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Effects of sub-lethal exposure of cadmium on histopathology of gills of Nile tilapia, *Oreochromis niloticus* and the mitigating effects of *Cladophora glomerata*

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Abstract: In this study, Nile tilapia, *Oreochromis niloticus* kept in the medium with or without green algae, *Cladophora glomerata* was exposed to sublethal concentrations of 0.1 mg/l and 1 mg/l Cd^{+2}. At the end of 15 and 30 day periods, fish gills were removed to investigate histolopathological alterations by light microscopy. As a result of cadmium application; in the gills, changes were observed such as curling and fusion in secondary lamellae, epithelial hypertrophy, epithelial hyperplasia, pillar cell breakage, edema, swelling, aneurysm, necrosis and increased mucus secretion. The severity of the alterations resulting from cadmium increased with dose-time dependent. Histopathologic effects were observed to be lighter in the groups contained algae. This suggests that algae-like organisms in the environment accumulate some of the cadmium in their bodies, causing fish to be less affected.

Keywords: Heavy metals, Histopatology, Gill, Green algae.

Introduction

Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface waters. However, they accumulate on a rising level in aquatic ecosystems due to anthropogenic activities. Sometimes, aquatic organisms are exposed to unnaturally high levels of these metals (Abdel-Warith et al., 2011).

Fishes can be considered as one of the most significant biomonitors in freshwater systems for the determination of heavy metal pollution (Rashed, 2001; Begum et al., 2005) and they offer several specific advantages in describing the natural characteristics of the aquatic systems and in assessing changes to habitats (Lamas et al., 2007; Chovance, 2003). Fish health may therefore reflect and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may only be evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance (Hinton and Lauren, 1993: Gernhofer et al., 2001).

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is recognized as a good biological model due to its easy handling, culture, and maintenance in the laboratory, as well as its capacity to adapt to pollutants in toxicological studies (Garcia-Santos et al., 2006; Saha et al., 2006).

Cadmium is a biologically nonessential metal and often becomes toxic to aquatic animals due to its presence in high concentrations in industrial and domestic sewage waste streams (Dunnick and Fowler, 1988; Kaviraj and Das, 1995). Studies on Cd exposure of fish indicate that it is accumulated in high concentrations in the intestine, kidney, liver, gill and muscle (Kalay and Canli, 2000; Cogun et al., 2003; Kim et al., 2004). Cadmium has been shown to cause several adverse effects on fish, such as decreased survival, growth and reproduction (Kumada et al., 1980; Dutta and Kaviraj, 2001; Szczerbiket al., 2006), histological changes in kidney, gills, liver and gastrointestinal tract, anemia (Thophon et al., 2003).

Histopathological examination has been increasingly recognized as a valuable tool for the assessment of the impact of environmental pollutants on fishes (Heath, 1995). Histological study appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gill. Exposure to heavy metals may cause many histopathological changes in the gill (Visoottiviseth et al., 1999; Yılmaz et

al., 2011; Kaoud et al., 2011; Jiraungkoorskul et al., 2006; Pratap and Wendelaar Bonga, 1993; Mekkawy et al., 2013). Fish gill histology could therefore serve as a model for studying the interactions between environmental factors and gill structures and functions (Gernhofer et al., 2001; Peebua et al., 2008).

Some algae show remarkable capability to adsorb metal ions from aqueous solution (Mehta and Gaur, 2005). This has opened up the possibility of their use in treatment of metal containing wastewaters (McHardy and George, 1990; Mehta and Gaur, 2005). Many green algae have been found as potential scavengers of heavy metals from water and wetlands (Singh et al., 2007; Laib and Leghouchi, 2012). In the present study, the green algae, *Cladophora glomerata* was chosen as a scavenger of Cd because it is widely distributed in the rivers of Turkey (Ünlü et al., 2009; Yalçın et al., 2008; Karadede and Ünlü, 2013). Hence, this study aimed to investigate the histopathological effects of sub-lethal exposure of cadmium to Nile tilapia and to determine mitigating effect of *C. glomerata* in the same medium.

Materials and Methods

The test fish, Nile tilapia fingerlings were obtained from Cukurova University (Adana, Turkey), Fisheries Faculty, Fresh Water Aquaculture Experimental Units. Green algae, *C. glomerata*, were collected from the Tigris River (37˚55'06"N, 40˚14'057"E, 580 m) near Diyarbakir City, Turkey. The mean total length and weight of the fish were 10.98±1.46 cm and 16.40±4.20 g, respectively.

