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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Determination of oxidative stress in common carp (*Cyprinus carpio*) treated with phenol

## Fenol uygulanan sazan (*Cyprinus carpio*)'da oksidatif stresin belirlenmesi

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**Abstract:** This study examined the effects of phenol exposure on oxidative stress and antioxidant defense mechanisms in common carp (*Cyprinus carpio*). A total of 120 common carp with an average body mass of 30 g were randomly allocated into four experimental sets, consisting of one untreated control and three groups subjected to phenol at 0.01, 0.1, and 1 ppm for 96 hours. Following the exposure period, samples of liver and gill tissues were obtained to assess oxidative stress markers, including malondialdehyde (MDA), and the enzymatic activities of catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), along with reduced glutathione (GSH) levels. The analysis revealed that MDA concentrations were markedly elevated in every group exposed to phenol relative to the control fish, demonstrating enhanced lipid peroxidation and heightened oxidative stress. Antioxidant enzyme activities (CAT and GSH-Px) and GSH levels significantly decreased in liver and gill tissues, while GST activity increased in a dose-dependent manner. These findings suggest that phenol disrupts the balance between oxidation and antioxidant defences, leading to cellular stress and potential tissue damage. The changes in enzyme activity imply compensatory mechanisms or detoxification responses triggered by phenol exposure. Since even low concentrations of phenol caused notable oxidative changes, this study highlights its potential toxicity to aquatic organisms. The results emphasize the importance of cautious use of phenolic compounds in aquatic environments and contribute to a broader understanding of phenol-induced oxidative stress in fish. Further research involving different species, exposure durations, and physiological markers is recommended to confirm and expand these findings.

**Keywords:** Antioxidants, fish, phenols, oxidative stress

**Öz** Bu çalışmada, fenolün sazanlarda (*Cyprinus carpio*) oksidatif stres ve antioksidan savunma mekanizmaları üzerindeki etkileri araştırılmıştır. Ortalama 30 g ağırlığındaki 120 balık, bir kontrol ve üç farklı deney grubuna ayrılmış; deney grupları 96 saat süreyle sırasıyla 0.01, 0.1 ve 1 ppm fenol konsantrasyonlarına maruz bırakılmıştır. Uygulama sonunda karaciğer ve solungaç dokuları incelenerek malondialdehit (MDA) düzeyleri ile katalaz (CAT), glutatyon peroksidaz (GSH-Px), glutatyon S-transferaz (GST) aktiviteleri ve redükte glutatyon (GSH) seviyeleri ölçülmüştür. Sonuçlar, tüm fenol uygulanan gruplarda MDA düzeylerinin kontrol grubuna göre belirgin şekilde yükseldiğini ve bunun artmış lipid peroksidasyonu ile oksidatif stresin göstergesi olduğunu ortaya koymuştur. Karaciğer ve solungaç dokularında CAT, GSH-Px aktiviteleri ve GSH düzeyleri anlamlı olarak azalırken, GST aktivitesi doza bağlı şekilde artış göstermiştir. Bu bulgular, fenolün oksidatif dengeyi bozarak hücresel stres ve potansiyel doku hasarına yol açtığını göstermektedir. Enzim aktivitelerindeki değişimler, organizmanın fenole karşı geliştirdiği telafi edici mekanizmaları veya detoksifikasyon yanıtlarını işaret etmektedir. Düşük konsantrasyonlarda dahi fenolün kayda değer oksidatif değişimlere neden olması, bu bileşiğin sucul organizmalar için yüksek toksisite potansiyeline sahip olduğunu göstermektedir. Çalışma, fenolik bileşiklerin su ortamlarında kullanımında dikkatli olunması gerektiğini vurgulamakta ve fenol kaynaklı oksidatif stresin balıklardaki etkilerinin daha kapsamlı anlaşılmasına katkı sağlamaktadır. Bu sonuçların pekiştirilmesi için farklı türler, maruziyet süreleri ve ek fizyolojik parametreleri içeren ileri araştırmalar önerilmektedir.

