

Oxidative Stress Mechanism Developed Against Environmental Conditions in Aquatic Organisms: Cyprinus Carpio L. (freshwater fish)

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**Abstract:** As a result of the effect of various factors on aquatic ecosystems, polluting agents such as many heavy metals are mixed, and as a result, the living creatures in the aquatic ecosystem are adversely affected because of contamination with these chemical agents. In recent years, it is important to monitor living organisms and their metabolic processes to determine the cleanliness of water. Therefore, in our study, malondialdehyde (MDA), glutathione (GSH), and Total Antioxidant Status (TAS) which is an oxidative stress marker in the gills, liver, and lungs of *Cyprinus Carpio L.*, which is widely found in the Obruk Dam Lake in Oğuzlar district of Çorum province? A total of 90 *Cyprinus carpio* specimens were collected from different parts of the Obruk Dam Lake. Relevant tissues of the samples were taken and homogenized. Methods suitable for spectrophotometric measurement were used to determine MDA, GSH and TAS levels. According to the results, it was determined that antioxidant biomarkers were higher in the liver and gills. According to the data obtained from our previous studies, it is reported that the water of the Obruk Dam Lake complies with the standards in terms of heavy metals. These data showed that the physiological events that occur in the relevant tissues to provide oxidative balance contribute to the stabilization of the aquatic ecosystem.

**Keywords:** Dam Lake, *Cyprinus carpio*, Malondialdehyde, Glutathione, Total antioxidant status

## 1. Introduction

One of the most important problems caused by industrialization and urbanization in nature is considered as environmental pollution (Appannagari, 2017). An undesirable situation arises when the need for food is proportional to the increase in the world population. One of these environmental problems is water pollution (Khatun, 2017). In addition to the various substances found in the natural structures of water, different substances are mixed during the cycle process between the place and the atmosphere, especially when people use them for various purposes. Density and mixtures of polluting agents in water can have serious adverse effects on biodiversity and vitality in the aquatic environment (Lopez-Lopez et al. 2011). Thus, the physical, chemical, and biological properties of water change positively and negatively. Naturally contaminated substances are polluting the water environment and water pollution occurs (Bashir et al., 2020). In this case, both living things in the water environment, people using this water, and other living things are adversely affected.

It is known that the intense release of pollutants into aquatic environments has negative effects on the environment and living organisms, which is important in terms of revealing oxidative stress situations in aquatic ecosystem creature affected by toxic substances (Soares et al. 2008, Karadag et al., 2014). Oxidative stress is defined as the imbalance between the antioxidant defense system and the production of free radicals that cause the peroxidation of the lipid layer of the cells. Proteins are less affected by free radicals than lipids (Shaw et al., 2022). The system that prevents cell damage due to free radicals is called the "antioxidant defense system". These molecules give the free oxygen radicals a hydrogen ion and bind these radicals to themselves. In this way, they turn them into weak molecules and prevent radical damage (Phaniendra et al., 2015; Sharifi-Rad et al., 2020). Superoxide dismutase (SOD), Total antioxidant activity (TAS), and Glutathione peroxidase (GSH) are the most important antioxidant enzymes (Sharifi-Rad et al., 2020; Shaw et al., 2022). Many living organisms, especially animals, are immediately affected by environmental change and respond to this process behaviorally or physiologically. Animals respond to temperature stress adaptation. These adaptive responses are purely survival, with behavioral displacement (hot/cold search) being observed. Again, at high environmental temperatures, animals reduce feed intake by changing the

feeding regime (Crawshaw, 2011; Mainwaring et al., 2017). Many oxidant antioxidant markers have been proposed to determine the effects of harmful pollutants in aquatic environments on the level of oxidative stress in the environment. (Borkovic' et al. 2005, Gul et al., 2004). Because fish generally store pollutants from the aquatic system, they are often used as bioindicators in environmental research. Therefore, within the scope of this study, total antioxidant status, glutathione (GSH), and malondialdehyde (MDA) are in some tissues (blood, gill, and liver) of carp fish (*Cyprinus carpio* L. 1758) in Obruk Dam Lake are determined and evaluated. We believe that these data will be the basis for further studies on the growth, reproduction, and metabolism of this species.

## 2. Materials and Methods

### 2.1. Study area and characteristic properties of Obruk Dam Lake

The Obruk dam's body volume is 12,000,000 m<sup>3</sup>, the height from the streambed is 127.00 m., the lake volume normal water level is 661.11 hm<sup>3</sup>, and the lake area at normal water level is 50.21 km<sup>2</sup>. While the dam provides irrigation services to an area of 5,538 hectares, it produces 473 GWh of energy annually with 203 MW of power (Fig. 1).



