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Comparison of Tissue Lesions in Two Species of Marine Fish (*Solea solea* and *Mugil cephalus*) Inhabiting Bardawil Lagoon.

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ABSTRACT

The present work reports the biochemical and histological changes in the gills, spleen and liver of *Solea solea* and *Mugil cephalus* habiting in Bardawil lagoon during three seasons of year 2012. Transferase enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] in the liver and muscles tissue of *Solea solea* and *Mugil cephalus* were measured, they are higher *in S. solea* than in *M. cephalus*. Histopathologically the gill, spleen and liver revealed varying degrees of alterations in these two fish species. The gills showed hyperplasia with fusion of adjacent lamellae, lamellar vasodilation and lamellar edema with epithelial detachment proliferation of mucus and chloride cells. The spleen showed enlargement of melanomacrophage centre (MMC), focal area of necrosis and spread of vacuoles filled with neutrophils. Dilation and congestion in hepatic sinusoids, vacuolar degeneration of the hepatocytes with necrotic focal areas and coagulative necrosis were observed in liver. Histopathology could therefore be considered an important effective tool in biomarkers of disease in fish.

Keywords: Bardawil lagoon, *Solea Solea*, *Mugil cephalus*, histopathology, ALT, AST.

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INTRODUCTION

Lake Bardawil is a large coastal lagoon on the Mediterranean Coast of Sinai, Egypt. It is one of the most important Lakes in Egypt as a source of good quality fish and wildlife. Lake Bardawil had been the subject of many studies during the last 20 years. Many of these, reports and papers had been published covering the geomorphology, morphometry, sediment logy, hydrology and water quality, macrophytes and phytoplankton, zooplankton and zoobenthos, fishes and fisheries, avifauna and others [1, 2]. The catch composition of the lagoon fish had greatly changed in the last decades. Contribution of the most economic species [seabream (*Sparus auratus*) and seabass (*Dicentrarx labrax*)] dropped from 56.5% in year1988 to 8% in year 2000, while other species with little economic value, such as crabs and shrimps have attained a noticeable percentage [3]. The decrease of some fish populations and partial loose of commercial fishing importance are among of the huge changes in Bardawil ecosystem. It is a well-known fact that biochemical, cellular, tissue, and organism modifications underlines different types of ecosystem changes; however the chronic biological effects of the Bardawil lagoon on fish organism are poorly studied. The fish, as a bio indicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment [4]. The exposure of fish to any stress induces a number of modifications in different organs, particularly gills, liver and spleen. Enzymes are necessary for normal cellular metabolism including that of the liver, and the degenerative changes due to the combined metal toxicity exhibited in the liver alter the level of a number of its enzymes. For example, lactate dehydrogenase (LDH) is released from the liver after its cellular damage and failure due to organophosphate insecticide intoxication [5]. LDH, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are released in acute and chronic liver disorders. These enzymes are biomarkers of acute hepatic damage, thus their bioassay can serve as a diagnostic tool for assessing necrosis of the liver cells [6, 7]. This study was made to investigate the incidence of biochemical and histological alterations in the gills, spleen, and liver of important fish *Solea solea* and *Mugil cephalus* collected from Bardawil lagoon.

MATERIALS AND METHODS

Study area

Lake Bardawil is located at southern Mediterranean coast (Fig. 1). It represents a transitional zone between land and sea and it separated from the Mediterranean along most of its length by a long narrow sand bar. It is connected to the sea via a three narrow artificial inlets, two of them man-made (western and eastern inlets), while the third one has been naturally closed [8].

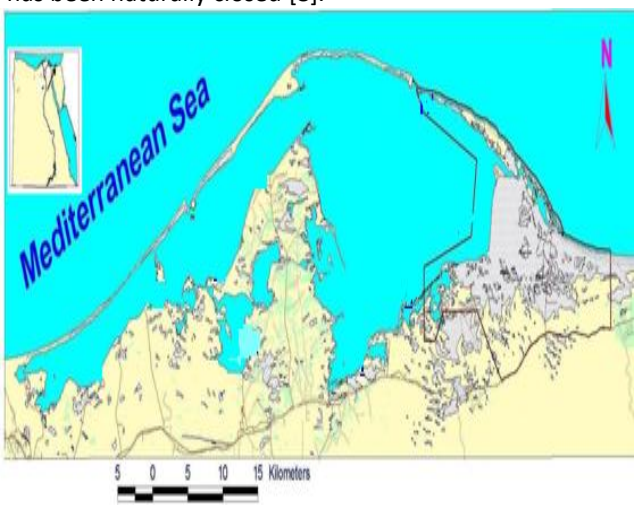

Figure 1: Map illustrating location of Bardawil lagoon, Egypt.

Sampling

Twenty samples of each of *Solea solea* (39-133g) and *Mugil cephalus* (462.8-847g) were collected bimonthly from March to November 2012 from Bardawil lagoon (Aghzawan area). After dissection of fish samples, parts of gills, spleen and liver were carefully removed and prepared for histological studies.

ALT and AST activity

Fish liver and muscles were collected during summer season, secluded and homogenized in 3 vol (v/w) of 10 mM Tris-HCl, 0.25M sucrose buffer (pH 7.4) at 40°C, using Teflon homogenizer. Homogenates were centrifuged at 1600 x g for 20minutes at 40°C. Supernatant was collected and used in the estimation of AST and ALT. Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) in liver and muscles were determined colorimetrically according to [9] using kits supplied from Biomerieux, France.

