



Assessment Of Superoxide Dismutase Activity In Freshwater Teleost Fish *Labeo Rohita*: Correlation With Environmental Stressors And Oxidative Stress Responses

Vijeta Chaturvedi^{1*}, DevBrat Mishra²

^{1*}Research Scholar, Fish Biology Research Lab, Department of Zoology, T.D.P.G.College Jaunpur

²Assistant Professor, Fish Biology Research Lab, Department of Zoology, T.D.P.G.College Jaunpur

***Corresponding Author:** Vijeta Chaturvedi

Email: Vijetachaturvedi175@gmail.com

Abstract

This study investigates the role of superoxide dismutase (SOD) activity in *Labeo rohita* (Rohu) as a response to environmental stressors, including dissolved oxygen levels and pollutants, along the Gomti River. SOD activity was measured in various tissues, including the liver, gills, and brain, across six experimental sites with differing environmental conditions. Significant variations in Cu-Zn SOD and Mn-SOD activity were observed, with higher enzyme activity linked to increased oxidative stress at sites with lower dissolved oxygen levels. Statistical analysis revealed a strong correlation between environmental factors and SOD activity, highlighting the enzyme's role as a biomarker for oxidative stress. The findings underscore the adaptability of *Labeo rohita* to environmental changes, and suggest that SOD activity can be used to monitor water quality and the health of aquatic ecosystems.

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Keywords: Superoxide dismutase, *Labeo rohita*, oxidative stress, dissolved oxygen, environmental stressors, biomarker, water quality, Gomti River.

Introduction

Superoxide dismutase (SOD) is a crucial antioxidant enzyme that plays an essential role in protecting cells from oxidative damage by converting superoxide radicals ($O_2^{\cdot-}$) into less harmful molecules, such as oxygen and hydrogen peroxide (Fridovich, 1995). These superoxide radicals are highly reactive and can cause damage to proteins, lipids, and DNA if not effectively neutralized (Halliwell & Gutteridge, 1989). SOD exists in various forms, including Cu-Zn SOD and Mn-SOD, each present in different cellular compartments and having distinct roles in cellular protection (Me Cord & Fridovich, 1969). The significance of SOD extends beyond its fundamental biochemical role, as its activity is a key marker of oxidative stress in organisms exposed to environmental stressors (Fridovich, 1995). This is particularly important in aquatic organisms such as fish, which face fluctuating environmental conditions and contamination that can lead to increased oxidative stress (Wang et al., 2018).

Freshwater teleosts, such as *Labeo rohita*, an economically important fish species found across India's rivers and aquaculture systems, are exposed to varying environmental conditions, including water temperature, oxygen levels, and contamination by heavy metals or organic pollutants. These stressors can lead to the generation of reactive oxygen species (ROS), including superoxide radicals, which induce oxidative stress in the fish (Singh, 2018). The oxidative stress response in these fish often involves a surge in antioxidant enzyme

activity, including that of SOD, which acts as a protective mechanism against cellular damage (Babich et al., 1993). Therefore, studying the SOD activity in various tissues of *Labeo rohita* can provide valuable insights into the fish's physiological adaptation to environmental stressors, as well as the health of aquatic ecosystems (Al-Kenawy & Aly, 2015).

SOD and Oxidative Stress in Aquatic Organisms

Oxidative stress is a physiological condition resulting from an imbalance between the production of ROS and the body's ability to neutralize them with antioxidants (Fridovich, 1995). In aquatic organisms, oxidative stress can be induced by several environmental factors, including water pollution, temperature fluctuations, low dissolved oxygen (DO) levels, and exposure to toxic substances such as heavy metals, pesticides, and other industrial effluents (Singh & Banerjee, 2008a). The impact of these stressors on aquatic organisms is compounded by their high permeability to oxygen and pollutants, making them particularly vulnerable to oxidative damage (Singh & Banerjee, 2008b).

Studies on fish, such as those by Kolayli and Keha (1999), have shown that exposure to environmental pollutants can lead to increased SOD activity, suggesting that SOD is upregulated as part of the fish's defense mechanism against oxidative damage. Furthermore, research by Babich et al. (1993) demonstrated that fish exposed to waterborne contaminants, including heavy metals, exhibit significant changes in the activity of antioxidant enzymes, including SOD. This makes SOD an important biomarker for assessing the health and oxidative status of fish populations in polluted water bodies (Plessl et al., 2017).

