INT. J. BIOL. BIOTECH., 20 (2): 183-193, 2023.

GLUTATHIONE S-TRANSFERASE AND PROTEIN CONTENTS AS A BIOMARKERS OF OXIDATIVE STRESS IN MAHSEER FISH (*TOR MACROLEPIS*)

Sajad Khan[1], Faiza Yaseen[1], Abid Salamat[1], Saba Zahra[1], Afshan Mehreen[1], Wajid Ali[1†], Muhammad Faisal[1], Shahnaz Salamat[1], Khadija Nawaz[2†], Delima Samanmali Rathnayake[1], and Syakirin Syafiqa Binti M Idris[2]

[1]*College of Marine Life Sciences, Ocean University of China, China*
[2]*Faisalabad Agriculture University, Faisalabad, Pakistan.*
Corresponding author: Shahnazsalamat704@yahoo.com
Equally contribution; †

ABSTRACT

Stress directly affects many biological processes and damaging lipid peroxidase and proteins membrane, inactivation of enzyme and DNA breakage. In the tissues of fish there are several antioxidants mechanisms (to say Catalase (CAT) and glutathione-S-transferase enzymes (GST) that are involved for the protection of the organism from oxidative stress. This study aims to explore GST as a biomarker of oxidative stress in fish Mahseer (*Tor macrolepis).* Different organ samples of the fish i.e., liver, heart, gills, kidney and muscle were collected from three different regions of River Jhelum. Liver showed high GST activity (8.0 ± 0.2 U/mL) while muscle showed low GST activity (1.1 ± 0.2 U/mL). The overall activity of glutathione-S-transferase was recorded at 340 nm wavelength through spectrophotometer. The protein contents were also estimated for all samples, Gills organs contained the highest protein contents (2.544 mg/mL), whereas muscle with low (0.762 mg/mL). The study concluded that evaluation of antioxidant enzyme i.e., GST is certainly useful to understand environmental stress in *Tor macrolepis.*

Key words: Heavy metals, Reactive oxygen species, Catalase, Oxidative stress.

INTRODUCTION

The aquatic environment can be changed by different kinds of factors which might be external or internal. The improper discarding and discharge of wastes substances into rivers increase the environmental pollution and cause stress in the aquatic organisms (Pandey *et al*., 2003). Many different substances such as pesticides, chemical substances, hydrocarbons and heavy metals are known as pollutants (Jabeen *et al*., 2011). This kind of pollutants can change the quality of water, which is living place of various aquatic organisms (Donohue *et al*., 2006). Thus, the organisms effected badly due to change in quality of water (Sarwar *et al*., 2007; Sabae *et al*., 2014).Pollution causing substances like pesticides can induce impact on organisms by creating free radicals of oxygen that initiate degenerative processes and cause oxidative stress (Tagliari *et al*., 2004). The phenomenon of higher reactive oxygen species (ROS) production and oxidative stress related to the toxicity in the fish liver, whereas the physiological and biochemical analysis of these ROS use for the indication of toxicity which is beneficial for diagnosing its effect and diseases (Tejeda *et al*., 2007). Many biological processes is affected by oxidative stress such as membrane damage of lipid peroxidase and proteins, inactivation of enzyme and DNA breakage (Halliwell and Gutteridge, 2007). Fish tissues are awarded with antioxidant defense mechanisms including CAT, GST enzyme for provide protection from the oxidative stress which is induced by metals (Bhasha and Rani, 2003). The use of biomarkers is a method to assess the damage caused by increasing pollution present in water bodies. Few biomarkers contain assays at the biochemical level to estimate oxidative stress damage due to (ROS) in macromolecules (Barata *et al*., 2005). Heavy metals including manganese, iron, nickel, copper, zinc cadmium, chromium and lead, these elements induce badly impact on the growth, physiology (Hayat *et al*.,2007).

In different organs like gills, liver, muscle and brain tissues of *Tor putitora* (Mahseer), Cypermethrin caused more changes in Lipid peroxidase, Glutathione-s-transferase, Peroxidase, Catalase and glutathione reductase (Ullah *et al*., 2014). Mahseer is present in the hilly areas streams and rivers of the Indo-Pakistan. In Pakistan it is found in River Jhelum, Mangla, Chenab, Marala, River Swat, lower parts of Bara River, River Chitral and River Panjkora (Mirza and Alam, 2000). Mahseer population has been extinct from some areas (Kashmir, Nainital) and rapidly decreased over the last few decades due to which it has 'threatened'

status in India (Khan and Sinha, 2000). Oxidative stress is not the only reason for overexploitation of mahseer population but also others developmental activities such as increasing number of hydroelectric irrigation projects that are fragmented and destroying its natural habitat etc. (Khan and Sinha, 2000).
GST is a family of prokaryotic and eukaryotic enzymes of phase II. These enzymes are commonly soluble and found in the cytosolic fraction of the liver (Henson *et al.*, 2001). It helps in detoxification in many ways. These enzymes speed up the bonding of glutathione (GSH) with xenobiotic and help in the transport of hydrophobic compounds and hydrocarbon anions (Townsend and Tew, 2003). Beside GSTs vital functions in the cell also to play important role in defense mechanism of oxidative damage. In the defense mechanism against oxidative stress GST enzymes are capable in detoxification of damaging endogenous compounds such as base propenals, hydroxyl-alkenals or electrophilic xenobiotics and DNA hydro peroxides (Cnubben *et al*., 2001). Therefore, it is considered that induction of these antioxidant enzymes is useful to control environmental stress (Van der Oost *et al*., 2003). Measurement of glutathione S-transferase activity can be used as a biomarker of oxidative stress (Martinez-Gomez *et al*., 2006). Therefore, goals of the current study are to explore GST as a biomarker of oxidative stress, to study the specific activity of GST and protein contents in different organs of fish, *Tor macrolepis.* Further, we are also interested in GST activity comparison of fish organs from the samples obtained in three different sites of river Jhelum.

