Histopathology and oxidative stress in the liver of *Chalcalburnus tarichi* living in lake Van, Turkey

Burak Kaptaner[1], Ertuğrul Kankaya[2], Abdulahad Doğan[1], İsmail Çelik[1]

[1.] Department of Biology, Science Faculty, Yüzüncü Yıl University, 65080 Van, Turkey
[2.] Fisheries Faculty, Yüzüncü Yıl University, 65080 Van, Turkey
bkaptaner@yahoo.com

Abstract: To assess the environmental quality in the Lake Van, *Chalcalburnus tarichi*, the only vertebrate and an endemic carp species of this lake basin, samples were collected from the lake, and their livers were examined histologically for this purpose. Histological alterations were recorded and evaluated semi-quantitatively based on the liver lesion categories and their severities. According to the results, five of a total of forty samples displayed normal (healthy) liver architecture, but the others displayed various histopathological alterations, including hepatocyte vacuolization, fatty degeneration, increase in melanomacrophage centers, necrosis, infiltration, congestion, fibrosis, single cell necrosis, bile duct alterations, glycogen depletion and nuclear pleomorphism. Liver index values were calculated and found to be changed between 2 and 50 (≤4 for fish with normal livers (FwNL) and ≥8-50 for fish with histopathological livers (FwHL). We also investigated oxidative stress biomarkers, lipid peroxidation (LPO) and antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) in FwHL as compared with FwNL. LPO and CAT were found to be significantly higher in FwHL, indicating increased oxidative stress. The results revealed that the observed histological and biochemical alterations in the livers of *C. tarichi* reflect the effects of environmental stressors present in the lake water causing pollution.
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1. Introduction

Aquatic organisms, including fish, are frequently exposed to a wide variety of environmental contaminants, such as industrial, agricultural and urban discharges leading to deleterious effects, especially when these contaminants are slightly decomposable, exhibit a high biological effectiveness and possess a high potential for accumulation or synergistic effects (Bernet et al., 1999; Au, 2004). In fish, pollutants can cause many health problems such as structural alterations and diseases at the population level (Schmalz et al., 2002; Lukin et al., 2011). Fish are often used as sentinel organisms to assess the biological impacts of contaminants and environmental quality because of their responses to low concentrations of toxic substances (Ayas et al., 2007). Histopathological alterations have been widely used as biomonitoring tools or biomarkers of health status of fish exposed to chemical compounds both in laboratory experiments (Thophon et al., 2003; Boran et al., 2012) and field studies (Stendiford et al., 2003). Fish liver plays an important role in the uptake, biotransformation and detoxification of pollutants (Gernhöfer et al., 2001), and studies on endemic fish species have demonstrated that fish liver histopathology is a useful reference biomarker of contaminant exposure (Stendiford et al., 2003; van Dyk et al., 2012; Oliva et al., 2013).

Environmental pollutants are present in the aquatic environment have the potential to cause toxic effects in aquatic organisms through free radicals and reactive oxygen species (ROS). ROS are derived from oxygen and capable of attacking biological macromolecules such as membrane lipids, proteins and DNA. There exists a balance between production and destruction of ROS in cells provided by their antioxidant defense systems, and toxicity of ROS is eliminated by the nonenzymatic and enzymatic components of this system. As a result of exposure to environmental stressors, ROS can overcome antioxidant defense and excessive production of ROS, damaging cell components and tissues, which is called oxidative stress and leads to adverse health effects and diseases. Therefore, assays of antioxidant defense and oxidative damage parameters are used as biomarkers of oxidative stress for evaluation of environmental stressors. In addition to the antioxidant defense system parameters, one of the most frequently used hallmarks of oxidative stress is damage to membrane phospholipids, which leads to formation of the secondary lipid peroxidation product malondialdehyde (MDA) (Valavanidis et al., 2006; Ben Ameur et al., 2012).

Chalcalburnus tarichi is an endemic fish species (Cyprinidae) inhabiting the Lake Van basin. Lake Van, located in Eastern Turkey, is the largest lake of the country, with a total surface area of 3574

km^2. This lake is known as the biggest soda lake of the world, and it has highly alkaline water with pH of 9.8 (Sari, 2008). *C. tarichi*, displaying anadromous character, is the only vertebrate species living in this lake, and it migrates to the rivers pouring into the lake for spawning at the reproduction period (April-June) and returns to the lake at postspawning (Danulat and Selçuk, 1992). Apart from the interesting biological properties, it is also economically important species because of amount of fishing (approximately 11.000 tons/year) (TurkStat, 2009). There are many large and small settlements around the lake discharging their waste waters to the lake, including sewage treatment plants, agricultural facilities and industrial activities, and there have been a few studies that have reported on the pollution in Lake Van. In a previous study, Bilgili et al., (1995) analyzed water quality criteria in the water of Lake Van and heavy metals in the muscle tissue of *C. tarichi*. They determined the water quality parameters were below the quality limits and reported high and hazardous levels for human health of Pb in the muscle tissue of *C. tarichi* as a result of terrestrial contamination. In a recent study, selected potential endocrine-disrupting chemicals were identified in the sediment and water samples of Lake Van and sewage treatment plant effluent (Oğuz and Kankaya, 2013). Ünal et al., (2007) already found decreased gonadosomatic index values, histopathological abnormalities and alterations in the testicular and ovarian tissues, inhibition of liver acetylcholinesterase activity and reduced plasma 17β-estradiol levels in *C. tarichi* that were causally related to endocrine-disrupting chemicals.

