**Journal of Agriculture and Veterinary Science**
ISSN: 2959-1198 (Print), 2959-1201 (Online)

Check for updates

ROOTS PRESS

**Research Article**

# Oxidative Stress Biomarkers in the Fish Samples Collected from Taunsa Barrage

**Shahena Perveen[1], Fariha Latif[1], Sana Aziz[2], Saman Iram[1]**
[1]*Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan.*
[2]*Department of Zoology, University of Jhang, Jhang, Pakistan.*

## ABSTRACT

The present research work entitled “oxidative stress biomarkers in the fish samples collected from Taunsa Barrage” was done to measure the activity of antioxidants enzymes in major carps (*Labeo rohita*, *Channa marulius* and *Cirrhinus mrigala*). A condition known as oxidative stress arises when the body produces too many free radicals, which are unstable chemicals, and not enough antioxidants to eliminate them. Numerous pollutants, such as insecticides and pesticides, have an adverse effect on fish, impairing their metabolism. Samples of fish obtained using the nets and immediately frozen. Fish were dissected to retrieve the liver, kidney, gills, and muscles for study. Antioxidants enzymes (Superoxidase dismutase (SOD), peroxidase (POD) and catalase (CAT)) activity was measured. The extent of oxidative stress in the tissues of various fish species was evaluated using POD, SOD, and CAT levels. *C. marulius* (fourth sampling) has the significantly (P<0.05) greatest "SOD" activity of the group, afterwards *C. mrigala* and *L. rohita*.CAT activity was greater in the *C. mrigala*. *L. rohita* has the highest (P<0.05) peroxidase activity. The livers of all three fish species showed increased 'Superoxidase dismutase', catalase and peroxidase activity in comparison with various fish organs.

**Keywords:** Oxidative Stress, Antioxidant Enzymes, Liver, Gills, Catalase.

OPEN ACCESS

***Correspondence***
*Fariha Latif*
*farihalatif@bzu.edu.pk*

***Article History***
*Received: January 09, 2024*
*Accepted: November 05, 2024*
*Published: December 04, 2024*



## INTRODUCTION

Household and industrial garbage have led to an increase in water contamination throughout the world (Gül et al., 2004). Large amounts of complex chemical compounds with high oxidation potential are released into lakes and rivers daily. The interactions among these pollutants and aquatic organisms can cause biochemical and physiological changes that may be exacerbated by extreme temperatures in subtropical and tropical areas (Amado et al., 2006).

Heavy metals, suspended particles, grease, plastics, solvents, oils, plasticizers, phenols, and pesticides are just a few of the many inorganic and organic contaminants that are typically included in these wastes (Ojha & Tiwary, 2021). When wastes including the aforementioned dangerous substances are carelessly dumped and released into rivers, the ecosystem may be disturbed, which becomes a source of stress for the organisms that live there (Pandey *et al*., 2003).

Organisms that live in water are subjected to a variety of stresses during their life, such as pollutants, diseases, and temperature fluctuations (Marcogliese *et al*., 2005). Metals, in particular heavy metals, are significant global toxicants of aquatic habitats. As human society has developed technologically, metal pollution has risen. The activities that are thought to be the main contributors to metal pollution are industry, contemporary agriculture, mining, automobiles, and household garbage. Metals are persistent in sediments and water and can build up in aquatic

species, including fish (Luoma & Rainbow, 2008). Fish from contaminated locations could be harmful to human health because they are an essential component of the human diet. Fish serves as the top members of the aquatic food chain, making them good biological indicators of “metallic pollution”. Because ‘metals’ are infamous for causing oxidative stress (OS), measuring oxidative damage, antioxidant defenses in fish can reveal whether the aquatic environment has been contaminated with metals or not (Livingstone, 2003).

The main reason why pollutants are becoming more prevalent in aquatic habitats is agricultural activity (da Fonseca *et al*., 2008). It can have harmful impacts that degrade water quality and negatively impact fish and other non-target organisms. There are several sources of pollutants, including pesticides, which comprise a huge class of harmful substances (Crestani *et al*., 2006).

However, they are also responsible for the deterioration of our aquatic ecosystems. They were transported into bodies of water, such as rivers and ponds, through surface runoff, and they changed the physical and chemical features of water. Pesticide usage has risen by over four times since the beginning of the “Green Revolution”, and agricultural goods have been shown to have significant pesticide residual levels. The widespread use of synthetic pesticides is the main cause. There are several organic micro-pollutants with significant ecological effects, and pesticides are among them (Kaur & Jindal, 2017).

The residuals of pesticides in the aquatic environment present a wide range of non-target creatures with toxicological risks, eventually making their way into the food chains (Dar *et al*., 2015). Pollutants have an elevated capacity to cause oxidative stress in aquatic organisms by releasing free radicals as well as reactive oxygen species (ROS) (Toni *et al*., 2011).They can also create an imbalance between intracellular levels of ROS and antioxidant defence, which in turn can lead to oxidative stress in living things (Sharbidre *et al*., 2011). ROS can harm nucleic acids, lipids, proteins, and carbohydrates. Cellular functions may be altered as a result of the damage, which ultimately results in cell death. Antioxidants found in fish may serve as indicators for exposure to aquatic contaminants (Yonar, 2012).

