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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Seasonal changes in antioxidant defense system indicators in the tissues of *Cyprinion macrostomus* (Heckel, 1843) caught from Göynük Stream (Bingöl, Turkey)

Göynük Çayı'nda (Bingöl) yakalanan *Cyprinion macrostomus* dokularında antioksidan savunma sistemi göstergelerindeki mevsimsel değişiklikler

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Abstract: In this study, antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD)) and malondialdehyde (MDA) levels occurring throughout the year were examined in *Cyprinion macrostomus* tissues (kidney, gill, liver and gonad) captured from Göynük Stream (Bingöl, Turkey). For this purpose, two locations (Ilıcalar and Garip) where fish can be caught regularly in summer, autumn, winter and spring were determined. Fish were caught regularly from these two locations every month and brought to the laboratory. Spectrophotometric methods were used to determine enzyme activities and MDA levels in the study. As a result of the study, it was determined that the MDA level and enzyme activities between Ilıcalar and Garip stations, in general, were statistically different from each other in all tissues. However, it was observed that there were important differences in general between the seasons at both stations. In addition, while GR and G6PD activities were lower than other enzyme activities throughout the study, CAT and SOD activities were higher.

Keywords: Doctor fish, freshwater systems, Murat River, Göynük Stream, oxidative stress

Öz: Bu çalışmada, Göynük Çayı'ndan (Bingöl, Türkiye) yakalanan *Cyprinion macrostomus* dokularında (böbrek, solungaç, karaciğer ve gonad) yıl boyunca meydana gelen antioksidan enzim aktiviteleri (süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz GPx), glutatyon redüktaz (GR) ve glukoz 6-fosfat dehidrojenaz (G6PD)) ve malondialdehit (MDA) seviyelerindeki değişimler mevsimsel olarak incelenmiştir. Bu amaçla yaz, sonbahar, kış ve ilkbaharda düzenli olarak balık yakalanabilecek iki lokasyon (Ilıcalar ve Garip) belirlenmiştir. Bu iki lokasyondan her ay düzenli olarak balıklar yakalanarak laboratuvara getirilmiştir. Çalışmada enzim aktivitelerini ve MDA düzeylerini belirlemek için spektrofotometrik yöntemler kullanılmıştır. Çalışma sonucunda genel olarak Ilıcalar ve Garip istasyonları arasındaki MDA düzeyi ve enzim aktivitelerinin tüm dokularda istatistiksel olarak birbirinden farklı olduğu belirlendi. Bununla berarber, her iki istasyonda da mevsimler arasında genel olarak anlamlı farklılıkların olduğu gözlemlenmiştir. Ayrıca çalışmada, GR ve G6PD aktiviteleri diğer enzim aktivitelerinden daha düşük iken, CAT ve SOD aktivitelerinin diğer enzim aktivitelerinden daha yüksek olduğu tespit edilmiştir.

Anahtar kelimeler: Doktor balık, tatlısu sistemleri, Murat Nehri, Göynük Çayı, oksidatif stress

INTRODUCTION

Today, one of the most important dangers for all living things in the ecosystem is environmental pollution. Environmental pollution has increased especially in parallel with the start of urban life and the realization of the industrial revolution. As a result, the aquatic ecosystem is affected the most from this pollution in our country as in the whole world (Sökmen et al., 2018; Güneş et al., 2019; Taysı et al., 2021; Kirici et al., 2022). Different pollutants that enter the aquatic environment and pose a great threat to fish, catalyze oxidative reactions; They lead to the formation of reactive oxygen compounds such as hydrogen peroxide, superoxide, singlet oxygen and hydroxyl radical. These radicals are highly reactive compounds and cause oxidation and impairment of the functions of important biological molecules such as deoxyribonucleic acid (DNA), protein and lipid (Yu, 1994; Castillo et al., 2002). The harmful effects of reactive oxygen compounds are neutralized by antioxidant defense systems. This system, known as antioxidant defense, includes the

enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD) (Figure 1), which are low molecular weight structures. It includes non-enzymatic structures such as vitamins A, E, C, carotenes, ubiquinone10. In a healthy cell, there is a physiological balance between reactive radicals formed as a result of metabolic reactions and the level of antioxidant molecules formed by various defense mechanisms (Finkel and Holdbrook, 2000; Yonar et al., 2016). Disruption of this balance towards oxidants is defined as oxidative stress (Sies, 1997). This may result in impairment of cell functions, apoptosis or necrosis. Therefore, the functionality of antioxidant defense systems and ensuring the balance of oxidants/antioxidants are vital for the cell (Nordberg and Arner, 2001). Increased free radicals also cause lipid peroxidation, causing impairment of cell membrane functions. As a result, malondialdehyde (MDA), which is the breakdown product of lipid peroxidation, is formed and is used as an indicator in determining the oxidative damage of lipids (Kasai, 1997).

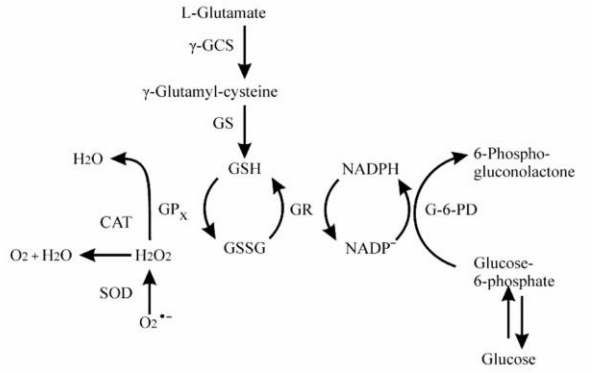

Figure 1. Key antioxidant enzymes and the reactions they catalyze are shown (Kehinde et al., 2016)

Oxidative stress in fish is affected by many factors. Temperature, salt adaptation, hunger, xenobiotics and diseases are the main ones. Increase in temperature increases metabolic activities in all living things. With increasing metabolism, the amount of oxygen needed increases and as a result, the total oxidant level increases. It has been reported that oxidative stress increases depending on the temperature in different living groups (Almroth et al., 2015). Fish with poikilotherm are strongly affected by water temperature; therefore, they constantly adjust their bodies to environmental conditions. They are widely used in biomonitor studies (Aleshko and Lukyanova, 2008). Many physiological changes are observed during salt adaptation in fish. These are increased energy metabolism, adjustment of ion balance, molecular and cellular changes, and hormonal regulations. Reactive oxygen species are formed in the tissues of fish during salt adaptation, both experimentally created and in the natural environment, causing oxidative damage (Liu et al., 2007; Wilson et al., 2014). Prolonged starvation has caused oxidative damage, particularly in the liver, where energy metabolism occurs in fish as well as in mammals (Morales et al., 2004; Bayir et al., 2011). It has been reported that foods with different contents in fish have an effect on the oxidative status in the liver. Especially foods with high lipid content increased antioxidant enzyme levels (Rueda-Jasso et al., 2004).

Cyprinion macrostomus (Figure 2) is distributed in West Asia, India, Afghanistan, Iran, Syria and Mesopotamia and is located in the Euphrates-Tigris system in our country. These two species are widely distributed, especially in thermal hot springs in the Euphrates and Tigris River Basin (Çelik and Güzel, 2017). It is known that these fish can easily survive in wide temperature ranges, they can survive in waters with a pH level of about 7.3 and isothermal, even in waters with temperatures around 35ºC throughout the year (Değirmenci and Ünver, 2021). It is known that such fish species help to heal some skin diseases (psoriasis, eczema and purulent wounds), the origin of their use for therapeutic purposes in spas goes back thousands of years and such practices continue today (Demir, 2009; Çelik and Güzel, 2017).

