International Journal of
Agriculture, Environment and Food Sciences
JAEFS
e-ISSN : 2618-5946
DOI: 10.31015/jaefs.2021.1.14
www.jaefs.com
Research Article
Int J Agric Environ Food Sci 5 (1):107-121 (2021)

A biomonitoring study: Using the biomarkers in *Cyprinus carpio* for the evaluation of water pollution in Sapanca lake (Sakarya, Turkey)

Gullu Kaymak[1,*] **Figen Esin Kayhan**[2] **Nazan Deniz Yon Ertug**[3]

[1]Kütahya Health Sciences University, Simav Vocational School of Health Services, Kütahya, Turkey.
[2]Marmara University, Faculty of Science and Art, Department of Biology, Istanbul, Turkey
[3]Sakarya University, Faculty of Science and Art, Department of Biology, Sakarya, Turkey

[*]Corresponding Authur: gullu.kaymak@ksbu.edu.tr

Abstract

This study aims to determine the toxic effects of heavy metal pollution on carp (*Cyprinus carpio*) in Sapanca Lake by biochemical and histological analyses. For this reason, fish and water samples were taken from the lake in 2015. Heavy metal (Cu, Fe, Zn, Pb, Cd) analyzes in the water column and tissues (muscle, liver, gill) were determined by ICP-OES. CAT, GSH and MDA levels, which are oxidative stress bioindicators in tissues, were measured by spectrophotometric methods. Histopathological findings in tissues were determined by Hematoxylin-Eosin staining. As a result, heavy metal concentrations in water were determined as Fe > Zn > Pb > Cu > Cd. The accumulation of Cu, Fe and Cd in the tissues of the fish were liver > gill > muscle, and the accumulation of Zn was gill > liver > muscle. CAT activity, MDA and GSH level of the tissues changed with the water temperature. General signs of destruction were observed in the gill tissues of the fish. Necrotic conditions in hepatocytes were observed. In conclusion, the presence of biochemical and histopathological findings in tissues suggests that the lake is not only affected by heavy metals but also by other pollutants.

Keywords: Heavy metals, Carp, Oxidative stress, Freshwater, Histopathology

Introduction

Due to the pollution in the air, water and soil, which are the basic elements of life, all living beings, especially humans, are damaged and negatively affected (Kahvecioglu et al., 2003; Katalay et al., 2005; Ozyürek, 2016). Increasing environmental problems cause undesirable changes in ecosystems. Industrial activities affect the air, soil and water ecosystem negatively, as well as environmental pollution in the air, not only affects the air negatively but also pollutes the water and soil ecosystems.

Turkey is surrounded by seas on three sides and it has 8333 km coastline, from more than 200 natural lakes, more than 1000 ponds, 706 reservoirs and 177 714 km long streams (Yılmaz, 2014). But when we assess Turkey in terms of the available water catchment area, it appears that Turkey is not a water-rich country. The amount of water is per capita per year in Turkey 1500 m^3. Turkey Statistical Institute (TUIK), in 2040 estimated population of 100 million will be achieved (TUIK, 2018), even in such a case the water of 1000 m^3 figures set for the issue urgently to ensure the necessary work will be important. For this reason, water pollution is one of the most important environmental problems.

Since the lakes from aquatic environments are more stagnant and open to human influence than rivers, they feel the impact of human activities more. The Sapanca Lake, which is an important drinking water resource for the provinces in the Marmara Region, is also an important water resource in terms of industrial water supply and aquaculture production. In recent years, the Sapanca Lake has suffered serious damage as a result of looting and destruction concentrated on and

Cite this article as:
Kaymak, G., Esin Kayhan, F., Deniz Yon Ertug, N. (2021). A biomonitoring study: using the biomarkers in Cyprinus carpio for the evaluation of water pollution in Sapanca lake (Sakarya, Turkey). J. Agric. Environ. Food Sci., 5(1), 107-121
Doi: https://doi.org/10.31015/jaefs.2021.1.14
Orcid: Gullu Kaymak: 0000-0001-6309-0208, Figen Esin Kayhan: 0000-0001-7754-1356 and Nazan Deniz Yon Ertug: 0000-0002-6830-8971
Received: 10 November 2020 **Accepted:** 09 February 2021 **Published Online:** 30 March 2021
Year: 2021 **Volume:** 5 **Issue:** 1 (March) **Pages:** 107-121
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

around it (Adasu, 2003; Çakır, 2010). In investigating the pollution in the lakes, it is important to evaluate the biological, physical and chemical parameters of the environment and their changes over time. Aquatic organisms that live in contaminated ecosystems and accumulate heavy metals in their tissues are used as bioindicators in biomonitor researches to determine the degree of pollution of their environment and the effects of contaminants (Chen and White, 2004; Sarkar et al ., 2006; Udroiu, 2006; Holt and Miller, 2011; Authman et al., 2015).

Previous studies on Sapanca Lake relates generally only to water quality and pollution (Altuğ and Okgerman, 2008; Altundağ et al. 2019). Unlike other studies, this research aims to determine the effects of heavy metal pollution in the water on heavy metal accumulation in carp tissues and biochemistry and histology of tissues. This study is also of great importance in determining the effects of pollution in the lake on human health through the food chain.

Material and Methods

Study Area, Test Organism and Sample Collection

The perimeter of the Sapanca Lake is 39 km, 26 km of it surround with the borders of Sakarya and 13 km of it surround by the borders of Kocaeli (Figure 1). The long axis of the lake is in the east-west direction and the short axis in the south-north direction. The average depth of the lake is 31–33 m, but its maximum depth is 61 m (Adasu, 2003; Çakır, 2010). A Study conducted in Sapanca Lake in previous years indicates that there are 32 fish species in the lake (Okgerman et al., 2006), but a recent study shows that this number has decreased to 22 (Kuş, 2012).

In this study, in which heavy metal pollution was investigated in Sapanca Lake, carp (*Cyprinus carpio*) was chosen, because of it was hunted heavily due to its economic importance and the best represents the effects of environmental pollutants due to its feeding habits (Singh et al., 2010). The fish samples were collected from Kırkpınar location between January-December 2015. The necessary permissions were obtained from The Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Fisheries and Aquaculture, and Marmara University Animal Experiments Local Ethics Committee. With the help of a professional fisherman, fish samples were caught in the fishnet and brought to the laboratory, and water samples were also taken to the laboratory on ice, in brown bottles. In the laboratory, after measuring of fish's weight and height, muscle, liver and gill tissues were taken from fish samples.

Figure 1. The location of the sampling site (X) on the map of the study area, Sapanca Lake, Turkey.

Heavy metal analyses

Samples stored in the freezer were subjected to wet-burning with the nitric acid (HNO_3) in the Milestone Start D (Italy) microwave oven equipped with a temperature control program. Metal concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Spectro Arcos, Germany). High Purity- ICP- 200-7-5 brand ICP-OES multi-element standard was used as standard, DORM-3 was used as certified reference material for fish tissues, NW-KEJIM-02 Soft Lake Water was used as reference material for Lake Water. Heavy metal analyzes in water samples taken in brown bottles were determined with ICP-OES by adding 65% HNO_3 at the rate of 10%.

Transfer Factor (TF) analyses

The Transfer Factor (TF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water. It gives an indication about the accumulation efficiency for any particular pollutant in any fish organ (Canpolat et al. 2016). TF was calculated according to Aboul Ezz and Abdel-Razek (1991) using the following equation:

$$TF = M_{tissue} / M_{water}.$$

Where; M_{tissue} is the metal concentration in fish tissue µg/kg and M_{water}, metal concentration in water µg/L.

Assessment of oxidative stress parameters

For the purpose of homogenization, the tissues taken from the freezer were thawed on ice. Tissues weighed for the purpose of homogenization of muscle, liver and gill samples were taken into eppendorf tubes with a 10% (w/v) cooled homogenate buffer and disintegrated in the homogenizer with the help of glass beads. Samples were preserved in ice at all stages of the studies. The homogenate was centrifuged for 20 minutes at 10000 rpm at 4°C. After the centrifuge, the supernatant part was taken and the pellet part was discarded.

Lipid Peroxidation (LPO)

The LPO in the tissue samples were measured using the thiobarbituric acid reaction according to the method described by Ledwozyw et al., 1986. The absorbance was determined at 535 nm and its concentration was expressed as nmol MDA/g tissue.

Catalase Enzyme Activity (CAT)

The enzyme activity was measured following the decrease of absorbance at 240 nm due to hydrogen peroxide (H_2O_2) consumption (Aebi, 1974). The activity was expressed as U/mg tissue.

Total Glutathione (GSH)

GSH concentration was measured with an assay using the dithionitrobenzoic acid (DTNB) recycling method described by Beutler (1975). GSH concentration was expressed as nmol GSH/g tissue.