Histological studies

Specimens from gills, spleen and liver were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4 - 6 µm then stained with haematoxylin and eosin according to the method described by [10].

RESULTS

Enzymes activity

AST and ALT values (Table 1) were recorded during summer season. They showed high values in the liver and muscles of the two examined fish species. ALT value was 142.93 µg /L in liver and 61.53 µg /L in muscle of *S. solea*. *M. cephalus* ALT values were 62.00 µg /L in liver and 59.87 µg /L in muscle. *S. solea* had AST values (88.2 and 108.72 µg /L) for liver and muscle, respectively. *M. cephalus* AST value was (78.07 and 94.73 µg /L) for liver and muscle, respectively.

Table 1: Alanine and aspartate aminotransferase enzymes in the liver and muscle of studied fishes inhabiting Bardawil Lagoon.

Fish species	organ	ALT	AST
Solea solea	liver	142.93±4.5	88.23±6.6
	muscle	61.53±3.3	108.72±3.7
Mugil cephalus	liver	62.00±5.8	78.07±7.4
	muscle	59.87±4.2	94.73±10.4

Data are represented as mean ± standard deviation

Histological Investigations

Gills

Figure (2) shows the normal histological structure of the gills. Several histopathological changes were seen in the gills of *Solea solea* and *Mugil cephalus* from Bardawil lagoon.

During spring season, the gills of *S. solea* showed lamellar edema accompanied with epithelial lifting, neutrophil infiltrations (Fig. 3) and hypertrophied chloride cells (Fig. 4). The pathological lesions were more severe in gills of *M. cephalus* where hemolysis of gill filament (Fig. 9) and secondary lamellae with necrotized cells aggregation (Fig. 10) were noticed.

During summer season, the gill lamellae stunned with hyperplasia of the epithelial cells leading to complete fusion and proliferation of hypertrophied chloride cells for *S. solea* fish (Figs. 5 and 6). The pathological changes of gill of *M. cephalus* showed severe degeneration, pillar system destroyed and coagulative necrosis (Fig. 11). Moreover, vasodilation with severe congestion and neutrophil infilteration of gill filament were observed (Fig. 12).

During autumn season, the histopathological changes in the gills of *S. solea* included elongated lamellae, vacuolted epithelial cells (Fig. 7) and Telangiectasia of lamellae (Fig. 8). The gills of *M. cephalus* have severe necrotic change and sloughing of secondary lamellae (Fig. 13) as well as dilation and congestion of the blood vessels of lamellae form aneurysm (Fig. 14).

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Spleen

Figure (15) shows the normal histological structures of the spleen. Several histological changes were seen in the spleen of the studied fish from Bardawil Lagoon during the three seasons.

During spring season, the spleen of *S. solea* showed melanomacrophage centres (MMC) enlargement (Fig. 16) and necrotic area (Fig.17). Moreover, the spleen of *M. cephalus* showed dilated vein and connective tissues around veins (Figs. 20 and 21).

During summer season, the histological alterations in the spleen of *S. solea* included dilated vein with aggregations of inflammatory cells and surrounded by connective tissues (Figs. 18 and 19) beside enlargement of MMC. In *M. cephalus***,** the spleen showed severe focal areas of large vacuoles filled with neutrophils (Fig. 22). Furthermore, large fat vacuoles and fibrous tissue between them were observed (Fig. 23).
During autumn season, the histological changes in spleen of *S. solea* showed the same pathological lesions that appeared in fish during summer season. Alterations exhibited in the spleen of *M. cephalus* include thrombosis formation in central vein and large number of enlarged MMC were spread (Fig. 24) as well as dilated veins filled with aggregated neutrophils and granuloma formed in between them (Fig. 25)

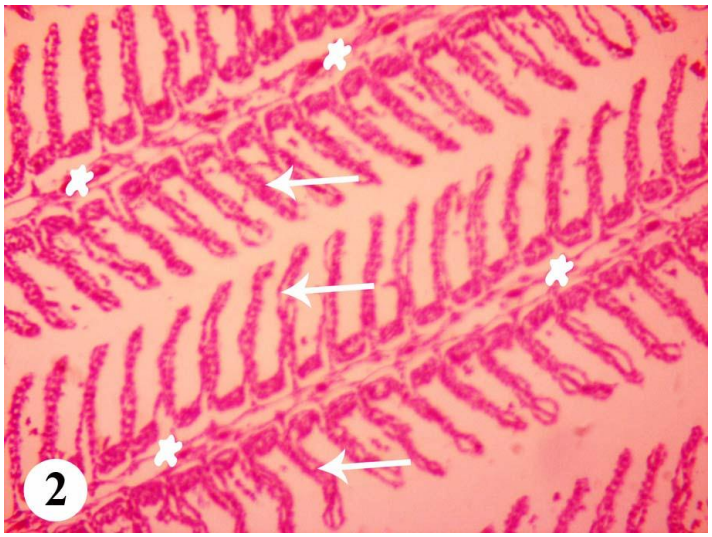
Liver

Figure (26) shows the normal histological structures of the liver of fish species. Several pathological lesions were seen in the liver of the studied fish from Bardawil lagoon.