Fish and algae were acclimatized to the laboratory conditions for 20 days prior to the experiment at 23±1°C, the temperature of the experimental conditions. The laboratory was illuminated under an 8 h light: 16 h dark period, with fluorescent lamps TL-D36 watt. The average values for tap water used in both acclimation and experiments were pH 7.94±0.505, dissolved oxygen 7.5±0.38 mg/l, total chlorid 42.6 mg/l, total hardness 287±2.35 mg/l $CaCO_3$, NO_3-N 2.1 mg/l, NO_2-N 0.002 mg/l and conductivity 7.94 Mmho/cm. The aquaria were aerated with air stones attached to an air compressor to saturate the water with oxygen the water quality parameters mentioned above were performed daily during the experiment.

Green algae, *C. glomerata* samples were washed in fresh water at the sampling site and transferred to the laboratory on the same day in polyethylene boxes under refrigeration (4°C). Upon arrival at the laboratory, they were rinsed with tap water to remove sand and particulate matter, epithytal and epifaunal species, and were rinsed once again with distilled water (Keskinkan, 2004). Taxonomic identifications were carried out according to John (2002).

During the experiment all fish were fed once a day with commercial pellet diet feed (ProAqua Nutrición S.A. composition: 45% protein, 22% lipids, 15% carbohydrates, 8.5% ash, 5% vitamin and mineral premix) at a rate of 1% body weight/day.

The study was performed in three replicates. Fish were randomly divided into six groups and placed in separate glass aquaria (35x40x40 cm containing 50 L) as follows, each group consisting of eight fishes: Group I, the control group (only fish) without Cd exposure, Group II, the control group (fish+green algae) without Cd exposure, Group III, treatment group (fish in 0.1 mg/L Cd), Group IV, treatment group (fish+green algae in 0.1 mg/L Cd), Group V, treatment group (fish in 1.0 mg/L Cd), and Group VI, treatment group (fish+green algae in 1.0 mg/L Cd).

A stock solution of Cd was prepared by dissolving 1632 mg $CdCl_2$ (analytical grade, Merck, Darmstadt, Germany) in 1 L double distilled water. The aquaria of the group II, group IV and group VI contained 50 g acclimatized green algae *C. glomerata* in each aquarium. The experiments were run for 15 and 30 days.

Three fish from each groups were removed for histopathological examinations after treatment periods of the 15 and 30 days. The fish were anesthetized in 50 mg/l MS-222 (3-aminobenzoic acid ethyl ester methane sulfonate salt; Sigma) solution and prepared for histopathological analysis. Gill of fish were dissected out and fixed in 10% formalin fluid for 24 hrs, washed with tap water. They were dehydrated through a graded series of ethanol, cleared in xylene. The tissues were then embedded in paraffin wax and 5 μm sections were taken with a rotary microtome. The tissues stained with hematoxylin and eosin, and then photographed (Digital Sight DS-2Mv, Nikon, Tokyo, Japan) and examined by light microscopy (Eclipse 80i, Nikon).

Results

In the control groups I and II, secondary lamellae are covered with a single layer epithelium. In the basal parts of the seconder lamellae, mucus cells and chloride cells

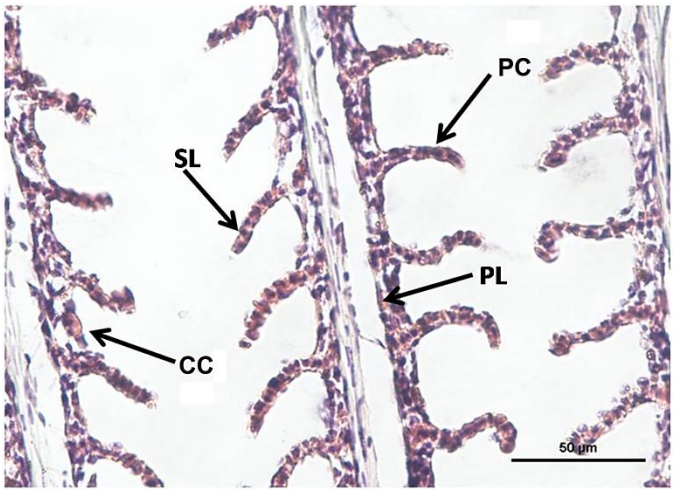

Figure 1. Control gill tissue of *Oreochromis niloticus*. PL; primer lamellae, SL; secondary lamellae, PC; pillar cell, CC; chloride cell (H&E).

are found (Fig. 1). No histological alteration was observed in the gills of the control group. However in fish representing experimental groups III, IV and V for 15 and 30 days showed many histopathological changes.