Histological and biochemical studies provide direct evidence of the adverse effects of foreign compounds in fish, and various histopathological alterations have been reported in the livers of fish species living in the aquatic environments receiving a wide range of pollutants such as heavy metals, polyaromatic hydrocarbons (PAHs) and endocrine-disrupting chemicals (Abdel-Moneim et al., 2012; Oliva et al., 2013; Kelly and Janz 2009; Gül et al., 2004; Marchand et al., 2009; Nero et al., 2006). The main objectives of this study are (1) to determine whether histological liver alterations occur in *C. tarichi*, (2) to characterize histopathological alterations, (3) to assess oxidative stress responses in fish with histopathological livers (FwHL) compared with fish with normal livers (FwNL) and (4) to provide baseline data and reference research for further studies related to environmental pollution in *C. tarichi*. This is a preliminary study evaluating the liver of the endemic fish, *C. tarichi*, to advance our understanding of pollution in Lake Van using quantitative histopathological and biochemical data.

2. Materials and Methods

2.1. Fish

C. tarichi samples (n=40) used in this study were caught from Lake Van (Figure 1) using gill nets, in March 2013. Live fish were transported to the laboratory with aerated containers. Each fish was anesthetized with MS222, and then fork length (19.78 ± 1.25 cm, mean ± SD) and total weight (72.07 ± 8.60 g, mean ± SD) were recorded before dissection. Then, a part of the liver tissue from each fish was dissected out and fixed for histological examinations. The other part of liver tissue of fish were stored at −80 ºC until biochemical analyses. The ages of the fish used in this study were determined using opercular bones according to the method described by Lagler et al., (1977) to be between 4+ years and 6+ years.

Figure 1. Geographic location of Lake Van and settlements around it. The sampling area is indicated with a red circle (STP: municipal sewage treatment plant of Van city).

2.2. Histological procedures

Liver samples were fixed immediately in 10 % neutral buffered formalin. After 24 h, the samples were washed in phosphate buffered saline (pH: 7.4), dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin wax. The 4-µm-thick sections taken from the paraffinized liver tissues were placed onto polylysine-coated slides (Menzel-Gläser, Germany). Sections were deparaffinized in xylene, rehydrated using a graded ethanol series and stained with hematoxylin-eosin (HandE), Mallory's trichrome (M-T) and periodic acid Schiff (PAS) for visualization of glycogen. After the staining procedures, the slides were dehydrated with a graded ethanol series, cleared in xylene and sealed under cover slips using Entellan. All preparations were examined under a Leica DMI 6000 B model microscope (Germany), and their photos were taken.

2.3. Qualitative and quantitative histological assessment

Histological preparations from liver tissue were examined under a light microscope (Leica DMI 6000 B) for the identification of histopathological alterations. Histological alterations were semi-quantitatively assessed using a modified protocol described by Bernet et al., (1999). This protocol can be applied any organ and allows a standardized quantification. Using lesions in the any organ, an index can be generated, and this index represents the degree of damage to an organ. A high index indicates a high degree of damage. Calculating the organ index allows a comparison between the degree of damage of the same organ in different individuals a population. Briefly, liver tissue was assessed by identifying histological alterations according to the six reaction patterns: (1) circulatory disturbances (CD), (2) regressive changes (RC), (3) progressive changes (PC), (4) inflammatory responses (IR), (5) foci of regressive and progressive changes (FRPC) and (6) neoplasia (N). If an alteration was identified, it was given an importance factor that represents the potential of the alteration to affect fish health: (1) minimal, alteration is reversible; (2) moderate, alteration is reversible if the stressor neutralized; and (3) marked, alteration is irreversible. A score value for every alteration was also assigned that represents the occurrence of alteration through the liver: 0 (absent), 2 (mild), 4 (moderate) and 6 (severe). The score value and the importance factor for each alteration were multiplied, and the results for all alterations were summed to calculate a liver index. The liver index values were used to identify biochemical differences between fish having normal liver architecture and fish having pathological alterations.

2.4. Biochemical analyses

The livers of fish with histopathological livers (FwHL) (n = 5) were used in the biochemical analyses for comparing oxidative stress indices with fish with normal liver (FwNL) architecture. The tissues were homogenized for 5 min in 50 mM ice-cold KH_2PO_4 solution (1:10 w/v) using a glass-porcelain ultrasonic homogenizer (Jencons Scientific Co.) for 5 min and then centrifuged at 10000 *g* for 30 min. All processes were carried out at 4 ºC. Supernatants were used to determine MDA content, superoxide dismutase (SOD) and catalase (CAT) activities.

Lipid peroxidation was determined by measuring MDA concentration, a product of lipid peroxidation, in the liver samples. MDA concentration was measured using the method described by Jain et al., (1989) based on thiobarbituric acid (TBA) reactivity.