During spring season, the liver of *S. solea* showed fatty degeneration with focal areas of necrosis (Fig. 27), haemorrhage and aggregations of inflammatory cells with heamosidrin in between the hepatocytes accompanied with large fat vacuoles (Fig. 28). In the liver of *M. cephalus*, coagulative necrosis and widened sinusoids with inflammatory cells infilteration (Fig. 31) as well as focal areas of necrosis were observed (Fig. 32).

During summer season, the histological alterations in the liver of *S. solea* included dilated veins and sinusoids (Fig.29), haemorrhage between the hepatocytes (Fig. 30). The most pronounced lesions in the liver of *M. cephalus* were coagulative necrosis, edema and pycnotic nuclei (Fig. 33). Dilation of central vein with haemorrhage was also noticed (Fig. 34).

During autumn season, the liver of both fish showed the same histological changes observed in the liver of the fish during summer season.



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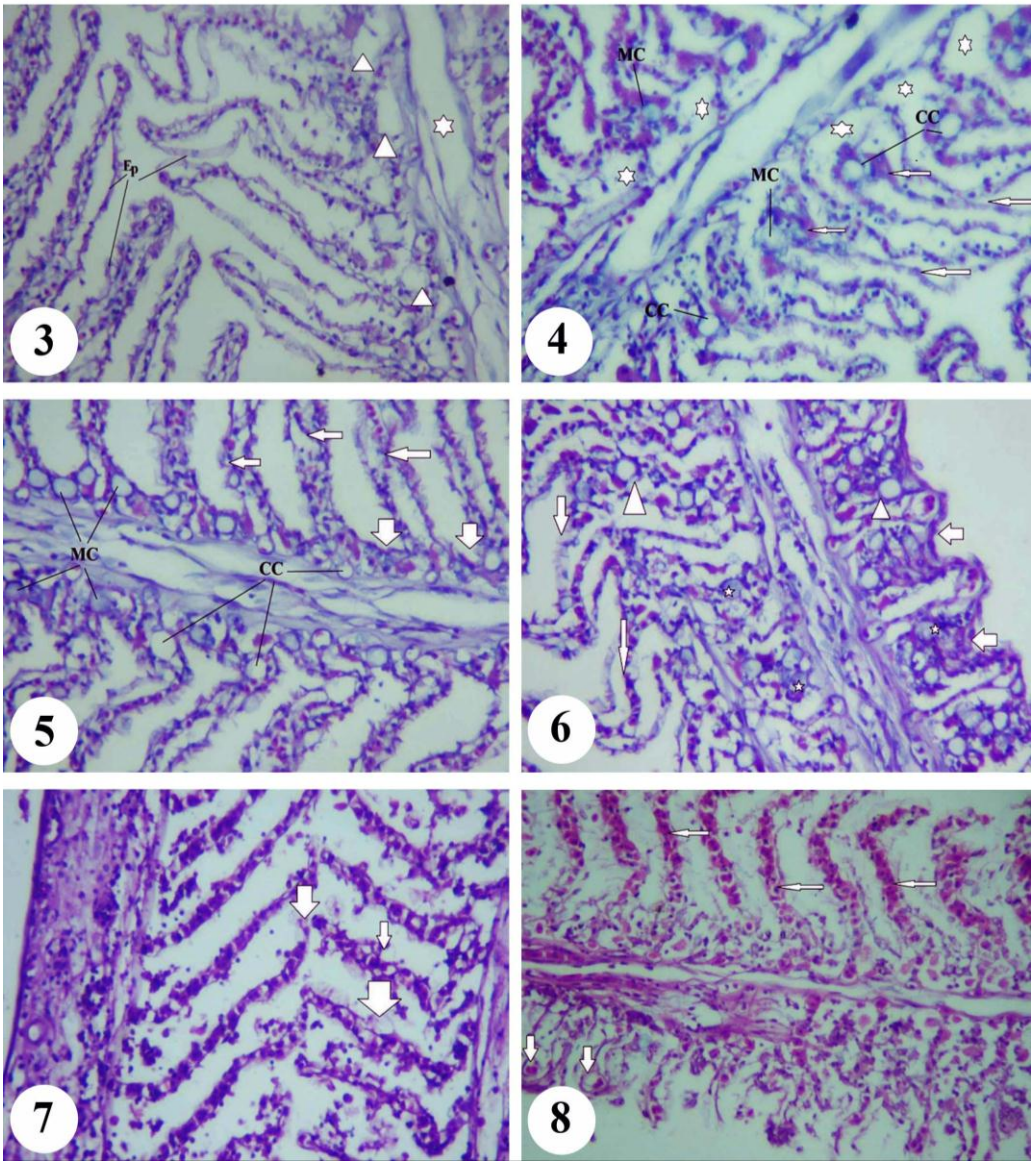

Figure 2: Gill of fish showing the normal histological structure of filaments (star) and lamellae (arrows). H&E (X100).

Figure 3: Gill of *S. solea* showing epithelial lifting (Ep), neutrophil, infilteraion and edema (head arrow). H&E (X100)(Spring season).

Figure 4: Gill of *S. solea* showing edema (star) and epithelial lifting (arrows), mucus (MC) and chloride cells (CC) hypertrophy. H&E (X400)(Spring season).

Figure 5: Gill of *S. solea* showing epithelial lifting and proliferated hypertrophied, mucus cells (MC) and chloride cells (CC), pillar system destroyed (long arrows). H&E (X400)(Summer season).