**Anahtar kelimeler:** Antioksidanlar, balık, fenoller, oksidatif stres

## INTRODUCTION

Aquaculture has experienced rapid global growth in recent decades, establishing itself as a key sector within the international food production industry. In addition to the oxygen absorbed from the environment, various stressors—including heavy metals, temperature changes, pesticides, parasites, and other pollutants—can lead to increased oxidative stress in fish. This heightened oxidative stress results in the excessive production of free radicals, which can cause physiological problems, weaken fish health, and ultimately lead to financial losses (Lou et al., 2011).

An imbalance between reactive oxygen species (ROS) generation and antioxidant defenses leads to oxidative stress, a condition in which cellular protective systems are overwhelmed by excessive oxidants. Although ROS are naturally generated during various metabolic processes and cellular respiration—and even serve essential roles in some physiological functions—excessive buildup can cause oxidative damage. This overproduction of ROS may harm cellular integrity by damaging proteins, lipids, and DNA, ultimately disrupting normal cellular functions (Niyogi et al., 2001).

In fish, two main types of defence mechanisms combat oxidative stress: enzymatic and non-enzymatic antioxidant systems. The enzymatic defence includes key enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), which work together to neutralize reactive oxygen species and prevent oxidative damage. Meanwhile, the non-enzymatic defence involves molecules such as glutathione (GSH), vitamin E, and vitamin C. These compounds help protect cells by scavenging free radicals and maintaining redox balance (Sies, 1997).

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A wide variety of disinfectants are used in aquaculture, among which phenol—an aromatic compound with the formula $C_6H_5OH$—is particularly important. Besides its use in aquaculture, phenol and its derivatives are widely employed in numerous industrial processes and can be released into aquatic environments in large amounts. High levels of phenol in water can cause acute or chronic toxicity in aquatic life. Additionally, the presence of phenol at elevated concentrations poses risks not only to fish but also to other organisms within the aquatic food web.

Numerous studies have reported that exposure to common disinfectants, including copper sulphate (Yonar et al., 2016), formalin (İspir et al., 2017; Yıldız and Kaptaner, 2025), and hydrogen peroxide (Seker et al., 2015), can provoke oxidative stress, leading to physiological impairments and alterations in biochemical indicators. Phenol is identified as a significant environmental stressor that causes oxidative damage, mainly by increasing lipid peroxidation, which leads to damage to cellular membranes. In addition, it induces cellular stress by suppressing the function of key antioxidant enzymes, including glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). As a toxicant, phenol has been shown to negatively impact various organ systems in fish, including the respiratory, central nervous, and renal systems. Once absorbed, phenol can interfere with numerous biochemical processes and trigger oxidative stress (Akram et al., 2021; Mahim et al., 2021).

Documented effects of phenol exposure in fish include growth retardation, behavioural abnormalities, increased mortality, and immunosuppression, all of which compromise fish health and welfare (Saha et al., 1999; Muthukumaravel et al., 2023). Despite the recognized toxicity of phenol, no studies to date have specifically examined phenol-induced oxidative stress in fish using antioxidant biomarkers as a primary investigative tool. In this regard, the present study offers a novel contribution to the field. By elucidating the oxidative stress responses triggered by phenol exposure, this research aims to assess the potential implications of phenol use in aquaculture and to promote strategies that support fish health. Moreover, this work addresses a current and relevant issue in aquaculture and is expected to provide valuable insights that may contribute to the sustainable management of aquatic animal health. Therefore, the study holds considerable scientific originality and significance.

## MATERIALS AND METHODS

The present thesis was conducted at the Aquatic Sciences Research Unit of Fırat University Experimental Research Center (FÜDAM). All experimental protocols were reviewed and authorized by the Local Animal Ethics Committee of Firat University (Approval ID: 2023/05-01, March 22, 2023). The experiment utilized a total of 12 aquaria, with four aquaria per treatment group and three replicates, thereby forming the complete setup. All tanks were disinfected in advance and covered with mesh screens to prevent fish from jumping out. The fish were allowed a 15-day acclimation period in the aquaria prior to the onset of the experiment.