At the end of the 15th day, the fish exposed to a concentration of 0.1 mg/l Cd (Group III; hypertrophy in the epithelium, increased mucus secration, subepithelial edema in the secondary lamellae, and hyperplasia in interlamellar epithelium (Fig. 2A), fish exposed to 0.1 mg/l Cd concentration (Group IV); hypertrophy in the epithelium was observed however the lesions were lighter due to the ability of the algae to retain heavy metals (Fig. 2B). In fish exposed to a concentration of 1 mg/l of Cd (Group V); fusing on the secondary lamellae, edema and aneurysms were observed in the secondary lamellae, and pillar cell breakage was also observed (Fig. 2C). Group VI (fish+green algae) exposed to 1 mg/l Cd; hyperplasia in the interlamidal epithelium and epithelial hypertrophy (Fig. 2D) but there has been less mild histopathologic changes.

At the end of the 30th day, high histopathological changes were observed in the gills after 30 days of treatment in fish exposed to subletal concentrations both 0.1 mg/l of Cd and 1 mg/l. Fibrosis and edema in the

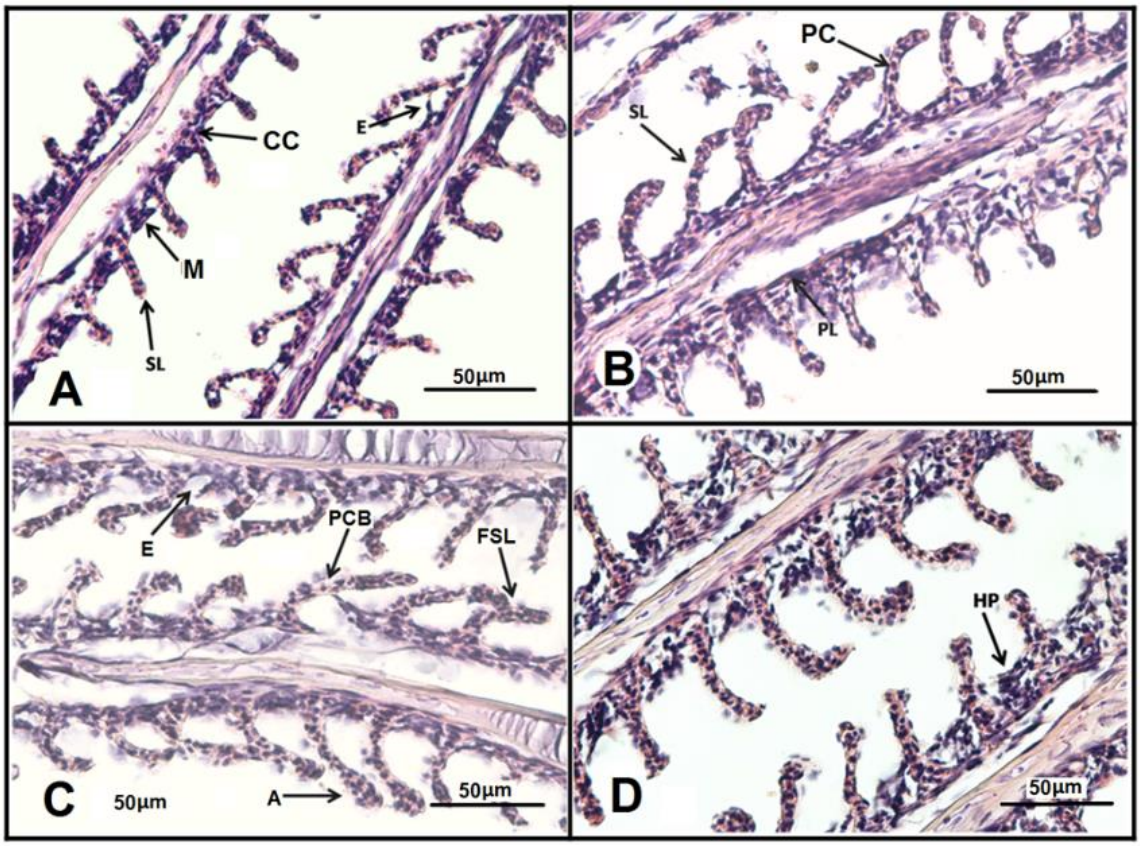

Figure 2. (A) 15th day, *Oreochromis niloticus* exposed to 0.1 mg/l of Cd. SL; secondary lamellae, E; edema, CC; chloride cell, M; mucus, (B) fish+algae exposed to 0.1 mg/l Cd, PL; primer filament, SL; secondary lamellae, PC; pillar cell, (C) fish exposed to 1 mg/l Cd, E; congestion and edema, PCB; pillar cell breakage, FSL; fusing on the secondary lamellae, A; aneurysm, and (D) fish+algae exposed to 1 mg/l Cd, HP; hyperplasia (H&E).

