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Histopathological Studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt

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Abstract: Lake Qarun is an inland lake occupies the lowest part of El-Faiyoum depression. It receives large amounts of contaminated drainage water from Faiyoum Province. The study was conducted to investigate the histological structures of the muscles, liver, gills, kidney and intestine of *Tilapia zillii* and *Solea vulgaris* obtained from Lake Qarun, Egypt during summer 2007 and winter 2008. Several histological alterations were observed in the muscles of both fish, including degeneration in muscle bundles with focal areas of necrosis, atrophy of muscle bundles and edema between muscle bundles. The liver showed vacuolar degeneration in the hepatocytes, focal areas of necrosis and fibrosis, aggregations of inflammatory cells between the hepatocytes, dilation and congestion in blood sinusoids and thrombosis formation in the central veins. In the gills, the pathological alterations included proliferative, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, edema in secondary lamellae, dilation and congestion in blood vessels of gill filaments and mucous cells proliferation. The kidney showed vacuolar degeneration in the epithelium of renal tubules, focal areas of necrosis, haemorrhage and haemosiderin between the renal tubules and edema in Bowman's capsules with atrophy in the glomeruli. In the intestine, degenerative and necrotic changes in submucosa and mucosa with edema between them, dilation in blood vessels of serosa and atrophy in the muscularis and submucosa are noticed. It was concluded that the environmental contamination of Lake Qarun induced several histopathological alterations in the tissues of *T. zillii* and *S. vulgaris*.

Key words: Pollution % Fish % Histopathology % Lake Qarun % Egypt

INTRODUCTION

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment having led to various deleterious effects on the aquatic organisms [1]. Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly *via* the food chain [2]. Lake Qarun is a closed system acts as a reservoir for agricultural and sewage drainage water of El-Faiyoum province. Previous studies reported that Lake Qarun components are polluted with heavy metals [3-5] and with a wide variety of pesticides (e.g. lindane, aldrin, some DDT analogues, malathion) [3,6]. Also, it was reported that the drainage waters discharged into the lake are high in solids, nutrients, pesticides, heavy metals and organics [7]. Moreover, a remarkable increase in the bacterial indicators of sewage pollution (total coliforms, faecal coliforms and faecal streptococci) in the lake was recorded [6,8]. Extensive evaporation of the water from such closed ecosystem increases concentration of heavy metals, pesticides and other pollutants, consequently, such contaminants changes the quality of water and affects the biology of the lake [6].

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics

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could initiate the formation of a specific enzyme that causes changes in the metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i.e. histopathological biomarkers on a tissue level [9]. Previous studies reported that the exposure of fish to pollutants (agricultural, industrial and sewage) resulted in several pathological alterations in different tissues of fish. Histopathological changes were observed in the muscle of fish as a result of exposure to different toxicants [10-14]. The liver, as the major organ of metabolism, comes into close contact with xenobiotics absorbed from the environment and liver lesions are often associated with aquatic pollution. Several histopathological alterations were observed in the liver of *Gymnocephalus cernua* collected from Elbe Estuary contaminated by domestic, industrial and agricultural pollutants [15], *Oreochromis niloticus* collected from the southern region of Lake Manzalah contaminated with domestic, industrial and agricultural pollutants [16], *Coregonus clupeaformis* exposed to nickel [17], *Corydoras paleatus* exposed to methyl-parathion [18], *Clarias gariepinus* exposed to lead [19] and *Oncorhynchus mykiss* exposed to copper sulphate [20].

Histopathological changes have been reported in the gills of many fish as a result of exposure to different toxicants [21-24]. Several pathological alterations have been reported in the kidney of *Cyprinus carpio* exposed to sewage [21], *Prochilodus lineatus* exposed to trichlorfon [25], *Lates calcarifer* exposed to cadmium [26], *Channa punctatus* exposed to zinc [27] and *Prochilodus lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes [23]. Histopathological alterations have been reported in the intestine of fish as a result of exposure to different toxicants [28-30]. The present study aimed to investigate the impact of the environmental conditions of Lake Qarun on the histological structures of the muscles, liver, gills, kidney and intestine of two commercially important fish, *Tilapia zillii* and *Solea vulgaris*.