Fig. 1. Sampling sites' location in the Dam Lake

### 2.2. Material Examples

The study was done on carp fish (500-650 g; 38-50 cm) caught at different times [2015- April (n:20), July (n:20), October (n:25) and 2016-January (n:25)] in Obruk Dam Lake (40° 46' 13.0044" and 34° 47' 16.9980").

A total of 90 *Cyprinus carpio* specimens were collected from different regions of Obruk Dam Lake. The liver and gill tissues of the samples were removed, and the tissues were immediately frozen in liquid nitrogen and stored at -80 ° C until use. Blood samples were taken at the relevant center under the supervision of the project manager, the serum was separated. Serums were protected against light and stored in a -80 0C freezer until the study was performed.

### 2.3. Homogenization of tissue samples

Tissue samples were homogenized using IKA T25 Homogenizer (Digital Ultra-Turrax-Germany) in ice-cold trichloroacetic acid (1g of tissue plus 10 ml of 10% trichloroacetic acid).

### 2.4. Determination of glutathione (GSH) level

The glutathione (GSH) level was determined by the modified Elman method (Aykac et al., 1985). The homogenate was centrifuged at 3,000 g for 10 minutes by Sigma 3-30k (Sigma Group Inc., Munich, Germany) high-speed centrifuge. 0.5 ml of supernatant was added to 1 ml of Tris-EDTA-SDS solution; vortexed at room temperature for 5 minutes and centrifuged at 10,000 xg for 5 minutes. Then 40 DTL of DTNB solutions was added and incubated for 15 minutes at 37 ° C. Absorbance at 412 nm was measured by spectrophotometer (Biochrom Libra S70 by Harvard Biosciences, Holliston, MA, USA).

### 2.5. Determination of malondialdehyde (MDA) level

The homogenate was centrifuged at 3,000 g for 15 minutes using high-speed centrifugation with Sigma 3-30k at 4 ° C. The supernatants were transferred to glass test tubes containing 1 ml of thiobarbituric acid-TCA-HCl and incubated for 15 minutes at room temperature. Samples were centrifuged at 10,000 × g for 5 min. BHT (10 µl) was added to the pellet, then heated in a boiling water bath at 100 ° C for 15 minutes, cooled, and centrifuged to remove the precipitate. The absorbance of each sample was read at 532 nm.

### 2.6. Determination of Total Antioxidant (TAS) Level

Serum and tissue extracts, the TAS Assay kit (Assay Rel Diagnostics®, Turkey) were evaluated for total antioxidant status according to the procedures described. The data were calculated according to a new automatic measurement method developed by Erel in 2004.

### 2.7. Statistical analysis

Statistical analyzes were performed using SPSS 20.0 software (SPSS Inc., USA). Data are shown as mean ± standard deviation. The statistical significance level of the tests was determined as 0.05. First, the distribution of normality (Kolmogorov-Smirnov and Shapiro-Wilk test) and data homogeneity of variances were determined. Then, when these assumptions were met, parametric t-test and ANOVA were used. Non-parametric tests such as Mann Whitney and Kruskal Wallis tests were used in cases where these assumptions were not met. A correlation test was used to determine the relationship between GSH and MDA parameters.

## 3. Results

A total of ninety fish from five different regions of Obruk Dam Lake were included in the study; blood, liver and gill tissues samples were taken from fish. The malondialdehyde (MDA), glutathione (GSH), and total antioxidant (TAS) activities were evaluated in study. Also, the water quality criteria were determined. Markers that are considered as water quality criteria are noteworthy in terms of seasonal values. Results were given in Table 1. The MDA, which are indicative of lipid damage, were higher in serum and liver tissue than in gill tissue. GSH enzyme, which is an antioxidant indicator, was found highest in liver tissue and the lowest level was obtained in serum. Total antioxidant indicator TAS levels were similar in liver, gill, and serum levels. There was no statistically significant difference between liver, gill, and serum levels for MDA (p = 0.243). A statistically significant difference was observed between liver, gill, and serum levels for GSH (p = 0.034). There was a statistically significant difference between liver, gill, and serum levels for TAS. The correlation coefficient between GSH and MDA (r = -0.399) and a negative strong correlation was determined.