The study involved 120 common carp (*Cyprinus carpio*), each weighing approximately 30 grams. These fish were divided into three groups, each containing 40 fish. All chemical reagents required for the lab tests were obtained from reputable commercial suppliers.

The fish were randomly placed into 12 separate aquariums, with 10 fish in each tank, and divided into four experimental groups as follows:

C: Control group

D1: Group exposed to 0.01 ppm phenol through immersion

D2: Group exposed to 0.1 ppm phenol through immersion

D3: Group exposed to 1 ppm phenol through immersion

The 96-hour experiment concluded with all fish being anesthetized using benzocaine at 25 mg/L and humanely euthanized following standard dissection protocols (Mişe Yonar et al., 2011). Tissue samples, including liver and gills, were taken from the dissected fish, wrapped in aluminum foil, and stored at −20 °C. All samples were analysed within 30 days of collection.

For the evaluation of oxidative and antioxidant indices, liver and gill samples were homogenized in a 1.15% potassium chloride buffer at a 1:10 (w/v) tissue-to-solution ratio. Following homogenization, the samples were placed into polypropylene tubes and subjected to centrifugation at 3200 rpm for 10 minutes at 4 °C. The clear supernatants were carefully separated and used in biochemical measurements. In these preparations, MDA levels, serving as an indicator of lipid peroxidation, were determined, along with the enzymatic activities of CAT, GSH-Px, and GST, and the concentration of GSH (Sakin et al., 2011; Sakin et al., 2012).

Tissue oxidative stress markers were quantified using established spectrophotometric methods. MDA concentrations, as an indicator of lipid peroxidation, were determined following a modified procedure of Placer et al. (1966). CAT activity was analysed based on the protocol of Aebi (1984). GSH-Px activity was evaluated using the approach proposed by Beutler (1975), whereas GST activity was measured according to the technique of Habig et al. (1974). GSH levels in the tissues were determined using the Ellman (1959) method. To normalize enzyme activities and oxidative stress parameters (CAT, GSH-Px, GST, MDA, and GSH), total tissue protein concentrations were measured based on the method reported by Lowry et al. (1951).

Data analysis was carried out using SPSS software (version 21.0). Group differences were assessed through one-way analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. For variables monitored across different time points, repeated-measures ANOVA was applied to evaluate changes within groups. When overall significance was detected, post hoc pairwise comparisons were performed using the Least Significant Difference (LSD) test. Results are presented as mean values ± standard error (SE).

## RESULTS

The changes in MDA levels observed in the liver and gill tissues of fish from both control and experimental groups are summarized below.

Compared to the control group, all phenol-exposed groups (D1, D2, and D3) showed a statistically significant increase in liver MDA levels ($p < 0.05$, Table 1). The increases in MDA levels were 81.75%, 123.72%, and 183.21% for D1, D2, and D3, respectively. Additionally, significant differences in liver MDA levels were observed among the D1, D2, and D3 groups when compared to each other ($p < 0.05$, Table 1). Similarly, all phenol-treated groups also had significantly higher gill MDA levels than the control group ($p < 0.05$, Table 2). The increases were 108.67%, 144.50%, and 190.17% for D1, D2, and D3, respectively. The analysis also revealed significant differences in gill MDA levels among the phenol-treated groups ($p < 0.05$, Table 2).

**Table 1.** Changes in liver MDA levels (nmol/g protein), CAT (U/mg protein), GSH-Px (U/mg protein), GST (U/mg protein) activities, and GSH levels (µmol/g protein) in phenol-exposed groups (K: Control; D1: 0.01 ppm phenol; D2: 0.1 ppm phenol; D3: 1 ppm phenol. Different letters in the same row indicate statistically significant differences)