CAT (EC 1.11.1.6) activity was determined by measuring the decrease in hydrogen peroxide (10 mM solution) concentration at 240 nm (Aebi, 1984).

SOD (EC 1.15.1.1) activity was measured at 505 nm and 37 ºC and calculated using the inhibition percentage of formazan dye formation (McCord and Fridovich, 1969).

2.5. Statistics

All data were analyzed using the software package SPSS version 16.0. Comparisons were performed with Student's *t* unpaired tests. Statistical significance was inferred at $P < 0.05$ and $P < 0.01$.

3. Results

3.1 Qualitative and quantitative histological assessment

Histological assessment of the liver slides from *C. tarichi* revealed that five of 40 fish had normal tissue structure (Figure 2A and Figure 4E), but the remaining individuals exhibited various histopathological alterations at varying frequency and prevalence (Table 1). Among these histopathological alterations, there was an increase in melanomacrophage centers (MMC) (Figure 2B); vacuolization of hepatocytes (Figure 2C); fatty degeneration (Figure 2D); perivascular necrosis (Figure 2E); diffuse necrosis (Figure 2F), focal necrosis (Figure 3A); single-cell necrosis (Figure 3B); infiltration in the parenchyma (Figure 3C) and periportal area (Figure 3D); congestion (Figure 3E); thickening of the tunica adventitia of blood vessels (Figure 3F); fibrosis (Figure 4A); bile duct-related alterations such as infiltration and congestion at the peribiliary area (Figure 4B), degeneration of bile duct epithelia (Figure 4C) and increase in the connective tissue of bile duct (Figure 4D); glycogen depletion (Figure 4E); and nuclear pleomorphism (Figure 5).

Liver index values were observed to be changed between 2 to 50 in all individuals. Index values were 4≤ in the fish (n = 5) demonstrating normal liver architecture and were ≥8-50 in the fish having displaying various pathological alterations. The distribution of liver index values belonging to all samples is shown in (Figure 6).

3.2. Biochemical analyses

The levels of oxidative stress biomarkers, including lipid peroxidation (MDA concentration), CAT and SOD activities in liver tissue, are summarized in Table 2. MDA concentrations were also significantly greater ($P < 0.01$) in fish with histopathological livers (FwHL) compared with fish with normal liver structure (FwNL). CAT activity was found to be significantly higher ($P < 0.05$) in FwHL compared with FwNL. Significant differences were not observed in the levels of SOD activity.

Table 1. Frequencies (F) and percentage prevalence (PP) of histopathological alterations determined in the liver samples of *C. tarichi*. The importance factor for each alteration was indicated in the parenthesis (*).

Reaction pattern	Fuctional unit of the tissue	Alteration (*)	F (n=40)	PP (%)
Circulatory disturbances	Liver	Congestion (1)	8	20
	Bile duct	Congestion at peribilliary area (1)	3	7.5
Regressive changes	Liver	Architectural and structural alterations (1)	2	5
		Increased in MMC (1)	33	82.5
		Vacuolization (1)	28	70
		Glycogen depletion (1)	15	37.5
		Nuclear pleomorphism (2)	2	5
		Single cell necrosis (2)	9	22.5
		Fatty degeneration (3)	5	12.5
	Bile duct	Architectural and structural alterations (1)	6	15
		Degeneration (necrosis) (3)	1	2.5
Progressive changes	Liver	Fibrosis (3)	9	22.5
Inflammatory response	Liver	Infiltration (2)	10	25
	Bile duct	Infiltration at peribilliary area (2)	5	12.5
Foci of regressive and progressive changes (FRPC)	Liver	Focal necrosis (3)	5	12.5
		Perivascular necrosis (2)	5	12.5
		Diffuse necrosis (2)	2	5
Neoplasia		Benign	0	0
		Malign	0	0

4. Discussion

Histopathological changes and oxidative stress indices in fish organs have been increasingly studied as biomarkers for assessing aquatic contamination in environmental monitoring studies (Kelly and Janz, 2009; Ben Ameur et al., 2012; Fricke et al., 2012). The fish liver plays a fundamental role in the biotransformation and detoxification of foreign compounds in the body and is thus a target organ of xenobiotics. In addition, it is a useful tool for describing and documenting the hazardous impacts of specific and nonspecific environmental stressors (Stendiford et al., 2003).

C. tarichi is an endemic fish species of Lake Van basin, and some contaminants, such as heavy metals (Bilgili et al., 1995) and endocrine-disrupting chemicals (Oğuz and Kankaya 2013), have been reported in its environment as a result of anthropogenic activity. Recently, gonadal abnormalities and altered sex hormone level most likely related to endocrine disruption were described by Ünal et al., (2007) in *C. tarichi* and in the another study, pesticides' (aldrin, dieldrin, 4-4'-dichlorodiphenyldichloroethane and 4-4'-dichlorodiphenyldichloroethylene) residues in the ovarian tissue of *C. tarichi* and organic compounds (benzaldehyde, bis(2-ethylhexyl) phthalate, diethyl phthalate, naphthalene and phenol) in the sediment samples of Lake Van were detected which suggests pollution in the lake related to household wastes and other reasons (Unal et al., 2014).