MATERIALS AND METHODS

Study Area

Current study is carried out in three different collection sites of Punjab province in Jehlum River (Azad Kashmir, Mangla and Chingus Rasul).

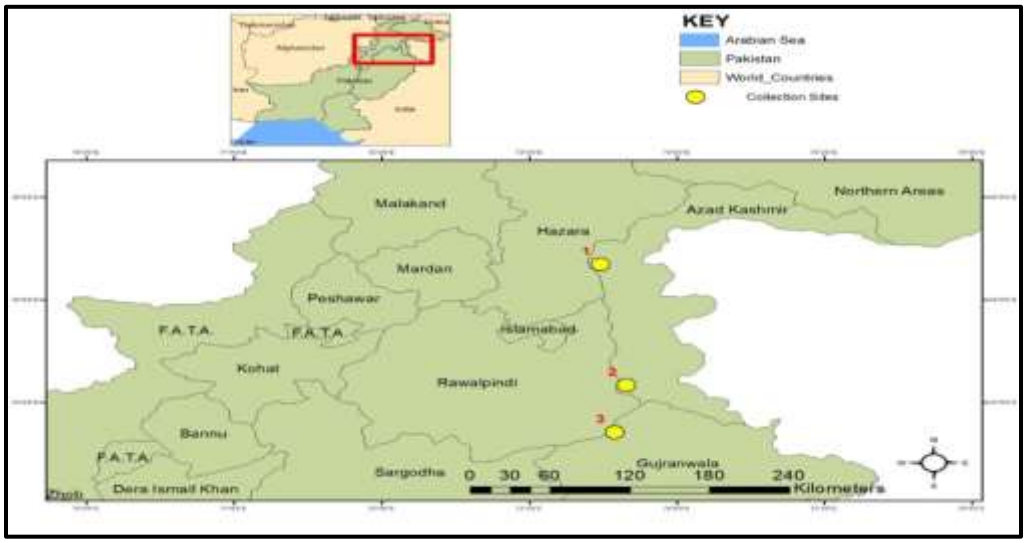

Fig. 1. Collection sites of current study.

Sample Collection

The different size of freshwater fish, Tor macrolepis samples were collected from three different sites (Azad Kashmir, Mangla and Chingus Rasul) of river Jhelum. Different organs i.e liver, gills, kidney, heart and muscle were extracted and stored in buffer.

Determination of Physico-chemical Parameters

Water temperature, dissolved oxygen and pH were measured and recorded by electronic meter HANNA HI-99301 and HANNA HI-8424. The rest of others conditions such as total hardness, calcium, magnesium, bicarbonate, total alkalinity and CO2 were measured by following the method of A. P. H. A. (1998).

Preparation of enzyme extract

All organs were grinded and then added one gram of organ in 4ml Tris HCl buffer, the mixture is then inserted in eppendrops. Clear supernatants were stored at -80 ºC for enzyme assay after centrifugation process.

Preparation of chemicals

Preparation of 0.3 M potassium phosphate buffer, pH 6.9

10.20675 g KH_2PO_4 was taken in a flask and then 13.0635 g K_2 H PO_4 was added in it and volume was made up to 500 mL in flask by the addition of distilled water and pH was adjusted 6.9.

Preparation of 30mM substrate

0.018g CNDB was taken in dark bottle and added distilled water to make volume upto 3mL.

Preparation of 30mM Glutathione

0.027g GSH was taken in black bottle and added water to make volume upto 3ml.

Protein Content Analysis

To estimate protein contents of samples Biuret method (Gornall *et al*., 1949) was used. 500ml solution of Biuret reagent was prepared by adding 3g of Copper sulfate pentahydrate ($CuSO_4.5H_2O_2$) and 12g of Sodium potassium tartarated pentahydrate with distilled water. The final solution was 1000mL after addition of 500mL of 10% NaOH. The procedure for protein analysis first involves obtaining a standard factor and then calculating the value of protein content of the sample. For standard factors enzyme sample was taken in concentration of 0.50 mL then 0.5mL of biuret reagent was added and mixed it and incubated at 37 ^oC temperature in water bath for 15 minutes. The Optical density (OD) was recorded with the help of spectrophotometer at 540 nm wavelength. The Readings were plotted in form of graph and a standard factor was obtained. The same procedure was also conducted for analysis of protein contents in the samples (Fig. 2, 3). The protein contents were calculated by the formula.