| Parameters | Experimental Groups | | | |
|---|---|---|---|---|
| | K | D1 | D2 | D3 |
| **MDA** | 2.74 ± 0.18 A | 4.98 ± 0.29 B | 6.13 ± 0.36 C | 7.76 ± 0.54 D |
| **CAT** | 3.92 ± 0.22 D | 3.11 ± 0.17 C | 2.73 ± 0.24 B | 2.08 ± 0.18 A |
| **GSH-Px** | 3.46 ± 0.11 D | 2.98 ± 0.14 C | 2.31 ± 0.16 B | 1.96 ± 0.13 A |
| **GST** | 115.37 ± 12.20 A | 137.65 ± 11.38 B | 159.04 ± 15.47 C | 188.00 ± 14.12 D |
| **GSH** | 86.93 ± 7.90 D | 61.07 ± 4.55 C | 50.83 ± 5.95 B | 39.29 ± 6.21 A |

The alterations in catalase (CAT) activity in the liver and gill tissues of fish from the control and experimental groups are described below.

A statistically significant reduction in liver CAT activity was observed across in all experimental groups treated with phenol compared with the control group ($p < 0.05$, Table 1). The reductions were 20.66%, 30.35%, and 46.93% in the D1, D2, and D3 groups, respectively. Moreover, significant differences in liver CAT activity were also identified among these groups ($p < 0.05$, Table 1).

Similarly, all phenol-exposed groups exhibited decreased CAT activity in gill tissues relative to the control ($p < 0.05$, Table 2), with reductions of 13.68%, 34.52%, and 51.79% in D1, D2, and D3, respectively. Significant differences were detected among the phenol-treated groups in gill CAT activity ($p < 0.05$, Table 2).

**Table 2.** Changes in gill MDA levels (nmol/g protein), CAT (U/mg protein), GSH-Px (U/mg protein), GST (U/mg protein) activities, and GSH levels (µmol/g protein) in phenol-exposed groups (K: Control; D1: 0.01 ppm phenol; D2: 0.1 ppm phenol; D3: 1 ppm phenol. Different letters in the same row indicate statistically significant differences)

| Parameters | Experimental Groups | | | |
|---|---|---|---|---|
| | K | D1 | D2 | D3 |
| **MDA** | 3.46 ± 0.19 A | 7.22 ± 0.34 B | 8.46 ± 0.57 C | 10.04 ± 0.69 D |
| **CAT** | 3.07 ± 0.17 D | 2.65 ± 0.12 C | 2.01 ± 0.20 B | 1.48 ± 0.10 A |
| **GSH-Px** | 2.87 ± 0.14 D | 2.10 ± 0.19 C | 1.56 ± 0.11 B | 1.07 ± 0.10 A |
| **GST** | 74.06 ± 8.92 A | 95.82 ± 10.06 B | 118.10 ± 13.51 C | 132.81 ± 16.01 D |
| **GSH** | 45.19 ± 5.46 D | 37.84 ± 4.12 C | 28.28 ± 3.22 B | 20.33 ± 2.10 A |

Compared to the control group, all phenol-exposed groups showed a statistically significant decrease in liver GSH-Px activity ($p < 0.05$, Table 1). The reduction rates were 13.87%, 33.23%, and 43.35% in the D1, D2, and D3 groups, respectively. The activity of liver GSH-Px showed significant group-dependent differences among D1, D2, and D3 ($p < 0.05$, Table 1). In gill tissues, a significant decline in GSH-Px activity was also recorded in all experimental groups treated with phenol compared with the control group ($p < 0.05$, Table 2). The decreases were 26.82%, 45.64%, and 62.71% for the D1, D2, and D3 groups, respectively. When comparing these phenol-exposed groups, statistically significant differences in gill GSH-Px activity were also found ($p < 0.05$, Table 2).

The variations in glutathione-S-transferase (GST) activity in the liver and gill tissues of fish from the control and experimental groups are shown below.

