Journal of Positive School Psychology http://journalppw.com
2023, Vol. 7, No. 6, 119-128

Comparative Study Of Catalase Activity Of Normal And Heavy Metal Stressed Liver Of Labeo Rohita

Amara Akhtar[1], Sajid Abdullah[1], Muhammad Ahmed Saqib[1] , Ayesha Nawaz[1], Tayyaba Mehmood[1] , Saima yasin[1], Hira Akbar[1], Areej[1]

[1]Department of Zoology, University of Agriculture, Faisalabad, Pakistan.
Corresponding Author : Amara Akhtar

ABSTRACT

Heavy metal contamination of water cause toxicity and production of ROS (reactive oxygen species) in the fish body which react with the vital biomolecules of the cells resulted in lethal effects and fish mortality. Catalase (CAT) is an oxido-reductase enzyme and important component of antioxidant defense mechanism, inhibit the production of these free radicals. The work is aimed to determine catalase (CAT) enzyme activity in heavy metal (Pb + Cr) exposed liver of Labeo rohita (L.rohita). Significant difference of about 15.3 % was found in specific activity of catalase (CAT) in normal and metal stressed fish liver with values upto 88.00 U mg^{-1} and 74.50 U mg^{-1}, respectively. The enzyme purity of metal stressed and normal liver was found upto 1.00-fold with 100 %age recovery and was 0.99-fold purified with 99 % age recovery, respectively. However, maximum enzyme activity was found at pH 7 and 25°C up to 119.5 U mL^{-1} and 129 U mL, pH range between 4 to 12. Statistical analysis showed all data significantly different with $P<0.01$.The main aim of this study was to determine the response of Labeo rohita fish liver catalase (CAT) enzyme to external stress induced by heavy metal stressor i-e chromium and lead in order to explore changes in general pattern of its activity. Catalase plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells and in cells to prevent the drug-induced consumption of O2 either for destroying H_2O_2 to oxygen or for direct interaction with the drug. Therefore, high catalase activity which is good indicator of oxidative stress might lead to the consequences of respective metal deposition in human body and will adversely affect their health.

INTRODUCTION:

Metals, especially heavy metals, are drastic contaminants of aquatic environments worldwide, which is in continuous increase due technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow 2008). Metal toxicity level in aquatic bodies depends on its types and its ability to dissolve in aquatic reservoir. Metals can influence the organisms living in aquatic environment both in positive as well as in negative metal. (Jezierska and Witeska 2001). PH is an important factor for the solubility of metallic compounds (Corey, 1990)

Many pollutants are present in aquatic environment. Fish are widely exposed to Xenobiotics as well as other groups of contaminants at the same time. There are different ways by which organisms can expose to metals and in turn to metal toxicity. Skin,

alimentary canal and gills are the sources by which fish can take up metals in the body (Kamunde et al., 2002).

As a result of oxidation of cells, reactive oxygen species (ROS) or free radicals are produced. These reactive oxygen species and free radicals may act as harmful agents for the cells and tissues. Different animals have different antioxidant defense systems which can be enzymatic as well as non-enzymatic (Crockett, 2008). Metals can cause cellular oxidation and it is very important to assess the harm caused by oxidation in fish which can give information about metal pollution in water bodies (Javed, 2015).

Catalase (EC 1.11.1.6 pH ≥7.0) is oxido-reductase tetramer enzymes that contain four equally sized subunits and each subunit contain one ferric heme prosthetic group. Primarily catalase is found in peroxisomes. Catalase act as catalyst in the breakdown of hydrogen peroxide (H_2O_2) to oxygen (O_2) and water (H_2O), and give defense against the lethal effects of radicals that are produced due to oxygen. In many oxidase catalyzed reactions, highly reactive oxygen species (ROS) is produced in large number as byproducts in electron transport chain. Catalase enzyme is present in all organisms that respire aerobically (Gaetani and Ferraris, 1996).

Sabullah and Ahmed, 2015 compared short and long time exposure effect with different concentrations of metal on fish by using sensitive biomarker and reported increase in biological risk increase particularly with increase in heavy metal load.

