Histological and Ultrastructural Changes in Gills of Tilapia Fish from Wadi Hanifah Stream, Riyadh, Saudi Arabia

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Abstract: Tilapia fish *Oreochromis sp.* were collected from polluted and unpolluted areas of Wadi Hanifah stream in Riyadh to study the histopathological and ultrastructural effects of pollution on fish gills. Light microscopic observations showed several pathological changes of fish gills collected from polluted area including disorganization of the secondary lamellae structure as well as cellular hyperplasia. The tips of the secondary lamellae exhibited abnormal malformations and partial fusion of some of them. Epithelial lifting of the respiratory lamellae accompanied by edema and lamellar aneurism were also observed. On the other hand, electron microscopic observations revealed accurate alterations in the polluted fish gills. Distinct degeneration, necrosis of pillar cells and damage of the capillary walls of the secondary gill lamellae have been noted. There was also congestion of blood spaces by erythrocytes with presence of different leucocytes and the pavement cells appeared irregular with a considerable loss of microridges. The Chloride cells appeared with dilated vesicles and damaged mitochondria while the mucous cells were completely filled with electron – dense vacuoles. The present study indicates that histopathological and ultrastructural alterations are good biomarkers for field assessment in areas that are subject to a multiplicity of environmental variations.
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Keywords: Environmental pollution, Tilapia fish, gills, histopathology, ultrastructure

Introduction

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies (Teh *et al*.,1997; Thophon *et al*.,2003;Kasherwani *et al*.,2009). One of the great advantages of using histopathological biomarkers in environmental monitoring is that it allows examining specific target organs. These include gills, kidney and liver, which are responsible for vital functions, such as respiration, excretion, accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001; Camargo & Martinez, 2007). Furthermore, alterations found in these organs are normally easier to identify than functional ones (Fanta *et al*., 2003), and serve as warning signs of damage to animal health (Hinton & Lauren, 1990; Sorour, 2001). Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as in fresh water habitats (Fernanders *et al*., 2007; Miron *et al*., 2008; Nwani *et al*.,2010).Metals in particular are one of the most deleterious environmental toxicants affecting fish gills by changing their morphology and ultrastructure (Wong & Wong, 2000; Machado & Fanta, 2003). The heavy metal ions interfere with respiration and osmoregulation causing cellular damage to gill cells (De Boeck *et al*., 2001; Pandey *et al*., 2008).

Wadi Hanifah stream is one of the most important water sources in Riyadh. It suffers from environmental contamination that have adversely effects on biological conditions of fish and other aquatic animals (Siddiqui and Al-Harbi,1995; Al-Ogaily *et al*.,1999).

Nevertheless, field studies using histopathology of fish as biomarker of aquatic contamination in Wadi Hanifah have not yet been reported. The present study was designed to characterize the histopathological and ultrastructural effects of pollution in gills of *Oreochromis sp.* from two sites in Wadi Hanifah varying in degree of chemical contamination in sediments.

2. Materials and Methods

Fourty-seven mature fish (Oreochromis *sp.*) weight between 250- 650 g were collected at February and October from highly polluted and less polluted (control) areas of Wadi Hanifah stream. Sites were taken according to Siddiqui and Al-Harbi (1995). Fish were dissected and the gills fixed in 10% neutral buffered formalin or in Bouin`s fluid, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin for histological evaluation. For transmission electron microscopic studies, small slices of the gills were immediately fixed in 3% glutaraldehyde cacodylate buffer (pH 7.3) for 2-4 hours and post- fixed in 1% osmium tetraoxide in the same buffer for 1-2 hours at 4 C. The specimens were dehydrated through graded series of ethyl alcohol and embedded in Epon 812. Semi-thin and ultrathin

sections were cut on LKB ultramicrotome and the semi-thin sections were stained with toluidine blue and examined with light microscope. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined with Jeol 100 S electron microscope at King Saud University.