Figure 6: Gill of *S. solea* showing epithelial lifting (long arrows) and stunned lamellae with complete fusion (short arrows), proliferated chloride and mucus cells (head arrows). H&E (X400)(Summer season).

Figure 7: Gill of *S. solea* showing elongated lamellae (arrow) and Vacuolated epithelial cells (short arrows). H&E (X400)(Autumn season).

Figure 8: Section in the gill of *S. solea* showing telangiectasia of lamellae (long arrows) stunned lamellar with vacuolated their tips (short arrows). H&E (X400) (Autumn season).

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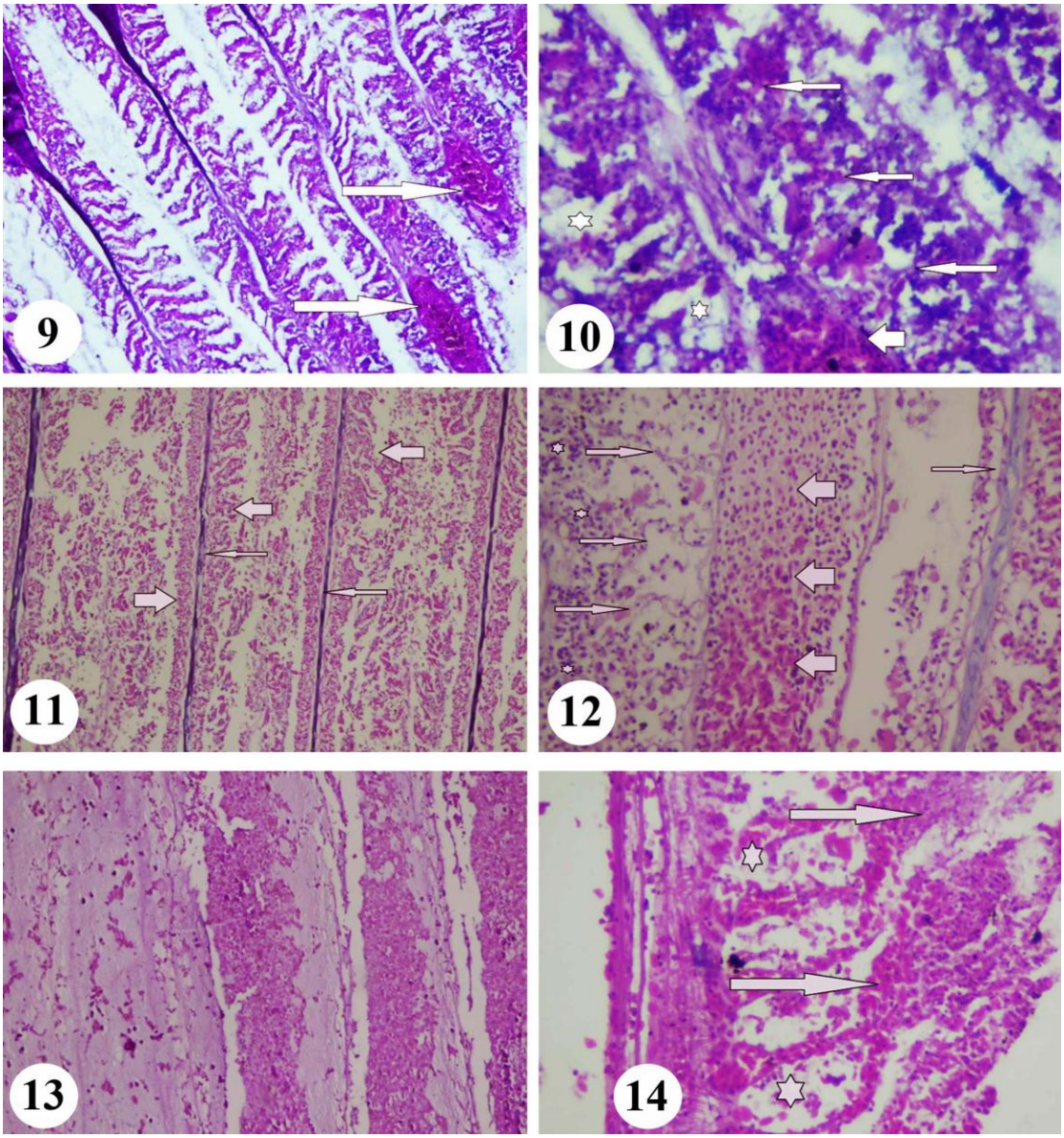

Figure 9: Gill of M. cephelus showing heamolysis of blood vessel of filament (arrows). H&E (X100)(Spring season).

Figure 10: Gill of M. cephelus showing coagulative necrosis (long arrows) and edema (star) and congestion of blood vessels (short arrow). H&E (X400)(Spring season).

Figure 11: Gill of M. cephelus showing destroyed pillar system, necrotic filament (long arrows) and coagulative necrosis (short arrows). H&E (X400)(Summer season).

Figure 12: Gill of M. cephelus showing heamolysis of filament (short arrows), neutrophil infiltration (star) and pillar system destroyed (long arrows). H&E (X400)(Summer season).

Figure 13: Gill of M. cephelus showing necrotic change of pillar system and lamellae. H&E (X400)(Autumn season).

Figure 14: Gill of M. cephelus showing vasodilation of the secondary lamellae (long arrows) and edema (star) and sloughing. H&E (X400)(Autumn season).