Protein contents = Ab x S.f x D.f

Protein contents $= \Delta \times$ standard factor $\times$ Dilution factor

Where**:**

Δ: Absorbance at 540 nm

Standard factor: 5.45

Dilution factor: Added distilled water

Estimation of Enzyme activity

The enzymatic activity was calculated by the following formula

$$A=1000\times (Eexp-Econt)^3/ 9.6xVxtxc$$

Where:

- A = Enzyme activity, conjugate mol/mg protein /min
- E_{exp} = Increase of the optical density at 340 nm of the sample
- E_{cont} = Increase of the optical density at 340 nm of the blank
- 1000 = coefficient
- 3= The total volume of the mixture, ml
- 9.6 = Molar coefficient of the conjugate formation
- V = Volume of the sample, mL
- t = time, min
- c = Protein concentration

Estimation of specific activity for GST enzyme

Specific activity of an enzyme increased after each step of purification i.e. from crude extract to dialysis. From desalted to ion exchange column chromatography. Specific activity of an enzyme was measured by using the following formula:

Specific Activity = Activity of Fraction / Protein contents of Fraction

Statistical analysis

Microcomputer software by following Steel *et al*., (1996) was used for the statistical analysis of the data obtained from the experiments. This experimental data was presented as mean ± standard deviation

(Mean ± SD). Statistical analysis was implemented in SPSS statistical package programs. Two-way ANOVA was used to compare GST or protein contents variables for different organs of the fish for various sites.

RESULTS

Determination of physico-chemical parameters

The physico-chemical parameters were determined after the collection of Samples from three different regions of river Jhelum i.e., Azad Kashmir, Mangla and Chingas Rasul. The average pH was recorded as 8.51 ± 0.01 for Azad Kashmir, 7.61 ± 0.02 for Mangla and 7.26 ± 0.01 for Chingas Rasul respectively. Similarly dissolved oxygen, temperature, total alkalinity, carbonates, bicarbonates, total hardness, calcium, magnesium and carbon dioxide were measured as presented in.

Correlation among physico-chemical parameters

After determination of physic-chemical parameters it was concluded that total alkalinity of Azad Kashmir site showed significantly higher ($p \leq 0.01$) negative correlation with carbon dioxide. Total hardness demonstrated significant ($p \leq 0.05$) and negative correlation with calcium and significantly high and negative correlation with pH. Calcium showed significant and positive correlation with pH. Temperature showed significant and positive correlation with dissolved oxygen (Table 1).

In Mangla site total hardness had highly significant ($p \leq 0.01$) and positive correlation with carbon dioxide. Calcium and dissolved oxygen showed highly significant and positive correlation with pH. Magnesium showed highly significant and positive correlation with carbonates and temperature showed highly significant and negative correlation with carbonates at the level ($p \leq 0.01$). Magnesium and temperature had highly significant and negative correlation (Table 1).

Table 1. Physico-chemical parameters determination for water sampled from Azad Kashmir, Mangla and Chingas Rasul sites of river Jhelum.

Physico-Chemical parameters	Azad Kashmir	Mangla	Chingas Rasul	Overall means
Temperature (^{0}C)	14.00 ± 1.00	23.00 ± 1.00	24.00 ± 1.00	20.00 ± 1.00
Dissolved oxygen (mgL^{-1})	8.80 ± 0.02	7.30 ± 0.02	7.10 ± 0.03	7.73 ± 0.02
Cabon Dioxide (mgL^{-1})	0.95 ± 0.11	0.81 ± 0.11	0.77 ± 0.11	0.84 ± 0.11
Total Hardness (mgL^{-1})	100.11 ± 1.01	110.12 ± 1.02	115.18 ± 1.01	112.43 ± 0.01
Calcium (mgL^{-1})	53.26 ± 1.03	57.31 ± 0.87	59.22 ± 1.02	56.59 ± 0.91
Magnesium (mgL^{-1})	18.66 ± 0.39	14.85 ± 0.30	16.20 ± 0.36	13.20 ± 0.30
Total Alkalinity (mgL^{-1})	100 ± 1.00	115 ± 1.00	120 ± 1.00	111.03 ± 1.02
Carbonates (mgL^{-1})	25.35 ± 1.02	35.15 ± 0.81	39.33 ± 1.22	33.27 ± 1.01
Caronates (mgL^{-1})	74.66 ± 0.28	58.82 ± 0.27	85.66 ± 0.30	73.04 ± 0.28
pH	8.51 ± 0.01	7.61 ± 0.02	7.24 ± 0.01	7.70 ± 0.01

Heavy metals concentration

EPA (Environmental protection agency laboratories) (2010) reported that in water sample of Azad Kashmir site, heavy metals were found to be manganese (0.041 mgL^{-1}), followed by copper (0.032 mgL^{-1}), cadmium (0.022 mgL-1), chromium (0.0 mgL^{-1}), zinc (0.012 mgL^{-1}), iron (0.314 mgL-1) and nickel (0.016 mgL^{-1}). For Mangla site heavy metals concentration were manganese (0.074 mgL^{-1}), copper (0.054 mgL^{-1}), cadmium (0.057 mgL-1), chromium (0.039 mgL^{-1}), zinc (0.0 mgL^{-1}), iron (0.092 mgL^{-1}) and nickel (0.451 mgL^{-1}). Heavy metals concentration in chingas Rasul site were present as manganese (0.071 mgL-1),

copper (0.041mgL^{-1}), (cadmium 0.024 mgL^{-1}), and chromium (0.0 mgL^{-1}), zinc (0.014 mgL^{-1}), iron (0.321 mgL^{-1}) and nickel (0.021 mgL^{-1}) (Table 1).

Table 1. Heavy metals concentration (mg/L) in three different sites of river Jehlum.