3. Results

Light microscopic observations of the gills of fish collected from less polluted area (the control area) showed uniform arrangement. Each gill consisted of a large number of gill filaments (primary lamellae) on which a series of alternately arranged secondary lamellae (respiratory lamellae) are projected (Fig.1). The primary lamellar epithelium is multilayered and contains many squamous epithelial cells, interlamellar cells, mucous and chloride cells. While, the secondary gill lamellae are lined by a squamous epithelium (pavement cell), below it there are lamellar blood sinuses separated by pillar cells. Histological changes could be observed in the primary and secondary gill lamellae of fish collected from the polluted area. Disorganization of the lamellar structure of the secondary lamellae as well as cellular hyperplasia were occasionally observed (Fig.2). The tips of the secondary lamellae showed peculiar malformations such as curving (Fig.3), globate structures (Fig. 4) and partial fusion of some of them (Figs. 5&6).The lamellae became near each other and the interlamellar space decreased in some parts (Fig. 7). A number of the secondary lamellae appeared with wrinkling epithelium (Fig.6), while others appeared short (Fig.2). The most common tissue modifications are dilatation of the blood capillaries "aneurism" (Fig.3) and the displacement of the epithelial layer of the secondary lamellae from the underlying connective tissue (Fig. 8). This epithelial lifting is accompanied by intraepithelial edema.

Transmission electron microscopic examination of gills of fish collected from control area revealed a double layer of epithelial cells that constituted the secondary lamellar epithelium. The pavement cells are flattened and are equipped with short microridges, where as the pillar contractile cells are separated the capillary channel. The blood vessels are in contact with the basal lamina and filled with erythrocytes (Figs. 9a & 9b).

Chloride cells are located in the primary filament at the base of the respiratory lamella. They have numerous apically located mitochondria and a basally located nucleus. The apical part of the cells formed a deep pit with clearly developed microvilli (Fig. 10). Mucous cells are apically located in the primary filament. They are characterized by the presence of large number of mucous containing vacuoles with variable electron density and basal nucleus (Fig.11).

Ultrastructural alterations appeared in the gills of fish collected from the polluted area. The primary and secondary gill lamellae exhibited hypertrophy and hyperplasia of the epithelial cells (Fig.12). The pavement cell appeared irregular with a considerable loss of microridges(Fig. 13). Vasodilatation in many areas of the secondary lamellae with break down of the pillar cell system appeared by degenerative and necrotic changes of the pillar cells (Fig.14). Moreover, congestion of blood spaces by erythrocytes with presence of different leucocytes have been observed (Fig.15). Occasionally, proliferation of chloride cells and mucous cells could be identified in the secondary lamella (Fig.16). The chloride cells appeared with dilated vesicles within the cytoplasm and damaged mitochondria, while the mucous cells were completely filled with electron- dense mucous containing vacuoles and no other organelles could be visible in this cell (Fig.17). It is worth to mention that all light and electron microscopic alterations observed in the gills during this study were not related to the seasonal variations.

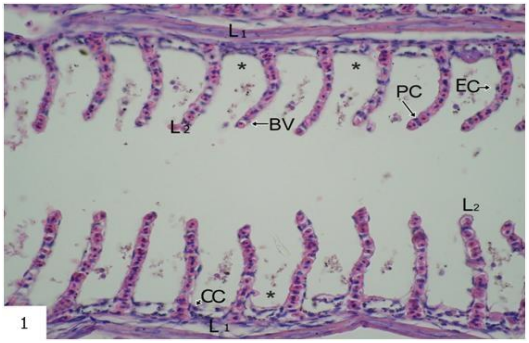


Fig.1: Light micrograph of gills of fish collected from control area showing the normal appearance of primary lamellae (L1) and secondary lamellae (L2). BV, blood vessel; CC, chloride cell; EC, epithelial cell; PC, pillar cell; *, the water channel H&E. X400.

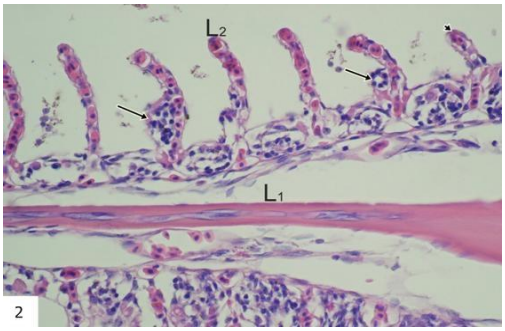


Fig.2: Light micrograph of gills of fish collected from polluted area. Note hyperplasia of the respiratory epithelium of the secondary lamellae (arrows), some of them appeared short (short arrow). L1, primary lamella; L2, secondary lamella. H&E.X500.