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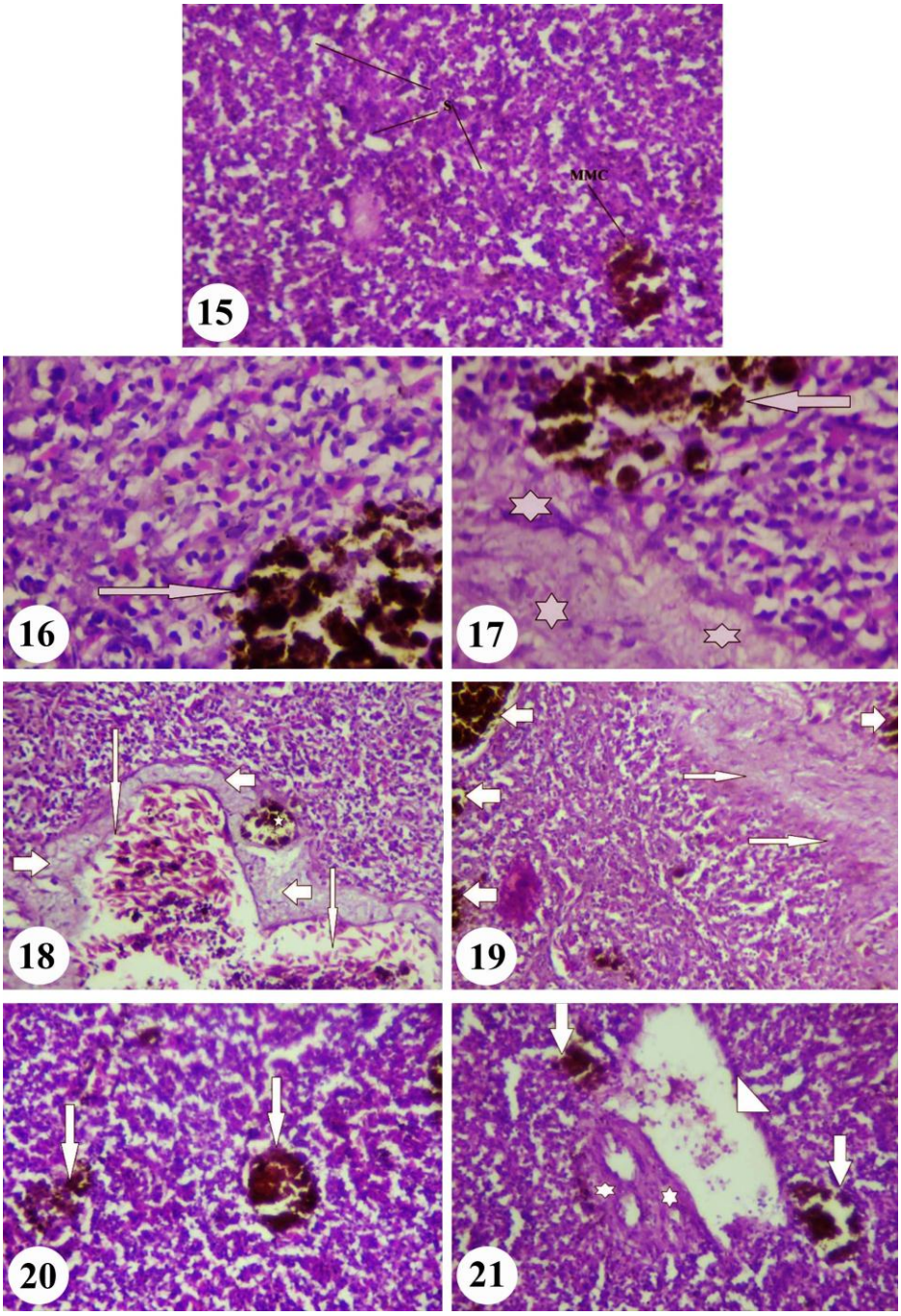

Figure 15: Spleen of fish showing the normal histological structure, melanomacrophage centres (MMC). H&E (X100).
Figure 16: Spleen of *S. Solea* showing MMC (arrow). H&E (X400)(Spring season).

Figure 17: Spleen of *S. Solea* showing showing MMC (arrow) and necrotic area (stars). H&E (X400)(Spring season).

Figure 18: Spleen of *S. Solea* showing dilated vein with inflammation (long arrow) and necrotic area around it (short arrow). H&E (X400)(Summer season).

Figure 19: Spleen of *S. Solea* showing spread of MMC (short arrow) and area of necrosis (long arrow). H&E (X400)(Summer season).

Figure 20: Spleen of *M. cephalus* showing spread of MMC (arrows). H&E (X400) (Spring season).
Figure 21: Spleen of *M.cephalus* showing MMC (arrows) and area of necrosis (head arrow) and connective tissues (star). H&E (X400)(Spring season).