Compared to the control group, all phenol-treated groups showed a statistically significant increase in liver GST activity ($p < 0.05$, Table 1). The increases were 19.31%, 37.85%, and 62.95% for the D1, D2, and D3 groups, respectively. Additionally, statistical analysis indicated significant differences in liver GST activity among the phenol-exposed groups themselves ($p < 0.05$, Table 1). Likewise, GST activity in gill tissues was significantly higher in all experimental groups treated with phenol compared with the control group ($p < 0.05$, Table 2). The increases observed were 29.38%, 59.46%, and 79.32% for the D1, D2, and D3 groups, respectively. Significant differences in gill GST activity were also present among the D1, D2, and D3 groups ($p < 0.05$, Table 2).

The changes in reduced glutathione (GSH) levels in liver and gill tissues of fish from the control and experimental groups are described below.

Compared to the control group, all phenol-exposed groups showed a statistically significant decrease in liver GSH levels ($p < 0.05$, Table 1). The reductions in GSH levels were 29.74%, 41.52%, and 54.80% for the D1, D2, and D3 groups, respectively. Furthermore, significant differences were also found among the D1, D2, and D3 groups in liver GSH concentrations ($p < 0.05$, Table 1). Similarly, phenol administration caused a statistically significant decline in gill GSH levels across in all experimental groups treated with phenol compared with the control group ($p < 0.05$, Table 2). The decreases in GSH levels were 16.26%, 37.41%, and 55.01% for the D1, D2, and D3 groups, respectively. Significant differences in gill GSH levels were also observed when comparing these groups with one another ($p < 0.05$, Table 2).

## DISCUSSION

Free radicals, created by the one-electron reduction of oxygen, can harm cells by breaking down essential biomolecules, including carbohydrates, proteins, lipids, and nucleic acids. Furthermore, these reactive species can inactivate cellular enzymes and impair membrane permeability. Malondialdehyde (MDA) is a by-product of the oxidative breakdown of polyunsaturated fats. It is one of the aldehydes formed during lipid peroxidation, a process caused by free radical activity. Therefore, MDA is widely recognized as a key marker for evaluating oxidative damage (Morales et al., 2004; Fontagné et al., 2006). In the present work, variations in MDA concentrations were evaluated as a biomarker of oxidative stress, providing insight into the extent of tissue injury induced by phenol exposure.

Multiple studies have demonstrated that phenol exposure induces stress, assessed through various physiological and biochemical methods. Das et al. (2016) investigated oxidative responses in common carp (*Cyprinus carpio*) exposed to sublethal phenol concentrations (0.26, 0.8, and 8 mg/L) for 15 days, monitoring samples from serum, liver, and ovary at intervals on days 1, 2, 4, 7, and 15. Their findings revealed marked elevations in hydrogen peroxide and hydroxyl radical production between days 1 and 7, after which levels stabilized, showing no further significant differences up to day 15. Similar effects have been documented in unfertilized eggs of *Gobiocypris rarus*, where exposure to 1 and 200 µg/L nonylphenol over 21 days elevated ROS production (Zhang et al., 2008). Correspondingly, increased ROS has also been reported in mammalian models. In rats, exposure to polychlorinated biphenyls (Aroclor 1254) elevated ROS in Leydig cells (Murugesan et al., 2007; Peltola et al., 1996; Homsby, 1989). In addition, exposure to all three phenol concentrations resulted in a marked elevation of MDA levels in liver and gill tissues, indicating enhanced lipid peroxidation in these organs. The rise in MDA likely reflects increased lipid peroxidation, presumably driven by excess reactive oxygen species resulting from phenol toxicity.