Singh et al., 2012; Saliu and Bawa-Allah, 2012; Han et al., 2013 reported environmental risk of heavy metal contamination in aquatic organisms on the basis of in vivo examination. Fish exposed to heavy metals of different concentrations and time duration reveal the induction of metal stress and production of free radicals lead to programmed cell death. However, Tripathi and Gaur, 2004 reported penetration of these toxicants in cells activate the detoxification function with the help of enzymes by neutralizing xenobiotics to non-toxic compounds. Antioxidant enzyme which are present in lysosomes plays a very vital role in protecting cells against free radicals production as well as in homeostasis balance to remove these reactive oxygen species.

Chromium compounds are used in ferrochrome production, electroplating, pigment production, and tanning. These industries, together with the burning of fossil fuels and waste incineration are sources of chromium in air and water. Chromium is ubiquitous in nature (WHO, 1988). The most biologically important oxidative states of chromium are trivalent (Cr III) and hexavalent (Cr VI). The trivalent and hexavalent forms of chromium are involved in redox cycling (Stohs and Bagchi, 1995). The hexavalent form can be reduced to the trivalent form. This transformation is considered to be a major means of detoxification of Cr (VI) in biological systems. Chromium (III) plays a stimulatory role in physiological glucose metabolism. Chromium (VI) actively enters cells through an anion (phosphate) transport mechanism. Chromium (III), meanwhile, is not able to use this mechanism (Valko et al., 2005).

Lead is a major environmental pollutant. Paint, cosmetics, human medicines, food supplements, and petroleum-based fuels are sources of lead pollution (Stohs and Bagchi, 1995). Lead accumulation in sediment is of significance for aquatic organisms. Lead is not a transition metal and cannot readily undergo valence changes. Lead can induce oxidative damage through direct effects on the cell membrane, interactions between lead and haemoglobin, which increase the auto-oxidation of haemoglobin, auto-oxidized δ-aminolevulinic acid, interactions with Glutathione reductase (GR) or through the formation of complexes with selenium, which decrease Glutathione peroxidase (GPx) activity (Ercal et al., 2001).

Antioxidant enzymes are focused in present study are present naturally in lysosome in liver and kidney. However, liver catalase enzyme is focused in present study due to its importance as all the vital biochemical processes or metabolism is carried out in liver. Catalase convert hydrogen peroxide in oxygen and water hence works in detoxification. Due to this activity catalase is more present in liver than any other organ as it aids liver in detoxification of Reactive Oxygen Species (ROS).

In the present study was aimed to investigate effect of chromium and lead by comparing catalase activity in controlled and metal stressed L. rohita liver due to its increasing concentration, anticipated role as indicator of biological stress and indirect hazardous health effects not only in near former mine sites, dumps, tailing piles, and impoundments, but also in urban areas and industrial centers. Moreover these heavy metals were selected for further examination due to its potential for human exposure and increased human health risk (McKinney and Rogers, 1992). It was emphasized by Gül et al., (2004) that catalase activity would be a sensitive biomarker for aquatic bodies oxidative stress before adversely affecting fish health. Therefore the present study was conducted to see the effect of lead and chromium on activity of catalase in L. rohita liver which is involved in antioxidant defense mechanism of the body and inhibits the production of free radicals by converting H_2O_2 in O_2 and H_2O.

1) Materials and Methods:

2.1 Experimental Animals:

The fingerlings of freshwater fish L. rohita were purchased from the Fish Seed Hatchery, Faisalabad and transferred to Fish Farm at University of Agriculture, Faisalabad. Fingerlings were acclimatized to the laboratory conditions for one week. The fish fingerlings were placed in glass aquaria with tap water having pH (7.0) temperature (25°C), dissolved oxygen (5.65 mg L^{-1}) and total hardness (200 mg L^{-1}) throughout study period by following methods of A.P.H.A (1998). The fish fingerlings were fed with standard fish food twice a day and were exposed with 12hrs light and dark conditions during the acclimatization.