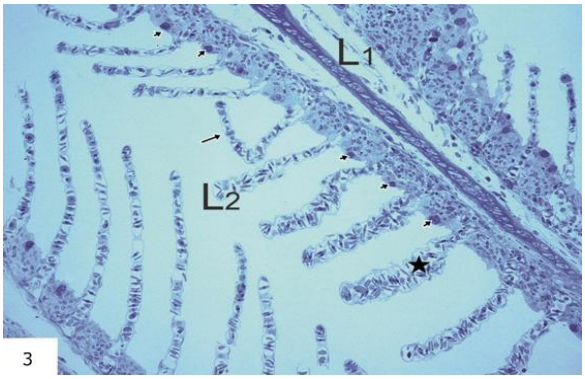


Fig.3: Light micrograph of gills of fish collected from polluted area showing curved tips of the secondary lamellae (arrow), mucous cells proliferation (short arrows), lamellar aneurism (*). L1, primary lamella; L2, secondary lamella. T.B. X400.

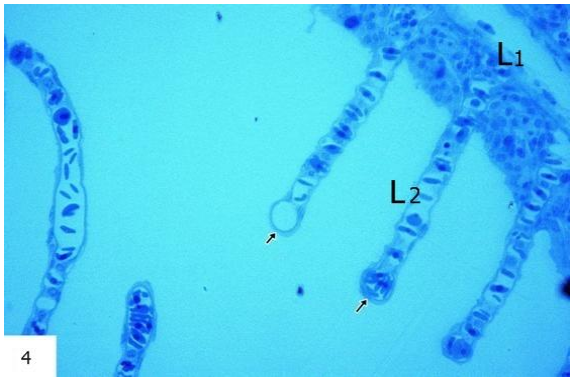


Fig.4: Light micrograph of gills of fish collected from polluted area showing globate structures at the tips of the secondary lamellae (short arrows). L1, primary lamella; L2, secondary lamella T.B.X600.

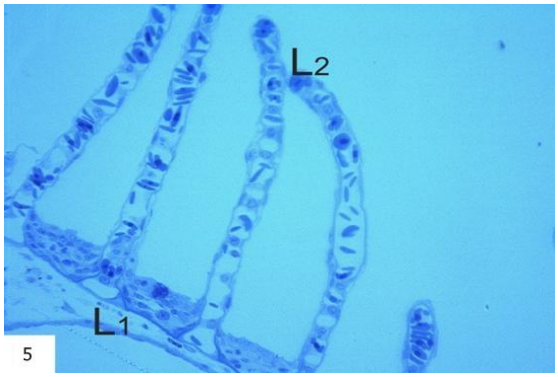


Fig.5: Light micrograph of gills of fish collected from polluted area showing fusion at the tips of the secondary lamellae. L1, primary lamella; L2, secondary lamella. T.B. X600.

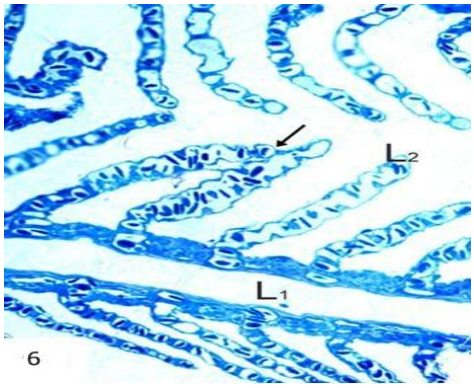


Fig.6: Light micrograph of gills of fish collected from polluted area showing wrinkling of the respiratory lamellae (L2) and fusion of their tips (arrow). T.B. X500.

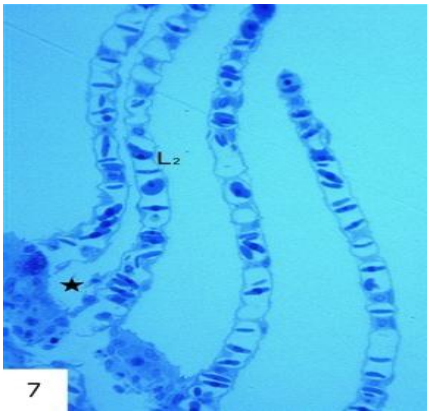


Fig.7: Light micrograph of gills of fish collected from polluted area. The respiratory lamellae (L2) become near each other and the interlamellar space is decreased (*). T.B. X600.