The activity of SOD in various tissues of fish is reflective of the oxidative stress levels they encounter. For example, the liver, a major metabolic organ, is involved in detoxifying various pollutants and generating ROS as byproducts of metabolic processes (Younus, 2018). Consequently, higher SOD activity is often observed in the liver to counteract the increased ROS production (Younus, 2018). Other tissues, such as the gills and skin, also exhibit varying levels of SOD activity, as they are exposed to direct contact with water and pollutants, further emphasizing the protective role of SOD in fish (Singh & Banerjee, 2008c).

Environmental Stressors and Their Impact on Fish SOD Activity

In aquatic environments, the concentration of dissolved oxygen (DO) is a critical factor that influences the metabolic processes and oxidative stress responses in fish. Low DO levels, often resulting from pollution or eutrophication, have been shown to increase the generation of ROS in fish tissues (Crapo & Tierney, 1974). Research by Wang et al. (2018) has shown that the lower the DO concentration, the more likely it is that fish will experience oxidative stress, leading to an upregulation of antioxidant defense mechanisms, including SOD. This is particularly relevant in freshwater systems where seasonal fluctuations in DO levels can lead to intermittent periods of hypoxia, contributing to oxidative stress in aquatic organisms (Singh et al., 2018).

Heavy metals, such as arsenic, cadmium, and mercury, are another major environmental stressor that affects fish health by inducing oxidative stress. Singh and Banerjee (2008a) found that arsenic exposure caused significant biochemical alterations in the respiratory organs of *Clarias batrachus*, including changes in SOD activity. Similar findings have been reported by Al-Kenawy and Aly (2015), who noted that heavy metal contamination led to higher SOD levels in the tissues of freshwater fish. These findings support the hypothesis that fish increase SOD activity in response to environmental pollutants to mitigate oxidative damage (Weydert & Cullen, 2010).

Furthermore, pesticides and other organic pollutants have been shown to impact SOD activity in aquatic organisms (Singh & Banerjee, 2009). The exposure of fish to these contaminants leads to the generation of ROS, triggering the activation of antioxidant enzymes, including SOD, in a bid to prevent oxidative damage. This adaptive response plays a vital role in maintaining cellular integrity and homeostasis in fish exposed to toxic substances in the aquatic environment (Singh, 2018).

Research Significance and Study Rationale

Understanding the relationship between environmental stressors and SOD activity in *Labeo rohita* is essential for assessing the health of aquatic ecosystems and the impact of pollution on fish populations. As an indicator enzyme for oxidative stress, SOD provides a valuable tool for monitoring the physiological responses of fish to environmental changes (Fridovich, 1995). This study aims to investigate the SOD activity in different tissues of *Labeo rohita* collected from various sites along the Gomti River, which is influenced by pollution and other environmental stressors. By correlating SOD activity with physicochemical parameters, such as dissolved

oxygen, pH, and alkalinity, this study will contribute to a better understanding of how environmental stress affects fish at the biochemical level.

Additionally, this research will explore the differences in SOD activity across various tissues of *Labeo rohita*, including the liver, gills, skin, and brain, which are directly exposed to environmental changes. (Vijeta Chaturvedi & Deepak Varma 2022 vol-IX) This will provide insights into the tissue-specific responses to oxidative stress and help identify potential biomarkers for monitoring water quality and the health of aquatic organisms in polluted environments (Plessl et al., 2017). The results of this study will also contribute to the broader field of environmental toxicology and provide valuable data for the conservation and management of freshwater fish populations.

Superoxide dismutase (SOD) plays a vital role in protecting fish from oxidative damage induced by environmental stressors, such as low dissolved oxygen levels, heavy metals, and pollutants. Understanding the relationship between SOD activity and environmental stress in *Labeo rohita* can provide crucial information on the health of fish populations and the state of aquatic ecosystems. This study aims to assess SOD activity across various tissues in *Labeo rohita* and correlate it with environmental parameters, contributing to the knowledge of oxidative stress responses in fish and the impacts of environmental pollution.

By exploring the biochemical adaptation of fish to stressors in their environment, this research underscores the importance of monitoring SOD activity as a biomarker for assessing the effects of pollution and other stress factors on aquatic life. The findings of this study will have significant implications for environmental conservation, water quality management, and sustainable aquaculture practices.

Literature Review

Superoxide dismutase (SOD) is a vital enzyme in the antioxidant defense system of many organisms, including aquatic species like fish. SOD plays an essential role in neutralizing reactive oxygen species (ROS), such as superoxide anions ($O_2^{\cdot-}$), which are by-products of cellular metabolism and can cause severe oxidative damage to lipids, proteins, and DNA if not controlled. In fish, SOD activity is crucial for maintaining cellular integrity and responding to environmental stressors such as pollution, oxygen depletion, and temperature fluctuations. This literature review explores the existing research on SOD, its role in oxidative stress, and the effects of environmental stressors on SOD activity in aquatic organisms, with a particular focus on *Labeo rohita*.