MATERIALS AND METHODS

Study Area: Lake Qarun is a closed elongated saline basin lying in the western Egyptian desert. It located between longitudes 30°24´ and 30°49´E and latitudes of 29°24´ and 29° 33´N in the lowest part of El-Faiyoum depression, about 80km Southwest of Cairo (Fig. 1). It has an irregular shape of about 40km length and about 6km mean width. The area of the lake is about 240km^2. The lake is shallow, with mean depth of 4.2m and most of the lake's area has a depth ranging between 5 to 8 meters. The water level of the lake fluctuated between 43 to 45m below mean sea level [31]. The lake receives agricultural and sewage drainage water from El-Faiyoum Governorate through a system of twelve drains, most of the drainage water reaches the lake by two main drains, El-Batts and El-Wadi, whereas there are minor drains poured its drainage water into the lake by means of hydraulic pumps but in small amounts. The small drains are recently connected with a larger drain, namely Dayer El-Birka, which transfers a part of wastewater to the lake by pumping stations [32].

Sampling: Samples of *T. zillii* and *S. vulgaris* were collected from Lake Qarun during summer 2007 and winter 2008, measuring about 10.0-15.2 and 13.0-24.2 cm in total length and 21.0-67.5 and 32.5-140.8g in weight, respectively. After dissection of fish samples, parts of muscles, liver, gills, kidney and intestine were carefully removed and prepared for histological studies.

Histological Investigations: Specimens from muscles, liver, gills, kidney and intestine were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4-6 µm then stained with haematoxylin and eosin and examined microscopically[33].

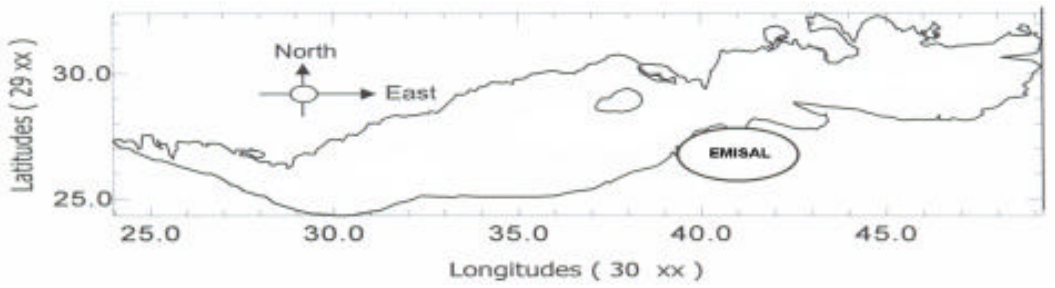

Fig. 1: Map of Lake Qarun

RESULTS

Muscles: Figure 2a and 2b show the normal structures of the muscles. Several histopathological alterations were seen in the muscles of *T. zillii* and *S. vulgaris* from Lake Qarun during summer and winter. The pathological findings included degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Also, vacuolar degeneration in muscle bundles and atrophy of muscle bundles were observed. Edema between muscle bundles and splitting of muscle fibers were seen.

Liver: Figure 3a shows the normal histological structures of the liver. The most common lesions in the liver of both studied fish were vacuolar degeneration in the hepatocytes, focal areas of necrosis and aggregations of inflammatory cells between the hepatocytes. Also, dilation and thrombosis formation in central veins, dilation and congestion in blood sinusoids and intravascular haemolysis in hepatic blood vessels and hepatoportal blood vessels were observed. Moreover, focal areas of coagulative necrosis and fibrosis were seen.

Gills: Figure 4a shows the normal histological structures of the gills. The histopathological alterations in the gills of *T. zillii* and *S. vulgaris* from Lake Qarun during summer and winter were more or less similar. They included proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, severe degenerative and necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucous cells proliferation. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also, dilation and congestion in blood vessels of gill filaments were observed. Atrophy of secondary lamellae was seen.

Kidney: Figure 5a shows the normal histological structures of the kidney. The histopathological alterations in the kidney of both fish included severe degenerative and necrotic changes in renal tubules with focal areas of necrosis and haemorrhage, haemolysis and haemosiderin between renal tubules. Aggregations of inflammatory cells were seen between renal tubules. Vacuolar degeneration (cloudy swelling) in the epithelium of renal tubules and dilation in the capillary tubes of renal tubules were observed. Also, edema in Bowman's capsules with atrophy in the glomeruli and dilation in renal blood vessels were observed.

Intestine: Figure 6a shows the normal histological structures of the intestine. The pathological findings in the intestine of *T. zillii* and *S. vulgaris* included atrophy in the muscularis, severe degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them and atrophy in the submucosa. Dilation was observed in the blood vessels of serosa.

