaz et al. (2011) found degeneration in seconder lamellae, necrosis and hydropic degeneration of chlorid cells in gills; and hydropic degeneration and necrosis in liver of *L. cephalus*. Bais and Lokhande (2012) observed hypertrophy, degeneration of lamellae, blood congestion in gills; and degeneration, necrosis and hypertrophy in liver of *O. striatus*. Omer et al. (2012) detected fatty vacuolation, necrosis and blood congestion in liver of *O. niloticus*. Selvanatan et al. (2013) found hyperplasia, fusion of seconder lamellae, degeneration of epithelial cells in gills; and vacuolation, necrosis in liver of *C. batrachus*. Younis et al. (2015) reported vacuolation and granulation in liver of *O. niloticus*.

In this study, survival was high (100%) during the acute trial (72 h) although high Cd concentrations were used. This survival may be obtained because of high water hardness and alkalinity. Carbonate and bicarbonate ions may tide up with Cd in water. They precipitated as white powder on the bottom of the aquarium. High hardness and alkalinity could protect fish from high Cd concentration and decrease Cd toxicity (Pascoe and Evans, 1986; Kiyani et al., 2013).

After recovery of chronic Cd concentrations, electric yellow cichlids did not become well exactly. Cd exposure severely affected fish visceral organs. It was observed that edema, aneurysm, hyperplasia in gills; vacuolar degeneration and necrosis in liver; granulomas and degeneration in spleen were still observed on the tissue samples, although clean water used instead of Cd concentrations. No recovery situation were seen in fish and survival was very low (30-60%) in this part.

In conclusion, this study was conducted to investigate the histological alterations of electric yellow cichlids after acute (0, 50, 60, 70, 80 mg l^{-1} for 72 h) and chronic (0.00, 0.25, 0.50, 1.00, 2.00 mg l^{-1} for 20 d) Cd toxicities and recovery (just water not containing Cd for 10 d). Cd effects were examined on several fish species previously. Many similarities are present between this study and previous ones (Thophon et al., 2003; Van Dyk et al., 2007; Liu et al., 2011; Yılmaz et al., 2011; Bais and Lokhande, 2012; Omer et al., 2012; Selvanatan et al., 2013; Younis et al., 2015). At 80 mg l^{-1} of Cd, severities of symptoms were increased such as edema, aneurysm in gills; and vacuolation and degeneration in liver; and granulomas and degeneration in spleen. Acute and chronic Cd concentrations may cause important disorders in gill, liver and spleen tissues. At 1.00-2.00 mg l^{-1} of Cd, severities of symptoms were increased. Three days is not enough time to see Cd damages in fish tissues. It is recommended that histological studies can be conducted more than 10 days to observe important pathological symptoms.

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