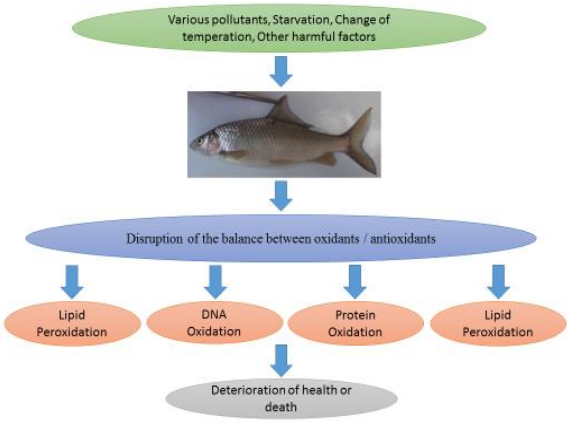

Figure 2. Schematic representation of major events in accrued damage of various harmful factors in *Cyprinion macrostomus*

In this study, 2 stations were determined from the Göynük Stream, which is a branch of the Murat River, which passes through the north of Bingöl Province Genç District and flows into the Keban Dam. These two stations were chosen because the fish could be caught regularly throughout the year. In addition, Ilıcalar station is a region where thermal springs are located and therefore has temperature values above seasonal norms. The other station is Garip station, where the water temperature is relatively cold, passing mostly through the residential area and close to Genç district. These stations were selected based on the migration criteria of these species, which tend to migrate to warm waters. The aim of this study was to investigate the seasonally the changes occurring antioxidant enzyme (SOD, CAT, GPx, GR and G6PD) activities and MDA levels in kidney, gill, liver and gonad tissues of *C. macrostomus* fish caught from Ilıcalar and Garip stations in the Göynük Stream. In addition, the suitability and sensitivity of fish oxidative stress biomarkers for early detection of the health of the freshwater ecosystem were evaluated.

MATERIAL AND METHODS

Study area and stations

In this study, 2 stations (Ilıcalar and Garip) were determined from the Göynük Stream, which is a branch of the Murat River, which passes through the north of Bingöl Province Genç District and flows into the Keban Dam (Figure 3).

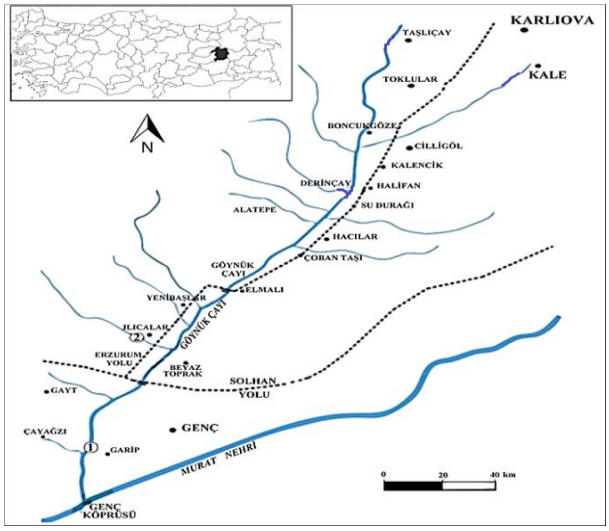

Figure 3. Stations (1. Garip; 2. Ilıcalar) (Modified from Koyun et al., 2018)

Homogenate preparation for enzyme activity determination

The fish were anesthetized using anesthetics. The abdomen was opened and the kidney, gonad, liver and gills were removed and treated with 0.9% NaCl to remove the blood from the tissues. After these procedures, the tissues were stored at -80ºC in the deep freezer until the time of use (Kirici et al., 2017).

10 g of each kidney, gonad, liver and gill tissue were weighed on a precision scale. The weighed tissue samples were thoroughly cut into small pieces with scissors or a scalpel. The fragmented tissue samples were taken into porcelain mortar cooled at -80ºC beforehand, and some liquid nitrogen was added on it and crushed until it became a dough. 3 times the amount of KH_2PO_4 buffer solution was added to the pulp tissue sample. After the samples were taken into the centrifuge tube, they were centrifuged at 13000 rpm for 2 hours at 4ºC. Enzyme activities were studied by taking the supernatant after centrifugation (Beutler, 1975).