Superoxide Dismutase and Its Function

Superoxide dismutase (SOD) is an enzyme responsible for catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen (Fridovich, 1995). This process is vital for mitigating oxidative stress caused by an imbalance between the production of ROS and the body's antioxidant defense mechanisms (Halliwell & Gutteridge, 1989). There are three main types of SOD: Cu-Zn SOD, Mn-SOD, and Fe-SOD, each localized in different compartments of the cell, including the cytoplasm, mitochondria, and extracellular matrix. Cu-Zn SOD is predominantly found in the cytoplasm, while Mn-SOD is located in the mitochondria (Fridovich, 1995). The enzymatic action of SOD prevents the accumulation of superoxide radicals, which could otherwise lead to significant cellular damage and contribute to the onset of various diseases (Wang et al., 2018).

In fish, the presence and activity of SOD have been linked to their ability to cope with oxidative stress, particularly in environments with fluctuating oxygen levels. According to Crapo and Tierney (1974), SOD plays a protective role in the lungs and other tissues of vertebrates by neutralizing superoxide radicals produced during respiration. Given that fish are highly dependent on oxygen and live in environments where oxygen levels can fluctuate, SOD plays a crucial role in protecting their cells from oxidative damage. The importance of SOD in preventing oxidative damage in fish has been well-documented, making it a key enzyme for understanding the physiological responses of fish to environmental stress (Singh, 2018).

Oxidative Stress in Fish

Oxidative stress occurs when there is an imbalance between ROS production and the body's ability to neutralize these reactive molecules. In aquatic environments, fish are particularly vulnerable to oxidative stress due to their high metabolic rate and exposure to a range of environmental factors that can increase ROS production, such as pollution, temperature variations, and changes in water quality (Singh & Banerjee, 2008b). Environmental pollutants, particularly heavy metals and pesticides, have been shown to elevate oxidative stress

in fish, leading to increased SOD activity as a defense mechanism (Al-Kenawy & Aly, 2015). These pollutants generate ROS either directly by interacting with cellular components or indirectly by disrupting cellular processes like mitochondrial respiration (Singh et al., 2018).

Heavy metals, such as arsenic, cadmium, and mercury, are known to cause oxidative damage in fish tissues by generating superoxide radicals, which in turn activate antioxidant enzymes like SOD (Singh & Banerjee, 2008a). The response of fish to these pollutants includes an increase in SOD activity, suggesting that SOD acts as a crucial line of defense in protecting against oxidative damage induced by environmental contaminants (Plessl et al., 2017). Furthermore, other environmental factors, such as temperature and dissolved oxygen (DO) levels, also influence the production of ROS in aquatic organisms. Fish exposed to low DO levels, a common scenario in polluted water bodies, experience increased oxidative stress, triggering a higher production of SOD to neutralize ROS and protect cells from damage (Wang et al., 2018).

Environmental Stressors and Their Impact on SOD Activity

Environmental stressors such as pollution, temperature fluctuations, and hypoxia significantly affect the health of aquatic organisms, including fish. According to Babich et al. (1993), fish exposed to pollutants, such as heavy metals and organic chemicals, show elevated levels of oxidative stress, which is reflected in increased SOD activity. The presence of these pollutants in aquatic environments can lead to the production of ROS, which overwhelms the body's natural antioxidant defenses and triggers the upregulation of enzymes like SOD. (Vijeta Chaturvedi & Sangam 2023 vol.48 ,0974- 8946) This adaptive response helps to mitigate cellular damage but can also indicate the environmental stress the fish are experiencing (Al-Kenawy & Aly, 2015).

The role of dissolved oxygen (DO) in modulating SOD activity in fish is critical. Fish that live in oxygen-poor environments, such as stagnant or polluted waters, experience a higher oxidative burden due to low oxygen availability (Crapo & Tierney, 1974). As a result, the activity of antioxidant enzymes like SOD increases to counterbalance the oxidative damage caused by the accumulation of superoxide radicals. Studies have shown that fish exposed to low DO levels exhibit higher SOD activity in their tissues, suggesting that SOD serves as a vital defense mechanism against the damaging effects of hypoxia (Singh & Banerjee, 2008b).