Total Protein Content

Total protein was determined according to the method of Bradford (1976). The intensity of the developed blue color was measured at 595 nm against the blank. Its concentration was expressed as µg/µL.

Histopathology analysis

The liver tissues were fixed in 10% neutral buffered formalin were dehydrated using a series of graded ethanol solutions (70–100%), cleared in xylene, embedded in paraffin and sectioned at 5 µm. The gill tissues were fixed again in Bouin's solution for 24 h for decalcification. Then the tissues were dehydrated and embedded in the paraffin wax and sectioned at 5 µm thickness and stained with Hematoxylin and Eosin (H&E) for standard histopathological evaluation. For each month, 5 secondary filaments from the inner section on 10 slides were analyzed. Slides were examined under the light microscopy.

Statistical analysis

Statistical analyzes were made using IBM SPSS Statistic 23 computer program. Study findings were expressed as mean ± standard error of mean (SEM). Comparisons between the two groups are the parametric Student's t-test and nonparametric Mann-Whitney U test in unequal variances, and comparisons between more than two groups are one-way ANOVA; in statistically significant results, Tukey's post hoc test was performed to compare the significant difference between groups following ANOVA. In all statistical comparisons, those with a significance level less than $p < 0.05$ were considered significant.

Results and Discussion

The amount of Cu, Fe, Zn, Pb and Cd metals in the water samples taken from Sapanca Lake was investigated in this study. The samples were collected between January and December 2015 (Table 1). The results of the reference material and the sample values were different. For example, Fe was found to be more than the reference in January and March, and quite low in other months. Cu showed an increasing trend throughout the year. In the first study on water pollution between 1978 and 1980 in our study area, Sapanca Lake, the water samples were taken seasonally was examined. It was emphasized that one of the drinking water resources could be lost (Sümer et al., 1996), in the following years, it is stated that the lake faces the danger of pollution (Yalçın and Sevinç 2001). In the study conducted on the streams flowing to Sapanca Lake before and after the Marmara earthquake on August 17, 1999, it was observed that the accumulation of lead and cadmium in Istanbul, Mahmudiye and Kuruçay streams increased after the earthquake (Dündar et al., 2003). In other studies carried out in Sapanca Lake, which is one of the important wetlands (Bakan and Balkas, 1999; Yalçın and Sevinç, 2001; Şişman et al., 2002; Duman et al., 2007), it was reported that the water quality is changing and the pollution in the lake has increased. Heavy metal levels determined in water samples taken from Mogan Lake, Turkey in 2009 were determined as Pb> Fe> Cu≥Zn> Cd (Dostbil, 2010). Zuo et al. (2018) showed that the ranges of the metal concentrations in Taihu Lake's (China) water in the following order Zn> Cu> Cr> Cd. As a result of this study, heavy metal concentrations in the water column of Sapanca Lake were determined as Fe> Zn> Pb> Cu> Cd. Mwamburi (2009) determined that Fe level in the water column of the Victoria Lake is far above the drinking water standards set by WHO and other parameters (Al, Mn, Zn, Cu, and Cr) are in compliance with the standards. When the water quality of the Sapanca Lake was evaluated according to SKKY (Regulation on Control of Water Pollution) (2004), for Fe and Zn were in Class I, for Pb was in Class III, for Cd was in Class II and for Cu was the first six months in Class I, the other months in Class II.

During this experiment, totally 41 *Cyprinus carpio* (25 ♂ and 16 ♀) were caught in the sampling period. The weight, length, fork length and condition factors of fish were shown in Table 2. Heavy metals were bioaccumulated at varying levels and were noticeable in different tissues of *Cyprinus carpio*. Zn and Fe were detected in all tissues every month as a result of heavy metal analysis of carp fish caught from Sapanca Lake (Table 3). Cu was not detected only in the muscle tissues of the fish in some months. Pb was not detected in the liver and gill tissues of carp in all months. Cd was detected in all tissues in some months. All results were found under the specified maximum limit of fish tissues by FAO and TFC. The evaluated heavy metal concentration can be ordered in muscle Fe>Zn>Cu>Cd>Pb, in liver and gill Zn>Fe>Cu>Cd>Pb. Because of not being an active organ, heavy metal accumulation in muscle tissue is thought to be at low levels. Altundağ et al. (2019) found the following levels of Cu< Fe< Zn in muscle tissue of carp caught from the Sapanca Lake. Junianto and Apriliani (2017) analyzed the accumulation of Zn, Cd, Pb and Hg in the muscle tissue of *C. carpio*, *Oreochromis niloticus* and *Pangasianodon hypophthalmus* in Cirata Dam (Indonesia), and determined the Pb value above of the determined standard values in fish. Mustafa, (2020) determined that Pb and Cd content in *Luciobarbus xanthopterus* caught from the Tigris River in

Baghdad and represented the general order of metals levels in different tissues as follows liver> kidney> muscle> gill. According to the analysis data revealed in this study made with carp samples, the organ with the heavy metal accumulation in all fish species was found to be the liver. Then the liver tissue was followed by gill and muscle tissues, respectively. Heavy metals usually accumulate more in non-lethal concentrations in the metabolically active organs of fish, such as the liver (Kargın and Erdem, 1992; Gülcü-Gür and Tekin-Özan, 2017). The high concentration of metal in the gill is due to the metals absorbed by breathing water adhering to the mucus in the gill and staying between lamellae (Heath, 1987; Al-Bairuty, 2013; Stanley and Preethe, 2016). In a study conducted by Uysal (2010), the Zn level in which the highest Zn accumulation in the tissues of the *C. carpio, Carassius carassius* and *Rutilus rutilus* species were found in the gill tissue. They reported that the Zn level in the muscle tissues of all species is lower than the value determined by the Turkish Food Codex (TFC). Likewise, this study found that the concentrations of the heavy metals were lower than the value determined by TFC and FAO.

The concentrations of metals in tissues of carp are much higher than in the water were found. The Transfer Factors (TF) from water to fish in case of *C. carpio* were in the muscle tissue in order of Fe> Zn> Cu> Cd; in the liver tissue in order of Zn> Fe> Cu> Cd; in the gill tissue in order of Zn> Fe> Cu> Cd> Pb (Table 4). The presence of metals in high levels in the fish environment does not indicate a direct toxic risk to fish if there is no significant accumulation of metals by fish tissues (Engin et al., 2016). On the other hand, TF from the water was higher than 1.00 which means that the *C. carpio* accumulated metals from water, especially Fe and Zn values. According to the accumulation of heavy metals in tissues, the TF can be listed as follows: for Cu, Fe and Cd; Liver> Gill> Muscle, for Zn; Gill> Liver> Muscle. This result agrees with many previous studies. Abdel-Baki et al. (2011) calculated TF of five heavy metals from water in Tilapia fish, results indicated that fish accumulated all metals (Pb> Cr> Cu> Hg> Cd) in its tissues from water. Canpolat et al. (2016) determined that the TF of heavy metals in *C. carpio* from Karakaya Dam Lake, Turkey. They were found that heavy metals accumulated in the muscle of *C. carpio* were higher than that in the surrounding water and explained that Fe was the greatest metal accumulated by *C. carpio* from water while the TF of Cu was the lowest. According to a previous study made with *C. carpio* captured from Karacaören (I) Dam Lake, Turkey, the highest organ/ water ratio was determined for Zn in the liver (Kır et al. 2016).

Table 1. The heavy metal accumulation in Sapanca Lake water results were expressed as Mean± SEM.

	Cu (μgL^{-1})	Fe (μgL^{-1})	Zn (μgL^{-1})	Pb (μgL^{-1})	Cd (μgL^{-1})
January	15.4±3.72^{b}	360.8±24^{a}	90.8±18.7	18.4±1.50^{b}	1.89±0.4^{b}
March	17.9±2.80	303.01±11.37	73.12±12.4	43.4±9.6^{a}	3.03±0.72^{a}
April	16.2±3.4	41.41±5.4	34.92±5.7	39.14±11.2	3.011±1.04
May	16.82±5.78	27.13±4.3	18.93±3.9	40.7±10.3	2.694±0.47
June	16.27±1.2	21.74±3.75	16.17±3.2^{b}	39.26±2.46	2.66±0.08
July	18.41±1.37	12.84±2.9^{b}	20.36±1.9	40.45±6.42	2.70±0.9
August	20.46±1.1	68.73±5.8	22.85±2	39.15±7.6	2.84±0.42
September	20.83±4.32	67.31±14.7	57.67±8.45	40.35±13.7	2.75±0.09
October	20.2±1.4	61.94±6.7	60.29±15.3	42.4±9.36	2.94±0.51
November	22.17±6.15	63.75±9.1	108.8±21.3^{a}	43.06±11.2	2.88±0.64
December	22.25±1.7^{a}	45.44±2.7	82.95±11.7	43.22±7.65	2.94±0.7
NW-KEJIM-02	18.78±0.4	119.3±8.5	19.63±2.84	38.19±0.59	2.6195±0.06

a: Maximum, b: Minimum

Table 2. The parameters results of carp were expressed as Mean± SEM (Standard Error of the Mean).