Table 2. Oxidative stress indices in the liver tissue of *C. tarichi* samples

Oxidativen Stress Indices		FwNL[a] (mean ± SE)		FwHL[b] (mean ± SE)
MDA (nmol/g)		54.89 ± 5.35		194.78 ± 23.83*
CAT (U/g)		1412.40 ± 253.66		2182.10 ± 206.33**
SOD (U/g)		4274.60 ± 17.82		4247.10 ± 55.35

* $P < 0.01$, ** $P < 0.05$ in Student's *t* unpaired tests compared to corresponding FwNL values

[a] : FwNL, Fish with normal liver

[b] : FwHL, Fish with histopathological liver

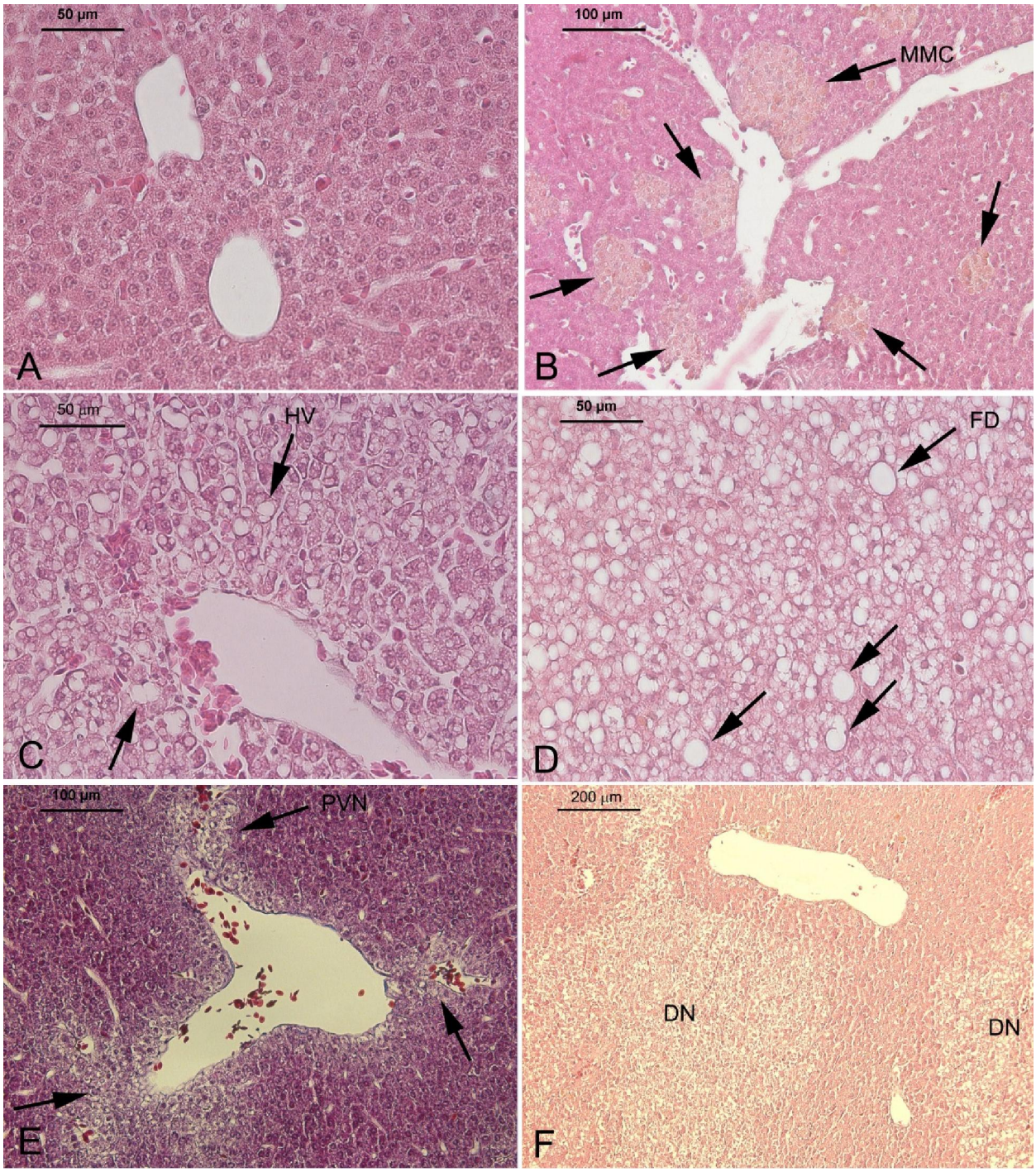

Figure 2. Histological sections of liver tissue of *C. tarichi* **A)** normal histological architecture of liver, (H&E) and **B)** increase in melanomacrophage centers (MMC, arrows), (H&E) **C)** vacuolization in hepatocytes (HV, arrows), (H&E) **D)** fatty degeneration (FD, arrows), (H&E) **E)** perivascular necrosis (PVN, arrows) (M-T) and **F)** diffuse necrosis (DN, pale stained areas) (H&E).