Heavy metals	Azad Kashmir	Mangla	Chingas rasul
Manganese (mg/L)	0.041	0.074	0.071
Copper (mg/L)	0.032	0.054	0.041
Cadmium (mg/L)	0.022	0.057	0.024
Chromium (mg/L)	0.00	0.039	0.00
Zinc (mg/L)	0.012	0.00	0.014
Iron (mg/L)	0.314	0.092	0.321
Nickel (mg/L)	0.016	0.451	0.021

Biochemical analysis

Fish samples that collected from different organs such as liver, gills, kidney, heart and muscles were analyzed biochemically for different activities i.e., Glutathione S-transferase enzyme activity, Protein content estimation and specific enzyme activity in different organs of fish.

Glutathione S-transferase enzyme activity

The Research work was conducted for the measurement of glutathione S-transferase activity in different organs i.e., liver, kidney, heart, gills, and muscle in fish *Tor macrolepis.* The study indicated that the mean activity of glutathione S-transferase in liver of fish was recorded as 8.0 ± 0.2 U/mL followed by heart 7.1 ± 0.2 U/mL, gills 6.9 ± 0.1 U/mL, kidney 4.8 ± 0.3 U/mL and muscle 1.1 ± 0.2 U/mL for Azad Kashmir, Mangla and Chingas Rasul, respectively (Table 2 and Table 3, Fig. 3).

Table 2. Replicates for the GST activity (U /mL) determination in different organs of *Tor macrolepis.*

Sampling sites	Liver	Kidney	Heart	Gills	Muscle
Azad Kashmir	8.5	4.4	7.6	7.4	1.1
	8.9	4.8	7.8	7.2	1.2
	8.1	4.6	7.1	7.6	1.4
Mean (± SD)	8.5 ± 0.4	4.6 ± 0.2	7.5 ± 0.3	7.4 ± 0.2	1.2 ± 0.1
Mangla	8.2	5.2	7.2	6.3	0.3
	8.4	5.6	7.6	6.1	0.93
	8.6	5.8	7.4	6.5	0.6
Mean (± SD)	8.4 ± 0.2	5.5 ± 0.3	7.4 ± 0.2	6.3 ± 0.2	0.6 ± 0.3
Chingas Rasul	7.1	4.6	6.3	7.2	1.6
	7.2	4.9	6.5	7.1	1.4
	7.5	4.1	6.9	7.4	1.8
Mean (±SD)	7.2 ± 0.2	4.5 ± 0.4	6.5 ± 0.3	7.2 ± 0.1	1.6 ± 0.2

Table 3. Mean (± SD) for GST activity (U /mL) in different organs of *Tor macrolepis.*

Sampling sites	Liver	Kidney	Heart	Gills	Muscle	Overall means
Azad Kashmir	8.5 ± 0.4	4.6 ± 0.2	7.5 ± 0.3	7.4 ± 0.2	1.2 ± 0.1	5.8 ± 0.2
Mangla	8.4 ± 0.2	5.5 ± 0.3	7.4 ± 0.2	6.3 ± 0.2	0.6 ± 0.3	5.6 ± 0.2
Chingas Rasul	7.2 ± 0.2	4.5 ± 0.4	6.5 ± 0.3	7.2 ± 0.1	1.6 ± 0.2	5.4 ± 0.2
Overall means	8.0±0.2	4.8 ± 0.3	7.1 ± 0.2	6.9 ± 0.1	1.1 ± 0.2	

Result showed highest activity of glutathione S-transferase in the liver whereas lowest in the muscle organ for all three sites i.e. Azad Kashmir, Mangla and Chingas Rasul. Further, according to results glutathione S-transferase showed lowest activity in more polluted area and it was comparatively higher in less polluted site. Thus, the results concluded that Glutathione S-transferase activity shows highly significant difference among organs of fish and sites of river (Table 4).

Table 4. Analysis of variance on GST activity (U /mL) in different organs of various sites for *Tor macrolepis.*

Source of variance	Degree of freedom	Sum of square	Mean square	F-Value
Organ	4	275.990	68.9975	967.89**
Site	2	1.241	0.6203	8.708**
organ*site	8	8.638	1.0798	15.15**
Error	30	2.139	0.0713	
Total	44	288.007		

** shows highly significant value at $p \leq 0.01$

The analysis of variance for the activity of GST was performed and results showed highly significant variation in activity among the comparison for the different organs of the fish and among the river sites because p value was ≤0.01.

Protein content estimation

The protein contents in different organs (liver, kidney, heart, gills, and muscle) of fish were also estimated in the current study. The study estimated the Mean protein contents in liver 1.8 ± 0.2 mg/mL, followed by gill 7.2 ± 0.1 mg/mL, heart 6.5 ± 0.3 mg/mL, kidney 4.5 ± 0.4 mg/mL and muscle 1.6 ± 0.2 mg/mL for Azad Kashmir, Mangla and Chingas Rasul, respectively (Fig. 2).

Result estimated higher protein contents in the gills (2.544 mg/mL) whereas lower in the muscle organ (0.762 mg/mL) for all three sites i.e., Azad Kashmir, Mangla and Chingas rasul. Further, according to results protein contents showed lower in more polluted water fish as compared to less polluted water fish. Thus, the results concluded that protein contents showed highly significant difference among organs of fish and sites of river (Table 5).

Table 5. Analysis of variance for protein contents (mg/mL) in different organs of *Tor macrolepis.*

Source of variance	Degree of freedom	Sum of square	Mean square	F-Value
Organ	4	26.2826	6.57064	877389**
Site	2	4.6281	2.31403	3089958**
organ*site	8	19.7071	2.46339	328940**
Error	30	0.0002	0.00001	
Total	44	50.6180		

** shows highly significant value at $p \leq 0.01$.

The analysis of variance for the protein content of GST was performed and results showed highly significant variation in protein content among the comparison for the different organs of the fish and among the river sites because p value was ≤0.01.