Months	n gender	Weight (±SE) (min.-max., g)	Total Length (±SE) (min.-max., cm)	Fork Length (±SE) (min.-max., cm)	Condition Factor (±SE) (min.-max.)
January	3 (2 ♂ +1♀)	282± 48.2[b] (200-367)	27± 1.73[b] (24-30)	24.3± 1.2[b] (22-26)	1.41± 0.026 (1.35-1.44)
March	6 (4♂+ 2♀)	623.8 ±77.96 (445-950)	33.83±1.3 (30-39)	31.9±0.63 (29.5-34)	1.57±0.057 (1.37-1.78)
April	2 (2♂)	2710±20 (2690-2730)	73.2±0.2[a] (73-73.4)	70.75±0.25[a] (70.5-71)	0.69±0.0005[b] (0.69-0.691)
May	6 (4♂+2♀)	1192.6±269.2 (188-1884)	40.25±4.48 (25-55)	36.75±4.34 (21-50.5)	1.73±0.34 (1.09-3.36)
June	3 (2♂+1♀)	455.66±92.4 (270-671)	28.83±1.92 (26-32.5)	27±2.08 (24-31)	1.81±0.05 (1.53-1.95)
July	3 (1♂+2♀)	1187±137.99 (949-1427)	43.3±2.18 (39-46)	41.16±2.08 (37-43.5)	1.455±0.086 (1.3-1.59)
August	3 (2♂+1♀)	2328±467.13 (987-4232)	53±8.326 (41-69)	51.16±8.207 (39.5-67)	1.4±0.062 (1.28-1.5)
September	3 (3♂)	2133±290.5 (1600-2600)	50.3±1.85 (48-54)	47±2 (45-51)	1.65±0.122 (1.44-1.86)
October	3 (1♂+2♀)	2897.6±162.54[a] (2643-3200)	53.3±1.85 (57-51)	50.3±1.33 (49-53)	1.915±0.094[a] (1.72- 2.02)
November	5 (2♂+3♀)	562.8±81.7 (322- 759)	33±1.51 (28- 36)	30.9±1.57 (26- 34.5)	1.51±0.06 (1.41- 1.77)
December	4 (2♂+2♀)	557±160.89 (949- 161)	30.5±3.37 (22- 38)	28.5±3.38 (20- 36)	1.76±0.16 (1.51- 2.25)

a: Maximum, b: Minimum

 DOI: 10.31015/jaefs.2021.1.14

Table 3. The average concentration of heavy metals in the tissues of *Cyprinus carpio*. Data are represented as mean ± SEM.

MUSCLE					
Months	Cu (μgkg^{-1})	Fe (μgkg^{-1})	Zn (μgkg^{-1})	Pb (μgkg^{-1})	Cd (μgkg^{-1})
January	16.18±3.72	825.5±64	674.91±58.69	BDL*	BDL
March	BDL	200.84±11.37^{b}	288.73±12.39	BDL	BDL
April	6.18±2.4	660.61±53.4	1042.95±95.7	BDL	BDL
May	15.02±3.5	881.4±64.3	587.93±43.9	BDL	BDL
June	BDL	225.6±43.75	299.04±35.2	BDL	BDL
July	3.06±1.2^{b}	689.48±67.9	343.76±41.9	BDL	BDL
August	12.82±5.1	759.12±65.8	575.37±26	BDL	BDL
September	5.14±2.4	360.52±26.7	334.65±45.3	BDL	BDL
October	4.69±1.7	500.79±39.1	268.43±61.3^{b}	BDL	BDL
November	12.92±4.21	329.5±18.6	298.87±28.4	BDL	0.71±0.03
December	462.13±35.2^{a}	1864±142.6^{a}	1344.89±112.3^{a}	BDL	0.96±0.213
LIVER					
Months	Cu (μgkg^{-1})	Fe (μgkg^{-1})	Zn (μgkg^{-1})	Pb (μgkg^{-1})	Cd (μgkg^{-1})
January	1343.49±98.5	5484.9±134.6	8385.5±240.6	BDL	BDL
March	882.3±47.3	3864.1±36.6	4287.6±321.8	BDL	BDL
April	1634.15±97.45^{a}	5220.1±351.3	21083±367.9^{a}	BDL	BDL
May	1559.81±250.6	8423.27±125	8937.5±75.8	BDL	BDL
June	1219.8±69.7	6066.3±305.2	4006.63±203.5	BDL	2.66±0.98
July	1470±109.4	8114.5±264.2	10852.9±409.7	BDL	4.58±1.43^{a}
August	946.65±109.4	8998.3±305^{a}	10854.32±679	BDL	0.65±0.08
September	1347.79±187.1	5707.68±217	12763.76±445	BDL	0.49±0.2^{b}
October	452.64±35.6	4587.65±123.3	3722.87±235.4	BDL	BDL
November	412.25±27.3^{b}	2937±219.2	2677.52±198.5	BDL	BDL
December	679.26±96.4	2428.43±218.4^{b}	2347.21±512^{b}	BDL	3.49±1.4
GILL					
Months	Cu (μgkg^{-1})	Fe (μgkg^{-1})	Zn (μgkg^{-1})	Pb (μgkg^{-1})	Cd (μgkg^{-1})
January	49.51±12.4	2872.34±421	10934.3±563.4	6.77±2.4	BDL
March	36.47±10.3	2418.13±219.3	19025.55±208.4	3.93±0.89^{b}	BDL
April	32.49±12.4^{b}	3248.03±176.4	30261.55±420.9^{a}	BDL	BDL
May	78.59±21.2^{a}	4933.7±101.3	26769.9±451.3	29.49±11	BDL
June	50.42±9.3	3418.39±202.4	8546.42±302.4	10.97±3.2	0.504±0.1
July	36.47±9.6	2583.2±408.2	14745±763.3	BDL	2.01±0.05
August	69.16±4.2	5324.28±191^{a}	27331±947.3	43.04±2.6^{a}	2.9±0.8
September	47.42±21.2	3721.79±219.2	21700.35±739.3	BDL	0.38±0.01^{b}
October	32.67±8.3	1037.18±85.4^{b}	17766.85±594.6	BDL	0.44±0.02
November	39.6±7.4	2254.5±32.6	11034.25±847.4	BDL	1.02±0.4
December	47.27±18.3	2838.69±263.4	8472.92±289^{b}	BDL	2.76±1.35^{a}
DORM-3	752.37±38.06	12252.1±750.6	1986.28±144.94	218.15±28.5	34.7±0.51
FAO(max.)	30.000	-	40.000	500	100
TFC (max.)	20.000	-	50.000	300	50

a: Maximum, b: Minimum

*BDL: Blow Detection Limit

Table 4. Transfer Factors of heavy metals from water to the tissues of *Cyprinus carpio*.

MUSCLE					
Months	Cu	Fe	Zn	Pb	Cd
January	1.05	2.28	7.43	*	*
March	*	0.66[b]	3.95	*	*
April	0.38	15.95	29.86	*	*
May	0.89	32.48	31.06[a]	*	*
June	*	10.37	18.49	*	*
July	0.16[b]	53.69[a]	16.88	*	*
August	0.62	11.04	25.18	*	*
September	0.24	5.35	5.80	*	*
October	0.23	8.085	4.45	*	*
November	0.58	5.16	2.75[b]	*	0.24
December	20.76[a]	41.02	16.21	*	0.32
LIVER					
Months	Cu	Fe	Zn	Pb	Cd
January	87.24	15.20	92.35	*	*
March	49.32	12.75[b]	58.63	*	*
April	100.81[a]	126.05	603.75[a]	*	*
May	92.73	310.48	472.13	*	*
June	74.97	279.04	247.78	*	1.02
July	79.84	631.97[a]	533.05	*	1.69[a]
August	46.26	130.92	475.02	*	0.23
September	64.70	84.79	221.32	*	0.18[b]
October	22.4	74.06	61.75	*	*
November	18.59[b]	46.07	24.6[b]	*	*
December	30.52	53.44	28.29	*	1.18
GILL					
Months	Cu	Fe	Zn	Pb	Cd
January	3.21	7.96[b]	120.42	0.36	*
March	2.04	7.98	260.19	0.09[b]	*
April	2.004	78.43	866.59	*	*
May	4.67[a]	181.85	1414.15[a]	0.72	*
June	3.09	157.23	528.53	0.28	0.19
July	1.98	201.18[a]	724.21	*	0.74
August	3.38	77.46	1196.1	1.09[a]	1.02[a]
September	2.27	55.29	376.28	*	0.14[b]
October	1.61[b]	16.74	294.68	*	0.15
November	1.78	35.36	101.4[b]	*	0.35
December	2.12	62.47	102.14	*	0.93

a: Maximum, b: Minimum

*: BDL values were not taken into account for TF determination.