2.2) Preparation of Metal Solution:

Stock solution of 1000ppm of metal was prepared by dissolving, 6.72 g and 8.95 g pure compounds of lead chloride ($PbCl_2$) and chromium chloride ($CrCl_2$), respectively in 1000ml of deionized water. For investigation of metal mixture effect on fish fingerling 18.34mL of this stock solution was introduced in the aquarium periodically within 6hrs duration to avoid fish mortality.

2.3) Isolation of Catalase enzyme:

Liver was dissected out after 2 weeks trial for isolation of catalase enzyme. For this purpose phosphate buffer (pH 7.4) has been added in 4 times greater as compared to the weight of tissue in 1:4 ratio. The mixture was homogenized with pestle and mortar for 15mins and homogenized tissue was extracted by passing through muslin cloth. After filtration with Whattman filter paper no 1, the filtrate was centrifuged in refrigerated centrifuge at 10, 000 rpm per for 15 minutes at 4°C. Both sediment and supernatant were stored after adding 10% glycerol at 4°C until further analysis. All experiments were performed in triplicates.

2.4) Total Protein Content Estimation:

Protein content was determined by biuret method by using bovine serum albumin (BSA) as standard protein. Briefly, for a given sample 1.0 ml was taken in test tube and 9ml of biuret reagent was added. Then it was allowed to stand at RT for 20 mins after immediate vortexing. Optical density (OD) was recorded with the help

of spectrophotometer at 540 nm wave length. (Gornall et al, 1949).

2.5) Analysis of catalase enzyme activity (assay):

The activity of catalase was determined by measuring its ability to decompose H_2O_2 concentration at 240 nm by using Bergmeyer (1974) modified method (Chance and Mehaly, 1977). Briefly, **Volume** of 50 mM phosphate buffer (pH 7.0) and **Volume** 10 mM hydrogen peroxide were added to prepare buffer substrate solution. Then 0.05 mL enzyme extract was added in test tube containing buffer substrate solution and allowed to stand for 3mins so that reaction could take place. The absorbance was taken against phosphate buffer solution as blank at 240nm with **1.0min** interval.

2.6) Purification of Catalase Enzyme:

The purification of catalase enzyme from liver was carried out by a) Partial purification by Ammonium Sulfate precipitation b) Ion exchange chromatography c) Gel filtration chromatography for comparison.

2.6.1) Partial purification by Ammonium Sulfate precipitations:

Ammonium sulfate precipitations was further carried out by Salting in and Salting out methods. (Nakamura et al 2000) Briefly, crude extract of enzyme was saturated with 25 % ammonium sulfate (powder) in salting in, refrigerated for four hours at 4 ^{o}C and was centrifuged at 10,000 rpm for 15 min at 4°C. Obtained pellet was stored at 4°C for further analysis. While supernatant was subjected to salting out method. Saturation was adjusted upto 50 % and 35 g of solid ammonium sulfate was added by shaking. The mixture allowed to stand at 4°C overnight and centrifuged at 10,000 rpm for 15 minutes. Obtained supernatants and residues were dissolved in phosphate buffer (pH 7.4). Obtained residue from salting out method were subjected to dialysis by using dialysis bag. Precipitated out protein samples was dialyzed against low ionic strength phosphate buffer (pH 7.4). All of the obtained supernatant, sediments and desalted sample were subject to enzyme essay for measuring enzyme activity and protein contents estimation.

2.6.3) Purification of enzyme by Ion Exchange Chromatography:

After dialysis, enzyme was purified by ion exchange chromatography following the method of (Zia et al. 2007).

The column of DEAE-cellulose (diethyl amino ethyl-cellulose) was prepared and used. After preparation, column was washed with 30 mL of 0.5 M NaOH solution by passing distilled water through the column to maintain pH at 7. The same procedure was repeated with 30 mL of 0.5 N HCl and distilled water. Equilibration of column was done with phosphate buffer of pH 7.4 (10 mM) by passing it through the column until the pH of elute become equal to that of the buffer (pH 7.4). 0.25 mL of the desalted sample was applied on top layer of the column bed to avoid column disturbance and allowed to penetrate. The sample was eluted out with the help of 10mM phosphate buffer (pH 7.4) for keeping the drop rate constant. Same procedure was adopted for collection and further analysis of 50 fractions with 2mL of elute.