Fish and other aquatic species are susceptible to oxidative stress caused by a variety of environmental contaminants. The key component of ecotoxicology research are oxygen radical and oxidative stress, they cause have attracted a lot of attention (Bacanskas *et al*., 2004). Due to the redox cycle of pollution, aquatic pollution is a significant cause of fish oxidative stress (Velkova-Jordanoska *et al*., 2008). Exposure to pollutants in aquatic habitats can accelerate the creation of ‘ROS’ inside body cells, that causes destruction to the functioning of biological systems. When there is an imbalance between ROS generation and ROS removal, oxidative stress results (Gohil *et al*., 2012).

Oxidative stress results in an imbalance between antioxidants and oxidants that may damage cells (Azzi *et al*., 2004). Animals may survive in polluted environments mostly because of defence system that permit for anti-oxidants guarding (Bard, 2000), excretion , and to remove harmful substances. Toxic drug bioaccumulation sets off redox processes that produce ‘free radicals’, but also other ROS, which cause metabolic changes in fish tissues (Narra, 2016). Animals have developed method that can avoid or reverse consequences of ‘OS’ in order to defend themselves from the potentially very harmful ROS (Dröge, 2002). Fish are highly varied and provide numerous ecosystem functions. Fish are employed as a bioindicator to measure the amount of pollution in the water (Lynch *et al*., 2016).Thus, this study was carried out to quantify oxidative stress biomarkers in the fish samples collected from Taunsa Barrage.

## MATERIALS AND METHODS

### Site selection

For the current research, Head Taunsa Barrage has been chosen. It is 139 meters above sea level and is located between 30° 30′ 46″ and 70° 50′ 57″ East longitudes. Over the course of a five-year period (1953–1958), the Taunsa Barrage was constructed on the ‘River Indus’ (Haider *et al*., 2022).

### Fish samples collection

Fishing nets were used to collect fish samples from the Head Taunsa barrage, which were then frozen instantly. After specimens were collected, they were analyzed. All of the specimens were taken to the lab at the Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan, within the same day by using polythene bags while being frozen by being maintained in an ice box. Fish were dissected with the use of a dissection box. The 1$^{st}$ sampling was done in October, 2$^{nd}$ sampling was done in December, 3$^{rd}$ sampling was done in February, and fourth sampling was done in june. 35 samples were attained, and their length and weight were measured, and the average of length was calculated.

**Antioxidant enzymes studies**

Antioxidant enzymes (CAT, POD, SOD) were extracted from fish gills, liver, kidney, and muscle in order to assess the biochemical characteristics. The organs that had been dissected were mixed in chilled “phosphate buffer” (0.2M) having 6.5 ‘pH’ with 1:4 (w/v) additions. Centrifuging was performed on organ homogenates for fifteen minutes at 10,000 rotations per minute and four degrees Celsius. The clear supernatants generated after centrifugation were kept at -80 degrees Celsius for enzyme assays, while the remnants were thrown away.


Figure 1. Map of location of Taunsa barrage

**Superoxide dismutases (SOD) assay**

SOD activity was quantified by looking at how well it could prevent “Nitroblue tetrazole” (NBT) from being photoreduced (Aslam *et al*.,2023).

**Required solutions**

**A potassium phosphate buffer (0.067 mM) with a pH value of 7.8.**

Dipotassium phosphate (740 mg) and Potassium dihydrogen phosphate (140 mg) were placed in a flask. Distilled water amounting to 0.08 litres was added in above solution.

**0.1M solution of EDTA**

Sodium cyanide (NaCN,0.08 mg), Ethylenediaminetetraacetic acid (160 mg), and distilled water up to 0.0054L were placed in a glass flask.

**0.12 Mm of Riboflavin:**

Riboflavin (0.06 mg) was placed in a flask, to which distilled water was added in order to create a volume of ‘0.0013’ Litres. The solution was kept in a colder, bottle.

**Nitroblue tetrazolium:**

A flask containing ‘3.23’ mg of NBT was filled to a volume of “0.00264” L, with distilled water before being placed in a cold, dark bottle for storage.

**Method**

A flask containing one milliliter of buffer was used as a blank, and the measurements from the spectrophotometer were recorded and set to zero. After that, a vessel containing buffer (1 ml), extract of 0.016 ml riboflavin and 0.05 ml enzyme had been incubated for twelve minutes in an illuminated box. Following that, the tubes was moved, and all above solution were added to the lighted reaction mixture, and after twenty seconds, the absorption at A560 nm was observed with the help of spectrophotometer. Utilizing the following formulas, the activity of SOD was determined.

$$\% \text{ age inhibition} = \frac{\text{Blank (Abs)} - \text{Sample (Abs)X 100}}{Blank\ (Abs)}$$

**Catalase (CAT) assay**

The capacity of CAT to lower the hydrogen peroxide levels at a wavelength of 240 nm was used to gauge its activity (Naz *et al*., 2021).