The primary function of the catalase (CAT) enzyme is to eliminate hydrogen peroxide ($H_2O_2$) radicals from the cytoplasm. It has been demonstrated that catalase metabolizes hydrogen peroxide and that its enzymatic activity is directly dependent on the concentration of $H_2O_2$ (Fornazier et al., 2002). As one of the first lines of antioxidant defence, catalase plays a pivotal role in maintaining cellular integrity by eliminating hydrogen peroxide through its conversion into water and oxygen. Beyond this primary function, catalase can also exert peroxidase-like activity, employing hydrogen peroxide as a co-substrate to counteract reactive molecules, thus reinforcing the antioxidant capacity of the cell. In a study conducted on *Brycon amazonicus*, fish were exposed to phenol for 96 hours, and recovery responses were evaluated in erythrocytes and liver tissues after one and two weeks (Avilez et al., 2008). The findings showed that catalase activity decreased in erythrocytes while remaining unchanged in the liver, and these trends persisted throughout the recovery period. Other studies have also reported that various phenolic compounds can suppress CAT activity (Roche and Bogé, 2000). For instance, researchers observed significantly lower CAT activity in the blood of sea bass following exposure to different concentrations of polyphenols compared to the control group. In the present study, investigating the potential toxic effects of phenol, catalase activity in both liver and gill tissues was found to be reduced in all experimental groups treated with phenol compared with the control group. The outcome corroborates the trends described in previous studies. The reduction in CAT activity may be explained by phenol's capacity to neutralize reactive species directly, or alternatively by a decrease in free radical generation as a result of phenol binding to and reacting with cellular biomolecules (Das et al., 2016). Furthermore, this decline may indicate insufficient catalase-mediated detoxification of hydrogen peroxide, which could potentially lead to increased oxidative stress resulting from phenol exposure. The inhibition of catalase activity in the presence of oxygen was previously described by Kono and Fridovich (1982).

Among peroxidase enzymes, GSH-Px plays a central role in cellular protection, primarily by eliminating hydrogen peroxide and thereby preventing oxidative damage to vital biomolecules. This enzyme facilitates the conversion of hydrogen peroxide and organic hydroperoxides into non-toxic compounds, utilizing glutathione as the electron donor. Therefore, this enzyme is considered essential in defending tissues against oxidative damage associated with lipid peroxidation (Yonar et al., 2019). In *Carassius auratus*, a notable reduction in hepatic GSH-Px activity was observed 24 hours after exposure to copper as copper sulfate pentahydrate—a disinfectant frequently used in aquaculture—at concentrations of 0.1, 0.5, 1, and 1.5 ppm. Moreover, this decline was found to be dose-dependent (Fontagné et al., 2006). In another study by Gaur and Mathur (2017) on *Labeo rohita*, it was found that fish exposed to 1/10th of the $LC_{50}$ value of phenol showed decreased GSH-Px activity in gill, liver, and kidney tissues, while an opposite trend—a rise—was observed in muscle tissue. Similarly, in *Brycon amazonicus*, exposure to phenol for 96 hours followed by a recovery period of 1 to 2 weeks resulted in a reduction in hepatic GSH-Px activity by the end of the second week (Avilez et al., 2008).

In this experiment, phenol exposure resulted in a marked suppression of GSH-Px activity in fish tissues. This reduction reflects a weakening of the enzymatic antioxidant defense system and is consistent with previous evidence showing that phenolic compounds impair peroxidase-dependent detoxification mechanisms. While some environmental pollutants or toxicants can increase glutathione peroxidase activity, a reduction in enzyme activity is also commonly observed under such stress conditions (Vinodhini and Narayana, 2009). The lower GSH-Px activity seen here is likely due to the toxic effects of phenol.

As a central phase II detoxification enzyme, GST catalyses the conjugation of glutathione with electrophilic molecules, thereby improving their water solubility and facilitating excretion (Hamed et al., 2003). Previous studies have reported variable GST responses depending on the toxicant and tissue involved. For example, Varó et al. (2007) observed only a minor, statistically insignificant elevation in gill GST activity of *Sparus aurata* exposed to copper sulfate for 24 h, whereas Paris-Palacios et al. (2000) demonstrated a significant increase in hepatic GST activity in zebrafish following longer-term copper exposure. Similarly, Gaur and Mathur (2017) documented a tissue-dependent pattern in *Labeo rohita*, where phenol exposure suppressed GST activity in the liver and gills but enhanced activity in kidney and muscle tissue.