Fish SOD Activity in Response to Environmental Contaminants

Research by Kolayli and Keha (1999) on antioxidant enzyme activities in rainbow trout exposed to seawater and freshwater conditions revealed that the activity of SOD and other antioxidants was higher in fish exposed to polluted waters. This increase in enzyme activity was attributed to the elevated oxidative stress induced by environmental contaminants. Similarly, Singh et al. (2018) found that fish exposed to arsenic contamination showed significant alterations in their respiratory organs, with a marked increase in SOD activity as a response to oxidative stress. These findings underscore the importance of SOD as a biomarker for environmental stress and pollution in aquatic ecosystems.

The ability of fish to modulate SOD activity in response to environmental stressors is a key factor in their survival. SOD activity can vary significantly across different tissues, depending on the level of exposure to oxidative stress. For instance, tissues like the liver and gills, which are involved in detoxification and respiration, show the highest SOD activity when exposed to high levels of pollution (Singh & Banerjee, 2009). On the other hand, less active tissues, such as the brain, may exhibit lower SOD activity, reflecting their relative susceptibility to oxidative damage (Fried, 1979).

SOD as a Biomarker for Environmental Monitoring

Due to its pivotal role in mitigating oxidative stress, SOD has been proposed as a reliable biomarker for monitoring environmental pollution and the health of aquatic organisms (Singh et al., 2009). Changes in SOD activity can provide valuable information on the physiological status of fish and the quality of the surrounding aquatic environment. Research by Plessl et al. (2017) suggests that SOD activity in fish can be used to assess the impact of trace element pollution in aquatic ecosystems. As SOD activity increases in response to oxidative stress induced by pollutants, it serves as an early indicator of environmental degradation, providing valuable data for environmental management and conservation efforts.

Conclusion

Superoxide dismutase (SOD) is a critical enzyme in the antioxidant defense system of fish, playing an essential role in protecting against oxidative damage induced by environmental stressors. The increase in SOD activity in response to pollutants, low dissolved oxygen levels, and other environmental factors highlights its importance as a biomarker for oxidative stress and pollution in aquatic ecosystems. This literature review underscores the significance of SOD in fish health and the potential of SOD activity to serve as an indicator of environmental pollution. The findings from previous studies on the effects of pollutants, including heavy metals and pesticides, on SOD activity in fish provide a solid foundation for understanding how environmental stressors influence oxidative stress responses. Future research on SOD activity in fish populations will help further elucidate the role of this enzyme in maintaining fish health and contribute to the development of effective strategies for monitoring and managing aquatic ecosystems in the face of increasing environmental pollution.

Results

This study assessed the superoxide dismutase (SOD) activity in various organs and tissues of *Labeo rohita* (Rohu) from different experimental sites along the Gomti River. The objective was to investigate the relationship between environmental stressors (such as dissolved oxygen levels) and oxidative stress responses in fish tissues. The analysis focused on the activity of total SOD, Cu-Zn SOD, and Mn-SOD in the liver, gills, adrenal glands, kidneys, cardiac muscle, skin, and brain. The results showed significant differences in SOD activity across experimental sites, with variations corresponding to the physico-chemical characteristics of the water at each site.

Physico-Chemical Parameters of Water Samples

The physico-chemical parameters of the water samples from six experimental sites are summarized in **Table 1**. The temperature, pH, alkalinity, and dissolved oxygen (DO) levels were recorded at each site. The highest DO levels were observed at Site 6, while Site 3 had the lowest DO levels.

Table 1: Physico-Chemical Parameters of Water

Experimental Sites	Temperature (°C)	pH	Alkalinity (mg/l)	Dissolved Oxygen (mg/l)
1	24.0 ± 1.35	7.3	194 ± 16.3	11.9 ± 1.83
2	24.2 ± 1.89	7.4	190 ± 17.8	11.4 ± 1.08
3	24.2 ± 1.18	7.5	199 ± 13.8	7.6 ± 0.98
4	23.8 ± 1.45	7.1	170 ± 16.8	11.3 ± 0.93
5	23.8 ± 0.21	7.0	169 ± 13.6	11.6 ± 0.53
6	24.1 ± 1.20	7.2	171 ± 13.9	12.0 ± 1.97

Dissolved Oxygen (DO) Levels across Sites

The **DO levels** were measured using Winkler's method, and the data is presented in **Table 2**. The DO levels ranged from 7.6 ± 0.98 mg/l at Site 3 to 12.0 ± 1.97 mg/l at Site 6. The low DO levels at Site 3 are particularly noteworthy as they may contribute to higher oxidative stress and, consequently, elevated SOD activity in the fish.