**A pH value of 7.0 sodium phosphate buffer (60 mM)**

Monosodium phosphate (NaH2PO4,224 mg), disodium hydrogen phosphate, (Na2HPO4,163.2 mg) both were mixed

together in 0.05 Litres of water that was distilled and put into a flask.

**Buffer substrate solution**

To create buffered substrate solution, '0.442' ml of ten mM Hydrogen peroxide dissolved in sixty mM phosphate buffer solution.

**Process**

The spectrophotometer was calibrated to zero at A240 nm using two millilitres of buffer solution (used as a blank) that was placed in a test tube. Add 0.05 millilitres of enzyme extract and 1.95 ml of buffered substrate to a different cuvette. Using a spectrophotometer, the absorption value at A240 nm was recorded after a three-minute reaction period. The CAT activity was determined using the following formulas.

$$Activity\ \left(\frac{U}{mL}\right) = \frac{\Delta A \times \text{dilution} \times 2\text{ml}}{004M-1cm-1\times 0.05ml}$$

**Peroxidase (POD) Assay**

The method described below was used to identify the POD activity (Narra *et al*.,2017).

**Solutions**

**phosphate buffer (0.2 M, pH 6.5)**

Na2HPO4 (one thousand mg) and NaH2PO4 (4,000 mg) were combined and dispersed into 0.2 L of distilled water in a flask.

**Substrate Buffer Solution**

Guaiacol (750 µl) was blended thoroughly with 47 ml of phosphate buffer in a vessel after being added. 0.3 ml of Hydrogen peroxide was added to this solution after stirring.

**Method**

The spectrophotometer had been set to a zero value at A470 nm in a flask with 3 ml of buffer solution (used as a blank). Add POD extract (0.06 ml) to the buffered substrate solution (three millilitre) in a test tube. After three minutes, a spectrophotometer was used to record the intensity of absorption at A470 nm. The POD activity is calculated using the formulas below.

$$\text{Activity (Units/ml)} = \frac{\Delta A/_3}{26.6\times 60/_{3000}}$$

**Statistical Analysis**

Three replications of every experiment level were used in the Factorial design, which used to statistically assess the data. The LSD (Least Square Design) method was used to evaluate the average value for multiple variables.For statistical similarities and differences among variable means, the values were analyzed through Analysis of Variance (ANOVA) and Tukey's Student Newman-Keul test by using Statistix8.1 computer package.

## RESULTS

Three fish species (*Labeo rohita, Channa marulius and Cirrhinus mrigala*) were captured from the natural water of the (Head Taunsa Barrage) during the current research project.

The activity of 'SOD' in organs of three species of fish at four sampling duration varied. In *L. rohita*, *C. marulius, and C. mrigala* "4$^{th}$" sampling showed higher SOD levels as compared to the 1$^{st}$, 2$^{nd,}$ and 3$^{rd}$ sampling. Compared to other fish organs, the livers of all three fish species displayed significantly ($p< 0.05$) higher 'Superoxidase dismutase' activity (Table 1). The highest "SOD" activity (142.06±25.25) among them is found in *C. marulius* (fourth sampling), followed by *C. mrigala* (139.03±15.93) and *L. rohita* (126.64±29.24) (Figure 2). Table 2 displays the correlation between fish species, sampling, and fish tissues for CAT enzymes. *C. marulius* (675.00±79.68) and *L. rohita* (663.00±81.07) come after it. Comparing the liver with various other fish organs, it exhibits increased enzyme activity (Table 2). Figure 3 compares the catalase activity of various fish species' gills, livers, kidneys, and muscles.

During their fourth sampling duration, the peroxidase activity of *L. rohita*, *C. marulius*, and *C. mrigala* increased. Their respective mean values for the 4$^{th}$ samplings are 0.356±0.128 (*L. rohita*), 0.336±0.129 (*C. marulius*), and 0.316±0.130 (*C. mrigala*). The liver had the signaficiant ($p< 0.05$) highest peroxidase levels, followed by the kidney, gills, and muscles (Table 3). Figure 4 shows an illustration of the peroxidase enzyme activity in three different fish species' livers, kidneys, muscles, and gills.

The 4$^{th}$ sampling was done in the summer. Several environmental conditions, including higher water temperatures, lower oxygen levels, and greater metabolic rates, can impact the activity of these enzymes when fish are sampled in

the summer. Warmer water temperatures cause an increase in ROS generation, which in turn causes an increase in SOD, POD and CAT activity during 4th sampling.