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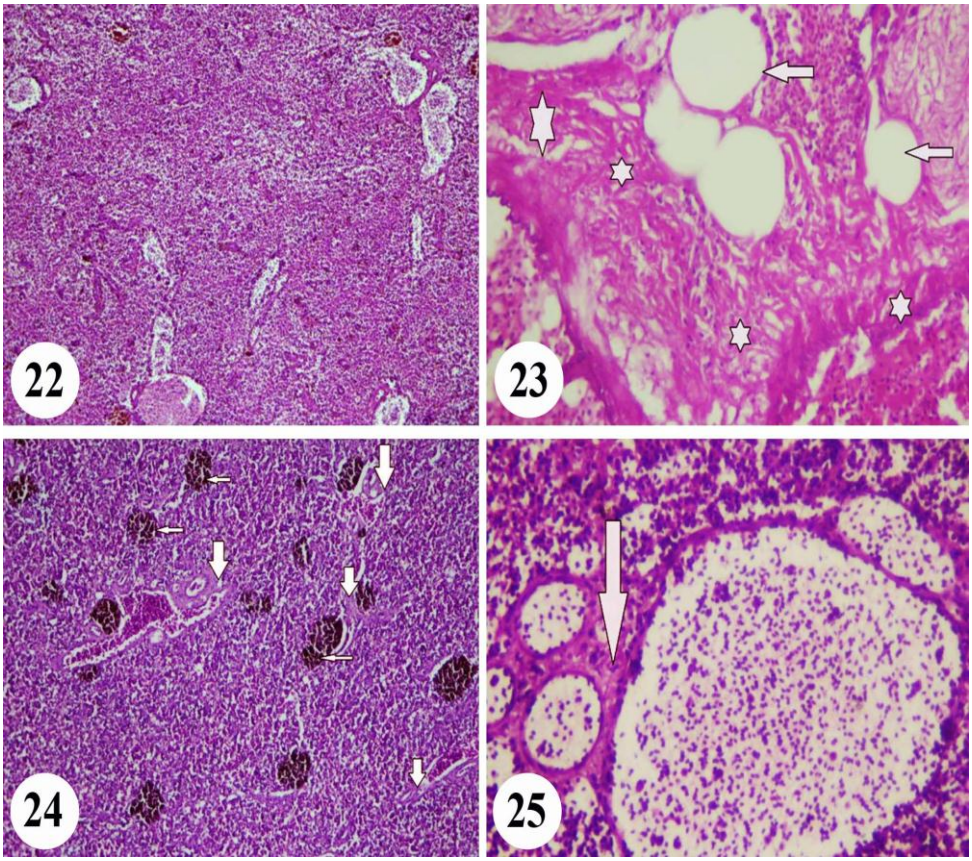

Figure 22: Spleen of *M. cephalus* showing dilated veins with neutrophil infilteration. H&E (X400)(Summer season).

Figure 23: Spleen of *M. cephalus* showing large fat vacuoles (arrows) and fibrosis (star). H&E (X400)(Summer season).

Figure 24: Spleen of *M. cephalus* showing focal areas of dilated vessel with congestion spread of MMC and granuloma (arrows). H&E (X100)(Autumn Season).

Figure 25: Spleen of *M. cephalus* showing dilated veins filled with neutrophil surrounded by connective tissues (arrow). H&E (X400)(Autumn Season).

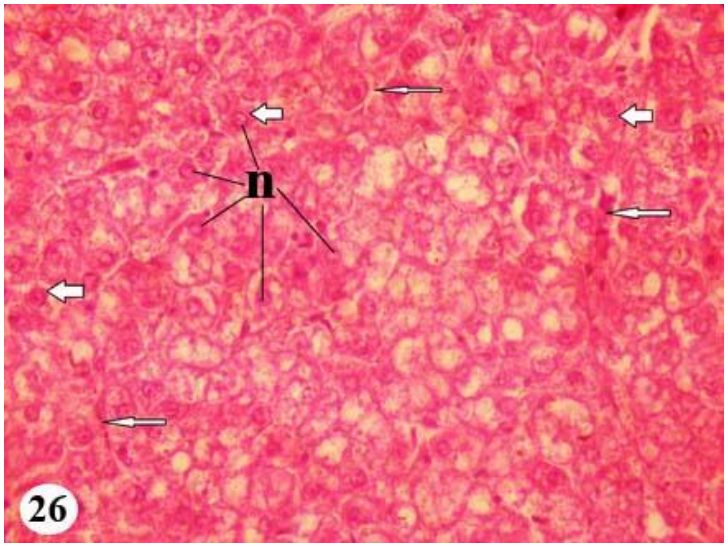

Figure 26: Liver of fish showing normal histological structure (hepatocytes filled with cytoplasm) and nucleus (n). H&E (X).

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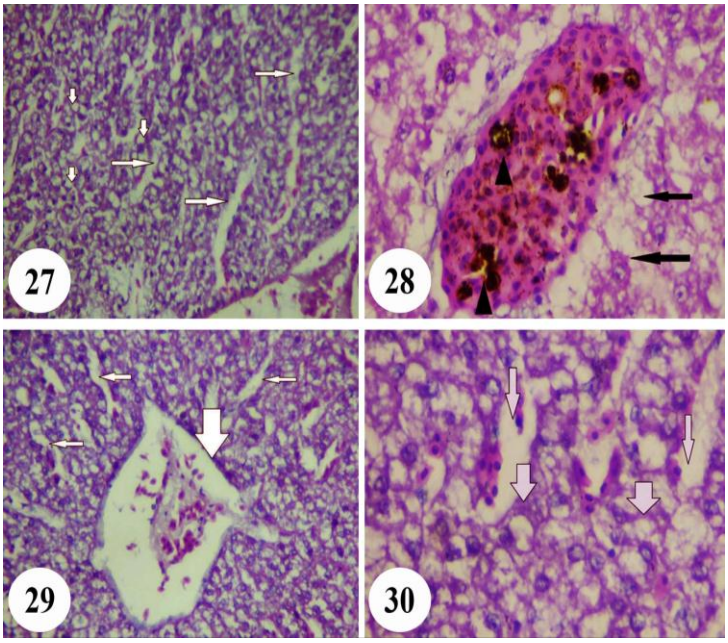

Figure 27: Liver of *S. Solea* showing widened sinusoids (long arrows) and fatty degeneration (short arrows). H&E (X400)(Spring season).