**Table 1. GSH, MDA, and TAS Levels in Cyprinus carpio L.**

Tissue parameters (Cyprinus carpio L.)	Mean ± SD	Standard Error	95% Confidence interval Min. Max.
<b>GSH</b>			
Liver	32.78 ± 1.83	0.69	31.09 - 34.47
Gill	28.59 ± 8.42	3.18	20.80 – 36.38
Serum	5.18 ± 0.44	1.61	4.77 – 5.58
<b>MDA (TBARS)</b>			
Liver	3.70 ± 3.55	1.34	0.41 -6.70
Gill	3.28 ± 1.10	0.41	2.27 – 4.30

Serum	3.85 ±1.00	0.38	2.92 -4.78
TAS			
Liver	5.82 ±0.12	0.47	5.70 – 5.93
Gill	5.32 ± 0.36	0.14	4.99 – 5.66
Serum	5.17 ± 0.44	0.16	4.77 – 5.58

Values are given as mean ± standard error.

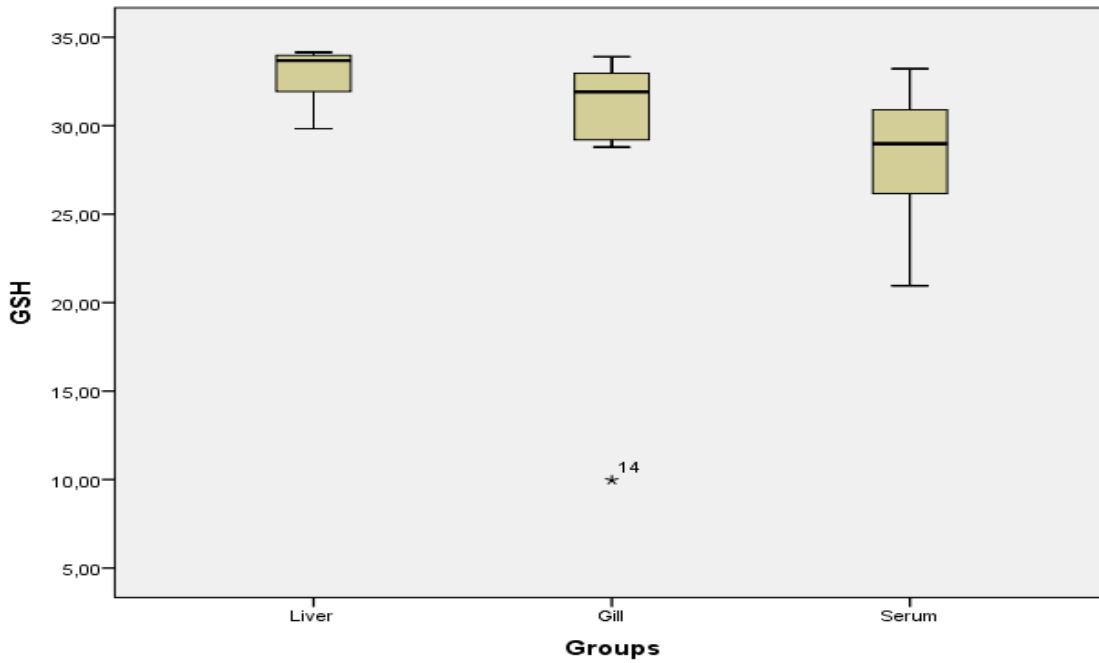


Figure 2. Statistical distribution of data Liver, Gill, and Serum of GSH Levels of *Cyprinus carpio* L.