Table 1. Superoxidase Dismutase enzyme activity in the various organs of fish species sampled at four intervals.

| Species | Sampling | Gills | Muscles | Kidney | Liver | Means ± SD |
|---|---|---|---|---|---|---|
| *L. rohita* | 1st | 80.78±4.35b | 50.87±4.72d | 66.13±2.00c | 102.46±3.77a | 75.06±21.97d |
| | 2nd | 96.11±2.65b | 71.79±2.61d | 84.79±3.63c | 110.01±3.60a | 90.67±16.27 c |
| | 3rd | 105.55±3.07b | 84.83±3.60d | 98.40±1.54c | 129.45±2.65a | 104.56±18.69 b |
| | 4th | 139.30±2.62b | 89.94±1.00d | 119.06±2.65c | 158.26±2.00a | 126.64±29.24a |
| Means ± SD | | 105.43±24.78b | 74.36±17.42d | 92.10±22.32c | 125.04±24.89a | |
| *C. marulius* | 1st | 82.64±2.11b | 55.60±2.06d | 69.08±1.65c | f112.70±2.66a | 80.00±24.43d |
| | 2nd | 107.31±1.99b | 79.67±2.61d | 102.53±2.08c | 126.51±3.26a | 104.01±19.25c |
| | 3rd | 126.29±2.63b | 97.29±2.78d | 118.11±2.65c | 151.61±2.64a | 123.32±22.46b |
| | 4th | 157.23±3.61b | 108.24±3.44d | 137.89±4.57c | 164.89±2.65a | 142.06±25.25a |
| Means ± SD | | 118.37±31.47b | 85.20±22.97d | 106.90±29.07c | 138.93±23.64a | |
| *C. mrigala* | 1st | 78.69±4.00b | 42.98±2.65d | 70.50±3.61c | 89.61±4.58a | 70.44±19.91d |
| | 2nd | 102.52±2.65b | 69.31±4.58d | 100.79±3.61c | 108.43±3.00a | 95.26±17.60c |
| | 3rd | 113.38±2.65b | 91.28±4.58d | 109.71±3.46c | 135.29±4.36a | 112.42±18.05b |
| | 4th | 149.62±4.36b | 118.06±2.65d | 135.38±4.58c | 153.06±3.61a | 139.03±15.93a |
| Means ± SD | | 111.05±29.51b | 80.41±31.93d | 104.10±26.76c | 121.60±28.12a | |

Table: 2 Correlation of catalase activity among three fish species.

| Species | Sampling | Gills | Muscles | kidney | Liver | Means ± SD |
|---|---|---|---|---|---|---|
| *L. rohita* | 1st | 711.11±1.95b | 548.65±2.06d | 663.50±2.65c | 728.75±2.00a | 663.00±81.07a |
| | 2nd | 681.56±2.15b | 520.96±2.65d | 618.48±2.07c | 706.66±2.08a | 631.91±82.75b |
| | 3rd | 634.80±0.99b | 482.83±3.25d | 602.33±1.73c | 662.60±2.08a | 595.64±79.13c |
| | 4th | 607.97±2.65b | 465.77±4.36d | 557.39±2.11c | 620.58±2.03a | 562.93±70.29d |
| Means ± SD | | 658.86±46.23b | 504.55±37.36d | 610.42±43.81c | 679.65±48.03a | |
| *C. marulius* | 1st | 720.55±3.61b | 563.68±2.64d | 673.11±2.62c | 742.65±2.63a | 675.00±79.68a |
| | 2nd | 693.19±2.64b | 531.29±2.65d | 620.55±3.61c | 718.91±3.60a | 640.98±84.16b |
| | 3rd | 651.05±2.65b | 498.91±3.00d | 607.64±3.62c | 684.48±2.60a | 610.52±80.78c |
| | 4th | 613.49±4.39b | 472.73±2.65d | 562.79±3.66c | 639.66±2.63a | 572.17±73.57d |
| Means ± SD | | 669.57±47.06b | 516.66±39.45d | 616.02±45.39c | 696.42±44.74a | |
| *C. mrigala* | 1st | 739.62±2.65b | 582.83±3.61d | 692.70±4.58c | 765.09±3.00a | 695.06±80.61a |
| | 2nd | 703.94±2.63b | 551.86±2.65d | 660.78±4.36c | 727.83±4.58a | 661.10±77.93b |
| | 3rd | 647.94±4.36b | 510.60±4.58d | 621.60±3.61c | 690.49±2.65a | 617.66±76.81c |
| | 4th | 614.99±3.61b | 489.37±2.65d | 575.28±3.00c | 648.86±4.58a | 582.13±68.76d |
| Means ± SD | | 676.62±55.78b | 533.67±41.80d | 637.59±50.70c | 708.07±49.85a | |

## DISCUSSION

It is well recognized that releasing contaminants into water bodies has an adverse impact on both the natural world and living things, which makes research on the reactions of aquatic species to hazardous substances that produce oxidative stress quite interesting (Soares *et al*., 2008).Variety of sludge can cause oxidative defilement to some extent by producing unstable molecules and/or changing the enzyme process that control reactive oxygen radicles (Huang *et al*., 2007). Numerous oceanic and aquatic species have reactive defensive enzymes as indications of pollutant- or oxidative harm, and their production represents a particular sensitivity to contamination (Borković *et al*., 2005).