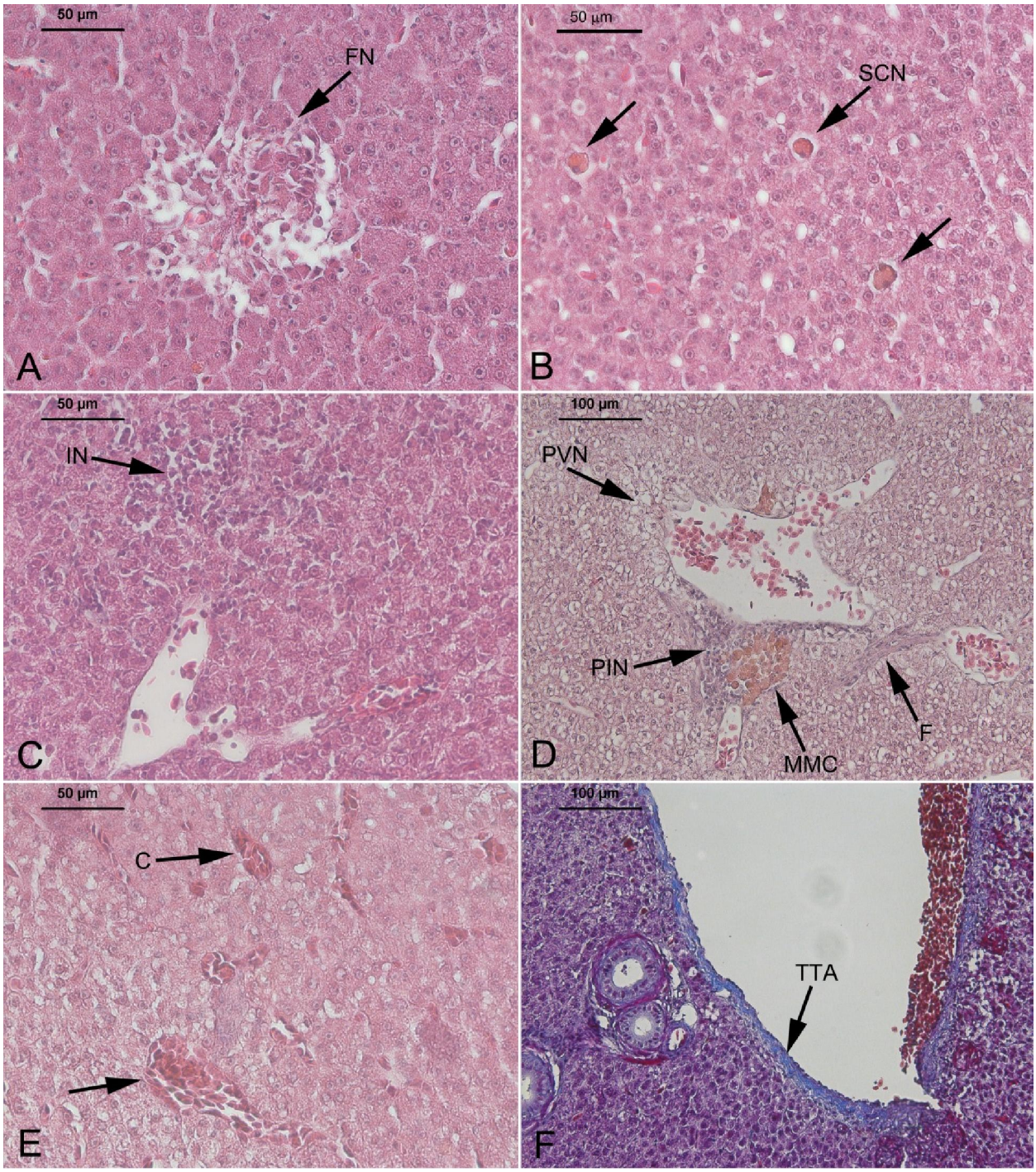

Figure 3. Histological sections of liver tissue of *C. tarichi* **A)** Focal necrosis (FN, arrows), (H&E) **B)** single cell necrosis (SCN, arrows), (H&E) **C)** infiltration in liver parenchyma (IN, arrow), (H&E) **D)** infiltration in periportal area (PIN, arrow) together with other alterations (melanomacrophage centers (MMC, arrow), perivascular necrosis (PVN, arrow) and fibrosis (F, arrow), (H&E) **E)** congestion (C, arrows), (H&E) **F)** thickening of tunica adventitia (TTA, arrows) of portal vessel, (M-T).

