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Structural alterations in Gills, Liver and Ovaries of Tilapia Fish (*Saratherodon galilaeus*) as a Biomarker for Environmental Pollution in Ismalia Canal

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ABSTRACT

The present work studied the histological changes in gills, liver and ovaries of Tilapia fish (*Saratherodon galilaeus*) subjected to disturbed urban stream (sewage and industrial contamination) of two sites (EL-aakada and EL-amerya) on Ismailia Canal during summer season. The most common lesions in gills were epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, and lamellar fusion. Liver hepatocytes showed hypertrophied, cytoplasmic and nuclear degeneration, inflammatory cells aggregates and focal areas of necrosis. The ovary showed widened vitellogenic space filled with huge debris, the oogonial cells and immature oocytes were in the process of degeneration particularly at the ooplasmic level. The most sever lesions were comparatively in the liver. It is concluded that histopathology can used as a biomarker for study the effect of pollutants in organs of fish.

Key words: Gills, histopathology, Ismailia Canal, liver, *S. galilaeus*, and ovary

INTRODUCTION

Ismailia Canal is one of the ecosystems most hit by the contamination resulting from human activity (Geriesh *et al*., 2008). Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids (Pandey *et al.*, 2003). Such contaminants change water quality and may cause many problems to fish, such as diseases and structural alterations (Chang *et al.*, 1998).

The effects of pollutants on fish can be properly evaluated if bioaccumulation is complemented with other biomarkers. Contaminants usually appear in the environment as very complex mixtures that can cause interactive effects, thus biomarkers offer an integrated measurement of these effects (Orbea *et al*., 2002; Ferreira *et al*., 2005). Among pollutants that can accumulate in fish, heavy metals are of great interest because they could trigger oxidative stress in fish (Bla´ha *et al*., 2004; Deviller *et al*., 2005), by reactive oxygen species (ROS) generation (Livingstone, 2003; Ferreira *et al*., 2005; Durmaz *et al*. 2006; Lesser, 2006).

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton, 1991; Thophon *et al.,* 2003) and field studies (Hinton *et al.,* 1992; Schwaiger *et al.*, 1997; Teh *et al.,* 1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is the allowance of examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.*, 2001). Furthermore, the 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 and Laurén, 1990).

The liver plays a primary role in the metabolism of xenobiotic compounds and it is a detoxification organ essential for excretion of toxic substances in fish (Hinton and Laure´n, 1990; Figueiredo-Fernandes *et al*., 2006). Gill is the osmoregulatory surface tissue and it is the primary site of uptake of waterborne pollutants (McDonald and Wood, 1993; Monteiro *et al*., 2005).

Chemical pollution is dangerous in disrupting the food chain and damage caused by chemical elements chemicals included under disruptors endocrine, which widely used in agriculture (as emulsifiers in the production of liquid pesticides) in plastics , elastomers and textile industries, the production of paper, detergents and deodorized products etc, have significant potential for release into the environment. With their lipophilic-nature quality, alkiphenols accumulate and deposit predominantly in fat tissues, liver, bile and kidneys, being possible to become available for human consumption (White, 1994).

A major consequence of endocrine disruptors' action on fish is represented by the process of male effeminate, caused by the induction and increase of the release of vitellogenin, reduction in number of eggs, spawned and their quality, low rate of fertility in females (Arukwe *et al*., 2000; Folmar *et al*., 1996, Le Guellec *et al*., 1988). The reason of this sensibility is long term exposure and easy way in which these substances get in the blood stream of the fishes trough the gills system.

The aim of the present study was to evaluate the occurrence of histological alterations in gills, liver and ovaries of the freshwater fish, (*Saratherodon galilus*), collected from two different polluted sites on Ismailia Canal.

MATERIALS AND METHODS

Collection of water and fish samples

Water samples were collected at 70 cm depth during summer season (2012) from two sites (EL-amerya and

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EL-aakada) on Ismailia canal. One liter plastic bottles were filled with water samples for chemical analysis.

Field observations

In *situ*, air and surface water temperatures (°C) were measured by a dry mercury thermometer, transparency (cm) by Secchi disc, electrical conductivity (EC) (μmohs/cm) by using conductivity meter.