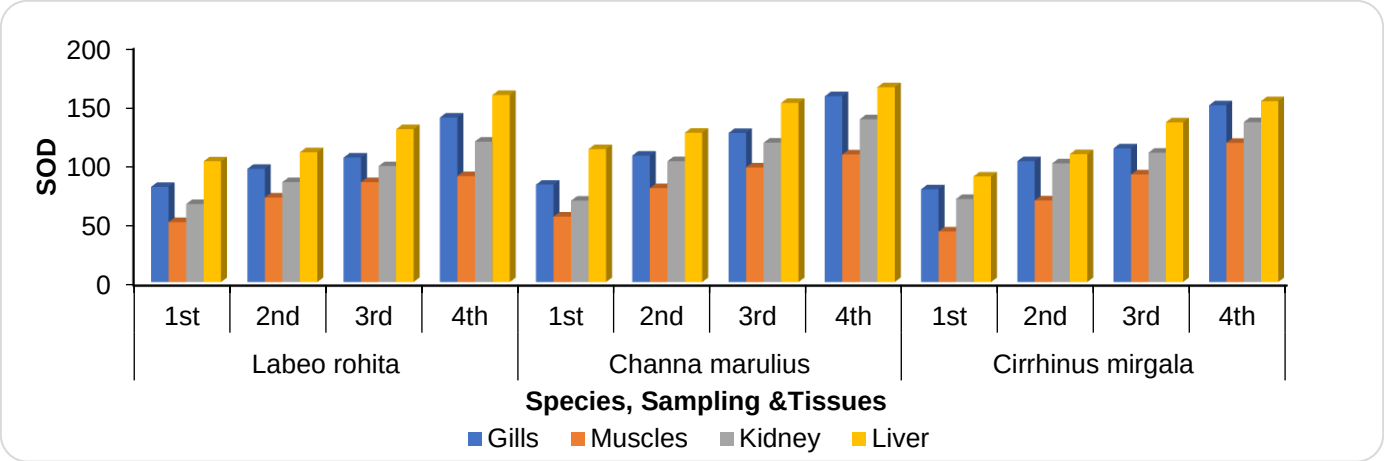


Figure 2. Comparison of 'SOD' activity among tissues of three species.

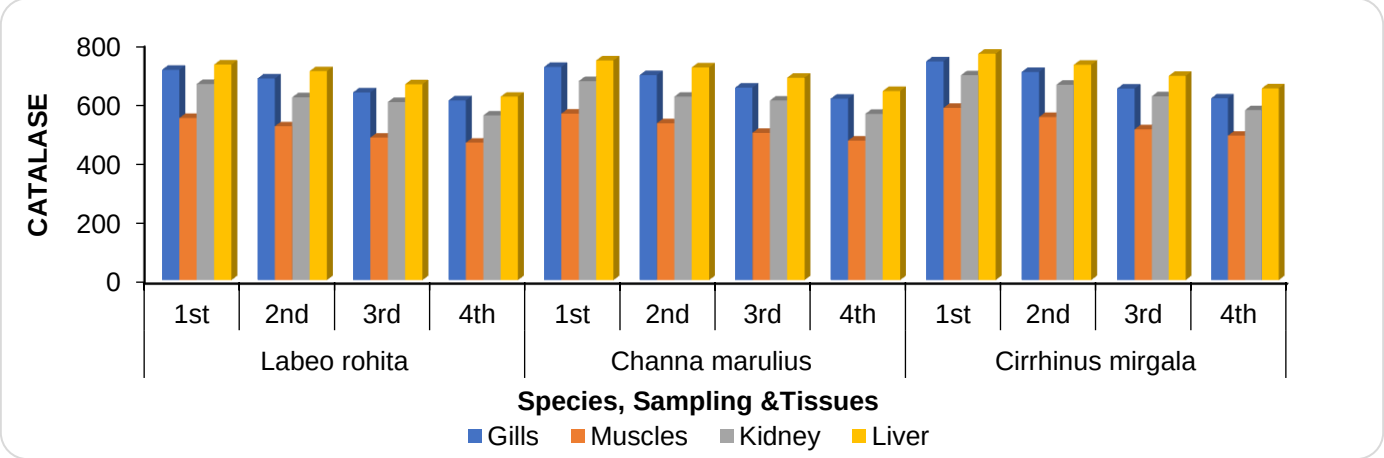


Figure 3: Study of catalase activity in three fish species.

Table 3. Comparison of peroxidase activity among three fish species.