DISCUSSION

Results of the present study revealed that *T. zillii* and *S. vulgaris* from Lake Qarun, manifest histopathological changes in muscles, liver, gills, kidney and intestine during summer and winter seasons. It is possible that the pathological alterations in the tissues of both studied fish could be a direct result of the heavy metals, pesticides, fertilizers, salts and sewage, which are entered to the lake with the drainage water [3-8]. The histopathological alterations in the muscles of both studied fish are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles [10-12]. Focal areas of myolysis were seen in the muscles of *O. spilurus* exposed to contra/insect 500/50E.C. [13]. In the same time, [14] observed destruction and vacuolation of the muscle cells in *Oreochromis* spp. exposed to chromium.

The organ most associated with the detoxification and biotransformation process is the liver and due its function, position and blood supply, it is also one of the organs most affected by contaminants in the water [23]. The liver of both studied fish showed vacuolar degeneration in the hepatocytes, focal areas of necrosis, thrombosis formation in central veins, dilation and congestion in blood sinusoids and fibrosis. These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals [30]. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system [34]. Oxygen deficiency as a result of gill degeneration being the most common cause of the cellular degeneration in the liver [35]. The vascular dilation, intravascular haemolysis and thrombosis

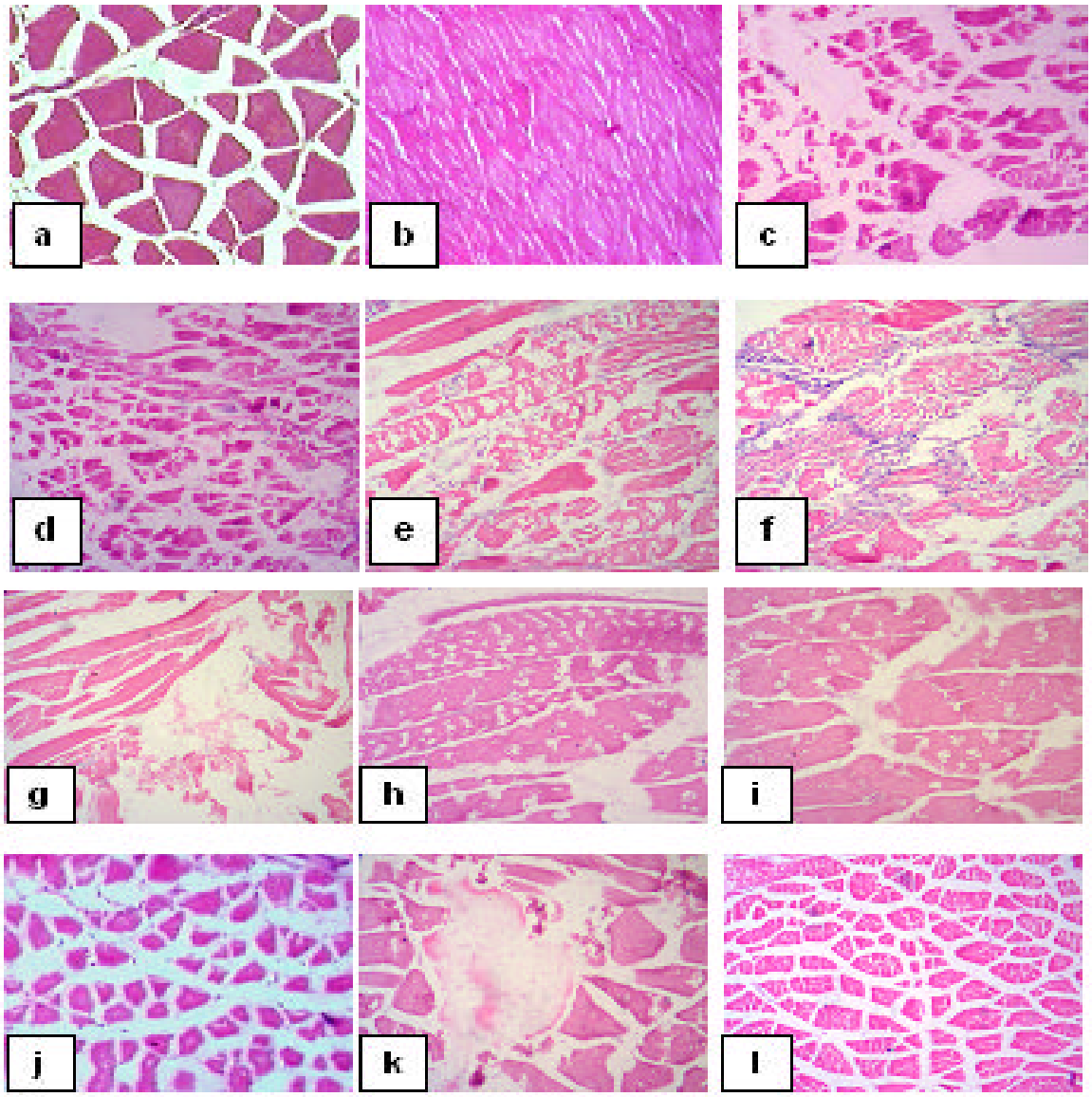

Fig. 2: Muscles of fish showing the normal (a,b)(X400), degeneration in muscle bundles (c,d{*T. zillii}*,e,f{*S.vulgaris*}{X400}), focal area of necrosis (g { *T. zillii*}{X400}), vacuolar degeneration in muscle bundles (h {*S.vulgaris*}, i { *T. zillii*}{X400}), atrophy of muscle bundles (j{*T. zillii*}{X400}), edema between muscle bundles (k { *S.vulgaris}{X400}),* splitting of muscle fibers (l { *T. zillii*}{X400})