Determination of lipid peroxidation

Lipid peroxidation product MDA level, absorbances at maximum 532 nm were measured in Shimadzu UV / VIS-1201 spectrophotometer as a result of color reaction with TBA reagent. In a tube, 200 µl of extracted tissue samples were taken and suspended on it with 800 µl phosphate buffer and 25 µl BHT. Then 500 µl of 30% TCA was added. The tubes were kept in a refrigerator at -20ºC for 2 hours by vortexing. It was then centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant was taken and transferred to other tubes. 75 µl EDTA Na_2H_2O and 250 µl TBA were added on top of this. After vortex mixing, the tubes were kept in a hot water bath (90ºC) for 15 minutes. Then it was brought to room temperature and its optical densities were read at 532 nm (Slater, 1984).

Measuring the levels of antioxidant enzymes

The amount of NADP reduced during its formation from glucose-6-phosphate and 6-phosphoglucanolac is directly proportional to the activity of the G6PD enzyme that catalyzes this reaction. The measurement of enzyme activity is based on the determination of the absorbance difference of NADPH formed during the reaction at 37ºC and 340 nm wavelength per unit time (Beutler, 1975).

GR activity was determined by the method of Carlberg and Mannervik (1985). The reaction mixture consisted of 100 µL 50 mM Tris-HCl + 1 mM EDTA (pH 8.0), 100 µL 20 mM GSSG, 100 µL 2 mM NADPH substrate in 600 µL distilled water in a total volume of 1 ml and supernatant containing 100 µL enzyme. The reaction was carried out by observing the absorbance change in every 1 minute for 3 minutes in quartz cuvettes with a light path of 1 cm at 340 nm wavelength of NADP, which is formed when the enzyme reduces GSSG in the presence of NADPH at 25ºC.

The SOD enzyme was determined by the method modified by Sun et al. (1988). The principle of this method is based on the reduction of nitroblue tetrazolium (NBT) by the xanthine-xanthine oxidase system, which is the superoxide producer. SOD activity was expressed as units $gram^{-1}$ (U g^{-1}) tissue protein.

GPx activity was studied according to the method of Beutler (1975). GPx catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) in the presence of hydrogen peroxide. In the presence of hydrogen peroxide, the GSSG formed by GPx is reduced to GSH with the help of GR and NADPH. GPx activity was calculated by reading the absorbance decrease at 340 nm during the oxidation of NADPH to NADP + and was expressed as units $gram^{-1}$ (U g^{-1}) tissue protein.

CAT activity was determined according to Aebi (1983) method. The method is based on reading the enzymatic degradation of the H_2O_2 substrate with CAT at 240 nm (Aebi, 1983).

Protein Determination

Tissue protein determination is performed by Lowry et al. (1951) was done spectrophotometrically by the method described. This method is based on the complex formation of peptide bonds of proteins with copper ions in an alkaline environment. The copper-peptide complexes react with the folin reagent to form a blue-purple color and are read in the spectrophotometer against blank at 750 nm.

Statistics

Data are expressed as mean ± standard error. Data were subjected to One-Way ANOVA and Duncan test was used to determine the significant difference between control and experimental groups using the SPSS 23.0 computer program. P<0.05 value was considered statistically significant.

RESULTS

While the difference between MDA level, CAT, GR, G6PD and GPx activities in kidney tissue between Ilıcalar and Garip stations was statistically significant in all seasons, the difference between SOD activities was not significant (P<0.05). The highest activity value in kidney tissue was detected in CAT activity in Ilıcalar station in summer, and the lowest in G6PD activity in Ilıcalar station in spring. However, the differences between the seasons in the levels of MDA and enzyme activities at the two stations were found to be statistically significant. Only at Garip station, there was no statistical difference between seasons in G6PD activities (P<0.05) (Table 1).