Figure 28: Liver of *S. Solea* showing heamosidrin (head arrow) and fat vacuoles (long arrows). H&E (X400)(Spring season).

Figure 29: Liver of *S. Solea* showing widened sinusiod (small arrows) dilated hepatoportal, blood vessel (large arrow). H&E (X100)(Summer season).

Figure 30: Liver of *S. Solea* showing focal areas of necrosis (short arrows) congestion between hepatocytes and widened sinusoids (long arrows). H&E (X400)(Summer season).

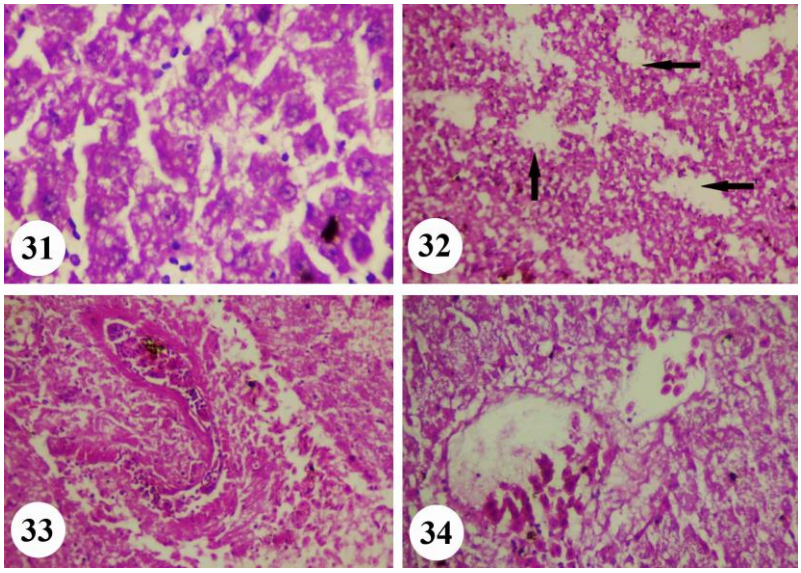

Figure 31: Liver of *M. cephalus* showing coagulative necrosis. H&E (X400)(Spring season).

Figure 32: Liver of *M. cephalus* showing focal areas of necrosis (arrows). H&E (X400)(Spring season).

Figure 33: Liver of *M. cephalus* showing coagulative necrosis, and edema. H&E (X400)(Summer season).

Figure 34: Liver of *M. cephalus* showing dilation of central vein with haemorrhage and nuclei disappear. H&E (X400)(Summer season).

DISCUSSION

The damaged cells release their contents (including aminotransferase) towards the blood stream and the level of these enzymes enhances in serum [11].

Transaminases play important roles in carbohydrate and amino acid metabolism in the tissues of fish and other organisms [12]. Alanine aminotransferase is a key metabolic enzyme released on the damage of hepatocytes. Liver injury was detected by a significant increase in liver and muscle tissues of studied fish, AST and ALT activities. This agreed with the study of [13] who found that changes in the antioxidant abilities of the liver and changes in the phospholipid structure of the cell membrane were accompanied by rising activities of ALT and AST as markers of liver damage. The increase of both aminotransferases activity was shown in common carp impacted by heavy metals (cadmium, lead, nickel and chromium) [14] and exposed to herbicide pendimethalin [15].

The present study revealed a variety of histological changes in the gills, spleen and liver of the two fish species (*S. Solea* and *M. cephalus*) from Bardawil lagoon. Previous studies reported that Bardawil lagoon is polluted with high concentration of the biological metabolites, such as *p*,*p*'-DDD from the parent OCPs in sediments depicted that the organochlorine pesticides contamination were mainly from the caged and weathered agricultural soils [16-18]. High concentration of heavy metals (especially Fe, Pb, Zn and Cu) were recorded by [19] in muscle of fish (*Sparus auratus* and *Dicentrarchus Labrax*) inhabiting in the Bardawil lake, also aminotransferase enzymes showed high values in the muscles of the two examined fish species [20] concluded that high concentrations of heavy metals in lake sediments contaminated fish, especially bottom feeders, it may be the case in the Northern lakes, since all of them receive the sewage discharges of major cities. Unfortunately, with the dwindling of the natural nutrient-rich Nile water discharge into the Mediterranean. The sewage discharges from coastal lakes and other land run-off have become the alternative source of nutrients for coastal fisheries [21].

In this study, the gill showed epithelial hyperplasia, lamellar fusion, epithelial lifting, necrosis, desquamation and congestion of the blood vessels of gill filaments as well as lamellar aneurysms were observed with excessive mucous secretion. Similar kinds of affects were noticed earlier in fish exposed to toxicants including heavy metals [22-29].