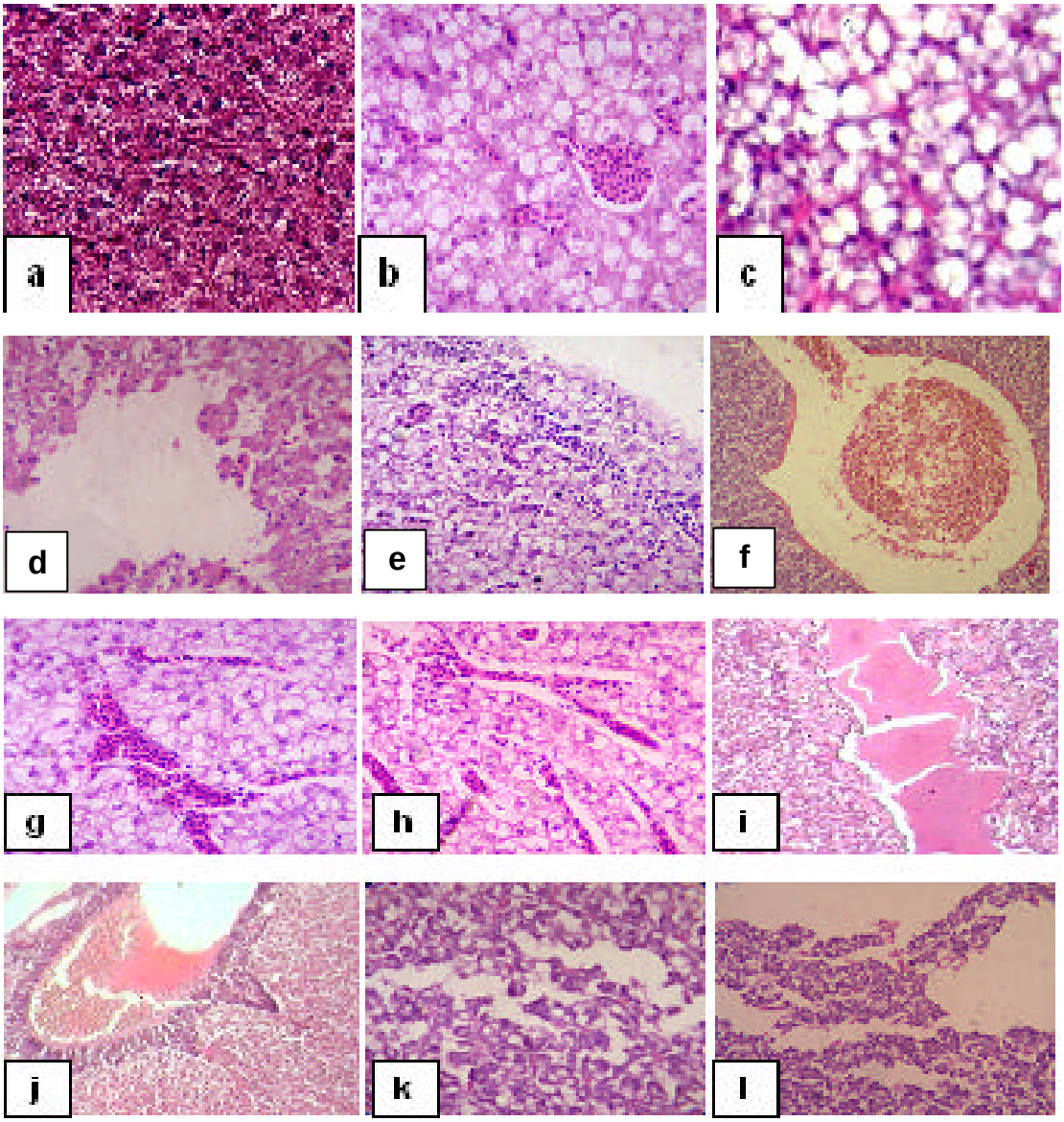

Fig. 3: Liver of fish showing the normal (a)(X400), vacuolar degeneration (b {*T. zillii*}, c {*S.vulgaris*}{X400}), focal area of necrosis (d {*S.vulgaris*}{X400}), aggregations of inflammatory cells between the hepatocytes (e {*T. zillii*}{X400}), dilation and thrombosis formation in central vein (f {*T. zillii*}{X100}), dilation and congestion in blood sinusoids (g {*T. zillii*}, h {*S.vulgaris*}{X400}), intravascular haemolysis in blood vessels (I,j {*T. zillii*}{X100}), coagulative necrosis (k {*S.vulgaris*}{X400}), fibrosis (l {*T. zillii*}{X400})