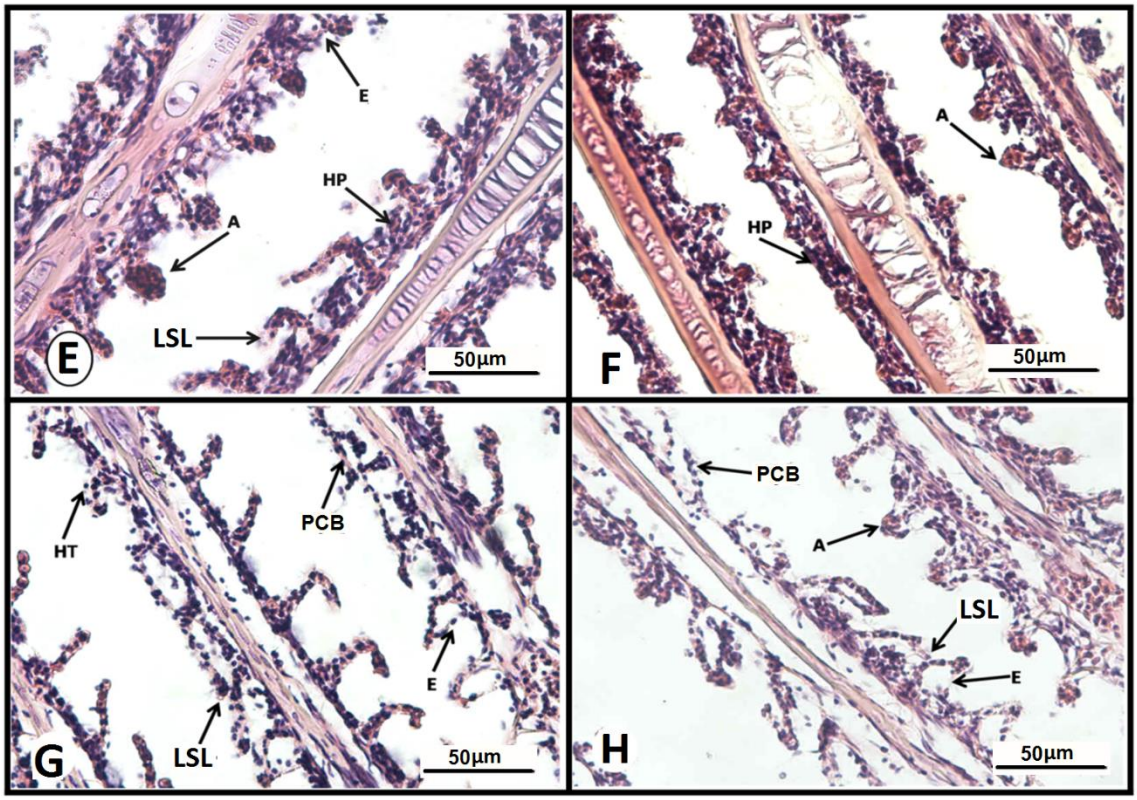

Figure 3. (E) 30 day, *Oreochromis niloticus* exposed to a concentration of 0.1 mg/l Cd (Group III), A; aneurysm, E; subepitelial edema, HP; hyperplasia, LSL; lamina breakage of the secondary, (F) fish+algae (Group IV) exposed to 0.1 mg/l Cd; A; aneurysm, HP; hyperplasia, (G) fish (Group V) exposed to 1 mg/l Cd, E; subepitelial edema, PCB; pillar cell breakage, LSL; laminae of secondary lamellar, HT; hypertrophy, and (H) fish+algae (Group VI) exposed to 1 mg/l Cd, A; aneurysm, E; subepitelial edema, PCB; pillar cell breakage, LSL; laminae of secondary lamellae (H&E).

secondary lamellae, hypertrophy in mucous cells, increase in pillar cell breakage and aneurysm (Fig. 3E). The lesions of fish+algae exposed to 0.1 mg/l Cd concentration were lighter due to algae, hyperplasia in the interlamidal epithelium and aneurysm in the primer lamellae (Fig. 3F). At the level of 1 mg/l Cd in the gills, the epidermal hypertrophy was observed in the laminae of the secondary, and fusion, edema, abrasion and rupture occurred in the secondary lamella due to increased mucus (Fig. 3G). When the lesions of fish exposed to 1 mg/l Cd concentration + algae were compared with those of the fish in the algal environment, histopathologic changes were milder and epithelial hypertrophy, fusion in the secondary lamellae, edema, pillar cell breakage and aneurysm were detected (Fig. 3H).