Laboratory analysis

Dissolved oxygen was measured using the modified winkler method and concentration of ammonia; nitrite and nitrate were determined by using the colorimetric techniques. All previous analysis were carried out according to the standared methods for examination of water and wastewater (APHA, 1998). The fish samples of *Saratherodon galilaeus* (20 fish) were collected from two main fishing sites on Ismailia Canal namely EL-aakada and EL-amerya, during summer season 2012.

Histopathological studies

Fish were dissected directly in the field, and the target organs (gills, liver and ovaries) were quickly removed. Histological techniques were performed according to Bucke (1994). After fixation in Bouin's solution for 24hr at room temperature, tissues were dehydrated and routinely processed for paraffin embedding. Then, 5–7 μ thick sections were made in a rotary microtome and stained with hematoxylin and eosin. Tissues were microscopically examined for a variety of histopathological features and lesions then photographed.

RESULTS

Water physico-Chemical Parameters of Ismailia Canal during summer season appreciably higher in EL-amerya station [NH3=137.4, No2=4.98, No3 = 65.42 and PO_4 = 92.4 μg/L] than EL-aakada station which were [129.2, 2.05, 35.9 and 72.6 μg/L respectively] and dissolved oxygen (DO) which was7.2 and 8.64 mg/L for EL-amerya and EL-aakada station respectivily. The present results showed depletion in ammonia, nitrate, nitrite and phosphate concentrations at EL-aakada station. The field observations such as EC value for EL-amerya and EL-aakada stations were (327 and 347 ms/cm respectively) and transparency values were (80 and 40 cm), while temperature value was 29.6°C for EL-amerya and 33°C for EL-aakada station.

Histopathlogical observations

Gills

Figures (1 and 2) show the histopathological alterations in the gills of *S. galilaeus* from EL-aakada station during summer season. The alterations included severe degenerative and necrotic changes in gill filaments and secondary lamellae. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Samples collected from EL-amerya station showed proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion and curling of secondary lamellae, mucous and chloride cells proliferation. (Fig. 7 and 8).

Liver

Figures (3 and 4) shows liver histological structure of *S. galilaeus* collected from EL-aakada station. The most common lesions were vacuolar degeneration in the hepatocytes, focal areas of necrosis and aggregations of inflammatory cells between the hepatocytes. On the other hand, dilation and thrombosis formation were in the central veins (Fig. 9) and focal areas of coagulative necrosis were observed at samples of EL-amerya station (Fig. 10)

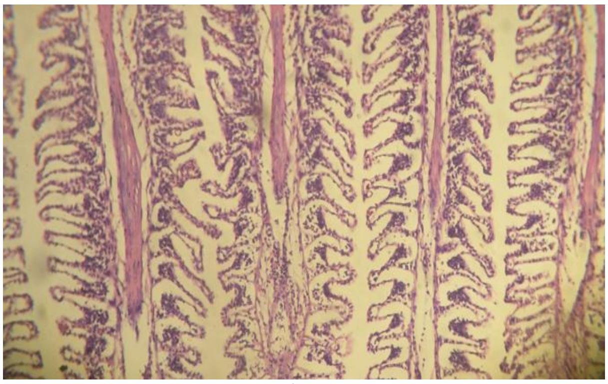

Figure (1): Section in gill of *S. galilaeus* showing epithelial lifting of secondary lamellea EL-aakada station. (X100).

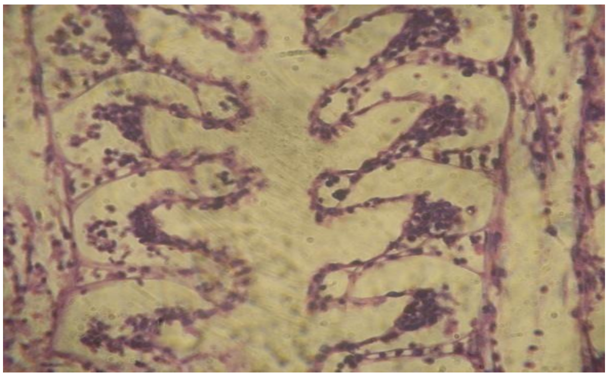

Figure (2): Section in gill of *S. Galilaeus* showing neutrophile infiltration, edema in secondary lamellae and gill filaments. (X400).

Ovary

Ovaries of fish samples of *S. galilus* collected from EL-aakada station frequently present large oocytes and cytoplasm loaded with tertiary vitellus, including the oocytes with secondry vitellus and a reduced number of small oocytes, with primary vitellus (Fig.5,6). Most examined ovaries presenting small size oocytes, with primary vitellus and spherical central nucleus occupied by numerous basophilic nucleolus with a peripheral deposition (Fig. 11,12).