Aquatic organisms, such as fish, are widely used to assess aquatic environmental quality and are considered as bioindicators of environmental pollution. Various organisms can be used to determine the mechanism of the effect of pollution on specific physiological functions. Güngördü et al., (2012) monitored the pollution in Meriç River Delta temporally and spatially by using carp as a biomonitor and showed that antioxidant enzyme parameters can be used as a useful biomarker in the evaluation of environmental contamination.

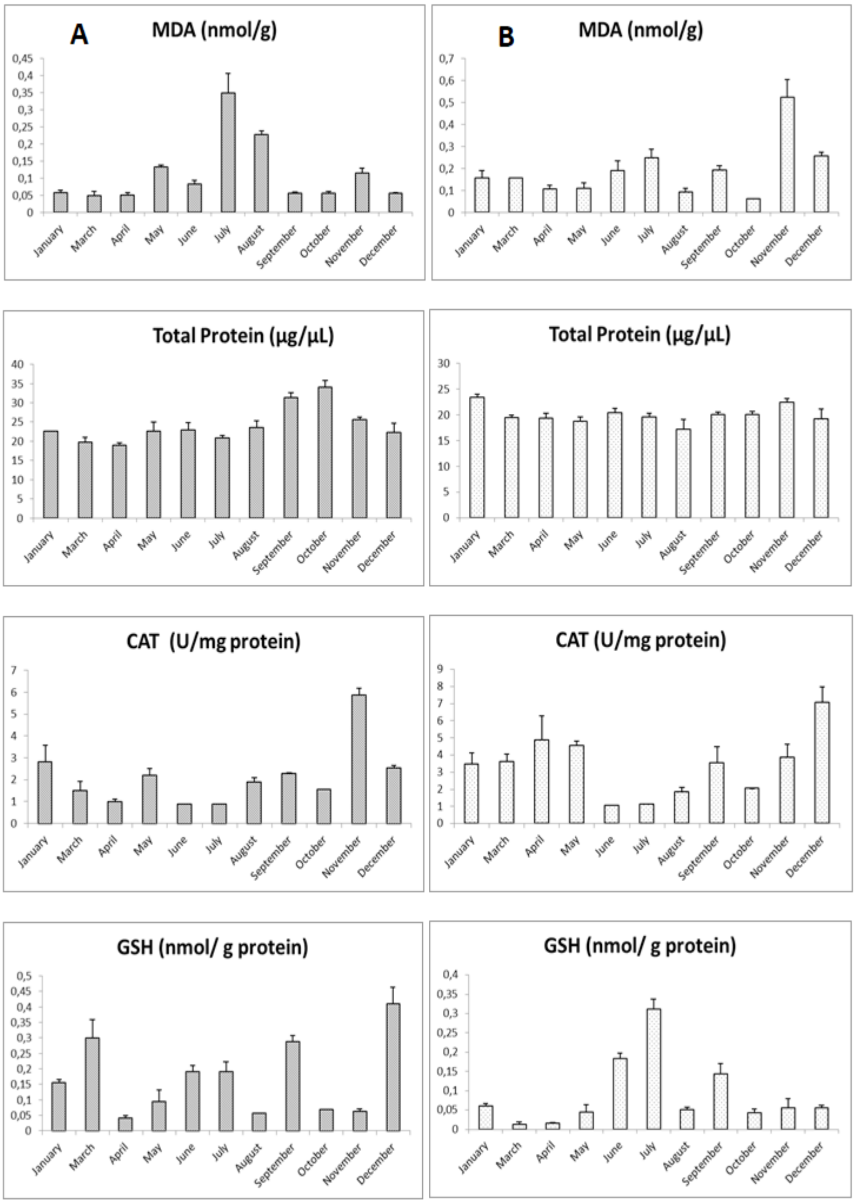

Figure 2. The responses of oxidative stress parameters in tissues (Liver (A) and Gill (B)) of carp. Lipid peroxidation (LPO; MDA), Catalase enzyme activity (CAT), Total glutathione Level (GSH). Data are represented as mean ± SEM.

Especially the temperature and nutrition increase oxygen consumption and cellular oxyradical production and enhance the antioxidant defense system to compensate these oxyradicals (Sheehan and Power, 1999; Javed et al., 2016). In this study, due to the increased sensitivity of fish tissues to oxidative stress, a decrease in the antioxidant defense system was observed in the summer months (Figure 2). Catalase (CAT) is one of the important intracellular antioxidant enzymes in the defense mechanism, and it is one of the peroxidases that provide H_2O_2 detoxification by breaking down the oxygen and water. It was determined that catalase gave different responses in xenobiotic effect (Mahboob, 2013; AnvariFar et al., 2018). It has been reported that excessive O_2 production can reduce CAT activity by its ability to inhibit CAT activity (Pandey et al., 2003). Karadağ et al. (2014) determined that increased CAT activity in carp samples were taken from the polluted area by untreated wastewaters, when compared to the relatively clean areas of Ataturk Dam Lake, Turkey. Padmini et al., (2008) determined the liver oxidative stress status of grey mullets living in heavy-metal-rich polluted Ennore Estuary compared with unpolluted Kovalam Estuary. CAT was significantly decreased in polluted estuary fish compared with unpolluted estuary fish. This indicates the inability of polluted estuary fish to cope with available antioxidant defense presumably due to the free radicals produced by pollutants. When the production of ROS is increased, the activity of antioxidant enzymes is intensified to reestablish the redox balance and health of the individual. The failure to reestablish this balance causes oxidative damage to biomolecules, compromising the ability of the organism to survive (Valavanidis et al., 2006).

Glutathione (GSH) is important in antioxidant defense as an oxygen radical scavenger. The change in the GSH level is an important indicator of detoxification ability. Although the metal-GSH conjugation process is the desired condition to provides the removal of metals with gall but reduces the antioxidant defense capacity due to the consumption of GSH (Cheung et al., 2001). This might be a factor responsible for the lack of elimination of toxic compounds that enter the fish and thus result in their accumulation, aggravating oxidative stress (Nammalwar, 1992). Fırat and Kargın, (2010) studied the effect of cadmium and zinc on *O. niloticus* was investigated in erythrocyte antioxidant systems, GSH and CAT activities were reported to increase in all groups. Yıldırım et al. (2011) determined decreased GSH levels in the *Capoeta trutta* samples were caught from the contaminated station when compared to the uncontaminated stations in Munzur River, Turkey. Hermenean et al. (2015) observed reduce GSH content the exposure to the polluted waters of the Tur River, Romania, on the *Leuciscus cephalus* compared to fish from reference river areas. In this study, the GSH level started to decrease in tissues due to the warming of the weather and reached the lowest levels in the summer months. Reduced GSH level is thought to serve as an indicator for determining the degree of pollution in fish exposed to pollutants.

Malondialdehyde (MDA) is one of the products formed as a result of lipid peroxidation (LPO) and is a commonly used parameter in demonstrating oxidative damage. The high amount of MDA indicates lipid peroxidation. Lack of lipid peroxidation or low levels is indicative of the protective effects of oxidative enzymes. Şahan et al., (2010) analyzed the levels of CAT, GSH, and LPO from biomarkers in carp to determine the level of agricultural and industrial pollution in the Ceyhan River. CAT activity and GSH levels were observed at high levels in the liver tissues of the fish in the polluted area. Besides, the amount of LPO was found to be quite high in the polluted area. Perez-Coyotl et al., (2019) specified increase LPO level in embryos of *C. carpio* was exposed to the water samples taken in The Madín Dam, Mexico. Rajeskumar et al. (2017) showed that metals exposure caused a time-dependent significant increase of LPO values in the fish exposed to multiple metal mixtures and it was almost double in 30 days group compared to controls. Heavy metals can interact with cell membrane structures and alter the normal physiology by stimulating LPO (Ahmad et al., 2006). In addition, because the typical response to oxidative stress is peroxidative damage to unsaturated fatty acids, an increase in the LPO level has been extensively used as a marker of oxidative damage in organisms. The reactive carbonyls produced during lipid peroxidation may diffuse from the original site of radical production, causing damage to inter- and intra-cellular targets (Yeşilbudak and Erdem, 2014).