Discussion

Histopathological biomarkers can be used as indicators of various anthropogenic contaminations in the study of fish population health exposed to environmental pollution in ecosystems (Gernhofer et al., 2001; Chovance et al., 2003; Stentiford et al., 2003; Hook et al., 2014). Many histopathological alterations were observed in fish exposed to heavy metal pollution (Kaviraj and Das, 1995; Hinton et al., 1993; Kaoud and El-Dahshan, 2010; Abdel-Warith et al., 2011; Mekkawy et al., 2013; Ahmed et al., 2014). According to Velkova-Jordanoska and Kostoski (2005), histopathologic changes can be used as biomarkers in tissue and cellular changes in the affected organism. Histopathologic studies are also thought to be the studies used to elicit the effects of chemical substances accumulated in the target organs of fish studied in laboratory conditions. As a result of cadmium exposes, slow down growth and changes in organs function have been observed in fish (Garcia-Santos et al., 2006; Almeida et al., 2001; Jiraungkoorskul et al., 2006; Kaoud et al., 2011)

As a result of the application of Cd in this study, significant histological changes were observed in gills (Kumada et al., 1980; Dutta and Kaviraj, 2001; Szczerbik

et al., 2006; Thophon et al., 2003). Yilmaz et al. (2011) reported that degeneration, desquamation, swelling in chloride cells, hydropic degeneration and necrosis of epithelial cells were detected in the seminal lamellar epithelium of the gills of the fish exposed to $CdSO_4$. Selvanathan et al. (2012) stated that an increase in mucus cells was observed with fusion of secondary mucus, hyperplasia on epithelial cell surfaces, cell separation from pillar system and disruption of gill filaments due to mucus hypersecretion in cadmium-treated gill tissues. Edema, separation of the respiratory epithelium and changes in lipid vacuolization were detected in the secondary lamellae. Mekkawy et al. (2013) observed irregularities in the pillar cell system resulting from toxic effects of cadmium, subepithelial edema in the secondary lamellae, and hypertrophy at the upper level in the secondary lamellae. It is thought that the pathological effects of cadmium accumulated negatively interfere with the transport of oxygen required for respiration by the gills (Omer et al., 2012).

Ahmed et al. (2014) found the histopathological changes that resulted from severe fish physiological events such as imbalances due to gills are covered with a thick mucus layer. Histopathologic changes such as fusion of the secondary lamellae, hypertrophy of the mucous cells and necrosis have also been identified (Ahmed et al., 2014). Gill is known to be one of the most affected organs in many types of toxicities since it is the port of entry of the toxicants dissolved in water. Degeneration of this organ, in general, results in unbalanced oxygen delivery, which may trigger functional problems in other metabolic organs such as liver and kidney (Yılmaz et al., 2011).

In fishes, various metabolite residues are thought to increase algal metabolism and increase metal accumulation in algae. Metabolic residues can increase the pH of the medium (Yalçın et al., 2008) and this situation leads to an increase in metal bioabsorption in algae. Chmielewska and Medved (2001) indicate that Ni, V, Cd, Pb and Cr have high accumulation ability in algae and green algae are important bioindicator species removing heavy metals from the environment. The sizes of the adsorbate are probably due to the algae cell surface width (Andrade et al., 2005) and then accumulate them in intracellular elements (Mehta and Gaur, 2005). Ünlü et al. (2009) and Karadede and Ünlü (2013) reported that Cd accumulation in fish and algae significant accumulation were observed.

Conclusion

The findings obtained from the studies suggest that there is a direct dose-time dependent correlation with histopathological alterations. The high level of cadmium in fish indicates that it causes fish deaths and even those who consume them have great risks for their health. It can be concluded that green algae have the ability to eliminate heavy metals in the environment and can be used to remove heavy metals from contaminated areas at low cost (Ünlü et al., 2009; Karadede and Ünlü, 2013).

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