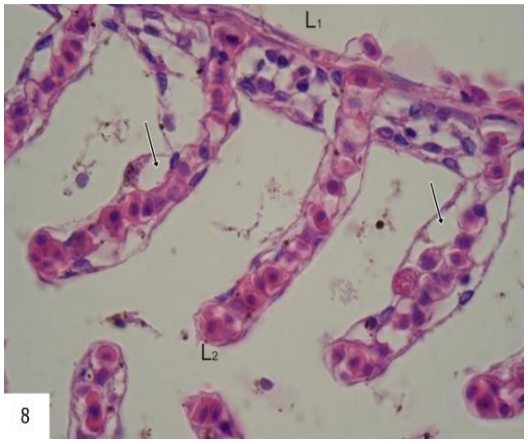


Fig.8: Light micrograph of gills of fish collected from polluted area showing epithelial lifting of secondary lamellae (arrow). H&E. X850.

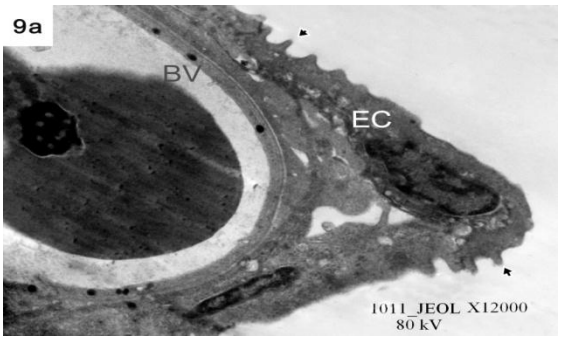
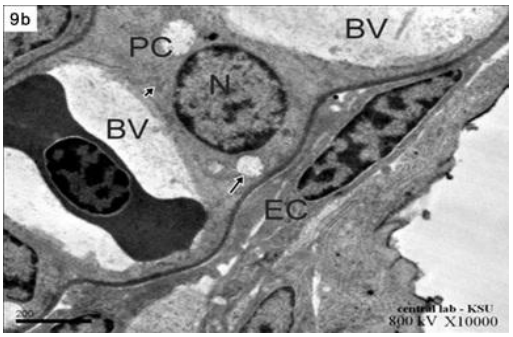


Fig 9a: Electron micrograph of gills of fish collected from control area showing the terminal part of the respiratory lamella.
The pavement cells (epithelial cells EC) are equipped with microridges (short arrows). BV, blood vessel. X12000.



9b: Electron micrograph of gills of fish collected from control area showing the normal features of the respiratory lamella. BV, blood vessel; short EC, epithelial cell; PC, pillar cell; Mitochondria (short arrow), vacuoles (arrow), X10000.

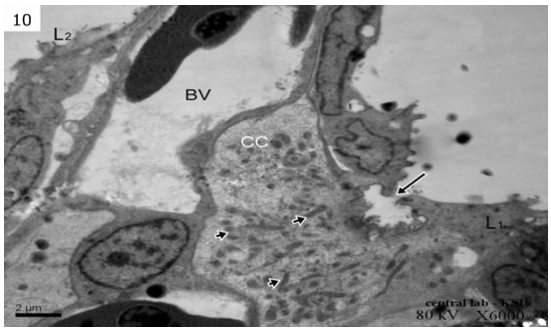


Fig.10: Electron micrograph of gills of fish collected from control area. The chloride cells (CC) are filled with numerous mitochondria (short arrows) and the apical part forming deep pit with microvilli (arrow). BV, blood vessel; L1, primary lamella; L2, secondary lamella. X6000.

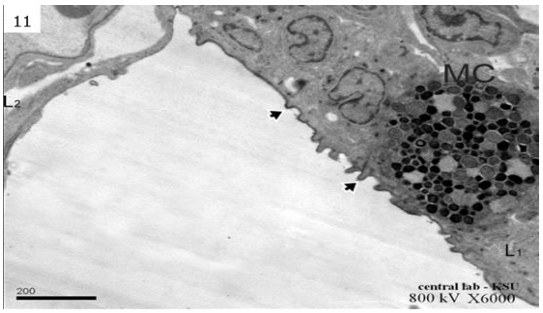


Fig.11: Electron micrograph of gills of fish collected from control area.
The mucous cells (MC) are containing large number of mucous vacuoles with variable electron density. L1, primary lamella; L2, secondary lamella; microvilli (short arrows). X6000.