Pollutants that enter the aquatic systems directly or indirectly through deliberate disposals or accidents adversely affect the various tissues and organs of living things in these environments. Basic data on these effects are provided by histopathological research, which is still the most reliable method. In addition to respiratory functions, gills that are in direct contact with the aquatic environment play an active role in the regulation of ion balance, osmoregulation and partial removal of nitrogenous wastes (Bury et al., 2003; Simonato et al., 2008; Hadi and Alwan, 2012). These are the structures that are primarily affected by all changes in ambient conditions (İşisağ Üçüncü et al., 2010; Uribe et al., 2011).

It is already well known that the histological structure of the gills shows high sensitivity to toxic chemicals in water (Brand et al., 2001; Rodrigues et al., 2010; Hesni et al., 2011). The results of this study also overlap with this information. In the gill tissues of carp caught from Sapanca Lake, general signs of destruction such as irregularity of lamellae, and hyperplasia were observed (Figure 3). Desquamation (spillage, deformity) in epithelial cells and edema in secondary lamellar tips were seen. Rarely, mutual fusions of secondary lamellae and vacuolization in primary lamellae have been observed. Also, mucus-like accumulation between lamellae has been encountered many times. As noted by Furia (2004) reported that hyperplasia, separation, and lamellar fusion in the lamellar epithelium, by increasing the distance, to some extent delay the passage of the toxic substance into the veins where it will be involved in toxicodynamic processes. The researcher also states that this defense response will cause damage to the respiratory, circulatory and osmoregulation systems, increase the risk of illness and death, and even more and more threaten the population. Hesni et al., (2011) also highlighted that gill histopathologies can reach levels that may affect the population. These general views are confirmed by the results of the presented study. Indeed, hyperplasia, hypertrophy, and even edema strengthen the cell-tissue barrier for chemicals in a biophyscochemical. However, as can be understood from desquamation, the barrier quality of the epithelium, which can be easily worn, is essentially weak. This natural barrier, which is enhanced with the increased mucus secretion, can be easily overcome by chemicals, bacteria, parasites, etc. (Fonseca et al., 2017). Fusion plays a major role at this point; because fused lamellae reduce the exposure surface. The labefaction due to the shortening in the fusion extension in the lamellar sizes can also be compensated by hyperplasia, hypertrophy, and edema (Ossana et al., 2019).

In histopathological studies, the liver and gill are the most appropriate target organs used to determine the effects of pollution. These organs are primary indicators of aquatic pollution. This study confirms all common views that these vital and complex organs are truly biomarkers (Figure 4). Accordingly, there is no lobular arrangement in the parenchyma of the liver, in some instances, regular polygonal-shaped hepatocytes, which are separated from each other by sinusoids, have formed clumps. However, hepatocytes and sinusoids have hemorrhage, which is evident with dark pink staining. Hepatopancreas cells contain zymogenic granules. In some samples, erythrocytes were seen in the hepatopancreas, which was separated from its environment by necrotic areas. In some cases, hepatopancreas is dysplasia and atrophic. Kuppfer cells, a type of macrophage specific to the liver, are evident in all samples. In some hepatocytes, nucleus and nucleolus can be easily selected, while in others it is significantly hypertrophic. In some examples, various nucleus dystrophies have been identified in the form of karyolisis and picnosis. As it is known, the main histopathological changes caused by the effects of various chemicals in the teleost liver, which has a special function of detoxification: steatosis, hypertrophy in hepatocytes and destruction in membranous structures; nucleus injuries such as pycnosis, karyorrhexis, karyolysis; necrosis and sometimes cancer. The other elements of the parenchyma are of course also affected by chemicals; dilatation and congestion, hemorrhagia and hyperemia can be observed in the vessels; the inflammatory response can be characterized by lymphocyte infiltration. Various changes in bile pigments can be seen in the extension of hepatocellular atrophy because of the liver's functions within the digestive system. In addition, fibrosis and cystic formations can occur. The melanomacrophage changes are also one of the main indicators of the immune response (Peters et al., 1987; Hinton and Lauren, 1990; Köhler et al. 1992; Hinton et al., 2001; Hinton et al., 2008). The excessive oil accumulation was detected in hepatocytes of *A. anguilla* samples collected from the contamination of the water with polychlorinated biphenyls, organochlorinated pesticides, PAH and heavy metals (Oliveira Riberio et al., 2005). Syasina et al., (2012) observed the large and heavily vacuolated hepatocytes, binuclearity, numerous nuclei of irregular shape, karyopyknosis and the accumulation of large amounts of lipids (lipidosis) in the liver of carp from the Bol'shoi Ussuriiskii Island.

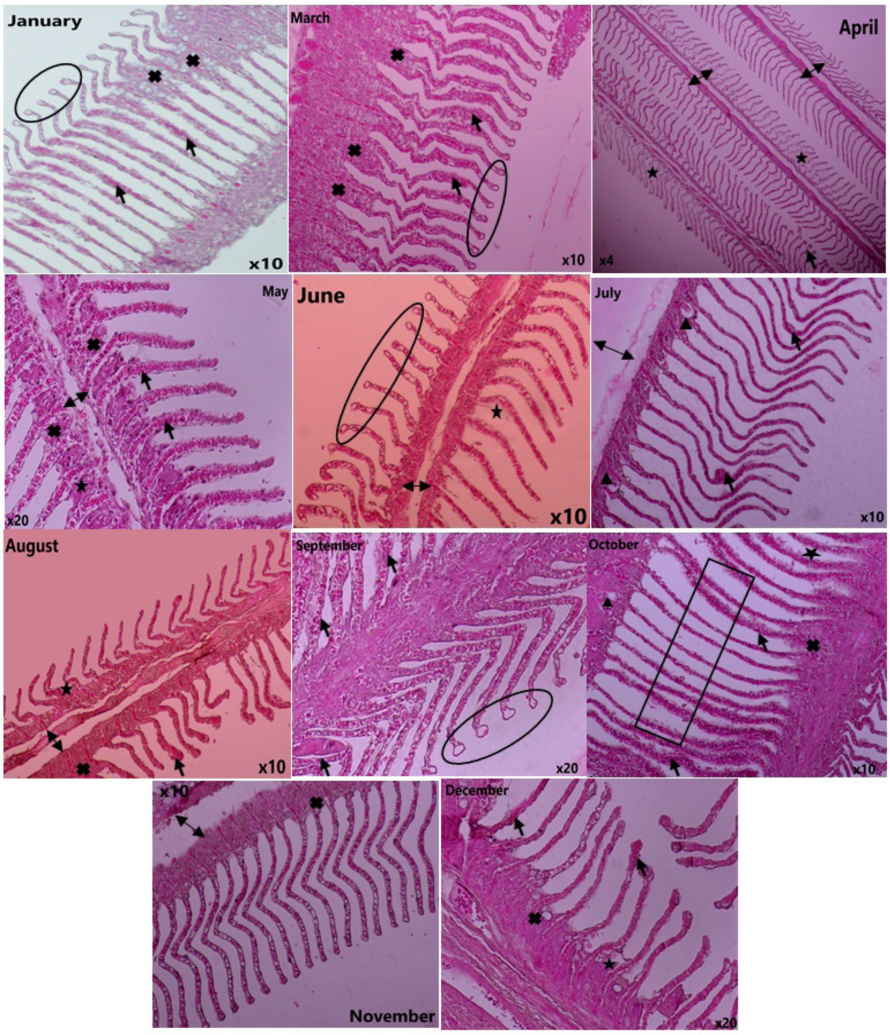

Figure 3. Histopathological results of carp fish gill tissue. Hyperplasia in the secondary lamellae (arrows), separating the primary lamellae (bidirectional arrows), desquamation in secondary lamellae (stars), the mutual fusion of secondary lamellae (rectangular), vacuolization in the primary lamellae (triangle), edema secondary lamella ends (ellipse), mucus-like accumulation (cross) between lamellar. H & E.