Specific enzyme activity

Specific activity of the glutathione S-transferase enzyme was calculated indirectly through dividing the activity of glutathione S-transferase with protein content of the fraction. The specific activity of glutathione S-transferase has highly significant values in different organs of the fish. During the study the Mean specific activity of glutathione S-transferase for different organs were calculated as 10.50 ± 0.02 U/mg for Heart, followed by liver 5.80 ± 0.02 U/mg, kidney 5.01 ± 0.02, gills 2.80 ± 0.03 U/mg and muscle 1.90 ± 0.03 U/mg in all three regions i.e., Kashmir, Mangla and Chingas Rasul (Table 6).

Table 6. Mean (± SD) for GST specific activity (U /mg) in different organs of *Tor macrolepis*

Sampling sites	Liver	Kidney	Heart	Gills	Muscle	Overall means
Azad Kashmir	4.65 ± 0.02	2.32 ± 0.02	7.05 ± 0.02	2.24 ± 0.03	2.34 ± 0.04	3.72 ± 0.02
Mangla	6.33 ± 0.03	1.36 ± 0.03	8.63 ± 0.03	3.45 ± 0.03	1.34 ± 0.02	4.20 ± 0.02
Chingas Rasul	6.56 ± 0.03	11.36 ± 0.03	16.06 ± 0.03	2.84 ± 0.03	2.13 ± 0.03	7.79 ± 0.03
Overall means	5.80 ± 0.02	5.01 ± 0.02	10.5 0± 0.02	2.80 ± 0.03	2.80 ± 0.03	1.90 ± 0.03

The current research calculated higher activity of glutathione S-transferase enzyme in the heart (10.50 ± 0.02 U/mg) whereas lower in the muscle organ (1.90 ± 0.03 U/mg) for all three sites i.e. Azad Kashmir, Mangla and Chingas Rasul. Specific activity showed highly significant difference among organs of fish and three sites of river Jhelum (Table 7).

Table 7. Analysis of variance for GST specific activity (U /mg) in different organs of *Tor macrolepis.*

Source of variance	Degree of freedom	Sum of square	Mean square	F-Value
Organ	4	409.980	102.495	97100.6**
Site	2	147.381	73.691	69812.1**
organ*site	8	184.061	23.008	21796.8**
Error	30	0.032	0.001	-
Total	44	741.455	_	-

** shows highly significant value at p≤0.01

The analysis of variance for the specific activity of GST was performed and results showed highly significant variation in specific activity among the comparison for the different organs of the fish and among the river sites because p value was ≤ 0.01.

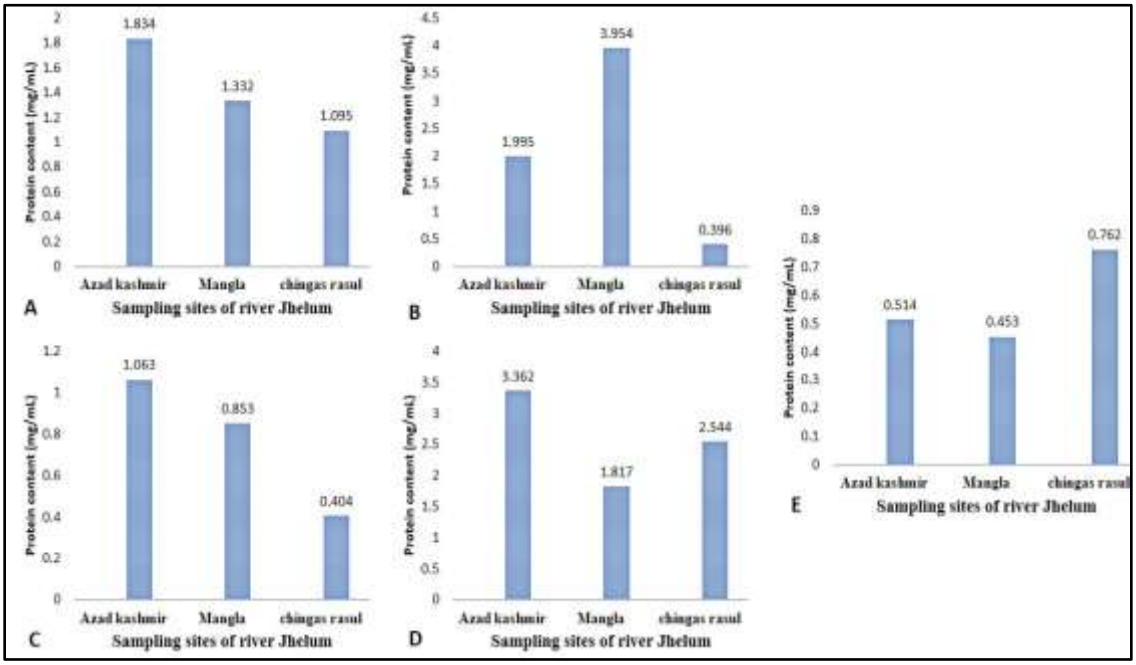

Fig 2. Graphical representation for protein contents in (A) Liver (B) kidney (C) heart (D) gills (E) muscle of *Tor macrolepis.*