Optical density of all the fractions were obtained at 280 nm against blank (buffer) and plotted a graph between fractions vs. absorbance. Fractions having higher peaks or having higher protein contents were select for enzyme assay and protein contents determination. The activity of catalase enzyme calculated as:

$$\text{Activity}\left(\frac{\text{Units}}{\text{mL}}\right) = \frac{\frac{\Delta A}{\text{min}} \times \text{dilution} \times 2\ \text{mL}}{0.04\ \text{mM} - 1\text{cm} - 1\ \times 0.05\text{mL}}$$

2.6.4) Purification of enzyme by Gel filtration Chromatography:

The column of Sephadex G-150 was prepared by the method of (Zia et al. 2007). Briefly, 1 g of dry Sephadex G-150 was suspended in 15 mL of phosphate buffer and heated in a water bath at 95°C for 3 hours without drying. The column (1×20 cm) was set in vertical position at some stable place. Outlet was filled with distilled water and the slurry was poured into it. It was left undisturbed overnight so that water and gel layer separated distinctly. 10 mM phosphate buffer (pH 7.4) was transferred at top of the column by using glass pipette. pH of the column was checked until pH of inlet buffer and outlet became equal. 0.25 mL of sample (with highest specific activity after ion exchange chromatography) was applied for analysis and after the penetration of the sample 50 fractions of 2 mL each were taken with 10 mM phosphate buffer (pH 7.4). Optical density of all the fractions at 280 nm against blank were noted and then a graph between fraction and absorbance was plotted. Enzyme essay and protein estimation was performed to get the activity and specific activity of the fractions showed highest beak and protein contents. The activity of catalase enzyme calculated as:

$$\text{Activity}\left(\frac{\text{Units}}{\text{mL}}\right) = \frac{\frac{\Delta A}{\text{min}} \times \text{dilution} \times 2\ \text{mL}}{0.04\ \text{mM} - 1\text{cm} - 1\ \times 0.05\text{mL}}$$

2.7) Physiochemical Properties of Purified Catalase Enzyme:

Effects of pH and temperature on maximum enzyme activity of the purified catalase enzyme from (controlled and Pb+Cr stressed rohu),were observed by conducting experiments at different pH ranging from 4-12 (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5 and 12.0), temperatures from 4-66°C (4, 16, 20, 25, 30, 37, 50, 60 and 66°C).

2.8. Statistical analysis

Statistical analysis of obtained data was done by using SPSS. One-way ANOVA was performed to compare variables among controlled and metal stressed samples, and significant difference was found with ($P<0.05$).

3. RESULTS AND DISCUSSION:

The present work is a comparative study of normal and metal stressed liver of L. rohita, which was performed in terms of catalase enzyme activity, to analyze the effect of heavy metals i-e lead and chromium on fish health which can ultimately effect health of human being. Investigation of metal effect on liver of L.rohita was correlated with determination of antioxidant activity of catalase enzyme due to its innate sensitivity towards heavy metal contamination. In present work, liver was chosen for the oxidative stress study as bioindicator because according to (Xiong and Spector, 2004: Radovanovic et al., 2010) liver is responsible for body metabolism regulation and xenobiotics detoxification and the change in catalase enzyme activity could be interpreted as change in antioxidant activity.

3.1 Ammonium Sulphate Precipitation

Crude extract of metal stressed liver has 7.36 % high activity as compared to controlled Labeo rohita liver. While lowest protein contents upto 0.85 mg mL^{-1} was estimated in desalted or dialyzed sample of metal exposed L. rohita liver. Protein contents were decreased sequentially at each and every step from crude extract to dialysis (Table 1). The trend regarding sequential decrease in protein content and enzyme activity is comparable with report of Nadeem et al., (2015) while working with purification and properties of liver catalase from water buffalo. Similarly, Hussein, (2012) also observed same behavior of protein content when purification and characterized thermo-alkali stable catalase from bacillus sp.