Table 1. Levels of oxidative stress parameters in *C. macrostomus* kidney caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	51.76 ± 8.04^{a,*}	4.10 ± 1.09^{a}
	Autumn	56.53 ± 11.29^{a,*}	10.20 ± 2.71^{b}
	Winter	40.14 ± 9.20^{b,*}	1.71 ± 0.82^{c}
	Spring	73.03 ± 15,61^{c,*}	2.90 ± 0.91^{c}
SOD	Summer	7.75 ± 0.87^{a}	8.06 ± 0.64^{a}
	Autumn	5.24 ± 0.70^{b}	4.98 ± 0.24^{b}
	Winter	4.75 ± 0.87^{b}	5.33 ± 0.41^{b}
	Spring	4.06 ± 0.57^{b}	4.41 ± 0.32^{b}
CAT	Summer	254.27 ±	30.72 ± 3.21^{a}
	Autumn	121.15± 24.11^{b,*}	62.74 ± 5.77^{b}
	Winter	150.44 ±	45.69 ± 4.45^{c}
	Spring	117.82 ±	48.63 ± 4.80^{c}
GR	Summer	0.21± 0.02^{a,*}	4.41 ± 0.43^{a}
	Autumn	0.33± 0.03^{a,c,*}	8.43 ± 0.72^{b}
	Winter	0.59± 0.04^{b,*}	2.14 ± 0.44^{c}
	Spring	0.39± 0.03^{c,*}	7.72 ± 0.59^{b}
G6PD	Summer	0.024± 0.001^{a,*}	1.56 ± 0.09
	Autumn	0.023 ± 0.004^{a,*}	1.59 ± 0.09
	Winter	0.039 ± 0.001^{b,*}	2.01 ± 0.23
	Spring	0.009± 0.0003^{c,*}	1.34 ± 0.56
GPx	Summer	68.31 ± 9.26^{a,*}	13.53 ± 1.29^{a}
	Autumn	71.32 ± 13.88^{a,*}	40.76 ± 2.72^{b}
	Winter	45.37 ±11.03^{b,*}	11.90 ± 1.84^{a}
	Spring	63.44 ± 12.49^{a,*}	38.89 ± 2.03^{b}

*P<0.05 when compared with values at Garip
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P<0.05)

In the gill tissue, there was no statistically significant difference in MDA level between stations in all seasons (P<0.05). However, the difference between seasonal values of SOD, CAT, G6PD and GPx activities between Ilıcalar and Garip stations was statistically significant (P<0.05). Although there was no statistically significant difference between the stations in the summer, autumn and winter seasons in GR activity, it was found higher at Garip station than at Ilıcalar station in the spring season, and the difference between them was found to be statistically significant. At the Garip station, there is a statistically significant difference between the values of MDA levels and enzyme activities between seasons. At Ilıcalar station, there were no statistically significant differences between the values of GR and G6PD activities between seasons, while the differences in other parameters were found to be statistically significant (P<0.05) (Table 2).

Table 2. Levels of oxidative stress parameters in *C. macrostomus* gill caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	7.86 ± 1.31^{a}	7.09 ± 1.64^{a}
	Autumn	4.15 ± 0.84^{b}	4.22 ± 0.85^{b}
	Winter	5.49 ± 1.19^{b}	4.09 ± 0.35^{b}
	Spring	4.09 ± 0.98^{b}	3.36 ± 0.29^{b}
SOD	Summer	34.46 ± 3.30^{a,*}	243.24 ± 21.04^{a}
	Autumn	15.71 ± 1.19^{b,*}	231.11 ± 29.84^{a}
	Winter	19.91 ± 1.24^{b,*}	317.00 ± 39.04^{b}
	Spring	11.60 ± 2.00^{b,*}	226.71 ± 21.01^{a}
CAT	Summer	111.49 ± 10.61^{a,*}	64.73 ± 24.39^{a}
	Autumn	318.58 ± 23.17^{b,*}	22.29 ± 7.91^{b}
	Winter	128.18 ± 11.72^{a,*}	43.89 ± 10.47^{c}
	Spring	141.82 ± 15.29^{a,*}	28.05 ± 9.87^{b}
GR	Summer	1.23 ± 0.19	1.56 ± 0.44^{a}
	Autumn	0.97 ± 0.20	1.53 ± 0.25^{a}
	Winter	1.57 ± 0.28	2.02 ± 0.39^{a}
	Spring	1.09± 0.33*	4.96 ± 0.97^{b}
G6PD	Summer	0.21 ± 0.002*	2.67 ± 0.35^{a}
	Autumn	0.19 ± 0.001*	1.10 ± 0.11^{a}
	Winter	0.21 ± 0.002*	4.28 ± 0.42^{b}
	Spring	0.26 ± 0.002*	6.85 ± 0.92^{c}
GPx	Summer	75.06 ± 8.20^{a,*}	37.68 ± 4.32^{a}
	Autumn	53.34 ± 4.11^{b,*}	17.49 ± 1.11^{b}
	Winter	72.33 ± 7.91^{a,*}	15.53 ± 1.75^{b}
	Spring	70.61 ± 7.73^{a,*}	20.97 ± 3.03^{b}