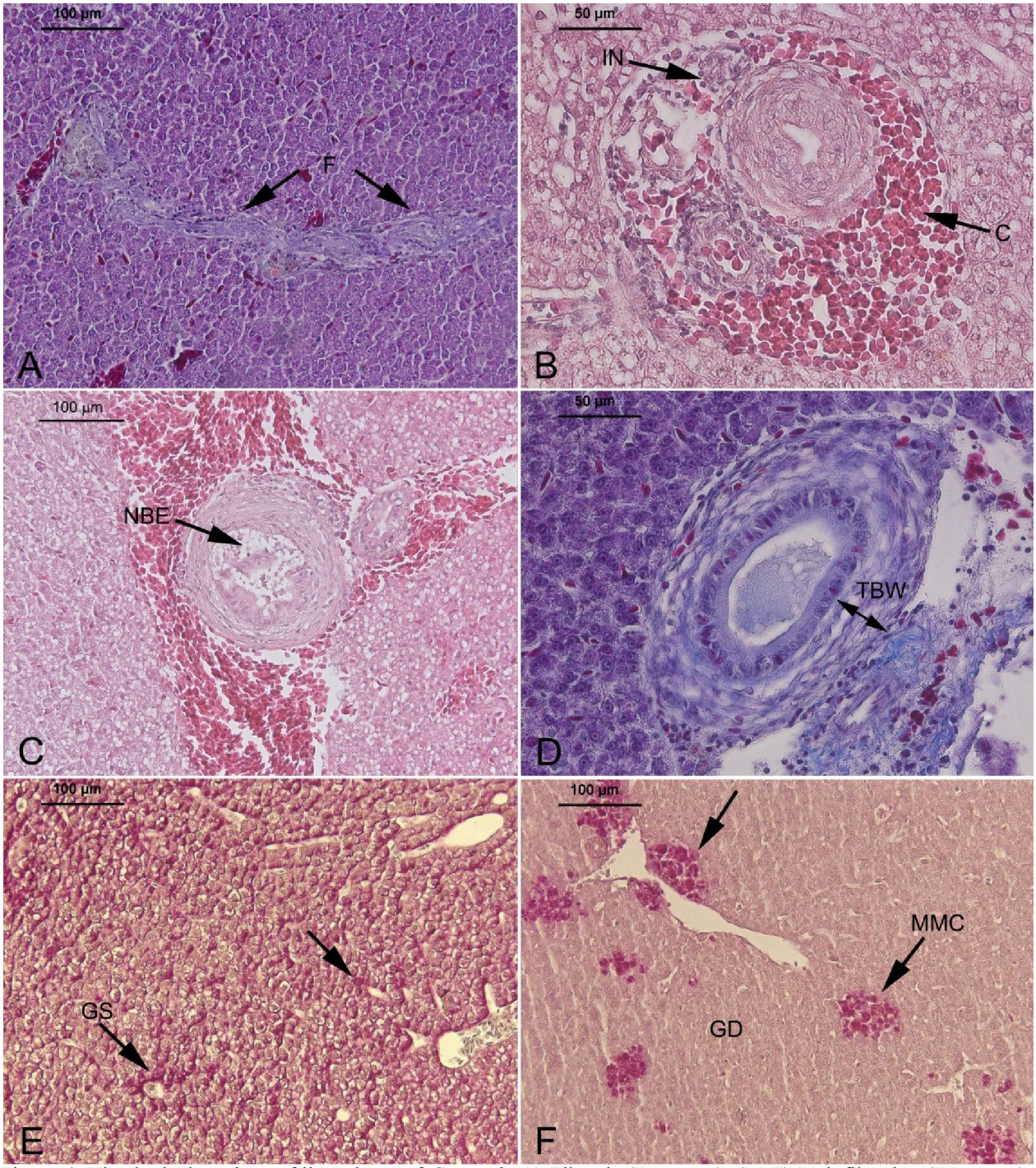

Figure 4. Histological sections of liver tissue of *C. tarichi* **A)** Fibrosis (F, arrows), (M-T) **B)** infiltration (IN, arrow) and congestion (C, arrow) at peribiliary area, (H&E) **C)** necrosis in bile epithelia (NBE, arrow), (H&E) **D)** thickening of bile duct wall (TBW, arrow), (M-T) **E)** glycogen staining (GS) in the normal tissue (arrows), (PAS) **F)** glycogen depletion (GD) in histopathological liver and PAS-positive melanomacrophage centers (MMC, arrows).

In agreement with the aforementioned studies, in the present study, the occurrence of histopathological lesions and oxidative stress as biomarkers of environmental pollution in the liver of *C. tarichi* was first determined. Data from the current study can be regarded as a baseline study, and the results may be used in future monitoring studies for further studies in Lake Van and *C. tarichi*. A wide range of histopathological changes were observed in the liver samples of *C. tarichi* (e.g., vacuolization,

increased in MMCs, necrosis, glycogen depletion, congestion, necrosis, fibrosis and other such changes). The variety in the lesion types might be due to the in the life history, contaminant exposure history and individual sensitivity to the contaminants. These also can be interpreted as nonspecific responses to the miscellaneous pollutants present in the lake water. The histological analysis of *C. tarichi* liver revealed vacuolization of hepatocytes. Similar alterations were observed in the hepatocytes of *Clarias gariepinus* living in contaminated areas with endocrine disrupters and heavy metals (Marchand et al., 2009). *Oreochromis niloticus* exposed to heavy metals in its environment displays the same histopathology (Abdel-Moneim et al., 2012). The vacuolization of hepatocytes in the liver was a more