Table 2: Dissolved Oxygen (DO) Levels across Sites

Experimental Sites	Dissolved Oxygen (mg/l)
1	11.9 ± 1.83
2	11.4 ± 1.08
3	7.6 ± 0.98
4	11.3 ± 0.93
5	11.6 ± 0.53
6	12.0 ± 1.97

SOD Activity in Various Tissues of *Labeo rohita*

The activity of **Total-SOD**, **Cu-Zn SOD**, and **Mn-SOD** was measured in various tissues of *Labeo rohita* across the six experimental sites. The data for the liver, gills, adrenal gland, kidney, cardiac muscle, skin, and brain are presented in the following tables.

Table 3: Total SOD Activity (Units/mg Protein) in the Liver

Experimental Sites	Total SOD Activity (Units/mg Protein)
1	10.2 ± 0.75
2	9.5 ± 0.67
3	8.2 ± 0.91
4	10.5 ± 0.80
5	9.9 ± 0.50
6	11.1 ± 1.03

The data for Total SOD activity in *Labeo rohita* tissues across the six experimental sites shows variation in response to environmental stressors. Site 6 exhibited the highest total SOD activity (11.1 ± 1.03 units/mg protein), suggesting that the fish in this location may be experiencing higher oxidative stress and, therefore, have upregulated antioxidant defense mechanisms. Conversely, Site 3 showed the lowest total SOD activity (8.2 ± 0.91 units/mg protein), which could indicate less oxidative stress or a lower need for antioxidant protection at this site. Sites 1, 2, 4, and 5 had intermediate levels of SOD activity, reflecting varying levels of environmental stress, possibly influenced by factors such as dissolved oxygen levels, water temperature, or contamination. Overall, these findings suggest that SOD activity is sensitive to environmental changes, and higher SOD activity is associated with increased oxidative stress in fish.

Table 4: Cu-Zn SOD Activity (Units/mg Protein) in the Liver

Experimental Sites	Cu-Zn SOD Activity (Units/mg Protein)
1	6.5 ± 0.42
2	6.1 ± 0.35
3	5.0 ± 0.60
4	6.7 ± 0.54
5	6.2 ± 0.45
6	7.3 ± 0.67

The Cu-Zn SOD activity in the liver of *Labeo rohita* varied across the six experimental sites, with Site 6 showing the highest activity (7.3 ± 0.67 units/mg protein). This suggests that fish at Site 6 may be experiencing elevated oxidative stress, prompting a stronger antioxidant defense response. Site 3 had the lowest Cu-Zn SOD activity (5.0 ± 0.60 units/mg protein), which may indicate relatively lower oxidative stress or a lower need for antioxidant protection in this environment. Sites 1, 2, 4, and 5 showed intermediate levels of Cu-Zn SOD activity, reflecting moderate oxidative stress in these locations. Overall, the variation in Cu-Zn SOD activity across sites suggests that the liver's antioxidant response is influenced by the specific environmental conditions at each site, with higher SOD activity generally associated with greater oxidative stress.

Table 5: Mn-SOD Activity (Units/mg Protein) in the Liver

Experimental Sites	Mn-SOD Activity (Units/mg Protein)
1	3.7 ± 0.50
2	3.4 ± 0.60
3	3.2 ± 0.55
4	3.8 ± 0.45
5	3.7 ± 0.42
6	3.8 ± 0.60

The Mn-SOD activity in the liver of *Labeo rohita* displayed relatively consistent levels across the six experimental sites, with values ranging from 3.2 ± 0.55 to 3.8 ± 0.60 units/mg protein. Sites 4 and 6 exhibited the highest Mn-SOD activity (3.8 ± 0.45 and 3.8 ± 0.60, respectively), indicating that fish in these locations may have higher levels of oxidative stress, leading to a more pronounced antioxidant response. Sites 1, 2, and 5 showed similar intermediate levels of Mn-SOD activity (3.7 ± 0.50, 3.4 ± 0.60, and 3.7 ± 0.42), while Site 3 had the lowest Mn-SOD activity (3.2 ± 0.55), suggesting a comparatively lower oxidative stress or antioxidant

demand. Overall, Mn-SOD activity in the liver remained relatively stable across all sites, reflecting a moderate, consistent response to oxidative stress in this organ.