The oogonial cells and immature oocytes were in the process of degeneration particularly at the ooplasmic level and these were devoid of vitellogenic oocytes. Wide vitellogenic space filled with huge debris mass of their dissolute components these features are noted in studied samples inhabiting EL-amerya station (Figs. 13 and14).

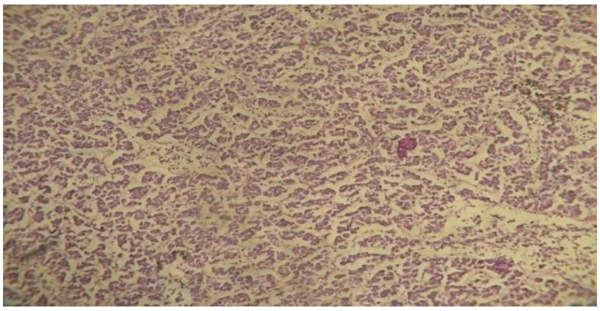

Figure (3): Section in liver of *S. Galilaeus* showing focal areas of necrosis and lymphocyte infiltration EL-aakada station. (X100).

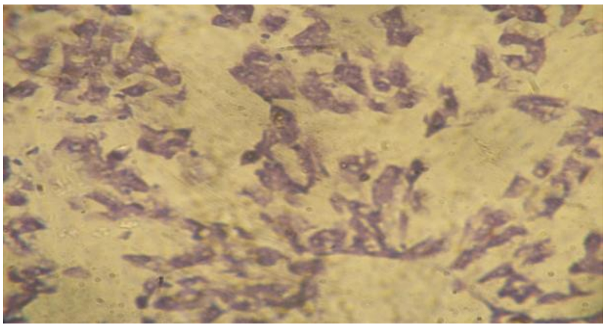

Figure (4): Section in liver of *S.galilaeus* showing severe edema. (X400).

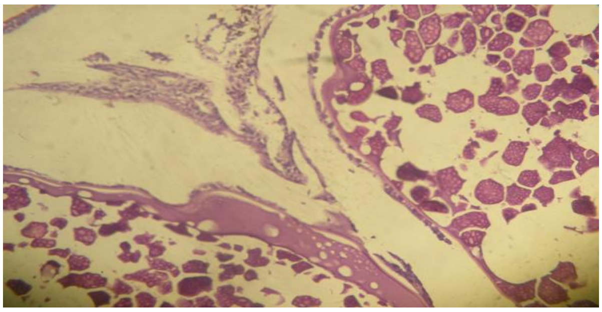

Figure (5): Ovary has large oocytes with Secondary vitellus and hyaline degeneration at the periphery. EL-aakada station (X100).

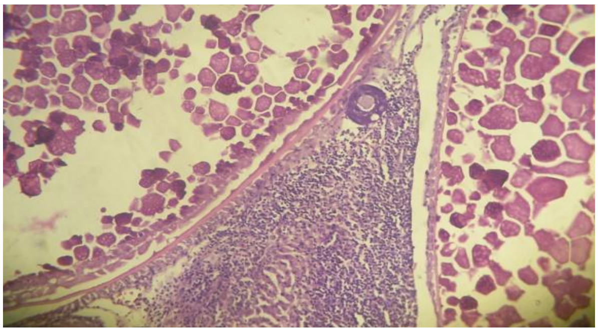

Figure (6): Ovary has formed predominantly of large oocytes, with secondary vitellus, and huge intrestitium cells in between. (X100).

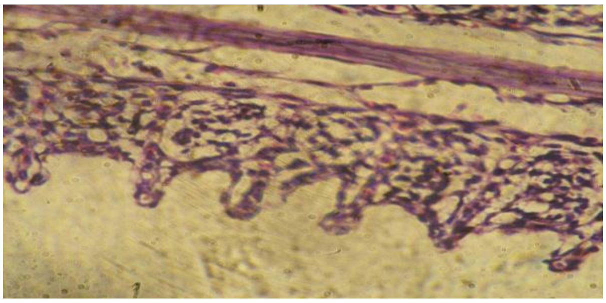

Figure (7): Section in gill of *S.galilaeus* showing epithelial cells hyperplasia, stunned secondary lamellea and fusion. (X400).