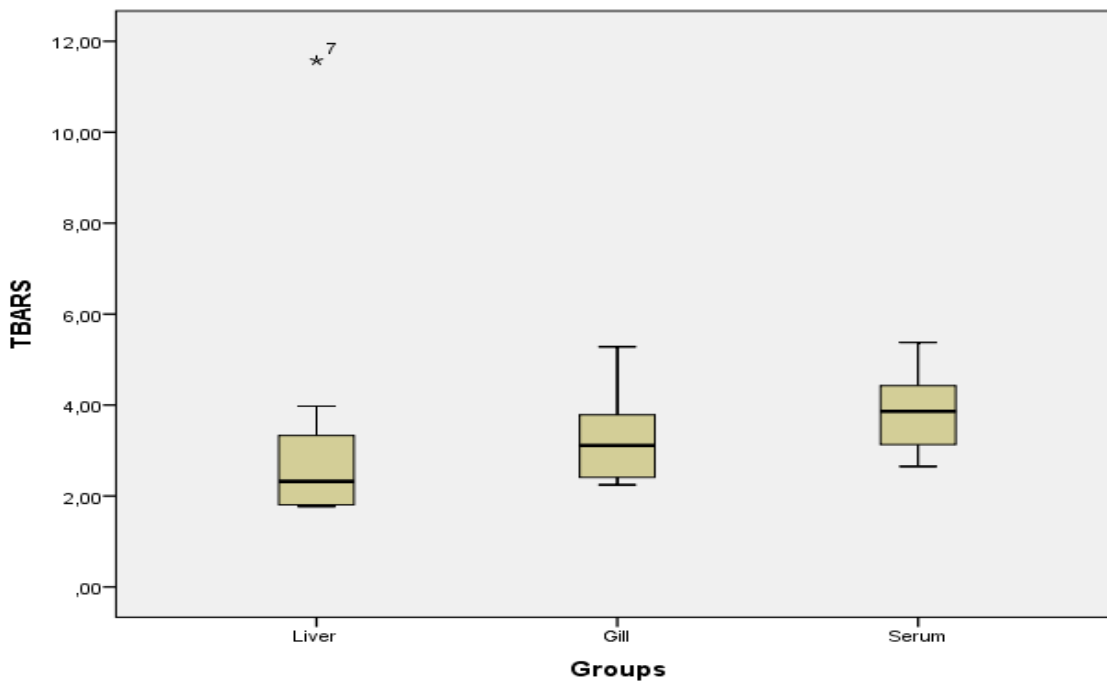


Figure 3. Graphical Representation of *Cyprinus carpio* L. Liver, Gill, and Serum MDA (TBARS) Levels.

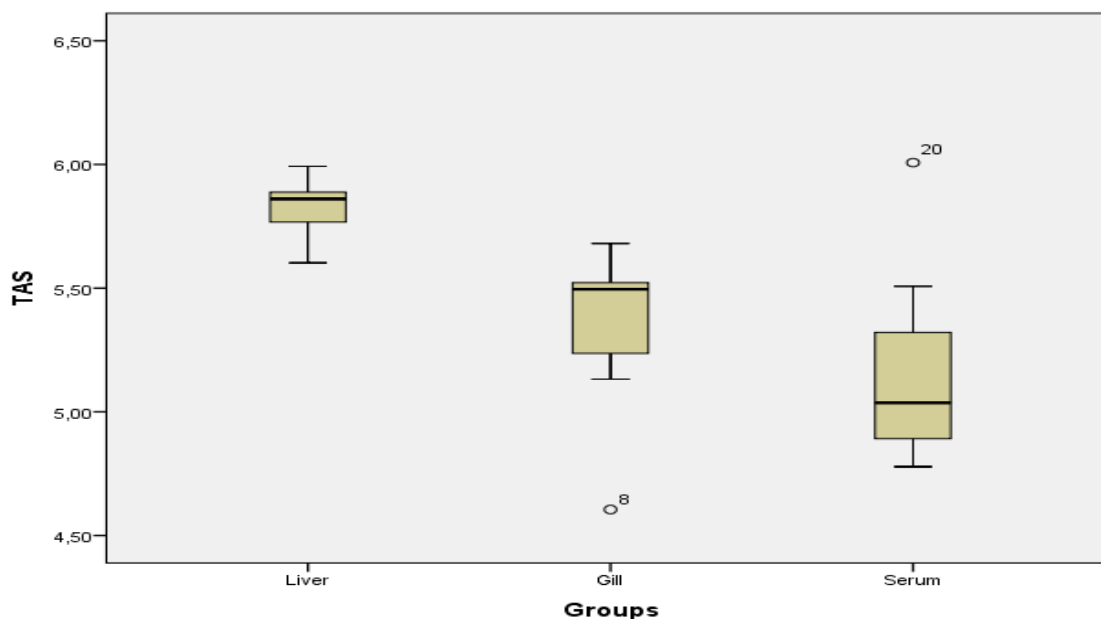


Figure 4. Graphical Representation of *Cyprinus carpio* L. Liver, Gill, and Serum TAS Levels.

#### 4. Discussion

In this study, it was planned to determine the oxidative stress susceptibility on the *Cyprinus carpio* (freshwater fish) population in the obruk dam lake and to reveal the effect of various polluting agents on the process. Pollutants entering the aquatic ecological environments directly or indirectly because of various factors negatively affect the entire aquatic ecology, especially the various tissues and organs of the living things living in these environments.