*P<0.05 when compared with values at Garip
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P<0.05)

Although no statistically significant difference was found between the stations in the spring season in liver tissue, MDA level and GR activity, it was found higher at Ilıcalar station than at Garip station in summer, autumn and winter seasons, and the difference between them was found to be statistically significant. Significant differences were found between stations in SOD and GPx activities in all seasons. In G6PD activity, the difference between the stations in the summer and autumn seasons is statistically significant, while the difference between the winter and spring seasons is not statistically significant. In addition, no difference was detected between stations in CAT activity. At Garip station, there was no significant seasonal difference in GPx activity. However, statistically significant differences were determined between the MDA level and the activities of enzymes in all stations as seasons (P<0.05) (Table 3).

Table 3. Levels of oxidative stress parameters in *C. macrostomus* liver caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	2.59 ± 0.53^{a,*}	0.65 ± 0.11^{a}
	Autumn	1.08 ± 0.69^{b,*}	0.40 ± 0.12^{a}
	Winter	4.12 ± 0.53^{c,*}	0.32 ± 0.09^{a}
	Spring	2.41 ± 0.45^{a}	1.58 ± 0.26^{b}
SOD	Summer	3.19 ± 0.74^{a,*}	18.53 ± 1.99^{a}
	Autumn	3.15 ± 0.59^{a,*}	39.76 ± 3.35^{b}
	Winter	2.97 ± 0.37^{a,*}	10.54 ± 1.09^{a}
	Spring	1.02 ± 0.70^{b,*}	14.76 ± 1.35^{a}
CAT	Summer	95.53 ± 21.19^{a}	101.78 ±
	Autumn	86.22 ± 16.37^{a}	84.97 ±8.05^{b}
	Winter	58.79 ± 13.04^{b}	63.28 ±2.66^{c}
	Spring	85.90 ± 22.48^{a}	72.80±5.17^{b,c}
GR	Summer	0.25 ± 0.009^{a,*}	5.15 ± 0.82^{a}
	Autumn	0.71 ± 0.054^{b,*}	6.71 ± 1.49^{a}
	Winter	0.31± 0.005^{a,*}	2.22 ± 0.27^{b}
	Spring	1.45 ± 0.177^{c}	2.09 ± 0.55^{b}
G6PD	Summer	4.28 ± 0.59^{a,*}	1.04 ± 0.29^{a}
	Autumn	6.72 ± 0.43^{b,*}	0.88 ± 0.08^{a}
	Winter	2.39 ± 0.09^{c}	2.80 ± 0.08^{b}
	Spring	2.51 ± 0.27^{c}	2.79 ± 0.57^{b}
GPx	Summer	51.19 ± 4.23^{a,*}	11.7±0.71
	Autumn	45.28 ± 7.11^{b,*}	10.4±0.57
	Winter	38.05 ± 8.10^{c,*}	9.7±0.38
	Spring	45.19 ± 10.06^{a,*}	10.7±1.08