In line with these findings, the present study revealed a consistent increase in GST activity within the liver and gill tissues of phenol-exposed carp. This upregulation likely represents a protective biochemical adaptation, aimed at mitigating oxidative stress and neutralizing phenol-derived reactive metabolites. The tissue-specific response highlights the complexity of GST regulation under xenobiotic challenge and suggests that GST activity may serve as a sensitive biomarker for evaluating chemical stress in fish. Unlike some earlier studies that reported both upregulation and suppression depending on the tissue and toxicant, our results point to a more uniform induction pattern in carp liver and gills, underscoring potential species- and tissue-specific differences in detoxification strategies.

GSH is a tripeptide that, although it does not possess enzymatic activity, plays a pivotal role as a cellular antioxidant. It neutralizes free radicals and peroxides, thereby safeguarding cells from oxidative damage. Beyond its direct antioxidant action, GSH serves as a cofactor for several glutathione-dependent enzymes. Furthermore, by maintaining the thiol groups of proteins in their reduced form, it helps preserve the structural integrity and activity of numerous proteins and enzymes (Hayes and McLellan, 1999). For example, glutathione peroxidase (GSH-Px) can only convert hydrogen peroxide ($H_2O_2$) into water when GSH is available, during which GSH is oxidized to form glutathione disulfide (GSSG). In a study where *Labeo rohita* were exposed to 1/10th of the $LC_{50}$ concentration of phenol, GSH levels in the gill and liver increased compared to controls. In contrast, kidney and muscle GSH levels decreased (Gaur and Mathur, 2017). In *Brycon amazonicus*, after 96 hours of phenol exposure, GSH levels in erythrocytes decreased and remained low during the 1- and 2-week recovery periods (Avilez et al., 2008). In the present study, GSH levels in the liver and gill tissues of phenol-treated fish also decreased. While these results align with some previous findings, they differ from others. These differences may stem from tissue- or organ-specific responses. The variation observed in this and similar studies can be attributed to several factors, including the fish species involved, as well as the method, concentration, and duration of phenol exposure.

In summary, the marked elevation of MDA levels in tissues of fish exposed to 0.01, 0.1, and 1 ppm phenol clearly demonstrates the oxidative stress-inducing effect of this compound. Concurrently, the activities of key antioxidant enzymes—including CAT, GSH-Px, and GST—together with the concentration of GSH, declined significantly in liver and gill samples across all phenol-treated groups. Interestingly, despite these reductions, GST activity showed a pronounced increase in the same tissues, suggesting an adaptive response to enhanced xenobiotic metabolism.

## CONCLUSION

Taken together, these findings indicate that phenol has toxic effects on fish even at very low concentrations. Phenol not only causes oxidative stress but also interferes with the antioxidant defense system, posing a threat to fish health. Therefore, caution is advisable when using this compound in aquatic environments. The current results should be confirmed through further studies. Future research is necessary to explore the effects of phenol on various aquatic species at different doses and exposure times, as well as across a broader range of physiological and biochemical parameters.

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This study is a condensed version of the MSc thesis by Didem Taşçı, completed as part of her graduate studies in Aquatic Sciences. The original thesis was submitted and approved at Firat University, serving as the scientific foundation for the experimental design, data collection, and analysis presented here. This manuscript emphasizes key findings and interpretations from the thesis, aiming to advance the broader academic understanding of phenol-induced oxidative stress in fish.

## AUTHORSHIP CONTRIBUTIONS

Didem Taşçı: Conceptualization, investigation, data collection, formal analysis, and original draft preparation. Muhammet Enis Yonar: Supervision, guidance on methodology, data interpretation, critical review, and editing of the manuscript, project administration. All authors have reviewed the manuscript thoroughly and approved its final version for submission.

## CONFLICT OF INTEREST STATEMENT

The authors state that no financial or non-financial conflicts

of interest are associated with this manuscript.

## ETHICS APPROVAL

All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Approval No. 2023/05-01, March 22, 2023). Throughout the study, procedures strictly adhered to established ethical standards for the care and use of laboratory animals.

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## DECLARATION OF AI USE

No generative AI model was used in any stage of this research or manuscript preparation.

## DATA AVAILABILITY

The data produced and/or analysed during this study can be obtained from the corresponding author upon reasonable request.

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