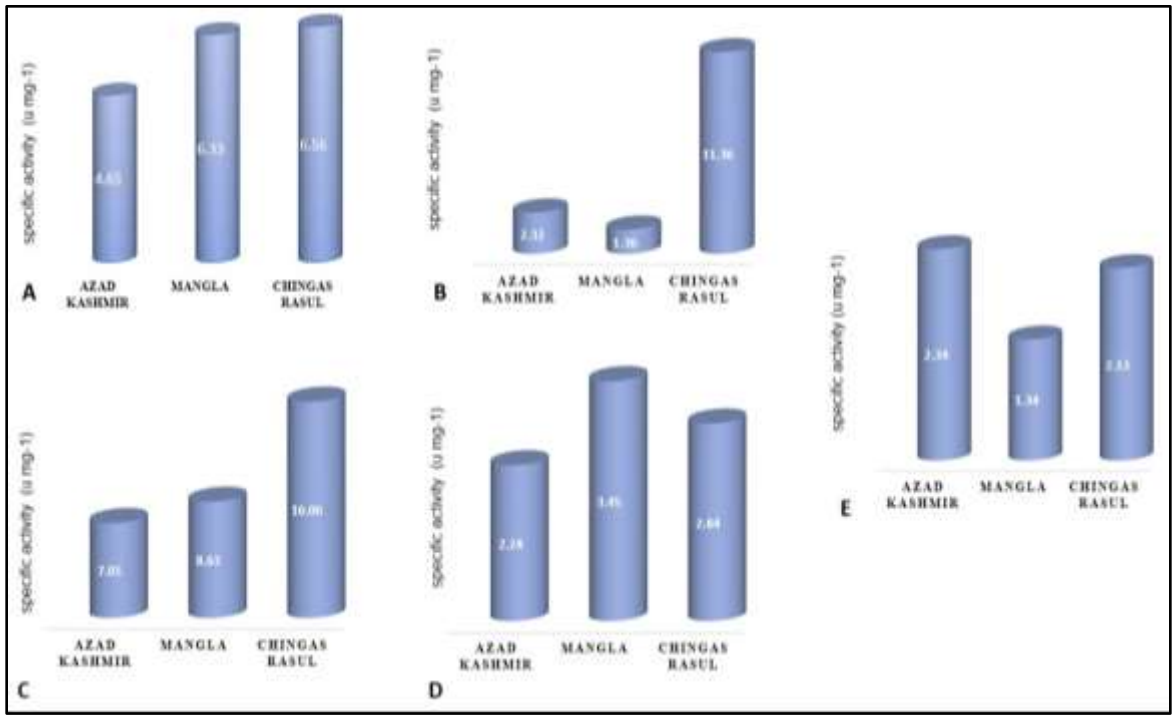

Fig 3. Graphical representation for GST specific activity in (A) Liver (B) kidney (C) heart (D) gills (E) muscle of *Tor macrolepis.*

DISCUSSION

In the current study we found that Glutathione S-transferase and protein contents act as a biomarkers of oxidative stress in fish Mahseer (*Tor macrolepis*). Different samples of fish organs were collected from three different regions i.e. Azad Kashmir, Mangla and Chingas Rasul. Although, all the measured conditions such as temperature, pH, total hardness, alkalinity and CO_2 etc. were varies from region to region and it might be due to some reasons e.g., temperature might be due to extra plants at that site, pH might be due to some factories expulsion, and low dissolved oxygen (DO) due to thermal conditions and vice versa. For the life water is one of the most significant components and life without it is not possible. Due to some natural processes and increasing anti-environmental human activities the quality of water is declining constantly and is affectation immense hazard to all forms of life (Donohue *et al*., 2006). When the basic harmony amongst antioxidants and oxidants is disturbed, Oxidative stress happens because of the reduction of oxidants or extreme aggregation of the responsive oxygen species (ROS), or both, prompting harm, it was reported by (Scandalios, 2005).

In fish tissues GST levels presented to toxins demonstrate an initial reduction took after by an expansion in its fixation. This biphasic reaction is measured as an adjustment to expanded detoxification movement (Chatterjee *et al*., 2000). Diminished enzyme action's reaction may go with a first contact to toxins, which can be trailed by prompting of antioxidant system. The presence of an inducible antioxidant system in this manner may reflect an adjustment of living beings, reported by (Dimitrova *et al*., 2001). During the current study from organs, fish liver showed highest activity of GST 8.0 U/mL followed by heart 7.1 U/mL, gills 6.9 U/mL and kidney 4.8 U/mL. Fish kidney was the organ that showed lowest activity of glutathione S-transferase 1.1 U/mL in three sites i.e., Azad Kashmir, Mangla and Chingas Rasul. The GST action in kidney tissue was observed to be lower in fish. In the study on hepatic antioxidants biomarker of freshwater catfish (*Channa punctatus* Bloch) demonstrated that activity of glutathione s-transferase is time dependent increased due to the exposure to effluent of paper mill, Pattern of antioxidants were different in kidney and gill, it showed that liver had more resistance to oxidative damage as compared to kidney and gills (Ahmad *et al*., 2000). Similarly, in 2006 Sureda and colleagues examined response of antioxidant enzymes to environmental caulerpenyne in liver of Labrid fish (*Coris julis*). The reactive oxygen species production increase with alterations in oxidant defenses levels and consequent damage to