| Species | Sampling | Gills | Muscles | kidney | Liver | Means ± SD |
|---|---|---|---|---|---|---|
| *L. rohita* | 1st | 0.218±0.004c | 0.092±0.002d | 0.239±0.003b | 0.272±0.003a | 0.205±0.078d |
| | 2nd | 0.264±0.004c | 0.118±0.003d | 0.286±0.004b | 0.360±0.001a | 0.257±0.101c |
| | 3rd | 0.321±0.005c | 0.146±0.004d | 0.371±0.005b | 0.412±0.002a | 0.313±0.117b |
| | 4th | 0.368±0.006c | 0.174±0.002d | 0.410±0.003b | 0.473±0.003a | 0.356±0.128a |
| Means ± SD | | 0.293±0.06551 c | 0.133±0.035379d | 0.327±0.077994b | 0.379±0.085117a | |
| *C. marulius* | 1st | 0.206±0.003c | 0.067±0.003d | 0.231±0.004b | 0.259±0.003a | 0.191±0.085d |
| | 2nd | 0.251±0.002c | 0.110±0.004d | 0.296±0.003b | 0.346±0.005a | 0.251±0.101c |
| | 3rd | 0.296±0.003c | 0.137±0.004d | 0.352±0.007b | 0.398±0.002a | 0.296±0.113b |
| | 4th | 0.328±0.004c | 0.160±0.003d | 0.392±0.005b | 0.462±0.005a | 0.336±0.129a |
| Means ± SD | | 0.270±0.053219c | 0.119±0.039954d | 0.318±0.069964b | 0.366±0.085807a | |
| *C. mrigala* | 1st | 0.179±0.002c | 0.088±0.004d | 0.206±0.003b | 0.226±0.003a | 0.175±0.060d |
| | 2nd | 0.237±0.003c | 0.103±0.002d | 0.273±0.004b | 0.315±0.004a | 0.232±0.091c |
| | 3rd | 0.275±0.004c | 0.126±0.003d | 0.341±0.002b | 0.384±0.006a | 0.282±0.112b |
| | 4th | 0.310±0.005c | 0.137±0.005d | 0.382±0.003b | 0.436±0.005a | 0.316±0.130a |
| Means ± SD | | 0.250±0.056c | 0.114±0.022d | 0.301±0.077b | 0.340±0.090a | |

This recent study sought to assess the production of "oxidative stress" in various organs of three fish species—

*L. rohita*, *C. marulius*, and *C. mrigala*—that exhibit different patterns of behaviour while residing in a contaminated environment. Polluting substances like insecticides are bioactivated and degraded by the liver, which is a critical process. Pesticides have been studied for a possible cause for their harm and disintegration, including the creation of oxidative stress-related situations in multiple organs (Oruç & Üner, 2000). By measuring the peroxidase, SOD, and CAT activity, the 'OS' was kept under observation.

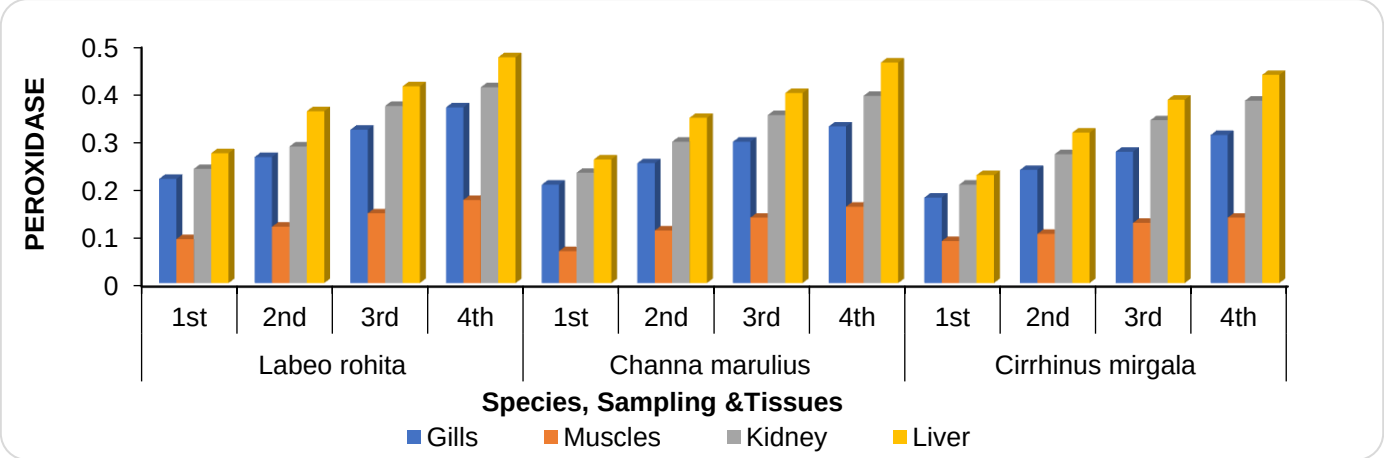


Figure 4. Comparison of Peroxidase activity of three fish species.

In order to protect from the destructive effects of oxygen, the superoxidase-catalase mechanism serves as the body's initial line of defence. The reactive superoxide particle is dismutated by SOD into H2O2 and H2O, whereupon CAT activity then detoxifies.

This kind of link was found in the current analysis. In our investigation, it was discovered that fish liver tissues taken from the site of contamination had significant levels of SOD and CAT activity. A number of researchers claimed that the fish from the polluted area had increased the amount of SOD enzyme, which indicated an elevated yield of super oxide ions (Das *et al*., 2004). Most people agree that fish are a good example of evaluating aquatic pollution and are employed as an ecological monitor for water toxins. In the current research additionally, the livers of all carps (*C. marulius*, *L. rohita*, and *C. mrigala*) had increased levels of catalase and SOD. While above enzymes levels were decreased in the fleshy gill, muscles and kidney. Following is the sequence of their enzymes' actions.

Liver>gills>kidney>muscles

This discovery concurs with those made by Pandey *et al*. (2003) regarding catfish. It was discovered that the gills' concentrations of enzymes that protect cells had reduced than liver.