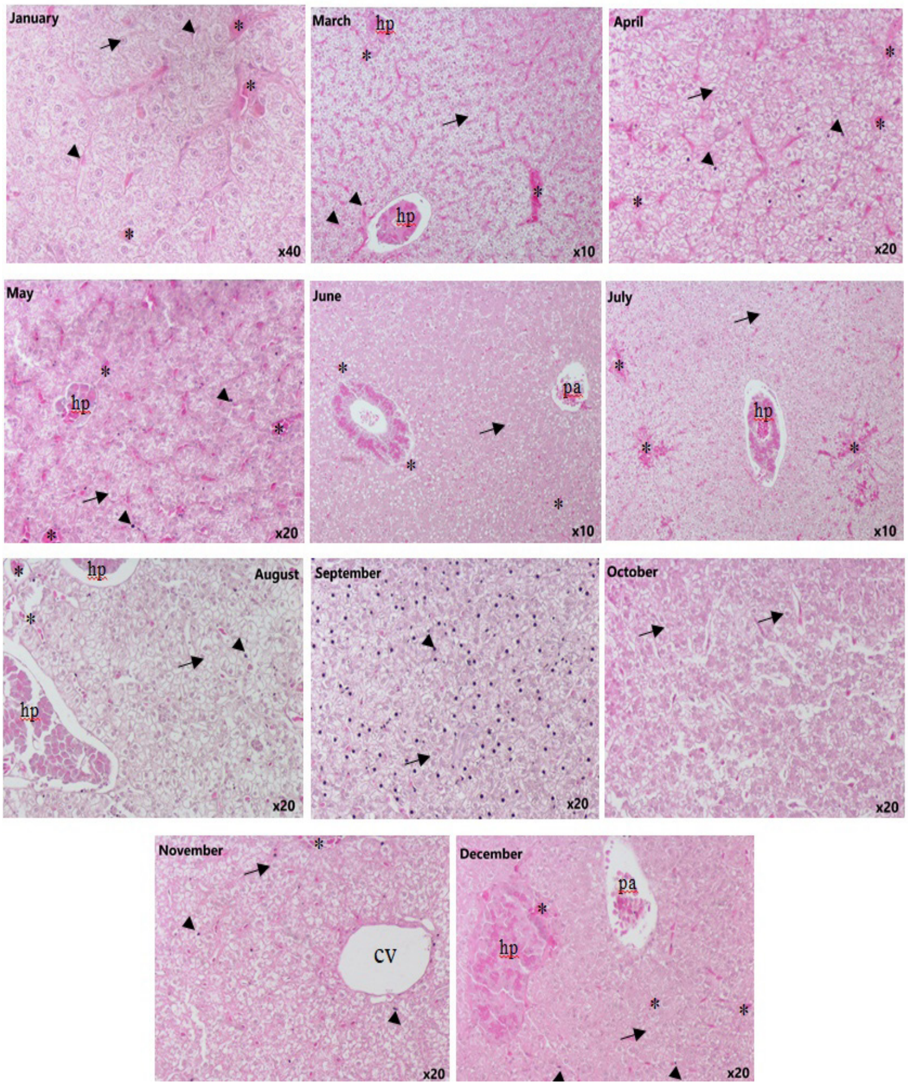

Figure 4. Histopathological results of carp fish liver tissue. Central vena (cv), hepatocytes (arrows), kuppfer cells (arrowheads), hepatopancreas (hp), portal area (pa) and red blood cells (star) in hepatocytes and sinusoids, H&E.

Conclusion

Increasing environmental pollution has become an important problem in the modern world due to industrial and domestic wastes. The recovery of lake waters polluted by the discharge of these wastes is very costly and adds an extra burden to the economy of the countries. Therefore, it is necessary to protect the water ecosystem from pollution and to protect public and environmental health. The histological and biochemical findings of this study showed that there is pollution in the lake, although heavy metal amounts are below specified standards in the analysis of water and fish tissues. These results suggest that Sapanca Lake may be under the influence of not only heavy metal pollution but also other pollutants.

Nowadays, many scientific researchers are conducted on the characterization and remediation methods of contaminated water systems and the sources polluting them, and the results are published. The aquatic ecosystem's organisms, such as fish, are commonly used to assess aquatic environmental quality and are considered as bioindicators of environmental pollution. Continuing to regularly monitor the water quality and biological parameters of the lake will help keep the condition of the lake under control. Future studies should pay more attention to what concentrations of pollution have become dangerous for aquatic organisms and humans. As a result, a clearer prediction should be provided about the measures to be taken by determining the in vivo and in vitro effects of the pollutants in the lake on the organic and inorganic environment.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Gullu KAYMAK carried out the collecting of samples, analysis, reporting, correction of literature sources, article writing, and publishing procedures. In addition to Nazan Deniz YON ERTUG, who was the thesis advisor, Figen Esin KAYHAN contributed to the reporting, analysis, and statistical analysis of the data, review, and editing of the article.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

This study was funded by Scientific Research Projects Commission of Sakarya University with project number 2015-50-02-007.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

We thank the TÜBİTAK-BİDEB 2211-C National Doctoral Scholarship Program for Priority Areas as it provides financial support during the doctoral thesis study.

References

Abdel-Baki A.S., Dkhil M.A., AlQuraishy S. (2011). Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. African Journal of Biotechnology, 10(13), 2541-2547. Doi: https://doi.org/10.5897/AJB10.1772

Aboul Ezz A.S., Abdel-Razek S.E. (1991). Heavy metal accumulation in the *Tilapia nilotica* L. and in the waters of Lake Manzalah. Egypt. J Applied Sci 6, 37-52.

Adasu General Directorate (2003). Sapanca Gölü Çevre Koruma Projesine Esas Bilgiler, Adapazarı (Basics of Sapanca Lake Environmental Protection Project, Adapazarı). (in Turkish)

Aebi H. (1974). Catalase *In vitro*. In: Methods of Enzymatic Analysis. Ed: Bergmeyer HU, 2nd ed, FL, 121-126.

Ahmad V.L., Maria M., Oliveira M., Pacheco M., Santos A. (2006). Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla* L. exposed to chromium with or without pre-exposure to betanaphthoflavone, Mutat. Res. 608, 16-28. Doi: https://doi.org/10.1016/j.mrgentox.2006.04.020

Al-Bairuty G.A. (2013). Histopathological effects of metal and metalic nanoparticles on the body systems of rainbow trout (*Oncorhynchus mykiss*). Doctoral Thesis, University of Plymouth.

Altug G., Okgerman H. (2008). Levels of some toxic elements in the surface sediment and some biota from the Sapanca Lake, Turkey. Fresenius Environmental Bulletin, *17*(1), 24-28. Retrieved from https://www.prt-parlar.de/download_feb_2008/1

Altundag H., Yıldırım E., Altıntıg E. (2019). Determination of some heavy metals by ICP-OES in edible parts of fish from Sapanca Lake and streams. Journal of Chemical Metrology, *13*(1). Doi: http://doi.org/10.25135/jcm.24.19.03.1219

AnvariFar H., Amirkolaie A.K., Jalali A. M., Miandare H.K., Sayed A.H., İşisağ-Üçüncü S., Ouraji H., Ceci M., Romano N. (2018). Environmental pollution and toxic substances: Cellular apoptosis as a key parameter in a sensible model like fish. Aquatic toxicology, 204:144-159. Doi: http://doi.org/10.1016/j.aquatox.2018.09.010

Authman M.M., Zaki M.S., Khallaf E.A., Abbas H.H. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. Journal of Aquaculture Research & Development, 6(4), 1-13. Doi: http://doi.org/10.4172/2155-9546.1000328

Bakan G., Balkas T.I. (1999). Enrichment of metals in the surface sediments of Sapanca Lake. Water Environment Research, 71 (1):71-74. Doi: http://doi.org/10.2175/106143099X121463

Beutler E. (1975). Glutathione in Red Cell Metabolism: A Manual of Biochemical Methods, 2nd ed., Grune and Stratton, NY, 112-114.

Bradford M.M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantitites of Protein Utilizing the Principle of Protein-dye Binding. Analytical Biochemistry, 72, 248-254. Doi: https://doi.org/10.1016/0003-2697(76)90527-3

Brand D.G., Fink R., Bengeyfield W., Birtwell I.K., McAllister C.D. (2001). Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stressrelated visceral lesions after 10-day exposure to sublethal concentrations of the water-soluble fraction of north-slope crude oil. Toxicologic Pathology, 9(5):574- 584. Doi: https://doi.org/10.1080/019262301317226384

Bury N.R., Walker P.A., Glover C.N. (2003). Nutritive metal uptake in teleost fish. The Journal of Experimental Biology, 206:11-23. Doi: https://doi.org/10.1242/jeb.00068

Çakır Z., Barka A., Akyüz S. (2010). Coulomb stress interactions and the 1999 Marmara earthquakes. İTÜ *Dergisi/d*, *2*(4):1-4. Retrieved from https://journals.tubitak.gov.tr/earth/abstract.htm?id=6021

Canpolat O., Eroglu M., Dusukcan M. (2016). Transfer factor of some heavy metals in muscle of *Cyprinus carpio*. Fresenius Environ Bull, 25(11), 4988-4994. Retrieved from https://www.prt-parlar.de/download_feb_2016/11

Chen G., White P.A. (2004). The mutagenic hazards of aquatic sediments. A Review. Reviews in Mutation Research, 567:151-225. Doi: https://doi.org/10.1016/j.mrrev.2004.08.005

Cheung C.C.C., Zheng G.J., Li A.M.Y., Richardson B.J., Lam P.K.S. (2001). Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of Marine Mussels, *Perna viridis*. Aquatic Toxicology, 52:189-203. Doi: https://doi.org/10.1016/S0166-445X(00)00145-4

Dostbil M. (2010). Determination of heavy metal concentrations in water and sediment of mogan lake; examination of effects on tissues of carp (*Cyprinus carpio*) and tench (*Tinca tinca*). Master's Thesis, Gazi University. (in Turkish).