Highest activity upto 61.00 and 68.33 and specific enzyme activity upto 88.00 and 74.50 U mg^{-1} was noted in the present during partial purification by ammonium sulphate precipitation of catalase enzyme from desalted liver fraction of controlled and metal stressed L. rohita liver, respectively. However, lowest specific activity about 62.47 U $mg^{-1}$was found from crude extract fraction of metal stressed L. rohita liver (Table 1 & 2).

Moreover, it was noted that enzyme activity of Pb+Cr exposed L. rohita liver was 10.72% higher as compared to controlled L. rohita liver of desalted/ dialyzed sample (Table 1 & 2) which could be associated with more hydrogen peroxide production. Atli et al., (2006) also described oxidative stress is reason for increased production of hydrogen peroxide and superoxide radical. The study of Wong and Whitaker, (2002) advocate results of present study by describing though high concentration of hydrogen peroxide is associated with rapid inactivation of catalase but stimulated catalase activity might be associated with activated antioxidant defense system against oxidative stress. Roche and Boge (1993) correlated catalase enzyme activity with nature of metal exposed while studying the catalase enzyme activity modification and stimulation in the sea bass erythrocytes as a result of in vitro exposure of Zn and Cr and inhibition in the presence of Cu.

Furthermore, Gül et al., (2004) and Avci et al., (2005) also observed stimulation in activities of antioxidant enzyme as a result of laboratory exposure of different organic and metal contaminants but with variation depending on species, enzymes and single or mixed exposures. It was further narrated by Roche and Boge (1993) that oxyradicals formation by metals may be linked with deterioration of the protective defense system, whereas catalase activity stimulated for compensation for other antioxidant enzymes activity deterioration.

Atli et al., (2006) concluded liver stronger candidate to face oxidative stress as compared to other tissues. This description could be supported by the fact that the liver is free radical generation and multiple oxidative reactions site. Similarly, Atli et al. (2006) reported that CAT help an animal against oxidative stress by studying CAT enzyme activity in brain, gills, liver, kidney and skin of freshwater fish tilapia L. rohita that was kept in different concentration of Ag^{+}, Cu^{+2}, Cd^{+2}, Zn^{+2} and Cr^{+6} for 96 hours and observed no CAT stimulation in liver when exposed in Ag^{+} while remaining metals stimulate liver CAT but decrease in CAT activity in kidneys and gills of L. rohita was noticed.

Moreover, fold purification which was done in present work by applying three steps including ammonium sulfate precipitation, ion exchange and gel filtration chromatography is measurement of how many folds an enzyme purified or how many times an enzyme purified after each and every step of purification from crude extract to dialysis in partial purification by using Ammonium sulfate as described by (Gornal et al. 1949).

Nonetheless, highest fold purification in the present study was noted upto 1.29 with percent recovery upto 51.32 % during partial purification by ammonium sulfate precipitation of catalase from controlled L. rohita liver desalted fraction while lowest fold purification was noticed upto 0.99 with percent recovery of 99% from crude extract fraction of again controlled fish liver. However, maximum percent recovery upto 100% was observed for crude extract of metal stressed liver. It was noticed that fold purification was increased with every step of partial purification. However, percent recovery was decreased after every step of partial purification, from crude extract to desalted sample (Table 1, 2 & 3).

Nadeem et al., (2015) reported percent recovery of catalase enzyme upto 82.9% at 1.48 folds purification of dialyzed sample after 70%

acetone saturated precipitation step while working on purification and properties of catalase from liver of water buffalo. However, Hussein, (2012) also achieved 128.5 U/mg specific activity at 1.45 fold purification with 80% product recovery as a result of ammonium sulfate precipitation.

3.2) Purification of Liver Catalase enzyme by using Ion exchange and Gel Filtration Chromatography

Fifty (50) samples of normal and metal stressed (each) L. rohita liver were subjected by Ion exchange chromatography. Among 50 samples each, sample # 30 and #17 showed highest activity and specific activity upto 51.66 U mL^{-1}, 53.66 U mL^{-1} and 249.99U mg^{-1} and 352.52 U mg^{-1} for controlled and metal stressed liver, respectively as a result of ion exchange chromatography with protein contents upto 0.218 mg mL^{-1} and 0.152mg mL^{-1}, respectively (Table 4).