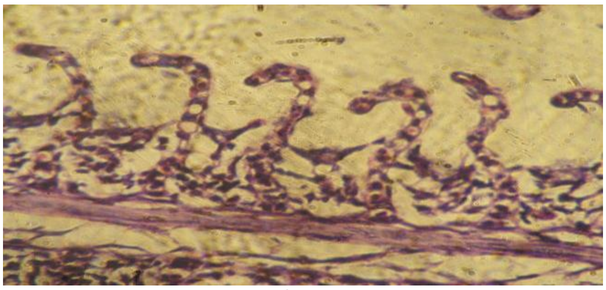

Figure (8): Section in gill of *S. Galilaeus* showing curling of secondary lamellae and edema, EL-amerya station. (X400).

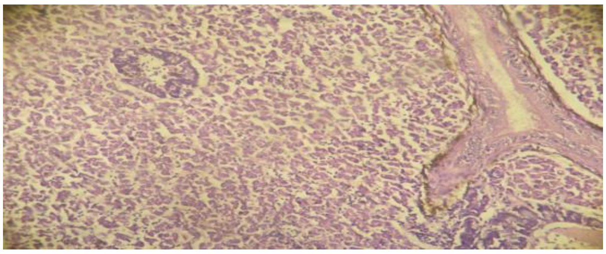

Figure (9): liver of *S. Galilaeus* showing Intravascular haemolysis in blood vessels, EL-amerya station. (X100).

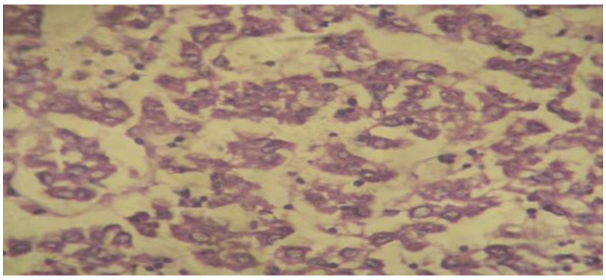

Figure (10) Liver of *S. Galilaeus* showing focal necrosis and lymphocyte cells infiltration. (X400).

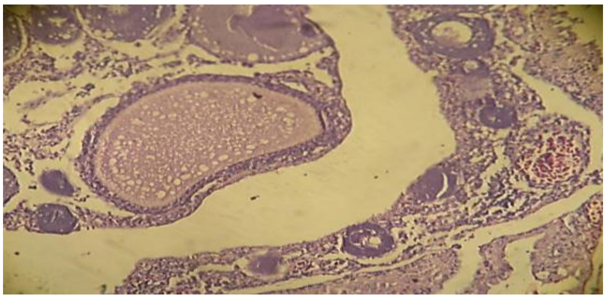

Figure (11): Section in ovary of *S. galilaeus* showing atretic

Young oocytes (perinucleolus stage), EL-amerya station (X100).

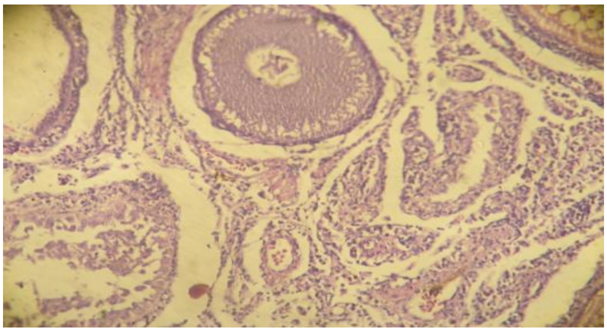

Figure (12): Ovary showing primary vesicle stage and huge macrophage cells (X100).

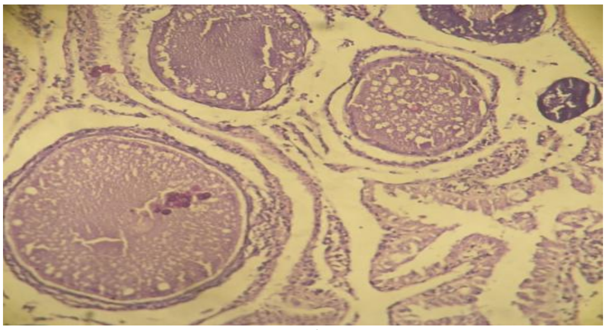

Figure (13): Section in ovary of *S.galilaeus* showing widened vitellogenic space and atretic oocytes, EL-amerya station. (X 400).