Table 6: Cu-Zn SOD Activity (Units/mg Protein) in the Gills

Experimental Sites	Cu-Zn SOD Activity (Units/mg Protein)
1	7.8 ± 0.68
2	7.4 ± 0.50
3	6.2 ± 0.45
4	8.1 ± 0.70
5	7.6 ± 0.60
6	8.3 ± 0.75

The Cu-Zn SOD activity in the gills of *Labeo rohita* varied across the six experimental sites, with Site 6 showing the highest activity (8.3 ± 0.75 units/mg protein), suggesting that fish at this site may be experiencing the highest oxidative stress, leading to a stronger antioxidant response. Site 4 also exhibited high Cu-Zn SOD activity (8.1 ± 0.70 units/mg protein), further supporting the idea that increased oxidative stress correlates with higher enzyme activity. In contrast, Site 3 had the lowest Cu-Zn SOD activity (6.2 ± 0.45 units/mg protein), which may indicate relatively lower oxidative stress or a less pronounced need for antioxidant defense. Sites 1, 2, and 5 displayed intermediate Cu-Zn SOD activity, reflecting moderate oxidative stress in these areas. Overall, the results highlight that Cu-Zn SOD activity in the gills is sensitive to environmental stressors, particularly oxidative stress, and varies accordingly across different sites.

Table 7: SOD Activity in the Brain

Experimental Sites	SOD Activity (Units/mg Protein)
1	2.5 ± 0.30
2	2.3 ± 0.25
3	1.8 ± 0.20
4	2.7 ± 0.35
5	2.5 ± 0.40
6	3.0 ± 0.50

The SOD activity in the brain of *Labeo rohita* showed relatively low levels across all experimental sites, with Site 6 exhibiting the highest activity (3.0 ± 0.50 units/mg protein). This suggests that, despite the brain's generally lower response to oxidative stress compared to other organs, fish at Site 6 might be experiencing higher oxidative stress, resulting in increased SOD activity in the brain. Site 3 recorded the lowest SOD activity (1.8 ± 0.20 units/mg protein), which could indicate a reduced need for antioxidant defense or lower levels of oxidative stress at this site. Sites 1, 2, 4, and 5 had similar intermediate SOD activity levels, reflecting moderate oxidative stress. Overall, the brain showed a more conservative response to oxidative stress in comparison to other organs, but SOD activity still varied with environmental conditions, suggesting the importance of this enzyme even in tissues with lower oxidative stress.

Statistical Analysis

The data from various tissues and experimental sites were analyzed using **Analysis of Variance (ANOVA)** to determine the significance of differences in SOD activity across sites. The results of the ANOVA for **Cu-Zn SOD** and **Mn-SOD** activities are shown in **Tables 5.3** and **5.4**, respectively. Both Cu-Zn and Mn-SOD activities exhibited significant differences across experimental sites ($P < 0.001$), indicating that environmental stressors such as dissolved oxygen levels have a profound impact on the antioxidant defense mechanisms in fish.

Table 5.8: ANOVA for Cu-Zn SOD Activity

Source	Sum of Squares (S.S.)	Mean Square (M.S.)	F-value	P-value
Total	6.0		27.7	<0.001
Between Experimental Sites	5.1	1.04		
Error	0.9	0.0373		

The ANOVA for **Cu-Zn SOD activity** reveals a statistically significant difference in enzyme activity between the experimental sites, with an F-value of 27.7 and a p-value of <0.001. This indicates that the environmental conditions at different sites significantly influence Cu-Zn SOD activity in *Labeo rohita*. The high F-value and low p-value suggest that the variation in SOD activity is not due to random error but rather to site-specific environmental factors.

Table 5.9: ANOVA for Mn-SOD Activity

Source	Sum of Squares (S.S.)	Mean Square (M.S.)	F-value	P-value
Total	5.70		21.43	<0.001
Between Experimental Sites	5.6	1.14		
Error	1.4	0.0539		

The ANOVA for Mn-SOD activity shows a significant difference between experimental sites, with an F-value of 21.43 and a p-value of <0.001. This indicates that the environmental conditions at different sites significantly affect Mn-SOD activity in *Labeo rohita*, with the variation being attributed to site-specific factors rather than random error.

Discussion

The results show significant variations in the SOD activity across different experimental sites, indicating the influence of environmental factors such as dissolved oxygen levels, pH, and alkalinity on the oxidative stress response in *Labeo rohita*. The liver and gills exhibited the highest levels of SOD activity, suggesting that these organs play a crucial role in protecting the fish from oxidative damage induced by environmental pollutants and other stressors. The brain, in contrast, showed the lowest SOD activity, possibly reflecting its limited ability to cope with oxidative stress compared to other tissues.

The findings from this study are consistent with previous research indicating that SOD activity in fish is influenced by environmental stressors, and SOD is a useful biomarker for assessing oxidative stress and water quality (Singh et al., 2009). Further studies are needed to explore the relationship between specific pollutants and SOD activity in different fish species and to assess the long-term effects of environmental stress on aquatic ecosystems.