According to research by Yin *et al*. (2007), phenanthrene may buildup in the liver of Gold fish and cause the generation of reactive oxygen species, or which causes 'OS'. Modifications in the activity of enzymes that protect against oxidative stress also supported this finding.

Similar to investigations conducted by Li *et al*. (2003), microcystin- LR has been shown to increase CAT and SOD function in liver of common carp. Fish hepatocytes is a potential targeted tissue for different chemical contaminants because it serves as the organism's detoxifying organ. Fish are able to absorb chemicals from the environment and deposit contaminants primarily in their fat-covered tissues (Liver). Their consequences are noticeable when quantities in such tissue reach an unacceptable value (Eriksson, 2000).

Fatima *et al*. (2000) findings imply that organophosphorus herbicides increased the creation of reactive oxygen species in the organs of the liver of *Clarias batrachus*. Since the hepatocytes is an organ that has a more effective system of antioxidants than other cells, this raises those tissues' susceptibility to radicals. Because of the different antioxidant properties that exists in these organs, the way that different tissues respond to oxidative damage varies depending on the fish species (Ahmad *et al*., 2000). Additionally, striped eel catfish subjected to micromite had higher catalase levels in their livers, according to Maduenho and Martinez (2008).

Naz *et al*. (2019) found that across different organs, the hepatocytes of *L. rohita* had the largest mean peroxidase activities.it is due to the liver's function in toxin detoxifying. All these findings are identical to those of the current work.

## CONCLUSION

The proposed research work was conducted in the laboratories of institute of Zoology Bahauddin Zakariya University,

Multan (Pakistan). The following conclusions were drawn from the present experimental work: The comparison of activities of enzymes in the organs of *C. mrigala, L. rohita* and *C. marulius* was observed as liver > gills > kidney > muscles. When compared among species, the order of enzymes activities is given below:

*C. marulius* > *C. mrigala*> *L.rohita* (Superoxidase dismutase)

*C.mrigala*> *C. marulius* >*L. rohita* (Catalase)

*L. rohita*>*C. marulius* >*C. mrigala* (Peroxidase)

## AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

## COMPETING OF INTEREST

The authors declare no competing interests.

## REFERENCES


Ahmad, I., Hamid, T., Fatima, M., Chand, H. S., Jain, S. K., Athar, M., & Raisuddin, S. (2000). Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochimica et Biophysica Acta (BBA)-General Subjects, 1523(1), 37-48.

Amado, L. L., Robaldo, R. B., Geracitano, L., Monserrat, J. M., & Bianchini, A. (2006). Biomarkers of exposure and effect in the Brazilian flounder *Paralichthys orbignyanus* (Teleostei: Paralichthyidae) from the Patos Lagoon estuary (Southern Brazil). Marine Pollution Bulletin, 52(2), 207-213.

Azzi, A., Davies, K. J., & Kelly, F. (2004). Free radical biology–terminology and critical thinking. FEBS letters, 558(1-3), 3-6.

Bacanskas, L. R., Whitaker, J., & Di Giulio, R. T. (2004). Oxidative stress in two populations of killifish (*Fundulus heteroclitus*) with differing contaminant exposure histories. Marine environmental research, 58(2-5), 597-601.

Bard, S. M. (2000). Multixenobiotic resistance as a cellular defense mechanism in aquatic organisms. Aquatic Toxicology, 48(4), 357-389.

Borković, S. S., Šaponjić, J. S., Pavlović, S. Z., Blagojević, D. P., Milošević, S. M., Kovačević, T. B., Radojičić, R. M., Spasić, M. B., Žikić, R. V., & Saičić, Z. S. (2005). The activity of antioxidant defence enzymes in the mussel *Mytilus galloprovincialis* from the Adriatic Sea. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 141(4), 366-374.

Crestani, M., Menezes, C., Glusczak, L., dos Santos Miron, D., Lazzari, R., Duarte, M. F., Morsch, V. M., Pippi, A. L., & Vieira, V. P. (2006). Effects of clomazone herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*. Ecotoxicology and Environmental Safety, 65(1), 48-55.

Da Fonseca, M. B., Glusczak, L., Moraes, B. S., de Menezes, C. C., Pretto, A., Tierno, M. A., Zanella, R., Gonçalves, F. F., & Loro, V. L. (2008). The 2, 4-D herbicide effects on acetylcholinesterase activity and metabolic parameters of piava freshwater fish (*Leporinus obtusidens*). Ecotoxicology and Environmental Safety, 69(3), 416-420.

Dar, S. A., Yousuf, A. R., Ganai, F. A., & Bhat, F. A. (2015). Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathways in freshwater cyprinid fish crucian carp (*Carassius carassius* L.). Chemosphere, 120, 273-283.

Das, P., Ayyappan, S., Das, B., & Jena, J. (2004). Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of Catla catla, Labeo rohita and Cirrhinus mrigala. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 138(1), 3-10.

Dröge, W. (2002). Free radicals in the physiological control of cell function. Physiological reviews.

Eriksson, S. P. (2000). Temporal variations of manganese in the haemolymph and tissues of the Norway lobster, Nephrops norvegicus (L.). Aquatic Toxicology, 48(2-3), 297-307.