macromolecules. Activity of Glutathione S-transferase was essentially higher in both Caulerpa stations contrasted with the *P. oceanica* reference site. Results concluded that in order to prevent oxidative damage the caulerpenyne production by Caulerpa species could be induce an antioxidant adaptation in the liver of *C. julis* (Sureda *et al*., 2006). Our results show high activity of Glutathione S-transferase for liver organ in all three locations that strengthen the evidence that liver perform a great role from the rest of other organs in response to oxidative stress. However, Ballesteros *et al*. (2009) studied the oxidative stress responses in various organs of *Jenynsia multidentata.* They assess cell reinforcement reactions of *Jenynsia multidentata* tentatively presented to sublethal groupings of endosulfan (EDS). The fundamental objective was to decide contrast in the reaction between various organs to evaluate which one was all the more extremely influenced. Results demonstrated that glutathione-S-transferase (GST) action was repressed in liver, and muscle of uncovered fish yet was instigated in cerebrum. Lipid peroxidation level was additionally expanded in cerebrum. They find that the cerebrum was the most delicate organ to oxidative harm (Ballesteros *et al*., 2009). We also estimated the protein contents during the study, the result demonstrated that in fish organs gills showed highest protein content of 2.574 mg/mL followed by kidney 2.115 mg/mL, liver 1.420 mg/mL and heart 0.773 mg/mL. Fish muscle was the organ that showed lowest protein content 0.576 mg/mL in three sites i.e., Azad Kashmir, Mangla and Chingas Rasul. The protein content was determined in fish as 1.753 mg/mL (Azad Kashmir), 1.681 mg/mL (Mangla) and 1.040 mg/mL (Chingas Rasul). The healthy condition and biological mechanism of metabolism in fish could also be analyzed by using proteins as biochemical parameters during stress caused by pollutants (Martinez *et al*., 2004). In our study the protein contents in more polluted water fish were lowered as compared to less polluted water fish. The previous study in fish *C. batracus,* also showed lowest protein contents when exposed to different toxicants or sublethal level (Rajput *et al*., 2012). Similarly, Flubendiamide exposure in L. rohita caused the changes in protein contents, reported by Nirmalaksllsgsdda and Rathnamma (2014). The diminution in the quantity of protein contents could be protective step for fish to adjust according to the new environmental circumstances that owing of stress response (Oruc *et al*., 2007). Proteins are very important. These are vital and have their preliminary importance in all cell types (Jha and Varma, 2002). Proteins play a significant role in the development of tissues and in the metabolic processes of organisms (Yeragi and Koli, 2003). Muthukumaravel *et al.* (2013) reported that proteins act as a source of energy during stressed conditions and are essential elements for growth of organisms.

Conclusion

Glutathione S-transferase (GST) is a phase II enzymes that help in detoxification in many ways. In fish such enzyme is used as a biomarker against oxidative stress. Pollutants are the main causes of oxidative stress that changes the quality of water, which is living place of aquatic organisms. In the defense mechanism against oxidative stress GST enzymes are capable of detoxification of damaging endogenous compounds. The production of such antioxidants enzymes is required for survival of the fittest fish as the function of pollution stress. It is considered that induction of these antioxidant enzymes is useful to control environmental stress. Our study has provided varied results with different organs for the antioxidative activity of GST.

REFERENCES

A. P. H. A. (American Public Health Association). (1998). *Standard method for examination of water and waste water* . (20th Edition). New York, p. 1193.

Ahmad. I., T. Hamid, M. Fatima, H. S. Chand, S. K. Jain, M. Athar and S. Raisuddin (2000). Induction of hepatic antioxidants in freshwater catfish (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure. *Biochim. Biophys. Acta*, 1523: 37-48.

Barata, C., I. Varo, J.C. Navarro, S. Arun ans C. Porte (2005). Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 140(2): 175-186.

Ballesteros, M. L., D. A.Wunderlin and M. A. Bistoni (2009). Oxidative stress responses in different organs of Jenynsia multidentata exposed to endosulfan. *Ecotoxicol. Environ. Saf.* 72: 199-205.

Basha, P. S. and A. U. Rani (2003). Cd-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicol. Environ. Saf.*, 56: 218-221.

Chatterjee, S. and S. Bhattacharya (2000). Detoxication of industrial pollutants by the glutathione S-transferase system in the liver of Anabas testudineus (Bloch). *Toxicol. Lett.* 22: 187-198.

Cnubben, N. H. P., I. M. C. M. Rietjens, H. Wortelboer, J. V. Zanden and P. J. V. Bladeren (2001). The interplay of glutathione-related processes in antioxidant defense. *Environ. Toxicol. Pharmacol.*, 10: 41-152.

Dimitrova, M. S. T., V. Tsinova and V. Velcheva (2001). Combined effect of zinc and lead on the hepatic superoxide dismutase–catalase system in carp (*Cyprinus carpio). Comp. Biochem. Physiol*., 108: 43-46.

Donohue, I., D. Style, C. Coxon and K. Irvine (2006). Importance of spatial and temporal patterns for assessment of risk of diffuse nutrient emissions to surface waters. *J. Hydrol., 304: 183-192*.

Gornall, A. G., C. S. Bardwill and M. M. David (1949). Determination of serum proteins by means of biuret reaction. *J. Biol. Chem., 177*: 751-766.

Halliwell B. and J. M. C. Gutteridge (2007). *Free radicals in biology and medicine.* New York: Oxford University Press, p. 963.