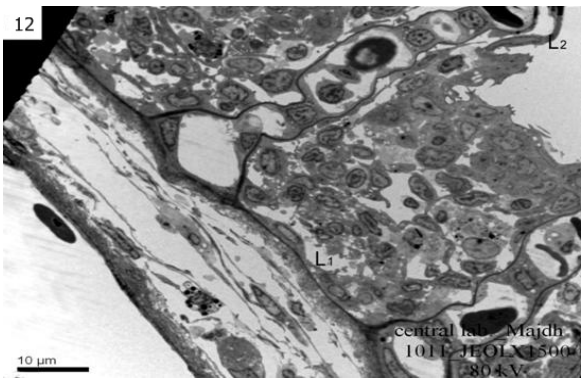


Fig. 12: Electron micrograph of gills of fish collected from polluted area showing epithelial hypertrophy and hyperplasia of gill lamellae.
L1, primary lamella; L2, secondary lamella. X1500.

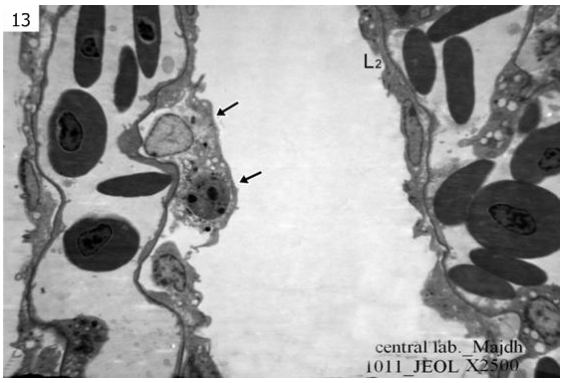


Fig. 13: Electron micrograph of gills of fish collected from polluted area showing the irregular appearance of the secondary lamellae (L2).
The pavement cells lost most of the microridges (arrows). X2500.

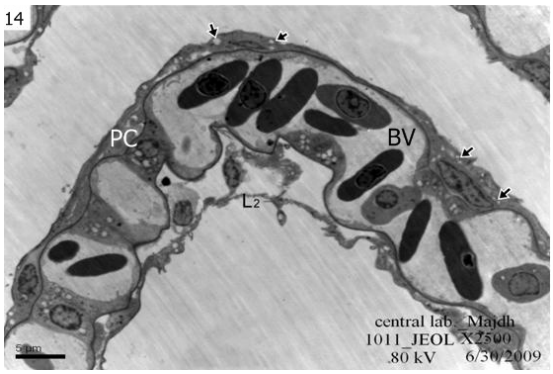


Fig. 14: Electron micrograph of gills of fish collected from polluted area showing dilatation of the blood vessel walls (BV) and degeneration of the pillar cells (PC). The surface epithelium is infolded at several points (short arrows); L2, secondary lamella.
X2500.

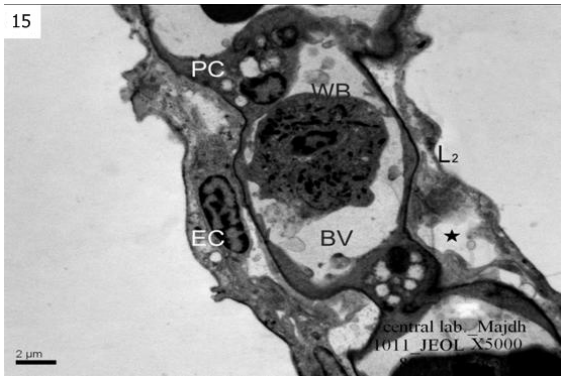


Fig. 15: Electron micrograph of gills of fish collected from polluted area showing necrotic changes of the pillar cells (PC), presence of leucocytes (WB) in the blood vessel (BV). Epithelial cell, EC; dilated intercellular space, *. X5000.

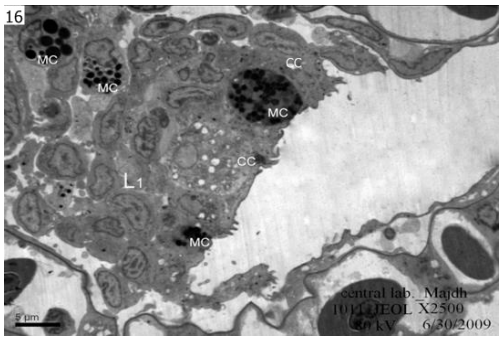


Fig. 16: Electron micrograph of gills of fish collected from polluted area. Proliferation of chloride cells (CC) and mucous cells (MC) at primary lamella (L1).
X2500.