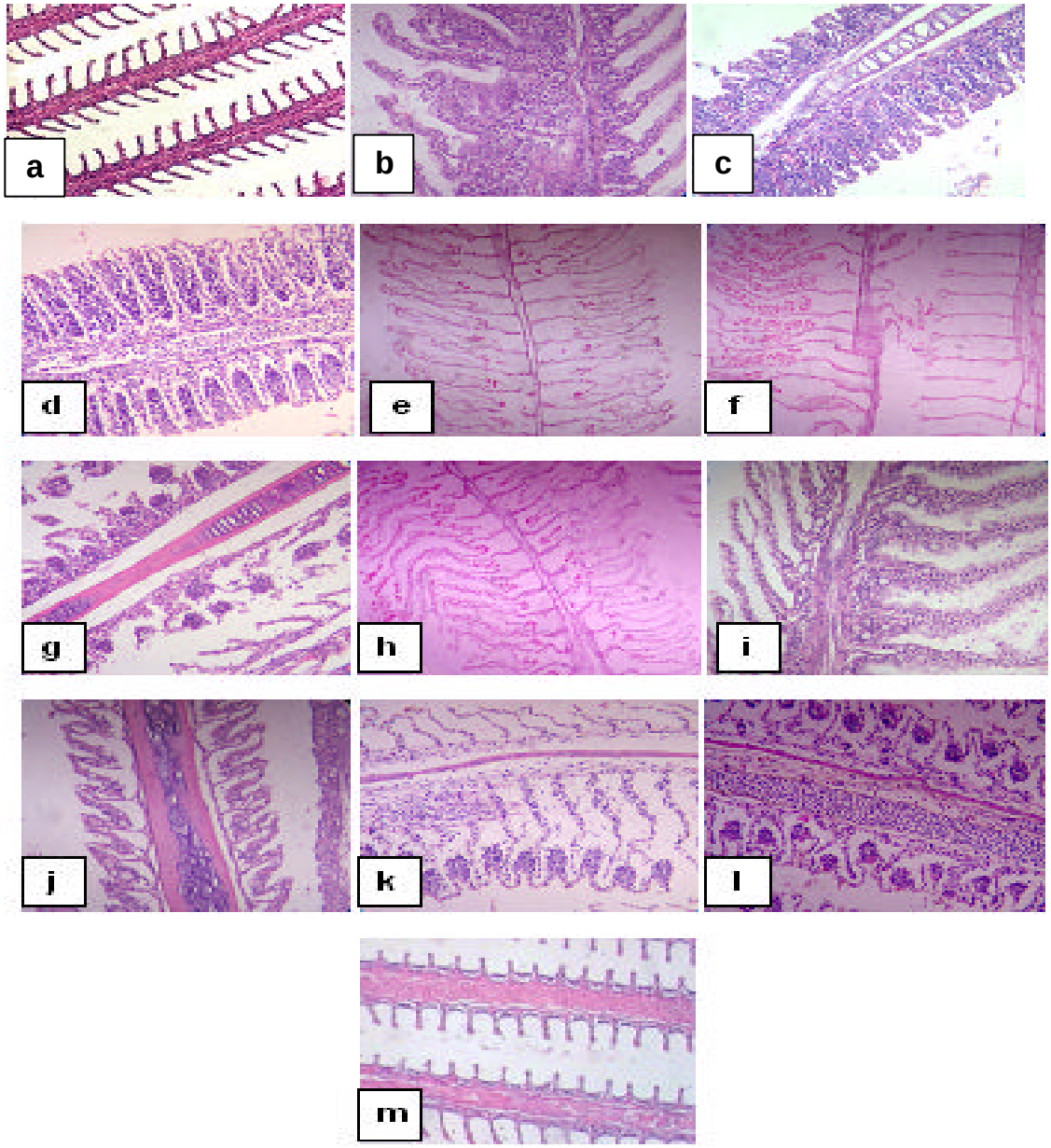

Fig. 4: Gills of fish showing the normal (a)(X100), proliferation in the epithelium of gill filaments and secondary lamellae (b,c {*T. zillii*},d {*S.vulgaris*}{X400}), degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae (e,f {*S.vulgaris*}, g{*T. zillii*}{X400}), curling of secondary lamellae (h{*S.vulgaris}{*X400}), proliferation of mucous cells (i{*T. zillii*}{X400}), edema in secondary lamellae and gill filaments (j{*T. zillii*},k {*S.vulgaris*}{X400}), aggregations of inflammatory cells in gill filaments (l{*S.vulgaris*}{X400}), dilation and congestion in blood vessels of gill filaments and atrophy of secondary lamellae (m{*T.zillii*}{X400})