Duman F., Sezen G., Nilhan Tug G. (2007). Seasonal changes of some heavy metal concentrations in Sapanca Lake water, Turkey. International Journal of Natural on Engineering Sciences, 1(3):25-28.

Dündar M.Ş., Altundağ H., Boz V., Akaya K., Sayın M. (2003). A Comparative Analysis of Same Trace Elements in Rivers Flow Through Sapanca Lake Before and After 17 August 1999 Marmara Earthquake. Anadolu University Journal of Science and Technology, 4 (2):205-210 (in Turkish).

Engin M.S., Uyanik A., Cay S., Kir I. (2016). Evaluation of trace metals in sediment, water, and fish (*Mugil cephalus*) of the central Black Sea coast of Turkey. Human and Ecological Risk Assessment: An International Journal, 22(1): 241-250. Doi: https://doi.org/10.1080/10807039.2015.1057685

Fırat Ö., Kargın F. (2010). Effects of zinc and cadmium on erythrocyte antioxidant systems of a freshwater fish *Oreochromis niloticus*. J. Biochem. Molecular Toxicology, 4:145-149. Doi: https://doi.org/10.1002/jbt.20327

Fonseca A.R., Fernandes L.S., Fontainhas-Fernandes A., Monteiro S.M., Pacheco F.A.L. (2017). The impact of freshwater metal concentrations on the severity of histopathological changes in fish gills: A statistical perspective. Science of the Total Environment, 599: 217-226. Doi: https://doi.org/10.1016/j.scitotenv.2017.04.196

Furia R.R. (2004). Preliminary aspects of water soluble fraction of diesel oil effects on gill histology of *Trachinotus sp.* behavior, physiology and toxicology interactions in fish. International Congress on the Biology of Fish. Manaus, Brazil, August 1-5, pp. 343-349.

Gülcü-Gür, B., Tekin-Özan, S. (2017). The investigation of heavy metal levels in water and sediment from Işıklı Lake (Turkey) in relation to seasons and physicochemical parameters. Journal of Aquaculture Engineering and Fisheries Research, 3(2):87-96. Doi: https://doi.org/10.3153/JAEFR17012

Güngördü A., Belda E., Dürdane K. (2012). Evaluation of spatial and temporal changes in biomarker responses in the common carp (*Cyprinus Carpio* L.) for biomonitoring The Meriç Delta, Turkey. Environmental Toxicology and Pharmacology, 33:431–439. Doi: https://doi.org/10.1016/j.etap.2012.01.003

Hadi A.A., Alwan S.F. (2012). Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, Exposed to Aluminum. International Journal of Pharmacy & Life Sciences, 3(11): 2071-2081.

Heath A.G. (1987). Water pollution and fish physiology. CRP Press Inc. 245 s. Florida.

Hermenean A., Damache G., Albu P., Ardelean A., Ardelean G., Ardelean D.P., Horge M., Nagy T., Braun M., Zsuga M., Kéki S., Costache M., Dinischiotu A. (2015). Histopatological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. Ecotoxicology and environmental safety, 119:198-205. Doi: https://doi.org/10.1016/j.ecoenv.2015.05.029

Hesni M.A., Savari A., Sohrab A.D., Mortazavi M.S. (2011). Gill histopathological changes in milkfish (*Chanos chanos*) exposed to acute toxicity of diesel oil. World Applied Sciences Journal, 14(10):1487-1492.

Hinton D., Lauren D. (1990). Liver structural alterations accompanying chronic toxicity in fishes: Potential biomarkers of exposure, in: Biomakers of environmental contamination, McCarthy, J. and Shugart, L. (Eds.), Lewis Publishers, Boca Raton, Florida.

Hinton D.E., Segner H., Au D.W.T., Kullman S.W., Hartman R.C. (2008). Liver toxicity. In: The toxicology of fishes, Di Giulio RT, Hinton DE (Eds), CRC Press Inc., Florida.

Hinton D.E., Segner H., Braunbeck T. (2001). Toxic responses of the liver. In: Daniel, S., Benson, W.H. (Eds.), Target organ toxicity in marine and freshwater teleosts, vol. 1, London.

Holt E. A., Miller S. W. (2011). Bioindicators: using organisms to measure environmental impacts. Nature Education Knowledge, 3(10), 8.

İşisağ Üçüncü S., Önen Ö., Ergen G., Üreten M., Boz E., Seferoğlu K., Gökçe B. (2010). The Effects of Dioctyl Adipate (DOA) on the gill histology of *Labidochromis caeruleus* Fryer, 1956 (Cichlidae, Teleostei). Kafkas Üniversitesi Veterinerlik Fakültesi Dergisi, 16(2):343-346. (In Turkish). Doi: https://doi.org/10.9775/kvfd.2009.933

Javed M., Ahmad I., Usmani N., Ahmad M. (2016). Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in *Channa punctatus* from heavy metal polluted canal. Chemosphere, 151:210-219. Doi: https://doi.org/10.1016/j.chemosphere.2016.02.080

Junianto Z., Apriliani I.M. (2017). Evaluation of heavy metal contamination in various fish meat from Cirata Dam, West Java, Indonesia. *AACL Bioflux*, *10*(2)242-248.

Kahvecioğlu Ö., Kartal G., Güven A., Timur S. (2003). Metallerin çevresel etkileri-I (environmental effect of metals-I). *Metalurji Journal* 136:47–53. (in Turkish)

Karadag H., Fırat Ö., Fırat Ö. (2014). Use of oxidative stress biomarkers in *Cyprinus carpio* L. for the evaluation of water pollution in Ataturk Dam Lake (Adiyaman, Turkey). Bulletin of Environmental Contamination and Toxicology, *92*(3), 289-293. Doi: https://doi.org/10.1007/s00128-013-1187-0

Kargın F., Erdem C. (1992). Metal accumulation of *Tilapia nilotica* (L.) in liver, gill and muscle tissues in copper-zinc interaction. Turkish Journal of Zoology, 16:343-348.

Katalay S., Parlak H., Arslan Ö.Ç. (2005). The accumulation of some heavy metals in the liver tissues of black gobby (*Gobius niger* L., 1758) living in the aegean sea. Ege University Journal of Fisheries& Aquatic Sciences, 22(3-4):385 – 388. Doi: https://doi.org/10.12714/egejfas.2005.22.3.5000156939

Kır I., Erdogan M., Engin M. S., Cay S. (2016). Trace metals in sediment, water and their bioaccumalation in Carp (*Cyprinus Carpio* L., 1758) samples, captured from the Karacaören (I) Dam Lake, Turkey. Fresenius Environmental Bulletin, 35-57. https://www.prt-parlar.de/download_feb_2016

Köhler A., Deisemann H., Lauritzen B. (1992). Histological and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. Marine ecology progress series. 91(1): 141-153. Doi: https://doi.org/10.3354/meps091141

Kuş U.Ş. (2012). Metazoan parasites of rudd *Scardinius erythrophthalmus* (Linnaeus, 1758) in lake sapanca. Master's Thesis, Marmara University. (in Turkish).

Ledwozyw A., Michalak D., Stepien A., Kadziolka A. (1986). The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clin Chim Acta*, 155(3), 275-283. Doi: https://doi.org/10.1016/0009-8981(86)90247-0

Mahboob S. (2013). Environmental pollution of heavy metals as a cause of oxidative stress in fish: a review. Life Sci. J, 10, 336-347. Retrieved from http://www.lifesciencesite.com/lsj/life1010s/056_20923life1010s_336_347.pdf

Mustafa S.A. (2020). Histopathology and heavy metal bioaccumulation in some tissues of *Luciobarbus xanthopterus* collected from Tigris River of Baghdad, Iraq. The Egyptian Journal of Aquatic Research. In Press. Doi: https://doi.org/10.1016/j.ejar.2020.01.004

Mwamburi J. (2009). Trace metal concentration in water and sediments of satellite lakes within Lake Victoria. Lakes & Reservoirs: Research & Management, 14(3):203–220. Doi: https://doi.org/10.1111/j.1440-1770.2009.00408.x

Nammalwar P. (1992). Fish bioassay in the Cooum and Adyar estuaries for environmental management In: Singh KP (ed.). Tropical Ecosystems. Ecology and Management. Wiley Eastern, Delhi. 359–370.