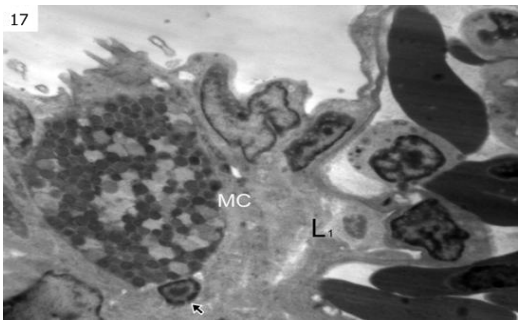


Fig 17: Electron micrograph of gills of fish collected from polluted area. Mucous cells (MC) are completely filled with electron-dense mucous vacuoles with shifting of the nucleus (short arrow).
L1, primary lamella.X8000.

4.Discussion

The present study revealed to histopathological changes as well as ultrastructural alterations in the gills of fish collected from polluted area compared to that found in gills of fish collected from less polluted area (control area).

The major changes are hypertrophy and hyperplasia of the epithelial cells, partial fusion of some secondary lamellae, lamellar aneurism, besides epithelial lifting and edema. This may be early responses of the gills to the harmful substances. These alterations are examples of defense mechanisms because the lifting lamellar epithelium and edema increased the distance between the external environment and the blood, thus serving as a barrier to the entrance of contaminants (Fernandes & Mazon, 2003). Similar alterations in the gills have also been reported in the fish exposed to metals (Oliveira-Ribeiro *et al*., 2000; Cerqueira & Fernandes, 2002), organic contaminants (Rosety-Rodriguez *et al*., 2002; Fanta *et al*., 2003) and after acute exposure to insecticides (Ortiz *et al*., 2003; Cengiz, 2006). According to Mallat (1985) such alterations are non-specific and may be induced by different types of contaminant.

The predominant ultrastructural alterations in the gills of fish collected from the polluted area consisted in a distinct degeneration and necrosis of pillar cells and consequently in a damage of the capillary walls of the secondary gill lamellae. Similar observations recorded by Schwaiger *et al*. (2004) who assumed that these gills alterations might interfere with normal respiratory functions and might lead to an impairment of the general health conditions of fish.

Damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilatation of the marginal channel, blood congestion or even an aneurism (Rostey-Rodriguez *et al*., 2002; Camargo & Martinez, 2007). The present electron micrographs show a reduction in the quantity of the microridges of the pavement cells in the gills of *Oreochromis sp.* collected from the polluted area. Such reduction was also observed by Wong &Wong (2000), Mazon *et al*.

(2002) and Biagini *et al*. (2009). Mallat (1985) suggested that the microridges are related with the retention of mucous on the epithelium as a way to protect it against environmental alterations. The proliferation of chloride cells in gills of fishes collected from polluted area could be explained by increased excretion or adaptive processes to different ionic environments (Laurent & Hebibi, 1998; Mc Donald *et al*., 1991).However, the increase of mucous containing vacuoles in the mucous cells are evident to the mucous function in protection of the gill epithelium from environmental impacts, infectious agents, toxic agents and particles in suspension (Powell *et al*., 1992; Biagini *et al*., 2009). Perry and Laurent (1993) stated that mucous cells can be efficient in seizing the toxic agents and thus help in the prevention of the entrance of these agents into the gills. So far, the inflammatory reactions of gills observed in the current study may be caused by direct contact of the respective epithelia with a toxin. This could be due to the high sensitivity of the gills to environmental stress and their capacity to react to low concentrations (Pawert *et al*., 1998; Pandey *et al*., 2008). However, it is more probably that these effects are secondary alterations to the pollutants action in receptors bond to the epithelial cell membranes (Evans, 1987). Lesions in the gill morphology could lead to functional alterations and interference in fundamental process such as maintenance of osmoregulation and antioxidant defense of gills (Pandey *et al*., 2008). According to Arellano *et al*. (2001) and Biagini *et al*.(2009) the histological alterations observed in fish gills are acknowledged as a fast and valid method to determine the damages caused by exposition to different pollutants .

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