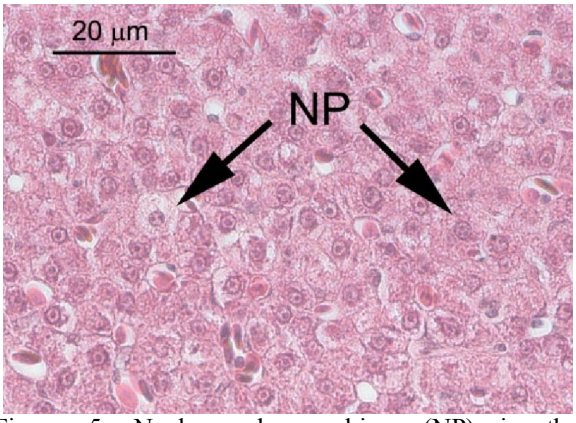

Figure 5. Nuclear pleomorphism (NP) in the hepatocytes (arrows) (H&E)

common pathology in the fish exposed to contaminants in their environments (Lukin et al., 2011; Kelly and Janz, 2009) and is associated with the inhibition of protein synthesis, energy depletion and accumulation of lipid responses to toxic substances (van Dyk et al., 2007), suggesting that vacuolization might be the result of the chemical substance exposure. The fatty degeneration changes observed in this study have been reported in other fish species living in contaminated areas (El-Naggar et al., 2009; Marchand et al., 2009; Fricke et al., 2012). This may arise from a decrease in the utilization of energy reserve or pathological synthesis (El-Naggar et al., 2009). Hepatic lipid accumulation has been reported to be associated with exposure to different toxic substances (Wolf and Wolfe, 2005) and can result from a general failure of lipid metabolism (van Dyk et al., 2007). Toxicant-induced lipid peroxidation may also cause such an effect. For example, channel catfish (*Ictalurus punctatus*) exposure to PAHs, polychlorinated biphenyls (PCBs) and metals via sediment increases lipid peroxidation (Di Giulio et al., 1993). A more common response of fish liver to the toxicants is glycogen depletion. The loss of glycogen can occur directly by intoxication, or it may occur due to decreased body condition due to starvation, stress or disease (Di Giulio et al., 1993). Some PAS-applied liver sections of *C. tarichi* display no or less glycogen staining. These phenomena have also been reported in fish experimentally exposed to endosulfan (Glover et al., 2007) and endocrine-disrupting chemicals (Schvaiger et al., 2000; Pawlovski et al., 2004). Increases in the MMC were observed in the histopathological liver samples of *C. tarichi*. These pigment-containing macrophage aggregates play a role in the storage of foreign material and increases in size and frequency in environmentally stressful conditions, suggesting they are reliable biomarkers for water quality in terms of deoxygenation and chemical pollution (Agius and Roberts, 2003). Similar to our findings, fish inhabiting areas contaminated with different types of pollutants such as PAHs and pesticides (Chang et al., 1998), urban stream (Camargo and Martinez, 2007), heavy metals (Abdel-Moneim et al., 2012) and bleached-kraft mill effluent (Couillard and Hodson, 1996) and waste water treatment plant effluent (Pinto et al., 2010) displayed an increase in the density of MMC in their liver tissues. The liver parenchyma display degenerative-necrotic changes affecting cells from groups to the single-cell level. Hepatic necrosis, an indicator of toxic injury by contaminants, has also been described in other fish living in polluted areas with heavy metals and endocrine disrupters (Marchand et al., 2009; Triebskorn et al., 2008; Fernandes et al., 2008). The necrosis of biliary epithelium was also observed as in this study and has been induced in rainbow trout exposed to the bile duct toxin alpha-naphthylisothiocyanate (Metcalfe, 1998). Necrosis is strongly associated with oxidative stress and free radical generation, which causes enzyme inhibition, cell membrane damage (lipid peroxidation) and inhibition of protein synthesis, resulting in increased cell death (Avci et al., 2005; Abdel-Moneim et al., 2013). There were slight fibrotic changes in the liver samples of *C. tarichi*. Fibrosis has been suggested to be a chronic tissue response to chemical injury (Blazer, 2002). Another histopathological finding was congestion in sinusoids and blood vessels. Other fish species captured from contaminated sites also have displayed this alteration in association with toxic substance exposure (Abdel-Moneim et al., 2012; Marchand et al., 2009; Paulo et al., 2012). Some fish displayed infiltration at the periportal and peribiliary area and liver parenchyma. This observation agrees with those of other researchers (Gül et al., 2004; El-Naggar et al., 2009; Marchand et al., 2009; Pinto et al., 2010) and suggests that the presence of stressors inducing inflammatory responses in Lake Van.

Nuclear pleomorphism is considered to be one of the lesions caused by exposure to PAHs and PCBs (Mikaelian et al., 1998; Myers et al., 1998) and has been discussed as an early signal of neoplastic alterations (Myers et al., 1987; Fricke et al., 2012). Various authors have reported the presence of nuclear pleomorphism in fishes inhabiting PAH- and PCB-contaminated areas (Bogovski et al., 1999; Lyons et al., 2004; Lang et al., 2006; Fricke et al., 2012). Thus, our finding indicates possible PAH and PCB contamination in the waters of Lake Van or other factors.