Other investigators have reported histopathological changes in the gills of different fish species exposed to pesticides, petroleum hydrocarbon, PCBs, PAHs, and heavy metals [4, 30-35]. These included several alterations similar to those of Nile tilapia exposed to contaminated sediment, such as hyperplasia and hypertrophy of chloride cells and mucous cells, edema of epithelial cells, clubbing of gill filament, and aneurysm. Lamellar fusion and clubbing of lamellae may have been protective in that they could diminish the vulnerable gill surface area [36]. These lesions also could be serving as defense responses because it increases the distance across which waterborne pollutants must diffuse to reach the bloodstream [37], [30] reported that histopathological changes of the gills likely resulted in hypoxia, respiratory failure problems with ionic and acid-base balance. The observed lamellar vasodilation can induce changes in pillar cell normal structure, with consequently loss of their support function and probably, was responsible for the emergence of lamellar aneurysms in fish. The induction of tissue lesions indicates bioavailability of contaminants released from the sediments. If exposure to contaminated sediment in the field extends to a longer term, physiological impairment of the individual would most likely increase with increasing prevalence and severity of pathologic changes [38].

In the present study, the spleen of the studied fish showed atrophy and hypertrophied MMC, focal areas of necrosis, dilated vein with congestion, large fat vacuoles, fibrosis, edema and early glanuloma. The utility of MAs as a histopathological bioindicator or biomarker has been criticized by some researchers as being too nonspecific; others consider that too many variables are involved in alterations of MA parameters to be of value. Even though several studies have indicated a limited usefulness of MAs as indicators of pollution, e.g. [39], several investigators have noted increases in various MA parameters in fish collected at contaminated versus those collected at reference sites or in laboratory exposures to individual contaminant. Alterations in the spleen may be useful as markers that indicate prior exposure to environmental stressors. Melanomacrophage centers (MMC) are physiological features in fish spleen and kidney [40]. They are believed to be functional equivalents of the germinal centres of spleen and lymph nodes in mammals [41]. MMC may

contain four types of brown pigments: melanin, lipofuscin, ceroid and hemosiderin [42]. [43] first suggested MMC as potential monitors of fish health. [44] found that stressful situations related to aquaculture practices have resulted in increased numbers of splenic and kidney MMC. Clinical studies have shown an association of MMC with highly resistant intracellular bacteria such as mycobacteria and renibacteria and parasites such as *Myxobolus* spp. [45]. [46] found hyperactivation of MMC in spleen of the monosex Nile tilapia *Oreochromis niloticus* that exposed to sub-acute concentration (1.46 g L^{-1}**)** of a pyrethroid insecticide, deltamethrin for 28 consecutive days. Similar alterations in the mosquito fish, *Gambusia affinis*, exposed to two sublethal concentrations of deltamethrin (0.25-0.50 µg L^{-1}) for periods of 10, 20 and 30 days, have been observed by [47]. Excessive tissue destruction or hemolysis that could result from exposure to contaminants would therefore lead to an increase in MA formation in the spleen. Correlations between the number of MAs per square millimeter and stressors were also observed in spleen [48]. Therefore, the pathological alterations in the spleen of the studied fish may be due to mycobacteriosis [49].

The liver is the vital organ of detoxification. The most common lesions in the liver of the studied fish were vacuolar degeneration of the hepatocytes, focal areas of necrosis, nuclear pycnosis, haemorrhage as well as aggregations of inflammatory cells between the hepatocytes, thrombosis formation in the hepatoportal blood vessels and coagulative necrosis. The alterations in liver due to toxicity impact are often associated with a degenerative necrotic condition [24, 28, 29].

The changes induced in hepatocytes such as vacuolization, necrosis and nuclear condensation were also reported for copper exposure [29]. The high accumulation potential of heavy metals was thought to be due to their close association with the transformation pathway. Accordingly, heavy metals enter the blood circulation through gill epithelia and finally accumulate in the liver [50]. Hepatic necrosis and inflammation, indicative of infection or toxic injury by contaminants, was prevalent in greenback flounders (*Rhombosolea tapirina*) exposed to contaminated marine Sediments [51].

The liver has the ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by elevated concentrations of these compounds which could subsequently result in structural damage [52]. The cellular degeneration in the liver might be due to oxygen deficiency as a result of gill degeneration [53]. [54] observed inflammation, central necrosis and cell degeneration in liver tissue of *Oreochromis aureus* juveniles while subjecting them to phenol. [29] reported that the hepatic parenchyma of fish exposed to copper showed cytopalsmic vacuolation and hepatocellular necrosis. Similar alterations were observed in the liver of *Tilapia mossambica* due to the toxicity of copper sulphate, lead nitrate and zinc sulphate [55], Nile tilapia *Oreochromis niloticus* exposed to alachlor [38]. Hepatocytic necrosis changes were observed in fish exposed to crude oil over one day periods [56]. Lipidiosis (the excessive accumulation of fat in the cytoplasm) is characteristic of many exposed livers, the large vacuole in the cell forces the nuclei to the periphery of the hepatocytic and this condition is usually accompanied by nuclear atrophy [51, 57].

The results indicated that the pathological lesions were severe during the three seasons and this may be revealed to polluted water of Bardawil lagoon.

CONCLUSION

This study concluded that fish of Bardawil Lagoon may be polluted with some heavy metals due to their concentrated levels in the sediment. Alterations in fish liver and muscle enzymes ALT and AST as well as histopathological alterations in different fish organs may be a result of the target tissue damage and dysfunction induced by the heavy metals and these parameters could be used as rapid and sensitive indicators for monitoring the impact of heavy metals on aquatic organisms.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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