Okgerman H., Elp M., Dorak Z., Yardımcı H.C., Yılmaz N., Yiğit S. (2006). Changes on the fish fauna of Lake Sapanca. In II National Workshop for Limnology, 6-8.

Oliveira Ribeiro C.A., Vollaire Y., Sanchez-Chardi A., Roche H. (2005). Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquatic Toxicology, 4:165-171. Doi: https://doi.org/10.1016/j.aquatox.2005.04.008

Ossana N. A., Baudou F.G., Castañé P.M., Tripoli L., Soloneski S., Ferrari L. (2019). Histological, genotoxic, and biochemical effects on *Cnesterodon decemmaculatus* (Jenyns 1842) (Cyprinodontiformes, Poeciliidae): early response bioassays to assess the impact of receiving waters. Hindawi Journal of toxicology,1-13. Doi: https://doi.org/10.1155/2019/4687685

Özyürek F. (2016). Heavy metal accumulation (Cd, Cr, Cu, Fe, Ni, Pb, Zn) in vegetables irrigated with different water resources in Nevşehir. Master's Thesis, Nevşehir Hacı Bektaşi Veli University. (in Turkish).

Padmini E., Vijaya Geetha B., Usha Rani M. (2008). Liver oxidative stress of the grey mullet *Mugil cephalus* presents seasonal variations in Ennore estuary. Brazilian Journal of Medical and Biological Research, 41(11):951-955. Doi: https://doi.org/10.1590/S0100-879X2008005000038

Pandey S., Parvez S., Sayeed I., Haque R., Bin-Hafeez Raisuddin S. (2003). Biomarkers of oxidative stress: A comparative study of River Yamuna Fish *Wallago attu* (Bl. & Schn.). The Science of The Total Environment, 309:105- 115. Doi: https://doi.org/10.1016/S0048-9697(03)00006-8

Pérez-Coyotl I., Galar-Martínez M., García-Medina S., Gómez-Oliván L. M., Gasca-Pérez E., Martínez-Galero E., Islas-Flores H., Pérez-Pastén B.R., Barceló D., López de Alda M.. Pérez-Solsona S., Serra-Roig M.P., Montemurro, N., Peña-Herrera, J.M., Sánchez-Aceves, L. M. (2019). Polluted water from an urban reservoir (Madín dam, México) induces toxicity and oxidative stress in *Cyprinus carpio* embryos. Environmental pollution, 251, 510-521. Doi: https://doi.org/10.1016/j.envpol.2019.04.095

Peters N., Köhler A., Kranz H. (1987). Liver pathology in fishes from the lower Elbe as a consequence of pollution. Diseases of Aquatic Organisms, 2:87-97.

Rajeshkumar S., Liu Y., Ma J., Duan H. Y., Li X. (2017). Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, *Cyprinus carpio* L. Fish & shellfish immunology, 70:461-472. Doi: https://doi.org/10.1016/j.fsi.2017.08.013

Rodrigues R.V., Miranda-Filho K.C., Gusmão E.P., Moreira C.B., Romano L.A., Sampaio L.A. (2010). Deleterious effects of watersoluble fraction of petroleum, diesel and gasoline on marine *Pejerrey odontesthesargentinensis* Larvae. Science of the Total Environment, 408:2054–2059. Doi: https://doi.org/10.1016/j.scitotenv.2010.01.063

Şahan A., Kurutaş E.B., Altun T. (2010). The determination of biochemical indicators (biomarkers) in the common carp (*Cyprinus carpio*) to the physicochemical parameters of the Ceyhan River (Adana-Turkey). Ekoloji, 19:8-14. Doi: https://doi.org/10.5053/ekoloji.2010.762

Sarkar A., Ray D., Shrivastava A.N. (2006). Molecular Biomarkers: Their significance and application in marine pollution monitoring. Ecotoxicology, 15:333-340. Doi: https://doi.org/10.1007/s10646-006-0069-1

Sheehan D., Power A. (1999). Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comp. Biochem. Physiol. 123C:193- 199. Doi: https://doi.org/10.1016/S0742-8413(99)00033-X

Simonato J.D., Guedes C.L.B., Martinez C.B.R. (2008). Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicology and Environmental Safety, 69:112-120. Doi: https://doi.org/10.1016/j.ecoenv.2007.01.012

Singh A.K., Pathak A.K., Lakra W.S. (2010). Invasion of an exotic fish—common carp, *Cyprinus carpio* L. (Actinopterygii: Cypriniformes: Cyprinidae) in the Ganga River, India and its impacts. Acta Ichthyol. Piscat. 40 (1): 11-19. Doi: https://doi.org/10.3750/AIP2010.40.1.02

Şişman I., Imamoglu M., Aydin A. O. (2002). Determination of heavy metals in roadside soil from Sapanca area highway, Turkey. Int. J. Environment and Pollution, 17 (4):306-311. Doi: https://doi.org/10.1504/IJEP.2002.000674

SKKY, (2004). Regulation on Control of Water Pollution, T.C. Official newspaper, 25687, 31.12.2004.

Stanley J., Preetha G. (2016). Pesticide toxicity to fishes: exposure, toxicity and risk assessment methodologies. In Pesticide Toxicity to Non-target Organisms (pp. 411-497). Springer, Dordrecht.

Sümer B., Ileri R., Gezbul H., Senol E. (1996). Investigation of trophic state of the Lake Sapanca. In Symposium on Drinking Water, Istanbul Water and Sewage Administration (ISKI), Istanbul, Turkey. (in Turkish)

Syasina I. G., Khlopova A.V., Chukhlebova L.M. (2012). Assessment of the state of the gibel carp *Carassius auratus gibelio* in the Amur River Basin: heavy-metal and arsenic concentrations and histopathology of internal organs. Archives of environmental contamination and toxicology, 62(3):465-478. Doi: https://doi.org/10.1007/s00244-011-9719-2

Turkey Statistical Institute (TUIK) (2018). Population Projections, 2018-2080. News Bulletin, 30567. Retrieved from https://tuikweb.tuik.gov.tr/PreHaberBultenleri.do?id=30567

Udroiu L. (2006). The micronucleus test in Piscine erythrocytes. Aquatic Tox., 79:201-204. Doi: https://doi.org/10.1016/j.aquatox.2006.06.013

Uribe C., Folch H., Enriquez R., Moran G. (2011). Innate and adaptive immunity in teleost fish: a Review. Veterinarni Medicina, 56(10):486-503. Doi: https://doi.org/10.17221/3294-VETMED

Uysal K. (2010). Heavy metal in edible portions (muscle and skin) and other organs (gill, liver and intestine) of selected freshwater fish Species. International Journal of Food Properties, 14:280-286. Doi: https://doi.org/10.1080/10942910903176378

Valavanidis A., Vlahogianni T., Dassenakis M., Scoullos M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicology and environmental safety 64:178-189. Doi: https://doi.org/10.1016/j.ecoenv.2005.03.013

Yalçın N., Sevinç V. (2001). Heavy metal contents of Lake Sapanca. Turk. J. Chem., 25:521-525. Retrieved from https://journals.tubitak.gov.tr/chem/issues/kim-01-25-4/kim-25-4-16-0010-37.pdf

Yeşilbudak B., Erdem C. (20149. Cadmium accumulation in gill, liver, kidney and muscle tissues of common carp, *Cyprinus carpio*, and nile Tilapia, *Oreochromis niloticus*. Bull. Environ. Contam. Toxicol. 92:546-550. Doi: https://doi.org/10.1007/s00128-014-1228-3

Yılmaz E. Türkiye'de İçsu Balıkçılığının Mevcut Durumu. Ziraat Mühendisliği, (361), 28-32. Retrieved from https://dergipark.org.tr/en/download/article-file/946517

Yildirim N.C., Benzer F., Danabas D. (2011). Evaluation of environmental pollution at Munzur River of Tunceli applying oxidative stress biomarkers in *Capoeta trutta* (Heckel, 1843). The journal of Animal & plant sciences, 21(1):66-71. Retrieved from http://thejaps.org.pk/docs/21(1)2011/EVALUATION-ENVIRONMENTAL.pdf

Zuo J., Fan W., Wang X., Ren J., Zhang Y., Wang X., Zahnag Y., Yu T., Li X. (2018). Trophic transfer of Cu, Zn, Cd, and Cr, and biomarker response for food webs in Taihu Lake, China. RSC advances, *8*(7), 3410-3417. Doi: https://doi.org/10.1039/C7RA11677B