*Cyprinus carpio* is one of the most important fish species cultivated in the world (Toni et al., 2011). It is the most dominant species in the Obruk dam lake in our country. Therefore, this fish was chosen for this research. Many studies have shown that toxic chemicals accumulate in different organs/tissues of fish and disperse in metabolism. Among these tissue deposits, liver and kidney tissue are the most common (Jiang et al., 2012). In the literature, it is stated that this toxicological accumulation in tissue may have a significant task in the pathophysiology of oxidative balance (Jaishankar et al., 2014). Uptake of toxic chemical agents into the liver tissue may lead to the formation of free oxygen radicals and further triggering oxidative stress. (Cichoż-Lach and Michalak, 2014). However, intracellular reduced glutathione (GSH) depletion in liver cells because of exposure to toxicological chemicals can change the intracellular redox state and promote the formation of oxidant agents (Jiang et al., 2011a). The GSH is an antioxidant that protects cells from the toxic effects of reactive oxygen species such as free radicals, peroxides, and heavy metals (Pizzino et al., 20147). The increase/decrease in the GSH level in animals is an important indicator of the detoxification ability of the creature (Cheung et al., 2001). Glutathione peroxidase is involved in the detoxification of pollutants by using glutathione as a substrate (Lubos et al., 2011). The amount of cellular Glutathione is important in maintaining cellular functions, and can be reduced in the case of detoxification and oxidative stress. However, in the case of ongoing stress, the GSH/GSSG ratio is oxidative with the effect of adaptive mechanisms, increases to resist stress (Zhang et al., 2005) Fish are exposed to potentially high levels of toxins during fertilization. In the ecological environment, some fish species have a high tolerance system that includes enzymatic and non-enzymatic antioxidant defense systems such as glutathione S-transferases against many chemical agents in their metabolism. (Liao et al., 2006; Liang et al., 2007). In studies on the subject, it has been reported that the level of lipid peroxidation product Malondialdehyde is increased in the liver tissues of fish in dam lakes contaminated with polluted water (Gül et al., 2004, Karadag et al., 2014). In our study, it was observed that not only liver tissue, but also gill and serum levels were high in terms of oxidative stress. TAS and GSH are among the most important antioxidants that protect from oxidative attack of active oxygen species such as MDA, as they act as reducing agents and free radical scavengers. There seems to be a balance between this antioxidant mechanism and oxidant molecules. In our study, it was observed that the level of gsh was higher in the liver, which plays a key role in the process, and plays a key role in the regulation of oxidative balance in the entire metabolism of all blaks. Similarly, in one study showed that high metal concentration increases the level of oxidative stress. Likewise, considering the samples taken from the regions where the total antioxidant levels are low, they explained that the

low level in this region is the oxidative stress is triggered as the cause of the highly toxic metal in the samples and this affects the energy metabolism (Alkan et al, 2021). In another study, the total oxidant clearance capacity of the digestive glands of mussels collected from chemically affected and clean areas was compared and it was seen that the total oxidant level was lower in mussels from polluted areas (Regoli et al, 2000). Some studies have been reported that organic pollutants mixed with aquatic systems cause accumulation of oxidative stress factor molecules in fish and decrease antioxidant systems in fish (Ghio, Silbajoris, Carson and Samet, 2002). In another study, it was stated that an increase in lipid peroxidation and higher antioxidant enzyme activities occur in proportion to water pollution (Ali et al., 2004). These effects were found to be most common in gill tissues, suggesting that the gills are most vulnerable to pollutants.

In our studies, we determined that the MDA level, which is a marker of lipid oxidation, and the GSH and TOS values in tissues of *Cyprinus carpio* (freshwater fish) which are antioxidant markers, show a proportional increase with each other. But we only observed that liver GSH Levels were higher in terms of enzymatic antioxidant response compared to other tissues. We think that this is because it has the main responsibility mechanism for the elimination of damage caused by oxidative stress on liver metabolism. It is seen that liver tissue, which plays an important role in detoxification, plays an important role in oxidant balance in fish exposed to water pollutants.

## 5. Conclusion

Malondialdehyde (MDA) levels and glutathione (GSH), which are the result of lipid peroxidation, oxidative stress product that may occur in some tissues (blood, gill, and liver) of the *Cyprinus carpio* (freshwater fish) commonly found in Obruk Dam Lake, and total antioxidant status (TAS) were determined and evaluated. When the current research is examined, it is concluded that oxidative stress markers can be used as an indicator of water pollution in their body especially in antioxidant systems of fishes. Therefore, it is concluded that the findings of our study support the current studies and shed light on the studies that will be done in the future. Fish are the living creatures affected by the smallest changes in the aquatic environment in which they live. Changes in water criteria have been found to cause stress in fish and make physiologically adaptive arrangements to overcome this stress and survive. This data will shed light on the fish health, physiology, and ecological change studies.

## 6. Acknowledgement

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