*P<0.05 when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

Table 4. Levels of oxidative stress parameters in *C. macrostomus* gonad caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	11.71 ±0.92^{a,*}	7.05 ± 1.02^{a}
	Autumn	26.54 ± 1.96^{b,*}	4.91 ± 0.49^{b}
	Winter	15.77 ± 1.00^{a,*}	1.22 ± 0.37^{c}
	Spring	10.30 ± 0.75^{a,*}	2.19 ± 0.25^{c}
SOD	Summer	27.92 ± 4.18^{a,*}	14.28 ± 0.91^{a}
	Autumn	38.93 ± 6.30^{b}	36.01 ± 3.74^{b}
	Winter	19.48 ± 5.47^{c}	17.79 ± 1.07^{a}
	Spring	13.54 ± 4.13^{c}	14.29 ± 1.78^{a}
CAT	Summer	23.09 ± 2.09^{a}	25.47 ± 8.54
	Autumn	29.21 ± 3.10^{b}	26.12 ± 3.19
	Winter	21.45 ± 2.17^{a}	22.99 ± 1.37
	Spring	23.40 ± 2.32^{a}	21.74 ± 1.54
GR	Summer	3.25 ± 0.64^{a,*}	0.64 ± 0.27^{a}
	Autumn	5.76 ± 1.03^{b,*}	0.53 ± 0.14^{a}
	Winter	6.82 ± 1.48^{b,*}	0.20 ± 0.01^{b}
	Spring	3.72 ± 0.76^{a,*}	0.22 ± 0.01^{b}
G6PD	Summer	1.64 ± 0.12	1.56 ± 0.03
	Autumn	1.87 ± 0.15	1.60 ± 0.03
	Winter	1.94 ± 0.39	2.04 ± 0.02
	Spring	1.78 ± 0.42	1.24 ± 0.01
GPx	Summer	5.80 ± 1.37^{a}	6.12 ± 0.93
	Autumn	6.30 ± 1.03^{a}	6.07 ± 0.33
	Winter	9.72 ± 1.76^{b,*}	5.04 ± 0.54
	Spring	5.85 ± 0.64^{a}	5.75 ± 0.47

*P<0.05 when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P<0.05)

The difference between stations in gonad tissue, MDA level and GR activity was found to be statistically significant. However, the difference between stations in CAT and G6PD activities was not found to be statistically significant. In addition, only the difference in SOD activity in summer was found significant among the stations, while the difference in GPx activity in winter was found to be statistically significant. Although the seasonal differences were determined to be significant in the stations in general, it was determined that the seasonal differences between the G6PD activities at the Ilıcalar station and the CAT, G6PD and GPx activities at the Garip station were not statistically significant (P<0.05) (Table 4).

DISCUSSION

Murat River is one of Turkey's most important water resources. It is polluted by domestic wastewater along with natural pollution and pesticides, which have cumulative negative effects. Biomarkers are frequently employed in ecotoxicology to assess the interaction of the biological system with a chemical, physical, or biological environmental agent. *In vivo* inhibition or induction of biomarkers can be used to evaluate xenobiotic exposure and potential effects on living organisms (Yonar et al., 2011; Yildirim et al., 2014; Kirici et al., 2016a). The use of a biochemical technique to provide early warning of potentially harmful alterations in stressed fish has been promoted. In the field of ecotoxicology, the use of oxidative stress biomarkers has exploded. Antioxidant enzymes are recommended as biomarkers because the first response to environmental effects is given by the antioxidant defense system and they are suitable and reliable for ecotoxicological risk assessment (Farombi et al., 2007; Alak et al., 2011; Yonar et al., 2012; Topal et al., 2014).

Fish are extremely sensitive to anthropogenic contamination, and certain of them can be used as biomonitors to assess the aquatic environment's ecological status. Many variables influence aquatic creatures' resistance to pollution, including their phylogenic location, ecological and biological traits, physiological circumstances, and the presence of effective detoxifying mechanisms (Hotard and Zou, 2008; Kirici et al., 2015; Kirici et al., 2016b). Detection of seasonal biomarker changes of *Cyprinion macrostomus* fish, which are a common species in the Göynük Stream, may be an indicator of their reproductive potential and river health. *C. macrostomus* was selected as sentinel organisms in this study due to their ease of sampling, good adaptability to environmental conditions, and high ecological and economic convenience.