Fractions with high optical density were further processed for gel filtration chromatography (Table 5). Results of present study is comparable with studies of different workers. Nadeem et al., (2015) reported percent recovery of catalase enzyme upto 30.5% at 123 folds purification after gel filtration chromatography while working on purification and properties of catalase from liver of water buffalo. However, Zhang et al., (2005) accomplished a 58.5 fold purification from crude extract of catalase by applying four step purification procedure including ammonium sulfate precipitation, ion exchange, gel filtration and hydrophobic interaction chromatography. Hussein, (2012) also achieved 1500 U/mg specific activity at 16.6 fold purification with 50% product recovery after gel filtration chromatography.

3.3) Physiochemical Properties of Purified Catalase Enzyme:

The purified enzyme from both controlled and metal stressed L. rohita liver showed maximum activity at pH 7 and 25°C temperature (Fig 1 & 2). Comparable results regarding optimum activity conditions were found from studies of different workers working on purification and characterization of catalase. Kandukuri et al., (2012); Al- Bar, (2012); Nadeem et al., (2015) reported maximum activity at pH 7 for catalase extracted from camel liver, black gram and water buffalo, respectively however latter reported comparable temperature 30°C for maximum catalase activity purified after extraction from water buffalo liver. Furthermore, Hussein et al., (2012) reported broad range of pH 6.0-10.0 for maximum activity of catalase extracted from bacillus sp.

Statistical analysis showed that there is a significant different with $p < 0.01$ between catalase activity of controlled and Pb+Cr stressed L. rohita liver (Table 3) that was kept in the aquarium with exposure of (Pb+Cr) showed higher catalase enzyme activity as compared to controlled L. rohita liver. Catalase activity also found significantly different at different pH and temperature (Table 6 & 7).

Conclusion:

The present study was conducted to explore importance of catalase enzyme activity by comparing activity and specific activity in controlled and metal stressed Labeo rohita liver in response of metal toxicity and its association with toxicity, aquatic organism sensitivity and health.

Wall and Hanmer. (1987) reported various fish antioxidant enzymes could be parameter for prediction of chronic or sub-lethal metal toxicity in aquatic organisms due to their higher sensitivity. Nonetheless, fish liver and kidney can be used as an alternative biomarker to analyse the metal toxicity level. According to Younis et al. (2013) primary stage of toxicity includes the

abnormalities in the morphology of parenchyma cells and cytoplasmic vaculation alongwith dilation depending on the time period. According to Papagiannis et al. (2004) when catalase activity was reduced due to metal exposure, its ability to protect cell from the destruction due to hydrogen peroxide was also reduced. Angel et al. (1999) also reported decrease in catalase enzyme activity with more metal polluted aquatic habitats. Therefore it was concluded that catalase activity is higher in heavy metal stressed fish than controlled fish which is antagonistically associated with specific activity of enzyme as a result of fold purification. Moreover, response of liver is variable depending on type and concentration of metal. This study emphasized that bioaccumulation of heavy metal in water bodies and in aquatic organisms can disturb whole food chain, food web and ultimately human health. It was concluded that that activity of catalase enzyme could be used as delicate biomarker for biomonitoring of aquatic habitats. Furthermore data of present study is useful contribution for future investigation, monitoring and evaluation of level of healthiness of aquatic habitats and aquatic fauna as well more specifically with respect to heavy metal effect on human health.

Acknowledgements:

AA and SSM wrote most of the manuscript. SA conceptualized the manuscript, contributed to parts and critically reviewed the manuscript. All authors read and approved the final manuscript.