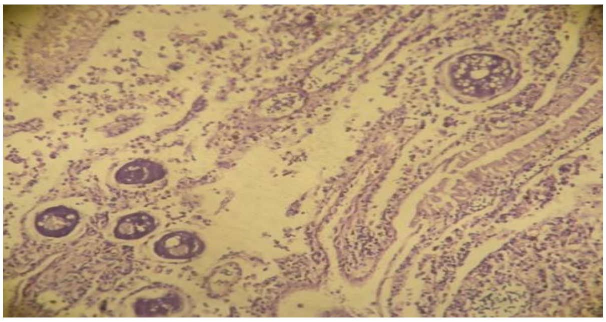

Figure (14): Ovary has crumpled young oocytes with huge number of macrophages. (X100).

Discussion

The physico-chemical parameters are considered the most important principles in the identification of the nature, quality and type of the water (fresh, brackish or saline) for any aquatic ecosystem (Abdo, 2005).

Dissolved inorganic nitrogen is the summation of the ammonia, nitrate and nitrite (Tayel *et al*., 2007; Osman *et al*., 2010). These parameters were found in high concentrations in EL-amerya station than in EL-aakada station which may be due to sewage outfalls, as recorded by Tayel *et al*. (2007). Thus, the higher contents of nitrite in El-amerya station are possibly indication of the microbial activity. Osman *et al*. (2010), reported that the increase of NO in Ismailia Lake comparing to that in River Nile water might be attributed to the fast conversion of NO-NO–ions by nitrifying bacteria. The increase in ammonia level in water samples collected from EL-amerya station is an indicator of the presence of pollutants of high activity e.g., sewage discharge and human activities could be attributed to the increase in the oxygen consumption of the decomposing organic matter and oxidation of chemical constituents (Elghobashy *et al*., 2001).

The increased organic chemicals (may be phosphates) as well organic matter bearing highest value of *T. coliform* and Colony counts may be contributed to some of liver severe lesions (Zapata-Perez *et al*., 2000) where they found out liver necrosis in Nile Tlapia (*O. niloticus*) after exposure to a sediment containing a variety of organic chemicals **(***Zakia et al*., 2012).

Results of the present study revealed that *S. galilus* inhabiting EL-aakda and EL-amerya stations manifest histopathological changes in gills, liver and ovaries during summer season. So, studied fish deformed organs from both stations on Ismailia Canal could be a direct result of the heavy metals, pesticides, fertilizers, salts and sewage, which are entered to the Canal with the drainage water, the present results were confirmed by Mansour and Sidky (2003) and Geriesh *et al*., (2008).

In addition, the current study showed the dilation of the lamellar blood sinuses and curling of the secondary lamellae with separation of the respiratory epithelia and proliferation of chloride cells in base lamellar regions. There was epithelial layer separation causing epithelial lifting and neutrophil infiltration. These changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface. The edematous separation of the respiratory epithelium and lamellar clubbing may be due to water born toxins and this depature from normal structure affect the functional efficiency of the gills for gas transportand ionic regulation (kantham and Richard, 1995).

Chloride cells proliferation due to an added function of oxygen transport due to injury to gill tissue proper. In certain abnormal conditions chloride cells may to be an oxygen transport functions (Tamse *et al*., 1995).

On the other hand, the observed alterations like proliferation of the epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defence mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Fernandes and Mazon, 2003; Poleksic and Mitrovic-Tutundzic, 1994). Fusion of lamellae decreases gill surface area, this may allow slower up take of toxins but create an anoxic condition that will ultimately harm the fish (Takashima and Hibiya, 1995).

Previous studies reported swelling and fusion of the gill lamellae in fish exposed to sublethal concentration of where metals bind strongly to the plasma membrane of the lamellar epithelial cells increaing their permeability to water and ions (Mallat *et al.,* 1995; El-Feki, 1998; Bin Dohaish, 2001). Also, heavy metals might inhibit ion carried in chlorid cells and thus, an increase in their number would be compensatory (El-Feki,1998).

The cellular damage observed in the gills in terms of epithelial proliferation, separation of the epithelial layer from supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation (Dutta *et al*., 1993). The observed oedematous changes in gill filaments and secondary lamellae may probably due to increased capillary permeability (Olurin *et al*.,2006).