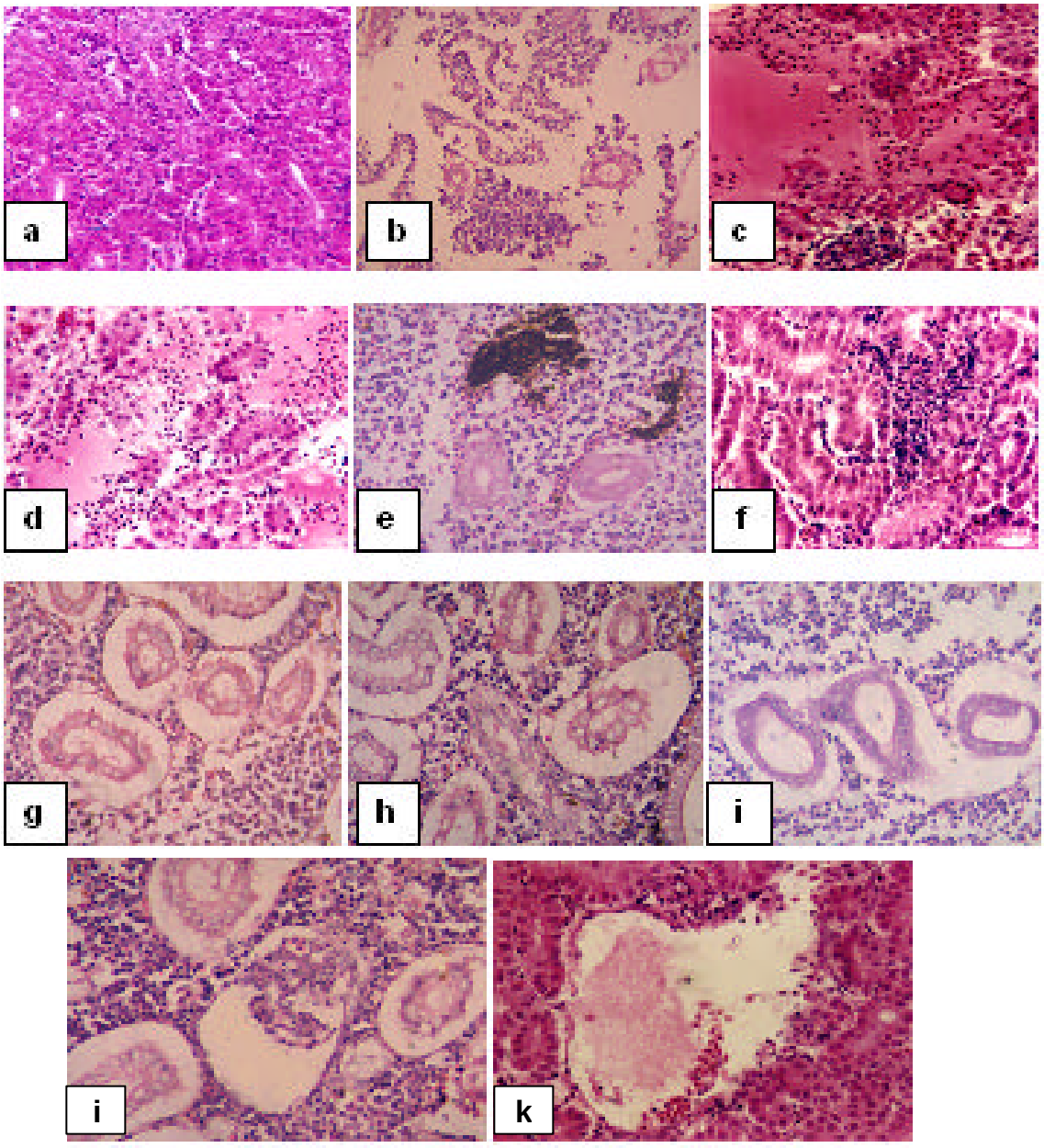

Fig. 5: Kidney of fish showing the normal (a)(X400), severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis (b {*S.vulgaris*}{X400}), haemorrhage, haemolysis (c {*T.zillii*}, d{*S.vulgaris*}{X400}), haemosiderin (e{*S.vulgaris*}{X400}) and aggregations of inflammatory cells (f{*T.zillii*}{X400}) between the renal tubules, vacuolar degeneration in the epithelium of renal tubules (g,h{*T.zillii*}{X400},i{ *S.vulgaris*}{X400}),dilation in the capillary tubes of renal tubules (i{ *S.vulgaris*}{X400}), edema in Bowman's capsule (j{*T.zillii*}{X400}), dilation in renal blood vessel (k{*T.zillii*}{X400})