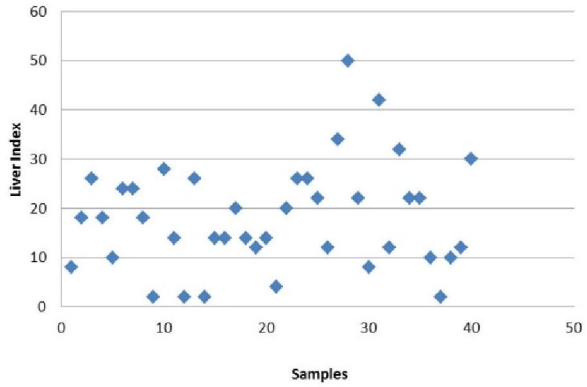

Figure 6. Distribution of Liver Index values among the samples

Many pollutants (PAHs, PCBs, metals, pesticides, among other) exert their toxicity via oxidative stress. When contaminant-stimulated ROS generation exceeds the antioxidant defense system capability, oxidative stress occurs. Therefore, antioxidant defense system biomarkers are commonly used useful biomarkers in both laboratory and field studies (Livingstone, 2003). In the current study, we investigated oxidative stress in the histopathological liver tissue of *C. tarichi* as compared with normal liver tissue using antioxidant defense system biomarkers (MDA, CAT and SOD). The elevation of lipid peroxidation is known to occur after the consumption of some xenobiotics and following superoxide overproduction, which produces dismutation singlet oxygen and H_2O_2, which can be easily converted later into the reactive $^{\cdot}OH$. Both singlet oxygen and the OH radical have a high potential to initiate free radicals chain reactions of lipid peroxidation. Furthermore, it is known that $^{\cdot}OH$ can initiate lipid peroxidation in tissues (Kang, 2002), and MDA is a major oxidation product of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of lipid peroxidation (Freeman and Crapo, 1981). Our study revealed higher lipid peroxidation levels (increased MDA concentration) in the fish with histopathological livers (FwHL) than fish with normal livers (FwNL). In agreement with our results, increased lipid peroxidation levels have also been reported in fish demonstrating histopathological features inhabiting contaminated environments (Gül et al., 2004; Ben Ameur et al., 2012; Abdel-Moneim et al., 2013). A remarkable increase in lipid peroxidation, simultaneously observed with liver histopathology, may be attributed to foreign compounds causing oxidative stress in the lake water. The SOD-CAT system provides the first line of defense against oxidative stress. The endogenous scavenger SOD catalyzes the dismutation of the highly reactive superoxide anions ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2), whereas CAT is mainly localized in peroxisomes, and it is responsible for the degradation of H_2O_2, a precursor of hydroxyl radical, to H_2O (Livingstone, 2003). Simultaneous increases in the activities of SOD and CAT are usually observed in the livers of fishes in the presence of environmental contaminants (Pandey et al., 2003; Dimitrova et al., 1994; Belge Kurutaş et al., 2009). In the present study, SOD activity unexpectedly did not display a significant increase together with significant CAT induction and remained stable in the histopathological livers of *C. tarichi*. Dimitrova et al., (1994) suggested that the superoxide radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity. Consequently, the lack of an observation of increase is expected in SOD activity parallel to CAT and may arise from a similar mechanism. Other reason for such an effect may also be an adaptive and protective response to environmental stressors as a result of chronic exposure. Unaltered and reduced liver SOD activities have also been reported in other fish species under contaminant exposure (Karakoç et al., 1997; Wilhelm Filho et al., 2001). In another study, the absence of SOD and induction of CAT activity, together with histological damage, have been found in the liver of cichlid fish (*Geophagus brasiliensis*) living in a polluted site of Benedito River, Brazil (Wilhelm Filho et al., 2001). In addition, an inverse relationship between SOD and CAT was reported in the histopathological livers of fish, *Oreochromis niloticus*, living in the polluted lakes of Egypt in that while SOD activity increased, CAT decreased (Abdel-Moneim et al., 2013). The higher CAT activity, together with histopathological lesions, observed in the present study can be attributed to the increase in the production of peroxide radicals that might cause subsequent lipid peroxidation, indicating oxidative stress in the liver.

Conclusion

In conclusion, this study reveals that Lake Van is undergoing a process of environmental degradation due to the high presence of histopathological alterations together with oxidative stress in the livers of *C. tarichi*. Causally, pollutants from domestic discharge, agricultural runoff and industrial effluents to the lake water are exerting environmental pressure on *C. tarichi*. Furthermore, our results provide a description of histopathological and biochemical alterations in the liver of *C. tarichi* that can be used as baseline information for further studies and suggest that *C. tarichi* is a useful bio-indicator organism for monitoring the effects of pollutants in Lake Van. Our study reinforces that the use of semi-quantitative histopathology together with biochemical indices is a useful tool for environmental quality studies. To determine the exact causes of damage in the liver tissue of *C. tarichi*, detailed investigations should be performed by measuring environmental contaminant levels in the fish tissues and water in future studies.

Corresponding Author:
Dr. Burak Kaptaner
Department of Biology
Science Faculty
Yüzüncü Yıl University
65080 Van, Turkey
E-mail: bkaptaner@yahoo.com

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