REFERENCES:

1. P. H. A. (American Public Health Association). 1998. Standard method for examination of water and waste water (20 th) Ed. New York, p. 1193.
2. Al-Bar, O. A. M. 2012. Characterization of partially purified catalase from camel (Camelus
3. dromedarius) liver. African Journal of Biotechnology., 11: 9633-9640.
4. Angel, D. L., U. Fielder, N. Eden, N. Kress, D. Adelung and B. Herut. 1999. Catalase activity in macro and microorganisms as an indicator of biotic stress in coastal waters of the eastern Mediterranean sea. Helgoland Marine Research., 53: 209-218.
5. Atli, G., O. Alptekin, S. Tükel and M. Canli. 2006. Response of catalase activity to Ag^{2+}, Cd^{2+}, Cr^{6+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish Oreochromis niloticus. Comp. Biochem. Physiol., 143: 218–224.
6. Avci, A., Kaçmaz, M., Durak, İ., 2005. Peroxidation in muscle and liver tissues from fish in a
7. contaminated river due to a petroleum refinery industry. Ecotoxicology and
8. Environmental Safety. 6, 101– 105.
9. Bergmeyer H, 1974. Methods of enzymatic analysis, Deerfield Beach, FL: Verlag Chemie,
10. 3: 273–82 .
11. Chance, M. and A. C. Mehaly. 1977. Assay of catalase and peroxidase. Methods Enzymol., 2: 764-817.
12. Corey, R.B., 1990, Physical-chemical aspects of nutrient availability, in Westerman, R.L., ed., Soil Testing and Plant Analysis, third edition, Soil Science Society of America, Madison, Wis., p. 1: 11-24.
13. Crockett, E.L. 2008. The cold but not hard fats in ectotherms: consequences of lipid
14. restructuring on susceptibility of biological membranes to peroxidation, a review.
15. Journal of Comparitive Physiology. B 178, 795–809.
16. Ercal, N., H. G. Orhan and N. A. Burns. 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal induced oxidative damage. Current Topics in Medicinal Chemistry., 1: 529–539

17. Gaetani G, Ferraris A, Rolfo M, Mangerini R, Arena S, Kirkman H (1996). "Predominant role of
18. catalase in the disposal of hydrogen peroxide within human erythrocytes". Blood **87** :
19. 1595–9.
20. Gornall, A.G., Bardwill, C.S. and David, M.M., 1949. Determination of serum proteins by means
a. of biuret reaction. Journal of Biological Chemistry., **177**: 751-766.
21. Gül, Ş., Belge-Kurutaş, E., Yıldız, E., Şahan, A., Doran, F., 2004. Pollution correlated
22. modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living
23. in Seyhan Dam Lake, Turkey. Environment International. 30, 605–609.
24. Han, J., Won, E. J., Hwang, D. S., Rhee, J. S., Kim, I. C. and Lee, J. S. 2013. Effect of copper exposure on GST activity and on the expression of four GSTs under oxidative stress condition in the monogonont rotifer, Brachionus koreanus. Comparative Biochemistry and Physiology, Part C 158 : 91-100.
25. Hussein .A .A. 2012 .Purification and characterization of thermo-alkali stable catalase from bacillus sp. International research journal of biotechnology. 3: 207-214.
26. Javed M, Usmani N, Ahmad I, Ahmad M (2015) Studies on the oxidative stress and gill
27. histopathology in Channa punctatus of the canal receiving heavy metal loaded
28. effluent of Kasimpur Thermal Power Plant. Environmental Monitoring and assessment 187: 417.
29. Jezierska B, Witeska M .2001. Summary of metal-induced disturbances in fish organism. In: Metal Toxicity to Fish. Wydawnictvo Akademii Podlaskej, Siedlce.2 :214–243.
30. Kandukuri SS, Noor A, Ranjini S, Vijayalakshmi MA (2012).Purification and characterization
31. of catalase from sprouted black gram Vigna mungo seeds. Journal of Chromatogr. B Analytical Technologies in the Biomedical and Life Sciences 15:889-890
32. Kamunde C, Clayton C, Wood CM .2002. Waterborne vs. dietary copper uptake in rainbow trout and the effects of previous waterborne copper exposure. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 283, R69–R78.
33. Luoma, S. N. and P. S. Rainbow. 2008. Metal Contamination in Aquatic Environments: Science and Lateral Management. Cambridge University Press, Cambridge. Chapter 7. Trace metal bioaccumulation. Uptake processes. pp. 129-141.
34. McKinney, James, and Rogers, Ron, 1992, Metal bioavailability: Environmental Science and Technology, v. 26, p. 1298-1299.
35. Nadeem MS, Khan JA, Murtaza BN, Muhammad K, Rauf A (2015). Purification and properties of liver catalase from water buffalo (Bubalus bubalis). S. Asian J. Life Sci. 3(2): 51-55.
36. Nakamura, K., Watanabe, M., Sasaki, Y. and Ikeda, T., 2000. Purification and characterization of liver catalase in acatalasemic beagle dog, comparison with normal dog liver catalase. International Journal of. Biochemistry and Cell Biology., 32: 89-98.
37. Papagiannis, I., I. Kagalou, J. Leonardos, D. Petridis and V. Kalfakaku. 2004. Copper and Zinc in four fresh water fish species from lake Pamvotis. Environment International., 30: 357-362.