Accumulation of Leukocytes infilteration in the sub epithelial spaces of secondary lamellae and necrotic gill tissues may be an inflammatory response to different chemical toxic substances (EL-Feki, 1998) or to phagocyte the toxicant particles and tissue debrises (Muhvich, *et al.,* 1995). Uniform changes in the hepatocytes of studied fish species were found such as degeneration, necrosis and hyperaemia. The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water (Mohamed, 2009).

Some authors also reported degenerative and necrotic changes in hepatocytes but in other species and under the influence of various pesticides and other toxic heavy metals (Jee *et al*., 2005; Velisek *et al*., 2007; 2009). Mohamed (2009) (Lake Quarun, Egypt), found degenerative, necrotic, inflammatory, hyperemic and thrombosed alterations similar in the liver of both *Tilapia zillii* and *Solea vulgaris.* El-Naggar *et al*., (2009) proved that bioaccumulation of some heavy metals (Fe, Cu, Zn, Mg, Pb and Cd) in the liver of *Oreochronis niliticus* in the Nile caused similar changes in the hepatopancreas confirming the current work results.

Camargo and Martinez (2007) stated that necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the water soluble fraction (WSF) from its body during the process of detoxification by the liver. The inability of fishes to regenerate new liver cells may also have led to necrosis. According to Saxena *et al*. (2008) lymphocytic infiltrations in liver were observed in fish after exposure to polluted water with heavy metals, the same alterations were present in EL-amerya fish samples.

The present study showed over-all reduction in oocyte development in ovaries of fish collected from EL-Amerya station. Presence of a few number of oogonia and immature oocytes and a few early degenerating vitellogenic oocytes with complete dissolution of ooplasmic and nuclear materials in ovaries. (Masud *et al*., 2003) reported that large degenerative changes in ovary and liver leading to decreased GSI and complete mortality of *C. carpio* were observed after 45 days exposure to 0.5 ppm $HgCl_2$, the ovaries of *C. Fasciatus* when exposed to sublethal concentration of nickel and *H. fossilis* exposed to textile mill effluent, the prominent changes were occurrence of atretic oocytes and increase in the interfollicular space (Srivastava and Srivastava,1994). They found the nuclei of the oogonia underwent complete karyolysis, vacuolation within nucleoli and shrinkage of nucleus in the ovary of treated fish. Jyothi and Narayan (1999) observed reduction in diameter of oocytes I, II and III. Prominent interfollicular space was observed in the ovaries which were probably formed due to shrinkage of the oocytes. Large numbers of atretic follicles were also observed in the ovaries of *H. fosillis* exposed to chloride conc. Baruah and Das (2002) studied the effect of carbaryl on the ovary of *Clarias batrachus* and observed vacuolation and necrosis, arrested ovarian recrudescence and interfollicular oedema. Sharma (2002) noted partial lysis, swelling, atersia and changes in nucleus and cytoplasmic organization after exposure of *Heteropneustes fossilis* to paper mill effluent for 20 days. They suggested that alterations were due to influence on the pituitary gonadal axis. Clumping, thickening of ovarian wall and disappearance of nucleus were observed in mature oocytes. The above results correlate with the findings of Ramachandra (2000); Wahbi El-Greisry (2007) and histological changes of the fish gonads as a response to environmental stress has shown to be a biomarker indicative tool to assist in the bio-monitoring process of aquatic ecosystems (Bhuiyan *et al*., 2001).

Muley *et al*. (2007) reported reduction in protein, glycogen and lipid in tissues of freshwater fish *Labeo rohita* induced by heavy metals from electroplating industry. Shoba *et al*. (2007), studied biochemical changes in freshwater fish, *Catla catla* on exposure to heavy metal toxicant cadmium chloride. Mastan, (2008) studied changes in protein levels of certain tissues of freshwater fish *Heteropneustes fossilis* induced by copper. The observations obtained from the present study demonstrated the presences of atresia of developing oocytes. Similar effects have been demonstrated in white perch in areas affected by domestic and industrial effluents (Richard *et al*., 2004) and in salmon chronically exposed to sewage effluent (Luis *et al.,* 2002).

Conclusions

Finally, fish histopathology could therefore make valuable contribution in the monitoring of aquatic ecosystems and should form an important part of environmental impact assessment in the environmental management process. In conclusion, the results showed that the fish inhabiting in EL-aakada station are healthier than those live in EL-amerya station which this is may be due to the presence of human activities in such area.

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