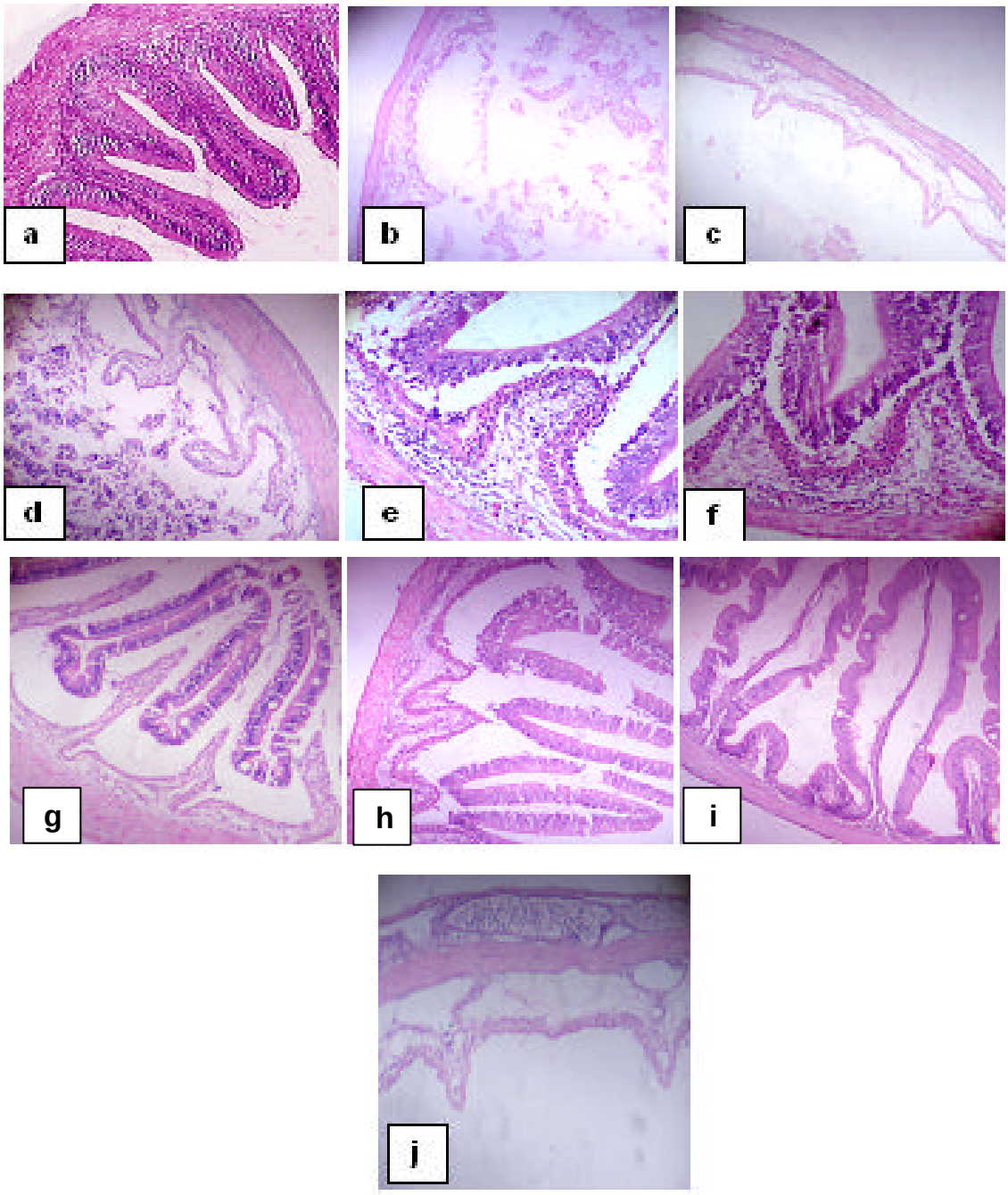

Fig. 6: Intestine of fish showing the normal (a)(X400), atrophy in the muscularis (b{*T.zillii*}, c{*S.vulgaeris*}{X400}), severe degenerative and necrotic changes in the intestinal mucosa and submucosa (d {*T.zillii*}{X400}), haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa (e {*T.zillii*}, f{*S.vulgaeris*}{X400}), edema between the intestinal submucosa and mucosa (g{*T.zillii*}, h{*S.vulgaeris*}{X400}) with atrophy in the submucosa (i{*S.vulgaeris*}{X400}), dilation in the blood vessels of serosa (j{*S.vulgaeris*}{X400})

formation observed in the blood vessels with subsequent stasis of blood may be also responsible for the cellular degeneration and necrosis in the liver [16]. The present results are in agreement with those observed by many authors who have studied the effects of different pollutants on fish liver [16-18]. Also, [19] observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead. Exposure of *Oncorhynchus mykiss* to copper sulphate was found to induce degeneration of hepatocytes, sinusoidal dilation and congestion in the blood vessels of the liver [20].

The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants [23,36]. The gills of both studied fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae, edema in gill filaments and secondary lamellae and congestion in blood vessels of gill filaments. These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface. The observed alterations like proliferation of the epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defense 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 [36,37]. 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 [38]. The observed edematous changes in gill filaments and secondary lamellae probably due to increased capillary permeability [22]. The present results are in agreement with those observed in other fish species under the influence of different pollutants [21,22]. In this respect, [23] observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes. Also, [24] noticed epithelial lifting, proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of *C. nasus* and *L. cephalus* from River Mures, Western Romania, polluted by heavy metals, faecal coliforms and streptococci bacteria.