Changes in concentrations and enzyme activity frequently represent cell damage in specific organs in toxicological investigations of acute exposure. The liver is an important organ for metabolic activities and xenobiotic detoxification. Heavy metals can build up to dangerous amounts in the liver and induce pathological alterations in some people. Fish liver tissues have been proposed as a better indicator of water pollution than other organs. Toxic compounds create a change in the fish's physiological state, which has an impact on

enzyme activity. Later, it causes disruption in cell organelles, which may result in an increase in enzyme activity (Vinodhini and Narayanan, 2009; Kaptaner and Dogan, 2019).

As a result of the study, statistically significant differences were determined between MDA levels and enzyme activities in all tissues between stations in general and between seasons in both fish. In the study, in *C. macrostomus* fish, the lowest activity was detected in G6PD enzyme in kidney tissue at Ilıcalar station in the spring season (0.009 ± 0.0003), and the highest activity value in CAT activity in gill tissue in Ilıcalar station in autumn (318.58 ± 23.17).

Gabryelak et al. (1983) and Palace and Klaverkamp (1993) suggested that the antioxidant defense in fish is stronger in spring and summer compared to colder winters. However, Ronisz et al. (1999) did not report an interaction between GPx and CAT activities and water temperature in eelpout *Zoarces viviparous* (L). In this study, it was found that SOD activity increased in the summer, especially in the liver, while the highest GPx activity was obtained in the summer, and Gabryelak et al. (1983) and Palace and Klaverkamp (1993). On the other hand, it was determined that there is a significant increase in CAT activity in muscle tissue in summer and autumn, in gills in winter, in liver in summer and winter, which differed from these studies. In another study, it was found that the basic liver antioxidant enzyme activities were not related to the rising temperature, but changed in autumn in all 3 fish species studied. It has been suggested that fish experience oxidative stress during this period and this has been associated with the pre-breeding period. In addition, it has been mentioned that a rapid decrease in temperature, an increase in daylight and an increase in precipitation can cause stress in fish (Aras et al., 2009). Again, the same researchers (Aras et al., 2009) found that SOD, GPx, CAT, G6PD, GR and GST activities were generally higher in the livers of the 3 different species studied compared to the gills in their study. In our study, especially SOD and GPx activities in the liver were found to be higher than other organs studied in all seasons. Can et al. (2017) examined the seasonal changes of CAT, GPx and SOD activities in their study with Munzur Alası in Munzur Stream. As a result of the study, they found that CAT, GPx and SOD activities increased in summer. However, they found that the difference between seasonal values of SOD activities in liver tissue was significant. They found the difference in GPx activities in muscle, gill and liver tissues between summer and other seasons statistically significant. In addition, they stated that the difference between the seasonal CAT activities in muscle, gill and liver tissues was significant ($P<0.05$).

CONCLUSION

The selected parameters are valuable biomarkers for monitoring aquatic systems, as they provide an early warning signal of xenobiotics that help counteract their adverse effects on aquatic organisms at molecular levels. This approach used in this study has significant potential for use in routine monitoring or evaluation studies of all other aquatic environments. The findings obtained in this study will shed light on the studies to be carried out on the cultivation of *C. macrostomus* in natural conditions, which are used as an alternative treatment method in the treatment of some skin diseases such as psoriasis, eczema and pus. Although oxidant and antioxidant enzyme activities vary according to the seasons, they have also been affected by location, gender, age, date, sexual maturity, contaminants, climatic parameters and reproductive period. Therefore, regular monitoring and evaluation should be done, and surveys should be used to discover the unknowns. It should focus on similar studies, taking into account the factors.

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AUTHORSHIP CONTRIBUTIONS

All authors took part in designing the research, collecting and writing the manuscript. Muammer Kırıcı analysed all data of the study statistically and writing the manuscript. Muammer Kırıcı and Mustafa Koyun prepared their field studies and references. Nurgül Şen Özdemir undertook the editing and application of the article. Muammer Kırıcı and Fatma Caf have edited the graphics and figures of the article. All authors took part in a part of the article. All authors approved the submission and publication of this manuscript.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest on this manuscript.

ETHICS APPROVAL

The research was approved by Bingöl University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with the decision number 06/5 at the meeting held on 13.10.2016. All researchers declare that all trials were conducted in accordance with ethical values.

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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