The results of this study demonstrate the significant role of superoxide dismutase (SOD) activity in responding to oxidative stress in *Labeo rohita*, with variations observed across different experimental sites along the Gomti River. The study highlights the correlation between environmental stressors, such as dissolved oxygen (DO) levels, and SOD activity in various tissues of the fish. The higher SOD activity observed at sites with elevated oxidative stress, such as Site 6, suggests that *Labeo rohita* upregulates antioxidant defense mechanisms, particularly Cu-Zn SOD and Mn-SOD, in response to environmental challenges. In contrast, lower SOD activity at sites with relatively better environmental conditions, such as Site 3, indicates a less pronounced oxidative stress response.

The ANOVA results for both Cu-Zn SOD and Mn-SOD activity confirmed the statistically significant impact of environmental factors on enzyme activity, reinforcing the importance of SOD as a biomarker for assessing oxidative stress and water quality. The liver, gills, and brain of the fish showed varying levels of SOD activity, with the liver and gills exhibiting higher activities due to their roles in metabolic processes and detoxification. Meanwhile, the brain, which generally shows lower SOD activity, indicated the tissue's limited but still significant antioxidant response to oxidative stress.

Overall, this study underscores the adaptive role of SOD in maintaining cellular homeostasis under oxidative stress and provides valuable insights into how fish respond to environmental stressors. By understanding these physiological mechanisms, SOD activity can be utilized as an effective biomarker for monitoring the health of fish populations and assessing the quality of freshwater ecosystems.

Conclusion

In conclusion, this study highlights the crucial role of superoxide dismutase (SOD) activity in *Labeo rohita* as a protective mechanism against oxidative stress induced by environmental stressors such as dissolved oxygen levels and pollutants. The significant variations in Cu-Zn SOD and Mn-SOD activity across different experimental sites indicate that environmental factors play a key role in regulating antioxidant defense mechanisms in fish. Higher SOD activity at sites with elevated oxidative stress underscores the adaptability of *Labeo rohita* to fluctuating environmental conditions. Overall, SOD activity serves as an effective biomarker

for assessing oxidative stress, providing valuable insights into the health of fish populations and the impact of environmental changes on aquatic ecosystems.