Hayat, S., M. Javed and S. Razzaq (2007). Growth performance of metal stressed major carps viz. *Catla* catla, Labeo rohita and *Cirrhina mrigala* reared under semi-intensive culture system. *Pak. Vet. J*., 27: 8-12.

Henson, K. L., G. Stauffer and E. P. Gallagher (2001). Induction of glutathione s-transferase activity and protein expression in brown bullhead (Ameiurus nebulosus) liver by ethoxyquin. *Toxicol. Sci.*, 62: 54-60.

Jabeen, G., M. Javed and H. Azmat (2011). Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. *Pak. Vet. J.*, 32: 107-111.

Jha, B. S. and B. P. Verma (2002). Effect of pesticidal mixture on protein content in the fresh water fish *Clarius batrachus*. *J. Ecotoxicol. Environ. Monit*., 12: 177-180.

Khan, M. A. and M. Sinha (2000). Status of mahseer fisheries in north and northeastern india with a note on their conservation. *Int. Fish. Soc*., 32: 28-36.

Martinez- Gomez, C., J. A. Campillo, J. Benedicto, . Fernandez, J. Valdes, I. Garcia and F. Sanchez (2006). Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra)* from the northern Iberian shelf after the Prestige oil spill. *Mar. Pollut. Bullet.*, 53: 305-314.

Martinez, C.B.R., M.Y. Nagae, C.T.B.V Zaia and D.A.M Zaia (2004). Morphological and physiological acute effects of lead in the neotropical fish, Prochilodus lineatus, *Brazilian Journal of Biology*, 64: 797.

Mirza, M. R. and S. K. Alam (2000). Ichthyoregions of Indus river. *Sci. Int*., 12: 143-149.

Muthukumaravel, K., B. Sivakumar, P. Kumarasamy and M. Govindarajan (2013). Studies on the toxicity of pesticide monochrotophos on the biochemical constitutents of the freshwater fish Labeo rohita. *Int. J. Curr. Biochem, Biotech*., 2: 20-26.

Nirmalakallagadda and V. Rathnamma (2014). Flubendiamide a pathalic acid diamide effect on protein metabolism of freshwater fish Labeo rohita (Hamilton*). Int. J. Recent Sci. Res.*, 5: 1554-1557.

Oruc, O.E. and D. Usta (2007). Evaluation of oxidative stress response and neurotoxicity potential of diazinon in different tissues of Cyprinus carpio, *Environ. Toxicol. Pharmacol*., 23: 48.

Pandey, S., S. Parvez, I. Sayeed, R. Haque, B. Bin-Hafeez and S. Raisuddin (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu. *Sci. Environ.*, 309: 105-115.

Rajput, V., S.K. Singh, Arpita, Kirti and Abhishek (2012). Comparative toxicity of Butachlor, Imidacloprid and Sodium fluoride on protein profile of the walking catfish Clarias batrachus. *Journal of Applied Pharmaceutical Science*, 2 (6): 121-124. DOI:10.7324/JAPS.2012.2629.

Sabae, S. Z., M. M. Sheekh, M. A. Khalil, W. A. E. Elshouny and H. M. Badr (2014). Seasonal and regional variation of physicochemical and bacteriological parameters of surface water in el-bahr el-pherony, menoufia, Egypt. *World J. Fish. Mar. Sci.*, 6: 328-335.

Sarwar, S., F. Ahmad and J. Khan (2007). Assessment of the quality of Jhelum river water for irrigation and drinking at district Muzaffarabad, Azad Kashmir. *Sarhad. J. Agri.*, 23: 1041-1046.

Scandalios, J. G. (2005). Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res*., 38: 995-1014.

Steel, R. G. D., J. H. Torrie and D. A. Dinkey (1996). *The principles and the procedures of Statistics*, 2nd Ed. McGraw Hill Book Co., Singapore. p. 627.

Sureda, A., A. Box, M. Ensenata, E. Alou, P. Taulera, S. Deuderob and A. Pons (2006). Enzymatic antioxidant response of a labrid fish (Corisjulis) liver to environmental caulerpenyne *Comp. Biochem. Physiol.,* 144: 191-196.

Tagliari, K. C., R. Cecchini, J. A. V. Rocha and V. M. F. Vargas (2004). Mutagenicity of sediment and biomarkers of oxidative stress in fish from aquatic environments under the influence of tanneries. *Mutat. Res*., 561: 101-117.

Tejeda, V. R., E. L. Lopez and J. E. S. Diaz (2007). Biomarkers and bioindicators of the health condition of Ameca splendens and Goodeaa tripinnis (Pisces: Goodeaidae) in the american river. *Mexico. Environ. Int.,* 33: 521-531.

Townsend, D. M. and K. D. Tew (2003). The role of glutathione s-transferase in anti-cancer drug resistance. *Oncogene*, 22: 7369-7375.

Ullah, R., A. Zuberi, S. Ullah, I. Ullah and F. U. Dawar (2014). Cypermethrin induced behavioral and biochemical changes in mahseer, *Tor putitora. J. Toxicol. Sci*., 39: 829- 836.

Van der Oost, R., J. Beyer and N. P. Vermeulen (2003). Fish bioaccumulation and biomarkers in environmental risk assessment. *Environ. Toxicol. Pharmacol*., 13: 57-149.

Yeragi, S. G. and V. A. Koli (2003). Effect of pesticide malathion on protein metabolism of marine crab Uca marionis. *J. Ecotoxicol. Envior. Monit.,* 10: 59-62.

(*Accepted for publication March 2023*)