38. Radovanovic, T. B., S. S. B. Mitic, B. R. Perendija, S. G. Despotovic, S. Z. Pavlovic, P. D. Cakic and Z. S. Saicic. 2010. Superoxide dismutase and catalase activities in the liver and muscle of barbel (Barbus barbus) and its intestinal parasite (Pomphoryinchus laevis) from the danube river, serbia. Archives of Biological Science ., 62: 97-105.
39. Roche, H., Boge, G., 1993. Effects of Cu, Zn and Cr salts on antioxidant enzyme activities in
40. vitro of red blood cells of a marine fish Dicentrarchus labrax. Toxicol. In Vitro 7, 623–
41. 629.
42. Sabullah, M. K., Ahmad, S. A., Sulaiman, M. R., Shukor, M. Y., Syed, M. A. and Shamaan, N.
43. 2015. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction
44. and proteomics approaches. International Food Research Journal 22: 435-454.
45. Saliu, J. K. and Bawa-Allah, K. A. 2012. Toxicological effects of lead and zinc on the antioxidant enzyme activities of post juvenile Clarias gariepinus. Resources and Environment 2 : 21-26.
46. Singh, D., Katiyar, S. and Verma, A. 2012. Role of copper sulphate on oxidative and metabolic enzymes of freshwater fish; Channa punctatus. Journal of Environmental and Analytical Toxicology 2: 121
47. Stohs, S. J. and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal ions, Free Rad. Biology and Medicine., 18: 321-336.
48. Tripathi, B. N. and Gaur, J. P. 2004. Relationship between copper and zinc induced oxidative stress and proline accumulation in Scenedesmus sp. Planta 219: 397- 404.
49. Valko, M., H. Morris and M. T. D. Cronin. 2005. Metals, toxicity and oxidative stress. Current Medicinal Chemistry., 12: 1161-1208
50. WHO, 1998. Environmental health criteria. Copper. WHO, Geneva.
51. Wall T W and Hanmer RW.1987. Biological testing to control toxic water pollutants. Journal of
52. Water Pollution Control Federation ; 59: 7 – 12.
53. Wong, D.W.S., Whitaker, J.R., 2002. Catalase. Handbook of Food Enzymology. New York, USA, p. 389.
54. Xiong Y, Ma W, Spector A, Ho D (2004). "Mice Lacking Catalase Develop Normally
55. but Show Differential Sensitivity to Oxidant Tissue Injury". The Journal of Biochemistry
56. **279** :32804–32812.
57. Younis, E. M., Abdel-Warith, A. A., Al-Asgah, N. A., Ebaid, H. and Mubarak, M. 2013.
58. Histological changes in the liver and intestine of Nile Tilapia, Oreochromis niloticus,
59. exposed to sublethal concentrations of cadmium. Pakistan Journal of Zoology 45 :
60. 833- 841.
61. Zhang XQ, Xue YF, Zhao AM, Du G, Xu Z, Chen J, Ma Y (2005).Purification and
62. characterization of a monofunctional catalase from an alkaliphilic Bacillus sp. F26.
63. Sheng Wu Gong Cheng Xue Bao21:71-7.
64. Zia, M. A., K. Rahman, M. K. Saeed and F. Anjum. 2007. Thermal characterization of hyperproduced glucose oxidase from Aspergillus niger BCG-5 mutant strain. Institute of Chemical and Medical engineering.Pp: 1950-1955.