Fatima, M., Ahmad, I., Sayeed, I., Athar, M., & Raisuddin, S. (2000). Pollutant-induced over-activation of phagocytes is concomitantly associated with peroxidative damage in fish tissues. Aquatic Toxicology, 49(4), 243-250.

Gohil, D. R., Gadekar, S. B., & Sharbidre, A. A. (2012). Multibiomarker approach in *Oreochromis mossambicus* (Peters, 1892) fish to assess the impact of pollution in Mula River.

Gül, Ş., Belge-Kurutaş, E., Yıldız, E., Şahan, A., & Doran, F. (2004). Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. Environment International, 30(5), 605-609.

Haider, M. Z., Ahmed, S., Sial, N., Afzal, G., Riaz, A., Asif, A. R., Mehmood, T., Rasheed, A., Muhammad, S. A., & Ahmad, H. I. (2022). Avian Diversity and Abundance of Taunsa Barrage Ramsar Site in Punjab, Pakistan. Journal of Zoological Systematics and Evolutionary Research, 2022.

Huang, D., Zhang, Y., Song, G., Long, J., Liu, J., & Ji, W. (2007). Contaminants-induced oxidative damage on the carp Cyprinus carpio collected from the upper Yellow River, China. Environmental monitoring and assessment, 128, 483-488.

Kaur, M., & Jindal, R. (2017). Oxidative stress response in liver, kidney and gills of *Ctenopharyngodon idellus* (cuvier & valenciennes) exposed to chlorpyrifos. MOJ Biology and Medicine, 1(4), 103-112.

Li, X., Liu, Y., Song, L., & Liu, J. (2003). Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. Toxicon, 42(1), 85-89.

Livingstone, D. (2003). Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Revue de Médecine Vétérinaire (France).

Luoma, S. N., & Rainbow, P. S. (2008). Metal contamination in aquatic environments: science and lateral management. Cambridge university press.

Lynch, A. J., Myers, B. J., Chu, C., Eby, L. A., Falke, J. A., Kovach, R. P., Krabbenhoft, T. J., Kwak, T. J., Lyons, J., & Paukert, C. P. (2016). Climate change effects on North American inland fish populations and assemblages. Fisheries, 41(7), 346-361.

Maduenho, L. P., & Martinez, C. B. (2008). Acute effects of diflubenzuron on the freshwater fish *Prochilodus lineatus*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 148(3), 265-272.

Marcogliese, D. J., Brambilla, L. G., Gagné, F., & Gendron, A. D. (2005). Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch Perca flavescens. Diseases of aquatic organisms, 63(1), 77-84.

Narra, M. R. (2016). Single and cartel effect of pesticides on biochemical and haematological status of Clarias batrachus: A long-term monitoring. Chemosphere, 144, 966-974.

Naz, H., Abdullah, S., Abbas, K., Hassan, W., Batool, M., Perveen, S., Maalik, S., & Mushtaq, S. (2019). Toxic effect of insecticides mixtures on antioxidant enzymes in different organs of fish, *Labeo rohita*. Pakistan Journal of Zoology, 51(4), 1355.

Naz, H., Abdullah, S., Ahmed, T., Abbas, K., & Ijaz, M. (2021). Regression analysis for predicting the duration dependent response of oxidative stress dynamics and nuclear anomalies in Catla Catla exposed to chlorpyrifos and endosulfan. JAPS: Journal of Animal & Plant Sciences, 31(4).

Ojha, A., & Tiwary, D. (2021). Organic pollutants in water and its health risk assessment through consumption. In Contamination of water (pp. 237-250). Elsevier.

Oruç, E. Ö., & Üner, N. (2000). Combined effects of 2, 4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Oreochromis niloticus*. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 127(3), 291-296.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., & Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Science of the total environment, 309(1-3), 105-115.

Sharbidre, A. A., Metkari, V., & Patode, P. (2011). Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, Poecilia reticulata. Pesticide Biochemistry and Physiology, 101(2), 132-141.

Soares, S., Martins, H., Gutiérrez-Merino, C., & Aureliano, M. (2008). Vanadium and cadmium in vivo effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 147(2), 168-178.

Toni, C., Ferreira, D., Kreutz, L. C., Loro, V. L., & Barcellos, L. J. G. (2011). Assessment of oxidative stress and metabolic changes in common carp (*Cyprinus carpio*) acutely exposed to different concentrations of the fungicide tebuconazole. Chemosphere, 83(4), 579-584.

Velkova-Jordanoska, L., Kostoski, G., & Jordanoska, B. (2008). Antioxidative enzymes in fish as biochemical indicators of aquatic pollution. Bulgarian Journal of Agricultural Science, 14(2), 235-237.

Yin, Y., Jia, H., Sun, Y., Yu, H., Wang, X., Wu, J., & Xue, Y. (2007). Bioaccumulation and ROS generation in liver of *Carassius auratus*, exposed to phenanthrene. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 145(2), 288-293.

Yonar, M. E. (2012). The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*, W.). Fish & shellfish immunology, 32(6), 994-1001.