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal wastes from blood but it is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoieses [39]. The kidney is one of the first organs to be affected by contaminants in the water [26]. The common alterations found in the kidney of both studied fish were severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis, haemorrhage between renal tubules and edema in Bowman's capsules with atrophy in the glomeruli. In the present study, kidney of the fish often showed vacuolar degeneration (cloudy swelling) in tubules cells, characterized by the hypertrophy of the cells. In more severe cases, the degenerative process leads to tissue necrosis. The necrosis of the renal tubules affects the metabolic activities and promotes metabolic abnormalities in fish [40]. The present results are in agreement with those observed in *C. carpio* exposed to sewage [21], *P. lineatus* exposed trichlorfon [25] and *L. calcarifer* exposed to cadmium [26]. Also, [41] observed necrosis of tubular epithelium, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman's capsules and contraction of the glomerulus in the kidney of *C. mrigala* exposed to fenvalerate. While, [23] found cloudy swelling degeneration in the epithelium of renal tubules in the kidney of *P. lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes.

The intestine of both studied fish showed severe degenerative and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them. According to Bhatnagar *et al.* [42], the observed irritation and destruction of the mucosa membrane of the intestine, hampering absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine [28,29]. Epithelial degeneration, inflammatory cells infiltration in the sumucosa as well as submucosal edema was seen in the intestine of tilapia fish exposed to carbofuran [30].

It could be concluded that the environmental contamination of Lake Qarun induced several histopathological alterations in the tissues of *T. zillii* and *S. vulgaris*. Consequently, it is recommended to coordinate different efforts to rescue Lake Qarun from the environmental pollution problems. The overcoming of these problems can be possible by subjecting the

drainage waters discharged into the lake to technical treatments that fulfill its safety.

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