References

1. Al-Kenawy, D. A., & Aly, N. A. (2015). Levels of some heavy metals in muscles and liver of freshwater farmed fish at Abbassa Abbassa. *International Journal for Aquaculture*, 8, 20–35.
2. Babich, H., Palace, M. R., & Stem, A. (1993). Oxidative stress in fish cells: In vivo studies. *Archives of Environmental Contamination and Toxicology*, 24(2), 173–178.
3. Bert, P. (1878). *Barometric pressure: Researches in experimental physiology*. In M. A. Hitchcock & F. A. Hitchcock (Trans.), *La Pression Barometrique*. Ohio: College Book.
4. Christine, J. W., & Cullen, J. J. (2010). Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue. *Nature Protocols*, 5(1), 51–66.
5. Crapo, J. D., & Tierney, D. L. (1974). Superoxide dismutase and pulmonary oxygen toxicity. *American Journal of Physiology*, 226, 1401–1407.
6. Doroshow, J. H. (1989). Role of oxygen radical metabolism in the cardiac toxicity of anticancer quinones. In C. Vigo-pel-ffey (Ed.), *Membrane Lipid Peroxidation* (pp. 303–314). Boca Raton: CRC Press.
7. Devbrat Mishra, Rajesh, Winkle & VB Singh: (2008-09) Distribution of Antioxidant Enzyme SOD in Gomti & Ganga River in liver & skin of Cirrhinus Mrigala (Ham.) *Asian J.Env. Sci.*3(2) 128- 130
8. Devbrat Mishra, Prashant Singh(2020) Biochemical Activity of Superoxide Dismutase Enzyme in Liver of Labeo rohita in River Gomti. *Indian Journal of Biology. Vol.7,(1) Jan – Jun 2020, 15- 19*
9. Evans, H. J., Steinman, H. M., & Hill, R. L. (1974). Bovine erythrocyte superoxide dismutase. Isolation and characterization of tryptic cyanogen bromide and maleylated tryptic peptides. *Journal of Biological Chemistry*, 249, 7315.
10. Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*, 64, 97–112.
11. Fried, R. (1979). Superoxide dismutase activity in the nervous tissue. *Journal of Neuroscience Research*, 4, 435–441.
12. Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine* (2nd ed.). Oxford: Clarendon Press.
13. Harman, D. (1971). Free radicals: Theory of ageing. *Journal of Gerontology*, 26, 451–457.
14. Harmon, D. (1986). Free radical theory of aging: Role of free radicals in the origination and evolution of life, aging and disease processes. In J. E. Johnson, R. Waford, D. Harman, J. Miquel (Eds.), *Free Radicals, Aging and Degenerative Diseases* (pp. 3–49). New York: Alan R. Liss, Inc.
15. Harris, J. I., & Steinman, H. M. (1977). The amino acid sequence homologies among SOD. In J. M. Michelson, J. M. McCord, & I. Fridovich (Eds.), *Superoxide and Superoxide Dismutase* (pp. 225). Academic Press.
16. Izokun-Etiobio, B. O., Oraedu, A. C., & Ugochukwu, E. N. (1990). A comparative study of superoxide dismutase in various animal species. *Comparative Biochemistry and Physiology - B*, 95(3), 521–523.
17. Kolayli, S., & Keha, E. A. (1999). A comparative study of antioxidant enzyme activities in freshwater and seawater adapted rainbow trout. *Journal of Biochemical and Molecular Toxicology*, 13(6), 334–337.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–268.
19. McCord, J. M., Keele, B. B. Jr., & Fridovich, I. (1971). An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. *Proceedings of the National Academy of Sciences USA*, 68, 1024–1027.
20. McCord, J. M., & Fridovich, I. (1969). The utility of superoxide dismutase in studying free radical reactions. I. Radical generated by the interaction of sulfite, dimethylsulfoxide, and oxygen. *Journal of Biological Chemistry*, 244, 6056–6063.
21. Otto, D. M., & Moon, T. W. (1995). 3,3', 4,4' tetrachlorobiphenyl effects on antioxidant enzymes and glutathione status in different tissues of rainbow trout. *Pharmacology and Toxicology*, 77(4), 281–287.
22. Plessl, C. L., Elick, O. Otachi, E. O., Körner, W., Oldewage, A. A., & Jirsa, F. (2017). Fish as bioindicators for trace element pollution from two contrasting lakes in the Eastern Rift Valley, Kenya: Spatial and temporal aspects. *Environmental Science and Pollution Research International*, 24(24), 19767–19776.
23. Singh, A. (2018). The nitric oxide: A molecule of possible therapeutic use against aquatic stress in fishes. *Biology and Medicine: Case Report*, 2(2), 7–8.

24. Singh, A. K., & Banerjee, T. K. (2008a). Arsenic-induced biochemical alterations in the respiratory organs (gills, ABOs, and skin) of the walking catfish *Clarias batrachus* Linnaeus. *Biological Research*, 41, 341–350.
25. Singh, A. K., & Banerjee, T. K. (2008b). Recovery of damages in the skin of arsenic exposed *Clarias batrachus* (Linn.) following withdrawal of the stress. *Iranian Journal of Science and Health Engineering*, 5, 217–224.
26. Singh, A. K., & Banerjee, T. K. (2008c). Toxic impact of sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) on skin epidermis of the air-breathing catfish *Clarias batrachus* (Linn.). *Veterinarski Arhiv*, 78, 73–88.
27. Singh, A. K., & Banerjee, T. K. (2009). A study on carbohydrate moieties of the gills and air-breathing organs of the walking catfish *Clarias batrachus* (Linn.) following exposure to arsenic. *Toxicological and Environmental Chemistry*, 91, 43–52.
28. Singh, A. K., & Banerjee, T. K. (2014). Histopathological and histochemical study on gills of the freshwater walking catfish *Clarias batrachus* (Linn.) following exposure and withdrawal of arsenic stress. *International Journal of Integrative Sciences, Innovation, and Technology*, 3(4), 12–19.
29. Singh, A. K., Sawale, S., & Chavan, S. (2016). Glycoconjugates study in surface secretions of a freshwater edible catfish, *Clarias gariepinus* in response to clove oil anaesthesia. *International Journal of Chemical and Biomedical Sciences*, 2(5), 34–41.
30. Vijeta Chaturvedi, Deepak Varma, DevBrat Mishra (2022) Histochemical Reaction in the Epithelial cells in different layers of epidermis of *Channa punctatus*. Vol- IX, Issue II (IV) 393- 404
31. Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology*, 217(6), 1915–1928.
32. Younus, H. (2018). Therapeutic potentials of superoxide dismutase. *International Journal of Health Sciences (Qassim)*, 12(3), 88–93.